ASPECTS OF STRESS WITH PARTICULAR REFERENCE TO MYTILID MUSSELS AND THEIR PARASITES

S C WEBB BSc MSc

Thesis Presented for the Degree of

DOCTOR OF PHILOSOPHY

in the Department of Zoology

UNIVERSITY OF CAPE TOWN

January 1999

ASPECTS OF STRESS WITH PARTICULAR REFERENCE TO MYTILID MUSSELS AND THEIR PARASITES

PART IV: ASPECTS OF STRESS THEORY

PART V: SUMMARY OF STRESS SURVEY: CONCLUSIONS, CONSEQUENCES AND HYPOTHESES

PART VI: TESTS OF THEORY

---O---

ABSTRACT

Eight new species of digenea were found in a survey of *Aulacomya ater*, *Choromytilus meridionalis, Perna perna and Mytilus galloprovincialis*. *Metacercaria notobucephala* sp. nov., a bucephalid from *Choromytilus* has locality-specific prevalences ranging from 0.23% to 4.46%. It is a severe reproductive stress and reduces the sperm count by a factor of 1000. *Metacercaria notobucephala* also causes *Choromytilus* to lose ninhydrin positive substances. The gymnophallid *Metacercaria perchorupis* sp. nov. occurs in 90.7% to 100% of *Choromytilus* and in 74.83% of *Perna*. *Metacercaria A* sp. nov., a renicolid, occurs in 78.86% to 90.33% of *Choromytilus* and 44.74% of *Perna*. *Metacercaria B* sp. nov., a lepocreadid, infects from 18.3% to 43.17% of *Choromytilus* and 3.51% of *Perna*. The combined effect of these three digenea causes 10% of the variation in emersion survival time of male *Choromytilus* ($P = 0.02$). Longer surviving mussels had fewer cysts; this suggests that these parasites constitute a somatic stress. *Metacercaria columbinesis* sp. nov., a zoogonid, was found in 28.6% of *Mytilus*. The fellodistomid *Metacercaria maculatopsis* sp. nov. occurred in 0.4% of *Choromytilus*. The gymnophallid, *Metacercaria ater* sp. nov. was found in 0.125% of *Aulacomya*. A rapid technique of semi-permanent staining and fixing with acetic orcein was developed to aid the description of these worms. Juvenile pycnogonids, *Nymphonella* sp., were found in 0.15% to 7.5%. of *Choromytilus*. Nemerteans, nematodes, copepods, polychaetes and other minor symbionts are quantified in these mussels. *Mastigocoleus* sp., a shell boring cyanophyte, colonises *Mytilus* with prevalences from 1% to 94% and can reduce shell strength by 43%. The relationship between stress (amount of alga on the shell) and strain (degree of damage) is demonstrated: 61.8% of variation in damage is attributable to the extent of infection ($P = 0.001$). Stress is defined as an agent that reduces the fitness of the organism. The degree of reduction is termed strain. Fitness is an ontological statement about the organism. Schemes are proposed for rendering these concepts operational. Individual and ecosystem concepts of stress are not homologous and therefore cannot be substantively integrated. In contrast, psychological and physiological stress are deemed integrable: a scheme for this is proposed. Physical (salinity), chemical (ammonia & phenol) and biological agents (scavenging whelks) are shown to be substantively integrable as stresses since they all inhibit shell gaping and byssus production. Mussels demonstrate clear and

appropriate closure responses when in the presence of salinity, ammonia levels and *Burnupena*: all agents that are likely to be of historical selective significance. In contrast, phenol, an unfamiliar agent, elicits an inconsistent response. It is hypothesised that this difference may help distinguish natural stresses from synthetic pollutants. As predicted, the valve opening dynamics of heat stressed mussels were qualitatively and invariably different from those of non-lethal voluntary movements. Dying dynamics have increasing velocity; voluntary dynamics show maximum velocity initially. Analysis of stimuli effects, often interpreted as eustresses, show that assays covering distal as well as proximal effects and those that tend towards whole body integrations are more likely to detect the agent as deleterious. Thus any notion of positive deflections or eustress must be treated with great caution and subject to longer term tests and with more complete integrations if it is to be accepted.

KEY WORDS

Aulacomya ater, byssus production, *Choromytilus meridionalis*, cercaria, cilia activity, digenea, dynamics, emersion, feedback, fitness, *Mastigocoleus*, metacercaria, mortality, mussels, mytilids, *Mytilus galloprovincialis*, ninhydrin positive substances, *Nymphonella*, parasites, parasitic castration, *Perna perna*, pycnogonid, shell damage, strain, stress.

PART I:

INTRODUCTION

CHAPTER 1: INTRODUCTION

RATIONALE FOR THE MIXING OF PARASITES AND STRESS

Stress and parasites have not been thrown together capriciously in this study. The two have strong connections, but despite this many previous stress studies have ignored parasites entirely. Even where parasitism was considered, it was often merely as a parameter that increased with increased imposition of stress (the literature is reviewed elsewhere). Parasitism is not just a result of stress: it *is* a stress in its own right. In terms of the host, parasitism may be subsumed under stress. If it were not, then parasitism could not be distinguished from other interspecific associations such as mutualism or inquilinism. The comments of Kinne (1984 p7) are to the point: "Unfortunately the terminology employed for describing organismic responses to environmental stress - including that due to coexisting foreign free living or parasitic organisms - is inconsistent". It follows that, among other things, stress and parasite studies must find a way to render equivalent the stress that parasites exert and that posed by non-living agents.

Clearly, the mixing of parasites and stress proposed here is not arbitrary. On the contrary, this study aims to put parasites in their rightful position as stresses. The following section surveys the parasites of mussels to find suitable species for the testing of this and other ideas.

GENERAL INTRODUCTION TO MYTILIDS

'Mussel' is not a strictly scientific term; it is a label for widely disparate bivalve forms that range from members of the Family Mytilidae to *Donax serra*, the white mussel of the Family Veneroida. It has even been used as a term for all bivalves except 'oysters' (Bayne 1976). 'Mussel' is derived from the Saxon 'musculus': that which retires quickly on being disturbed. This is generally apt, but not when applied to *Perna perna,* in whose case, overt assault on the exposed mantle is required to elicit adduction. Mussels may usefully be defined as certain members of the Family *Mytilidae* which inhabit rocky shores, where they cling to hard substrata by means of byssus. Mussels may, in addition, have the faculty of forming tightly-knit beds on soft substrata. Thus not all the *Mytilidae* are mussels in this sense. Those such as *Botula* spp. and *Lithophaga* spp. bore into rocks. And the coral symbiont, *Fungicava eilatensis,* lives intimately surrounded by coral growth (Yonge 1976).

Mussels are generally triangular wedge-shaped bivalves with equal but asymmetric valves. They tend to a heteromyarian condition - one adductor muscle, the posterior, is larger than the other. The extreme of this tendency is manifest in *Choromytilus* where deletion of the anterior adductor muscle has resulted in the monomyarian condition.

A byssus anchorage is characteristic of mytilids. These threads consist of tanned protein which hardens on exposure to sea water. A gland at the side of the foot releases the unhardened protein, which runs to its tip along a posterior groove. The foot attaches the byssus threads; a pair of pedal retractor muscles and the foot's intrinsic musculature, which includes an outer layer of circular fibres, facilitate this. Once attached to the substratum, movement of the byssus is independent of the foot. Instead, it is controlled by two sets of retractor muscles: a number of posterior and a single anterior retractor.

Female mytilids have a darker gonad/mantle. Females may be yellow, salmon-pink and dark chocolate brown; males may be off-white to yellow-cream. In both sexes at maximum development the gonad spreads into the mantle and ramifies throughout the viscera and mesosoma (pers. obs.). The latter, in particular, assumes the colour of the

gonad. The advent of a new biopsy sexing technique by Jabbar & Davies (1987) has contributed to the accuracy of non-fatally sexing these animals.

Mytilids in South African waters

Some 27 mytilid species are recorded from South African coasts (Kilburn & Rippey 1982). Four are the subjects of study in this thesis. These are: *Mytilus galloprovincialis* (Lamarck), an alien newcomer; *Choromytilus meridionalis* (Krauss), the black mussel; *Perna perna* (Linnaeus) the brown mussel, and *Aulacomya ater* (Molina) the ribbed mussel. These mussels form beds of significant size in the intertidal and subtidal zones of rocky shores. These four species may be identified and distinguished from one another using the table in van Erkom Schurink & Griffiths (1990). In addition, colour figures of *Choromytilus, Mytilus* and *Perna* are to be found in Grant, Cherry & Lombard (1984).

Identification of these mussels is not quite straightforward. Although shell shape is sometimes a useful diagnostic feature, it may vary greatly in any one species. Causes of variation include crowding and age. These are described in *Mytilus edulis* by Seed (1978), whose findings are also applicable to South African mytilids. A further distortion of shell shape occurs when *Mytilus galloprovincialis* is translocated for culture. This, along with variations in shell colour, has been seen in South African waters and has added to the confusion. This is dealt with later in this chapter.

Aulacomya ater

Aulacomya may grow to 90mm (Branch, Griffiths, Branch & Beckley 1994) and forms extensive beds at the low tide mark (Day 1969). It occurs from Port Elizabeth westwards all the way up to the border with Namibia and beyond. Populations of *Aulacomya* occur in their greatest densities on the West Coast (van Erkom Schurink & Griffiths 1990). *Aulacomya ater* also occurs on the West Coast of South America (Webb pers. obs. seen at Viña del Mar, Chile). It is also reported from the Falkland Islands on the East Coast of South America (Davenport, Davenport & Davies 1984).

Perna perna

In South Africa this intertidal to subtidal mussel grows to 125mm (Branch *et al.* 1994). *Perna* occurs in the greatest density between Cape Agulhas and East London

(van Erkom Schurink & Griffiths 1990) where it is often the dominant mytilid. Very few occur to the west of Cape Point and even these may go unreported as they are often in association with *Mytilus galloprovincialis* which also has a brown form (pers. obs.; Grant *et al.* 1984). *Perna* resumes its distribution in Namibia after a gap of some thousand kilometres (Branch *et al.* 1994). *Perna* is distributed around Africa from Morocco to Mozambique (Kensley & Penrith 1973) and is also found around Malagasy, in the Mediterranean and the Red Sea. *Perna perna* also extends to Brazil (Lunetta 1969; Umiji, Lunetta & Leonel 1976) and the Caribbean, were it has been reported from Venezuela (Vélez & Epifanio 1981).

Three species of *Perna* are extant (Siddall 1980): *Perna viridis* (L.) from Asian waters, *Perna canaliculus* (Gmelin) from New Zealand (also mentioned by Hickman & Illingworth 1980) and *Perna perna* as reported above. Shafee (1989), however, reports differences in the *Perna* from the Atlantic coasts of North Africa and the Mediterranean and adopted the name *Perna picta* for *Perna* from this area. Siddall (1980) does not mention *Perna indica* Kuriakose & Nair (1976, in Stephen & Shetty 1981), which occurs on the Mangalore Coast on the west of India along with *Perna viridis*.

Choromytilus meridionalis

Choromytilus grows to 150mm (Branch *et al.* 1994) and occurs from Port Edward on the Transkei-Natal border, westwards to the border with Namibia (van Erkom Schurink & Griffiths 1990). It occurs particularly where there is upwelling of cool water (Grant *et al.* 1984) and thus its greatest population densities are on the West coast (van Erkom Schurink & Griffiths 1990). *Choromytilus* is more resistant to sand inundation than other mussels and is commonly found at the bottom of gullies and tide pools rather than on more exposed areas. The genus *Choromytilus* is predominantly from the Southern hemisphere. It occurs in South Africa, South America and Australasia. Another noteworthy member of the genus is *Choromytilus chorus* which occurs in estuaries in southern Chile (Navarro 1988) and semi-exposed headlands (Lasiak 1991).

Mytilus galloprovicialis

Mytilus reaches 140mm (Branch *et al.* 1994) and occurs intertidally from East London westwards to the border with Namibia (van Erkom Schurink & Griffiths 1990; Branch *et al.* 1994). Its greatest population densities occur on the West Coast (van Erkom Schurink & Griffiths 1990). This invasive alien has settled in South Africa, probably in the last three decades (Grant & Cherry 1985). See Hockey & van Erkon Schurink (1992) for more information on the invasive biology of this species in southern Africa. It is native of the Mediterranean Sea (Hepper 1957), Adriatic and Black Seas (Seed 1978). It has also been reported from the British Isles where it appears to be less tolerant of low salinity than the native *Mytilus edulis* (Hepper 1957). It also occurs along the Atlantic coast of France as far north as the Cherbourg Peninsula (Seed 1978).

The species status of *Mytilus galloprovincialis* is in dispute. For instance, in comparative studies of its mitochondrial DNA with that of *Mytilus edulis,* Edwards and Skibinski (1987, p547) say..."it seems inappropriate to regard them as good biological species". This is supported by King, McGrath & Gosling (1989 p355) who assert that "currently *Mytilus galloprovincialis* is not considered to merit separate specific status". And despite Seed's (1978 p466) report that, "In certain localities reproductive isolation has resulted in the evolution of two quite distinctive mussels" he adds that, "Elsewhere, however, it appears that varying degrees of interbreeding may occur and the concomitant mixing of individual characteristics can then make the separation of these mussels difficult or even impossible". Hepper (1957), in contrast, found that besides a difference in salinity tolerance *Mytilus galloprovincialis* is also considerably more resistant than *Mytilus edulis* to infestation by *Mytilicola intestinalis.* This suggests that there may be significant differences between the two.

In consequence we can treat the name as a convenient label rather than as a species *sensu stricto*. *Mytilus edulis* has been reported once in South African waters at 420m depth (Knudsen 1980). He suggested that they had become detached from the hull of a ship. The *Mytilus edulis* complex is the most widespread species in the genus. Its ambiguous relationship with *M. galloprovincialis* render its distribution of interest here. Representatives occur around the Americas from the Arctic to South Carolina on the east coast and from Alaska to California on the west Coast (Lutz & Darling

Centre 1980). It is found in Europe from the Arctic to North Africa (Seed 1978) but is absent from the Mediterranean. *Mytilus edulis* is also reported from Australia, where it occurs from Perth on the West Coast, all along the South Coast to Port Stephens on the East Coast (Wallis 1975).

Reported subspecies include *Mytilus edulis planulatus* Lamarck, found on the coasts of Australia and New Zealand (Macpherson & Gabriel 1962; in Grant *et al.* 1984). *Mytilus edulis platensis* Orbigny occurs on the eastern shores of South America (Soot-Ryen 1955 in Grant *et al.* 1984). The same mussel (as *Mytilus platensis*) is reported by Morris (1976) from Argentina. *Mytilus edulis chilensis* Hupé occurs on the western shores of South America. As *Mytilus chilensis* Hupé it is also reported from the Falkland Islands on the East Coast of South America (Davenport *et al.* 1984). *Mytilus edulis diegensis* Coe is reported from California. *Mytilus desolationis* Lamy is reported from Kerguelen in the southern Indian Ocean. *Mytilus crassitesta* Lischke is reported from Japan (Seed 1976). *Mytilus californianus* Conrad is reported by Denny (1987) from the mid intertidal zone of rocky shores of the Pacific North west.

Other mytilid genera in South Africa

Gregariella petagne, the half-hairy mussel, grows to 18mm (Branch *et al.* 1994). It occurs singly in rock crevices and on seaweed. *Barbatia obliquata,* the oblique ark shell, grows to 58mm and is found in crevices or under rocks in tidal pools (Branch *et al.* 1994). These two species may be found on beaches in the Western Cape, but they are not nearly so common as the main subjects of this study. *Arcuatula capensis* (Krauss), the estuarine mussel, grows up to 76mm and is found in brackish and estuarine water (Branch *et al.* 1994). *Brachidontes (Musculus) virgiliae* (Barnard) grows to 25mm in low salinity estuarine water. It is also a dominant species in a number of Southern Cape coastal lakes (Davies 1982, 1984). See Davies (1980) for descriptions and distributions of these two mytilids. *Septifer bilocularis* (Linn.), the ledge mussel, grows to 43mm from mid-tide down, singly or in small groups. *Modiolus auricularis,* the ear mussel, commonly grows to 50mm in low-shore pools but it can grow to over 80mm in Mozambique. Both of these mussels occur on Eastern Cape shores and progress eastwards (Branch *et al.* 1994).

MORPHOLOGICAL VARIABILITY OF *MYTILUS GALLOPROVINCIALIS* IN SOUTH AFRICA: PROBLEMS WITH IDENTIFICATION

Although environmentally mediated shell morphology changes have been noted previously in *Mytilus edulis* (Seed 1968, 1978), *Argopecten gibbus* (Clark 1976) and in *Penitella penita* (Evans 1968), this case in *Mytilus galloprovincialis* is of particular interest because the new morphs are liable to cause confusion with common native mussels. Translocation of specimens from an area of moderate wave action and tidal range to one of no wave action and small tidal range resulted in a growth step (Figures 1A, 2B, 2C, 2D & 2E). This is followed by production of darker, smooth shell. Figure 1B shows the normal profile for comparison.

Mytilus is farmed commercially in Saldanha Bay (33°S 18°E) on the West Coast of South Africa. Seed mussels are collected from pilings supporting an iron-ore loading jetty. They are then suspended on culture ropes in a sea water dam at the base of the jetty. The pilings are subject to moderate wave action and a tidal range of about two metres. The dam, which covers several hectares, is connected to the sea by a 1.5 metre diameter pipe. Wave action in the dam is absent and the tidal range is about 1 metre. In the dam, mussels are continuously submerged to maximise the growth rate.

Seed mussel shells were originally pitted and blue over most of their surface. On harvesting the mussels after approximately four months of immersion on culture ropes it was noted that much of the new shell growth was smooth and black. A step in the shell marks a growth discontinuity coincident with the time of translocation. The appearance of the growth step is very similar to, but even more marked than, that found by Seed (1968) for similarly translocated specimens of *Mytilus edulis*. This step appears to be a region where growth increments to the valve margin are in a predominantly lateral rather than distal direction. Thus the shell increases in volume without adding appreciably to its length. Spacing between growth lines in the step between the two normal shell contours indicates that here, growth rate occurs unequally. More occurs around the posterio-ventral margin of each valve. As growth proceeds, the anterio-posterior axis of each of the pre-translocated valves becomes splayed laterally and dorsally, their axes intersecting at the anterior. New growth

beyond the step approximates in form to that before translocation and the region of maximum growth rate, indicated by the greatest separation of growth lines, returns to its posterior direction.

The metallic black of the new still-water growth is very similar to the shell form of the native black mussel *Choromytilus meridionalis* and raises thoughts of possible hybridisation. This resemblance is all the more startling in mussels raised entirely in still, subtidal, conditions (Figure 2A) because their shell texture and colours are uniform and the shell step is lacking. This resemblance is particularly interesting in the light of controversy surrounding the origin and identity of *Mytilus galloprovincialis* on the South African coast (Lombard & Grant 1986; Grant *et al.* 1984).

Less commonly, a brown form of *Mytilus* was found and this bore a close resemblance to the native brown mussel *Perna*. Griffiths & Blaine (1994) report that this morph of *Mytilus* has been thus confused. No change in shell contour was evident and the mussel is uniformly coloured. Grant, *et al.* (1984) report that this form has been previously considered as a possible hybrid between *Perna* and *Choromytilus* or as a cold water ecotype of *Perna*. But they electrophoretically demonstrated it to be related to neither, and it is in fact a *Mytilus*. This form could be analogous to those found in *Mytilus edulis* (Newkirk 1980), or possibly the brown shell pigment is derived from the iron-ore fallout (clouds of iron-ore dust are released during loading) which settles over both the dam and the jetty. Despite these superficial resemblances to other species, morphological examinations revealed, in all cases, anatomical features specific only to *Mytilus galloprovincialis*.

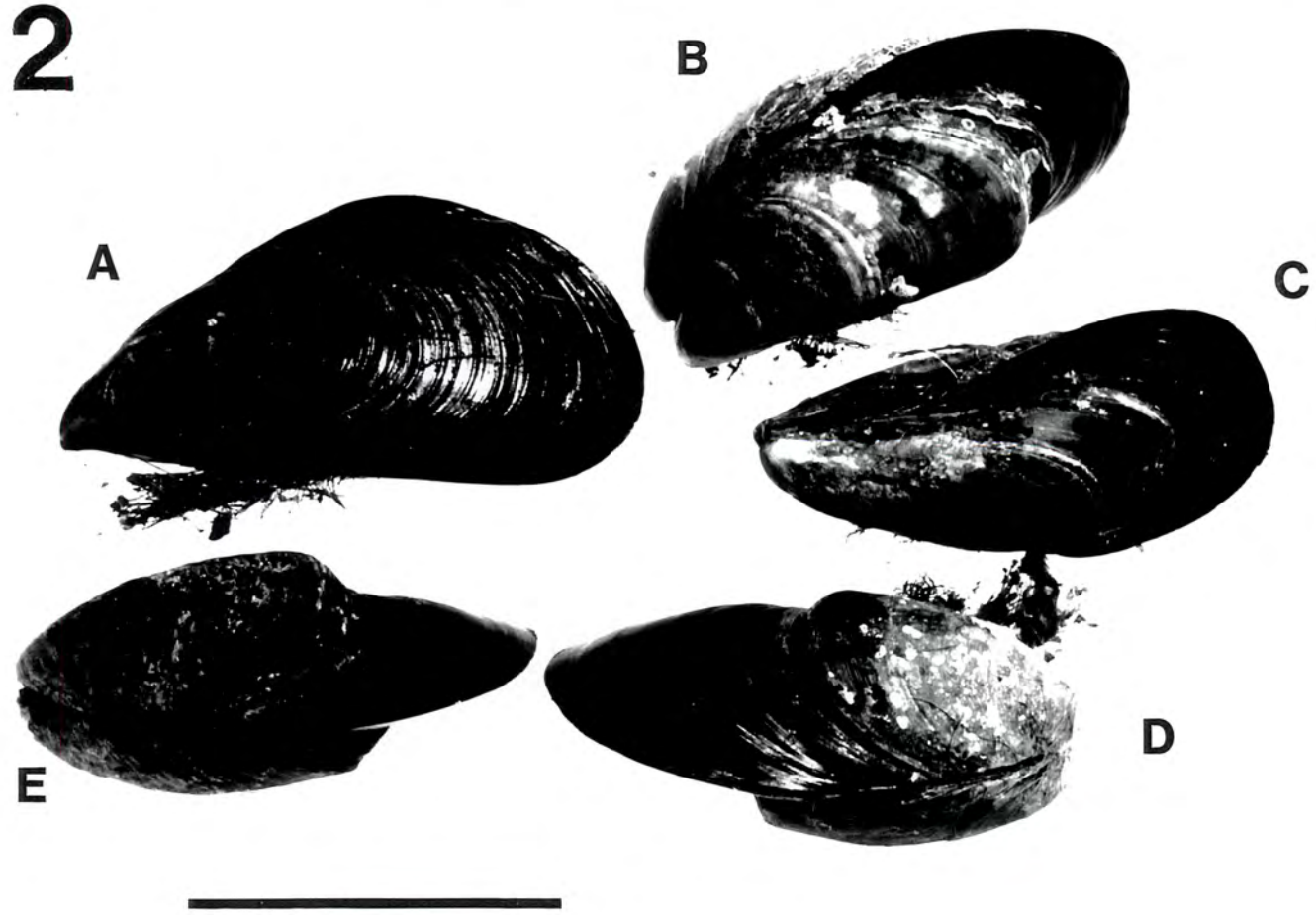


Figure 1. *Mytilus galloprovincialis*, A: transplanted mussel showing the shell step, B: the normal shell profile. The scale bar equals 25mm.

Figure 2. *Mytilus galloprovincialis*, A: showing the shiny black shell resulting from entirely subtidal development. B, C, D & E: showing the growth step and subsequent glossy shell surface after translocation of mussels from intertidal to subtidal conditions. The scale bar equals 50mm.

PART II:

DIGENEAN TREMATODE PARASITES FROM SOUTH AFRICAN MARINE MYTILIDS

CHAPTER 2: INTRODUCTION, MATERIALS & METHODS AND STUDY LOCALITIES

INTRODUCTION

Published works on the parasites of South African marine mytilids are sparse. Faust (1926) described the gasterostome *Bucephalopsis modiolae* in *Arcuatula capensis* (Krauss). This bivalve lives on rocky substrata close to mud deposition zones in the lower reaches of estuaries and ranges from Hermanus, west of Cape Agulhas, to the coast of Mozambique (Davies 1980). Lasiak (1989) reported widespread infections of the brown mussel *Perna perna* with *Proctoeces* sp. an unencysted fellodistomid metacercaria. Lasiak (1993) also reported bucephalid infections in *Perna*. Calvo-Ugarteburu (1996) reports similarly infected *Perna* in samples taken from Tsitsikamma on the South coast all the way to Durban in Natal. Other pathological organisms in South African mytilids include *Mastigocoleus* sp., a blue green alga that weakens shells of *Aulacomya, Choromytilus, Mytilus, and Perna* (Webb & Korrubel 1991, 1994).

Other works on marine digenea in South Africa include the description of an adult zoogonid *Lecithostaphylus spondyliosomae* from *Spondyliosoma blochii* by Fantham (1938). In addition, Bray (1984) reports on adult digenea of the Bucephalidae, Haplosplanchnidae, Mesometridae, Fellodistomidae, and (Bray 1987) Opecoelidae, all from fish. In the Indian Ocean, Toman (1977, 1989, 1992a & 1992b) described adult digenea in fish of the Seychelles.

MATERIALS AND METHODS

Collections

Only general materials and methods are given here. Specific protocols are described elsewhere as appropriate. Collections of mussels took place at spring low tides when mussel beds were exposed for convenient collection. Mussels were kept at 15^{o}C in a through-flow seawater aquarium with aeration.

Measurement

Length is the greatest linear dimension over the posterior-anterior axis.

Dissection

Mussels were opened by pushing a scalpel blade into the byssus aperture and cutting backward to sever the posterior adductor muscle. The mussel was then laid in a petri dish and the surface of the internal organs was viewed by dissecting microscope. Both sides of the gills and palps were examined and then moved aside to expose the mantle. After this, the water released by the mussel was examined microscopically (45x). Discoloration, unusual structures or atrophy of normal tissues was investigated by examination of a sample of tissue under a compound microscope. The mussel was then removed from the shell and the mantle next to the shell was scanned at 45x. The mussel was then progressively disrupted with forceps and the macerated tissue suspended in water. Sex of the mussel was determined by gonad colour in the mantle and mesosoma and by the presence of gametes. Pearls and other inclusions were noted.

Preparation of parasites

Where possible, living parasites were drawn and measured. Heat-killed and fixed specimens (10% formalin in seawater) were also measured. The problems of measurement, as described by Lauckner (1983), are obviated by ensuring that the parasite was not subject to pressure from the cover slip. Such pressure occurred by surface tension as the water evaporated from the preparation and it could be prevented by ensuring that a drop of water rests on the slide by the side of the cover slip at all times. Capillary action will draw enough water under the cover slip to create sufficient space for the specimen between slide and slip.

Since moribund parasites often show more detail, live worms were re-examined after about an hour. Specimens were heat-killed by placing the slide on a 60W light bulb for a few seconds. Hot-formalin fixed specimens were often distorted, had greater variability and were much less representative of living cercariae. Thus, cold formalin was used in preference. Glacial acetic acid (Berland 1982) fixes, clears and relaxes cercariae.

Numerous stains were used, including acetic-orcein stain after the method of Webb (1991b), inspired by Bergan (1955), which is described below. Other methods are described fully in Webb (1985 & 1991a). This new use for acetic-orcein stain is reported here as an interpolation.

USE OF ACETIC ORCEIN AS A FAST METHOD TO RELAX, FIX, CLEAR, AND STAIN CERCARIAE FOR LIGHT MICROSCOPY.

SUMMARY

Acetic-orcein stain may be dropped onto the specimen before applying a cover slip. This single reagent reveals cercarial structures that would otherwise be made visible only by complicated and time consuming procedures.

INTRODUCTION

Although numerous specialisations and refinements in staining cercariae have been made since, the most common techniques in use remain largely the same as those set out by Stunkard (1930). He advocated haematoxylin, counterstained by eosin or one of the carmines for fixed, sectioned material, and staining with neutral red for *intravitam* preparations. More complicated procedures such as Mallory's triple stain were recommended for histological work. James (1964), in his extensive use of stains, mentions advanced histochemical procedures, but he also advocates the use of methyl-green and bromocresol-blue as vital stains.

Common techniques used for the preparation of cercariae show a dichotomy between the short lived but easy to apply vital stains on one hand, and permanent but more complex staining, counter staining and multiple application procedures on the other. This work will attempt to show how the gap may be bridged by the use of acetic-orcein stain, whose usual application is in the staining of chromosomes.

MATERIALS AND METHODS

Cercariae and metacercariae of several digenean (Trematoda) taxa, including those of microphallids, gymnophallids, and gasterostomes were treated. Specimens in Figures 1 & 2 are gymnophallid metacercariae (described in Chapter 4) found encapsulated in the mantle of the black mussel *Choromytilus*. The specimen in Figure 3 is an undescribed cercaria found in the wedge clam *Donax hanleyanus* from the coast of Uruguay.

The stain is `Solution A' according to La Cour's (1941) formula: orcein 1g, glacial acetic acid 45 ml, and distilled water 55 ml. The distilled water is added after the mixture has been heated to near boiling and cooled. The reagent is then shaken and filtered. The living specimens of cercariae and metacercariae, in a drop of seawater (those tested were of marine origin), are placed on a microscope

slide. Acetic-orcein may be dropped onto the specimen and then covered by a glass slip, whose edges should be sealed with petroleum jelly to reduce evaporation. The preparation is ready for observation but its definition improves during the following hour.

RESULTS AND DISCUSSION

The subjects gave uniformly good results: they are relaxed by the reagent and appear similar to the moribund stage in untreated cercariae, *i.e.* internal organs are prominent and distinct. This condition, which is short in untreated specimens, is prolonged by fixative action of acetic acid in the reagent. Disintegration of the cercaria is prevented and the specimen is usable for three days or more. A drawback in the use of other fixatives is that they may leave specimens distorted, contracted, and internally cloudy. Formalin in its various solutions is particularly poor in this respect. In contrast, acetic-orcein has a clearing action and proved effective even on specimens previously fixed and stored for over a year in formalin-seawater. A further function of this reagent is to dissolve the refractile granules commonly found in the excretory system of metacercariae. This system is often extensive and the granules frequently dense. Their removal allows unhindered observation (Figures 1 & 2, A before, and B, after treatment).

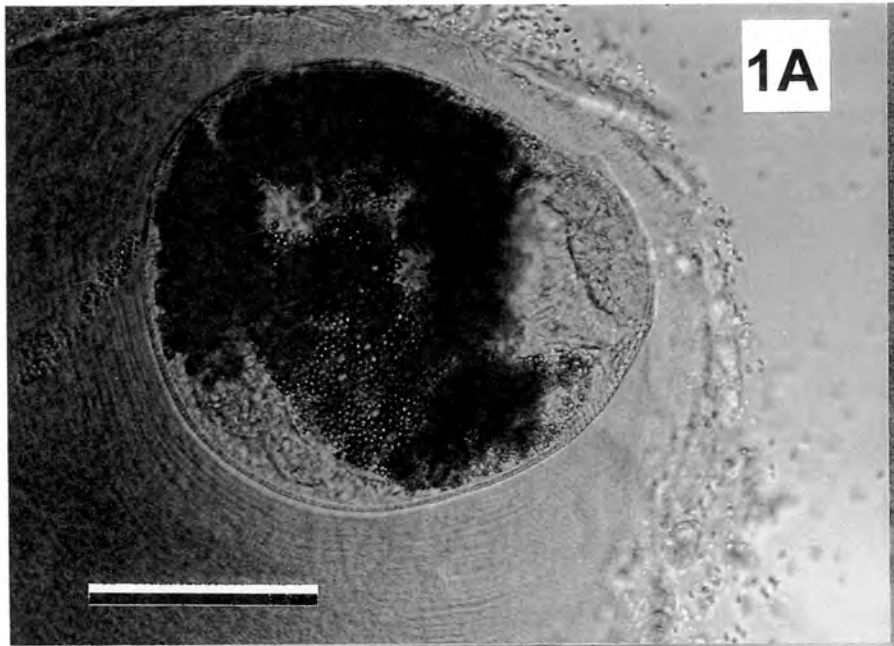
These foregoing effects enhance the appearance and detail shown by the stain. Nuclei appear red and, as is common in cercariae, they are often densely packed, with little cytoplasm between. In consequence, the nuclei-free areas, such as those in gland ducts and cavities - e.g. gut and bladder - are more easily seen (Figure 3, A before and B after treatment).

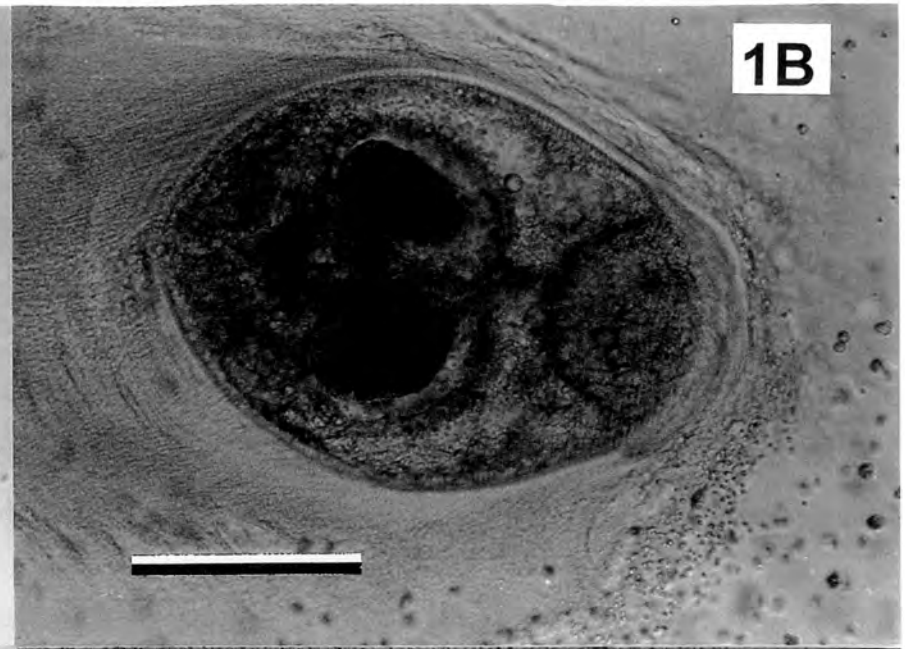
The ease of application and breadth of benefits are a significant advantage to the study of cercarial morphology. This technique affords a swift anatomical overview and, by indicating where to look, it will facilitate observations of living specimens. In conjunction with current methods, use of this reagent promises to ease the work of parasitologists.

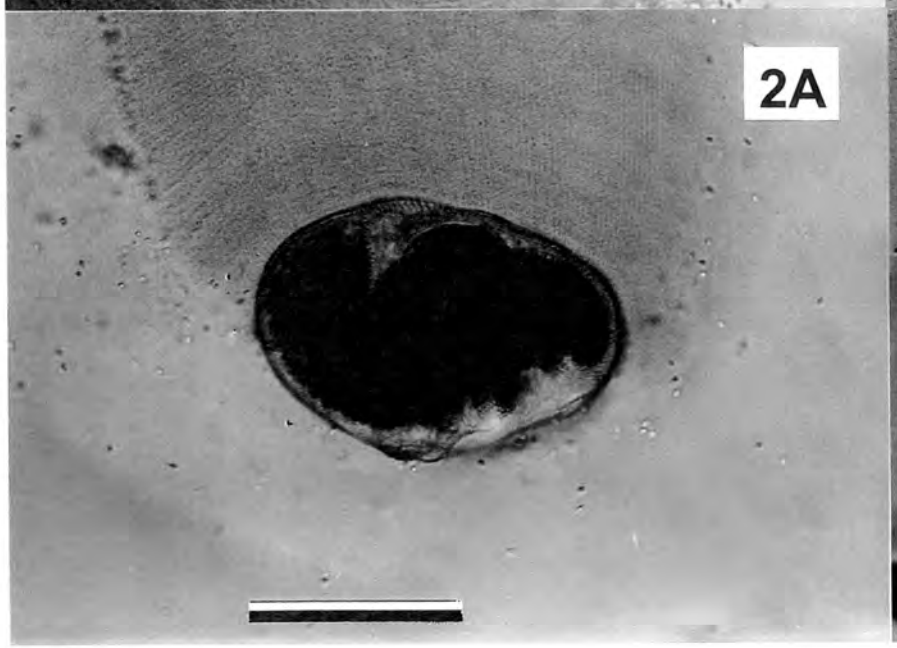
Other general methods

Small pieces of infected tissue were fixed in 4% glutaraldehyde in seawater. They were then dehydrated and critical-point dried. Tissue specimens were fractured to provide a fresh face and then mounted on stubs.

Epidemiological parameters such as prevalence, mean intensity and abundance are as defined in Williams & Jones (1994) and Margolis, Esch, Holmes, Kuris & Schad (1982). Prevalence is the proportion of organisms that are infested, expressed as a percentage of the whole sample. Mean intensity indicates the mean number of parasites (of each type) in each infected organism. It is the total number of parasites divided by the total number of infected hosts. Abundance is the mean number of







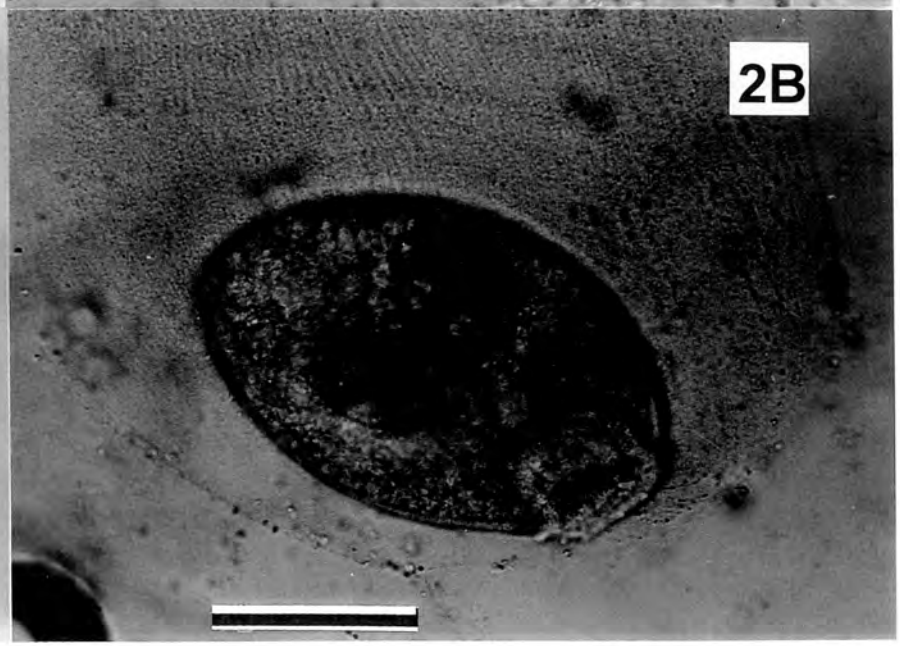


Figure 1: A, living gymnophallid metacercaria showing the opaque excretory system; B, the same metacercaria after treatment with acetic orcein. Note the layered structure of the gelatinous capsule. The scale bar equals 100µm.

Figure 2: A, another living encapsulated gymnophallid metacercaria; B, the same metacercaria after treatment with acetic orcein. Note the layered structure of the capsule. The scale bar equals 100µm.

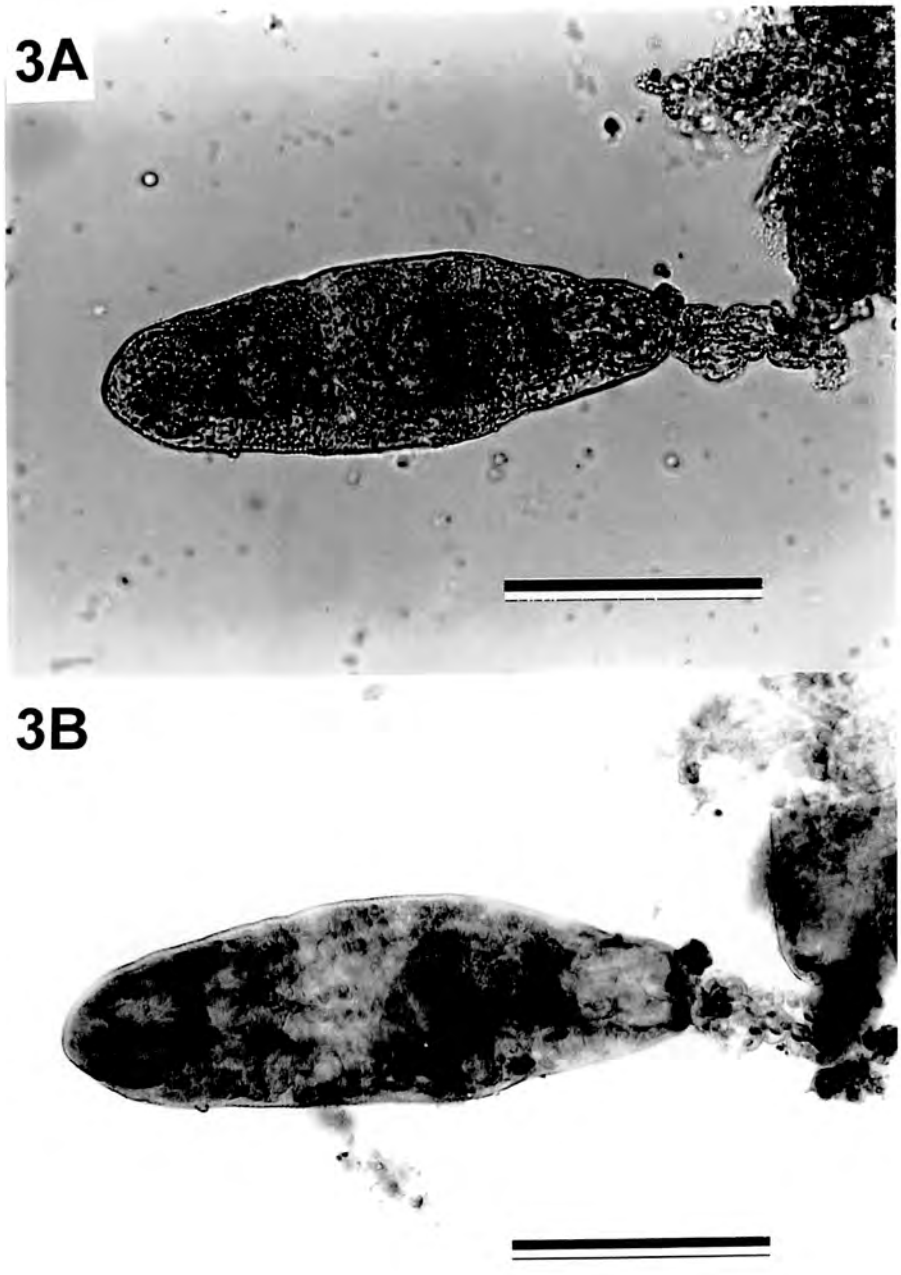


Figure 3: A formalin fixed cercaria; B the same cercaria after treatment with acetic orcein. The scale bar equals 100μm.

parasites of each type in any population of organisms. It is the total number of parasites divided by the total number of hosts (infected and uninfected).

In some infections these parameters were further subdivided. Some parasites may occur in the palps or the mantle, or both, of the host. Prevalence of infection in the palps is derived from the total number of mussels with infected palps divided by the total sample multiplied by 100. Prevalence of infection in the mantle is derived from the total number of mussels with infected mantle divided by total sample multiplied by 100. Mean intensity of infection in the palps is derived from the total number of worms in the palps divided by total number of hosts with infected palps. Mean intensity of infection in the mantle is derived from the total number of worms in the mantle divided by total number of mantle infected hosts. Abundance of parasites in the palps is derived from the total number of worms counted divided by the total number of (infected and uninfected) hosts. Abundance of parasites in the mantle is derived from the total number of parasites in the mantle divided by total number of (infected and uninfected) hosts.

LITERATURE CONSULTED

The following works were consulted to assist in identification: Erasmus (1972), Combes (1980), Cheng (1967), Ito (1964), Lauckner (1983), Cable (1956, 1963) Faust (1926), Stunkard (1932, 1983), Bray & Gibson (1980) and Holliman (1961). Faust (1919) dealt with aspects of taxonomy of the digenea with reference to their excretory system. Again Faust (1932) discussed the excretory system as a method of classification of digenetic trematodes and this was amplified by La Rue (1957). Despite Lauckner's (1983) assertion that, so far, there is no agreement between accepted generic features and the excretory system, details of this system still have considerable taxonomic utility. Gibson (1987) raises several questions in digenean systematics and evolution. The significance of these works will be discussed where appropriate.

Nomenclature using life-cycle stages as generic names.

Cheng (1967), Stunkard (1932) and Lauckner (1983) present named and described cercariae and metacercariae by using the life-cycle stage as a quasi-generic term. The

new species is given a specific term and the life-cycle stage is used instead of the genus. Loos-Frank (1971) uses the same formula but does not put it in italics. It is considered here that to italicise is more explicit. Use of this formula conveys that the adult stage has not been identified and described or that it has not been connected experimentally with the larva. As mentioned elsewhere, identification of many cercariae and metacercariae to the level of genus is often difficult or impossible. Thus the above nomenclature transmits considerable amounts of information without committing the author to the use of a generic name.

STUDY LOCALITIES

Table 1. Collection localities.

locality	latitude	longitude
Blouberg	33°48'S	18°27'E
Cape Columbine	32°50'S	17°15'E
Dido Valley	34°12'S	18°28'E
Kleinmond	34°20'S	18°49'E

DIGENEAN AUTHORITY NAMES AND ANATOMICAL TERMS

Anatomical terms follow those used by Erasmus (1972), for example the acetabulum is called the ventral sucker. In addition, it is convenient that the main component of the excretory system, the excretory vesicle, be subdivided as by Bray & Gibson (1980) with a stem and arms as appropriate. The excretory vesicle is connected to secondary collecting tubes. In the stenostomate condition (Erasmus 1972) these recurve posteriorly from an anterior insertion to the vesicle. In the mesostomate condition (Erasmus 1972); posterior and anterior collecting vessels insert near the mid-point of the vesicle. Tertiary ducts or flame cell tubes connect to the secondary ducts. Although stenostomate and mesostomate examples may be seen in the same sub-family (Erasmus 1972), the difference may be of use to distinguish species.

The following descriptions and discussions include many authority names that can be confused with published works. To avoid this confusion, works cited will be placed in positions other than immediately after names of taxa.

CHAPTER 3: SPOROCYST AND CERCARIA OF *CERCARIA NOTOBUCEPHALA* SP. NOV.

HOSTS AND LOCALITY

This parasite was found in *Choromytilus meridionalis* from Blouberg and Dido Valley, and in *Venerupis (= Tapes) corrugatus* (Gm.) from Blouberg.

TYPE SPECIMENS

Nomenclature of type specimen, host and locality follows that recommended by Williams (1986). There are only paratypes; no holotypes are extant as the specimens disintegrated during description. Specimen number A29427, Marine Biology Department, South African Museum, Queen Victoria Street, Cape Town, RSA. Type host: *Choromytilus meridionalis.* Type locality: Blouberg.

DESCRIPTION

The mother sporocyst was not seen. Daughter sporocysts are always unbranched (Figure 1, 3 & 4). They occur throughout the tissues of the host and infection intensity is always high. Large numbers of daughter sporocysts are concentrated in the viscera (digestive gland) and mantle (gonad). In consequence, the infected mantle was often yellowish. Sporocysts lack birth pores and the tegument is non-spinous. They are often highly convoluted and their circular transverse sections vary haphazardly. No movement was noted: the subtegumental tissue has sparse musculature, which appears as longitudinal and transverse striations.

Germ balls, immature cercariae (Figure 1B and 3), and cercariae occur with the sporocysts. Some germ balls stain heavily with bromocresol-blue, others less so. They (Figures 1, 3, 5, 6, 7, 8, & 10) consist of sub-units each about 2.4μm in diameter. Germ balls also stain with haematoxylin, which suggests that they are a concentration of nuclei with little cytoplasm. Cercariae, at all stages are less densely staining. Numerous bodies (2.4μm-5μm diameter) in the sporocyst wall that stain yellow with acetic-orcein are possibly nuclei (Figure 1).

Several unsuccessful attempts were made to obtain naturally shed cercariae. At each attempt, some hundred mussels would be left overnight in small jars of seawater a few

degrees warmer than normal. Different light regimes were used. Mussels were opened afterwards and examined to ensure that infected specimens were indeed in each sample. Since naturally shed cercaria were not available, only fully developed cercaria from dissected mussels were described and measured.

Flat elliptical plates in staggered rows cover the body tegument of this tailed gasterostome cercaria (Figures 15 & 18). The subterminal anterior sucker (it is not an oral sucker: the ventral sucker is the oral sucker in this cercaria) stains deeply with neutral red. Neutral red is non-toxic to this cercaria but stains heavily some glandular tissue. The anterior sucker lips are in cruciform arrangement (Figures 2B, 6, 16 & 17) when fixed, and also in life. Two pairs of glands that stain with Nile blue sulphate open into this sucker. The short pair of glands terminate just behind this sucker; the long glands terminate just before the patches of gonad tissue (Figure 2A). Two pairs of globular gland cells that stain with Nile-blue sulphate lie just posterior to a pharynx-like structure. This structure is rendered visible by haematoxylin and appears as a hollow sphere of dark staining nuclei. It may be associated with a crescent-shaped orifice on the ventral surface of the cercaria (Figures 6 & 17). This orifice is some 7μm in length and lies about 20μm behind the anterior sucker. The circular mouth, which is ventral and about 60% of the way from the anterior, is surrounded by a radial pattern of tegument plates. The mouth leads into a bag-like gut whose contents stain purple with Nile blue sulphate. Genital primordia appear as two diffuse patches which stain with acetic orcein. The cercarial body also contains many, possibly cystogenous, gland cells (Figure 13). There is a contractile sac-like bladder, which has four small lateral diverticulae. Nine pairs of flame cells (Figure 2) occur in an arrangement that suggests the formula:

$$2\,[(3 + 2) + (2 + 2)] = 18 \text{ flame cells.}$$

Each of the tail furcae has great powers of contraction and extension. They are transversely grooved and non-spinous. The furca, in transverse section, has a thick cuticle over about three-quarters of its circumference. The other quarter is occupied by two rows of eosinophilic structures (Figure 2C & 10) and a longitudinal groove may be seen between the rows. No nuclei occur in these eosinophilic areas. Similar eosinophilic structures occur on the posterior of the tail stem on which the furcae are anchored (Figure 8).

Table 1. Measurements (μm) of gasterostome sporocysts and cercaria of *Cercaria notobucephala*: A living, B fixed in formalin and C heat-killed.

A

	mean	SD	*n*	max.	min.
body length	262	27.4	10	297	206
body width 25% from front	30.7	7.2	10	43	19
body width 50% from front	38.4	12.4	10	53	19
body width 75% from front	39.8	9.9	10	53	19
anterior sucker length	45.5	7.7	10	56.4	36.8
anterior sucker width	28.7	5.5	10	39.2	22.1
tail stem length	34.6	9	10	49	24.5
tail stem width	56.6	11.7	10	75.9	36
width of tail filament	13	4.7	10	22	5
germ ball diameter	37.9	16.1	10	67	19
sporocyst width	135.4	61.5	10	288	67

B

	mean	SD	*n*	max.	min.
body length	154	10.4	11	345	99
body width 25% from front	45.8	10.4	10	63	28
body width 50% from front	54	17	10	77.5	30
body width 75% from front	47.5	8	10	57.5	32.5
anterior sucker length	42	9	10	62.5	32.5
anterior sucker width	29.5	5.1	10	40	22.5
tail stem length	32.7	5.7	10	43	25
tail stem width	57.8	14.1	10	92.5	45
tail furca width	21.8	3.2	10	25	17.5
cuticle thickness	2.5	0.7	10	3.8	1.3
germ ball diameter	23.8	6.5	10	32.5	15
sporocyst width	96.3	50.4	10	187	49

C

	mean	SD	*n*	max.	min.
body length	236	17.9	10	257	206
body width 25% from front	39	5.4	10	48	31.9
body width 50% from front	48.6	13.6	10	83.3	36.8
body width 75% from front	42.4	7.9	10	61.3	36.8
anterior sucker length	45.8	5.9	10	53.9	34.3
anterior sucker width	26	4.8	10	36.8	19.6
tail stem length	34.8	4	10	36.8	24.5
tail stem width	67.6	6	10	76	58.8
tail furca width	12	1.5	10	14.7	9.8
germ ball diameter	30.9	10	10	53.9	17.2
sporocyst width	171	48.4	10	242.6	102.9

Cercariae are in constant movement, in particular, the region of the body between the anterior sucker and the mouth is often twisted during stretching and contraction. They exhibit leech-like movement but cannot swim or leave the substratum. The tail furcae exhibit extreme contraction and extension.

GAMETE PRODUCTION

Infection does not always abolish gamete production in *Choromytilus*. Figure 13 shows the anterior of a cercaria surrounded by host sperms.

EPIDEMIOLOGY

The following prevalences at each collection locality are aggregated from a series of collections. Size dependent prevalences for *Choromytilus* are represented in Figures 19 & 20 for females and males respectively.

Table 2. Prevalences of infection with *Cercaria notobucephala*.

	infected	*n*	prevalence
Choromytilus meridionalis Blouberg			
males	21	337	6.23%
females	7	303	2.31%
unidentified	1	10	10%
total	29	650	4.46%
Choromytilus meridionalis Dido Valley			
total	2	870	0.23%
Venerupis corrugatus Blouberg			
total	2	10	10%

Table 3A. Mean lengths of sub-populations of *Choromytilus* from Blouberg.

	mean length	SD	*n*	SE
male infected	55.63	8.08	21	1.763
male uninfected	55.05	11.14	316	0.627
female infected	65.87	17.85	7	6.748
female uninfected	54.09	8.99	296	0.522
unidentified	49.55	---	1	---
uninfected	44.78	4.84	9	1.613
total			650	

Table 3B. Mean lengths of *Choromytilus* sub-populations from Dido Valley.

	mean length	SD	*n*	SE
male infected	71.95	4.25	2	3.01
male uninfected	58.14	10.43	412	0.514
female uninfected	58.17	10.33	428	0.499
unidentified	34.78	10.52	25	2.104
hermaphrodite	51.8	14.52	3	8.383
total			870	

Figures 19 & 20 depict size dependent prevalence of *Cercaria notobucephala* in female and male *Choromytilus* from Blouberg. Figures 21 & 22 depict monthly variation in prevalence of *Cercaria notobucephala* in female and male *Choromytilus* from Blouberg.

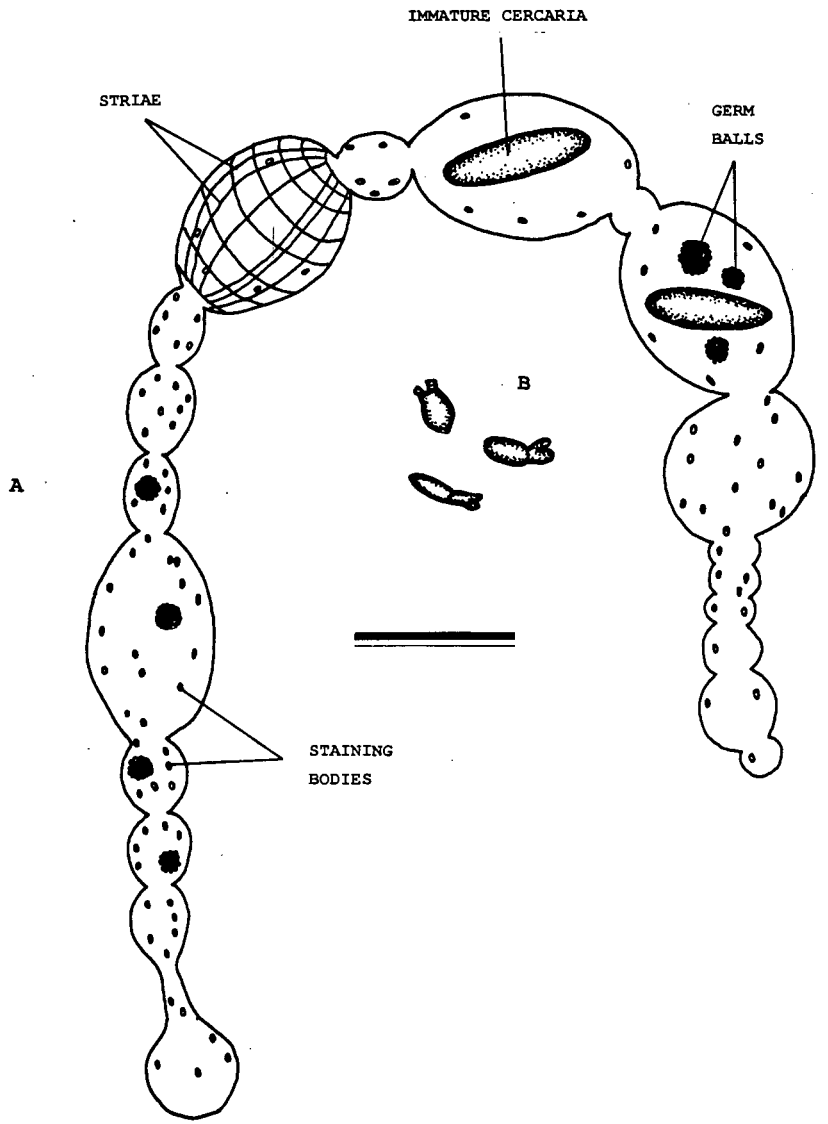


Figure 1. *Cercaria notobucephala*. A, mature sporocyst; B, immature cercariae, scale bar = 240μm.

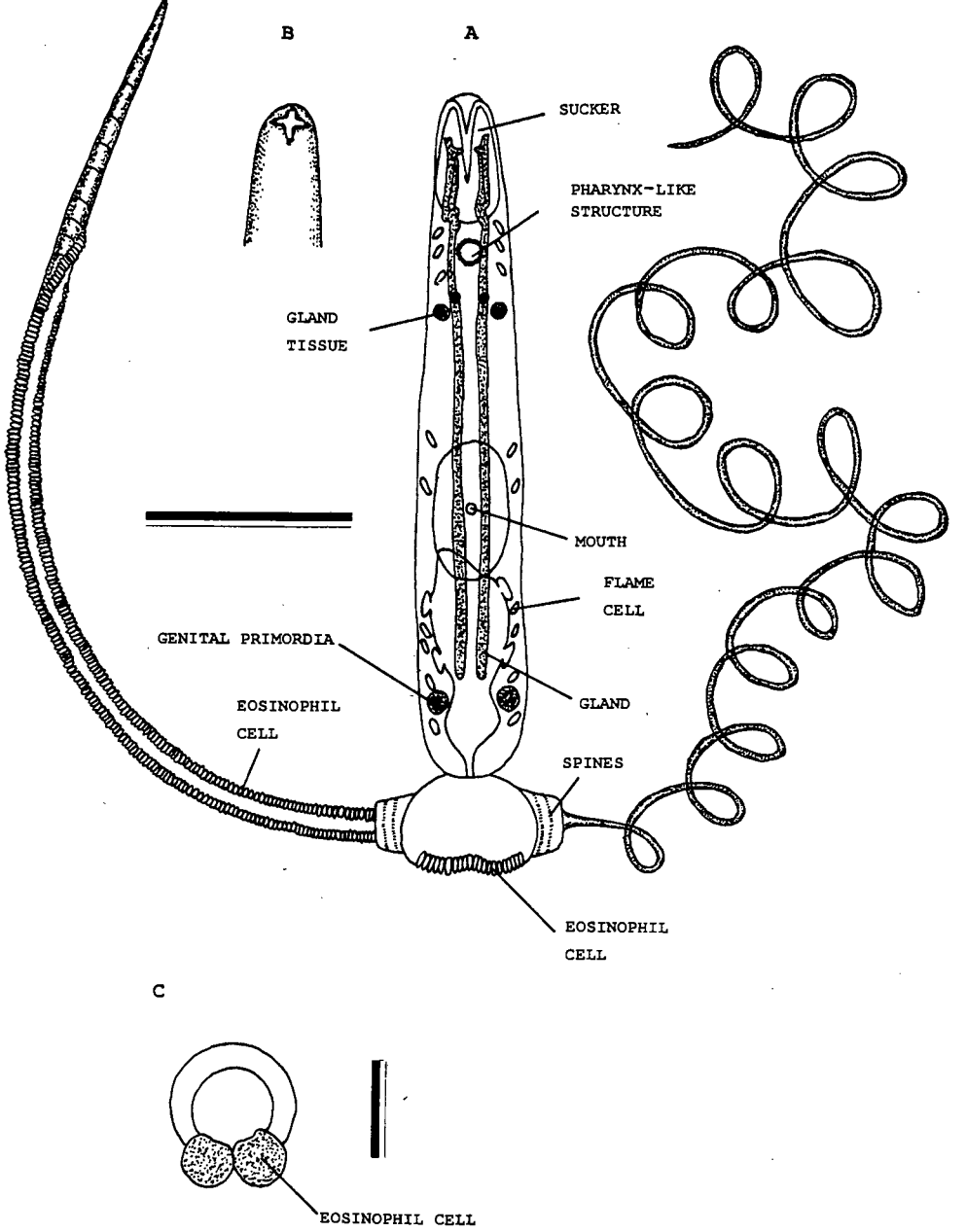
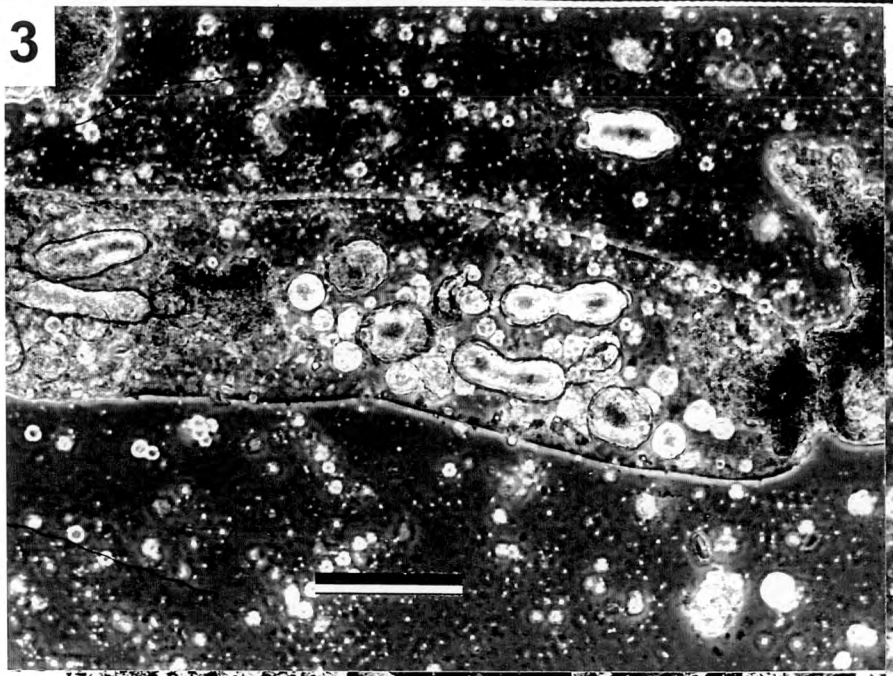
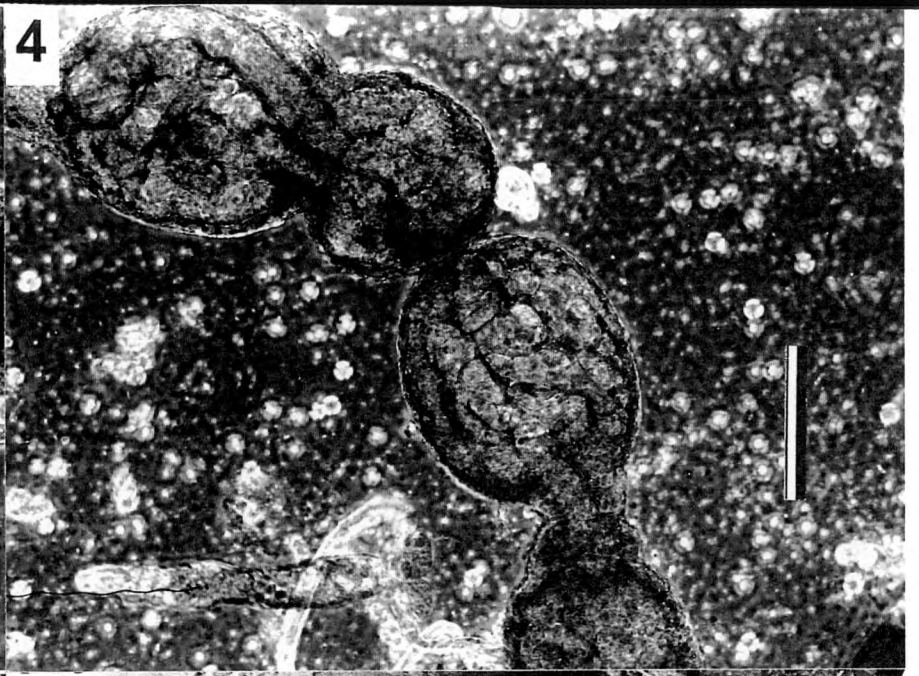
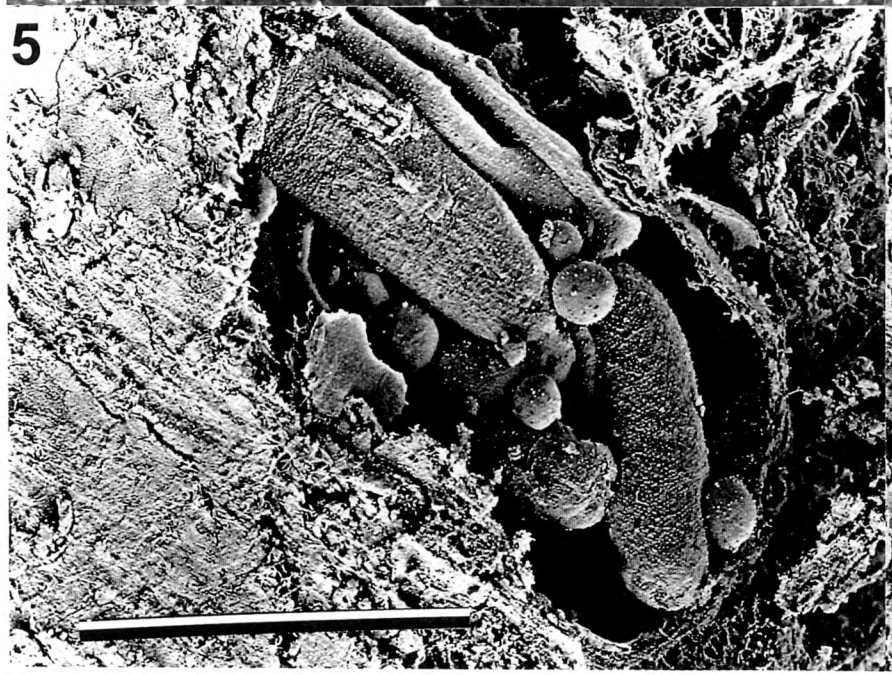


Figure 2. *Cercaria notobucephala*. A, anatomy of the cercaria; B, external features of anterior sucker; C, transverse section of tail filament, scale bars A & B = 40μm, C = 10μm.







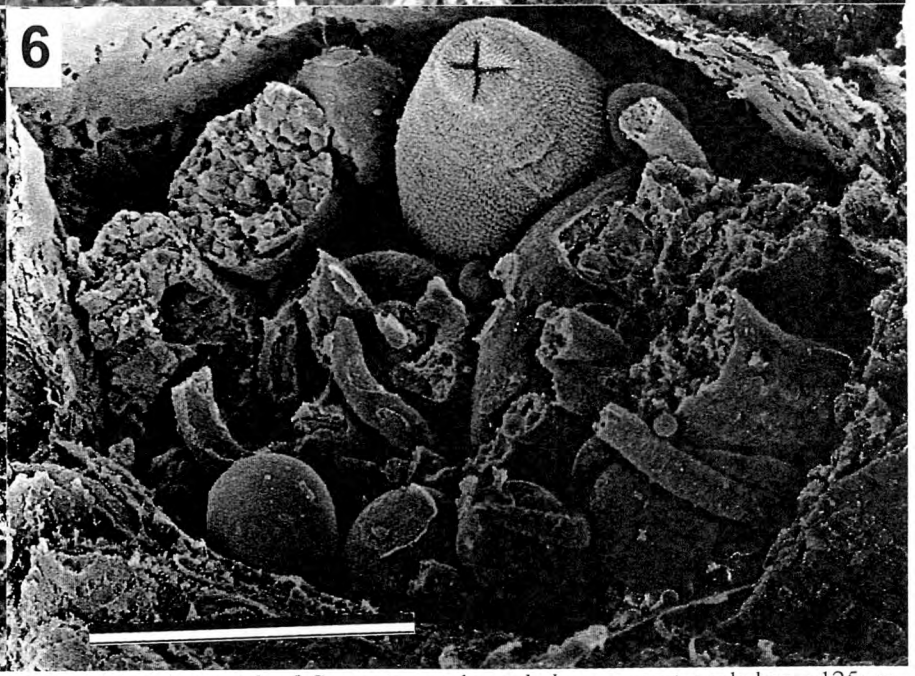


Figure 3. Light micrograph of *Cercaria notobucephala* sporocyst, scale bar = 125μm.

Figure 4. Light micrograph of *Cercaria notobucephala* sporocyst, scale bar = 125μm.

Figure 5. SEM. *Cercaria notobucephala* sporocyst, scale bar = 100μm.

Figure 6. SEM. *Cercaria notobucephala* sporocyst, scale bar = 50μm.

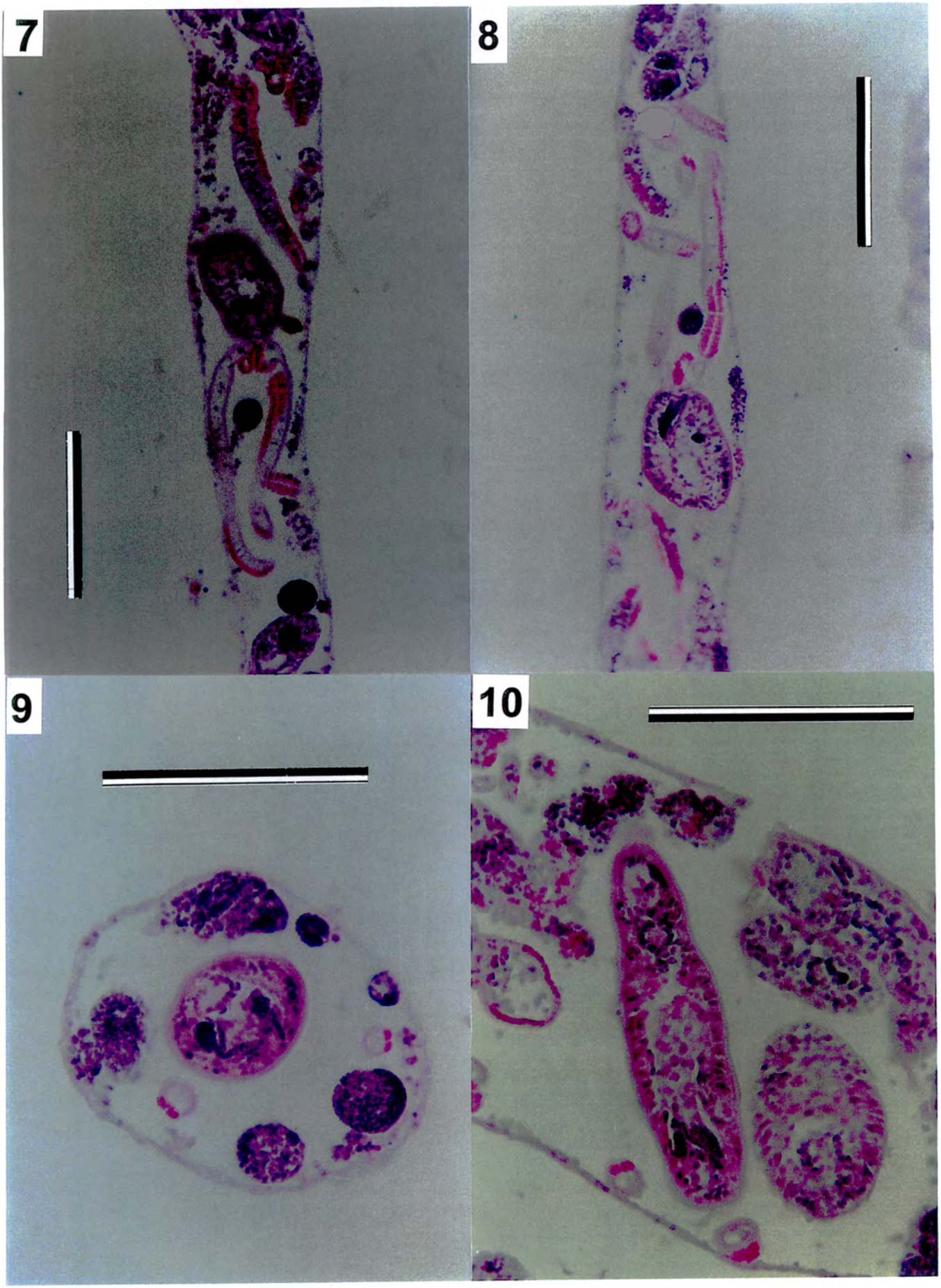


Figure 7. Light micrograph of *Cercaria notobucephala* sporocyst, L.S. stained with haemotoxylin and eosin, scale bar = 100µm.

Figure 8. Light micrograph of *Cercaria notobucephala* sporocyst, L.S. stained with haemotoxylin and eosin, scale bar = 100µm.

Figure 9. Light micrograph of *Cercaria notobucephala* sporocyst, L.S. stained with haemotoxylin and eosin, scale bar = 100µm.

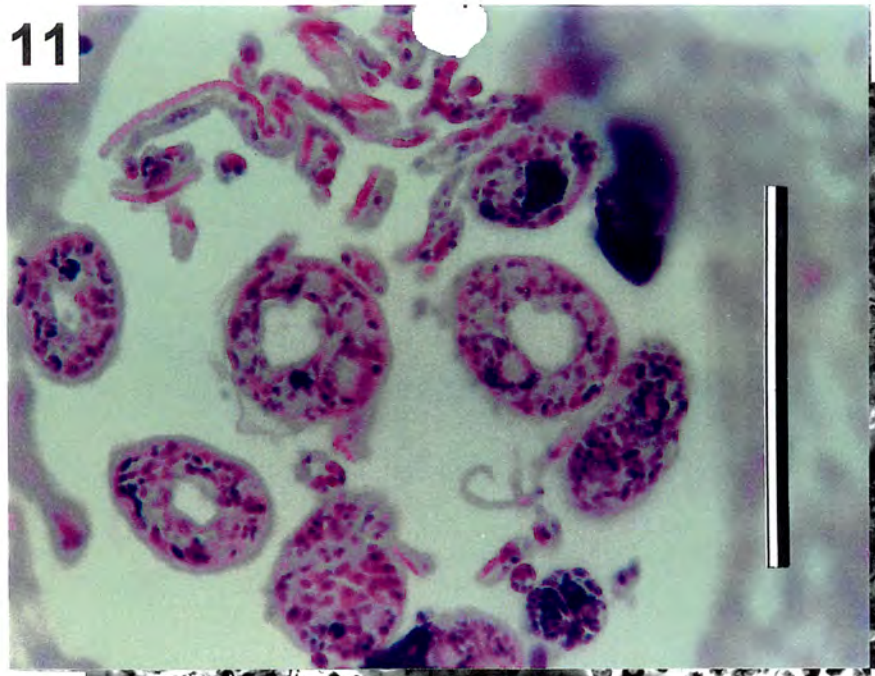
Figure 10. Light micrograph of *Cercaria notobucephala* sporocyst, T.S. stained with haemotoxylin and eosin, scale bar = 100µm.

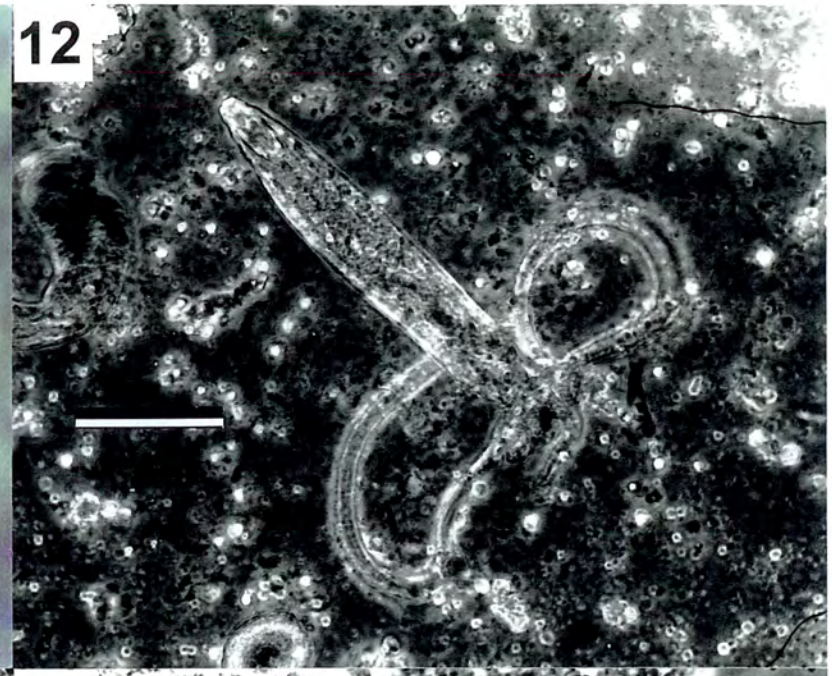
Figure 11.
Light micrograph of *Cercaria notobucephala* sporocyst, T.S. stained with haematoxylin and eosin, scale bar = 100µm.

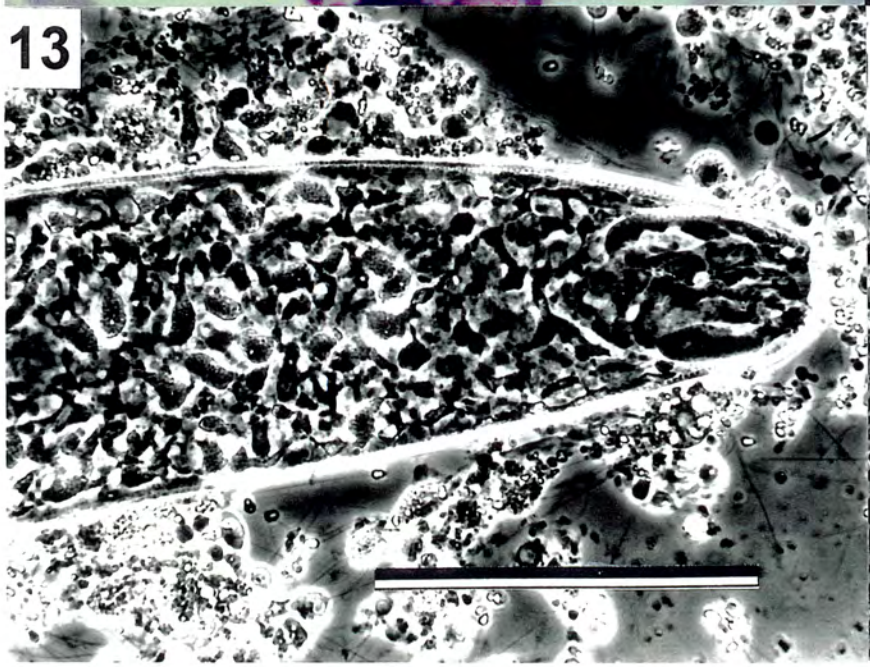
Figure 12.
Light micrograph of living *Cercaria notobucephala*, scale bar = 100µm.

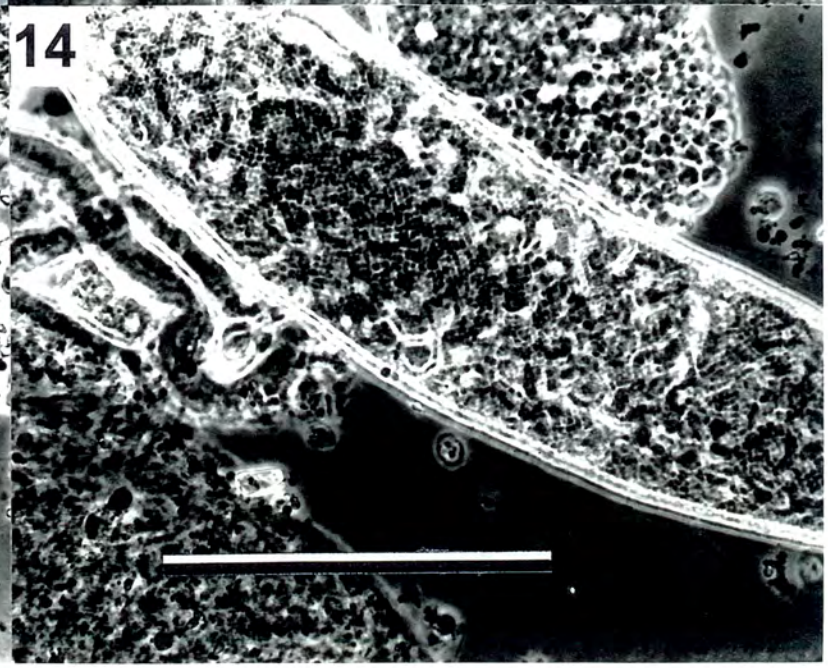
Figure 13.
Light micrograph of *Cercaria notobucephala* anterior, showing the numerous gland cells and the presence of mytilid sperm, scale bar = 100µm.

Figure 14.
SEM. *Cercaria notobucephala*, showing the pattern of spines on the cercarial tegument, scale bar = 100µm.









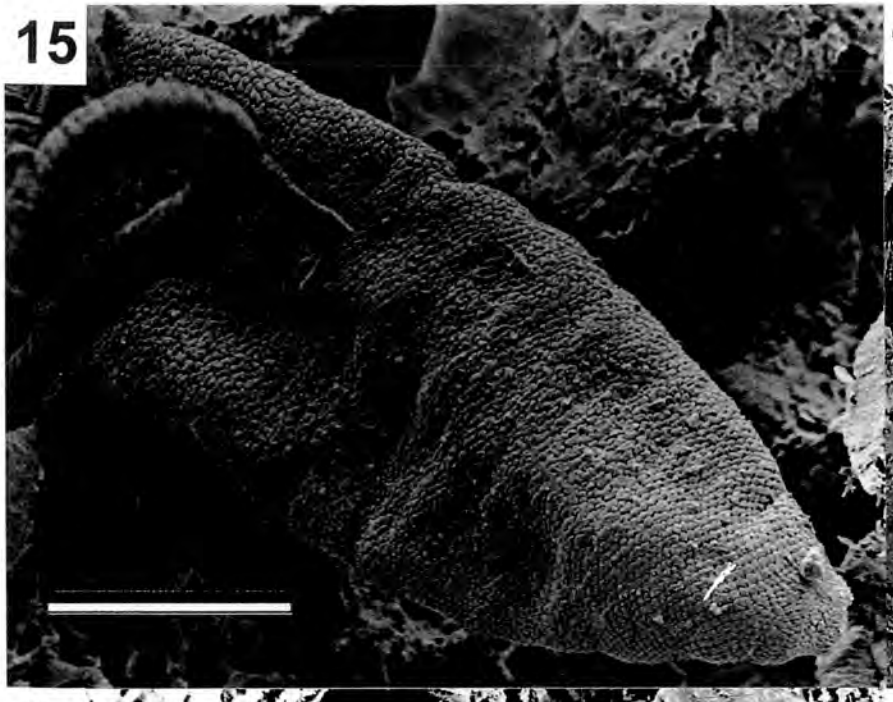


Figure 15.
SEM. *Cercaria notobucephala*, dorsal aspect of the anterior, scale bar = 35μm.

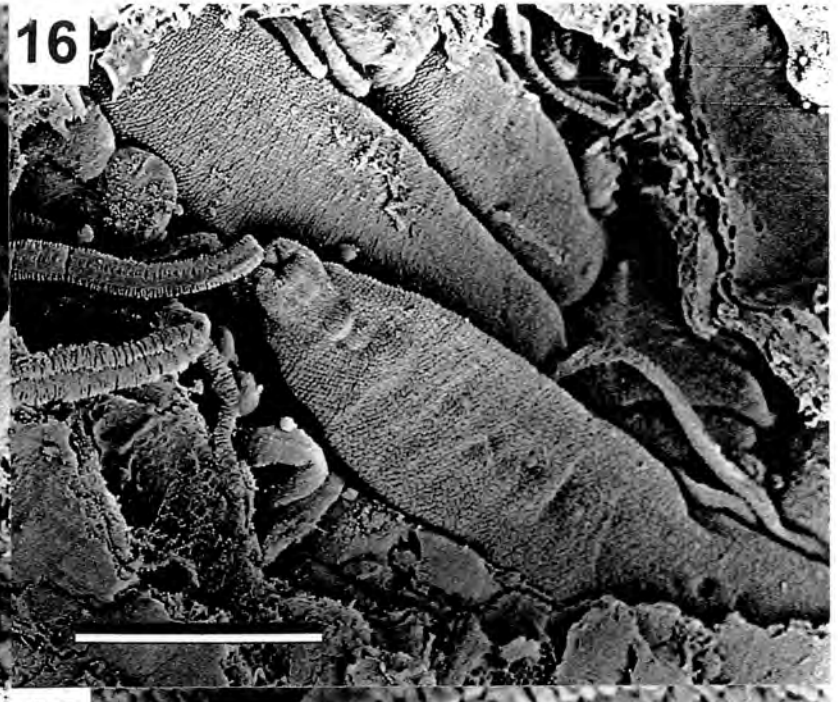


Figure 16.
SEM. *Cercaria notobucephala*, ventral aspect of the anterior, scale bar = 50μm.

Figure 17.
SEM. *Cercaria notobucephala*, ventral aspect of the anterior, scale bar = 50μm.

Figure 18.
SEM. *Cercaria notobucephala*, showing cercarial tail structure and pattern of spines on the cercarial tegument, scale bar = 20μm.

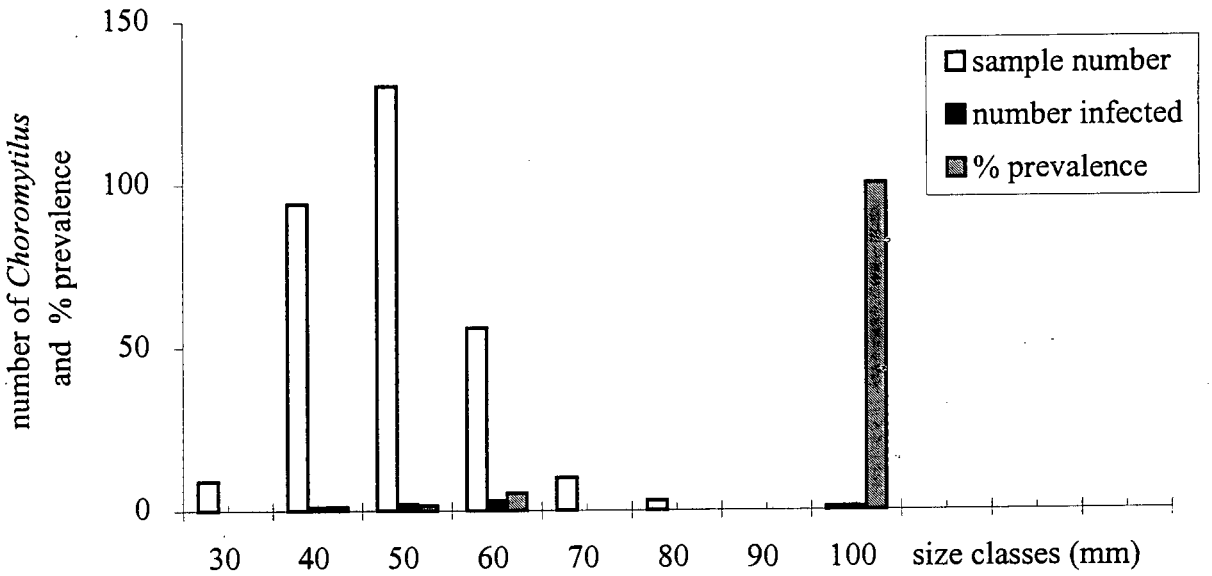


Figure 19. Size dependent prevalence of *Cercaria notobucephala* in female *Choromytilus* from Blouberg.

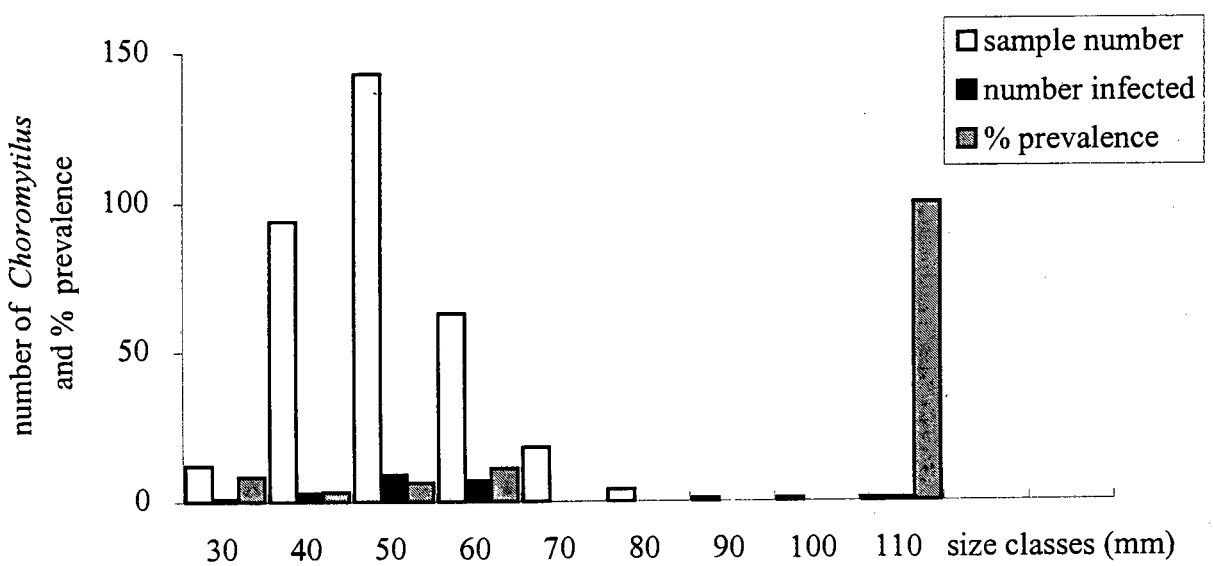


Figure 20. Size dependent prevalence of *Cercaria notobucephala* in male *Choromytilus* from Blouberg.

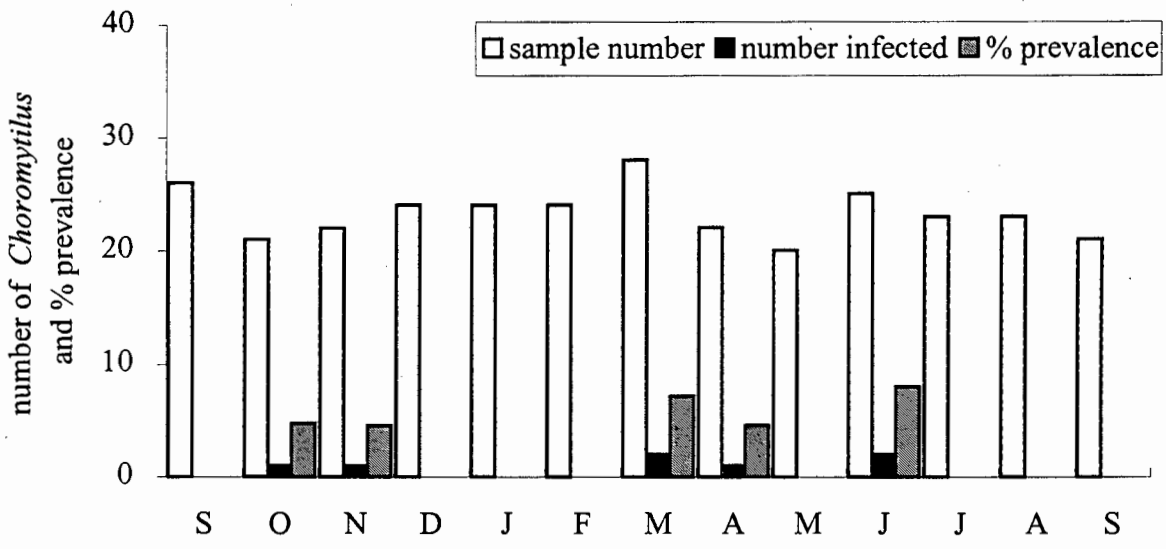


Figure 21. Monthly variation in prevalence of *Cercaria notobucephala* in female *Choromytilus* from Blouberg.

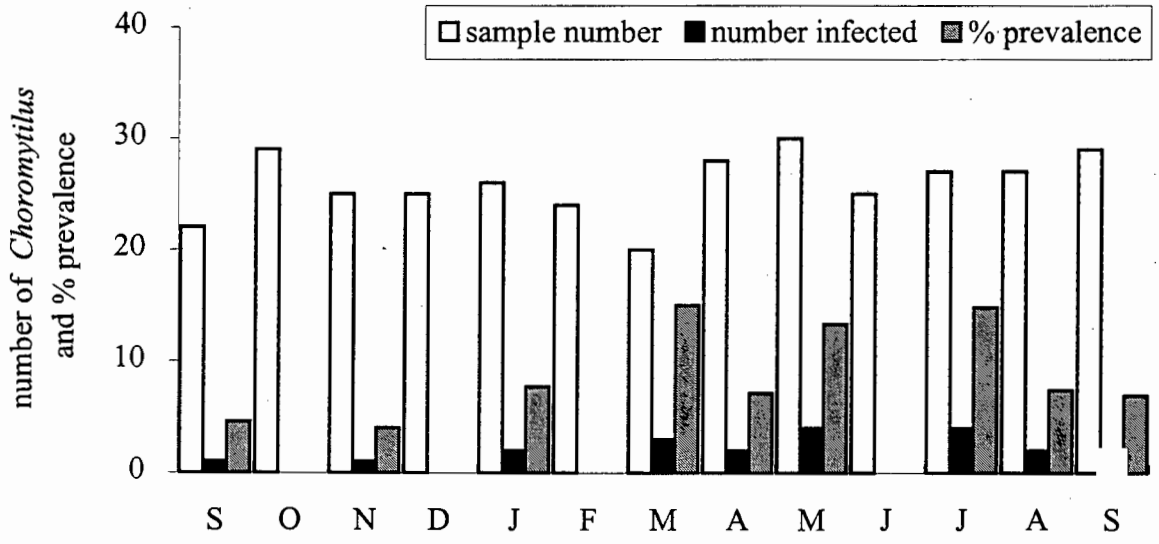


Figure 22. Monthly variation in prevalence of *Cercaria notobucephala* in male *Choromytilus* from Bouberg.

DISCUSSION

In contrast to other reports such as those by Gibson (1987) and Taskinen, Valtonen & Gibson (1991), where gasterostome cercaria are reported to have been shed in large quantities, no naturally shed *Cercaria notobucephala* were available in this study. Thus, specimens were obtained after dissection of the host. Despite Stunkard's

(1930) reservations about the validity of descriptions from extracted, rather than naturally emerged cercariae, it is considered here that he overstates the problem. Ito (1964) deprecates any great difference between naturally released and extracted cercariae. Moreover, Matthews (1973) extracted cercariae of the bucephalid *Prosorhynchus crucibulum* (Rudolphi, 1819) Odhner, 1905, [syn. *Rudolphinus crucibulum* (Rudolphi, 1819) Stunkard, 1974] from dissected *Mytilus edulis* L. that were infective in life cycle experiments with the fish host *Scophthalmus maximus* L. Pekkarinen (1986) also used extracted cercariae for successful infection experiments. When Pike & Erasmus (1967) collected cercariae from dissected sporocysts, these were able to encyst. Similarly, allocreadid cercariae were observed (Webb 1985) to encyst after extraction. Furthermore, microphallid cercariae extracted from sporocysts in a snail host are capable of penetrating and surviving in a crustacean, the putative next host, for a longer period than they would if simply left in sea water after extraction (Webb 1985). That all these cercariae exhibit evidence of viability after extraction suggests that similarly obtained specimens of *Cercaria notobucephala* might also be acceptable subjects for morphological studies. This is considered especially likely if only the most developed cercariae are selected for study.

Taxonomic affinities and etymology

Comparison of this cercaria with extant cercariae from the literature suggests that it is a new species. The name *Cercaria notobucephala* - the cercaria of the southern bucephalus- is suggested. This name should be seen as a provisional label. It thus serves the immediate purpose of identifying this parasite in the rest of this thesis. The specific epithet may be eventually applicable to a described adult derived from life cycle experiments - if it has not been previously described elsewhere. No attempt is made here to place *Cercaria notobucephala* in any particular genus: Lasiak (1991, 1993) and Lauckner (1983) report that bucephalid sporocysts or cercariae cannot be identified to the generic level. For reviews on the bucephalids see Lauckner (1983) and Calvo-Ugarteburu (1996).

Identification of *Cercaria notobucephala* as a new species is based on various unusual features of its morphology and host specificity. In contrast to other bucephalids, *Cercaria notobucephala* sporocysts are always unbranched. This indicates a significant and possibly familial difference between *Cercaria notobucephala* and

other bucephalids. Branching sporocysts are reported universally when features of this group are enumerated: Lauckner (1983), Erasmus (1972), Ito (1964), Faust (1926), Stunkard (1983, 1974), Combes (1980), Holliman (1961), Lasiak (1991, 1993), Cole (1935), Baturo (1977), Calvo-Ugarteburu (1996), Matthews (1973), Heasman, O'Connor & Frazer (1996), Umiji Lunetta & Leonel (1976), Taskinen, Valtonen & Gibson (1991) and Princep, Bigas & Durfort (1996). Another feature that distinguishes *Cercaria notobucephala* is the presence of long paired gland cells that run almost the length of the cercarial body from the anterior sucker. A further reason for declaring *Cercaria notobucephala* a new species is based on bucephalid host specificity. Baturo (1977) asserts that bucephalids are particularly specific with respect to the first intermediate host. Lauckner (1983 p636) says, "...specific identification of larval forms parasitizing bivalves is facilitated by the fact that bucephalids are markedly specific with respect to the choice of their first intermediate hosts". These factors of morphology and host specificity strongly suggest that *Cercaria notobucephala* is indeed a new species.

Nevertheless, descriptions of the following gasterostome cercariae were examined. Where available, their flame cell formulae were compared with that of *Cercaria notobucephala.* No reference was found to a bucephalid cercaria of the same flame cell formula or even flame cell number as that in *Cercaria notobucephala.*

Combes (1980) reports the flame cell formula of 2[(1+2)+(2+1)] = 12 in *Bucephaloides gracilescens* (Rudolphi, 1819) Hopkins, 1954. Holliman (1961) reports the flame cell formula of 2[(2+2+2)] = 12 in *Cercaria apalachiensis* Holliman. The flame cell number was given but the formula was ascertained from the figure. *Cercaria apalachiensis* also has a more muscular ventral sucker and occurs in branched sporocysts. Its anterior sucker is surrounded by five lips, rather than four as in *Cercaria notobucephala.*

Stunkard (1974), reports that *Bucephalopsis hexaglandulata* Pandey, 1970, *Bucephalopsis multiglandulata* Pandey, 1970, *Bucephaloides caecorum* Hopkins, 1956, *Bucephalopsis oxygasteri* Pandey, 1970, and *Bucephalus cynoscion* Hopkins, 1956, all have 2[(2+2)+(2+2)] = 16 flame cells. The same formula is reported by Stunkard (1983) in *Rhipidocotyle transversale* Chandler, 1935, and *Rhipidocotyle*

lintoni Hopkins, 1954. Stunkard (1974) reports the formula of 2[(2+2+2)+ (2+2+2)] = 24 in *Bucephalopsis clara* Komiya, 1943, and *Bucephalus cuculus* McCrady, 1874. The formula is also reported by Cable (1956) in *Cercaria caribbea XLII* Cable, 1956, and Combes (1980) reports it in *Parabucephalopsis prosthorchis* Tang *et* Tang, 1963. The same number - the formula is not given - is reported by Madhavi, Lakshmibai, & Rao (1994) in *Cercaria chilkaensis III* Madhavi, Lakshmibai, & Rao.

Combes (1980) reports a formula of 2[(3+3+3)+(3+3+3)] = 36 in *Bucephaloides baeri* Maillard, 1976, and *Bucephalus longicornutus* (Manter, 1954) Howell, 1966. This is also reported in *Bucephalus polymorphus* Baer, 1827, by Wallet & Lambert (1984), *Cercaria scoti* Woodhead, 1936, in Stunkard (1974), *Cercaria argi* Woodhead, 1936, in Stunkard (1974) and *Rhipidocotyle septpapillata* Krull, 1934, in Stunkard (1974). The same number of flame cells is shared by *Prosorhynchus crucibulum* [syn. *Rudolphinus crucibulum*] but the formula differs. It is simplified here using instead Stunkard's (1974) and ignoring Dubois' (1944) system of flame cell formula presented in Matthews (1973): 2[(3+2+4)+(3+3+3)] =36. *Prosorhynchus squamatus* Odhner, 1905, in Matthews (1973) [spelt *squamatus* and *squamata* in Stunkard (1974)] is simplified, ignoring Dubois' (1944) system using instead Stunkard's (1974): 2[(3+4+4)+(3+4)] = 36.

Stunkard (1974) reports that *Bucephaloides strongyloides* Hopkins, 1954, and *Rhipidocotyle lepisostei* Hopkins, 1954, both have formulae of 2[(4+4+4)+ (4+4+4)] = 48. *Bucephalus polymorphus* Baer, 1827, in Baturo (1977) has 2[(4+4+4+4)+(4+4+4)] = 56. *Bucephalus haimenus* Lacaze-Duthiers, 1854, in Combes (1980) has 2[(3+3+6+6+3)+(4+4+3)] = 64. *Rhipidocotyle illense* (Ziegler, 1883) in Baturo (1977) and *Rhipidocotyle campanula* (Dujardin, 1845) Dollfus, 1968, in Combes (1980) both have 2[(5+5+5+5)+(5+5+5)] = 70. *Labratrema minimum* Stossich 1887 (Maillard 1975) in Combes (1980) has 2[(6+6+6)+(6+6+6)] = 72. *Rhipidocotyle papillosa* Woodhead, 1929, Eckmann 1932, in Stunkard (1974) has 2[(5+5+5+5)+(5+5+5+6)] = 82. *Bucephalus elegans* Woodhead, 1929, in Stunkard (1974) has 2[(7+7+7)+(8+8+9)] = 92. Besides other morphological differences, that none of these cercariae share the flame cell formula - or even flame cell number - rules them out as possible homologues of *Cercaria notobucephala*.

The following cercariae require closer scrutiny because of either a lack of published flame cell formulae or because they are found more locally. The bucephalid cercaria from *Perna* (Calvo-Ugarteburu 1996) differs in that its sporocysts in *Perna* are branched, in contrast with those of *Cercaria notobucephala*. Furthermore, no bucephalid infection in *Perna* has been reported from Cape Peninsula beaches. If this were the same species as *Cercaria notobucephala* one would expect it to also infect *Perna* at Dido Valley, where there is significant prevalence of *Cercaria notobucephala* in *Choromytilus*.

Another local bucephalid must be eliminated as a possible previous description of *Cercaria notobucephala*. Faust (1926) reports the gasterostome *Bucephalopsis modiolae* Faust, 1926, in *Arcuatula (Modiola* or *Lamya) capensis* (Krauss), a mytilid found on rocky substrata associated with mud deposition zones in lower reaches of estuaries. This mytilid has been reported from Hermanus, west of Cape Agulhas, to the coast of Mozambique (Davies 1980). *Bucephalopsis modiolae* is not the same as *Cercaria notobucephala*. This is despite no flame cell formula for *Bucephalopsis modiolae* having been ascertained. The anterior sucker of *Bucephalopsis modiolae* is more muscular and some 25% larger than that in *Cercaria notobucephala*. Its ventral sucker is much more muscular and Faust (1926) does not report the excretory diverticulae seen in *Cercaria notobucephala*.

Why does *Cercaria notobucephala* occur in *Choromytilus meridionalis* and *Venerupis corrugatus* but not in the other mytilids? If one accepts Fahrenholz's Rule, one would expect more closely related hosts to have more closely related parasites. Fahrenholz's rule (Rohde 1993, p100) "states that the classification of some groups of parasites parallels that of their hosts, which implies that ancestors of extant parasites must have been the parasites of the ancestors of extant hosts, or in other words, that the evolution of hosts and parasites must have been in parallel." One can infer that phylogenetically closer organisms have phylogenetically closer parasites. If this is conflated with host specificity, one would expect closely related hosts to be more likely to share the same parasites than less related hosts. This does not appear to be happening here. Organisms separated by a greater phylogenetic distance such as *Choromytilus* and *Venerupis,* apparently share a very specific parasite but the phylogenetically closer

Choromytilus, Mytilus, Perna and Aulacomya do not. How can we interpret the results in such a way that makes sense?

Two alternatives must be considered. The first is that lack of infection in other mytilids is due to niche separation and thus an ecological barrier to infection. The second is that the parasites in *Choromytilus* and *Venerupis* are really sibling species each with their own host specificity, neither of which includes the other mytilids. If we look at the first option, it can be seen that *Choromytilus* and *Venerupis* share a similar niche and would have a similar exposure to the parasite. Both occur in tidal pools and are often buried in sand. Other mussels are less tolerant of this and they do not occur in this niche. At Blouberg, *Aulacomya* is more sub-tidal and occurs in areas of greater wave action. *Perna* is extremely uncommon (but not absent pers. obs.) at Blouberg. At Dido Valley it tends to occur on non-horizontal surfaces and is emersed for longer than most *Choromytilus*. The same can be said for *Mytilus* and that it is non-indigenous may confer some resistance by the parasite having less familiarity with it, but would one be justified in expecting total absence of the parasite? In addition, there is some niche overlap between these mussels but there is no proportional level of infection. Thus this option only partly explains the difference in infection susceptibility between the mussels.

The hypothesis that the parasites are sibling species is plausible if one considers that, (Ito 1964, p399) "A very small morphological difference may be observed between cercariae of closely related species and it may frequently occur that cercariae considered to be apparently one species are proved to be more than two species after detailed observation on the adult flukes obtained by experimental infection". Thus it could be that both *Venerupis* and *Choromytilus* harbour the same morphological species (*Cercaria notobucephala)*, but perhaps the parasites of *Venerupis* and *Choromytilus* are sibling species.

If true, such a hypothesis could also help to reinterpret Lasiak's (1991) report about bucephalid infections of *Perumytilus purpuratus* (Lamarck, 1819) [1% prevalence] and *Semimytilus algosus* (Gould, 1850) [20%-32% prevalence] that occurred adjacent to uninfected *Choromytilus chorus* (Molina, 1782) in Southern Chile. This juxtaposition of infection and hosts and apparent immune *Choromytilus chorus* might

be better understood if two host specific infections were postulated, one for *Perumytilus purpuratus* and one for *Semimytilus algosus* with no species present that infects *Choromytilus chorus*.

Neither hypothesis can be substantiated without further work and meanwhile a label for the parasite is required. It is argued here that for the sake of parsimony that the first hypothesis be provisionally accepted. These hypotheses can be tested by methods mentioned in the final comments.

Epidemiology

The infection is usually of very high intensity. An attempt at estimating relative intensities is made in Chapter 47. A problem lies in deciding whether the sporocyst or the cercaria is the unit of infection. Thus, analyses of prevalences, only, are presented here.

Prevalences varied (Table 2) from 10% in *Venerupis* (n=10) to 4.46% in *Choromytilus meridionalis* (n=650) at Blouberg and 0.23% in *Choromytilus* (n=870) from Dido Valley. Only in *Choromytilus* from Blouberg was the infection prevalence significantly high enough, and the sample size large enough, to merit graphic presentation of data. Griffiths (pers. comm.) found bucephalids - now suspected to be *Cercaria notobucephala* - in *Choromytilus* from False Bay where 7 out of 78 were infected giving a prevalence of 9%.

Figures 19 & 20 depicting size dependent prevalence in *Choromytilus* from Blouberg suggest that prevalence in host size-groups increases with host size. In both sexes the very largest mussels have 100% prevalence. That infection is size dependent is supported, in part, by inspection of the mean sizes of (Tables 3A & B) infected and uninfected *Choromytilus*. For females from Blouberg the values were for infected and uninfected mussels 65.87mm (SE 6.75) and 54.09mm (SE 0.52) respectively. In male *Choromytilus* from Blouberg the mean size of infected mussels was very close to that of uninfected mussels: 55.63mm (SE 1.76) and 55.05mm (SE 0.63) respectively. Infected and uninfected male [there were no female infected mussels] *Choromytilus* at Dido Valley were 71.95mm (SE 3.01) and 58.14mm (SE 0.51) respectively. This evidence points to a size dependent trend in infection with this parasite. Calvo-

Ugarteburu & McQuaid (1998) and Lasiak (1993) also found a positive correlation between infection prevalence with *Bucephalus* sp. and host size in *Perna.*

Male *Choromytilus* from Blouberg appear to suffer prevalence (Table 2) some three times higher than females. The sample size of over 300 mussels of each sex suggests that this difference is significant. The sample population from Dido Valley contains only two infected mussels, both male. These findings suggest a contrast with the assertion of Lauckner (1983) that both sexes of the bivalve host are affected to a similar extent.

Size comparison of uninfected male and female *Choromytilus* from Blouberg suggests no sex difference in size distribution. These are 55.05mm (SE 0.63) and 54.09mm (SE 0.52) respectively. Comparison of uninfected male and female *Choromytilus* from Dido Valley also suggests no sex difference in size distribution. These are 58.14mm (SE 0.51) and 58.17mm (SE 0.5) respectively. Thus it may be expected that any sex differences in epidemiology are due to causes other than sex-linked differences in morphometrics.

Figures 21 and 22 suggest no marked seasonal change in prevalence. This is consonant with the findings of Calvo-Ugarteburu (1996) and Calvo-Ugarteburu & McQuaid (1998) for the bucephalid in *Perna.* They, however, report that to detect any seasonal changes a monthly sample would need to be taken at a locality with a higher prevalence. This probably holds also for this study of *Cercaria notobucephala.* Lasiak (1993) found seasonal differences in prevalence of the bucephalid in *Perna.* Nevertheless, she suggested that this might reflect a seasonal shift in host mean size. This does not appear to be so in the Western Cape for *Cercaria notobucephala.*

The seasonal change in mean size of mussels *Choromytilus* and *Perna* (See Chapter 5 Figures 40, 41 & 42) shows that though it may change, month to month the sexes remain very close in size. This further suggests that there is no detectable sexual dimorphism as measured in this way. Figures 19 and 20 show that the size distributions in samples of males and females both approximate to normal but with a

longer tail on the larger end of the distribution. This may be explained in part by the omission of smaller mussels whose sex could not be identified and whose omission here cuts off the lower tail of the distribution. Figures 19 and 20 suggest that there is some size-dependent prevalence; but it must be noted that the upper prevalence value of 100% for both females and males at their largest size is based on a sample of one specimen each. The association of infection and host size raises the issue of whether such parasites cause gigantism or whether the increased prevalence is a function of age of the mussel. Age, in turn, may determine size. Thus, larger, and therefore older, mussels will have had greater opportunity for becoming infected. This has been discussed by Calvo-Ugarteburu (1996) and Calvo-Ugarteburu & McQuaid (1998) in bucephalid infections of *Perna*. They argue that the parasites afflict large mussels more and that the bucephalid affects reproduction (by parasitic castration) rather than growth.

Webb (1991a) reviews aspects of parasitic castration and gigantism as it refers to a microphallid infection in *Bullia digitalis,* the nassariid surfing snail. The conclusions are also applicable to infections of *Cercaria notobucephala* in *Choromytilus*. See also Princep, Bigas, & Durfort (1996) for details of the mechanism of parasitic castration in the bucephalid *Prosorhynchus squamatus* Odhner, 1905 in *Mytilus edulis*. Parasitic gigantism is characteristic of semelparous rather than iteroparous species. Since *Bullia digitalis* (Dillwyn) and *Choromytilus meridionalis* are both iteroparous, one would not expect parasitic gigantism. Instead, as Lauckner (1983) asserts, larger molluscs have higher prevalences because of their longer exposure time.

The infection in *Choromytilus* never fails to reduce gamete production and infection is always advanced with the entire mantle being ramified by sporocysts. That no lighter or heavier infections were seen, might be accounted for by the small numbers of parasitised mussels examined. On the other hand, it may indicate that the disease progresses rapidly to a stable and thus long-lived and common condition. A third possibility is that the infection is fatal once the mussel has been parasitically castrated. For example, Heasman, O'Connor & Frazer (1996) assert that after parasitic castration the parasite may spread to other organs causing increased mortality and weakening of the adductor muscle. Thus, heavy infections would not appear in the samples of live mussels taken in this study.

The bucephalid infecting *Perna* had no effect on mortality or gaping behaviour of the mussels when it was exposed to air, although water loss increased and adductor muscle strength was diminished (Calvo-Ugarteburu 1996). In addition, there was no significant effect on filtration rate or oxygen consumption. Thus, somatic effects on *Perna* are far less significant than reproductive effects. In *Choromytilus* the reproductive effect of *Cercaria notobucephala* is apparently milder than that of the infection in *Perna*. Could it be expected that the somatic effects in *Choromytilus* are correspondingly milder? Attempts will be made to ascertain this in later chapters.

Lauckner (1983) asserts that bucephalids are among the most harmful metazoan parasites of marine bivalves. Such infection reduces (Lauckner 1983) the host's resistance to environmental stress -It is significant that Lauckner (1983) says that the flesh yield of infected bivalves may be improved at the outset of the infection followed by a decline. Is this increase indicative of an increase in mussel fitness? Is it a eustress? Such apparent fluctuations are discussed in Chapter 16 and elsewhere. The deleteriousness of bucephalids is also asserted by Princep, Bigas & Durfort (1996) and Lasiak (1993) who found that infections can result in increased mortality.

Epidemiology of other bucephalid infections

Lasiak (1993) reported a prevalence of 2-12% in *Perna* from beaches of the Eastern Cape, Transkei and Natal in South Africa. Calvo-Ugarteburu & McQuaid (1998) reported a maximum prevalence of 49% in the bucephalid from *Perna* on Eastern Cape beaches but usually it was lower than this with typical figures of 6%, 12% and over 20%. Some of these prevalences are higher than for *Cercaria notobucephala.*

Heasman, O'Connor & Frazer (1996) report prevalences of *Bucephalus* sp. in *Pecten fumatus* Reeve (5.1% to 63%) and *Chlamys asperrima* (Lamarck) (4.5% to 24%). Infection was size dependent, reaching 66% in *Pecten fumatus* and 40% in *Chlamys asperrima* over 80mm and 75mm shell height respectively. Lauckner (1983) reports that prevalences, among other parameters, of bucephalids vary widely with locality and season. An extreme example is his report of 100% prevalence in *Semimytilus algosus* (Gould, 1850) from Chile.

CHAPTER 4: *METACERCARIA PERCHORUPIS* SP. NOV.- A GYMNOPHALLID IN A GELATINOUS CYST

HOSTS AND LOCALITY

This parasite occurs in *Perna perna* at Dido Valley and *Choromytilus meridionalis* at Dido Valley and Blouberg. It was also found in *Venerupis corrugatus* from Blouberg.

TYPE SPECIMENS

Paratypes: Specimen number A29428. Marine Biology Department, South African Museum, Queen Victoria Street, Cape Town, RSA. Type host: *Choromytilus meridionalis*. Type locality: Blouberg.

DESCRIPTION

The metacercaria lies extended within its gelatinous cyst (Figures 1 & 2) embedded in the mantle next to the shell. Several cysts may abut one another forming polyhedra. Host tissue, which stains (acetic-orcein) much more densely than the parasite, sometimes adheres to the cyst. The cyst wall is concentrically striated with fine particles (Chapter 2 Figures 1 A, B, & 2 A, B,). The width and pitch of these striae measure from one to a few micrometres. Striae resemble the pattern of spines on the tegument and may possibly be made by the passage of mucus from the cystogenous glands over the spines. These glands (Figure 3), whose ducts open at the surface of the tegument, underlie the entire tegument and stain with methylene-blue or bromocresol-green. Gentian-violet stimulates the glands to throw off a "shell" of mucus. Tegument spines at the anterior tend to be large and blunt (Figure 5). Midway between oral and ventral suckers the spines become truncated. Further to the posterior the spines are more pointed and are about half the pitch and size of those at the anterior. Between the genital pore and the ventral sucker, a distance of just over 20µm, the spines double in pitch and length.

The oral sucker is subterminal. On its lip there are usually ten papillae, four rounded (dorsal) and six sharp (ventral). More laterally, one large, blunt, horn-like lateral papilla lies on either side of the oral sucker (Figures 3, 4 and 5). Eight gland cells, each about 2.5µm by 5µm, lie radially in the oral sucker musculature. The sucker interior is paved with cells, each about 2µm across. Five pairs of penetration gland

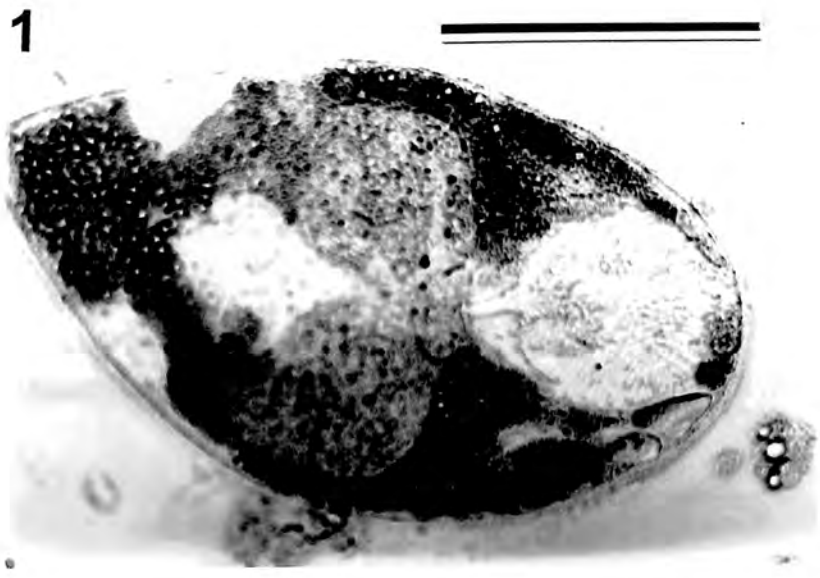


Figure 1. Light micrograph of *Metacercaria perchorupis,* scale bar = 150μm.

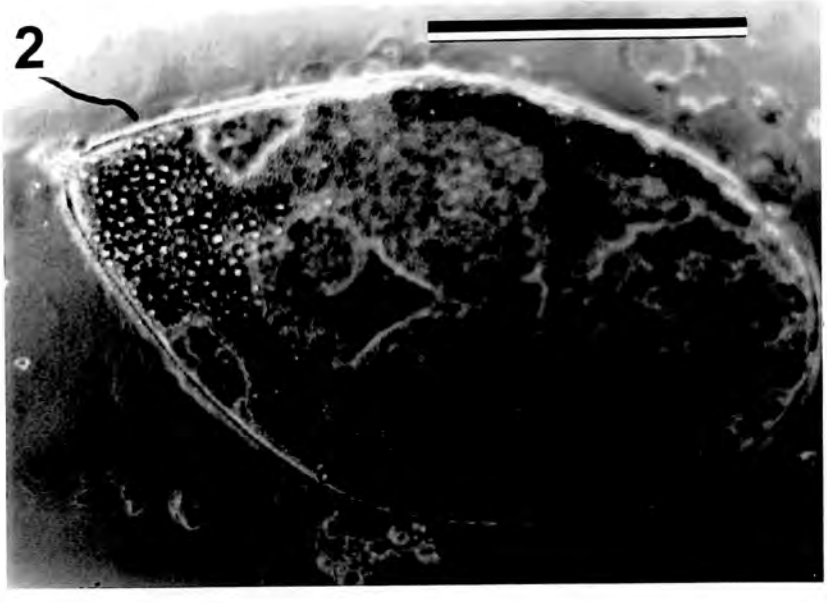
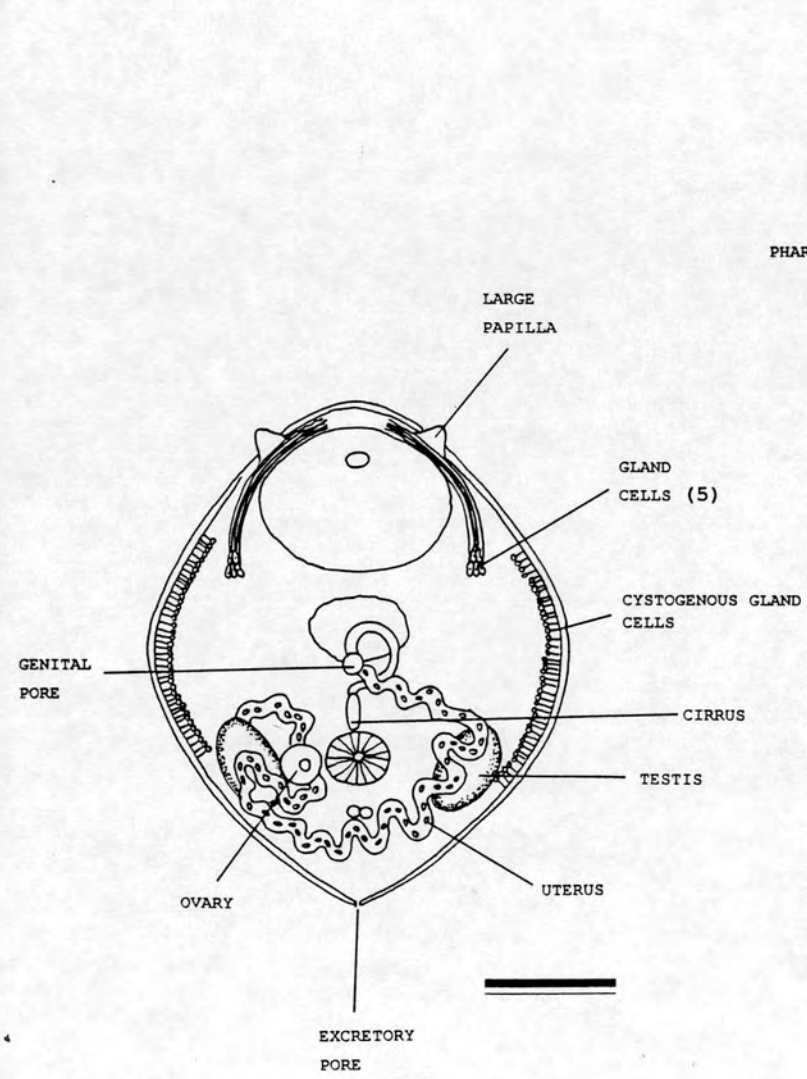


Figure 2. Light micrograph of *Metacercaria perchorupis,* scale bar = 150μm.

Figure 4. *Metacercaria perchorupis,* showing the excretory system and the digestive tract, scale bar = 50μm.



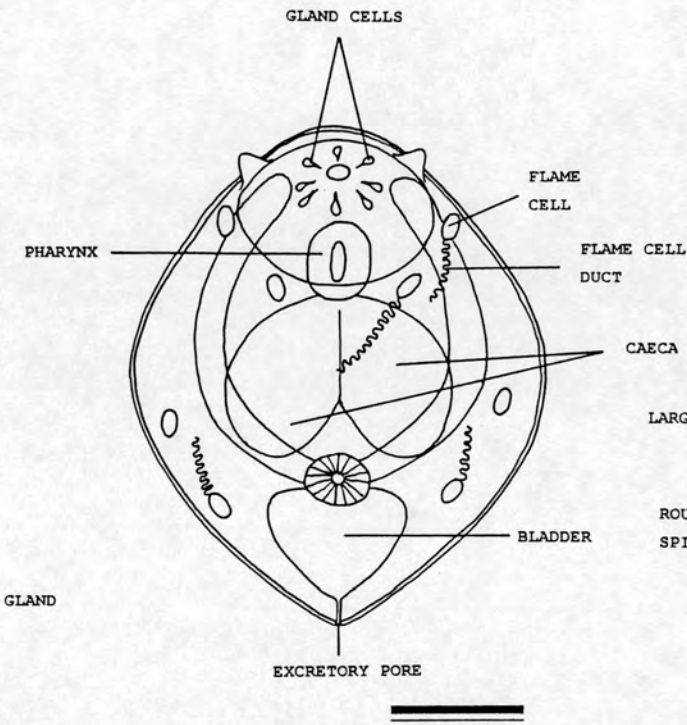


Figure 3. *Metacercaria perchorupis,* details of the reproductive system, scale bar = 50μm.

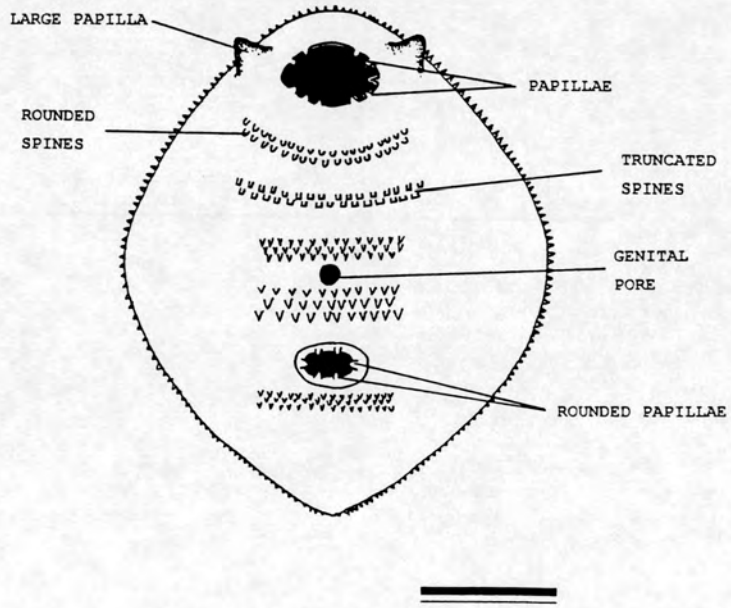


Figure 5. *Metacercaria perchorupis* details of tegument, scale bar = 50μm.

cells (Figure 3) enclose the oral sucker longitudinally and open at its mouth.

The pre-pharynx has marked longitudinal grooves and is about half the length of the pharynx. The translucent yellow, short and fat caeca terminate at about the mid-point of the body. They are filled with granules or oil droplets that are covered by minute hair-like filaments. These droplets can be dissolved by acetone but this is only partially successful in rendering the caeca clear. Instead of being carried away by the acetone the oil was redeposited in adjacent tissues. Acetic-orcein or glacial acetic acid does not remove the oil even though it does clear the excretory vesicle. Gentian violet causes convulsions that void the contents of the caeca through the oral sucker, thus clearing the digestive tract. Each caecum has a sphincter near the junction with the oesophagus. Measurements of the caeca are given below: length is the extreme anterio-posterior extent; width is the extreme lateral extent of the caecum.

In life, the ventral sucker is deeper than long and lies about two thirds of the way from the anterior. Its opening is guarded by six large inward pointing (Figure 5) papillae: two point forwards, two point backwards and one on either side points towards the body centre line. The excretory pore is at the posterior extremity of the worm and has a diameter of about 4μm (5μm in a formalin-fixed specimen). The bladder, which is roughly conical with the apex posterior, appears lined with epithelium. A short duct leads forward from the bladder and branches into the two primary ducts at the level of the ventral sucker. The ducts proceed along the lateral margins to terminate either side of the oral sucker. The entire excretory system is filled with globular refractive particles (Figures 1A & 2A in Chapter 2). These may be cleared by glacial acetic acid or acetic-orcein (Figures 1B & 2B in Chapter 2). Eight flame cells are visible (Figure 4): six occur laterally and two more medially. The arrangement of flame cell ducts suggests the mesostomate formula:

$$2\,[(2) + (2)] = 8 \text{ flame cells.}$$

Testes lie dorsal and laterally about halfway between the ventral sucker and the posterior extremity. The cirrus lies between the ventral sucker and the genital pore (Figure 3). Overlapping and anterior to the genital pore is the dark staining genital atrium. A canal from the cirrus loops around the genital pore and joins it anteriorly. A single ovary about two thirds of the size of a testis lies slightly medial and anterior to one of the testes on the left of the body (ventral side up). The uterus, whose width

is about 5μm, proceeds from the ovary and fills the posterior part of the body with its convolutions. It is filled with small eggs and joins the genital pore posteriorly but just before this it swells slightly. The uterus starts at about mid-depth in the body at the posterior and as it approaches the genital pore it becomes more ventral.

Table 1. Measurements (μm) of *Metacercaria perchorupis*. A living, B formalin fixed, C heat killed.

A

	mean	SD	*n*	max.	min.
body length	185	46.8	13	281	122
width 25% from front	117	18.4	10	159	102
width 50% from front	155	24	10	189	123
width 75% from front	114	15.4	10	150	103
oral sucker length	53.8	15	10	70	30
oral sucker width	58.7	14.6	10	78	33
oral sucker depth	68.6	9.1	10	78.4	53.9
ventral sucker length	18.4	9.1	10	34	7
ventral sucker width	23.4	8.6	10	35	13
ventral sucker depth	27	4.4	10	30	22
pharynx length	26.7	8.3	10	47	17
pharynx width	22.9	4.7	10	30	15
cuticle thickness	2.8	0.6	10	4	2
anal subtegument	9	-	2	10	8
cuticle spines length	1.7	0.7	10	2.5	0.6
cuticle spines width	2	0.5	10	2.5	1
cuticle spines pitch	2.5	0.76	10	4.5	1.7
genital pore diameter	6.84	2.0	10	9.8	4
flame cell length	9.1	2.7	10	12	5
flame cell width	5	1.3	10	7	3
testis length	34.9	7.3	10	48	25
testis width	21.3	3.8	10	30	17
testis subunits dia.	5.5	1.7	10	7.4	2.5
excretory granules	3.4	1.8	10	7.4	1.3
granules in caeca	4.8	2.2	10	10	2.5
caeca to end of body	68.1	15.1	10	90	50
caeca length	60.8	10.6	10	68.6	42
caeca width	45.9	8.6	10	65	36.8

B

	mean	SD	*n*	max.	min.
length	91.4	31	10	232.8	159.3
width 25% from front	113.2	16.5	10	134.8	85.8
width 50% from front	157.5	12.2	10	176.4	134.8
width 75% from front	119.8	13.7	10	134.8	98
oral sucker length	58.6	7.1	10	68.6	49
oral sucker width	68.4	9.6	10	88.2	53.9
oral sucker depth	74.7	-	2	71.1	78.4
ventral sucker length	27.69	2.3	10	24.5	31.9
ventral sucker width	31.85	3.3	10	36.8	27
pharynx length	27	7.2	10	36.8	17.2
pharynx width	22.5	4	10	27	17.2

cuticle thickness	2.5	0.48	10	3.7	1.7
cuticle spines length	2.9	0.7	3	3.7	2.5
cuticle spines width	1.8	0.7	4	2.5	1.2
cuticle spines pitch	1.7	0.6	4	2.5	1.2
genital pore diameter	5.2	1.8	4	7.4	3.7
testes length	29.7	8.6	10	51.5	22.1
testes width	21.1	3.1	10	24.5	17.2
excretory granules	4.34	2.4	10	9.8	1.2
granules in caeca	5.4	1.4	10	7.4	2.5
caeca to end body	65.2	9.5	10	24.5	17.2
caeca length	58.3	6.2	10	24.5	17.2
caeca width	53.4	22	10	93.1	27

C

	mean	SD	*n*	max.	min.
body length	197.9	17.2	10	225.4	176.4
width 25% from front	115.4	13.3	10	137.2	98
width 50% from front	144.3	13.3	10	166	122.5
width 75% from front	116.1	11.4	10	134.8	102.9
oral sucker length	67.1	9.4	10	83.3	51.5
oral sucker width	74.2	5.8	10	83.3	67.3
ventral sucker length	29.9	3.8	10	36.8	27
ventral sucker width	29.4	6.2	10	39.2	22.1
ventral sucker depth	29.4	5.7	10	36.8	22.1
pharynx length	21.3	4.3	10	29.4	14.7
cuticle thickness	2.1	0.6	10	2.5	1.2
cuticle spines length	1.8	0.6	7	2.45	1.23
cuticle spines width	2.1	0.6	7	2.45	1.23
cuticle spines pitch	2.3	0.6	7	2.94	1.23
genital pore diameter	5.8	1.3	10	7.4	3
testes length	28.2	4.8	10	36.8	194.6
testes width	21.3	2.5	10	24.5	17.2
excretory granules	3.55	1.58	10	6.13	1.23
granules in caeca	6.13	2.83	10	9.8	2.45
caeca to end of body	72.3	12.5	10	90.7	58.8
caeca length	59.5	13.1	10	73.5	36.8
caeca width	54.9	15	10	58.8	36.8

EPIDEMIOLOGY

Prevalences

Table 2A. Prevalences of *Metacercaria perchorupis* in sub-populations of *Choromytilus meridionalis* from Blouberg.

	infected	sample no	prevalence
males	81	81	100%
females	68	68	100%
unidentified	1	1	100%
total	150	150	100%

In all cases prevalence was 100%. The size distributions and their mean intensities for females and males are represented in figures 6 & 7 respectively.

Table 2B. Prevalences of *Metacercaria perchorupis* in sub-populations of *Choromytilus meridionalis* from Dido Valley.

	infected	sample no.	prevalence
males	68	73	93.15%
females	68	75	90.67%
unidentified	0	2	0%
total	136	150	90.67%

Table 2C. Prevalences of *Metacercaria perchorupis* in sub-populations of *Perna perna* from Dido Valley.

	infected	sample no.	prevalence
males	51	76	67.11%
females	55	64	85.94%
hermaphrodites	1	3	33.33%
total	107	143	74.83%

Table 2D. Prevalences of *Metacercaria perchorupis* in *Venerupis corrugatus* from Blouberg.

	Infected	sample no.	prevalence
total	16	20	80%

Host morphometrics, intensity and abundance

Table 3A. Morphometrics of *Choromytilus meridionalis* and intensity of infection with *Metacercaria perchorupis* at Blouberg. All *Choromytilus meridionalis* from Blouberg were infected. Thus, abundance and intensity are the same.

	mean length mm	SD	SE	*n*	intensity	SD	SE
males	60.28	6.55	0.73	81	35.53	23.02	2.56
females	60.48	7.69	0.93	68	58.66	39.2	4.75
immature	41.65	---	---	1	3	---	---
total	60.24	7.23	0.59	150	45.8	33.57	2.74

Table 3B. Morphometrics of *Choromytilus meridionalis* at Dido Valley.

	mean length mm	SD	SE	*n*
male infected	62.75	6.44	0.78	68
male uninfected	61.60	1.53	0.68	5
male total	62.67	6.23	0.73	73
female infected	61.59	6.02	0.73	68
female uninfected	57.77	4.41	1.66	7
female total	61.23	5.99	0.69	75
total infected	62.17	6.26	0.54	136
total uninfected	55.90	9.79	2.62	14
total	61.60	6.90	0.56	150

Table 4. Abundance and intensity of infection with *Metacercaria perchorupis* in *Choromytilus meridionalis* at Dido Valley.

	abundance	SD	intensity	SD	SE
total	11.34	10.197	12.507	10.005	0.86
females	14.08	11.366	15.53	10.953	1.33
males	8.836	7.965	9.49	7.871	0.95

Table 5. Morphometrics of *Perna perna* at Dido Valley.

	mean length mm	SD	SE	*n*
male infected	54.12	7.26	1.02	51
male uninfected	55.61	9.74	1.95	25
male total	54.61	8.19	0.94	76
female infected	54.78	8.65	1.17	55
female uninfected	54.61	10.27	3.42	9
female total	54.76	8.89	1.11	64
total infected	54.33	8.1	0.79	106
total uninfected	55.14	9.66	1.59	37
total	54.53	8.53	0.71	143

hermaphrodites have been omitted

Table 6. Abundance and intensity of infection with *Metacercaria perchorupis* in *Perna perna* at Dido Valley.

	abundance	SD	intensity	SD	SE
total	3.28	5.42	4.38	5.87	0.57
females	4.41	6.30	5.13	6.52	0.88
males	2.45	4.44	3.65	5	0.7

DISCUSSION

Taxonomic affinities

Besides the general works on cercariae mentioned in Chapter 2, the following deal specifically with gymnophallid metacercariae: Loos-Frank (1971), Calvo-Ugarteburu (1996), Bowers, Bartoli, Russell-Pinto & James (1996), Pekkarinen & Ching (1994), Pekkarinen (1986), Machkevsky (1989), Cable (1953), Seed (1991), Tharme, Webb & Brown (1996), Kyle & Noblet (1985), Stunkard & Uzmann (1958), James (1964), Lee, Chai, & Hong (1993), Yu, Chai & Lee (1993), Campbell (1985), Ching (1973 a & b), Stunkard (1932), Lee, Choi, Seo & Chai (1995), Bowers & James (1967), Tharme, Webb & Brown (1996) and Tharme (1988). These were also consulted when seeking previous description of this worm.

The presence of a mucus envelope around the metacercaria was initially thought to be significant but the literature revealed that it is not uncommon. It occurs around (Lauckner 1983) *Parvatrema duboisi* (Dollfus) Bartoli, 1974, which is a parasite of *Mytilus galloprovincialis*. The worm is found at the base of gill lamellae, and between mantle and shell. Similar mucus envelopes are found around *Meiogymnophallus sp.* in (Lauckner 1983) and *Metacercaria perlingena* Palombi, 1940, in Cheng (1967, p222, Fig 132). A very similar gelatinous capsule embedded with calcium particles is found (Cheng 1967) surrounding cysts of *Gymnophallus margaritarum* (Dubois, 1901) and *Cercaria megalocoela* Palombi, 1934.

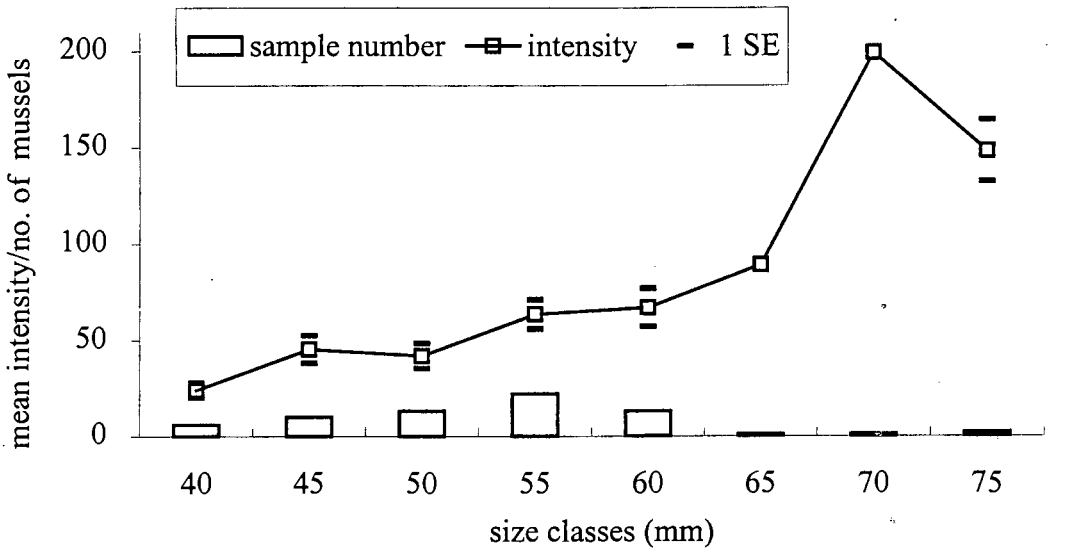


Figure 6. Size dependent mean intensity of infection with *Metacercaria perchorupis* and sample number in female *Choromytilus* from Blouberg.

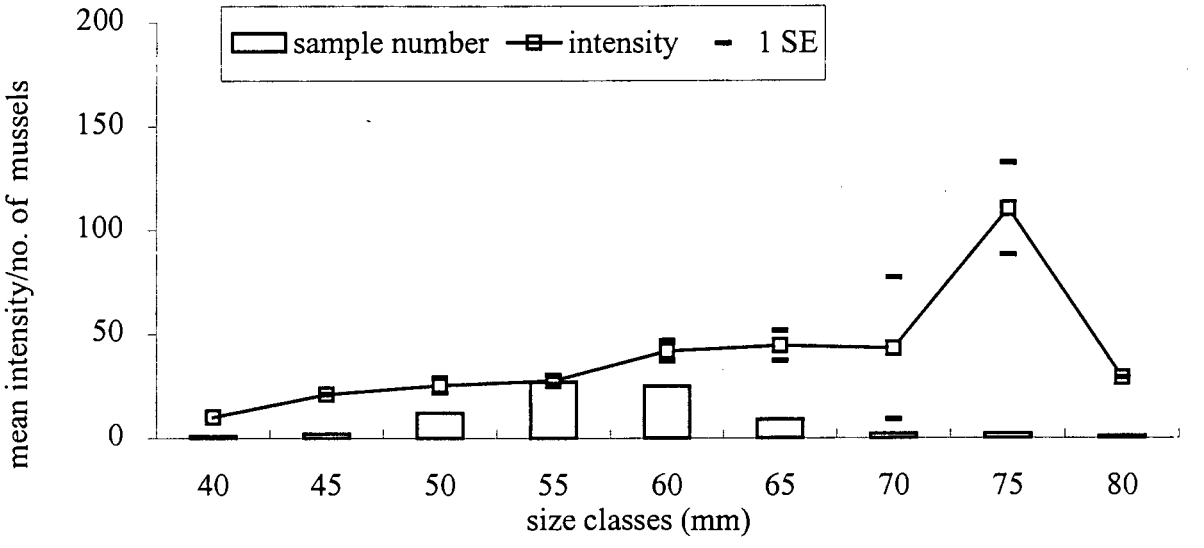


Figure 7. Size dependent mean intensity of infection with *Metacercaria perchorupis* and sample number in male *Choromytilus* from Blouberg.

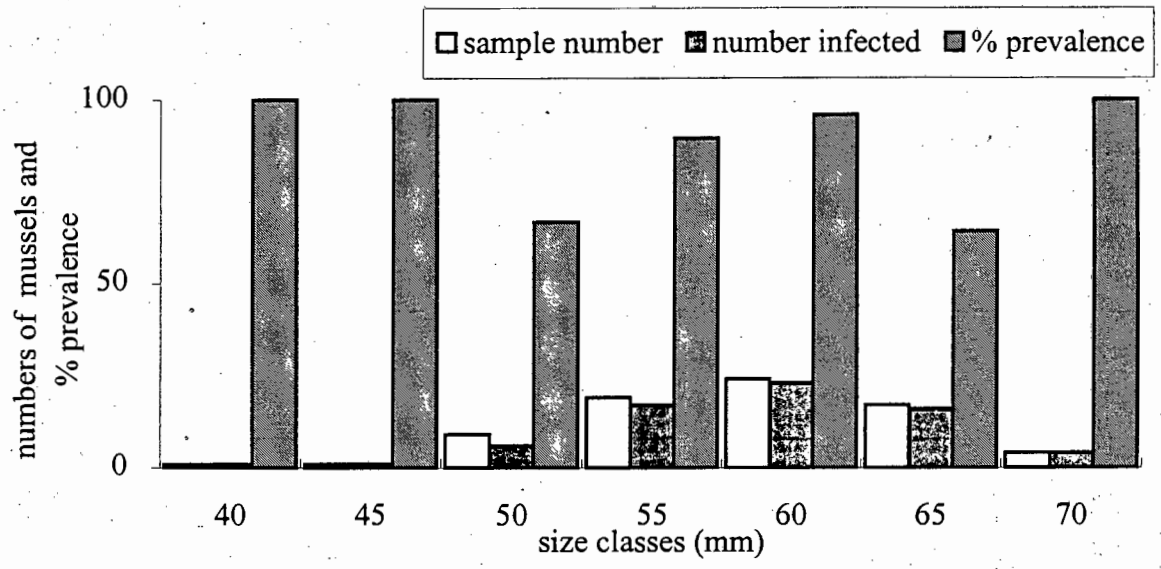


Figure 8. Size dependent prevalence, number infected, and sample number of female *Choromytilus* infected with *Metacercaria perchorupis* from Dido Valley.

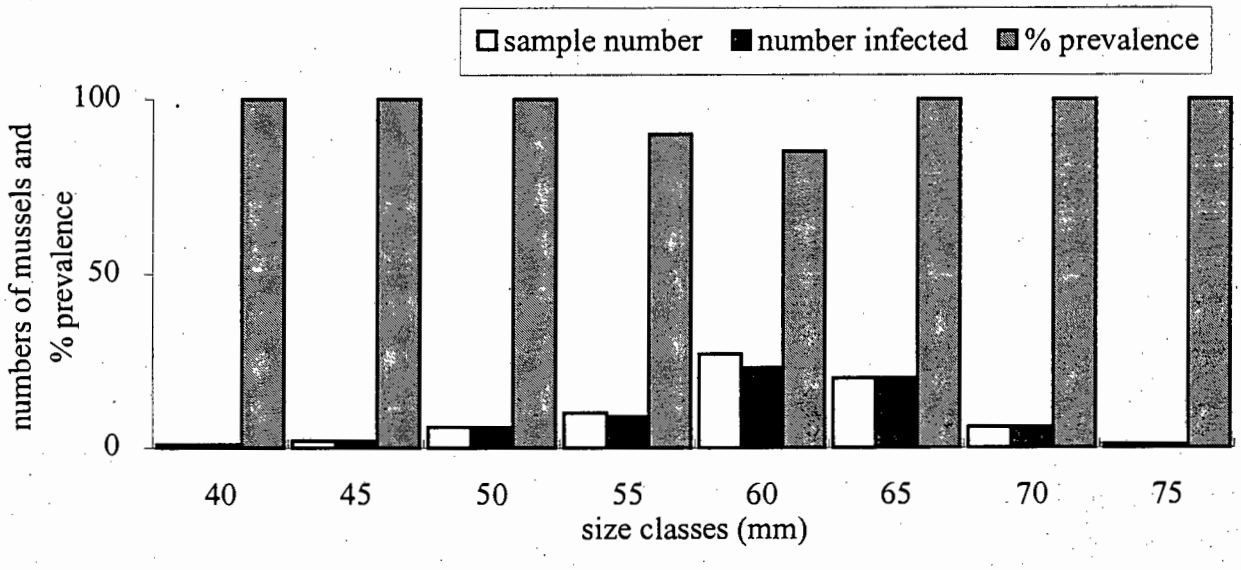


Figure 9. Size dependent prevalence, number infected, and sample number of male *Choromytilus* infected with *Metacercaria perchorupis* from Dido Valley.

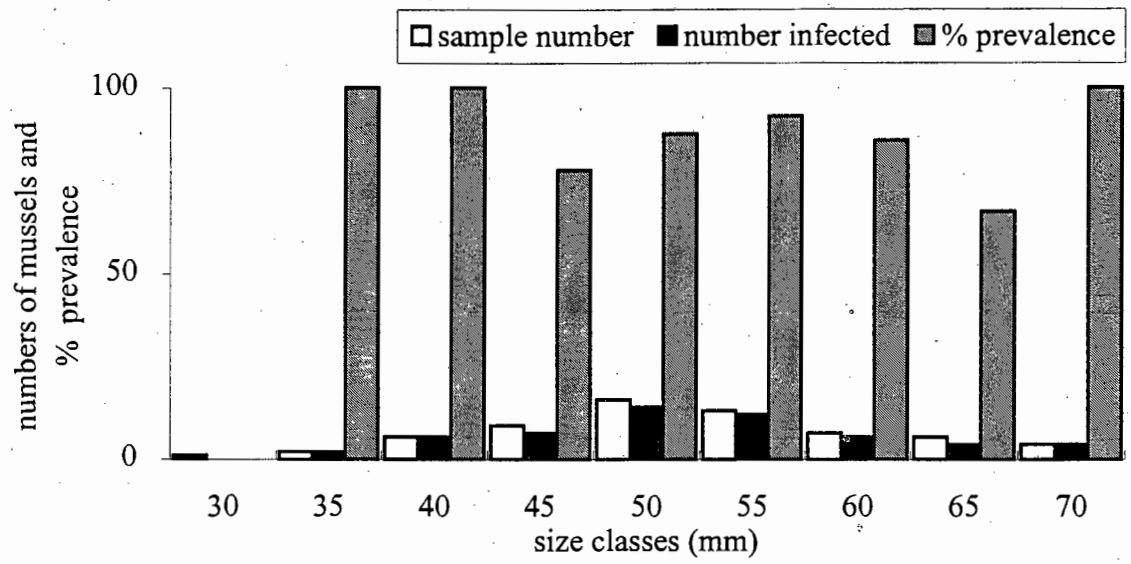


Figure 10. Size dependent prevalence, number infected, and sample number of female *Perna* infected with *Metacercaria perchorupis* from Dido Valley.

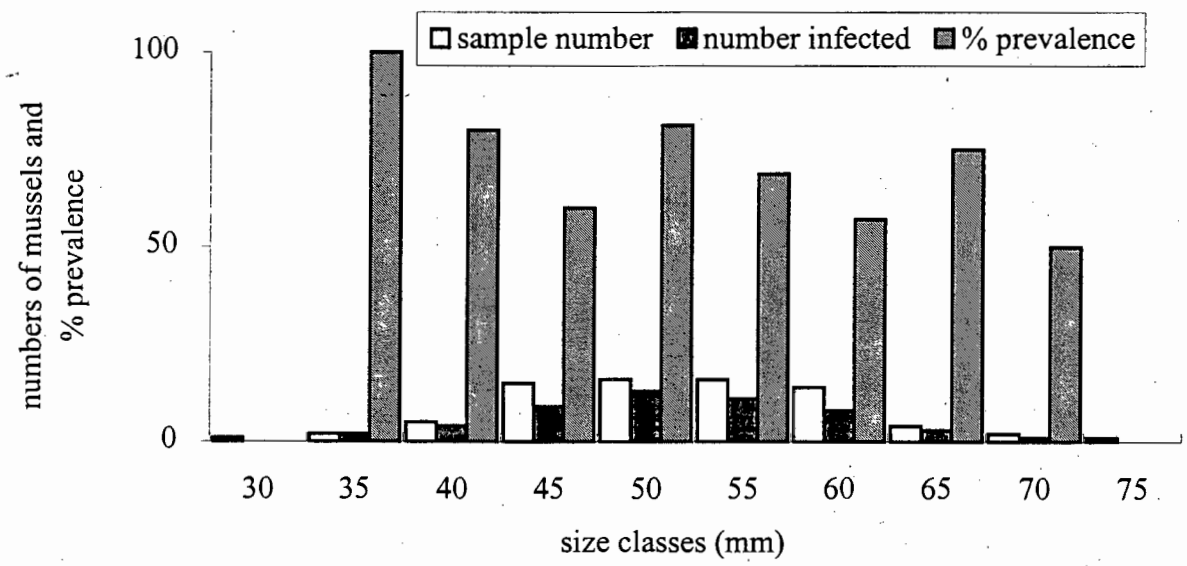


Figure 11. Size dependent prevalence, number infected, and sample number of male *Perna* infected with *Metacercaria perchorupis* from Dido Valley.

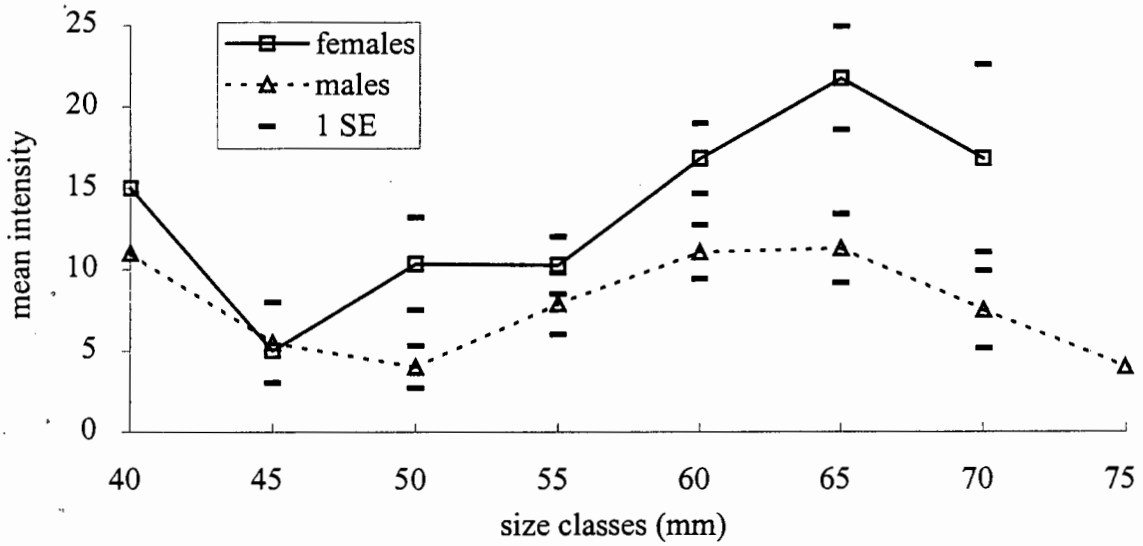


Figure 12. Size dependent mean intensity of *Metacercaria perchorupis* in female and male *Choromytilus* from Dido Valley.

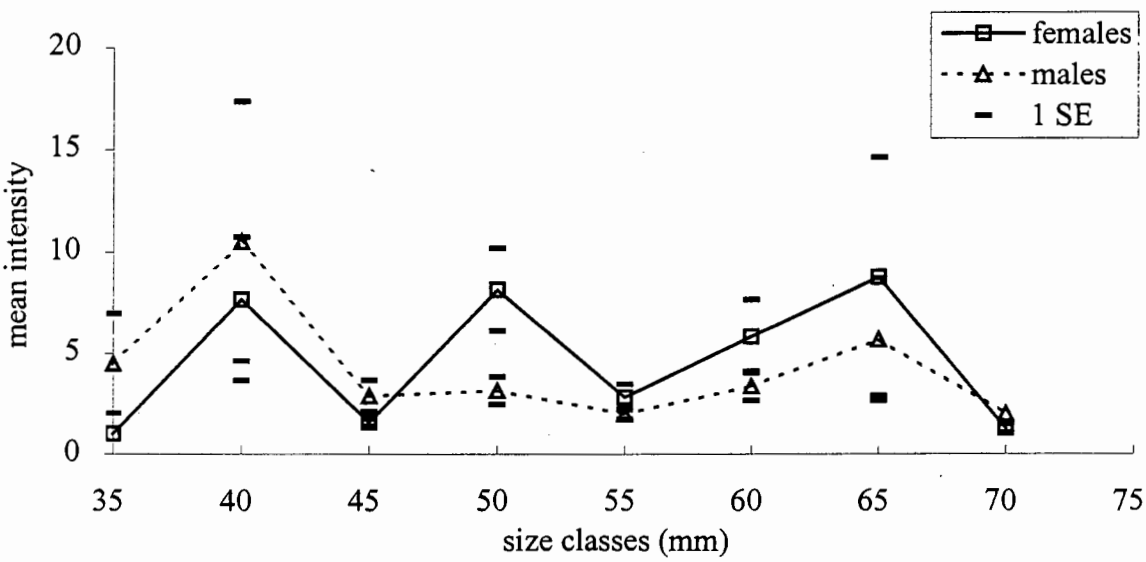


Figure 13. Size dependent mean intensity of *Metacercaria perchorupis* in female and male *Perna* from Dido Valley.

Griffiths (pers. com.) found this worm in *Choromytilus* from Bailey's Cottage at False Bay and at Blouberg. It has also been reported by Calvo-Ugarteburu (1996) in *Perna* from several collection localities near Port Elizabeth in South Africa. Here prevalence varied from a minimum of 80% to a maximum of 100% depending on locality.

According to James (1964) the Gymnophallidae can be split in two sub-families. The excretory vesicle in the Gymnophallinae is Y-shaped; in the Parvatreminae it is V-shaped. Since it has a Y-shaped excretory vesicle *Metacercaria perchorupis* appears to have more affinity for the Gymnophallinae. Even so, *Metacercaria perchorupis* differs in the flame cell formula and number from either sub-family.

The literature revealed some gymnophallids that merit further comparison. According to James (1964) a V shaped excretory vesicle is typical of *Parvatrema.* An exception is *Parvatrema borinquenae* Cable; but even here, Cable (1953) says that the stem is very short. In addition, the flame cell formula for the Genus *Parvatrema* is given by James (1964) as 2[(2+2)+ (2)] = 12. Bowers & James (1967) add an option of 2[(2+2)+ (2+2)] = 16. Both of these differ from the flame cell number in *Metacercaria perchorupis* and thus may be eliminated. The metacercariae of *Parvatrema duboisi* in Machkevsky (1989) are very similar to *Metacercaria perchorupis* but, typical of members of *Parvatrema,* it has a V-shaped excretory duct. Other differences include a tendency to form aggregations of several-dozen metacercariae in gelatinous cysts. In contrast, *Metacercaria perchorupis* occurs, at the most, in half-dozens. The calcareous concretions associated with the gelatinous cysts are much bigger in *Parvatrema duboisi.* Moreover, *Parvatrema duboisi* is found (Machkevsky 1989) in *Mytilus galloprovincialis.* Thus if it were the same as *Metacercaria perchorupis* it would be curious if it did not infect *Mytilus galloprovincialis* in South Africa. Thus, one can exclude *Parvatrema duboisi* from being *Metacercaria perchorupis.*

Metacercaria perchorupis also resembles *Lacunovermis macomae* (Lebour) in Pekkarinen (1986). They differ in the number and disposition of the papillae on the anterior sucker; the absence of a ventral pit and a smaller genital atrium, and absence of papillae around the genital pore in *Metacercaria perchorupis.* Other similar but

distinguishable metacercariae include those of *Meiogymnophallus minutus* (Cobbold, 1859) in Bowers & James (1967), metacercariae of *Gymnophallus strigatus* (Lebour, 1908) cited in Bartoli (1974a), and the members of the Genus *Gymnophallus* Odhner, 1900, cited in Cable (1953): each have 2[(2+2)+ (2+2)] = 16 flame cells. Metacercariae of *Cercaria granosa* Holliman, cited in Holliman (1961) has 2[(2+2)+ (2)] = 12 flame cells, it differs from *Metacercaria perchorupis* in flame cell formula, number of papillae around the anterior and ventral suckers, the caeca of *Cercaria granosa* are consistently more angular than those of *Metacercaria perchorupis* and the number of gland cells around the oral sucker differs. Although the flame cell formulae of *Cercaria fragosa* Holliman, and *Cercaria fimbriata* Holliman, cited in Holliman (1961) is the same as *Metacercaria perchorupis,* they differ from *Metacercaria perchorupis* in that they are both described as furcocercariae not as metacercariae. Such furcocercariae have not been seen in South African bivalves. Moreover, there are significant differences in the disposition of gland cells and papillae on their ventral suckers. Metacercariae of *Gymnophallus somateridae* (Levinsen, 1881) cited in Ching (1973a) have 2[(2+2+2)+ (2+2)] = 20 flame cells. Loos-Frank (1971) provides a list of extant gymnophallids and their hosts.

Cercaria serrae Tharme, Webb & Brown in Tharme, Webb & Brown (1996) also bears consideration because it is found within a few kilometres of the infections of *Metacercaria perchorupis*. It may be eliminated, however, because of its flame cell formula 2[(3+2)+(2)] = 14 and its excretory vesicle is V shaped with two pairs of diverticulae.

Problems with host specificity

The infectivity of *Metacercaria perchorupis* to *Perna, Choromytilus* and *Venerupis* is not remarkable. Gymnophallid metacercariae are not particularly specific in choice of second intermediate host. Indeed, Lauckner (1983) states that *Gymnophallus rebecqui* Bartoli, 1983, has two bivalve hosts, *Parvatrema duboisi* has three bivalve hosts, *Gymnophallus strigatus* has eight bivalve hosts, *Gymnophallus fossarum* Bartoli, 1965, has nine bivalve hosts and *Gymnophallus rostratus* Bartoli, 1982, has sixteen bivalve hosts. Thus one might expect *Metacercaria perchorupis* to be similarly non-specific. What is puzzling is that, although it occurs in *Choromytilus, Perna* and *Venerupis,* it is absent in *Mytilus galloprovincialis* and *Aulacomya ater* . If

it is so non-specific, why does it not infect *Mytilus* or *Aulacomya*?

That *Mytilus* is non-indigenous perhaps confers some resistance to the local parasites. *Aulacomya* may escape because it occurs more sub-tidally and in greater wave exposure at Blouberg. These two reasons may be enough to expect a drop in prevalence and intensity but not a complete absence. Moreover, the niche difference between *Perna* and *Mytilus* appears to be much smaller than the niche difference between *Mytilus* and *Venerupis*. One might expect some level of infection but there is none.

The alternative to a weak hypothesis of niche separation is an equally weak argument for individually host specific sibling species. Perhaps the worms in *Choromytilus*, *Perna* and *Venerupis* are sibling species, each with its own host specificity that does not include *Mytilus* or *Aulacomya*. Bowers *et al.* (1996) report very similar sibling species of the genus *Meiogymnophallus: Meiogymnophallus minutus* (Cobbold, 1859), *Meiogymnophallus strigatus* (Lebour, 1908), *Meiogymnophallus fossarum* (Bartoli, 1965) *and Meiogymnophallus rebecqui* (Bartoli, 1983). Furthermore, Lee, Chai and Hong (1993) report that metacercariae of different species or even genera of gymnophallids may be indistinguishable. There is thus a choice of niche separation or sibling species with host specificity. Neither option is totally satisfactory so the first is supported on the grounds of parsimony. One new species is more supportable than three that are indistinguishable. This provisional decision can be tested by methods suggested in final comments. In addition, it may be possible to find the mollusc first host. A study of its distribution on the shore may suggest a differential exposure and therefore infection probability of the different mytilids. This would appraise the niche separation hypothesis. Alternatively the gastropods could be used as a source of cercariae for infection experiments in mussels of different species to test the host specificity hypothesis.

It is established that no previous description from the literature matches that of *Metacercaria perchorupis*. The name *Metacercaria perchorupis* is a compound of *Perna*, *Choromytilus* and *Venerupis*. This name has the double advantage of being informative about its reported hosts and is unlikely to have been coined previously, thus forestalling duplication.

Epidemiology

100% of *Choromytilus* from Blouberg are infected. Hence, their data are presented slightly differently from those for other species and localities.

Prevalences

Prevalences decline from 100% for *Choromytilus* at Blouberg, to 90.67% (males 93.15%; females 90.67%) in *Choromytilus* at Dido Valley, to 80% in *Venerupis* at Blouberg, and 74.83% (males 67.11%; females 85.94%) in *Perna* at Dido Valley. Size dependent prevalences in male and female *Choromytilus* and *Perna* at Dido Valley (Figures 8, 9, 10 & 11 respectively) show no strong trends.

Intensities

Locality dependent intensities appear to follow the trend of prevalences. Populations with high prevalences also have high intensities. Prevalence and intensity may be integrated into the value of abundance. If the mussel population numbers are the same at both localities, it implies that parasite numbers are much higher at Blouberg than at Dido Valley. In *Choromytilus* from Blouberg, abundance is 45.8. That is 13.96 times higher than for *Perna* at Dido Valley, which has and abundance of 3.28. *Choromytilus* from Dido Valley has an abundance of 11.34, which is 3.46 times higher than that in *Perna* at Dido Valley.

Sex linked intensity

Females (Tables 3A, 4 & 6) have higher mean intensities than males. At Blouberg, *Choromytilus* males and females had a mean infection intensity of 35.53 (SE 2.56) and 58.66 (SE 4.75) respectively. At Dido Valley, *Choromytilus* males and females had a mean infection intensity of 9.49 (SE 0.95) and 15.53 (SE 1.33) respectively. At Dido Valley, *Perna* males and females had a mean infection intensity of 3.65 (SE 0.7), and 5.13 (SE 0.88) respectively. In addition, inspection of the figures 6, 7, 12 & 13 show that the females of *Choromytilus* and *Perna* appear to have a higher intensity of infection.

Size dependent intensity

At Blouberg, *Choromytilus* mean infection intensities appear to increase with host size (Figures 6 & 7). The decline in intensity at the upper extreme of host size in both sexes suggests that larger mussels may become more resistant. Similar but less marked trends of increase with host size are seen in *Choromytilus* from Dido Valley (Figure 12). The results (Figure 13) for *Perna* show fluctuations, but no general size dependent trends.

Sex linked differences in mean size of infected hosts

At Blouberg, *Choromytilus* males (100% prevalence) had a mean size of 60.28mm (SE 0.73) and females had a mean size of 60.48mm (SE 0.93). At Dido Valley, *Choromytilus* infected males had a mean size of 62.75mm (SE 0.78) and infected females had a mean size of 61.59mm (SE 0.73). At Dido Valley, *Perna* infected males had a mean size of 54.12mm (SE 1.02) and infected females had a mean size of 54.78mm (SE 1.17). These results all strongly suggest that there is no significant difference in size between the two sexes. Thus, the higher intensity in females is probably not a size dependent phenomenon.

Sex linked differences in mean size of uninfected hosts

At Dido Valley, *Choromytilus* uninfected males had a mean size of 61.6mm (SE 1.53) and uninfected females had a mean size of 57.77mm (SE 1.66). At Dido Valley, *Perna* uninfected males had a mean size of 55.61mm (SE 1.95) and uninfected females had a mean size of 54.61mm (SE 3.42). These results strongly suggest that there are no significant differences in size between male and female uninfected mussels.

Infection linked host size differences

In *Choromytilus* at Dido Valley the total infected mean size is 62.17mm (SE 0.54); the total uninfected mean size is 55.9mm (SE 2.62). In *Perna* at Dido Valley the total infected mean size is 54.33mm (SE 0.79); the total uninfected mean size is 55.14mm (SE 1.59). These results suggest, particularly in the *Perna* sample that there is no significant size difference between infected and uninfected mussels.

Other gymnophallid infections

Campbell (1985) reported *Gymnophallus rebecqui* Bartoli, 1983, in *Abra tenuis* and *Cerastoderma glaucum* at prevalences varying from 5% to 100% with intensities of 1 to 30. Bowers and James (1967) reported *Meiogymnophallus minutus* in *Cardium (= Cerastoderma) edule.* They reported that intensity increases with size, age and as salinity approaches the full marine value from brackish. Prevalence also increases with salinity to a maximum in normal seawater. In normal seawater they reported 96%-100% prevalence and mean intensities of 31.9 to 113.3. They also reported a close correlation between seasonal intensity and numbers of final hosts on the shore. The final host is the oystercatcher *Haematopus ostralegus occidentalis* and there are more of them on the shore in the northern winter, when there also more parasites.

Pekkarinen (1986) reported *Lacunovermis macomae* and other gymnophallids in *Macoma balthica* in brackish water. Prevalences are 100% (derived from intensity data) and intensity varies from 34 to 68. Pekkarinen (1986) adds that infection intensities of about 40 had no effect on the condition of the host.

Bowers *et al.* (1996) investigated the microhabitat (site) of *Meiogymnophallus minutus* (Cobbold, 1859), *Meiogymnophallus strigatus* (Lebour, 1908), *Meiogymnophallus fossarum* (Bartoli, 1965) *and Meiogymnophallus rebecqui* (Bartoli, 1983) in members of the genus *Cerastoderma.* They report very high prevalences close to 100% and found that infections of *Meiogymnophallus minutus* and *Meiogymnophallus fossarum* resulted in pathology and heavy host mortality especially from damage to the hinge and shell edge. This is in contrast to gymnophallid infections from South African mytilids where there is no evidence of such damage.

For size dependent intensity of infection with *Metacercaria perchorupis,* and sample number, of female and male *Choromytilus* from Blouberg see Figures 6 & 7. For size dependent prevalence, number infected and sample number, of female and male *Choromytilus* infected with *Metacercaria perchorupis* at Dido Valley see Figures 8 & 9. For size dependent prevalence, number infected and sample number, of female and male *Perna* infected with *Metacercaria perchorupis* at Dido Valley see Figures 10 & 11. For size dependent intensity of *Metacercaria perchorupis* in female and male

Choromytilus at Dido Valley see Figure 12. For size dependent intensity of *Metacercaria perchorupis* in female and male *Perna* at Dido Valley see Figure 13.

The effect of *Metacercaria perchorupis* on emersion mortality in *Choromytilus* is quantified in Chapter 45.

CHAPTER 5: *METACERCARIA A*: SP. NOV.

HOSTS AND LOCALITY

This parasite occurs in *Perna perna* at Dido Valley and in *Choromytilus meridionalis* at Dido Valley, Kleinmond, Blouberg and Cape Columbine.

TYPE SPECIMENS

Paratypes: Specimen number A29429. Marine Biology Department, South African Museum, Queen Victoria Street, Cape Town, RSA. Type host: *Choromytilus meridionalis*. Type locality Blouberg.

DESCRIPTION

There is a concentration of host nuclei around the cyst (Figures 5 & 8) and these could indicate a host response. Alternatively, it could be caused by the host tissue being compressed by the growing cyst. Apart from this minor effect the host tissues appear normal. The cyst is rigid and spheroid (Figures 1, 2, 3, 4 & 5). Its wall has four layers, all of which stain deep violet with Nile-blue sulphate. The inner layer is thin, smooth, transparent and apparently homogeneous; the next layer is thicker, granular, dark and less refractile; the third layer consists of radiating columns and numerous cavities and the fourth (outer) layer is similar to the second layer but is about one half of its thickness. The inner surface of the cyst is paved with triangular plates each about 4.3µm high with a 2.5µm base and a pitch of 5µm. Similar but half-size plates lie between them.

The metacercaria (Figure 9) was released by rolling the cyst under pressure until the wall fatigued. The worm could then be expelled by application of pulsing pressure on the cover slip. A drop of water was then placed at the side of the coverslip to relieve any pressure to allow the worm to assume its uncompressed shape. *Metacercaria A* is leaf-shaped. Tegument spines stain deeply by gentian-violet stain and occur only on the anterior. Three pairs of gland cells, whose bodies lie laterally but as far back as the ventral sucker, have long ducts, which terminate near the mouth. The sub-terminal oral sucker is lined with numerous triangular flat spines. The pre-pharynx is about a third of the length of the pharynx. The caeca, whose contents stain with neutral red, branch close to the junction with the pharynx and there is thus no

measurable oesophagus. They terminate close to the lateral margin in the first half of the body. The digestive tract is a shallow inverted Y. The ventral sucker lies about half way from the front. The tegument surrounding the ventral sucker is armed with spines, each about 3.7μm long.

The excretory pore is at the extreme posterior. The vesicle expands into a bladder, which is slightly smaller than the ventral sucker. This bladder is supplied by a stem, which branches just posterior to the ventral sucker. The two arms of the vesicle terminate next to the oral sucker. Secondary ducts meander laterally and join the arms at their anterior extremity. Secondary ducts are traceable back to level with the caeca. Seven flame cells, each about 7.5μm by 2.5μm, were seen (Figure 9). Numerous cells in the tegument stain violet with Nile-blue sulphate. This stain also stimulates activity of the metacercaria while it is still in the cyst.

Table 1. Measurements in μm of *Metacercaria A*. A. Living, B, Formalin fixed.

A

	mean	SD	*n*	max.	min.
cyst wall thickness inner	2.7	0.9	15	4.9	1.2
cyst wall thickness outer	11.3	3.6	13	15.9	5.5
cyst diameter internal	160.9	28.1	15	213	116
cyst diameter external	169.4	22.2	34	211	131
length	332	41.1	4	367	294
width 25% from front	106.9	14.4	10	122.5	83.3
width 50% from front	123.7	19.7	10	171.5	102.9
width 75% from front	106.2	22.3	9	129.9	73.5
oral sucker length	54.3	13.8	10	72	27
oral sucker width	55.8	11.4	10	66.2	30
oral sucker depth	39.2	4.8	10	49	34.3
ventral sucker length	46.6	8	10	58.8	36.8
ventral sucker width	56.1	8.5	10	61.3	44.1
ventral sucker depth	45.7	7.4	10	60	35
pre-pharynx length	4.9	2.0	4	7.4	2.5
pharynx length	20.0	4.5	10	29.4	14.7
pharynx width	16.4	2.3	10	19.6	14
excretory spheres	3.1	0.83	10	4.2	1.23
eggs length	16.2	1.85	4	18.4	14.7
eggs width	9.8	0.99	4	11.03	8.6
cuticle spines length	6.16	2.04	10	9.8	2.45
cuticle spines pitch	6.86	2.02	10	9.8	4.9
cuticle spines width	1.66	0.51	10	2.45	0.98

B

	mean	SD	*n*	max.	min.
cyst wall thickness	12.8	1.79	10	14.7	9.8
length	273.8	39.7	3	319	257
width 25% from front	97.75	21.7	10	137.2	73.5

width 50% from front	112.9	11.3	10	129.9	93.1
width 75% from front	108.41	18.9	10	147	76
oral sucker length	55.62	6.8	10	66.2	46.6
oral sucker width	52.8	3.9	10	58.8	46.6
oral sucker depth	41.9	7	10	51.5	33.1
ventral sucker length	39	9.8	10	58.8	24.5
ventral sucker width	53.1	3.8	10	58.8	49
ventral sucker depth	39.8	7.6	10	51.5	29.4
pharynx length	20.9	4.8	10	27	14.7
pharynx width	14.8	2.2	10	19.6	12.3
spines length	3.84	1.27	10	5.39	2.45
spines pitch	6.3	1.92	10	9.8	3.7
bladder length	--	--	2	49	34.3
bladder width	--	--	2	49	41.7

EPIDEMIOLOGY

Prevalences

Table 2A. Prevalences of *Metacercaria A* in *Choromytilus meridionalis* from Blouberg.

	infected	sample no.	prevalence
males	279	315	88.57%
females	257	277	92.78%
unidentified	6	8	75%
total	542	600	90.33%

Table 2B. Prevalences of *Metacercaria A* in *Choromytilus meridionalis* from Dido Valley.

	infected	sample no.	prevalence
males	215	280	76.78%
females	255	311	81.99%
unidentified	4	10	40%
total	474	601	78.86%

Table 2C. Prevalences of *Metacercaria A* in *Choromytilus meridionalis* from Kleinmond.

	infected	sample no.	prevalence
total	5	19	26.3%

Table 2D. Prevalences of *Metacercaria A* in *Perna perna* from Dido Valley.

	infected	sample no.	prevalence
males	156	356	43.82%
females	109	231	47.19%
hermaphrodites	2	7	28.57%
unidentified	1	5	20%
total	268	599	44.74%

Mean intensities

Table 3A. Intensities of *Metacercaria A* in *Choromytilus meridionalis* from Blouberg.

	no. cysts	no. mussels	intensity	SD	SE
males	1299	279	4.66	4.21	0.25
females	1692	257	6.58	5.11	0.32
unidentified	39	6	6.5	4.35	1.78
total	3030	542	5.59	4.76	0.20

Table 3B. Intensities of *Metacercaria A* in *Choromytilus meridionalis* from Dido Valley.

	no. cysts	no. mussels	intensity	SD	SE
males	1369	215	6.37	8.43	0.57
females	2173	255	8.52	11.29	0.71
unidentified	20	4	5.0	3.54	1.77
total	3562	474	7.51	10.11	0.46

Table 3C. Intensities of *Metacercaria A* in *Perna perna* from Dido Valley.

	no. cysts	no. mussels	intensity	SD	SE
males	424	156	2.72	2.72	0.22
females	285	109	2.61	2.69	0.26
hermaphrodites	2	2	1	0	---
unidentified	4	1	4	0	---
total	715	268	2.66	2.67	0.16

Abundances

Table 4A. Abundances of *Metacercaria A* in *Choromytilus meridionalis* from Blouberg.

	no. cysts	no. mussels	abundance	SD
males	1299	315	4.12	4.23
females	1692	277	6.11	5.21
unidentified	39	8	4.88	4.70
total	3030	600	5.05	4.82

Table 4B. Abundances of *Metacercaria A* in *Choromytilus meridionalis* from Dido Valley.

	no. cysts	no. mussels	abundance	SD
males	1369	280	4.89	7.86
females	2173	311	6.99	10.74
unidentified	20	10	2	3.32
total	3562	601	5.93	9.49

Table 4C. Abundances of *Metacercaria A* in *Perna perna* from Dido Valley.

	no. cysts	no. mussels	abundance	SD
males	424	356	1.19	2.25
females	285	231	1.23	2.26
hermaphrodites	2	7	0.28	0.45
unidentified	4	5	0.8	0
total	715	599	1.19	2.24

Prevalence, intensity and abundance in the palps and the mantle.

Table 5A. *Choromytilus meridionalis* from Blouberg, morphometrics and numbers of *Metacercaria A* cysts in palps and mantle.

	males	females	unidentified	total
n	315	277	8	600
cysts in palps	1153	1129	22	2304
cysts in mantle	146	563	17	726
total	1299	1692	39	3030
mean host size mm	55.19	54.78	45.96	54.88
SD mm	9.88	9.41	3.72	9.65
SE mm	0.56	0.56	1.31	0.39

Table 5B. Mean size difference between *Choromytilus meridionalis* from Blouberg, infected and uninfected with *Metacercaria A.*

	mean size mm	SD	SE
infected	55.35	9.7	0.42
uninfected	50.44	7.92	1.04

Table 6A. *Choromytilus meridionalis* from Dido Valley, morphometrics and numbers of *Metacercaria A* cysts in palps and mantle.

	males	females	unidentified	total
n	280	311	10	601
cysts in palps	1015	1216	17	2248
cysts in mantle	354	957	3	1314
total cysts	1369	2173	20	3562
mean host size mm	58.76	58.23	35.07	58.09
SD	10.35	11.33	13.77	11.34
SE	0.62	0.64	4.35	0.46

Table 6B. Mean size difference between *Metacercaria A* infected and uninfected *Choromytilus meridionalis* from Dido Valley.

	mean size mm	SD	SE
infected	60.39	10.41	0.48
uninfected	49.49	10.51	0.93

Table 7A. *Perna perna* from Dido Valley, morphometrics and numbers of *Metacercaria A* cysts in palps and mantle.

	males	females	unidentified	hermaphrodites	total
n	356	231	5	7	599
cysts in palps	397	275	4	2	676
cysts in mantle	29	10	0	0	39
total	426	285	4	2	715
mean host size mm	54.74	52.58	36.47	51.2	53.7
SD	9.43	8.64	16.89	5.94	9.38
SE	0.5	0.57	7.55	2.25	0.38

Table 7B. Mean size difference between *Metacercaria A* infected and uninfected *Perna perna* from Dido Valley.

	mean size mm	SD	SE	n
infected	56.68	8.675	0.53	268
uninfected	51.38	9.270	0.51	331

Table 8A. Prevalence, abundance and intensity of infections of *Metacercaria A* in sub-populations of *Choromytilus meridionalis* from Blouberg.

	male	SD	SE	female	SD	SE	all	SD	SE
prevalence palps %	88.25			89.53					
prevalence mantle %	17.46			54.15					
mean intensity palps	4.15	3.68	0.22	4.55	3.13	0.2	4.34	3.43	0.15
	n=278			*n*=248			*n*=526		
mean intensity mantle	2.65	1.94	0.26	3.75	2.98	0.24	3.47	3.43	0.24
	n=55			*n*=150			*n*=205		
abundance palps	3.66	4.08		4	3.49				
abundance mantle	0.46	2.03		1.45	2.80				

Table 8B. Prevalence, abundance and intensity of *Metacercaria A* infections in sub-populations of *Choromytilus meridionalis* from Dido Valley.

	male	SD	SE	female	SD	SE
prevalence palps	75.36%			78.78%		
prevalence mantle	20.71%			44.05%		
mean intensity palps	4.81	5.246	0.36	4.96	6.985	0.45
	n=211			*n*=245		
mean intensity mantle	6.1	7.549	0.99	6.99	8.96	0.77
	n=58			*n*=137		
abundance palps	3.63	5.0		3.91	4.82	
abundance mantle	1.26	4.23		3.08	6.884	

Table 8C. Prevalence, abundance and intensity of *Metacercaria A* infections in sub-populations of *Perna perna* from Dido Valley.

	male	SD	SE	female	SD	
prevalence palps	43.82%			47.19%		
prevalence mantle	2.81%			3.03%		
intensity palps	2.53	2.32	0.19	2.52	2.62	0.26
	n=156			*n*=100		
intensity mantle	2.9	3.27	1.03	1.43	0.73	0.28
	n=10			*n*=7		
abundance palps	1.11	1.98		1.19	2.20	
abundance mantle	0.08	0.73		0.04	0.28	

For size dependent prevalence of *Metacercaria A* in female and male *Choromytilus* from Blouberg see Figures 10 & 11; for *Choromytilus* from Dido Valley see Figures 12 & 13 and for *Perna* from Dido Valley see Figures 14 & 15. For size dependent prevalence of infections of *Metacercaria A* in palps and mantle of the female and male *Choromytilus* from Blouberg see Figures 16 & 17; for *Choromytilus* from Dido Valley see Figures 18 & 19 and for *Perna* from Dido Valley see Figures 20 & 21. For

monthly variation in prevalence of infections of *Metacercaria A* in female and male *Choromytilus* from Blouberg see Figures 22 & 23; for *Choromytilus* from Dido Valley see Figures 24 & 25 and for *Perna* from Dido Valley see Figures 26 & 27. For size dependent mean intensity of infections of *Metacercaria A* in female and male *Choromytilus* from Blouberg see Figure 28; for *Choromytilus* from Dido Valley see Figure 29 and for *Perna* from Dido Valley see Figure 30. For size dependent mean intensity and abundance of infections of *Metacercaria A* in palps and mantle of the female and male *Choromytilus* from Blouberg see Figures 31 & 32; for *Choromytilus* from Dido Valley see Figures 33 & 34 and for *Perna* from Dido Valley see Figures 35 & 36. For monthly variation in mean intensity of infections of *Metacercaria A* in female and male *Choromytilus* from Blouberg see Figure 37; for *Choromytilus* from Dido Valley see Figure 38 and for *Perna* from Dido Valley see Figure 39. For monthly variation in mean samples size of males and females of *Choromytilus* from Blouberg see Figure 40, for *Choromytilus* from Dido Valley see Figure 41 and for *Perna* from Dido Valley see Figure 42.

DISCUSSION

Taxonomic affinities and etymology

The literature was surveyed to find other worms of similar morphology and life-cycle. Particular attention was paid to those with a bivalve second intermediate host. These include the following families: (Identification authorities of the mesostomate or stenostomate condition are given after each family name.) Fellodistomidae and Psilostomidae are stenostomate (Erasmus 1972). Mesostomate families include Monorchiidae (Erasmus 1972), Microphallidae, (Erasmus 1972), Lepocreadidae (Erasmus 1972), Plagiorchida (Erasmus 1972) Troglotrematidae (Erasmus 1972 & Cable 1956), Zoogonidae (Erasmus 1972) and Renicolidae (Erasmus 1972). In the Gymnophallidae; the mesostomate condition was confirmed by inspection of flame cell formulae in James (1964).

There appear to be affinities of *Metacercaria A* with *Renicola roscovita*. These will be discussed after the other possible candidates are examined and discarded. Fellodistomids, in such works as Bray (1983) and Bray & Gibson (1980) included no examples even remotely similar to *Metacercaria A*. Particular non-fellodistomid distinctions in *Metacercaria A* include caeca that terminate well before the ventral

sucker and a ventral sucker that is considerably smaller than the oral sucker. Psilostomids differ by having a subcutaneous network of collecting vessels in the excretory system (Dawes 1946, Loos-Frank 1968) - see also *Metacercaria B* (Chapter 9) since it has psilostomid affinities. In addition, the main stem proceeds anterior to the ventral sucker. The cyst wall is much thinner in *Psilostomum brevicolle* (Loos-Frank, 1968) and the caeca are much more elongate than in *Metacercaria A.* Monorchiids have caeca that extend to mid-body and overlap the ventral sucker. *Metacercaria A* has a longer pre-pharynx than is typical of monorchiids (Dawes 1946). The ventral sucker is typically anterior to the mid-point of the body in monorchiids; it is at the mid-point in *Metacercaria A.* Furthermore, in contrast to *Metacercaria A,* monorchiids have a tendency for the ventral sucker to be larger than the oral sucker. Microphallids share a number of features with *Metacercaria A* but its ventral sucker is atypically large, the pre-pharynx is too short and the caeca are too short. Another contrast is that microphallids are typically heavily spinous (Etges 1953, Cable & Kuns 1951). Further circumstantial evidence to discount *Metacercaria A* as a microphallid is that they commonly encyst in arthropods (Erasmus 1972). Lepocreadids are spinous (Dawes 1946) and they typically have much larger digestive caeca that reach to the extreme posterior (See Cheng 1967). The pre-pharynx is usually longer than in *Metacercaria A.* In plagiorchids the digestive caeca extend far to the posterior. And according to Holliman (1961), they encyst in crustaceans rather than molluscs. Troglotrematids (Dawes 1946) typically have feeble suckers and their caeca often extend into the posterior half of the worm. Gymnophallids have been discussed previously - see *Metacercaria perchorupis* (Chapter 4). Gymnophallids typically do not encyst in a hard-shelled cyst. Their excretory system is much more expansive and their tegument is often heavily spinous.

The cyst of *Metacercaria A* resembles that of *Renicola roscovita* (see Lauckner 1983 fig.13-109). That it is not, may be established by the flame cell formulae of *Renicola roscovita*:

2[(1+1+1)+ (1+1+1)] early cercaria

2[(3+3+3)+ (3+3+3)] late cercaria (Stunkard 1964)

In both *Metacercaria A* and *Renicola roscovita* the cysts are thick walled and the granules in the excretory system obscure much detail in the cyst. However, the excretory system (Stunkard 1964) in *Renicola roscovita* is much branched. This

contrasts with the simplicity of the *Metacercaria A*. Nevertheless, the basic configuration of a Y-shaped excretory vesicle is common to both. Similarly the caeca in both reach barely to the ventral sucker. Another commonality is that both are found in the palps of the host. According to Lauckner (1983) the palps are the preferred site for encystment of *Renicola roscovita* in *Mytilus edulis*: its second preference appears to be the mantle margin. This is also similar to the distribution in hosts found in South Africa. *Renicola roscovita* is not particularly host specific; it occurs in such disparate bivalves as *Mytilus edulis, Cardium (Cerastoderma) edule, Cardium (Cerastoderma) lamarcki and Mya arenaria.* Stunkard (1964) reports enormous numbers of encysted *Renicola roscovita* metacercariae in the gills, mantle and other tissues of *Mytilus edulis* and *Pecten irradians*. The lack of host specificity in *Renicola roscovita* is echoed by *Metacercaria A* in this study. But this raises question about the lack infections in *Aulacomya* and *Mytilus*. *Aulacomya* may escape infection by being more sub-tidal and *Mytilus*, because it is non-indigenous, may therefore be resistant. Besides this, it may be that *Metacercaria A* is specific to *Perna* and *Choromytilus* only. There is no out-group that has the infection to raise questions of host specificity. This argument is also applicable to the host specificity of *Metacercaria B* (Chapter 9).

It is concluded that *Metacercaria A* has renicolid affinities. This is despite it being stenostomate in contrast to renicolids, which Erasmus (1972) asserts are mesostomate. Erasmus (1972) also says that mesostomate and stenostomate configurations may occur in the same sub-family. Thus *Metacercaria A* may be the first recorded stenostomate renicolid. This provisional conclusion must be tested by life-cycle experiments.

Epidemiology

Mean size of mussels: infected versus uninfected

At Blouberg, infected *Choromytilus* had a mean size of 55.35mm (SE 0.42) and uninfected mussels had a mean size of 50.44mm (SE 1.04). At Dido Valley, infected *Choromytilus* had a mean size of 60.39mm (SE 0.48) and uninfected mussels had a mean size of 49.49mm (SE 0.93). At Dido Valley, infected *Perna* had a mean size of 56.68mm (SE 0.53) and uninfected mussels had a mean size of 51.38mm (SE 0.51). All the results suggest that infected mussels may be significantly larger than

uninfected.

Prevalences

In common with infections of *Cercaria notobucephala* and *Metacercaria perchorupis,* the Blouberg sample has a much higher prevalence than Dido Valley. Again, *Perna* has a lower prevalence than *Choromytilus* from either of these collection localities. Prevalences decline from 90.33% (males 88.57%; females 92.78%) for *Choromytilus* at Blouberg to 78.86% (males 76.78%; females 81.99%) in *Choromytilus* at Dido Valley to 44.74% (males 43.82%; females 47.19%) in *Perna* at Dido Valley to 26.3% in *Choromytilus* at Kleinmond.

Size dependent prevalences

Size dependent prevalences in males and females of *Choromytilus* and *Perna* from Blouberg and Dido Valley (Figures 11, 12, 13, 14 & 15) show a trend of increasing prevalence with size of host mussels.

Prevalences in palps and mantle

Tables 8A, B & C show that *Cercaria A* occurs more often in the palps than the mantle. The Figures (16, 17, 18, 19, 20 & 21) also show that the prevalence is always higher in the palps. The difference is most marked in *Perna* (Table 8C) where the great majority of cases are in the palps. The Figures (16, 17, 18, 19, 20 & 21) also show clear trends of increasing prevalence with host size in palps and mantle in all samples, except for the females of *Perna* at Dido Valley.

Seasonal prevalences

Of the graphs (Figures 22, 23, 24, 25, 26 & 27) showing variation of prevalence with season in males and females of *Choromytilus* from Blouberg and Dido Valley and *Perna* from Dido Valley, only Figures 26 and 27 (*Perna*) exhibited a hint of a dip, and that occurred around March-May.

Intensities

At Blouberg, *Choromytilus* males had a mean infection intensity of 4.66 (SE 0.25) and females had a mean intensity of 6.58 (SE 0.32). At Dido Valley, *Choromytilus* males had a mean infection intensity of 6.37 (SE 0.57) and females had a mean

intensity of 8.52 (SE 0.71). This suggests that, at least in *Choromytilus* from Blouberg, females have a higher intensity of infection. In *Perna* at Dido Valley, this is not so. Males had a mean infection intensity of 2.72 (SE 0.22) and females had a mean intensity of 2.61 (SE 0.26). Figures 37 & 38 show that for *Choromytilus* the intensity of infection in females generally exceeds that of the males at all host sizes. This does not appear to be so for *Perna* (Figure 39).

Mean intensities in palps and mantle

Inspection of table 8 A, B & C reveals that mean intensities do not appear to be consistently higher in the palps or in the mantle. Mean intensities in male *Choromytilus* at Blouberg were, in the palp 4.15 (SE 0.22) and in the mantle 2.65 (SE 0.26). Mean intensities for female *Choromytilus* at Blouberg were, in the palp 4.55 (SE 0.2) and in the mantle 3.75 (SE 0.24). Mean intensities for male *Choromytilus* at Dido Valley were, in the palp 4.81 (SE 0.36) and in the mantle 6.1 (SE 0.99). Mean intensities for female *Choromytilus* at Dido Valley were, in the palp 4.96 (SE 0.45) and the mantle 6.99 (SE 0.77). Intensities for male *Perna* at Dido Valley were, in the palp 2.53 (SE 0.19) and in the mantle 2.9 (SE 1.03). Intensities for female *Perna* at Dido Valley were, in the palp 2.52 (SE 0.26) and in the mantle 1.43 (SE 0.28).

Size dependent intensities

Figures 28, 29, 30, 31, 32, 33, 34, 35 & 36 all show a general trend of increasing intensity with host (*Choromytilus* and *Perna*) size. Those data points that appear outside this trend are often based on a single value. They can be identified as such by their lack of error bars.

Seasonal variation in intensity

In both host species there appears to be a hint of a dip around March-May (Figures 37 & 38 for *Choromytilus* and Figure 39 for *Perna*). Inspection of the seasonal variation in host size shows no commensurate variation in intensity due to size/intensity relationships. If there is a significant difference in intensity at different times of the year, it is due to other factors.

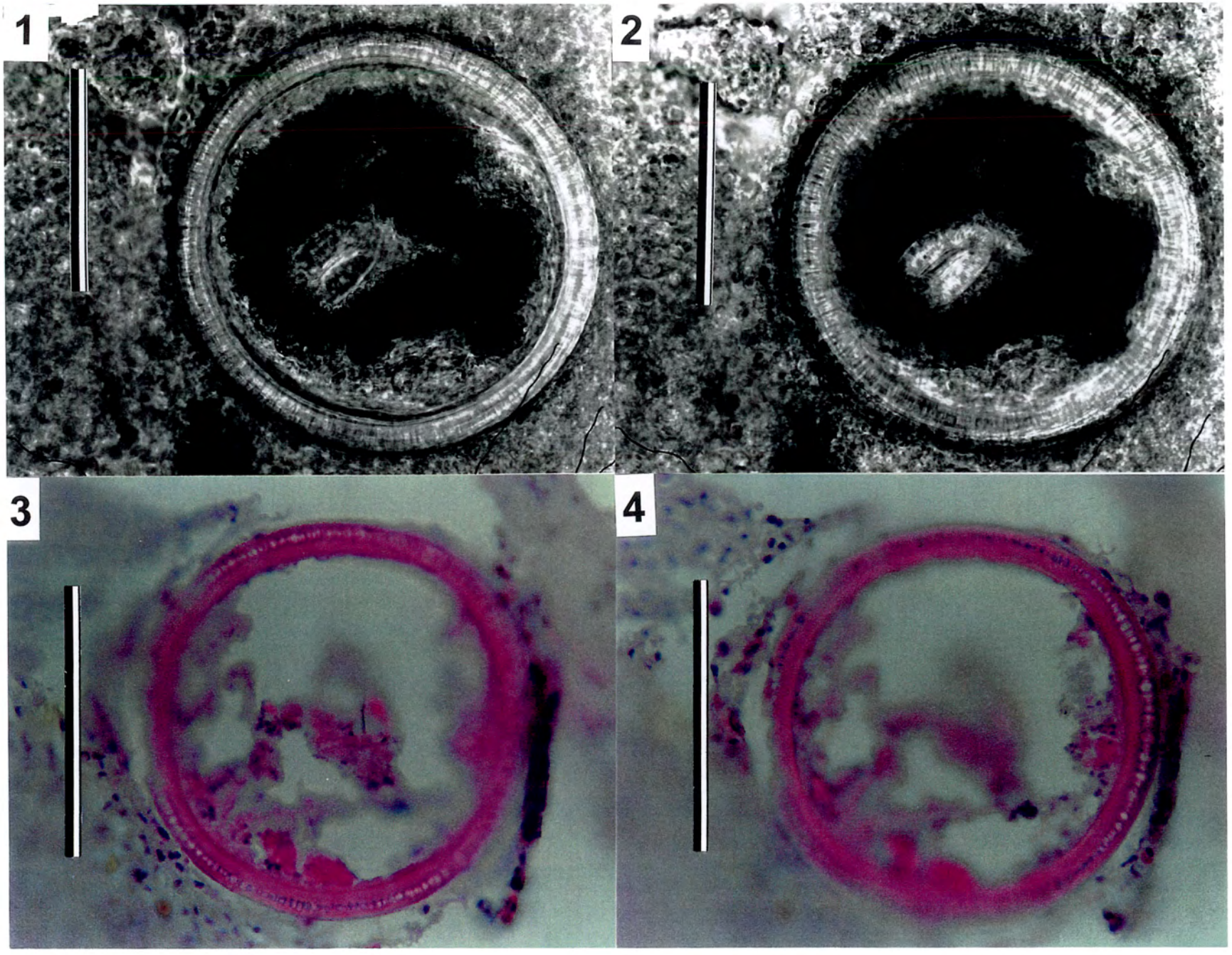
Abundance

Perna from Dido Valley has an abundance of 1.19. The abundance of *Choromytilus*

from Blouberg is 5.05. This is 4.24 times higher than that in *Perna*. The abundance in *Choromytilus* from Dido Valley is 5.93, which is 4.98 times higher than that in *Perna perna*.

Sex-linked morphometrics

Comparisons of size distributions of males and females of *Choromytilus* from Blouberg (Figure 40) and Dido Valley (Figure 41) and *Perna* (Figure 42) from Dido Valley shows close correspondence between males and females. This suggests that there is no size distribution difference between males and females.



Figures 1 to 4. Light micrographs of *Metacercaria A* showing cyst wall layers, scale bars = 100μm.

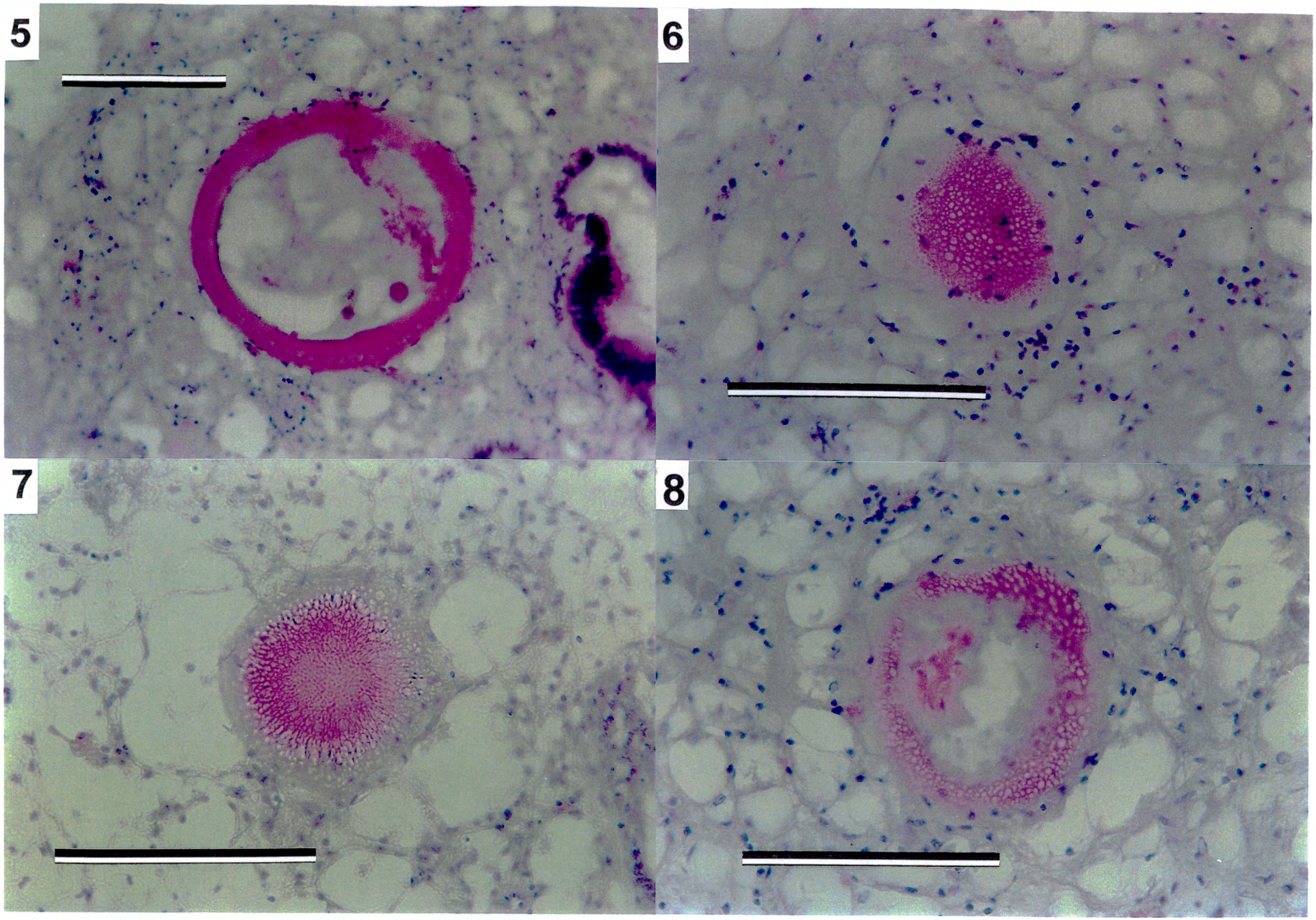


Figure 5. Light micrograph of *Metacercaria A* showing cyst wall layers.
Figures 6 to 8. Serial sections through cyst of *Metacercaria A*, scale bar = 100μm for figures 5 to8.

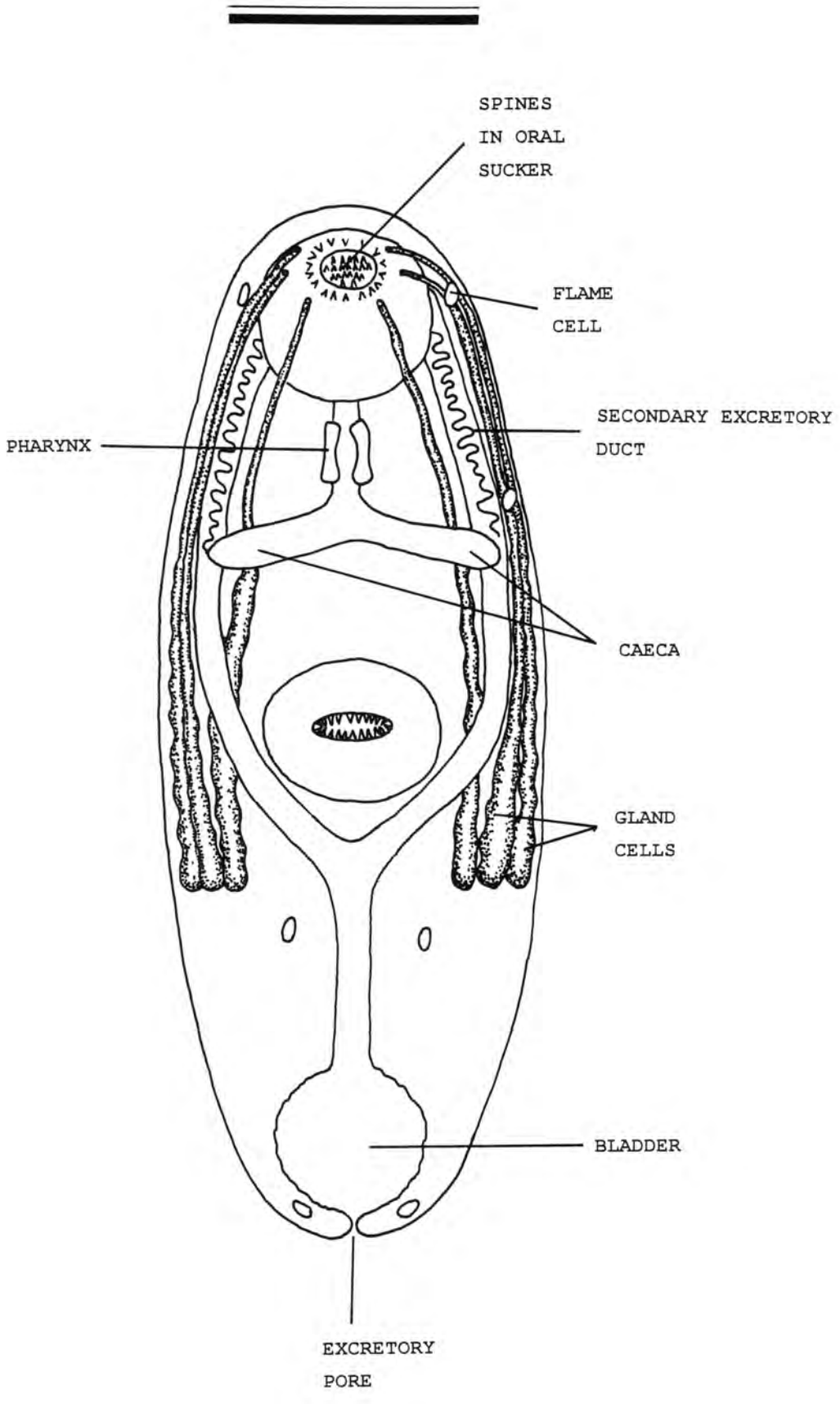


Figure 9. Excysted *Metacercaria A,* scale bar = 80μm.

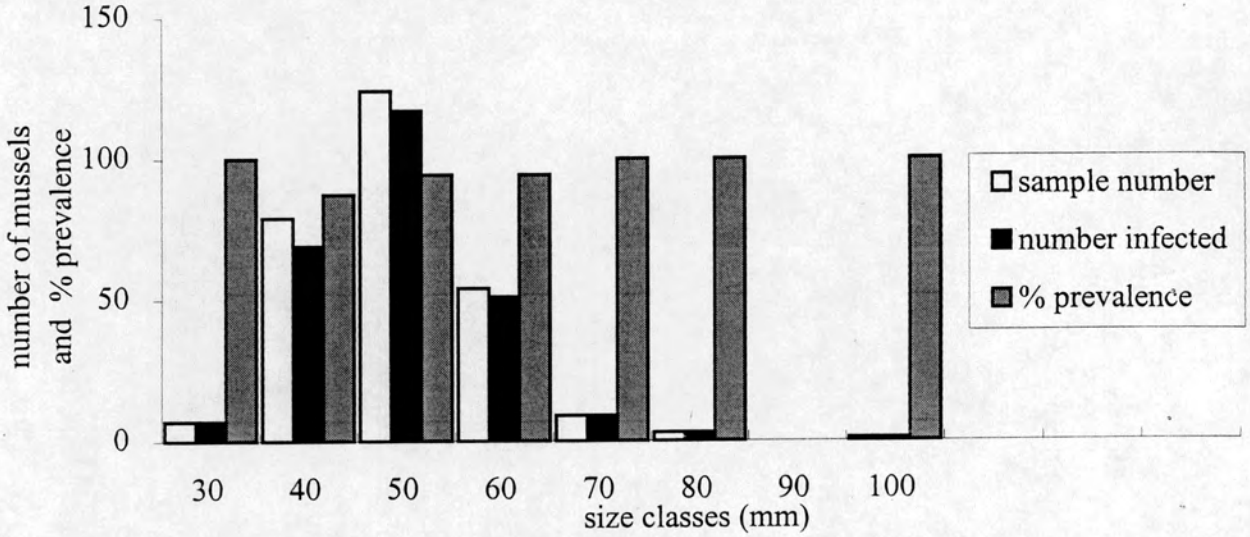


Figure 10. Size dependent prevalence of *Metacercaria A* in female *Choromytilus* from Blouberg.

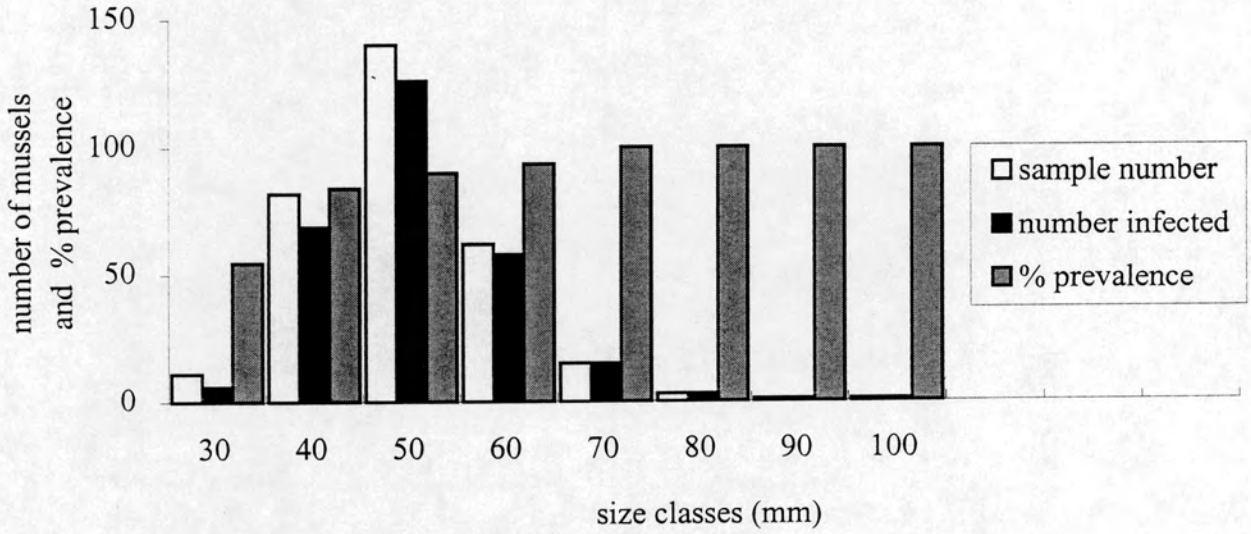


Figure 11. Size dependent prevalence of *Metacercaria A* in male *Choromytilus* from Blouberg.

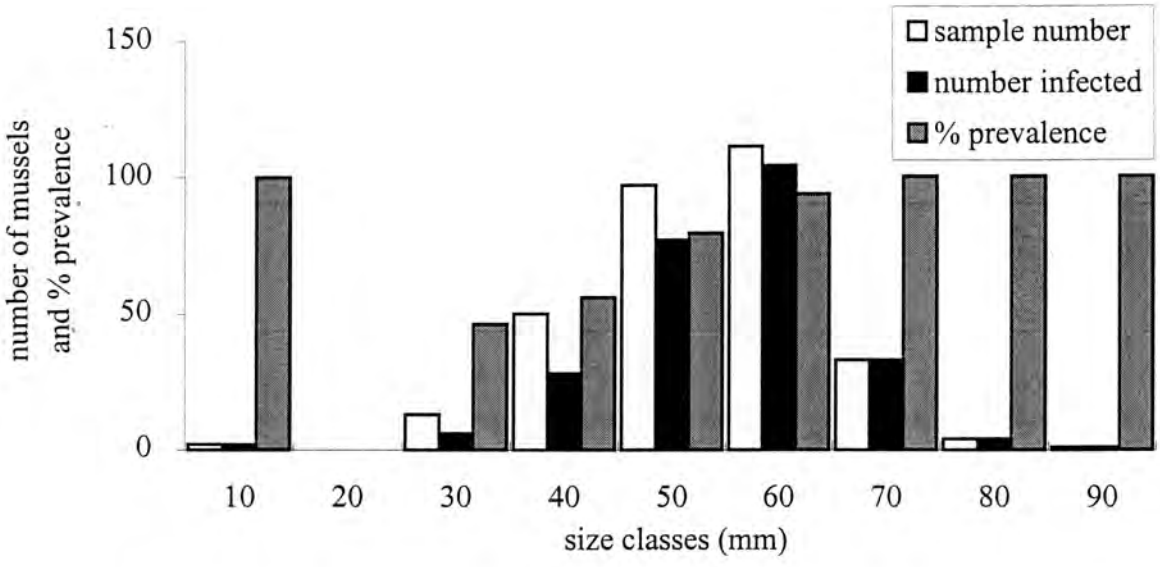


Figure 12. Size dependent prevalence of *Metacercaria A* in female *Choromytilus* from Dido Valley.

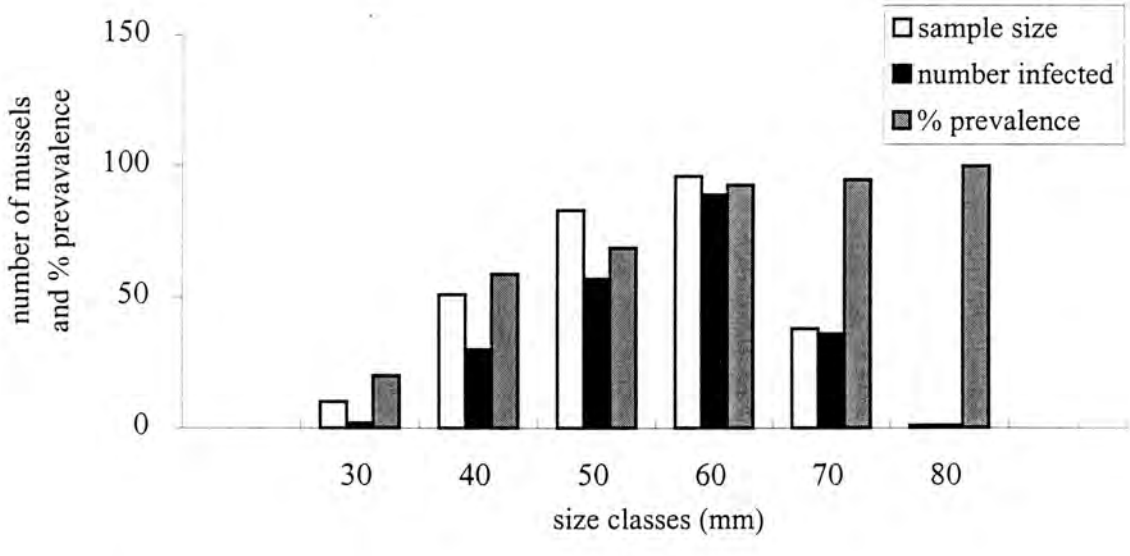


Figure 13. Size dependent prevalence of *Metacercaria A* in male *Choromytilus* from Dido Valley.

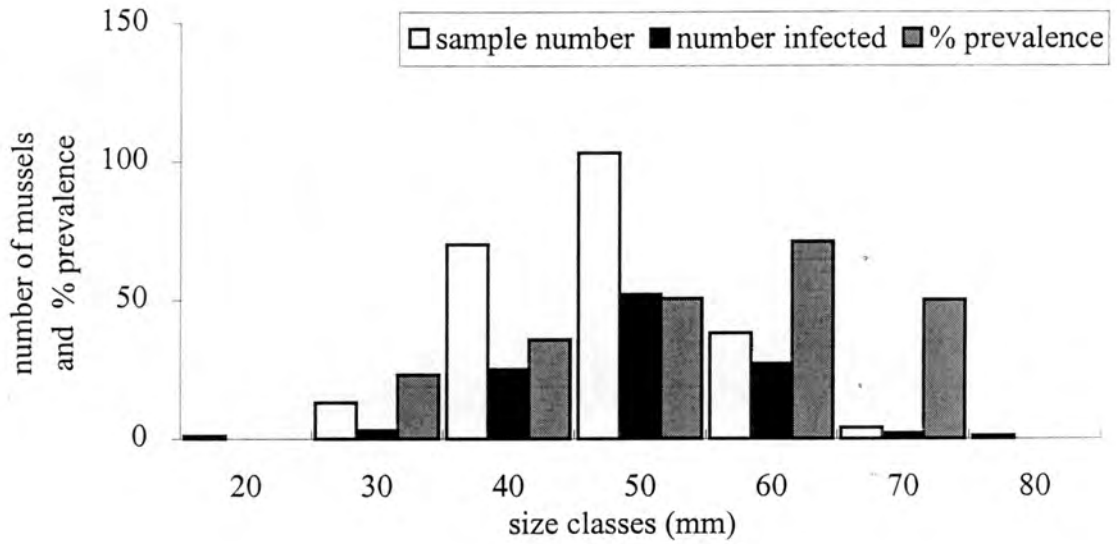


Figure 14. Size dependent prevalence of *Metacercaria A* in female *Perna* from Dido Valley.

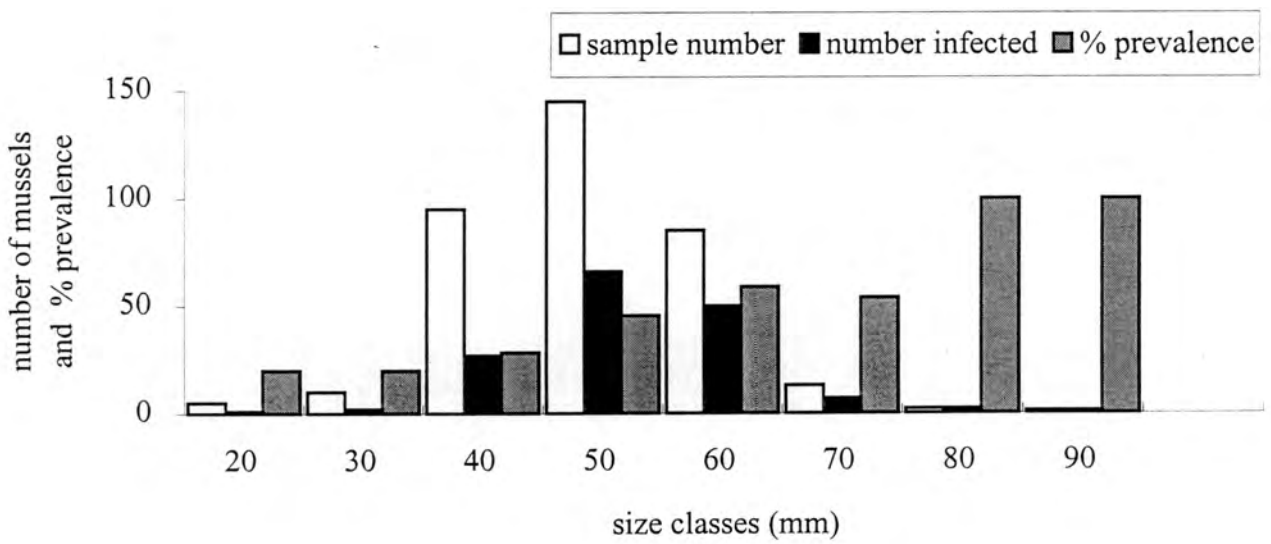


Figure 15. Size dependent prevalence of *Metacercaria A* in male *Perna* from Dido Valley.

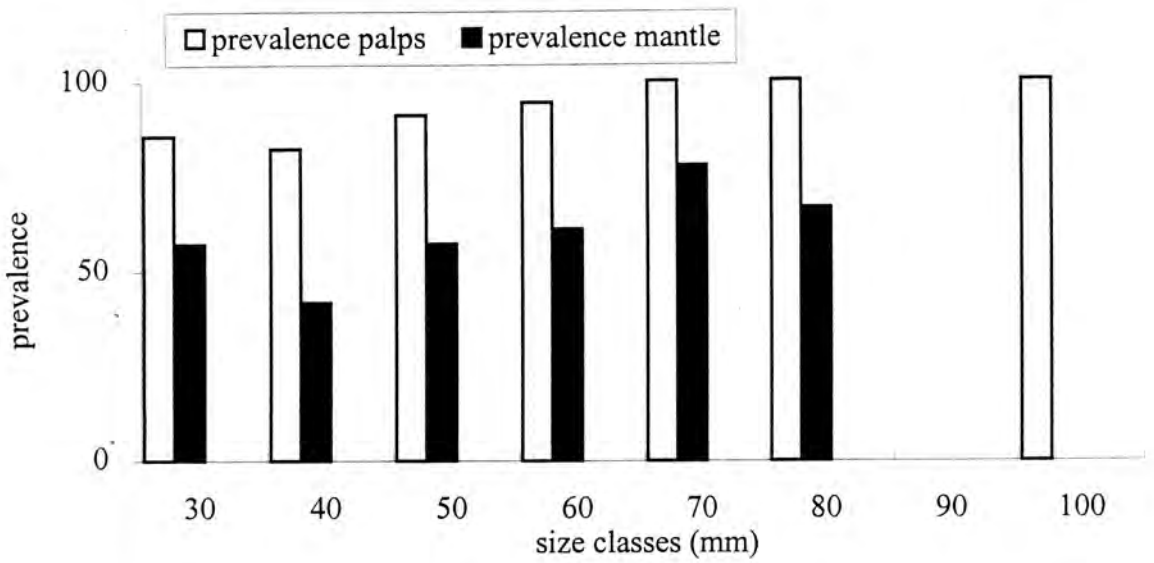


Figure 16. Size dependent prevalence of *Metacercaria A* in palps and mantle of female *Choromytilus* from Blouberg.

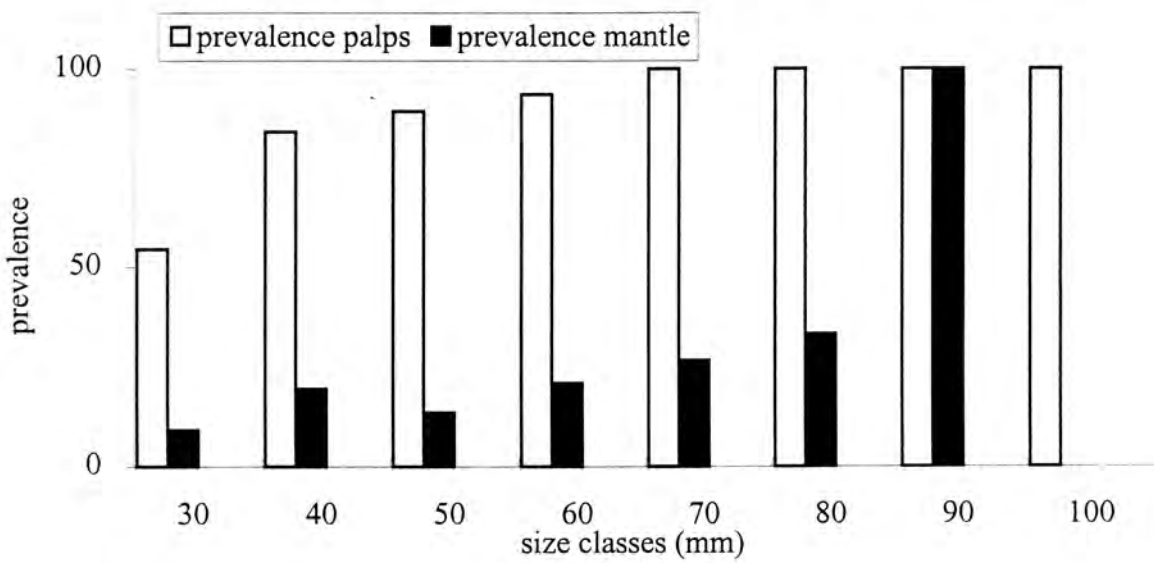


Figure 17. Size dependent prevalence of *Metacercaria A* in palps and mantle of male *Choromytilus* from Blouberg.

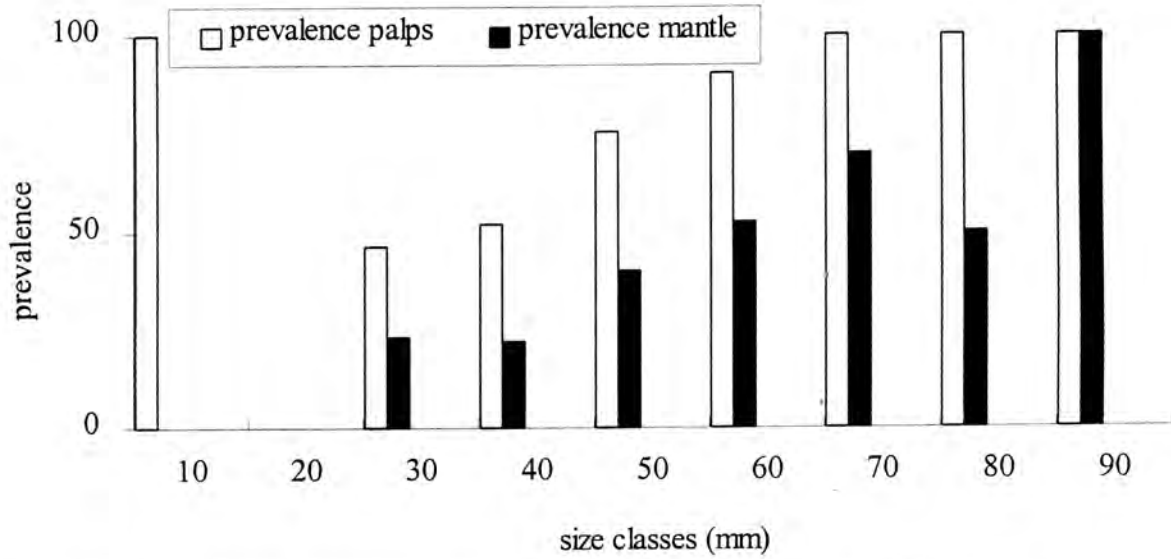


Figure 18. Size dependent prevalence of *Metacercaria A* in palps and mantle of female *Choromytilus* from Dido Valley.

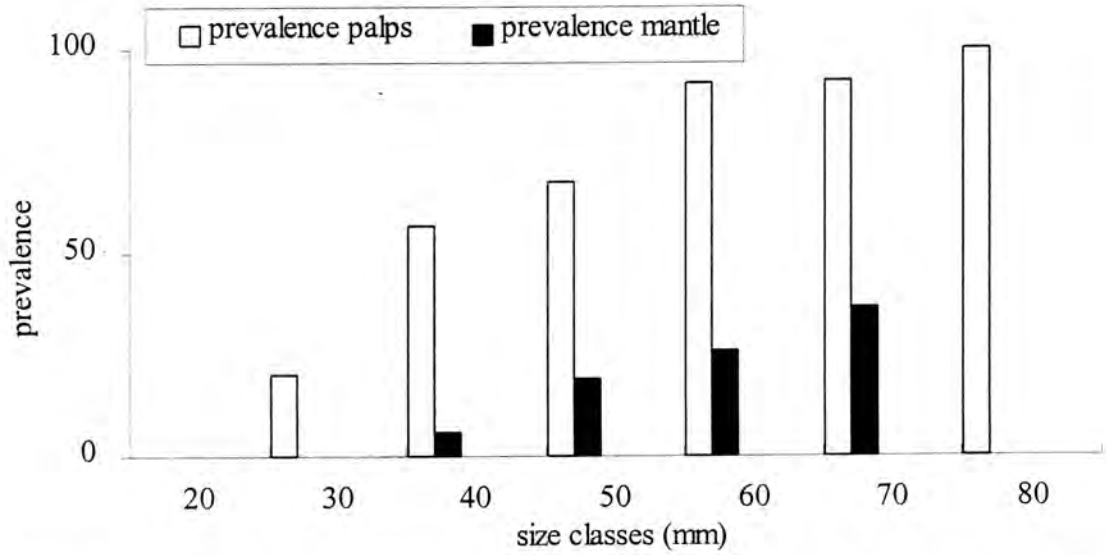


Figure 19. Size dependent prevalence of *Metacercaria A* in palps and mantle of male *Choromytilus* from Dido Valley.

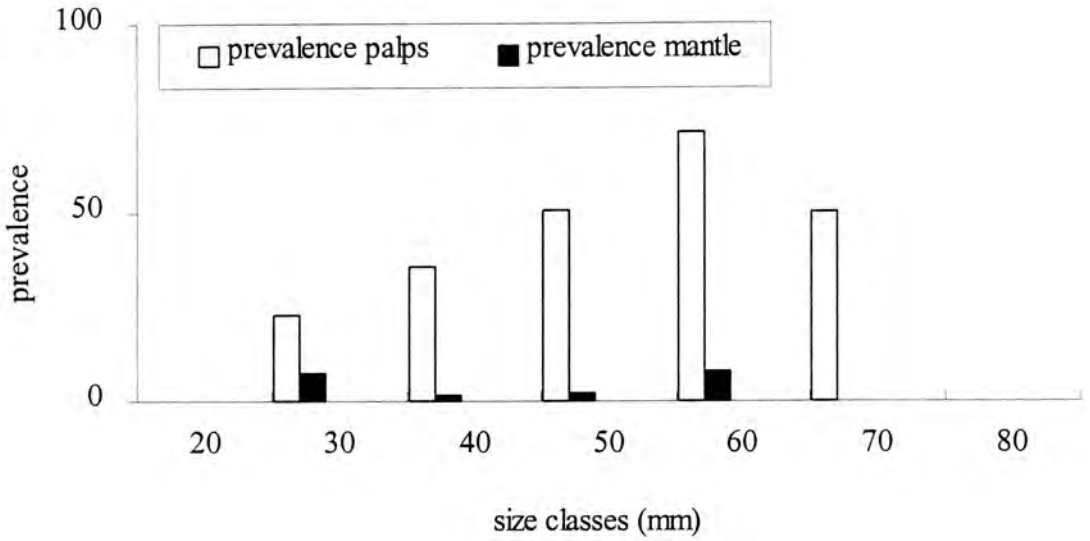


Figure 20. Size dependent prevalence of *Metacercaria A* in palps and mantle of female *Perna* from Dido Valley.

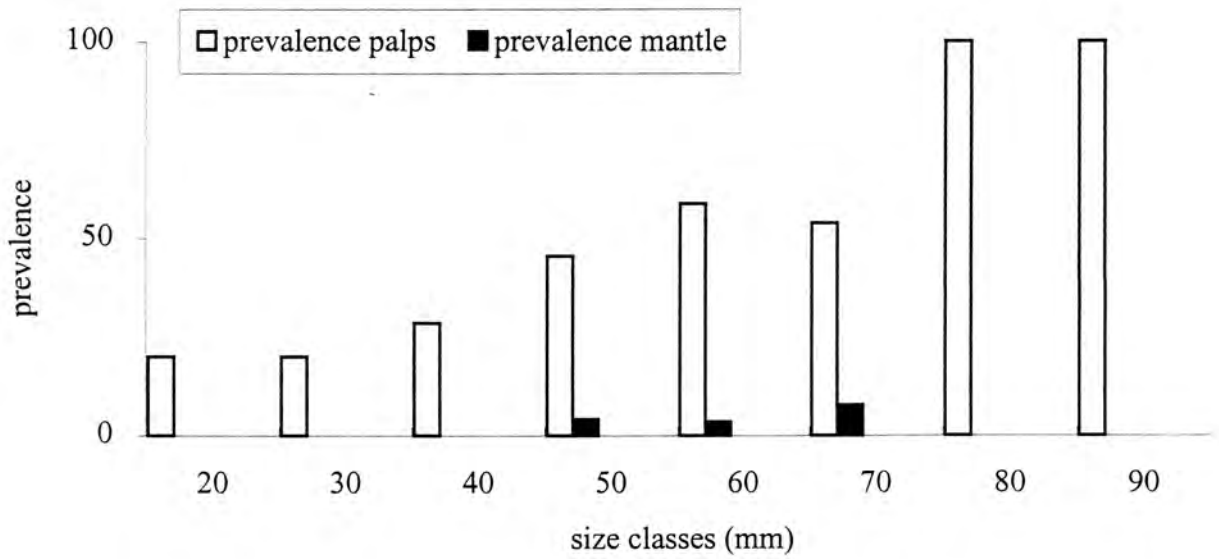


Figure 21. Size dependent prevalence of *Metacercaria A* in palps and mantle of male *Perna* from Dido Valley.

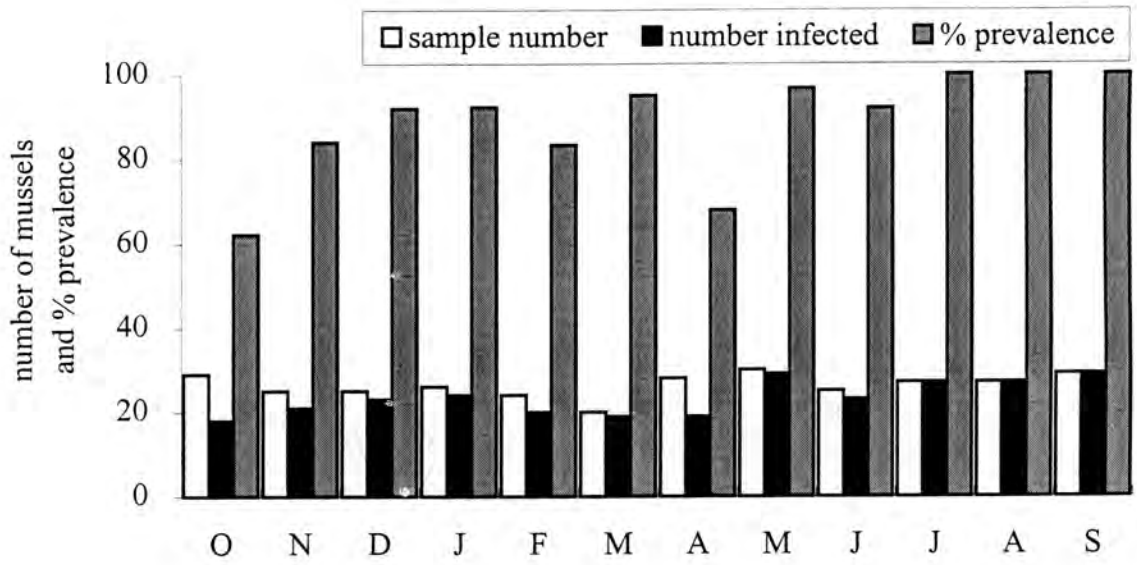


Figure 22. Monthly variation in prevalence of *Metacercaria A* in female *Choromytilus* from Blouberg.

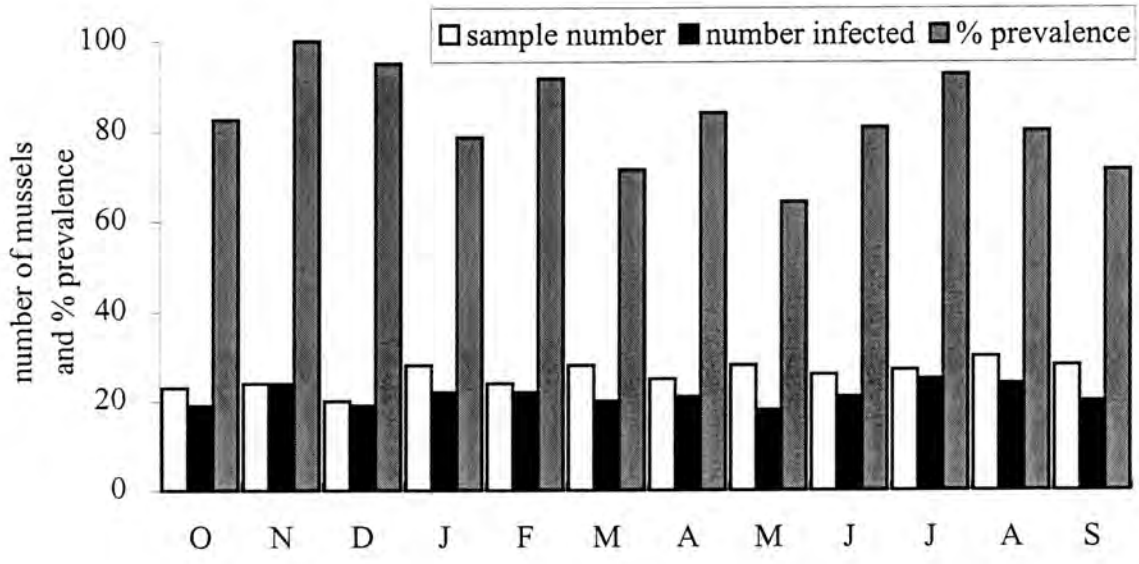


Figure 23. Monthly variation in prevalence of *Metacercaria A* in male *Choromytilus* from Blouberg.

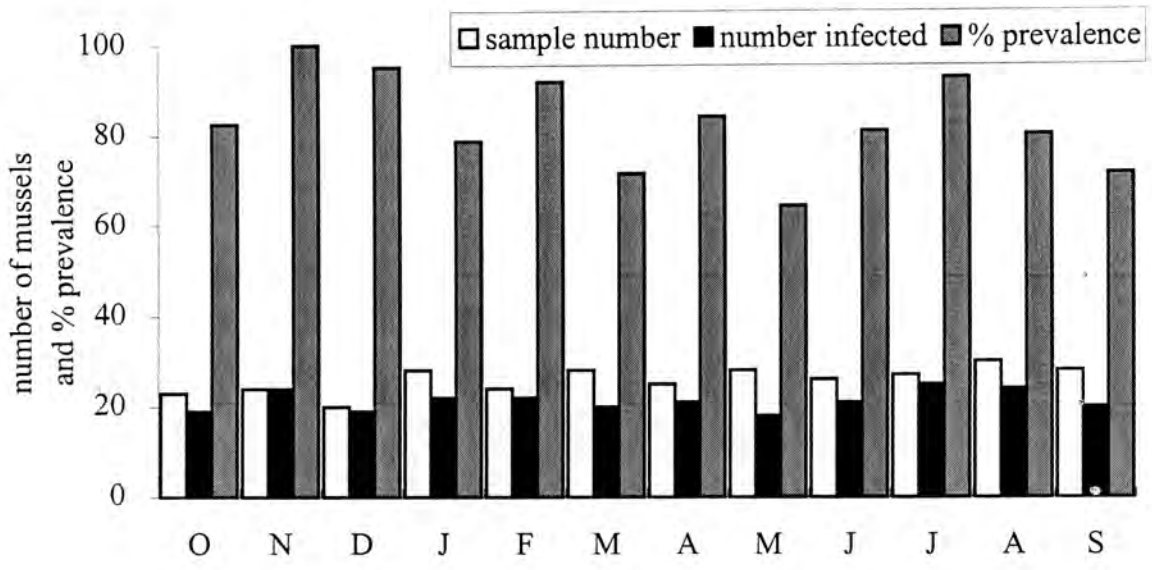


Figure 24. Monthly variation in prevalence of *Metacercaria A* in female *Choromytilus* from Dido Valley.

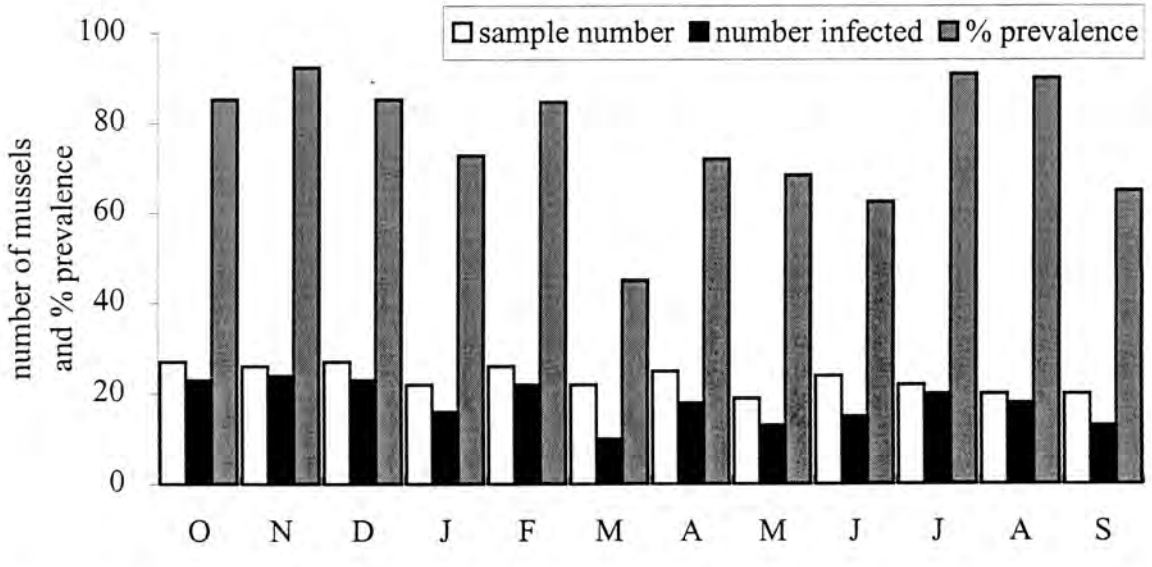


Figure 25. Monthly variation in prevalence of *Metacercaria A* in male *Choromytilus* from Dido Valley.

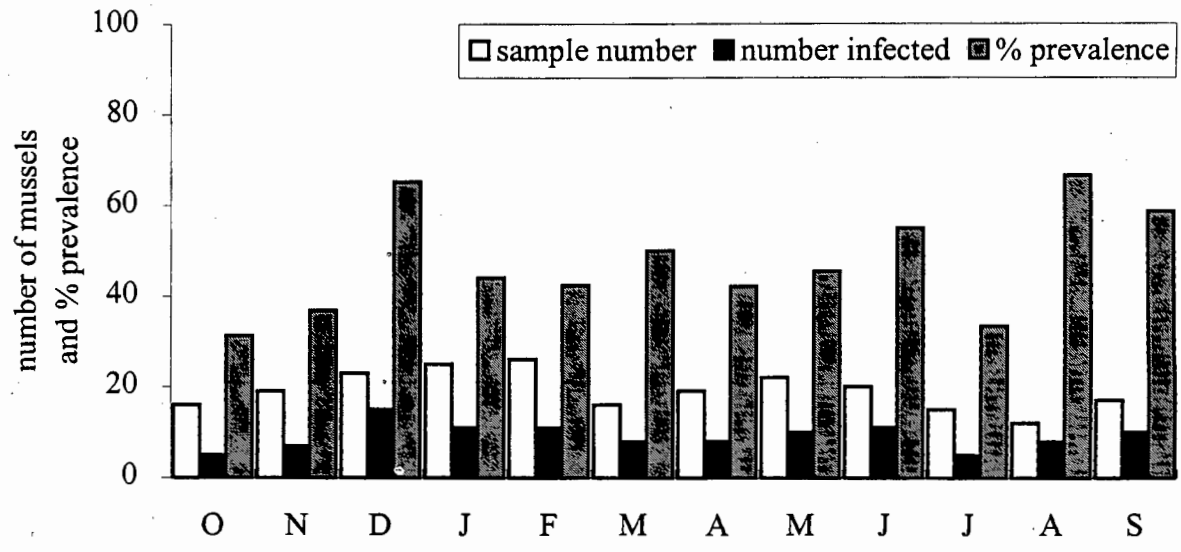


Figure 26. Monthly variation in prevalence of *Metacercaria A* in female *Perna* from Dido Valley.

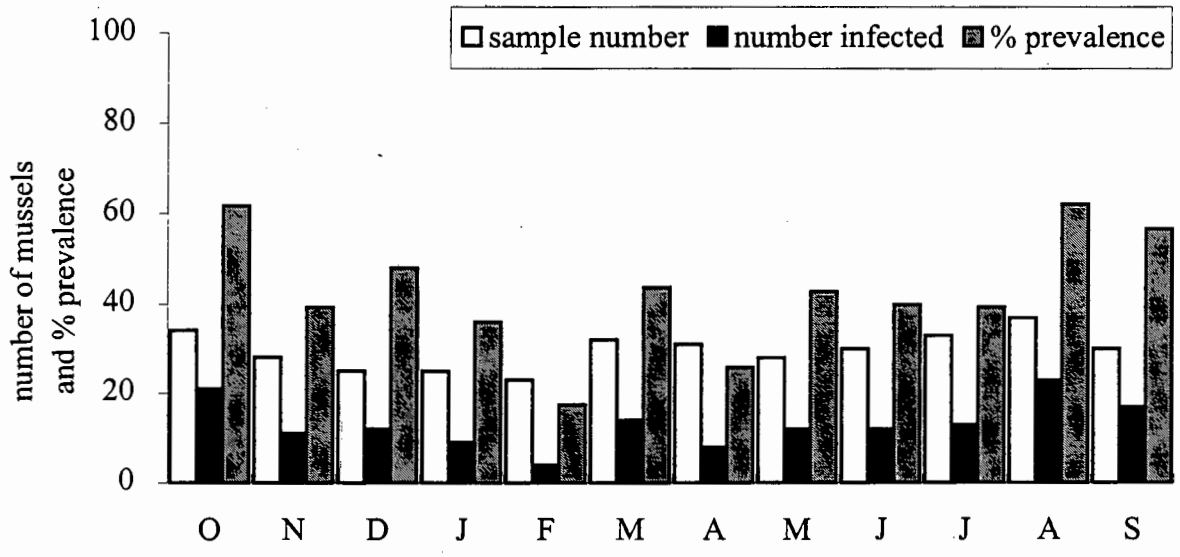


Figure 27. Monthly variation in prevalence of *Metacercaria A* in male *Perna* from Dido Valley.

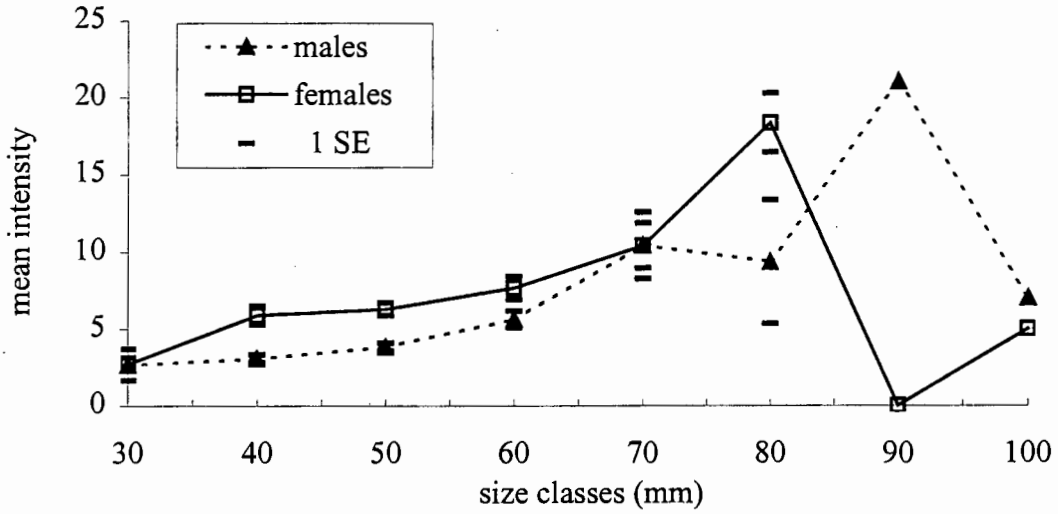


Figure 28. Size dependent mean intensity of infections of *Metacercaria A* in female and male *Choromytilus* from Blouberg.

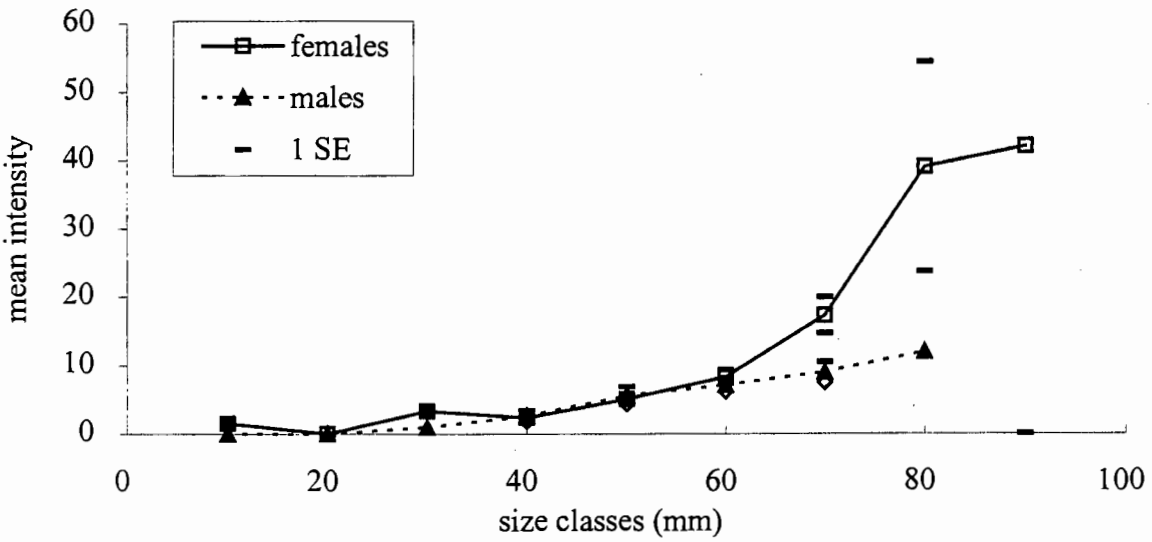


Figure 29. Size dependent mean intensity of infections with *Metacercaria A* in female and male *Choromytilus* from Dido Valley.

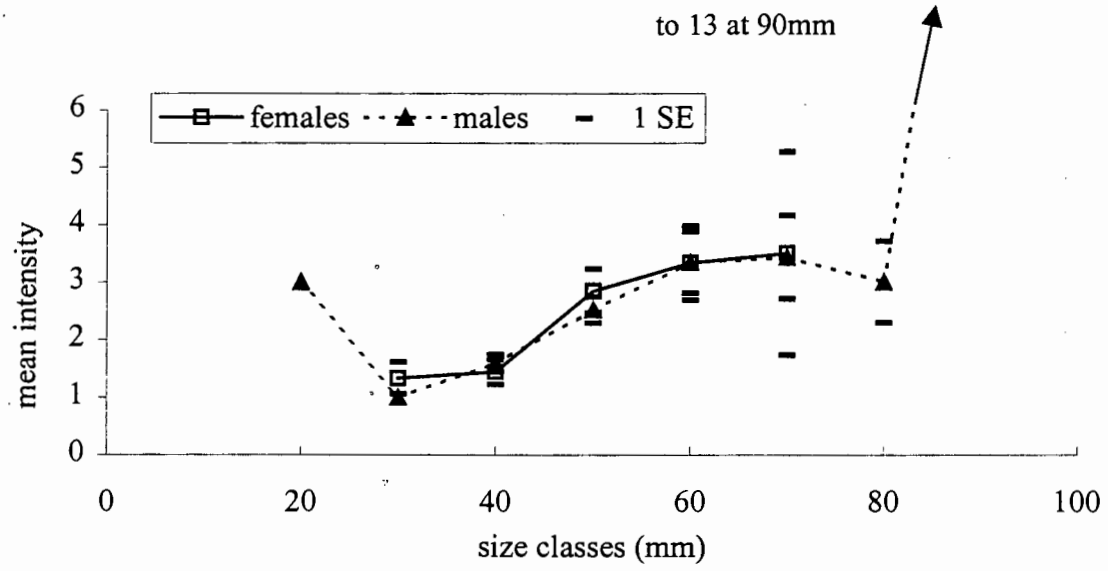


Figure 30. Size dependent mean intensity of infections of *Metacercaria A* in female and male *Perna* from Dido Valley.

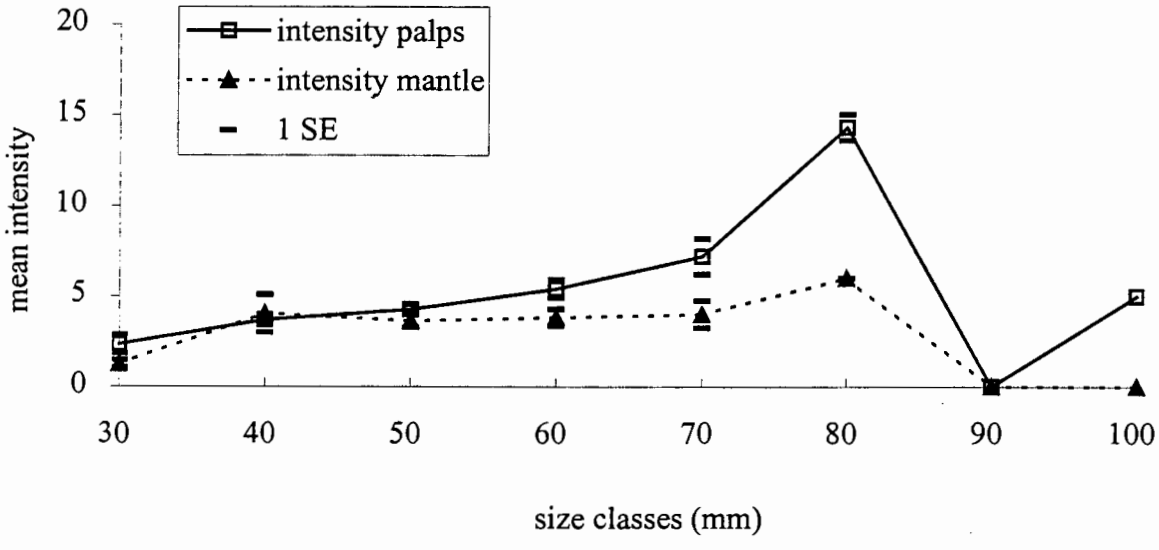


Figure 31. Size dependent mean intensity of *Metacercaria A* infections in palps and mantle of female *Choromytilus* from Blouberg.

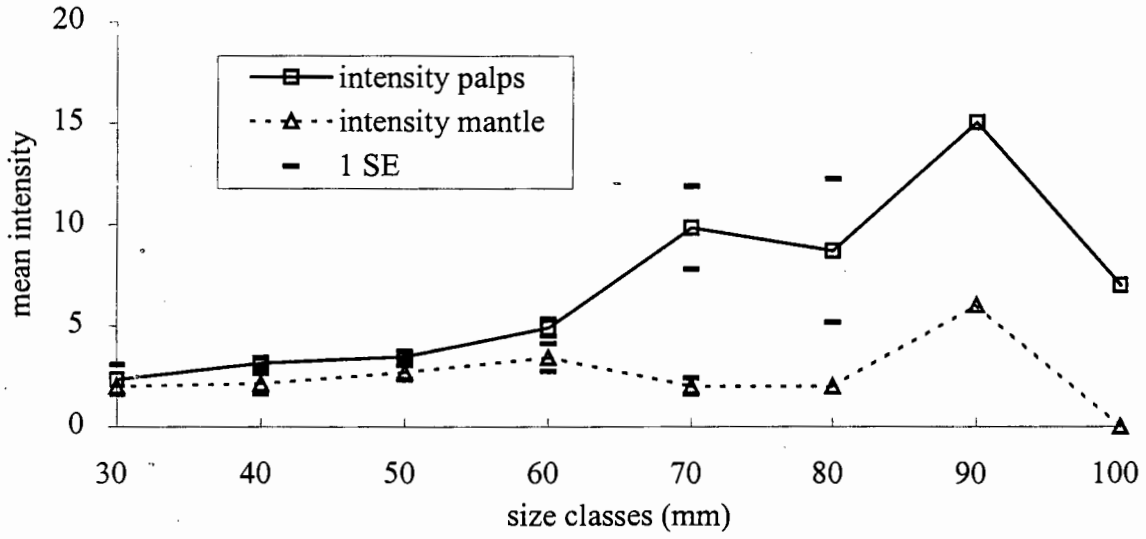


Figure 32. Size dependent mean intensity of *Metacercaria A* infections in palps and mantle of male *Choromytilus* from Blouberg.

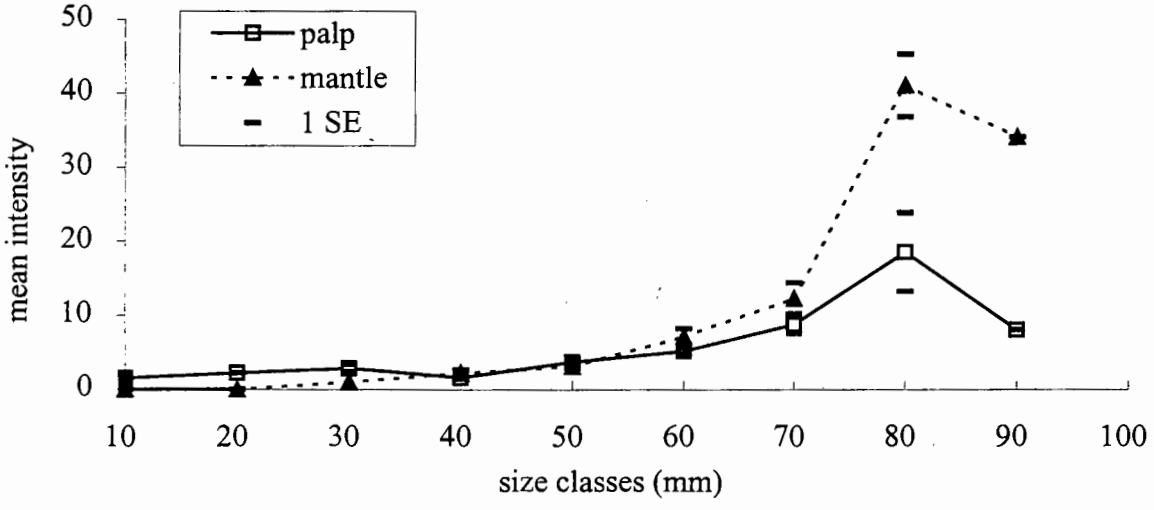


Figure 33. Size dependent mean intensity of *Metacercaria A* infections in palps and mantle of female *Choromytilus* from Dido Valley.

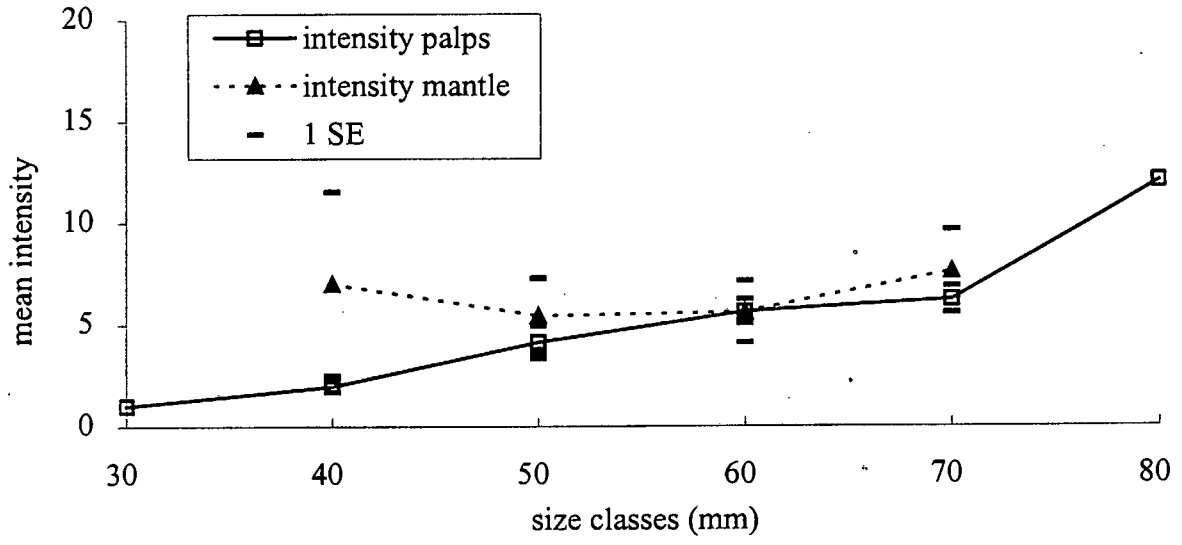


Figure 34. Size dependent mean intensity of infections with *Metacercaria A* in palps and mantle of male *Choromytilus* from Dido Valley.

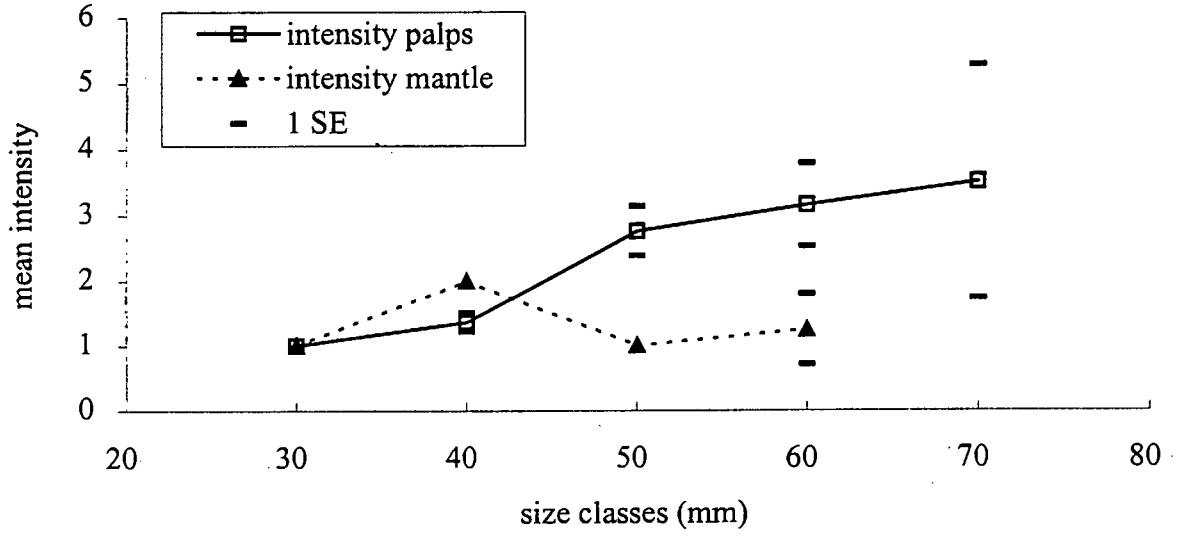


Figure 35. Size dependent mean intensity of infections with *Metacercaria A* in palps and mantle of female *Perna* from Dido Valley.

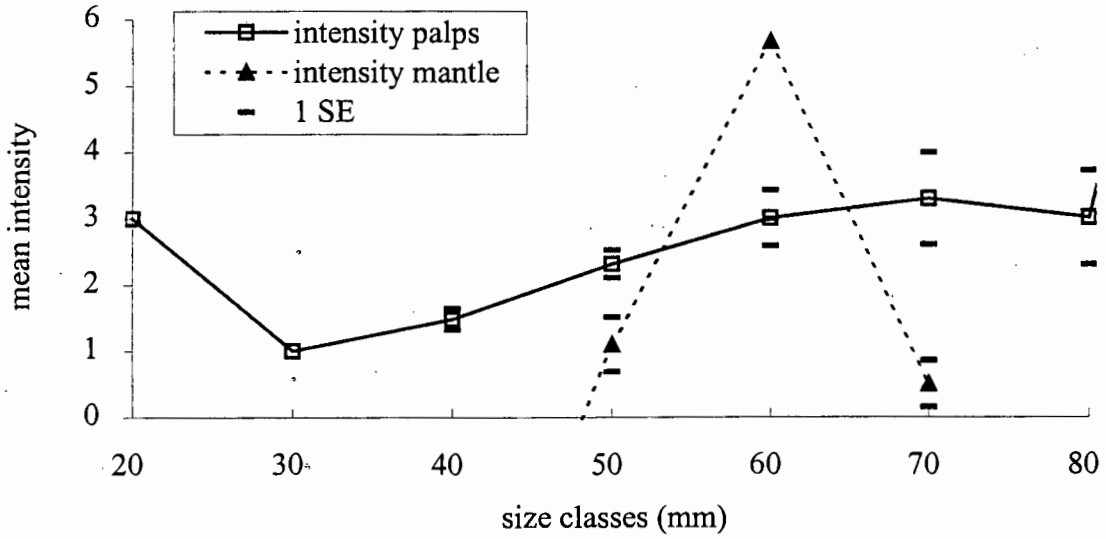


Figure 36. Size dependent mean intensity of infections with *Metacercaria A* in palps and mantle of male *Perna* from Dido Valley.

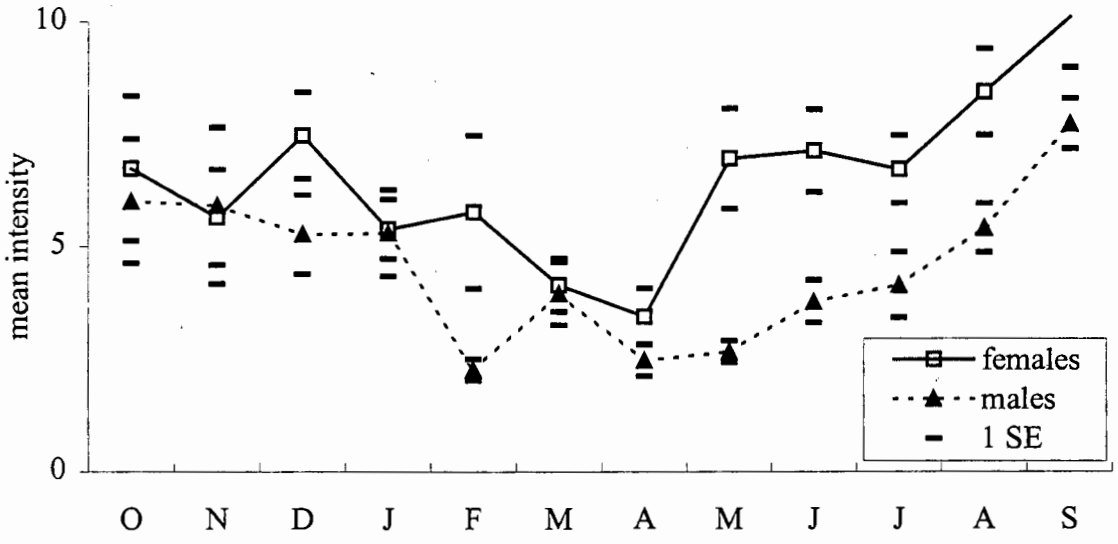


Figure 37. Monthly variation in mean intensity of infections with *Metacercaria A* in female and male *Choromytilus* from Blouberg.

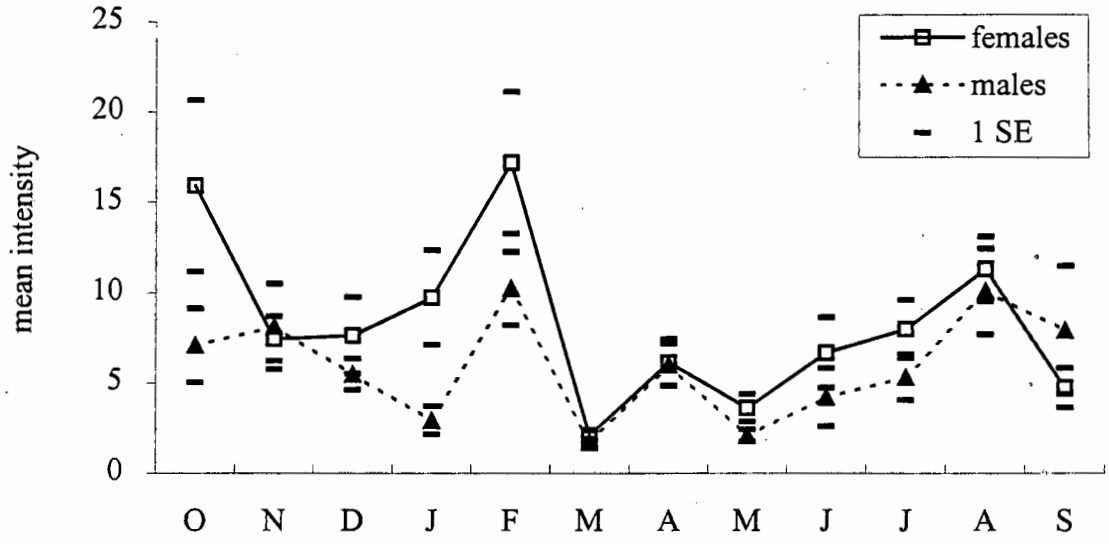


Figure 38. Monthly variation in mean intensity of infections with *Metacercaria A* in female and male *Choromytilus* from Dido Valley.

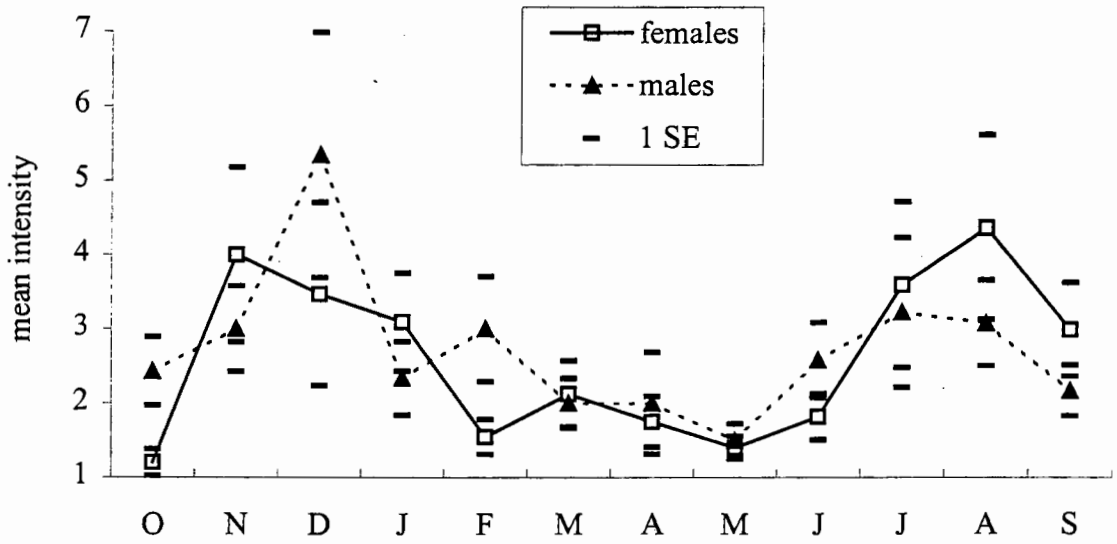


Figure 39. Monthly variation in mean intensity of infections with *Metacercaria A* in female and male *Perna* from Dido Valley.

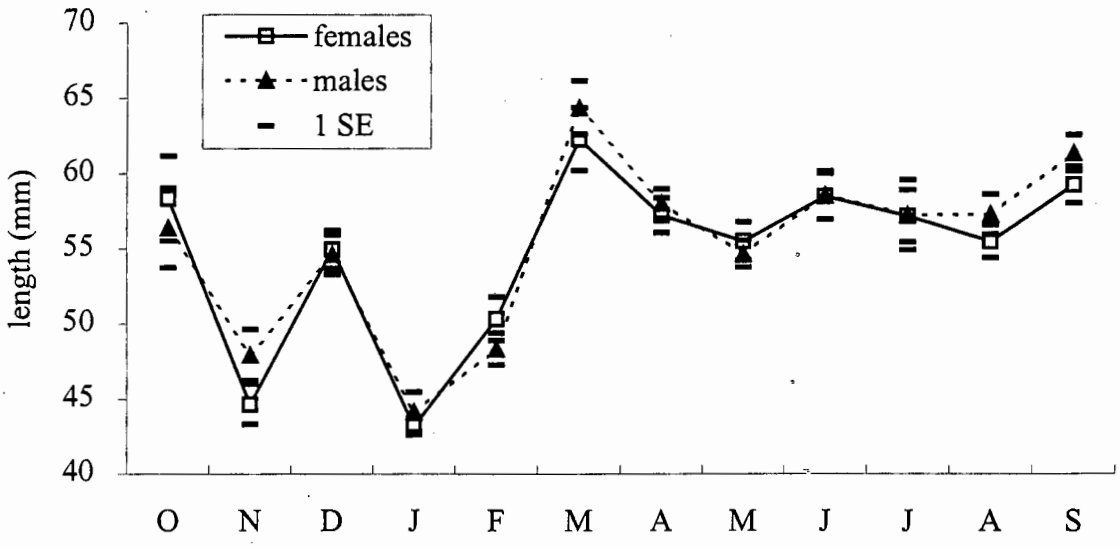


Figure 40. Monthly variation in mean sample size of female and male *Choromytilus* from Blouberg.

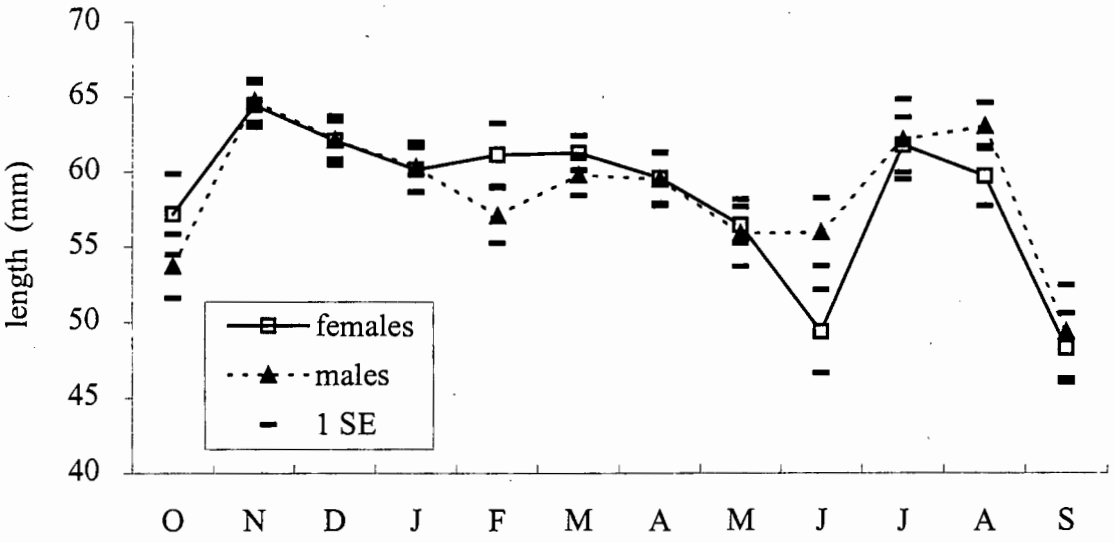


Figure 41. Monthly variation in mean sample size of female and male *Choromytilus* from Dido Valley.

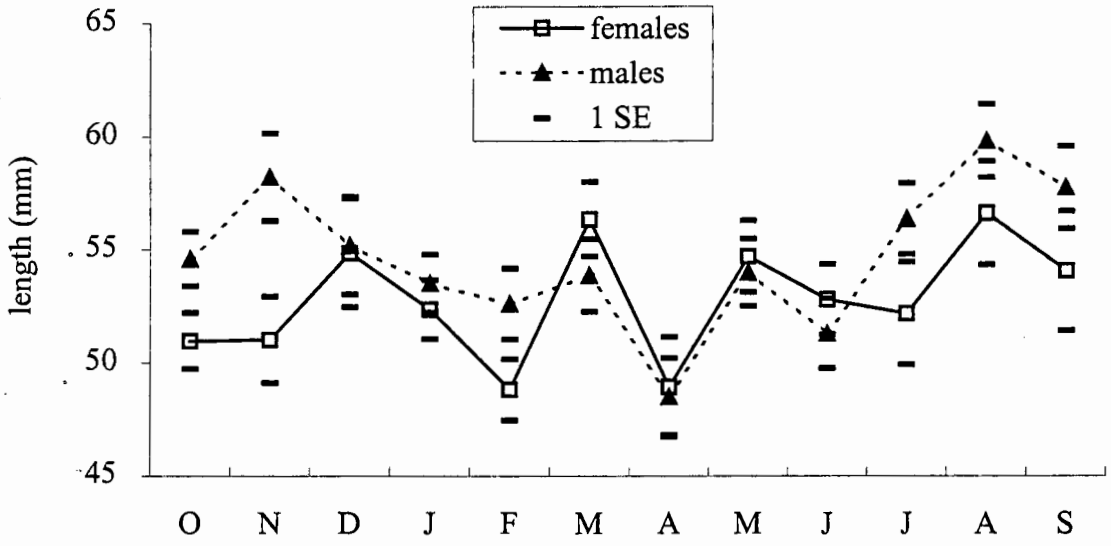


Figure 42. Monthly variation in mean sample size of female and male *Perna* from Dido Valley.

CHAPTER 6: *METACERCARIA COLUMBINENSIS* SP. NOV.

HOSTS AND LOCALITY

This parasite was found in *Mytilus galloprovincialis* at Cape Columbine.

TYPE SPECIMENS

Paratypes: Specimen number A29430. Marine Biology Department, South African Museum, Queen Victoria Street, Cape Town, RSA. Type host: *Mytilus galloprovincialis*. Type locality: Cape Columbine.

DESCRIPTION

The cyst is ellipsoidal, and there is a dark area around the metacercaria when it is encysted (Figure 1). Figures 2A & B show the metacercaria and the cyst wall in greater detail. The wall is irregular in thickness but there appears to be an external boundary separating it from the unaffected host tissue. The metacercaria was extracted from the cyst by gently rolling it between a microscope slide and a cover slip. Figure 3 shows the typical posture of the extracted metacercaria. The entire cuticle is covered with papillae, which have curious knob-like endings. The sub-terminal oral sucker is about two-thirds the size of the ventral sucker. The opening of the oral sucker into the pre-pharynx is initially stepped (1.2μm per step) before becoming parallel as it passes out of the oral sucker musculature. The pharynx is about one third of the length of the oral sucker and is a truncated cone with its base to the posterior. The oesophagus is thicker than the pre-pharynx and consists of columnar cells. The oesophagus and the caeca are similar in structure. Caeca terminate about half-way to the posterior. The excretory pore is at the extreme posterior. The bladder lies about 25μm anterior to this pore and is visible because of the contained excretory granules or spheres; no bladder wall was seen. The rest of the excretory ducts are invisible except for the ends of the primary excretory vessels, which terminate just posterior to the junction of the oesophagus and the caeca. There are ten flame cells. The ovary is not visible. A uterus containing eggs runs posteriorly away from what appears to be a testis. It then curves around behind the bladder and passes another testis. Close to both of the testes are large, apparently single celled, glands. The uterus continues forwards and is lost at about the level of the ventral sucker. No movement was noted.

Table 1. Measurements (μm) of living *Metacercaria columbinensis*.

	mean	SD	*n*	max.	min.
inner cyst wall thickness	7.4		1		
cyst diameter	163.8	33.43	14	258	126
metacercaria length	411	---	2	434	388
width from front 25%	66.7	4.11	3	71	61
width from front 50%	86		1		
width from front 75%	93	---	2	93	80
oral sucker length	60.29	8.78	7	78	47
oral sucker width	64.14	7.1	7	75	51
ventral sucker length	90	9.91	6	108	74
ventral sucker width	96	---	2	96	88
ventral sucker depth	87.5	11.3	5	103	71
thickness muscle layer	18		1		
distance to rear	61		1		
pre pharynx length	1.5		1		
pharynx length	23.32	5.9	5	35	19.6
pharynx width	23.22	6.07	6	35	17
cuticle thickness	1.8		1		
cuticle wrinkles pitch	0.8	---	2	1	0.6
papillae length	1.3		1		
papillae pitch	2.18	---	2	2.45	1.9
bladder length	27.5	---	2	30	25
bladder width	58.25	---	2	67.5	49
excretory pore width	2.5		1		
excretory granules dia.	3.7		1		
eggs length	4		1		
eggs width	3		1		
uterus width	3		1		
caecal epithelium width	2.5		1		
genital pore	7.5		1		

EPIDEMIOLOGY

Table 2. Host mean lengths of *Mytilus galloprovincialis* (1988 collection).

	mean length	SD	*n*	SE
male	75.21mm	8.97	21	1.96
infected male	73.28mm	9.68	8	3.42
uninfected male	76.42mm	8.27	13	2.29
female	76.81mm	9.34	21	2.04
infected female	84.15mm	9.96	4	4.98
uninfected female	75.09mm	8.29	17	2.01

Table 3. Prevalences, mean intensities and abundances of *Metacercaria columbinensis* in *Mytilus galloprovincialis*. Normal intensity was 1 or 2 in the palps but a maximum of 18 was counted from one host (1988 collection).

sex	prevalence	intensity	SD	SE	abundance	SD	SE
male	38.1%	4	5.52	1.95	1.524	3.92	0.86
female	19.1%	1.25	0.43	0.22	0.24	0.53	0.12
total	28.6%	2.92	---	---	0.83	---	---

Table 4. Mean lengths of *Mytilus galloprovincialis* (1997 collection).

	mean length	SD	*n*	SE
male	69.50mm	5.66	52	0.78
infected male	71.95mm	---	1	---
uninfected male	69.61mm	5.71	51	0.8
female	69.59mm	6.19	48	0.89
infected female	69.40mm	---	1	--
uninfected female	69.60mm	6.25	47	0.91

Table 5. Prevalences, mean intensities and abundances of *Metacercaria columbinensis* in *Mytilus galloprovincialis* (1997 collection).

	prevalence	intensity	abundance
male	1.92%	1	0.021
female	2.08%	1	0.019
total	2%	1	0.02

For size dependent prevalence and intensity see Figures 4 & 5.

DISCUSSION

Taxonomic affinities and etymology

Metacercaria columbinensis was the only digenean found in *Mytilus galloprovincialis* during this survey. And despite surveys of this mussel in other parts of South Africa, it has been found infected with this parasite only at Cape Columbine. Thus the name *Metacercaria columbinensis* is proposed for this worm. The paucity of parasites in South African *Mytilus galloprovincialis* is more than matched by reports that samples from Spain were free of trematodes (Calvo-Ugarteburu 1996 and Calvo-Ugarteburu & McQuaid 1998). This suggests that *Mytilus galloprovincialis* may have a lower level of parasitism than other mytilids. This could be investigated by comparing parasite loads of this mussel with those of other mytilid species from a range of localities around the world. *Metacercaria columbinensis* occurs in the labial palps of the

mussel in a similar manner to infections of *Metacercaria A* and *Metacercaria B* in *Choromytilus* and *Perna*. See Chapter 10 for discussion of why the palps may be a choice site for infection.

Metacercaria columbinensis was initially considered to be a gymnophallid but it is ungymophallid-like because it has a pre-pharynx and a large ventral sucker in the posterior half of the body. *Metacercaria columbinensis* exhibits a number of zoogonid features. Comparison with *Zoogonus* sp. showed that *Zoogonus* had a much longer pre-pharynx, and the bifurcation of the digestive tract was posterior to the ventral sucker. The pharynx was too large in proportion, and the ventral sucker was not far enough to the posterior. Nevertheless, the habit of the posterior extremity is very similar - see Stunkard (1941). When encysted, *Zoogonus* sp. occurs in a tough, thin colourless cyst wall. This contrasts with that in *Metacercaria columbinensis* (Figures 1 & 2). A final difference is the flame cell number. *Zoogonus rubellus* (Olsson, 1868) Odhner, 1902 has 16 flame cells and encysts in annelids. *Zoogonoides laevis* Linton, 1940, also has 16 flame cells and encysts in annelids. *Metacercaria columbinensis* appears to have a closer affinity with *Zoonogenus vividus* Nicoll, 1913, in Dawes (1946). In both, the caeca terminate before they reach the large ventral sucker, which is in the posterior part of the body.

The only other larval zoogonid reported from the Western Cape coast is *Cercaria hapax* Brown & Webb in Brown & Webb (1994). This was found as cercariae and sporocysts in *Bullia digitalis.* It was found once in 3000 whelks. It is a xiphidiocercaria, which suggests that it encysts in an arthropod intermediate host. An adult zoogonid *(Lecithostaphylus spondyliosomae)* has been reported previously (Fantham 1938) in a fish from South African waters. Comparison of an adult with a metacercaria is not entirely satisfactory but the small size and forward position of the ventral sucker in *Lecithostaphylus spondyliosomae* and the caeca that proceed well beyond it suggests significant difference. Furthermore, the genital pore was much further forward in *Lecithostaphylus spondyliosomae* which had no pre-pharynx and whose testes were also far more anterior. These differences are sufficient to rule out the identification of *Metacercaria columbinensis* as a larva of *Lecithostaphylus spondyliosomae.*

Epidemiology

There is (Figures 4 & 5) no marked size dependent intensity of infection in males or females. Scattergrams of shell length versus the number of cysts give r^2 of 0.115 (d.f.=19) for females and r^2 of 0.072 (d.f.=19) for males. From the 1988 (Tables 2 & 3) collection, males exhibit a higher prevalence (38.1%) than females (19.1%). Males also have a higher mean intensity (4, SE 1.95) than females (1.25, SE 0.22), however, the standard errors suggest that this may not be significant at this sample size. From the 1997 collection, the sexes exhibit a similar prevalence and identical intensity (Tables 4 & 5). The two collections in this study were taken eight years apart. Prevalences of 28.6%, and intensities of 2.92 were noted in the first and prevalence of 2% and intensity of one were noted in the second. This attests to a decline but at these sample sizes this may be questionable.

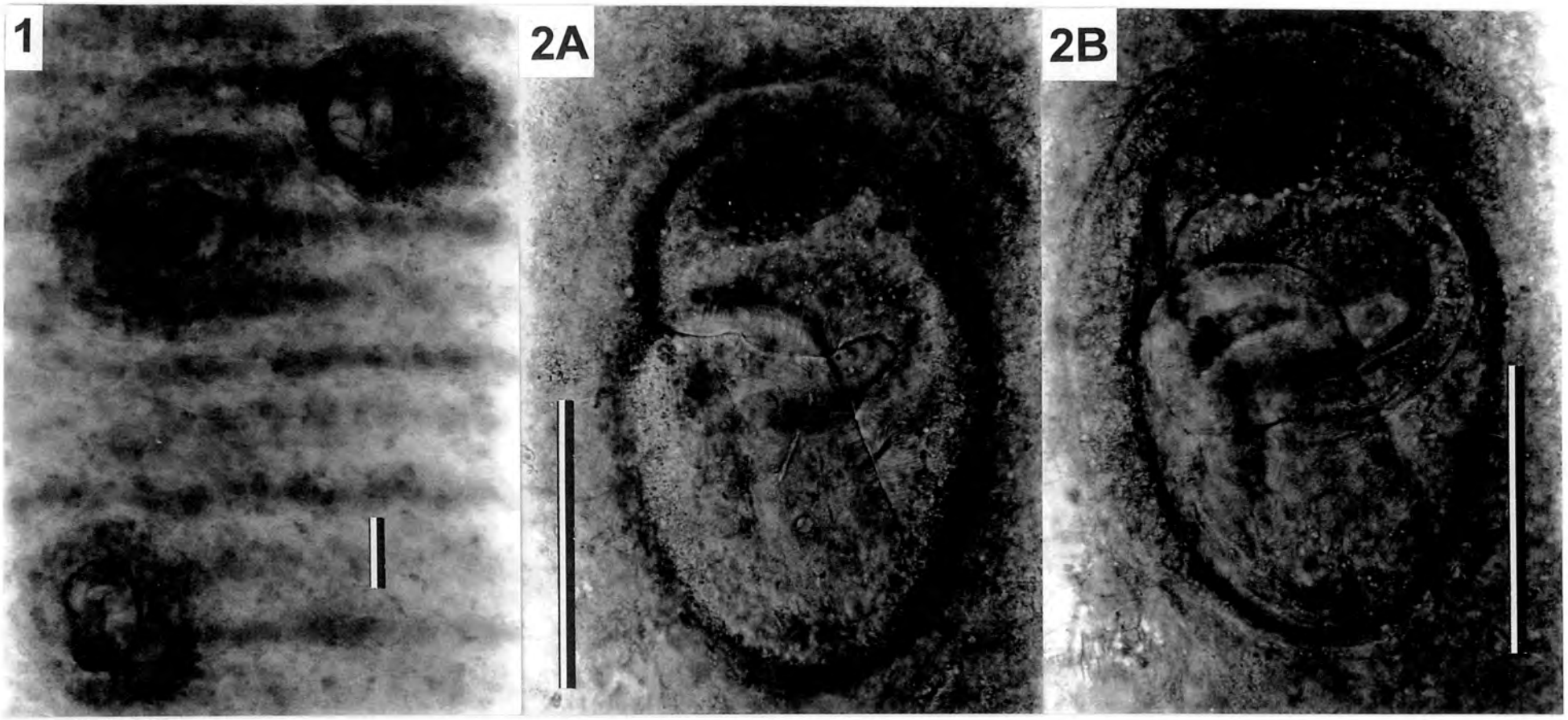


Figure 1. Light micrograph of encysted *Metacercaria columbinensis* showing discoloured tissue around the cyst, scale bar = 100µm.
Figure 2A & B. Light micrographs of encysted *Metacercaria columbinensis* at different depth of focus, scale bar = 100µm.

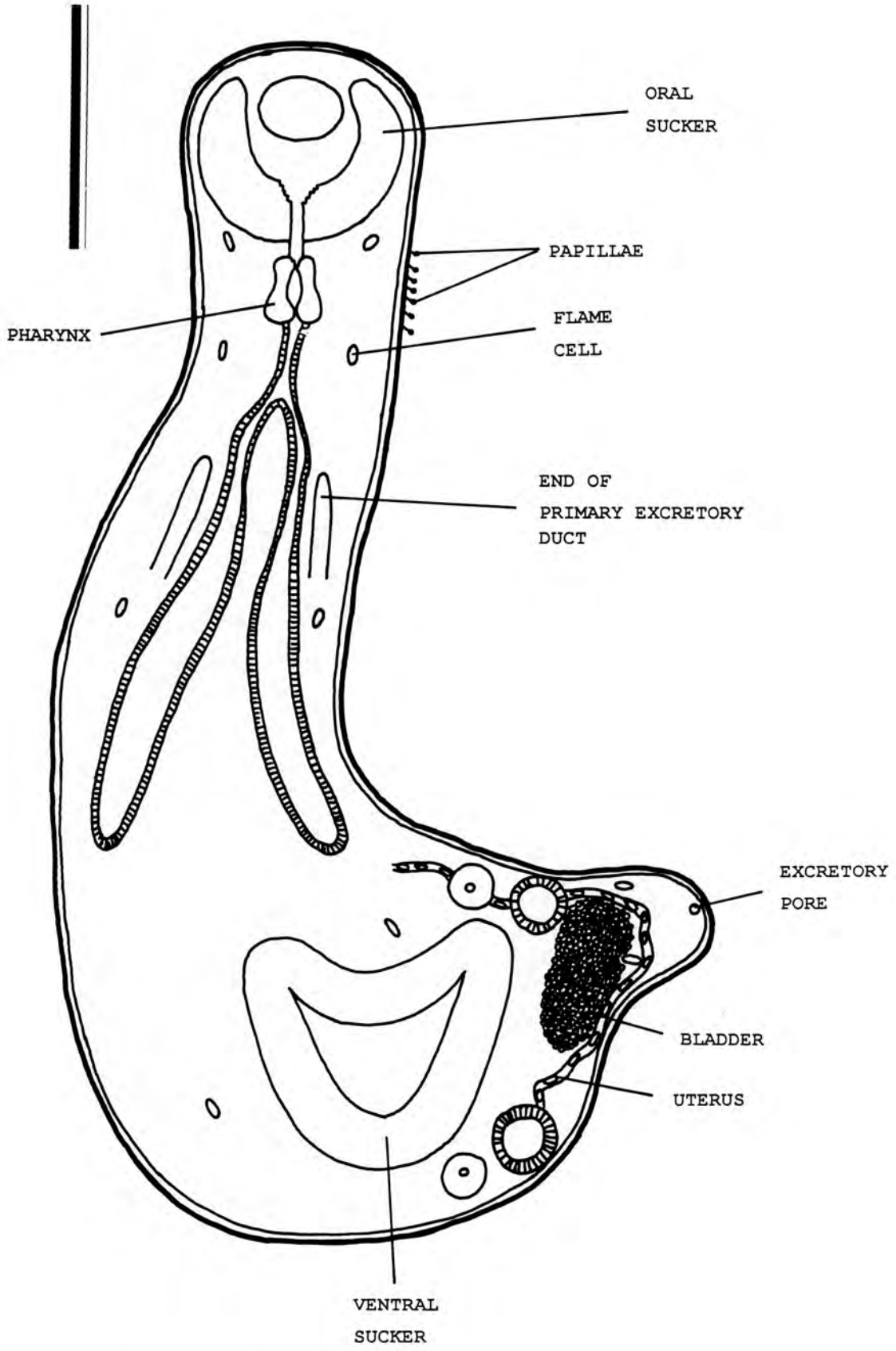


Figure 3. Excysted *Metacercaria columbinensis*, scale bar = 80μm.

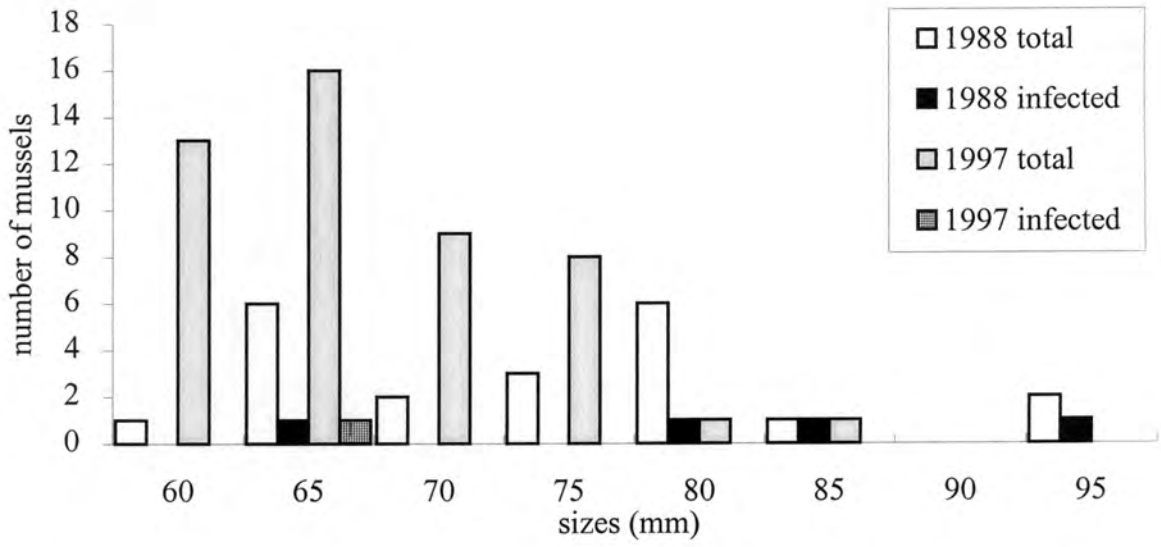


Figure 4. Size distributions of collection samples (1988 & 1997) of female *Mytilus* and their infected sub-populations from Cape Columbine.

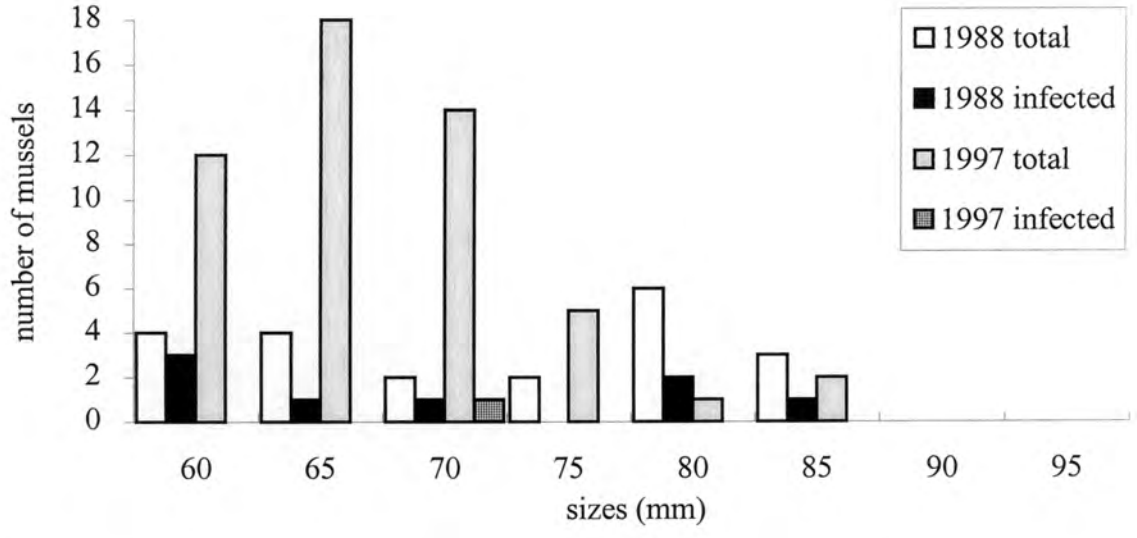


Figure 5. Size distributions of collection samples (1988 & 1997) of male *Mytilus* and their infected sub-populations from Cape Columbine.

CHAPTER 7: *METACERCARIA MACULATOPSIS* SP. NOV.

METACERCARIA MACULATOPSIS I

Host and locality

This worm was found in the mantle cavity of a male *Choromytilus meridionalis* (length 48.8mm) at Dido Valley.

Type specimen

Holotype: Specimen number A29431. Marine Biology Department, South African Museum, Queen Victoria Street, Cape Town, RSA. Type host: *Choromytilus meridionalis*. Type locality: Dido Valley.

Description

No sporocysts or rediae were observed. This worm is a large opaque, green coloured and slightly flattened fusiform distome (Figures 1 & 2). Fine wrinkles, each about 5µm wide, cover the tegument. They show a tendency to coalesce into much larger wrinkles around both suckers and the excretory pore. The oral sucker opens sub-terminally (Figures 3 & 4); the mouth is surrounded by an irregular array of nodular papillae. The lining of the oral sucker consists of diamond shaped areas, each of about 6µm per side. There is no pre-pharynx - the pharynx is elongated and about the same length as the oral sucker but narrower. Digestive caeca arise directly from the pharynx and run almost to the posterior end of the body. They are broad and lie halfway from the mid-line to the lateral margin of the body. The ventral sucker is about twice the width of the oral sucker and lies slightly less than one third of the body length from the front. The cirrus is directly behind the ventral sucker. Two testes lie staggered behind the cirrus; the vas efferens from each testis is not visible but a vas deferens is discernible posterior to the ovary. This duct leads to the cirrus pouch by a circuitous route. Inside the cirrus pouch it leads into a seminal vesicle before running to the cirrus. The genital atrium is long and runs approximately from the level of the mid-point of the ventral sucker to the genital pore, which lies 240µm from the nearest part of the ventral sucker. The ovary, which is about 30% smaller than a testis, lies anterior to the testes and on the right of the cirrus. Other features of the reproductive system are hidden by the masses of green lobular vittellaria. These

give the body a mottled appearance (Figure 2). They contain a huge number of small spheres, each with two long filaments (See Webb 1985). The filaments arise from the spheres at about the eleven and one o'clock positions. Before release, these filaments are tightly wound round the sphere. On exposure to seawater by rupturing the worm they spring apart. The excretory system is stenostomate and the pore is terminal. No bladder is visible but the medial stem is probably extensible as it is in *Metacercaria maculatopsis II.* The vesicle bifurcates to the anterior of the testes, about 2/3 of the way to the posterior. Each duct then curves out dorsally and laterally to lie above the caeca. At the anterior extent of the primary excretory ducts they curve back in to lie more medially. The secondary duct arises just anterior to the ventral sucker at the termination of the primary duct and terminates just beyond mid-way to the posterior. No flame cells could be seen through the vitellaria. The eggs are thick walled and lemon shaped.

Table 1. Measurements (μm) of *Metacercaria maculatopsis I.* A (living), B formalin fixed.

A

length	1750-3700
width 25% from front	650-1400
width 50% from front	825-1200
width 75% from front	625-1000
oral sucker length	204-250
oral sucker width	200-291
ventral sucker length	369
ventral sucker width	407
pharynx length	233-250
pharynx width	150-175
cirrus length	534
cirrus width	78
egg length	49
egg width	38
cuticle wrinkles	4.9
excretory pore width	25

B

length	3589
width 25% from front	1229
width 50% from front	1024
width 75% from front	896
oral sucker length	174
oral sucker width	253
ventral sucker length	313
ventral sucker width	354
pharynx length	202
pharynx width	131
cirrus length	454

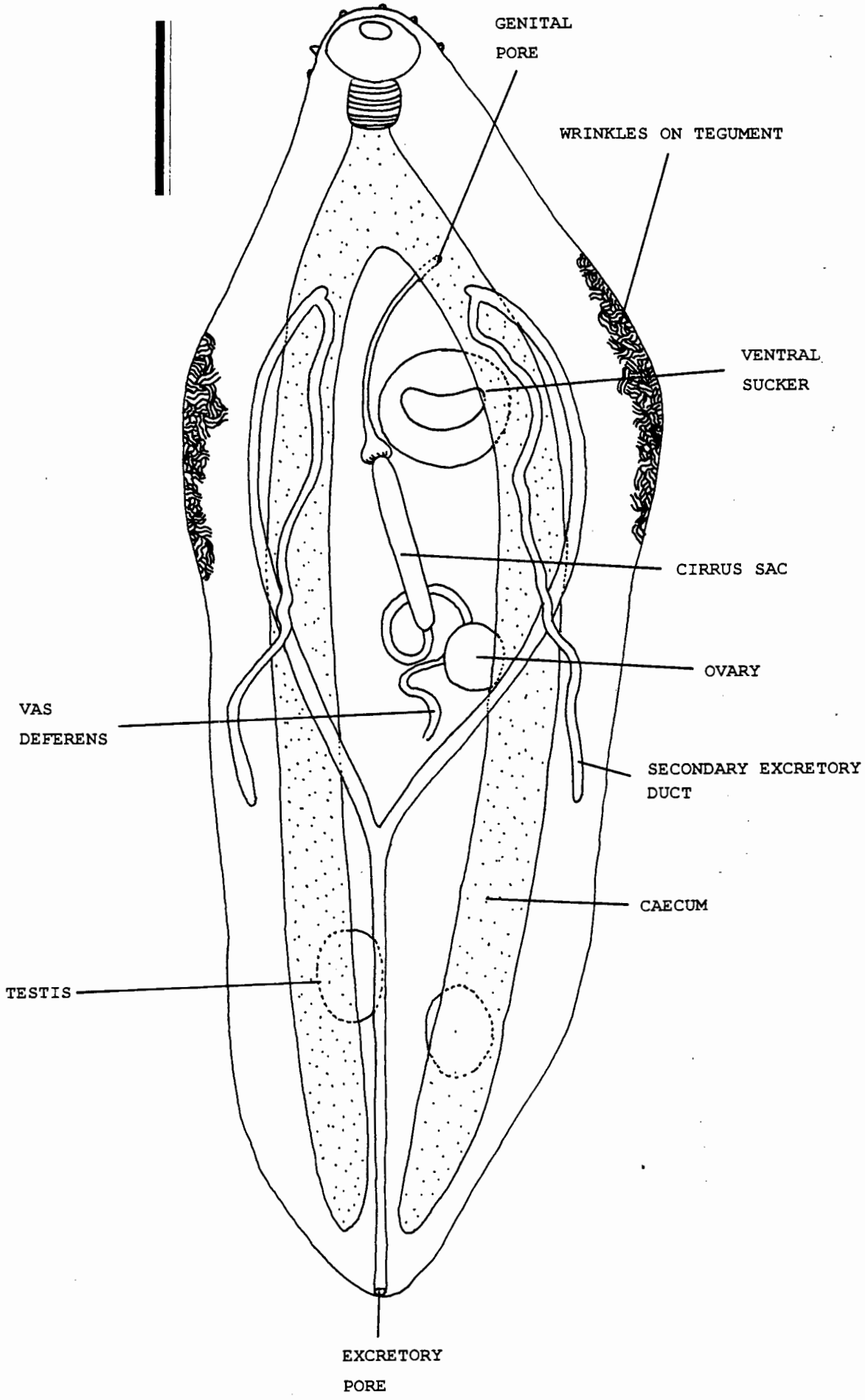


Figure 1. *Metacercaria maculatopsis type I*: ventral view of internal structure, scale bar = 500μm.

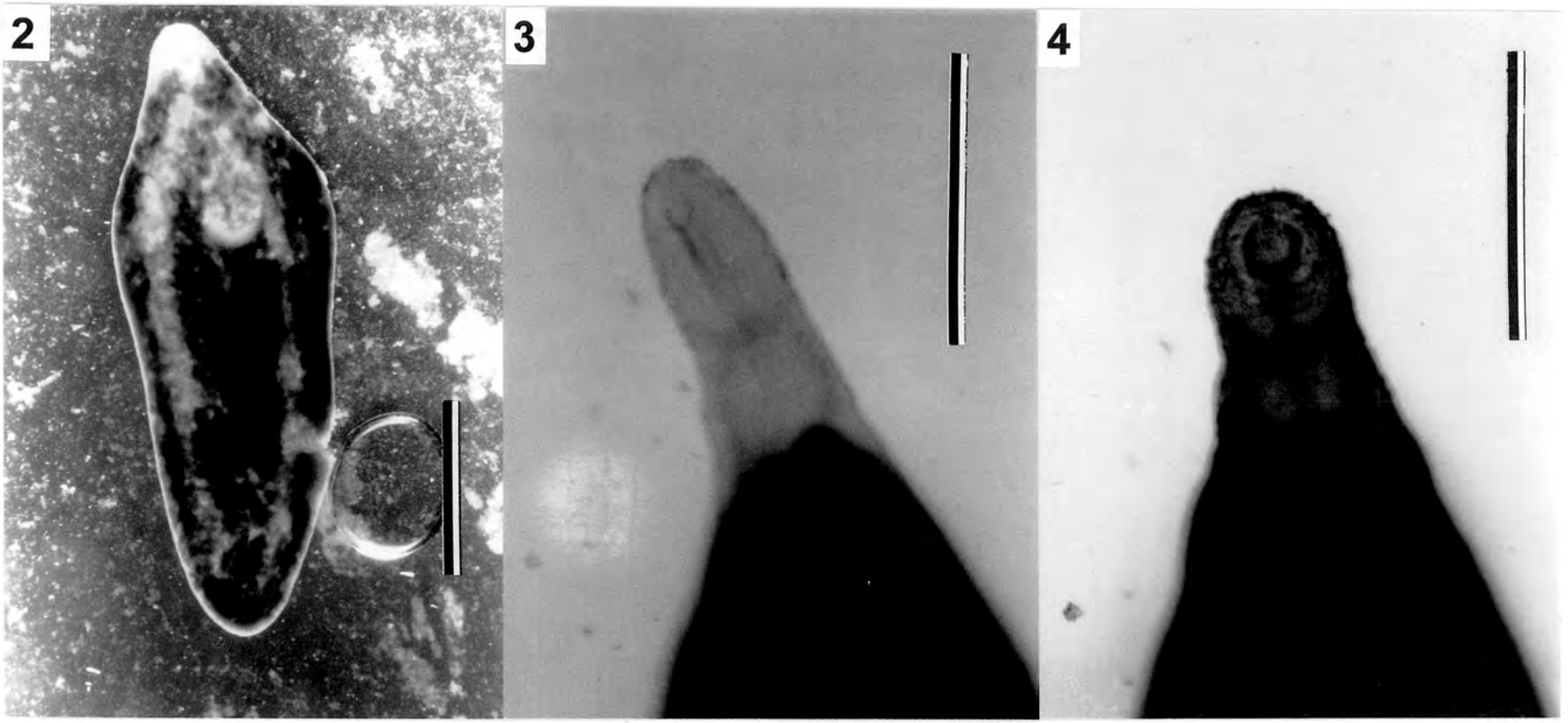


Figure 2. Light micrograph of *Metacercaria maculatopsis type I*, scale bar = 1000μm.
Figure 3. Light micrograph of *Metacercaria maculatopsis type I*, anterior of metacercaria, scale bar = 500μm.
Figure 4. Light micrograph of *Metacercaria maculatopsis type I*, anterior of metacercaria, scale bar = 500μm.

cirrus width	61
testis right length	252
testis right width	162
ovary length	182
ovary width	141

One infected *Choromytilus* was found in a sample of 850 from Dido Valley giving a prevalence of 0.12%. This worm has also been reported (Webb 1985) in *Bullia pura.*

METACERCARIA MACULATOPSIS II

Host and locality

This worm was found in *Choromytilus meridionalis* at Dido Valley.

Type specimens

Syntype series: specimen numbers: A29432 : KII to KIV.

Marine Biology Department, South African Museum, Queen Victoria Street, Cape Town, RSA. Type host: *Choromytilus meridionalis.* Type locality: Dido Valley

Description

No sporocysts or rediae were found in any host. The metacercaria is a large, slightly dorso-ventrally flattened fusiform worm (Figures 5 & 6). The tegument appears smooth but is covered by many small nodules. It shows extensive wrinkling during contraction (Figure 7B shows the pattern of wrinkling). The oral sucker opens sub-terminally (Figure 8). The junction between sucker and pharynx is always open but it is marked by finger-like projections into the lumen (Figure 9). There is no pre-pharynx. Cerebral ganglia lie on either side of the oesophagus at the posterior extremity of the pharynx. The oesophagus is extensible but it is usually very short; the caeca normally appear to emerge from the pharynx; they extend to the extreme posterior close to the excretory pore. The blind ends of the caeca are attached to the posterior by thin fibres running to the tegument. Both the caeca and the oesophagus appear to be lined with columnar cells. The entire digestive tract appears murky and brown. Some five pairs of gland cells, that stain deeply turquoise with Nile-blue sulphate, originate next to the cerebral ganglia and lead forward to the periphery of

the oral sucker opening. The ellipsoid ventral sucker is about twice the diameter of the oral sucker (Figures 5, 8 & 9). The ventral sucker shows considerable powers of mobility. Four groups of gland cells are symmetrically grouped around the ventral sucker (Figure 9); two groups lie anterior to the sucker and two posterior. They stain turquoise with Nile blue sulphate and appear to empty at the opening of the sucker (Figure 8). The sucker opens through a fold in the tegument that is almost the width of the sucker. The cirrus lies on the mid-line in the (Figure 10) body, dorsal to the ventral sucker. A short left, and longer right, vas efferens join to form a convoluted vas deferens which then enters the cirrus pouch. Inside the cirrus pouch the seminal vesicle is also convoluted. The testes lie laterally and staggered; they have the appearance of being composed of bundles of fibres. The ovary is anterior to and smaller than the testes and adjacent to the cirrus on the right hand side of the body. The uterus is coiled and the ascending limb runs from the cirrus to the bladder where it recurves. The descending limb of the uterus leads from the ovary and is packed with eggs. The first dozen or so eggs proximal to the ovary appear about half the size of more distal eggs. The Mehlis gland, vitelline ducts and Laurer's canal are not visible.

The excretory system is stenostomate. The entire excretory vesicle has a dark granular appearance; the granules are 2.4μm or less in diameter. Only the extreme posterior of the bladder (a swelling in the median stem of the vesicle) is clear of granules. This swelling occupies the rear tenth of the body and just anterior to the bladder is a sphincter in the median stem. Anterior to this the vesicle bifurcates (Figure 7) at the level of the testes and each arm proceeds forwards to the mid-point between the ventral and the oral sucker. Here they terminate in a bulge where secondary excretory ducts enter laterally. Cilia lie in the lumen at the junction between the primary and secondary ducts. These cilia beat in a manner similar to flame cells found elsewhere in the body. Secondary ducts run posteriorly and terminate very close to the excretory pore. There are four flame cells on each side of the body.

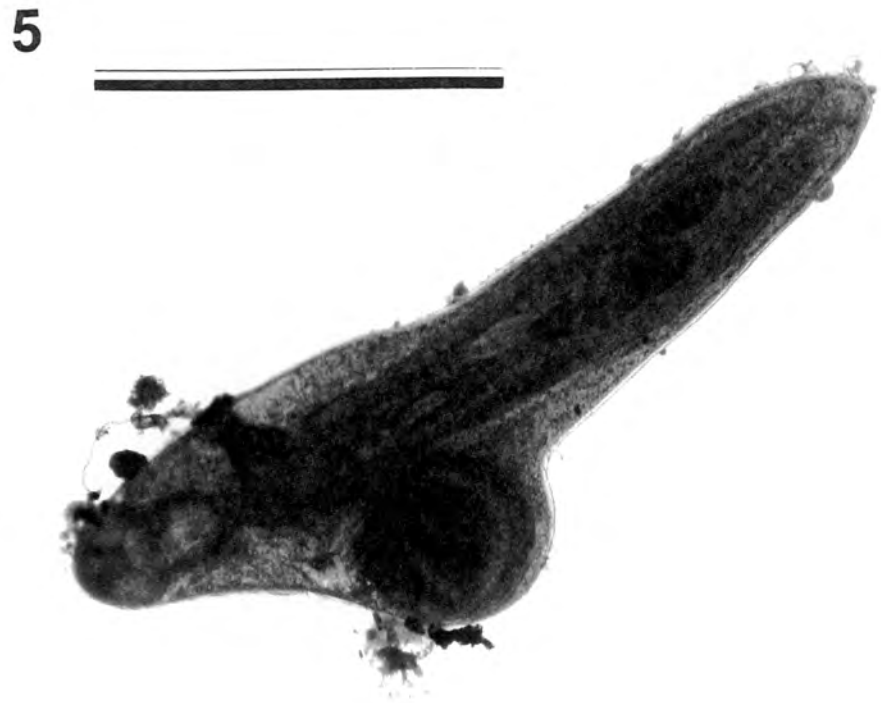


Figure 5. Light micrograph of *Metacercaria maculatopsis type II* (K1), scale bar = 1000m.

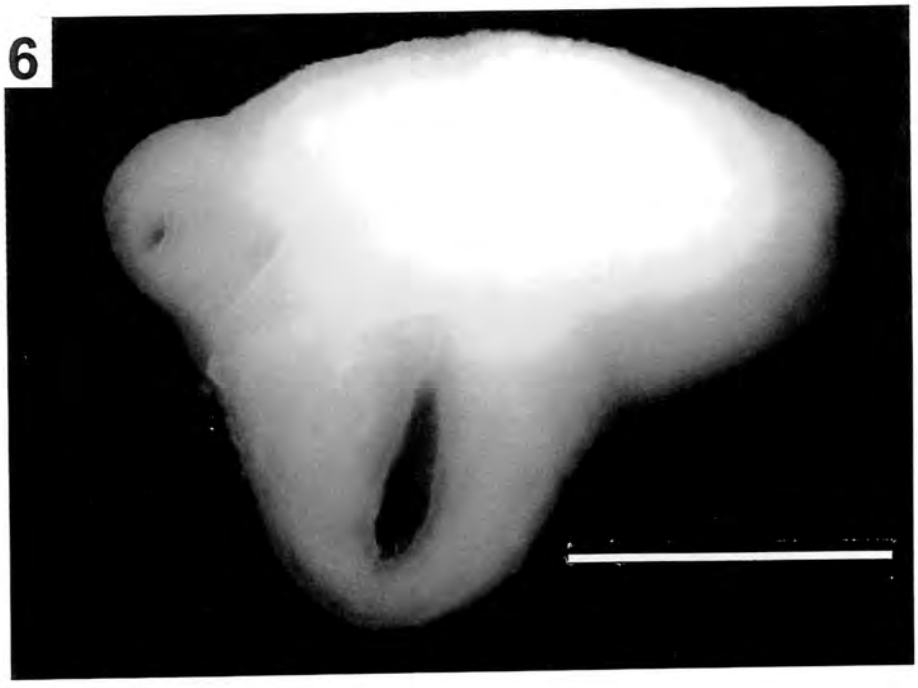


Figure 6. Light micrograph of *Metacercaria maculatopsis type II* (K2), scale bar = 500µm.

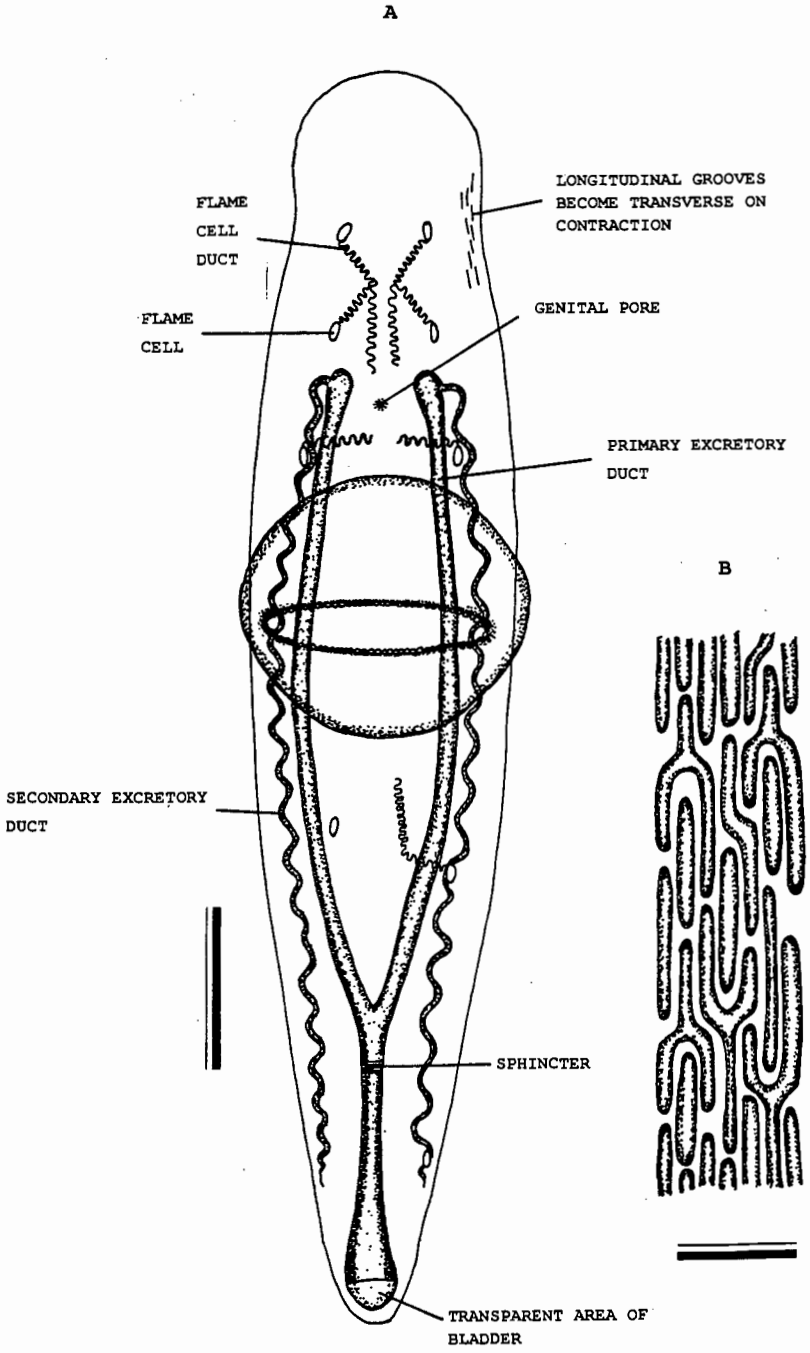


Figure 7. *Metacercaria maculatopsis type II* (K1). A: excretory system, scale bar = 300μm. B: tegument patterns, scale bar = 35μm.

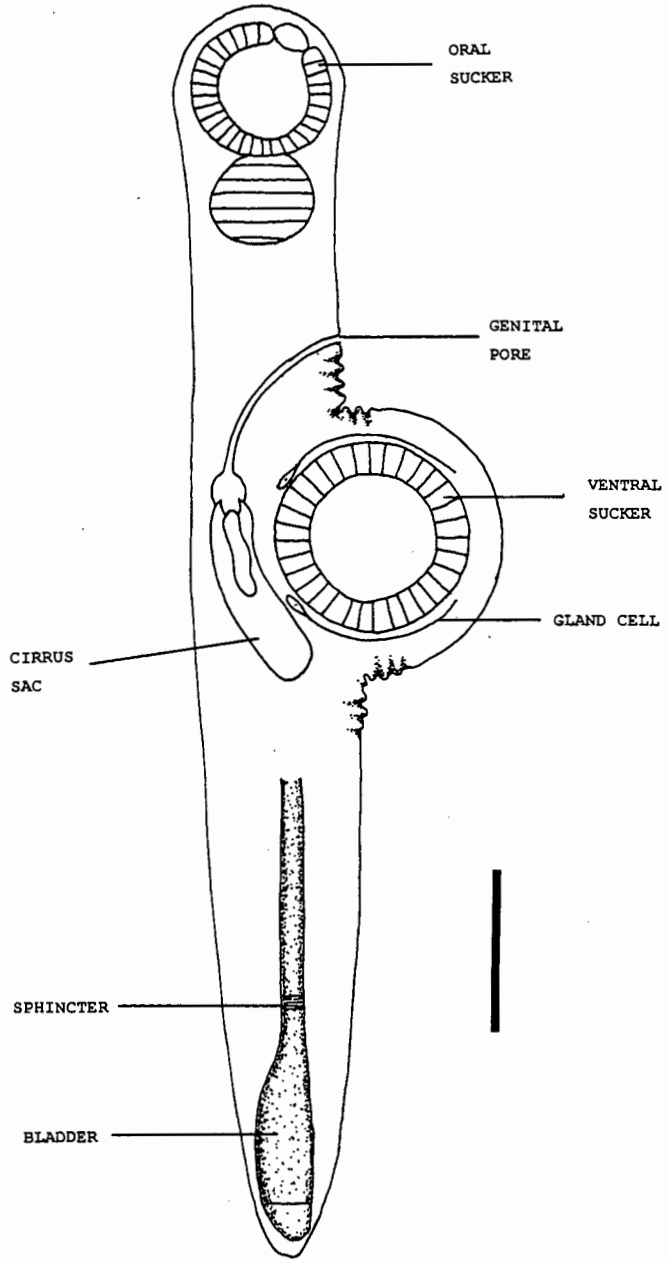


Figure 8. *Metacercaria maculatopsis type II* (K1), side view, scale bar = 300μm.

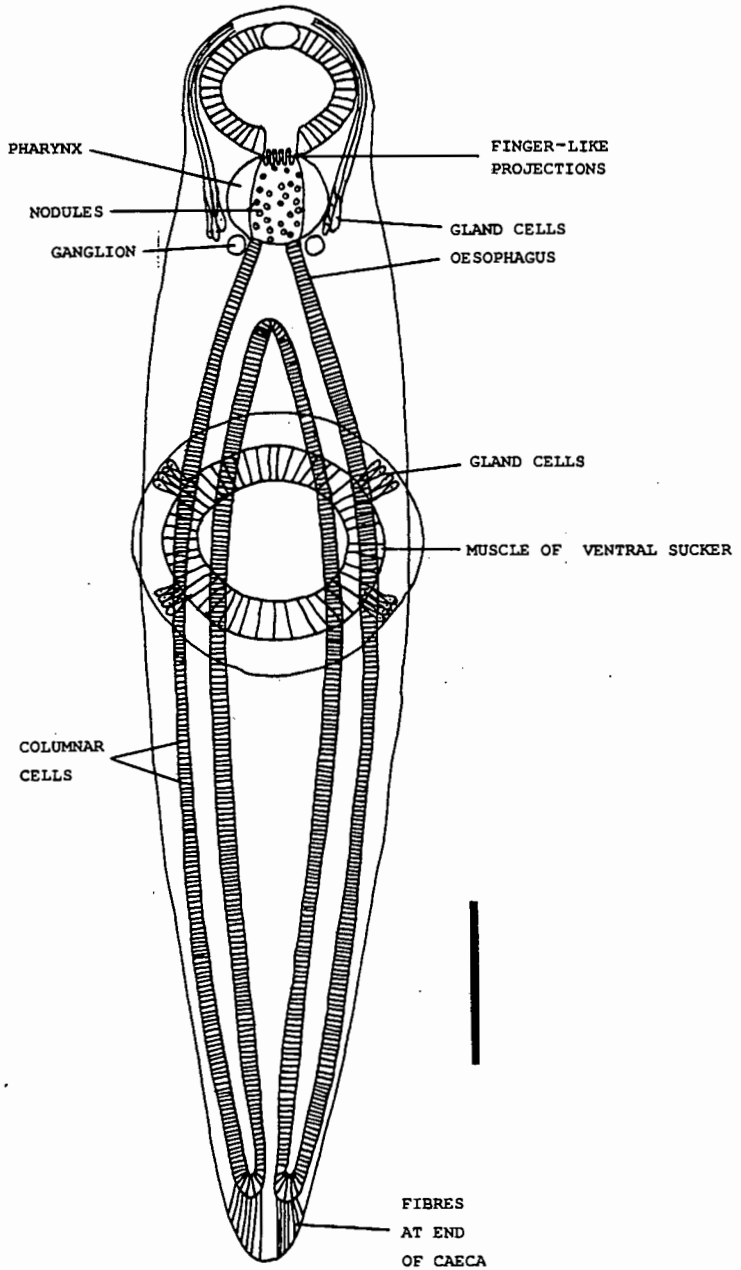


Figure 9. *Metacercaria maculatopsis type II* (K1), details of digestive system and suckers, scale bar = 300µm.

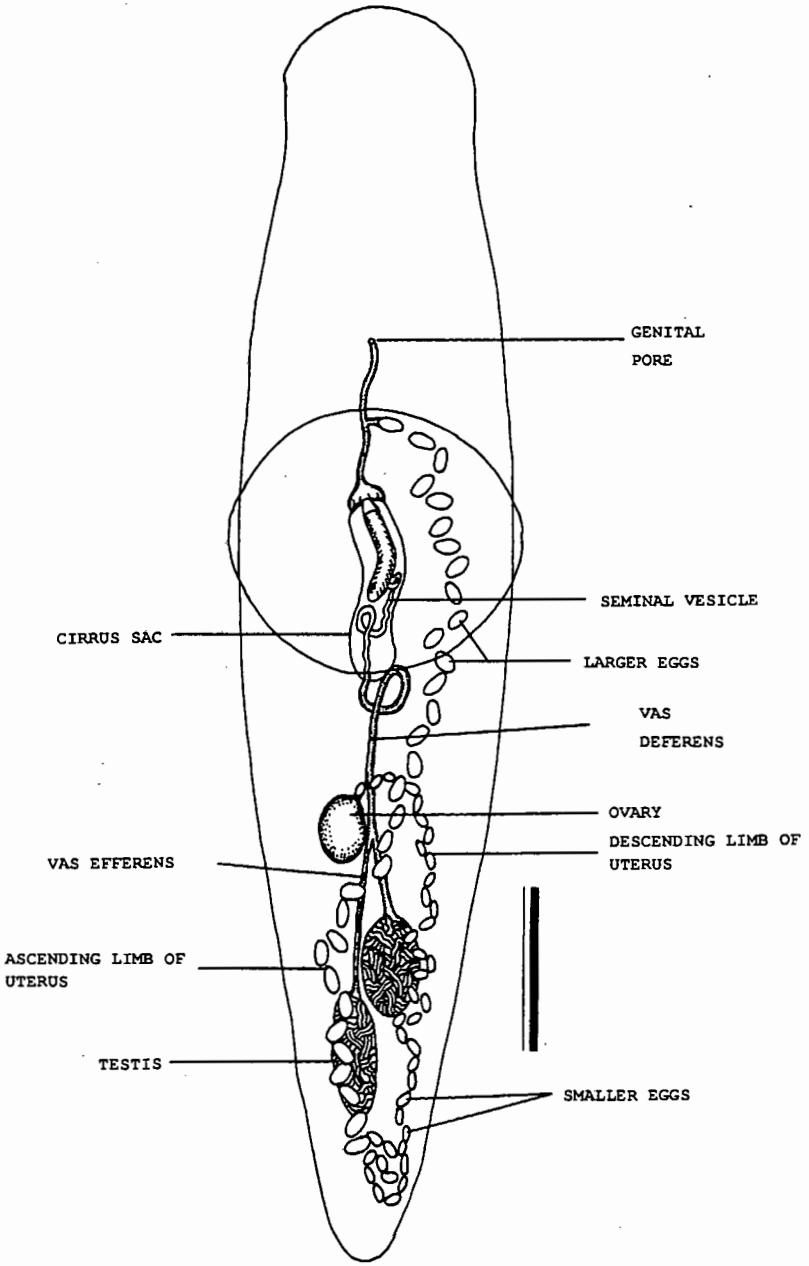


Figure 10. *Metacercaria maculatopsis type II* (K1), details of reproductive system, scale bar = 300µm.

Table 2. *Metacercaria maculatopsis II* measurements (μm); A of living worms, B formalin fixed.

A

	KI	KII	KIII	KIV
length	2187	1175	2200	1680-2036
width 25% from front	194		728	345
width 50% from front	292	975	970	592
width 75% from front	170		728	592
oral sucker length	154		291	316
oral sucker width	154	243	340	295
ventral sucker length	307 - 336	486	485	276
ventral sucker width	346		601	424
thickness of muscle layer	77			
pharynx length	192	272	213	197
pharynx width	115	194	281	167
oesophagus length			116	
oesophagus width			87	
testis front length	223	194	155	
testis front width	264	107	146	
testis rear length		213	194	
testis rear width		233	97	
ovary length	168	97	136	
ovary width	106	146	116	
cirrus length	96	262	243	
cirrus width	24	107	49	
tegument thickness			20	
genital pore diameter		58		
excretory pore diameter		29.4	14.4	
caeca width		97		
papillae height		9.7		
papillae diameter		9.7		
columnar epithelium in caeca length			25	
columnar epithelium in caeca width			10	
eggs: length	55.1			
eggs: width	30.6			
excretory pore diameter			4.4	

B

	KI	KIV
length	2309	3156
width 25% from front	454	739
width 50% from front	496	966
width 75% from front	345	858
oral sucker length	238	285
oral sucker width	281	325
ventral sucker length	367	463
ventral sucker width	410	542
thickness of muscle layer	65	
pharynx length	172	237
pharynx width	184	207
testis front length	194	
testis front width	108	
testis rear length	306	

testis rear width	250	
ovary length	238	
ovary width	86	
egg length	55.1	
egg width	30.6	
cirrus sac length	324	1085
cirrus sac width	86	138
cirrus length	152	
cirrus width	43	

Movement

This worm is very sluggish. Little attempt is made at locomotion. Leech-like movement using the suckers was occasionally seen. The worm is quiescent when adhering to the tissue with its ventral sucker; it is active, bending, stretching and contracting when detached. It shows no propensity to adhere to glass. Neutral-red and Nile-blue sulphate stains are non-toxic.

Epidemiology

This worm was found only at Dido Valley. Below are summarised details of the hosts. *Metacercaria maculatopsis II* specimen KI (Figure 5) was found in a female *Choromytilus* of length 106.75mm. *Metacercaria maculatopsis II* specimen KII (Figure 6) was found in a male *Choromytilus* of length 61.1mm. *Metacercaria maculatopsis II* specimen KIII was found in a male *Choromytilus* of length 60.25mm. *Metacercaria maculatopsis II* specimen KIV was found in one female *Choromytilus*.

A total of 1000 mussels were examined of which four (two males and two females) contained this worm. This gives a prevalence of 0.4%. Only one worm was found in any host; this gives an intensity of 1. In *Burnupena lagenaria* (Lam.) from Dido Valley, one whelk was found infected out of 124 giving a prevalence of 0.81%.

Discussion

Metacercaria maculatopsis has been found in two forms, one larger than the other. They are designated here *Metacercaria maculatopsis I* and *Metacercaria maculatopsis II.* The general plan of *Metacercaria maculatopsis II* is very similar to that of *Metacercaria maculatopsis I,* and it is considered here to be a less mature stage of *Metacercaria maculatopsis I.* The two differ in that the secondary excretory ducts

proceed further to the posterior in *Metacercaria maculatopsis II* and the eggs are different in shape and smaller than those of *Metacercaria maculatopsis I.* These differences could be due to differential growth and development. *Metacercaria maculatopsis I* may be likened, in outline and general habit, to *Proctoeces maculatus* (Looss, 1901) Odhner, 1911; see Lang & Dennis (1976) and Stunkard & Uzmann (1959). It also shares the density of vitellaria exhibited by *Proctoeces ostreae* Fujita, 1925, in Cheng (1967). *Metacercaria maculatopsis II* is smaller than *type I,* colourless and shows considerable resemblance to *Proctoeces* sp. (Calvo-Ugarteburu 1996 Figure 2.14). *Metacercaria maculatopsis I* and *II* differ from *Proctoeces maculatus* in that the main stem of the excretory vesicle is longer in *Metacercaria maculatopsis* and the excretory system of *Proctoeces maculatus* appears to have no recurved secondary excretory ducts (see Wardle 1980). Instead, the flame cell ducts empty into short stubs of secondary excretory ducts. Another contrast is that the excretory system in *Cercaria maculatopsis* appears to be stenostomate: the Y-shaped excretory vesicle collects from two secondary ducts, into which the flame cell ducts flow. *Cercaria maculatopsis* also differs from *Proctoeces* by its vasa efferentia that unite some distance from the base of the cirrus sac. This is in contrast to the vasa efferentia that unite at the cirrus sac, which is a diagnostic feature of the Genus *Proctoeces* (Bray & Gibson 1980). Another difference is that the caeca in *Cercaria maculatopsis* continue to the extreme posterior whereas those in *Proctoeces maculatus* (Bray & Gibson 1980) go only as far as half way beyond the rear testis and the posterior. Thus it can be concluded that while *Cercaria maculatopsis* has much in common with *Proctoeces maculatus,* it is unlikely be of the same genus. Because this worm has close affinity with *Proctoeces maculatus* the name *Cercaria maculatopsis* is proposed in recognition of this resemblance (maculatopsis = looks like maculatus).

Lasiak (1989), Calvo-Ugarteburu (1996) and Calvo-Ugarteburu & McQuaid (1998) report the presence of *Proctoeces* (probably *maculatus*) in *Perna* in collections from Sodwana Bay to Cape Agulhas. It is significant that they found no *Proctoeces maculatus* in the Western Cape collections. This agrees with findings here when over 800 *Perna* were examined, also with no results. *Metacercaria maculatopsis* has been found in *Choromytilus meridionalis, Bullia digitalis, Bullia pura,* and *Burnupena lagenaria.* It is curious that they have not been found in other mussels. The low prevalences of infection may mean that all have them and that they have not been

detected. A larger sample may solve the problem. An alternative way to investigate this would be to find the first intermediate host, which is most probably a gastropod, and conduct infection experiments on a range of gastropods and bivalves.

Metacercaria V (Metacercaria maculatopsis II) & Metacercaria U (Metacercaria maculatopsis I) were found in association in the same individuals of the whelk *Bullia pura* (Webb 1985). *Metacercaria V* was found singly or in groups of two or three; while *Metacercaria U* was found either singly or in pairs. A graduation in size from the smallest *Metacercaria* V to the largest *Metacercaria U* in the same host suggests that they are a development continuum. It is curious that *Metacercaria maculatopsis I & II* were found only at Dido Valley. Several thousand molluscs [*Bullia* spp. examined by Webb (1985) and *Donax serra* examined by Tharme (1988) and the mytilids in this study] have been taken for parasitological examination from collection localities on False bay and on the West coast but large sub-adult to adult flukes have never been seen from West coast sites.

CHAPTER 8: *METACERCARIA ATER* SP. NOV.

A single immature *Aulacomya ater* (length 45.8mm), collected from Blouberg beach was found containing a single metacercaria in its viscera. There are no type specimens.

DESCRIPTION

The worm has a circular transverse section and when extended it often appeared to have a waist. The oral sucker is globular and is almost twice the size of the ventral sucker (Figure 1). The oral sucker opening is sub-terminal, gland cells lie within its musculature and have ducts leading to the mouth. Other gland cells lie outside the sucker at its junction with the pharynx. There is no pre-pharynx; the pharynx is ellipsoid and is about one quarter of the size of the oral sucker. It leads into a concertina-like oesophagus. Midway along the oesophagus is what appears to be a subsidiary pharynx. Aft of this structure, at the junction of the oesophagus with the caeca, are three sphincters, each capable of independent action; they allow any permutation of isolation or communication of the components. The caeca, which are larger in diameter and their walls are thicker than those of the oesophagus, terminate at about 60% of the body length from the front. There is no distinct bladder and the vesicle is V-shaped. Two arms arise directly at the excretory pore and pass forward until they lie adjacent to the oral sucker. The arms are broad and their excretory granules obscure much detail in the body. Because of this, no secondary excretory ducts were seen and only four flame cells could be discerned. Two flame cells occur bilaterally just posterior to the mid-point of the body and the other two occur on the left side next to the oral sucker gland cells. The testes lie laterally behind the ventral sucker. The ovary lies equidistant ahead of the ventral sucker on the left side. The tegument is spinous from the anterior to about the level of the subsidiary pharynx. On the ventral surface (from just behind the junction of the oesophagus and caeca) is a series of three rows, each of four to five large papillae. Between these rows lie smaller papillae arranged in a staggered formation. The last row of large papillae is bordered posteriorly by an infolding of the tegument to form a sucker-like depression. This depression is bounded to the rear by four further rows of four large papillae (Figure 1). It is not known if these are specific features as only one worm was available for examination. The tegument has a similar depression behind the ventral

sucker, but this one is not surrounded by papillae.

Table 1. *Metacercaria ater* measurements (μm) A: living worm, B: moribund.

A

length	245-408
oral sucker length	97-109
oral sucker width	82-139
ventral sucker length	60-39
ventral sucker width	49-61
pharynx length	25-34
pharynx width	23-61
subsidiary pharynx length	9
subsidiary pharynx width	13
cuticular spines length	0.6
cuticular spines pitch	1.3

B

length	449
width from front 25%	184
width from front 50%	245
width from front 75%	204
left testis length	51
left testis width	37
right testis length	52
right testis width	41

Movement

A wave of extension starts at the anterior, when the body becomes longer and thinner. This wave passes posteriorly. The body returns to its original shape after about 40 seconds. The metacercaria was unable to swim or leave the substratum.

DISCUSSION

Taxonomic affinities

After referring to works mentioned above, those of La Rue (1957), Dawes (1946) and Erasmus (1972) were also consulted: no description of this worm was found. It is thus named *Metacercaria ater* (= the metacercaria of *Aulacomya ater*). It is assigned to the Gymnophallidae, Sub-family Gymnophallinae. Its structure suggests affinities with *Lacunovermis conspicuus* Ching, 1965; this is largely because of the ventral pit present in *Lacunovermis conspicuus* and absent in both *Meiogymnophallus multigemmulus* Ching, 1965, and *Gymnophallus somateridae* (Levinsen, 1881).

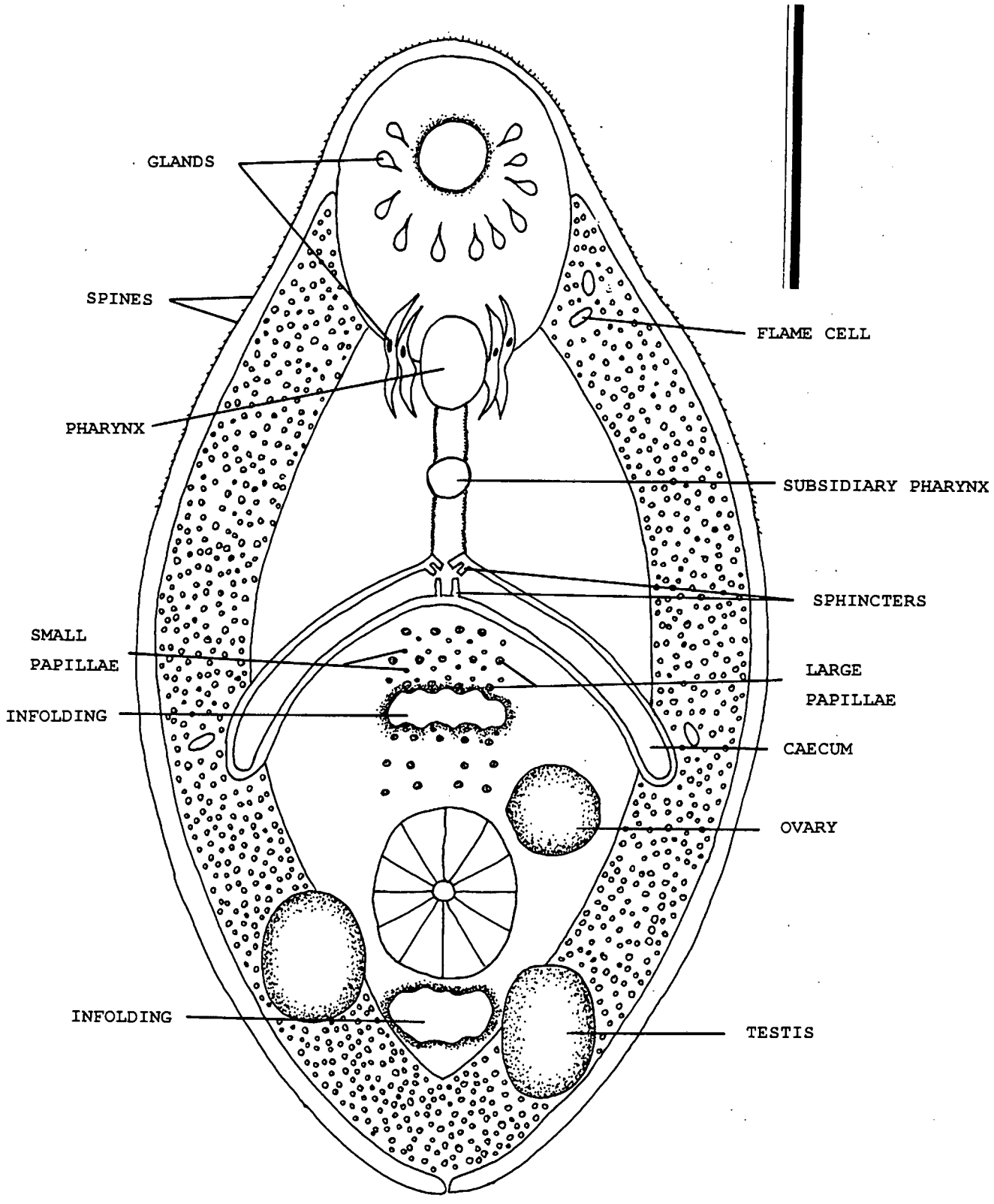


Figure 1. *Metacercaria ater,* scale bar = 100μm.

It should be stressed that with only one specimen, not much detail could be ascertained. The most distinctive feature of the worm at this taxonomic level is the presence of ventral pits. No genital pore was seen so it is inferred that this pore is small and inconspicuous. *Gymnophalloides seoi* Lee, Chai & Hong 1993, in Lee, Chai & Hong (1993) shows considerable similarity. The absence of large papillae on the side of the oral sucker is evidence that, though *Metacercaria ater* is related, it is probably not in the same genus. The subsidiary pharynx of *Metacercaria ater* is also noteworthy. *Metacercaria ater* also has similarities with *Gymnophallus bursicola* Odhner, 1900 in Cheng (1967) but in *Metacercaria ater* the testes lie further back and there is no swelling at the bladder; also in *Metacercaria ater,* the excretory vesicle is V-shaped in contrast to the Y-shaped vesicle of *Gymnophallus bursicola.* See *Metacercaria perchorupis* (Chapter 4) for more details of the gymnophallids.

Epidemiology

Only one worm found in a sample of 800 collected at 50 per month. This suggests that this worm may be more usual in another host. And since it is a gymnophallid, one might expect to find it also in other bivalves. Its absence in the mytilids examined here and also in *Donax serra* examined by Tharme (1988) suggests that its normal host may be one of the lesser bivalve fauna.

CHAPTER 9: *METACERCARIA B* SP. NOV.

HOSTS AND LOCALITIES

Metacercaria B was found in *Perna perna* at Dido Valley and *Choromytilus meridionalis* at Kleinmond, Blouberg, Dido Valley and Cape Columbine.

TYPE SPECIMENS

Paratypes: Specimen number A29433. Marine Biology Department, South African Museum, Queen Victoria Street, Cape Town, RSA. Type host: *Choromytilus meridionalis*. Type locality: Blouberg.

DESCRIPTION

The cyst is spheroid and is most commonly found in the palps. There is no obvious effect on the host except for some mild local compression of tissue (Figures 1, 2 & 3). The cyst has a thin eosinophilic wall with (Figures 1, 2 & 3) no distinct layers. The wall is flexible and may be considerably distorted under cover slip pressure. Inside the cyst the metacercaria is very active and it releases excretory granules from its bladder into the cyst. The metacercaria (Figure 4) was removed by gently rolling the cyst between microscope slide and cover slip until the cyst wall fatigued and released the entire worm. In the unfixed specimen the inner layer of the wall is a very elastic membrane of about 4µm thickness. Once released from the cyst the worm becomes non-motile but shows no evidence of damage. *Metacercaria B* is flattened and elongated. Numerous pink (unstained) bodies each about 5µm long are dotted throughout the body. Its entire tegument is covered with a staggered diamond pattern of spines. Each spine is broader than long, flattened and shield-shaped. The tegument also bears papillae in rows, each row being about 8µm apart. These cover the tegument from the anterior back to the level of the ventral sucker. This sucker is pear-shaped with the opening at the small end. The oral sucker is comparable in size with the ventral sucker. Two pairs of glands open at the mouth of the oral sucker. Cell bodies of these glands lie about half way between the oral sucker and the pharynx. The oral sucker is elongated and opens sub-terminally. Immediately behind the oral sucker on either side of the pre-pharynx lie the cerebral ganglia. The pre-pharynx is very variable in length; so also is the oesophagus which varies from one to two lengths of the pharynx and branches to give two caeca, each of which proceed along

the lateral margin of the body to the extreme posterior. The bladder is an irregular sac; its multiple layered wall is thin and difficult to see. The excretory granules (Figure 4) are much more visible and it is these that indicate the extent and position of the bladder. The single main stem to the bladder branches into primary ducts just anterior to the ventral sucker; they terminate level with the posterior of the oral sucker. Secondary ducts arise at the ends of the primary ducts. The secondary ducts run posteriorly to level with the bladder. No flame cell formula can be suggested as out of all the worms examined only a single flame cell was seen in one specimen.

Two testes, each about 45μm long were seen next to and partially obscured by the bladder. They are staggered; the anterior is on the left. An ovary of about half the diameter of the ventral sucker lies about 60μm behind and to the right of the ventral sucker. Between the ventral sucker and the ovary lies the cirrus sac (60μm long) containing a cirrus (25μm long). From the cirrus sac leads a long genital atrium whose end closest to the genital pore was not visible.

Table 1. Measurements (μm) of *Metacercaria B*: A alive and B (Formalin fixed).
A

	mean	SD	*n*	max.	min.
cyst wall thickness	9.6	3.8	10	15.9	3.7
external diameter of cyst	285.2	126.3	10	625	189
length	981.4	545.1	5	1750	405
width 25% from front	127.6	46	10	220.5	85.8
width 50% from front	164.4	59.4	10	294	98
width 75% from front	206.5	64.4	10	306	125
oral sucker length	95.7	38.6	10	184	54
oral sucker width	59.9	15.5	10	98	42
oral sucker depth	47	8.37	10	64	32
ventral sucker length	69.4	18.8	10	98	50
ventral sucker width	66.9	14.3	10	103	54
ventral sucker depth	61.6	15.4	10	78.4	37
pre-pharynx length	133.8	64.9	10	245	41.7
pharynx length	66.6	23.5	10	110	49
pharynx width	40.5	12.9	10	67.9	19.6
bladder length	146.8	64.2	10	269.5	74
bladder width	90.3	24.7	10	129.9	46.6
granules in bladder	6.1	2.2	10	8.6	2.45
tegument papillae length	9.8	2.12	11	12.25	6.13
tegument papillae width	2.16	0.53	10	2.94	1.23
tegument papillae pitch	7.9	4.29	11	4.7	2.45
tegument spines length	3.55	0.76	10	5	2.5
ovary length	30		1		
ovary width	25		1		

B

	mean	SD	*n*	max.	min.
cyst wall thickness	5.15	1.49	10	7.35	2.45
external diameter of cyst	251.1	34.7	10	318.5	203.4
width 25% from front	182.5	37.3	10	233	122.5
width 50% from front	206.4	40.6	8	250	134.8
width 75% from front	212.9	56.9	7	294	125
oral sucker length	119.4	19.5	10	160	98
oral sucker width	79.1	9.7	10	100	68.6
oral sucker depth	58.3	10.1	10	71.1	36.8
ventral sucker length	75.5	14	10	98	66.2
ventral sucker width	83.1	8.4	10	98	73.5
ventral sucker depth	72.1	6.4	10	83.3	61.3
pre-pharynx length	67.8	40	10	122	12.3
pharynx length	76.2	8.3	10	86	61.3
pharynx width	34.9	7.1	10	42.9	19.6
bladder length	171.6	85.3	10	318.5	85.8
bladder width	141.2	37.9	10	215	90.7
granules in bladder	3.9	1.7	10	7.35	2.45
tegument spines length	1.51	0.42	10	2.2	1
tegument spines width	1.96	0.44	10	2.7	1.4
tegument spines pitch	2.14	0.53	10	2.94	1.17

EPIDEMIOLOGY

Prevalences

Table 2A. Prevalences of *Metacercaria B* in *Choromytilus meridionalis* from Blouberg.

	infected	sample no.	prevalence
males	123	315	39.05%
females	134	277	48.38%
unidentified	2	8	25%
total	259	600	43.17%

Table 2B. Prevalences of *Metacercaria B* in *Choromytilus meridionalis* from Dido Valley.

	infected	sample no.	prevalence
males	51	280	18.21%
females	58	311	18.65%
unidentified	1	10	10%
total	110	601	18.3%

Table 2C. Prevalences of *Metacercaria B* in *Perna perna* from Dido Valley.

	infected	sample no.	prevalence
males	11	356	3.08%
females	10	231	4.33%
hermaphrodites	0	7	0%
unidentified	0	5	0%
total	21	599	3.51%

Mean intensities

Table 3A. Mean intensities of *Metacercaria B* in *Choromytilus meridionalis* from Blouberg.

	no. of cysts	sample *n*	intensity	SD	SE
males	217	123	1.76	1.66	0.15
females	246	134	1.84	2.23	0.19
unidentified	2	2	1	---	---
total	465	259	1.8	1.97	0.12

Table 3B. Mean intensities of *Metacercaria B* in *Choromytilus meridionalis* from Dido Valley.

	no. of cysts	sample *n*	intensity	SD	SE
males	99	51	1.94	2.64	0.37
females	94	58	1.62	1.99	0.26
unidentified	2	10	0.2	0	0
total	195	118	1.65	2.316	0.21

Table 3C. Mean intensities of *Metacercaria B* in *Perna perna* from Dido Valley.

	no. of cysts	sample *n*	intensity	SD	SE
males	16	11	1.45	1.437	0.43
females	12	10	1.20	0.4	0.13
total	28	21	1.33	1.08	0.05

Abundances

Table 4A. Abundances of *Metacercaria B* in *Choromytilus meridionalis* from Blouberg.

	no. of cysts	sample *n*	abundance	SD
males	217	315	0.69	1.35
females	246	277	0.89	1.8
unidentified	2	8	0.13	---
total	465	600	0.78	1.57

Table 4B. Abundances of *Metacercaria B* in *Choromytilus meridionalis* from Dido Valley.

	no. of cysts	sample *n*	abundance	SD
males	99	280	0.35	1.35
females	94	311	0.30	1.07
unidentified	1	10	0.1	0
total	194	601	0.32	1.20

Table 4C. Abundances of *Metacercaria B* in *Perna perna* from Dido Valley.

	no. of cysts	sample *n*	abundance	SD
males	16	356	0.05	0.36
females	12	231	0.05	0.26
hermaphrodites	0	7	0	---
unidentified	0	5	0	---
total	28	598	0.05	0.32

Other epidemiological values

Table 5. Prevalences, abundances and intensities of *Metacercaria B* in *Choromytilus meridionalis* from Dido Valley.

	males	SD	SE	females	SD	SE
prevalence						
palps	46 in 280 = 16.43%			57 in 311 = 18.33%		
mantle	9 in 280 = 3.21%			1 in 311 = 0.32%		
mean intensity						
palps	1.89	2.2	0.32	1.89	2.5	0.33
mantle	1.33	0.47	0.16	2	---	
abundance						
palps	0.31	1.23	0.07	0.3	1.07	0.06
mantle	0.04	0.25	0.01	0.006	0.11	0.006

Table 6. Prevalences of *Metacercaria B* in *Choromytilus meridionalis* from Kleinmond.

no. infected	sample no.	prevalence
16	19	84.2%

Epidemiological figures

For size dependent prevalence of infections of *Metacercaria B* in female and male *Choromytilus* from Blouberg see Figures 5 & 6; see Figures 7 & 8 for corresponding results from Dido Valley. For size dependent prevalence of infections of *Metacercaria B* in female and male *Perna* from Dido Valley see Figures 9 & 10. For size dependent prevalence of infections of *Metacercaria B* in palps and mantle of female and male *Choromytilus* from Dido Valley see Figures 11 & 12. For monthly variation in prevalence of infections of *Metacercaria B* in female and male *Choromytilus* from Blouberg see Figures 13 & 14. For monthly variation in prevalence of infections of *Metacercaria B* in female and male *Choromytilus meridionalis* from Dido Valley see Figures 15 & 16. For monthly variation in prevalence of infections of *Metacercaria B* in female and male *Perna perna* from Dido Valley see Figures 17 & 18. For size dependent mean intensity of infections of *Metacercaria B* in female and male *Choromytilus* from Blouberg see Figure 19. For size dependent mean intensity of infections of *Metacercaria B* in female and male *Choromytilus* from Dido Valley see Figure 20. For size dependent mean intensity of infections of *Metacercaria B* in female and male *Perna* from Dido Valley see

Figure 21. For monthly variation in mean intensity of infections *Metacercaria B* in female and male *Choromytilus* from Blouberg see Figure 22. For monthly variation in mean intensity of infections of *Metacercaria B* in female and male *Choromytilus* from Dido Valley see Figure 23. For monthly variation in mean intensity and abundance of infections of *Metacercaria B* in female and male *Perna* from Dido Valley see Figure 24.

DISCUSSION

Taxonomic affinities and etymology

This metacercaria possesses features of two families, the Psilostomatidae and the Lepocreadidae, both of which are reported (Lauckner 1983) to have bivalve hosts. According to Erasmus (1972), however, the Lepocreadidae typically have arthropod intermediate hosts. And Cheng (1967) says of *Lepocreadium album* that it can encyst in *Aplysia punctata* - a nudibranch, *Tapes decussatus* and *Tapes aureus*. The cyst of *Lepocreadium album* differs from *Metacercaria B* in that the wall and gelatinous part of the cyst is thicker than in *Metacercaria B.* The lepocreadid genera *Lepidapedon, Opechonis* and *Stephanostomum* appear to be the most likely relatives of *Metacercaria B. Metacercaria B* has features common with these such as the great length of the pre-pharynx and the caeca that reach to the extreme posterior. Erasmus (1972) reports that lepocreadids have a typically mesostomate excretory system, and Dawes (1946) states that they have a spinous cuticle. In *Metacercaria B* the cuticle is papillate and spinous. Lepocreadids, as shown in Dawes (1946), are elongated worms that have a long pre-pharynx and long caeca that reach to the to end of the body. *Lepocreadium album* Stossich, 1890 has a long excretory vesicle that reaches to the ventral sucker. The cysts have a diameter of 300µm, and the metacercaria has eyespots. The following, all in Dawes (1946) are worthy of comparison: *Lepidapedon rachion* (Cobbold, 1858) Stafford, 1904, *Lepidapedon elongatum* (Lebour, 1908) Nicoll, 1915, *Opechona bacillaris* (Molin, 1859). Two other cercaria to be compared are in Cable (1963): *Cercaria caribbea LXV* Cable, 1963, and *Cercaria caribbea LXVI* Cable, 1963. Of all these, *Metacercaria B* most resembles *Lepocreadium album.*

Metacercaria B also has some affinities with Psilostomatids (See Loos-Frank 1968

and Dawes 1946). In the Family Psilostomatidae Odhner 1911 emend. Nicoll 1935, the excretory system forms a network of vessels (Dawes 1946). The tegument is smooth, the cirrus pouch curves around the ventral sucker. This is not so in *Metacercaria B*. The oesophagus and pre-pharynx in psilostomatids are both short. In *Psilostomum brevicolle* (Creplin, 1829) the cyst wall is thin as in *Metacercaria B* and the caeca go to the end of the body. The pre-pharynx is shorter than the oesophagus, unlike *Metacercaria B*. The adult worm has almost no pre-pharynx and the testes are much larger and closer to the mid-point of the body than in *Metacercaria B*. The excretory system of *Psilostomum brevicolle* is diffuse and net-like, this contrasts with that of *Metacercaria B*.

Metacercaria B is similar to *Cercaria misa* Komiya, 1951, (See Ito 1964) but it differs in that *Cercaria misa* has eyespots and far more flame cells: 2[(6+7+8)+(8+8+8)]=90 or 2[(6+8+8)+(8+8+8)]=92 arranged in a stenostomate excretory system typical of psilostomatids (Erasmus 1972). It also has a spinous armament around suckers that is absent in *Metacercaria B*. *Metacercaria B* has a thin-walled cyst as does (Lauckner 1983) *Psilostomum brevicolle* (Creplin, 1829). Likewise, its excretory bladder is full of granules and is localised to the extreme posterior. Loos-Frank (1968) shows that, in the cercariae, there are glands around the oral sucker of *Psilostomum brevicolle* that are similar to those in *Metacercaria B*. However, in contrast to *Metacercaria B* the pre-pharynx is short (Dawes 1946) in *Psilostomum brevicolle*. *Metacercaria B* is morphologically more alike *Lepocreadium album* than other lepocreadids and certainly more than the psilostomatids. Until the adult stage is obtained it is concluded that this metacercaria is of lepocreadid affinity.

Epidemiology

Prevalences

Prevalences (Table 2) decline from 43.17% (males 39.05%; females 48.38%) in *Choromytilus* at Blouberg, to 18.3% (males 18.21%; females 18.65%) in *Choromytilus* at Dido Valley to 3.51% (males 3.08%; females 4.33%) in *Perna* at Dido Valley. Thus the females in all three populations show a slightly higher prevalence.

Prevalences in palps and mantle

Metacercaria B was found only in the palps of *Choromytilus* from Blouberg and *Perna* from at Dido Valley. *Choromytilus* at Dido Valley had infections also in the mantle. Prevalences of *Metacercaria B* in *Choromytilus* at Dido Valley were 16.43% in the palps and 3.21% in the mantle for males, and 18.33% in the palps and 0.32% in the mantle for females (Table 5).

Size dependent prevalences

Female *Choromytilus* from Blouberg (Figure 5) show a trend of prevalence increase with size except for the first (30mm) data point. This point, however, may be given less weight than the others because its sample number is lower. Male *Choromytilus* from Blouberg also show (Figure 6) a trend of increase in prevalence with size, as do both sexes of *Choromytilus* from Dido Valley (Figures 7 & 8). Female *Perna* from Dido Valley females show (Figure 9) a trend of increase in size dependent prevalence but in males (Figure 10) it is not obvious.

Size dependent prevalences in palps and mantle

Only in *Choromytilus* from Dido Valley was there an infection in the mantle. In females, only one such infection was noted (Figure 11). In males, prevalence in the mantle increases with host size but at a lesser rate than in the palps (Figure 12).

Seasonal variation in prevalence

No apparent seasonality of prevalence was discernible in any of the samples.

Mean intensities of infection

Choromytilus males (Table 3A) from Blouberg, had a mean infection intensity of 1.76 (SE 0.15) and females had a mean intensity of 1.84 (SE 0.19). At Dido Valley, *Choromytilus* males had a (Table 3B) mean infection intensity of 1.94 (SE 0.37) and females had a mean intensity of 1.62 (SE 0.26). At Dido Valley, *Perna* males had a mean (Table 3C) infection intensity of 1.45 (SE 0.43) and females had a mean intensity of 1.2 (SE 0.13). The standard errors suggest that no significant difference exists between the sexes.

Abundances

Perna from Dido Valley had an abundance of 0.05. *Choromytilus* from Blouberg had an abundance of 0.78, which is 16.5 times higher than in *Perna*. *Choromytilus* from Dido Valley had an abundance of 0.32, which is 6.9 times higher than in *Perna*.

Mean intensities in palps and mantle

Choromytilus males at Dido Valley, had mean intensities (Table 5) of 1.89 (SE 0.32) in the palp and 1.33 (SE 0.16) in the mantle. In females these were 1.89 (SE 0.33) and 2(SE ---) respectively. The apparent anomaly of female intensity being higher here than in the previous part dealing with intensity for each sex occurs because the mean intensity in the palp is calculated as the mean number of cysts in mussels that have cysts in the specified part.

Size dependent intensity

Choromytilus from Blouberg show an upward trend in males and females (Figure 19). *Choromytilus* from Dido Valley females show no tendency but there is a trend upwards in males (Figure 20). Mixed results were also obtained from *Perna* at Dido Valley where females show a possible upward trend (Figure 21) but males show no trend.

Seasonal variation in intensity

In Figures (22, 23 & 24) the error bars indicate no significant change of intensity with season, or between the sexes in *Choromytilus* from Blouberg and Dido Valley and *Perna* from Dido Valley. This metacercaria has also been reported from *Perna* by Calvo-Ugarteburu (1996 Figure 2.6) who reports prevalences of 18% to 100% from different localities. In contrast to results here, she reports that prevalence is seasonal with the higher figures in winter from over 70% in winter, to just over 40% in summer.

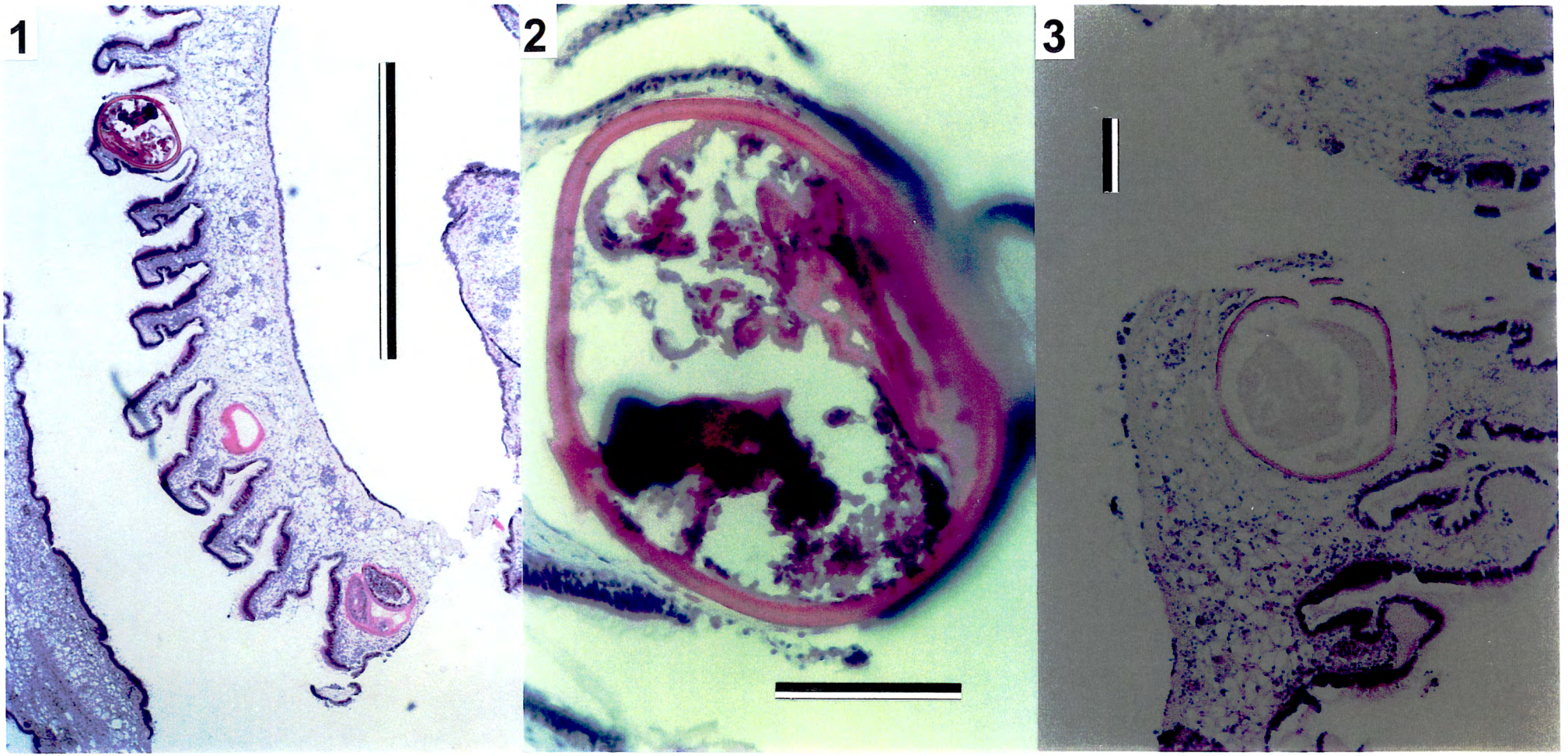


Figure 1. *Metacercaria B* in palp, scale bar = 1000μm.
Figure 2. *Metacercaria B* in palp showing eosinic cyst wall,scale bar = 100μm.
Figure 3. *Metacercaria B* in palp showing metacercaria and cyst wall, scale bar = 100μm.

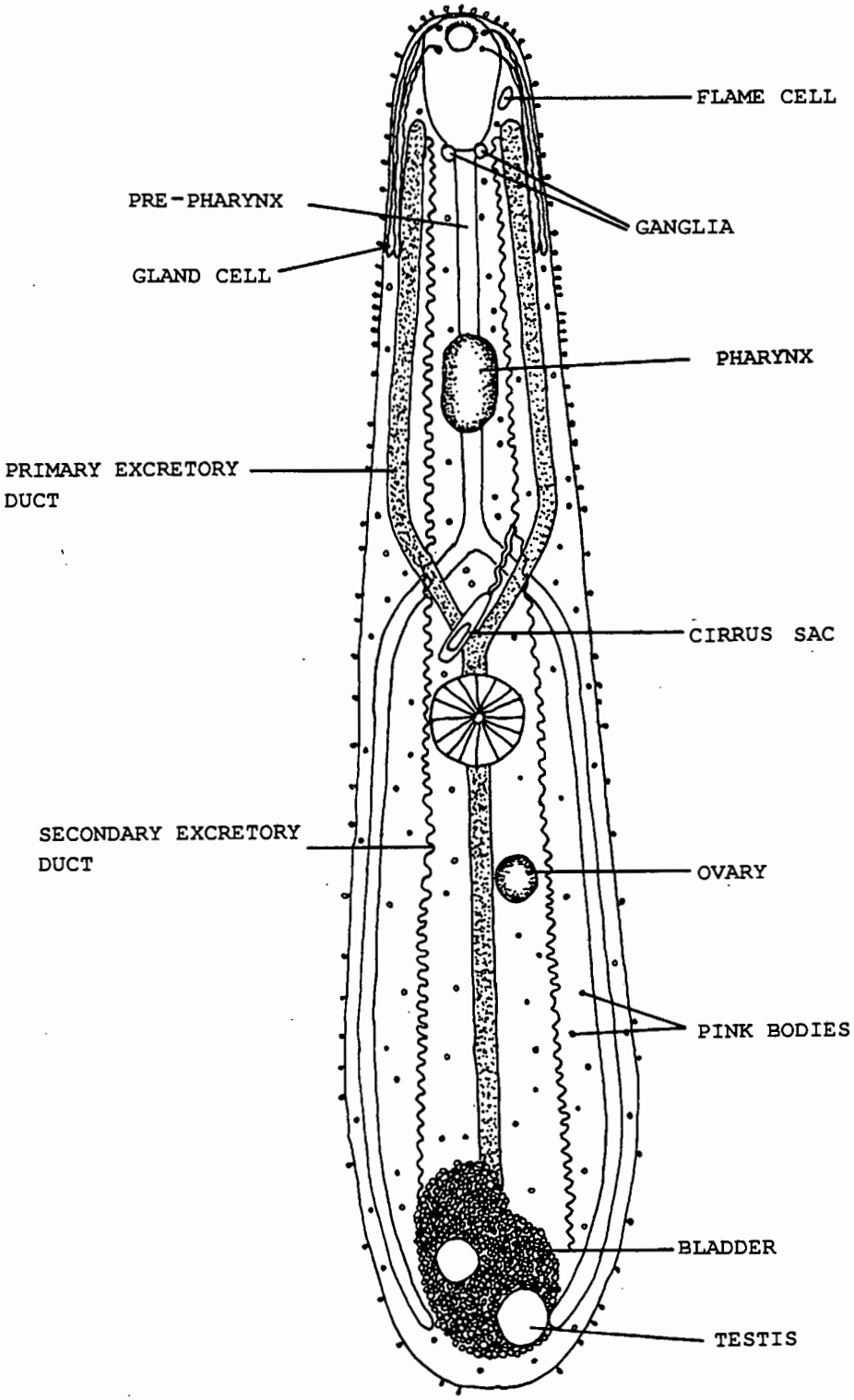


Figure 4. Excysted *Metacercaria B* top view; scale bar = 200μm.

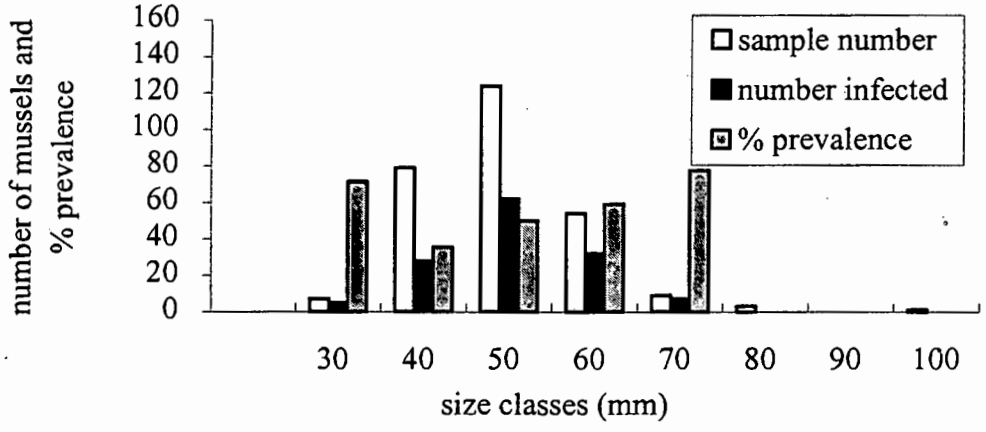


Figure 5. Size dependent prevalence of *Metacercaria B* in female *Choromytilus* from Blouberg.

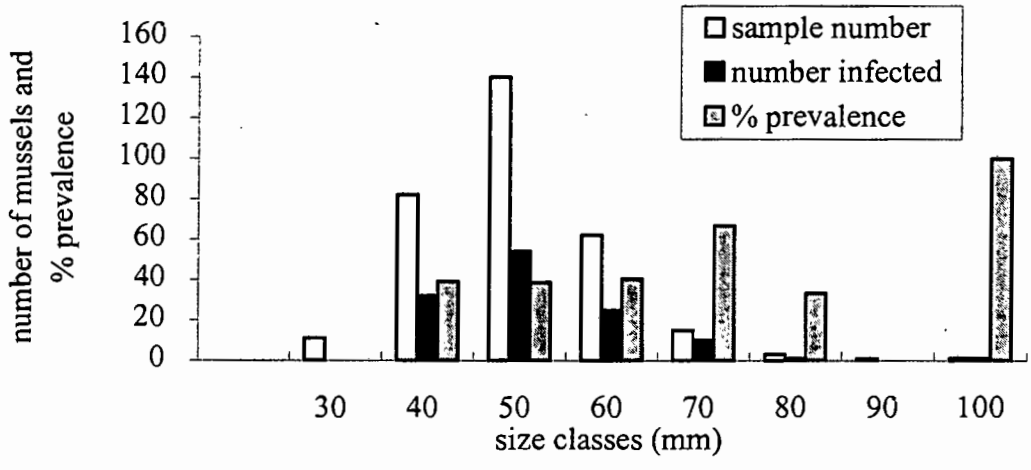


Figure 6. Size dependent prevalence of *Metacercaria B* in male *Choromytilus* from Blouberg.

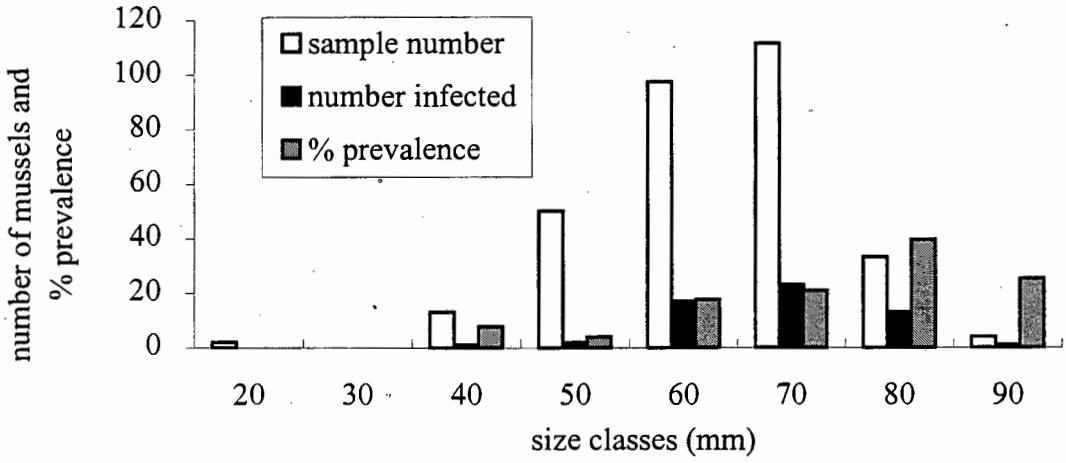


Figure 7. Size dependent prevalence of *Metacercaria B* in female *Choromytilus* from Dido Valley.

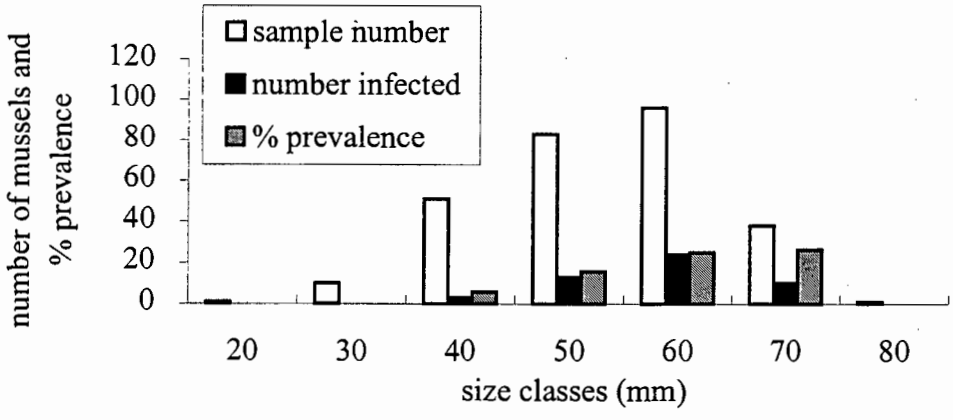


Figure 8. Size dependent prevalence of *Metacercaria B* in male *Choromytilus* from Dido Valley.

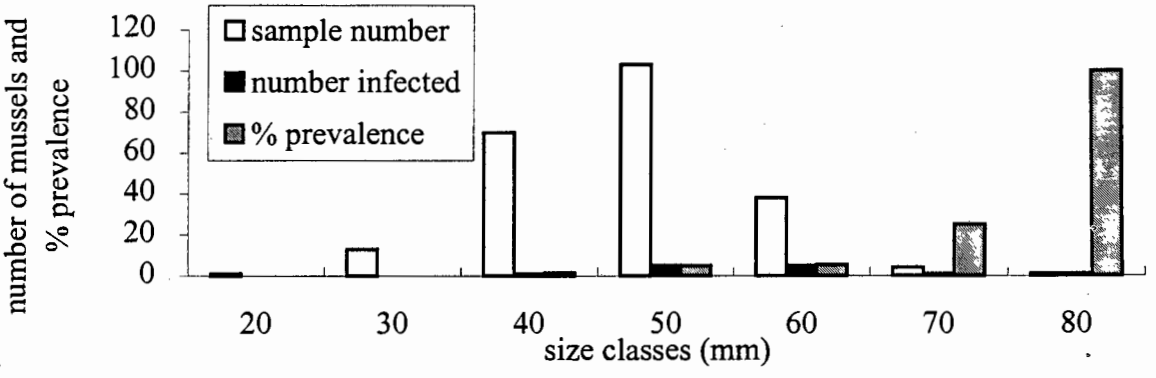


Figure 9. Size dependent prevalence of *Metacercaria B* in female *Perna* from Dido Valley.

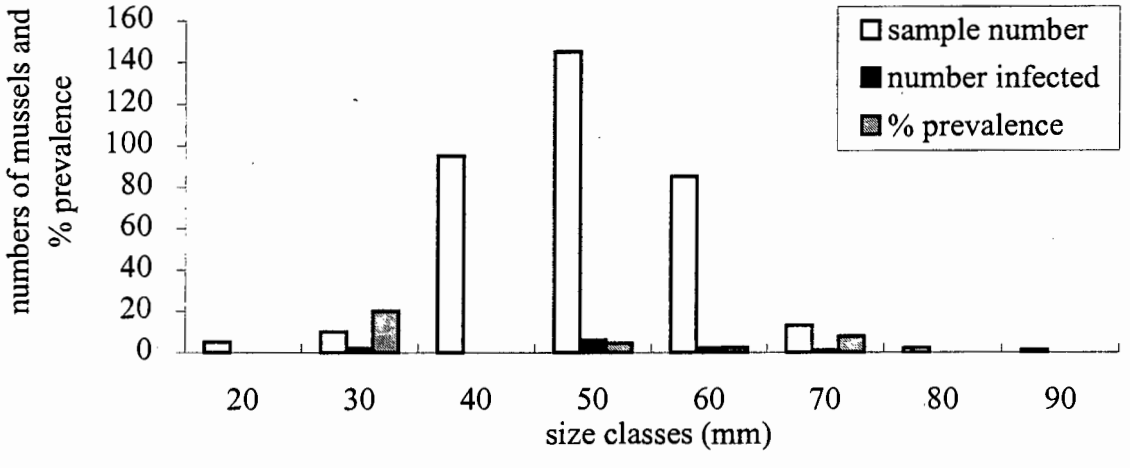


Figure 10. Size dependent prevalence of *Metacercaria B* in male *Perna* from Dido Valley.

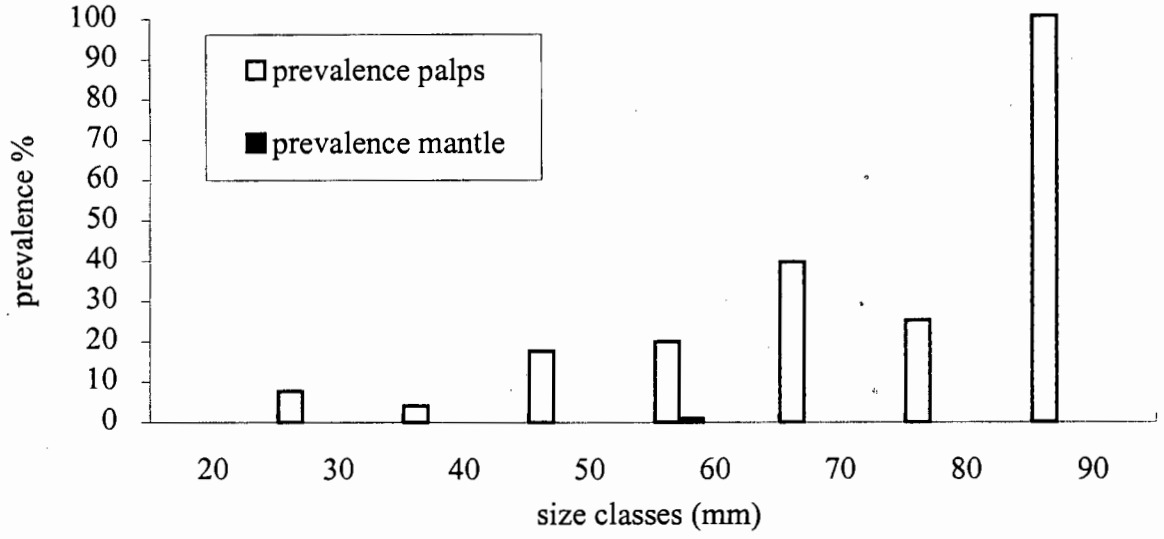


Figure 11. Size dependent prevalence of *Metacercaria B* in palps and mantle of female *Choromytilus* from Dido Valley.

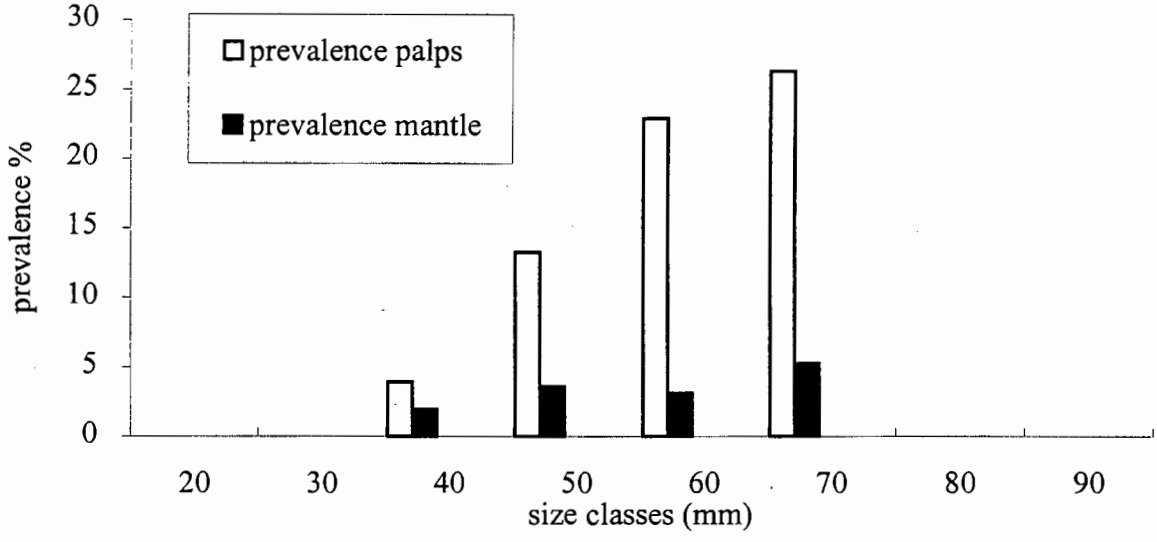


Figure 12. Size dependent prevalence of *Metacercaria B* in palps and mantle of male *Choromytilus* from Dido Valley.

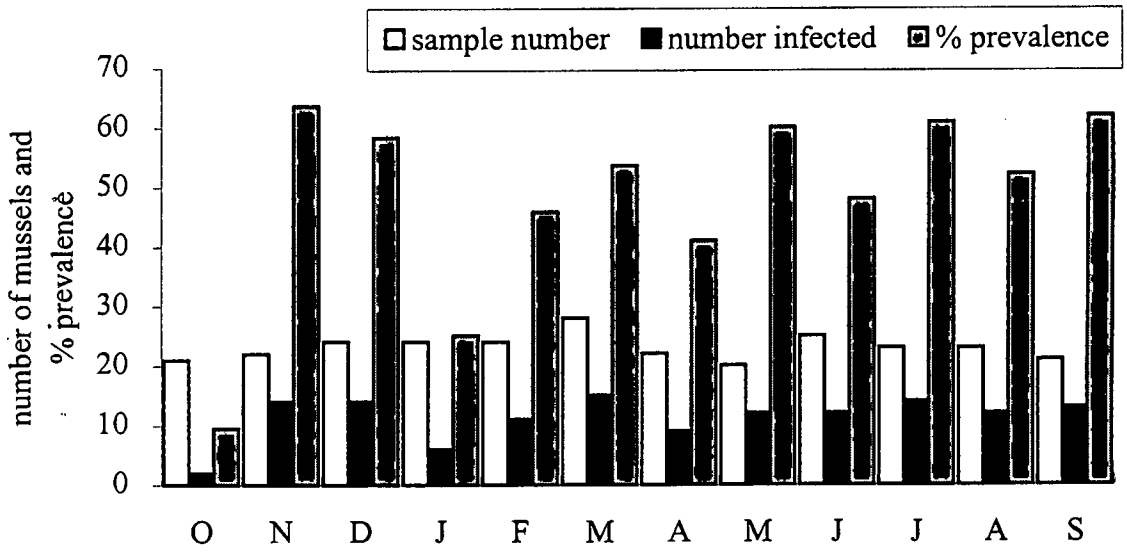


Figure 13. Monthly prevalences of *Metacercaria B* in female *Choromytilus* from Blouberg.

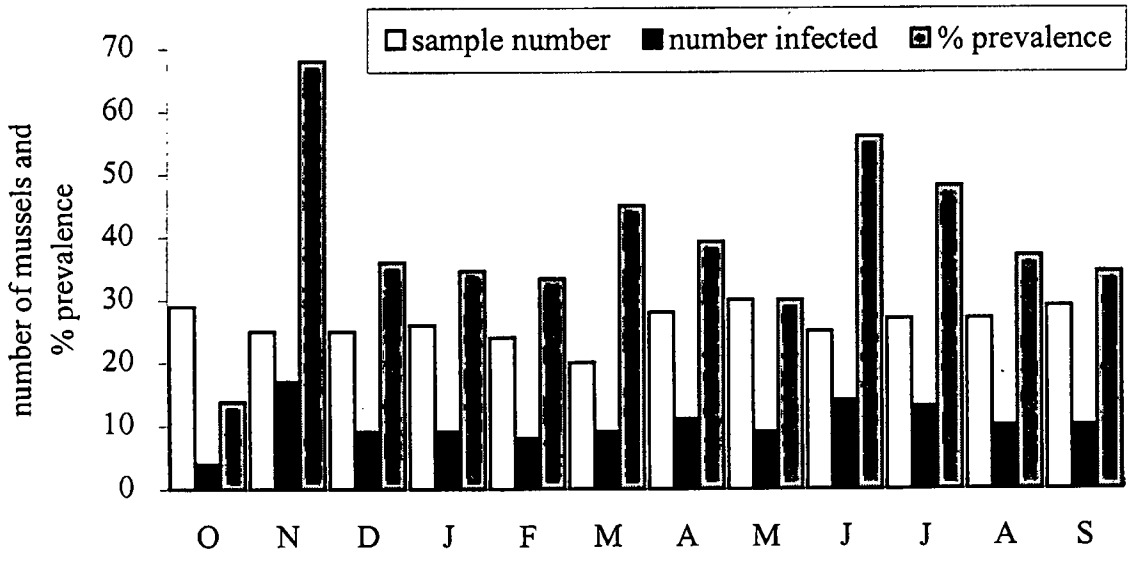


Figure 14. Monthly prevalences of *Metacercaria B* in male *Choromytilus* from Blouberg.

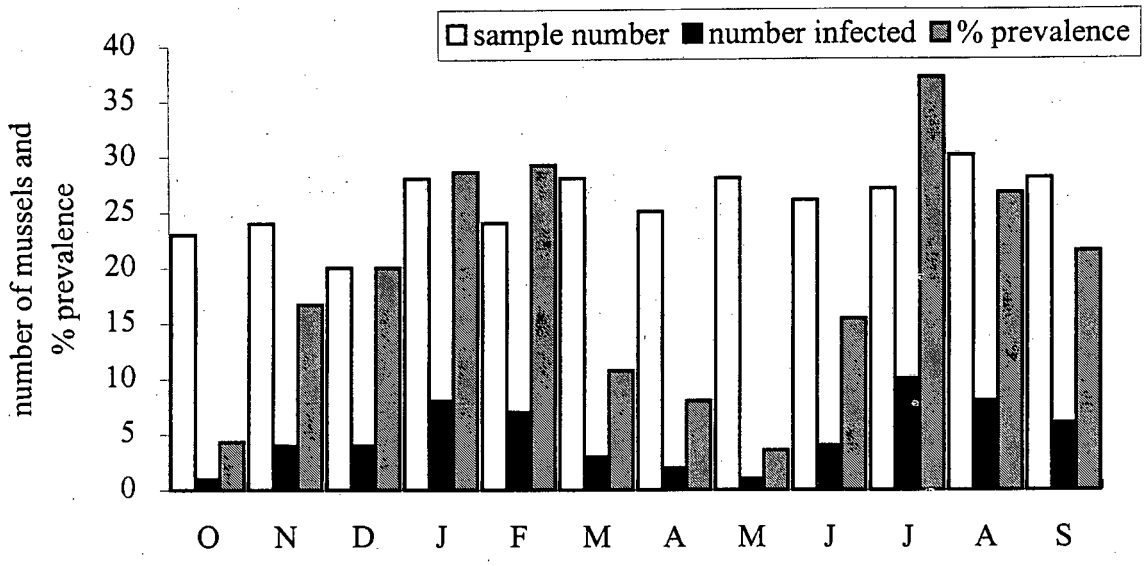


Figure 15. Monthly prevalences of *Metacercaria B* in female *Choromytilus* from Dido Valley.

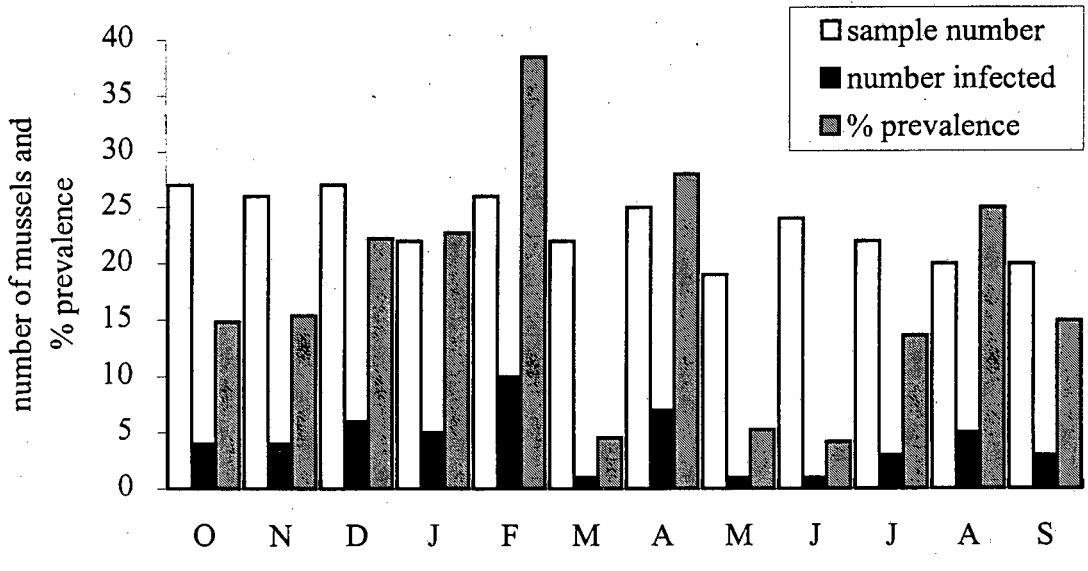


Figure 16. Monthly prevalences of *Metacercaria B* in male *Choromytilus* from Dido Valley.

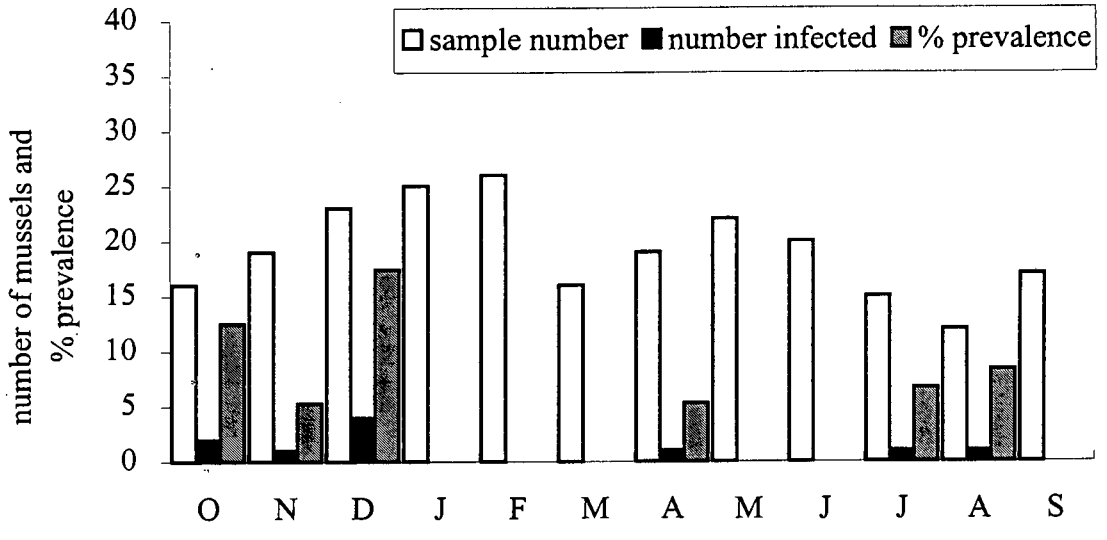


Figure 17. Monthly prevalences of *Metacercaria B* in female *Perna* from Dido Valley.

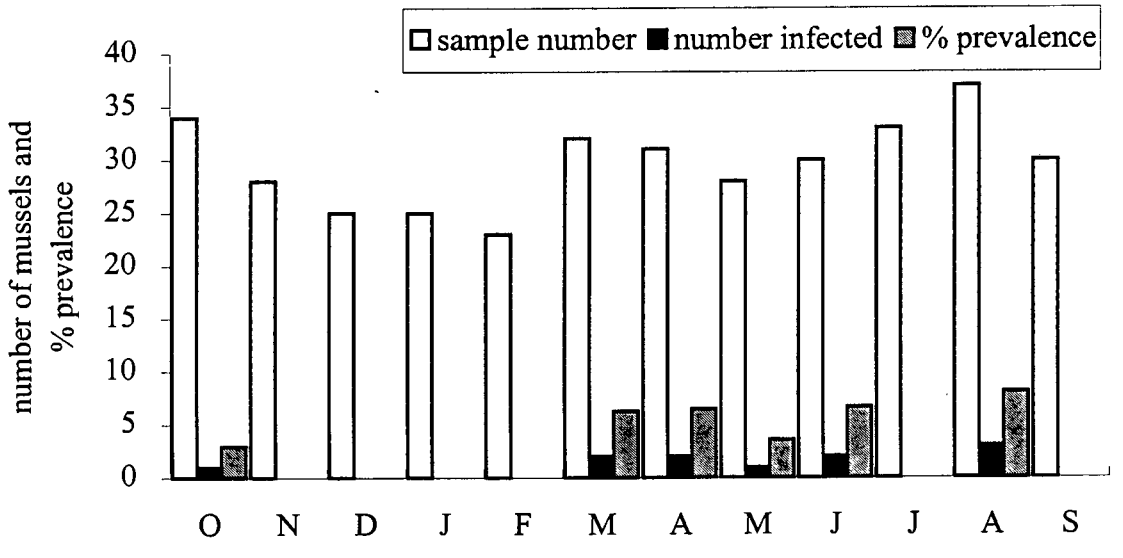


Figure 18. Monthly prevalences of *Metacercaria B* in male *Perna* from Dido Valley.

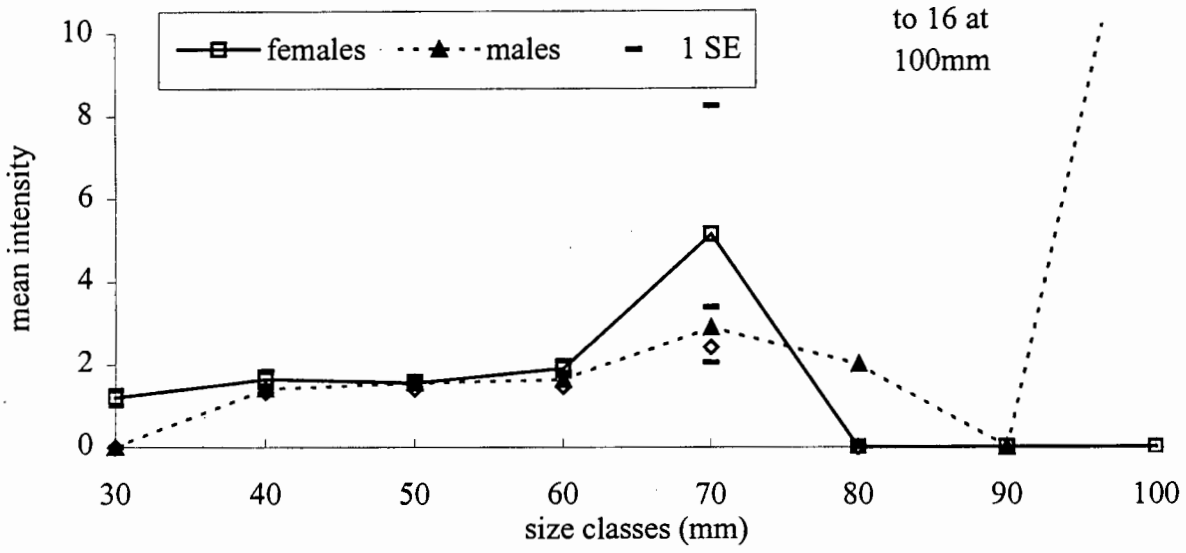


Figure 19. Size dependent mean intensity of *Metacercaria B* in male and female of *Choromytilus* from Blouberg.

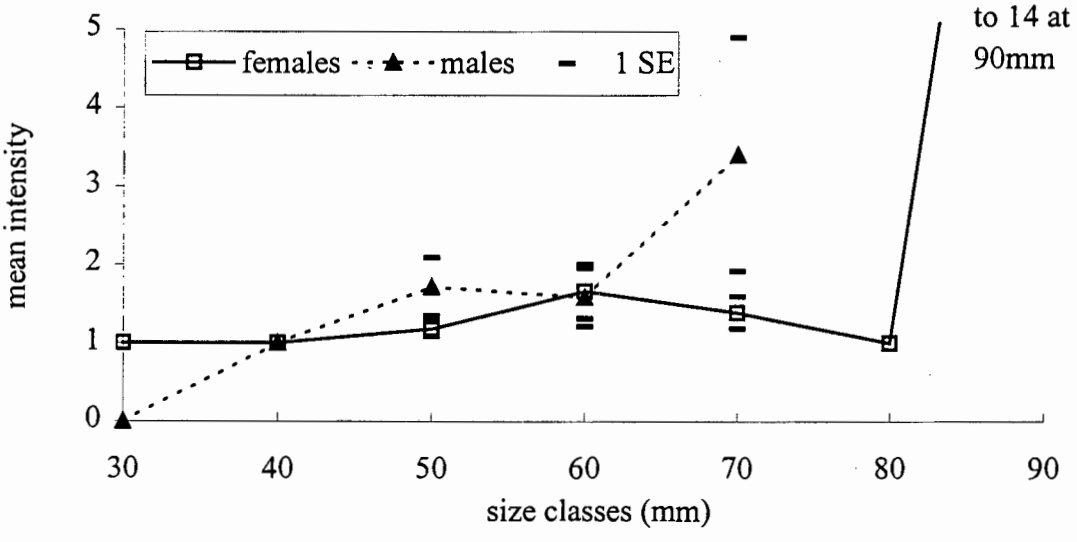


Figure 20. Size dependent mean intensity of infections of *Metacercaria B* in female and male *Choromytilus* from Dido Valley.

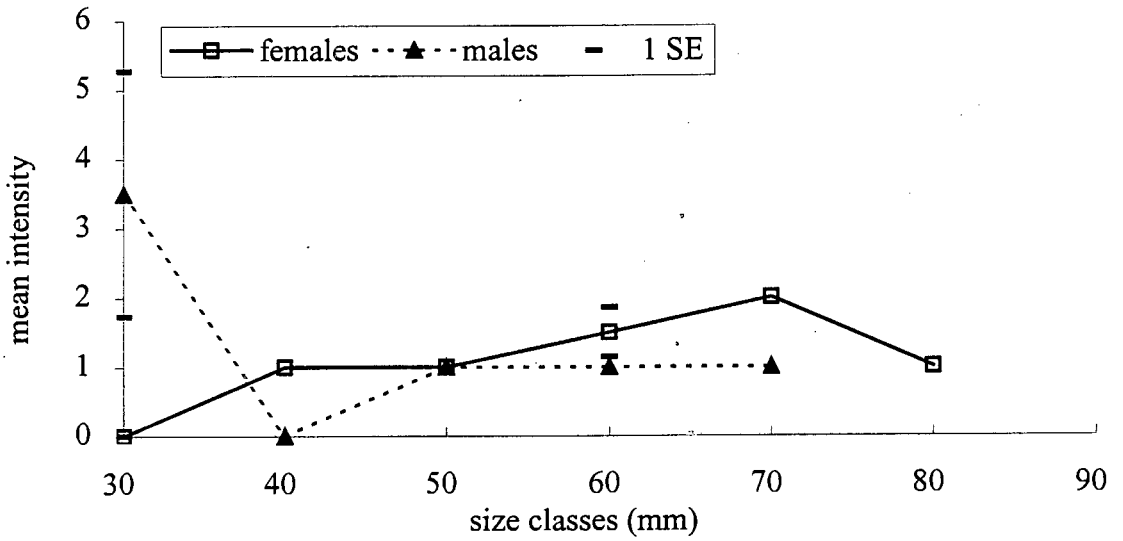


Figure 21. Size dependent mean intensity of infections of *Metacercaria B* in female and male *Perna* from Dido Valley.

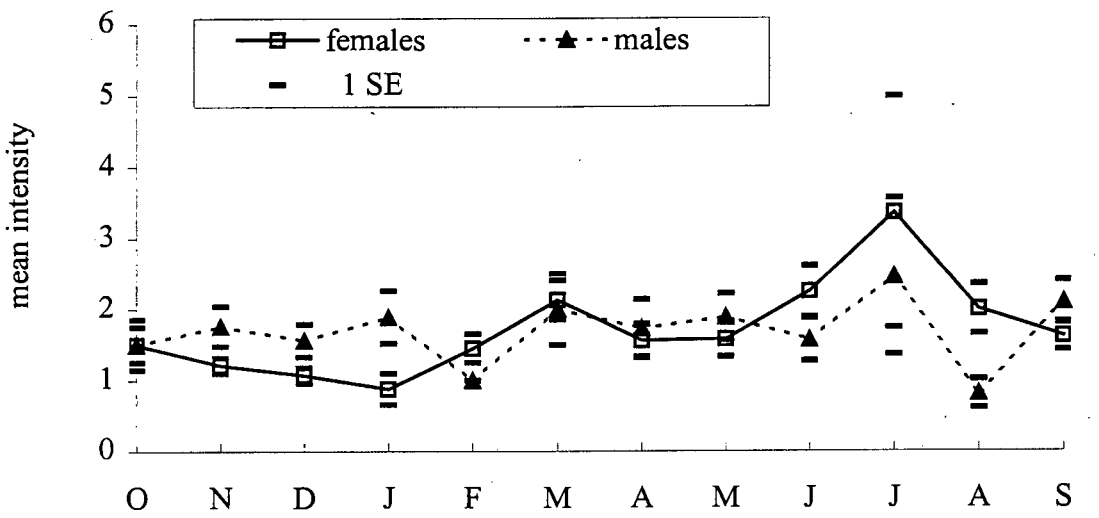


Figure 22. Monthly variation of mean intensity of infections with *Metacercaria B* in female and male *Choromytilus* from Blouberg.

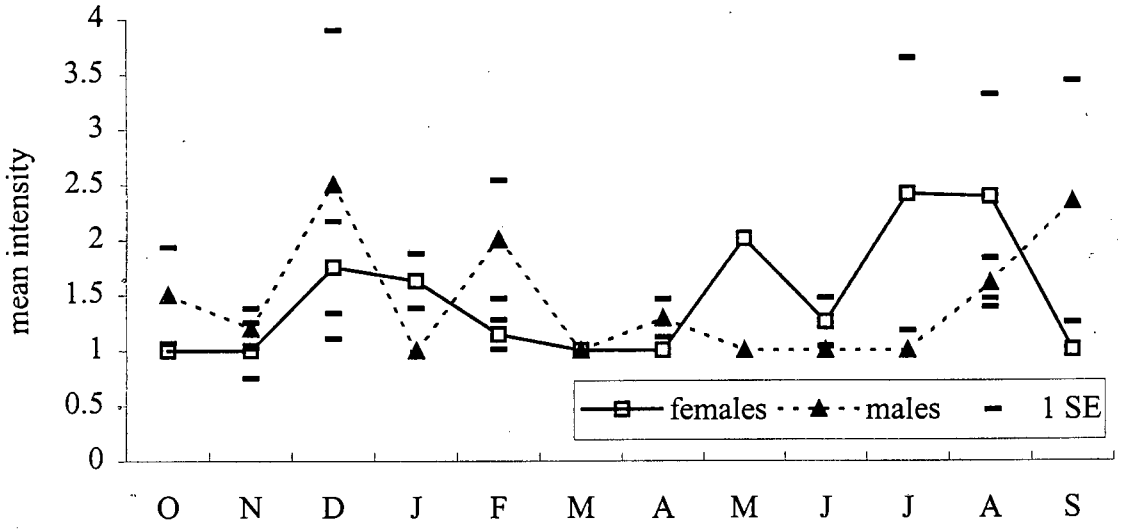


Figure 23. Monthly variation in mean intensity of infections with *Metacercaria B* in male and female *Choromytilus* from Dido Valley.

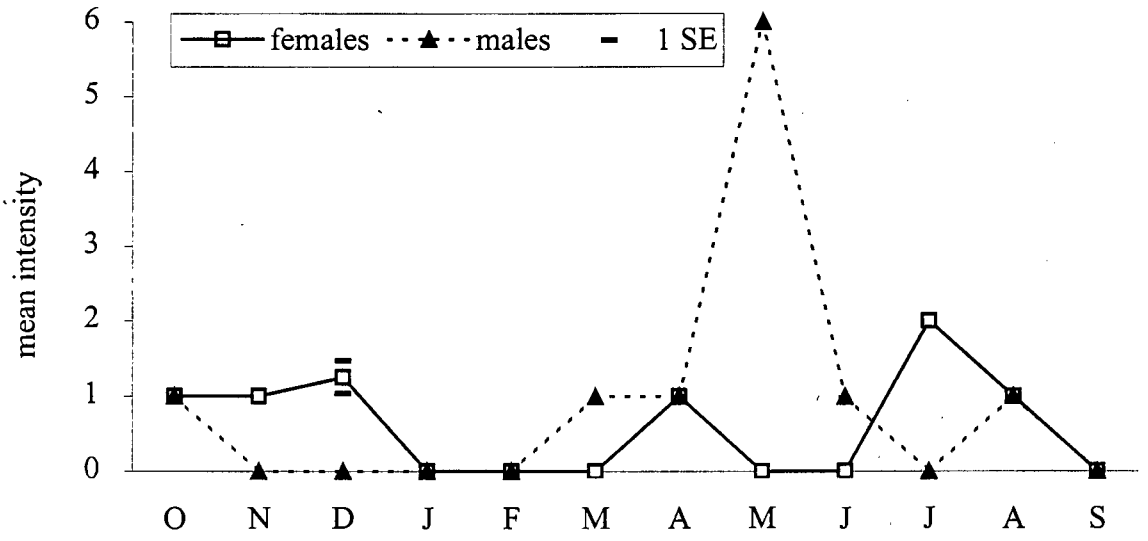


Figure 24. Monthly variation in mean intensity of infections with *Metacercaria B* in male and female *Perna* from Dido Valley.

CHAPTER 10: GENERAL COMMENTS

FURTHER IDENTIFICATION OF PARASITES

Identification of these larval worms could be facilitated by a molecular biology approach similar to that used by Mattiucci, Nascetti, Cianchi, Paggi, Arduino, Margolis, Brattey, Webb, D'Amelio, Orecchia & Bullini (1997) in their work on differentiating sibling species of the nematode *Anisakis simplex* complex. In addition, the methods of Calvo-Ugarteburu (1996) could be adopted along with the aid of principal components analysis (PCA) as proposed by Gibson, Taskinen & Valtonen (1992). Using these techniques, comparisons should be made with those larvae and adults that appear to have some morphological affinity. Described adults from nearby waters include those in works such as Toman (1977, 1989, 1992a 1992b), Bray (1984 & 1987) and Fantham (1938). Once the molecular approach has tied together adults and larvae, it has also identified the hosts at each stage. This should facilitate the completion of the life cycle by experiment and thus confirm that the adult and larvae are the same species. Gibson (1987), in his review of problems in digenean systematics and evolution, does not mention molecular biological methods. He says (p432) that, "currently the only practical species definition is that of the morphological species". He advocates the paramouncy of adult morphology. But concedes that: "Nevertheless it is valuable to take account of other life history stages and, indeed, the life history pattern itself, although ....evidence from these sources should be treated with caution." If molecular biological methods can be used to help tie together life cycles then they will greatly assist without usurping the authority of morphological taxonomy.

CYSTS IN PALPS

The propensity for cysts to invade the palps of mussels is curious. One possible reason would be for them to gain access to energy supplies in the palp. Lenoir, Robbins, Matheiu, Lubet & Gabbott (1989) describe the isolation of glycogen containing vesicular connective tissue cells from the labial palps and mantle of *Mytilus edulis*. That digeneans can exploit this resource is suggested by the report of Coustau, Renaud, Delay, Robbins & Matheiu (1991) who found that *Prosorhynchus squamatus* Odhner, 1905 has a factor that stimulates mobilisation of glycogen from host glycogen cells in other parts of the body. A histopathological study of the effect

of metacercarial cysts on these cells may shed more light on this possibility.

WORM BURDENS IN DIFFERENT HOSTS

Choromytilus from Blouberg has the highest worm burden - as measured by intensity and prevalence - and *Perna* has the lowest. Even the least worm burden in *Choromytilus* (From Dido Valley) is some three times higher than that in *Perna*. One reason for this might be that *Choromytilus* often occurs in horizontal substrata such as at the bottoms of crevices and rock pools where it is much more likely to be in contact with still water, and consequently exposed to infective digenean larvae. *Perna,* in contrast, is found often on more vertical surfaces, which would be more likely to be exposed at low tide or subject to stronger currents during tidal movements.

LOCALITIES AND DIFFERENT PREVALENCES

Parasite loads in *Choromytilus* populations differ greatly between collection localities. At Blouberg, *Choromytilus* have 19 times higher prevalence of *Cercaria notobucephala* and abundance of *Metacercaria perchorupis* is four times higher than that at Dido Valley. Abundance of *Metacercaria A* shows similar values at both beaches but the prevalence at Blouberg is some 10% higher than at Dido Valley. *Metacercaria B* at Blouberg has an abundance that is some 2.5 times higher than at Dido Valley. Reasons for these differences are likely to become clear only after the life cycle has been elucidated for each parasite and the numbers of other hosts on each beach assessed.

PART III:

MISCELLANEOUS ORGANISMS ASSOCIATED WITH MYTILIDS

CHAPTER 11: A PYCNOGONID FOUND IN *CHOROMYTILUS MERIDIONALIS* FROM BLOUBERG

On Western Cape beaches, pycnogonids that parasitise mytilids are sparse and their distribution is patchy. Of a mixed sample of *Choromytilus meridionalis*, *Aulacomya ater, Mytilus galloprovincialis* and *Perna perna* taken in 1988 only one was infested out of 4763, an overall prevalence of 0.02%. The single host, a male *Choromytilus meridionalis* from the August collection at Blouberg, contained one sub-adult pycnogonid (Specimen number A43470 Marine Biology Department, South African Museum, Queen Victoria Street, Cape Town RSA). Prevalence from the Blouberg sample of 650 *Choromytilus* is 0.15%. A further three pycnogonids (Two may be found under specimen number A43471, Marine Biology Department, South African Museum, Queen Victoria Street, Cape Town RSA) were recovered In 1997 from a sample of 40 *Choromytilus* (prevalence = 7.5%) also from Blouberg.

A loose association has been reported between the free-living pycnogonid *Nymphon gracile* and *Mytilus edulis* by Lintas & Seed (1994) on British beaches. Thus mere association of pycnogonid and mussel is not enough; evidence of parasitic activity or deleterious effect on the host is required to characterise the association. In *Choromytilus,* with the exception that in two infections the pycnogonid is fixed firmly to its host mantle by the chelicerae, the effect of this pycnogonid on its host is not obvious. The mussel gonad was sparse in the mantle when compared with other *Choromytilus* of the same size, but this is not conclusive: small numbers of uninfested *Choromytilus* of similar size also had sparse gonad. The comparison sample was taken from mussels that were free of infection with bucephalid sporocysts and cercariae, as these parasites also disrupt the gonad.

The lack of tubercular structures on the body segments and legs identify this pycnogonid (Figures 1 to 4) as a sub-adult. In addition, the legs are short in comparison with the body; also short are the undeveloped ovigers. The chelifores (Figure 3) are large, but sub-adult pycnogonids commonly have better developed chelae than adults. Change in proportions of body parts is a frequent feature of pycnogonid development. This change in proportions is evident by comparison of Figure 1 (immature) and Figure 2 (mature) *Nymphonella tapetis* in Oshima (1933a).

It is also evident in Oshima (1935) and Arita (1936).

With the exception of atypically large chelifores, the pycnogonids from *Choromytilus* approximate to *Nymphonella tapetis* as seen in Oshima (1933a) Figure 1. Oshima (1938) erected the Family Nymphonellidae with *Nymphonella* as the type genus. The taxon Nymphonellidae was superseded (Stock 1959) by Ammotheidae, now containing the Genus *Nymphonella. Nymphonella* spp. may be found on a range of substrata, as the following reports show. Oshima (1927a & b, cited in Oshima 1933a) found immature *Nymphonella tapetis* infesting the clams *Paphia philippinarum* and *Protothaca jedoensis* from South Japan. Infestation intensity was commonly from one to three with a maximum of seven. The host molluscs are intertidal sand-dwelling clams (Ogawa & Matsuzaka 1985). *Nymphonella tapetis* adults are found free-living in sand (Oshima 1933a). Since then the veneracean *Paphia philippinarum* has been renamed (Kikuchi 1976) *Tapes philippinarum* which in turn became (Ogawa & Matsuzaka 1985) *Ruditapes philippinarum.* Lauckner (1983) refers to *Protothaca (Venus) jedoensis.* Kikuchi (1976) found immature *Nymphonella tapetis* in the tellinid *Theora lata* [*Theora fragilis* in Ogawa & Matsuzaka (1985)] and in *Tapes philippinarum. Theora fragilis* (Semilidae), is a surface deposit feeder on sublittoral muddy bottoms (Ogawa & Matsuzaka 1985). *Nymphonella tapetis* was also found in the bivalve *Hiatella orientalis* (Hiatellidae) which has an, "epifaunal habit on hard substrate in the intertidal zone" (Ogawa & Matsuzaka 1985).

In South African waters, Stock (1959) reports an adult free-living *Nymphonella lambertensis*, but does not mention larval or immature parasitic forms. But immature pycnogonids resembling closely *Ammothea* sp. (see Oshima 1933b) have been reported in the sandy-beach bivalve *Donax serra* Röding (Tharme 1988 and Tharme, Webb & Brown 1996). From Namibia, in *Semimytilus algosus,* the bisexual mussel, Branch (pers. comm.) found sub-adults subsequently identified (pers. obs.) as *Nymphonella* sp.

The pycnogonid *Achelia chelata* is commonly associated with mytilids. Benson & Chivers (1960) and Ricketts & Calvin (1968) [cited by Lauckner (1983)] report mature and immature *Achelia* in *Mytilus californianus. Achelia* is unmistakably different from *Nymphonella:* the body shape of *Achelia* is much more disc-like, as is

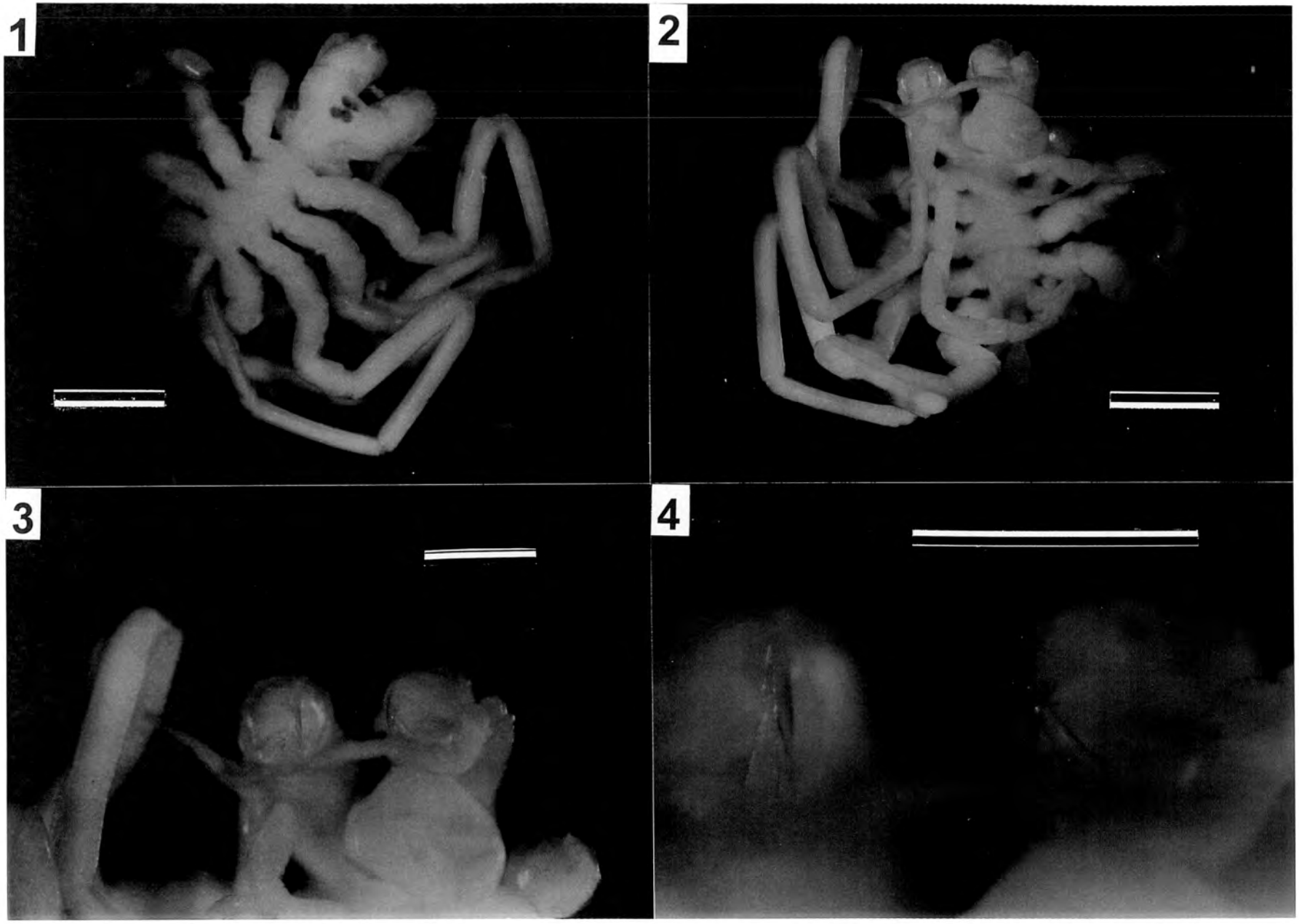


Figure 1. *Nymphonella* sp. dorsal view. Scale bar = 1mm.
Figure 2. *Nymphonella* sp. ventral view. Scale bar = 1mm.
Figure 3. *Nymphonella* sp. ventral view of chelifores. Scale bar = 0.5mm.
Figure 4. *Nymphonella* sp. ventral view of chelifores. Scale bar = 0.5mm.

Achelia bullosa in Child (1996) and *Achelia dentata* in Day (1969), than elongated and the walking legs are sub-chelate, in contrast to those of *Nymphonella*. Free-living *Achelia* spp. adults have been collected from the South African West coast (Stock 1959). These included *Achelia quadridentata*, *Achelia brevicauda*, *Achelia barnardi* and *Achelia* sp. See King (1973) for a list of records of associations of pycnogonids with other invertebrates.

In their review, Arnaud & Bamber (1987) cite three pycnogonid parasites of bivalves, *Nymphonella tapetis*, *Achelia chelata*, and *Ascorhynchus* sp. The first two have been dealt with above. *Ascorhynchus* sp. was reported from *Tellina perna* collected off the coast of Madagascar. Arnaud (1978) describes a new species of *Ascorhynchus* and compares it with other parasitic pycnogonids. His arguments for the differentiation of *Ascorhynchus* are also valid for the differentiation between *Nymphonella* in *Choromytilus meridionalis* and *Ascorhynchus endoparasiticus*. In particular, the body habits of the two differ and the proboscis is proportionately larger in *Ascorhynchus endoparasiticus* (See Figure 1 A & B in Arnaud 1978). It also has a markedly different shape from that of *Nymphonella*. The legs of *Nymphonella* are proportionally longer and thinner, and this is for a sub-adult *Nymphonella* as compared with an adult *Ascorhynchus endoparasiticus*. This difference can only become larger as the *Nymphonella* matures. The lateral processes of this *Nymphonella* remain approximately the same breadth from anterior to posterior. This is in contrast to *Ascorhynchus endoparasiticus*, where they taper markedly in breadth to the posterior.

CHAPTER 12: *MASTIGOCOLEUS* SP. (NOSTOCHOPSIDAE), A SHELL WEAKENING BLUE-GREEN ALGA FOUND INFESTING MARINE MYTILIDS

INTRODUCTION

Shells of living *Mytilus galloprovincialis* - the introduced Mediterranean black mussel - on the Western Cape Coast of South Africa have recently been found with grey corroded patches caused by burrows of the filamentous cyanophyte *Mastigocoleus* sp. These infested patches are often weak, and in extreme cases may fracture to produce large holes in the shell (Figure 1).

Endolithic (rock burrowing) micro-organisms are important bio-erosive agents of animal hard-structures and limestone (Peyer 1945). For reviews see Kobluk & Risk (1977) and Lauckner (1983). Noteworthy work on the identification of marine endolithic algae from their burrowing patterns has been done by Golubic (1969) who in collaboration (Golubic, Brent & Le Campion 1970) also pioneered study techniques and has proposed a new scheme of terminology for endolithic organisms (Golubic, Friedmann & Schneider 1981). Although infestation of mollusc shells by endolithic micro-organisms is common, serious structural weakening attributable solely to *Mastigocoleus* sp. has not been reported. Furthermore, direct damage resulting from algal penetration of bivalve shells is usually negligible (Lauckner 1983). And although Raghukmar, Sharma & Lande (1991) reported that bivalves, including the mytilid *Perna viridis,* suffered shell weakening when infested with boring algae including *Mastigocoleus* sp., they did not, however, report any extreme effects such as the production of fracture holes in the shell. This is consonant with their findings that the burrows did not penetrate deeply into the shell. Nevertheless, burrows by this alga up to two millimetres deep have been reported in intertidal calcareous rocks (Anand 1937).

More recently *Mastigocoleus testarum* has been reported (Le Campion - Alsumard, Golubic & Hutchings 1995) in dead and denuded skeletons of the coral *Porites lobata*. Also in French Polynesia (Mao Che, Le Campion - Alsumard, Boury Esnault, Payri,

Figure 1. *Mytilus galloprovincialis* showing shell damage and fracture holes caused by *Mastigocoleus* sp., scale bar 30mm.

Golubic & Bezac 1996) report that the shell of the black pearl oyster *Pinctada margaritifera* var. *cummingii* suffers minor damage from infesting *Mastigocoleus testarum*. The oyster shells are reported as becoming so brittle that they may break during culturing operations but no mortalities were noted in undisturbed oysters. Mao Che *et al.* (1996) also describe the succession of endolithic organisms in the shell, their specific locations and the degree of damage caused by each.

Mastigocoleus sp. and other cyanophyte protophytes are widespread in marine habitats (Fogg 1973) and endolithic algae occur in all types of carbonate substrata. According to Humm & Wicks (1980) this genus contains only the species *Mastigocoleus testarum* Lagerheim, and only this species is mentioned in the literature. *M. testarum* is a common species found mostly in temperate areas, but Lawson & John (1982) consider its distribution to be world-wide. The closest previous reports to South Africa are from Sierra Leone (John & Lawson 1977, Aleem 1980).

Endolithic cyanophytes share this niche with chlorophytes (Harris, da Silva, Bolton & Brown 1986), rhodophytes (Nolan 1991), fungi - in lichens (Griffiths, pers. comm.), and some sponges (Lauckner 1983), particularly of the genus *Cliona.* Nolan (1991) reports an infestation by a conchocelis phase, probably of the red alga *Bangia* sp. or *Porphyra* sp. in shells of the limpet *Nacella concinna* from the South Orkney Islands. This infestation affects the density of the shell and furthermore induces other grazers to abrade and sometimes perforate the shell in an attempt to harvest the alga.

Harris *et al.* (1986) in South Africa, detail the interaction between the sandy-beach whelk *Bullia digitalis* (Dillwyn) (Gastropoda, Nassariidae) and the endolithic chlorophyte *Eugomontia sacculata.* Also in South Africa, lichens of the genus *Pyrenocollema* have been observed (Griffiths pers. comm.) infesting a number of molluscs, including mytilids and limpets. Although *Pyrenocollema* appears to pose a threat to the physical integrity of shells, it has not been noted on any of the mussels examined here. In this study we examine *Mastigocoleus* sp. infestations in *Mytilus galloprovincialis* for three reasons: it seems to be the most heavily infested of the

mussel species on these coasts; it is an important maricultural component of the industry centred in Saldanha Bay on the West coast of South Africa and, not least, *M. galloprovincialis* has proved to be a vigorous invader. In places it has become a dominant intertidal species. Thus, the ecological and economic significance of this mussel renders it particularly interesting.

MATERIALS AND METHODS

Collections of mussels were taken from four localities at Saldanha Bay (33° 02'S, 18° 00'E) which is about 120 km north of Cape Town, South Africa. These localities comprise one high intertidal of mixed rock and sand at Saldanha Beach with light to moderate wave action (collected on 19/7/90), and three (collected on 18/7/90) at Sea Farm - an aquaculture enterprise located in a dam next to the Saldanha-Sishen ore jetty. The dam, at which the tidal range is about one metre, covers several hectares. Wave action is nil to very light. Flow in and out of the dam is conducted by a 1.5 metre diameter pipe to the bay. Wave action at the high intertidal locality on the outer (sea) side of the dam wall is moderate. The differing tidal levels at collection localities provided a range of different mussel growth rates varying from very fast in the subtidal, to slow in the high intertidal (van Erkom Schurink & Griffiths 1993).

Other localities examined for infested mussels were Simonstown (34° 10'S, 18° 25'E); this is the only locality not on the West Coast, it lies on the western shore of False Bay about 30km south of Cape Town. Blouberg (33° 48'S, 18° 27'E) is about 20km north of Cape Town, and Stompneusbaai (32° 45'S, 17° 55'E) is just north of Saldanha. Duiwegat (31° 30'S, 18° 05'E) is about 270 km north of Cape Town, and Port Nolloth (29° 16'S, 16° 52'E) lies another 330 km further north from Duiwegat.

Shell fragments were prepared for SEM examination by fixation in 3% glutaraldehyde, then dehydrated in an alcohol series and critical-point dried. Shell pieces were then fractured to expose a fresh face and mounted on stubs before they were coated with gold-palladium. Decalcified shell specimens were obtained by immersion in 10% acetic acid overnight. Algal filaments could then be teased apart for examination.

Except for mussels from the culture ropes, a representative $0.1m^2$ quadrat sample of mussels from the Saldanha collection localities was taken which included all mussels down to the rock surface; each mussel was measured. Cultured mussels were taken randomly from ropes brought in for harvesting. Shell length is as defined in Seed (1968): the greatest measurement along the anterior-posterior axis.

To determine the location of the infestation patch on the shell, each valve was divided into areas A, B and C (Figure 6A) corresponding to areas designated 3, 2 and 1 on the shells of *Mytilus edulis* by Laihonen & Furman (1986). Extent of the infestation patch was graded out of 5 on each of these divisions: clear areas being graded 0 and covered areas 5. The overall extent of the infestation patch on each mussel was determined as the sum of values, each out of 5, for divisions A, B and C (maximum 15).

Location of the infestation patch on the valve surface was plotted by hand onto the diagram of a shell as an infestation patch perimeter line for each individual. Contours reflecting 10%, 50% and 80% frequency of infestation were obtained by evaluating groups of ten superimposed infestation patch perimeter line plots from the same locality. Individual perimeter lines were then counted from the outer margin of the valve to a point at its centre. Counting was aided by drawing a series of lines radiating from the nominated centre of the shell (Figure 6B). The 10% contour is the outermost (first) line. It signifies that the area between the 10% and 20% lines is encroached upon in 10% of cases. The other contour lines have corresponding meaning. In samples of fewer than ten, the distance between the first and last line was split *pro rata* and percentage contour lines constructed. These composite contours were then combined with others similarly constructed until the sample size for each collection locality was achieved. Probability contours for holes broken in the shell were similarly derived.

Shell penetration strength in Newtons was obtained by use of a spike penetrometer over the adductor muscle area. The figures given are for comparison of infested with uninfested shells. Absolute values of force per unit of shell area are not available as a conical spike was used. Two mussel samples, one lightly and one heavily infested,

were taken from each collection locality and their strengths compared using Student's one-tailed t-test. Equal numbers of infested and uninfested mussels were used, and each was matched to an equal sized counterpart, thus ensuring an identical size distribution and mean size of population. This is to eliminate any bias that change in shell strength with size might introduce. In the case of the sample from the outer side of the Sea Farm dam wall, it was not possible to match all mussels with equal sized counterparts. Here, mussels of size within one millimetre were paired.

In this work an infestation of *Mastigocoleus* is defined as any presence of the alga as indicated by grey roughening of the shell in areas where the periostracum is absent. This grey roughening has been demonstrated by pilot decalcification studies to be invariably associated with the presence of *Mastigocoleus* filaments in the shell. The identification *Mastigocoleus* sp. was confirmed by H. Stegenga (pers. comm.): it is a cyanophyte of order Stignematales and family Nostochopsidaceae. See descriptions in Humm & Wicks (1980), Lawson & John (1982) and Fogg, Stewart, Fay & Walsby (1973). Because the genus has only one previously recorded species, *M. testarum*, it is likely that this study also deals with this species. Identification to specific level was not confirmed and in consequence the subject of this study is referred to as *Mastigocoleus* sp.

RESULTS

Figures 2 and 3 depict the exposed *Mastigocoleus* sp. filaments in a decalcified piece of infested mussel shell. Scanning electron microscopy (Figures 4 & 5) revealed that the filamentous thallus of this endolithic blue-green alga had honeycombed the shell with burrows about 8μ in diameter. In severe infestations they may occur in such density as to weaken the shell matrix to the point of disintegration. The burrows are invisible to the naked eye, and light microscopy of the shell did not disclose them.

Mastigocoleus sp. also affects the brown mussel, *Perna perna*, and the black mussel *Choromytilus meridionalis* but with less severity (pers. obs.). *Aulacomya ater*, the ribbed mussel, was largely uninfested except at Port Nolloth where severe shell

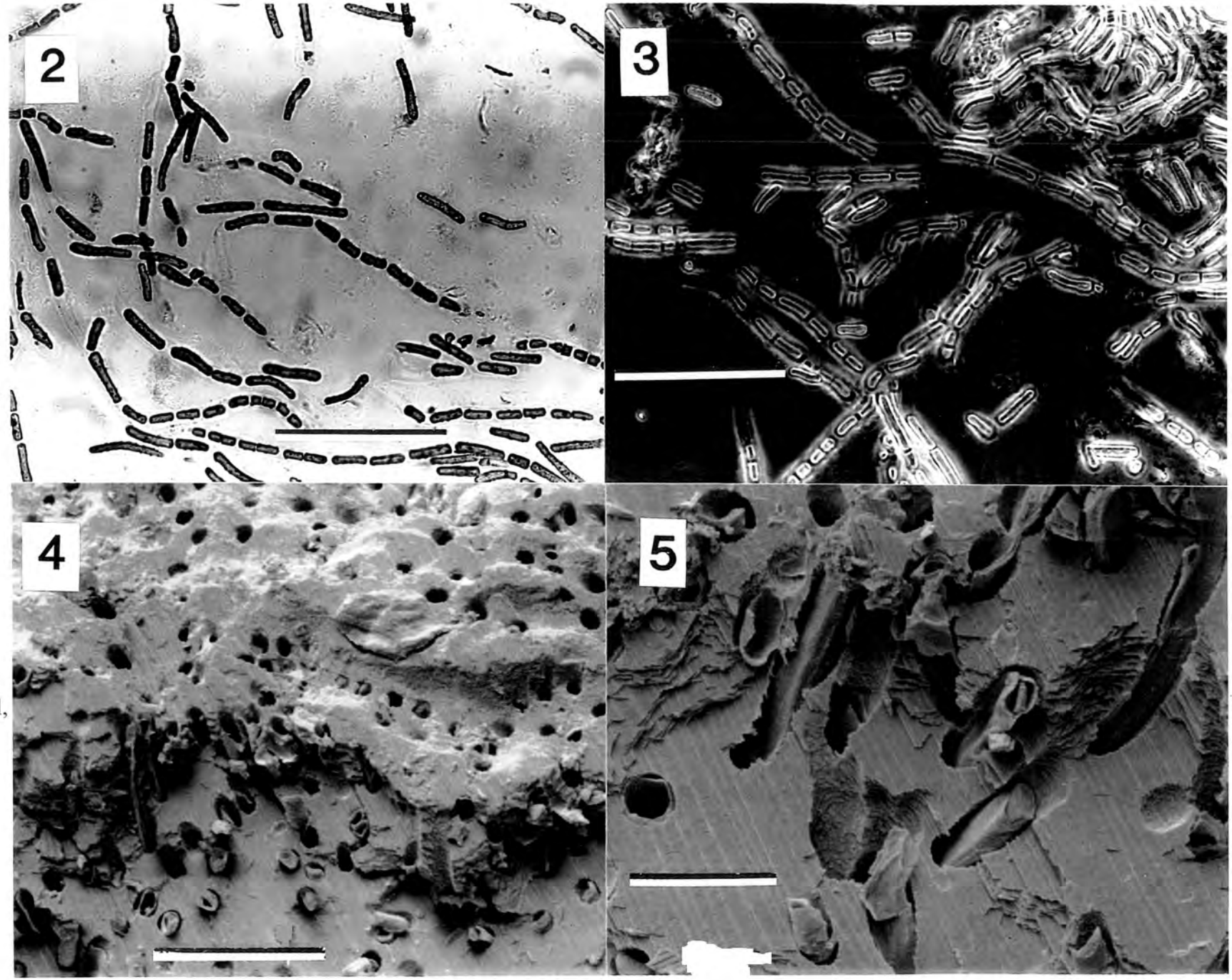


Figure 2.
Mastigocoleus sp. filaments after being released from the shell by decalcification, scale bar 100µm.

Figure 3.
Mastigocoleus sp. filaments (phase contrast) after being released from the shell by decalcification, scale bar 100µm.

Figure 4.
SEM. *Mastigocoleus* sp. infested mussel shell, scale bar 50µm.

Figure 5.
SEM. Details of *Mastigocoleus* sp. tunnels in mussel shell, scale bar 20µm

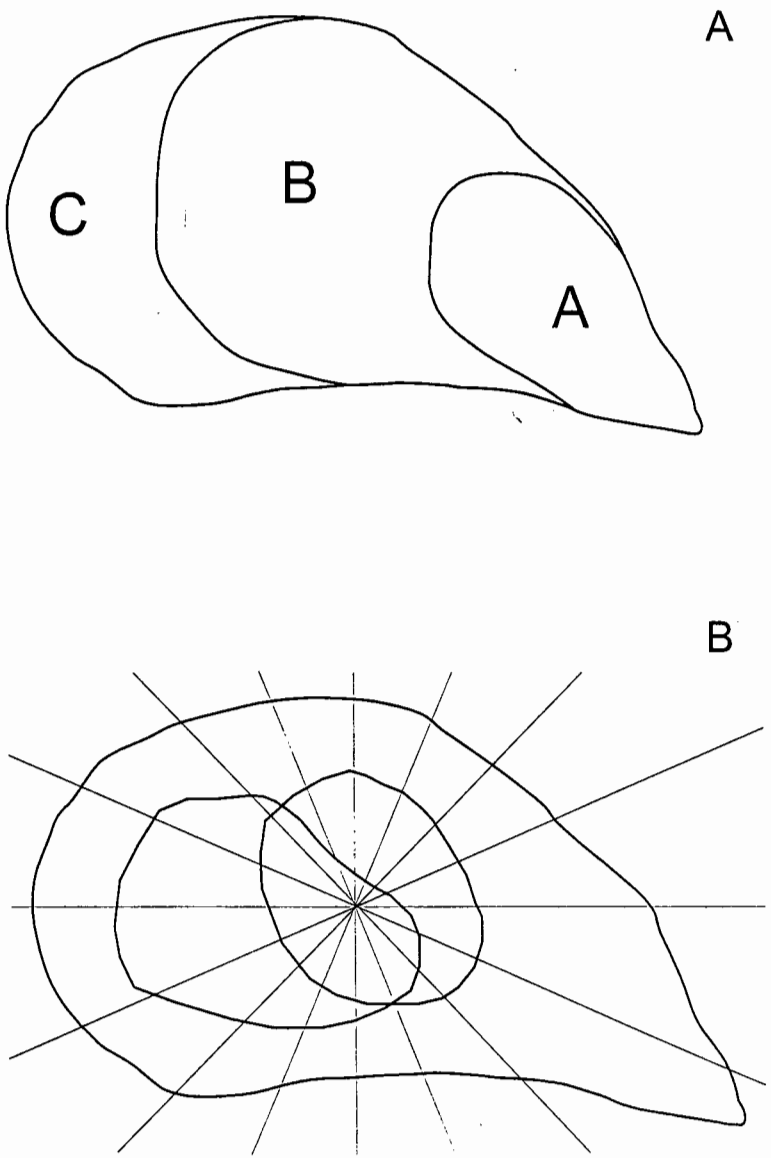


Figure 6. A: valve areas nominated A, B & C. B: the radiating lines show the contour method for determining infestation patch size and location of *Mastigocoleus* sp. on the shell.

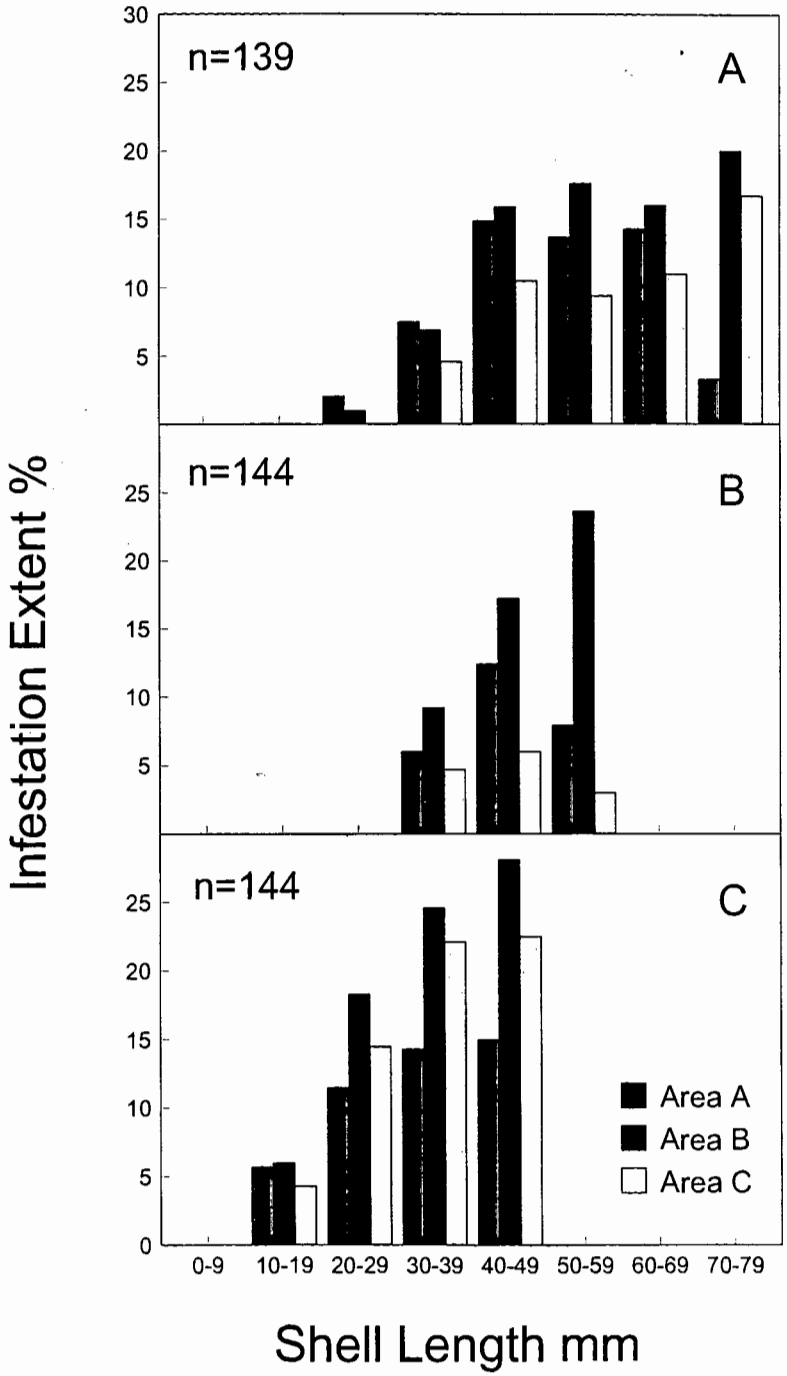


Figure 7. A: Extent of *Mastigocoleus* sp. infestation on each of the three shell areas (collection from the inner side of Sea Farm dam wall). B: Extent of *Mastigocoleus* sp. infestation on each of the three shell areas (collection from the outer side of Sea Farm dam wall). C: Extent of *Mastigocoleus* sp. infestation on each of the three shell areas (collection from Saldanha Beach).

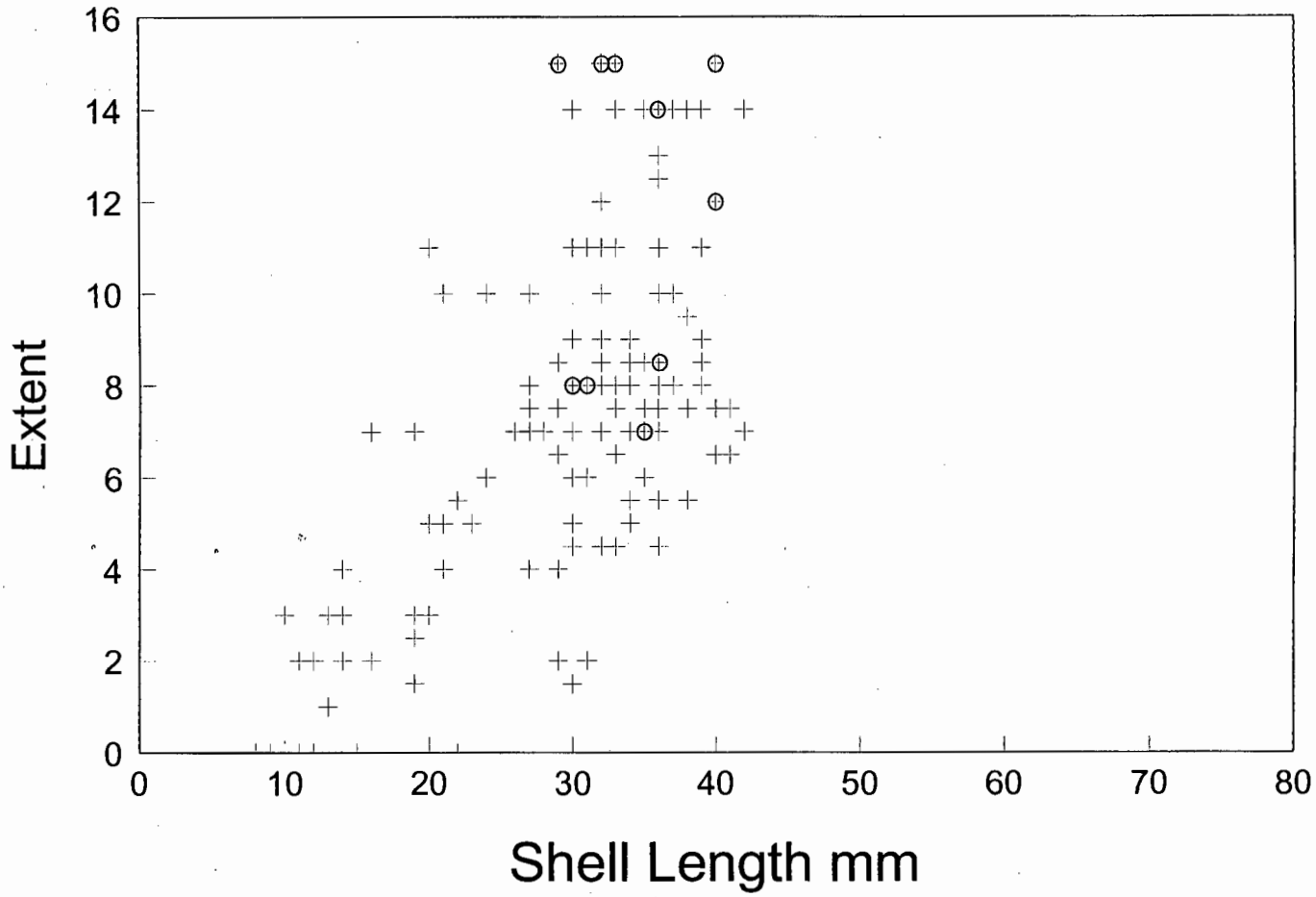


Figure 8. Extent of shell coverage by *Mastigocoleus* sp. versus shell length of *Mytilus galloprovincialis* from Saldanha Beach. Holed individuals are circled.

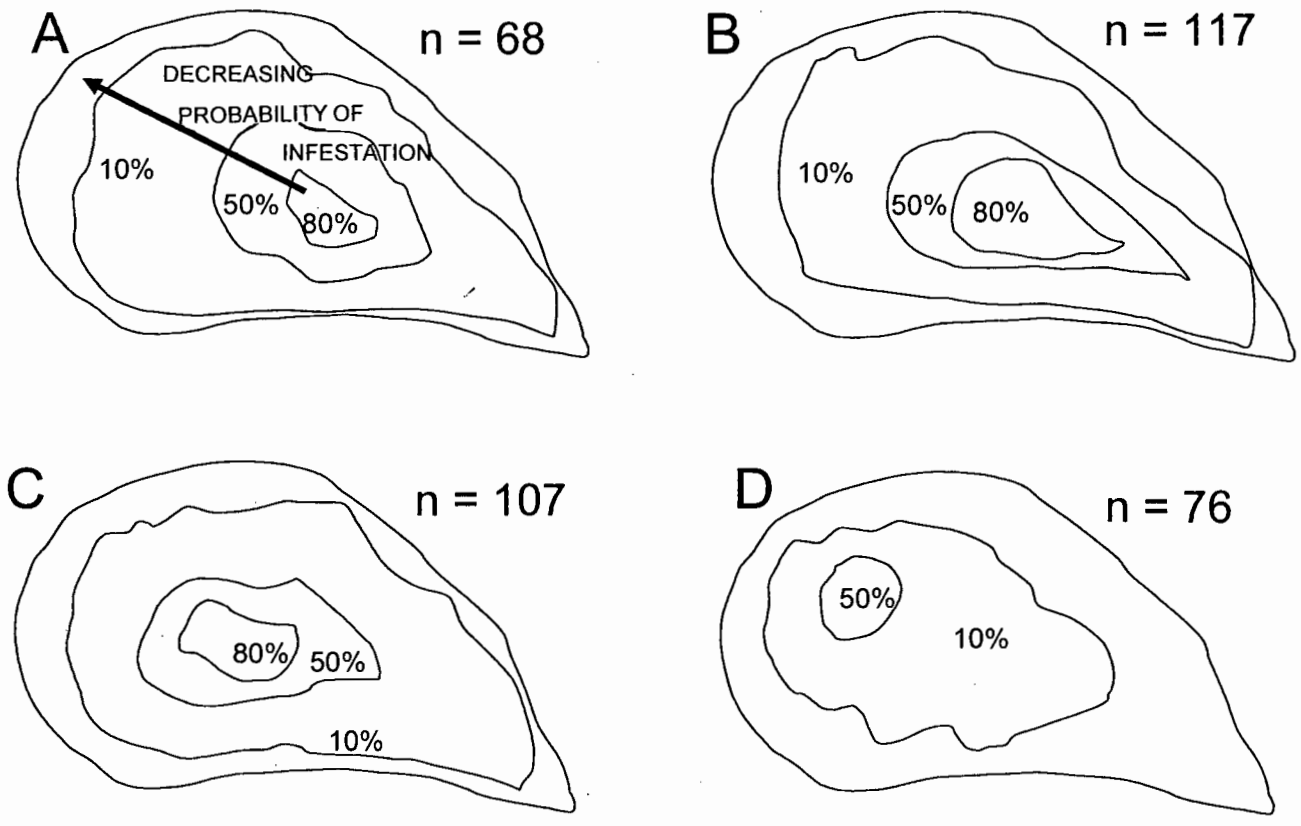


Figure 9. Areas and locations of *Mastigocoleus* sp. infestations at three localities. A: Low intertidal - inner causeway; B: High intertidal - outside causeway (Sea Farm); C: High intertidal - Saldanha Beach; D: Area and location of holes in shells of *Mytilus galloprovincialis* collected from Saldanha Beach.

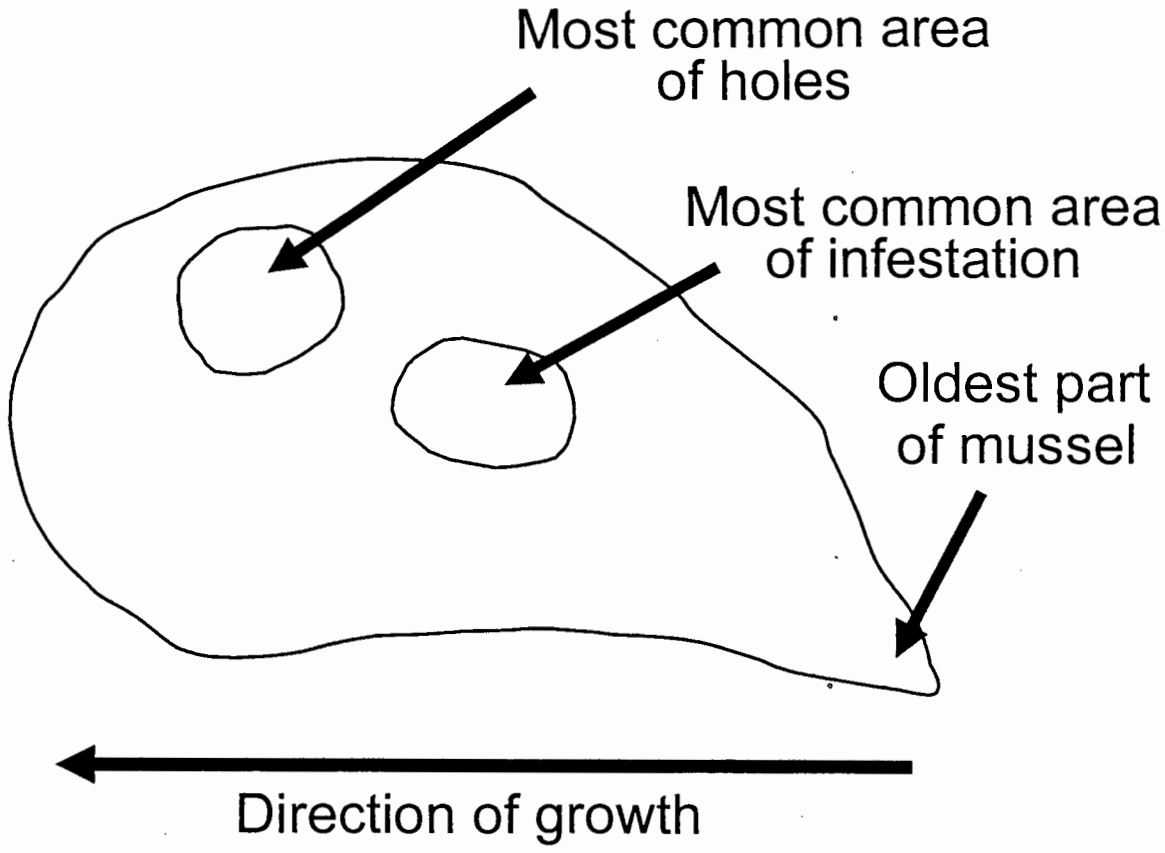


Figure 10. Summary diagram indicating the most common area of *Mastigocoleus* sp. infestation on the shell, and the most common area where holes occur.

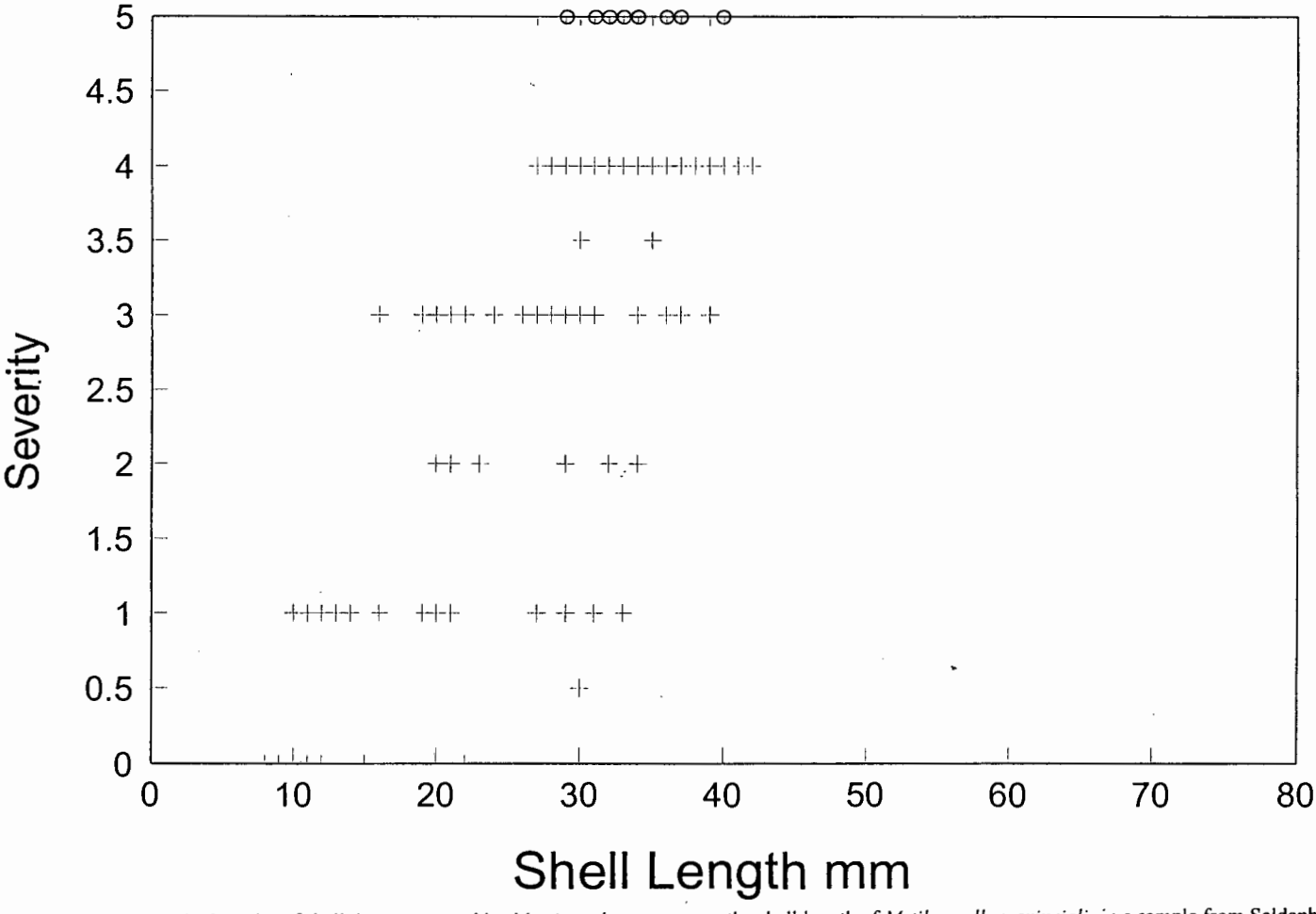


Figure 11. Severity of shell damage caused by *Mastigocoleus* sp. versus the shell length of *Mytilus galloprovincialis* in a sample from Saldanha Beach. Individuals with fracture holes in their shells are circled.

weakening was common (pers. obs.).

Mean length, population density and prevalence in *M. galloprovincialis* are summarised in Table 1. Subtidal mussels attain a much greater maximum size than those from intertidal populations; in fact, very few intertidal mussels exceeded 70mm, which is a common size for cultured mussels.

Table 1. Population density, mean shell length, and prevalence of infestation by *Mastigocoleus* sp. in *Mytilus galloprovincialis* from different collection localities.

locality	mean shell length (mm)	population density/m^2	prevalence	*n* for prevalence
outer causeway	18	15240	51.4%	142
Saldanha	19	7120	94.4%	144
inner causeway	45	380	66.2%	139
culture ropes	53	-	0.96%	104

Prevalence, the proportion of mussels in a population showing signs of infestation, was ascertained (Table 1) from the data on extent and severity. When these were both nil the mussel was deemed uninfested.

Intensity, the number of infesting individuals per host, could not be measured directly, but aspects of it were ascertained under the headings of extent, severity and shell penetration strength. Extent, the area of infestation patch coverage on the shell (Figure 9 A, B & C), the extent of shell fracture holes (Figure 9D), and the most common areas of both are given on Figure 10. The mean extent in each size group for each of the three shell areas at the different collection localities is given in Figure 7 A, B & C. Infestation extent at each locality increased with increasing mussel length. Figure 8 shows a typical plot. Extent shows a trend that increases with increasing shell length (Figures 7 & 8).

Shells were graded for severity - the amount of damage caused by the infestation - from 0 (free) to 5 (holed). The amount of damage apparent was always ascertained on the

most heavily affected valve, as any perforation will critically reduce survival. Regression equations are given below; their significance in all cases is P = 0.001.

At the outer side of the Sea Farm dam wall (high water) 50% of variation in severity is accounted for by shell length:

$$Y = 0.10979X - 2.30436,\ r^2 = 0.50\ (df = 141)$$

At Saldanha (intertidal) 64.1% of variation in severity is accounted for by shell length:

$$Y = 0.13346X - 0.75587,\ r^2 = 0.64\ (df = 142)$$

At the inner side of the Sea Farm dam wall (low intertidal) 38.2% of variation in severity is accounted for by shell length:

$$Y = 0.047953X - 0.97872,\ r^2 = 0.38\ (df = 137).$$

Thus, severity increases with shell length (Figure 11). Regression analyses show that components of this relationship are highly significant. The smallest mussels are often undamaged, whereas larger ones tend to suffer deep pits, or even have holes broken in their shells.

Mussels whose shells are severely infested with *Mastigocoleus* sp. have weaker shells than those of the same length with lesser infestations. The mean penetration strength of infested samples is reduced by between 36% to 43%. Furthermore, t-tests comparing penetration strengths of shells with light and heavy infestations show that they are significantly different.

The Saldanha Beach mussels had the highest prevalence (Table 1) and severity (Figure 11), and were the only sample to contain mussels with holes broken in their shells. Saldanha mussels also had the most marked strength differences: samples of infested and uninfested mussel shells differed in mean penetration strength (P = 0.005; one-

tailed t test). Shell strength was 76.9N and 134.3 N for heavy and light infestations respectively. The sample size was eight of each class of mussel and the mean sample shell lengths in both classes were 34.51mm with a Standard Deviation in both samples of 2.601mm. The critical region $t_s \geq 2.977$; the test statistic = 3.1389 and the degrees of freedom = 14.

At the outer side of the Sea Farm dam wall, samples of infested and uninfested mussel shells differed in mean penetration strength (P= 0.025, one-tailed t-test). Shell strength was 97.2 N and 152.6 N for heavy and light infestations respectively. The sample size was nine of each class of mussel. The mean sample shell lengths were 46.89 mm (S.D. = 3.923 mm) for heavily infested mussels and 46.87 mm (S.D. = 3.911 mm) for those with light or no infestation. These figures were very close, so no size dependent differences may be expected. The critical region $t_s \geq 2.12$; the test statistic = 2.46 and the degrees of freedom = 16.

At the inner side of Sea Farm dam wall, samples of infested and uninfested mussel shells differed in mean penetration strength (P = 0.005; one-tailed t-test). Shell strength was 63.4 N and 99.6 N for heavy and light infestations respectively. The sample size was sixteen of each class of mussel. The mean sample shell lengths were 52.86 mm for each class with a Standard Deviation of 6.39 mm in both cases. The critical region $t_s \geq 2.75$; the test statistic = 3.1389 and the degrees of freedom = 30.

A study of infestation distribution on the shell valves (Figures 7, A, B & C) shows that it is predominantly the mid-area on the outer surface of each shell valve that is most commonly infested by the alga and that the extent of infestation tends to rise with increasing shell length. Figures 9 & 10 indicate specific areas of infestation and the location of holes. Note that the pattern of infestation is similar, irrespective of sample localities, and also that the 80% infestation area does not correlate with the oldest part of the mussel.

Only one mussel out of 104 from the culture ropes at Sea Farm was infested with *Mastigocoleus* sp. This infestation had an extent and severity rating of less than 1, and it occurred in the typical area on the valve. In contrast, the Saldanha Beach sample contained shells with holes broken in them. Holes were most common over the adductor muscle insertion points, which fall outside of the 80% infestation probability contour. Figures 8 & 11 show that shells with holes in them tend to be larger than most.

DISCUSSION

Within populations, the prevalence of *Mastigocoleus* sp. infestations rises with increasing shell length (Figures 8 & 11): any score above 0 signifies an infestation. These figures also show that the smallest mussels are commonly disease free. If one assumes a correlation between shell length and age in any collection, then the larger mussels are older: the duration of opportunity for infestation increases with age and therefore size (Lauckner 1983).

When we compare the relationship of mean shell length and prevalence among the populations (Table 1), the converse of the above is suggested: populations with larger mean shell lengths suffer lower prevalences. The explanation appears to rest with differential growth rates and shell abrasion caused by tidal conditions. Faster growing mussels may outrun the growth of the alga resulting in a lesser degree of infestation severity and extent on the shell. The sample with the smallest mean shell length - that from the outer side of the Sea Farm dam wall - was subject to the highest wave action and was also high on the intertidal. The sample with that largest mean size was from the most favourable conditions: subtidal culture ropes with no wave action.

Faster growth of the subtidal mussels is attributable to the unbroken feeding time provided by permanent immersion (van Erkom Schurink & Griffiths 1993). Moreover, such favourable conditions, lacking either emersion or mechanical threat by wave action, allow the mussels to put more effort into growth even at the expense of shell thickness (Raubenheimer & Cook 1991).

Mastigocoleus sp. is mainly restricted to shell areas where the periostracum has worn away. This is also supported by Raghukmar *et al.* (1991) who found the same for the mytilid *Perna viridis*. It seems that an intact periostracum layer may be an important factor in preventing infestation. Indeed, according to Bottjer & Carter (1980) this conchiolin layer, in living bivalves, offers some protection from epibionts.

Subtidal cultured mussels had relatively thin shells, but they still had an intact and shiny periostracum. And coincidentally, they had a very low prevalence of *Mastigocoleus* sp. Intertidal mussels, at their points of maximum shell width, often had patches where the periostracum was worn through. This is coincidentally the area of most frequent *Mastigocoleus* sp. infestation (Figures 7, 9, & 10). This damage may be caused by wave action in combination with water borne sediments and exacerbated by high mussel population densities. Closely packed mussels will suffer more abrasion of the periostracum at the point of maximum width because this area is more likely to rub against that of the neighbouring mussel when mussels are moved on their byssus by wave action.

Though the most common area of infestation on the shell is not the oldest part (Figure 10), absence of heavier infestations in the older regions of valves may be explained by these parts usually being wedged in between neighbouring shells and buried under sand where there may be insufficient light for algal growth.

Holes caused by fracture of the shell, as shown in Figure 1, are indicative of extreme shell weakening. The Saldanha Beach sample contained some holed specimens and they were usually larger mussels (Figures 8 & 11). It is curious that these fracture holes lie outside of the most common area of infestation and instead are frequent at the point of adductor muscle insertion (Figure 10). The shell here is thinner because there is no nacreous layer beneath the point of muscle insertion. In consequence, the shell here is more highly stressed and any weakening coupled with the concentrated force applied by the adductor muscle may be critical. Other parts of the shell may be more severely infested, and thus be weaker, but they are not so subject to mechanical forces.

This work demonstrates that burrows of *Mastigocoleus* sp. can drastically weaken the shells of mussels. Indeed, some of the more heavily infested mussels may be crushed between finger and thumb (pers. obs.). This weakening is likely to render them more vulnerable to predation and mechanical effects of wave action. As yet there is no explanation for the outbreak or for its unusual destructiveness, but the intensity of *Mastigocoleus* sp. infestations has worsened considerably over the past several years (pers. obs.) and further work is needed to gauge its impact.

CHAPTER 13: A SURVEY OF SYMBIONTS AND PEARLS IN *CHOROMYTILUS MERIDIONALIS, MYTILUS GALLOPROVINCIALIS, AULACOMYA ATER* AND *PERNA PERNA*

INTRODUCTION

This chapter records prevalences of minor symbiotic fauna from each mussel species. They are deemed minor in terms of their numbers or apparent impact on the host; but this is not to deny that some may present a pathological threat to mussels elsewhere. Thus such parasites as *Polydora* and *Mytilicola* are treated here as minor symbionts. Other organisms encountered that may have higher prevalence and intensity, are explicitly parasitic, or cause damage to the mussel host have been described previously.

The prevalence of pearls is also included here despite evidence (Seed 1991) that, at least in some cases, the growth of pearls is stimulated by gymnophallid metacercariae. That pearls are included here rather than in the chapters (2, 4 & 6) concerning these parasites is because of the difficulty in assessing their deleterious effect on the host and also in ascribing them to the appropriate digenean. See Seed (1991) for references to structure, composition, and factors governing the occurrence and abundance of pearls.

STUDY LOCALITIES, MATERIALS AND METHODS

These are described previously in the survey of digenean parasites. The categories of symbionts are not all the same taxonomic rank; thus mytilicolids and other copepods are entered separately, as are polydorids and other polychaetes. This is because categories were chosen for their potential biological significance to the host rather than according to a strict taxonomic hierarchy. Percentage prevalences for each category and the number of categories found in each mussel sample are given below.

RESULTS

Table 1. Percentage prevalences of symbionts in *Choromytilus meridionalis* from Blouberg and Dido Valley.

Categories	Blouberg *n* = 650 %	Dido Valley *n* = 850 %
mesozoa	0.3	--
pearls	1.4	3.1
nemertea	--	0.2
nematoda	0.5	2.7
copepoda	0.5	0.7
isopoda	0.3	--
polydorids	--	0.6
other polychaeta	0.3	1.8
clams	--	1.7
gastropoda	--	0.1
N° of categories	6	8

Table 2. Percentage prevalences of symbionts in *Mytilus galloprovincialis* from Blouberg and Saldanha Sea Farm ropes.

Categories	Blouberg *n* = 800 %	Saldanha Sea Farm ropes *n* = 850 %
mesozoa	--	0.1
pearls	5.9	1.7
turbellaria	0.1	--
nemertea	0.1	--
nematoda	1.6	0.2
polydorids	0.4	1.5
other polychaeta	1.6	0.4
oligochaeta	0.1	--
copepoda	0.3	0.2
isopoda	0.3	--
amphipoda	0.5	--
nauplius larvae	0.1	--
ostracoda	0.1	--
mussels	0.6	--
clams	4.4	--
N° of categories	14	6

Table 3. Percentage prevalences of symbionts in *Aulacomya ater* and *Perna perna* from Blouberg and Dido Valley respectively.

Categories	*Aulacomya* Blouberg $n = 800$ %	*Perna* Dido Valley $n = 750$ %
mesozoa	--	0.1
pearls	6.4	7.7
turbellaria	0.3	--
nemertea	0.4	4.8
polydorids	0.8	0.5
other polychaeta	7.1	3.9
oligochaeta	0.5	--
mytilicolids	--	0.1
other copepoda	2.6	1.3
isopoda	0.4	0.4
amphipoda	0.6	--
ostracoda	0.3	--
nauplius	--	0.1
mites	--	0.1
clams	16	1.9
mussels	0.8	--
gastropoda	--	0.3
N° of categories	12	12

DISCUSSION

Nemerteans, turbellarians, and polydorids

Nemerteans varied in prevalence from 0 to 4.8% and nematodes from 0 to 2.5%. This is comparable with their occurrence in beds of *Mytilus edulis* in Wales where they are reported to be (Lintas & Seed 1994) fairly common. Turbellarians varied from 0 to 0.3% prevalence. This is considerably lower than 24.7% prevalence of rhabdocoel turbellarians in the gut lumen of *Tapes philippinarum* from British Columbia (Bower, Blackbourne, & Meyer 1992). *Tapes philippinarum* is mentioned here because it is an introduced species to British Columbia and thus bears comparison with the South African *Mytilus galloprovincialis*. It is noteworthy that both these introduced species have an extensive symbiotic fauna.

Prevalence of polydorids varied from 0% to 1.5% and only in one sample (from the ropes at Sea Farm) did it exceed 1%. In contrast, Ambariyanto & Seed (1991) report

prevalences from 32% to 66% in Welsh *Mytilus edulis*. They found values for *Polydora ciliata* in *Mytilus edulis* highest in estuarine conditions; in the purely marine environment prevalence was 38%. The South African survey reveals low prevalences also in comparison with that by Pregenzer (1983) in Australia who reports a range of values from 0.6% to 24.2%. From 14 collection localities only two had prevalences below 1%. Four were above 10% and seven were above 5%.

Crustacea

Up to 0.1% of mussels contained mytilicolids. This contrasts with a mean of 3.9% in *Tapes philippinarum* in British Columbia (Bower, Blackbourne, & Meyer 1992). In South African mussels other copepods occurred with 0.2% to 2.6% prevalence depending on locality. This was comparable to a mean of 2.5% in *Tapes philippinarum* from British Columbia (Bower, Blackbourne & Meyer 1992) but far lower than that in Australian *Mytilus edulis* (Pregenzer 1983) where a prevalence of over 50% was recorded.

Although Calvo-Ugarteburu (1996) found two pea-crabs (pinnotherids) in "a few thousand" *Perna perna* during a study based to the east of the present work, none was found in the six populations studied here. This low South African prevalence contrasts with a mean prevalence of 13.7% in *Tapes philippinarum* from British Columbia (Bower, Blackbourne & Meyer 1992) and prevalences varying from 0% to 80% in Australian *Mytilus edulis* (Pregenzer 1983).

Mites

Mites occurred at a prevalence of 0.1%. This is not unexpected. Lintas & Seed (1994) reported a number of mite taxa from a *Mytilus edulis* bed in North Wales.

Nestling molluscs

These gastropods and pelecypods are found inside living mussels. Gastropods, up to 2mm long, were found in some 0.3% of mussels. The pelecypod population consisted of mytilids and clams. The mytilids, all less than 1mm long, were found at a prevalence of up to 0.8%. Nestling clams were more common with prevalences ranging to 16%. They appear to be minute examples (less than 1mm) of *Venerupis (Tapes) corrugatus*. *Venerupis* is common (pers. obs.) at Blouberg in the substratum

beneath the predominantly *Choromytilus* mussel beds in tidal pools.

Pearls

All four species of mussel had pearl bearing individuals. From 1.7% to 5.9% of *Mytilus,* 1.4% to 3.19% of *Choromytilus,* 7.7% of *Perna* and 6.4% of *Aulacomya* contained pearls. It is surprising that *Mytilus* should contain pearls since it has not been found harbouring any digenea at Blouberg. And although gymnophallids have been found in *Choromytilus, Perna* and *Aulacomya*, the latter was found infected only once, yet its prevalence of pearls is higher than that in *Choromytilus* which has up to 100% prevalence of a gymnophallid (Chapter 4). The relationship between parasites and pearls obviously requires clarification.

Comparison of variety and prevalences of minor symbionts between species and localities

It is noteworthy that *Mytilus* from Blouberg has the greatest diversity of minor symbionts with 14 categories. Second are *Aulacomya* from Blouberg and *Perna* from Dido Valley, each with twelve. These are followed by *Choromytilus* from Dido Valley with eight; *Choromytilus* from Blouberg and *Mytilus* from the Sea Farm ropes each have six.

That *Mytilus* and *Aulacomya* should be among those with the greatest diversity of minor symbionts when they have very few parasites compared with *Choromytilus* is curious. The low numbers of symbionts in *Mytilus galloprovincialis* from the Sea Farm may be attributable to its isolation on the culture ropes. This could be accounted for by the ropes having a smaller resident habitat fauna and thus there being less opportunity for these to get into the mussels. Support for this comes from the report of Pregenzer (1983, p387) who noted pea-crabs, copepods, larval digenea and polydorid polychaetes in *Mytilus edulis.* He says: "cultivated mussels are generally less infected due to their distance from the bottom of the waterbody and time in the water".

This diversity raises questions about the nature of the symbionts. Do they gain access to the mussel by chance or do they seek it? If it is the latter, then why is *Mytilus*

galloprovincialis from Blouberg the most occupied? Is it the most accommodating? Obviously, one must first establish that *Mytilus* really does have a higher occupancy than the other mussels. To achieve this a number of independent samples should be taken from each locality for each mussel species and the diversity of symbionts subject to statistical analysis after each organism has been identified more rigorously than that done here. The species of symbionts could be keyed out to see if they are the same for each mussel species. If after this, the difference between species appears statistically significant, then we must ask if there is evidence of active entry to the mussel. A way of testing this would be to compare the number and types of co-fauna in the external habitat with the number and types of symbionts. If the organisms found in the mussels are true symbionts with some degree of dependency on the host, and not just accidents, then one might expect to find fewer of the symbionts represented in the external co-fauna. If numbers outside and inside are proportional, then it could be inferred that the process is passive. If the numbers outside and inside are not proportional, it suggests that the fauna are actively entering the mussels.

Comparison of the symbiont numbers between *Mytilus* samples from Blouberg and from Saldanha Sea Farm ropes shows a marked difference. The mussels from the ropes contain considerably fewer species and at lower prevalences. This suggests that the high numbers in *Mytilus* from Blouberg are a result of the greater numbers and diversity of the fauna there and the proximity of the fauna and the mussels - they are in a mussel bed rather than culture ropes. Results here suggest that there is some proportionality between numbers of fauna outside the mussels and those getting in.

The aggregate prevalence of symbionts appears higher in *Choromytilus* at Dido Valley than at Blouberg. In addition, at Dido Valley the aggregate prevalence of symbionts in *Perna* was comparable with that of *Choromytilus*. Prevalence at Blouberg in *Aulacomya* was also higher than that in *Choromytilus*. *Aulacomya* is perhaps a more suitable host than *Choromytilus* or it may be that symbionts have greater opportunity for entry into *Aulacomya* because it is the most subtidal of the mussel species examined. This could be tested by using caged samples of the different mussel species at different tidal levels to see if the numbers tend to equalise over time in the same conditions.

This survey could be refined using multiple sampling techniques and more rigorous classification of the organisms. Since, however, the thrust of the survey was to identify suitable parasites for practical integration into a scheme of stress and none of these organisms was considered significant enough to merit inclusion, this account remains a collation of data gathered as a contingency to other work.

PART IV:

ASPECTS OF STRESS THEORY

ASPECTS OF STRESS THEORY

PREFACE

The following chapters (14 to 30) explore aspects of biological stress. Chapters 14 to 17 survey some philosophical approaches to the nature of the organism and its general context with stress studies. Chapters 18 to 24 examine stress in detail. The most significant models of stress are analysed, compared and contrasted. This is to see where the disparate applications and usages may be integrated. Equally importantly, it is hoped to delineate where usages such as individual and ecological stress are non-integrable. Chapters 25 to 30 proceed to develop ideas of stress through their logical ramifications and to account for a few miscellaneous but allied concepts such as selection. It is hoped to arrive at new approaches to investigation and conceptualisation of the field.

The resulting survey of stress literature provides a base on which to develop ideas and speculations. In chapters 31 to 40 the salient findings of this survey are summarised and developed. Suggestions are made for further experimental work.

The text is comprehensively referenced. Work drawn from other authors is duly cited. Where other work has stimulated ideas set out here, it too, has been cited. Any other schemes, constructs or insights not credited are the work of the author of this thesis.

CHAPTER 14: INTRODUCTION

"There is a distinct lack of agreement among experts on any particular definition of the concept of stress" (Bailey & Bhagat 1987, p207). "Stress is a significant but elusive concept in biology, and, like any concept, it can be misunderstood, misinterpreted, or abused" (Sindermann 1990, p219). "We cannot really explain what stress is" (Moss 1973, p27). "Although the term stress is commonly used, its meaning is often obscure" (Ivanovici & Wiebe 1981, p14). "Nor is there general acceptance of what the word stress means" (Hattingh 1988, p731). "The quasi-technical use of the word 'stress' as a referent for a bewildering range of phenomena continues to produce semantic, linguistic and taxonomic confusion" (Turkkan, Brady & Harris 1982, p153). "As more theorists attempt to examine and define stress, this concept becomes more diffuse and complex" (Zegans 1982, p137).

These are among the more useful statements made about stress. They point not to what we think we know but rather to our present state of ignorance and confusion. Current usage of stress in biology is as a label for deleterious but often ill-defined conditions or change. So ill-defined is it that Pearlin (1982, p369) says: "Almost all stress researchers experience some confusion and despair about the field." We would thus do well to heed the exhortation of Broom & Johnson (1993, p63) that: "Research on the fundamental biology of stress and welfare would benefit from a rationalization of its terminology".

What is stress? What is its origin? What does it do? Where does it act? Is it the response shown by an organism when exposed to unfavourable conditions? Or does stress refer to the degree of exposure to a noxious agent? Or is the organism stressed by its response to the agent? Is stress, thus, a cause or an effect, a combination of both, or something else? It is unfortunate that stress, in the literature, appears to cover all these. Stress is now doing so much duty that one could suspect it of being a "weasel word" (Hull 1988, p7): one with "plasticity of meaning that helps researchers to buy time to develop their positions". It is high time to "define the term accurately and try to unravel its biology" (Broom & Johnson 1993, p173).

A further complication is that the term stress occurs in widely different fields of study.

Cameron & Meichenbaum (1982, citing Cannon 1942) mention anthropological stress. Other stresses include biological stress (Selye 1956), cultural stress (Zborowski 1969), ethological stress (Tinbergen 1974), and psychological stress (Lazarus 1966). Similar or overlapping fields may also claim divergent usages. For instance, the medical concept of stress (Levitt 1980, p3) "is quite different from both the biological and the physical concepts".

Stress is sometimes a label for cause: "Stress has also been defined as any force that pushes the functioning of a critical subsystem beyond its ability to restore homeostasis" (Meier 1972, in Auerbach 1981, p30). Sometimes stress is a label for an effect: "Stress has been viewed as a response to external or internal processes which reach those threshold levels that strain psychological and physiological integrative capacities situated close to, or beyond, their limits" (Auerbach 1981, p30).

The term stress is itself in danger of being stressed. Stressed so much that Begon, Harper & Townsend (1990, p862) have observed: "The word (stress) is often used confusingly in two senses, both to describe force and the condition induced in the organism by the force - a confusion of stimulus and response. We have tried not to use the word." Charlton (1991) advocates that it not be used at all.

One thing that we can say about the word stress is that it is here and that it will not go away. It is futile to attempt to suppress a word that has obvious utility. So instead perhaps we should try to find a more focused and enlightening meaning for it. Charlton's critique is valuable. There is circularity in much stress thinking. Here it has been straightened out, except when postulating a feedback loop. Charlton says that essentially dissimilar things need essentially dissimilar names. The task here is to demonstrate an essential similarity behind all legitimate uses of the word stress. Thus the present diverse usages of stress obviously need critical examination.

Here, stress phenomena will be re-appraised so that a useful theoretical construct may be produced. As Derogatis (1982, p287) urges, "One of the most demanding requirements that can be placed on any construct is that it serve as the basis for effective operational measurement". Accordingly, the principal aim is to devise an operational relationship of

stress, strain and fitness.

A provisional definition of stress is here proposed. Stress is a term denoting agents and phenomena of change in the organism, population, lineage or ecosystem. It is usually deleterious but sometimes appears beneficial.

This definition may over-generalise but this is to ensure that the phenomena of interest are included in its ambit. To begin sharpening this definition let us start by looking more closely at the nature of the organism. We must establish what the organism is so that we can ascertain how stress relates to it.

Organisms are dynamic systems: during their life cycles they may exhibit many changes. These changes may be interpretable as fluctuations in fitness or, at least, may interfere with indicators that we associate with fitness levels in the organism. Thus if stress phenomena are to be detected and interpreted we must have a clear idea of what constitutes "normal fluctuations". One must also remember that the fluctuations may not be around a fixed datum.

The contextual meaning of stress and the hazards of misreading its signs and symptoms are underlined by Livingstone, Widdows & Fieth (1979, p51). They report that homeostatic capacities of *Mytilus* may fluctuate according to the season: "the results imply that at different times of the year, or at different stages of the gametogenic cycle, certain tissues are less able to use ninhydrin positive substances as the solute for isosmotic regulation". Also in bivalves Richardson (1988) reports that endogenous rhythms of shell formation occur in the clam *Tapes philppinarum*. Nelson & Demas (1996) report seasonal changes in immune function in a wide range of vertebrates.

Baseline fluctuations also occur in mammalian adrenal cortex activity. Here, the activity is apparently cyclic and the reactivity of the animal is dependent on its stage in the cycle (Broom & Johnson 1993). We must also be aware of the possibilities of similar changes during ecological successions and in lineages as they undergo evolution. It may thus be more difficult to talk of return to normal levels. It follows that we should know more about the

organism or biological system we are studying, or at least be explicit about our assumptions of stability in organic processes. Clearly, a deeper consideration of the general nature of organisms is essential.

The following section explores various conceptualisations of the organism. The aim is to devise a model of the organism that most appropriately accommodates stress phenomena.

CHAPTER 15: THE ORGANISM: WHAT IT IS OR WHAT IT DOES?

PROBLEMS IN DEFINING AN ORGANISM

Since we are interested in the phenomenon of stress and the living organism, we must examine the biological meaning of what it is to be alive. In addition, we must identify key characteristics of "aliveness". Among the more important of these are structure, function and process. If structures typify life, then what structures are most important? Similar questions also may be asked of functions and processes.

Approach to these questions requires that we define our terms, starting with an appraisal of definition. A definition (*Chambers English Dictionary*, 1988) is: "a description of a thing by its properties, or an explanation of the exact meaning of a word, term or phrase". This definition requires in turn that the meaning of property be defined. *Chambers English Dictionary* (1988) says that a property is "that which is always present: a characteristic: an essential detail".

Clearly, problems may arise when looking for properties that are always present in, for instance, a trematode throughout its life cycle. There are many stages and they differ markedly. In a trematode life cycle it is possible that the only constant features would be the presence of identifiable proteins or genetic components such as the DNA complement, associated enzymes and the karyotype. Though the excretory system may be of help, the life cycle stages of trematodes are often connectable only by experiment and observation. In consequence, no simple formulation of constant features can identify the different stages of an organism throughout its life.

The first characteristic of life mentioned above, structure, is defined by *Chambers English Dictionary* (1988) as "the arrangement of parts". We must be aware, though, that this term may be ambiguous. To Young (1993) an organ can have structure or it can be seen as a structure; the first refers to form, the second refers to the actual organ. Structure is here understood as the latter.

The second characteristic, function, is: "the doing of a thing; the vital activity of a tissue,

organ or cell," (*Chambers English Dictionary* 1988). Function implies, if only weakly, a goal oriented action; it can profitably be replaced by 'process', which has fewer teleological connotations. Process is defined (*Chambers English Dictionary* 1988) as a series of actions or events; the series may be causally related but this does not imply purpose. Thus, processes are just a sequence of events. Some processes may enhance continued existence in organisms and some may compromise it. The problem with function arises because it tends, implicitly, to denote only those processes that enhance the organism's ability to continue existence. Other processes are often called malfunctions. Functions thus acquire ontological significance. This is not a serious error but since existence is often seen as somehow 'better' than non-existence this criterion of judgement must be made explicit.

Biological thinking is, of necessity, existocentric. This may be detected by a tendency to intrusion of normative as well as descriptive assessments of organisms: the less fit are not as 'good' as the more fit. Good or otherwise is not the issue here; we can only note the persistence of different organisms.

TELEOLOGY

Function connotes teleology and teleology has an interesting but uncomfortable relationship with biology. Kant deals with it in his *Critique of Pure Reason* (Trans., Smith 1973, p520). Though he ultimately rejects it, Kant says of the teleologically based physico-theological proof (argument from design): "It enlivens the study of nature, just as it itself derives its existence and gains ever new vigour from that source. It suggests ends and purposes, where our observation would not have detected them by itself, and extends our knowledge of nature by means of the guiding-concept of a special unity, the principle of which is outside nature".

Ruse (1989) deals with teleology in biology. Although he argues for teleology in biology on the utility grounds that it has great heuristic value -as did Kant 202 years before- he does recognise (p53) that: "Perhaps logically, in the interests of conceptual purity one might eliminate the teleology of biology". His grounds for this assertion are more reasons for dropping the term function. Furthermore, because we are attempting a fresh appraisal of stress phenomena, a consciously non-teleological approach has been selected.

Current wisdom, taking into account the implications of Heisenberg's (1927) Uncertainty Principle, and Chaos Theory (Prigogine & Stengers, 1985; Schaffer & Kot 1986; Berryman & Millstein 1989; Gleick 1991; Stewart 1993) can furnish us with either a statistical treatment of the probability of future events or with a system which, though rigidly deterministic, is so complex that it cannot be used as a basis for long term prediction. Prediction is rendered even more difficult by the advent of complex or deep chaos. Moore (1990) says that this form of chaos is unpredictable even if the starting conditions are known exactly.

Phenomena have emergent properties, which in principle cannot be predicted. Yet organisms often have the adaptability to cope. If there is a designer then the designer must be able to predict the unpredictable. If so then why all the waste? In consequence, a teleological approach that refers to the future to explain the present may be approaching the problem from the wrong end. This echoes Kierkegaard's observation on our approach to life: "Life must be understood backwards but...it must be lived forwards" (Dru, [Ed. and trans.] 1938, p127).

The noted stress researcher Selye (1956, p243) is a strong proponent of causation and teleology. He critically comments that: "many of the most outstanding investigators of our time" believe: "that one can, and should, merely register scientific observations, refraining from all considerations of causality." In this he disagrees and asserts that sensations of causality and purpose are inherent in the structure of the human brain.

This cannot go unanswered. Inferring the nature of biology from the structure of the human brain may not tell the whole story. And one should not confuse biology with biological thinking. Moreover, causality and teleology need not be coupled as Selye asserts. We can discard a teleological approach to science, at least temporarily. In contrast, it would be difficult to discard causation; it is, for better or worse, the currency of the mind. Nevertheless, causation may only be an essential artefact. It is in the mind of the beholder; as Hume's (1898) argument suggests. Selye (1956) sees purposeful causation because he observes the world through teleologically tinted glasses. Things that persist by way of processes may look purposeful to us, but they cannot help that.

The confusion of biology with biological thinking requires more comment. The subject

matter of biology is what exists; it is thus existocentric. The root of biology in this context is selection, which is based on elimination (Holloway, Sibly & Povey 1990) it is eliminocentric. Objectively, one is the mirror image of the other. But the terms conjure up different world-views that do not have the symmetry of mirror image and object. Therein lies the potential for distortion and tendency to see such things as aims and goals.

Selye's (1956) notion of teleological centres is unnecessarily complex. His assertion (Selye 1956, p246) that the formation of teleological centres within the universe "appears to be one of the great laws of nature" borders on the "centrocentric". Natural phenomena may be better explained by describing what they are and what their propensities are, rather than by postulating a centre of self-interest or any vitalist force.

The problems of teleology in biology have been reviewed extensively. These include works by Nagel (1961; 1979), Ruse (1973; 1989), Ayala (1970), Mayr (1974), and Wright (1976). Little remains to be said but one can add that teleological explanation commonly posits more than a simple descriptive inductive explanation: it requires an understanding and identification of motive. This may not be attainable. Human motives are difficult enough to ascertain, even after much investigation and insight. The motives (if they have them) of more distantly related phyla are likely to remain inscrutable.

Thus, in this study of stress the teleological view is discarded. Phenomena will be judged as to whether they enhance the persistence of the organism or its lineage. An organism is thus studied as a bundle of interconnected phenomena, which through their interactions may or may not keep it alive. These interactions may appear teleological but this is a by-product of our processes of perception and understanding.

THE SYSTEM PROPERTY VIEW

What alternatives are there to teleology? The system-property view is an organismic approach and thus interprets phenomena in terms of processes inherent in the organisation. Nagel (1979, p288) refers to the system-property view of goal directed processes: "It is evident that being goal directed is a property of the system in virtue of the organisation of its parts'. He adds: "the origins of the programs are left to be explained by evolutionary theory".

This last sentence should finally lay to rest hopes for the existence of any *élan vital.* Teleological explanations are thus more acceptable if their ontology is elucidated - apparently goal directed phenomena can come about by their being part of the process that contributes to persistence of the organism. Once the underlying assumptions are made explicit the rest will fall into place without giving offence.

It is appropriate here to consider the theoretical basis of our interpretations concerning the nature of life. In this respect, two competing ideas, mechanism and organism, deserve scrutiny.

MECHANISM AND ORGANISM

According to Lincoln, Boxshall & Clark (1985, p149), mechanistic theory is: "That natural processes are mechanistically determined and can be fully explained by the laws of physics and chemistry". Thus, mechanism is devoid of teleology if one considers that anything that happens is by definition pre-determined. The machine runs on and it is not goal directed unless the original design (if it was designed) inclined the mechanism to a certain course of action. Nevertheless this apparent teleology would in effect involve looking backwards at its causation and not forwards to where it is going.

The organismic approach dwells on the hierarchical nature of biological organisation and the new phenomena that arise from new structures. "In substance, therefore, the organismic standpoint is a variant of the doctrine of emergent evolution, for, like the latter, it maintains that traits exhibited by a hierarchically organised system cannot be reduced to, or explained by, the properties of parts of the system whose mode of organisation occurs on a lower rung of the hierarchy" (Nagel 1979, p264). This is further supported by Sudakov (1996) who in a stress study says that the body is a "total combination of interacting systems of different levels of organisation". This alludes to a dialectical process between components within the body. And Stewart (1993, p3) says that life is characterised by complex systems and, dynamically speaking, such systems lie at "the edge of chaos". Such systems are not predictable from a simple knowledge of their interacting components. Perhaps we should consider the advice of Lewin (1993, p5). He urges that it is necessary to look at "the whole system even if it means taking a crude look, and then allowing possible simplifications to

emerge from the work".

A moderate organismic approach that makes allowance for mechanism is proposed here. Analysis of components based on assumptions of mechanism is not, and cannot be, excluded from biological study and explanation. Such assumptions should, however, be seen in an organismic context.

The transition from mechanism to organism is suggested by the dialectical Law of Transformation of Quantity into Quality (Somerville 1946, p172). This states that change phenomena are "not merely a mechanical accumulation of quantitative factors, but always involve the emergence of new qualities, that is of complexes of properties not possessed before". As Nagel (1961, p434) says: "Organismic biologists are therefore on firm ground in maintaining that mechanistic explanations of all biological phenomena are currently impossible."

To progress coherently from mechanist to organicist paradigms, one would need a greater understanding of the sciences of chaos and complexity. And even then, the phenomena contingent on new hierarchies of complexity are likely to require empirical discovery of their own general laws. This is because new levels of complexity lead to new phenomena that are not implicit (using our present investigative philosophies and methods) in lower orders of hierarchy. A mechanist attempt to predict the nature of an organism requires an extrapolation from the more basic sciences such as physics, chemistry, biochemistry and physiology. In addition, it must also allow for the influence of hierarchical organisation, complexity and chaos. Thus we can conclude that if we wish to know more about organisms, it is far easier to study their phenomena directly.

This is supported in part by Calow (1989, p175). He says "sound predictions can only be based upon mechanistic understanding of the interaction between system and input - and this usually entails representing the system of interest in terms of mechanisms at lower levels in the hierarchy - individuals in terms of physiology, populations in terms of individuals and so on." He continues: "The extent to which the physiological properties of individuals dominate the influence of ecological interactions between individuals in population dynamics would

seem to be a matter for empirical exploration."

The organismic approach advocated here is intended to be instrumental and empirical rather than dogmatic. It thus allows that mechanism may be more applicable if the above objections are met.

THE ORGANISM AS A PROCESS

Life is typically characterised by the phenomena of irritability, respiration, reproduction, excretion, nutrition, movement, and growth. If a definition of an organism by such phenomena is to be scientific, then such phenomena should be present constantly, or at least as frequently as required to satisfy the meaning of definition. Such features should also be widespread or, ideally, universal in the population.

A problem emerges here. For an organism such as an anchovy its most common propensity is to be eaten. In fact more anchovies are eaten than reproduce. It would, however, be unsatisfactory to define an anchovy in terms of its most common destiny, which is death by predation. Instead we would be more tempted to define it in terms of the long-lived reproductive individual - a statistical oddity. Thus, a meaningful definition of an anchovy, based on what it does, would be in terms of the statistical oddity. The greater number are then defined in terms of the actions or fate of a minority. But which minority should be used for the definition and why not some other? And what are the criteria of judgement? A further difficulty arises: if any organism is defined by what it does or what happens to it, then one that does differently is liable to be identified as different.

Problems may thus arise if an organism is defined by its fate or by what it does. There are enough problems in defining a thing by its properties (Putnam 1975); even essential properties may vary in weighting of importance. Clearly, basing definition of an organism on its actions or experiences is asking for trouble. These problems may be eased by redefining the organism in terms of what it <u>is</u>. Does this mean structure?

THE ORGANISM AS A STRUCTURE

Why choose structure? Because structure is more basic than process. That structure is

ontologically anterior to process can be inferred by consideration of the nature of the grey area that lies between life and death: suspended animation. A definition of death would be useful here. It is the total, and irreversible cessation of all life processes (Lincoln *et al.* 1985, p63). It is obvious that suspended animation (such as the storage of sperm at cryogenic temperatures) is not death, yet it is sparse in the processes that typify life. It is an example of structure without significant process. On the other hand it is almost redundant to state that there are no examples of process divorced from structure.

These points suggest an ontological hierarchy beginning with matter and form. Together they create structure (including morphometrics and genetics). And from structure and movement arise life processes. If this is accepted, then processes, being only contingent on structure, are subject to an extra level of uncertainty than are structures. Thus the structure of an organism is, in the main, more concrete than its processes; and definition of the organism by its structure rests more securely on fewer levels of inference.

How can stress affect structure? Physical stress breaks bones. Cuts, burns, and the denaturing of proteins by such agents as heavy metal ions, salts and pH shifts are examples of other structural changes. Another example of structural change is that wrought by carbon monoxide on the conformation of the haemoglobin molecule. Carboxyhaemoglobin is produced which does not transport oxygen in the normal way. Similar changes to protein structures occur in enzymes when they are inactivated by inhibition. Stress, evidently, disorders structure.

Disorder can be in the context of entropic (absolute) disorder, which entails degradation towards randomness. Or disorder can be relative. A parasite living within an organism is (in absolute terms) highly ordered, but in terms of the host it is an agent of disorder.

Biological structures are often extremely complex and this leaves them vulnerable to entropic degradation. In consequence, persistent organisms are supported by structure-maintaining processes. We must, then, develop our model and entertain the possibility that stress can affect both structure and process and that these changes can also act as stresses.

Clearly, organisms may be considered as an interaction of structure with process. In consequence, an interim definition of life is proposed. It is the continuity of complex structure-sustaining chemical reactions in a structured body. Structure leads to processes which dialectically change the structure and result in a structure/process complex. Structure is mutable by process and vice versa. This definition borrows much from the organismic approach (Nagel 1961 & 1979). It dispenses with vitalism and de-emphasises mechanism.

Selye's (1956, p248) definition of life supports this in the critical area of maintenance: "Life is perhaps best defined by the degree to which it has developed certain characteristics, particularly those of recreating its own kind (growth, reproduction) out of less highly organised materials and of maintaining its structure tenaciously..."

We can thus develop our model of the organism and now we consider it as an interaction of structure and process.

THE ORGANISM AS A STRUCTURE/PROCESS COMPLEX

Being alive entails a degree of physicochemical separation between the organism and its environment and even between components of the organism. Because of this, many unlikely gradients of order and concentration exist - internally between organs as well as externally. They persist with the help of processes. Perhaps we can say that, all other things being equal, the more gradients and structural order in an organism and the more energy and matter flowing through it the more alive it is.

We can now consider the organism as a structure/process co-ordination whose primary qualities are that it exists and that it has some likelihood of continued existence - as an individual, as a lineage and as a species. These are the qualities that will be examined when fitness is discussed in a later section.

Persistent organisms are sustained by appropriate anabolic processes; usually at the expense of energy supplying catabolism. This anti-entropic maintenance requirement is underlined by Kleiber (1975, p253): During starvation "life presumably goes on as long as continual changes toward a condition, unfit for life, are reversed by metabolic processes. These life

maintaining metabolic processes require energy, and changes toward death become irreversible when the organisms lacks the power of restoration." In effect, entropic damage is shifted to expendable energy stores: "pumping out the disorder" as Odum (1985, p420) would put it.

Life thus depends on the favourable balance between anabolism and catabolism such that the organism is maintained in a viable condition. It is suspected that the operational meanings of strain and fitness reside in the co-ordination of these processes and manifest themselves in the structure/process complex. Stress may be viewed as a causal agent, which impairs this co-ordination - i.e. stress causes disorder. Here we can sharpen our definition of disorder. Disorder in this biological sense may be seen as a shift of structure or process from a condition which enhances persistence to a less favourable one.

The terminal effects of stress, says Sindermann (1990, p220) are a "...failure of critical biochemical functions leading to physiological and morphological disorders and death". Disorder is mentioned but its significance is not sufficiently emphasised. **Clarification of the stress issue hinges on the thesis that disorder is the underlying and universal effect of stress.**

Despite the manifest complexity of living organisms, simple processes and their fluctuations are often accepted as stress indicators in research. Inference is drawn from the fluctuation that there has been a change in the underlying structure/process complex of the organism. Vincent & Leahy (1997) achieve a good correlation between heart rate fluctuations and subjective assessments of excitability in dogs. This was done with a view to assessing levels of welfare. But in the main, as Broom & Johnson (1993, p93) say: "Care must be taken to consider the biology of the animal when using heart rate changes as an indicator of welfare". Any single measure is thus likely, at best, to be only an indirect indicator of the condition of the organism. Furthermore: "A combination of measures is preferable if valid comparisons of conditions affecting the welfare of animals is to be made" (Broom & Johnson 1993, p108). As an example, core body temperature in some animals fluctuates diurnally (Broom & Johnson 1993, p94-95). On this fluctuation may be superimposed the effects of disturbance. And even the timing of the disturbance during the fluctuation may be decisive in determining

whether the temperature rises or falls. For instance, the handling and transport of calves causes increased adrenal cortex activity, a concomitant may be increased body temperature. The body temperatures of laboratory rats may increase in response to disturbance by a storm.

Conversely, body temperatures may decrease in response to stresses. Infant monkeys after prolonged separation from their mothers have depressed body temperatures. Bradycardia and depressed temperatures are also found in stressed tree shrews - those that had lost fights (Broom & Johnson 1993). Single processes may thus not suffice as stress indices.

Zegans (1982, p137) warns, "we must avoid reducing the rich concept of stress to the older single entity, mechanistic model of disease". Single process rates may have their uses but it is important to ascertain what precisely is affected in the structure/process complex and how this affects fitness. Only then can we judge changes in rates harmful or otherwise. We must also remain conscious of the extra stages of inference that research based on change of process requires as opposed to change of structure. With each inference is an attendant possibility of error.

Nevertheless, simple physiological tests - because of their brevity - are common even though the effect of stress may be better ascertained by a broader study of the organism's living processes. This broader approach is advocated by Widdows (1978, p125): "the animals' response is not in terms of individual physiological rates but rather as a whole organism". Furthermore, Koehn & Bayne (1989, p158) state: "the effects of stress will be an integrated response involving all levels of functional complexity within the organism (molecular, cellular and physiological)". This integration is more nearly approached if the organism is considered as a co-ordination of living processes and their underlying structures. Physiological integrations may include scope for growth and growth efficiency (Navarro 1988 and Widdows 1978).

The concept of co-ordination presented here is inspired by analogy with the Pythagorean belief that the soul is an adjustment of extremes. Simmias refers to it in the *Phaedo* of Plato (Translation, Tredennick, 1983, p140). Echoes may be heard in Cannon, (1939) cited by Selye (1982, p8) when he speaks of homeostasis as: "the co-ordinated physiologic processes

which maintain most of the steady states in the organism". In organic co-ordination there is also an adjustment: one of structures and processes. Each makes the appropriate contribution to the stability of others and to the stability of the organism as a whole.

Selye (1956, p55) also uses the term adjustment, but his is one of opposition. He says "the condition of biologic stress is essentially an adjustment, through the development of an antagonism between an aggressor and the resistance offered to it by the body". The nature of the adjustment is influenced by what he calls conditioning factors. These are events and propensities that may influence the way in which the organism responds to a particular situation. He mentions two categories: internal conditioning factors are those which "have become part of the body" (Selye 1956, p95). In these he includes heredity and experiences. External conditioning factors include diet, climate and other current experiences.

From the interplay of Selye's (1956) conditioning factors, stress and the organism we can see that the dialectic of structure, process and environment can occur. An adjustment, it certainly is, but in reality it is not antagonism. It is rather, as he says above, that the stress becomes incorporated into the organism. When a stress acts, a new process is initiated which does not optimise persistence of the organism.

It must be understood that organic co-ordination does not aim at stability of the organism: the tendency towards stability is inherent in any persistent organism. Those without the tendency do not persist and are thus not so easily available for study. Moreover, organisms exhibiting processes which enhance their persistence are not programmed from any inner urge to persist. This urge, if it existed, is very defective, as the rate of mortality in all organisms suggests.

Co-ordination is also evident in Levitt (1980, p538): "all stresses may produce a primary indirect injury due to a metabolic disturbance. These disturbances are of two main types - a deficiency of an essential metabolite or an excess of a toxic metabolite". In addition: "Thus, a low temperature stress may simply decrease the rates of all metabolic processes reversibly, but not all may be decreased to the same degree. Therefore, if the stress is maintained for a long enough period, the strain may conceivably lead to an accumulation of toxic intermediates or a deficiency of essential intermediates".

Interpretation of performance fluctuations is clearly easier if one uses the structure/process complex model of the organism. Such a holistic approach keeps in mind the importance of integration of all living processes. To such an organismic system-property view we should, however, add extra emphasis to the influence of the environment. In this respect Selye's (1956) conditioning factors are a good starting point. In consequence, two aspects of stress should be considered: its instantaneous effect on the processes of life and a historical effect by becoming part of the new co-ordination of the organism. Clearly, we must now consider a larger role for the environment.

THE NATURE OF THE ORGANISM AS A STRUCTURE/PROCESS/ENVIRONMENT COMPLEX: ASEITY

If discussion is required on the nature of the structure/process/environment complex from the point of view of the organism then, for the sake of brevity, our first task is to propose a concise name for it: aseity.

The word aseity is taken from the noun used in theology to denote self-origination: that which is endogenous or comes from oneself. Aseity is the instantaneous quality of existence or beingness of the organism. It is a consequence of the dialectical interaction of the organism's phenotype with its environment. Phenotype is here understood as the structure/process complex outlined above.

That previous influences of the environment may become part of the aseity is supported not only by Selye's (1956) conditioning factors but also by Yousef (1985, p4) who says: "the animal and its environment form a system in which both act and react on each other". Cameron & Meichenbaum (1982, p696 quoting Levine 1975) agree: "...there may be critical development periods during which different types of interactions between organisms and their environments result in, "permanent and profound" individual differences in adrenocortical responses to environmental stress that persist into adulthood".

In an evolutionary sense, Grime (1989a, p6) says: "the form and extent of response to contemporary challenge may be strongly conditioned by ancestral evolutionary attunements

to earlier stresses." He continues, "It is also evident that the exposure of the organism to stress and its scope for phenotypic or genetic adjustment will depend critically upon its nutritional mode, life history and breeding system".

Aseity is an organism's propensities made manifest in any given conditions. And aseity is mutable by different conditions; such changes may precipitate different outcomes, some of which would compromise survival.

Phenotypic plasticity

Phenotypic plasticity could be seen as an aspect of aseity. Phenotypic plasticity is "the range of phenoyptes that can be developed by an organism if exposed to heterogeneous or changing environments" (Kammenga, Van Koert, Koeman & Bakker 1997). The plasticity allows optimal fitness in the new environment.

This may not be so in the other aspect of aseity. This aspect is manifest in shell deformities of *Crassostrea gigas* held in acid sea-water (Bamber 1990) and *Mytilus edulis* in brackish sea water contaminated with heavy metals (Sunila & Lindstrom 1985). Here it is less easy to see if the phenotypic change confers any fitness benefit on the bivalves. Is it a different phenomenon? Is there a contamination level at which phenotypic plasticity (with implied fitness benefit) occurs?

Developmental instability and fluctuating asymmetry

These phenomena may be related to aspects of the above and are other aspects of aseity. According to Moller (1996) developmental instability is manifest as the inability of the genotype to follow stable development to a normal phenotype when assailed by adverse environmental conditions. Fluctuating asymmetry is taken as a manifestation of developmental instability. Fluctuating asymmetry appears as bilateral asymmetry of morphological traits (Naugler & Ludman 1996). The degree of asymmetry, they say, is thought to correlate with the severity of the applied stress. Parsons (1990a, p141-142) concurs, "Fluctuating asymmetry is a useful trait for monitoring stress in the laboratory and in natural environments. Increased fluctuating asymmetry is a reflection of poorer developmental homeostasis at the molecular, chromosomal and epigenetic levels".

Parasitism and developmental instability have been documented by Escos Alados, Emlen & Alderdstein (1995) in hake *(Merluccius productus)* infected by the myxozoan *Kudoa* spp. They found that the otoliths were asymmetrical in shape, width and weight. These differences they interpreted as fluctuating asymmetry, which, they say, indicates developmental instability. They also found that asymmetry was significantly related to parasitism. Moller (1996) reviews the effect of parasites on the developmental instability of hosts and finds that generally parasites increase developmental instability as measured by fluctuating asymmetry.

Campbell & Emlen (1996), however, found fluctuating asymmetry an inconclusive indicator in chinook salmon *Onchorhychus tshawytscha* that were infected by vertical transmission with bacterial kidney disease. Two other recent works examine fluctuating asymmetry and arrive at no solid conclusion. Both discuss fluctuating asymmetry, stress and fitness. Forbes, Leung & Schalk (1997) look at fluctuating asymmetry in the damsel fly *Coenagrion resolutum,* they find that there is some relationship between fluctuating asymmetry and fitness, but that it is inconclusive. Leung & Forbes (1996) report some relationship between fluctuating asymmetry and fitness and between fluctuating asymmetry and stress. Again they find that the relationship is as yet inconclusive. These results may be improved upon if the recommendations of Naugler & Ludman (1996) are followed in their critique.

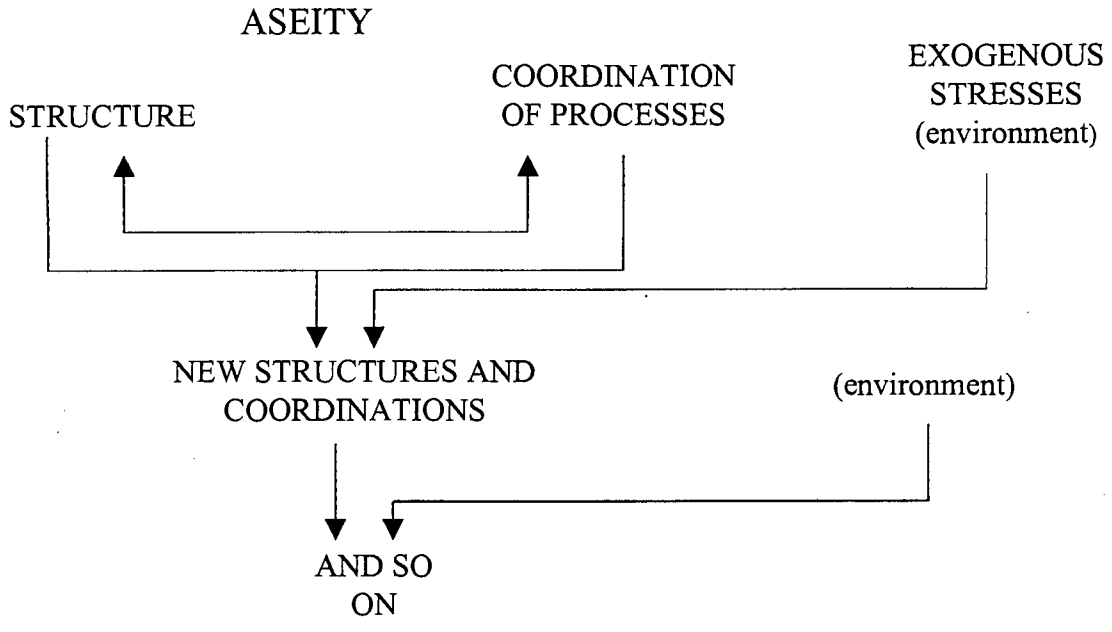


Figure 1. The interaction of stresses, aseity and the environment.

This model (Figure 1) posits that the future experience of the organism is influenced by its condition, and that of the environment. Not by what it 'wants' to become. It is, thus, not in pursuit of any goal: it is being what it is. With this model, organisms have no need of explanation by their goals or ends. Their actions may instead be likened to water; it follows the route of least resistance. Beware: least resistance does not imply no resistance. Persistent organisms are able to negotiate the worst stresses; but only at the cost of dealing with others.

Aseity is evident in developmental biology. Development takes place according to a recipe (epigenesis) rather than a teleological (preformation) blueprint (Dawkins, 1986): body components are not placed in their position but rather develop because of their nature. Aseity in individuals is closely analogous with evolution in lineages. The following also applies to aseity: "evolution is exactly what its etymology implies: it is an unfolding, an indeterminate, and in principle, inexplicable unfolding" (Ollason 1991, p92).

CHAPTER 16: DYNAMICS AND DELETERIOUSNESS

BENEFICIAL STRESS (EUSTRESS)

Notions of beneficial stress must be refuted and dismissed. Eustress (Selye 1982, p16) - the "pleasant stress of fulfilment", positive stress, and positive deflections are problematic. Such apparently beneficial aspects of stress have also been examined by Perkins (1982); Glass & Singer (1972); Esch, Gibbons & Bourque (1975); Haan (1977) - who proposes that gains as well as losses accrue from stress; and Zegans (1982) who says that stress can be deleterious or beneficial.

Conversely, Broom & Johnson (1993 p63), assert: "...the concept that stress can be beneficial...must be explicitly refuted." How can these be reconciled? How can the application of a stress sometimes appear beneficial? Stress is broadly understood to be deleterious, so how can it be admitted that it is sometimes the opposite?

Two explanations may dispel this confusion. First, the changes could be misinterpreted as beneficial when they are really the opposite. Second, an examination of the temporal regime of any particular stress phenomenon may bring clarity.

STRESS AND PROCESS RATES

Change in process rate, as we have seen previously, is not a sure indication of harm or benefit to the organism. Even widespread increases in physiological rates (which might be misinterpreted as beneficial stress) may be deleterious. Two examples follow.

The condition of malignant hyperpyrexia in man may occur on administering the anaesthetic halothane F_3-CHClBr (Meyers, Jawetz & Goldfien 1980). "Halothane inhibits the oxidation of diphosopyridinenucleotide-linked substrates in the mitochondrion. This inhibitory effect decreases oxidative phosphorylation and increases the activity of the glycolytic system. The increased demand for adenosine triphosphate production by a glycolytic system that has an inherently higher rate of 'futile cycling' than normal imposes a demand for energy on a very inefficient system. The high rate of lactate production that ensues calls for (via a feedback mechanism) a release of norepinephrine by the peripheral adrenergic nerves to compensate

for the shift in metabolism, and thereby a vicious cycle develops. The presence of a high peripheral resistance and the development of an intense peripheral vasoconstriction (mediated via norepinephrine) serves to accelerate further the release of norepinephrine, lactate production, tissue acidity, electrolyte changes, heat retention and muscle rigor. A summation of these effects leads to cellular death and the development of an irreversible syndrome" (Williams 1976, p25). This is also an example of positive feedback instability about which more will be said later.

Another example to show just how misleading simple changes in physiological rates can be is provided by the action of 2,4-dinitrophenol. This compound (Lehninger 1973) uncouples oxidative phosphorylation from electron transport. Respiration rates in tissue samples may stay the same or even increase but the production of ATP from ADP is blocked. A dose of 3-5mg dinitrophenol per kg body weight can increase the metabolic rate by more than 20% for over 24 hours (Meyers *et al.* 1980). This apparent increase in performance is offset by lowered efficiency of ATP production and a raised likelihood of damage to liver and kidneys, cataracts, neuritis, and anaemia. This agent is clearly harmful.

The notion of stress causing a loss of co-ordination (rather than a diminution of simple performance) may explain such misinterpretations. Thus, positive stress indications should be held suspect. Stress, if it is to be a useful concept, must at some point be deleterious. This brings us to a consideration of temporal regimes and stress.

STRESS AND TIME SCALES

If, despite the above, stress is still held to be correctly interpreted as causing a beneficial change in co-ordination, then we should examine the temporal regime. The reason may be that although stress does cause a reduction in fitness, it may also stimulate change in the organism to make it more resistant to stress in future.

Rapport, Reiger & Thorpe (1981, p270) say of Selye's eustress: "In the context of human medicine, not all stresses lead to reduced viability. Indeed, some stresses may challenge the system in such a way as to evoke an adaptive response that enhances human welfare". It is unfortunate that Rapport, Reiger & Thorpe (1981) refer only to Selye (1974) in which the

eustress concept is not developed; only stress and distress are discussed. Selye (1976) deals more fully with eustress.

Another example of apparent enhancement by exposure to noxious substance is given by Roesijadi & Fellingham (1987 in Gosling 1992, p392): "Exposure of mussels to Cu or Cd can induce metallothioneins which can then serve to 'protect' the animal during subsequent exposure to mercury". Metallothioneins, says Gosling, (1992, p442) "mainly function to maintain low levels of free heavy metal cations in cells". In both of these cases there is a later enhancement of fitness to deal with a toxic threat. It can be seen that the production of such metallothioneins would incur a metabolic cost and so would be a stress initially.

Calow (1989) discusses proximate and ultimate responses to stress; the proximate response being the immediate effect of a stress and the ultimate responses being understood as the evolution of stress tolerance, in the lineage, by the stress acting as a selection pressure for stress tolerance. Thus, proximate and ultimate responses reside in a lineage and not in a single individual. Although Calow (1989, p173, p179) asserts that individuals are important units of study for a synthesis of proximate and ultimate effects, these, for reasons given above, cannot be seen in the same individual.

An adaptation of the concept is here proposed so that it may be applied to individuals as well as, or instead of, lineages. In individuals it is proposed that these responses be termed proximal instead of proximate and distal instead of ultimate. Ultimate effects in individuals always end in death which is perhaps too ultimate for our purposes.

These temporal effects may be seen in the aerobic training effect of increasing the heart/lung physiological capacity. The proximal effect of this stress is strain in the organism. During training the subject is working close to capacity and, then, is less capable of meeting further demands. Thus the fitness of the organism is temporarily reduced. Later, though, physical fitness may be increased as this strain may also stimulate an adaptation response, the distal response to this stress at individual level. This is analogous to the increase in lineage fitness, which results from undergoing the strain of reproduction. The opposite temporal regime might be seen by the effect of performance-enhancing drugs such as some steroids and

amphetamines. These may have positive proximal effects and become deleterious only later. It is obvious that consideration of the temporal regime is vital if cost/benefit analysis is to be meaningful. Different time scales may disclose different outcomes, so we must be explicit about the time scale. Stress, to be meaningful, must reduce fitness over any given period. It is granted, though, that fitness may be increased over another period as a result of the application of stress. But it will not suffice to label agents as beneficial or deleterious unless the time scale is considered.

Although as Calvo-Ugarteburu (1996) says that parasites generally degrade their host's fitness, Combes (1997) reports that parasites can take care of the health of their hosts. He says that larvae of *Drosophila melanogaster* parasitised by wasp larva *Leptopilina boulardi* are more successful competitors than their uninfected counterparts. Their proximal fitness is enhanced but their distal fitness is destroyed. If one considers the parasitised individuals in terms of reproductive fitness they do not exist. But in terms of their proximal fitness they are enhanced. Clearly, we must have a biological vocabulary to deal with reality. The larvae exist and they are more competitive than if they were uninfected. They may have no genetic future but they are definitely here now. Thus, they must have some quality of fitness to which we can refer.

Calow (1989, p173) says: "Environmental stress causes reductions in survival probability...". Since survival parameters for an individual cannot include a survival rate, one can only speak of survival duration or a probability. This establishes the importance and significance of a probabilistic assessment of stress as applied to individuals. An attempt at quantifying this probability will be pursued later.

Temporal stress considerations are also raised by Sindermann (1990, p223): "...the stress responses are part of the array of homeostasis preserving defenses of animals against short term traumas". It is important that we be careful in our definitions of long and short term: we should seek a significant phenomenological difference between them so that the dichotomy may be more than arbitrary.

One aspect of this temporal dichotomy arises in Breznitz & Goldberger's (1982, p5) review of

arguments supporting the view that different kinds of events (mostly social and psychological) produce a cumulative deleterious impact only if they follow one another at a rate above a certain critical level. Though it deals with psychological stress we may consider how this temporal aspect may also apply to physiological stresses.

Concepts such as overstress and distress (Selye 1982, p16; Ewbank 1984; Hattingh 1988) may now be considered redundant. They have no essential difference from stress except for intensity. Although Selye (1982 p8, 1974) refers to distress (damaging stress) as always unpleasant and contrasts this with the "general concept of stress, which also encompasses experiences of intense joy and pleasure of self expression". Again, a cost/benefit analysis is necessary to establish how deleterious these are.

Broom & Johnson (1993, p61) deal with Selye's (1973, 1982) ambivalent approach to stress by drawing a distinction between 'stress' and stimulation. Stress, they say, is to be avoided but stimuli are an unavoidable part of life. Indeed, they say that a minimum level of stimulation is desirable. If this is missing then presumably "understress" (Selye 1982) may be said to occur. Such a model offers limited utility. The distinction between stimulus and stress is hazy. And it can be defined only in terms of the effect of these causes. Thus what may be a stimulus to one may be a severe threat to another. Independently from the organism, there is no way of distinguishing one from the other. It would be better to consider the two agents as differing only in intensity. Whether an agent is a stress or a stimulus must be ascertained by cost/benefit analysis.

Any biological activities, and inactivities, may cause strain (i.e. deleterious effect). It is only through summing the strain levels of each action over a specified period for a specified biological unit (cell, organ, individual, population, lineage, etc.) that cost or benefit is ascertained. Assessments of stress phenomena are meaningless unless the temporal regime is made explicit.

MAINTENANCE OF ORDER IN THE ORGANISM: NON-LINEARITY, FEEDBACK AND CHAOS

From organelles all the way up to the level of ecosystems there is evidence for the involvement of feedback, non-linearity and chaos in stress phenomena. Moreover, feedback, non-linearity and chaos, are implied by one another. The nature of this implication and ontological hierarchy are examined here. Examples are described and an attempt is made to establish causal connections between these concepts.

Why are these concepts important in stress phenomena? Let us look at the essential nature of life. It is non-random extreme but flexible self-sustaining chemical complexity. No inorganic system even remotely approaches biological complexity. Such complexity, as Stewart (1993, p3) puts it, may be "at the edge of chaos" but not quite in it. In this zone (Stewart 1993), complex systems are poised between the two extremes of a clockwork universe and chaos. Living processes are thus potentially on the brink of the catastrophically unstable dynamics of chaos. If the dynamics of the organism are usually non-chaotic then the shift to chaos may precipitate disorder. Chaotic dynamics are non-linear and may be rooted in positive feedback; as the following account will attempt to demonstrate.

As established previously, the effect of stress is to increase disorder, i.e. degradation of persistence enhancing structure or process. This degradation must take place under some temporal regime. An example of the temporal aspect of stress phenomena is that the effect of stress may persist after the stress itself has gone. The stress has become part of the organism: in accordance with the postulated characteristic of aseity.

Selye (1955, p625) illuminates this: "A man may die from a single exposure to ionising rays, a rheumatic heart, or an infectious nephritis long after the original cause of his illness is no longer present in his body". This can be understood as stress altering the co-ordination of the body to one that is less favourable to fitness. As Levitt (1980, p6) says: "The importance of the time factor becomes obvious in plastic strains. The plastic stretch of a wire may be just as dependent on the time exposed to the stress as on the stress itself". Plastic strain in organisms is defined as, "An irreversible physical or chemical change" (Levitt 1980, p6). We can extend the analogy of biological stress with that of the wire - into the region of plastic

deformation in the stress/strain relationship where non-linear dynamics occur. First we need some definitions.

Disorder

Disorder may arise in structure, or process, or both, where its manifestations may interact. Structural disorders occur from the molecular level all the way up to ecosystems. Disorders of process are illuminated by Gleick (1991, p292): "Some physiologists speak of dynamical diseases: disorders of systems, breakdowns in co-ordination and control. Systems that normally oscillate, stop oscillating or begin to oscillate in a new and unexpected pattern, and systems that normally do not oscillate, begin oscillating". Gleick (1991) cites breathing disorders such as Cheyne-Stokes syndrome and infant apnoea.

Since all disorders must occur over time (assuming that nothing happens instantaneously this side of quantum mechanics) progress from order to disorder must have dynamics. These are characterised by linearity or otherwise. As posited previously, disorder is any shift of structure or process from a condition which enhances persistence to a less favourable one. To this we can add that it may embrace any biological unit such as a molecule, organelle, organ, organism and ecosystem. And it reduces the persistence of that unit as a biological entity.

This definition is intended to be all embracing, but in making it so, disorder has become difficult to characterise. On closer examination of the consequences of such a definition we find that an absolute measure of disorder may be impossible. Do we assume that organisms have one mode of action and one structure that constitutes peak efficiency and that all others are disorder? Or is there a suite of conditions of similar efficiency? Previous discussions on the organismic, rather than mechanistic nature of life may mean that in principle the state of disorder may only be inferred from the ultimate outcome. This is unsatisfactory. Then for practical purposes we must attempt to spot and catalogue salient features of disorder; especially those which are general in organisms. Thus, to detect and measure disorder, it may be useful to make comparative studies and to make inferences about disorder indicators. These are, perforce, incomplete and may border on the inadequate, but this may be the best we can do. One thing that we can do well, however, is to be explicit about the assumptions we make.

Disorder in organisms may be random or chaotic. Though it cannot be denied that random disorder is a stress it is probably minor. The emphasis on chaotic disorder is laboured here because it is probably more important. Why is this? Non-linear reactions with feedback (the product of the reaction has an influence on the reaction that produces it) in living systems are "virtually the rule" (Prigogine & Stengers 1985, p153). And positive feedback, a principal cause of chaos, is a common feature of disease dynamics. Examples of positive feedback have been previously mentioned. Other examples follow.

Further evidence can be adduced from the nature of the organism as a complex, highly ordered structure with many far-from-equilibrium conditions at many structural levels. These range from ionic concentration gradients across membranes to highly ordered and energy-rich proteins, carbohydrates and nucleic acids. The organism is, energetically speaking, at the top of a hill and, though it may rest in a depression at the summit, it is in fact only metastable. In far-from-equilibrium conditions - and what better an example than the organism? - "we find that very small perturbations or fluctuations can become amplified into gigantic, structure-breaking waves" (Prigogine & Stengers 1985, pxvii). It is proposed that we look here for clues about stress phenomena.

Despite the operational problems outlined above, the goal of any stress assessment must be to ascertain the rate and degree of change in total order in the organism. But indicators of order may give only a partial and inaccurate view. Not least because disorder may be unequally distributed throughout the organism. Indicators must therefore be assessed to allow for this.

Chaos

Chaos is a sustained dynamic pseudoperiodic pattern which, though it does not repeat itself, fluctuates about a centre. "Chaotic systems are those that do not settle into equilibria or simple cycles, but rather exhibit irregular and non-repetitive dynamics" (Berryman & Millstein 1989, p26). The fluctuations may appear random but this is not so; mathematical models of chaos are strictly deterministic (Schaffer & Kot 1986). In chaos dynamics there is "sensitive dependence on initial conditions" (Schaffer & Kot 1986, p59). Thus "nearby trajectories on average separate exponentially. Consequently, small differences in initial

conditions are amplified, and since one can never specify a system's state with infinite precision, long term forecasting becomes impossible" (Schaffer & Kot, 1986 p59). Thus: "Simple non-random laws may lead to complicated, unpredictable behaviour" (Stewart 1993, p2). "Chaotic dynamics emerge when positive/negative feedback systems are dominated much of the time by positive feedback growth processes" (Berryman & Millstein 1989, p26).

Non-linear dynamics

Chaos is characterised by non-linearity. This may arise in organisms if the rates of processes are not constant. How might this come about? Process rates may change in response to an agent, but the response may not be directly proportional to the change of intensity of the agent. If the rate of change is directly proportional then the dynamic would be termed linear. Should the rate of increase be more than directly proportional then the dynamics are non-linear (superlinear). Likewise the dynamics are non-linear if the rate of increase is less than directly proportional (sublinear).

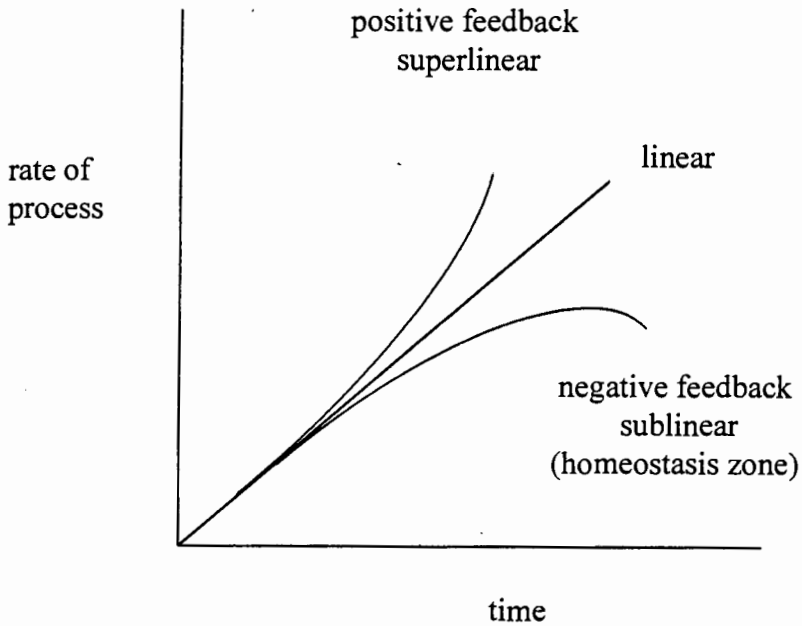


Figure 1. Examples of dynamics.

Feedback

Feedback can cause non-linear dynamics. Feedback in homeostasis is: "The effect of a system output, in response to a system input, which modifies that input by reducing it (negative feedback) or enhancing it (positive feedback)" (Broom & Johnson 1993, p176). They say that feedback is a response, after the fact, to changes in body parameters. Negative feedback tends to annul the effect of the change, thus returning conditions to the original point. Positive feedback tends to increase the effect of the change thus pushing the parameters even further away from the origin. Negative feedback tends to stability or maintenance of original order; positive feedback tends to instability or loss of order. Positive feedback may cause non-linear dynamics, and these are characteristic of chaotic systems (Berryman & Millstein 1989).

A related concept, feedforward, has been erected by Broom & Johnson (1993, p176). It is the "effect of a system output which, prior to any input, modifies the state of the system in such a way that the effect of an input is partly or wholly nullified." It may thus be considered as a homeostatic process. The organism anticipates a change before it occurs, thus minimising disruption of homeostatic set points. Thus the organism is adapting to a predicted change in the future rather than to a historical or current change. Feedforward must be taken into account when fluctuations, apparently without cause, occur in the organism.

EVIDENCE FOR CHAOS AND RELATED CONCEPTS IN STRESS PHENOMENA

It has been shown that stress may precipitate positive feedback. This prompts the suspicion that the state of strain or fitness of an organism may be assessable by examination of the linearity of its dynamics. Do non-linear dynamics become more apparent as stress becomes more intense? Or do characteristic damping processes occur, and if so by what means? If the organism is as suggested above then homeostasis must have a damping capacity which is capable of interposing a negative feedback dynamic over any positive feedback tendency.

This is indeed what happens in integration of positive and negative feedback loops in a crayfish muscle (Bush pers. comm.) See Skorupski, Vescovi & Bush (1994) for details. Although Prochazka, Gillard & Bennet (1997b, p3243) say, "Positive feedback is normally associated with instability" below are examples of how it can be used and stabilised. The role

of positive feedback in neuromuscular control systems has also been reported by Cannone & Bush (1982 & 1983), Prochazka, Gillard & Bennet (1997a & 1997b), Skorupski, Rawat & Bush (1992) and Skorupski, (1992). Mechanisms are cited that control positive feeback in these cases. Cannone & Bush (1982, p365) say, "The thoracic-coxal muscle receptor organ (T-C MRO) in crabs mediates concurrent reflex excitation (positive feedback) and inhibition (negative feedback)". Cannone & Bush (1982, p376) say, "The positive feedback loop is controlled independently". Cannone & Bush (1983, p310) say, "The inhibitory input acting concurrently with a T-fibre mediated excitation imparts stability to the dynamic positive feedback". Prochazka, Gillard & Bennet (1997a, p3235) say "positive force feedback is stabilised by delays in the pathway." and "positive force feedback can produce stable load compensations with properties that complement negative displacement feedback". Prochazka, Gillard & Bennet (1997b, p3237) say, "The models also show how positive force feedback is stabilised by concomitant negative displacement feedback and by delays in the positive feedback pathway". The ß-loop is "the least disputable example of positive feedback in mammalian reflexes". The system is stabilised because the loop is "nested inside a negative feedback loop controlling muscle displacement". Prochazka, Gillard & Bennet (1997b, p3234) say, "Another factor that tended to stabilise positive force feedback was concomitant negative displacement feedback".

If the organisms cannot interpose such a negative feedback dynamic then homeostasis would reach its limit and positive feedback could no longer be damped; it would run away to the destruction of the organism. What evidence is there for this? A specific instance of positive feedback dynamics has already been given in the description of malignant hyperpyrexia. And this is not the only evidence. Positive feedback may also be inferred from Selye (1955, p629): "temporary overdosage with desoxycorticosterone can initiate a self-sustaining hypertension, which can eventually lead to death, long after the hormone administration has been discontinued". Furthermore: "Irreversible shock may occur after excessive blood loss leading to death even if the loss is made up" (Lippold & Winton 1979, p478). This is supported by Choudry & Baue (1982), who refer to irreversible circulatory deterioration after blood loss in mammals.

Positive feedback also occurs when the physiological response to psychological threats

causes a degradation of the organism. Prolonged exposure to such a stress can result in self-induced peptic ulcers, raised blood pressure, fatty deposits on blood vessel walls and lowered disease resistance (Archer 1979). The risk of cardiovascular illness is increased by raised catecholamine levels (Fisher 1984) and these levels are stimulated by demanding situations that require effort. This condition may lead to ruptured blood vessels and may, over long periods, cause hardening of blood vessels in the kidneys. These physiological conditions can arouse psychological reactions of anxiety which further exacerbate the original condition. This potential for feedback is underlined by Selye (1955, p631): ..."It appears very probable that corticoids secreted during stress also have an important influence on nervous and emotional reactions. Conversely, it is now definitely established that nervous stressors (pain and emotions) are particularly conducive to the development of the somatic manifestations of the stress syndrome; thus stress can both cause and be caused by mental reactions".

Destructive positive feedback is also evident in psychological interactions of self-image and quality of performance. "Degradation of one leads to degradation of the other and so on to the point of crisis" (Fisher 1984 pxxiii). Though it may be a psychological stress, its effect is the same in principle: co-ordination of the processes that maintain fitness is degraded. In all these examples one problem causes another and so on.

Stress induced positive feedback has been invoked in the progress of BSE by Purdey (1996). The original corruption of the prion proteins may have occurred *in utero* by an organophosphate insecticide containing pthalimide. Long after the insecticide residues have been removed from the body, corrupt prions remain in the neurons. Corrupt prion proteins are less accessible to enzyme mediated breakdown. They thus build up in cells. Any stress event in adult life then is thought to induce a nerve growth factor mediated synthesis of normal prion proteins in the cells. These proteins are then corrupted by the pre-existing prions. Thus there is a build up of non-functioning prions in the cells. Note that the stress affects the aseity of the organism. Thus the effect remains after the stress agent has gone.

Even where positive feeback is used by the organism, its outcome may be destructive. Frisch, Vuori, Kelaita & Sicks (1996) give an example of a putative positive feedback mechanism that triggers apoptosis.

POPULATION AND ECOSYSTEM PHENOMENA

In populations, non-linear responses have been discussed by Underwood, Denley & Moran (1983). And although Berryman & Millstein (1989, p27) say "the evidence from modeling exercises generally, but not always, supports the contention that ecological systems do not normally behave chaotically," they do concede that, if sufficiently perturbed, ecological systems have the potential for chaos. This could be seen as an analogue of the same dynamics in the individual. On the other hand, chaos phenomena may be rare because such events in an ecosystem are likely to change its nature rapidly and thus its identity. Chaotic ecosystems would effectively self-destruct and reconstitute as some other system. If so, chaotic systems clearly would be rare specimens for study.

Broom & Johnson (1993) give an unequivocal example of positive feedback dynamics in a population when they describe flight response and alarm calls in broiler house chickens spreading to all the others in a population; leading to mass hysteria. This condition can occur also in other social species such as man.

Another potential for positive feedback is the transmission of disease in a crowded population. Lowered disease resistance is associated (Fisher 1984) with high ACTH and corticoid levels. This is echoed by Sindermann (1990), who says that hypersecretion of corticosteroids as a stress response may in turn lead to a higher probability of infections by their effect of lowering the efficiency of the immune system. Furthermore, says Sindermann (1990), even short periods of raised adrenal activity can result in sufficient immunosuppression for a pathogen attack to be successful. And as Broom & Johnson (1993, p121) say: "Animals encountering difficult conditions such as poor housing often show some degree of immunosuppression". Specifically, they are less able to produce antibodies following antigen challenge (Broom & Johnson 1993). Schneiderman & McCabe (1985) report that both corticosteriods and catecholamines can be inhibitory to a range of immune response cells. In man (Schneiderman & McCabe 1985, p25) say, "research that has related behavioural variables to immune deficiencies has supported the view that exposure to intense, unavoidable stress may increase susceptibility to disease".

Overcrowding is a potent stress. Mice stressed by overcrowding have shown a decrease in

immune response to some antigens. Esch *et al.* (1975, p345) say, "fighting (i.e. social aggression) decreases resistance to parasitism, presumably through the action of corticosteroids". Esch *et al.* (1975, p347) say of crowding that "stress may be the outcome" and "the biological outcome of the stress will be reduced host resistance to further infection...". This decrease is coupled with a higher probability of pathogen transmission facilitated by the crowding. And thus a weaker organism is assailed by more pathogens which in turn may weaken it further. The potential for positive feedback is evident.

CHAOS AT THE LEVEL OF THE ORGAN: THE VERTEBRATE HEART

Central to the normal dynamics of the heart is the orderly conduction of impulses from the pacemaker cells to the rest of the myocardium. During normal rhythm a wave front of contraction (systole) sweeps over the entire heart, and this is followed by a period of relaxation (diastole). During the first part of the diastole the heart muscle is refractory and thus unresponsive. The next systole normally occurs once the entire heart has passed out of its refractory period. This is one type of heart dynamic. Another type is fibrillation: when individual muscle fibres contract out of synchrony. This is chaotic disorder. Fibrillation is, in principle, a deviation of a process from its normal timing; and it compromises persistence of the organism.

How can it occur? Numerous agents, such as drugs, electric shock and ischaemia may predispose the heart to an unstable rhythm. Chaotic problems arise when a conduction wave front runs into tissue still refractory from a previous irregular systole. The front may split as it goes either side of the area and contraction will thus begin to lose synchrony. As these two fronts separate and travel over different distances they are likely to desynchronise further thus creating more out-of-time refractory areas for other wave fronts to strike and so further divide. Soon the entire heart, down to individual muscle fibres, may be beating out of synchrony. In this condition the heart uses energy as fast as a normal heart but no blood is being pumped: co-ordination has gone. Other aspects of positive feedback come into play as the heart, because of this loss of co-ordination, deprives itself of blood (Lippold & Winton 1979). This further weakens and disrupts the contraction process.

DISORDER AND SIZE

It is curious that susceptibility to disorder may also be size dependent. Smaller hearts, all other things being equal, have less need of complex conduction fibre pathways to prevent chaotic and random breakdown of the synchronised systole. This phenomenon appears to be influenced by the rate of conduction through the heart muscle and the length of the refractory period. The conduction of the contraction impulse is broadly the same speed in all hearts (temperature being equal) and completes its journey in a time proportional to the distance it has to cover. The refractory period of cardiac tissue is also of a similar order in all hearts. In smaller hearts there is, thus, less likelihood of some areas of the heart remaining refractory while others are coming out of it.

Not only is this tendency for stability to be size dependent evident in organs; it is found also in organisms. Small sized species are common in stressed areas (Gray 1989, and Moore 1972). It could be simply a replacement of K-selected species by r- selected species. Or is it that smaller species are less susceptible to the onset or effects of the stress? Does stress in this context mean initiator of uncontrolled non-linear events? Is there, perhaps, a lower frequency of non-linear events in smaller organisms? Smaller mussels, for example, are less stressed by, and more tolerant of, high temperatures (Widdows 1978; Bayne, Widdows & Worrall 1977). Furthermore (Gosling 1992, p400-2) states that for mytilids: "Generally, adults appear to be > 10-fold more sensitive than larvae with respect to copper, petroleum hydrocarbons, and sewage sludge and approximately 4-fold for tributyl tin...The explanation for this lower sensitivity in the early larval stage may be a combination of their reliance on energy reserves provided by the parent rather than on direct feeding, and the absence of a developed nervous system, which is an important site of toxic action". They add: "there is now a growing body of evidence which demonstrates that the early larval stages are not necessarily more sensitive than adults to a wide range of pollutants". According to Moore & Folt (1993, p180) "...Studies have generally shown that large cladocerans and copepods are more sensitive to a variety of toxicants than small cladocerans, copepod nauplii and rotifers." And (Moore & Folt 1993, p180): "The large cladocerans were more sensitive to Carbaryl than were the small cladocerans, rotifers and *Chaborus*" (a dipteran predator). Moore & Folt (1993, p181) add: "Lehman (1988) suggests that large individuals are more susceptible to elevated temperatures because the metabolic demand of their larger volume increases more

rapidly than the efficiency of food collecting. And "The physiological mechanism causing the reduction in growth and body size may be the increased costs of respiratory maintenance under conditions of toxic stress" (Barber, Baird & Calow 1990, reported by Moore & Folt 1993, p181). Moore & Folt (1993, p181) continue: "Reduced body size in a community also results when larger individuals or species succumb to toxicants. In a field study of metal body burdens in three species of crustacean zooplankton, Arts & Sprules (1987) found that the smallest species was least sensitive to copper, nickel and aluminium. In addition, the lipid content of individuals was reduced at the higher metal concentrations for the two larger species, but not for the smaller species".

Odum (1985, p421) asserts: "small organisms outcompete large organisms, both under conditions of enrichment and toxic stress". And, "Although large organisms are often efficient feeders when resources are scarce, they are subject to bioaccumulation of toxins, have vulnerable life history stages, or are otherwise more sensitive than are small organisms".

We would expect manifestations of instability in more complex organisms to be particularly evident as a result of agents that have played little part in selection in the lineage of the organism. Lineages of organisms that have encountered specific agents are likely to have physiological adaptations. It follows that the more complex are likely to have more complete adaptation to agents with which they are selectively familiar. It is suggested here that an unfamiliar perturbation in a complex system has the potential to do more entropic damage than could the same perturbation in a simple system. Thus in a selection of organisms from simple to complex, if all of them are equally evolutionarily unfamiliar with a deleterious agent, one would expect the most complex to be the most susceptible.

This finds some support in the differential lethality of ionising radiation. Ionising radiation at high dose is a good example of an unfamiliar agent. According to Carter & Orr (1970) mammals are killed by a radiation dose that is 100 times less than that lethal to fruit flies. They report that bacteria and viruses are even more resistant than fruit flies. This suggests a gradient of radiation lethality that has some proportionality to complexity.

Further support, by analogy, for simplicity being more stable comes inadvertently from

Berryman & Millstein (1989) who in criticism declare that as applied to ecosystems the logic of the chaos hypothesis is questionable. They say that if ecosystems were chaotic, then complex ones should be more so than simple ones, this they say is because chaos is more likely in higher order systems. Theory and evidence may clash for ecosystems but it appears fitting at the level of the organism.

The above combination of theory and observation suggests that in some circumstances to be smaller and simpler is to be more stable. Large and complex is an advantage only when the advantage of the size outweighs the disadvantage of instability. Large organisms might benefit from being so by being better predators, more difficult prey, or more efficient at temperature regulation. But it is postulated that no organism just becomes big and complicated: such increments must add a benefit not otherwise attainable. Stewart (1993, p3) says: "A central tenet of complexity theory is that selection or learning drives systems towards this edge of chaos. Systems that are too simple do not survive in a competitive environment because more sophisticated systems can outwit them by exploiting their regularities. But systems that are too random do not survive either. It pays to be as complicated as possible, without becoming totally structureless".

In dealing with the problem of size and instability we make the following assumptions. Increased size implies increased complexity. And increased complexity implies increased potential for entropic energy/order loss. There is more to go wrong. If this were so we would expect to see new types of mechanisms for stability in more complicated organisms. Thus, this hypothesis may be testable.

THE ULTIMATE EFFECT OF STRESS: DYING

Although it has been shown that there are many kinds of disorder caused by stress, death is the only universal result. We must, then, consider its nature and dynamics. By what processes does death occur? Is dying a gentle drifting away or does it end in an increasing rush? Is it linear or non-linear?

These questions can be conveniently discussed against the scheme of Wilson (1980, in Sindermann 1990, p224, Figure 45) who presents a graphic relationship of disturbance of

function with dose of contaminant (See Figure 1). For our purposes we may also read these as strain and stress respectively. The disturbed function may not be the same as disorder, however, unless it approaches a whole-body integration. The ultimate limit, death, is operationally acceptable but the lower limit (normal) must be clearly defined. Moreover, this lower level, contrary to available evidence, appears to omit the possibility of process rates above normal.

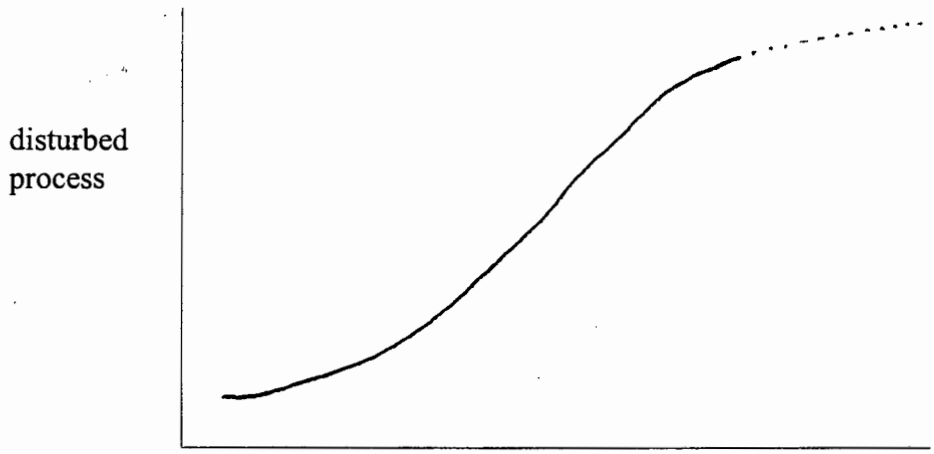


Figure 2. Relationship of disturbance of function with dose of contaminant, after Wilson (1980).

Nevertheless, this model (Wilson 1980) is particularly useful because it allows a critical assessment of stress/death dynamics. The following questions may be posed. Is the curve really sigmoid? How does the rate of progress towards death decrease as death is approached? What negative feedback homeostatic mechanisms can account for this?

If positive feedback is as significant as has been asserted here, then the process must accelerate. Instead of being sigmoid, the curve should become steeper as it approaches death. After all, the organism is becoming more disordered and homeostasis is becoming less effective. If so, then the rate of breakdown must increase. Reduction in co-ordination of the maintenance processes will result in acceleration of loss of order. This, in turn, will damage

maintenance and so on. The substance of the body - an aggregation of highly ordered and often unstable energy containing molecules - thus becomes increasingly vulnerable to runaway chemical breakdown. If this does not occur (an unlikely alternative) then one would have to assume that most of the degradation occurs before death. Or, perhaps, homeostatic processes increase efficiency to make the final part of the curve more shallow; but by what mechanism? Common sense prompts the conclusion that the final process towards death should be an accelerating loss of order. This may easily be rendered operational.

EMPIRICAL GROUNDING AND CAUSAL CONNECTEDNESS OF CHAOS PHENOMENA

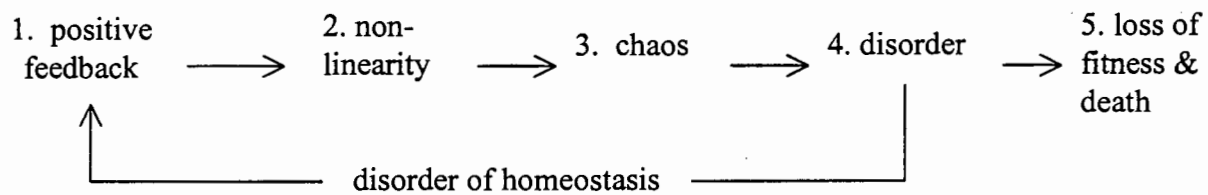


Figure 3. The causal relationships of various aspects of stress dynamics.

Figure 3 outlines the ontological hierarchy of these concepts. The feedback loop (4 → 1) describes how the level of disorder might non-linearly increase: the homeostatic mechanisms may be fully employed but are themselves becoming disordered. In an approach to making these concepts operational the following inferences are proposed.

1. Disorder is caused by feedback exhibiting non-linearity.
    a) These tend to be positive feedback.
    b) Negative feedback mechanisms tend to stability unless they over-correct, when they can be a source of instability (Berryman & Millstein 1989).
2. Characteristics of linearity or otherwise indicate the nature of the feedback.
    a) Super-linear dynamics are evidence of positive feedback.
    b) Sub-linear dynamics are evidence of negative feedback.
    c) Linear dynamics rule out feedback.

3. Evidence of feedback may indicate the possibility of non-linearity.
4. Evidence of non-linearity is strongly associated with disorder. This inference is stronger as one approaches a whole-body integration.
5. Evidence of feedback is also (but less strongly) associated with order/disorder.
6. Order may be inferred from measurable condition states of the organism.
7. Chaos presupposes non-linear dynamics - either positive feedback or over-compensating negative feedback.

We now move on to considering the organism and its relation to stress in terms of energy flowing through it.

CHAPTER 17: STRESS, ENERGY FLOWS, ANTIDOTES AND POTENTIATORS

THE ORGANISM AS AN ENERGY FLOW

If the organism is considered as a locus of energy flow, then stresses may be seen as dams or diversions. Energy is thus misused or its flow obstructed. The vehicle for this energy flow is organised matter: the substance of the organism. A change in the nature of the energy flow implies a change in the condition of the organism and *vice versa*. Ivanovici & Wiebe (1981, p15) say that Lugo (1978) underlined this when he "re-emphasized an important characteristic of stressed systems, namely that, in them energy expenditure is increased or potential energy is decreased" - this being applicable to systems and organisms. Bayne (1973) found similarly in the mussel *Mytilus edulis* where energy was lost as alpha-amino nitrogen. Strain (the effect of stress) can be measured in this model as a loss or diversion of energy away from persistence enhancing processes. Ecosystem energy flow has been examined *inter alia* by Rigler (1975) and Odum (1968). Where differences, significant to stress studies, exist between ecosystem and organism they will be appraised.

Figure 1. Energy flow in a healthy organism.

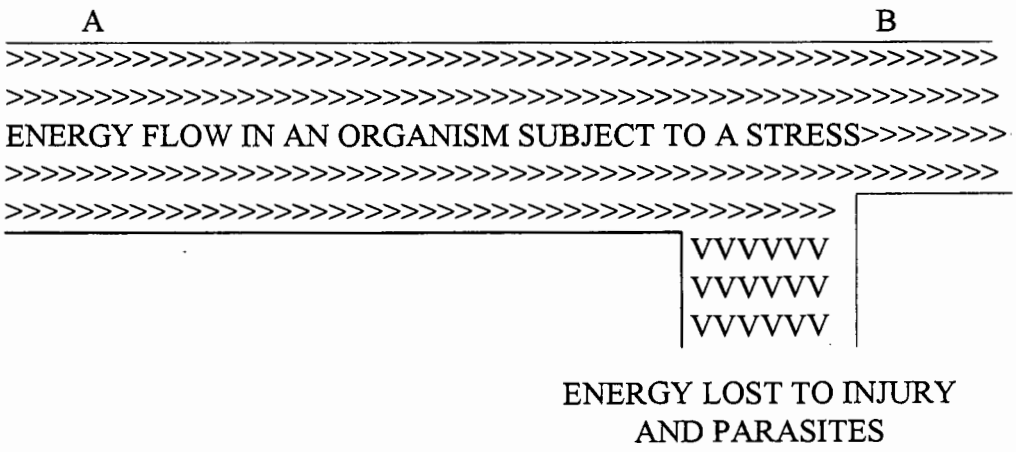


Figure 2. Energy loss by diversion in a stressed organism. Examples of diversion effects would include loss of blood or body fluids to parasites, or by injury.

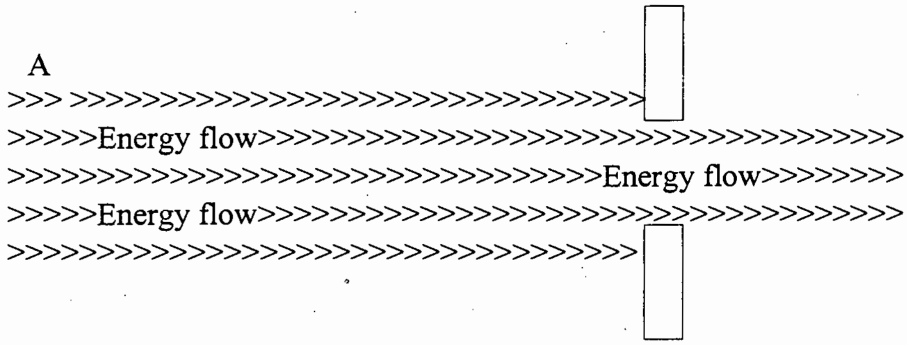


Figure 3. Energy lost in a stressed organism by damming. Examples of damming effects include the action of 2,4 DNP and the effect of lowered temperature. The latter reduces metabolic rate according to the temperature dependent enzyme kinetic curve. A parasite may also become a dam stress; Munger & Karasov (1989) report that the tapeworm *Hymenolepis citelli* in mice decreases host digestive efficiency.

Figure 2 represents energy loss by diversion in a stressed organism. If the energy flow at B is to be maintained then energy input at A must increase. This extra energy requirement implies higher risk as the organism must spend more time foraging. As Sibly & Calow (1989, p102) say: "a food limited animal can sometimes increase its growth rate by foraging in more dangerous places, or at more dangerous times of the day, so increasing growth rate at the cost of increased mortality rate". (Are we talking about an individual or a population? It is urged that for an individual we refer to an increase in mortality probability.) Increased risk from foraging cannot, however, be avoided without incurring another risk: exhaustion of energy reserves.

Organisms that persist find more energy before they run out, and thus avoid losing too much to parasites or all to predators on the way. Ivanovici & Wiebe (1981, p15) agree: "The discussions by Odum, Finn, & Franz, (1979) and Lugo (1978) support the earlier definitions of stress, by reinforcing the concept that stress places an organism or a system at a disadvantage, as continued expenditure of excess energy is incompatible with survival". The persistent organism is so because it crosses from one source of energy to another without running out. Crossing this gap costs energy and may expose the organism to risks such as predation. If foraging or metabolic costs rise, then the reserves for contingencies become depleted and persistence becomes less likely.

The concept of energy flow (as energy flux per unit biomass with time) is an operational measure of activity and allows comparison of organisms. It may indicate how much work an organism can perform and thus provides a gauge of health. This has been formulated as scope for growth (SFG) (Koehn & Bayne 1989; Bayne & Newell 1983).

The above model of energy flow does not consider mass flow through the organism. This may also be significant and should be ascertained. Fitness parameters could include biomass turnover, and consideration of its importance as structure and not just as an energy store. More biomass, all other things being equal, is suggestive of higher fitness.

Odum (1985, p420) says: "To summarize, community respiration per unit biomass tends to increase and biomass accumulation to decrease as organisms cope with the disorder created by unusual exogenous disturbance". And that stressed ecosystems will tend to have a "decreased ratio of biomass to energy flow, or a low efficiency of converting energy to organic structure".

THE ORGANISM AS A FIGURE OF TOLERANCE TO STRESS

The concepts of one and two-way stress must be introduced at this point so the figure of tolerance can be constructed.

One and two-way stresses

Stresses may be of the one or two-way type. A two-way stress has a medial optimum and is stressful at upper and lower extremes. A one-way stress has an optimum (no stress?) at one extreme and becomes increasingly stressful towards the other (the lethal limit).

One-way stresses

Such stresses are exemplified by the toxicity of those heavy metals that are not required as trace elements; such as cadmium and mercury (Gosling 1992). The action of one-way stresses may be represented as shown in Figure 4.

Two-way stresses

Examples of two-way stresses include: maximum and minimum temperature; the inhibitory,

beneficial and toxic effects of essential trace elements (Simkiss & Mason 1983); and the concentration of glucose in blood. Two-way stresses may be represented as shown in Figure 5. Sibly & Calow (1989) also give examples of copper concentrations where the least toxic concentration is not the least concentration. Here, toxicity rises either way: lower and higher concentrations are both harmful. Essential trace metals (Simkiss & Mason 1983) such as molybdenum, manganese, cobalt, copper, zinc, chromium, nickel and tin are required at low concentrations. At lower concentrations deficiency diseases may occur. At concentrations above normal the metals become increasingly toxic. This phenomenon is termed hormesis (Lincoln *et al.* 1985, p118) It is: "The stimulus afforded by exposure to non-toxic concentrations of a potentially toxic substance". Or as defined by Parsons (1989, p183) it "occurs when a substance or an environmental perturbation is stressful at high levels and beneficial at low".

Exercise is also a two-way stress. But this is also a temporal relationship. An optimal level of exercise will result in an increase in distal fitness - at the expense of immediate proximal fitness. Too little exercise is proximally less stressful but no fitness increase can be expected. Too much is proximally stressful and also raises the possibility of distal stress through damage. The optimum would depend on other stress conditions of the organism such as its nutritional status.

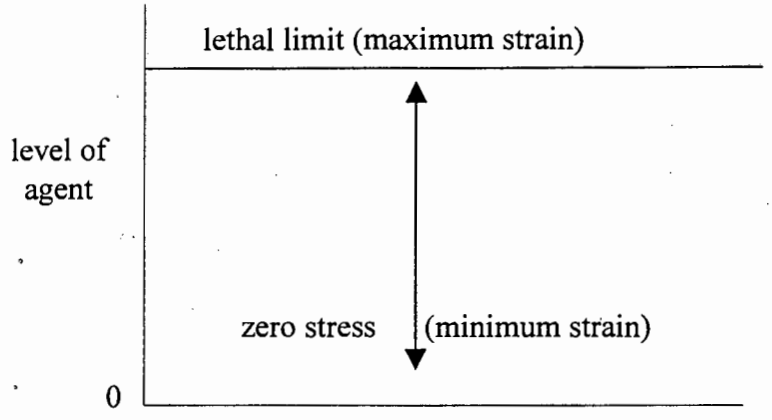


Figure 4. One-way stress.

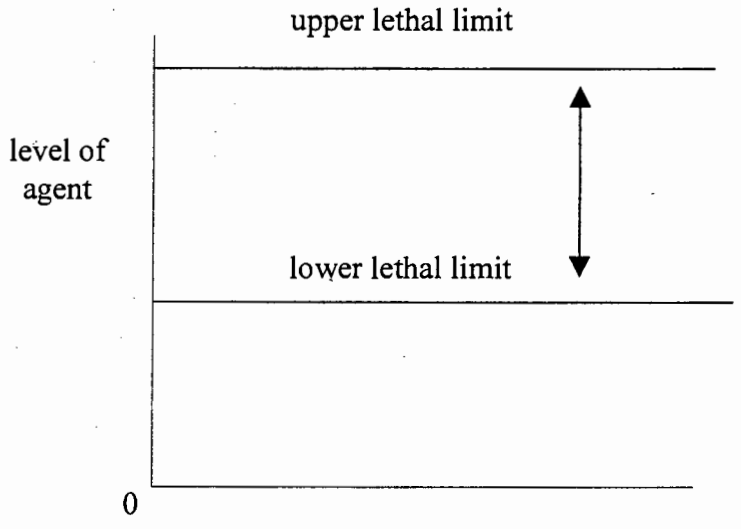


Figure 5. Two-way stress.

One and two-way stresses may be illuminated by the following energy balance relationships. If the level of stress is plotted against energy production, then one might expect the curves A, B & C (Figure 6):

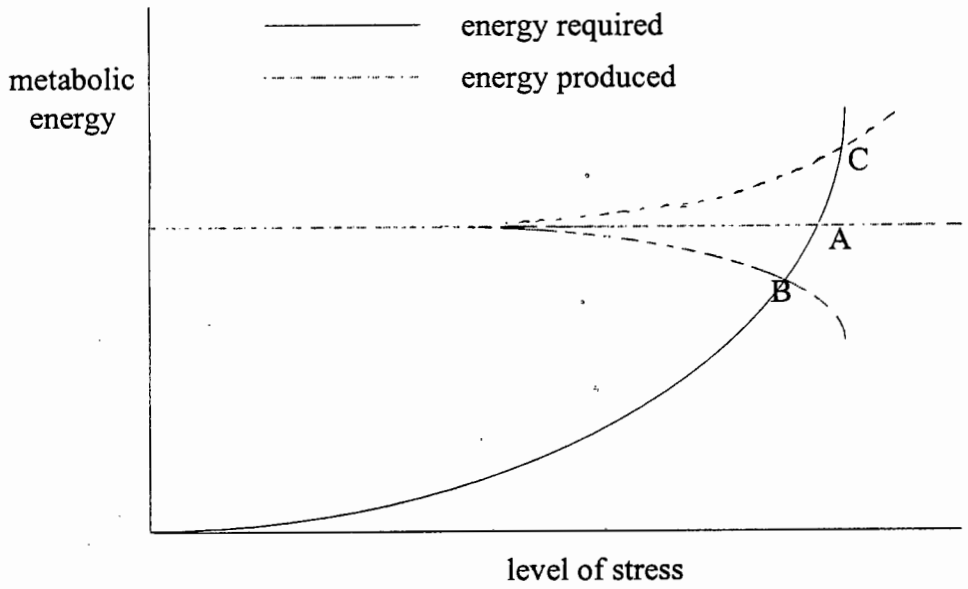


Figure 6. Energy balance and one-way stress (possible responses)

As the stress level increases so does the energy requirement to maintain co-ordination. If total energy production is constant, as in A (Figure 6), then death ensues at the point where energy required exceeds energy produced. If as in B (Figure 6) energy production is dammed, or limited, then death will occur at the point where line B meets the line depicting energy requirement. At C, energy production may increase but the ultimate capacity of the organism is limited. Either production will eventually fall away or the energy requirement will rise to meet, and exceed, supply. Death results.

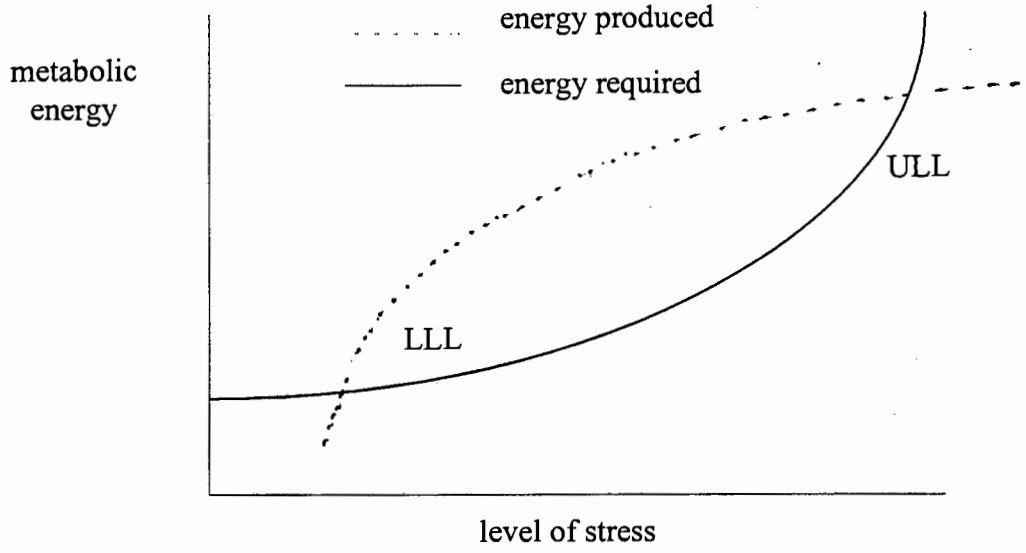


Figure 7. Energy balance and a two-way stress such as temperature. ULL = upper lethal limit; LLL = lower lethal limit.

Figure 7 shows the energy balance in an organism subject to a two-way stress such as temperature. The trends in Figure 7 are suggested by the relationship of salinity and energy flux described by Koehn & Bayne (1989, p169, figure 6). Lower lethal limit (LLL) to upper lethal limit (ULL) is the range of positive scope for growth. Temperature governs metabolic rate and this in turn determines energy supply. The energy supply/temperature relationship may, moreover, not be linear, as a change in temperature may impair the co-ordination of enzyme suites, resulting in lower metabolic efficiency. Energy requirement for body maintenance is also temperature dependent but requirement and supply do not keep in step. Death ensues at the temperature where energy supply falls short of that required. These points are the upper and lower lethal temperatures.

Now that the concepts of one and two-way stress have been elucidated we can use them to help construct a figure of tolerance whose surface represents the lethal limits of stress to the organism. This may provide a useful supplement to the definition of the organism. The figure may be multidimensional with axes such as pH, temperature and ammonia concentration, or temperature, time and salinity as used by McLusky, Bryant & Campbell (1986). Its surface defines the lethal limits (or other biologically significant index) of any particular stress on that axis. Between the axes the figure charts the tolerance of the organism

to combinations of stresses. Thus, the interactions of three stresses could easily be visualised using a figure of three axes. More than three dimensions could be used for a more comprehensive but mathematical rather than visual approach.

Similar multivariable analyses are found in the surface response figures of McLeese (1956) and Alderdice (1965) which have been discussed by Sprague (1970). These are taken here as a starting point. The figure of McLeese (1956 in Sprague 1970, p15) is not entire. It would be more complete if the three axes had been extended until the untested upper/lower extremes of lethality were also delineated. Alderdice's figure (1965) is similar to the three axes ellipsoid figure proposed here but it differs in the following respects. The toxicant has no independent axis. Thus, comparison of a situation of toxicant and non-toxicant would require a control experiment. This would not be needed if the toxicant had its own axis. A stress may just be another environmental condition and thus an axis of its own would give information about its effect at different doses.

The scheme proposed here takes Alderdice's (1965) figure and, along with the above comments, adds to it the notion of a meaningful volume and also of partition coefficients within the volume. The speculation that there may be partition coefficients is inspired in part by Baillieul, Selens & Blust (1996) who refer to partitioning of assimilated energy in the organism, particularly between survival, growth and reproduction. "Trade-offs" they say determine the amount allocated to each. It is speculated that these trade-offs are not completely plastic and that beneath the margins of plasticity there may be a fixed partition of energy and resistance. The characterisation of this may help in our identifying and understanding of the particular nature of that species.

The figure of tolerance, proposed here indicates the boundaries of stress intensities and mortality. In addition, each figure has a volume and this has a degree of constancy. The possible significance of this volume is alluded to by Selye (1956) who says that at the stage when resistance to a particular agent is at a peak, resistance to other agents is reduced below normal. It appears that during the resistance phase the resources of the organism are focused on the current problem.

By positing a constant but flexible figure volume, this model matches the evidence that an increase in one environmental extreme can reduce tolerance to others. Parasites, poisons, inclement environmental conditions and other stresses may reduce tolerance to other extremes as if by stretching the figure. It follows that tolerance for other stresses must contract.

On one hand other stresses may make organisms more susceptible to parasitic infection. In marine fish, for instance, chronic exposure to petroleum hydrocarbons (Khan 1990) leads to an increase in the prevalence and intensity of trichodinid ciliate parasites. Khan (1990) reports that the mean intensity of infection of parasites in oil treated fish *(Myxocephalus octodecemspinosus)* was 17 times higher than in controls. Khan (1990, p761) reviews evidence in the literature and concludes that, "a variety of pollutants suppress the immune response causing fish to become susceptible to an infection".

On the other hand the parasitic infection may reduce the organism's tolerance to other stresses. The oyster *Crassotrea virginica* when infected with *Haplosporidium nelsoni,* has an increase in oxygen consumption of almost 70% when subject to a sudden temperature rise (Littlewood & Ford 1990). They conclude that external stress may exacerbate the deleterious effect of parasites. Lauckner (1984) reports that heavy infestations of the digenean trematodes *Himasthla elongata* and *Renicola roscovita* impair byssus thread production in mussels *(Mytilus edulis)* and affects the burrowing ability of cockles *(Cerastoderma edule).* In addition, longevity and resistance of these bivalves to enviromental -particularly thermal- stress are reduced. Williams & Jones (1994) review a number of cases of helminth parasites in fish that reduce the tolerance of the hosts to other stresses.

Other stresses may interact in the same way. More than 20ppm carbon dioxide can be tolerated in some fish at normal oxygen levels (Piper, Mc Elwain, Orme, McCraren, Fowler, & Leonard 1982). But if there is less than 3-5ppm ambient oxygen, such carbon dioxide concentrations are harmful. In addition, elevated temperatures can leave fish more vulnerable to extremes of pH. Jones (1975) gives examples of the interactions of salinity, temperature and heavy metals on mortality in aquatic isopods.

So what is the significance of this figure volume? Is it constant, or can stresses reduce it as

well as distort it? Is it definable in strain units? Does it represent the reserve resistance of the organism? How much of the volume can be mobilised to deal with the effects of stress? Is the amount which is not mobilisable from one stress to another accountable as a sort of partition coefficient? Such a coefficient would tell us about the adaptability of the organism and hence should give an indication of its potential fitness.

The figure shape would be influenced by whether the stresses are of the one or two-way type. If three two-way stresses were arranged with their axes at right angles in three dimensions then the volume bounded by the plot would make an entire and closed ellipsoidal figure. One one-way and two two-way stresses would make a one half ellipsoid figure. Two one-way stresses and one two-way stress make a one quarter ellipsoid. And three one-way stresses would make a one eighth ellipsoid. Examples of two-way stresses are described below.

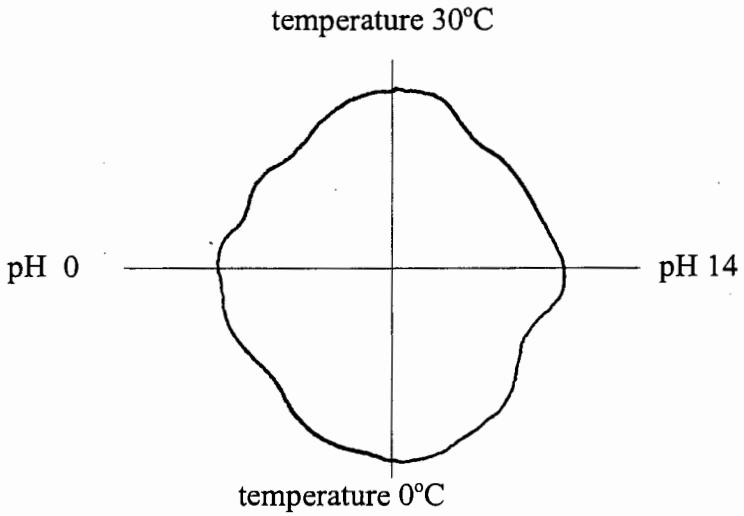


Figure 8. An example of the figure of tolerance showing how two two-way stresses (pH and temperature) interact to give a plane surface. This is similar to the figure from Costlow, Bookhout & Monroe (1960) but is simplified to enhance its clarity and allow for further development.

If a third axis, (PO_2) at right angles to the other two axes, is added and the whole viewed obliquely then three intersecting plane surfaces of interaction will be apparent:

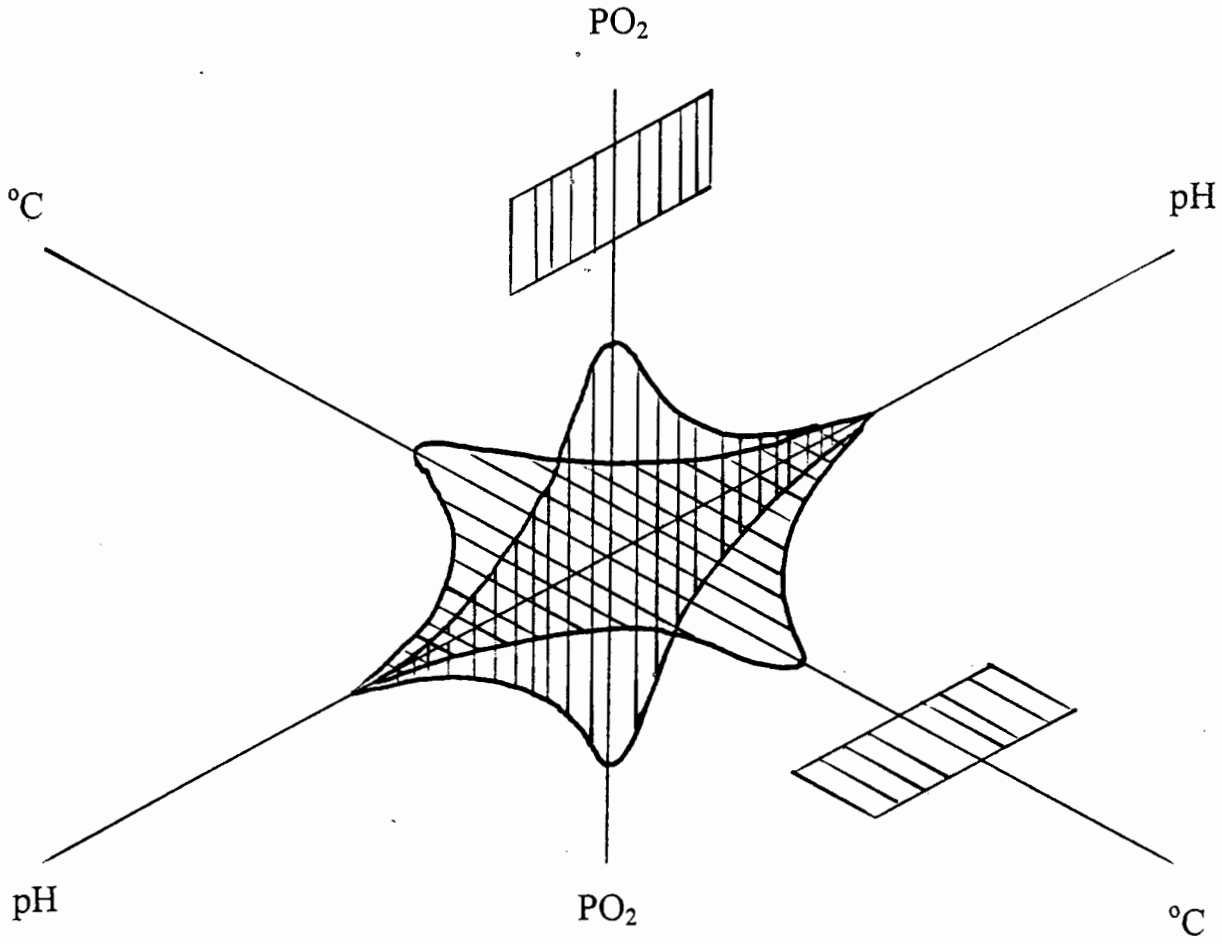


Figure 9. Plane surfaces of the interaction of three two-way stresses: pH, PO_2 and temperature. Outside the plane surface is death.

If each curve and its bounded plane surface is integrated with the others a figure is obtained. In this figure the volume bounded by the limits indicates the tolerance of the organism in as many dimensions as there are axes:

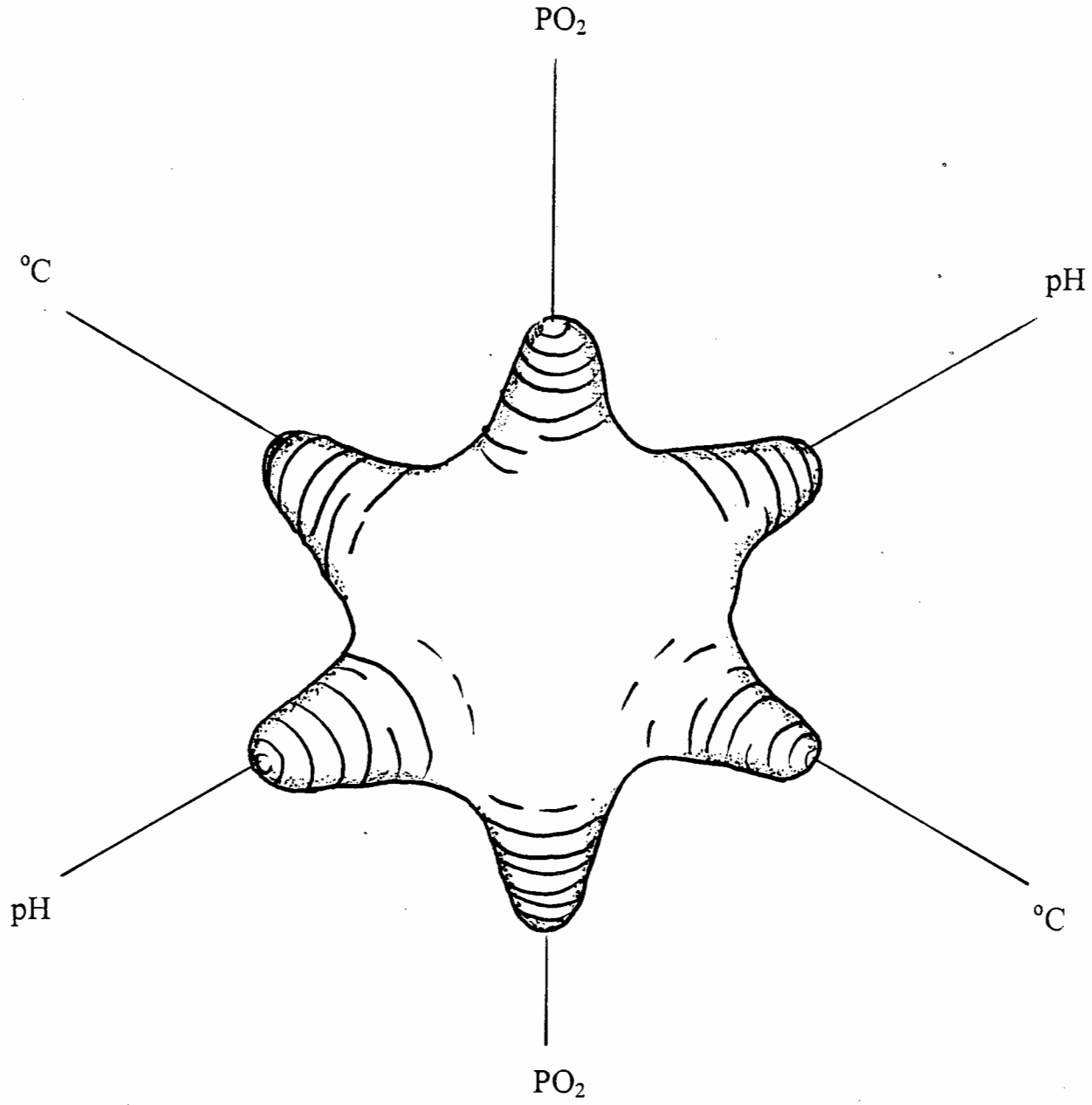


Figure 10. A figure of tolerance resulting from interacting levels of three two-way stresses: pH, PO_2 and temperature.

The environment as a figure of stress extremes

Such a figure can also characterise the environment. Here the figure surface would chart environmental extremes. When matching figures of environment and organism, the organism

would be judged viable only when its figure surface envelopes that of the environment, i.e. if its tolerance extends beyond the extremes of the environment. A stress, from the aspect of the environment may be seen as an increase in the volume of its figure causing a divergence of some of its extremes. From the point of view of the organism, a stress may be seen as an agent that reduces the volume of the organism figure or narrows its tolerance extremes.

Utility of the figure and significance of its volume

The larger the organism figure-volume, the greater the diversity of habitats that the organism can tolerate. This finds support in Boesch & Rosenberg (1981, p198): "Eurytolerance of species in inconstant environments also seems to extend to sources of stress such as pollution which are not regular features of their milieu".

The figure of stress tolerance shows potential for providing a model of multiple stress interactions. On the other hand, though it promises utility in such events, it is limited to few axes before complexity defeats this purpose. Nevertheless, a picture of interactions of only three stresses can have utility. The lethal limits of stress in the organism are, it is posited here, the thresholds where damping processes are overpowered by non-linear runaway processes.

INTERACTIONS OF STRESSES

The above stress figures present surfaces of lethality, which describe interactions of stresses. A consideration of the nature of these interactions is now appropriate. Why is tolerance to some combinations of stresses different from others? Are naturally occurring suites of stresses such as ammonia, temperature and oxygen tension more interdependent than unnatural suites such as copper, oxygen tension and mercury? Although Carefoot (1994, p579) talks of "natural stresses" such as stranding, exposure and low salinity, is there, besides their origin, any difference between these and unnatural stresses?

There is considerable ignorance concerning the interactions of toxic substances. As Doi (1994, p290) says: "The factors causing complex mixture toxicity are uncertain". Hermens (1986, p294) concurs: "Usually the mechanisms of toxic action responsible for death are not known. In some cases the observed phenomenon can only suggest the mechanism".

Toxic substances are only a subset of stresses and if this is the state of knowledge about toxic interactions then how much less we must know about stress interactions in general. Considering these uncertainties it may be useful to speculate on the modes of interactions of stresses.

Why are some stresses additive, some more than additive and others less than additive? The categories additive, more than additive, less than additive, and antagonistic are based on toxicity work presented by Gaddum (1948) in Sprague (1970, p5). A mixture toxicity index MTI scheme proposed by Könemann, (1981) contains the categories: antagonism, no addition, partial addition, concentration addition and supra-addition. Another category, independent action, (Doi 1994, p290) occurs "when the effluent toxicity is correlated with the toxicity of an individual toxicant regardless of the other chemicals in the complex mixture". Heuristic models of these interactions, as they might apply to stresses in general, are suggested below.

Additive

a) Both agents affect the same site.

b) The agents affect different processes in such a way that they do not interact to create a multiplier effect (see below for an illustration of the multiplier effect).

For example (Widdows & Johnson 1988, Widdows & Donkin 1991, cited by Gosling 1992) report interactions between structurally unrelated toxicants such as petroleum hydrocarbons and copper in mussels to be simply additive in their effect on shell growth and clearance rate.

More than additive

a) This may be due to combination and potentiation external to the organism, e.g. pH, ammonia and temperature.

b) A more than additive effect may also be attributable to potentiation occurring inside the organism. The multiplier effect may occur by disruption of homeostatic mechanisms at different levels in the process chain thus resulting in a cascade of increasing instability. In other words, stress (**x**) may cause disequilibrium at one point, and stress (**y**) may do so at another point in the same process chain. The first

effect is multiplied by the second. Possibly this is what happened when McLusky, Bryant & Campbell (1986) reported that the mortality resulting from a combination of increased temperature and elevated cadmium concentration was greater than the sum of mortalities caused by the either agent alone. Another example is the potentiation of toxicity in Man of tetrachloromethane by ethanol (Meyers, Jawetz & Goldfien 1980).

Less than additive

a) Disequilibrium caused by one agent is inhibited by the other.

b) One agent may hit a damaged process again but at a different site. When this occurs the effect is the same whether either or both stresses have been applied. Thus there is a less than additive effect.

c) Those agents, which, in combination, tend to neutralize one another in the external environment, e.g.

$$NaOH + HCl \text{ ----> } NaCl + H_2O$$

For most organisms, sodium chloride and water are far less stressful than sodium hydroxide or hydrochloric acid.

Another example of antagonism is given by Gosling (1992), between tributyl-tin (TBT) and some hydrocarbons.

Antagonistic (antidote)

a) Those antidotes that are always non-poisonous may affect the stress agent before strain occurs or before the agent gains access to the organism. Alternatively, such antidotes may affect only defective processes in the organism.

b) Those antidotes that are safe only when another stress is present may have some compensatory effect, which is in itself stressful but their net effect is to reduce strain. An example of this is protection from the effects of ionising radiation protection in mammals that is afforded by a sublethal state of anoxia (Tobias 1970). It is thought that this reduces the number of oxygen free-radicals that can be produced in the body by ionising radiation. Agents such as sodium cyanide and carbon monoxide have been found as efficacious antagonists in mice and rats.

Other examples of antagonistic action follow. During the exposure of mussels to mixtures of copper, cadmium and lead, there is a reduction in the uptake of cadmium and lead (Theede & Jung 1989, in Gosling 1992, p391-2). This is, "probably as a result of relatively rapid rate of uptake of copper and its toxic effects on ventilation rate". Ethanol outcompetes methanol for access to human metabolism and thus is used to treat methanol poisoning (Meyers, Jawetz & Goldfien 1980). Sodium thiosulphate mitigates the effect of cyanide in Man by supplying sulphur to the liver where it turns cyanide to thiocyanate (Meyers, Jawetz & Goldfien 1980). Nitrite may be used to treat cyanide poisoning. Nitrite makes available iron in ferric form from the haemoglobin. This takes up the cyanide preferentially to the cytochrome system (Meyers, Jawetz & Goldfien 1980).

PHYSICOCHEMICAL ESTIMATION OF TOXICITY

According to Hermens (1986, p287): "Quantitative structure-activity relationships (QSAR) enable the toxicity of aquatic pollutants to be predicted from their physicochemical properties". Gosling (1992, p407) goes further and states that, "QSAR's provide a unified approach to modelling and predicting the environmental behaviour, fate and effects of structurally diverse organic contaminants from their physicochemical properties". QSAR's may thus be predictive of the additive effects of complex mixtures of structurally related toxicants.

Gosling (1992, p410) says: "Once established, a QSAR between a physicochemical descriptor (e.g. K_{OW} - Octanol water partition coefficient) and a biological response can then play an important role in: (a) predicting the toxicity of untested but structurally related compounds, (b) identifying potentially toxic environmental contaminants, which need to be incorporated into chemical monitoring programmes, (c) the systematic comparison of relative sensitivities of different organisms to classes of toxicants, thus enabling extrapolation from a test organism (e.g. mussels) to other species..." Connel (1994) reviews the significance of the octanol/water partition coefficient.

Hermens (1986) surveys the physicochemical properties and other chemical descriptors used in QSAR analyses for toxicity and lists hydrophobicity, electronic factors, steric factors and miscellaneous factors as the characteristics, which are determined by the physicochemical

property of the compound. Hydrophobicity is ascertained by octanol-water partition coefficient, chromatographic retention indices and water solubility. Electronic factors are identified by examination of Hammett constant, dissociation constants for acids and bases, molar refractivity, and dipole moments. Steric factors are identified by Taft constants and by molecular volume. Miscellaneous factors are identified by number of carbon atoms and by molecular connectivity indices.

Molecular connectivity *mc* (Brown 1986, Wynberg, Brown & Hole 1989) has been found to predict, with greater or lesser accuracy, the toxicities of organic pollutants. It is entirely non-empirical and therefore saves much time-consuming experimental work. "Its calculation is based on a count of groupings of skeletal atoms and the number of valence electrons of each such atom" (Brown 1986, p109). It is thus an independent assessment of a stress.

QSAR's and their utility are reviewed by Donkin (1994). Donkin (1994) proposes a non-specific narcotic effect, which is a universal biological response. He also says that narcosis can be highly correlated between organisms as diverse as bacteria and fish. Besides narcotic effect there are specific effects caused by such groups as neurotoxins, esters, respiratory uncouplers, and herbicides. Gosling, (1992, p411, reporting Widdows & Donkin 1991) says, "many toxicants induce effects via more than one mechanism of toxicity. Pentachlorophenol, for example, is an uncoupler of oxidative phosphorylation, which results in enhanced oxygen consumption, and simultaneously induces narcosis which reduces ciliary feeding activity in mussels".

Hermens (1986, p294) says, "Because a high quality QSAR is an indication of simplicity in mode of action, another role QSAR can play is in classifying the great number of aquatic pollutants into a limited number of groups of similarly acting compounds".

Now that we have looked at some of the possible underlying phenomena, let us return to what has been said about stress.

CHAPTER 18: STRESS

We must, "define the term and try to unravel its biology" (Broom & Johnson 1993, p173.). Problems begin here. "There is substantial disagreement over the definition of stress". And, according, to Breznitz & Goldberger (1982 p3), stressors are "external events or conditions that affect the organism". Breznitz & Goldberger (1982) also discuss the inadequate taxonomy of stressful situations. They appreciate that different species and individuals may have different responses to the same stress agent.

After further examination of the current usages of stress, some will be discontinued and the rest, where possible, will be integrated. A more focused working definition of stress will then be proposed and its subdivisions examined.

We proceed heedful that inferences in stress studies are particularly prone to circularity. Cassel (1967) in Moss (1973, p55) warns that this hazard may surface "when subjective response is taken as evidence both that the situation is stressful and that stressful situations produce undesirable symptoms".

WHAT IS STRESS?

Sindermann (1990, p219) says: "stress represents the sum of morphological, physiological biochemical and behavioural changes in individuals which result from actions of stressors". To Dillon & Lynch (1981, p228): "...clearly an altered physiological state cannot by itself constitute stress. The missing criterion is measured proof that the altered state results in a decreased chance of survival or a diminished ability to adapt to further environmental change. Only then can the organism be considered to be stressed".

We can adduce that stress must entail some form of harm to the organism, which in turn means a reduction in fitness. Any notion that stress can be harmful without reducing fitness is either an absurdity or it indicates that the term fitness needs clarification. Broom & Johnson (1993, p67) miss the point when they say, "the effect of stress occurring is to cause some or all of the control systems within the individual to work too hard for effective functioning, that is, to over tax them". The effect of stress is better interpreted as disruption

of processes which enhance the persistence of the organism, population or lineage.

As discussed previously, stress has a probabilistic (stochastic) aspect. It also indicates an increased probability of unfavourable outcomes. "It is necessary to use indicators that suggest that fitness is likely to be reduced" (Broom & Johnson 1993, p67). They continue, "stress exists when there is a high probability that the fitness of the individual will be reduced, as well as when the reduction has been proven to have occurred." Stress as a probability will be discussed further when incremental (deterministic) and (probabilistic) stochastic strain is examined.

We now posit that stress be used as a label for agents that cause or increase the probability of harmful changes in the organism that result in loss of fitness. The reduction in the fitness of the organism, the effect of the stress, is here termed strain. Stress is thus the cause or perturbation, which brings about a condition of strain in the organism. The previous examination of aspects of the organism, have presented diverse ways of exposing this definition to scientific investigation.

Stress as used here means the same as Selye's (1973) "stressor", a term that will occur only in quotation from other works. Stress as understood here may include Selye's (1973) stress response if the response itself is harmful to the organism. Selye's (1973) stress response may also be embraced by the term strain if the response is measurable as a loss of fitness. An unnecessary complication is the notion that: "the response is important and not the stressor" (Hattingh 1988). He asserts that stress elicits a reaction by the animal and it is in response to this reaction that the animal exhibits symptoms of strain. Assertions recur that this response also has some universal characteristic (Wedermeyer 1981; Pickering 1981). What is this reaction if it is not an intermediate stress - one of Levitt's (1980) secondary or tertiary stresses?

If the concept of strain is to be useful it must also, in some way, indicate deleteriousness, which implies a reduction of fitness. The degree of strain is posited here as varying inversely with fitness: increased strain means much the same as decreased fitness and vice versa. This is inspired in part by Koehn & Bayne (1989, p158): "Stress may be defined as any

environmental change that acts to reduce the fitness of an organism". Thus, an integration of all the manifestations of strain in an organism should vary inversely with its fitness and it should be possible to quantify strain and fitness with equivalent units. If so then strain and fitness can be understood as two words dealing with the same concept but from different directions.

Strain would be assessed by the condition of an organism. Fitness could only be assessed in hindsight by observing the reproductive and somatic performance of the organism. The problem is to connect these terms convincingly, both theoretically and operationally.

The theoretical framework to be proposed is constructed to admit these assertions over the range of physiological and psychological stress phenomena occurring in the individual. Thus, a large area of the stress field may then be integrated into a coherent system as urged by Hattingh (1988) but at the same time it takes into account the warnings of Van Der Steen (1993) about pseudointegration. Van Der Steen (1993, p259) warns that, "allegedly overarching theories of stress which are meant to unite biology and psychology, upon analysis, turn out to represent terminological rather than substantive unity. They should be replaced by more specific local theories."

Marmot & Madge (1987), who attempt to deal with social, psychological and biological aspects, reject the unitary underlying stress process as espoused by Selye (1976) and propose multiple stress terms. Considering the breadth of their study, this is not surprising. It is not intended to integrate social or ecological stress in this thesis. The reasons for this will be set out later.

STRESS AND CAUSATION

Stress as a cause

In analogy to Hooke's law of physics, stress may be seen as the stimulus (Cannon 1935; Levitt 1972; Ulanowicz 1978) causing a response, termed strain. Engel (1985) emphasises that biological stress is only an analogy of Hooke's law and shows that the looser definition of biological stress may lead to woolly thinking. Engel's (1985, p4) criticism of the stress

enterprise may be justified when one considers his argument: "Claude Bernard defined stress as an adaptive response to an external stimulus; while the equally distinguished 20th century physiologist Walter Cannon defined stress as the stimulus". It is posited here that the biological use of the term stress will give less offence if we are explicit that it is biological stress and that we sharpen our meaning as far as possible.

Further arguments follow to establish that though biological and physical stress overlap in meaning they are largely analogues. Biological stress is not just a force. Moreover, in organisms the response to a simple force may differ diametrically from that in an inanimate object. A physical stress applied to an inanimate object, even if considerably below the yield strength of the material may eventually cause fatigue failure. But in living material such as bone, muscle, tendon and ligament, such stress may actually cause the tissue to become stronger such as in human heart muscle (Tamargo 1995). The occurrence of work hardening in some metals (Thomas 1968) does not undermine this argument; work hardening is a considerably different phenomenon and occurs after plastic deformation (i.e. beyond the yield point) of the material. A further difference is that repair occurs only in living material. And finally, for a living material such as bone the absence of physical stress for any length of time may cause it to weaken. This is in sharp contrast to inanimate materials.

Stress, (Calow 1989, p174) is "any environmental influence that impairs the structure and functioning of organisms such that their neo-Darwinian fitness is reduced. The latter incorporates survival probability, developmental rate and fecundity..." Sibly & Calow (1989, p102) also propose stress as a cause of reduced fitness. They define stress as an "environmental condition that, when first applied, impairs Darwinian fitness; for example, it reduces survivorship (S) and/or fecundity (n) and/or increases the time between life cycle events." Sibly & Calow (1989) in using the phrase "when first applied" show, as discussed elsewhere, that they are aware of the temporal aspect of stress.

According to Bradshaw & Hardwick (1989, p138), "stress is anything which reduces growth or performance...in a situation where a particular stress operates, there must be a reduction in fitness - defined in the normal Darwinian sense as the ability to contribute to the next generation". Koehn & Bayne (1989, p157), "consider stress as an environmental change that

results in reduction of net energy balance (i.e. growth and reproduction)". Selye (1956) also says that stress in man may stunt growth. Koehn & Bayne (1989, p157) continue: "We consider that any reduction in production (somatic growth, reproduction or both) in response to an environmental change signifies reduced Darwinian fitness, and therefore represents a result of environmental stress". They go on: "Stress may be defined as any environmental change that acts to reduce the fitness of an organism. ...three general considerations should be borne in mind. First, the effects of the stress will be an integrated response involving all levels of functional complexity within the organism (molecular, cellular and physiological). Secondly, the stress response is dynamic, and involves an alteration in functional properties over time. And thirdly, a potential stress may be neutralised by homeostatic physiological compensation, although these processes may themselves be metabolically costly. It is when compensation for an environmental change is incomplete or, in the extreme, impossible, that lasting effects are measurable as a decline in the organism's fitness or, ultimately, as death."

Since Koehn & Bayne (1989, p157) "consider stress as an environmental change that results in reduction of net energy balance", the neutralisation of a potential stress by a "metabolically costly" homeostatic physiological compensation can only be a reduction, not an elimination, of that stress. Though harm may be minimised, that which is "metabolically costly" must be a stress, even if it is of lesser magnitude.

Wedermeyer (1981) and Pickering (1981) see stress in vertebrates as a stimulus which elicits a specific response, the response being characteristic irrespective of the nature of the stimulus.

"Environmental stress (Calow 1989, p173) is a somewhat elusive term since it is both level and subject dependent." Calow (1989, p173) does, however, characterise environmental stress as causing "reductions in survival probability, growth rates and reproductive outputs." This approach admits a probabilistic (stochastic) element in stress studies; it is also entertained by Broom & Johnson (1993, p170) who allow the concept of a potential stress in an environment: "that is, factors that can be measured without the animals being present."

To Levitt (1980, p3) stress is a term, "for any environmental factor potentially unfavorable to

living organisms. A biological stress may, therefore, be defined as any environmental factor capable of inducing a potentially injurious strain on living organisms." Grime's (1989a, p4) stress describes "external constraints limiting resource acquisition, growth or reproduction of organisms." Grime (1989a) thus ignores internal phenomena such as disease and ageing. Parsons (1996), Beaumont & Toro (1996), and Dahlgaard & Loeschke (1997) also understand stress as a cause.

Stress as an effect

Stress is often understood as an effect: Esch *et al.* (1975, p340) say, "In our definition, stress is identified as the product and not the cause (stressor) of the change in homeostasis or environmental stability". Pearlin (1982, p369) says, "there is general agreement that stress refers to a response of the organism to a noxious or threatening condition." "Stress can be defined extrinsically in terms of resulting effects" (Boesch & Rosenberg 1981, p179). "Stress is the effect of any force which tends to extend any homeostatic or stabilizing process beyond its normal limit at any level of biological organization" (Esch *et al.* 1975, p340). This also suggests that stress is the same thing at different ecological levels. This suggestion will be examined later.

Esch *et al.* (1975, p340) go on: "The definition recognises that stabilizing forces operate within an ecosystem and that these are analogous to those operating to maintain stability (homeostasis) in an individual organism". How did these ecological stabilizing forces come about and how can they be selected for? What is the unit of inheritance in an ecosystem? The analogy is, at best, very loose.

As Calow (1993) says, "Ecosystems do not reproduce as unitary wholes and do not have unitary genetic memories and therefore are not subject to this form of selection." Calow (1993) also has an existocentric approach to ecosystem health: "On the other hand healthy ecosystems might be defined as those that persist through time. This relates to stable states, but not ones achieved in the active cybernetic sense."

Stress (Sindermann 1990, p219), "represents the sum of morphological, physiological, biochemical, and behavioural changes in individuals which result from actions of stressors".

Brett (1958 in Ivanovici & Wiebe 1981, p15) defines stress as, "A state produced by an environmental or other factor which extends the adaptive responses of an animal beyond the normal range, or which disturbs the normal functioning to such an extent that, in either case, the chances of survival are significantly reduced". Thus, Brett (1958) also recognises the importance of probability in stress studies.

Stress, to Mikhail (1985, in Van Der Steen 1993, p264), "is a state which arises from an actual or perceived demand capability imbalance in the organism's vital adjustment actions and which is partially manifested by a non-specific response". According to Franz (1981, p50), "An organism is in a state of stress when some measure of its performance falls below par". Fisher (1977 in Boesch & Rosenberg 1981, p179) says: "Most man-induced stresses are similar to those which occur naturally, but they may differ in intensity or quality. Even those stresses that are caused by exotic, synthetic toxins must be dealt with via existing physiological and behavioral mechanisms." Weinstein (1997), Feltmate & Williams (1991), Auerbach (1981) and Perkins (1974) also see stress as an effect.

Stress as both cause and effect and/or an intermediate part of the chain of causation

If we accept causation as a model for understanding phenomena, we can see that the chain of causation may have numerous links. Each link is both a cause and an effect depending on whether it is antecedent or precedent. This chain is evident in Levitt (1980, p533), who says for plants, "(1) in nearly all cases the primary stress gives rise to a secondary and sometimes even a tertiary, stress, each of which is capable of producing its own strains within the plant. (2) These strains whether produced by the primary, secondary or tertiary stress, may be direct or indirect and elastic or plastic. The elastic inhibit growth and metabolism reversibly, the plastic are irreversible and therefore injure the cell. (3) The plant may develop resistance, either by stress avoidance or stress tolerance. (4) Tolerance to a stress may be due to either strain avoidance or strain tolerance." Stress may thus be seen as an intermediate event. This, with complications, is also implied by Hattingh (1988).

The chain of causation also underlies reports by Zegans (1982), Archer (1979) and Koehn & Bayne (1989). Zegans (1982, p137) says, "Stress, therefore, is an organismic state that can contribute...to changes in body function, which if intense or chronic may lead to disease".

Archer (1979) defines stress as, "the prolonged inability to remove a source of potential danger, leading to activation of systems for coping with danger beyond their range of maximal efficiency." This definition refers to the, "strain or force applied to the organism, the physiological response to such a strain", and to the psychological aspect of stress. Thus, Archer (1979), by so burdening the word stress has attempted to integrate disparate stress phenomena under one definition, possibly, though, to the detriment of understanding.

Koehn & Bayne (1989, p158) more accurately assert that "an organism's response may be seen on different time scales with an implied cascade of cause and effect". Stress precipitates a cascade of cause and effect, some of which may be further stressful to the organism. These facets of stress phenomena are embraced by the concept of stress causing a loss of co-ordination. The stress cascade is evident in the account by Akberali (1980) in the bivalve *Scrobicularia plana*. Hyposaline water elicits shell closure. This eliminates food intake and initiates anaerobic metabolism. In turn the calcium of the shell is dissolved and thus the shell is weakened. Energy is required later to replace this calcium.

This is also apparent in activity of the adrenal glands. A cascade of causes and effects, some with beneficial and some with deleterious effects, is mediated by the adrenal hormones. The adrenal hormones are primarily adrenaline (epinephrine) and noradrenaline (norepinephrine) - the catecholamines - from the adrenal medulla; and cortisol is produced in the cortex. Adrenomedullary hormones (Broom & Johnson 1993, p98) make available more glucose, and cortisol makes more amino acids and fatty acids available as substrates -presumably for energy production- in an emergency.

"Glucocorticoid levels rise in response to many short term problems in life and their measurement gives valuable information about the welfare of animals" (Broom & Johnson 1993, p117-118). Cortisol and corticosterone are the main glucocorticoids. In some animals both may be produced. Cortisol is predominant in primates, dogs, cats and most ungulates (Broom & Johnson 1993); corticosterone predominates in rodents and chickens. Stressful events, such as pain, association of conditions with previous pain, fighting and handling, may elevate plasma cortisol levels (Broom & Johnson 1993). But these elevations may be masked or obscured by cyclic fluctuations in baseline levels of the hormone. Moreover, (Broom &

Johnson 1993, p98) "the magnitude of the response can vary according to the stage of this cycle".

Cortisol deficiency leads to hypoglycaemia; its excess leads to hyperglycaemia and produces resistance to insulin. Cortisol also promotes the metabolism of proteins and affects nitrogen balance and growth (Schmidt-Nielsen 1990, p496).

Release of catecholamines into the blood from the adrenal medulla is a common mammalian stress response. Broom & Johnson (1993, p116) review the activity of the adrenal medulla. They say "activation of the adrenal medulla is an effective response only to short term problems." Half lives of catecholamines in the blood are shorter than those of cortical products. The adrenal medulla releases these two catecholamines within a couple of seconds of the initiating stimulus being perceived (Broom & Johnson 1993).

Adrenaline (Moss 1973, p103) stimulates carbohydrate metabolism and glycogenolysis in the muscles. "It dilates arterioles of the heart, brain and skeletal muscle, and it speeds heart rate and increases its output. Oxygen consumption and carbon dioxide production increase with elevated body temperature. It can relax the smooth muscles of the gastrointestinal tract while producing constriction of the pyloric and ileocecal sphincters. It also dilates bronchial musculature." Adrenaline is released in greater quantities in situations of fear and passive reaction to a stress.

Noradrenaline, (Moss 1973, p103) "constricts arterioles and raises blood pressure with much less influence than epinephrine on blood glucose and heart rate, both cause an increase in circulating free fatty acids". Moss (1973) suggests that noradrenaline acts as a stimulant in the brain and contributes to alertness, pleasure, euphoria, anger and fear. In contrast to adrenaline, noradrenaline (Broom & Johnson 1993) is produced in response to conditions associated with aggression and physical activity.

Stress as neither cause nor effect

Zegans (1982, p137) considers stress first as a state of the organism, then as a force and then as a causative agent, before settling for the view of Lazarus (1966) that "stress is a relational

or transactional concept describing certain kinds of adaptive commerce between any organism and its environment."

McGrath (1976, p1352) says of person to person (social-psychological stress): "there is a potential for stress when an environmental situation is perceived as presenting a demand which threatens to exceed the person's capabilities and resources for meeting it, under conditions where he expects a substantial differential in the rewards and costs from meeting the demand versus not meeting it." McGrath (1976) considers arousal as an operational definition of stress and infers arousal level from the pulse rate. He asserts that arousal is influenced by (a) the uncertainty of outcome and (b) the consequences of successful versus unsuccessful handling of the situation.

Franz (1981, p51) considers stress phenomena to be system-environment misfits. By this he means that in ecosystems, stress may be seen as, "a U-shaped function of goodness of fit centred on (S - D) = 0". S is the supply of resources and D is the demand for the resources. Thus, stress is at a minimum when these two are well matched. This systems-approach concentrates on the goodness of fit of supply and demand between organism or system and environment. And so stress may be defined as a misfit resulting in excess or deficit of supply relative to demand.

Holroyd & Lazarus, (1982, p22) "define stress relationally by reference to both the person and the environment". And..."At the psychological level, which is our primary focus here, mediational processes involving evaluation and judgement are crucial to the stress reaction".

Stress as neither cause nor effect is predominantly a concept used in psychological, ecological and social contexts. It is thus a side issue in this study. But because it overlaps certain areas of interest it will be dealt with where necessary. A large body of work, which can be categorised as treating stress as effect and an intermediate part of the chain of causation is that by Selye. Selye's contribution is so extensive that it deserves major consideration.

CHAPTER 19: SELYE'S STRESS

Selye's definitions of stress are discussed first. The significance of terms and consequences immediately springing from this are then discussed before his General Adaptation Syndrome (GAS) and other concepts are examined in detail.

SELYE'S DEFINITIONS OF STRESS

Selye (1955, p626) says, "stress may be defined as a non-specific deviation from the normal resting state, it is caused by function or damage and it stimulates repair". Selye (1955, p626) adds:..."stress is not necessarily the result of damage but can be caused by physiologic function and...it is not merely the result of a non-specific action but also comprises the defence against it." It is important to note that stress, as he sees it, includes the response of the body to it. It is thus a combination of cause and effect. These responses, he says, may also be damaging to the organism.

Selye (1956, p47) defines stress as "...the state which manifests itself by the General Adaptation Syndrome GAS." It is (Selye 1956, p54) "the state manifested by a specific syndrome which consists of all the non-specifically induced changes within a biologic system". "Thus stress has its own characteristic form and composition but no particular cause." These non-specific features according to Selye (1955, p625) might include, "...the feeling of being ill, loss of appetite and vigor, aches and pains, loss of weight..."

"In tissues more directly affected by the stress, there develops a local adaptation syndrome (LAS)" (Selye 1956, p47). The LAS is the manifestation of stress through time in one part of the body and its three stages are characterised (Selye 1956) by inflammation, degeneration and death of cells in the affected area.

The terms strain and stress appear in Selye's (1955, p626) discussion but he does not usefully distinguish them. Later Selye (1956) declares that a stressor is an agent that causes stress. Selye (1976, p50) explains that he chose the term stress in ignorance (due to his then poor command of English) of its relationship to strain. By the time he became aware of his mistake, the term stress in its new meaning had already become established. In consequence

he coined the term stressor as the causal agent.

Selye's definitions of stress continue to flow: "Stress (Selye 1956, p274) is the sum of all the wear and tear caused by any kind of vital reaction throughout the body at any one time." **This definition is edging towards stress as damage**. And (Selye 1956, p61) "stress always manifests itself by a syndrome, a sum of changes not by one change." "Whether (Selye 1973, p693) a man suffers from severe loss of blood, an infectious disease, or an advanced cancer...he loses his appetite, his muscular strength and his ambition to accomplish anything...". These conditions and the above-mentioned sum of changes all exhibit degraded co-ordination; this is the common feature.

We may reinterpret Selye's (1982, p8) statement "In all forms of life it would seem that there are common pathways that must mediate any attempt to adapt to environmental conditions and sustain life". Why postulate pathways when in all organisms the common result of stress is actually disorder, hence the similarity.

Stress is not a non-specific reaction (Selye 1956). But in contrast Selye (1973, p692) defined stress as "the non-specific response of the body to any demand made on it". How do the terms reaction and response differ? This is made more obscure because Selye (1955; 1956) interchanges the terms actions, responses, and changes without usefully distinguishing them. Although on closer examination of the context one can discern the subtle distinction intended, this latent ambiguity is not an isolated occurrence. Despite these difficulties, however, useful inferences can be made; for instance Selye (1982) adds to his definition of stress by saying that the effect may be mental or somatic. As we have seen elsewhere, the cause may also be mental or somatic and that these two causes may dialectically interact.

The stress effect (Selye 1973, p693) is: "the non-specific demand to readjust to an entirely new situation". And, "...No matter what kind of derangement is produced, all these agents have one thing in common: they also make an increased demand upon the body to readjust itself" (Selye 1982, p8). Stress as a loss of co-ordination is alluded to but not explicit in Selye's work. But he does identify stress with a derangement and recognises that readjustment is necessary.

What Selye says stress is not

"Stress is not (just) any deviation from homeostasis." Selye (1956, p53) says that any biological function can do that. Concerning this, Selye (1956, p53; 1973, p693) emphasises that "Stress is not the non-specific result of damage." However, later Selye (1974, p18) says: "Stress is not **always** the non-specific result of damage". This weakens his argument. What can damage be but derangement of structure or processes in living matter?

"Stress is not a non-specific reaction...The pattern of the stress reaction is very specific" says Selye (1956, p54). Although Selye (1956; 1973) mentions that the stress response may also occur in lower animals and plants, he also makes much of the significance of its highly selective effects on certain organs (Selye, 1956, p54) such as the adrenal, the thymus, the gastrointestinal tract and the "hypothalamus-hypophysis-adrenocortical axis" (Selye 1973, p695) or as Broom & Johnson (1993, p119) call it: "the hypothalamic-pituitary-adrenal cortex axis" (HPA).

According to Selye (1973, p694) the omnipresent signs of damage to the body when under disease attack are: "adrenal enlargement, gastrointestinal ulcers, and thymicolymphatic shrinkage." He adds: "the three changes thus became the objective indexes of stress and the basis for development of the entire stress concept." This is too limited to apply to all other organisms. Obviously, Selye's scheme is wholly applicable only to those organisms possessing such a neuroendocrine configuration. This limits the universality of his theory.

Selye (1973) states that various derangements in the secretion of hormones can lead to maladies he calls diseases of adaptation. Some of these are inflammatory diseases, which are characterised by the body wasting energy in processes that also damage it. In this context it is difficult to distinguish derangement from loss of co-ordination.

SPECIFIC AND NON-SPECIFIC STRESS RESPONSES

Selye's (1955; 1956) dichotomy of specific and non-specific stress responses undermines assertions about the universality of a stress response. "Stress shows itself as a specific syndrome, yet it is non-specifically induced" Selye (1956, p56). And: "A non-specifically

formed change (Selye 1956, p56) is one that affects all, or most, parts of a system without selectivity. It is the opposite of a specifically formed change that affects only one, or at most, few units within a system. A non-specifically caused change is one that can be produced by many or all agents. It is the opposite of a specifically caused change that can be produced by only one or at most by few agents. It is important to keep in mind that specificity is always a matter of degree. Both among changes and among causes, there are fluent transitions between the least and the most specific."

Selye postulates a universal response to stress and then erects distinctions and exceptions to preserve the validity of his construct. He then says that these distinctions are really a matter of degree. This raises more doubts; explicit criteria must be supplied to distinguish one from the other. Unfortunately Selye is less than explicit about specific responses. We can only infer that he means the direct result of application of the damaging agent. He (Selye 1976, p471) says, "The term specific has no meaning unless we indicate whether it refers to the change itself or to its causation".

He provides four categories of change: The specifically formed change is one that affects a single or a few units in the system and with great selectivity. The non-specifically formed change affects all, or most, parts of a system without selectivity. The specifically caused change is a change that can be produced by one or only a few agents. For example, "the development of a calcified or iron incrustated *(sic)* nodule (Selye 1976, p67) as only relatively few agents will produce it". The non-specifically caused change is one that can be caused by many or all agents. An example (Selye 1976, p67) is: "simple inflammation which can be produced by any irritant that enters the body."

Non-specifically caused changes are manifest in the general adaptation syndrome. Selye, however, says that the GAS is highly specific (he must mean in form, but if this is what he does mean then it is not truly general but more of a disseminated Local Adaptation Syndrome, see next page)

Instead of a specific response it is argued here that across the spectrum of organisms the outcome of application of a stressful stimulus is a change in the aseity to one that is less

persistent. It is posited that universal symptoms originate from an increase of disorder, positive feedback and homeostatic instability. It is contended that the specific response is a consequence of the fundamental effect of stress, and that is disorder.

OUTLINE OF THE ADAPTATION SYNDROMES

The General Adaptation Syndrome is driven by the secretion of adrenocorticotrophic hormone (ACTH) from the anterior hypophysis. "Neural activity in the hypothalamus results in the secretion first of interleukin 1ß and then corticotrophin releasing factor (CRF), this is followed by (ACTH) release and then glucocorticoid secretion" (Broom & Johnson 1993, p117-118). Selye (1956, p33) also characterises the GAS in space: "three fixed points (Selye favours triads) have been established as being part of a co-ordinated syndrome: the adrenal, the thymicolymphatic, and the intestinal changes."

The General Adaptation Syndrome of Selye (1955) has three temporal phases. It starts with the alarm reaction, followed by the stage of resistance and ends with the stage of exhaustion. These (Selye 1956) can also be applied to the Local Adaptation Syndrome (LAS).

Selye (1973, 1974) says that during the alarm phase if the stress is sufficiently strong then death may result. Then in this case only one phase is exhibited. A further problem is that according to Selye (1982) the organism need not go through all the stages but instead may progress through the first two stages many times in its life. These examples both erode assertions of universality of the triphasic GAS. Therefore the GAS does not tell the whole story. Perhaps it would be better to say that stress causes disorder and a response to the disorder is the GAS, if the organism lives long enough. Clearly, disorder is the result of stress; and the GAS is a secondary phenomenon.

Phases of the GAS

Selye (1956, p87) presents a figure of the relationship of general resistance to injury with time. He asserts that in the alarm reaction general resistance "falls way below normal" (Selye 1956, p87, 1955). It then climbs to the plateau of the phase of resistance where it is above normal, here the organism is said to be adapted.

The alarm reaction (Selye 1956, p31) probably represents: "the bodily expression of generalized call to arms of the defensive forces of the body." During this reaction, "the cells of the adrenal cortex discharge their microscopically visible granules of secretion (which contain the hormone) into the blood stream". At the phase of resistance which Selye (1956) says follows the alarm reaction, the cortex accumulates a reserve of secretory granules. At the third phase, exhaustion, "the stores of the gland were depleted" (Selye 1956, p31). Selye (1956) says that the symptoms of exhaustion are in many respects similar to those of the alarm phase.

By analogy Selye (1955, 1982) argues for the similarity of the triphasic stress response with the apparent triphasic (another triad) resistance of the body during development from childhood to senility. This terminal loss of resistance, he asserts, is physicochemically obscure and he suggests the term adaptation energy for what is lost.

ADAPTATION ENERGY

Adaptation energy (Selye 1976, p463) is: "The energy necessary to acquire and maintain adaptation, apart from caloric requirements." "It is as though, at birth, each individual inherited a certain amount of adaptation energy" (Selye 1956, p66). In consequence, ageing, says (Selye 1956) is caused by loss of adaptation energy. Nothing is known (Selye 1955) of the nature of this energy and "We still do not know precisely what is lost, except that it is not merely caloric energy: food intake is normal during the stage of resistance" (Selye 1982, p10).

Selye (1976, p429) divides adaptation energy into superficial and deep categories. Superficial adaptation energy is apparently ready to use but the deep adaptation energy can be transferred to the superficial energy pool only slowly. This, says Selye, prevents squandering of resources.

Can such a concept as non-caloric energy be made operational? It is suggested that the loss of adaptation energy be better conceptualised as loss of order in structures and processes, particularly those involved in the reorganisation and repair of the organism. Thus, ageing could be more profitably seen as increasing disorder in living structures and processes. It is

contended here that the GAS with the exception of the end point is a proximal stress response. Any part of it must be contextualised by giving some indication of time-frame.

EUSTRESS

Although eustress has been previously discussed it is necessary here to re-examine it in the light of the more detailed exposition of Selye's stress. Selye (1974, p20) says that: "Pleasant as well as unpleasant emotional arousal is accompanied by an increase in physiological stress (but not necessarily in distress)". Selye (1974) asserts that distress is harmful, unpleasant stress; he does not present the concept of eustress here (Selye 1974). Distress (Selye 1976, p465) is "unpleasant or disease producing stress" And eustress is (Selye 1976, p466) "pleasant or curative stress."

But Selye (1974, p15) says: "From the point of view of its stress producing or stressor activity, it is immaterial whether the agent or situation we face is pleasant or unpleasant..." He echoes this when he (Selye 1974, p66) declares that there is a stereotyped physical pattern of the body's response **to stress of any cause**. He then, in self-contradiction, says that "The outcome of our interactions with the environment depends just as much upon our reactions to the stressor as upon the nature of the stressor itself". He is, in effect, saying that the body responds in a stereotyped way except when it does not.

One can speculate how the categories of distress/stress and eustress would fare if the contrasting effects of chemotherapy and cocaine were considered. Chemotherapy is unpleasant but curative and cocaine, so it is said, is pleasant but hazardous. Distress/ eustress categories break down here. But if proximal and distal effects were noted, i.e. the time-frame of the stress phenomena were noted, then the situation would easily be resolved.

Selye (1974, p67) says that "depending on our reactions, meeting a challenge may result in a gain or loss...On the automatic, involuntary level the gain is accomplished through chemical responses (immunity, destruction of poisons, healing of wounds, etc.)." These are not gains; they are burdens. The opportunity for repair is nothing more than an extra drain on energy reserves. It may be the lesser of evils but it is not an advantage. A stress of lesser magnitude cannot become a eustress.

Another problem is that Selye hints at stress having an optimal level above or below which the organism suffers. Selye (1956, p54) says of stress: "countless people have actually suffered or benefited from it." And, "Stress, applied in moderation is necessary for life" (Selye 1956, p300). Selye (1956, p299) furthermore says, "The goal is certainly not to avoid stress. Stress is part of life. It is a natural by-product of all our activities". This is apparently supported by Broom & Johnson (1993, p61), who state that glucocorticoids are also "released in response to situations that are not normally regarded as stressful, including courtship, copulation and hunting". These situations require re-evaluation.

For instance, the physiological and anatomical preparations for courtship (Dawkins 1986) may result in an increase in predation probability and use up somatic energy. The same may be said for copulation, but one must remember the findings of Gwynne (1989) who asserts that for some organisms there may actually be a survival advantage during mating. These findings do not change the facts that in some spiders and mantids copulation results in death of the male and hence in zero somatic fitness. Hunting pushes somatic fitness parameters such as oxygen transport and consumption towards their limits. A predator may have lower defence capability immediately after a hunting session until it has "got its breath back". Hence situations which result in glucocorticoid release probably have some fitness implications. These should be interpreted in the light of proximal and distal responses to stresses. It is not a good/bad but a bad/less bad dichotomy. This is better covered by a cost/benefit analysis.

PRIMARY AND SECONDARY CHANGES

Selye (1956, p58) asserts: "stress causes two types of change: a primary change, which is non-specific both in its form and in its causation (it can be induced anywhere and by any kind of damage or function), and a secondary change, which has the specific pattern of the GAS. The first acts as a common signal which can elicit the second from any part of the body." Can we infer that the GAS is specifically formed but has non-specific causation? This is rather complicated. Again, the events ascribed to Selye's stress can more completely be embraced by treating its effects as a loss of co-ordination.

On distinguishing primary and secondary change, Selye (1956, p59) says, "We recognise this difference in principle, but it is often impossible to do so in practice". So how can we distinguish and measure manifestations of these concepts? Selye (1956) also suggests that specific and non-specifically caused changes are on a continuum; this weakens the argument for their utility.

DIRECT AND INDIRECT PATHOGENS

Positing the existence of a response - especially one that is universal - raises difficulties which are only partially offset by referring to direct and indirect pathogens (Selye 1973) - Selye's pathogen is here understood as a stress. Difficulties still exist because such a dichotomy implies a further denial of universality of response.

An indirect pathogen (Selye 1976, p467) is, "An agent causing disease by stimulating an inappropriate or excessive defensive response (e.g., immune reactions, inflammation)" and (Selye 1974, p149) "excessive mental irritation and tension". Indirect pathogens elicit a harmful response similar to that posited by Hattingh (1988): they subvert the system and make it damage itself. Such pathogens would include irritants and inflammatory agents. It is interesting though that indirect pathogens tend to be more concerned with the changing of processes.

Direct pathogens cause damage regardless of the reaction of the body. A direct pathogen Selye (1976, p465) is: "An agent causing disease through its direct effect." Examples of direct pathogens include strong acids, alkalis and boiling water. In contrast to the process-changing indirect pathogens, direct pathogens change structure.

Direct pathogens may be less likely to cause a response. For instance, during some fatal stresses the response of the organism may be non-existent. If an organism is crushed, chopped into pieces or incinerated, it may be dead before any of its stress response repertoire is mobilised. Thus a response may occur but it is not necessarily universal. As previously asserted the only universal response to stress in this context is disorder with an end at death. This approach embraces Hattingh's (1988) intermediate responses and Selye's (1973) dichotomy of pathogens.

SYNTOXIC AND CATATOXIC RESPONSES

Syntoxic reactions are triggered by syntoxic stimuli. They, "act as tissue tranquilizers, creating a state of passive tolerance which permits a kind of symbiosis or peaceful coexistence with aggressors" (Selye 1973, p697). The anti-inflammatory corticoids such as cortisone inhibit inflammation and the immune response (Selye 1973).

Catatoxic reactions are triggered by catatoxic stimuli. These agents, "cause chemical changes (mainly through the production of destructive enzymes) which lead to an active attack upon the pathogen, usually by accelerating its metabolic degradation" (Selye 1973, p697). "Catatoxic hormones are proinflammatory and include the mineralocorticoids" (Selye 1976, p56).

Selye (1976, p56) says, "the response to stress has a tripartite (another triad) mechanism consisting of: (1) the direct effect of the stressor upon the body; (2) internal responses which stimulate tissue defense or help to destroy damaging substances; and (3) internal responses which cause tissue surrender by inhibiting unnecessary or excessive defense. Resistance and adaptation depend on a proper balance of these three factors."

SELYE'S REACTON THEORY

Selye (1956) proposes a theory of stress that unites biology and psychology. It is based on "**reactons**" which are reminiscent of Leibniz's (1898) monadology. These are, (Selye 1956, p233) "the smallest possible biologic target which can still respond selectively to stimulation". It is, (Selye 1956, p310) "the primary sub-cellular unit in living matter, which still exhibits the property of responding selectively to stimulation". Reactons can enlarge and multiply but they, (Selye 1956, p236) "may not have visible limits; they may merely be focal points of interactions between the constituents in living matter". Selye (1956) alleges that the reacton concept opens to experimental analysis that range of units between the cell and the chemical element. Each reacton (Selye 1956) can give only one kind of response and it is the sum of all reactons responding to events that give the characteristics of each organismic reaction.

Questions remain unanswered: does every species have its own reactons? If so then we need a taxonomy of reactons. What is the genealogy of reactons? Is there a stem lineage of undifferentiated reactons? How do they react with one another? What is the outcome of these reactions, new reactons? So far, reactons have been loosely defined; a few examples would help.

The concept of reactons appears too diffuse to have any operational meaning. Moreover, its reductionist approach may overlook that the nature of organisms is not merely the result of a simple agglomeration of subordinate events. Organisms present new phenomena at higher levels of complexity. Reductionism does not take into account the increasing numbers of boundary conditions that may arise as a result of increasing complexity. As Brown (1994, p143) says "a level of organisation cannot be fully understood in terms of the factors involved in the level below it".

A use for reductionism?

Calow & Sibly (1990), nevertheless, propose a reductionist programme to link response to toxicants at the individual level to population numbers. They go further and propose to tie molecular or cellular responses to population numbers. This hinges, they say, on assessment of the relationship between survival probability and metabolic rate as measured by oxygen consumption. This approach has the wisdom of seeing the organisms in terms of survival probability, which is an existocentric term. Possibly this approach may be sufficient to explain and predict the salient events of population dynamics. Calow & Sibly (1990) propose three models of the relationship between scope for metabolism and survival probability. The first has a metabolic threshold, above which, survival is independent of scope for metabolism and below which the organism is dead. In the second, survival probability rises in linear proportion to scope for metabolism. In the third, survival probability rises to an asymptotic maximum. If the relationship is either the second or the third then the reductionist programme is possible. If it is best represented by the first then there are likely to be difficulties in predicting survival. Baillieul, Selens & Blust (1996) found that a measure of metabolic potential (scope for growth) is not correlated with different capacities to cope with future stress. Further work should be done to assess the relationship between oxygen consumption and scope for growth. If they are equivalent in survival terms, it suggests that

indeed the first relationship is likely to hold. This in turn casts doubt on the possibility of predicting survival.

SELYE'S STRESS QUOTIENT

Selye (1956) suggests that stress is an equalizer of activities. He says that it helps to prevent one-sided overexertion. This needs definition. Thus, no part of the body becomes excessively overused compared with any other part. To further describe this Selye (1956, p267) proposes a stress quotient, It would have been interesting if he had suggested units and values:

$$= \frac{\text{Local stress in any one part}}{\text{Total stress in the body}}$$

OTHER COMMENTS ON SELYE'S STRESS

Broom & Johnson (1993, p59) say, Selye's stress "is not sufficiently precise to form a basis for theoretical arguments". One must agree that Selye creates a number of new concepts and ideas, many of which are difficult to render operational. Broom & Johnson (1993, p63) put it succinctly: "the concept and vocabulary of stress are best solved by simplification not complication". Furthermore, Broom & Johnson (1993, p60) say: "It is clear that there is no single stress response, but rather a wide range of physiological and other changes which, although overlapping in some components, are usually quite specific to circumstances. The biological response to stress is considerably less uniform than the response proposed as the central tenet of Selye's theory."

Central to the GAS is the adrenal response, which Broom & Johnson (1993, p60) claim is inconsistent. They say: "whilst cold conditions increased the activity of the adrenal cortex of rhesus monkeys, other unpleasant and sometimes life-threatening situations which the monkeys would avoid if they could, did not lead to this response. In fact, gradual overheating caused a decrease in cortex activity and there was no change in response to bleeding, confinement after a period, or during the regime of intake of a normal bulk but nutrition free diet." In addition, Broom & Johnson (1993, p60) review evidence, "to show that the

neuroendocrinological and other biological responses to adversity are varied and stimulus dependent". For this and other reasons Broom & Johnson (1993, p61) conclude: "single adrenal indices must be considered questionable indicators of stress in many circumstances".

The GAS comes under further attack: Esch *et al.* (1975, p341) say: "environmental stressors often cause changes which extend the physiological or behavioural capabilities beyond homeostatic limits, without evoking the GAS. For example, some environmental stressors may induce changes in feeding behaviour, which would not necessarily result in an alarm reaction. Changes in diet and in environmental temperature are both capable of changing immunologic responsiveness without apparently evoking the GAS."

Transmission of the alarm response creates problems. Selye (1982) reports that the afferent carriers of the alarm signal have yet to be identified. He speculates that the afferent signals take many forms and at least some of them are mediated by the nervous system and hypothalamus, resulting in psychogenic stress (strain). Selye (1982, p14) acknowledges that, "psychological arousal is one of the most frequent activators". He emphasises, though, that this is not the only activation pathway, as stress reactions can also occur in patients who are physically traumatised while under deep anaesthesia. We must also be aware that even anaesthetics are stresses. Non-nervous initiators, he thinks, are "metabolic (p11) products released during activity or damage or that they are made evident by the lowering of levels of some vital substance consumed whenever any demand is made upon an organ." This is supported, he says, by experiments with deafferented rats which still show the syndrome. This, Selye (1982, p11) suggests, shows that "it is probable that often, if not always, the signals travel in the blood".

There is support for the notion that signals may be transmitted by a depression of normal levels of a messenger substance. Chalones appear to work in this way. Chalones are thought to be glycoproteins (Abercrombie, Hickman, Johnson & Thain, 1990). Brauner and Fitzpatrick (1971) report that chalones inhibit mitosis and are lacking in some cancers. Each tissue (Grinker 1974) produces its own chalone. Cell proliferation is inhibited when the concentration of chalone reaches a threshold.

The chalone concept is not, however, without controversy. The term chalone has been discontinued in the 14th collective index (1997) of Chemical Abstracts published by the American Chemical Society. Nevertheless, it has been recently used in studies on growth inhibiting factors by Ohnishi, Nakamura, Arakaki, and Daikuhara (1997) and by Harkava, Afonina, Zadorina and Bryuzhyna (1995).

The response of rats to a cold water swim after application of other stresses shows that the rats have no other general resistance; resistance that should be there according to Selye's model. Can we assume that normally, organisms are completely unstressed in terms of GAS? If so then the triphasic model would hold. If not then we would expect the triphasic response to be masked by the pre-existence of another stress. The level of stress absence would be indicated by the magnitude of the rise of the alarm reaction. A prealarmed organism must be in one of the later stages and cannot be alarmed again.

Konarska, Stewart & McCarty (1989a in Broom & Johnson 1993, p117) report: "after repeated foot-shock or restraint of rats for 27 days there was a steady decline in the increase in plasma catecholamine levels which occurred following the initial experiences, that is, there was habituation" (but only specific habituation as the following makes plain). "However, the catecholamine response of these animals to a new experience, for instance a cold water swim, was higher than that produced by a cold water swim in animals which had not been subjected to prior unpleasant experiences." It appears that the rats can be in a phase of resistance for one stress but are still subject to alarm from another. This casts suspicion on this model of GAS or it suggests that more than one GAS may run in the same organism. This damages notions of universality. If there were a general stress response one would expect it to have a general benefit but this does not appear to be so. The animals habituate to one stress such as foot shock or restraint for 27 days but are still responsive to the stress of a cold swim.

Some stresses may not fit into Selye's triphasic model. For instance, Selye (1955) cites a self-sustaining condition of hypertension that leads to death. The effect of asbestos or other carcinogenic poisoning also raises problems. Such problems are even more acute in Donkin's (1994, p339) report: "Some unreactive chemicals can be converted to reactive forms by metabolism within the organism. Perhaps the best known are polyaromatic hydrocarbons

which can be converted to carcinogenic metabolites by monooxygenase enzymes." The compound is not a stress until the body makes it so. Can one fit a triphasic curve to the effect of a self-manufactured carcinogen? How would such a process fit in to the inverted U shaped scheme of fluctuating resistance? Can one identify the resistance phase of such a process? What is the organism resisting? It is proposed that these difficulties could be circumvented by replacement of a triphasic stress response by the concepts of proximal and distal effects of stress and stress as an agent causing disorder.

"No agent produces only stress. Hence, in actual experimentation, the stress response is invariably complicated by certain superimposed specific changes....These factors tend to mask or deform the typical stress response" (Selye 1955, p626). This is an apologia for the confusion such a stress approach engenders.

Selye (1952, 1955) erects specific and non-specific stress responses, General Adaptation Syndrome and Local Adaptation Syndrome (Selye 1955, 1982), syntoxic and catatoxic responses (Selye 1973, 1982), direct and indirect pathogens (Selye 1973), primary and secondary changes (Selye 1956), and superficial and deep adaptation energy (Selye 1974, p28). These serve to explain why his original universal response is not so universal. We are presented with a plethora of exceptions. It is concluded that Selye's work has shed much light on stress phenomena to the benefit of our understanding. It is suggested, however, that he invented too many subsidiary concepts in his attempt to maintain the viability of his construct.

CHAPTER 20: STRESS SINCE SELYE

BROOM AND JOHNSON'S STRESS, WELFARE, SUFFERING, FITNESS AND ADAPTATION

It is now opportune to consider the construct offered by Broom and Johnson. Their model partly replaces, partly supplements and certainly clarifies Selye's stress approach.

Broom & Johnson's (1993, p178) stress, "is an environmental effect on an individual which overtaxes its control systems and reduces its fitness or appears likely to do so. Fitness reduction involves increased mortality and failure to grow or reproduce." Does not the statement referring to overtaxing imply loss of fitness? Is there an example of an organism with overtaxed control systems not being in a state of degraded fitness? Broom & Johnson (1993, p72) add, "There will normally be a reaction on the part of the individual to such an effect". This is a response to stress, or a stress response, and the immediate and short term consequences of the stress are strain. They define strain as "the short term consequences of stress" (p72), which is not very informative.

Brief or minor events such as transient heating and minor injury which are unlikely to reduce fitness would not be called stress. But on the other hand, prolonged but minor events, say Broom & Johnson (1993, p72), such as limited immunosuppression, or low level infection might be called stresses if they reduced fitness or appeared likely to do so. "Sufficiently innocuous" events, they say, would not constitute stress. Is this not necessarily implicit in the word "Sufficiently"?

Broom & Johnson (1993, p57) amplify the meaning of stress: "stimulation beyond the capacity for complete adaptation is a phenomenon which is referred to...as stress". This definition, Broom & Johnson (1993, p62) say, is better than that used by "some people" who define stress as, "anything that causes an adrenal cortex response". This is because harm or benefit (as a fitness change) as a result of adrenal fluctuations may be interpreted either way, unless the time-frame is specified.

Broom & Johnson (1993, p73) propose that, "A distinction is therefore made between minor

disturbances to an animal's equilibrium, which may result in the use of energy to correct them but have no consequences for fitness, and those disturbances which do, or are likely to, reduce fitness". We must ask how minor? And we must ask, over what time span? Even some physical damage can be made good given time and energy. Although during the repair phase fitness may be markedly degraded, later fitness indicators may be very optimistic. The above division of stresses into those that may be overcome entirely by increased energy and those that cannot, is not a dichotomy. It is a continuum with two poles. Energy loss, as shown above, is also a loss of fitness; either physical or probable.

Though they discuss summation of welfare parameters, Broom & Johnson (1993, p43) underestimate the possibility that individually insignificant stresses can add up to become measurable. This is despite their assertion that "the simultaneous imposition of numbers of minor stimuli, any one of which might be imperceptible...(can)...collectively cause drastic disturbances to animal functions". This summation may be of simultaneous events or over time. Thus, time-frames should be considered for fitness assessment. In transient events, fitness parameters may be only temporarily depressed and thus long term fitness may appear unaffected.

Caution is urged before ruling out the presence of a stress; damage may occur even without overtly deleterious symptoms. Normal activities..."can produce considerable stress without causing conspicuous damage" (Selye 1973, p693). And we have already seen (Koehn & Bayne 1989) that a stress may be neutralized but the process of neutralisation may impose a metabolic cost, a cost that is clearly due to the applied stress. Koehn & Bayne (1989) suggest that stress may reduce the range of environmental variation in which organisms can maintain positive production values. Calow (1989) supports this when he reports that stress responses at molecular or cellular level may not be apparent at the level of the organism because of homeostasis. Glass & Singer (1972, p11) agree: "The subject may adapt to the threat and show fewer overt physical symptoms of the flight or fight response yet the adaptation may take its toll". They add that after-effects such as physical and mental disease, psychosomatic disorders, performance and learning deficits may occur. Thus: "in spite of adaptation, a stress may leave its imprint on behaviours occurring after the stimulation has ceased" (Glass & Singer 1972, p11). Clearly the conceptual divide between stresses and innocuous stimuli is

far from defined.

Broom & Johnson (1993) reject the notion that stress refers to just any displacement from the optimum. They (Broom & Johnson 1993) deprecate such an assertion by Block (1985). Block's (1985) optimum, unfortunately, lacks precise definition; it could refer to a maximal rate of some single body process. Or it could apply to a suite of processes. Although its utility would be limited in the first instance it may become increasingly accurate as more processes are taken into account. Confusingly Block (1985, p135) uses the term optimum in two senses. First he implies that stimulus describes a change in the environment; and the response is a deviation from an internal optimum: "Stress in biological terms may be considered to occur when there is a deviation from the optimum of a particular parameter in response to a stimulus." Then he (Block 1985, p135) shifts the meaning of optimum: "In respect of cold and hot, dry and wet conditions, an organism may alter its level of activity of a particular physiological process, such as respiration, in response to deviations about the optimum condition." Here the optimum refers to external conditions.

The confusion is compounded when Block (1985, p135) says, "Freezing is a major stress of polar plants and invertebrates". Is freezing a response? Or is it a stimulus? He then says that their response is, "to either avoid ice formation in their cells or..." So freezing must be a stimulus? If so, then stress in this instance is a stimulus? What is gained here by separating stress from stimulus?

If we set aside these objections to Block's appreciation of stress we could, as do Broom & Johnson (1993, p63), conclude that Block's stress includes any perturbation inflicted on the homeostasis of the organism. We do not, however, have to accept Broom & Johnson's (1993, p63) conclusion that it has no real value. This is because all such perturbations carry with them a cost to the organism. Any response has a cost; so too does failure to respond. Any deviation of homeostasis equilibrium is attended by requirement to restore it; and this requires the diversion of resources. If it is not restored then further imbalances may come about due to such events as differential change in enzyme activity. All perturbations thus impose a load on the organism.

This is illustrated by Fry (1947, in Widdows 1978), who asserts that stress, acts to reduce the zone of tolerance: that zone where the effects of stress are not clinically apparent. This reduction is in itself deleterious: it narrows the range of conditions over which the organism may be able to withstand further perturbation. Clearly even in the zone of tolerance there are more and less favourable locations. Consequently, fitness, at least in a probabilistic (stochastic) sense, changes with position even in the zone of tolerance.

Any agent that makes demands on the organism to adapt to it does so in some proportion to the quantity of stress such as the amount, duration or intensity of application. Thus more stress demands more adaptation. If so, there is a limit at which the organism cannot adapt any further and then lack of adaptation will become more rapidly apparent as some change of fitness in the organism. It follows that an intensity of stress just below the limit of adaptation is more disadvantageous than one much lower. If only because the reserves of adaptation are now almost depleted and a random, or stochastic, component of fitness may supervene.

This may be illustrated by a graph of stress versus fitness (Figure 1). The zone of tolerance, or coping, etc. is indicated by a line of very shallow (perhaps undetectable) gradient. At a threshold the gradient becomes detectable and then we may say that the agent has become a stress. Thus stress and coping may be distinguished by a change in gradient. In the zone of coping, apparent fitness changes either insignificantly or only slightly with increase in stress. In the zone of strain the loss of fitness is much greater per unit of stress. By that criterion they are different, but they are still part of the same line signifying a change of fitness. The difference depends on how distinct the change of gradient is.

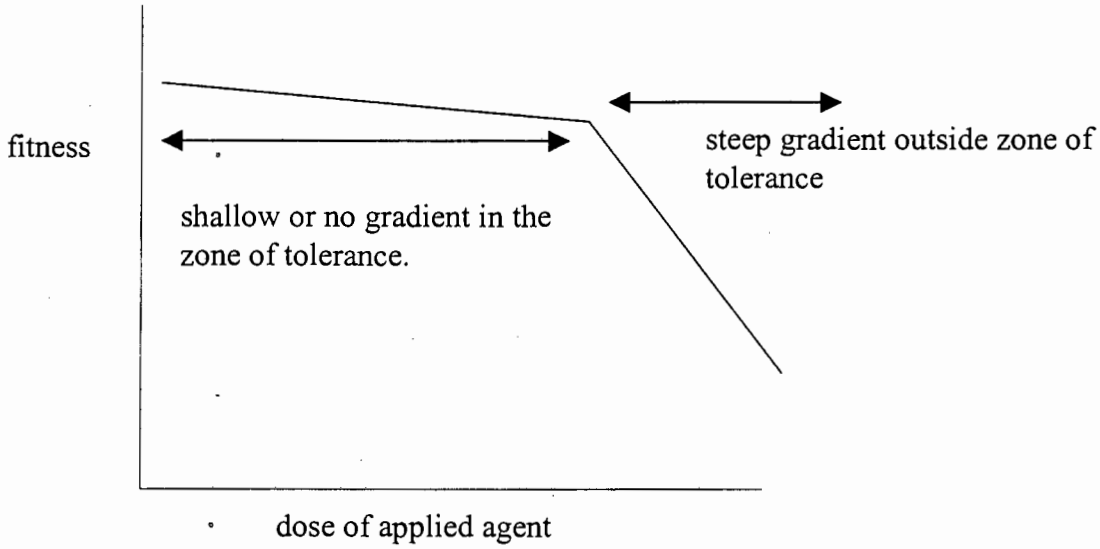


Figure 1. A possible biphasic response to varying intensity of a stress. Adapted from Koehn & Bayne (1989, p160).

Broom & Johnson (1993, p63) ask, "how great must the regulatory attempts or the consequences be before the word stress should be used?" Perhaps we should take an instrumentalist view and say as soon as it is detectable; no other distinction is as clear cut. When fitness is reduced, then by the amount that it is reduced is an index of the magnitude of stress.

Broom & Johnson (1993) lay particular emphasis on welfare, coping and suffering, which they see as connected with stress. In consequence, these terms must also be appraised.

Welfare

Welfare, (Broom & Johnson 1993, p178) is "the state of the individual as regards its attempts to cope with its environment". Puppe (1996) adds to this definition that the organism must be able to make an emotional assessment of the result. Broom & Johnson (1993, p77) list the following as measures of poor welfare:

Reduced life expectancy
Reduced ability to grow or to breed
Body damage

Disease
Immunosuppression
Physiological attempts to cope
Behavioural attempts to cope
Behaviour pathology
Self narcotization
Extent of behavioural aversion shown
Extent of suppression of normal behaviour
Extent to which normal physiological processes
and anatomical developments are prevented.

These examples could all be interpreted as affecting fitness so why invent another term? In fairness, however, it must be conceded that behavioural changes are probably the most difficult of the above indicators to incriminate as a loss of fitness. So we must examine them in more detail. These behaviours often take the form of stereotypies (Broom & Johnson 1993), which include bar biting, or sham chewing, tongue rolling in calves, crib biting and route tracing. Stereotypies are "repeated relatively invariant sequences of movements which have no obvious functions" (Broom & Johnson 1993, p77). Actions without functions (fitness enhancing processes are preferable to functions) are, by implication, a waste of energy and to waste energy is to compromise fitness. Even behavioural changes can be incriminated as deleterious to fitness. So does the term welfare tell us anything new?

However, according to Broom & Johnson (1993) there are many circumstances in which welfare is poor without there being any effect on biological fitness. This is in direct opposition to the usage of Dawkins (1976) that welfare is defined as chances of survival. Nevertheless, Broom & Johnson, (1993, p76, citing Wiepkema 1987) claim to verify their assertion by consideration of, for example, animals that are in pain, feel fear, or have difficulty controlling their interactions with their environment. This difficulty may be "because of (a) frustration, (b) absence of some important stimulus, (c) insufficient stimulation, (d) over-stimulation or (e) too much unpredictability". These do not support their assertion. They all, at least potentially, reduce fitness.

Broom & Johnson mention pain as a condition where welfare might be poor without there being any effect on biological fitness. Let us, then, look at the effect of pain. Broom & Johnson (1993, p76) say: "If...individuals in one situation are in slight pain but those in another are in severe pain, then welfare is poorer in the second situation even if the pain or its

cause does not result in any long term consequences such as a reduction in fitness". Two objections can be raised. Fitness fluctuation may occur over the short term. Moreover, pain causes physical and mental changes, which do result in deleterious outcomes.

The deleterious effect of pain should not be underestimated. Sternbach (1968, p58) lists the following general responses to pain: "Gastrointestinal motility is inhibited or movements are smaller and more frequent. The second symptom may precede complete blocking of contractions. Alveolar ventilation rate increases and hyperventilation may occur. There is increased skeletal muscle tension, especially in the region of the stimulus. Commonly, diastolic and systolic pressures increase; this may be accompanied by raised pulse rate and increased stroke volume." In addition, "to state the obvious, pain is disruptive of behavior. Learning, reaction time, and hand steadiness are examples of performance impaired by painful stimulation. Pain may also elicit aggressive behavior, and social stimuli previously associated with pain can come to elicit such hostility alone" (Sternbach 1968, p77). A result of pain is anxiety and Moss (1973, p36) reports that, "anxiety can cause disturbances of perception".

Pain (Selye 1955) elicits the stress syndrome and in this connection Broom & Johnson (1993, p97) assert that pain may elevate cortisol levels in the plasma. These are potentially damaging responses. Pain is clearly linked to loss of fitness as well as to welfare. Furthermore, one can say that pain is, normally, a cause of suffering. Broom & Johnson (1993, p80 cite Dawkins 1990): "that suffering occurs when unpleasant subjective feelings are acute or continue for a long time because the animal is unable to carry out the actions that would normally reduce risks to life and reproduction in those circumstances". This is clearly an effect on fitness.

Broom & Johnson also mention fear as affecting welfare. Let us now look at an aspect of fear. As examples of degraded welfare without stress Broom & Johnson (1993, p82-83) mention human phobias. They say that no effect on fitness occurs unless the response itself adversely affects fitness. Phobias are mental aberrations: they cause values to be allotted to entities which are not consonant with reality. In consequence, trivia may be overvalued and important things undervalued. This can lead to inappropriate actions prejudicial to fitness.

Moreover, fear and anxiety caused by phobias elicit physiological responses, such as adrenaline production, which are, on balance, only beneficial in the face of real threats. Secondary mental degradation due to fear is also liable to reduce performance and thus lead to a loss of fitness. Clearly this loss of welfare is also loss of fitness.

Broom & Johnson (1993, p109) do admit that, "an extreme response or a series of relatively extreme responses may affect fitness. In an extreme situation, a substantial startle response could lead to a heart attack, or to some cardiac tissue damage which makes subsequent heart attacks more likely to be fatal." Are not such startle responses likely to occur in the phobic subject when challenged by the object of phobia?

Broom & Johnson (1993) also assert that welfare can range into the positive as well as being negative. Before we can agree we must establish exactly what we mean by welfare. Then we must provide operational parameters of pure welfare. In the light of the above discussion, achieving this would be problematical.

Broom & Johnson (1993, p75) refer to a continuum of welfare states. But the examples they give are all on a continuum of decreasing fitness. Broom & Johnson (1993, p75) tell us that welfare is poor when there is inability or difficulty in coping. But they also say that "failure to cope implies fitness reduction and hence stress". It follows that, for the organism, being nearly unable to cope is more deleterious than easily coping. Therefore even this pre-threshold condition is, at least probabilistically, more stressful.

In an effort to uncouple welfare and subjective experiences Broom & Johnson (1993, p82) say, "welfare should not be defined solely in terms of subjective experiences". They give an example: "if an animal is injured, say by a bone breakage, a cut in the skin or an ulcer in the stomach, its welfare is poorer than that of an individual that is not injured." They furthermore say: "Even if the individual with the injury is asleep or anaesthetized, and hence is not suffering, there is an effect on welfare." Yes, but it is indistinguishable from loss of fitness.

Perhaps welfare could be used more profitably to indicate the direction of change of fitness of the organism? Alternatively welfare could be conceived as being dependent on the state of

health of the organism compared with its expected state of health at that stage in its life. The expected state must take into account the consequences of natural processes such as senescence and programmed cell death. Such considerations would include the following sort of questions. If one is dying on schedule is one's welfare bad? In this respect, what would be the welfare of a person in the process of dying at age 90? Is it better or worse than the welfare of someone dying at age 20?

An application of the above suggestion could be applied to Broom & Johnson's (1993, p113) statement that sub-optimal living conditions can cause reduced life expectancy. In farm animals they say that life expectancy can effectively be measured by potential rather than real life expectancy. This is where welfare as a relationship of actual to expected fitness comes into its own. This is because farm animals are often killed long before the onset of natural causes of death. They also say that (p113) "meaningful comparisons of life expectancy of husbanded and wild animals are difficult because wild animals have to contend with predators, parasites and pathogen challenges, which are avoidable in captivity. An estimate of life expectancy in the wild should be made by considering individuals which are not eaten by predators or severely affected by diseases and parasites." Such an estimate would be one sided. We must ascertain what the typical welfare level, including pathogens, is in the wild. This assumes of course that the organism in question is a viable wild species. It is possible that fitness/welfare parameters of husbanded animals is in fact higher than those in the wild.

Broom & Johnson's (1993) claim that degraded welfare does not of necessity indicate loss of fitness is not substantiated by the evidence. Even if their claim is accepted it still indicates vulnerability; which can be interpreted as a stochastic stress. Which is a probabilistic loss of fitness.

Coping

Coping is, on examination, another fitness parameter. Broom & Johnson (1993, p175) assert that coping is to "have control of mental and bodily stability." In consequence any erosion of coping ability means threatened or actual loss of body stability. This can only mean loss of actual or probabilistic fitness.

Coping has a cost. Broom & Johnson (1993, p170) refer to judgement of how long a particular level of coping activity can be tolerated and whether it will lead to a reduction in biological fitness. If a particular level of coping activity can be maintained only over a finite time then it means that something is being expended. This expenditure is obviously fitness related. The judgement, Broom & Johnson (1993, p170) refer to, they say, is difficult to make confidently as there is a paucity of published information on such parameters.

Broom & Johnson (1993) say that when an animal is having difficulty in coping or is failing to cope, its welfare is poor. Indications of failure to cope, they say, include impaired life expectancy and reduced ability to reproduce. These are fitness parameters.

We have already shown that poor welfare implies compromised fitness. Moreover, failure to cope (independently from the welfare argument) implies fitness reduction and hence the imposition of stress. It should also be noted that the difference between difficulty in coping and failure to cope is a matter of degree not of quality.

Broom & Johnson (1993, p73) state: "There are many occasions when individuals find coping difficult, but succeed without long-term adverse consequences (but there can be short term consequences - proximate fitness depression) by, for example using a brief adrenal response or a behavioural change of some kind." Furthermore Broom & Johnson (1993, p73) assert: "A minor injury or a period of illness might have no effect on the fitness of an individual." Is it deemed minor because of its effect? If so, we have circularity. How else would we ascertain its magnitude? One must define fitness, physically and temporally, before that can be asserted - some fitness parameter must be depressed for some period otherwise injury and illness as terms become meaningless.

Broom & Johnson (1993, p74) say, "The animal may succeed in its attempts to cope with the conditions in which it finds itself, in which case it has adapted to those conditions. Sometimes it may succeed only with great difficulty. Alternatively, it may fail to cope or seem likely to fail eventually to cope, and is stressed." Success with difficulty implies cost and such a cost must ultimately be to fitness.

Broom & Johnson (1993, p74) say: "It is important to emphasize that a small reduction in fitness may have less of an effect on welfare than very considerable and prolonged coping difficulties which do not affect fitness". Do such things exist?

Broom & Johnson (1993, p108) persist "Although it is clear that the individual is having difficulty in coping, it is not clear that there will be an effect on fitness". Yes, there will be an effect, at least on probabilistic fitness as explained above. Coping is a burden: the organism must do something. It is granted that during the coping phase fitness may not be measurably depressed and outside of the coping phase, fitness loss is marked, thus producing a biphasic curve. Nevertheless to move along the curve of coping to the point where it becomes non-coping is from higher fitness to lower fitness. Therefore even coping has its costs.

It is proposed that coping may be more profitably used to describe the rate of change and direction of the fitness/stress relationship.

Suffering

"On all occasions when there is any kind of suffering, there is an effect on welfare even if there is no likely effect on individual fitness" (Broom & Johnson, 1993, p73). It has been demonstrated above that welfare and fitness are inextricably linked. It will now be demonstrated that suffering also implies a loss of fitness.

Suffering (Broom & Johnson 1993, p82) is "an unpleasant subjective feeling which is prolonged or severe". Broom & Johnson (1993, p80) also assert: "...suffering has clear links with the definition of welfare..." Suffering, at least, causes mental disruption. If suffering is externally caused then it must have some correspondence with an objective and real agent such as injury or damage. If so then physical fitness is demonstrably and obviously lower and the organism is stressed: what else would it be? If on the other hand suffering has no objective cause, then this is evidence of poor mental fitness. Such mental disruption causing inappropriate decisions and errors can obviously compromise fitness. So either way suffering entails compromised fitness.

By implication a suffering animal must be stressed: if its response is appropriate it is responding to a threat to its fitness i.e. it is stressed. If its response is inappropriate then it is failing to tell harmful from innocuous, which also has fitness implications. Paradoxically, a degree of mental detachment from reality may in fact help increase fitness. For instance, Mechanic (1968, in Moss 1973, p47) considers that "the ability to control one's emotions is beneficial to the coping process. Whether these defenses restrict, distort, or falsify reality has no important meaning in and of itself as long as it facilitates coping." A further example is supplied by Fisher (1984, p247-248) who says: "There are two conflicting elements that are built in to a depression resistant decision making model: 1. I can always do something to change a situation I dislike 2. I am never totally responsible for my own failures." If these two are used they may serve to protect the sense of self so that it can continue to operate. Thus, in certain circumstances, it may be less than optimal for fitness to have an objective assessment of reality. This holds only as long as the distorted world view facilitates motivation and actions that are survival enhancing.

Is any awareness of deficiency (i.e. dissatisfaction) a cause of suffering? Or, in a Marxian sense, is it possible to suffer but not to know it? Suffering cannot simply be pain as "those born without the ability to feel pain" (Lippold & Winton 1979, p491) may suffer burns and loss of limbs etc. because they are not aware of their condition. It appears that the word "suffering" is being used in two senses here. Perhaps we are expecting too much from an everyday word of vague connotation.

Of course, problems arise if one examines the fringe phenomena. What about self dosing to death with one of the "recreational" drugs? Here one paradoxically suffers from too much pleasure! We can say, however, that the following occurs: a) immediate pleasure; b) loss of longevity and c) loss of lineage (probably). If we have an existocentric (as opposed to hedonistic) approach then such a drug would cause the suffering of loss of normal life cycle events. The hedonist, on the other hand, may opt for the drug. The biological goods of pain and pleasure can thus be assessed from different standpoints. A cost/benefit relationship would help in the characterisation of suffering; as would a characterisation of the costs and the benefits.

It is suggested that suffering be defined as any subjective mental state made manifest by behaviour disturbance which is strong enough to interfere with appropriate attention to fitness sustaining processes. It is clear that for suffering to be more accurately mapped it may be necessary to subdivide it into proximal and distal suffering etc. Perhaps it is better to use the term loosely with its limitations in mind and abandon attempts to define it. It currently stands as being a useful if woolly area of subjective experience on the stress/fitness relationship.

Adaptation

Broom & Johnson (1993, p175) refer to three uses of the word adaptation. The first is as the reduction in response of a cell or organ to a stimulus over time, such as that demonstrated by some nerve cells. The second is the use of homeostatic and other mechanisms at the level of the individual to minimise the effect of environmental conditions. The third refers to the process of evolutionary development of biological material, or to the name of a particular change, which better enables it to deal with environmental conditions and thus maximise its fitness.

Broom & Johnson (1993) are on the right track, but in ascertaining levels of adaptation we move into a less easily defined area. For instance, a better-adapted organism would be expected to have a higher survival probability. But in probability lies the uncertainty of predicting fitness from evident and expected capacities of the organism.

Elsewhere, adaptation is defined (Bligh & Johnson 1973) as an organismic change which acts to reduce the physiological strain produced by a stressful component of the environment. They say that the change may be phenotypic, i.e. it may occur within the lifetime of an organism or it may be genotypic - the result of genetic selection. They go on say that phenotypic adaptation occurs in individual organisms in response to stressful stimuli during their lifetime and that genotypic adaptation is a genetically fixed faculty, or it evolves in a species or subspecies and facilitates survival in a given environment. Whilst on the subject of evolution, Bradshaw & Hardwick (1989, p153) say that it is: "an almost inevitable outcome of stress. Because stress automatically gives rise to natural selection, evolutionary change will occur providing that the appropriate genetic variation is present."

Begon *et al.* (1990) suggest that the term adaptation, as used to denote the genotypic adaptation mentioned above, be replaced by abaptation. The prefix 'ab-' is more fittingly backward looking and emphasises that the heritable characteristics of an organism are consequences of the past and *not* an anticipation of the present or future. Begon *et al.* (1990) argue that the prefix ad in adaptation implies 'going to' or anticipation of subsequent events. They raise an interesting point; the prefix "ad" may indicate how deeply teleology is embedded in some biological thinking.

Dillon & Lynch (1981, p228) also discuss adaptation: "When changes in the environment occur, individual organisms either resist the change (resistance adaptation) or adapt it in a compensatory manner (capacity adaptation) at a rate and to a degree that is within their particular scope of achievement. When this ability is overly taxed, in either duration or in magnitude, the organism is said to be stressed (strained in the sense used here) and less competitive. Changes in populations and communities are predicated on the fact that individual organisms have become less competitive or environmentally fit."

BARNARD & HURST'S ASSESSMENT OF WELFARE AND SUFFERING

Barnard & Hurst (1996, p405):"contend that welfare can be interpreted only in terms of what natural selection has designed an organism to do and how circumstances impinge on its functional design". They say that: "each organism should be considered in terms of its (p408) environment of evolutionary adaptation". Thus Barnard & Hurst (1996) propose the useful concept of adaptive expendability which links welfare of the organism with its ability to participate in actions and live in an environment where its reproductive potential is maximised.

In reference to suffering they say (p406), "The degree of unpleasantness of negative subjective experiences might therefore be expected to relate to the magnitude of the cost of the organism's current state and the extent to which circumstances frustrate its ability to prioritise its activities. Thus suffering is measure of powerlessness". Then they say (p408), "Suffering-like states are viewed as generalized subjective states that are geared to avoiding deleterious circumstances with which the organism does not have specific adaptive mechanisms to deal". Here it is a general aversive response. Finally they say (p409), "we

make the reasonable assumption that suffering is an adaptive attribute of the organism shaped by natural selection to help it avoid circumstances that compromise its reproductive potential. Thus they say that suffering may have some utility.

Barnard & Hurst (1996) criticise assessment of individual fitness as an approach to welfare: (p411)"'Fitness' is therefore more accurately construed as a property of alleles coding for alternative strategies of response and thus life history investment. Responses that preserve the individual may be adaptive where reproductive success depends on longevity, but expendability and self sacrifice are the expected features of many other life histories." "The use of fitness at the individual level in welfare arguments is thus not just inaccurate but actively misleading since it obscures consideration of what selection has actually designed organisms to do and what their life history priorities might be. We refer to this anthropomorphic concern with the survival and maintenance of the individual as *the fallacy of individual preservationism.*" (their italics). Nevertheless, they cover themselves (p412), "The argument against individual self preservationism does not, of course, rule out self preservation as an important component of of most life history strategy." Their argument against the fallacy of individual preservationism describes well the ruthlessness of the biological process. But it ignores the possibility of suffering in organisms that are no longer biologically significant. Individuals suffer, not alleles.

They refer to (p411), "what selection has actually designed organisms to do and what their life history priorities might be". Let us look at what selection has designed organisms to do. The unfolding phenomena of lineal descent may be described as retrospective reference to the genetic instructions that allowed existence in the last generation in the context of the current environment. This is like looking out of the rear window of a car while driving it forwards: the potential for a mismatch -and consequent suffering- is obvious. The predictive value of such a system is questionable.

Suffering is a by-product of living. Organisms have genetically determined drives and responses to the environment; these do not go away until satisfied. The demands are thus open-ended. All that matters is that the demands be met. Those organisms that have the strongest motivating drives are likely to have the best chance for continued existence, thus

those with the stronger drives have the better chance of passing on their genetic material. The selection criterion is existence, not absence of suffering. Existence is the result of the process, suffering is subsidiary to it.

Existence is primary; suffering is contingent on existence. Therefore suffering or avoidance of it is of lower priority than existence. It follows that avoidance of suffering is important only when suffering impedes continued existence (or lineage). Thus suffering is a by-product and sometimes it is biologically desirable to limit it. A selection pressure against suffering in this context is clearly possible.

Barnard & Hurst's (1996) adaptive expendability fits well with the biological process if seen in this context. "From an evolutionary viewpoint, however, individuals are expendable commodities in the pursuit of reproductive success". Thus (p411), "an individual is simply an expendable vehicle for the perpetuation of strategies of response to the environment through the relative impact of different strategies on reproductive success". They argue that suffering is minimised and welfare maximised if the organism can spend itself in this successful pursuit.

In other circumstances suffering occurs but is biologically irrelevant. During senescence, for instance, suffering is inescapable and no longer relevant to biological continuity. This suffering is a matter of biological indifference and has selective neutrality. Terminal senescence cannot be a pleasant experience yet Barnard & Hurst's (1996) adaptive expendability appears to embrace this. A common alternative is to be torn apart by a predator this is also (endorphins aside) not a desirable experience.

Barnard & Hurst 's (1996) adaptive expendability is a valuable concept but it does not relieve us of the responsibility for minimising the suffering of organisms whose decision rules we have changed. It has been shown that although suffering has utility in some circumstances, in others it has none. In these cases its lack of biological significance rules out selection against it. Thus we have a dead end of useless suffering. Such states may be acceptable in nature but are they acceptable in a in a man-manipulated organism?

How can one ascertain the decision rules of adaptive expendability in a domestic animal? We, by artificial selection, [Barnard & Hurst (1996) discuss the difference between artificial and natural selection but the genotype and phenotype must get on with what it has] have to some extent perverted its nature if compared with the closest wild relative. Are its present decision rules of adaptive expendability appropriate? By what criteria? Or are the decision rules of adaptive expendability of its closest wild relative any better? Or are human synthesised rules the answer? How can one choose? Is there one valid set of decision rules of adaptive expendability for each species or sub-species or population or even sub-population? Or is it *ad hoc* from individual to individual?

A further problem is to place a suffering value on non-reproductive organisms. Are they not susceptible to a dead end of useless suffering? If as Barnard & Hurst (1996, p414) say, "The organism's priority is to maximise reproductive success by efficient self expenditure. Good welfare management policies should therefore strive to maintain natural or acclimatised strategies of self expenditure." And (p425), "All organisms are designed to survive and reproduce." What happens if the organism's reproductive ability is abolished as in many farm animals and domestic pets? What have they left to look to but to maximise somatic fitness? Can they reprioritise? Do they fall into limbo? What would appropriate decision rules of adaptive expendability be in their case? Perhaps welfare ought to be divided into reproductive and somatic welfare, and suffering should be likewise divided. But by what welfare criteria can we use? Possibly we should not discard fitness indices as they may yet provide useful corroboration.

The organism is thus torn between two behavioural poles. It will pursue a hedonistic existence maximising pleasure and minimising pain, which may be a good option for fitness of the individual. In addition, organisms, through evolution, have a repertoire of behaviour that allows persistence of the lineage. Such activities as display, fighting, mating and reproduction are of importance to the lineage but are likely to be hazardous to the individual performing them. In consequence, hedonism and existocentrism are in conflict and must be reconciled. Remember that existence must take priority, making the maximising of pleasure a secondary consideration. Thus the organism is motivated to take part in these activities by a system of bribes. Furthermore the bribes are the minimum necessary to get the organism to

do what it must to continue the lineage. Note that this is not a forward looking process, all organisms without such are less likely to pass on their genes; it follows that the population is more likely to accumulate existocentric traits. It is proposed here that to expedite this, behaviour is controlled (or coerced) by a series of "trapdoor" behavioural processes. Less energy is required to bribe the organism to perform one act that then commits it to a series of further acts. Thus the first step is an irreversible trapdoor into a behavioural repertoire that is less favourable to the individual fitness of the organism.

Such a bribe is the sex act in humans. Its successful completion is bolstered by a positive feedback mechanism. The completion of the act results in an irreversible process that requires increasing diversion of resources over the long term. The drive lasts just long enough to get the organisms to perform it. After the culmination of the act the motivation may not be merely sated but there may be elements of aversion; but now it is too late; the bribe has had its effect and no further bribe is necessary until the next time. Because there is no such necessity at this stage there is no evolved protocol for a tidy ending to it.

This is only one argument and the field should be developed. It is argued that the antagonism between existocentric and hedonistic poles of motivation should be investigated before a suitable model of welfare can be constructed.

FINAL COMMENTS ON WELFARE, COPING, SUFFERING AND ADAPTATION

Suffering leads to coping efforts, the outcome of which feeds back and influences suffering. These efforts may succeed or fail. In either event, energy is expended in coping efforts and thus there will be loss of fitness as measured by energy deficit. Loss of fitness may be ascertained by an effort reward relationship of coping outcome and energy loss.

Welfare quality may be understood to be indicated by the relationship of observed fitness and expected fitness at some specified time in the life cycle; taking into account somatic and reproductive fitness components. It follows that, to be more specific, welfare should also be subdivided. Expected fitness is the fitness an organism would be expected to have in an

optimum environment. To this end the concept of adaptive expendability proposed by Barnard & Hurst (1996) should be considered as a valuable basis for further investigation. The above objections to Barnard & Hurst (1996) could be met at least to a fair approximation and their ideas would give a more holistic approach to the problem.

CHAPTER 21: STRESS REDISSECTED

SUBDIVISIONS OF STRESS

Before attempting a new theoretical construct, stress in its different kinds should be allowed to fall into appropriate categories. Some of these categories may be dichotomous but they are not necessarily hierarchical. When they are hierarchical, simpler categories may be compounded into more operational entities. An evaluation of the subdivisions of stress is vital if fuzziness and ambiguity are to be minimised.

The stress subdivisions may have different ontogenies and these are not consistent; some being identified by origin and others by effect. For the sake of a comprehensive synthesis this inconsistency is unavoidable. This state of affairs will be allowed to stand unless it causes problems in theory.

Simpler categories include physical nature, origin, point of action and effect. More complex categories include somatic and reproductive stress. A stress might reduce bodily fitness and thus be a somatic stress or it might impede reproduction and thus be a reproductive stress.

The physical nature of the stress would include such properties as chemical composition, temperature, light intensity, mass and concentration. These are properties intrinsic to the stress entity (even though they are stresses only in the context of the affected organism) and exist independently of the organism.

In contrast, other stresses cannot be said to exist independently of the organism. These include its point of application and origin. In origin, the stress may be endogenous or exogenous. Some sub-divisions such as somatic, reproductive, endogenous and exogenous stress are so classified according to their manifestations as strain. Strain and its subdivisions are given detailed consideration in a later section. And those stresses that are closely dependent for their identity on the resultant strain will also be considered there. Stresses may also be classified by their dose response relationships; such subdivisions would be one-way and two-way as outlined above.

STRESS UNITS

A stress unit quantifies the amount of stress applied to the organism and is thus a concentration or dose or degree of environmental or internal (causative) change. This includes parameters such as ppm, pH, and temperature. We should also consider the toxic unit concept outlined by Sprague (1970), Doi (1994) and Marking & Dawson (1975) in Mathew & Menon (1992); and its developments such as the MTI mixture toxicity index which is a quantification of toxicity of diverse mixtures of toxicants (Könemann 1981).

Temporal regimes of stress exposure (Sprague 1970; Underwood 1989) and the concept of degree-days - where dose is integrated with time- should be further explored. As demonstrated above, temporal considerations are important. Levitt (1980) underlines this in botanical studies. The effects of plastic strains, as mentioned earlier -those strains or changes to the organism that are irreversible but may be repairable- emphasise the importance of time. "Similarly, injury to an organism is just as dependent on the time exposed to a high temperature stress as on the high temperature" (Levitt 1980, p6). Two different poles of temporal stress regimes (Underwood 1989) are possible: press-stresses, which persist chronically, and pulse-stresses, which are intermittent, temporary, acute stresses.

A stress unit must integrate three aspects of the stress; its quality, intensity and time of exposure. At this stage we must recognise that all stresses act under a time regime. Some act quickly and others take longer. Such arbitrary distinctions as long and short term and acute and chronic may have some utility but they must be clearly defined. No environment is stress free and thus the organism is always under a pre-existing stress regime. There may be a time threshold beyond which the stress is lethal and there may also be an intensity of the stress beyond which death ensues quickly. Responses to a stress regime (in terms of proportionality of response per time unit of exposure) may be additive, more than additive or less than additive. One approach to elucidating the progression of such a regime is to consider that the aseity of the organism may be conditioned by previous stress to respond to present conditions. Thus, there may be habituation and less than additive effects; or the opposite, sensitisation, may ensue. Given the number of degrees of freedom in an organism and the number of permutations of stress conditions the outcome of such interactions is not in principle determinable *a priori*. Thus we must explore the responses of organism over a

controlled range of stress conditions, in particular we must be explicit about the character of the stress and the time of exposure.

CHAPTER 22: STRESS AND ECOLOGY

We now turn to examine the nature of ecological stress phenomena to see if they can be included in this scheme of stress.

DEFINITION OF ECOSYSTEM

If ecological stress is to be appraisable, then the ecosystem must be soundly defined. Watt's (1973) definition (in Barrett 1981, p7) of an ecosystem is: "a level of organisation that embraces not only the total array of plant and animal species in an environment, but also the matter which cycles through the system and energy which is used to power the system". This definition indicates that ecosystems and organisms differ in significant ways. These differences must be reconciled before we can treat stress as the same phenomenon in both. A start would be made by considering the following questions. If stress degrades fitness (as argued for organisms) what does this mean to an ecosystem? What is ecosystem fitness? Do ecosystems die? Is an ecosystem one of a kind with analogues or are groups of ecosystems homologous? Can this be demonstrated? If not, then how does this affect the rationale behind comparing similar ecosystems? Can an ecosystem be considered as an individual? As such, can it be grouped into higher taxa with the same rigour as that applied to organisms? Does selection operate on ecosystems? What is the reproductive unit of an ecosystem? Even at the level of community there are conceptual problems: "Considerable debate centres around whether communities are more than just random assemblages of species...We must explicitly and consistently define community structure" (Lafferty, Sammond & Kuris 1994). Comparison of features that characterise individuals, populations, communities and ecosystems suggests that, in their manifestations of stress phenomena, there may be considerable scope for difference.

DEFINITION OF ECOLOGICAL STRESS

"Stress (Boesch & Rosenberg 1981, p179), exerts an energy cost and interferes with the normal functioning of the system". Stress (Barrett 1981, p4), is "a perturbation that is applied to any system by a stress which is foreign to that system or which is natural to it but in the instance concerned is applied at an excessive level". The word 'excessive' carries normative weight and with it some danger of circularity.

In contrast, Grime (1989a) raises the issue of whether the term stress is at all appropriate in ecological contexts. Furthermore, Grime (1986, p653), in symposium discussion, quotes the objection by Harper, who asserts that stress should not be used in ecology because it cannot be defined with precision as it can in physics. Harper also objects to the use of the word disturbance in ecology as it may not easily be made operational. The same is true for stress in this context and Harper warns of the danger of circularity: "Ideally, I suspect, we should aim to measure and compare the effects of various forces on individual fitness." This suggestion is followed later.

Underwood (1989, p52) defines stress as, "an environmental change that causes some response by the population of interest". Stresses (Underwood 1989) are a subset of perturbations; a sufficiently intense perturbation may become a stress. Perturbations (Underwood 1989, p52) are "any natural, accidental or deliberately induced changes in environments". Underwood (1989, p53) thus treats stress as a cause: "for the purposes of this discussion, the existence of a stress is determined by the existence of a response; that is, by some change in abundance (or density) of a population following a perturbation. That is to say, stresses are perturbations causing a response in a population." Franz (1981, p53) says, "stress is an energy drain which is reflected in structural changes at the community level". Does this mean that a stress may change the nature of the community? Can stress overthrow communities? Is stress a causal factor in ecological succession?

At this point we should consider the statement of Bayne (1975, p15 in Ivanovici & Wiebe 1981): stress is "a measurable alteration of a physiological (or behavioural, biochemical, or cytological) steady state which is induced by an environmental change, and which renders the individual (or the population or the community) more vulnerable to further environmental change".

The first point to be raised is that we must remain aware that the central tendency of the steady state he mentions may naturally drift during development in the individual, as explained earlier, and during ecological succession. Secondly, if Bayne's (1975) assertion really does apply to ecological stress then does the new community have a reduced resistance to further change? For instance, a woodland stressed by fire, wind and radiation regimes

(Woodwell 1967) tends to regress to smaller dominant life forms; plants such as grasses replace trees as the dominant ground cover. Is this new community more, or less, susceptible to further perturbation? Or does the stress/strain level reset to zero as a new community arises, and if so, then at what specific point? Does Bayne's (1975) statement about reduced resistance to further change hold?

Probabilistic stress

Probabilistic stress as a concept is suggested by the following use of the term potential stress. According to Underwood (1989, p51): "Experimental analysis of responses to stress must include distinction between potential stresses (environmental perturbations that might not cause stress) and actual stress (phenomena that cause a response by the population)." In addition, Underwood (1989) provides an introduction to the possibility of predation stress when he discusses stresses due to fishing which usually remove the larger members of fisheries. We must ask at which level is this stress acting? Is it the population or individual that is stressed? As a population stress, the symptom is loss of certain size classes. To the individual, this would be an all or nothing phenomenon. Or it could be interpreted as a predation probability.

STRESS AND OTHER TERMS

Underwood (1989, p52), in his review of stress terminology for populations, outlines the relationship of stress to other perturbations: "Apart from stresses (i.e. perturbations causing a response in a population), there are perturbations that cause no response". Underwood (1989) cites Sutherland (1981) as defining these as Type I perturbations and defined stress as Type II (causing a temporary change from which the population recovers) or Type III (causing a permanent or, at least, long term change in a population). "Finally there are perturbations that exceed the possibility of measuring a response by a population - because the population and its habitat are destroyed...these perturbations can be described as catastrophes" [McGuinnes (1988) cited by Underwood (1989, p52)].

Stability, persistence, resistance, resilience, and inertia are terms used to characterise stress-related effects on communities. Stability (Underwood 1989, p51) is the, "rate of recovery (of population numbers) following a stress". Orians (1975) subdivides stability into persistence,

resistance and resilience as proposed by Boesch (1974). Persistence is the degree of constancy in the community over time, regardless of the degree of stress applied. Resistance refers to the capacity of the community to remain unchanged by applied stress. Resilience refers to the ability of the community to return to a persistent state. Underwood (1989) defines resilience as a measure of the magnitude of stress response from which a population can recover. He also uses persistence as the constancy of population numbers, without passing judgement on the presence of a perturbation or on the inertia of the population. Inertia of a population is the lack of response to a perturbation and can be measured (Underwood 1989, p54) as "the maximal magnitude of a particular type of perturbation that causes no response (which is therefore equal to the smallest magnitude of that particular type of stress)".

SYMPTOMS OF ECOLOGICAL STRESS

"The primary variable of interest when considering stress and a population is the abundance of organisms" (Underwood 1989, p53). This, he says, is usually ascertained by a study of population density. Gray (1989, p19) goes further: "Three clear changes in community structure occur in response to stressors. These are reduction in diversity, retrogression to dominance by opportunist species, and reduction in mean size of the dominating species. Statistically significant reductions in diversity occur rather later in the sequence of increased stressor impact...Species which dominate in heavily stressed habitats are often species complexes..."

This is in accord with Warwick & Clark (1995) who find that taxonomic distinctness decreases with increasing stress. Thus, species diversity may be preserved in a stressed environment but the taxonomic distinction between them may diminish. This suggests that anatomy and life style are connected and that in the heavily stressed environment one would expect a lower niche diversity. How could this be measured?

Gray (1989) states that unstressed benthic sites are characterised by many rare species and few common species. Stressed sites, in contrast, are dominated by a small number of very common opportunist species and have few rare species. Other symptoms in ecosystems have also been posited, but Gray (1989) suggests that they may be less reliable. Rapport, Reiger &

Thorpe (1981, p272) state: "Rapid shifts in community species composition, increases in population fluctuations, and the reduction in size in dominant life forms may well constitute an ecosystem's alarm reactions".

Stress effects and symptoms of ecosystem pathology in the Gulf of Bothnia are discussed by Rapport (1989, p33). These are, "early signs of eutrophication in local and coastal waters, formation of local abiotic zones, reduction in species diversity, reduction in genetic diversity (particularly in salmonids), reduced size of biota, increased dominance by opportunist species, increased disease prevalence and bio-accumulation of toxic substances". Furthermore (Rapport 1989, p34): "While the specific indicators of ecosystem response to stress differ to some extent among these studies, there are broad similarities in the following aspects: (1) there appears to be a reduction in the efficiency with which ecosystems process energy (reflected variously as a decline in community respiration, primary production, or net landscape production); (2) there is an increase in the horizontal flow rates of nutrients; that is terrestrial systems lose nutrients and aquatic systems accumulate them; (3) changes in community structure appear to favour biota that are shorter lived, smaller, exotic, and have high reproductive rates. Such features often characterize 'weedy' or 'pest' species; (4) ecosystem development appears to reverse in direction, that is to 'retrogress' to resemble earlier stages in which self regulatory functions are less developed, species diversity is reduced etc." Woodwell (1967) supports this by reporting a tendency towards smaller dominant life forms, under stress regimes (this echoes what was said earlier about smaller organisms having more stability). Here, plants such as grasses replace trees as the dominant ground cover.

In contrast to Rapport (1989), Odum (1985, p419) says that in the absence of stressful perturbations: "net community production (tends to) decrease." Odum continues (1985, p420), "in practice, respiration increase does not provide a very good early warning distress signal because it is difficult to detect small increases in large open systems". Odum (1985, p421) also says that a decrease in species diversity is not a reliable index of stress because: "a disturbance affecting the structure of the system (e.g. patch cutting in a forest) often increases the diversity of species of both plants and animals". Though Rapport (1989) quotes Odum (1985) he does not comment on these disagreements.

Assessment of anthropogenic degradation in the Gulf of Bothnia is complicated; not least because climatic conditions and low salinities have resulted in the development of biotic communities with many of the features of stressed ecosystems - such as low aquatic species diversity (Rapport 1989). This is explained, suggests Rapport (1989), by the geologically recent origin of the Gulf. Recent origin also means recent disturbance; the Gulf was formed by inundation of the old terrestrial habitat. This pre-existing stress factor of recent disturbance would make it difficult to detect subsequent anthropogenic stress. This is further complicated because: "the detection of a stress acting on a natural population is clearly fraught with difficulty because of the intrinsic spatial variability and lack of equilibria of real populations" (Underwood 1989, p62).

Note that this also supports what has been said previously about shifting equilibria making it difficult to identify a steady state. Fisher's (1984, p107) comment, "...a number of quite complex biological and psychological rhythms exist that are likely to provide a fluctuating base for incoming influences." lends weight to this. The phenomenon of succession in ecological studies and concomitant shifts of equilibria make such assertions also relevant for ecological stress.

COMPARISON OF STRESS EFFECTS AT DIFFERING ECOLOGICAL LEVELS

Can stress operating at different ecological levels (ecosystems, communities, populations and individuals) be compared? Lugo (1978) says yes and asserts that biological material, from cells to ecosystems, follows a similar pattern of responses to stress. Boesch & Rosenberg (1981, p198) state: "Resistance to stress is effective at the individual or population level". Franz (1981), however, emphasises that Selye's concept of stress (1952, 1956) was never intended to apply to ecosystems. Selye (1982, p16) nevertheless hints - in echoes of Pascal (1961, Trans. Cohen) - that it may be applicable: "At first it seems odd that the laws governing life's responses at such different levels as the cell, the whole person, and even the nation should be so essentially similar. Yet this type of uniformity is true of all the great laws of nature." This may or may not be true but it will not be substantiated by adducing laws about laws.

Gray (1989, p19) is also sceptical about the applicability of Selye's model: The "responses of

individual organisms to a stressor are not appropriate for describing effects at the population or community level". In contrast, Sindermann (1990) discusses stress as it applies both to individuals and populations alike.

Nevertheless, as Odum (1985, p419) says: "I do not mean to imply a strict analogy with Selye's (1973) general stress syndrome"... (but) ..."there are interesting parallels between physiological and community levels". This obviously deserves further scrutiny.

Regardless of the above tendencies to lump together stress phenomena from different levels, their ontological roots may be unrelated. Different effects of stress may be seen at different levels (Calow 1989). At lower levels it is manifest in changes to the molecular and cellular processes, at higher levels the population density, dominance, diversity, primary production and community respiration may be affected.

There is no consensus that stresses at different ecological levels are equivalent. Nevertheless, we can accept that individuals and higher assemblages may be comparable in as much as they may both be subject to the effects of deleterious agents - so long as we define our terms.

Nisbet, Gurney, Murdoch & McCauley (1989) attempt to relate the effects of stress on the individual and population by using a structured population method and review attempts made to integrate these two levels. Nevertheless, it seems that response at one ecological level is hard to correlate with phenomena at other levels - either up or down. Underwood & Peterson, (1988) agree: "Any perturbation that does not cause an immediate effect on the abundance of a population, because it is buffered by responses at lower grades of organization (physiological, behavioural, cellular, etc.) has the potential to cause rapid changes once the stress becomes measurable as a change in abundance. For this reason, it is unlikely that monitoring of abundances of populations, on its own, is a useful tool for detecting and predicting stresses in natural habitats." "Unfortunately, to link such monitoring to physiological, cellular or biochemical assay, depends on knowing that these aspects of the biology of the species, are in fact, predictive of future changes in abundances" (cited by Underwood 1989, p65). Another structured population model, that of Metz & Diekmann (1986), is mentioned in Gosling (1992).

Sastry & Miller (1981, in Sindermann 1990) propose a scheme of relating pollutant stress and its effects at different ecological levels and time scales. These range from immediate to decades, and from biochemical to community and ecosystem effects. Bald assumptions that notions of stress can be used at different levels, as above, without any special caution are still evident in the literature. Now is the time to attempt to settle this one way or the other. We can start by posing a question: can an agent be a stress at one level and not at another? If the answer is yes then the universality falls apart.

Underwood (1989) suggests that stress does mean different things at different levels. He argues that, for some hypothetical population of meiofaunal invertebrates, hydrocarbons may not cause a population response even though individuals were stressed. This he explains by asserting that they could cope via physiological adaptation. So in this instance hydrocarbons are a stress to individuals but not to populations.

Odum (1985, p419) says: "My hypothesis is that a disordering disturbance to which a community is not adapted arrests and in many cases reverses these autogenic (succession) developments." Therefore, for lower levels of communities, succession is itself a stress.

The same is true for the assertion that "...an ungrazed system in the grasslands is to be considered as a stressed system. So, to be unstressed the grassland must be grazed" (Van Dyne 1981, p61). Surely grazing arrests succession as it maintains the grassland; in addition grazing damages individual plants. If succession is arrested, then we must also have a condition of ecosystem stress. Grazing is favourable only to the comparative (not absolute) fitness of grasses over competing succession species. This is borne out by Verkaar (1986, p169) who found that in all but "very particular circumstances" grazing is in fact deleterious to individual plants. There is a further interaction: we can see that grazing prevents "stress" (strain) in grassland, but at what level within the grass population are we ascertaining the effect of stress? Are we talking about persistence of grass dominance, or the persistence of grass-plant individuals? What if the collective strain of grazed grass and non-grazed grass were compared with the successional changes arrested by one and experienced by the other? Is the life-span of individual grass plants and their reproductive output (sexual and vegetative) influenced by grazing? Could it be that grass population fitness is degraded by

discontinuation of grazing whereas individual grass plant fitness actually increases, at least in the short term, until they are shaded out by taller competitors?

It has been demonstrated that an agent can be a stress at one level and not at another. And even on the same level the action of the stress, though equally lethal, may be along different pathways for different species. This is illustrated by Sprague (1970, p25): "Even if lethal levels are similar for fish and invertebrates, it must not be assumed that the mode of action is the same. For example, lobster larvae have about the same resistance to pulp mill waste as salmon, but are apparently affected by quite different mechanisms."

A further complication is that even among individuals of the same species there is a wide variation in susceptibility due to natural variation. The origin and nature of biological variability at different levels is discussed by Brown (1993). Universality of stress action at different ecological levels is thus refuted.

Similar stress phenomena manifest at different ecological levels may be no more than analogous. Thus it can be concluded that stress is in fact a series of ecological level dependent local concepts. In the interests of clarity, individual and ecological stress phenomena must be more sharply differentiated. It is easy to blur the distinctions between individual, population, community and ecosystem stress phenomena. Although this blurring may be generally convenient, one should not forget that different levels of complexity can create different phenomena. Accurate and meaningful use of the term stress requires that its hierarchical level be explicitly stated.

For a balanced view of the health of an ecological unit one would do well to heed Underwood & Peterson (1988, p233) that: "a complete assessment of any episode of pollution must include accurate measures of the biological effects of the pollution at a number of different scales." For a review of ectoxicology in the tropical marine environment see Peters, Gassman, Firman, Richmond & Power (1997).

EUSTRESS IN ECOLOGY

Any agent that causes a change in the succession biota is a stress to the *status quo* community

and eustress to the incoming community. It, thus, is two opposing entities at the same time. This contradicts the logical law of non-contradiction. How can this be reconciled?

If the succession community is seen as a stress to the incumbent then removal of the succession community is not eustress but removal of a pre-existing stress. No eustress as a concept is required. Otherwise we would have to declare that pollution is a eustress for nematodes and other r- strategists. Nevertheless, Odum (1985) says that a disturbance may be detrimental and beneficial at the same time on different levels. He mentions that for chaparral, fire is not a stress at the ecosystem level. He says that, conversely, absence of fire would be a stress for chaparral. It is contended that fire, in close proximity, is generally a stress to organisms. An exception would be to admit that heat exposure stimulates germination in some seeds. Otherwise one can say with some confidence that exposure to fire is a stress.

Perhaps it would be better to consider the differential effects of a stressful agent. If two populations living side by side are differentially sensitive to an agent then in the context of one that has the higher mortality we would term the agent a stress. The second population may have lower mortality. At this moment the fitness of the second population is higher than that of the first. Thus the agent may appear stressful to the first and eustressful to the second. This difference is enhanced if the effects of differential reproduction rates are allowed to follow the effect of the agent. It is contended here that the stress/eustress argument is based on misconception of the phenomena into a false good/bad dichotomy. The dichotomy is better seen as bad/ not so bad; and differential mortality is the mechanism.

This may be operationalised by measuring the initial effect of the agent on the mortality of the organisms in question. Stress that kills the opposition is eustress: "An enemy of my enemy is my friend", so the saying goes.

The statement that: "The derivation of the term and general usage dictate that stress should refer to negative deflections" (Odum, Finn, & Franz 1979, in Ivanovici & Wiebe 1981) is now not so clear. To identify a negative deflection we must be able to identify a positive deflection. A single change in any life process, increase or decrease, does not necessarily

indicate benefit or otherwise. No matter whether one is dealing with ecological or individual stress, it must be corroborated by examining concurrent processes. Only then can we hope to determine whether it is beneficial or not.

This raises questions about terms such as subsidy and eustress. Subsidy (Odum *et al.* 1979, Odum 1985) and eustress (Larkin 1974) are sometimes used to signify "favourable" deflections or positive responses in the performance of ecological systems. Performance, they say, may thus be enhanced by subsidy or diminished by stress. Augmentation of performance is also possible by impoverishment of an environmental factor which is reduced to the optimal range of the organism (Funk & Gibbons 1979).

Further study is, however, required to ascertain if a specific performance is directly proportional to fitness. Otherwise fitness degradation, as discussed earlier in organisms, could be a loss of co-ordination or a system mismatch. It is also clear that performance in an ecological context must be defined before it can play a meaningful role in any conceptual argument.

Other problems may arise if performance fluctuations are uncritically accepted as indicators of stress. Larkin (1974) comments that a stress such as nutrient loading might enhance the fish productivity of oligotrophic lakes, (it does this by eliminating a pre-existing stress - nutrient deficiency) thus contributing to a more 'desirable' ecosystem state. This is not a positive deflection or eustress it is, in effect, a restoration.

A further objection may be raised. If increased productivity means more fish, then more fish means that more will encounter stress. And this encounter is inevitable for every organism at least at the point of death. So how can this be desirable? In addition, production beyond the carrying capacity of the habitat can also be a stress because it may lead to crowding, competition, oxygen depletion and malnutrition. It is obvious that we must be explicit about temporal regime, location and identity of subject before we can discuss ecological stress with any meaning.

Two-way ecological stress

Populations may show a two-way response to a stress, similar to that in organisms. Underwood (1989, p70) reports: "examples of this occur in some plants that are affected by fire so that reproduction is enhanced by a minor stress due to fire, but excessive fire would destroy the seed bank and no increased production can occur." Again one must weigh up many factors before one can judge the harm or benefit that a possible stress agent can cause.

CHAPTER 23: PSYCHOLOGICAL STRESS

INTRODUCTION

"Stress is neither a noun, nor a verb, nor an adjective. It is an escape from reality" (Engel 1985, p10). "There is disagreement about the meaning of the term, There is disagreement about how it should be measured, and there is lack of understanding about how aspects of the psychosocial environment might actually make a person ill. The absence of a consensual definition of psychosocial stress provides a fundamental empirical difficulty" (Marmot & Madge 1987, p6).

"Ivancevich & Matteson (1980 in Beehr & O'Hara, 1987, p79) have gone so far as to compare stress to sin, since both terms are emotionally charged but different people think they mean different things". Mason's (1971, 1975a, 1975b) position is reviewed by Leventhal & Tomarken (1987, p28), who say: "There is no such thing as a unitary stress state" (as espoused by Selye), "extremely complex and varied physiological responses are associated with environmental stressors". Zegans (1982) makes the point that the psychological stress response is a new level of complication beyond that of Selye's GAS. Leventhal & Tomarken (1987) propose multiple stress terms; they say (p28): "Stress can be conceptualised at the social, psychological and biological levels." The social level includes "classes of institutional and role relationships that are correlated with these psychological and biological processes. Leventhal & Tomarken (1987, p50) add: "A major point stressed throughout was the importance of a well developed biopsychosocial model of the stress process". According to Moss (1973, p56) "A great variety of different environmental conditions is capable of producing a stress state." Moss (1973, p56) says: "We agree with Syme (1967) that we should abandon the concept of stress in the study of the relations between social, psychological, and physiological phenomena and look instead at these phenomena with an eye for fresh concepts". According to Moss (1973, p38): "However, as Caudill (1958) suggests, we must take the whole man and his biological, psychological, and sociological dimensions into account if we are to properly understand both the sources and effects of stress". Kaminoff & Proshansky (1982) adopt the person-environment fit model or congruence. They say that the optimum conditions are maximal fit and the worst are minimal fit.

Wolff (1953, in Moss 1973, p33), defines stress as "the internal or resisting force brought into action in part by external forces or loads"..."Stress becomes the interaction between external environment and organism, with the past experience of the organism as a major factor". Wolff refers to inappropriate physiological responses to social and psychological threats. Is this a loss of mental co-ordination?

Moss (1973, p45) gives Gross's (1970) definition of stress as "the failure of routine methods for managing threats". Threats are defined as "an imagined possible future deprivation of something one values". Moss (1981, p3) also says: "In the discussion of management stress presented here, the concept of a stressor as an extraordinary pressure rather than as an ordinary stimulus is preferred".

Regardless of its incomplete genealogy, psychological stress is an important and expensive issue. Beehr & O'Hara (1987, p80) estimate a yearly $17 thousand million loss of productivity by stress induced mental dysfunction and a yearly loss of from $75 thousand million to $90 thousand million in productivity by stress induced poor health in the USA.

STRESS AS A CAUSE

Let us look at some usages of stress as a cause. A stressor is (Beehr & O'Hara 1987, p79), "an environmental characteristic or event thought to produce an adverse reaction (either psychological or physiological)". "Strain (Beehr & O'Hara 1987, p79) refers specifically to the adverse reaction of the individuals to the environmental event or stressor". Kessler (1987, p113) says: "The most fundamental question asked by stress researchers is whether stress causes ill health"..."We still have only limited information about this question". Djawdan, Rose & Bradley (1997) say that environmental stress causes a reduction in fitness of the organism.

Leventhal & Tomarken (1987, p40) say: "Psychological stressors may be subdivided into discrete events - life change units, ongoing role strains, daily hassles". Moss (1973, p55) quotes Mechanic (1968), who says that stress stimuli include: "electric shocks, adrenaline injections, physical restraint, battle conditions, impending surgery, rapid cultural change,

intense competition, loss of loved ones, demotion at work, floods, tornadoes, earthquakes, acute illness, injury, failure and isolation".

STRESS AS AN EFFECT

Kaminoff & Proshansky (1982, p380) define stress as, a "pattern of psychological, behavioral and physiological responses of the individual to the demands of the physical and social environment that exceed his capacity to cope effectively..." Leventhal & Tomarken (1987, p28) say, ..."the term stress has served to label a research area that investigates the way in which economic, social, and psychological variables reduce an individual's resistance to disease or serve to precipitate or promote disease...the stress process and/or the responses comprising it can be conceptualised as distinct from the situational stimuli or stressors that initiate it." Thus, they say that the state of stress is distinct from the causal stressor.

"Stress involves an interaction of person and environment...which presents a person with a demand, or a constraint, or an opportunity for behavior" (McGrath 1976, p1352). The individual's perception of the stressful demand is the catalyst in McGrath's definition. McGrath (1976) says broadly that the extent to which a demand upon a person is stressful depends upon whether or not it is perceived as such. In addition, the degree of stress depends on the subject's perceived ability to deal with the threat and also depends on the consequences of success or otherwise in dealing with it. This definition implies that, for stress to exist, the stressful demand must be out of balance with the perceived capability of the individual. McGrath (1976) adds that this imbalance may be in either direction.

Stress, according to Leventhal & Tomarken (1987, p39) is, "*not* simply the stimulus: the response to the stimulus is the key to the generation of the stress process. Thus, stress is a product of the representation of the stimulus (how it is perceived and thought about), the responses taken to cope with it (responses to change the situation or to deny or alter its interpretation), and the appraisal of outcomes (successful or unsuccessful)."

Hobfoll (1989 in Broom & Johnson 1993, p62) sees stress as "a reaction to the environment in which there is threat of -or real- loss of resources, or a lack of gain following an investment of resources". Such an emphasis on threat and anticipation is also evident in Lazarus (1966).

And Moss (1973, p56) says: "stress is probably best conceived as a state of the total organism under extenuating circumstances rather than as an event in the environment."

SYMPTOMS OF STRESS

"It is impossible to test causal hypotheses about the relationship between events and disease outcomes if one cannot generate independent assessments of the stimulus, the process, and the disease; such tests are the *raison d'etre* of stress illness research" (Leventhal & Tomarken 1987, p39). Van Der Steen (1993, p264) says: "To make empirical research sensible, independent definitions of stimulus and response are needed." These important recommendations are broadly applicable to any field of stress research.

What symptoms can we observe? Stressful experiences in higher vertebrates often suppress T-lymphocyte function; the effect of various agents is reviewed by Broom & Johnson (1993). "In man, depressive symptoms may become manifest after events such as bereavement and among medical students before and during examinations. Depressive mood has been shown not only to increase morbidity but also to worsen prognosis and to prolong recovery from any disease" (Leigh 1982, p738). "Signs of depression include sleep disturbances, loss of appetite and weight loss, loss of sexual interest, fatigue and vague aches and pains" (Leigh 1982, p739). Depression of NK cell activity in mice exposed to prolonged high noise levels has also been noted. In addition, sleep deprivation has been reported (Schneiderman & McCabe 1985) to compromise aspects of the human immune response.

Moss (1973, p49) asserts: "there is poor correlation between subjective states such as anxiety, discomfort fear and physiological processes". But Bailey & Bhagat (1987, p210) dissent: "If the stress continues and we have no other outlet, chronic stress diseases may result such as migraine, ulcers or colitis".

"There is every indication that the linkages between environmental exposure and disease outcomes consist of innumerable specific aetiological processes. The specific nature of these linkages, not surprisingly, depends on the characteristics of the exposure and the set of risk factors...The assumption of a common pathway is quite difficult to sustain and at best represents a gratuitous assumption of dubious verifiability...It has been impossible to identify

and agree upon a criterion or, more appropriately, a set of criteria for identifying the presence of the state of stress and calibrating its intensity and duration. All classes of indicators which have been proposed - performance decrements, experienced distress, biological reactivity...have their own large set of other determinants and the unique additional contribution of stress is difficult to pin down" (Kasl 1987, p311). Kasl (1987), by questioning the commonly held views of psychological stress workers, brings the possibility of integrating it into the scheme proposed in this thesis.

Moss (1973) reviews stress models such as those of Selye (1956), Wolff (1953), Basowitz, Persky, Korchin & Grinker (1955), Dohrenwend (1961), Lazarus (1966), Gross (1970), Mechanic (1962; 1968) and Scott & Howard (1970). Moss (1973, p52) says that though these purport to integrate some of the factors in stress phenomena, he finds them all lacking in one aspect or other and says, "None has adequately embraced the whole spectrum from social to physiological".

Although Moss (1973, p57) states that there are limitations to a successful theory that links social behaviour, physiological processes and health, the term stress as defined above is applicable to all these. If stress is related to fitness, which is in turn related to existence, it is suggested that all stress phenomena of individual organisms may in principle be integrated.

Perkins (1982) finds positive correlation in the relationships between psychological stress assessed by life events scales and deleterious physical or psychological conditions. Zegans (1982) cites instances of disease being caused by disruption of the immune system, endocrine fluctuations, autonomic system disruption causing cardiovascular respiratory and secretory malfunctions. He also mentions disruption of sleep with consequent dislocation of protein metabolism and endocrine functions, changes to the neuroendocrine functions in the brain resulting in behavioural changes such as in eating, drug consumption and accident prone behaviour.

That psychological stress ought to be considered alongside physiological stress is suggested in the following statement: "It is not always at all obvious when a physical effect becomes a psychological one" (Broom & Johnson 1993, p62). Indeed Dantzer, Mormede, Bluthe, &

Soissons (1983) go further and propose that it is the subjective experience of an aversive stimulus that leads to the physiological response." This may be so in some circumstances but it is not the invariable pathway. Stress effects can occur in anaesthetised higher organisms and they also occur in plants. This would require a mental process which, firstly, is not available at that time and secondly does not exist.

PSYCHOLOGICAL EUSTRESS

"Stress is not necessarily harmful. Some degree of stress is normal and is in fact necessary for day to day functioning" (Moss 1981, p3). Moss (1973, p36), furthermore, infers from Basowitz *et al.* (1955) that, "Stress comprises stimuli that produce anxiety in most individuals, resulting in a number of physiological, psychological, and behavioural changes, possibly pathological but also possibly leading to higher levels of functioning and new forms of adjustment." This is admissible if one takes into account the possibility of proximal and distal stress.

Strümpfer (1983) discusses the concepts of dystress *(sic)* and eustress, both of which he refers to as demands. The former are unpleasant, harmful and disabling demands; the latter are pleasant, positive and energising. Strümpfer (1983) argues on etymological grounds that dystress is more appropriate than distress. All he has done is to increase the possibility for confusion by adding an extra, and very similar, word to the stress vocabulary.

Eustress predominates (Strümpfer 1983) when the subject experiences a sense of competence to act and thus cope with the conditions; when conditions are seen as a challenge and an opportunity for development rather than a threat; and the subject perceives life to be coherent. The latest stressful event is seen as just one of life's hurdles towards an optimistic future. Thus the subject has commitment to find meaning in the current conditions and to affirm the value of dealing with them. And stresses are approached with sense of control as opposed to a sense of powerlessness. The importance of a sense of control in helping to maintain psychological equilibrium is also demonstrated by Astin (1997). White (1959 in Strümpfer 1983, p23) proposes a concept of "effectance motivation": "competence behaviour cannot be explained in terms of other unlearned, primary drives, like the needs for food or water". "Effectance motivation" he says, is "just part of the way the nervous system is and how it

works." Aspects of this are dealt with in "Stress and the perception of control" in this Chapter.

Strümpfer (1983, p26) refers to two opposing types of psychological coping; one is regressional and the other is transformational. The former is retreating and avoidance behaviour. The latter, "involves the person in active interaction with the life events such that cognitive appraisal is optimistic and decisive action alters the events to make them less stressful". "A person who uses transformational coping, does not avoid or shrink from the dystressful event but confronts it head on and transforms it in to a productive, growth promoting experience." So when is it dystressful?

The terms eustress and dystress cause problems when Strümpfer (1983, p12) tries too rigorously to relate them. He accepts uncritically the inverted U curve proposed by Selye (1976) for the General Adaptation Syndrome. Strümpfer (1983, p12) asserts that this curve may be used to depict the relationship of dystress to performance. He says the curve was originally described by Yerkes & Dodson (1908) "who demonstrated by experiments on learning in mice, that as dystress increased, so did efficiency and performance. However, if dystress continued to increase, efficiency and performance declined." This may be contrasted with another of his statements: "The rising portion of the inverted U curve, (Strümpfer 1983, p12-13) plus the plateau at the top where the person is coping with the task (where resistance to stress is increasing) can be described as eustress". The latter section where resistance is declining he terms dystress. This stresses logic. According to this, one can have eustress and dystress at the same time. This also means that if the organism can remain in the rising portion of the curve it will experience only eustress. Are either of these statements true?

A further problem arises with this interpretation of the inverted U curve. According to Selye (1956) "adaptation energy" is used up during the stress experience. And the loss of this eventually results in the decline of resistance and death. If so then any part of the curve must be dystressful. Strümpfer's eustress and dystress curve is better covered by proximal and distal effects.

Strümpfer (1983, p12) also cites an inverted U curve by Nixon (1976) as a "human function curve" where performance is related to arousal. But this is a different consideration as it does not follow Selye's stress relationship of performance with time. Strümpfer (1983) would be more accurate if he used Figure 1 from Selye (1974, p20). This is an upright U curve with the vertical axis labelled stress and the horizontal axis an experience continuum from extremely unpleasant on the left to extremely pleasant on the right. The point of minimum stress lies medially to these two extremes. One could with more justification label the pleasant side eustress and the unpleasant as stress or distress. Yerkes & Dodson (1908) actually depicted a series of upright U shaped curves. These curves related the number of tests required to complete a learning task with the strength of electric shock applied for making errors.

Obviously, as shown in the physiological and ecological sections, notions of eustress, stress and dystress do not go well together. It is clearly better to deal only with different levels of deleteriousness and integrate them into a cost/benefit analysis of defined temporal regime.

APPRAISAL AND TELEOLOGY

If teleology belongs anywhere in stress studies it is in the division of psychological stress. We all have mental aims of some sort, and we can conclude that we, at least sometimes, act and think in pursuit of an aim. Some effects of psychological stress can arise when we perceive that things are not the way we want them to be. As Mechanic (1962 in Moss 1973, p46) says of stress, it, "refers to the difficulties experienced by individuals as a result of perceived challenges".

Moss (1973, p187) states: "information incongruities between the expected and experienced may exert strong influence on subjective feelings and appraisals of situations. This in turn can alter the subjects' involvement with communications networks and increase susceptibility to disease." A communication network is defined (1973, p242) as: "A configuration of interacting people transmitting and modifying a body of information. (This body of information includes language vocabularies - particular conceptions of the natural environment - norms and values, and preferred patterns of interaction.)"

Perception of the stress is commonly held as the activating factor in psychological stress. This notion is often stretched in attempt to assert that all stresses are mediated by appraisal of the agent as a stress. According to Holroyd & Lazarus (1982, p23): ... "appraisal serves as a final common pathway through which diverse personal and environmental variables influence the outcomes of stressful encounters". This is weakened by Broom & Johnson's (1993) report that adrenal cortex responses are associated with tissue damage even when anaesthetic was used. Nevertheless positive or negative appraisal is important to the efficacy of the coping process. According to Holroyd & Lazarus (1982, p24): "...The individual who responds to the potential for gain in a stressful encounter and is challenged is likely to fare better than the person who responds primarily to the potential for harm or loss". This, they say, parallels Selye's (1976) division of constructive and destructive stress (eustress and distress?). Harm, according to Moss (1973, p42), is: ... "the subjective evaluation of motive thwarting stimuli." "The subjective interpretation of the situation determines which situations produce physiological changes."... "Physiological responses to subjective definitions follow fairly consistent patterns within individuals, but vary between individuals" (Moss 1973, p59).

FEEDBACK

The possibilities for psychogenic feedback are obvious when one considers that Kubzansky, Kawachi, Spiro, Weiss, Vokonas & Sparrow (1997) have determined that high levels of worry may predispose some population sectors of men to a higher risk of coronary heart disease. If you are in the group at risk that is really something to worry about!

"Brain chemistry and brain morphology determine behavior, but brain chemistry and brain morphology are just as clearly determined by the behavior they determine!" (Moss 1973, p8 quoting Krech 1969). This opens the possibility for feedback dynamics. Lemke (1996) argues that chronic fatigue syndrome may be approached by a physical route or by a psychological route. Thus psychological fatigue leads to physical debility which leads to physical fatigue. This in turn can cause psychological fatigue. The possibility for a feedback loop is obvious.

The potential for such dynamics is also evident in the following. According to Reason (1993,

p416): "days in which a negative mood state predominates are associated with increased error rates and conversely". Moss (1981, p8) says that a cycle of defensiveness and compromised effectiveness can assume positive feedback dynamics; "worry does not necessarily promote constructive thinking-through of problems". Behavioural responses to stress (Moss 1973, p55) might include "increased reaction time, erratic performance rates, malcoordination, error increase and fatigue. Inferences of emotional states indicating stress include tremors, stuttering, exaggerated speech characteristics and loss of sphincter control." According to Mandler (1982 p101):..."practically any kind of stress, failure experience, or uncontrollable noise will impair short term memory retrieval". The above results of stress can in turn become stressful. A specific example of a stressful situation is given by Coulter (1970 in Fisher 1984), who found that if performance is shown to be relevant in reducing the frequency of electric shock received, improved performance is associated with reduced anxiety.

"Depression with helpless and hopeless feelings often follows severe anxiety; in such cases, the individual assumes that he is helpless about the stressor. Depressive mood has been shown not only to increase morbidity but also to worsen prognosis and to prolong recovery from any disease" (Leigh & Reiser 1980, cited by Liegh 1982, p738). This in turn can increase anxiety. And anxiety can cause disturbance of perception (Basowitz *et al.* 1955 in Moss 1973, p36). In addition, Moss (1973, p36) infers from this that anxiety can cause ACTH release from the pituitary. In turn ACTH production, according to Amkraut & Solomon (1975 cited by Fisher 1984, p105), "may increase the risk of physical illness by lowering the biological antibody defence system". Furthermore, in psychological testing, a high anxiety score has shown a positive correlation with long term onset of illness (Russek & Schwarz 1997).

The psychiatric condition known as endogenous depression is associated with deviation from normal adrenal regulation. Here, Hanin, Frazer, Croughhan, Davis, Katz, Koslow, Maas & Stokes (1985, in Broom & Johnson 1993) report that the hypothalamus-pituitary-adrenal axis is hyperactive and that feedback control by elevated levels of glucocorticoids is defective, resulting in continued higher than normal levels of glucocorticoids. A similar apparent malfunction occurs in subordinate baboons. They appear to have higher cortisol levels than

dominants; and this is associated with findings reported by Sapolsky (1983, in Broom & Johnson 1993) that subordinates have a less effective feedback regulation of cortisol levels by the hypothalamus and pituitary.

The hypothalamus co-ordinates information from homeostatic functions and is also influenced by higher mental process. For instance (Mandler 1982, p100), thought processes can lead to arousal with attendant "stress and coping activities". This is echoed by Zegans (1982, p146): "imagination can produce its own stressors and produce a neuroendocrine-autonomic response that itself poses a real threat to the organism". This would constitute an endogenous stress.

STRESS AND THE PERCEPTION OF CONTROL

The perception that control is possible has been shown by Houston (1972; in Holmes & Houston 1974) to reduce reported anxiety and also physiological arousal in advance". In addition, (Fisher 1984, p54) reports that, "appraisal by the individual that a degree of control may be exercised over the stressful event is more likely to increase noradrenaline relative to adrenaline, whereas the reverse occurs when perceived loss of control is an outcome". Frankenhaeuser (1971, in Fisher 1984, p71) adds: "adrenaline secretion has been shown to decrease successively as the degree of situational control exerted by the subject is systematically varied from a state of helplessness to ability to master the disturbing environmental influences". Noradrenaline, on the other hand, appears to be the "kick hormone" (Carruthers 1974, in Fisher 1984) that some individuals seek in performing the more spectacular of the action sports such as auto racing.

Higher adrenal adrenaline levels occur in animals which had more confrontations, and fights in which they were defeated. Sgoifo, Deboer, Haller, and Koolhaas (1996) report that noradrenaline levels in rats are elevated proportionally more than adrenaline in situations in which physical exercise is required. Adrenaline levels increase proportionally more with fear, and emotions experienced during limited or lacking coping capabilities. They found that proportionally higher noradrenaline levels are found in aggressive animals than in passive animals.

A similar condition (Hanin *et al.* 1985, in Broom & Johnson 1993, p120) is seen in rats subject to inescapable electric shock, whereas it is absent in rats that can escape. This according to Broom & Johnson (1993, p117) suggests that, "adrenaline production is associated with fear and anxiety but noradrenaline production is associated with greater motor activity."

According to Broom & Johnson (1993, p103), "Exposure of laboratory rats to severe treatment such as inescapable electric shock, rapid rotation or swimming in water at 4°C for 3 minutes can result in depletion of hypothalamic noradrenaline (Swenson & Vogel 1983; and Roth, Mefford, & Barchas 1982)". They also relate similar occurrences in chickens and man under difficult situations.

These relative production rates of adrenaline/noradrenaline production are reversed at the extremity of death. In rats after decapitation, plasma adrenaline concentrations increase ten times but noradrenaline concentrations increase 80 times (Popper, Chiueh & Kopin 1977, cited by Broom & Johnson 1993). This is the reverse of what one would expect. But these are extreme conditions where no further control is possible.

Broom & Johnson (1993, p119): "Repeated exposure to different unpleasant stimuli may sensitize the hypothalamic-pituitary-adrenal cortex axis so that a test with a novel disturbing stimulus elicits a greater response than such a test would normally". They say that this may be due to greater enzyme synthesis activity or other facilitation in the hypothalamic-pituitary-adrenal cortex axis. This they ascribe to a higher responsiveness to the same stimulus with ACTH. In support Grandin (1997) reports that while sheep and cows may habituate to novel but not unpleasant experiences they do not habituate to aversive experiences such as falling down in transit and inversion. In cattle this is reflected in cortisol levels that decreased in the first and remain elevated in the second instance.

In addition, Broom & Johnson (1993, p119) report that crowded pigs and those which had been defeated had higher cortisol production for the standard ACTH dose than did those that were less crowded or were either submissive or won their fights. It appears that it is more

stressful to attempt to change conditions than it is to acquiesce. Is this increased sensitivity beneficial to the pig? How would this fit the triphasic response of Selye?

According to Fisher (1984), learned helplessness and other impairments of capabilities may occur after repeated unpleasant experiences over which the subject has no control. Although Fisher (1984) reports some conflicting results it seems that animals with no control over unpleasant and challenging conditions are more likely to suffer ulcers. Broom & Johnson (1993) also refer to learned helplessness in rats, where after repeated exposure to unpleasant stimuli which they cannot avoid, the rats may cease to respond to events around them. Broom & Johnson (1993) also cite various possible behavioural and hormonal pathways which would account for the lack of mental adaptability in the rats. According to Broom & Johnson (1993, p143), "People who are overloaded with decision making are often unwilling to explore or attempt to learn new skills. Reduced exploratory behaviour is also a possible response to overload in other species".

Mendl (1990 in Broom & Johnson 1993, p144) found that animals that have experienced a prolonged period in an environment over which they have little control may not show any effort to change environments when a choice is presented. This is ascribed to apathy (Broom, 1986, 1987) or learned helplessness (Overmier, Patterson & Wielkiewicz 1980, Overmier 1996) stemming from prolonged experience of lack of control. This may result in loss of fitness and thus have a positive feedback component.

Overreaction is the opposite of learned helplessness. "Inappropriate engagement and struggle when the problem is un-solvable may be as unbalanced as helplessness when it is solvable" (Fisher 1984, pxxiii). Inappropriate responses may include stereotypies of behaviour. This is defined (Broom & Johnson 1993, p139) as: "a repeated, relatively invariate sequence of movements with no obvious function." Clearly such behaviour is waste of time and energy. There may thus be a pressure for the assumption of learned helplessness. If nothing is achievable one might just as well do nothing.

Opioids and undereaction

"It has been suggested that animals in unavoidable suboptimal conditions may be using endogenous opioids to help them cope" [Broom, (1986, 1987) in Broom & Johnson (1993, p141)]. Broom & Johnson (1993) review aspects of self narcotizing by opioids and they cite Zanella, Broom & Hunter (1992), who found that unresponsive sows have a higher density of μ (endorphin) receptors in the hypothalamus and striatum than responsive ones. Broom & Johnson (1993) and Schneiderman & McCabe (1985) report that ß-endorphin and other opioids may be released at the same time as ACTH in some circumstances, such as when under stress. They report that elevated plasma levels of these substances have been measured in animals after they have been subject to experiences such as electric shock or restraint. Schneiderman & McCabe (1985) report that chronic conditions such as arthritis are often accompanied by elevated levels of opiates and increase in the number of opiate receptors in the CNS.

AROUSAL AND PERFORMANCE

McGrath (1976), in common with Näätänen (1973, in Fisher 1984), criticises the inverted U shaped curve as the basis for the relationship of performance to arousal -or, in consequence, stress. Näätänen (1973) found that level of activation, as measured by heart rate and caused by dynamometer exercise had no influence upon reaction time or upon speed of arm movement.

McGrath (1976) considers arousal as an operational definition of stress. It is indicated by heart rate and is a product of consequences and uncertainty or perceived difficulty. If, for any task, it is assumed that the consequences remain constant, then the level of arousal is dependent on the level of uncertainty. This, he says, is maximal when perceived difficulty is equal to perceived ability. Arousal is thus lower when the task is seen to be either too hard or too easy. McGrath (1976) found that the relationship of performance to arousal is linear and rising. Thus, performance will reduce when the task is too hard or too easy. He says that, conversely, the relationship of performance to difficulty is linear and negative.

McGrath's (1976) findings may be summarised: 1) Performance increases with arousal. 2) Arousal increases with perceived uncertainty and also increases with perceived importance

of consequences. 3) Easy or difficult tasks are less arousing because the outcome is more certain. 4) It is not the performance to arousal relationship that has an inverted U shape; it is rather the performance related to perceived difficulty.

According to McGrath (1976) increase in arousal can only increase performance. Which means, in his terms, that an increase in stress can only increase performance. This tends to be supported by the go-getting "switched on" A type of personality. A study of performance, arousal and adrenaline - noradrenaline balance may shed more light on this subject.

BIOSOCIAL RESONATION

Moss (1973) rejects the concept of stress. He (Moss 1973 p25) says: "Some (such as Mechanic 1962) have insisted that the term stress applies only to physiological conditions and cannot be applied to social and psychological phenomena". Hence of his own work Moss (1973, p4) says: "The purpose of this work is to conceptualise some possible links between social behaviour, physiological processes and health." Among other models Moss (1973) presents one of psychological stress devised by Dohrenwend (1961) and based on Selye's physiological model. Though he praises Dohrenwend's (1961) ingenuity, Moss (1973) decries its paucity of practical value and its lack of integration with physiological phenomena.

Instead of stress, Moss (1973) proposes a "Biosocial Resonation" model to account for stress phenomena. Resonation being (Moss 1973, p241): "The continued reciprocal influences of physiological processes and social behavior in social interaction." "This perspective (Moss 1973, p10) is formulated in an effort to break away from arbitrary physical, psychological and social distinctions, especially in stress research, in which efforts to apply the concept of stress to these three categories as though they existed somewhat independently may have some heuristic value but tend to distract us from the proper appreciation of the wholeness of man."

According to Moss (1973, p13): "Disease definitions based on a biosocial resonation model would label particular configurations of resonating physical, social, and eventually, environmental variables. There would no longer be physical, and mental illnesses, but one classification of resonating configurations embracing physical and social-psychological processes and states in their social and environmental contexts. It would focus on neither

cause nor effects but embrace both in a resonating pattern."... "A more pressing need now is to develop a conceptual framework for categorising relations between perceptions of the situation, physiological responses, and disease manifestations within a social context." Resonation opens the possibility of feedback; cause and effect are very soon swallowed up in the dialectic of interaction.

Moss (1973, p188) reviews homeostasis in the light of psychological interactions and he quotes Ruff & Korchin (1967): "Stressors are inputs which force variables beyond the normal range controlled by homeostasis". Moss (1973, p188) adds that the French physiologist, Claude Bernard, sees disease as the, "outcome of attempts at homeostasis in which adaptive responses to noxious forces, although appropriate in kind were faulty in amount". Chapman, Hinkle & Wolff (1960, in Moss 1973, p188) say: "disease should be viewed as occurring when the adaptive responses of the organism to stimuli are inappropriate in kind or amount or both". Moss (1973, p191) however considers that "the concept of homeostasis is not adequate for social systems".

He thus proposes homeomaistre (p242) and defines this as a "Communication network's process for maintaining recognisable continuity in information content, preserving effective interaction patterns while modifying portions of the network's information no longer adequate, and acquiring new information". "Homeomaistre (Moss 1973, p195) is not a passive response or adaptation to the environment but the active resonation with it." "Individuals perceiving the environment and testing and modifying their information in it are resonating with the environment."

"The subjective interpretation of the situation determines (Moss 1973, p59) which situations produce physiological changes." Holroyd & Lazarus (1982, p25) say: "...health outcomes are a product of effective coping rather than simply a consequence of the presence or absence of stress". Fitness is thus contingent on coping.

The homeomaistre model of Moss is an interesting holistic view of stress. It does however raise some problems about the nature of the units of study. The resonating networks without doubt exert much influence on the targets of stress. But it is difficult to organise a coherent

classification of these networks so that by induction we can formulate predictive general laws about their nature.

OTHER OBSERVATIONS ON PSYCHOLOGICAL STRESS

All stresses can be defined as agents that compromise the continued existence of the biological entity such as the cell, organism, species, population, or community. Stresses impair the co-ordination of maintenance processes. The effect of psychological stress, which is an organism level stress, is to disorder thought processes such that fitness is compromised either concretely or probabilistically. In this respect one can cite Selye (1982, p14), who refers to "psychological mismanagement": is this poor co-ordination of psychological processes?

The principal symptom of psychological stress is that values are allotted to entities that are not appropriate to their potential for influencing the fitness of the appraiser. This is an entry point for the possibility of integrating it with physiological stress.

CHAPTER 24: REMARKS AND CONCLUSIONS ON THE DEFINITION OF STRESS

According to Lefcourt (1986 in Hattingh 1988) an absolute definition of stress is impossible because of the fluid context and assumptions about its utility. Since utility has teleological implications it is, as has been advocated previously, excluded in the model proposed in the present work. This study aims to be descriptive rather than teleological or normative. We ask not, what is stress for? but rather, what does stress do?

Lefcourt (1986) is nevertheless right, about the meaning of stress being difficult to pin down. This is because stress is stressful only in terms of the environment and the response of the particular organism it affects. Different types of organism thus find different conditions stressful: Hattingh (1988, p731) cites Mason (1971) as saying that reactions to a stress are "individual and stressor specific". And hence: "no objective measures of stress are available". For example, a fish and a mouse respond rather differently if placed in water. So too do obligate aerobic and anaerobic bacteria in the presence of oxygen. What might be a stress to one organism may be an essential condition for another. Moreover, even stresses evincing similar lethal levels in disparate taxa may have different pathways of action; as the example given above from Sprague (1970) demonstrates. In consequence there can be no universal scheme of stress (as cause) units.

Van Der Steen (1993, p264) strongly supports this position: "stress stimuli as stimuli do not have anything in common beyond producing the same kinds of internal states or responses". And he says that it makes no sense in trying to develop general laws of stress (I assume he means as stimuli). One must agree in principle but one should soften his statement and say that stress stimuli *may* not have anything in common. This is because a quantification of stress (as a stimulus), despite lacking universality, may still be useful in comparative studies of ecologically or taxonomically related organisms. And it may be a way of classifying disparate agents on a basis of equivalent noxiousness. Nevertheless, the equivalence can be determined only with reference to the effect on the organism. And in ascertaining this effect, a holistic approach is preferable. "It is realised that like 'critical species' and 'indicator organisms' physiological indices of stress are specific with respect to stressor, the organism

and the physiological process. Although an effort has been made to segregate responses for organisational purposes, the reader is urged to reflect in the light of the whole animal reacting to the total environment" (Dillon & Lynch 1981, p229). Stress might be made operational in terms of concentration, exposure regime, etc. But it must be emphasised that these indices have only local significance.

Since stresses can only be identified as such by responses in the organism, it is understood that this necessarily leads to such stimuli, by definition, causing responses. This circularity means that the causal link must be taken as assumed rather than proved. Although this does not solve the problem it does shift the burden to the term strain (as the inverse of fitness- not any particular response) which may be more easily measured. Fitness is a universal quality in organisms, and fitness should therefore be universally definable. An assessment of the universality of fitness parameters and how they may be subject to measurement is presented later.

To sum up, stress should be adopted as the term for a deleterious causal agent; the effect exhibited by the organism will be termed strain - a reduction in fitness. Responses are seen merely as indicators of strain and, as Dillon & Lynch (1981) say, they must be carefully interpreted before they can be equated with it. As urged by Van Der Steen (1993), independently grounded definitions of stimulus (stress) and response (strain) have now been provided. Thus the relationship between stress and strain is open to empirical study.

So far, stress and strain have both been given working definitions: they conceptualise aspects of deleterious phenomena. But is that the whole story? Stress phenomena deal only with limiting factors. Is there more to the organism than just this? What about the innate creative capabilities attributable to the organism such as its ultimate reproductive rate? What new can these capabilities tell us?

CHAPTER 25: STRAIN AND FITNESS

INTRODUCTION

When considering fitness one must ask: is it fitness of the individual, or the lineage, or the species? What does this mean to selection at individual or group level? Do more copies of genes mean greater fitness, and what about quality rather than quantity? Who will decide what quality is in this case? Fitness, in common with stress and strain, may be applicable to individuals and populations with varying degrees of interchangeability. In this work it is postulated that fitness means fitness for continued existence - what it is that continues existence must be explicitly stated, i.e. individual, species, lineage, etc.

Numerous questions about fitness arise. Is there such thing as ultimate fitness? Can it be identified? Is fitness descriptive or predictive? Similarly, how can the fitness of the ecosystem be judged? What is it fit for? Is this another ontological problem? How does it differ from individual fitness? Definitions of fitness usually refer to properties which influence the persistence of the organism or its lineage. It is an indicator of existence. Fitness is thus an ontological term. Before continuing with the ramifications of this question, usage of the words strain and fitness will be reviewed.

CURRENT DEFINITIONS OF STRAIN AND FITNESS

Strain is what happens to the organism when subject to a stress (Levitt 1980). It is: "Any physical or chemical change in a living organism produced by a stress" (Lincoln *et al.* 1985, p236).

Current definitions of fitness follow. Begon *et al.* (1990, p852) see it as the, "contribution made to a population of descendents by an individual, relative to the contribution made by others in the present population". Ollason (1991, p81) proposes, "that fitness is nothing more than the production of offspring". "Furthermore since there is no way that the environment can be defined independently of the presence of the animal there is no way that the quality of the animal can be assessed"..."Potential fitness could be defined as the maximum reproductive rate of the individual, manifested fitness as the observed reproductive rate" (Ollason 1991, p89). Ollason then begins to subdivide fitness into physical and reproductive

fitness and further characterises physical fitness as respiratory fitness (are there not other aspects of physical fitness?). Ollason (1991, p91) also shows that "the problem with evolutionary fitness is that there is no possibility in principle of establishing a mapping in physical and chemical terms from the phenotypic properties of the animal to its reproductive output". Stearns (1992 in Kozlowski 1993, p84) claims that, "fitness is only a problem solving tool and that no general definition of fitness has been found". Kozlowski (1993, p84) cautions however that "choosing a fitness measure for a given evolutionary problem cannot be arbitrary".

Broom & Johnson (1993, p176) define fitness as: "Success of an individual in passing on genes to future generations. Fitness expresses lifetime reproductive success, which is reduced by mortality, delay in breeding and reduced offspring production per breeding. Many indicators reveal when fitness reduction is likely." The last sentence is important as it indicates the uncertainty attached to discovering fitness. Therefore when devising fitness parameters, consideration must also be given to a quantification of probability.

Inclusive fitness is defined (Levins 1968) as the capacity (how is capacity measured?) to produce a maximum of vigorous well adapted adult offspring. What about fitness as the ability to get to the next generation at the same stage in the life cycle? Levins (1968) also says that the ultimate measure of fitness in ants is the production of a queen, and cites Andrewartha and Birch's definition of fitness as the intrinsic capacity to increase. "The biological fitness of an individual is the relative contribution of that individual to the ancestry of future generations. Usually measured by the number of offspring surviving to reproductive age relative to the population mean" (Mayo 1983, p34). Lincoln *et al.* (1985) define inclusive fitness as the sum of an individual's fitness quantified as the reproductive success of the individual and its relatives, with the relatives devalued in proportion to their genetic distance.

Neo-Darwinian fitness is the change in the relative frequencies of alleles. Fitness may also be the ability to get genes into the next generation (to the point of offspring reproduction?). Fisher (1932) and Haldane (1930), both cited by Calow (1983), deal with neo-Darwinian fitness. Such an approach, which ascertains the change in frequency of genes, is less applicable when attempting to ascertain the fitness fluctuations during the lifetime of an

individual organism. Lincoln *et al.* (1985) also define fitness as the relative competitive ability between two genotypes.

While it can be seen that relative frequency of genes, alleles, or genotypes in a population is important, it does overlook the possibility that absolute numbers of organisms may change. Surely fitness must take into account absolute numbers? For instance, in a total population of 20 organisms the relative fitness of any organism can at most be 20/20 = 1. If however the population is 100 then even with a relative fitness of only 0,25 the organism is more numerous than its relatively more successful counterpart in a smaller population.

It is urged that fitness should also take into account carrying capacity of the environment. And Levins (1968) gives a definition of fitness as the carrying capacity of the environment for a given genotype (K). Perhaps a more fundamental measure of fitness would be to ascertain the ratio of living to non-living material in the ecosystem.

Settle (1993) states the importance of linking the design (his word, hopefully without teleological connotations) of organisms or their components and their fitness enhancing appropriateness to the environment. Perhaps the emphasis should be more on the expected persistence of the said organ or organism and/or its lineage in a given environment.

Lincoln *et al.* (1985, p93) define fitness as: "the relative competitive ability of a given genotype conferred by adaptive, morphological, physiological or behavioural characters expressed and usually quantified as the average number of surviving progeny of one genotype compared with the average number of surviving progeny of competing genotypes; a measure of the contribution of a given genotype to the subsequent generation relative to that of other genotypes". This definition is still vague in some respects. Should it be measurable over just one generation? It does not address the problem of offspring fertility. Extension of the definition to production of the greatest number of fertile offspring into an arbitrarily chosen n^{th} generation would eliminate uncertainty about offspring fertility.

According to Mayo (1983, p37): "Fitness, in the sense of ultimate reproductive success is evidently only measurable after the fact, i.e. over particular known lineages for defined times. Indeed it could be argued that it is only known precisely for extinct lineages or organisms

which leave no descendents" **and then it must be zero**. Any other nominated period must be more or less arbitrary. Thus, the fitness of the ammonites is zero regardless of their great span of persistence. Clearly, fitness must take into account something more.

To be more optimistic, Van Der Steen (1993, p270) claims: "A great variety of factors (collectively captured by the concept of fitness) affect reproductive success". This suggests that an estimate of fitness may be obtained from the integration of different factors. These factors are dealt with here as the fitness counterparts of stress subdivisions previously presented. These include reproductive, somatic, incremental (deterministic) and risk (probabilistic) elements of fitness.

Calow (1989, p180) also suggests that such an integration may be possible: "...the allocations to catabolism and somatic and reproductive anabolism can, in principle, be translated into effects on survival probability, growth and hence time between developmental events and reproductive investment. Hence though the molecular, cellular and physiological traits associated with stress resistance may be very diverse, they can, at least in principle, be related to general metabolic properties of organisms that can be linked in turn to demographic variables that influence population dynamics." Calow also refers to fitness components such as survival probability, fecundity and time between developmental events.

Feltmate & Williams (1991) also refer to fitness components. In this case they are fitness components of stone flies such as nymphal density, head width (as an index of size) and condition (mass/head width). The first is a population fitness parameter the second and third are individual fitness parameters. It shows that fitness parameters may be divergent.

We must not become oblivious to the problem previously mentioned that the nature of events in a higher order of complexity may not be implicit by looking at the nature of the components. Clearly, we must elucidate the configuration of the relative hierarchies and develop local laws that are applicable at each level before we can hope to make progress.
The above definitions show that the word fitness has been stretched over a wide expanse of meaning. But they all have in common some notion of replicative and survival capacities; capacities that are basically descriptive of existence. They may be divided into those

definitions of fitness which are comparative and those which are absolute. Because in this work it is posited that strain and fitness are facets of the same quality, and that strain is an absolute concept, we must also reformulate fitness in absolute terms. This has been achieved by defining it ontologically: in terms of the qualities and quantities of the organism's existence.

IS FITNESS A DEFINITION OF THE ORGANISM OR ITS ENVIRONMENT OR BOTH?

We have already seen that Levins (1968) has commented on the significance of the environment on fitness. It is now time to give more consideration to the importance of the environment. Sibly & Calow (1989) suggest that, "resistant strains (lineages) are fitter in stressed environments, and susceptible strains fitter in unstressed environments". Is a long-lived organism "A", in a non-challenging environment with no opposition, fit? Is a long-lived organism which could outcompete "A" but is not in the same environment potentially or latently fit? What about the individual which produces a myriad of viable offspring into the wrong environment; is a reduction of expected fitness likely? Obviously, fitness must be defined with reference to the environment or likely environment. With the latter qualification (likely) one can see that probability can creep in to any estimate for the future.

What is an environment? Lincoln *et al.* (1985, p80) define it as "the complex of biotic, climatic, edaphic and other conditions which comprise the immediate habitat of an organism, the physical, chemical and biological surroundings of an organism at any given time". Kojima (1971) asserts that an isolated organism cannot have its fitness ascertained. Furthermore, Andrewartha & Birch (1986) say of the environment that it is everything that might influence an animal's chance to survive and reproduce. Thus by their definition, the environment must be taken into account when ascertaining fitness. As Henderson (1913) declares of the environment, it, besides the organism, must also be fit. But the fitness of the environment must be ascertained in the context of the organism.

Fitness of the organism thus means little without reference to the environment. For instance, in the absence of aphids, plant resistance to them is a disadvantage and more susceptible plants are better competitors (Windle & Franz 1979). Similarly in the mosquito *Culex*

quiquefasciatus some are resistant to infection with *Bacillus sphaericus* but the non-resistant *C. quiquefasciatus* showed higher fitness than resistant mosquitoes in the absence of the bacteria (Rodcharoen & Mulla 1997). This is echoed by Parsons (1990b) who says that plants selected for heavy metal resistance may be otherwise competitively inferior to normal plants.

Even taking the environment into account, fitness can only be described in approximate terms of probability, as the environment is often unpredictable. Again it seems that fitness is hard to ascertain except possibly in retrospect.

Earlier we have seen the possibility of constructing three-dimensional representations of the ranges of parameters encountered in an environment. Can we go further and give the environment an abstract fitness value?

CHAPTER 26: COMMON SUBDIVISIONS OF STRAIN AND FITNESS

Strain is used by Beehr & O'Hara (1987, p79) to refer specifically to the adverse reactions of the individuals to the environmental event or stressor. It is taken further here and strain is defined as the resultant loss of fitness.

EXOGENOUS AND ENDOGENOUS FACTORS

This dichotomy is suggested by the statement of Meier (1972) cited in Auerbach (1981, p30): "Stress has been viewed as a response to *external* or *internal* processes which reach threshold levels sufficient to strain capacities to, or beyond, their limits" (Italics mine). Exogenous strain may be caused by environmental factors that restrict growth or reproduction or both. It is the result of any external stress that disturbs the internal equilibrium of a system or its processes. It leads to a reduction in the likelihood of the organism's physical persistence. Exogenous strain thus implies a suboptimal environment in the context of the organism in question. The causes of exogenous strain embrace the terms predator, malentity and modifier as used by Andrewartha & Birch (1986). They also embrace the extrinsic stresses: pollutants, predators, radiation and osmotic stress mentioned by Sibly & Calow (1989).

Endogenous strain is manifest through internal inadequacy; it may affect reproductive and somatic processes. Such an internal somatic inadequacy might be age related susceptibility to hypothermia in man (Young & Lee 1997). In *Mytilus edulis,* Hole, Moore & Bellamy (1995) found that recovery, post emersion, from hypoxia and hyperthermia, of lysosomal integrity and metabolic rate is dependent on age; the youngest having the fastest recovery.

Such internal inadequacies are expanded on in Sibly & Calow (1989, p113, quoting Calow 1978): "It is possible to view ageing as a process involving the accumulation of cellular and molecular damage through the lifetime of the organism; i.e. due to intrinsic processes such as thermal noise, auto-oxidation, racemization, somatic mutation and errors in synthesis". Sibly & Calow (1989, p115) refer to these ageing processes as intrinsic stresses. Senescence results in, among other things, reduced ability to tolerate extremes of the environment. See Medvedev (1990) for a classification of the various theories of ageing.

A definition of senescence adopted by Edney & Gill (1968, p281) is: "the total effect of all changes which occur in an organism as it ages and which render it more vulnerable or less viable." Senescence, they say, results from: "(1) extrinsic causes including mechanical wear and, (2) intrinsic causes which are genetically built in" (p282). Edney & Gill (1968) define specific longevity as the time from birth to 99% mortality in a cohort. In attempting to derive specific longevity from senescence one must also take into account the increased risk of mortality with time from such events as predation, desiccation and starvation. Thus, they say, that specific longevity is determined by: random accident (increased risk), environmentally caused extrinsic senescence and intrinsic senescence. The first two of these together constitute the hazard factor.

Reproduction may precipitate endogenous somatic strain in the individual as energy is expended to the detriment of somatic resources. This increase in endogenous somatic strain may be mitigated, however, by increased reproduction, which maintains the fitness of the lineage even at the expense of the individual. As Broom & Johnson (1993, p112) put it: "Body resources are often apportioned to reproductive effort even at the expense of basic body maintenance".

Endogenous strain may also be inherited. For example, the faculty of flight may reduce individual fitness in some environments; hence the evolution of flightlessness in some island dwelling birds such as rails. An unneeded ability can be expensive and may therefore be selected against. In the absence of antagonists (assuming no role in intra-specific interactions) defensive armour, arms and poisons are a waste of energy and thus constitute endogenous strains.

This statement finds support from Sibly & Calow (1989, p102): "Many examples of personal defence, such as escaping predators, neutralising and excreting toxic chemicals, or pumping out excess water or ions, consist of active processes that consume power and hence energy". "...There are, therefore, good *a priori* grounds for believing that defence is generally expensive" Sibly & Calow (1989, p103). Sibly & Calow (1989, p101) "use an optimality approach to examine how animals are likely to respond to different levels of stress." They discuss the use of mortality/growth trade-off curves to maximise Darwinian fitness.

DETERMINISTIC AND STOCHASTIC FACTORS

Deterministic strain is measured by the degree of damage. Stochastic strain is measured by increased risk. In both cases, strain increases in proportion to the duration and intensity of applied stress. Deterministic strain is manifest as a shortened life span, or reduced performance or reserves or any combination. Stochastic strain is manifest as an increased risk of early death by a raised probability of meeting a critical inadequacy. The two sorts of strains may be described by analogy. Stochastic strain is like playing Russian roulette, it has no long term effects if you are not shot. On the other hand, deterministic strain is more like a lifetime of heavy drinking: the system will show evidence of damage even if it is not fatal. Thus, one may have no long-term effect if predation is avoided but the other may be measurable despite being sublethal.

It is recognised that deterministic and stochastic strains are two poles of a continuum. Sibly & Calow (1989, p105) say, "Other stresses, such as many chemical toxins, might simultaneously reduce the survival chances and the growth rates of organisms exposed to them". These agents would thus cause both stochastic strain and incremental (deterministic) strain. Despite this continuum it is maintained that the terms have utility in apportioning fitness values for the purpose of making more accurate predictions. This is dealt with later.

Deterministic strain is more easily studied: it could be represented by, for instance, a bioassay. Stochastic strain, in contrast, is less accessible as it is more of an all-or-nothing phenomenon; it does not have a concrete existence and can only be expressed as a probability. As Auerbach (1981, p35) states: "Death is a stochastic process with the probability of dying inversely related to the yearly growth increment". Stochastic strain could be ascertained for a population and then reduced to give a probability of mortality per unit time for a given individual.

Predation is one aspect of stochastic strain. Perkins (1974 in Auerbach 1981, p30) states: "It is recognised that stress [strain as understood here] results from normal ecosystem structures (e.g. predation or competition) as well". The threat of predation means that though the organism has no increased contact with somatic or reproductive stress, its potential fitness or its fitness probability is degraded, which is another way of saying that its mortality

probability is increased.

Other reports which may fall under stochastic strain include those by Dillon & Lynch (1981, p228) and Calow (1989, p173). According to the former, "the altered state results in a decreased chance of survival or a diminished ability to adapt to further environmental change". The latter states: "Environmental stress causes reductions in survival probability".

Stochastic strain is exemplified by a parasitised animal that must feed for longer and thus extend its exposure period to predators, thus increasing its risk. This may often be an all or nothing risk. However, a predator may merely injure its host causing a real decrease in fitness and increased probability of death. This is exemplified by Griffiths & Blaine's (1994) report of drilling whelks *(Nucella cingulata)* that drill shells of *Mytilus galloprovincialis.* In some cases the proboscides of the whelks are too small to reach any vital organ of the mussel. Mussels have been found with multiple boreholes and evidence of repair to the holes and thus survival of the mussels post-attack. As Griffiths & Blaine (1994, p347) say, "Clearly the probability that the injury inflicted by a drilling whelk will be fatal increases with relative size of predator to prey. A further issue worth investigating is if post attack mortality is also proportional to the relative size of predator to prey.

Parasites may also increase risk by causing aberrant host behaviour. This is seen in the effect of the digenean trematode *Parvatrema affinis* on the clam *Macoma baltica* (Swennen 1969 in Lauckner 1983). The behaviour in itself may not be energetically costly but the subsequent predation, should it happen, is catastrophic. Stochastic strain may be attributable to stochastic malentities, as used by Andrewartha & Birch (1986) and also their categories of predation and aggressive malentities. Modifiers, as defined by Andrewartha & Birch (1986) might be understood to cause deterministic or stochastic strain or both.

The concepts of growth stress (that which decreases growth rate) and mortality stress (that which increases mortality) as expounded by Sibly & Calow (1989) are embraced by deterministic and stochastic strain. Sibly & Calow (1989) say that mortality stress describes factors that impair survivorship, thus a pure mortality stress might be a predator. Growth stress describes factors that inhibit production such as fecundity and the interval between life

cycle events, a pure growth stress might be a temperature reduction in poikilotherms (within the physiological range) and possibly PO_2. Sibly & Calow (1989) liken their mortality stress and growth stress to Grimes's (1979) disturbance and stress respectively. In the scheme of Sibly & Calow (1989, p101), "optimal stress responses are calculated for different levels of growth and mortality stress, and are found to depend critically on the shape of the trade-off curve relating mortality to growth rate". For individuals, rates would have to be replaced by probabilities.

CATEGORIES OF STRESS AND STRAIN RESISTANCE IN PLANTS

According to Grime (1989b) whole-plant stress responses are either morphological or physiological. Morphological plasticity occurs usually at the time of cell differentiation and is considered (Grime 1989b, p43) as "...the predominant form of stress response in productive habitats. Cellular acclimation is characteristic of long lived tissues of plants occupying habitats of consistently low productivity".

Levitt (1980, p20) proposes a law of stress resistance in plants: "Whatever the stress, a plant may achieve stress resistance to it by either an avoidance or tolerance mechanism. These two mechanisms of resistance may be developed at any one of three levels - the stress level, the elastic strain level, or the plastic strain level." Stress resistance (Levitt 1980, p3) in plants is "the ability of the plant to survive the unfavorable factor and even grow in its presence.

Levitt (1980) also asserts that some plants may be stress evaders by completing their life cycle and reverting to a resistant dormant stage before stressful climatic conditions return.

REPRODUCTIVE AND SOMATIC STRAIN

If the effect of somatic and reproductive stresses on fitness were integrated, would one expect net fitness to remain approximately constant as somatic and reproductive fitnesses fluctuate? Or would another dynamic be expected? Is there any constancy in fitness in a lineage?

One possible speculative scenario in fluctuation of somatic and reproductive fitnesses would be that in a semelparous animal. As eggs are laid, somatic fitness declines but reproductive fitness increases. When the eggs hatch reproductive fitness declines (Figure 1) but somatic

fitness of the offspring starts to increase. In an iteroparous animal, somatic fitness would not decline to zero: the adult survives egg laying to produce subsequent broods.

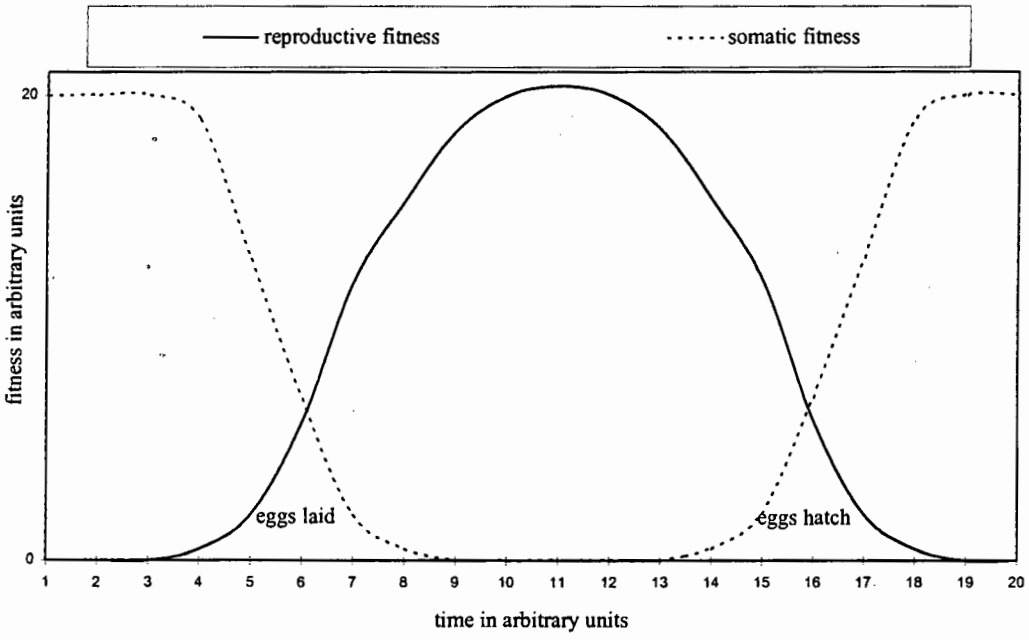


Figure 1. Possible fluctuation of somatic and reproductive fitness in a semelparous animal.

Is the sum of these fluctuations the net fitness? How does conversion of somatic to reproductive fitness, and back, occur? Is there a conversion loss - or gain? As somatic fitness wanes, does reproductive fitness increase and vice versa? And how can this be tested? To return to eggs: how does one gauge the fitness of an egg? Is a developing egg getting fitter, (not if it is judged by energy balance) and by what criteria? A developing egg is becoming more complex. Thus, examination of the rates of anabolism and catabolism in the egg may tell us about its order/disorder dynamics. A clear and precise definition of fitness in each context may be the prerequisite to answering these questions.

Somatic strain

Somatic strain is a reduction of the body's capability to serve its own needs and is manifest as an increase in mortality or lowered tolerance to further stress. Somatic stresses may be endogenous, such as diversion of energy into reproduction at the expense of the body, or exogenous. Somatic stresses may be negotiated by avoidance or tolerance. Somatic fitness

may be ascertained by using indices of tolerance, performance, resistance, maximum throughput of energy, most stored energy or least wasted energy. Activities which increase somatic fitness immediately include feeding and movement out of danger. Activities which increase somatic fitness later include foraging, shelter building and encystment.

Spawning may be considered as a natural stress, Calvo-Ugarteburu (1996) states that mussels may have reduced filtration rate and be depleted of energy reserves by spawning. In some females the concept of somatic strain may be complicated by the exogenous influence of fertilization which causes changes in the body of the female, some of which could be described as a somatic strain. This raises the interesting question: is fertilization a somatic stress? If so then it may cause a reduction in somatic fitness concurrently with an increase in reproductive fitness.

Fertility compensation occurs (Calvo-Ugarteburu 1996) when an organism increases its reproductive output as an initial response to exposure to a parasite that if it becomes established in the host usually castrates it. Thus the potential host appears to be anticipating future loss of reproductive fitness by a sudden burst of reproductive output. Such an *ad hoc* output may be inferred to come from the somatic reserves of the body. If so it would be a somatic stress. This phenomenon has been seen in mussels that have been exposed to parasites. See Calvo-Ugarteburu (1996) for further references.

Reproductive strain/fitness

Reproductive fitness may be estimated once it has been defined. Is it the lifetime output of propagules? Or is it energy invested in viable offspring? Or perhaps it is the maximum output of peak condition offspring? Reproductive strain on the other hand is a decrease in reproductive output or its potential. Energy deficit is an example of a reproductive strain. Bayne, Holland, Moore, Lowe & Widdows (1978, p838) say, ..."as a result of environmental stress, fecundity was reduced approximately in proportion to the decline in energy available for gamete production".

Sometimes a somatic strain apparently enhances reproductive fitness. Gosling (1992, p209) says: "Lifetime egg production in mussels would be maximised under suboptimal conditions

if, associated with a reduction in adult body size, reproductive effort is increased. This helps to explain accounts of enhanced reproductive effort following experimental starvation and increased aerial exposure". "Evidence therefore indicates that (Gosling 1992, p209) reproductive output is maintained at the relative expense of somatic tissues under moderately unfavourable conditions. Only when the stress becomes greater, is reproductive effort reduced, helping to preserve structural integrity." A starting point for the study of reproductive and somatic stress would be the equation of Koehn & Bayne (1989), which takes into account somatic and reproductive energy production.

CHAPTER 27: STRAIN INDICATORS

GENERAL INDICATORS OF STRAIN

Wedermeyer (1981), Pickering (1981) and Wedermeyer & Mcleay (1981) have, in vertebrates, divided the stress response (i.e. strain) into three categories. Primary responses include neuroendocrine activity such as the release of ACTH, catecholamines and corticosteroids. Secondary responses include changes in blood and tissue composition, hyperglycaemia, hyperlactaemia, leucopenia, depletion of liver glycogen, increase in nitrogen metabolism and diuresis. Tertiary responses are integrations of the whole animal and include behaviour, reduced growth, increased susceptibility to disease and higher mortality.

Examples of tertiary responses include the orienting response (OR) and defensive reaction of Archer (1979), and the inhibition of burrowing response in the sandy beach whelk *Bullia digitalis* (Brown 1982). Archer (1979) also reviews the neuroendocrine responses in mammals. Broom & Johnson (1993, p88) say, "the first behavioural responses to environmental change are orientation reactions". Broom & Johnson (1993, p88) also say, "Orientation reactions are common to many types and intensities of stimulation and are not themselves indicators that the animal is encountering a problem".

The orienting response is an appraisal reaction so that the organism can decide whether it is faced by a threat or not. A more marked and dramatic response is the startle response (Broom & Johnson 1993); this, they say, is preceded by the orientation response. The orientation and startle responses of Broom & Johnson (1993, p88) broadly overlap the orienting response and defensive reactions of Archer (1979). The startle response includes, "postural changes, jumps and vocalisations"; their intensity is said to be related to the amount of disturbance that the organism has experienced. Though the startle response may enhance the capability of the organism to deal with the new development it does so at the cost of discontinuing its normal behaviour, the length of the interval before it resumes normal behaviour may, says Broom & Johnson (1993), be a useful measure of disturbance level. It follows that, though the startle response may increase fitness by optimising the organism's power to cope with a sudden event, it may do so at the cost of loss of benefits accruing from continuation of normal behaviour.

Primary responses might reasonably be expected to give the earliest indication of stress. But, as Sprague (1971) broadly agrees, avoidance responses may in practice be detectable sooner, and have the advantage of being integrated responses. Further work is needed to ascertain if this is generally applicable to all organisms.

Heat shock proteins [hsp] (Prosser 1993 p120-121) are reportedly produced in response to elevated temperatures. These proteins, ranging in molecular weight from 15-90 kD are universal in all eucaryotes and procaryotes. Although their role is not known it is thought that it may be structural because they accumulate around, "specific organelles such as the cytoskeletal network, nucleolus, and others during periods of intense stress. If heat stress is applied to induce hsp synthesis and accumulation, cells and organisms can tolerate a temperature that would otherwise be lethal in the absence of heat shock proteins. Thus hsp can confer a transient thermotolerance." Not only heat stress can elicit the production of hsp. It is reported that such agents as "ionising radiation, amino acid analogs, lysergic acid (LSD), sodium arsenate and viral infections" can also elicit the production of heat shock proteins.

It is, therefore, not surprising that Lakhotia (1998) in a review of some aspects of heat shock proteins, proposes that they be termed stress proteins. He argues that their production is more universal than just in response to heat shock. He finds also that stress proteins may have a protective function such as in offering protection to brain and heart tissue from the effects of ischemia.

Other works dealing with these proteins include those by Whyard, Wyatt & Walker (1987), Chen & Li (1988 cited by Prosser 1993), Currie & White (1983 cited by Prosser 1993), Ketola-Pirie & Atkinson (1983) and Lindquist (1981). More specifically, these proteins are studied by: Dean & Atkinson (1985) in Japanese quail *Coturnix coturnix japonica*; Koban, Graham & Prosser (1987) in channel catfish *Ictalurus punctatus*; Kothary, Burgess & Candido (1984) in cultured cells of rainbow trout; Lindquist, (1980 & 1981) in the fruit fly *Drosophila melanogaster* and yeast *Saccharomycetes cerevisiae,* and Whyard, Wyatt & Walker (1986) in the locust *Locusta migratoria.*

STRAIN INDICATORS IN BIVALVES

Under this heading, indicators of the effect of stresses, as they occur in the literature, will be reviewed and other possible indicators will be suggested.

Heat shock proteins have been studied in mytilids (Hofmann & Somero 1996 and Roberts, Hofmann & Somero 1997). In the first, those of *Mytilus trossulus* and *Mytilus galloprovincialis* were studied. They determined that the more northerly species, *Mytilus trossulus,* is the more sensitive to elevated temperatures. In other words *Mytilus trossulus* is the more cold adapted of the two species. In the second work they found in *Mytilus californianus* that although heat shock protein expression differed with temperature, it also differed with season. Thus they say the stress response is modulated by environmental factors in addition to body temperature. Thus caution is required before extrapolating results from laboratory to the field.

The breakdown of intestinal epithelium is a common effect of exposure to such diverse agents as pollutants, salinity fluctuations and malnutrition (Thompson, Ratcliffe & Bayne 1974; Pipe & Moore 1985a; 1986; Sunila 1986; Moore, Livingstone, Widdows, Lowe, & Pipe 1987; Lowe, 1988; Lowe & Clarke 1989; in Gosling 1992, p450).

Ivanovici & Wiebe (1981, p22) propose adenylate energy charge AEC as an indicator. They say that stress (strain) is: "a significant reduction of AEC which is induced by an environmental perturbation". Energy availability is also discussed by Hoffmann & Parsons (1989, p117) who assert that, "the availability of metabolic energy provides a general measure of the environmental stress that can be tolerated by organisms, leading to the hypothesis that increased tolerance to a range of environmental stresses will be associated with a reduction in metabolic rate in *Drosophila* and many other organisms".

Oxygen consumption is lower in *Mytilus edulis* when it is under stress (Gabbott & Bayne 1973). But Gosling (1992, p411) says that dibutyl-tin (DBT) may inhibit oxidative phosphorylation, thus reducing the respiration rate, and tributyl-tin (TBT) may uncouple oxidative phosphorylation thus increasing the respiration rate. Clearly, interpretation is required to tell what is beneficial or stressful.

Gosling (1992, p209) asserts that strain may also be indicated by reduced ultimate body size, increased intolerance to further environmental change and changes in age specific gametogenesis. The related phenomena of growth rate and scope for growth are commonly cited indicators. It must be noted that scope for growth is, however, considered less sensitive as a stress indicator for short term changes (Gilfillan, Page, Foster, Vallas, Gonzalez, Luckerman, Hotham, Pendegast, & Herberts 1984). Juvenile mussels may be particularly suitable for growth rate experiments. This is because their growth rate is higher than that of adults and it is not compromised by gametogenesis (Gosling 1992).

Gosling (1992, p412) considers energy balance as an indicator of stress: "Field and mesocosm studies have provided confirmation that the long-term consequences to growth and survival of individuals and the population can be predicted from measured effects on energy balance observed at the individual level".

Filtering efficiency and filtering volume may be affected. Gill cilia activity changes markedly when some extraneous chemicals are added to the water (Davenport & Fletcher 1978; Basha, Swami & Pushpanjali 1988). Gosling (1992) says that non-specific narcosis caused by hydrocarbons may affect ciliary feeding activity. In addition, neural control of the gill cilia may be disrupted by neurotoxic effects, e.g. TBT, dinoflagellate toxins and copper. The change may not always be a depression. This will be dealt with in a later part of this thesis.

Adductor muscle strength, valve sealing efficiency, shell gape width, frequency, and shutting in response to stress are all indicators worthy of consideration. Marshall & McQuaid (1993) consider that in mussels an abnormally wide shell valve gape more than 11mm and insensitivity to prodding internally indicates stress or death. To this may be added the failure of the mantle to respond. Similar criteria are used by Mathew & Menon (1992). Gosling (1992) indicates that, though valve closure looks promising, it has been found to be sensitive only at pollution levels near lethal. The magnitude of valve opening width is apparently also insensitive. So long as the mussel continues to gape, however, its heart rate may be simply ascertained by observation of pulses in the plicate membranes. This further investigated in Chapter 42.

Although flesh weight/shell length ratio and lipid content of flesh and flesh weight/shell volume ratios look promising, Gosling (1992, p403) cautions that "there is no tight coupling between shell growth and other growth components such as somatic or gonad growth". To this Gosling (1992) adds that the body condition index, such as flesh wet weight/shell volume and flesh wet weight/total wet weight ratio, is a poor or inconsistent indicator of pollution effects.

Increased leakage of ions and molecules across membranes may be expected to occur in stressed bivalves. Excess mucus production is caused by some agents such as phenol (pers. obs.). This may result in loss of proteins and/or mucopolysaccharides. The possible loss of carbohydrates across membranes should not be ignored. Ammonia and amino acid production and leakage may increase: "Significant amounts of alpha-amino-N are lost from the body at all times and especially during stress" (Bayne 1973, p39). In *Mytilus edulis* "the O:N ratio increases during stress" (Bayne 1973, p39). Calcium ion loss may occur: bivalve body fluids are saturated with calcium (Lauckner 1983). And calcium may be lost from valves during anaerobiosis under stress conditions (Akberali 1980). Assessment of shell area/weight and shell weight/length ratios or shell thickness may detect anaerobic shell thinning.

Moore (1991 in Gosling 1992) reports that in marine molluscs, lysosome membranes increase in permeability proportionally to the magnitude of the applied stress. Lysosomal responses change and lipofuscin granules may be deposited in digestive gland cells (Regoli 1992) in *Mytilus galloprovincialis* exposed to heavy metals.

Other sublethal stress responses to heavy metals in *Mytilus edulis* and *Mya arenaria* include the effect of Pb, Cr, Cu, Ag, Zn and Cd burdens on specific activities of aspartate amino transferase in protein metabolism, and glucose-6-phosphate dehydrogenase in carbohydrate metabolism (Page, Gilfillan, Hanson, Hotham & Foster 1984). According to Swartz (1987) some sublethal responses at biochemical or physiological level have no obvious ecological significance as they cannot easily be extrapolated to effects at population or community level. Nevertheless, sublethal stress indicators (especially behavioural changes), according to Swartz (1987), do have an advantage as indicators because they occur quicker than the results

of acute mortality tests.

Parasitised mussels may indicate strain in various ways. *Mytilus edulis*, exhibits increased gaping on emersion (Lauckner 1983). Other indicators include decreased efficiency of shell brushing by the foot (Lauckner 1983) and reduction of the host's resistance to freezing (Kanwisher 1955), salinity and temperature tolerance (Lauckner 1983). The trematode parasites appear less resistant to freezing and transfer this susceptibility to the host.

Gosling (1992, p455) has suggested: "antioxidant enzymes, free radical scavengers, end points of biological damage such as lipid peroxidation and genotoxicity" as less specific molecular stress indices. Though "the normal fate of molecular oxygen is (Gosling 1992, p443) tetravalent reduction to water, coupled to oxidative phosphorylation and the production of energy...small amounts are continually partially reduced by endogenous and xenobiotic-stimulated processes to reactive oxygen species, so-called oxyradicals" such as superoxide, hydrogen peroxide and the hydroxyl radical. "Oxyradicals (Gosling 1992, p444) are implicated in oxidative tissue damage and free radical pathology." Antioxidant defences include free radical scavengers and antioxidant enzymes. Once these defences are overwhelmed then one can expect the damage to become apparent. Small molecular weight free radical scavengers such as glutathionine, vitamins C, A and E and carotenoids such as beta carotene have been detected in mytilids (Gosling 1992). Exposure of mussels to various chemical stresses have resulted in fluctuations in the levels of these enzymes and free radical scavengers (Gosling, 1992) often in an upward direction as a response to the onslaught.

An interesting occurrence is the increase in total carotenoid content of *M. galloprovincialis* after exposure to mineral oil and hypoxia (Gosling 1992, reporting Karnaukhov, Milovidova & Kargopolova 1977). Is it the same as the yellow/orange colour associated with gasterostome infestations and the brownish coloration around some metacercarial cysts? Is it indicative of elevated levels of carotene? And if so, is it an antioxidant response to some effect of the parasite?

The rate of byssus thread secretion may be another indicator of strain. This is impaired by metacercarial infection (Lauckner 1983). It could be ascertained by measuring byssus weight

and strength after a standard growing time. The reproductive process is vulnerable to parasitic disruption; perhaps it is vulnerable also to other stresses. This would be measured by assessment of the gonad/somatic index and the gonad condition index. Or perhaps by assessment of sperm performance and mortality. Haemocyte counts and other histological change such as mitotic figures in molluscan blood cells (Cheng 1981) are also worthy of examination.

CHAPTER 28: STRESS/STRAIN RELATIONSHIPS

Figure 1, below, recapitulates the proposed relationship of stress, strain and fitness.

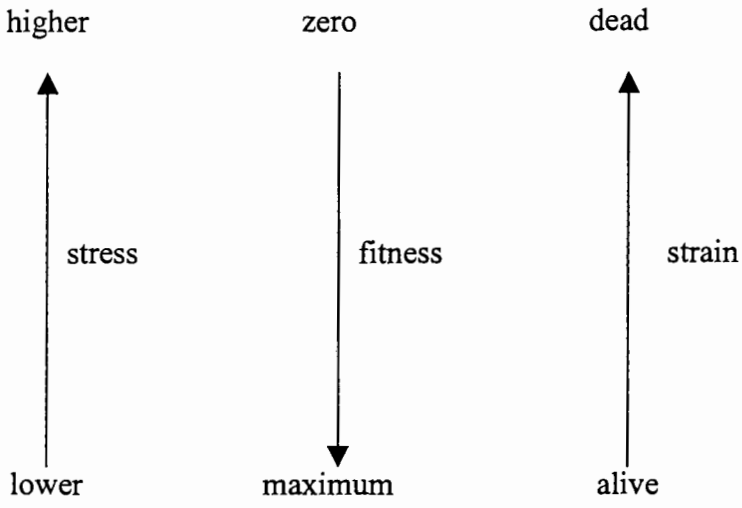


Figure 1. The relationship of scales of stress, fitness and strain.

The scale of fitness reaches from death (nil fitness or maximal strain) to a, so far, undefined point. As has been repeatedly urged, units of strain, as represented by performance fluctuations, must be defined for a particular organism at a particular time in its life cycle. The relationship of strain and fitness is tautological. The relationship of either of these to stress depends on the nature of the organism. An index of toughness or susceptibility may be constructed by relating the degree of stress required to elicit a given degree of strain. This may be comparative between individuals and species. Such an index would be constructed thus: if 8 units of stress cause 2 units of strain then the modulus is 8/2 = 4, which denotes a robust organism. If 2 units of stress cause 2 units of strain then the modulus is 2/2 = 1, which is not so robust.

When comparing organisms with overlapping environmental niches, a stress to strain relationship may indicate that one organism is more robust than another, i.e. the increase of strain for some organisms may be more per unit stress. "Quantifiable as the stress necessary to produce a specific strain" (Lincoln *et al.* 1985, p236) who also term the nature of this relationship of stress to strain as strain resistance.

One may assume that short term exposure is likely to be less stressful than long term. And therefore it should be possible to define stress in terms of units/time. In consequence consideration should be given to a scheme that integrates stress, strain and time.

As mentioned earlier, the stress/strain relationship may not be linear. If so, it is important to ascertain at which point on the curve the organism is observed. An example of non-linearity is given by Fry (1947) who proposed two zones of response to a stress: resistance [note that this is different from the meaning of resistance as posited by Levitt (1980) for plants], where there is negative scope for growth and the organism cannot survive indefinitely, and tolerance where there is positive scope for growth.

CHAPTER 29: SELECTION

Lincoln *et al.* (1985) define selection as non-random differentiated reproduction of different genotypes in a population. The selection coefficient (Lincoln *et al.* 1985) is a measure of the intensity of natural selection calculated as the proportional reduction in gametic contribution of one genotype compared with that of a standard genotype denoted by S. The coefficient may have any value from zero to one.

Selection appears to mean that "desired" organisms are selected to the detriment of others. This is not so. It is not that some are selected for, but that others have higher mortality. Surely elimination pressure is a more apt term than selection pressure? Mortality differential and consequent reproduction inequality is the active principle in selection. Selection selects against, not for. Although selection is selection out, it is easy to perceive it as selection in. Those that remain after selection have no traits that compromise fitness in the context of that selection criterion. It looks like design but it is the result of elimination not construction. Thus, selection is an opportunity to produce a viable organism. Those which did not take the opportunity did not survive. There is no pressure to do so. Selection is thus analogous to diffusion and not pumping.

Further to this, Grime (1989a, p6) refers to the organic evolution of earth as "emerging from a crucible of physical and chemical constraints..." Is it not more accurate to see opportunities rather than constraints? The significant organisms are those that could exploit the environment, not those that were constrained out of existence. We are, after all, studying the results of what happened - not what could not happen.

Comparative selection quotients would include the ratios of stress to mortality levels, strain quotient to individual numbers in next generation and stress level to numbers in the next generation. These could also be indices of strain or derived from it. It appears that causes of selection are also stresses. It is clearly profitable to clarify the relationship of selection to strain.

CHAPTER 30: RATIONALE FOR FURTHER WORK

Various indices of fitness/strain have been proposed: the task of making them operational will be left to a subsequent section. As Hull (1988, p278) says: "Theories that explain phenomena are necessary to raise them above the level of curiosities, but theories also need data if they are to be taken seriously".

The following basis for investigation is proposed: an explanation is sought that best covers the facts and is tolerably predictive. Any hypotheses proposed will be grounded in and in general agreement with previous empirical results. Hypotheses will be held corroborated if most of the evidence supports them, and if predictions based on them are largely accurate. If predictions are falsified it will be assumed that this is the product of unknown interactions. This is admittedly "ad hockery" but it can, it is believed, be justified if hypotheses are erected and tested to ascertain the nature of these interactions. Thus, any exceptions will be used as a base for further examination of the phenomena.

Where possible, corroboration should be sought by pursuing logical consequences of the hypothesis and seeing if these also square with observations. This has the weakness that it can lead to multiplication of excessively specific and decreasingly credible supporting hypotheses. But this weakness can be turned to advantage when it is realised that untenable positions are the more obvious when opposing theories are made to compete. The ideal in this regard would be to seek confrontation between theoretical models as in multicornered testing (Anderson 1987). Selection between them will be decided by Ockham's Razor (Lacey 1986) viz. "entities are not to be multiplied beyond necessity". Selye (1955) puts this into the stress context neatly when he says that: "the best theory is that which necessitates the minimum number of assumptions to unite the maximum number of facts." In the next section the above ideas will be examined to see if they may be empirically grounded by testable hypotheses.

PART V:

SUMMARY OF STRESS SURVEY: CONCLUSIONS, CONSEQUENCES AND HYPOTHESES

The following Chapters (31 to 40) summarise salient conclusions from the examination of stress phenomena in the last (14 to 30) chapters. For brevity, references are here kept to the minimum. Particularly important original derivations and corollaries are rendered in bold. Conclusions reached in the review are used as starting premises for a further examination of stress phenomena. The premises are developed and some of the consequences are framed as testable hypotheses or suggestions for further research. An attempt is also made to sharpen concepts and suggest units for some of them. Some ideas explored here are investigated experimentally in Chapters 41, 42, 43, 44, 45, 46 & 47.

CHAPTER 31: THE NATURE OF THE ORGANISM

Organisms are dynamic, changing systems. These changes may be interpretable as fluctuations in fitness; or they may interfere with indicators that we associate with fitness. Fisher's comment about biological rhythms that are likely to "provide a fluctuating base for incoming influences", is particularly relevant. Thus to detect and interpret stress phenomena correctly we must be clear about what constitutes normal fluctuations and we must ascertain if the datum around which they fluctuate is indeed fixed. For instance, homeostatic capacities in some mussels have been shown to fluctuate seasonally, and mammalian adrenal cortex activity is cyclic. Thus, assumed indicators of stress may not be so easily interpreted. Clearly, the response of the organism is dependent on its stage in the cycle or fluctuation. It follows that this stage must be identifiable. Similar changes are possible during ecological successions. These make it more difficult to talk of return to normal levels after application of a stress. We must, therefore, know more about the organism we are studying or at least be explicit about our assumptions of its homeostatic competence.

THE NATURE OF STRESS

Stress implies deleteriousness; it therefore poses a threat to life. In consequence, the biological significance of being alive has been examined. Among the key characteristics of 'aliveness', structure, function and process have been appraised. Structure is defined here as the structure an organ is, not the structure it has. Function implies goal-oriented action. In consequence, it is replaced here by 'process', which has fewer teleological connotations. A process is just a series of actions or events; the series may be causally related but this does not imply purpose: some processes enhance continued existence in organisms and some compromise it.

Function is a problem because it tends to denote only those processes that enhance the organism's ability to continue existence. Other processes are often called malfunctions. This makes 'function' an ontologically loaded term. Biological thinking is, of necessity, existocentric; but one can detect intrusion of normative as well as descriptive assessments of organisms: the less fit are not as 'good' as the more fit. Good or otherwise is not at issue; we are interested only in the persistence of organisms. If use of the word 'function' is to continue

its meaning must be made explicit.

TELEOLOGY

Function connotes teleology, which has had an uncomfortable relationship with biology and an unfortunate relationship with stress. Although it has great heuristic value, it is a mistake to take teleology seriously as an organising and driving force in biology. It has been shown (Chapter 23) that teleology belongs only in psychological stress. To postulate teleology in stress studies, other than in psychology, is mistaken. Such an approach that refers to the future to explain the present may be tackling the problem from the wrong end. Nevertheless, teleology has its proponents. The noted stress researcher Selye asserts that sensations of causality and purpose are inherent in the structure of the human brain. Even if he is correct, we would be rash to infer the nature of biology merely from the structure of the human brain - it may not tell the whole story. Moreover, biology and biological thinking are not the same thing. On top of this, Selye's coupling of causality and teleology need not be so rigid. We can discard a teleological approach to science - it has already been ejected from the physical sciences - but it would be difficult to discard causation entirely. Causation is essential currency of the mind - counterfeit currency perhaps, but until we get a better model of reality we cannot do without it.

Teleological explanation commonly posits more than a simple descriptive-inductive explanation: it requires an understanding and identification of motive, but this may not be attainable. Even after much investigation, human motives can be impossible to divine. What chance, then, is there of accounting for more distantly related phyla? It is conceded, however, that teleological explanations are more acceptable if their ontology is elucidated. This is because apparently goal-directed phenomena can arise by their being part of the process that contributes to persistence of the organism.

Nevertheless, a consciously non-teleological approach is advocated in this study of stress. One reason for this is that previous over-employment of teleology as a heuristic tool is responsible for at least some of the current confusion in the field.

ORGANISM VERSUS MECHANISM

Although an organismic rather than mechanistic view of living things is advocated, such an approach should be pragmatic. In consequence, mechanistic explanations, despite their deficiencies, have a place in the organismic approach advocated here. This is because analysis of components based on assumptions of mechanism is not, and cannot be, excluded from biological study and explanation. Such assumptions, however, should be seen in an organismic context.

ONTOLOGICAL LEVELS IN THE ORGANISM

Structure is the primary identity of the organism. It is considered here as more basic than process. An ontological hierarchy beginning with matter and form together create structure. Structure and movement give rise to processes. Processes, being only contingent on structure, are subject to an extra level of uncertainty than are structures. Thus, what an organism is, is more concrete than what it does. Therefore, a definition of the organism by its structure rests more securely on fewer levels of inference. Based on these inferences, life may be provisionally seen as the continuity of complex structure-sustaining chemical reactions in a structured body. However, structure leads to processes and these interact with structure in a structure/process complex. Structure is mutable by process and vice versa. Thus it is more accurate to consider the organism as a structure/process co-ordination whose primary qualities are that it exists and that it has some likelihood of continued existence.

ASEITY

Two temporal aspects of stress require consideration: its instantaneous effect on the processes of life and a historical effect by becoming part of the new co-ordination of the organism. It is clear that we cannot consider stress until we assess the fitness of the organism and for that we must assess its environment. **Thus we should now consider the organism as a structure/process/environment complex, an entity with numerous degrees of freedom and emergent properties. These properties when manifest are here termed its aseity.** It is characteristic of an organism in any given conditions and is mutable by different conditions. Such changes may precipitate different outcomes, some of which would compromise survival. Aseity is thus a convenient label for the 'nature of the structure/process/environment complex'. It is proposed that operational meanings of strain

and fitness may be found in the interactions within this complex. **Stress is the agent that gives rise to a less persistent aseity.** Aseity embraces the phenomena of fluctuating asymmetry and phenotypic plasticity. The former is a degradation of the organism with no implied fitness conservation outcome. The latter implies that fitness is conserved to some degree by the changed condition of the organism.

Stress disorders an aseity optimal for persistence of the organism. Disorder can be absolute or it can be contextual. It can be entropic, absolute disorder, which entails degradation towards randomness. Alternatively, disorder can be relative. A parasite living within an organism is, in absolute terms, highly ordered, but in terms of the host it is disorder.

Interpretation of stress indicators

Single processes are often used as stress indices but they may not be representative of the whole organism. This is not only because of fluctuations in homeostasis baselines, but also because changes of process require extra stages of inference before one can arrive at an assessment of deleteriousness. For instance, ammonia at some concentrations increases mytilid gill cilia current speed but this is not a beneficial change. Misinterpretations such as this may be forestalled by seeing stress as causing a loss of co-ordination rather than a diminution of simple performance. Thus the benefit of "positive" stress indications should be held suspect and investigated. Perhaps there is insufficient level of integration of the process being monitored. Stress, if it is to be a useful concept, must at some point be deleterious. If stress is still held to be correctly interpreted as causing a beneficial change in co-ordination, then we should examine the temporal regime (see Chapter 16).

It follows that the best way to assess a loss of co-ordination is to examine a complex process in the organism rather than a single simple process, e.g. organ and system processes require more co-ordination than organelle or cellular processes. It follows that whole body integrations offer the prospect of more informative indicators. Evidence for is examined in Chapter 48.

SUGGESTIONS FOR EXPERIMENTAL WORK

A selection of naturally fluctuating processes could be mapped throughout the life of the organism (or over any other defined period). Thus the influence of these fluctuations on the response to incoming stress can then be better elucidated. Because it may not be linear, the entire stress (as cause)/strain (as effect) relationship should be charted at numerous points during this period. Any effects should be seen merely as indicators of fitness until they have been calibrated as fitness parameters. Once this has been done one may be able to estimate fitness by integration of relevant measurable factors. These factors are posited as the strain/fitness counterparts of the stress subdivisions that have previously been presented (See also Chapter 39). The following are suggested as guidelines:

1. Correlate the magnitude of a single response to change in fitness/strain. By this is meant relating the degree of response, such as change of process rate, to change of fitness/strain. Ultimate fitness/strain values can be ascertained by mortality experiments.
2. Integrate a number of responses (or fitness/strain parameters) to see if this is a more accurate indicator of fitness/strain than just one parameter.
3. Ascertain if different fitness/strain indicators give similar results from the same stress.

CHAPTER 32: STRESS AND TIME SCALES

PROXIMAL AND DISTAL STRESS

In an attempt to apprehend temporal aspects of stress, an adaptation of Calow's scheme of temporal stress is proposed. It has been adapted to individuals instead of lineages. In individuals it is proposed that these responses be termed proximal instead of proximate and distal instead of ultimate: ultimate effects in individuals imply death. Any less-than-ultimate appraisals are arbitrary, with attendant ambiguity, unless temporal parameters are explicitly defined. Thus, though biological activities, and inactivities, may be stressful it is only through summing the deleterious effects of each event, action or process over a specified period that cost or benefit is ascertained. **Assessments of stress phenomena are meaningless, unless the temporal regime is made explicit.**

POSITIVE FEEDBACK, CHAOS AND NON-LINEARITY

Feedback

In addition to the examples of deleterious positive feedback in individuals (physiological) and populations (behavioural) given in Chapter 16, it is also manifest in psychological stress: behaviour is influenced by the chemistry and morphology of the brain; and the chemistry and morphology of the brain are influenced by behaviour. This circularity opens the door for feedback.

Chaos

Smaller hearts (Chapter 16) have less need of complex conduction fibre pathways to prevent breakdown of the synchronised systole to chaotic fibrillation. The tendency for stability to be size dependent is also found in organisms: small species are common in stressed environments. It appears that size and complexity are advantageous only when the advantage of the size outweighs the disadvantage of instability. **Increased complexity implies increased potential for entropic loss of energy and order. This can be tested by seeking new mechanisms for stability in more complicated organisms**; as are found in the conduction apparatus of larger mammalian hearts compared with smaller amphibian hearts.

Non-linear dynamics of dying

It is postulated that negative feedback and associated damping dynamics are diagnostic of healthy organisms and that stressed organisms are likely to show a degradation of damping and an enhancement of runaway positive feedback dynamics. "Damping" indicates the tendency for the rate of deviation from equilibrium to slow in proportion to the distance from the equilibrium. "Runaway" indicates that the rate of change of the process increases proportionally with the distance from the equilibrium. Damping dynamics delay deviation from the equilibrium; runaway dynamics accelerate it.

Towards the lethal threshold it is postulated that stress sets in motion a series of events, each of which exacerbates the others, resulting in acceleration away from the equilibrium that characterises life. **Thus, approach to the lethal condition is by way of positive feedback dynamics.** Death is therefore not a gentle drifting away but a confluence of mutually promoting events, which lead to further deviation from the living equilibrium condition. As the organism departs the living equilibrium it is, of course, approaching a new equilibrium.

SUGGESTIONS FOR INVESTIGATION

Proximal and distal effects

The apparent stimulus of an agent may be evaluated by observation of its action over a longer period and comparing it with a control. One can then apply the concept of proximal and distal stress. In mussels, ammonia appears to stimulate gill cilia activity (See Chapter 41). One can examine proximal and distal effects by using selected fitness indicators over an extended period to see if ammonia confers an unmixed fitness benefit to the mussel. The following hypotheses could be tested:

1. **Proximal and distal fitness changes will be smaller as one approaches whole body integrations**, i.e. single process rates of isolated organs are likely to be less informative than responses by the intact organism. This is examined in Chapter 48.
2. The sum of fitness over any time is always highest for organisms at optimal conditions and lower for organisms at other conditions even if they are stimulated proximally.

By using agents such as ammonia and temperature, changes in mussels can be measured by assessing process rates such as cilia activity, byssus production and gaping frequency. The following results (Figure 1) might be expected:

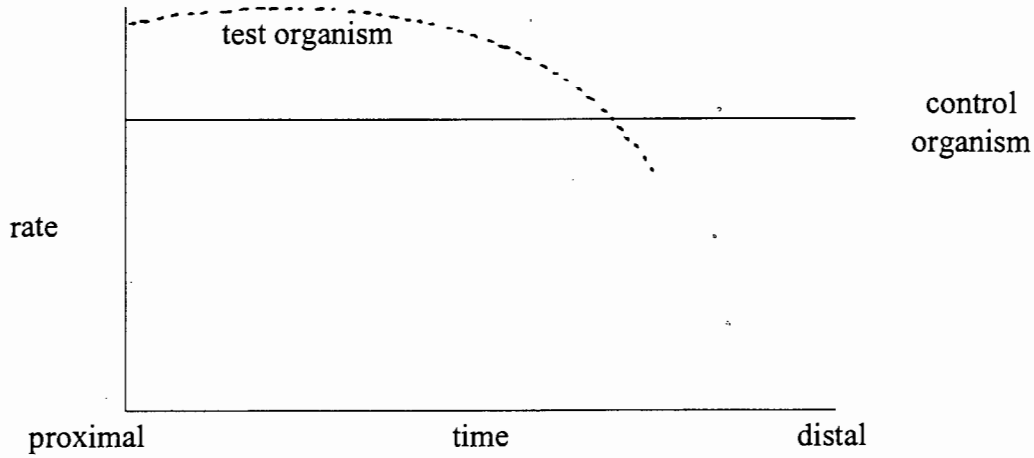


Figure 1. Comparison of a fitness/performance parameter in a healthy organism and one subject to a stress over time showing the expected difference.

Thus though the test organism has an elevated proximal response, its distal response is lower than that of the control organism.

Feedback, chaos and non-linearity

If positive feedback plays a large role in stress phenomena, then we can expect to see it manifest in processes that lead towards death. Once homeostasis is overcome, the final process towards death should be an accelerating loss of order. This is examined in Chapter 16 & 44. It must be emphasised that it is the rate of change that is important rather than the absolute value. Process rates may increase or decrease and either may be lethal ultimately, but it is the dynamic of the change that indicates whether the change is under control or not (compare the dynamics in Figures 2 & 3).

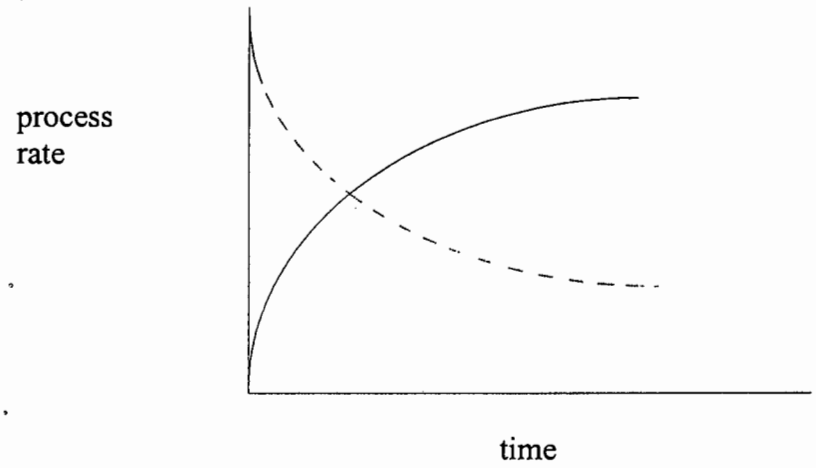


Figure 2. If a perturbation occurs, homeostasis will slow down the rate of change before reversing the perturbation. This slowing down is represented by the curve becoming horizontal.

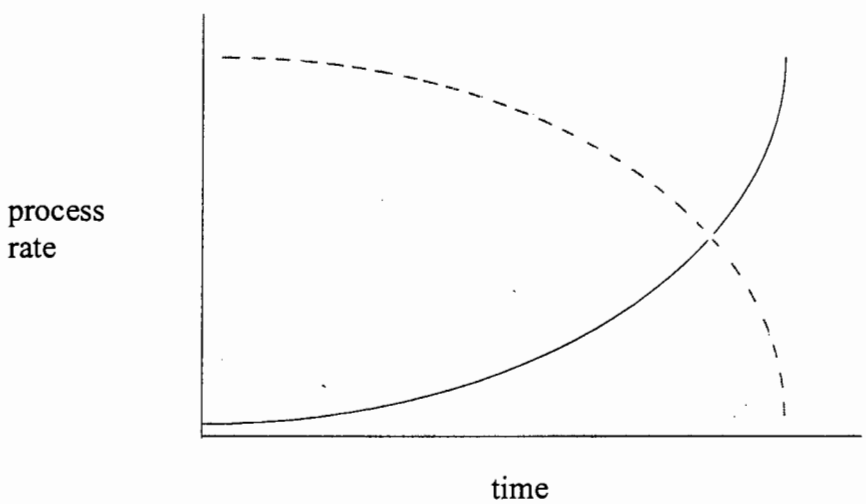


Figure 3. If homeostasis is overcome and positive feedback takes over, then the process rate will deviate from equilibrium at ever increasing speed. The curve will become vertical.

A further suggestion for investigation

Homeostatic competence at different levels of fitness (for instance during starvation) could be measured. At high levels of fitness one would expect prompt recovery from a perturbation (such as shock of salinity change followed by a return to normal salinity). At lower levels of fitness one would expect to see less vigorous recovery (or more mortality in a population fitness assessment). As fitness declines one might also expect a smaller perturbation to require longer to stabilise. Smaller and smaller fluctuations in salinity should cause increasing mortality as starvation progresses.

CHAPTER 33: DISORDER AND ENERGY FLOW

MEASUREMENT OF ORDER AND DISORDER

Order as structure

An operational estimate of order may lie in the following:

1. biomass of the organism
2. calorific value of the organism
3. calorific value per unit mass of organism
4. estimation of the number of cells in the organism
5. estimation of the number of cells per unit mass of the organism
6. number of molecular species at a given pH by electrophoresis

These can be estimated before and after imposition of the stress or between control and experimental subjects.

Order in gradients

It was concluded that the 'aliveness' of an organism could be assessed by the quality of the gradients of energy and energy containing compounds and structural order it maintains. This may be supplemented by assessment of the direction and quantity of its energy and matter flow. Energy gradients may be ascertained as the ratio of total energy density inside the organism to total energy density outside it. Units such as kj/cm^3 could be used. Order/disorder values must be calibrated against manifestations of fitness quality in the test organisms. This is further pursued in Chapter 40.

ENERGY FLOW IN THE ORGANISM

Dam stress

If the organism is considered as a locus of energy flow, then stresses may be seen as dams or diversions. Energy for maintenance or growth is diverted or its flow is obstructed. The vehicle for this energy flow is organised matter. This model of energy flow should also, perhaps, consider mass flow through the organism. Fitness parameters could then include biomass turnover and consideration of its importance as structure and not just as an energy store. More biomass and biomass turnover, all other things being equal, suggests higher fitness.

A dam stress prevents energy from getting into (or flowing through) the organism. This in mussels might be because of valve closure caused by salinity or other water-borne stresses. It could also be caused by damage to the feeding apparatus. Such a stress would be defined in terms of input energy deficit compared with a normal organism in a normal environment.

Diversion stress

A diversion stress is a loss of energy. It may be lost in mucus production, seepage of energy rich molecules across membranes, faeces, higher metabolic rate, higher food/calorific value of the pseudofaeces, and gamete production (viable and non-viable). Parasite metabolic rate, growth and offspring production are also diversion stresses. A diversion stress can be expressed as a quotient of energy wasted to energy used. If it is assumed that the parasite confers no fitness advantage then the mass of the parasite divided by the mass of the host gives an indication of biomass misapportionment. If we assume that the parasite and host have the same tissue calorific value then the portion taken by the parasite is part of the energy diversion.

CHAPTER 34: STRESS INTERACTIONS: ADDITIVE AND OTHERWISE

USE OF THE FIGURE OF TOLERANCE TO CHART INTERACTIONS OF STRESSES

A three dimensional figure could be constructed on a variety of stress axes. This figure would characterise the interaction of these stresses in any particular organism. The figure might also have a biologically significant volume.

Figure volume: degree of constancy

The figure volume is defined in cubic strain/fitness units. Units of the different stresses applied to the organism under test can be converted to equivalent strain/fitness units. Thus all stresses are comparable in strain/fitness units.

One- and two-way stresses

The concepts of one and two-way stress were proposed in Chapter 17. These can be used to help construct a figure of tolerance outside of whose surface represents the lethal limits of stress to the organism. This will provide a descriptive supplement to the definition of the organism. This scheme adapts Alderdice's (1965) figure, and adds to it the notion of a constant volume and also of partition coefficients within the volume. By positing a constant but flexible figure volume, this model matches the evidence that an increase in one environmental extreme can reduce tolerance to others. Parasites, poisons, inclement environmental conditions and other stresses may reduce tolerance to other extremes. It is also possible to construct similar three-dimensional representations of the ranges of parameters encountered in an environment. By so doing it may also be possible to give the environment an abstract fitness value.

HYPOTHESES AND SUGGESTIONS FOR FURTHER INVESTIGATION

Additive interactions

Additive interactions may occur when different agents affect the same site or process in the organism. These interactions may also occur when different processes are affected but neither confers instability on the other. A possible interaction in Man would be that between carbon monoxide and sodium nitrite. They convert haemoglobin to

carboxyhaemoglobin and methemoglobin respectively. Both represent a loss of oxygen carrying capacity but through different causes at the same metabolic level. They should exhibit additive interaction. As should EDTA, oxalate and citrate since in mussels they would all remove calcium ions.

More than additive interactions

More than additive interactions may occur when the combination of the two stresses occurs outside the organism, such as pH, ammonia and temperature. If the interaction occurs inside the organism, then it suggests a multiplier effect at two different homeostatic levels. **The effect of one disrupts the homeostatic competence of the next process, which is thus disproportionately affected.** For example, ouabain is a sodium-pump poison: sodium will not be pumped out of the axon. If the axon is then poisoned with condylactis toxin, this allows the sodium channels to open but delays closure - sodium will flood into the nerve. The deleterious action of these two may be more than additive.

Less than additive interactions

In less than additive interactions one agent may hit a damaged process again but at a different site, thus no extra instability is incurred. One might expect a less than additive interaction between hydrogen cyanide (a cytochrome poison) and carbon monoxide (a haemoglobin poison). Both compounds block normal transfer of oxygen. A blockage at any one part should be sufficient to halt aerobic metabolism. Similarly, cyanide and 2-4 dinitrophenol affect different links in the same chain. If these are the only toxic effects of these agents, then one would expect there to be a less than additive effect. EDTA may harmfully chelate trace elements as it chelates calcium ions. But it may also protect the organism against toxic effect of some heavy metals. In the presence of toxic heavy metal ions the deleterious effect of EDTA may be less than additive.

CHAPTER 35: DEFINITION OF STRESS

Stress in a biological sense should be treated as a term different from the stress of physics. Biological stress must entail some form of harm to the biological entity, which in turn means a reduction in its fitness. Any notion that stress can be harmful without reducing fitness is either an absurdity or it shows that 'fitness' needs clarification. Fitness is an ontological term; it speaks of the state of existence of the organism. **Thus stress is a label for agents that compromise the continued existence of the organism. This reduction in fitness is here termed strain. Stress thus causes strain and strain is posited to vary inversely with fitness.**

A stress might reduce bodily fitness and thus be a somatic stress. Or a stress might impede reproduction and thus be a reproductive stress. The physical nature of the stress would include such properties as chemical composition, temperature, light intensity, mass and concentration. These are properties intrinsic to the stress entity (even though they are stresses only in the context of the affected organism) and exist independently of the organism. In contrast, other stresses cannot be said to exist independently of the organism. These include its point of application and origin. In origin, the stress may be endogenous or exogenous to the organism. Stresses may also be classified by their dose/response relationships, such as the one- and two-way subdivisions outlined previously.

A stress unit must integrate three aspects of the stress: quality, quantity and exposure regime. The first is the identity and nature of the agent, the second is the amount, intensity or concentration of the agent, and third is the length of exposure or number of exposures of the agent. **A typical stress unit must embrace:**

Stress unit = Agent X, of quantity Q, per time T.
This is the independent operational definition of a stress unit.

CHAPTER 36: SELYE, BROOM & JOHNSON AND BARNARD & HURST

SELYE'S STRESS

The centrepiece of Selye's scheme is its claimed universality. He says that stress responses are similar because there are common pathways that must mediate any attempt to adapt to environmental conditions and sustain life. It is contended here that there are no dedicated stress pathways. Why postulate common pathways when organisms have nearly identical biochemistry? In this respect, living things vary from the similar to the almost identical. **In all organisms, the universal effect of stress is disorder.** If similar systems are disordered, why should it be surprising to see similar results, especially in the near equilibrium (homeostatic) region? Moreover, his scheme is applicable only to those organisms possessing an approximation of a mammalian neuroendocrine configuration.

Universality is invoked again: good or bad, pleasant or unpleasant, are all the same says Selye:"from the point of view of its stress producing or stressor activity, it is immaterial whether the agent or situation we face is pleasant or unpleasant". There is, he says, a stereotyped physical pattern of the body's response to stress of any cause. But he contradicts himself when he says "the outcome of our interactions with the environment depends just as much upon our reactions to the stressor as upon the nature of the stressor itself". All this tells us is that the body responds in a stereotyped way except when it does not.

Selye is, unfortunately, not sparing with his terminology. He uses the terms, 'actions', 'responses', 'changes' and 'reactions' without clear distinction. He talks about specific and non-specific responses but he is less than explicit about specific responses. Non-specifically caused changes, he says, are manifest in the general adaptation syndrome (GAS). Selye, however, says that the GAS is highly specific. He must mean in form. If this is what he does mean then it is not truly general but more of a disseminated local adaptation syndrome (LAS).

Selye's primary and secondary changes, he admits, are often impossible to distinguish in practice. He also suggests that specific and non-specifically caused changes are on a continuum, but this weakens their utility. Positing the existence of a response - especially

one that is universal - raises difficulties, which are only partially offset by referring to direct and indirect pathogens. In reality, if an organism is crushed, chopped into pieces or incinerated, it may be dead before any of its stress response repertoire is mobilised.

Selye says that during the alarm phase if the stress is sufficiently strong then death may result. Then only one phase of his universal three phase GAS is exhibited. A further problem is that, according to Selye, the organism need not go through all the stages. It may, instead, progress through the first two, many times in its life. These examples erode assertions of universality of the triphasic GAS. It means that most of the time the theoretical triphasic GAS does not occur. Perhaps it would be better to say that stress causes disorder and a response to the disorder is the GAS - if the organism lives long enough. Clearly, disorder is the result of stress; and the GAS, except for the end point, is a proximal stress response. Any part of it must be put into a temporal context.

The GAS fails to predict the response of pre-stressed rats to cold. The alarm reaction caused by a cold water swim in previously stressed (by other stresses) rats, shows that the rats have no general resistance. But this resistance should be there according to Selye's model. It appears that the rats can be in a phase of resistance for one stress but are still subject to alarm from another. This casts suspicion on this model of GAS. Alternatively, it suggests that more than one GAS may run in the same organism. Either way the GAS becomes less general and more specific. This questions its universality - even in higher vertebrates.

Some stresses may not fit Selye's triphasic model. For instance, Selye himself mentions a self-sustaining condition of hypertension that leads to death. The effect of asbestos or other carcinogenic poisoning also raises problems. Can one fit a triphasic curve to the effect of a self-manufactured stress agent such as cholesterol? Where are the alarm and resistance phases? These difficulties could be bypassed if we look beyond a theoretical triphasic stress response and instead look at proximal and distal effects.

Selye's non-caloric adaptation energy is difficult to measure. What passes for loss of adaptation energy is perhaps better seen as loss of order in structures and processes involved in the reorganisation and repair of the organism. Another of his concepts, that of reactons,

appears too diffuse to have any operational meaning. Moreover, its reductionist approach may overlook the fact that the nature of organisms is not merely the result of a simple agglomeration of subordinate events.

Selye proposes eustress as beneficial stress but eustress as a category breaks down under examination. It is clearly better to deal only with different levels of deleteriousness and integrate them into a cost/benefit analysis of defined temporal regime. Any stress such as eustress may be the lesser of evils but that does not make it an advantage.

Selye erects specific and non-specific stress responses, General Adaptation Syndrome and Local Adaptation Syndrome, syntoxic and catatoxic responses direct and indirect pathogens, primary and secondary changes, and superficial and deep adaptation energy. These are all to explain why his asserted universal response is not so universal. Selye's work has shed much light on stress phenomena to the benefit of our understanding, it is suggested, however, that he invented too many subsidiary concepts in attempts to maintain the viability of his construct.

BROOM & JOHNSON'S STRESS

Broom & Johnson critically review Selye's stress and propose some new concepts. Their critique is appropriate but the new concepts are of questionable utility. Also questionable is their interpretation of some old concepts. For instance, they define stress as "an environmental effect on an individual which overtaxes its control systems and reduces its fitness or appears likely to do so". Does not overtaxing imply loss of fitness? Is there an example of an organism with overtaxed control systems not being in a state of degraded fitness?

They say that, "there will normally be a reaction on the part of the individual to such an effect". This is a response to stress but the consequence of stress is strain. So we can infer that stress causes strain. But their definition of strain as "the short term consequences of stress" leaves us none the wiser. They say that brief or minor events such as transient heating and minor injury are unlikely to reduce fitness. Thus they would not be called stress. On the other hand, they say that prolonged but minor events might be called stresses if they

reduced fitness or appeared likely to do so. "Sufficiently innocuous" events, they say, would not constitute stress. Is this not necessarily implicit in the word "sufficiently"?

Broom & Johnson reject the notion that stress refers to just any displacement from the optimum. However, it has been shown that all perturbations impose a load on the organism. Clearly, even in the zone of tolerance, there are more and less favourable locations. It follows that an intensity of stress just below the limit of adaptation is more disadvantageous than one much lower. We must thus be sceptical of their claim that, "A distinction is therefore made between minor disturbances to an animal's equilibrium, which may result in the use of energy to correct them but have no consequences for fitness, and those disturbances which do, or are likely to, reduce fitness". It has been demonstrated that division of stresses into those that may be overcome entirely by increased energy and those that cannot, is a continuum not a dichotomy. Energy loss is also a loss of fitness.

Welfare

Broom and Johnson's concept of welfare requires clarification. Welfare, is "the state of the individual as regards its attempts to cope with its environment". They refer to a continuum of welfare states. But the examples they give are all on a continuum of decreasing fitness. Their examples most likely to approach pure welfare may be subsumed under behaviour and even these can be deleterious to fitness.

Broom & Johnson mention pain as an example of poor welfare without degraded biological fitness. But pain causes physical and mental changes, which do result in deleterious outcomes. Pain elicits the stress syndrome and in this connection Broom & Johnson (oblivious that this statement weakens the last) assert that pain may elevate cortisol levels in the plasma. This is a potentially damaging response. Pain is thus linked to loss of fitness as well as to welfare. Furthermore, one can say that pain is, normally, indicative of damage or loss of serviceability of an organ, which clearly affects fitness. Pain is discussed further under suffering below.

If welfare is to be taken seriously we must have some operational parameters, but they are unavailable. Broom & Johnson's claim that degraded welfare does not of necessity indicate

loss of fitness is not substantiated by the evidence. Loss of welfare is also loss of fitness. Why then have two words for one concept?

Perhaps welfare could be used more profitably to indicate the direction of change of fitness of the organism. Alternatively, welfare quality may be understood as the relationship of observed somatic fitness and expected somatic fitness at some specified time in the life cycle. The units would be in percentage above or below expected somatic fitness. In the control (i.e. the expected fitness) organism welfare would be constant throughout life regardless of absolute fitness values.

Coping

Broom & Johnson's 'coping' is another fitness parameter. They say that when an animal is having difficulty in coping or is failing to cope its welfare is poor. And we have already shown that poor welfare implies compromised fitness. Therefore even coping has fitness costs. **It is proposed that coping may be more profitably used to describe the nature of the curve (rate of change and direction) of the stress/strain relationship.** Coping would either be positive or negative. When fitness is improving (strain is decreasing), coping is positive. When fitness is getting worse coping is negative. Coping would thus be an absolute parameter and it would change with time. An organism that is dying of old age is failing to cope but its welfare may be unaffected if it is dying on schedule.

Suffering

Suffering also implies loss of fitness. By implication, a suffering animal must be stressed. If its response is appropriate, it is responding to a threat to its fitness; i.e. it is stressed. If its response is inappropriate, then it is failing to distinguish what is harmful from what is innocuous. This also has fitness implications.

Suffering leads to coping efforts, the outcome of which feeds back and influences suffering. These efforts may succeed or fail but in either event, energy is expended in coping efforts. Thus, there will be loss of fitness as measured by energy deficit. Such loss of fitness may be ascertained by analysis of the cost/benefit relationship of energy expenditure and coping outcome.

In an attempt to use the word suffering scientifically Broom & Johnson are, perhaps, expecting too much from an everyday word of vague connotation. Perhaps it would be better to use the term loosely with its limitations in mind and abandon attempts to define it. It currently stands as being a useful, if woolly, area of subjective experience in the stress/fitness relationship. **However, if we are to use the word scientifically, it is suggested that suffering be defined as any subjective mental state made manifest by behaviour disturbance which is strong enough to interfere with appropriate attention to fitness sustaining processes.**

Suggestions for investigation

Suffering could be ascertained experimentally by examination of the following:

1. Avoidance, withdrawal or defensive posture instead of normal (control) activity. The units would be percentage of time in avoidance activity compared with the control.
2. Lowered speed of defensive response compared with control. Units would be percentage of normal speed.
3. Degraded accuracy of orientation response compared with control.

BARNARD & HURST'S ADAPTIVE EXPENDABILITY

Barnard & Hurst say that each organism must be considered in terms of its environment of evolutionary adaptation. Suffering, they say, has the utility of helping organisms to avoid circumstances in which they cannot realise their reproductive potential. Suffering is minimised and welfare maximised if the organism can spend itself in successful pursuit (adaptive expendability). This is good for the lineage but it does not attest to any natural mechanisms that would minimise suffering - Darwinian notions of overproduction and competition guarantee suffering. Neither does adaptive expendability deal with the suffering experienced by biologically irrelevant organisms such as those undergoing post-reproductive senescence.

Their concept of adaptive expendability is valuable in assessing the welfare of organisms but only if we know the decision rules of their adaptive expendability strategy. Thus they say, "Good welfare management policies should therefore strive to maintain natural or

acclimatised strategies of self expenditure". When one considers the factors to which agricultural animals might be subject, such as constraint, crowding, abolition of reproduction; and change of diet, one wonders just how much reprioritizing an animal can do so as to acclimate to a reasonable state of welfare.

It is urged here that the tension between existocentric and hedonistic poles of motivation should be investigated before a suitable model of welfare can be constructed.

CHAPTER 37: ECOLOGICAL STRESS

Stress operating at different ecological levels such as ecosystems, communities and individuals may not be comparable. Organisms and higher assemblages may be comparable only in as much as they may both be subject to the effects of deleterious agents. It can be accepted that stabilising forces operate within an ecosystem but these are probably mere analogues of those operating to maintain stability in organisms. Until the following questions can be answered, stress phenomena at different levels should remain conceptually separate.

1. How did these ecological stabilising forces come about and how can they be selected for?
2. What is the unit of inheritance in an ecosystem?
3. How accurate and precise is the ecological stabilising mechanism? Is it comparable to homeostasis in individuals?
4. Does an ecosystem die in the same way that an organism dies?
5. Is there any ecological analogy with senility?
6. What is a generation in an ecosystem?

These questions point to the great differences between individuals and ecological assemblages. Answers to them are likely to further separate concepts treating ecological and individual stress phenomena. Notions (although popular in the literature) that stress can be used at different levels without any special caution, can be refuted by asking: can an agent be a stress at one level and not at another? The following facts settle the issue. Some hydrocarbons are a stress to individuals but not populations. For lower levels of communities, ecological succession is itself a stress. Grazing arrests succession as it maintains the grassland; in addition, grazing damages individual plants. If succession is arrested, then we must also have a condition of ecosystem stress. So an agent can be a stress at one ecological level and not at another. Stresses at different ecological levels are thus unlikely to be homologous.

This discussion encroaches onto the stress/eustress dichotomy. Eustress is also redundant in ecology. If the succession community is seen as a stress to the incumbent, then removal of

the succession community is not eustress but removal of a pre-existing stress. The stress/eustress argument is based on misconception of the phenomena as a false beneficial/harmful dichotomy when it is really a dichotomy of harmful and less harmful. Differential mortality is the mechanism of succession. It is, therefore, obvious that we must be explicit about temporal regime, location and identity of subject before we can discuss ecological stress with any meaning.

Stress currently embraces a series of ecological level-dependent local concepts. Accurate and meaningful use of the term stress requires that its hierarchical level of action be explicitly stated and notions of deleteriousness at each level must be defined with rigour.

CHAPTER 38: PSYCHOLOGICAL STRESS

If there is a place for teleology in stress studies, it is in psychological stress only. Psychological stress is an organism-level phenomenon; it disorders mental processes such that individual fitness is compromised. Values are allotted to entities that are not appropriate to their potential for influencing the fitness of the appraiser. This opens the possibility of integrating it with physiological stress. **If an index of psychological stress can be related to fitness, then all stress phenomena of individual organisms may, in principle, be integrated.**

Psychological stress may have a bipolar effect. At one extreme it could increase the probability of making a fatal or injurious error but without slowing the decision making process. The other extreme is that it could reduce speed of mental processing performance without affecting accuracy of processing. This could have no effect on survival unless the need to make decisions exceeds the processing speed of the stressed brain. In reality, the effect of stress may impact on both of these probabilities.

SUGGESTIONS FOR INVESTIGATION

A stress such as a noise, or electric shock is administered during a series of tasks in a scenario that carries the theoretical possibility of personal injury. The scenario could be a series of exercises in a driving simulator. Psychological strain is measured as an error rate of choices between options entailing low or high mortality probability while driving. One would expect the error rate to increase and the decision speed (measured by default penalties) to decrease as the stress applied increases in intensity. These results can be represented by survival probability values. These in turn can be translated into a stochastic strain/fitness value (see Chapter 39), which may be made compatible with other strain/fitness values.

CHAPTER 39: STRAIN AND FITNESS

Stress is here used as the term for a deleterious causal agent and its effect is strain. Strain is defined as the resultant loss of fitness. Responses and strain must not be uncritically interchanged; responses should be seen merely as indicators of strain until they have been calibrated in terms of fitness. Because stresses can only be identified as such by changes in the organism and because organisms are different in their responses to various agents, it is unlikely that there is a universal scheme of stress (as cause) units. Instead, this necessarily leads to stress being defined in terms of its effects. These effects are measurable independently of the operational definition of stress given in Chapter 35. Though stress and strain are defined in terms of one another they are now given independent empirical grounding.

Strain and fitness are inverse assessments of the same quality. This raises the problem that strain is an absolute concept while fitness can be comparative. So here, fitness must be reformulated as an absolute quality. This can be achieved by defining it in terms of the organism's existence. The organism rather than a higher or lower biological unit is chosen as the focus of this study: it is in the individual where stress interactions take place.

Fitness must be further defined if it is to be meaningful. For instance can we talk of the fitness of an individual oak tree in the same way as the fitness of an individual oak gall wasp? Precise definition is obviously essential. Nevertheless, fitness is an ontological term and is universally applicable; it should therefore be universally definable - if not necessarily convertible. But, as has been demonstrated, fitness can only be known with certainty after the fact, i.e. over particular known lineages for defined times. Thus it can only be known precisely for extinct lineages or organisms which leave no descendants, and then it must be zero. Death is an easily defined but unsatisfactory point as the result and its fitness measurement will always be the same - thus it tells us very little. Any other timetable will be either arbitrary or based on less definite events. Consequently, judging the appropriate event on which to stop and start the clock becomes more difficult. If the researcher decides to stop the clock at the point of production of viable offspring then we are in a morass of definition of what viable means in terms of organism and environment. These complications prompt

the conclusion that there is no convenient absolute event time-frame on which to hang strain/fitness.

In contrast, any fitness assessment that deals with still living organisms or lineages is likely to have little utility unless it can tell us something about the probability of continued existence, thus bringing in an element of uncertainty. Nevertheless, some estimate of fitness would be useful. Such an estimate may be derivable because, as we have seen, a large number of measurable factors affect continued existence of the organism. These include exogenous, endogenous, reproductive, somatic, deterministic and stochastic elements, which are discussed below. These probabilistic elements, mentioned last, are also important as any projection of fitness assessment is subject to uncertainty. An attempt will be made to quantify this uncertainty in Chapter 40. It is recognised that the categories below are sometimes heterogeneous and sometimes overlapping. They are nevertheless suggested as the starting point for an integration of factors. The weighting given to each must be determined by experiment. Some methods are suggested below and in the next chapter.

EXOGENOUS STRAIN/FITNESS

This may be caused by environmental factors that restrict growth or reproduction or both. It is the result of any external stress and leads to a reduction in the likelihood of the organism's physical persistence or reproductive ability. Exogenous strains may be measured by any of the strain/fitness categories.

ENDOGENOUS STRAIN/FITNESS

Endogenous strain is manifest through internal inadequacy in reproductive or somatic processes. Endogenous strain may also be inherited. It concerns the fluctuation of response with age or time in the life-cycle. Thus it may be necessary to map such fluctuation throughout the lifetime of the organism. Any of the strain/fitness parameters suggested elsewhere may be used. Reproduction may cause endogenous somatic strain in the individual when energy is expended on reproduction to the detriment of somatic resources.

SOMATIC STRAIN/FITNESS

Somatic strain is a reduction of the body's capability to serve its own needs; it may be endogenous or exogenous. It is manifest as an increase in mortality or lowered tolerance to further stress. If we go beyond the organism it may be seen that an increase in reproductively caused endogenous somatic strain may be offset by increased reproduction that will maintain the fitness of the lineage. But this is at the expense of the individual and we must be sure to distinguish between individual and lineage fitness.

REPRODUCTIVE STRAIN/FITNESS

This comparative index measures reproductive output when in the presence of stress compared with expected output for normal conditions. It may also be considered as the percentage loss of reproductive output in any period. Indicators include gonadosomatic indices, sperm motility, sperm and egg counts, and gamete viability (FID_{50}). Aspects of this are examined in Chapter 47.

DETERMINISTIC STRAIN/FITNESS

Deterministic strain is measured by the degree of damage or disruption. It is manifest as a shortened life span, reduced performance or reserves, or any combination. Deterministic strain/fitness is the condition of the organism usually measured by one or more of its processes. It may be measured, for instance as percentage fitness/strain between normal condition and death.

STOCHASTIC STRAIN/FITNESS

It is manifest as an increased risk of early death by a raised probability of meeting a critical inadequacy. Stochastic strain/fitness is often an all-or-nothing phenomenon; all run the risk but only few pay the price at any particular time. Units include percentage mortality probability in any period and probability of suffering a given percentage change of somatic fitness/strain in any period. A parasite may do the same for reproductive stochastic strain. Units would be percentage sterility probability in any period and probability of suffering a given percentage reproductive fitness/strain in any period.

INTEGRATION OF DETERMINISTIC AND STOCHASTIC STRAIN/FITNESS IN MUSSELS

Deterministic and stochastic strains are two poles of a continuum. Despite this continuum, the terms have utility in apportioning fitness values for the purpose of making more accurate fitness predictions. Deterministic fitness could be characterised as somatic fitness of which 100% would be taken as the normal performance level. In byssus production, for instance, this would be the mean number of threads produced each day. Half the number would signify 50% fitness. Stochastic fitness could be taken as the daily number in the population dying as a percentage of that population in specified conditions. To integrate these two strain/fitness parameters it is necessary to demonstrate a relationship between percentage fitness and mortality probability. If stochastic fitness is seen as percentage mortality probability, this must be defined carefully. It is the percentage dying over a given time or after exposure to a given dose of a specified stress. The transition from individuals to populations is fraught with difficulties that may be overcome in part by the study of cloned individuals. This may allow derivation of a conversion factor between levels of somatic fitness in the individual and mortality probability in a genetically identical population.

CHAPTER 40: PROPOSED STRAIN/FITNESS MODELS

It has been argued (Chapter 16) that stress causes disorder in organisms. This disorder degrades fitness of the organism. It is advocated here that loss of order is loss of fitness. However, different types of disorder may have different fitness implications. In consequence, an independently grounded assessment of order must be shown to be convertible with an independently grounded assessment of fitness.

INSTANTANEOUS FITNESS

Since any convenient time frame as applied to fitness is arbitrary, one approach may be to consider discarding time frames altogether and instead attempt to ascertain instantaneous fitness. This may take the form of an index of 'aliveness' or co-ordination. This is the condition of the organism as compared with the condition at the threshold of death. Some possible fitness/strain indices are listed below:

Fitness/strain as an index of organisation

1. Fitness/strain as the ratio of negative feedback to positive feedback dynamics in metabolism.
2. A fitness index may be derived as a ratio of energy available to the amount required to maintain order.
3. Fitness as an index of entropy production. Strain and disorder are relative in as much as when there is increasing strain there is increasing disorder.
4. Quantification of free radicals in tissues and fluids.
5. The presence of abnormal molecular species from non-enzyme mediated reactions.

Fitness/strain described in terms of:

1. Reserves
2. Tools and Abilities
3. Anticipation of changing conditions
4. Probability
5. Environmental conditions

In other words, what can the organism do now? Integrate 1, 2, 3, and 4 in the context of 5 to give fitness. Each attribute must be assessed for fitness value for the particular organism. It would also be necessary to ascertain the subminima that are required of each attribute. If probabilistic uncertainty is the most important of the four, then other differences between the species will count for little. To proceed we must therefore devise ways to measure the uncertainty in order to be able to reduce it. This will be dealt with later in this chapter.

Process rates may be used as an index of fitness/strain:

A rate of 0 indicates death

A rate of 10 indicates normal health

above 10 indicates enhancement of normal process

20 = process 100% above normal

This index, to be meaningful, must be placed in context with an estimate of the effect on the co-ordination of the organism, otherwise increases may be interpreted as enhancements when in fact they are not. Co-ordination may be lost even though one process rate may be elevated.

Fitness/strain as the rate of energy wasted in proportion to the rate used.

The units would be Kj intake/Kj wasted: the higher the number the less strain.

10/ 2 = 5

10/ 5 = 2

10/10 = 1 fatal (lowest no.)

SUGGESTIONS FOR INVESTIGATION

Dam stress and diversion stress

Dam stress and diversion stress could be incorporated into scope for growth. Then it would be possible to ascertain somatic and reproductive energy requirements and also determine strain as a ratio of energy available to energy required. To do this it is necessary to know the energy requirement at each stage in the life cycle. It would also be necessary to know the seasonal fluctuation in energy requirement and apportionment in mussels of different sizes

and sexes. This may be difficult and time consuming. Consequently, an investigation of fitness/strain parameters of starving mussels to ascertain the reproductive and somatic effects is suggested. A large number of standard sized mussels are kept in food-free water. They are assayed for various strain/fitness parameters and mortality at each stage. Both somatic and reproductive fitness parameters could be investigated. Control mussels from the collection site could also be examined. Assuming that the cause of condition loss is starvation it should be possible to calibrate the minimum energy condition (that just before death) in terms of strain/fitness indicators. In addition, it should be possible to see if the effect of parasites is to reduce reproductive fitness only or somatic fitness as well. It may be possible to allot a fitness/strain value to the parasite for different levels of starvation. This may be assessed through fitness/strain parameters and also through mortality. Fitness parameters could then include biomass turnover, and consideration of its importance as structure and not just as an energy store. More biomass, all other things being equal, suggests higher fitness. This can be related to other fitness/strain indices for confirmation.

Order/disorder

An estimate of order/disorder is an instantaneous fitness assessment and may be in units or percentage of 'aliveness', using indices of disorder such as the numbers of free radicals in tissue and fluids (as assessed by redox potential?). The second possibility would be the detection of abnormal molecular species from non-enzyme mediated reactions such as L or D forms of molecules when they should not be there. It is speculated that they may be detectable by their polarising effect on transmitted light. If this is feasible, it may be possible to detect the effect of stress non-destructively. Perhaps amino acid fragments and polypeptides could be identified by electrophoresis. A healthy sample of tissue or fluid could be used as control. Order may be also indicated by a balance of catabolic and anabolic processes. Catabolic processes may be detected by a profusion of smaller fragmentary molecules. Anabolic processes may be detected by large amounts of complex molecules and relatively few species of small precursors. These may be detectable using electrophoresis. Considerable development will be needed to identify which electrophoresis conditions are required to show the most representative molecular indicators. Abnormal amounts of amino acids may be lost from the organism across membranes or through excretion. The last possibility is tested in Chapter 46.

Suggested methods to render a parasite equivalent to other stresses

The following will assist in characterisation of the stress that parasites exert on the mussel and help quantify its effect as a reduction of fitness. It would be hoped to show that this reduction is proportional to parasite intensity/numbers or amounts.

1. Quantify the proportions of parasite to host: Count parasites in a given wet mass of mantle using a counting slide. Estimate percentage of host occupied by the parasite. This could be done by inspection of histological sections. Count the parasite as biomass but do not count its contribution to activity and other fitness parameters. Thus mean activity will be less than an organism without a parasite.
2. Estimate the metabolic rate of the parasite and therefore its energy consumption, this can be reflected as part of the energy wasted in a fitness/strain assessment. The parasite may cause one of three effects: It may reduce fitness less than it reduces energy availability or it may reduce fitness by as much as it reduces energy availability, or it may reduce fitness more than it reduces energy availability.
3. Treat the parasite as a disorder in terms of extra molecular species detectable by electrophoresis.
4. Consider the interaction of the parasite and other stresses such as temperature, osmotic shock, poisons and emersion. The last suggestion has been investigated in Chapter 45.
5. Examine the relationship of structural integrity of the host with the intensity of parasitic infection. Aspects of this are assessed in Chapters 12 & 48.
6. Investigate freezing survival of the mussels using ice and calcium chloride mixture.
7. Show that parasitised/stressed mussels may be detected by their decreased fitness as indicated by performance parameters such as heart rate, cilia current speed, byssus production and sperm count. The last parameter was inspected in Chapter 47.

Values for fitness/strain assessed by the above indicators may be integrated into a fitness value Te presented below.

A PROPOSED FITNESS VALUE Te

Fitness may be expressed in units of time and defined as the time to death of the individual or extinction of the lineage: Te. This may be estimated by examination of the rate of change of fitness parameters (calibrated as somatic strain/fitness) with time and integrating stochastic fitness assessments. From this it will be possible to project when the process rate (and therefore fitness) will be zero. If it indicates an infinite time to death it means that the organism has no foreseeable time to death. Fitness parameters might include those mentioned above: level of organisation, rate of change and lethal level; it would be measured in time units giving the span of existence. The duration may be arbitrary with units such as seconds, hours, years, up to one generation. The formula is also applicable to lineages but we must specify the fitness values that are descriptive of assemblages rather than individuals. This requires among other things that survival numbers of each stage be ascertained as well as survival time. In addition we must also deal with conversion between somatic and reproductive fitness.

To derive Te, the time left until extinction, we must first ascertain the rate and direction of change of fitness = G, over time Tm:

$$G = D_2 - D_1/Tm$$

D_1 = initial condition of strain/fitness, D_2 = final condition of strain/fitness, Tm = time between D_1 and D_2

Te may then be derived:

$$Te = D/G.$$

This assumes linearity. The validity of this must be ascertained by experiment and any deviation must be built in to the equation. Condition D may be derived from assessment of the organisation and the reserves of the organism. The rate of change of condition G may be estimated by examining the rate of disorganisation or loss of reserves of the organism (this is the part of the assessment of welfare of the organism as defined earlier (See Chapter 36). If, for simplicity, it is assumed that the relationship is linear then the derivation of Te can be shown as below:

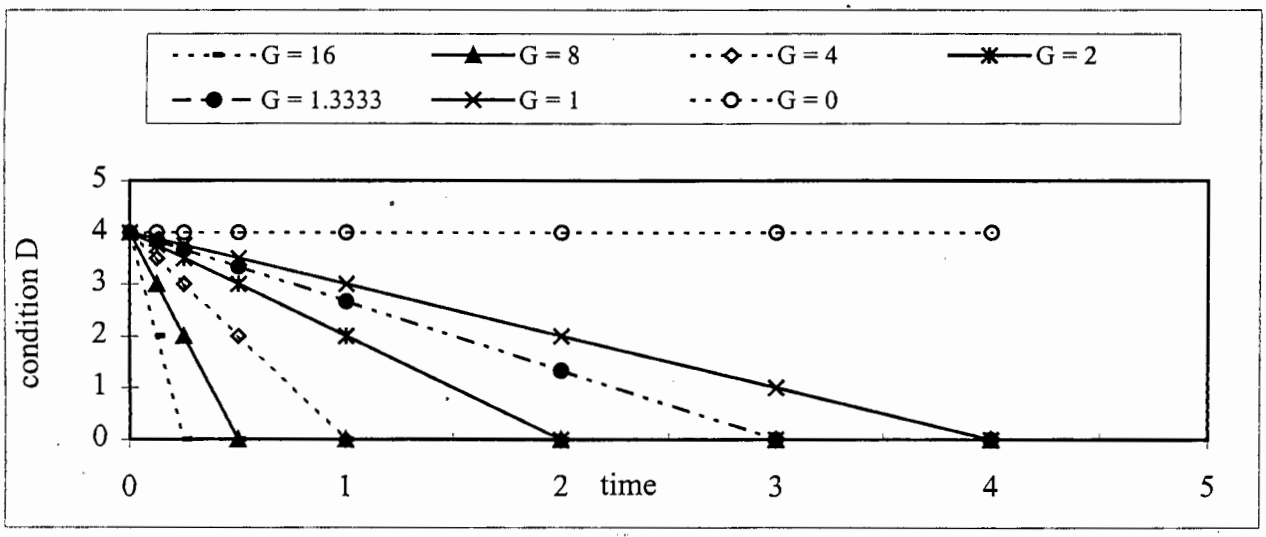


Figure 1: The relationship between D (condition), G (rate of change of condition) and Te, the fitness time span.

In Figure 1, gradient G values of infinity, 0, 1, 1,333, 2, 4, 8 and 16 are given as examples. The condition value of 4 is an arbitrary starting point. The condition value of 0 is the threshold of non-existence - the degree of disorganisation, below which continued life is impossible. If the slope is horizontal (gradient 0:1) then Te = 4/0 = infinity: at this point the organism will exist indefinitely. All slopes up have negative gradient and for these Te would also equal infinity.

The following gradients are examples of downward slopes:

Te = D/G
Te = 4 /0 = infinity
Te = 4 /0,25 = 16
Te = 4 /0,5 = 8
Te = 4 /1 = 4
Te = 4 /2 = 2
Te = 4 /4 = 1
Te = 4 /infinity = 0

Thus in this scheme fitness consists of a condition and rate of change which can be integrated to give a duration. Fitness in this case is thus a measure of time Te to go before the organism reaches extinction. This time may vary from 0 to infinity and its units are arbitrary.

Statistical confidence in the projection

In measuring the rate of change of condition over a given time it is also possible to derive an

estimate of statistical uncertainty for the rate. For example, Te is a regression line and thus it will be possible to calculate the usual regression confidence limits. These may be used to predict the confidence that we may have in any projection into the future.

An alternative, and perhaps simpler method, is to use an index here nominated the coefficient of confidence. This might be derived from a coefficient of variance (CV) for each period and be related thus:

$$CC = 100\% - CV$$

The CC has utility in that, for example, a CC of 99% would suggest a more precise estimate than one with a CC of 80%. More importantly the CC could be used to integrate longer periods and give meaningful estimates of statistical significance. Thus to extrapolate beyond the measured period it is suggested that for each extra period the CC should be multiplied by itself, e.g. for three periods each with a CC of 80%:

$$80\% \times 80\% \times 80\% = 51.2\%$$

Five periods each with a CC of 80% give a summed CC of 32.8%. Thus the confidence of the extrapolation decreases drastically with time and any estimate that is extrapolated for long would have a CC that indicates great latitude. This is presented as a broad example of what may be possible, it is recognised that the CC for each period would probably require correction factors to make it compatible with the next so that a useful value of probability might be derived from any linkage.

A strength of this approach is that the maximum profitable projection is indicated by the divergence of the CC limits with time. The only way to make the projection less subject to wide probabilities is to assess the fitness/strain accurately and subdivide the fitness parameters so that a more accurate representation of important factors may be arrived at. A selection and careful weighting of fitness parameters that come from an increased knowledge of the particular organism being studied and its responses at different times in its life cycle may give the optimum results with minimum of variability. Thus it would add to the value of the prediction by narrowing the confidence limits. This method may also be applied to lineages, but here fitness must be divided into reproductive and somatic components and the fitnesses of overlapping generations integrated at the appropriate time.

This approach owes something to the actuarial method of life expectancy, upon which Settle (1993) has cast doubt. Fitness, he correctly asserts, is a more complicated statistical property than life expectancy. But one can make predictions only with reference to previous data. The following suggestions may meet some of his criticisms. The estimate must make allowance for stochastic events, whose usual effect will be to blur underlying trends. It is hoped that through careful experimental design it may be possible to reach useful conclusions despite, or perhaps with the help of assessments of stochastic events. The condition of the organism and its state of matching to the environment must also be ascertained. This assessment must be approached by an attempt to determine the factors that cause loss of fitness.

It is understood that this presents two possibilities for faulty inference. One is the argument of *post hoc ergo propter hoc*; the fallacy on which, as Hume (1898) implies, any inference of cause and effect is built. This inference, in consequence, may or may not be correct and great care must be taken to reduce the likelihood of error. The second possibility for erroneous inference is in the choice of experimental subjects (and their circumstances) from which the cause and effect relationship is built. Are these subjects and their responses homologous or merely analogous with the organisms for which we are attempting to ascertain fitness? So we have to infer cause from effect in one organism or organisms and then we have to use this cause (or causes) to infer the effect (fitness) in another conspecific or otherwise. Tortuous though this process is there appears to be no other way if we wish to make informed predictions of fitness. These limitations having been clearly stated may help in avoiding the more likely errors.

SUGGESTIONS FOR CONVERSION OF FITNESS PARAMETERS IN INDIVIDUALS AND LINEAGES

We cannot be sure that fitness in individuals and in lineages has exactly equivalent and convertible meaning. What it is that continues existence must be explicitly stated, i.e. individual or species or lineage. The following points may assist when attempting to formulate a conversion from one ecological level to another:

1. The instantaneous fitness of an individual may be taken as its somatic fitness.
2. The instantaneous fitness of a lineage is its mean somatic fitness and number of

individuals, allowing for mortality and recruitment.

3. Somatic fitness of individuals (instantaneous fitness and fitness over different life cycle stages) can be broken into different stages such as egg, larva and adult for weighting of importance.

4. Reproductive fitness in individuals and assemblages is taken as output (number) of gametes, divided by two if dioecious. Or it could be the number of gametes that survive to fertilisation divided by two. Reproductive fitness is just the means to somatic fitness and should not be taken into account except as a connecting and multiplying phase.

FINAL COMMENTS

The problem of "pseudo-integration" (Van Der Steen 1993) has been considered and some of his more damning objections have been met. Although the present work was not specifically intended to integrate physiological stress with its psychological counterpart, it is apparent that the theoretical framework provided here does allow substantive integration of these two stresses. They now share the same definition; both are agents that cause a loss of co-ordination leading to a reduction in fitness. An inherent problem of integration: overloading a few concepts with too much burden of meaning has been anticipated. Thus stress has been subdivided into relevant, but connected, aspects. These unburden it and give the new subconcepts only sufficient load to give them clear meaning. For the same purpose, strain and fitness have been similarly subdivided. The details of each phenomenon are here dealt with by use of more "local" theories as suggested by Van Der Steen, (1993). In addition, we have unloaded stress into different areas. For instance, ecological stress is no longer classified with individual stress. The general theoretical construct presented here is capable of embracing all biological stress phenomena up to the level of the individual, without doing violence to any parts of theory to make it fit. As urged by Van Der Steen, (1993), independently grounded definitions of stimulus (stress) and response (strain) have now been provided. Thus the relationship between stress and strain is open to empirical study.

PART VI:

TESTS OF THEORY

SELECTION OF EXPERIMENTAL PARASITES

The next seven chapters (41 to 47) experimentally examine aspects of stress in the light of the foregoing survey and critique of current theory. Attempts are made to gauge the effect of parasites on *Choromytilus meridionalis* in Chapters 41, 45, 46 & 47. The aim is to establish that parasites are indeed stresses and to quantify their effect so that they may be rendered equivalent to other stress agents. *Cercaria notobucephalus* (Chapter 3) has been selected because of its extreme infection intensity. Because of this it, more than any of the other digenea, is likely to produce measurable effects. Its infections are usually associated with an apparent decrease in host gamete numbers. This will now be quantified; in addition, any somatic stress effects will be measured. Although it is not so common as some other parasites surveyed, *Cercaria notobucephalus* is sufficiently available for study. That *Cercaria notobucephalus* infections are either heavily infected or clear, with no intermediate levels may be useful in eliminating any overlap in effect between uninfected and lightly infected individuals. Thus a small sample of gamete counts should be sufficient to render a significant difference.

Three other digeneans have higher prevalences than *Cercaria notobucephalus,* but all at lower intensities. They are, thus, mass for mass, much smaller in proportion to their hosts and one might expect such infections to have a correspondingly smaller influence with a diminished prospect of measurable effect. This expectation is tested in the investigation of emersion survival where the effects of *Metacercaria A* (Chapter 5), *Metacercaria* B (Chapter 9) and *Metacercaria perchorupis* (Chapter 4) are assessed. Some of these occur also in *Perna perna* but at considerably lower intensities such that a deleterious effect may not be measurable. Thus it was decided to study their effect only in *Choromytilus meridionalis*.

Other parasites have been reported from the other mussels: *Metacercaria columbinensis* in *Mytilus galloprovincialis* (Chapter 6), *Metacercaria maculatopsis* in *Choromytilus meridionalis* (Chapter 7) and *Metacercaria ater* in *Aulacomya ater* (Chapter 8). These parasites have not been selected because they are much less common or are found only in

remote localities which makes them less convenient to collect. None of them, moreover, occur in high intensity and thus may be less likely to produce a measurable effect. For these reasons they are omitted.

The deleterious effects of parasites are thus studied in only two of the four mytilid species surveyed: *Choromytilus meridionalis* for its digenea and *Mytilus galloprovincialis* for the effect of the shell borer *Mastigcoleus* sp. This cyanophyte has been demonstrated to drastically weaken mussel shells, sometimes to the point of fracture. Discussion of the effect of the cyanophyte, *Mastigcoleus* sp., is pursued in the Synthesis (Chapter 48) where some of the data from Chapter 12 is reanalysed to obtain a stress/strain relationship. It is fortunate that (in contrast to *Cercaria notobucephalus*) *Mastigcoleus* occurs in a range of infection intensities which allows a quantification of the stress and the strain and a mathematical characterisation of the relationship between them.

CHAPTER 41: GILL CILIA ACTIVITY OF *CHOROMYTILUS MERIDIONALIS* AND STRESS AGENTS

INTRODUCTION

Choromytilus gill cilia activity is assessed as an indicator of chemical, physical and biological stress agents. Ammonia, varying salinity and *Cercaria notobucephala* are used respectively for these agents. Some of these agents are also combined to see if gill cilia activity can integrate disparate stresses.

Significant works on gill cilia and bivalve feeding include the following: Aiello (1974) overviews ciliary control in the metazoa. Jørgensen (1990) deals with bivalve filter feeding. Micallef & Tyler (1990) examine control of cilia activity in the mytilids. Aiello, Hager, Akiwumi & Stephano (1986) examine cilia control with particular emphasis on *Mytilus edulis*. Paparo (1985) in his investigation on the influence of innervation on ciliary activity in *Mytilus edulis* concluded that the visceral ganglia afford coarse control and the cerebral ganglia fine control over ciliary beating. Jones & Richards (1993) deal with the effect of various neurotransmitters on the mussel pump and in particular their effect on the different types of cilia in *Mytilus edulis*. Jørgensen, Larsen, & Riisgard (1990 p98) discuss the effect of temperature on the mussel pump but curiously they report that for the lateral cilia of *Mytilus edulis* : "The increase in beat frequency of the lateral cilia with temperature...had no clear-cut effect on the pumping rate". This shows that ciliary activity may not be a whole body integration: an increase in one lower order process does not necessarily translate into advantage at a higher level of integration. This is pursued further in the discussion in this chapter and also in the Synthesis (Chapter 48).

MATERIALS AND METHODS

The first part of this work assesses the operating parameters and limitations of this simplified gill cilia activity assay. It thus explores the variation of cilia activity: on different parts of the gill; with mussel size; over time; and according to the sex of the mussel. The second part ascertains the effect of various agents, such as: salinity, ammonia, mussel parasites and combinations of these agents, on gill cilia activity.

Choromytilus were from Blouberg, and in each comparison the mussels were collected at the same time and held under the same conditions before the experiment. They were acclimated at 15°C for about two weeks before being used but they were studied at room temperature which was measured at each experiment. This approximates to the procedure of Aiello, Hager, Akiwumi & Stephano (1986) who held *Mytilus edulis* at 5°C and allowed them to come to a room temperature of 20-22°C.

Gill cilia activity was estimated using a simplified method of Davenport & Fletcher (1978) which is derived from Gray (1923). Although, in contrast to Davenport & Fletcher (1978), the mussel is cut in two, the procedure used here is nevertheless less drastic than that of Clark (1966) used by Micallef & Tyler (1990) for *Mytilus edulis* in which the gill lamella is excised before the experiment. After the adductor muscle was cut and the valves parted, the mussel was sagittally bisected. A half-shell was placed gill-side up on a plastic cradle with the margin of the shell on the horizontal plane. The shell was then filled with the test solution and gill cilia current speed was estimated by timing passage over the gill of fine carmine particles suspended in the test solution. A dissection microscope with graduated eyepiece was used to monitor particles over 2 millimetres and the time of passage was noted. The particle speed is taken to represent the current speed and therefore is an indicator of gill cilia activity. Ten timings were obtained for each treatment.

Consideration was given to the possibility that there may be an optimum area on the gill for experiment. Criteria for this would be consistency of results and particle speed. Speed is important; if it is too slow then a complete assay may require longer than the period of stability of cilia activity in the control. In addition, the site must be big enough for accurate timing and it must be away from the edge of the gill where the current turns and runs along the longitudinal axis. The need for this investigation was prompted in part by the findings of Jørgensen (1990 p8 Fig4) who noted considerable variation in current direction over the mytilid gill in the intact mantle cavity.

Six areas on the *Choromytilus* gill were nominated:

		outer		
	1A	2A	3A	
anterior				posterior
	1B	2B	3B	
		inner		

Carmine particles were timed across the midpoint of each gill area. After each timing the gill was purged by pipette with fresh test solution. This also ensured that the gill was well irrigated throughout the experiment. Although Davenport & Fletcher (1978) used chalk and Micallef & Tyler (1990) used sand, carmine was used in this experiment because of the visibility, inertness, and size constancy of its particles; it was also readily available. Ten timings were made in each area, for each gill preparation the data were put into a bar chart with each bar representing mean particle speed in a part of the gill.

In the salinity experiments standard salinity (100%) was determined as that to which the mussels had acclimated in the aquarium. Lower salinities were obtained by dilution with distilled water. Solutions were allowed to equilibrate to ambient temperature. Ammonia concentrations, given here in parts per million (ppm), were obtained by the appropriate dilution with sea water of BDH analysed reagent 26.24% ammonia solution.

RESULTS AND DISCUSSION

Particle speed on different parts of the gill

Particle speeds on the six parts of the gill were determined (at 20.5°C) for three mussels. Figures 1, 2 & 3, show the considerable variation in particle speed on different parts of the gill. This contrasts with *Mytilus edulis* (Ajana 1975) where particle speed was not area specific. The optimum site on the *Choromytilus* gill was identified after assessing the variation in consistency and speed. Section A, which lies nearest the marginal groove, generally exhibits a higher particle speed than the more proximal section B. Evidence for this is strongest in Figures 2 and 3. Part 2A was

selected for experiment as here the current is linear, it is sufficiently broad and particle speed is high enough to allow rapid timings. Thus, in all experiments, particles were timed on part 2A over a course of 2mm that started 4mm from the gill (inner demibranch) marginal groove. This timing distance stops short of the gill distal margin as here the current curves to the anterior (pers obs).

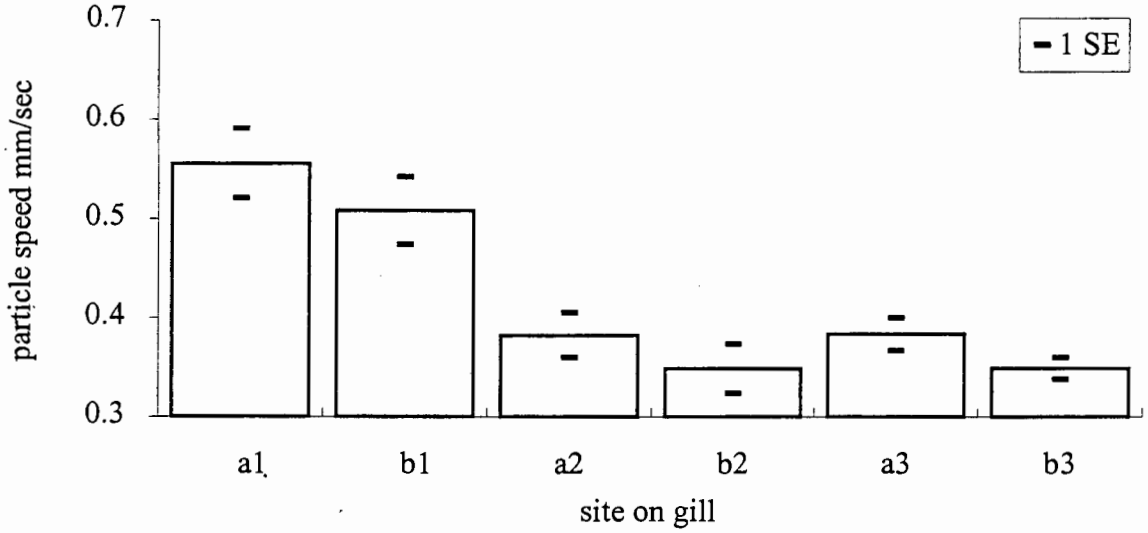


Figure 1. Site dependent variation in particle speed over the gill of a male *Choromytilus* (67.7mm) at 20.5°C.

Figure 2. Site dependent variation in particle speed over the gill of a female *Choromytilus* (69.1mm) at 20.5°C.

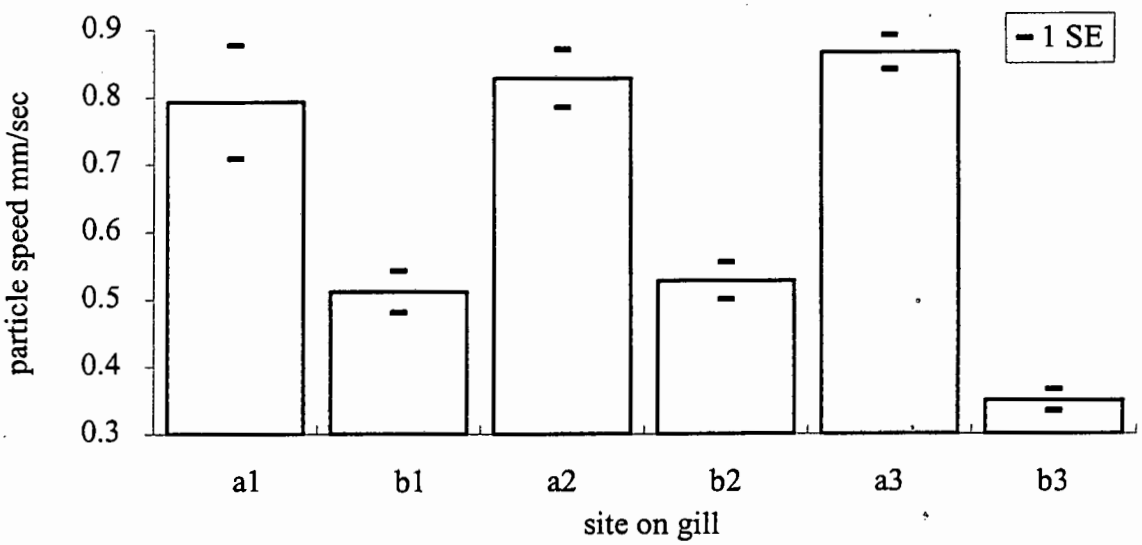


Figure 3. Site dependent variation in particle speed over the gill of a male *Choromytilus* (71.2 mm) at 20.5°C.

Mussel size and particle speed

Since Taylor & Brand (1975), Bayne & Livingstone (1977), Bryan & Uysal (1978) and van Erkon Schurink & Griffiths (1992) assert that size is important in physiological processes of bivalves, it was considered prudent to assess variation of particle speed in different sized mussels. Thus particle speeds in twelve mussels ranging from 61.5mm to 73.3mm were compared (Figure 4). The correlation coefficient (r = -0.359, n = 10) is significant at P = 0.001. The regression coefficient (r^2 = 0.129) indicates that only 12.9% of variation in particle speed is attributable to mussel size. And although the slope (-0.0043) shows that particle speed does indeed reduce with mussel size, over the range (11.8mm) of mussels in the sample, this reduction would be only 0.050mm/sec. Variation in size, though a significant factor, is responsible for only very minor variation in particle speed. Its effect is put into perspective when one considers that mean (n = 10) particle speed may differ by up to 0.28mm/sec between valves of the same mussel (Figure 5).

Variation between valves from the same mussel was examined for four *Choromytilus* (Figure 5) by use of student's t-tests. It is surprising that in no case was significant difference found between valves of the same mussel:

mussel length	Calculated t	t from table
69.80mm	1.307	3.922 t (0.001,18)
71.95mm	1.677	3.922 t (0.001,18)
73.15mm	0.361	3.922 t (0.001,18)
75.55mm	1.448	3.922 t (0.001,18)

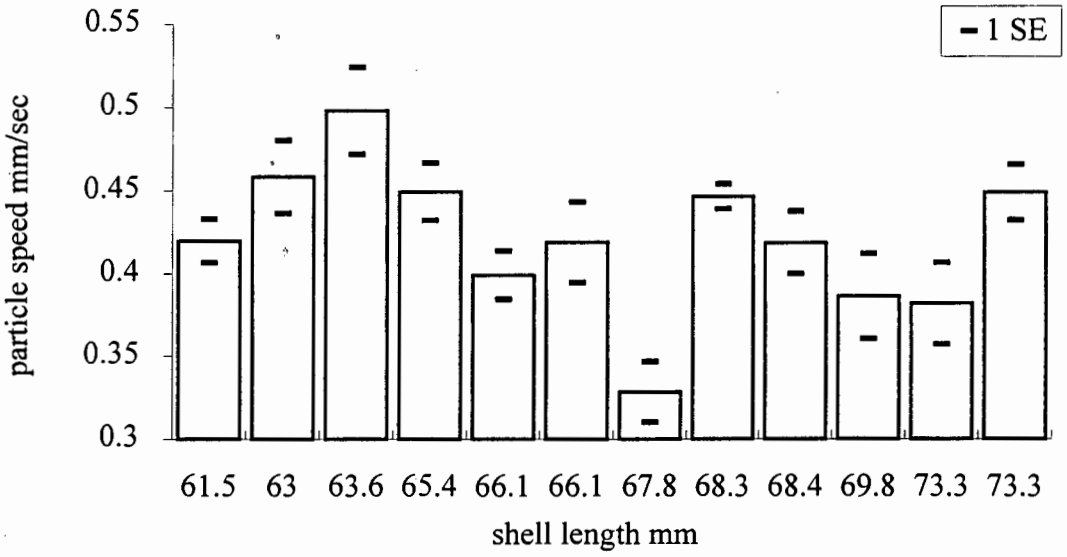


Figure 4. Size dependent variation in particle speed over the gills of a range of *Choromytilus* single valves at 15°C; $r^2 = 0.128$, slope = -0.00425.

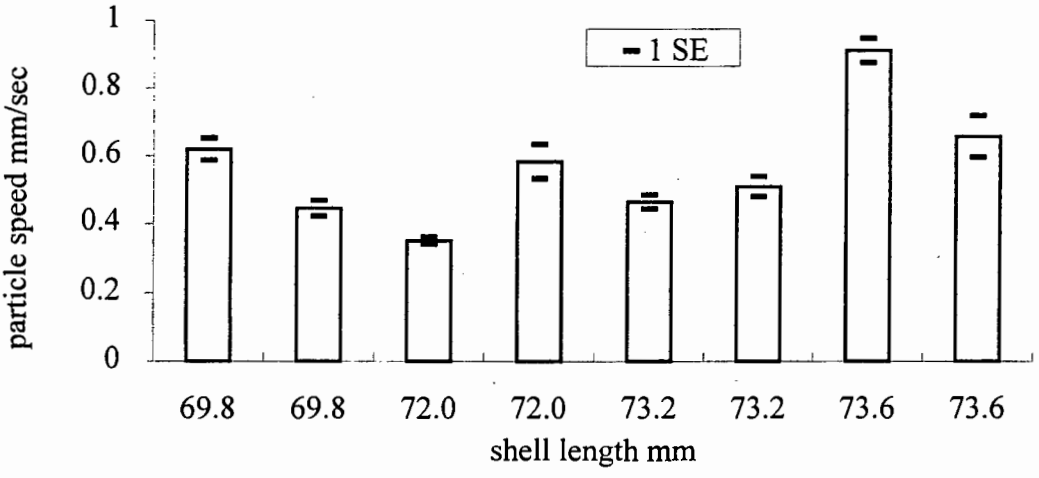


Figure 5. Size dependent variation in *Choromytilus* particle speed measured separately on both valves of four mussels at 23°C.

That these results are insensitive to a large difference when the regression detects a smaller difference demands explanation. This can be accounted for by there being

more data in the size assay (d.f. = 108) compared with the valve difference tests (d.f. = 18). It is evident that with smaller suites of data, size dependent differences in particle speed are likely to be lost in statistical noise. It must be remembered that this assay is intended to be simple and so use the fewest readings to discriminate levels of deleterious agents. Thus a minor effect such as mussel size is likely to be unimportant. This is supported by the absence of any detectable relationship between shell length and particle speed in the bivalves *Donax serra* (Viljoen 1990) and *Venerupis corrugata* (Carissan 1992) during experimental regimes similar to the one used here.

Particle speed and sex

Sex difference in particle speed was not anticipated and in consequence no experiment dedicated to detecting it was performed. Thus evidence must be adduced from other data. Figures 1 (male), 2 (female), 3 (male), and 5 (male and female) show that particle speeds overlap with no overt sex linked bias. This evidence is probably sufficient to relegate any difference (if it exists) to the statistical noise mentioned earlier. Moreover, even if a difference existed it would not hinder the aim of this study which is to use mussel preparations to detect significant changes in the same preparation, not absolute speeds. Thus experimental subjects were taken from either sex.

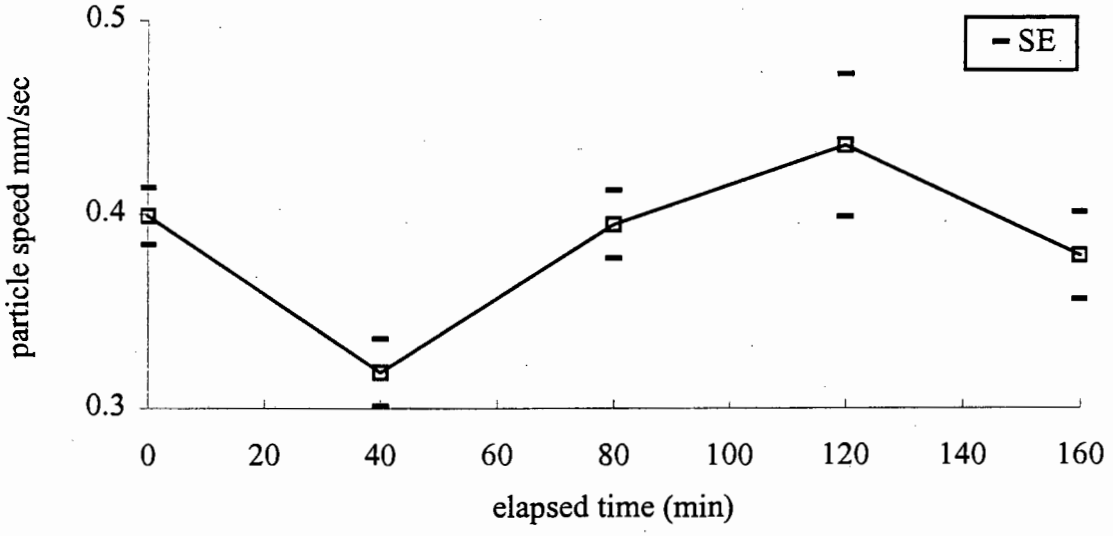


Figure 6. Variation in particle speed over 160 minutes in a male *Choromytilus* (66.1mm) at 15°C.

Stability of particle speed with time

Because ten readings are taken for each level of agent, the assay used here may take up to an hour to record particle speeds over a useful treatment range in one mussel. This period coupled with the extreme simplicity of the apparatus and the bisecting of the test mussel raised concerns about the temporal stability of the preparation. Thus, temporal variation of particle speed was assessed in a control. This would allow greater confidence in ascribing which fluctuations in subsequent assays are due to the agent applied.

In the first experiment, particle speed was measured ten times at each of five 40 minute intervals from 0 to 160 minutes (Figure 6). ANOVA was used to detect any significant difference between timings. The null hypothesis was that there is no difference between the means; the alternative hypothesis was that not all the means were the same.

Summary of ANOVA

	SS	d.f.	MS
total	0.33289	49	0.00679
groups	0.09123	4	0.02281
error	0.24166	45	0.00537

$$F = \frac{\text{groups MS}}{\text{error MS}} = 4.248$$

$F_{0.0025\,(1)\,4,45} = 4.83$

F calculated from the data = 4.248

Thus the null hypothesis is accepted: all the means are the same. Over 160 minutes there is no significant (P = 0.0025) variation in particle speed. Thus during the experimental period, no significant fluctuation occurred in the control. Inference is drawn that in subsequent assays the mean particle speed in normal sea water (the initial reading) of each preparation can be used as the control: any fluctuation is due to the treatment regime.

To investigate the longer term stability of the preparation another assay was made; this time comparing the activity over 24 hours in both valves. Valves were nominated 1 and 2 and the fluctuation in particle speed of each valve was ascertained for time 0, after 20 minutes and at 24 hours (Figure 7).

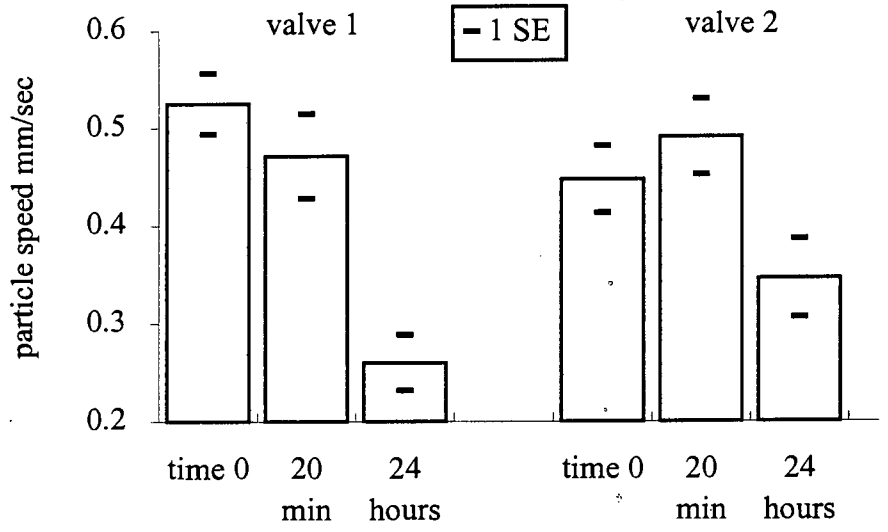


Figure 7. Variation in *Choromytilus* particle speed over 24 hours. Female mussel (66.45mm) at 23°C.

ANOVA (Zar 1984) was used to detect any significant difference within or between valves. The null hypothesis was that there is no difference between the means. The alternative hypothesis was that not all the means were the same.

Summary of ANOVA

	SS	d.f.	MS
total	0.579172	59	0.00982
groups	0.50323	5	0.1006
error	0.075942	54	0.00141

$$F = \frac{\text{groups MS}}{\text{error MS}} = 71.34$$

$F_{0.0025\,(1)\,5,54} = 4.30$
F calculated from the data = 71.34

Thus the null hypothesis is rejected: not all the means are the same. Tukey's test (Zar 1984) was used to differentiate the means.

rank	1	2	3	4	5	6
mm/sec	0.2599	0.3459	0.447	0.4712	0.4904	0.525
time	24hrs	24hrs	0	20 min	20 min	0
valve	1	2	2	1	2	1

SE = 0.01186
$q_{(0.005,\,54,\,6)} = 5.465$
q values for those rank comparisons below 5.465 showed that 6 = 4, 5 = 3 and 4 = 3.

In valve 1 there is no significant difference between time 0 (rank 6) and 20min. (rank

4) but there is a significant difference between both of these and 24 hours (rank 1). The same occurs in valve 2; there is no significant difference between time 0 (rank 3) and 20min. (rank 5) but there is a significant difference between both of these and 24 hours (rank 2). Thus in both valves the gill preparation gives significantly lower particle speeds after 24 hours but over the first 20 minutes readings are stable. The results also show that ranks 1 and 2 are significantly different from the other ranks and from one another. This comparison of the particle speeds after 24 hours demonstrates that difference between valves can be significant.

The experiment was repeated, the fluctuation in particle speed of each valve was ascertained for time 0 and at 24 hours (Figure 8).

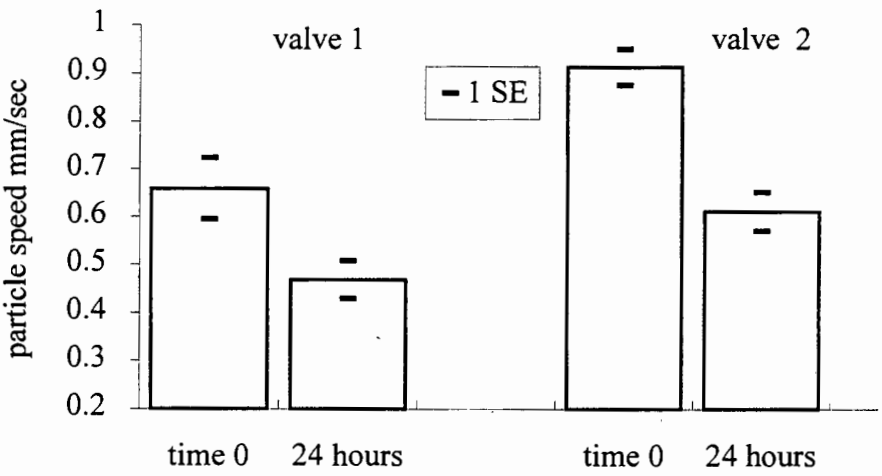


Figure 8. Variation in *Choromytilus* particle speed over 24 hours in both valves of a male (73.55mm) at 23°C.

ANOVA (Zar 1984) was used to detect any significant difference within or between valves. The null hypothesis was that there is no difference between the means. The alternative hypothesis was that not all the means were the same.

Summary of ANOVA

	SS	d.f.	MS
total	0.14165	39	0.03632
groups	0.629	3	0.2097
error	0.7875	36	0.02188

$$F = \frac{\text{groups MS}}{\text{error MS}} = 9.58$$

$F_{0.0025\,(1)\,3,36} = 5.80$

F calculated from the data = 9.58

The null hypothesis is rejected: not all the means are the same. Tukey's test (Zar 1984) was used to differentiate the means.

rank	1	2	3	4
mm/sec	0.468	0.61	0.6583	0.9117
time	24hrs	24hrs	0	0
valve	1	2	1	2

SE = 0.04677

$q_{0.005,\,36,4} = 5.181$

q values for those rank comparisons below 5.181 showed that 3 = 2 and 2 = 1.

There is significant difference between time 0 (rank 3) and 24 hours (rank 1) in valve 1. This is the same in valve 2; there is significant difference between time 0 (rank 4) and 24 hours (rank 2). Thus in both valves the gill preparation gives significantly lower particle speeds after 24 hours. The differences between valves is again noteworthy. At time 0 valves 1 and 2 (ranks 3 & 4) have significantly different speeds. At 24 hours the valves 1 and 2 (ranks 1 & 2) are not significantly different. The inconsistency between valves at different times emphasises the importance of treating each valve (and mussel) as a comparative assay only.

Figures 7 & 8 and their tests point out the instability of this preparation over 24 hours, but it is stable over 160 minutes which is ample time for the assays used here. This is comparable to the findings of Watkins & Simkiss (1988) for *Mytilus edulis* whose ciliary activity does not vary significantly over three hours. *Donax serra* (Viljoen 1990) and *Venerupis corrugata* (Carissan 1992) both exhibit a similar stability for at least 90 minutes when following an experimental regime similar to the one used here. Stability in *Choromytilus* would probably be extended if the experiment were run at 15°C or lower rather than 23°C used here but with the penalty of a considerably lower particle speed.

A more stable arrangement is undoubtedly achievable but at the cost of increased complexity and inconvenience. For instance, Ajana (1975) cited in Davenport & Fletcher (1978) says that ciliary activity showed no appreciable alteration over 24

hours at 15 °C. This is probably due to Ajana's apparatus attending to water flow and aeration requirements of the gill much more completely than that here. In addition, Ajana's apparatus kept the haemolymph and nerve supplies intact. This is contrasted with separation of the valves performed here.

Particle load on the gill and speed

Jørgensen (1990) reports that heavy particle loads stimulate secretion of mucus, and that different sized particles have different velocities and trajectories. Similarly, Newell (1979) reports that mucus is secreted in proportion to the load of particles on the gill filaments. This was seen here in *Choromytilus*, as was the size dependent difference in particle speed (pers. obs). To prevent variation from such sources, carmine particles were applied sparingly and particles of similar size were timed. In addition, gills were purged with suspension-free water after each count. That this has been successful may be confirmed by the lack of dispersion in the data sufficient to allow differentiation of speeds under varying conditions.

Particle speed and salinity

Single valve preparations from two mussels were examined. Timing was discontinued at 20% and 10% salinity in Figures 9 & 10 respectively as particle speed below this had either stopped or was too slow to measure. In both cases particle speed reduces with decreasing salinity.

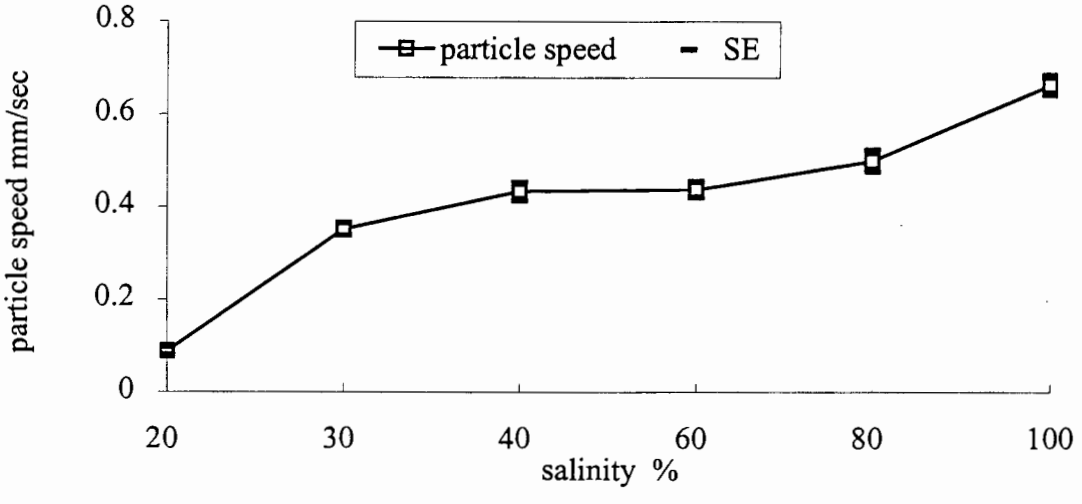


Figure 9. Salinity dependent particle speed in a female *Choromytilus* (63.1mm) at 24°C.

The results in Figure 9 were subject to a one-way ANOVA test (Zar 1984). The mean particle speeds were compared over the range of salinity from 100% (normal sea water) to 20%. The null hypothesis was that there is no difference between the means. The alternative hypothesis was that not all the means were the same.

Summary of ANOVA

	SS	d.f.	MS
total	2.04033	59	0.03458
groups	1.81474	5	0.36295
error	0.22554	54	0.00418

$$F = \frac{\text{groups MS}}{\text{error MS}} = 86.83$$

$F_{0.0025\,(1)\,5,54} = 4.30$
F calculated from the data = 86.83

Thus the null hypothesis is rejected: not all the means are the same. Tukey's test (Zar 1984) was used to differentiate the means.

rank	1	2	3	4	5	6
mm/sec	0.089	0.352	0.434	0.438	0.501	0.666
salinity %	20	30	40	60	80	100

SE = 0.0204367
$q_{0.005,\,54,6} = 5.465$
q values for those rank comparisons below 5.465 showed that 5 = 3, 4 = 2 and 3 = 2.

Rank 6, the control, (100% salinity) has a particle speed significantly different from rank 5 (80% salinity). Ranks 5 to 3 and 4 to 2 are not significantly different. Rank 1 (20% salinity) is significantly different. This assay can detect 80% salinity.

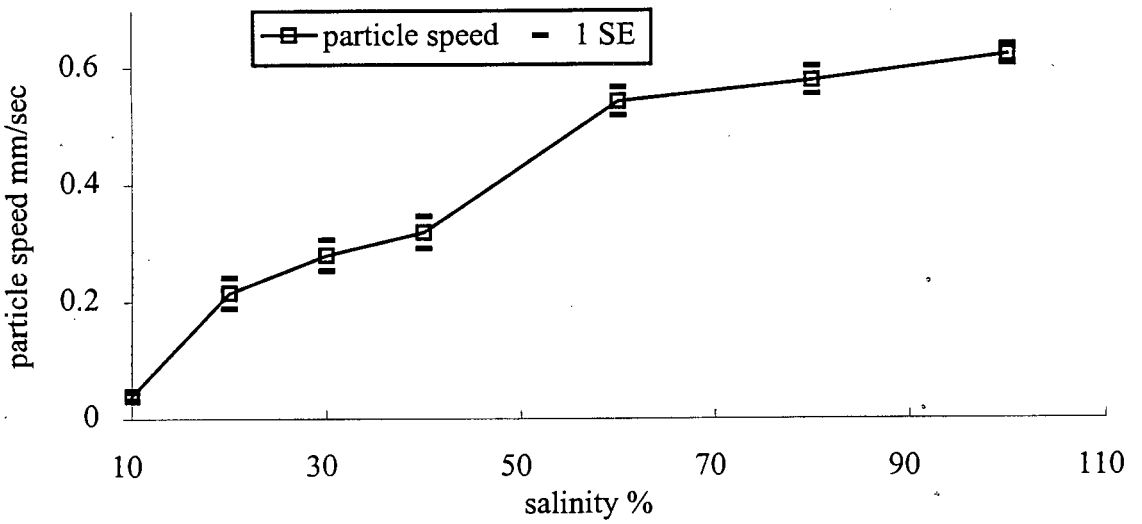


Figure 10. Salinity dependent variation in particle speed in a male *Choromytilus* (59.55 mm) at 24.5°C.

The assay was repeated (Figure 10). The data were subject to a one-way ANOVA test (Zar 1984). Mean particle speeds were compared over the range of salinity from 100% to 10%. The null hypothesis was that there is no difference between the means. The alternative hypothesis was that not all the means were the same.

Summary of ANOVA

	SS	d.f.	MS
total	3.132811	69	0.0454
groups	2.8061	6	0.4677
error	0.326741	63	0.0052

$$F = \frac{\text{groups MS}}{\text{error MS}} = 89.94$$

$F_{0.0025\,(1)\,6,63} = 3.87$

F calculated from the data = 89.94

Thus the null hypothesis is rejected: not all the means are the same. Tukey's test (Zar 1984) was used to differentiate the means.

rank	1	2	3	4	5	6	7
mm/sec	0.039	0.215	0.279	0.318	0.541	0.575	0.618
salinity %	10	20	30	40	60	80	100

SE = 0.022772

$q_{0.005,\,63,7} = 5.454$

q values for those rank comparisons below 5.454 showed that 7 = 5, 6 = 5, 4 = 2 and 3 = 2.

Rank 7, the control (100% salinity) is significantly different from rank 4 (40% salinity). Rank 4 is significantly different from rank 1 (10% salinity). This assay can detect 40% salinity and below.

Particle speed and ammonia

Single valve preparations from two mussels (Figures 11 & 12) were examined in this experiment.

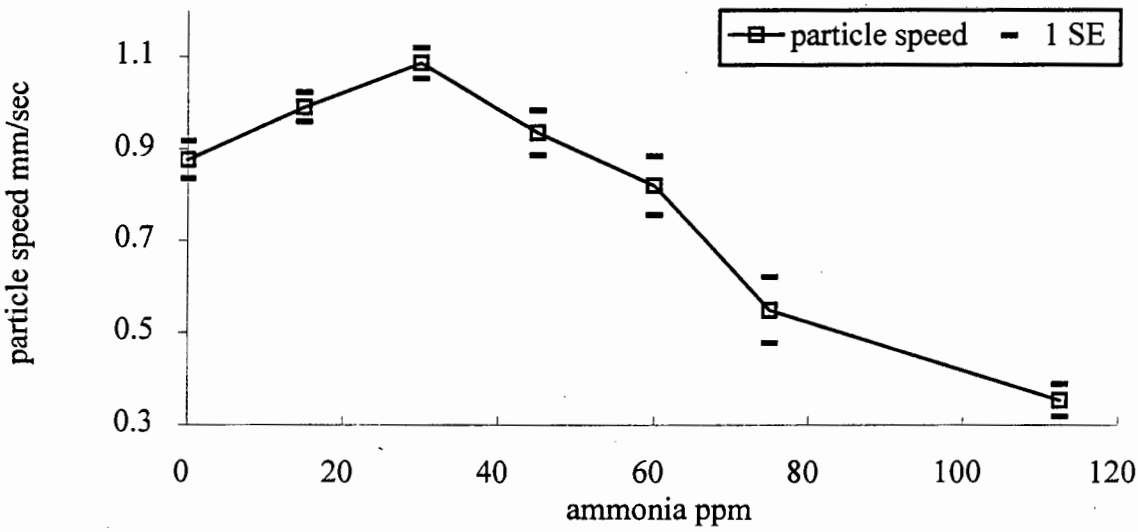


Figure 11. Variation in particle speed with ammonia concentration in a female *Choromytilus* (66.65 mm) at 22°C.

The results in figure 11 were subject to a one-way ANOVA test (Zar 1984). The mean particle speeds were compared over the range of ammonia concentrations of 0ppm to 112.5ppm. The null hypothesis was that there is no difference between the means. The alternative hypothesis was that not all the means were the same.

Summary of ANOVA

	SS	d.f.	MS
total	5.56033	69	0.0806
groups	4.0602	6	0.6767
error	1.50013	63	0.0238

$$F = \frac{\text{groups MS}}{\text{error MS}} = 28.43$$

$F_{0.0025\,(1)\,6,63} = 3.87$
F calculated from the data = 28.43

Thus the null hypothesis is rejected: not all the means are the same. Tukey's test (Zar 1984) was used to differentiate the means.

rank	1	2	3	4	5	6	7
mm/sec	0.353	0.548	0.819	0.876	0.935	0.991	1.086
NH_3 ppm	112.5	75	60	0	45	15	30

SE = 0.048785
$q_{0.005,\,63,7} = 5.454$
q values for those rank comparisons below 5.454 showed that 7 = 4, 6 = 3, 5 = 3, 4 = 3, 3 = 2 and 2 = 1.

Rank 4, the control, (0ppm NH_3) has a particle speed that is significantly different from that in rank 2 (75ppm NH_3). The control is not significantly different from any of the elevated cilia speeds. This assay can detect ammonia as an inhibitor of cilia activity at 75ppm and above.

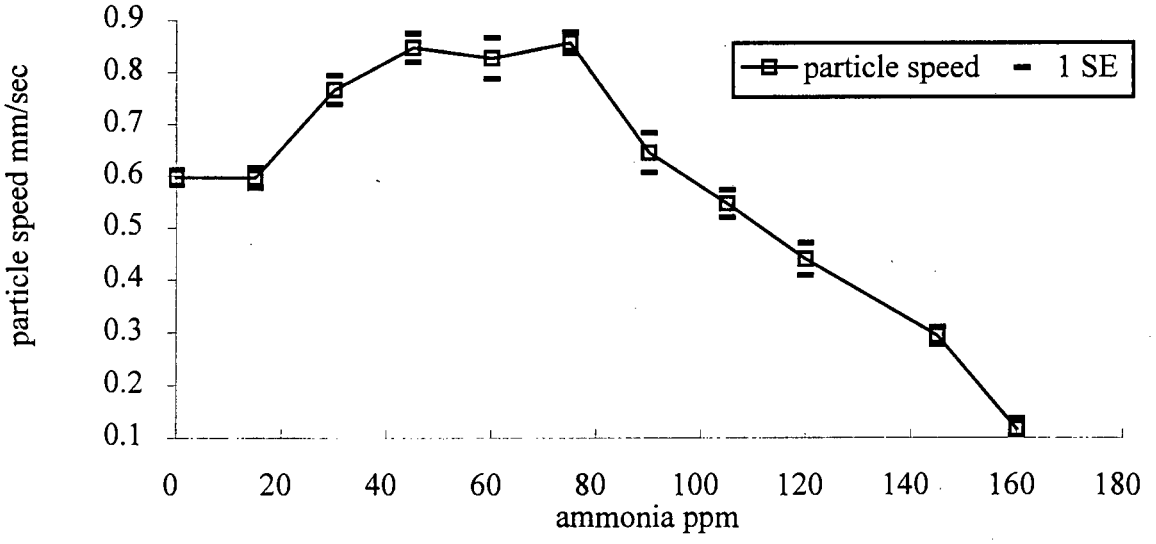


Figure 12. Variation in particle speed with ammonia concentration in a male *Choromytilus* (61.95mm) at 24°C.

The results in Figure 12 were subject to a one-way ANOVA test (Zar 1984). The mean particle speeds were compared over the range of ammonia concentrations from 0ppm to 160ppm. The null hypothesis was that there was no difference between the means. The alternative hypothesis was that not all the means were the same.

Summary of ANOVA

	SS	d.f.	MS
total	2.54549	109	0.02335
groups	1.7545	10	0.17545
error	0.70042	99	0.00707

$$F = \frac{\text{groups MS}}{\text{error MS}} = 24.82$$

$F_{0.0025\,(1)\,10,\,99} = 3.01$
F calculated from the data = 24.82

Thus the null hypothesis is rejected: not all the means are the same. Tukey's test (Zar 1984) was used to differentiate the means.

rank	1	2	3	4	5	6	7	8	9	10	11
mm/sec	0.1167	0.2935	0.4391	0.546	0.5964	0.5966	0.6443	0.766	0.8265	0.8465	0.8562
NH_3 ppm	160	145	120	105	15	0	90	30	60	45	75

SE = 0.02659
$q_{0.005,\,99,11} = 5.841$
q values for those rank comparisons below 5.841 showed that 11 = 8, 10 = 8, 9 = 8, 8 = 7, 7 = 4, 6 = 4, 5 = 4, 4 = 3, 3 = 2 and 2 = 1.

The control (rank 6) is not significantly different from rank 5 (15ppm NH_3), rank 7 (90ppm NH_3), or rank 4 (105ppm NH_3). But this does not make them equally innocuous. The significance of this will be discussed in the Synthesis (Chapter 48). The control (rank 6) is significantly different from rank 8 (30ppm NH_3), rank 9(60ppm NH_3) rank 10 (45ppm NH_3) and rank 11 (75ppm NH_3). All these concentrations elevate particle speed. It was noted that the cilia were moving somewhat more erratically but this had no effect on speed. The control (rank 6) is significantly different from rank 3 (120ppm NH_3) rank 2 (145ppm NH_3) and rank 1 (160ppm NH_3). All these concentrations are inhibitory. This assay can detect the stimulus threshold of 30ppm, and the inhibitory threshold of 120ppm ammonia.

The noteworthy feature in common with both Figures 11 and 12 is the biphasic influence of ammonia on particle speed. In Figure 12 both phases are significantly different from the control. Such an elevation might tempt one to interpret it as a eustress. The significance of this elevation is conflated with results from other experiments in the Synthesis (Chapter 48).

Particle speeds and combinations of ammonia and salinity

Half shell preparations of two mussels were used, the experiment began with normal sea water, after which the ammonia concentration was raised to 10ppm and the salinity was simultaneously lowered by 10%. This was continued in stages of 10 units of each until 80ppm NH_3 and 20% salinity was reached. Figure 13 shows the results for both mussels.

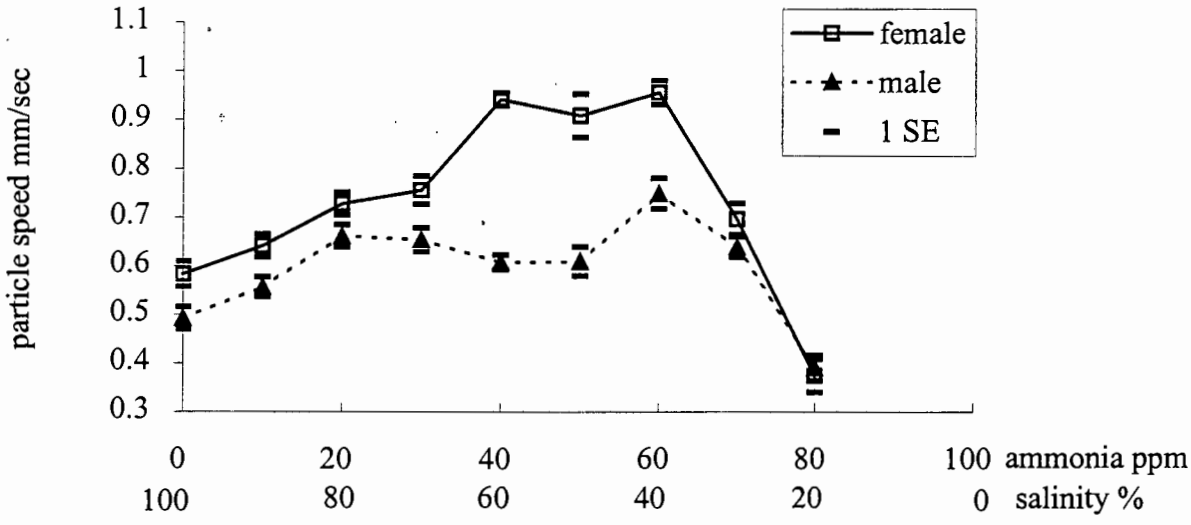


Figure 13. Variation in particle speed with rising ammonia concentration and falling salinity in a male *Choromytilus* (68.8mm) and a female *Choromytilus* (63.8mm) at 24°C.

Data for the female were examined first by a one-way ANOVA test (Zar 1984). The null hypothesis was that there is no difference between the means. The alternative hypothesis was that not all the means were the same.

Summary of ANOVA

	SS	d.f.	MS
total	6.0513	89	0.068
groups	5.38122	8	0.6727
error	0.67008	81	0.0082

$$F = \frac{\text{groups MS}}{\text{error MS}} = 82.03$$

$F_{0.0025\,(1)\,8,\,81} = 3.32$

F calculated from the data = 81.03

Thus the null hypothesis is rejected: not all the means are the same.

Tukey's test (Zar 1984) was used to differentiate the means.

rank	1	2	3	4	5	6	7	8	9
mm/sec	0.1077	0.583	0.641	0.696	0.727	0.755	0.908	0.941	0.955
NH_3 ppm	80	0	10	70	20	30	50	40	60
salinity %	20	100	90	30	80	70	50	60	40

SE = 0.02876

$q_{0.005, 81,9} = 5.673$

q values for those rank comparisons below 5.673 showed that 9 = 7, 8 = 7, 7 = 6, 6 = 3, 5 = 2, 4 = 2, and 3 = 2.

The control (rank 2) is not significantly different from ranks 3 (10ppm NH_3 & 90% salinity), 4 (70ppm NH_3 & 30% salinity) and 5 (20ppm NH_3 & 80% salinity). The control is significantly different from rank 6 (30ppm NH_3 & 70% salinity), 7 (50ppm NH_3 & 50% salinity), 8 (40ppm NH_3 & 60% salinity) and rank 9 (60ppm NH_3 & 40% salinity). All these are stimuli whose effect is significant. Rank 1 (80ppm & NH_3 20% salinity) is also a significantly different from the control but this is an inhibitor.

This assay can detect ammonia at 30ppm as a stimulant whose effect remains until 60ppm and only at 70ppm NH_3 does the difference become insignificant. At 80ppm NH_3 is detectable as an inhibitor. Salinities of 40%, 50%, 60%, and 70% can support particle speeds above that of control. Salinities of 30% and 80% and 90% cause no significant difference. Salinity of 20% is inhibitory.

The data for the male were analysed by a one-way ANOVA test (Zar 1984). The null hypothesis was that there is no difference between the means. The alternative hypothesis was that not all the means were the same.

Summary of ANOVA

	SS	d.f.	MS
total	1.3477	89	0.01514
groups	0.8756	8	0.10945
error	0.47217	81	0.00583

$$F = \frac{\text{groups MS}}{\text{error MS}} = 18.77$$

$F_{0.0025\ (1)\ 8, 81} = 3.32$

F calculated from the data = 18.77

Thus the null hypothesis is rejected: not all the means are the same.

Tukey's test (Zar 1984) was used to differentiate the means.

rank	1	2	3	4	5	6	7	8	9
mm/sec	0.39	0.492	0.556	0.607	0.609	0.639	0.653	0.661	0.748
NH_3 ppm	80	0	10	40	50	70	30	20	60
salinity %	20	100	90	60	50	30	70	80	40

SE = 0.02414

$q_{0.005, 81, 9} = 5.673$

q values for those rank comparisons below 5.673 showed that 9 = 6, 8 = 3, 7 = 3, 6 = 3, 5 = 2, 4 = 2, 3 = 2, and 2 = 1.

The control (rank 2) is not significantly different from ranks 1 (80ppm NH_3 & 20% salinity), 3 (10ppm NH_3 & 90% salinity), 4 (40ppm NH_3 & 60% salinity) and 5 (50ppm NH_3 & 50% salinity). The control is significantly different from rank 6 (70ppm NH_3 & 30% salinity), 7 (30ppm NH_3 & 70% salinity), 8 (20ppm NH_3 & 80% salinity) and rank 9 (60ppm NH_3 & 40% salinity). All these are stimuli to particle speed whose effect is significant. Thus a number of combinations are stimuli: none are inhibitors.

This assay can detect ammonia at 20ppm NH_3 as a stimulant whose effect remains until 60ppm and only at 80ppm NH_3 does the difference become insignificant. This is similar to the limits in the ammonia assay (Figure 12). Salinities of 30%, 40%, 70% and 80% can support particle speeds above that of control. Salinities of 20%, 50% and 90% cause no significant difference.

Analysis of combinations of salinity and ammonia

The above data have been simplified and summarised below. The dotted line ---- is the control datum, values (ppm or % salinity) on the line are not significantly different from the control. Values above the line are significant stimulants and values below are significant inhibitors. The sources of data are given under each representation.

The effect of ammonia only, all values are ppm ammonia:

stimulatory		**30**	**45**	**60**	**75**					
control	**15-***15*	*-30-*	*-45-*	*-60-*	-----	**-90-**	**-105-**	-------	**-115-**	-----
inhibitory					*75*			*112.5*		**120**

italic = data from Figure 11
bold = data from Figure 12

The effect of ammonia when combined with decreased salinity, all values are ppm ammonia:

stimulatory		*20*	*30* **30**	**40**	**50**	*60* **60**	*70*	
control	-**10**-*10*-	-**20**-	------	-*40*-	-*50*-	-------	-**70**-	-*80*-
inhibitory								**80**

italic = data from Figure 13 male
bold = data from Figure 13 female

Decreased salinity appears to have no marked influence on stimulatory or inhibitory effect of ammonia.

The effect of salinity only, all values are % salinity:

control	-----	----20-----	----30-----	----40----	-	--60-**60**-60-	---	-80-**80**-80-
inhibitory	10 **10**	20 **20** *20*	30 **30** *30*	40 **40** *40*		*60*		*80*

italic = data from Figure 9
bold = data from Figure 10
underlined = data from Figure 15 uninfected
font 8 = data from Figure 16 uninfected

The effect of salinity when combined with ammonia, all values are % salinity:

stimulatory	*20*	*30*	*40* **40**	**50**	**60**	*70* **70**	*80*	
control	-------	--**30**--	------	--*50*--	--*60*--	--------	--**80**--	--*90*-**90**--
inhibitory	**20**							

italic = Figure 13 male
bold = Figure 13 female

Ammonia appears to maintain elevation of particle speed as if the inhibitory salinity stress was not here. This interaction is obviously less than additive, or is even antagonistic. There appears to be an antidote relationship between elevated ammonia and decreased salinity at the level of the gill cilia activity.

Particle speed and parasites

A sample of eight *Choromytilus,* four infected with *Cercaria notobucephala* (see Chapter 3) and four uninfected, was taken and the particle speed from one valve in each was determined. There is (Figure 14) no apparent distinction between parasitised and unparasitised mussels. It can be concluded from this that particle speed cannot be used as an indicator of the presence of this parasite.

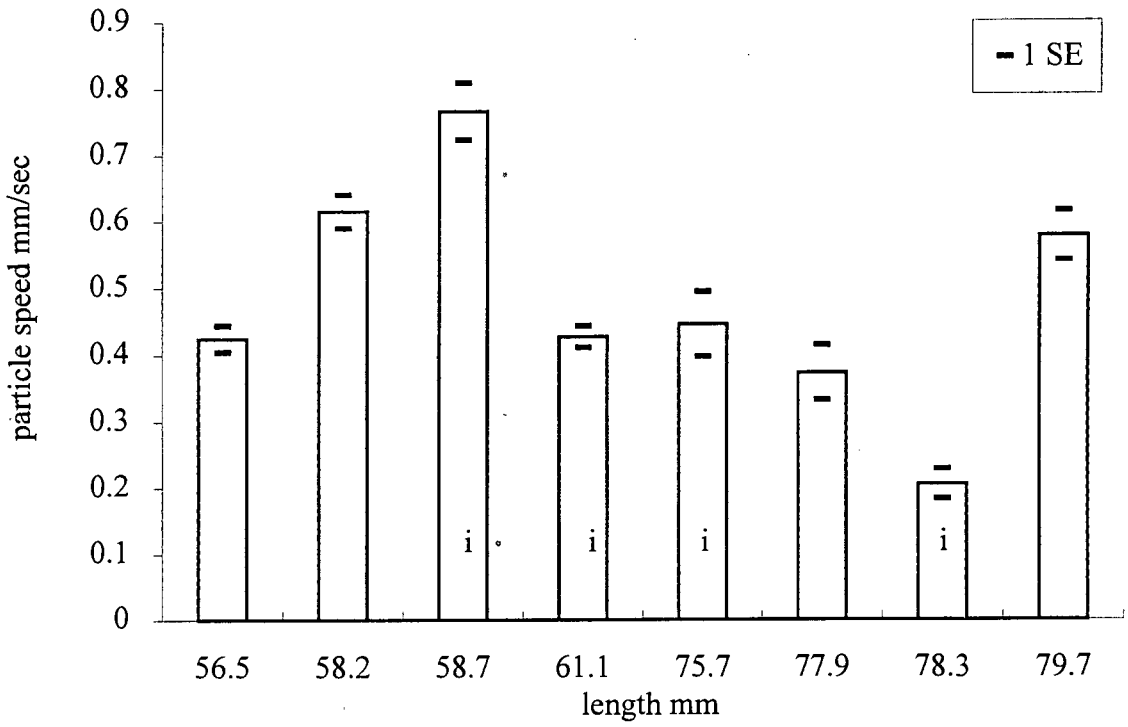


Figure 14. Variation in particle speed over the gill of *Choromytilus* and parasitism with *Cercaria notobucephala* (i = infected) in single valve preparations at 23°C.

The lack of difference between parasitised and healthy mussels is not surprising since Calvo-Ugarteburu (1996) found that infections of *Bucephalus* sp., a close relative of *Cercaria notobucephala*, had no effect on in the filtration rate of algal cells by *Perna perna*. Calvo-Ugarteburu (1996) also found no increase in oxygen intake concomitant with these infections. Thus in the case of *Perna perna* there was no increase in food intake or a decrease in oxygen consumption that could be ascribed to the parasites. Thus no extra energy is used to compensate for the loss that goes into parasite biomass. This is probably because reproductive energy allocation is used instead. This is discussed with reference to *Cercaria notobucephala* in the Synthesis (Chapter 48).

Particle speed and salinity: comparisons between infected and uninfected mussels

It was speculated that perhaps any deleterious loss of particle speed would only show if mussels were assailed by another stress as well as the parasite. To test this, two experiments were conducted in which the change in particle speed with salinity was

compared in infected and uninfected mussels.

The responses of two mussels, one infected and the other not, were compared when both were subject to salinity change (Figure 15).

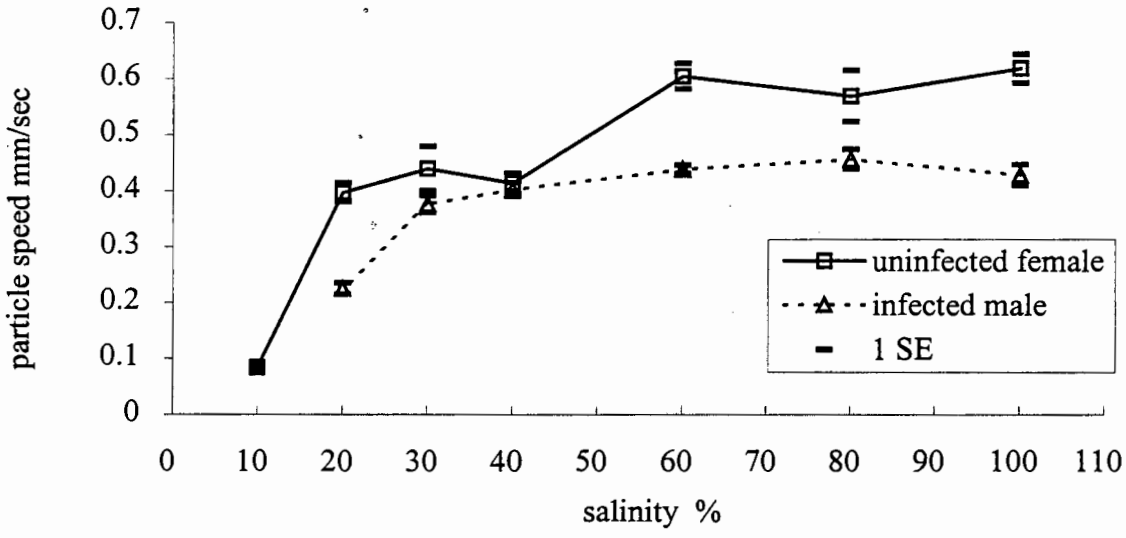


Figure 15. Comparison of variation in *Choromytilus* particle speed with salinity and parasitism with *Cercaria notobucephala* at 23°C.

The results for the uninfected mussel were examined first by a one-way ANOVA test (Zar 1984). The null hypothesis was that there is no difference between the mean particle speeds at different salinities. The alternative hypothesis was that not all the means were the same.

Summary of ANOVA

	SS	d.f.	MS
total	2.50757	69	0.03634
groups	2.02626	6	0.33771
error	0.481311	63	0.00764

$$F = \frac{\text{groups MS}}{\text{error MS}} = 44.2$$

$F_{0.0025\,(1)\,6,\,63} = 3.87$

F calculated from the data = 44.2

Thus the null hypothesis is rejected: not all the means are the same. Tukey's test (Zar 1984) was used to differentiate the means.

rank	1	2	3	4	5	6	7
mm/sec	0.0837	0.397	0.4133	0.4392	0.567	0.603	0.615
salinity %	10	20	40	30	80	60	100

SE = 0.02764

$q_{0.005, 63, 7} = 5.454$

q values for those rank comparisons below 5.454 show that 7 = 5, 6 = 5, 5 = 4, 4 = 2, and 3 = 2.

The control (rank 7) is not significantly different from ranks 6 (60% salinity) or 5 (80% salinity). The control is significantly different from rank 4 (30%) rank 3 (40%) 2 (20%) and 1 (10%). Thus, this uninfected mussel assay can detect salinities of 40% and below.

The results for the infected mussel were examined by a one-way ANOVA test (Zar 1984). The null hypothesis was that there is no difference between the mean particle speeds at different salinities. The alternative hypothesis was that not all the means were the same.

Summary of ANOVA

	SS	d.f.	MS
total	0.4569	59	0.00774
groups	0.3527	5	0.07054
error	0.10420	54	0.00193

$$F = \frac{\text{groups MS}}{\text{error MS}} = 36.55$$

$F_{0.0025\,(1)\,5, 54} = 4.30$

F calculated from the data = 36.55

Thus the null hypothesis is rejected: not all the means are the same.

Tukey's test (Zar 1984) was used to differentiate the means.

rank	1	2	3	4	5	6
mm/sec	0.225	0.3764	0.4021	0.4266	0.4373	0.4545
salinity %	20	30	40	100	60	80

SE = 0.01389

$q_{(0.005, 54, 6)} = 5.465$

q values for those rank comparisons below 5.465 show that 6 = 5, 5 = 4, 4 = 2, and 3 = 2.

The control (rank 4) is not significantly different from ranks 2 (30% salinity) 3 40% salinity, 5 (60% salinity) and 6 (80% salinity). The control is significantly different from rank 1 (20%). Thus this infected mussel assay can detect 20% salinity.

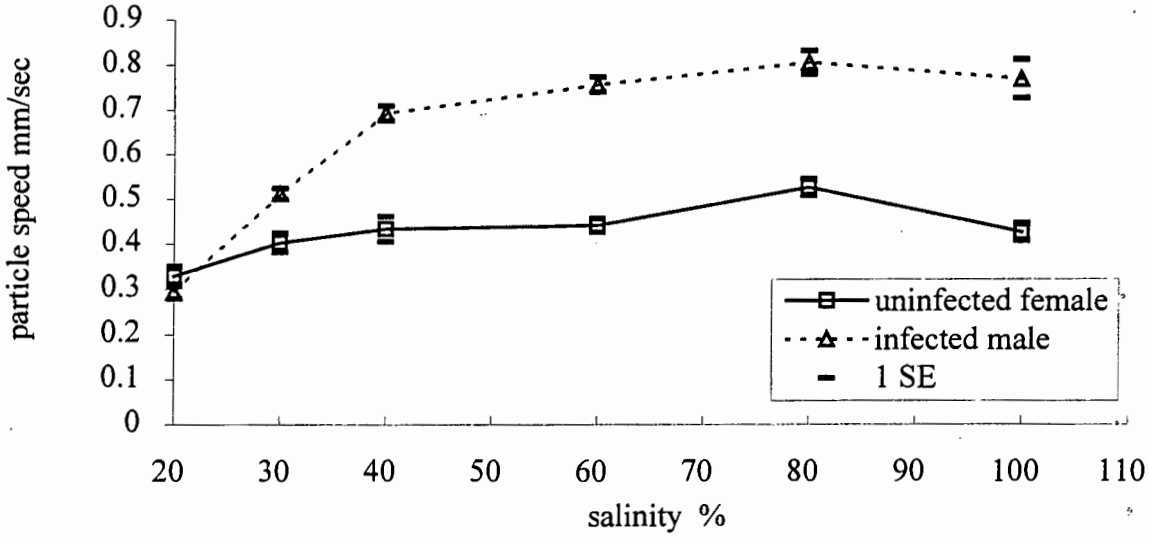


Figure 16. Comparison of variation in *Choromytilus* particle speed with salinity and parasitism with *Cercaria notobucephala* at 23°C.

Another pair of mussels were compared (Figure 16). Data for the uninfected mussel were examined first by a one-way ANOVA test (Zar 1984). The null hypothesis was that there is no difference between the mean particle speeds at different salinities. The alternative hypothesis was that not all the means were the same.

Summary of ANOVA

	SS	d.f.	MS
total	0.43774	59	0.007419
groups	0.1982	5	0.03964
error	0.23954	54	0.00444

$$F = \frac{\text{groups MS}}{\text{error MS}} = 8.93$$

$F_{0.0025\,(1)\,5,\,54} = 4.30$

F calculated from the data = 8.93

Thus the null hypothesis is rejected: not all the means are the same. Tukey's test (Zar 1984) was used to differentiate the means.

rank	1	2	3	4	5	6
mm/sec	0.329	0.402	0.424	0.432	0.44	0.524
salinity %	20	30	100	40	60	80

SE = 0.02724

$q_{(0.005,\,54,\,6)} = 5.465$

q values for those rank comparisons below 5.465 showed that 6 = 2, 5 = 1, 4 = 1, 3 = 1, and 2 = 1.

The control (rank 3) is not significantly different from any of the other ranks. Only rank 6 (80% salinity) is significantly different from rank 1 (20% salinity). Thus this

uninfected mussel assay cannot detect any salinity induced difference in particle speed.

Data for the infected mussel were then examined by a one-way ANOVA test (Zar 1984). The null hypothesis was that there is no difference between the mean particle speeds at different salinities. The alternative hypothesis was that not all the means were the same.

Summary of ANOVA

	SS	d.f.	MS
total	2.2578	59	0.0383
groups	1.9527	5	0.3905
error	0.30511	54	0.00565

$$F = \frac{\text{groups MS}}{\text{error MS}} = 69.12$$

$F_{0.0025\,(1)\,5,\,54} = 4.30$

F calculated from the data = 69.12

Thus the null hypothesis is rejected: not all the means are the same. Tukey's test (Zar 1984) was used to differentiate the means.

rank	1	2	3	4	5	6
mm/sec	0.293	0.511	0.6911	0.755	0.766	0.8029
salinity %	20	30	40	60	100	80

SE = 0.02377

$q_{0.005,\,54,\,6} = 5.465$

q values for those rank comparisons below 5.465 show that 6 = 3, 5 = 3, and 4 = 3.

The control (rank 5) is not significantly different from ranks 3 (40%), 4 (60%) and 6 (80%). The control is significantly different from ranks 2 (30%) and 1 (20%). Thus this infected mussel assay can detect salinity induced difference in particle speed at 30%.

Direct comparison of particle speeds between infected and uninfected mussels are meaningless. In Figure 15 the uninfected mussel has the higher particle speed. In Figure 16 the infected mussel has the higher particle speed. A comparison of significant detection limits (40% and no detection at 20% in uninfected mussels compared with 30% and 20% in infected mussels) also fails to disclose any marked differences between infected and uninfected mussels. In fact, infected mussels appear to be slightly more tolerant to lower salinity. This lack of difference is not surprising since one would expect particle speed to be a somatic indicator. Gasterostomes such

as *Cercaria notobucephala* are major reproductive stresses (see Chapters 3 & 47). One might expect their effect on somatic processes to be more muted and this is even more likely for such an incomplete integration as gill cilia activity.

An integrated response from a bisected mussel?

The limitations of this assay must be recognised: a bisected animal cannot give an integrated response. To put it more comprehensively, "The intact gill preparation does not offer a surrogate for the whole animal studies to assess the effects of contaminants in the environment" (Bayne & Thurberg 1988 p133). So why use this assay? It is used here primarily to demonstrate that more complete integrations are more sensitive. In consequence this assay will be compared with others in the Synthesis (Chapter 48).

This assay lacks integration but despite it being a cellular, or organ level phenomenon it may still have utility as an indicator of bioactive substances in the environment. It is quick, simple and convenient. Consequently it may be performed where others cannot, and any monitor is better than none - as long as its limitations are acknowledged. It should not be used to indicate safety but rather to detect that something may be wrong. Then it would be appropriate to use other more expensive, time consuming and accurate methods to characterise the problem.

CHAPTER 42: SHELL GAPING FREQUENCY AND STRESS AGENTS

INTRODUCTION

Shell closure is the principal defence of mussels and thus has implications for fitness. In consequence, this chapter charts an investigation of the effect of various agents on the closure response. Gaping and closure activity is monitored in the presence of physical, chemical and biological stress agents. It is hoped that these agents, regardless of origin, may be substantively integrated and rendered equivalent.

Immersed, undisturbed mussels in the laboratory exhibit (pers obs) a pattern of gaping and closure, and each mussel does so independently. Gaping goes on much longer than closure. Closure is usually without any apparent cause but in clean, aerated seawater, the mussels reopen promptly. Physical disturbance and chemical agents may also trigger a closure reaction and so isolate the mussel from its environment but here, reopening may be less prompt.

On one hand, closure obstructs the researcher. It restricts study of the effects of various agents on the mussel because a closed mussel is isolated from the agent. Furthermore, aspects of its physiological condition such as heart rate - whose pulse is seen in the fimbriae of the exposed mantle - are no longer visible. On the other hand, this loss is balanced by the whole body integration that the closure itself provides for study.

A mussel is safest when it is closed - noxious agents are kept out. But it must balance security with other needs; it must gape long enough to meet nutritional, excretory and gas exchange requirements. Any curtailment of gaping may thwart these requirements and so compromise fitness. Valve closure can be a favourable course of action only when it prevents a greater fitness loss by poisoning or predation. Effective management of these conflicting demands - security and openness - requires that the mussel allot appropriate fitness values to different agents. We might expect selection for an accurate cost/benefit analysis faculty in the mussel. But we would see this only in response to agents that occur in the environment and have influenced selection.

Thus unfamiliar agents may be expected to elicit responses that are not necessarily appropriate to optimal fitness.

MATERIAL AND METHODS

There are four main lines of investigation:

1. Varying salinity is chosen to assay closure response in *Mytilus galloprovincialis*, *Perna perna* and *Choromytilus meridionalis*. This will ascertain if a change in gaping activity can be used to detect fluctuation in levels of a familiar physical agent.
2. Similar experiments are run on the effect of varying phenol (an unfamiliar agent) concentration on the gaping frequencies of *Mytilus*, *Perna* and *Choromytilus*.
3. The effect of a constant concentration of phenol (100ppm) is investigated in *Choromytilus* over a period of 10 hours.
4. The time-dependent effects of constant levels of a variety of stresses (phenol, ammonia and *Burnupena* sp. - a scavenging whelk) on *Choromytilus* are investigated.

Freshly collected mussels were laid in containers side by side with the valve junction vertical to allow easy observation of valve opening. Groups of ten mussels were placed in 5 litres of water; groups of 30 were placed in 10 litres. No aeration was provided during the experiment because the disturbed surface of the water would have made observation difficult. In addition, switching off the air, or removal of the supply pipes and air stones would also have constituted a disturbance with attendant artefacts of closure stimulus. As an alternative, the water was aerated just before use and it was changed and/or aerated at intervals of 15 or 30 minutes depending on the experiment. It is unlikely that water quality would degrade significantly in that time; in some experiments, controls are provided to confirm this.

Salinity and closure response

In each test, a group of ten mussels was placed in five litres of normal salinity

seawater. After 15, 20 and 25 minutes the number open was noted. Thus three values for number of mussels gaping out of ten were obtained for each salinity. Salinity was changed by pouring off all the water into another tank and diluting it with distilled water to the correct percentage. The decreased salinity is expressed as a percentage of normal seawater salinity. The starting volume (5 litres) of the water at the new salinity was aerated and returned to the test tank; this decanting ensures that the water is well mixed. The mussels were left for 15 minutes and the number gaping were counted as above. This was repeated after five and ten minutes. The process was repeated for water of the next salinity value and so on.

The three values for each concentration were averaged and the standard error calculated. The points were then plotted on a graph as the mean number open at each salinity percentage. A control sample of ten mussels was also examined in the *Choromytilus* experiment. In the control the starting (100% normal seawater) salinity was kept constant and the number open was plotted at the same time as the varying salinity sample. The control water was also decanted, aerated, and replaced in the container to simulate the agitation received by the salinity experiment.

Data from the *Choromytilus* experiment were analysed statistically. Gaping frequency from the control was compared with gaping frequency at 100%, 90%, 80%, 70%, 60% and 50% salinity of normal sea water by ANOVA. This was then differentiated using Tukey's test (Zar 1984).

Phenol concentration and gaping response

Groups of ten *Mytilus*, and *Perna* were subject to a range of 0-500ppm phenol. Three values were obtained for each concentration as was done in the salinity experiment and their means were plotted with error bars. The experiment was repeated with 30 *Perna* at phenol concentrations of 0-200ppm, and with 30 *Choromytilus* at phenol concentrations of 0-400ppm. The effect of a constant concentration (100ppm) on gaping frequency was investigated in *Choromytilus* over 10 hours.

Comparison of physical biological and chemical agents

Four experiments were run concurrently: samples of 30 *Choromytilus* were exposed to either 10ppm phenol, 20ppm ammonia, the whelk *Burnupena* sp. or a sea water control. It was hoped that some of the *Choromytilus* might have been parasitised with *Cercaria notobucephala* (Chapter 3) to give some indication of their effect. But this trematode had recently become very scarce.

This experiment demonstrates that disparate agents can cause measurable effects on the same strain index (shell closure in this case). Salinity is the physical stress; mussels might be expected to meet this in their natural environment and they might be accustomed to dealing with it. Ammonia and phenol are two chemical stresses. The former might be expected to be a natural problem in rock pools. The latter is probably a novel stress. Thus there may be a difference in response to these two. *Burnupena* sp. is a scavenging whelk whose presence was seen to inhibit shell opening in pilot studies (pers obs). A control group in seawater was also monitored. The concentrations of the stresses used were suggested by the previous experiments in this chapter. Ammonia of 20ppm was chosen because it is below the detection threshold for cilia particle speed: in Chapter 41 it was found that ammonia concentrations of 30ppm to 100ppm stimulated gill cilia activity in *Choromytilus*. Thus ammonia at 20ppm was used to see if this whole body integration was more sensitive. Phenol at 10ppm was used to see if the depression of gaping frequency at that concentration as shown in Figure 4, 5 & 6 is stable over the longer term.

The experiment was run for 10 hours and gaping mussels were counted at 30 minute intervals. No replicates were taken because of the large number of mussels to be counted at each time. The results are plotted and the totals from each treatment averaged over the exposure period. Difference between the groups was detected by ANOVA; Tukey's test (Zar 1984) differentiated the groups.

RESULTS

Salinity

Relationships of percentage salinity and number of mussels open out of 10 were plotted for *Mytilus* (Figure 1), *Perna* (Figure 2) and *Choromytilus* (Figure 3).

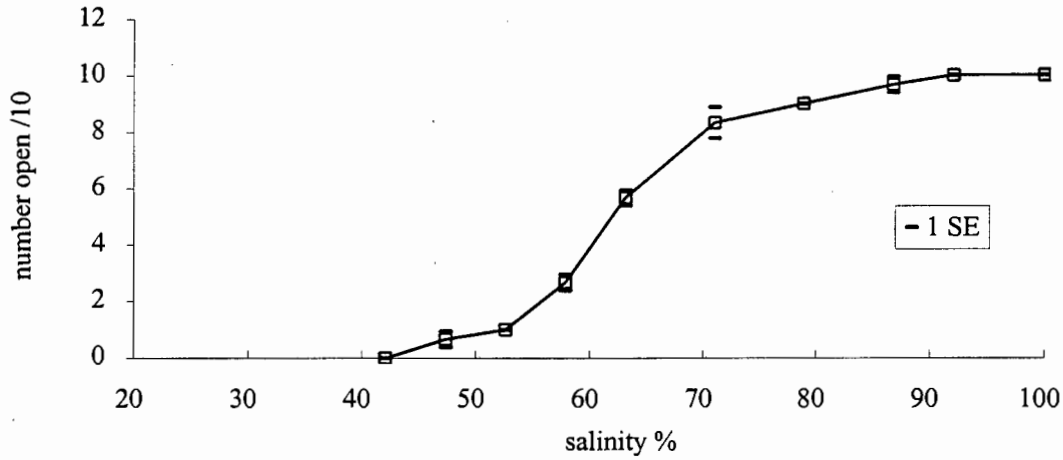


Figure 1. The effect of varying salinity on gaping frequency in *Mytilus* from Saldanha at 19°C.

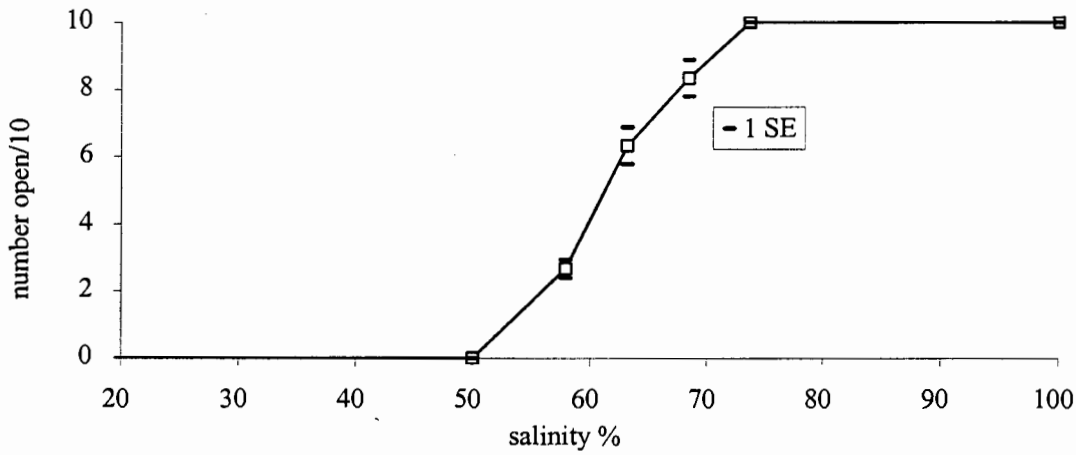


Figure 2. The effect of varying salinity on gaping frequency in *Perna* from Dido Valley at 15°C.

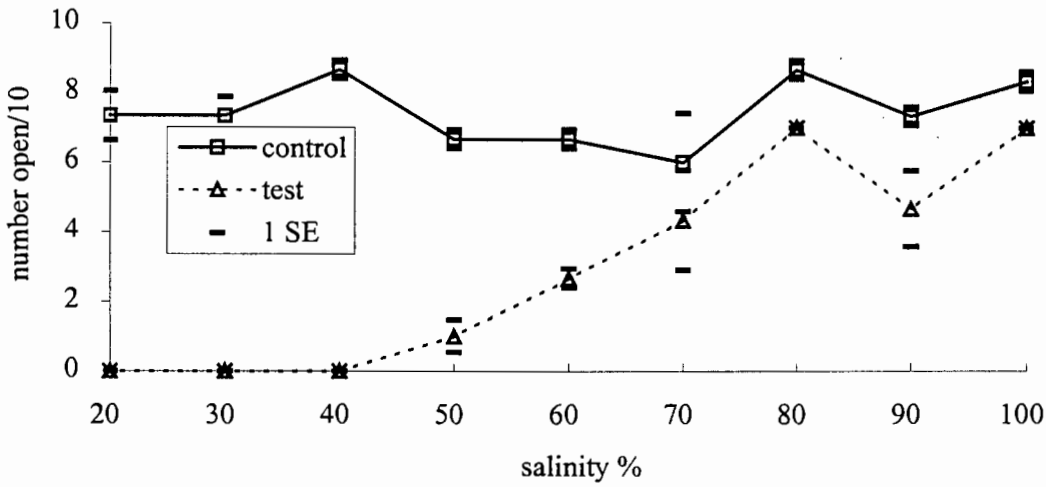


Figure 3. The effect of salinity on numbers gaping in a sample of *Choromytilus* from Blouberg at 15°C.

Data from Figure 3 were analysed by ANOVA and then differentiated by Tukey's test. The null hypothesis is that all salinities have the same effect on gaping numbers. The alternative hypothesis is that not all have the same effect on gaping numbers.

Summary of ANOVA

	SS	df	MS
total	221.56	26	8.5215
group	188.88	8	23.61
error	32.68	18	1.815556

$F = \frac{GMS}{EMS} = \frac{23.61}{1.815556} = 13.004$

F calculated from the data = 13.004

$F_{0.0025\,(1)\,8,\,18} = 4.89$

The null hypothesis that all salinities have the same effect is rejected. Tukey's test (Zar 1984) was used to differentiate the samples.

rank	1	2	3	4	5	6	7	8	9
salinity %	100	80	90	70	60	50	40	30	20
no. gaping/10	7	7	4.66	4.33	2.67	2.33	0	0	0

SE = 0.7779

$q_{0.005\,18,\,9} = 6.554$

q values for those rank comparisons below 6.554 showed that 1 = 6, thus the control does not differ from salinities down to and including 50%. Thus this test can distinguish 40% salinity as a significant cause of shell closure.

During the salinity fluctuation test in figure 3 a seawater control was also run to see if the valve opening activity changed significantly over time. The data were subject to ANOVA. The null hypothesis is that all samples of the control (seawater) have the same effect on gaping numbers. The alternative hypothesis is that not all samples of the control (seawater) have the same effect on gaping numbers.

Summary of ANOVA

	SS	df	MS
total	50.67	26	1.9488
group	21.31	8	2.6638
error	29.36	18	1.6311

$F = \frac{GMS}{EMS} = \frac{2.6638}{1.6311} = 1.6331$

F calculated from the data = 1.6331

$F_{0.0025\,(1)\,8,\,18} = 4.89$

The null hypothesis that all the control samples are the same is accepted.

Opening response and varying concentrations of phenol

Figure 4 shows the number of *Perna* and *Choromytilus* open out of ten, figure 5 shows the number of *Perna* open out of 30, all in response to different concentrations of phenol. Figure 6 shows the number of *Choromytilus* open out of 30 in response to different concentrations of phenol. The relationship between number open and the concentration of phenol was examined statistically between the concentrations of 175ppm - 400ppm from figure 6:

r^2	0.6699
n	30
P	0.001

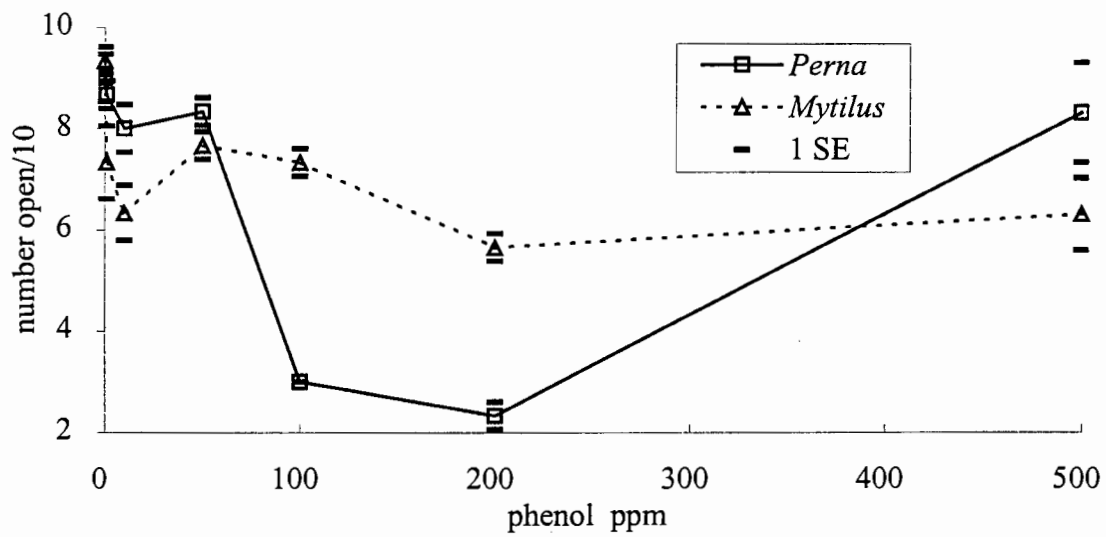


Figure 4. The effect of varying phenol concentrations on gaping frequency in *Mytilus* from Saldanha and *Perna* from Dido Valley at 19°C.

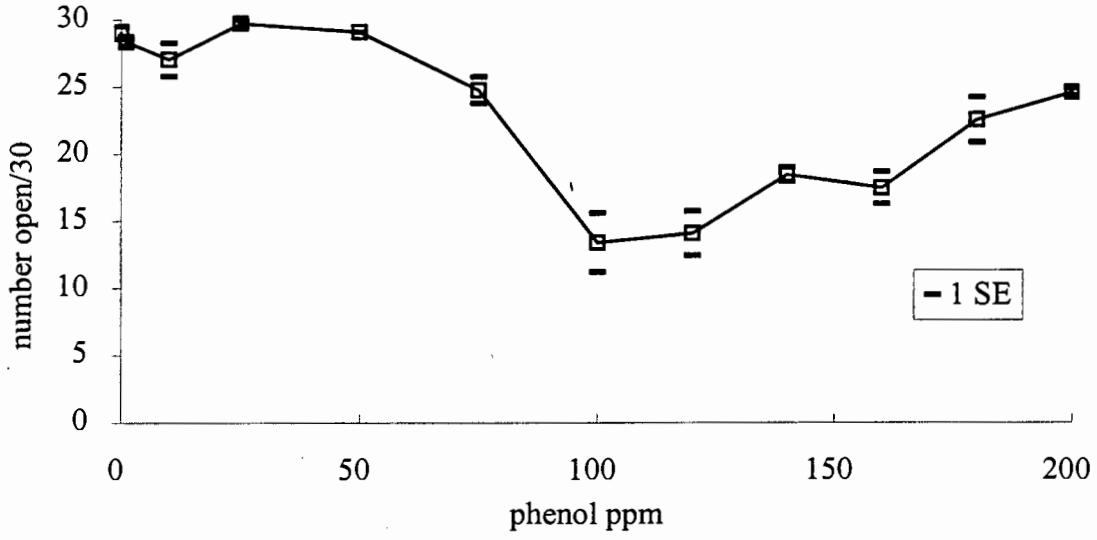


Figure 5. The effect of phenol on gaping frequency in *Perna* at 19°C.

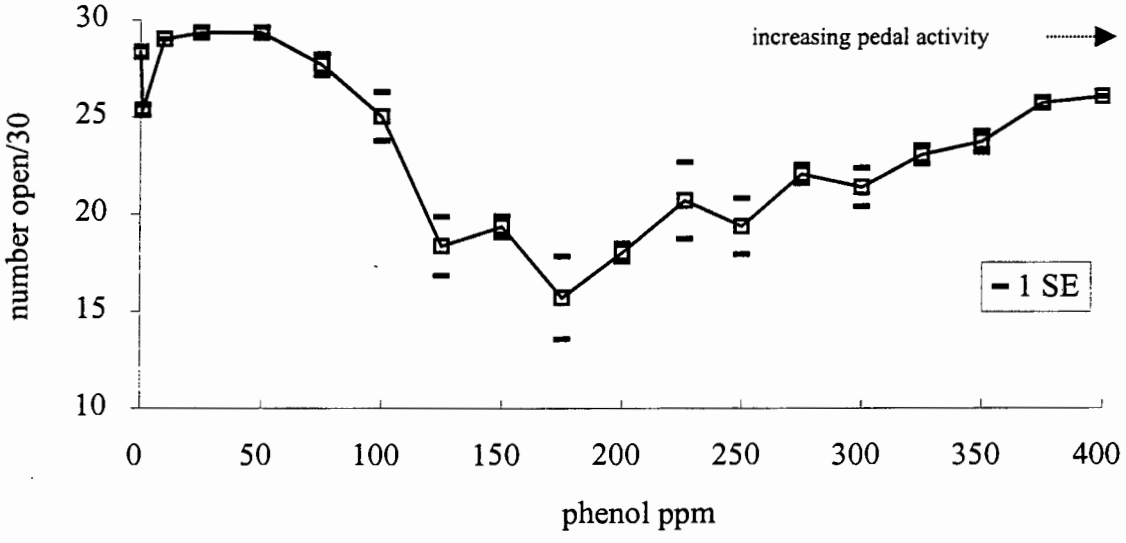


Figure 6. The effect of varying concentrations of phenol on gaping frequency in *Choromytilus* at 19°C.

Figure 7 shows the time dependent gaping response of 30 *Choromytilus* to a fixed concentration 100ppm of phenol. The data from figure 7 were analysed statistically:

r^2 = 0.246656
n = 63
P = 0.001

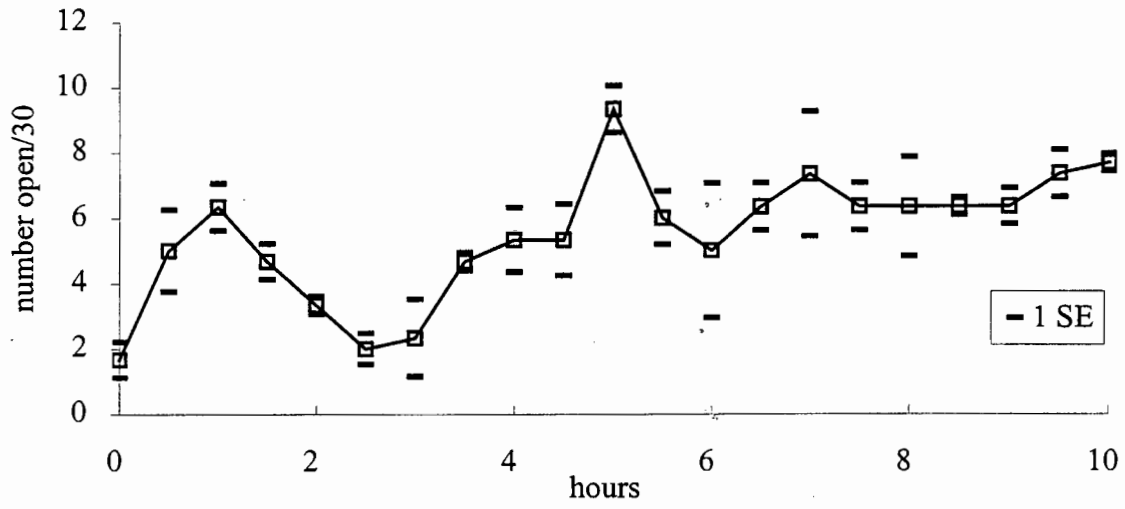


Figure 7. Temporal effect of constant 100 ppm phenol on numbers of *Choromytilus* gaping at 19°C.

The effect of chemical, physical and biological agents

Each *Choromytilus* was measured; the mean sizes of the four samples, each of thirty, are shown below. There is very little difference between the sample means and inspection of the standard errors suggests it is not significant. Thus the experiment is not biased by any possible size dependent difference in response.

	Mean length mm	SE	n
Control	65.603	0.5163	30
Phenol 10ppm	64.923	0.4969	30
Ammonia 20ppm	64.918	0.5190	30
Whelks	65.460	0.5238	30

Figure 8 shows the number of *Choromytilus* gaping in each of four samples (control, phenol, ammonia and *Burnupena*) at 30 minute intervals over a ten hour period. Figure 9 depicts the mean number, with error bars, gaping in each sample over the ten hours of the experiment and it suggests that they may be significantly different.

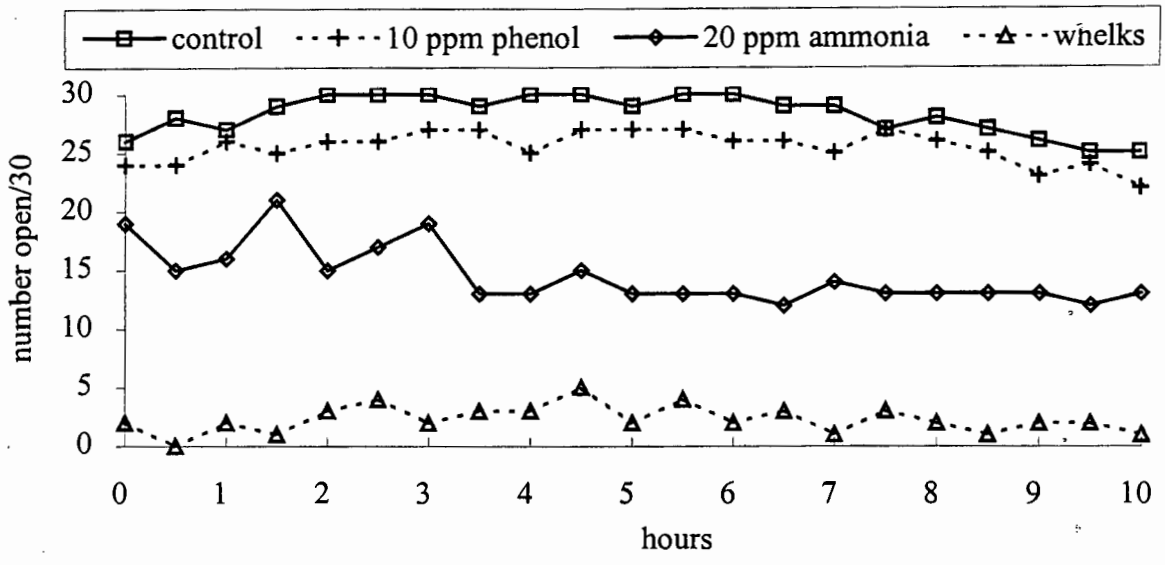


Figure 8. The temporal effect of various agents on gaping frequency in *Choromytilus* at 15°C.

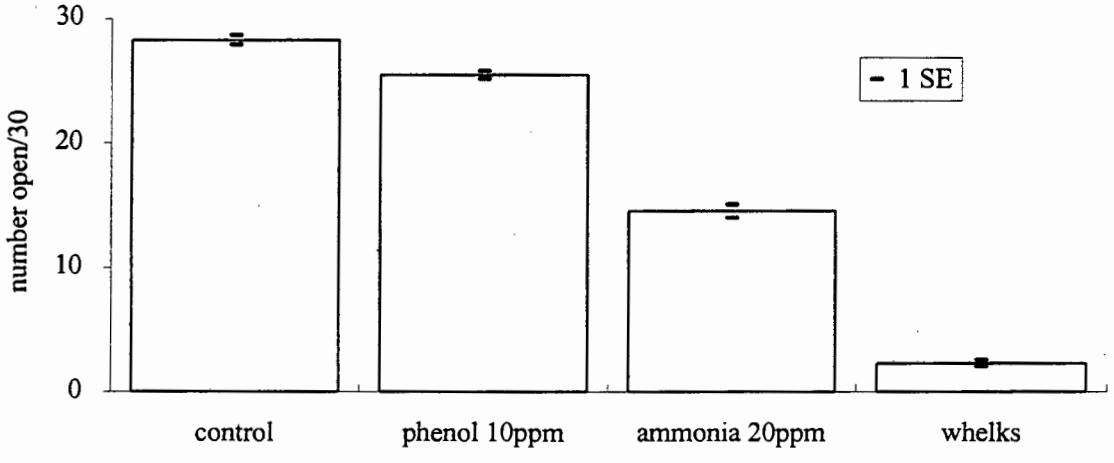


Figure 9. The effect of various agents on mean numbers of *Choromytilus* gaping over 10 hours at 15°C.

A one-way ANOVA test (Zar 1984) was performed to confirm this. The null hypothesis was that all treatments result in the same numbers gaping. The alternative hypothesis was that not all treatments result in the same numbers gaping.

Summary of ANOVA

	SS	DF	MS
total	9081.290	83	
groups	8824.234	3	2941.41
error	257.056	81	3.1736

$$F = \frac{GMS}{EMS} \quad \frac{2941.41}{3.1736} = 926.84$$

Critical region $F_{0.0025\,(1)\,3,\,81} = 5.19$
Calculated from data F = 926.84
The null hypothesis is rejected: not all treatments result in the same numbers gaping.

Tukey's test (Zar 1984) was used to differentiate the treatments:

rank	1	2	3	4
agent	whelks	ammonia 20ppm	phenol 10 ppm	control
mean gaping/30	2.2857	14.5238	25.476	28.2857

SE = 0.38875
$q = {}_{0.005\,81,\,4} = 4.928$

q values for all rank comparisons were more than 4.928, thus none of the ranks are equal. This separates all of the above agents: from the control and from each other. Whelks are the most stressful and 20ppm ammonia is more stressful than 10ppm phenol.

DISCUSSION

Salinity

All three species of mussel (Figures 1, 2 & 3) exhibit proportionality of response to varying salinities. Thus in principle the response/salinity slope could be ascertained and compared for each species. In this case only Figures 2 & 3 are strictly comparable because the variation of gaping and salinity in the *Mytilus* experiment was conducted at 19°C, those for *Choromytilus* and *Perna* were conducted at 15°C.

The control in Figure 3 shows that for *Choromytilus* in seawater, the gaping numbers remain substantially the same over the experimental period. Thus the depression of numbers open is not time dependent. It is reasonable to expect the same of the other two species.

All three showed a sigmoidal curve, with *Perna* being the most sensitive to low salinity. In *Perna*, maximum opening (10/10) persisted from 100% to 70% salinity. Between 70% and 50% salinity, gaping decreased to 0/10. In *Mytilus* and

Choromytilus the transition is much more gradual. The control (Figure 3) did not fluctuate significantly (P = 0.0025) and it shows no perceptible increasing or decreasing trend. This experiment can detect a difference in gaping frequency in water of 40% salinity.

Phenol

Perna and *Mytilus* both show (Figure 4) a dip in gaping frequency at low concentrations, followed by a recovery at about 50ppm. Thereafter the gaping numbers decline. But from 200ppm to 500ppm, gaping numbers increase as narcosis occurs. Test mussels were placed in flowing seawater after the experiment but nine out of ten of each were dead within 24 hours.

The experiment was repeated with 30 *Perna* (Figure 5) from 0-200ppm. The smaller range allowed scrutiny of the dynamics of depression, stimulation and narcosis. It was also hoped that the lower concentration would be sub-lethal. Again, the basic shape of the curve is as before with a small depression under 20ppm. At 75ppm the mussels cast off their byssus. From 20ppm to 100ppm, gaping numbers decline. Thereafter mussels begin to open between 100ppm and 200ppm. Excess mucus production begins at about 180ppm. Mussels were returned to clean flowing water after the experiment and all survived from 20th to 24th August 1997. Thus 200ppm is indeed sub-lethal.

The experiment was repeated with *Choromytilus* (Figure 6) over a range of 0-400ppm phenol. A similar pattern of response was seen: mucus production began at 150ppm, the concentration of least gaping was 175ppm, which was also where byssus was cast off. Pedal activity increased from 250ppm and higher. From this it can be concluded that the narcotic effect is limited to adductor muscles and not the foot musculature. The mussels were returned to clean flowing seawater and all were alive after 24 hours. The results show that 400ppm is not lethal to *Choromytilus*.

The pattern of response to varying concentrations of phenol is similar in all three species of mytilids (Figures 4, 5& 6). The depression of gaping at about 20ppm is

recurrent in four separate experiments and appears to signify sensitivity; perhaps initial detection of phenol causes this small depression. At 50ppm, valve opening increases, apparently as a flushing response to an irritant. This irritant response is used to induce faster valve activity in Chapter 44. Increased closure at higher concentrations (up to about 100ppm) may be ascribed to sensitivity of mussels to the toxic effects of the phenol. At concentrations beyond this, a gradual increase in gaping may be caused by narcosis. That it is induced by increased concentration rather than exposure time is evident by examination of the regression coefficients slopes of figure 6 (from 175pp-400ppm) and figure 7. In the former $r^2 = 0.6699$ which means that 66.99% of variation in gaping is attributable to concentration. In the latter $r^2 = 0.2466$ which means that only 24.66% of variation in gaping is attributable to period of exposure. In both cases the values are significant to P = 0.001.

In the 30 *Choromytilus* in constant 100ppm phenol (Figure 7) over 10 hours, most remained closed. There was a shallow trend to increasing gaping with time which suggests a minor degree of narcosis but this must be balanced against the fact that even after 10 hours, most of the mussels identify 100ppm phenol as a threat and isolate themselves from it. After returning to clean water all the mussels were open and were still alive 24 hours later.

Chemical, biological and physical stresses

In a comparison of gaping frequency in a control group, 10ppm phenol, 20ppm ammonia and *Burnupena* sp., none of the four curves overlapped (Figure 8). Only once did the curves touch: the control and 10ppm phenol. When the total mean gaping frequency was charted for the four groups (Figure 9) the standard errors suggested that all the groups may be significantly different with perhaps the exception of the control and 10ppm phenol. On testing by ANOVA and differentiation with Tukey's test it was found that they are all significantly different (P = 0.005) from one another. This means that all the agents had an effect significantly more than the controls; thus they can all be construed as a stress by the same criteria. If the control gaping frequency is taken as a normal value and assigned 100% then 10ppm phenol

causes 90.07% of normal performance. Percentages attributable to the other agents are 51.35% for 20ppm ammonia and 8.08% for *Burnupena* sp.

As mentioned in Chapter 27, the utility of valve gaping as a detector of pollutants was reviewed by Gosling (1992) who asserts that valve closure is sensitive only at concentrations near lethal. Gosling did not mention the species of mussels to which this applies. In contrast, the results in this chapter show that in *Choromytilus* valve closure can detect phenol at 10ppm, when concentrations of up to 400ppm have been shown to be non-lethal even after several days.

The water control (Figure 8) showed a slight hump in opening frequency. Its maximum opening was from 2 hours to 6 hours in the 10 hour experiment. This hump was also discernible in the 10ppm phenol (Figure 8). It is more important to note that there is no trend of increasing gaping frequency suggestive of irritation or narcosis at 10ppm phenol. The 20ppm ammonia (Figure 8) sample started with large fluctuations from time 0 to 3 hours, 30 minutes after which it became fairly stable. The sample with predatory whelks had a very low level of opening with a slight fluctuation and slight humping. It can be concluded that any time dependent fluctuation is not significant over the duration of the experiment. The relevance of these results is further discussed in relation to results from other chapters in the Synthesis (Chapter 48).

CHAPTER 43: AGENTS THAT INFLUENCE BYSSUS THREAD PRODUCTION IN *CHOROMYTILUS MERIDIONALIS*

INTRODUCTION

"Rate of thread formation is an easily measured and informative index of activity" (Van Winkle 1970, p143). As discussed in Chapter 27 & 40, byssus production is a short-term process that approaches the ideal of a whole body integration in respect to stress effects in mytilids. Sensory co-ordination, energy and anabolic synthetic capacity are all required for the production of byssus material from the byssus gland and for the physical act of placing it with the extensible muscular foot. Other aspects of the sensory system are also integrated as byssus attachment cannot be performed if defensive valve closure has been stimulated by any perceived threat. Such wide integration of somatic processes allows numerous entry points for the influence of deleterious agents on rates of byssus formation.

Mussels show a strong propensity to attempt reattachment to the substratum. This is not surprising as increased survival probability accrues from such action. Unattached mussels cannot feed efficiently and they are at the mercy of currents. Thus any agent that depresses byssus production in detached mussels may be construed as deleterious to fitness and is in consequence a stress. Disparate agents, in principle, can be quantified in the same units and integrated as stress agents.

Byssus is vital to the mytilid mode of life. For example in *Mytilus edulis* its byssus provides attachment of the mussel to the substratum and byssus musculature orients the mussel to optimise its exposure to water currents (Dolmer, Karlsson, & Svane 1994). Besides providing an anchorage, byssus allows the mussel to move by casting off old threads and attaching new ones, leaving a trail of byssus adhesion patches and broken threads. By this method, *Choromytilus meridionalis* and *Mytilus galloprovincialis* in the aquarium can climb more than thirty centimetres in twenty-four hours (pers. obs). Further details of the mytilid byssus apparatus may be found in Yonge (1962), Bayne (1976) and Burzio, Fuente, Gutierrez, Saez, Brito, Burzio, Burzio, Weiss, & Pardo (1989). In particular, Burzio *et al.* (1989) discuss the

adhesive properties of the byssus attachment protein (polyphenolic protein PPP) in *Aulacomya ater*, *Mytilus galloprovincialis*, *Choromytilus chorus* and *Perumytilus purpuratus*. They found that adhesion of these proteins to glass, slate, ceramic and plastics is influenced by such conditions as pH, and temperature. Inoue, Takeuchi, Miki, & Odo (1994) describe the structure and sites of production of the polyphenolic proteins produced by *Mytilus galloprovincialis*.

Besides its importance in attachment, orientation and transport, byssus may also be used in defence. Day, Barkai & Wickens (1991) report this in *Choromytilus meridionalis* and *Mytilus galloprovincialis* against the predatory gastropod *Nucella* sp. and to a lesser extent against *Burnupena* sp. from the West Coast of South Africa. Petraitis (1987) gives a similar report for *Mytilus edulis* against *Nucella lapillus* on the Atlantic coast of USA. The gastropods are immobilised by byssus threads from surrounding mussels. Other biological threats to mussels include parasites, and Lauckner (1983) decries the paucity of studies linking byssus production with parasitism. He remedies this by reporting (Lauckner 1984) that byssus thread production is detrimentally affected in *Mytilus edulis* when heavily infected with metacercarial cysts of trematodes.

The effects of trematodes were not examined here because the only severe infection (*Cercaria notobucephala*) had become considerably less common, thus requiring a much greater array of samples in each experiment to give any hope of including an infected mussel - much less a representative sample. Such numbers of mussels in each experiment would have been impracticable. Although other parasites are common in the mussels, experiments such as emersion survival (Chapter 45) have shown their effect on somatic fitness to be marginal. Since there are numerous other agents that are worthy of testing with the promise of more clear cut results, parasites are not included in these byssus studies. In consequence, the following series of experiments compares the rates of byssus production in the presence of chemical (ammonia and phenol), physical (lowered salinity) and biological (*Burnupena* - scavenging whelks) agents.

Ammonia was chosen because it is likely to be a physiologically familiar stress; phenol is an unfamiliar stress. Any difference in response to these two agents may shed light on mechanisms of dealing with familiar and unfamiliar stresses (this will be dealt with in the Synthesis at the end of the thesis). Lowered salinity is likely to be a familiar stress, as is the presence of *Burnupena.*

Burnupena was chosen as the representative biological stress despite the report of Day, Barkai & Wickens (1991) that mussels attach byssus to *Burnupena* sp. less readily than to *Nucella* sp. It can be inferred that the mussels either perceive *Nucella* sp. to be the greater threat or that these whelks are more vulnerable to this form of defence than *Burnupena* sp. *Nucella* sp., clearly, would be the preferable subject for this experiment but only specimens of *Burnupena* sp. were found in association with the mussels collected. In addition, pilot studies revealed that *Burnupena* sp. stimulated valve closure. Thus because of their availability and status as natural co-fauna with the mussels, their effect on byssus production is examined here.

MATERIALS AND METHODS

Mussels were placed singly, anterior down, in 250ml beakers. A test tube (150mm x 15.5mm) was placed in each beaker to help orient the mussel in a natural position and to ensure that its foot could reach easily a surface for byssus attachment. Ten such beakers, tubes and mussels were allotted to each test bucket. The buckets were then filled to the 10 litre level and supplied with vigorous aeration. Beakers, tubes and mussels remain submerged at the bottom of the bucket. The beakers separate individual mussels while allowing them all to be subject to the same regime. Mussels are negatively geotropic; any that attempt to climb up the beakers will stop at the rim, which is submerged. If mussels had been placed loose in the bucket, they might have climbed to the water surface, thus reducing their exposure to the agent and, more importantly, it would have allowed greater confusion during counting as byssus tracks would cross and mussels would be likely to form clumps in which counting of byssus is difficult. Each mussel was identified by a roman numeral scratched on the shell. Corresponding beakers were numbered using a diamond pencil.

Byssus growth was assessed after each 24 hours. Byssus anchor patches on the beaker or on the test tube were counted rather than just intact threads. This is because mussels break their own byssus as they climb, and so the total number of patches is a more accurate indication of byssus production than intact threads. It was also sometimes necessary to count byssus anchor patches on the bucket wall and on the shells of other mussels when they had aggregated. All byssus patches were removed after each counting. In contrast to the findings of Van Winkle (1970), who found that up to 50% of the byssus were attached to the mussels' own shell, it is a relief to report (pers. obs.) that *Choromytilus* showed little propensity to affix byssus threads to their own shells.

All mussels were included in the calculation of average byssus production whether or not they produced byssus. This contrasts with Van Winkle (1970) who omitted non-producing mussels from the data. Clearly, non-production is even more of an indication of deleteriousness than merely depressed production. In experiments that continued for more than 24 hours, the water (and agent) was changed every 24 hours. Thus each bucket had one mussel per litre of water that was constantly aerated and changed every 24 hours. All experiments were performed at a constant temperature of 15°C.

RESULTS AND DISCUSSION

Byssus growth over 24 hours

All mussels were used immediately after collection from the shore. They had, therefore, not been subject to similar tests on previous days but they all had been subject to one byssus detachment. Tests on some of these mussels continue over several days and these extra results are presented elsewhere. The mean number of byssus threads produced daily from each sample of 10 mussels in different treatment regimes was compared using ANOVA and then differentiated with Tukey's test (Zar 1984).

One way ANOVA test:

H_o: all means of byssus production are equal
H_1: not all means are equal
P = 0.0025
Critical region $F_{[0.001\ (1)\ 9.90]} = 3.13$
Calculated from data F = 18.43
H_o is rejected: not all the means of byssus production are equal.

Summary of ANOVA

	SS	df	MS
total	3152.51	99	31.844
groups	2043.81	9	227.09
error	1108.7	90	12.3189

$$F = \frac{227.09}{12.3189} = 18.434$$

ANOVA shows that some treatment regimes differ significantly from others, Tukey's test is used to identify these.

Tukey's test

$q_{0.005\ 90,\ 10} = 5.762$
SE = 1.099

Rank	1	2	3	4	5	6	7	8	9	10
Treatment	50% H_2O	40ppm NH_3	30ppm phenol	whelks	10ppm NH_3	15ppm phenol	80% H_2O	control	control	5ppm phenol
mean size mm	73.4	72.3	75.9	75.9	78.7	76.1	72.8	70.9	78.6	79.2
no.byssus	0.4	0.5	1.2	1.9	4.3	7.1	7.1	8.7	9.6	14.9

The means of 10 mussel samples, each of 10 individuals, are ranked above. Tukey's test shows that: 10 = 8, 9 = 5, 8 = 5, 7 = 3, 6 = 3, 5 = 1, 4 = 1, 3 = 1, & 2 = 1 (P = 0.005).

Detection limits for the 24 hour byssus growth experiment

This assay using ten mussels in each sample will detect 50% salinity or less, more than 40ppm ammonia, more than 30ppm phenol, and the presence of whelks *Burnupena* sp. in the same container. These disparate agents all cause a measurable change in the same biological process and they can thus all be integrated as stresses. Phenol at 5ppm appears to be a stimulus but Tukey's test is ambivalent in this result.

It cannot distinguish 5ppm phenol from one control but it can distinguish it from the other control and all the other treatments. The stimulus effect of 5ppm phenol is further examined below.

Six day byssus growth experiments

Byssus production by individuals

Daily byssus production was plotted for each mussel over 6 days. Figures 1A and 1B depict five each of mussels in control conditions - for clarity, only five mussels are plotted on each graph. Figures 1C and 1D depict five each of mussels from 5ppm phenol and Figures 1E and 1F depict five each of mussels from 10ppm ammonia. In all figures byssus production tends to decrease with time. It was hoped that individual mussels under stress would show an exponential drop of byssus production with time when compared with the controls. Although an exponential drop was evident in some of the results, recovery of byssus forming capacity also occurred. Also unexpected was increased production on the second day in the controls.

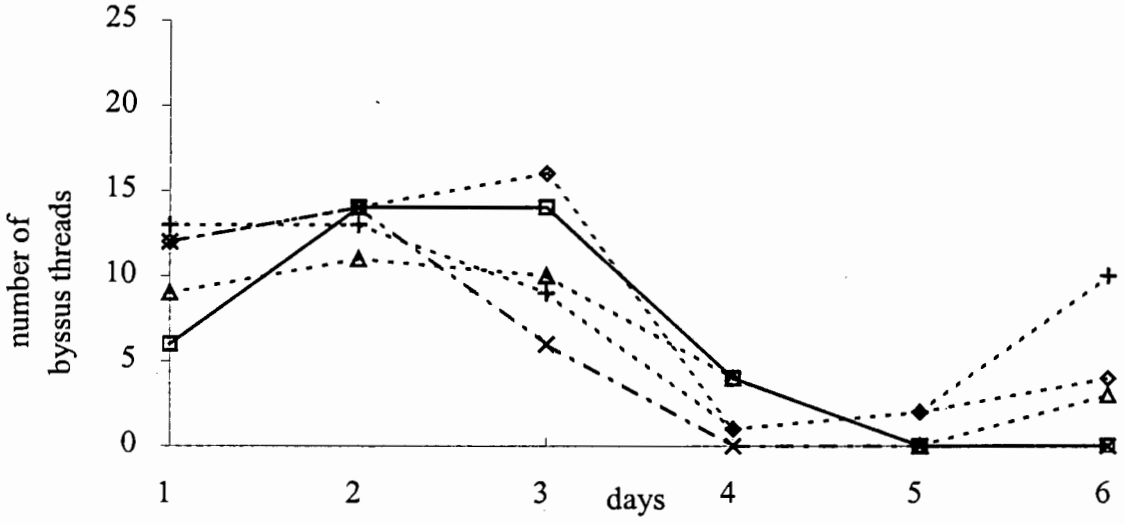


Figure 1 A. Daily variation in number of byssus threads produced by a sample of 5 individual *Choromytilus* held in control conditions over six days.

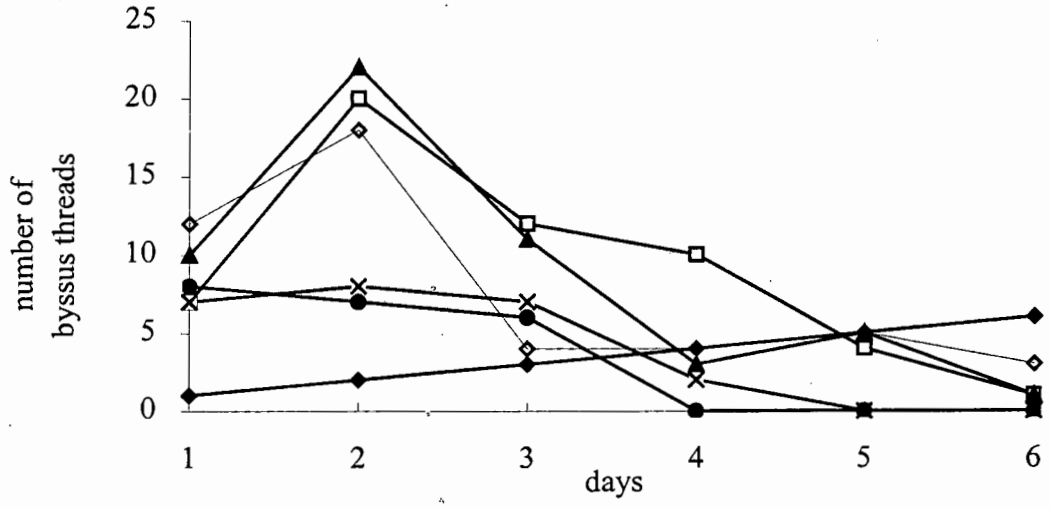


Figure 1 B. Daily variation in number of byssus threads produced by 5 individual *Choromytilus* held in control conditions over six days.

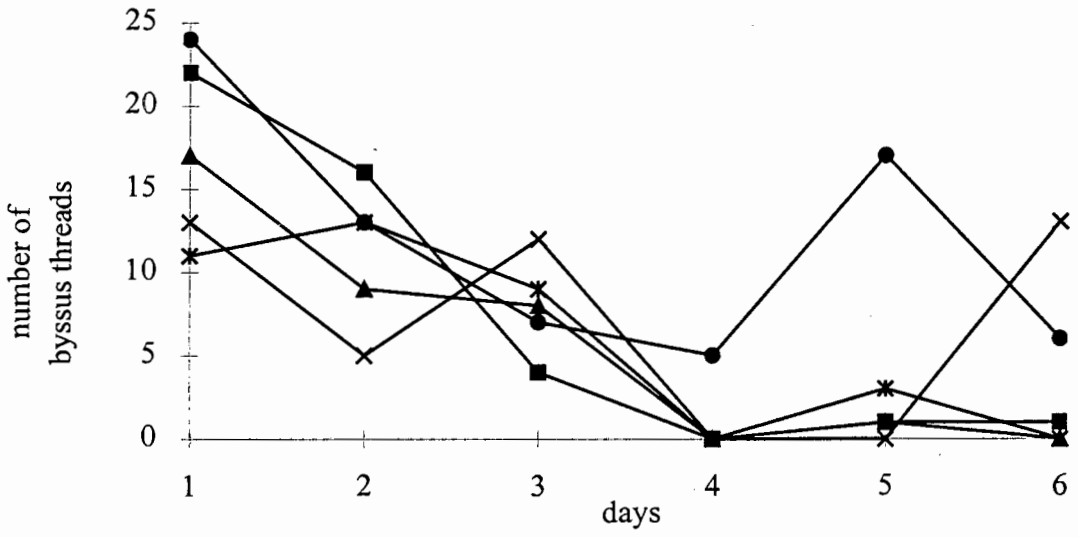


Figure 1 C. Daily variation in number of byssus threads produced by 5 individual *Choromytilus* held in 5ppm phenol for 6 days.

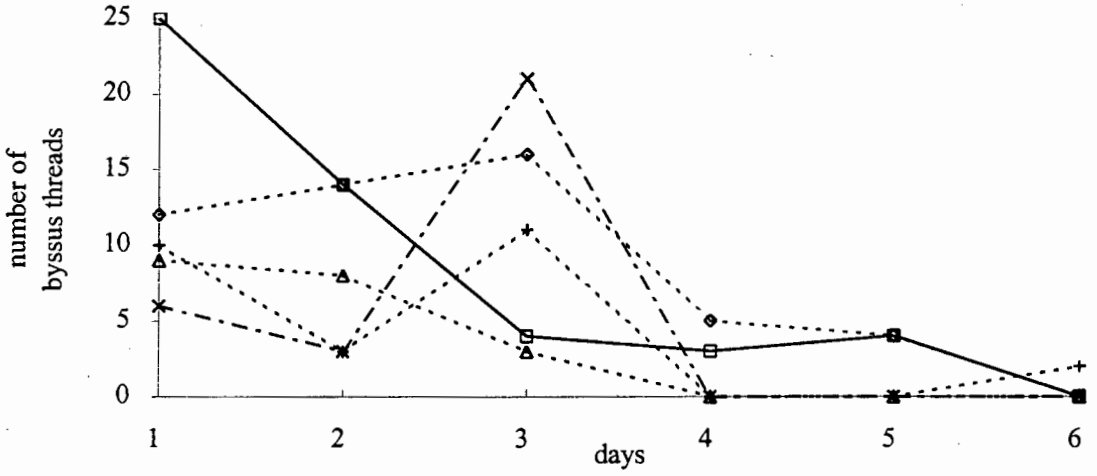


Figure 1 D. Daily variation in number of byssus threads produced by 5 individual *Choromytilus* held in 5ppm phenol for 6 days.

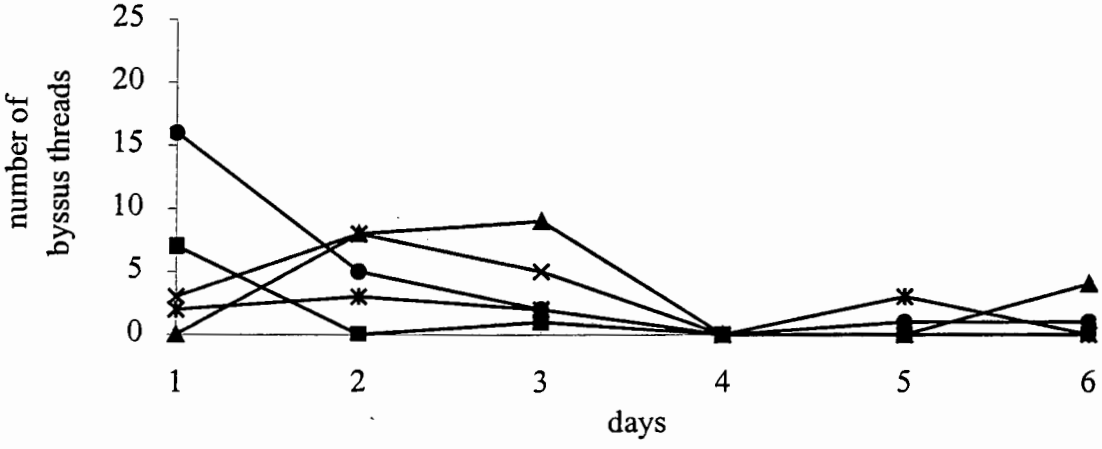


Figure 1E. Daily variation in number of byssus threads produced by 5 individual *Choromytilus* held in 10ppm ammonia for 6 days.

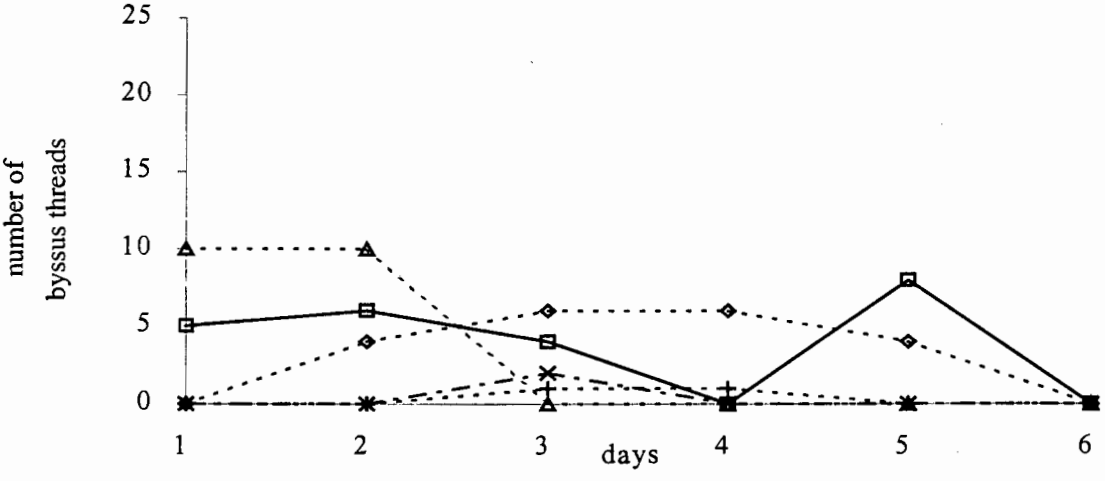


Figure 1 F. Daily variation in number of byssus threads produced by 5 individual *Choromytilus* held in 10ppm ammonia for 6 days.

Group byssus production fluctuations over six days

The results shown in Figures 1A to 1F were aggregated according to treatment regime. Mean byssus production per day over 6 days for each treatment group of mussels was plotted: Figure 2A (control), Figure 2B (5ppm phenol) and Figure 2C (10ppm ammonia). In the control it appears that there is an elevation of byssus production on the second day and then it declines. Byssus production declines from the outset under the other two treatment regimes (Figure 2B - 5ppm phenol & Figure 2C - 10ppm ammonia).

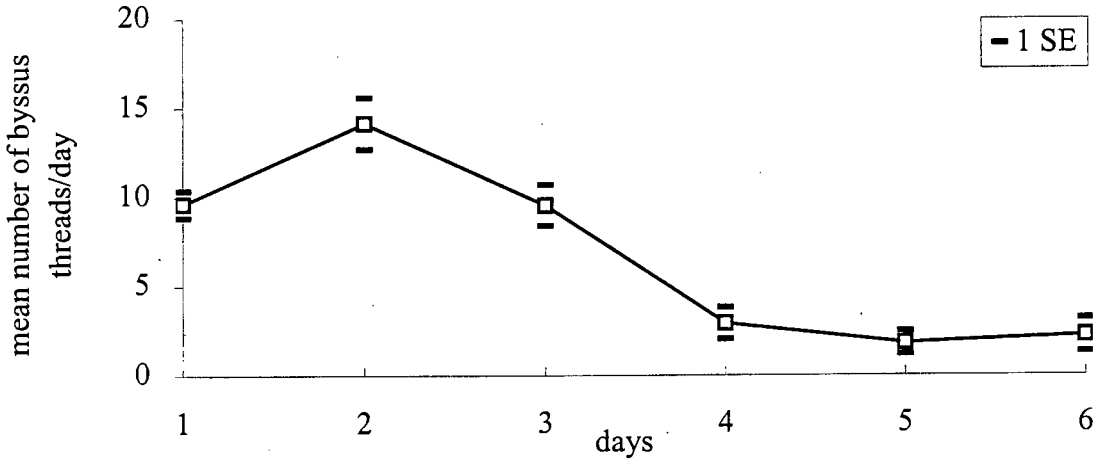


Figure 2A. Variation in mean daily byssus production by a sample of 10 *Choromytilus* held in control conditions over six days.

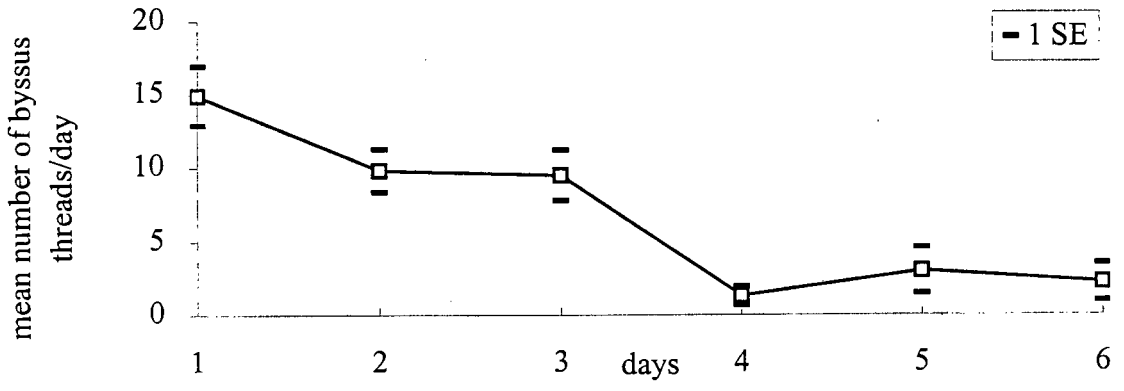


Figure 2B. Variation in mean daily byssus production by a sample of 10 *Choromytilus* held in 5ppm phenol over six days.

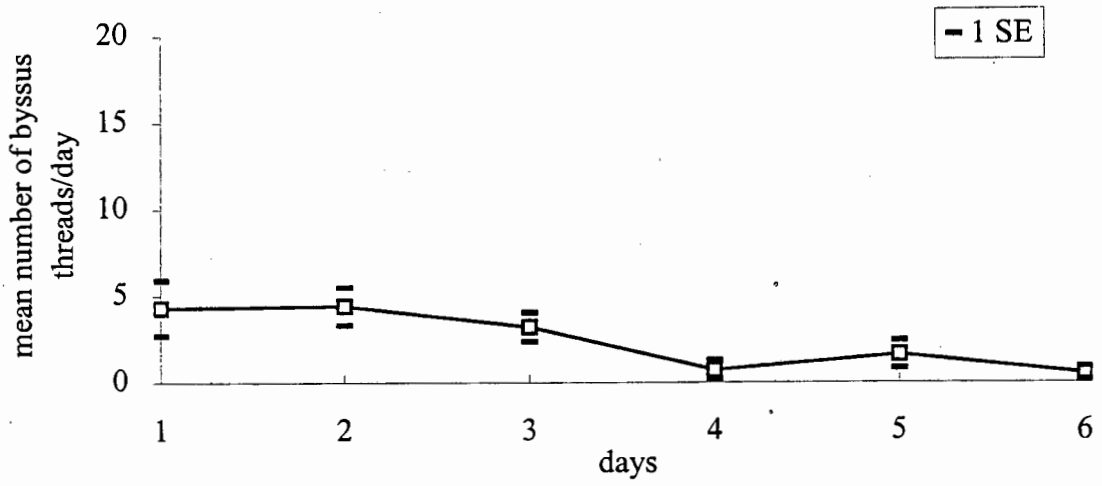


Figure 2C. Variation in mean daily byssus production by a sample of 10 *Choromytilus* held in 10ppm ammonia over six days.

Overall mean byssus production for each group

The data for mean daily production of byssus threads over six days for the entire sample of controls, mussels in 5ppm phenol and mussels in 10ppm ammonia were analysed by ANOVA to see if there were any differences in byssus production between treatments. Tukey's test (Zar 1984) was used to differentiate them.

One-way ANOVA test

H_o: there is no significant difference in mean daily byssus production over six days for samples of ten mussels.
H_1: there is significant difference.
P = 0.0025
Critical region $F_{[0.0005\ (2)\ 2.177]} = 6.22$

Summary of ANOVA

	SS	df	MS
error	5365.079	177	30.3118
groups	734.167	2	367.084
total	6099.246	179	34.074

$$F = \frac{367.084}{30.3118} = 12.11$$

H_o is rejected. Mean byssus production by samples in the different regimes is not equal.

Tukey's test

$q_{0.005\ 177,\ 3} = 4.523$
SE = 0.70177

Rank	1	2	3
agent	NH_3 10ppm	Sea water	phenol 5 ppm
Byssus	2.45	6.6833	6.7833
Mean shell length mm	78.74	78.59	79.15

Tukey's test shows that 3 = 2 (P = 0.005).

The mean value of daily byssus production over six days can detect a difference between the control and 10ppm ammonia. This is four times more sensitive than in a 24 hour assay. Phenol at 5ppm is not detectable; although over 6 days the apparent stimulus of phenol as exhibited over 24 hours is almost eliminated.

The stress of byssus breakage

Byssus production declines each day even in the supposedly unstressed controls (Figures 1A, 1B & 2A). Although water quality (it is not running but it is changed every day and it is kept aerated) may have some influence, it is suspected that the decline is determined by the incapacity of the mussel to continue byssus production at the initial replacement rate. It appears that byssus production declines with the number of times the byssus is broken. Thus one could conclude that byssus breakage is in itself stressful as it places demands on the mussel to reattach itself.

The influence of mussel size on byssus production

Size dependent rate of byssus production was assessed in a sample of ten *Choromytilus meridionalis* kept in control conditions - constant aeration of static water changed every 24 hours. Two sets of descriptive statistics were obtained: the first for byssus production over the first 24 hours and the second for the mean 24 hourly byssus production over 6 days. Mussels in the sample ranged from 75.3mm to 88.2mm.

	1st 24 hour mean	24 hour mean production over 6 days
r	-0.405	-0.823
r^2	0.164	0.677
slope	-0.258	-0.399
n	10	60
P	not significant	0.002

Table 1. Correlation coefficient r, regression coefficient r^2, slope and sample numbers of size dependent byssus production in mussels.

The column on the right shows that larger mussels have a significant tendency to produce fewer threads. This accords with reports on other mytilids such as that by Van Winkle (1970) on *Modiolus demissus,* and those of Glaus (1968) and Reish and Ayers (1968) in *Mytilus edulis*.

In the 6 day experiment, 67.7% of change of byssus production is attributable to shell length with a slope indicating that daily byssus production reduces by 0.4 threads for each millimetre increase in shell length. The significance of this size dependent difference in byssus production rate must now be assessed as there was considerable difference in the mean size of mussel samples. It is thus important to ascertain if the results from different samples are comparable. Although this effect might be construed to undermine the results presented here, it actually reinforces more than it detracts. For instance the 24 hour byssus production results for the control sample (rank 9, mean size 78.6mm) and 5ppm phenol (rank 10, mean size 79.2mm) samples would be even higher if they were not considerably larger than the mean size of mussels in the entire experiment. On the other hand, byssus production in 10ppm NH_3 (rank 5, mean size 78.7mm) should be more to allow for the larger than average sample length of the tests mussels. This would imply that ammonia at this concentration is less deleterious than it has been detected to be. This, however, should all be balanced by the implications of the results for byssus production of controls. The control at rank 8 (mean size 70.9mm: the smallest mean sample size) exhibits lower byssus production rate than that for the control of larger mean size (rank 9 mean size 78.6mm). This suggests that the effect of the differing sized samples is obscured by statistical noise.

Even after allowing for possible effect of size differences, the results from the six day growth experiment remain credible. The depression of mean byssus production from 6.6833 to 2.45 between the control (rank 2) and 10ppm NH_3 (rank 1) can hardly be caused by a difference of 0.15mm in mean shell length between the samples. According to the slope, 0.15mm would cause a difference of only 0.06 threads- not 4.233 given here. Furthermore the increase in mean size of 0.56mm should have caused a decrease of 0.223 byssus threads per day between the seawater control and phenol at 5ppm. Instead byssus production increased by 0.1 threads per day.

Thus, inspection of the sample means shows that the results would be even more marked and that some of the statistical differences are in fact larger than the figures would suggest. It is concluded that the treatment regime is the dominant determinant of byssus production rates. However, this lack of standard sizes in each sample does require more care in judgement to extract the meaning of the results and it is suggested that the findings be made unequivocal by the selection of standard-sized mussels in future.

The phenomenon of reduced byssus production with size raises some other interesting points. For mussels in any particular conditions, size is a function of age. Favourable conditions allow faster growth which in turn means faster loss of byssus producing capacity. Thus such a favourable (stress free) environment allows the expression of endogenous stress sooner. Furthermore, age can be correlated with the increasing stress of poor byssus anchorage. Thus age itself can be directly calibrated as a cause of fitness loss. Clearly, there is scope for much research on the fluctuation of byssus production capacity in response to mussel size and age at different localities and in relation to age/size dependent fecundity. From this it may be possible to gain greater insight on the roles and interactions of somatic and reproductive exogenous and endogenous stresses.

The effect of standing water

Van Winkle (1970) found that *Modiolus demissus* in standing sea water had a 43% reduction in byssus formation compared with *Modiolus* in running sea water. He

attributes this to increased metabolite or decreased oxygen concentration. Indeed oxygen appears to play a role as Reish and Ayers (1968 in Van Winkle 1970) found that oxygen concentrations below 0.6ppm depressed byssus production significantly. Similarly, *Mytilus edulis* held at a flow rate of 0.002l/sec had a 33% reduction when compared with those in flow rates of 0.02 and 0.2l/sec (Glaus 1968 in Van Winkle 1970).

The effect of standing sea water on byssus production was suspected here and thus copious aeration and a large volume of water was provided for each test mussel. Despite the possible drawbacks, standing sea water was used to obviate the necessity of elaborate and expensive dosing equipment that would be necessary to allow flowing water experiments to run for 1 to 6 days. In consequence the possibility that standing water conditions may have influenced the results cannot be denied in this experiment. Any such effect can, however, be largely discounted since the experiments are comparative rather than absolute. All mussels were subject to the same standing sea water regime and significant differences were still obtained. A correction factor, if deemed necessary, might be derived by comparing byssus production between controls in standing water and running water.

Byssus growth will be further discussed and integrated with the results of the other experiments in the Synthesis.

CHAPTER 44: POSITIVE FEEDBACK DYNAMICS AND STRESS

INTRODUCTION

In Chapter 16 it was hypothesised that stress produces positive feedback dynamics and as the intensity of stress increases, this will overpower the negative feedback of homeostasis. This is developed in Chapter 32 where it is argued that negative feedback of homeostasis and the positive feedback of strain are both non-linear. In a hypothetical homeostatic (negative feedback) controlled dynamic, the rate of displacement is highest initially and the rate decays to zero (Figure 2, Chapter 32). In contrast, the expected dynamic of dying in a lethally stressed organism (Figure 3, Chapter 32) would have a low initial rate of displacement that accelerates exponentially. This postulated difference is here demonstrated empirically.

The theoretical basis of this experiment was stimulated by the asserted relationship of disturbance of 'function' with dose of contaminant (Figure 2, Chapter 16) as presented by Wilson (1980). This figure shows a sigmoid relationship between dose of deleterious agent and disturbance of process leading to death. This curve implies that the rate of progress towards death declines as death is approached (This part appears as a dotted line of extrapolation in the original graph). Consideration of the consequences of uncontrolled feedback suggests that no such reduction of rate could occur before death.

MATERIALS AND METHODS

Freshly collected *Choromytilus meridionalis* from Blouberg were placed individually in a beaker on a cradle made from a microscope coverslip box (Figure 1). The coverslip box supported the shell with the plane of the valve margin horizontal and with no tendency to wobble. A 20cm length of 0.8mm diameter wire with a tight loop at one end was threaded through a hole about 2cm from the axis of a GWO isotonic lever transducer. The other end of the wire was rested on the upper valve and the transducer arm was arranged to be horizontal or slightly dipping. This gave the most proportional angular movement in response to shell gaping and closure. The transducer was connected to the oscillograph and the entire transducer rotated on its

axis by slackening the retort clamp. It was rotated until the oscillograph gave an upward sweep on the paper in response to an upward movement of the valve. This was checked over the total possible upward movement of the valve. This precaution is necessary because when the lever arm moves through certain angles, the polarity of output of the transducer switches. It is thus vital that the movement of the arm does not encroach on this angle or the trace would show a spurious reversal of valve movement. The apparatus was thus set up to give a proportional displacement of the pen for any valve opening. The length of wire allowed separation between the transducer and the liquid in which the mussel rested. This helped to minimise condensation on the transducer and it also facilitated use of different sized beakers.

The trace paper has a curved vertical axis which allows linear displacement to be depicted as a curve of radius of the pen arm. This makes negative feedback curves look more curved and straightens positive feedback curves - any positive feedback curve is likely to be more exponential than it looks.

A trace of terminal dynamics was obtained by starting the oscillograph with the mussel in a beaker with a small amount of sea water. The beaker was then filled with hot water at about 95°C while the oscillograph traced valve displacement with time. A longer term trace of terminal dynamics was made by warming a mussel in a beaker of sea water from ambient by placing a 60W light bulb close to the beaker.

Phenol or ammonia was used to enhance the voluntary valve opening response. A few drops of stock solution were added to the beaker until a response was elicited. Both cause an increase in gaping and closure after the manner of an irritant, but for a number of reasons their deleterious effect is considered to be minor. The mussel regains control of the displacement as shown by repeated ability to close its valves so it can be inferred that these are not terminal dynamics. Moreover, previous experiments with irritant levels of these agents has shown no tendency to increase mortality of these mussels as compared with untreated controls (pers obs).

RESULTS

Figures 2A to 2F all have the same valve displacement (vertical) and time axes (horizontal). Figures 2A to 2E show positive feedback dynamics of shell movement after sudden immersion in hot water. Figure 2F shows controlled shell opening, negative feedback, dynamics start at each arrow. Figures 3A & 3B have the same scale axes; the former depicts positive feedback dynamics as a result of hot water immersion, the latter shows voluntary movement (arrowed). Figures 4 & 5 show positive feedback dynamics after hot water immersion. Figure 6 shows the positive feedback dynamics of shell movement during steady warming over 15 minutes. Figures 7A & 7B have the same axes; Figure 7A shows the positive feedback dynamics of shell movement after hot water immersion, in contrast, Figure 7B shows controlled, negative feedback, shell opening (arrowed). The first part of 7B shows unstimulated opening activity and the second part shows phenol stimulated activity. Figures 8A to 8D have the same scale axes. Figures 8A & B show positive feedback dynamics after the mussel has been immersed suddenly in hot water. Figure 8C shows (arrowed) the negative feedback dynamics of voluntary movement and Figure 8D shows (arrowed) the negative feedback dynamics of phenol stimulated movement. Figures 9A & 9B have the same scale axes. Figure 9A shows positive feedback dynamics of shell movement after sudden immersion in hot water. Figure 9B shows negative feedback dynamics of ammonia induced activity.

In Figures 2A, 2B, 2C, 2D, 2E, 3A, 4, 5, 7A, 8A, 8B & 9A, an artefact, seen as a perturbation (labelled P) on the curve, indicates when the hot water is added. It is merely a detection of movement of the whole mussel as the water is poured in and does not represent any displacement of the valves.

DISCUSSION

In all cases, fatal heat stress causes an exponential increase in valve opening speed: the curve tends to the vertical. In contrast, all examples of controlled opening show that the speed of valve movement decelerates and the curve tends to the horizontal. The exponential part of the lethal curve can be most clearly seen in Figure 2A between points X and Y. Although the curve from Z onwards in Figure 2A appears to

turn and become more horizontal, thus suggesting negative feedback dynamics - these are post death dynamics. This part of the curve indicates where the valve movement runs into mechanical limits of the heat denatured muscle tissue after death.

The upward sweep can also be seen in Figures 2B, C, D & E and this exponential curve is also evident over a longer period. Figure 6 shows the same dynamic when a mussel in sea water is slowly warmed over 15 minutes. In contrast, Figure 2F shows a series of voluntary valve openings (arrowed). Here, each one begins at the highest rate of displacement and decelerates. Opening is arrested when the adductor muscle pulls the valves together. These two contrasting and characteristic curves are also seen in Figures 3A (lethal) & 3B (controlled).

Phenol and ammonia are used here at irritant rather lethal concentrations. This is evident because, especially for ammonia, the mussels would have sealed themselves off from the agent by valve closure (pers. obs.) at higher concentrations. Further evidence for sub-lethality of these agents lies in comparison of voluntary movements (8C & the first part of 7B), with those caused by the irritant, phenol (8D & the second half of 7B). These figures show that phenol merely accelerates the rate of opening and closing of the shell valves: the essential shape of the negative feedback curve is unaltered.

Two curves of opposite tendency are experimentally demonstrated. These results support the contention that the dynamics of dying are non-linear and of increasing speed, and in contrast, the dynamics of controlled movement show the opposite non-linear tendency. These tendencies, it is argued here, represent the poles of feedback dynamics. It is urged that consideration of these dynamics be not restricted to shell opening in mussels; previous discussions provide strong circumstantial evidence that they may have a wider biological relevance.

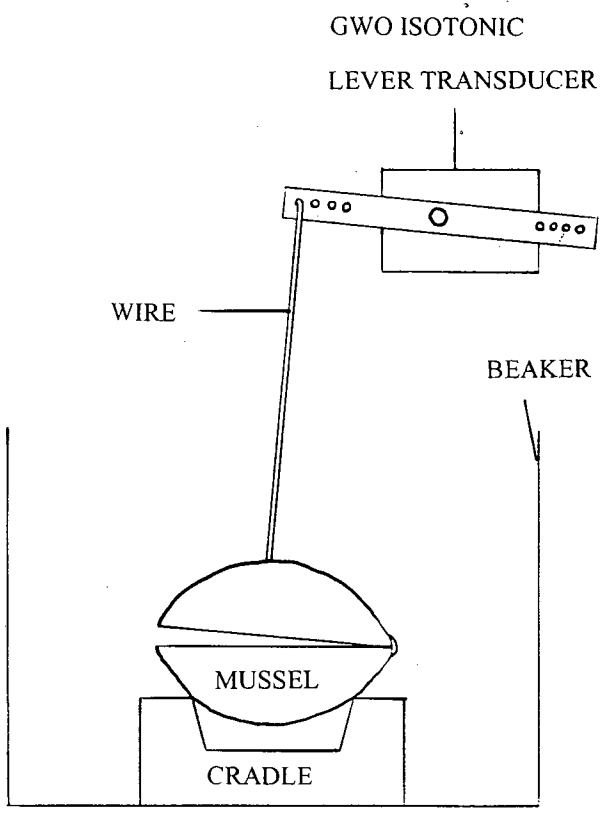


Figure 1. Diagram of apparatus to measure valve movement.

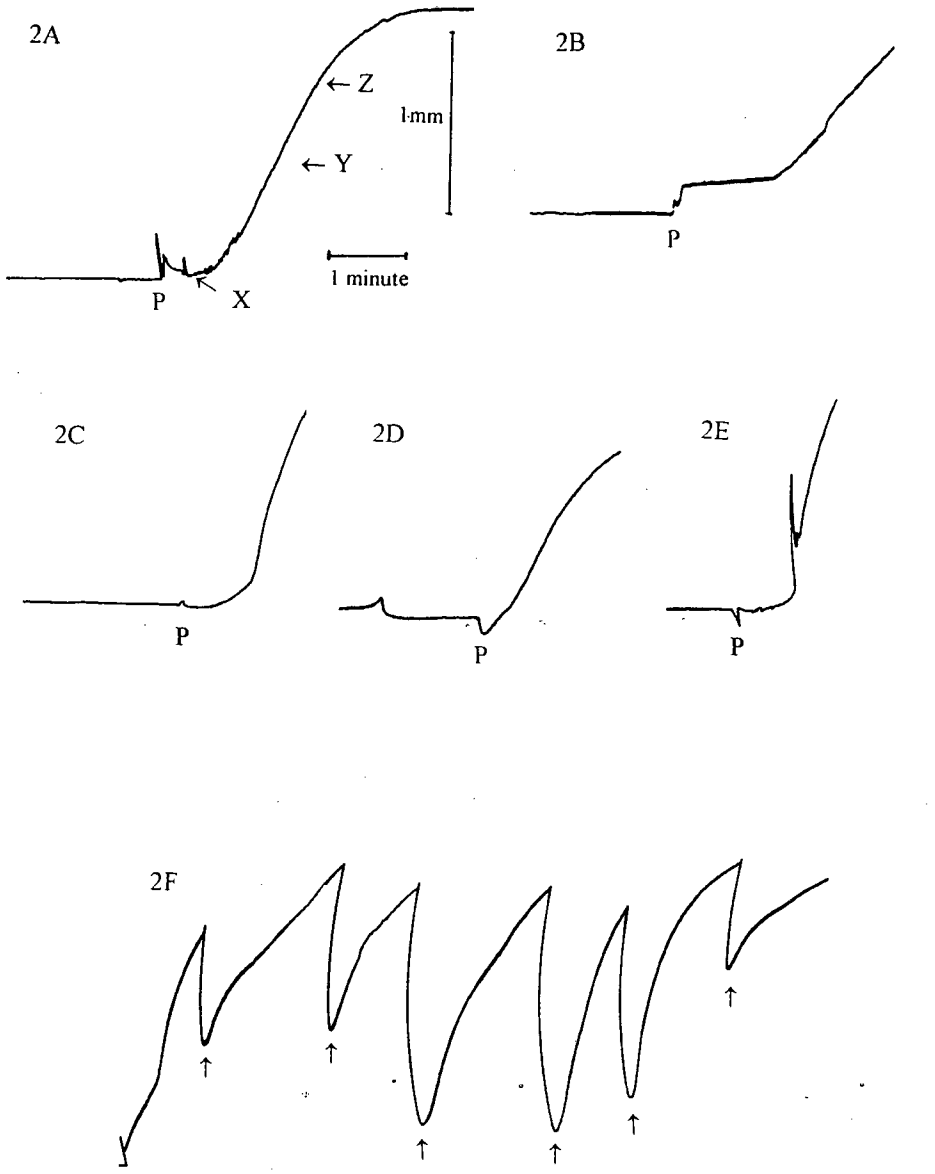


Figure 2. 2A to 2E show the dynamics of shell movement after the mussel has been immersed suddenly (time P) in hot water at about 95°C. 2F shows controlled shell openings, each marked with an arrow. All have the same valve displacement (vertical) and time axes (horizontal).

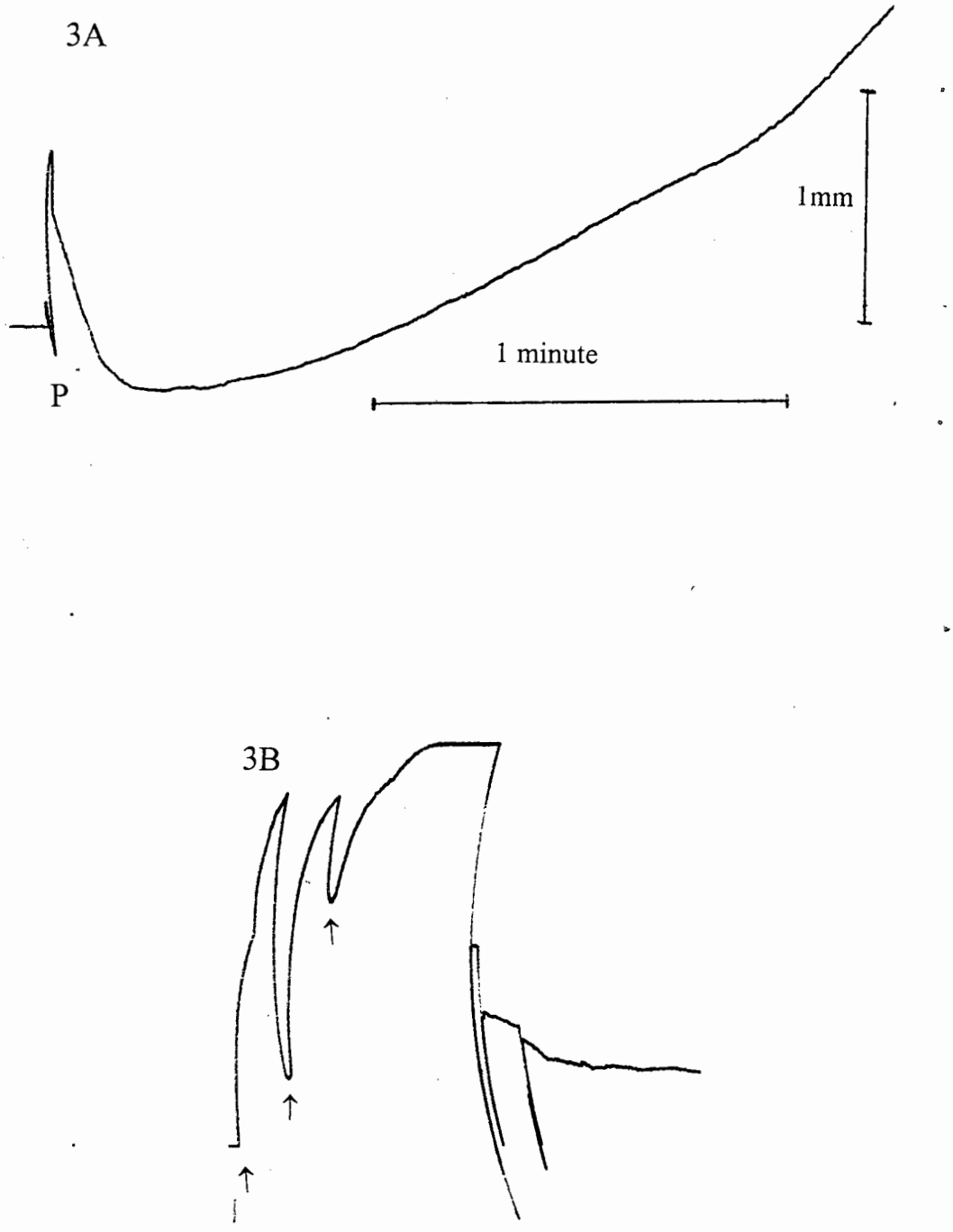


Figure 3. 3A shows the dynamics of shell movement after the mussel has been immersed suddenly (time P) in hot water at about 95°C. 3B shows controlled shell opening. All have the same valve displacement (vertical) and time axes (horizontal).

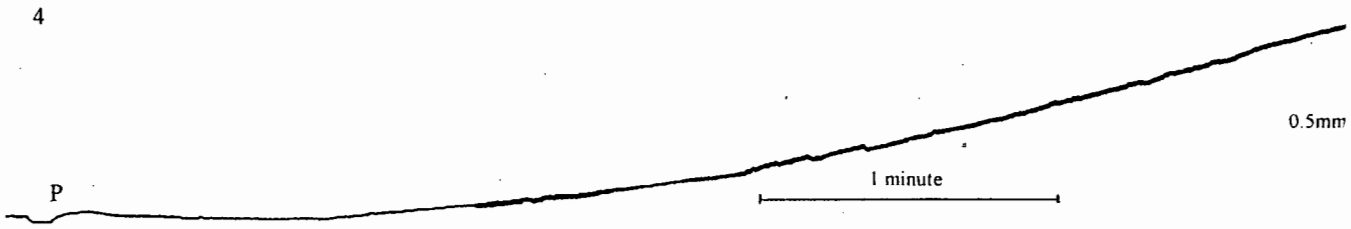


Figure 4. Dynamics of shell movement after the mussel has been immersed suddenly (time P) in hot water at about 95°C.

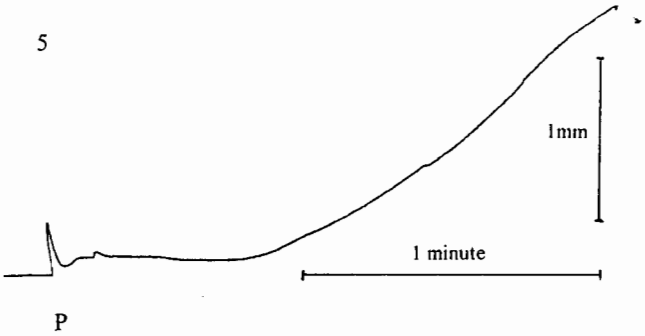


Figure 5. Dynamics of shell movement after the mussel has been immersed suddenly (time P) in hot water at about 95°C.

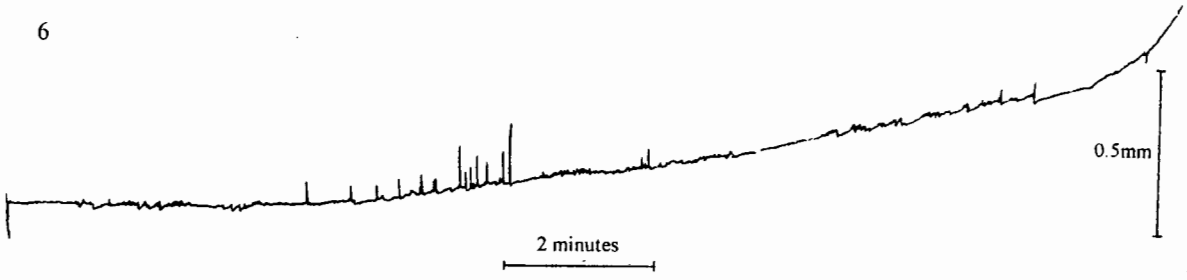


Figure 6. Dynamics of shell movement of a mussel subject to steady warming of the water over 15 minutes.

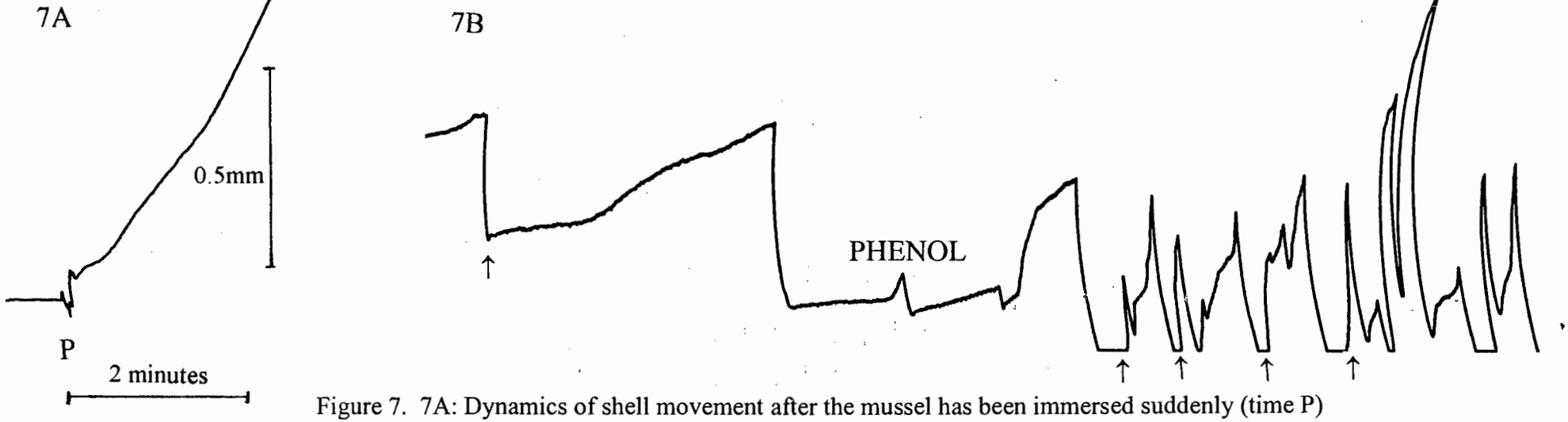


Figure 7. 7A: Dynamics of shell movement after the mussel has been immersed suddenly (time P) in hot water at about 95°C. 7B shows controlled shell opening (arrowed); the first part of 7B shows unstimulated opening activity, the second part shows phenol stimulated activity. 7A &7 B have the same valve displacement (vertical) and time axes (horizontal).

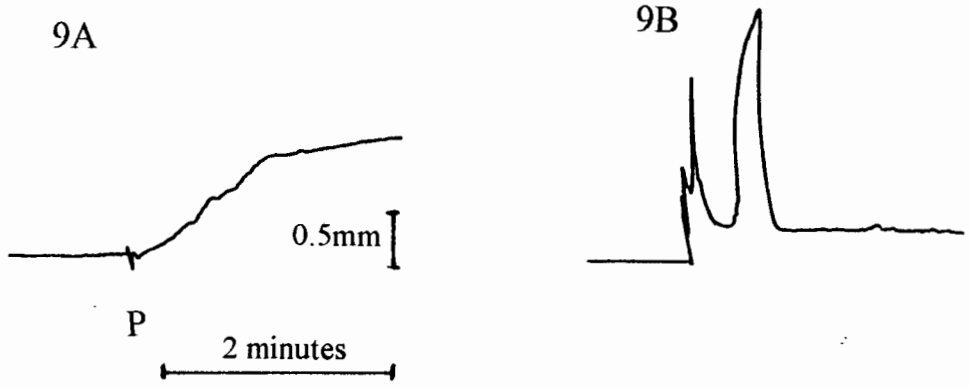


Figure 9. 9A shows the dynamics of shell movement after the mussel has been immersed suddenly (time P) in hot water at about 95°C. 9B shows the dynamics of ammonia induced activity. 9A & 9B have the same valve displacement (vertical) and time axes (horizontal).

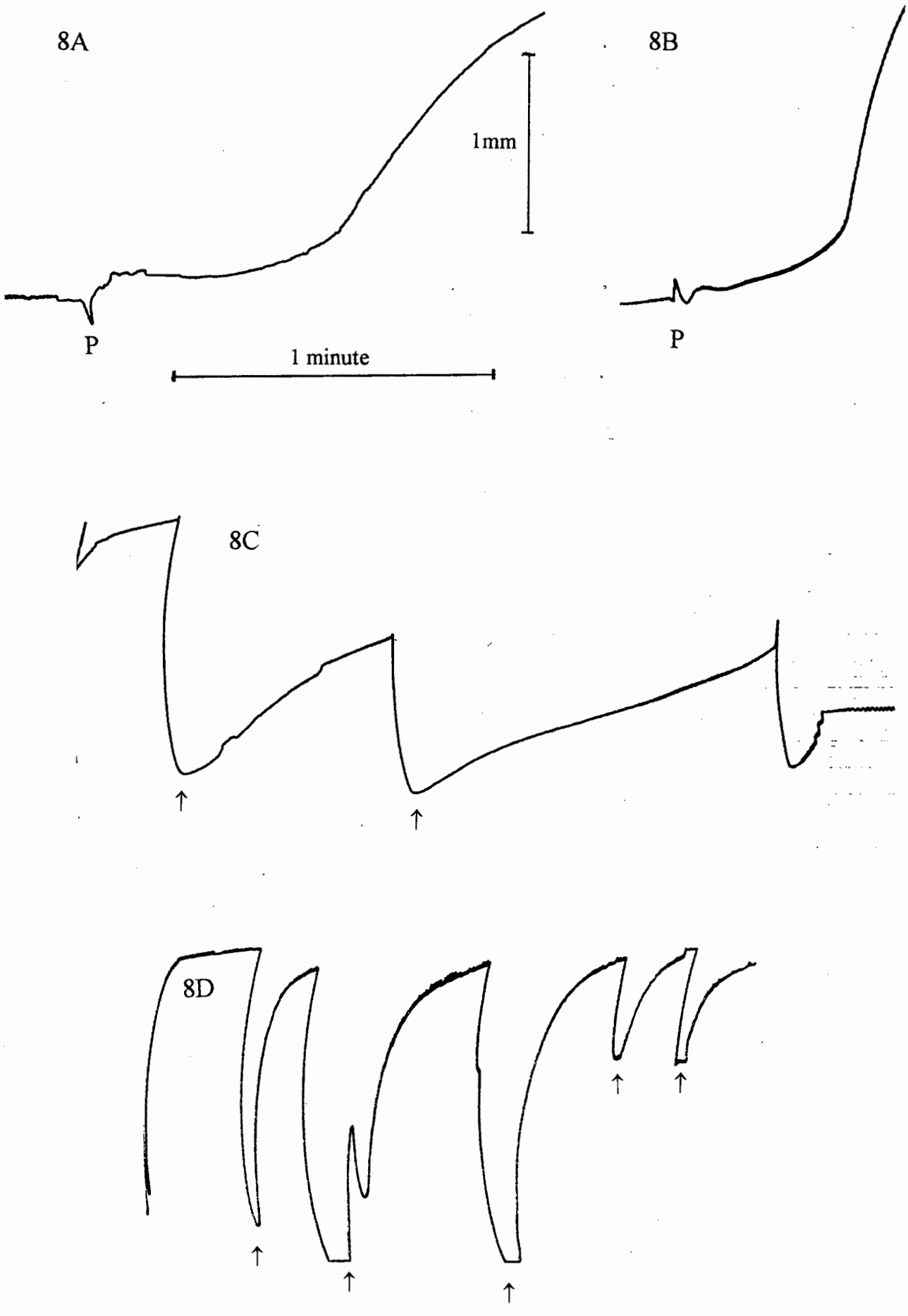


Figure 8. 8A & 8B show the dynamics of shell movement after the mussel has been immersed suddenly (time P) in hot water at about 95°C. 8C shows (arrowed) the dynamics of voluntary activity. 8D shows (arrowed) the dynamics of phenol stimulated activity. 8A to 8D have the same valve displacement (vertical) and time axes (horizontal).

CHAPTER 45: EMERSION AND MORTALITY IN *CHOROMYTILUS MERIDIONALIS*

INTRODUCTION

Emersion imposes demands on all sessile intertidal organisms and may thus be considered a stress. Survival time of emersed organisms may be a measure of resistance to this stress. This period is here correlated with such potential influences as size, sex and parasite loads of mussels. In addition, the mortality dynamics of emersed populations are examined, as are any relationships between survival time and condition at mortality.

Prochazka & Griffiths (1991) performed similar emersion experiments in which they related survival of *Choromytilus meridionalis* and *Mytilus galloprovincialis* with temperature, re-watering (returning to sea water holding tank for varying periods after harvesting) and harvesting method. This was to ascertain optimal treatments for transport and storage of mussels. They thus studied exogenous factors. This study assesses the influence of endogenous factors on survival.

MATERIALS AND METHODS

Three collections were made: June, August and September 1997 (See Tables 1, 2 & 3). Mussels were exposed immediately after collection without re-immersion. They were placed on their sides and lay same-side up throughout. The first and second collections were held at ambient temperature (17-20°C) on the laboratory bench. The third collection was held at 15°C in a constant temperature room with a day/night lighting regime. Only mussels with intact byssus were used; previous studies (pers. obs.) indicated that mussels with no byssus have higher mortality. Missing byssus is probably indicative of internal damage incurred during detachment from the rocks.

Dead mussels were removed and examined every 24 hours. Death criteria vary considerably in the literature. To Prochazka & Griffiths (1991) mussels are dead if they assume their original gape after the valves are squeezed together for five seconds and then released. They consider a lesser gape after this treatment to indicate a living mussel. Marshall & McQuaid (1993) defined death as an abnormally wide gape (more

than 11mm) where the mussels then showed no valve movements on prodding internally. The death criterion selected here differs from those above. It is the failure of valves to remain shut after ten compressions to closure between finger and thumb. Incomplete closure on stimulus was considered more deleterious than a sluggish complete closure response. This is because incomplete closure would leave the mussel exposed to the attentions of the many scavenging and predatory whelks that occur in its habitat (See Chapter 42). In addition, incomplete closure would allow much greater water loss through evaporation. For this reason the criterion of uncontrolled (if minor) gaping was selected in preference to those proposed by previous workers. Nevertheless, the criterion chosen here is merely an identifiable event in the death process rather than death itself: 'dead' mussels often exhibited gill cilia activity.

Mussels deemed dead were measured and opened. Sex was determined and reproductive condition estimated. For the criteria of condition see Chapter 47. In the first experiment, parasites were then identified and counted. See Chapter 2 for details of materials and methods. Those parasites noted in the emersion experiment were: *Metacercaria perchorupis* (Chapter 4), *Metacercaria A* (Chapter 5), *Metacercaria B* (Chapter 9), and *Cercaria notobucephala* (Chapter 3).

The worm burden parameters of abundance and intensity are used in the results and discussion. Of these, only intensity refers to parasite numbers in a particular individual mussel. Abundance is a population derived value; it is the total number of worms counted in the mussel sample divided by the number of mussels, infected and uninfected, in the sample. Thus individual intensity may be represented as a datum on a figure, but abundance is used in summary statistics of populations.

RESULTS

Figures 1 to 6 are scattergrams showing the relationship of shell length and emersion survival time for males and females from the three collections.

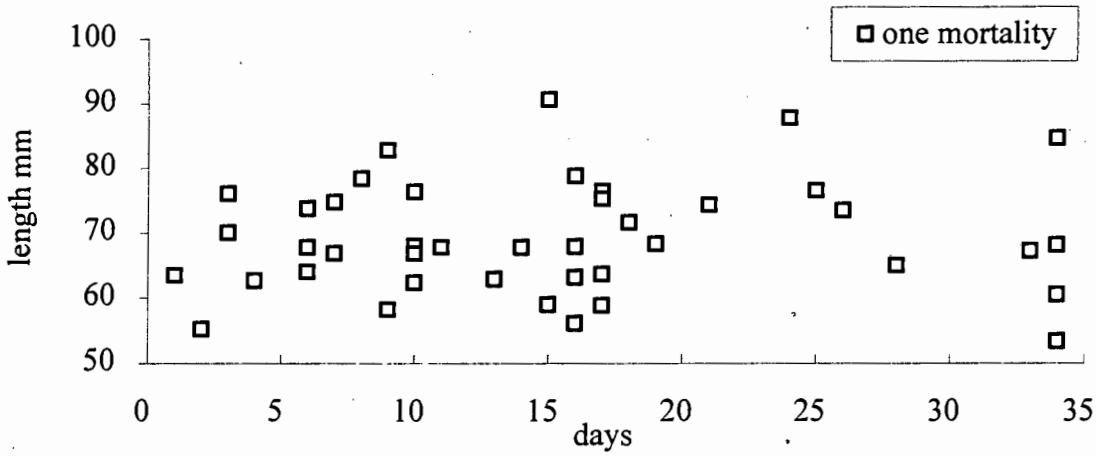


Figure 1. The relationship of shell length at mortality with survival time during emersion of female *Choromytilus* from the Blouberg June 1997 collection.

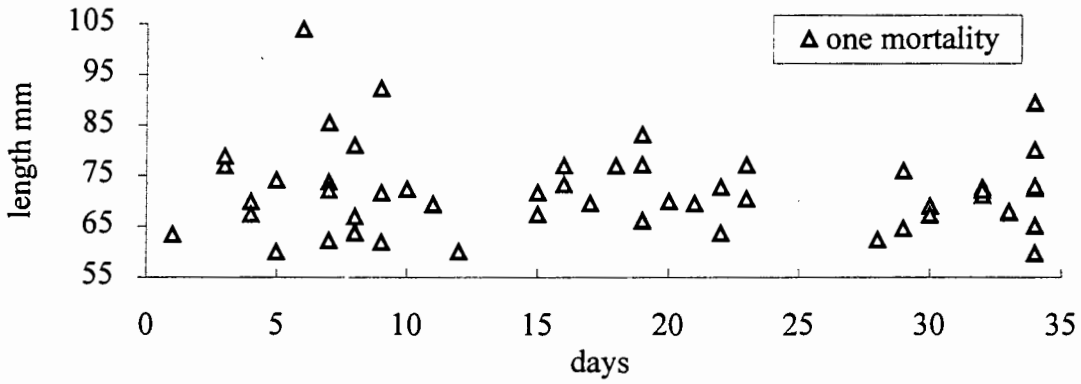


Figure 2. The relationship of shell length to mortality with survival time during emersion of male *Choromytilus* from the Blouberg June 1997 collection.

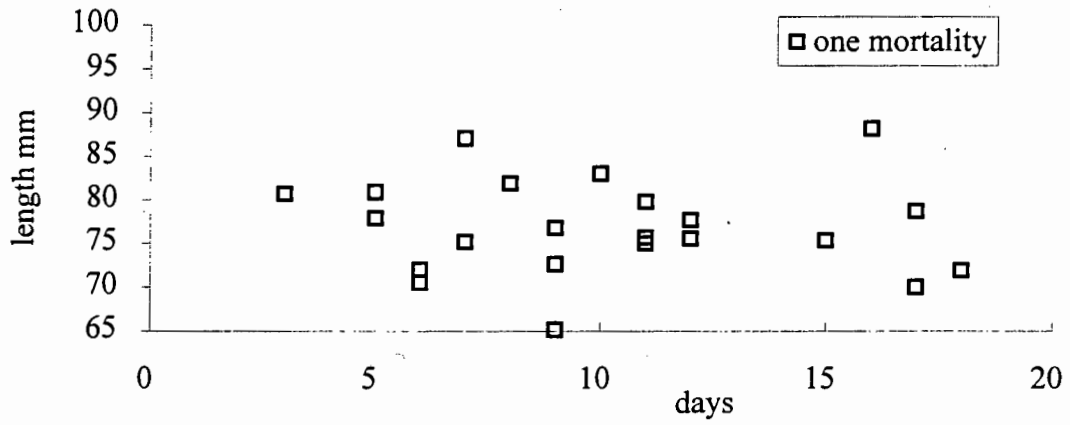


Figure 3. The relationship of shell length at mortality with survival time during emersion of female *Choromytilus* from the Blouberg August 1997 collection.

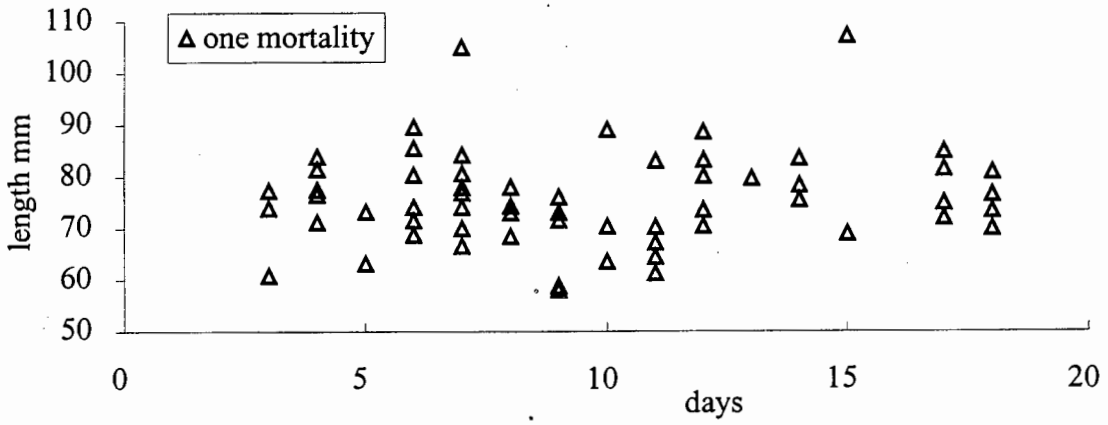


Figure 4. The relationship of shell length at mortality with survival time during emersion of male *Choromytilus* from the Blouberg August 1997 collection.

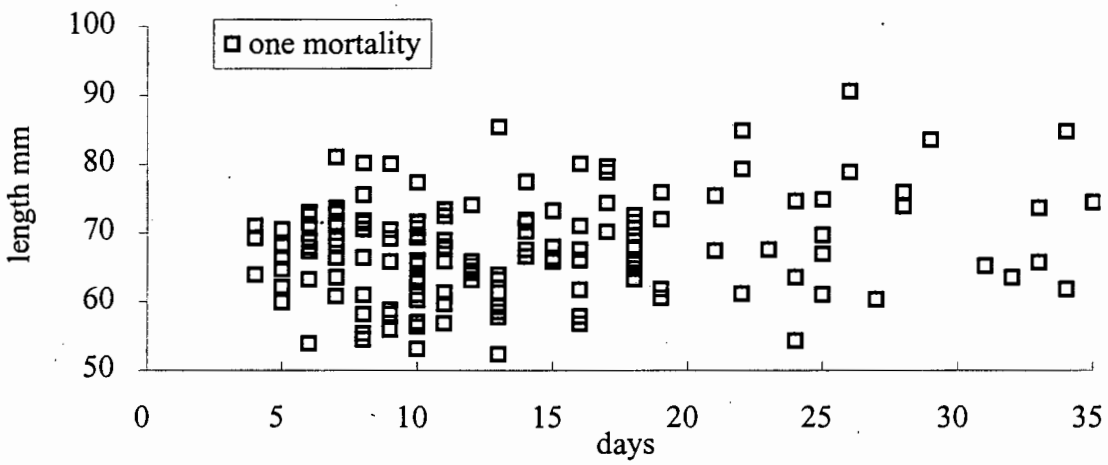


Figure 5. The relationship of shell length at mortality with survival time during emersion of female *Choromytilus* from the Blouberg September 1997 collection.

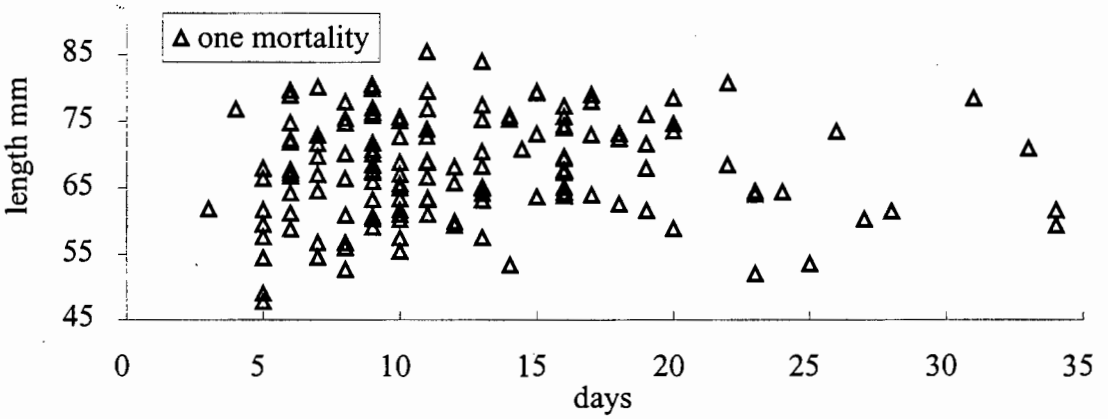


Figure 6. The relationship of shell length at mortality with survival time during emersion of male *Choromytilus* from the Blouberg September 1997 collection.

Tables (1, 2 & 3) below give the relevant descriptive statistics of these samples.

	female	male
sample number	44	54
mean length (mm)	69.026	72.037
SD	8.425	8.694
SE	1.270	1.183
mean survival (days)	15.023	18.185
SD	9.347	11.051
SE	1.409	1.504
r	0.065	- 0.021
r^2	0.004	0.0004
slope	0.059	-0.026

Table 1. Descriptive statistics relating size and survival period in male and female *Choromytilus* from the June 1997 collection.

	female	male
sample number	24	60
mean length (mm)	76.348	75.703
SD	5.6205	9.2667
SE	1.1473	1.1963
mean survival (days)	10.2917	9.6333
SD	4.4378	4.3994
SE	0.9059	0.568
r	-0.1060	0.1019
r^2	0.0125	0.0104
slope	-0.0837	0.0484

Table 2. Descriptive statistics relating size and survival period in male and female *Choromytilus* from the August 1997 collection.

	female	male
sample number	153	149
mean length (mm)	67.749	68.009
SD	7.0557	7.7611
SE	0.5704	0.6358
mean survival (days)	13.7386	12.456
SD	7.2889	6.279
SE	0.5893	0.514
r	0.2064	0.048
r^2	0.0426	0.0023
slope	0.2132	0.0389

Table 3. Descriptive statistics relating size and survival period in male and female *Choromytilus* from the September 1997 collection.

None of the *r* values in Tables 1 & 2 indicate that the relationship between mussel length and survival time is significant. However, with a larger sample in Table 3 the *r* value for females indicates that the relationship between mussel length and survival time is significant (P = 0.02), but only 4.26% of variation of survival time is attributable to shell length.

Morphometric differences between the sexes?

Tables 1, 2 & 3 show apparent sex differences in the mean size of mussels; but inspection of standard errors suggested that this difference was not significant. This was confirmed with Student's t-tests (Zar 1984).

June collection

H_0: males and females have the same mean shell length.
H_A: males and females do not have the same mean shell length.
P = 0.005
Critical region: ts = greater than or equal to $t_{[0.005\ (2)\ 96]} = 2.873$
ts calculated from data = 1.733
H_0 is not rejected: male and female mean sizes are not significantly different.

August collection

H_0: males and females have the same mean shell length.
H_A: males and females do not have the same mean shell length.
P = 0.005
Critical region ts = greater than or equal to $t_{[0.005\ (2)\ 82]} = 2.885$
ts calculated from data = 0.318
H_0 is not rejected: male and female mean sizes are not significantly different.

September collection

H_0: males and females have the same mean shell length.
H_A: males and females do not have the same mean shell length.
P = 0.005
Critical region ts = greater than or equal to $t_{[0.005\ (2)\ 300]} = 2.825$
ts calculated from data = 0.305
H_0 is not rejected: male and female mean sizes are not significantly different.

Mean survival times of males and females

Tables 1, 2 & 3 show apparent sex differences in mean survival times. Inspection of standard errors suggested that this difference was not significant. This was confirmed by Student's t-tests (Zar 1984).

June collection

H_0: males and females have the same mean survival time.
H_A: males and females do not have the same mean survival time.
P = 0.005
Critical region ts = greater than or equal to $t_{[0.005\ (2)\ 96]} = 2.873$
ts calculated from data = 1.509
H_0 is not rejected: mean survival times of the sexes are not significantly different.

August collection

H_0: males and females have the same mean survival time.
H_A: males and females do not have the same mean survival time.
P = 0.005
Critical region ts = greater than or equal to $t_{[0.005\ (2)\ 82]} = 2.885$
ts calculated from data = 0.618
H_0 is not rejected: mean survival times of the sexes are not significantly different.

September collection

H_0: males and females have the same mean survival time.
H_A: males and females do not have the same mean survival time.
P = 0.005
Critical region ts = greater than or equal to $t_{[0.005\ (2)\ 300]} = 2.825$
ts calculated from data = 1.641
H_0 is not rejected: mean survival times of the sexes are not significantly different.

Metacercaria B infection and mortality

Figure 7 shows the relationship of intensities of *Metacercaria B* infections to the emersion survival time.

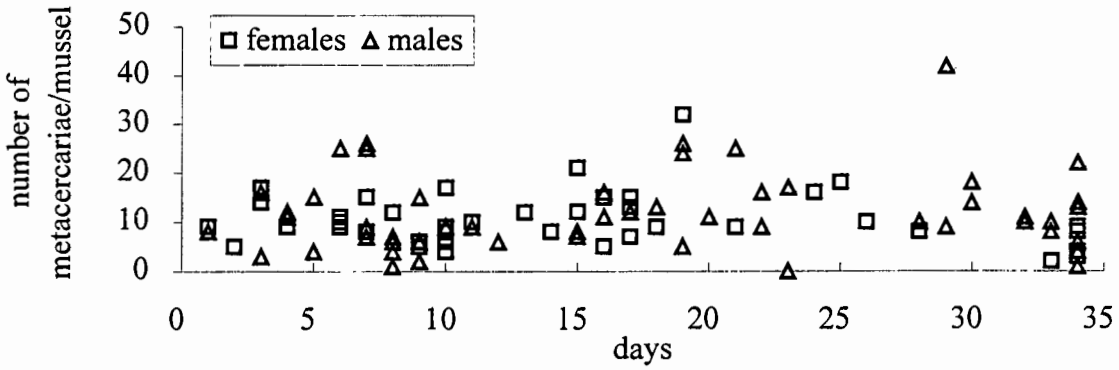


Figure 7. The relationship of infection intensity with emersion survival time in *Choromytilus* from Blouberg infected with *Metacercaria B*. June 1997 collection.

Table 4 gives descriptive statistics of the relationship.

	Female	Male
n	44	54
abundance	10.2955	11.7593
SD	5.4212	7.87621
r	-0.11537	0.07354
r^2	0.01331	0.00541
slope	-0.19665	0.10222

Table 4. Descriptive statistics concerning the relationships between intensity of infection with *Metacercaria B* and emersion survival period for male and female *Choromytilus*. The *r* values indicate that the relationship between infection intensity and survival time is not significant in either sex.

Intensity of infection with *Metacercaria A* and mortality

Figure 8 shows the relationship of intensity of *Metacercaria A* infections to the emersion survival time.

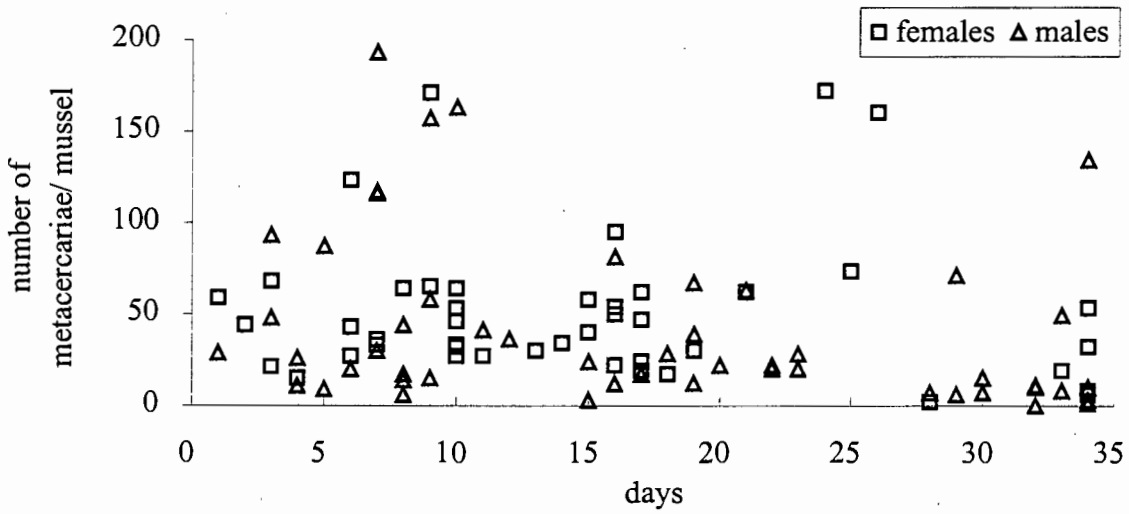


Figure 8. The relationship of total *Metacercaria A* infection intensity with emersion survival time in *Choromytilus* males and females from Blouberg. June 1997 collection.

Table 5 gives descriptive statistics of the relationship.

	females	males
n	44	54
abundance	55.454	40.129
SD	39.2321	44.6322
r	-0.06	-0.3333
r^2	0.0036	0.1112
slope	-0.0141	-0.0817

Table 5. Descriptive statistics concerning the relationship between total intensity of infection with *Metacercaria A* and survival period of the host for male and female *Choromytilus*. The *r* values indicate that the relationship between infection intensity and survival time is significant only in males (P = 0.02). Longer surviving mussels have lower intensity. The total number of *Metacercaria A* in each mussel accounted for 11.1% of the variation in survival time.

The results from Figure 8 are subdivided into cysts in palps (Figure 9) and cysts in mantle (Figure 10).

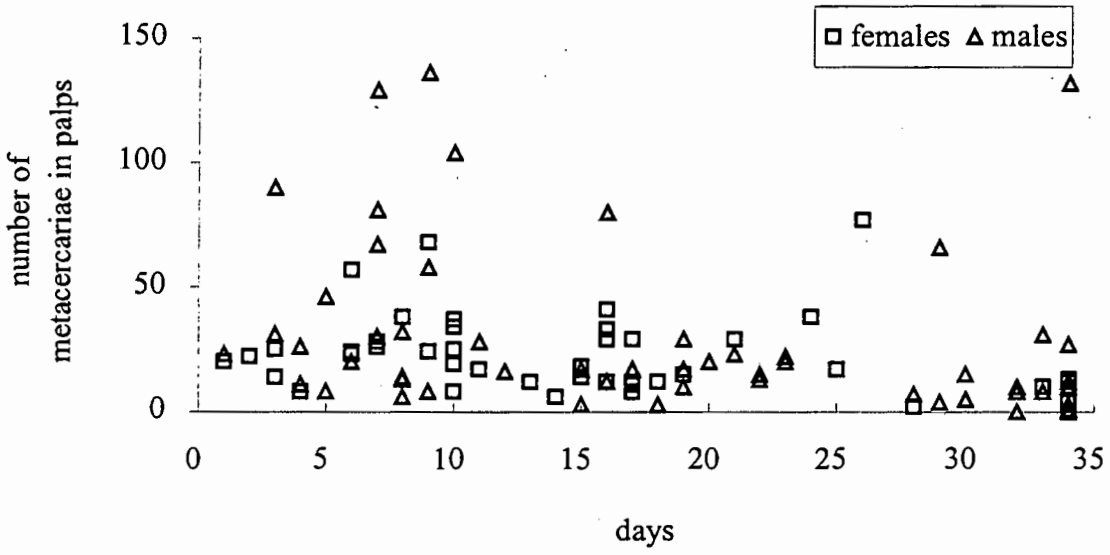


Figure 9. The relationship of *Metacercaria A* infection intensity in palps with emersion survival time in *Choromytilus* males and females from Blouberg. June 1997 collection.

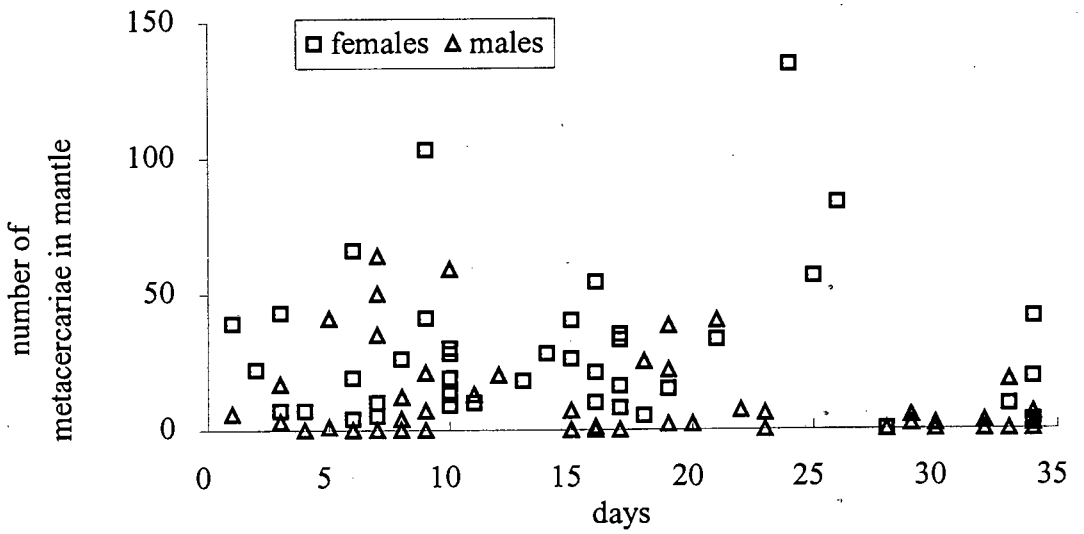


Figure 10. The relationship of *Metacercaria A* infection intensity in the mantle with emersion survival time in *Choromytilus* males and females from Blouberg. June 1997 collection.

Descriptive statistics of these relationships are given in Tables 6 and 7 respectively.

	females	males
n	44	54
abundance	22.6136	10.1852
SD	15.744	15.9223
r	-0.2222	-0.2887
r^2	0.04939	0.0832
slope	-0.1304	-0.0923

Table 6. Descriptive statistics concerning the relationship of intensity of infection with *Metacercaria A* in the palps and survival period in male and female *Choromytilus*. The *r* values indicate that the relationship between infection intensity and survival time is significant (P = 0.05) only in males: longer surviving mussels have lower intensity. The number of *Metacercaria A* in the palps of mussels accounted for 8.32% of the variation in survival time.

	females	males
n	44	54
abundance	27.8409	40.1296
SD	26.8132	44.6323
r	0.0428	-0.3147
r^2	0.00183	0.0990
slope	0.01474	-0.2164

Table 7. Descriptive statistics concerning the relationship of intensity of infection with *Metacercaria A* in the mantle and survival period in male and female *Choromytilus*. The *r* values indicate that the relationship between infection intensity and survival time is significant (P = 0.02) only in males. Longer surviving mussels have lower intensity. In males the number of *Metacercaria A* in each mussel accounted for 9.9% of the variation in survival time.

***Metacercaria perchorupis* and mortality**

Figure 11 describes the relationship of infection intensity with *Metacercaria perchorupis* and time of mortality in both sexes of mussel.

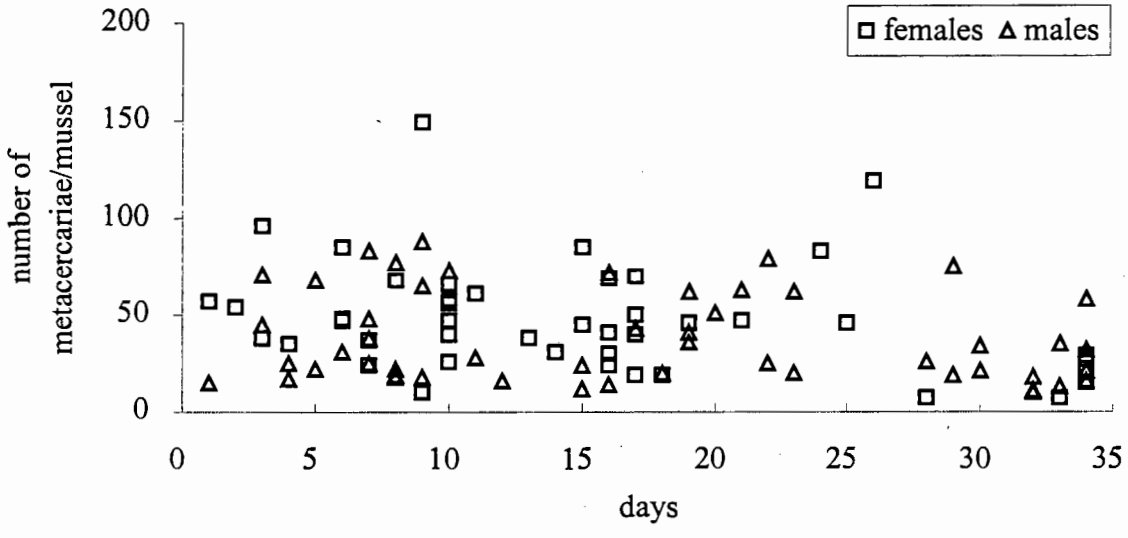


Figure 11. The relationship of *Metacercaria perchorupis* infection intensity with emersion survival time in *Choromytilus* males and females from Blouberg. June 1997 collection.

Descriptive statistics of these relationships are given in Table 8.

	females	males
n	44	54
abundance	47.9318	37.2593
SD	28.4887	23.0114
r	-0.2925	-0.208
r^2	0.08556	0.0433
slope	-0.0949	-0.099

Table 8. Descriptive statistics concerning the relationship of infection intensity with *Metacercaria perchorupis* and survival period in both sexes of *Choromytilus*. The *r* values indicate that only in females is intensity and survival time significantly (P = 0.05) related. The number of parasites per mussel at time of mortality accounted for 8.56% of the variation in survival time.

Total intensity of infection and mortality

Figure 12 describes the relationship of total number of parasites per mussel at time of mortality.

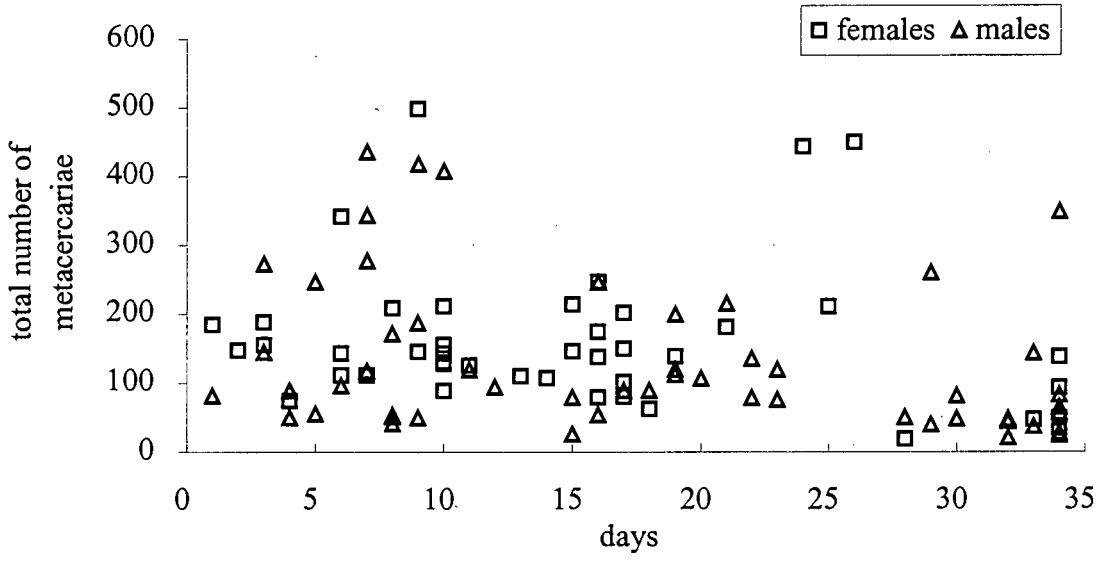


Figure 12. The relationship of total infection intensity (all metacercariae) with emersion survival time in *Choromytilus* males and females from Blouberg. June 1997 collection.

Descriptive statistics of these relationships are given in Table 9.

	females	males
n	44	54
abundance	159.159	129.278
SD	101.716	107.062
r	-0.1344	-0.3172
r^2	0.0187	0.1006
slope	-0.0122	-0.0324

Table 9. Descriptive statistics concerning the relationship of total infection intensity (all of the above metacercariae) and survival period in both sexes of *Choromytilus*. The *r* values indicate that only in males it is the relationship significant (P = 0.02). The total number of parasites per mussel at time of mortality accounted for 10.06% of the variation in survival time. The longer surviving mussels have fewer cysts.

Reproductive condition at mortality

	females	males
n	44	54
r	-0.471	0.0076
r^2	0.2218	0.000018
slope	-0.0115	0.00006

Table 10. Descriptive statistics concerning the relationship between reproductive condition at mortality and survival period in male and female *Choromytilus*. The *r* value is significant only in females (P = 0.001). The longer surviving mussels have decreased condition.

Mortality distribution over time

Figure 13 appears to show that mortality was highest just after high tide and lowest just before high tide. This prompted a repeat of the experiment and again the results (Figure 14) show a similar pattern of differential mortality before and after high tides. To substantiate these findings a third, larger, collection was taken in September (Figure 15).

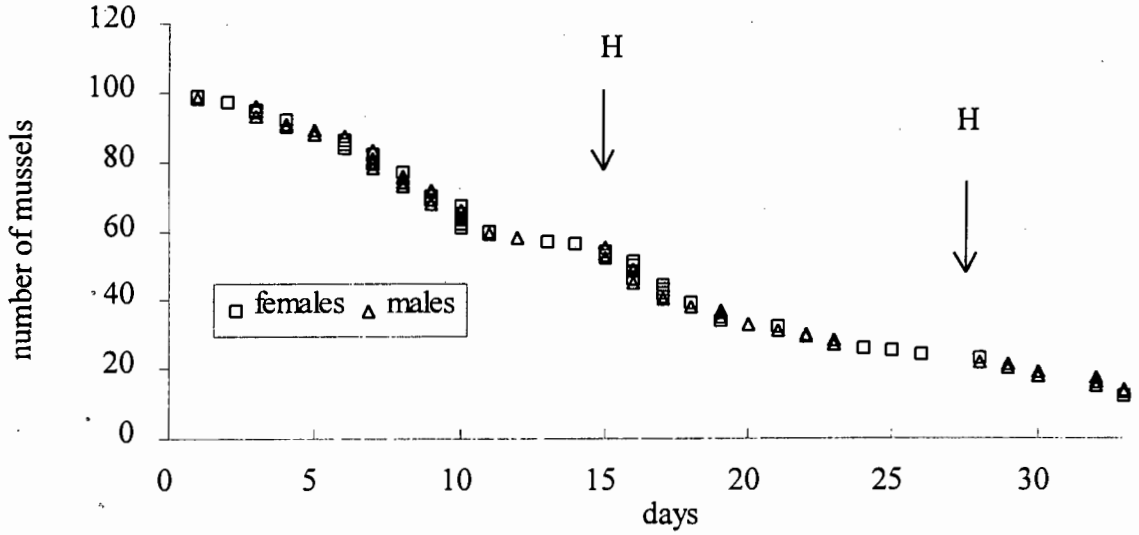


Figure 13. The relationship of mortality with time emersed in *Choromytilus* males and females from Blouberg. June 1997 collection. H = high water spring tide.

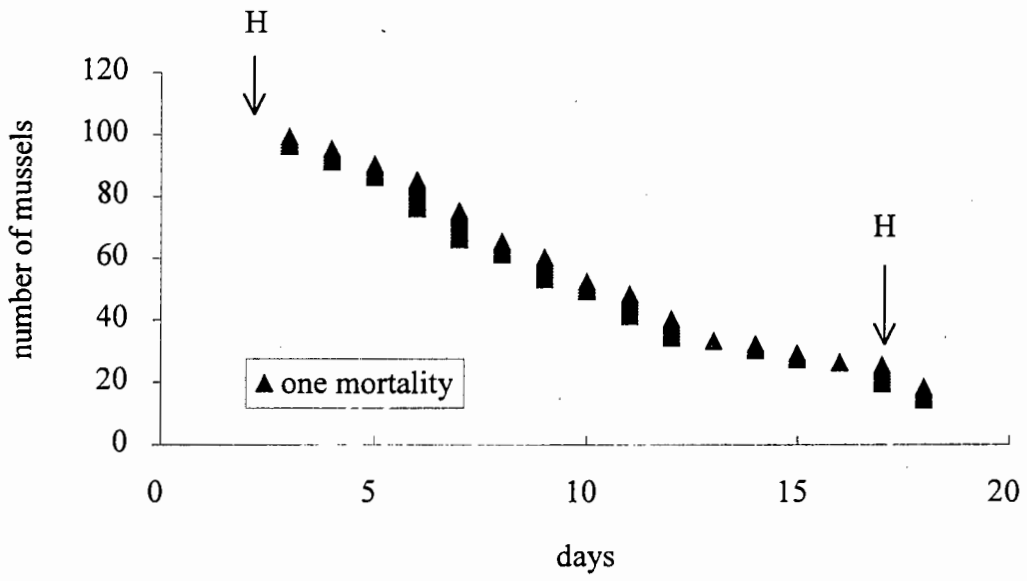


Figure 14. The relationship of mortality with time emersed in *Choromytilus* (both sexes) from Blouberg. August 1997 collection. H = high water spring tide.

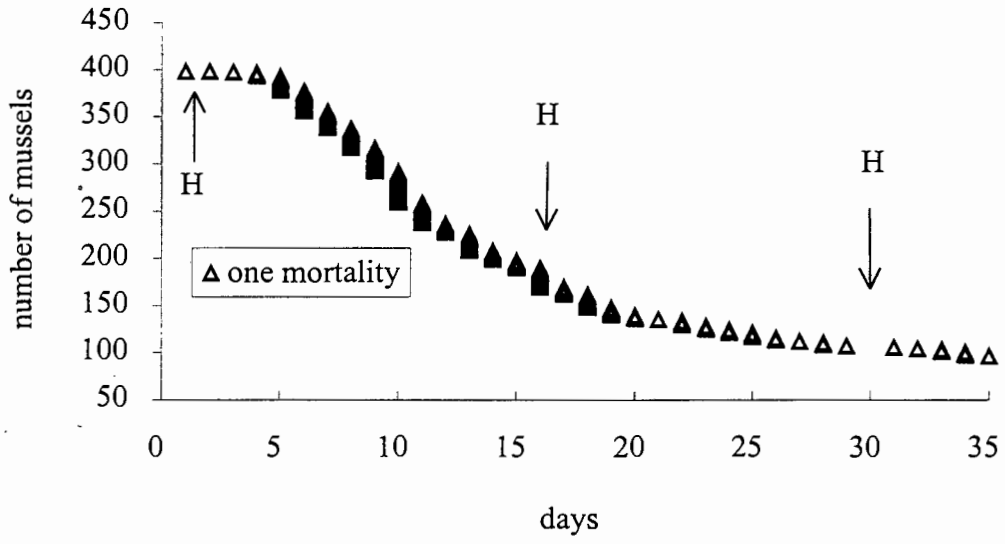


Figure 15. The relationship of mortality with time emersed in *Choromytilus* (both sexes) from Blouberg. September collection. H = high water spring tide.

DISCUSSION

Length and survival time

Regression parameters derived for the six samples: males and females from each of the three collections (See Tables 1, 2 and 3) were significant only in the case of females from the September collection (Table 3 referring to Figure 5). The *r* value indicates that this relationship is significant (P = 0.02) but only 4.26% of variation of survival time is attributable to shell length.

Sex differences in morphometrics and survival time

Student's t-tests between males and females of the three collection sites disclosed no significant sex linked difference in length between the sexes. Likewise, the results of t-tests between males and females of the three collection sites disclosed no significant sex linked difference between sex and survival time.

Sex dependent reproductive condition at mortality

After the above tests detecting no difference between the sexes it is surprising to find the *r* value in Table 10 to be significant in females (P = 0.001) but not males. Longer surviving females have declining condition; 22.18% of variation in condition can be

attributed to survival time. It is difficult to decide if reproductive condition or survival time is the independent factor. Do mussels in poor condition live longer or do those that live longer end in poorer condition? It could be argued that females mobilise their reproductive condition to extend survival - thus losing condition with time. This would explain the preponderance of poor condition mussels towards the end of the experiment. But this does not explain why there is a preponderance of females in good reproductive condition dying early in the experiment. This should not happen if they can convert reproductive condition into extended survival. If we reject this argument what alternative is there?

First let us consider that male mussels do not exhibit such a condition/survival time relationship. Why they do not remains to be explained. A study of energy flows and storage during conditioning in males and females may suggest a reason for this difference. This difference, for whatever reason, shows that in males, reproductive condition is not dependent on time. Perhaps also in females the loss of condition is not a temporal phenomenon. If so, then perhaps survival time is a condition dependent event. Thus one might conclude that females in poor reproductive condition last longer than those in better condition. This raises the possibility that good reproductive fitness does not confer good somatic fitness- as the facts coupled with this argument suggests here.

Metacercaria B and mortality

The relationship between *Metacercaria B* infection intensity and survival time is not significant in either sex. Thus, infections of *Metacercaria B* at the intensities encountered in this experiment are not a measurable somatic stress. This finding is, moreover, for abnormally high infection intensities; values determined in this experiment are considerably higher than those determined in the survey of mussel parasites (Chapter 9). This coupled with the lower intensities of *Metacercaria B* infections when compared with other parasites reported here prompts the conclusion that the deleterious potential of *Metacercaria B* can be discounted.

Metacercaria A and mortality

The relationship between total infection intensity and survival time is significant ($P = 0.02$) only in males. Longer surviving mussels have lower intensity. The total

number of *Metacercaria A* in each mussel accounted for 11.1% of the variation in survival time. When these results are broken down to infections in palps and mantle the *r* values in Table 6 & 7 indicate that the relationship between infection intensity and survival time at these sites is also significant (P = 0.05). The number of *Metacercaria A* in the palps and mantle of each mussel accounted for 8.32% and 9.9% of the variation in survival time respectively. That the r^2 value for the combined infection intensity is higher than either of the subdivisions into mantle infection or palp infection indicates that it is the total number of cysts and not their site that is important. If the r^2 value had been higher in either of the sites than the combined result then it would be obvious that the deleterious effect is site specific.

Metacercaria perchorupis and mortality

The *r* values in Table 8 indicate that the relationship between infection intensity with *Metacercaria perchorupis* and survival time is significant in females. It is concluded that *Metacercaria perchorupis* is a somatic stress to females at the intensities of infection found in this experiment.

Cercaria notobucephala

There was a disappointing paucity of mussels infected with *Cercaria notobucephala.* Only two mussels out of a total of 484 were infected with *Cercaria notobucephala* (0.41% prevalence). This contrasts with the prevalence of 4.46% in studies previously from Blouberg (Chapter 3). Of these mussels, one was the tenth to die out of 84 in the August collection and the other was 25th out of 302 in the September collection. That both infections occur in the early mortalities of the mussel suggests that *Cercaria notobucephala* might be an extra somatic load. The low prevalence with this parasite prevented further investigations.

This possible deleterious somatic effect (in addition to its overt reproductive effect) of *Cercaria notobucephala* is less surprising in the light of the report by Calvo-Ugarteburu (1996) on *Perna perna* that it shows weakened adductor muscles when infected with *Bucephalus* sp. Calvo-Ugarteburu (1996) reports that in *Perna perna* infected with *Bucephalus* there was a significant water loss after 12 hours exposure to air. However, there was no differential mortality over this period even up to the 80-90 hours that it took the whole sample to die. Calvo-Ugarteburu (1996, p104) reports

"Although bucephalid sporocysts do not directly affect mortality of the mussels (*Perna perna*), it is possible that, through weakening the adductor muscle, this parasite is responsible for high death rates due to causes that have yet to be assessed".

Total parasites and mortality

Significant relationship between total parasite infection intensity and survival occurs only in males. The total number of parasites per mussel at time of mortality accounted for 10.06% (P = 0.05) of the variation in survival time. The longer surviving mussels have fewer cysts. *Metacercaria A* appears to account for the majority of these effects

Two sex-linked conundrums

Males, though infected at lower intensity, show a significant intensity/mortality relationship, those dying sooner had more cysts (P = 0.02, $r^2 = 0.1006$). Chapters 3, 4, 5, 7 & 9 demonstrate that there is a general tendency for intensity to rise with mussel size and in consequence one might expect a tendency for larger males to be subject to higher mortality. In contrast, larger females have a tendency to lower mortality. Their survival time and shell length was significantly correlated (P= 0.02) but only 4.26% of variation in survival time was dependent on shell length. Thus males show parasite intensity dependent mortality and this, by inference, has a tendency to increase with mussel size. Meanwhile, females show significantly decreasing mortality with size. How can this be reconciled with data that shows the sexes to have no significant difference in survival time? How can there be an age/size differential mortality in females while overall mortality is the same as in males? Is it balanced by the parasite induced mortality in males? It would appear that large males are likely to be selected against as are small females. There appears to be selection pressure for sex-linked morphometric difference but examination of several hundreds of these mussels has failed to disclosed any sex bias in morphometrics.

Why do males outnumber females? The sex ratio is 1.227 to 1 and the ratio of infection abundances of males and females is 129.3 to 159.2. Though there are more males they have fewer worms each. If they had the same burden as the females they would suffer higher mortality. What mechanisms can be invoked for this lower intensity? Comparison of total worm burden carried by each sex as a separate

population produces an interesting result: multiplication of abundance in males by the ratio of their excess over the females will derive an equivalent worm burden for each sex. Thus for males one must multiply the abundance 129.3 by the ratio of excess of males (1.227) the result is 158.65. The value in females is 159.2. Is this coincidence? By what mechanism could such an equitable apportionment be effected between the sexes?

Mortality distribution over time

Figures 13, 14 & 15 strongly suggest a tidally determined mortality pattern. Further discussion concerning the significance of these results will be found in the synthesis (Chapter 48).

CHAPTER 46: DIFFERENTIAL LOSS OF NINHYDRIN POSITIVE SUBSTANCES IN PARASITISED AND NON-PARASITISED MUSSELS

INTRODUCTION

This work aims to ascertain any association between *Cercaria notobucephala* infections in *Choromytilus meridionalis* and elevated loss of ninhydrin positive substances such as ammonia, amines and amino acids. *Choromytilus* is the only mytilid on Western Cape beaches that harbours *Cercaria notobucephala* (Chapter 3). Because of its greater intensity of infection it is deemed to be potentially more disruptive of the host than other parasites found in mytilids on Western Cape beaches. *Choromytilus* mantle tissue may contain from 63 to 127 cercariae per cubic millimetre (Chapter 47). This is comparable to the entire body worm burdens of other parasites. See Chapters 4 to 9 for epidemiological details of other parasites.

It is fortuitous that, for any given level of food concentration and temperature, *Choromytilus* has higher excretion rates than *Perna perna*, *Mytilus galloprovincialis* or *Aulacomya ater* (van Erkom Schurink & Griffiths 1992). Thus, any results are more likely to represent production by the mussel than background production by co-fauna and flora living on mucus and pseudofaeces.

Of the ninhydrin positive substances, even loss of ammonia may be deleterious. Ammonia is both an end product and a substrate for protein metabolism in *Mytilus edulis* (Sadok, Uglow & Haswell 1995). Elevated loss can indicate an increase in excretion, which implies catabolic processes involving nitrogen; it can indicate a loss of ammonia (as a protein precursor); and it can indicate a loss of amino acids. Thus, all other things being equal, one would expect an organism that loses ninhydrin positive substances faster than another to be the more fitness-compromised. Such loss indicates inability of the mussel to maintain normal concentration gradients. This loss of order has been postulated (Chapters 16 & 33) as the result of stress. For further references to loss of nitrogen compounds from stressed bivalves see Chapter 27. Bucephalid parasites are notorious as reproductive stresses; members of the family have been implicated in

parasitic castration. Moreover, some of them even kill their hosts (Chapter 3). It is therefore likely that the stress inflicted by *Cercaria notobucephala* on *Choromytilus* also has a somatic component.

MATERIALS AND METHODS

Two experiments were run; each involved 15 mussels. Total wet mass was determined for each mussel and they were placed singly in beakers of aerated sea water that had been sterilised and filtered to standards sufficient for axenic seaweed culture (Levitt, pers. comm.). This was achieved by heating the water to 85°C for 3 hours and cooling to ambient. Sterilisation should minimise production of ninhydrin positive substances by microfauna and flora in the water.

After 6.5 hours, two 1ml samples of water were taken from each beaker and processed according to the experimental procedure outlined by Moore & Stein (1954). Colorimetric absorbance was measured by a Spectronic 20 spectrophotometer. A calibration curve of leucine concentrations (0, 5, 10, 20, 25, 50, & 100 micrograms per millilitre) was derived from standards made up with sea water. Use of sea water discounts any background levels of ninhydrin positive substances in the mussel immersion water and thus indicates only that produced by the mussel or by its attendant co-fauna and flora. Absorbance values from the mussel samples were converted into micrograms per millilitre of leucine on the standard curve. The mean of the two values was calculated and it was then corrected to give the production in micrograms of ninhydrin positive substances per millilitre of sample water per gram, wet mass, of the mussel and its shell. The mass-specific production of the 15 mussels was ranked and the ranking was divided into parasitised and non-parasitised groups. Difference between the groups was analysed by the Mann-Whitney U-test (Zar 1984), this being suited to testing small dissimilar sized samples.

RESULTS

The first experiment was performed at room temperature (22°C). The leucine standards gave the following absorbances:

leucine µg/ml	absorbance
0	0
5	0.087
10	0.179
20	0.33
25	0.395
50	0.84
100	1.7

The regression parameters of these values are:

constant	-0.0048
standard error of Y	0.0139
r	0.9998
r^2	0.9996
P	0.0005
no. of observations	7
degrees of freedom	5
X coefficient	0.01698
standard error of coefficient	0.00016

A shortage of uniform beakers necessitated placement of the mussels in differing volumes of water (80, 100 or 150 millilitres) for the experimental period; beaker allocations are presented below. Where possible, large mussels were placed in large volumes and small mussels in small volumes. The results were corrected accordingly.

host wet mass g	uncorrected mean µg/ml	corrected mean µg/ml	beaker volume ml	mean µg/ml/gram of host
14.83	3.387	3.387	80	0.228
11.44	3.946	3.946	80	0.345
19.05	4.182	4.182	80	0.220
13.63	4.771	4.771	80	0.350
16.76	4.182	5.227	100	0.312
14.31	5.302	5.302	80	0.371
21.14	3.093	5.799	150	0.274
23.84	4.654	5.817	100	0.244
17.25	4.978	6.222	100	0.361
19.32	5.478	6.847	100	0.354
23.43	5.508	6.885	100	0.294
19.27	6.923	8.651	100	0.449 parasitised
40.55	5.894	11.046	150	0.272
28.83	7.776	14.580	150	0.506 parasitised
26.90	23.563	44.183	150	1.643 DEAD

Mann-Whitney U test

The null hypothesis was that parasitised mussels do not produce higher concentrations of ninhydrin positive substance than non-parasitised mussels. The alternative hypothesis was that parasitised mussels produce higher concentrations of ninhydrin positive substances.

Ranking of values:

parasitised	non-parasitised
(13) 0.449	(1) 0.220
(14) 0.506	(2) 0.230
	(3) 0.244
	(4) 0.272
	(5) 0.274
	(6) 0.294
	(7) 0.312
	(8) 0.345
	(9) 0.350
	(10) 0.354
	(11) 0.360
	(12) 0.371
$n_1 = 2$	$n_2 = 12$
$R_1 = 27$	$R_2 = 78$

$U' = 24$

$U_{0.025\,(1)\,2,12} = 23$

Since the calculated value U' (24) is larger than U (23), the null hypothesis is rejected: parasitised mussels produce higher concentrations of ninhydrin positive substances.

The second experiment was also performed at room temperature (22.5°C). The following absorbances were obtained from the standards.

leucine µg/ml	absorbance
0	0
1	0.008
2	0.034
5	0.109
10	0.18
15	0.233
20	0.256

The regression parameters of these values:

constant	0.01381
standard error of Y	0.02601
r	0.97526
r^2	0.95113
P	0.0005
no. of observations	7
degrees of freedom	5
X coefficient	0.01365
standard error of coefficient	0.00138

All mussels were placed in 150 ml of water.

mass of host g	mean µg/ml	mean µg/ml/gram
11.56	8.902	0.770
19.89	8.902	0.448
39.96	9.415	0.236
20.27	9.416	0.465
18.54	10.001	0.539
12.86	10.478	0.815
16.84	10.551	0.627
33.05	11.284	0.340
17.12	11.284	0.659
21.82	11.430	0.524
18.77	12.126	0.646
10.9	12.566	1.153 parasitised
19.55	13.189	0.675
22.77	13.262	0.582
20.05	15.717	0.784 parasitised

Mann-Whitney U test

The null hypothesis was that parasitised mussels do not produce higher concentrations of ninhydrin positive substance than non-parasitised mussels. The alternative hypothesis was that parasitised mussels produce higher concentrations of ninhydrin positive substances.

Ranking of values:

parasitised	non-parasitised
(13) 0.784	(1) 0.236
(15) 1.153	(2) 0.341
	(3) 0.448
	(4) 0.465
	(5) 0.522
	(6) 0.539
	(7) 0.583
	(8) 0.627
	(9) 0.646
	(10) 0.659
	(11) 0.674
	(12) 0.770
	(14) 0.815
$n_1 = 2$	$n_2 = 13$
$R_1 = 28$	$R_2 = 92$

$U' = 25$

$U_{0.05\,(1)\,2,\,13} = 24$

Since the calculated U' (25) is greater than U (24), the null hypothesis is rejected: parasitised mussels produce higher concentrations of ninhydrin positive substances.

DISCUSSION

In both experiments, parasitised mussels showed a significant (P = 0.025 & 0.05) tendency to produce more ninhydrin positive substances. The method in its present development lacks precision between experiments and is sensitive enough only to show a comparative difference in the same assay. This, however, is sufficient for the purpose of this work. A refinement would be to derive the production of ninhydrin positive substances per unit dry mass of flesh or flesh and shell. In addition, more consistent results might be obtained by other methods such as the phenol-hypochlorite (Widdows & Johnson 1988), the Solorzano method using phenol-hypochlorite (Navarro 1988), ninhydrin (Sadok, Uglow & Haswell 1995), and Indophenol blue method (van Erkom Schurink & Griffiths, 1992). The ninhydrin method of Moore & Stein (1954) was used in this experiment because it was the most convenient, equipment was available and it was sufficiently sensitive.

The dead mussel in the first experiment provides an interesting datum. A comparison of the different categories of dead, infected and non-infected mussels shows a declining production. The dead mussel produced a mean of 1.643 µg/ml/gram, parasitised mussels produced a mean of 0.478 µg/ml/gram and non-infected mussels produced a mean 0.30 µg/ml/gram. Although one dead mussel is too small a sample for inference, it does prompt speculation that the rate of loss is an indication of the aliveness of the mussel.

At least some of the increase in the dead mussel must come from loss of control of membranes *post mortem* rather than just decomposition. This mussel was freshly dead; this is clear because all were inspected before immersion and the criterion of health was tight closure of the valves on handling. The experiment in its present form cannot distinguish the contribution made by decomposition and that by loss of gradient control at the membranes. To achieve this the experiment could be repeated with a sample containing non-parasitised, parasitised and freshly killed mussels. The freshly killed sample could itself be subdivided, some mussels could be used without further treatment and the others could be treated with an antibiotic or by a bacteriostat. By subtraction, this would give indication of the contribution made by decomposition over the immersion period. A problem is to find a method of killing the mussels without damaging or denaturing the membranes across which nitrogenous substances are lost. This requirement might be met by ionising radiation or a metabolic poison.

CHAPTER 47: THE EFFECT OF *CERCARIA NOTOBUCEPHALA* ON REPRODUCTIVE FITNESS IN *CHOROMYTILUS MERIDIONALIS*

INTRODUCTION

As discussed in Chapter 26, one might expect a separation of stress into components that affect either somatic or reproductive fitness. The taxonomic affinities of *Cercaria notobucephala* reviewed in Chapter 3 suggest that it presents a predominantly reproductive stress on *Choromytilus meridionalis* and its effects are those of parasitic castration. A measurable reduction in reproductive fitness is thus to be expected. It will be assessed here by comparison of gamete numbers per cubic millimetre of mantle tissue in infected and uninfected mussels. The effects of *Cercaria notobucephala* on somatic fitness are examined elsewhere. See Griffiths (1977), van Erkom Schurink & Griffiths (1991) and Lasiak (1986) for a survey of normal reproductive phenomena in South African mytilids.

MATERIALS AND METHODS

Seven uninfected and five infected male *Choromytilus* from Blouberg were examined. Means and descriptive statistics of each group are given below.

	mean length	SD	SE	n
uninfected	84.64mm	10.24	3.87	7
infected	80.57mm	7.28	3.25	5

Gamete counts were done on a Neubauer counting slide. The slide has two grids, each 3mm by 3mm of 0.1mm depth, giving 9 squares each of 1mm^2 (designated A in Figure 1). The central mm^2 is divided into 25 squares, each 0.2mm x 0.2mm = 0.040mm^2 (designated C). Each of these squares is in turn divided into 16 squares of 0.05mm x 0.05mm = 0.0025mm^2 (designated D). The peripheral 8 squares, each of 1 mm^2, are subdivided into 16 squares of 0.25mm x 0.25mm = 0.0625mm^2 (designated D).

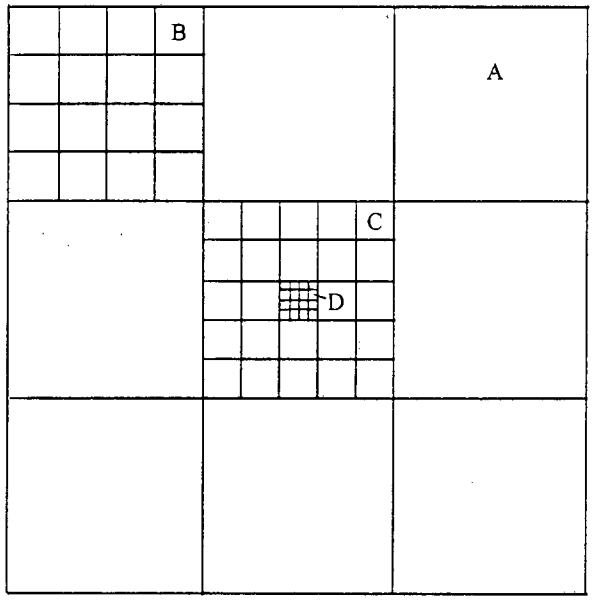


Figure 1. Details of subdivisions of one of the two grids on the Neubauer counting slide.

A scalpel stroke from the byssus aperture backwards severed the adductor muscle and the valves were separated. The entire mantle was removed from each valve by an incision that started just inside and parallel with the fimbrications, it continued around the shell and medially ran along the junction of mantle with the ctenidia. Excess water was blotted from the mantle tissue and it was fed into a graduated syringe of $1cm^3$. After manipulating the syringe contents to expel trapped air a $0.1cm^3$ sample was extruded into a measuring cylinder and topped up with sea water to $10cm^3$. This volume was decanted into a cubic plastic cell where the tissue could be macerated to release all the sperm from the tissue. The process was considered complete when the tissue ceased issuing clouds of sperm.

A drop of the supernatant was deposited on each grid, covered with a coverslip, and counts taken from ten D squares. The other grid was examined similarly. Thus, sperm were counted on twenty D squares for each tissue sample. If sperm heads overlapped the left or top sides of a D square they were counted; they were ignored if they overlapped the right or bottom sides.

A standard pattern was used for each grid: one D square from each C square was counted for the first two rows of C squares from the top. In each C square the D square second down and second from the left was counted. If this square was

obscured by detritus, then the corresponding D square in the 3rd or 4th row of C squares was observed. This gives a count of ten D squares per grid. The process was repeated once from this valve with another $0.1cm^3$ tissue sample. The other valve was examined similarly using two tissue samples. Thus a total of 20 x 4 sperm counts (80) was obtained for each mussel. Allowance was made for the tissue sample size, the dilution factor and the volume of sample liquid examined. The counting cell is 0.1mm high and its area depends on which square is chosen. The counts were converted to number of sperm per mm^3 of tissue and the mean count per mm^3 of tissue for each mussel was calculated, as were standard deviations and standard errors.

An indication of the reproductive condition was ascertained for each of the non-infected mussels. Females were not examined because no infected females were found after a search in over 1000 mussels. The condition index is comparative and based on the thickness of the mantle:

Condition 1	Thick mantle with lens-like thickening that deviates from the contours of the inner shell.
Condition 0.75	Thick mantle that follows the contour of the inside of the shell.
Condition 0.5	Gonad fills about half of the mantle
Condition 0.25	Gonad fills less than half of the mantle
Condition <0.25	Gonad very sparse

Cercarial counts per mm^3 of mantle were made in parasitised mussels. Cercarial numbers are more representative of infection intensity than those of sporocysts because sporocysts have no easily defined units that can be counted. The entire 3mm x 3mm grid was used and four grids were counted for each tissue sample. Four tissue samples – two from each valve were examined, thus sixteen cercaria counts were performed for each mussel. Cercariae that overlapped left or upper sides were counted; those that overlapped right or lower edges of the 3mm x 3mm grid were ignored.

RESULTS

Figure 2 compares sperm counts of infected and uninfected mussels. The log. scale is used to accommodate both meaningfully on the same graph. Figure 3 shows the sperm counts for uninfected mussels and their standard errors. Figure 4 shows the sperm counts of infected mussels and the standard errors of the values. Figure 5

relates the sperm count with the cercaria count in infected mussels.

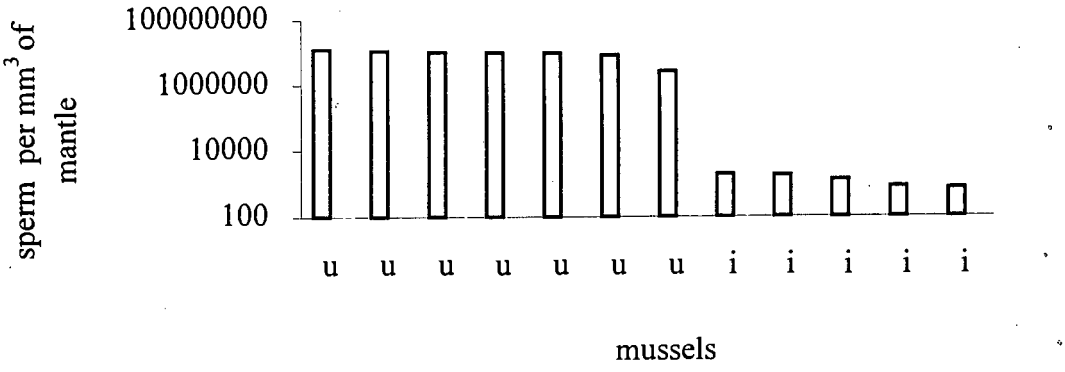


Figure 2. Comparison of sperm counts per mm^3 of uninfected (u) and infected (i) *Choromytilus* mantle.

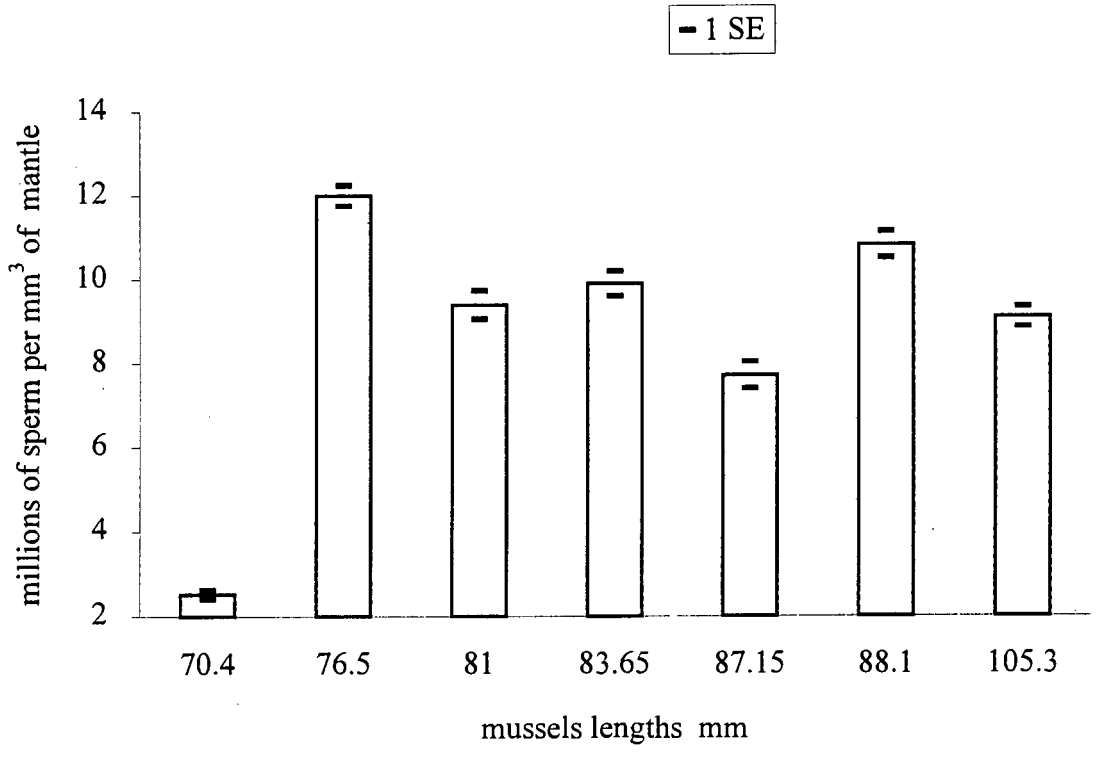


Figure 3. Sperm count and mussel size in uninfected *Choromytilus*.

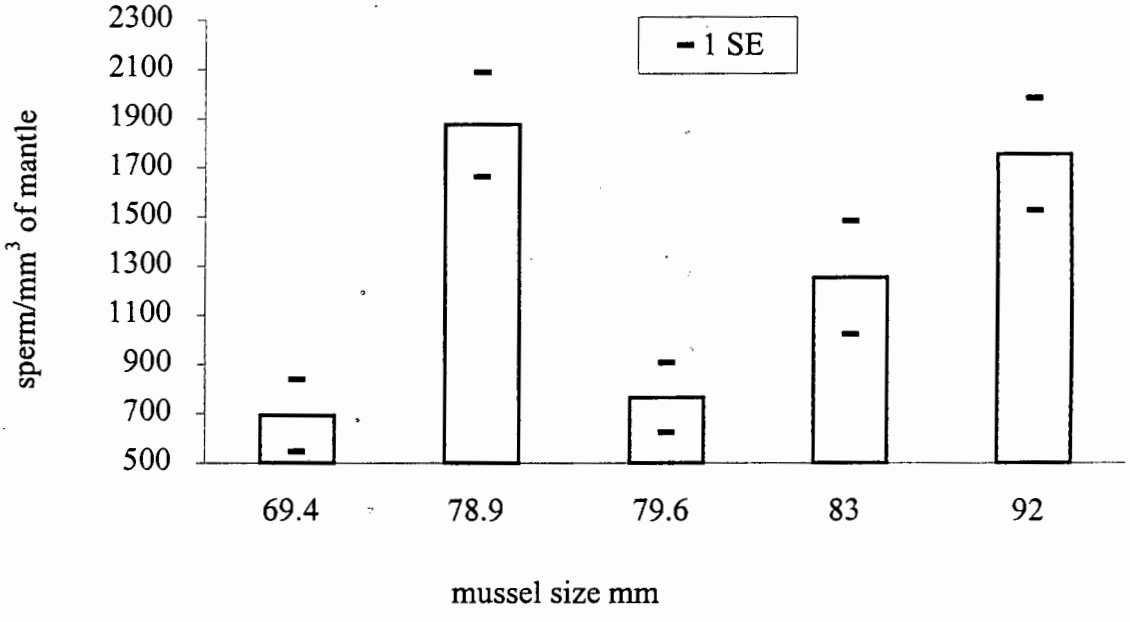


Figure 4. Sperm count versus mussel size in *Cercaria notobucephala* infected *Choromytilus*.

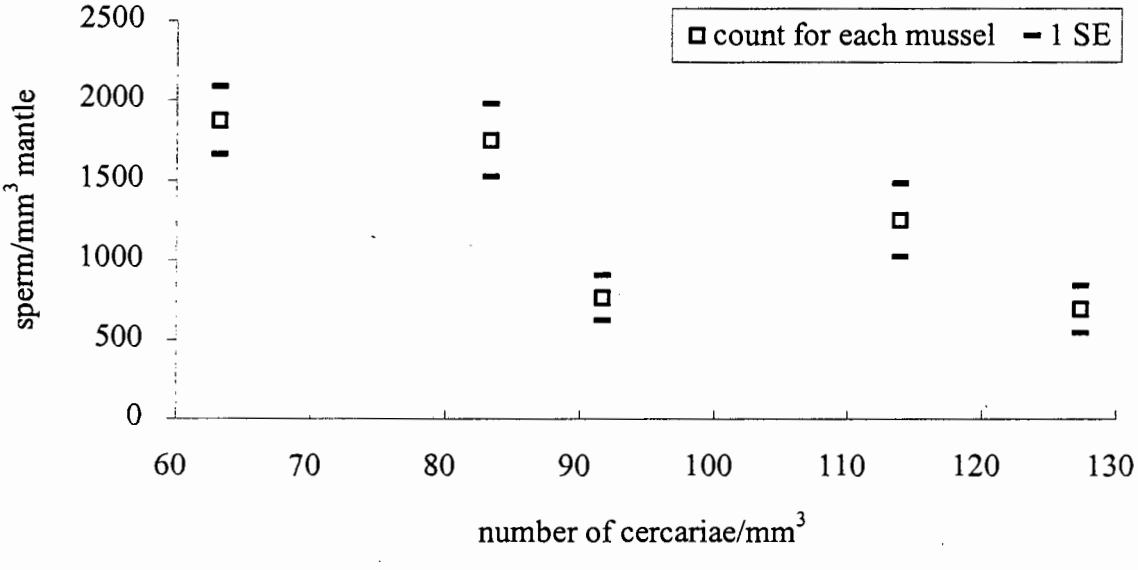


Figure 5. The relationship between sperm count and number of cercariae in infected *Choromytilus*.

DISCUSSION

Size ranges of infected and uninfected mussels overlapped. Although there were larger and smaller uninfected mussels than those in the infected sample, neither extreme accounted for any extreme in sperm count. The lowest sperm count in the uninfected mussel sample is exhibited by the smallest mussel. That mussel was selected as an example of poor condition (less than 0.25). There was also less volume

of mantle tissue in this mussel than in those with higher sperm counts. Its mantle was thinner even than infected mussels. Of the other uninfected mussels one had a condition of 0.75 and the others had a condition of 1. Sperm counts of the other six uninfected mussels (Figure 3) show no size dependence. Similarly sperm count shows no size dependent trends in infected (Figure 4) mussels. Thus any variation in sperm count can be attributed to condition rather than size.

Parasitism by *Cercaria notobucephala* reduces the sperm count drastically. The logarithmic scale on Figure 2 actually reduces the apparent difference between infected and uninfected mussels. The lowest count among the uninfected mussels was 2.5 million sperm/mm^3 (Figure 3); the highest count in the infected mussels was 1875 sperm/mm^3 (Figure 4). This is a minimum difference of over 1300 times; the other healthy mussels, had sperm counts of about 10 million/mm^3 (Figure 3). There is such great separation between the two samples that mere examination of the standard errors of the means is sufficient to show that no statistical test is necessary for confirmation of this difference.

Sperm count is depressed by *Cercaria notobucephala* but not abolished. This is evident also in Figure 13 of Chapter 3. In contrast, Calvo-Ugarteburu (1996 p112) reports that *Bucephalus* sp. in *Perna perna* causes "complete castration" and, "no trace of sex products are left".

Figure 5 suggests that there may be an inverse relationship between the sperm count and the number of cercariae per cubic millimetre of mantle tissue. It is tempting to discard the datum for 91.7 cercariae/ mm^3; if it were, then there would be evidence for a non-linear relationship of decreasing sperm count with increasing numbers of cercariae. However, at these low sperm numbers this relationship may be biologically irrelevant. During this part of the work, infected mussels became rather rare: less than one infected mussel in 200. Were it not for the sudden paucity of infected mussels at Blouberg it would have been possible to collect more data to substantiate this.

It can be concluded that infection with this gasterostome reduces sperm numbers in gonad tissue by a factor of over 1000. This parasite can thus be classified as a reproductive stress.

PART VII:

SYNTHESIS

CHAPTER 48: SYNTHESIS

This synthesis highlights where hypotheses and evidence coincide. It demonstrates various aspects of the stress field and shows that even disparate agents can be substantively integrated as stresses. Studies in this thesis have signposted the landscape of stress for further exploration. The following outlines the work.

1. A survey of the common intertidal mytilids has revealed eight new species of digenean trematodes and their epidemiology (Chapters 3 to 10). Contingent on this was the development of a technique (Chapter 2) for the preparation of helminths. The survey also revealed the parasitic pycnogonid, *Nymphonella* sp. (Chapter 11) in *Choromytilus meridionalis* and a shell-boring cyanophyte, *Mastigocoleus* sp. (Chapter 12).
2. The stress literature has been reviewed (Chapters 14 to 30). Previous concepts have been analysed and, where necessary, clarified or discarded. Suggestions have been made to integrate the disparate fields in which the term stress is used. Integrable and non-integrable areas have been identified.
3. Concepts and hypotheses concerning stress and its effects are propounded (Chapters 31 to 40). And some of these hypotheses have been tested (Chapters 41 to 47). In particular the action of parasites and other biological stresses have been shown to be integrable as, and made equivalent to, other stresses. Salient findings follow.

PARASITES ARE STRESSES

The following parasites are measurably deleterious:

Metacercaria notobucephala

As a somatic stress

Mussels parasitised with *Metacercaria notobucephala* lose ninhydrin positive substances significantly faster (Chapter 46). The one fortuitous mortality during the assay lost ninhydrin positive substances even faster. This loss apparently caused by parasites is not by the puncturing of the mantle as no cercaria were released. Other results for this parasite suggest that it may be a moderate somatic stress (Chapter 45) and a severe reproductive stress (Chapter 47).

As a reproductive stress

Cercaria notobucephala is a severe reproductive stress (Chapter 47) to *Choromytilus*. Infected mussels have about 1000 sperm per cubic millimetre of mantle/gonad compared with 10 million sperm per cubic millimetre in uninfected mussels. Parasites reduce the sperm count to 1/10 000 of the uninfected level.

Metacercaria A* and *Metacercaria perchorupis

Infection with these worms reduced emersion survival time. *Choromytilus* males surviving longer had fewer cysts ($P = 0.02$) of *Metacercaria A* (Chapter 45). *Choromytilus* females surviving longer had fewer cysts ($P = 0.05$) of *Metacercaria perchorupis*. Since these parasites compromise survival they are somatic stresses.

A quantified stress/strain relationship

Shell damage caused by *Mastigocoleus* sp. was quantified in *Mytilus galloprovincialis* (Chapter 12). To clarify the stress/strain relationship, stress (here defined as extent of the infection on the shell) is correlated with the strain (the severity of damage on the shell) and shows that there is a proportionality between the amount of alga and the amount of damage. Stress, the independent variable, was determined by division of the mussel into three parts and then judging each of them out of five (See Chapter 12) for coverage by the alga. Each of the units out of 15 indicates approximately 6.67% of the shell area.

Strain, the dependent variable, is indicated here by the degree of damage and graded from 0 (nil) to 5 (holed). Thus condition 5 indicates zero fitness and condition 0 is normal fitness. Holes in the shell caused by the alga are a result of weakening of the shell over the adductor muscle insertion point followed by the mussel pulling the shell through at that spot. This results in uncontrollable gaping and the mussel can no longer close to defend itself.

Stress and strain have thus been characterised by independently grounded criteria. The following statistics describe the relationship:

r	0.786
r^2	0.618
slope	0.29
n	144
df	142

The amount of damage is dependent on the degree of coverage by the agent. This relationship is highly significant (P = 0.001): 61.8% of variation in the damage to the shell is attributable to the extent of the infection on the shell. Thus from the slope it can be calculated that one unit of stress (6.67% of coverage) causes 0.29 units (out of 5) of strain. This means that 1% increase in stress causes 0.87% increase in strain. That even 100% coverage would produce 87% of mussels with holes is due in part to the catastrophic nature of hole production. The weakening process may be gradual but its progress increases the likelihood that the shell will fracture. Thus there is an element of stochastic stress/strain in this relationship (See Chapter 39).

SUBSTANTIVE INTEGRATION OF PHYSICAL, CHEMICAL AND BIOLOGICAL STRESS AGENTS

Salinity, ammonia, phenol, and scavenging whelks are all integrable as stresses since they all inhibit shell gaping (Chapter 42) and byssus production (Chapter 43). Thus one physical, two chemical and one biological agent are shown to be substantively integrable by two different assays.

AN ENDOGENOUS STRESS IDENTIFIED: AGEING IN MUSSELS

Mussel size may be correlated closely with age: large mussels are older. And larger mussels have a lower rate of byssus production (Chapter 43). If byssus production contributes to somatic fitness of mussels, then older mussels with lower rates of production are less fit. Consequently, age is an endogenous somatic stress (discussed in Chapter 26).

How significant is this intrinsic stress? It is assumed that strength of individual threads remain constant with age of the mussel and that the rate of byssus production is important to make up for breakage during physical stress of anchorage. The area of the mussel increases at the square of its length and thus hydrodynamic drag increases

at the square of length or the square of the age. This is one age limiting factor. The other is that the number of byssus threads reduces with age. Thus the force resistance of the mussel decreases with age. It follows that the relationship between force applied and force withstood as the mussel ages follows a non-linear trajectory. Thus the risk of meeting a critical inadequacy rises sharply with increase in mussel length.

IDENTIFICATION OF AN UNFAMILIAR STRESS BY THE INAPPROPRIATE RESPONSE IT ELICITS

Stresses to which mussels might be familiar such as whelks, salinity shifts or ammonia produce decisive and organised responses (Chapter 42). Phenol, which would be an unfamiliar stress, does not (Chapter 42). At 100ppm (a sublethal concentration) phenol, mussels appear to have no effective response. The narcotic effect of phenol increases gaping, which can only increase exposure to further narcosis, which is clearly deleterious. Such an inappropriate response may be because mussels have not been exposed to phenol as an agent of selection. It is speculated that if it were, then the mussels would close at low concentrations and remain isolated from the ambient water, as they do with familiar agents. In the presence of agents that one might reasonably expect to be of selective significance, mussels respond with clear and appropriate actions. Thus unfavourable salinity, ammonia levels or the threat of *Burnupena* sp. produce a decisive and unipolar increase in closure likelihood.

It is proposed that this difference may be exploited as a method of detecting novel stress agents as opposed to selectively familiar agents. Novel agents would be expected to evoke a less decisive and organised pattern of response in the test organism.

EMERSION SURVIVAL: DO MUSSELS 'HOLD ON' UNTIL THE NEXT SPRING HIGH TIDE?

Figures 13, 14 & 15 (Chapter 45) all show a reduction in mortalities just before spring high water and an increase on or just after. This suggests that mussels have intrinsic fluctuating responses (see Chapter 14) that manifest as changes in mortality. Alternatively, it suggests that mussels have a tide table and are able to 'hold-on' until the next spring high water. How mussels do this is worthy of further investigation.

LEVELS OF INTEGRATION AND PROXIMAL & DISTAL EFFECTS: NOTIONS OF EUSTRESS ARISE WHEN THESE ARE IGNORED

The particle speed assay (Chapter 41) is the least integrated of experiments performed in this work and it shows that some ammonia concentrations elevate particle speed (i.e. they are so-called eustresses). In Figure 12 (Chapter 41) particle speed exhibited by the control is significantly lower than in 30ppm, 45ppm, 60ppm & 75ppm ammonia, which are all elevated (P= 0.005). Although ammonia at 40ppm is a stimulus in the particle speed assay, it is a significant inhibitor in the byssus assay (Chapter 43). Thus the apparent stimulus of cilia by ammonia at 40ppm is not supported by the more integrated response of byssus production at 40ppm. Furthermore, at 20ppm, ammonia is not detectable in the particle speed assay but in the 10-hour gaping experiment it is an inhibitor. In the byssus 6-day experiment, ammonia is significantly inhibitory even at 10ppm. Thus cilia can detect 120ppm ammonia as a threshold of deleterious effect but the six day byssus assay is twelve times more sensitive. Ammonia at 10ppm is not detectable by byssus growth over 24 hours, but it is over 6 days (Chapter 43, Tables 1 & 2). Thus what might appear to be a proximal benefit (as measured by particle speed) or harmless (by 24 hr byssus growth) is identifiably harmful over a longer period and/or by a more integrated assay.

It is noteworthy that the byssus assay (the most integrated of the assays used here) identifies none of the agents as significant stimuli. The closest is phenol (5ppm), which in the 24 hour experiment is associated with a higher (but not significant) byssus production than the controls. The integrating effect of time is evident when this excess is substantially reduced in the 6-day byssus experiment.

Thus any notion of positive deflections or eustress must be treated with great caution and subject to longer-term tests and with more complete integrations if it is to be accepted.

A FATAL STRESS CAUSES POSITIVE FEEDBACK DYNAMICS

The experiment in Chapter 44 that characterises the dynamics of shell gaping confirms the prediction in Chapter 32 (Figures 1 & 2) that a lethal stress may stimulate an exponential increase of displacement from equilibrium.

FINAL COMMENTS ON STRESS

Parasites may now take their place as stresses in their own right. And it has been shown that physical, chemical and biological agents can all be integrated as stresses. In addition, a method has been outlined to include psychological stress in a general scheme of individual stress.

The future of applied stress studies, it is urged, lies in greater attention to temporal aspects of stress phenomena and, above all, dynamics. Organisms are exquisitely complex detectors with a consequent potential for a huge response repertoire. A greater awareness of the temporal aspects of stress phenomena will enable us to detect and interpret more of these indicators.

ACKNOWLEDGEMENTS

This work was supported, in part, by a grant from the Foundation for Research Development.

I thank my supervisor, Professor A C Brown, for his patience and restraint in the face of delay and prevarication. This delay is attributable, at least in part, to the development of my thesis in accordance with the organismic view of life therein expounded. It thus has more of the character of an emergent property than a predetermined goal. I am thus indebted to Professor Brown for not demanding an early and specific indication of where the work was going.

A number of people assisted in the *Mastigocoleus* project. Thanks go to Associate Professor C L Griffiths for his support and advice. I thank students of the Zoology third year field camp 1990 for their assistance. In particular, Mr J L Korrubel prepared figures 6, 7, 9 and 10 that appear in Chapter 12 of this thesis. He also assisted with a literature survey on *Mastigocoleus*. Thanks also to Dr Candy Lang of the Department of Materials Engineering, University of Cape Town, for instruction on the use of the penetrometer for testing shell strength. I am indebted to Mr Neville Eden for his help and advice with photographic techniqes and also the Electron Microscope Unit UCT. Thanks to Dr Brian Bush, University of Bristol for his advice on feedback dynamics.

More generally, I am indebted to the late Mr R K Brooke of the Percy Fitzpatrick Institute of African Ornithology, University of Cape Town, for numerous and valuable discussions on the philosophy of science. I thank my wife, Kathy, for tolerating prolonged and unseasonable demands on time and resources, especially in the closing stages of preparation of this thesis.

REFERENCES

References that have been cited from other works occur here as quoted, and may be less complete than references that have been seen. If the reference has subsequently been seen then it is cited in full.

Abercrombie, M., Hickman, M., Johnson, M.L. & Thain, M. 1990. *The New Penguin Dictionary of Biology.* 8th edition, Penguin Books, 600pp.

Aiello, E. 1974. Control of ciliary activity in metazoa. In*: Cilia and Flagella.* (Sleigh, M. A. ed.) pp. 353-376. Academic Press, London.

Aiello, E., Hager, E., Akiwumi, C. & Stephano, G.B. 1986. An opioid mechanism modulates central and not peripheral dopaminergic control of ciliary activity in the marine mussel *Mytilus edulis*. *Cellular and Molecular Neurobiology* 6(1) 17-30.

Ajana, A.M. 1975. *Effect of Temperature and Salinity on the Ciliary Activity of Bivalves*. MSc. Thesis, University of Wales.

Akberali, H.B. 1980. ^{45}Calcium uptake and dissolution in the shell of *Scrobicularia plana* (da Costa). *Journal of Experimental Marine Biology and Ecology* 43, 1-9.

Alderdice, D.F. 1965. Analysis of experimental multivariable environments related to the problem of aquatic pollution. *Biological Problems in Water Pollution, Trans. 3rd Seminar,* 1962, pp. 320-325. U.S. Public Health Service Publication No. 999-wp-25.

Aleem, A.A. 1980. Marine Cyanophyta from Sierra Leone, (West Africa). *Botanica Marina* 23: 49-51.

Almada-Villela, P.C. 1984. The effects of reduced salinity on the shell growth of small *Mytilus edulis* L. *Journal of the Marine Biological Association of the United Kingdom* 64: 171-182.

Ambariyanto & Seed, R. 1991. The infestation of *Mytilus edulis* Linnaeus by *Polydora ciliata* (Johnston) in the Conwy estuary, North Wales. *Journal of Molluscan Studies* 57, 413-424.

Amkraut, A. & Solomon, G.F. 1975. From the symbolic stimulus to the parthophysiologic response of immune mechanisms. *International Journal of Psychiatry in Medicine* 5, 541-563.

Anand, P.L. 1937. An ecological study of the algae of the British chalk cliffs I. *Journal of Ecology* 25: 153-188.

Anderson, M.L. 1987. A re-appraisal of method: the way of confrontation. *South African Journal of Science* 83, February, 89-93.

Andrewartha, H.G. & Birch, L.C. 1986. *The Ecological Web: More on the Distribution and Abundance of Animals*. University of Chicago Press, 506pp.

Archer, J. 1979. *Animals Under Stress*. (Institute of Biology. Studies in biology; no. 108 ISSN 0537-9024). Edward Arnold Ltd, London, 60pp.

Arita, K. 1936. Ein überzähliges bein bei einer pantapoden-art *(Nymphonella tapetis* Oshima). *Annotationes Zoologicae Japonenses* 15:4 469-479.

Arnaud, F. 1978. A new species of *Ascorhynchus* (Pycnogonida) found parasitic on an opisthobranchiate mollusc. *Zoological Journal of the Linnean Society* 63: (1 & 2) 99-104.

Arnaud, F. & Bamber R.N. 1987. The biology of Pycnogonida, *Advances in Marine Biology* 24: 1-96.

Arts, M.T. & Sprules, W.G. 1987. Energy reserves of three zooplankton species from two lakes with different metal concentrations. *Canadian Journal of Fisheries and Aquatic Sciences* 44(2), 458-466.

Astin, J.A. 1997. Stress Reduction through Mindfulness Meditation: Effects on Psychological Symptomatology, Sense of Control, and Spritual Experiences. *Psychotherapy and Psychodynamics* 66:97-106.

Auerbach, S.I. 1981. Ecosystem Response to Stress: A Review of Concepts and Approaches. Chapter 3 in Barrett, G.W. & Rosenberg, R. (Eds), *Stress Effects on Natural Ecosystems.* John Wiley & Sons Ltd. 305pp.

Ayala, F.J. 1970. Teleological Explanations in Evolutionary Biology. *Philosophy of Science* 37, 1, 1-15.

Bailey, J.M. & Bhagat, R.S. 1987. Meaning and Measurement of Stressors in the Work Environment: An Evaluation. Chapter 8 in Kasl, S.V. & Cooper, C.L.,

(Eds), 1987. *Stress and Health: Issues in Research Methodology.* John Wiley & Sons, Winchester, New York. 321pp.

Baillieul, M., Selens, M. & Blust, R. 1996. Scope for growth and fitness of *Daphnia magna* in salinity stressed conditions. *Functional Ecology* 10:227-233.

Bamber, R.N. 1990. The effects of acidic seawater on three species of lamellibranch mollusc. *Journal of Experimental Marine Biology and Ecology* 143: 181-191.

Barber, I., Baird, D.J. & Calow, P. 1990. Clonal variation in general responses of *Daphnia magna* Straus to toxic stress, II Physiological effects. *Functional Ecology* 4, 409-414.

Barnard, C.J. & Hurst, J.L. 1996. Welfare by design: the natural selection of welfare criteria. *Animal Welfare* 5: 405-433.

Barrett, G.W. 1981. Stress Ecology: An Integrative Approach. Chapter 1 in Barrett, G.W. & Rosenberg, R. (Eds), *Stress Effects on Natural Ecosystems.* John Wiley & Sons Ltd. 305pp.

Bartoli, P. 1974. Recherches sur les Gymnophallidae F.N. Morozov, 1955 (Digenea), parasites d'oiseaux des cotes de Carmargue: Systematique, biologie et ecologie. These, Univ. Aix-Marseille.

Basha, S.M., Swami, K.S. & Pushpanjali, A. 1988. Ciliary and cardiac activity of freshwater mussel, *Lamellidens marginalis* (Lamarck) as an index of evaluating organophosphate toxicity. *Journal of Environmental Biology* 9(3) (suppl.) 313-318.

Basowitz, H., Persky, H., Korchin, S. J. & Grinker, R.R. 1955. *Anxiety and Stress: An Interdisciplinary Study of a Life Situation.* New York: McGraw-Hill (Blakiston).

Baturo, B. 1977. *Bucephalus polymorphus* Baer, 1827 and *Rhipidocotyle illense* (Ziegler, 1883) *(Trematoda, Bucephalidae)*: morphology and biology of developmental stages. *Acta Parasitologica Polonica* 24: 203-220.

Bayne, B.L. 1973. Physiological changes in *Mytilus edulis* L. induced by temperature and nutritive stress. *Journal of the Marine Biological Association of the United Kingdom* 53, 39-58.

Bayne, B.L. 1975. Aspects of physiological condition in *Mytilus edulis* L., with respect to the effects of oxygen tension and salinity. *Proceedings of the Ninth European Marine Biology Symposium*, pp. 213-238.

Bayne, B.L. (Ed.) 1976. *Marine Mussels: Their Ecology and Physiology.* Cambridge University Press, 506pp.

Bayne, B.L., Holland, D.L., Moore, M.N., Lowe, D.M. & Widdows J. 1978. Further studies on the effects of stress in the adult on the eggs of *Mytilus edulis*. *Journal of the Marine Biological Association of the United Kingdom* 58, 825-841.

Bayne, B.L. & Livingstone D.R. 1977. Response of *Mytilus edulis* L. to low oxygen tension: acclimation to the rate of oxygen consumption. *Journal of Comparative Physiology* 114: 129-142.

Bayne, B.L. & Newell, R.C. 1983. Physiological energetics in marine molluscs. pp. 407-515 in Saleudden A.S.M. & Wilbur K.M., (Eds), *The Mollusca 4.* Academic Press, New York. 523pp.

Bayne, B.L. & Thurberg, F.P. 1988. Physiological measurements on *Nucula tenuis* and on isolated gills of *Mytilus edulis* and *Carcinus maenas*. *Marine Ecology Progress Series* 46:129-134.

Bayne, B.L., Widdows, J. & Worrall C.M. 1977. Some temperature relationships in the physiology of two ecologically distinct bivalve populations. pp. 379-400 in Vernberg, F.J., Calbrese, A., Thurberg, F.P., & Vernberg W.B. (Eds), *Physiological Responses of Marine Biota to pollutants.* Academic Press, New York. 462pp.

Beaumont, A.R. & Toro, J.E. 1996. Allozyme genetics of *Mytilus edulis* subjected to copper and nutritive stress. *Journal of the Marine Biological Association of the United Kingdom* 76(4): 1061-1071.

Beehr, T.A. & O'Hara, K. 1987. Methodological Designs for the Evaluation of Occupational Stress Interventions, Chapter 4 in: Kasl, S.V. & Cooper, C.L., (Eds), 1987. *Stress and Health: Issues in Research Methodology.* John Wiley & Sons. 321pp.

Begon, M., Harper, J.L. & Townsend, C.R. 1990. *Ecology: Individuals, Populations and Communities* (2nd Edn). Blackwell Scientific Publications. 945pp.

Benson, P.H. & Chivers, D.C. 1960. A pycnogonid infestation of *Mytilus californianus*. *Veliger* 3: 16-18.

Bergan, P. 1955. Aceto-orcein and Feulgen stains for anatomy and cytology of trematodes. *Stain Technology* 30: 305-310.

Berland, B. 1982. "Technology as applied to museum parasite collections" Workshop pp. 1-16, 5th International Congress of Parasitology, Toronto, Canada, 12 August 1982.

Berryman, A.A. & Millstein J.A. 1989. Are Ecological Systems Chaotic? - And if not why not? *Trends in Ecology and Evolution* 4, no 1, January, 26-28.

Bligh, J. & Johnson, K.G. 1973. Glossary of terms for thermal physiology. *Journal of Applied Physiology* 35, 941.

Block, W. 1985. Survival on land. *Biologist* 32, 3, 133-138.

Boesch, D.F. 1974. Diversity, stability and response to human disturbance in estuarine ecosystems. pp. 109-114 in *Proceedings of the First International Congress of Ecology.* Centre for Agricultural Publishing and Documentation, Wageningen, the Netherlands. 414pp.

Boesch, D.F. & Rosenberg, R. 1981. Response to stress in Marine Benthic Communities. Chapter 13 in Barrett, G.W. & Rosenberg R., (Eds). *Stress Effects on Natural Ecosystems,* John Wiley & Sons Ltd. 305pp.

Bottjer, D.J. & Carter, J.G. 1980. Functional and phylogenetic significance of projecting periostracal structures in the Bivalvia (Mollusca). *Journal of Paleontology* 54: 200-216.

Bower, S.M., Blackbourne, J. & Meyer G.R. 1992. Parasite and symbiont fauna of Japanese littlenecks, *Tapes philippinarum* (Adams and Reeve 1850), in British Columbia. *Journal of Shellfish Research* 11(1) 13-19.

Bowers, E.A., Bartoli, P., Russell-Pinto, F. & James, B.L. 1996. The metacercaria of sibling species of *Meiogymnophallus*, including *M. rebecqui* comb. nov. (Digenea: Gymnophallidae), and their effects on closely related *Cerastoderma* host species (Mollusca: Bivalvia). *Parasitological Research* 82: 505-510.

Bowers, E.A. & James, B.L. 1967. Studies on the morphology, ecology and life-cycle of *Meiogymnophallus minutus* (Cobbold, 1859) comb. nov. (Trematoda: Gymnophallidae). *Parasitology* 57: 281-300.

Bradshaw, A.D. & Hardwick, K., 1989. Evolution and stress - genotypic and phenotypic components. *Biological Journal of the Linnean Society* 37, 137-155. In: Calow, P. & Berry, R.J. (Eds), *Evolution, Ecology and Environmental Stress* Academic Press, London. 187pp.

Branch, G.M., Griffiths, C.L., Branch, M.L. & Beckley, L.E. 1994. *Two Oceans: a Guide to the Marine Life of Southern Africa.* David Philip, Cape Town & Johannesburg. 360pp.

Brauner, G.J. & Fitzpatrick, T.B. 1971. Dermatology: Medicine, p480, *Britannica Book of the Year.* William Benton Publisher.

Bray, R.A. 1983. On the fellodistomid genus *Proctoeces* Odhner, 1911 (Digenea), with brief comments on two other fellodistomid genera. *Journal of Natural History* 17:321-339.

Bray, R.A. 1984. Some helminth parasites of marine fishes and cephalopods of South Africa: Aspidogastrea and the digenean families Bucephalidae, Haplosplanchnidae, Mesometridae and Fellodistomidae. *Journal of Natural History* 18:271-292.

Bray, R.A. 1987. Some helminth parasites of marine fishes of South Africa: family Opecoelidae (Digenea). *Journal of Natural History* 21:1049-1075.

Bray, R.A. & Gibson, D.I. 1980. The Fellodistomidae (Digenea) of fishes from the northeast Atlantic. *Bulletin of the British Museum (Natural History) Zoology Series* 37(4):199-293.

Brett, J.R. 1958. Implications and assessments of environmental stress. In *The Investigations of Fish-Power Problems, H.R. Macmillan Lectures in Fisheries* (Ed. P. A. Larkin) 69-83. University of British Columbia Press, Vancouver, Canada: Illustr.

Breznitz, S. & Goldberger, L. 1982. Stress Research at a Crossroads. Chapter 1 in Goldberger, L. & Breznitz, S., (eds) *Handbook of stress: theoretical and clinical aspects.* New York Free Press. 804pp.

Broom, D.M. 1986. Indicators of poor welfare. *British Veterinary Journal* 142, 524-526.

Broom, D.M. 1987. Applications of neurobiological studies to farm animal welfare. Chapter 3. in *Biology of Stress in Farm Animals an Integrative Approach*, (eds

Wiepkema, P.R. & van Adrichchem P.W.M.). *Current Topics in Veterinary Medicine and Animal Science*, Martinus Nijhoff, Dordrecht, pp. 101-110.

Broom, D.M. & Johnson, K.G. 1993. *Stress and Animal Welfare*. Chapman & Hall Animal Behaviour Series, London. 211pp.

Brown, A.C. 1982. Pollution and the sandy beach whelk *Bullia*. *Transactions of the Royal Society of South Africa* 44, 4, 55-562.

Brown, A.C. 1986. Molecular connectivity indices of organic pollutants: correlation with cessation of burrowing in the whelk *Bullia digitalis* (Dillwyn). *Transactions of the Royal Society of South Africa* 46, 2, December, 109-114.

Brown, A.C. 1993. Variability in biological systems, *South African Journal of Science* 89, July, 308-309.

Brown, A.C. 1994. Is biology a science? *Transactions of the Royal Society of South Africa* 49, 2, 141-146.

Brown, A.C. & Webb, S.C. 1994. Organisms associated with burrowing whelks of the genus *Bullia*. *South African Journal of Zoology* 29(2) 144-151.

Bryan, G.W. & Uysal, H. 1978. Heavy metals in the burrowing bivalve *Scrobicularia plana* from the Tamar estuary in relation to environmental levels. *Journal of the Marine Biological Association of the United Kingdom* 58: 89-108.

Burzio, L.O., Fuente, E. de la, Gutierrez, E., Saez, C., Brito, M., Burzio, L.A., Burzio, V.A., Weiss, R. & Pardo, J. 1989. *Marine bioadhesives: Projections in medicine and industry.* Program of the first marine biotechnology conference, Tokyo (Japan) 4-6 September.

Cable, R.M. 1953. The life cycle of *Parvatrema borinquenae* gen. et sp. nov. (Trematoda: Digenea) and the systematic position of the sub-family Gymnophallinae. *Journal of Parasitology* 39:408-421.

Cable, R.M. 1956. Marine Cercariae of Porto Rico. *New York Academy of Sciences Scientific Survey of Porto Rico and the Virgin Islands* Volume XVI(4): 490-577.

Cable, R.M. 1963. Marine Cercariae from Curacao and Jamaica. *Zeitschrift für Parasitenkunde* 23: 429-469.

Cable, R.M. & Kuns, M.L. 1951. The trematode family microphallidae with the description of *Carneophallus trilobatus* gen et sp. nov., from Mexico. *Journal of Parasitology* 37:507-514.

Calow, P. 1978. Bidder's hypothesis revisited: a solution to some key problems associated with the general molecular theory of ageing. *Gerontology* 24, 448-458.

Calow, P. 1983. *Evolutionary Principles*. Blackie, Glasgow and London 108pp.

Calow, P. 1989. Proximate and ultimate responses to stress in biological systems. *Biological Journal of the Linnean Society 37*, 173-181. In: (Eds) Calow, P. & Berry, R.J. *Evolution, Ecology and Environmental Stress.* Academic Press, London. 187pp.

Calow, P. 1993. Ecosystems not optimised. *Journal of Aquatic Ecosystem Health* Dordrecht 2 (1): 55.

Calow, P. & Sibly, R.M. 1990. A physiological basis of population processes: ecotoxicological implications. *Functional Ecology* 4 283-288.

Calvo-Ugarteburu, G. 1996. *Parasitism and Invasive Species: An Ecological Study of Mussel Populations*. Ph.D. Thesis, Rhodes University, 200pp.

Calvo-Ugarteburu, G. & McQuaid C.D. 1998. Parasitism and introduced species: epidemiology of trematodes in the intertidal mussels *Perna perna* and *Mytilus galloprovincialis*. *Journal of Experimental Marine Biology and Ecology* 220:47-65.

Cameron, R. & Meichenbaum, D. 1982. The Nature of Effective Coping and the Treatment of Stress Related Problems: A Cognitive-Behavioral Perspective. Chapter 42 in Goldberger. L. & Breznitz, S. (eds) *Handbook of Stress: Theoretical and Clinical Aspects.* New York, Free Press. 804pp.

Campbell, D. 1985. The life cycle of *Gymnophallus rebecqui* (Digenea: Gymnophallidae), and the response of the bivalve *Abra tenuis* to its metacercariae. *Journal of the Marine Biological Association of the United Kingdom* 65:589-601.

Campbell, W.B. & Emlen, J.M. 1996. Developmental instability analysis of BKD-infected spring chinook salmon, *Oncorhychus tshawytscha*, prior to sea water exposure. *Oikos* 77(3):540-548.

Cannon, W.B. 1935. Stresses and strains of homeostasis. *American Journal of Medical Science* 189 (1): 1-14.

Cannon, W.B. 1939. *The Wisdom of the Body.* Norton: New York. 333pp.

Cannon, W.B. 1942. Voodoo Death. *American Anthropologist* 44, 169-181.

Cannone, A.J. & Bush, B.H.M. 1982. Dual reflex motor control of non-spiking crab muscle receptor. I Positive feedback tonically reduced and dynamically stabilised by concurrent inhibition of Rm1. *Journal of Comparative Physiology* 148: 365-377.

Cannone, A.J. & Bush, B.H.M. 1983. Dual reflex motor control, of non-spiking crab muscle receptor. II. reinforcement of Rm1 mediated positive feedback by dual afferent excitation of Rm2. *Journal of Comparative Physiology* 153: 309-320.

Carefoot, T.H. 1994. Effects of environmental stressors on glucose blood levels in sea hares, *Aplysia dactylomela. Marine Biology* 118, 579-583.

Carissan, F. 1992. *Study of Toxicity of Various Compounds Assessed by the Ciliary Activity of* Venerupis corrugatus *(Gm.) and Compared with Previous Works on* Donax serra *(Röding) and* Choromytilus meridionalis *(Kr.).* Honours Project, Zoology Department, University of Cape Town, 61pp.

Carruthers, M. 1974. *The Western Way of Death.* London: Davis-Poynter.

Carter, T.C. & Orr, C.O. 1970. Radiation: biological effects II. B2. *Encyclopaedia Britannica* Vol. 18 p1028.

Cassel, J.T. 1967. Appraisal and Implications for Theoretical Development. In Syme & Reeder (Eds), *Social Stress and Cardiovascular Disease. The Millbank Memorial Fund Quarterly* Vol. XLV, April 1967. p41.

Caudill, W. 1958. Effects of Social and Cultural Systems on Reactions to Stress. *Social Sciences Research Council Pamphlet No 14*, June.

Chambers English Dictionary, 1988. W. & R. Chambers Ltd, Edinburgh. 1792pp.

Chapman, L.F., Hinkle, F.E. Jr. & Wolff, H.G. 1960. "Human Ecology, Disease, and Schizophrenia". *American Journal of Psychiatry* 117, 193-204.

Charlton, B. 1991. Stress? Who needs it? *New Scientist* no 1775, 29 June, p63.

Chen, J. & Li, G. 1988. Heat shock proteins in ovary cells of *Tilapia. Journal of Cellular Physiology* 134, 189-199.

Cheng. T.C. 1967. Marine molluscs as hosts for symbioses with a review of known parasites of commercially important species. *Advances in Marine Biology* 5 424pp.

Cheng, T.C. 1981. Bivalves, in: *Invertebrate Blood Cells* Vol. 1 pp. 233-300, Eds Ratcliffe, N.A. & Rowley, A.F. Academic Press, New York.

Child, C. A. 1996. Pycnogonids of the western Pacific islands, XIII. Collections from Indonesia, Melanesia, and Micronesia. *Proceedings of the Biological Society of Washington* 109(3):540-559.

Ching, H.L. 1965. Life cycles of *Lacunovermis conspicuus* n. gen., n. sp. and *Meiogymnophallus multigemmulus* n.gen., n.sp. (Gymnophallidae: Trematoda) from *Macoma inconspicua* and diving ducks from Vancouver, Canada. *Proceedings of the Helminthological Society of Washington* 32: 53-67.

Ching, H.L. 1973a. Description of *Gymnophallus somateriae* (Levinsen, 1881) from *Macoma inconspicua* and diving ducks from Vancouver, Canada. *Canadian Journal of Zoology* 51(8): 801-806.

Ching, H.L. 1973b. *Paragymnophallus odhneri* gen. n., sp. n. (Trematoda: Gymnophallidae) for *Gymnophallus somateriae sensu* Odhner (1900, 1905). *Canadian Journal of Zoology* 51(8): 807-810.

Choudry, I.H. & Baue A.E. 1982. *Pathophysiology of Shock, Anoxia and Ischemia*, pp. 203-219. Williams & Williams, Baltimore MD.

Clark, G.R. II. 1976. Shell convexity in *Argopecten gibbus*: variation with depth in Harrington Sound, Bermuda. *Bulletin of Marine Science* 26: 4 605-610.

Clark, R.B. 1966. *A Practical Course in Experimental Zoology*. pp21-22. John Wiley, London.

Cole, H.A. 1935. On some larval trematode parasites of the mussel *(Mytilus edulis)* and the cockle *(Cardium edule)*. *Parasitology* 27:276-280.

Combes, C. 1980. Atlas Mondial des Cercaires. *Memoirs du Museum National d'histoire Naturelle*, Nouvelle Serie A, Zoologie 115, 235pp.

Combes, C. 1997. Fitness of Parasites: Pathology and Selection. *International Journal for Parasitology,* 27(1): 1-10.

Connel, D.W. 1994. The Octanol-Water Partition Coefficient. Chapter 13 in Calow, P. (Ed.) (1994) *Handbook of Ecotoxicology* Volume 2. Blackwell Scientific Publications, Oxford. 416pp.

Costlow, J.D., Jr, Bookhout, C.G. & Monroe R. 1960. The effect of salinity and temperature on larval development of *Sesarma cinereum* (Bosc) reared in the laboratory. *Biological Bulletin* 118: 183-202.

Coulter, X. 1970. The effect of performance relevance and feedback upon resistance to anticipatory stress. U.S. Army Aeromedical Research Laboratory, Naval Aerospace Medical Research Laboratory, *USAARL Report* No 6190.

Coustau, C., Renaud, F., Delay, B., Robbins, I. & Mathieu, M. 1991. Mechanisms Involved in Parasitic Castration: In Vitro Effects of the Trematode *Prosorhynchus squamatus* on the Gametogenesis and the Nutrient Storage Metabolism of the Marine Bivalve Mollusc *Mytilus edulis*. *Experimental Parasitology* 73:36-43.

Currie, W. & White, L. 1983. Heat shock proteins in rat tissues. *Canadian Journal of Biochemistry* 61, 438-446.

Dahlgaard, J. & Loeschke, V. 1997. Effects of inbreeding in three life stages of *Drosophila buzzatii* after embryos were exposed to a high temperature stress. *Heredity* 78 (4): 410-416.

Dantzer, R., Mormede, P., Bluthe, R.-M. & Soissons, J. 1983. The effect of different housing conditions on behavioural and adrenocortical reactions in veal calves. *Reproduction, Nutrition and Development* 23, 67-74.

Davenport, J., Davenport, J. & Davies, G. 1984. A preliminary assessment of growth rate of mussels from the Falkland Islands. [*Mytilus chilensis* (Hupé) and *Aulacomya ater* (Molina)]. *Journal du Conseil International pour l'Exploration de la Mer* 41: 2 154-158.

Davenport, J. & Fletcher J.S. 1978. The effects of simulated estuarine mantle cavity conditions upon the activity of the frontal gill cilia of *Mytilus edulis*. *Journal of the Marine Biological Association of the United Kingdom* 58: 671-681.

Davenport, J. & Manley, A. 1978. The detection of heightened sea-water copper concentrations by the mussel *Mytilus edulis*. *Journal of the Marine Biological Association of the United Kingdom* 58: 843-850.

Davies, B.R. 1980. The identification of the mytilids *Musculus virgiliae* Barnard, *Arcuatula capensis* (Krauss) and *Brachidontes variabilis* Krauss, with corrections to the literature and a note on their distribution. *Transactions of the Royal Society of South Africa* 44: 2 225-236.

Davies, B.R. 1982. Studies on the zoobenthos of some Southern Cape coastal lakes. Spatial and temporal changes in the benthos of Swartvlei, South Africa, in relation to changes in the submerged littoral macrophyte community. *Journal of the Limnological Society of Southern Africa* 8(1) 33-45.

Davies, B.R. 1984. The zoobenthos of the Touw River flood plain. Part 1: The benthos of the Wilderness Lagoon, Touw River and the Serpentine, and the effects of submerged plant cutting. *Journal of the Limnological Society of Southern Africa* 10(2) 62-73.

Dawes, B. 1946. *The Trematoda*. Cambridge University Press 644pp.

Dawkins, M.S. 1990. From an animal's point of view: motivation, fitness, and animal welfare. *Behavioural and Brain Sciences* 13, 1-61.

Dawkins, R. 1976. *The Selfish Gene*. Oxford University Press Oxford, 352pp.

Dawkins, R. 1986. *The Blind Watchmaker*. Longman, Harlow, Essex, 332pp.

Day, J.H. 1969. *A Guide to the Marine Life on South African Shores*. A.A. Balkema, Cape Town. 300pp.

Day, R.W., Barkai, A. & Wickens, P.A. 1991. Trapping of three drilling whelks by two species of mussel. *Journal of Experimental Marine Biology and Ecology* 149: 109-122.

Dean, R.L. & Atkinson, B.G. 1985. Synthesis of heat shock proteins in quail red blood cells following brief, physiologically relevant increases in whole body temperature. *Comparative Biochemistry & Physiology* 81B (1): 185-191.

Denny, M.W. 1987. Lift as a mechanism of patch initiation in mussel beds. *Journal of Experimental Marine Biology and Ecology* 113: 231-245.

Derogatis, L. R. 1982. Self-report Measures of Stress. Chapter 17 in Goldberger, L. & Breznitz, S. (Eds) *Handbook of stress: theoretical and clinical aspects.* New York Free Press, 804pp.

Dillon, T.M. & Lynch, M.P. 1981. Physiological Responses as Determinants of Stress in Marine and Estuarine Organisms. Chapter 16 In Barrett, G.W. &

Rosenberg R. (Eds). *Stress Effects on Natural Ecosystems,* John Wiley & Sons Ltd. 305pp.

Djawdan, M., Rose, M.R. & Bradley, T.J. 1997. Does selection for stress resistance lower metabolic rate? *Ecology* 78(3): 828-837.

Dohrenwend, B.P. 1961 "The Social Psychological Nature of Stress: A Framework for Causal Inquiry." *J. Abnor. Soc. Psychol.* 62, 294-302.

Doi, J. 1994. Complex Mixtures. Chapter 12 in Calow, P., (Ed.) 1994. *Handbook of Ecotoxicology* Volume 2. Blackwell Scientific Publications, Oxford. 416pp.

Dolmer, P., Karlsson, M. & Svane, I. 1994. A test of rheotactic behaviour of the blue mussel *Mytilus edulis* L. *Special Publication, Phuket Marine Biological Center, Phuket*: 13: 177-184. Workshop of the Tropical Marine Mollusc Programme (TMMP), Phuket (Thailand), 27 October-2 November.

Donkin, P. 1994. Quantitative Structure Activity Relationships. Chapter 14 in Calow, P. (Ed.) 1994. *Handbook of Ecotoxicology* Volume 2. Blackwell Scientific Publications, Oxford. 416pp.

Dubois, G. 1944. A propos de la specifite parasitaire des Strigeida. *Bulletin de la Societe neuchatelois des Sciences Naturelles* p69.

Edney, E.B. & Gill, R.W. 1968. Evolution of Senescence and Specific Longevity. *Nature* 220, 5164, 281-282.

Edwards, C.A. & Skibinski, D.O.F. 1987. Genetic variation of mitochondrial DNA in mussel (*Mytilus edulis* and *M. galloprovincialis*) populations from South West England and South Wales. *Marine Biology* 94: 547-556.

Engel, B.T. 1985. Stress is a noun! No, a verb! No, an adjective! Chapter 1 In Field, T.M., McCabe, P.M. & Schneiderman, N. (Eds.). *Stress and Coping.* Lawrence Erlbaum Associates, Hillsdale, New Jersey. 354pp.

Erasmus, D.A. 1972. *The Biology of Trematodes.* Edward Arnold, 312pp.

Esch, G.W., Gibbons J.W. & Bourque, J.E. 1975. Analysis of the relationship between stress and parasitism. *The American Midland Naturalist* 93 (2) 339-353.

Escos, J., Alados, C.L., Emlen, J.M. & Alderdstein, S. 1995. Developmental Instability in the Pacific Hake Parasitized by Myxosporeans *Kudoa* spp. *Transactions of the American Fisheries Society* 124, 943-945.

Etges, F.J. 1953. Studies on the life histories of *Maritrema obstipum* (Van Cleave and Mueller, 1932) and *Levinseniella amnicolae* n sp.(Trematoda Microphallidae). *Journal of Parasitology* 39: 643-662.

Evans, J.W. 1968. Growth rate of the rock boring clam *Penitella penita* (Conrad 1837) in relation to hardness of rock and other factors. *Ecology* 49: 619-628.

Ewbank, R. 1984. Behavioural responses to stress in farm animals. pp. 71-79 in Moberg, G.P. (Ed), *Animal Stress.* American Physiological Society. Bethesda, Maryland.

Fantham, H.B. 1938. *Lecithostaphylus spondyliosomae* n.sp. a trematode parasite of the hottentot fish, *Spondyliosoma blochii*, found in South African waters. *Transactions of the Royal Society of South Africa* 26: 387-393.

Faust, E.C. 1919. The excretory system in digenea. I. *Biological Bulletin* 36: 315-344.

Faust, E.C. 1926. Further observations on South African larval trematodes. *Parasitology* 18: 101-126.

Faust, E.C. 1932. The excretory system as a method of classification of digenetic trematodes. *Quarterly Review of Biology* 7: 458-468.

Feltmate, B.W. & Williams, D.D. 1991. Evaluation of predator induced stress on field populations of stoneflies (Plecoptera). *Ecology* 72 (5): 1800-1806.

Fisher, N.S. 1977. On the differential sensitivity of estuarine and open ocean diatoms to exotic chemical stress. *American Naturalist* 111, 871-895.

Fisher, R.A. 1932. *The Causes of Evolution.* Oxford Press.

Fisher, S. 1984. *Stress and the Perception of Control.* Lawrence Erlbaum Associates Ltd, London. 285pp

Fogg, G.E. 1973. Physiology and ecology of marine blue-green algae. In: *The Biology of Blue-Green Algae,* Eds N.G. Carr and B.A. Whitton. Blackwell, Oxford.

Fogg, G.E., Stewart, W.D.P., Fay, P. & Walsby, A.E. 1973. *The Blue Green Algae.* Academic Press, London.

Forbes, M., Leung, B. & Schalk, G. 1997. Fluctuating asymmetry in *Coenagrion resolutum* (Hagen) in relation to age and male pairing success (Zygoptera: Coenagrioindae). *Odonatologica* 26(1): 9-16.

Frankenhaeuser, M. 1971. Behaviour and circulating catecholamines. *Brain Research* 31, 241-262.

Franz, E.H. 1981. A General Formulation of Stress Phenomena in Ecological Systems. Chapter 5 in Barrett, G.W. & Rosenberg R., (Eds) *Stress Effects on Natural Ecosystems,* John Wiley & Sons Ltd. 305pp.

Frisch, S.M., Vuori, K., Kelaita, D. & Sicks S. 1996. A role for Jun-N-Terminal kinase in anoikis; suppression by bcl-2 and crma. *Journal of Cell Biology* 135 (5):1377-1382.

Fry, F.E.G. 1947. Effects of the environment on animal activity. *University of Toronto Studies: Biological Series*, No 55, 1-62. Publications of the Ontario Fisheries Research Laboratory. University of Toronto Press.

Funk, W.H. & Gibbons, H.L. 1979. Lake restoration by nutrient inactivation, pp. 141-151 in *Lake Restoration.* US Environmental Protection Agency, Office of Water Planning and Standards, Washington D.C. 254pp.

Gabbott, P.A. & Bayne, B.L. 1973. Biochemical effects of temperature and nutritive stress on *Mytilus edulis* L. *Journal of the Marine Biological Association of the United Kingdom* 53, 269-286.

Gaddum, J.H. 1948. *Pharmacology* (3rd ed.). O.U.P London.

Gibson, D.I. 1987. Questions in digenean systematics and evolution. *Parasitology* 95: 429-460.

Gibson, D.I., Taskinen, J. & Valtonen, E.T. 1992. Studies on bucephalid digeneans parasitising molluscs and fishes in Finland.II. The description of *Rhipidocotyle fennica* n. sp. and its discrimination by principal components analysis. *Systematic Parasitology* 23: 67-79.

Gilfillan, E.S., Page, D.S., Foster, J. C., Vallas, D., Gonzalez, L., Luckerman, A., Hotham, J.R., Pendergast, E. & Herbert, S. 1984. A comparison of stress indicators at the biological organismal and community level of organisation. pp. 503-504 in *Responses of Marine Organisms to Pollutants, Marine Environmental Research* 14, Eds Stegman J.J. & Heath G.W., Elsevier Applied Science Publishers London and New York.

Glass, D.C. & Singer, J.E. 1972. *Urban Stress: Experiments on Noise and Social stressors*. Academic Press, New York and London. 182pp.

Glaus, K.J. 1968. Factors influencing the production of byssus threads in *Mytilus edulis*. *Biological Bulletin* 135: 420.

Gleick, J. 1991. *Chaos: Making a New Science.* Cardinal Books, 325pp.

Golubic, S. 1969. Distribution, taxonomy and boring patterns of marine endolithic algae. *American Zoologist* 9: 747-751.

Golubic, S., Brent, G. & Lecampion, T. 1970. Scanning electron microscopy of endolithic algae and fungi using multipurpose casting embedding technique. *Lethaia* 3: 203-209.

Golubic, S., Friedman, E.I. & Schneider, J. 1981. The lithobiontic ecological niche, with special reference to micro-organisms. *Journal of Sedimentary Petrology* 51: 475-478.

Gosling, E. (Ed), 1992. *The Mussel Mytilus: Ecology, Physiology, Genetics and Culture.* Developments in Aquaculture and Fisheries Science 25. Elsevier, Amsterdam, 589pp.

Grandin T. 1997. Assessment of Stress During handling and Transport. *Journal of Animal Science* 75, 249-257.

Grant, W.S. & Cherry M.I. 1985. *Mytilus galloprovincialis* Lmk. in Southern Africa. *Journal of Experimental Marine Biology and Ecology* 90, 179-191.

Grant, W.S., Cherry, M.I. & Lombard, A.T. 1984. A cryptic species of *Mytilus* (Mollusca: Bivalvia) on the West Coast of South Africa. *South African Journal of Marine Science* 2: 149-162.

Gray, J. 1923. Mechanisms of ciliary movement. III, effect of temperature. *Proceedings of the Royal Society* (B) 95: 6-15.

Gray, J. S. 1989. Effects of environmental stress on species rich assemblages. *Biological Journal of the Linnean Society* 37: 19-32. In (Eds) Calow, P. & Berry, R.J. *Evolution, Ecology and Environmental Stress.* Academic Press, London. 187pp.

Griffiths, C.L. & Blaine, M.J. 1994. Non-fatal cropping of large mussels by drilling whelks, *Nucella cingulata* (Linnaeus, 1771). *Journal of Molluscan Studies* 60:346-348.

Griffiths, R.J. 1977. Reproductive cycles in littoral populations of *Choromytilus meridionalis* (Kr.) and *Aulacomya ater* (Molina) with a quantitative

assessment of gamete production in the former. *Journal of Experimantal Marine Biology and Ecology* 30: 53-71.

Grime, J.P. 1979. *Plant Strategies and Vegetation Processes.* Chichester: Wiley & Sons.

Grime, J.P. 1986. The circumstances and characteristics of spoil colonization within local flora. *Philosophical Transactions of the Royal Society, London,* B 314, 637-654.

Grime, J.P. 1989a. The stress debate: symptom of impending synthesis? *Biological Journal of the Linnean Society* 37, 3-17. In: (Eds) Calow, P. & Berry, R.J. *Evolution, Ecology and Environmental Stress.* Academic Press, London. 187pp.

Grime, J.P. 1989b. Whole plant responses to stress in natural and agricultural systems. *Plants under Stress: Biochemistry, physiology and ecology and their application to plant improvement.* Jones H.G., Flowers T.J. & Jones M.B. (Eds). SEB Lancaster Meeting, March 1988. pp. 31-46. 257pp.

Grinker, R.R. 1974. Chalones: Medical Research, Medicine, p459, *Britannica Book of the Year.* Helen Hemingway-Benton, Publisher.

Gross, E. 1970. "Work, Organization and Stress," In Levine and Scotch (Eds.), *Social Stress.* Chicago: Aldine, pp. 45-110.

Gwynne, D.T. 1989. Does Copulation Increase the Risk of Predation? *Trends in Ecology and Evolution* 4 (2) 54-56, February.

Haan, N. 1977. Coping and Defending: Processes of Self-Environment Organization. New York: Academic Press.

Haldane, J. B. S. 1930. *Genetical Theory of Evolution.* Longmans.

Hanin, I., Frazer, A., Croughhan, J., Davis, J.M., Katz, M.M., Koslow, S.H., Maas, J.W. & Stokes, P.E. 1985. Depression: implications of clinical studies for basic research. *Federation Proceedings* 44, 85-90.

Harkava, K.H., Afonina, H.B., Zadorina, O.V. & Bryuzhyna, T.S. 1995. Lymphocyte chalone effect on functional activity of lymphocytes in antioxidant insufficiency and its correction with vitamin E. *Fiziolohichnyi Zhurnal (Kiev)* 41(5-6):27-32.

Harris, S.A., da Silva, F.M., Bolton, J.J. & Brown, A.C. 1986. Algal gardens and

herbivory in a scavenging sandy-beach nassariid whelk. *Malacologia* 27 (2) 299-305.

Hattingh, J. 1988. The semantics of "animal stress". *South African Journal of Science* 84, 731-732.

Heasman, M.P., O'Connor, W.A. & Frazer, A.W.J. 1996. Digenean (Bucephalidae) infections in commercial scallops, *Pecten fumatus* Reeve, and doughboy scallops, *Chlamys (Mimachlamys) asperrima* (Lamarck), in Jervis Bay, New South Wales. *Journal of Fish Diseases* 19:333-339.

Heisenberg, W. 1927. Über den anschaulichen inhalt der quantentheoretischen kinematik und mechanik. *Zeitschrift Für Physik* vol. 43. heft 3&4 p172-198.

Henderson, L.J. 1913. The fitness of the environment. An inquiry into the biological significance of the properties of matter. Macmillan (1927). 317pp.

Hepper, B.T. 1957. Notes on *Mytilus galloprovincialis* Lamarck in Great Britain. *Journal of the Marine Biological Association of the United Kingdom* 36 33-40.

Hermens, J.L.M. 1986. Quantitative structure activity relationships in aquatic toxicology. *Pesticide Science* 17, 287-296.

Hickman, R.W. & Illingworth, J. 1980. Condition cycle of the green lipped mussel *Perna canaliculus* in New Zealand. *Marine Biology* 60:27-38.

Hobfoll, S.E. 1989. Conservation of resources: a new attempt at conceptualizing stress. *American Psychologist* 44, 513-524.

Hockey, P.A.R. & van Erkom Schurink, C. 1992. The invasive biology of the mussel *Mytilus galloprovincialis* on the southern African coast. *Transactions of the Royal Society of South Africa* 48 (1): 123-140.

Hoffman, A.A. & Parsons, P.A. 1989. An integrated approach to environmental stress tolerance and life history variation: desiccation tolerance in *Drosophila*. *Biological Journal of the Linnean Society* 37, 117-136. In: (Eds) Calow, P. & Berry, R.J. *Evolution, Ecology and Environmental Stress*. Academic Press, London. 187pp.

Hofmann, G.E. & Somero, G.N. 1996. Interspecific variation in thermal denaturation of proteins in the congeneric mussels *Mytilus trossulus* and *M. galloprovincialis*: evidence from the heat-shock response and protein ubiquitation. *Marine Biology* 126, 65-75.

Hole, L.M., Moore, M.N. & Bellamy, D. 1995. Age related cellular and physiological reactions to hypoxia and hypothermia in marine mussels. *Marine Ecology Progress Series* 122, 173-178.

Holliman, R.B. 1961. Larval trematodes from the Apalachee Bay area, Florida, with a check-list of known marine cercaria arranged in a key to their superfamilies, *Tulane Studies in Zoology* 9: 1-74.

Holloway, G.J., Sibly, R.M. & Povey, S.R. 1990. Evolution in toxin-stressed environments. *Functional Ecology* 4, 289-294.

Holmes, D.S. & Houston, B.K. 1974. Effectiveness of situation redefinition and affective isolation in coping with stress. *Journal of Personality and Social Psychology* 29, 212-218.

Holroyd, K.A. & Lazarus, R.S. 1982. Stress, Coping and Somatic Adaptation: Chapter 3 in Goldberger, L. & Breznitz, S. (Eds) *Handbook of stress: theoretical and clinical aspects.* New York Free Press, 804pp.

Houston, B.K. 1972. Control over stress, locus of control and response to stress. *Journal of Personality and Social Psychology* 21, 249-255.

Hull, D.L. (Ed.) 1988. *Science As A Process: An Evolutionary Account of the Social and Conceptual Development of Science.* University of Chicago Press. 586pp.

Hume, D. 1898. *A Treatise of Human Nature and Dialogues Concerning Natural Religion,* Vol. 1, pp. 372-394, (Eds) Green, T. H. & Grose, T.H., Longmans Green & Co. 565pp.

Humm, H.J. & Wicks, S.R. 1980. *Introduction and Guide to the Marine Blue-Green Algae.* Wiley-Interscience Publication.

Inoue, K., Takeuchi, Y., Miki, D. & Odo, S. 1994. Primary structure of two types of polyphenolic proteins of the mussel, *Mytilus galloprovincialis.* Tromsoe University: Tromsoe (Norway) Third International Marine Biotechnology Conference 1994: *Program, abstracts and list of participants*, p59.

Ito, J. 1964. A monograph of cercariae in Japan and adjacent territories. *Progress of Medical Parasitology in Japan* 1: 398-550.

Ivancevich, J.M. & Matteson, M.T. 1980. *Stress and Work: A Managerial Perspective.* Glenview IL, Scott, Foresman.

Ivanovici, A.M. & Wiebe, W.J. 1981. Towards a Working "Definition" of "Stress": A Review and Critique. Chapter 2 In Barrett, G.W. & Rosenberg R. (Eds). *Stress Effects on Natural Ecosystems.* John Wiley & Sons Ltd. 305pp.

Jabbar, A. & Davies, I.J. 1987. A simple and convenient method for sex identification in the marine mussel, *Mytilus edulis* L. *Journal of Experimental Marine Biology and Ecology* 107: 39-44.

James, B.L. 1964. The life cycle of *Parvatrema homeotecnum* sp. nov. (Trematoda: Digenea) and a review of the family Gymnophallidae Morozov, 1955. *Parasitology* 54: 1-41.

John, D.M. & Lawson, G.W. 1977. The Marine Algal Flora of the Sierra Leone Peninsula. *Botanica Marina* 20: 129-135.

Jones, H.D. & Richards, O.G. 1993. The effects of acetylcholine, dopamine and 5-hydroxytryptamine on water pumping rate and pressure in the mussel *Mytilus edulis* L. *Journal of Experimental Marine Biology and Ecology* 170: 227-240.

Jones, M.B. 1975. Synergistic Effects of Salinity, Temperature and Heavy Metals on Mortality and Osmoregulation in Marine and Estuarine Isopods (Crustacea). *Marine Biology* 30:13-20.

Jørgensen, C.B. 1990. *Bivalve Filter Feeding: Hydrodynamics, Bioenergetics, Physiology and Ecology.* Olsen & Olsen Denmark 140pp.

Jørgensen, C.B., Larsen, P.S. & Riisgard, H.U. 1990. Effects of temperature on the mussel pump. *Marine Ecology Progress Series* 64:89-97.

Kaminoff, R.D. & Proshansky, H.M. 1982. Stress as a Consequence of the Urban Physical Environment. Chapter 23 in Goldberger, L. & Breznitz, S. (Eds) *Handbook of stress: theoretical and clinical aspects.* New York Free Press, 804pp.

Kammenga, J.E., Van Koert, P.H.G., Koeman, J.H. & Bakker, J. 1997. Fitness consequences of toxic stress evaluated within the context of phenotypic plasticity. *Ecological Applications* 7(2): 726-734.

Kant, I. 1973. *Critique of Pure Reason.* Translation by N. K. Smith, The Macmillan Press Ltd. 681pp.

Kanwisher, J.W. 1955. Freezing in intertidal animals. *Biological Bulletin* 109, 56-63.

Karnaukhov, V.N., Milovidova, N.Y. & Kargopolova, I.N. 1977. On the role of carotenoids in tolerance of sea molluscs to environmental pollution. *Comparative Biochemistry and Physiology* 56A, 189-193.

Kasl, S.V. 1987. Methodologies in Stress and Health: Past Difficulties, Present Dilemmas, Future Directions. Chapter 11 in Kasl, S.V. & Cooper, C.L. (Eds) 1987. *Stress and Health: Issues in Research Methodology*. John Wiley & Sons.

Kensley, B. & Penrith, M-L. 1973. The constitution of the intertidal fauna of rocky shores of Moçamedes, Southern Angola. *Cimbebasia* (A) 2:113-123.

Kessler, R. C. 1987. The Interplay of Research Design Strategies and Data Analysis Procedures in Evaluating The Effects of Stress on Health in Kasl, S.V. & Cooper, C.L. (Eds) 1987. *Stress and Health: Issues in Research Methodology*. John Wiley & Sons.

Ketola-Pirie, C.A. & Atkinson, B.G. 1983. Cold and heat-shock induction of new gene expression in cultured amphibian cells. *Canadian Journal of Biochemistry and Cell Biology* 61, 462-471.

Khan, R. A. 1990. Parasitism in Marine Fish after Chronic Exposure to Petroleum Hydrocarbons in the laboratory and to the Exxon Valdez Oil Spill. *Bulletin of Environmental Contamination and Toxicology* 44, 759-763.

Kierkegaard, S. 1938. (Dru, A., Ed. and trans.) *The Journals of Søren Kierkegaard* (1843). Oxford University Press, London and New York. 603pp.

Kikuchi, T. 1976. Rediscovery of the bivalve-infesting pycnogonida *Nymphonella tapetis* Oshima 1927, in Amakusa. *Publications Amakusa Marine Biological Laboratory* 41: 51-56.

Kilburn, R. & Rippey, E. 1982. *The Sea Shells of Southern Africa*. Macmillan: South Africa.

King, P.A., McGrath, D. & Gosling E.M. 1989. Reproduction and settlement of *Mytilus edulis* on an exposed rocky shore in Galway Bay, West Coast of Ireland. *Journal of the Marine Biological Association of the United Kingdom* 69: 355-365.

King, P.E. 1973. *Pycnogonids*. Hutchinson, London.

Kinne, O. 1984. *Diseases of Marine Animals IV Part 1,* Introduction, Pisces. Biologische Anstalt Helgoland.

Kleiber, M. 1975. *The Fire of Life.* Revised Edition, Robert E. Krieger Publishing Co. Inc., Huntingdon, N.Y. 453pp.

Knudsen, J. 1980. *Mytilus edulis* L. (Bivalvia) recorded from South Africa. *Steenstrupia* 6: 21-26.

Koban, M., Graham, G. & Prosser, C.L. 1987. Induction of heat shock protein synthesis in teleost hepatocytes: effects of acclimation temperature. *Physiological Zoology* 60, 2, 290-296.

Kobluk, D.R. & Risk, M.J. 1977. Rate and nature of infestation of a carbonate substratum by a boring alga. *Journal of Experimental Marine Biology and Ecology* 27: 107-115.

Koehn, R.K. & Bayne, B.L. 1989. Towards a physiological and genetical understanding of the energetics of the stress response. *Biological Journal of the Linnean Society,* 37, 157-171. In Calow, P. & Berry, R.J. (Eds) *Evolution, Ecology and Environmental Stress.* Academic Press, London. 187pp.

Kojima, K. 1971. Is there a constant fitness value for a given genotype? No! *Evolution* 25, 281-285.

Konarska, M., Stewart, R.E. & McCarty, R. 1989. Sensitization of sympathetic-adrenal medullary responses to a novel stressor in chronically stressed laboratory rats. *Physiology and Behavior* 46, 129-135.

Könemann, H. 1981. Fish toxicity tests with mixtures of more than two chemicals: a proposal for a quantitative approach and experimental results. *Toxicology* 19, 229-238.

Kothary, R.K., Burgess, E.A. & Candido, E.P.M. 1984. The heat-shock phenomenon in cultured cells of rainbow trout: hsp70 mRNA synthesis and turnover. *Biochimica et Biophysica Acta* 783, 137-143.

Kozlowski, J. 1993. Measuring Fitness in Life History Studies, *Trends in Ecology and Evolution* 8, 3, 84-85.

Krech, D. 1969. Does Behavior Really Need a Brain? In Praise of William James: Some Historical Musing, Vain Lamentations and a Sounding of Great

Expectations. In *William James: Unfinished Business*, McLeod, R.B. (Ed.), Washington D.C., American Psychological Association. pp. 1-11.

Kubzansky, L.D., Kawachi, I., Spiro, A.-III, Weiss, S.T., Vokonas, P.S. & Sparrow, D. 1997. Is worrying bad for your heart? A prospective study of worry and coronary heart disease in the normative aging study. *Circulation* 95(4): 818-824.

Kuriakose, P.S. & Nair N.B. 1976. The genus *Perna* along the coasts of India with description of a new species *Perna indica*. *Aquatic Biology* 1: 25-36.

Kyle, D.E. & Noblet, G.P. 1985. Occurence of Metacercariae (Trematoda:Gymnophallidae) on *Amphirite ornata* (Annelida: Terebellidae). *Journal of Parasitology* 71(3): 366-368.

La Cour, L. 1941. Acetic Orcein: a new stain for chromosomes. *Stain Technology* 16, 169-174.

La Rue, G. 1957. The Classification of Digenetic Trematoda: A Review and a New System. *Experimental Parasitology* 6: 306-349.

Lacey, A.R. 1986. *A Dictionary of Philosophy* (2nd ed.). Routledge & Kegan Paul, London. 266pp.

Lafferty, K.D., Sammond, D.T. & Kuris, A.M. 1994. Analysis of larval trematode communities. *Ecology* 75(8): 2275-2285.

Laihonen, P. & Furman, E.R. 1986. The site of settlement indicates commensalism between blue mussel and its epibiont. *Oecologia* (Berlin) 71: 38-40.

Lakhotia, S.C. 1998. Stress Biology: A Challenging Area in Integrated Biology. *Biology International* No. 36, 18-30.

Lang, W.H. & Dennis, E.A. 1976. Morphology and seasonal incidence of infection of *Proctoeces maculatus* (Looss, 1901) Odhner, 1911 (Trematoda) in *Mytilus edulis* L. *Ophelia* 15(1): 65-75.

Larkin, P.A. 1974. *Freshwater Pollution, Canadian Style*. Mc Gill-Queen's University Press, Montreal Canada: xii + 132pp., illustr.

Lasiak, T. 1986. The reproductive cycles of the intertidal bivalves *Crassostrea cucullata* (Born, 1778) and *Perna perna* (Linnaeus, 1758) from the Transkei coast, southern Africa. *The Veliger* 29(2):226-230.

Lasiak, T. 1989. The prevalence of *Proctoeces* (Trematoda: Fellodistomidae) metacercarial infections in the brown mussel *Perna perna* (Bivalvia: Mytilidae) around the southern African coast. *South African Journal of Zoology* 24(3): 178-186.

Lasiak, T. 1991. Bucephalid trematode infections in mytilid bivalves from the rocky intertidal of Southern Chile. *Journal of Molluscan Studies* 58: 29-36.

Lasiak, T. 1993. Bucephalid trematode infections in the brown mussel *Perna perna* (Bivalvia: Mytilidae). *South African Journal of Marine Science* 13: 127-134.

Lauckner, G. 1983. Diseases of Mollusca: Bivalvia. In *Diseases of Marine Animals Vol. 2 Bivalvia to Scaphopoda* (O. Kinne, Ed) pp477-961. Biologische Anstalt Helgoland, Hamburg.

Lauckner, G. 1984. Impact of trematode parasitism on the fauna of a North Sea tidal flat. *Helgoländer Meeresuntersuchungen* 37, 185-199.

Lawson, G.W. & John, D.M. 1982. *The Marine Algae and Coastal Environment of Tropical West Africa.* J. Cramer, Vaduz.

Lazarus, R.S. 1966. *Psychological Stress and the Coping Process.* New York: McGraw-Hill.

Le Campion - Alsumard T., Golubic S. & Hutchings, P. 1995. Microbial endoliths in skeletons of live and dead corals: *Porites lobata* (Moorea, French Polynesia). *Marine Ecology Progress Series* 117: 149-157.

Lee, S.H., Chai, J.Y. & Hong, S.T. 1993. *Gymnophalloides seoi* n. sp. (Digenea: Gymnophallidae), The first report of human infection by a gymnophallid. *Journal of Parasitology* 79(5): 677-680.

Lee, S.H., Choi, M.H., Seo, M. & Chai, J.Y. 1995. Oysters *Crassotrea gigas*, as the second intermediate host of *Gymnophalloides seoi* (Gymnophallidae). *Korean Journal of Parasitology* 33(1): 1-7.

Lefcourt, A.M. 1986. Usage of the term "stress" as it applies to cattle. *Vlaams Diergeneeskundige Tijdschrift* 55, 258-265.

Lehman, J.T. 1988. Ecological principles affecting community structure and secondary production by zooplankton in marine and freshwater environments. *Limnology and Oceanography* 33, 931-945.

Lehninger, A.L. 1973. *A Short Course in Biochemistry.* Worth, N.Y. 452pp.

Leibniz, G. 1898. *The Monadology*. Trans. R. Latta. Oxford University Press.

Leigh, H. 1982. Evaluation of Management Stress in General Medicine: The Psychosomatic Approach. Chapter 44 in Goldberger, L. & Breznitz, S. (Eds) *Handbook of stress: theoretical and clinical aspects.* New York Free Press, 804pp.

Leigh, H. & Reiser, M.F. 1980. *The Patient: Biological, Psychological and Social Dimensions of Medical Practice.* Plenum, New York. 351pp.

Lemke, M.R. 1996. The chronic fatigue syndrome: Psychiatric aspects. *Fortschritte der Neurologie Psychiatrie* 64 (4): 132-141.

Lenoir, F., Robbins, I., Mathieu, M., Lubet, P. & Gabbott, P.A. 1989. Isolation, characterization and glucose metabolism of glycogen cells (= vesicular connective-tissue cells) from the labial palps of the marine mussel *Mytilus edulis*. *Marine Biology* 101: 495-501.

Leung, B. & Forbes, M.R. 1996. Fluctuating asymmetry in relation to stress and fitness: effects of trait type as revealed by meta-analysis. *Ecoscience* 3(4): 400-413.

Leventhal, H. & Tomarken, A. 1987. Stress and Illness: Perspectives from Health Psychology. Chapter 2 in Kasl, S.V. & Cooper, C.L. (Eds) 1987. *Stress and Health: Issues in Research Methodology*. pp. 27-55. John Wiley & Sons.

Levine, S. 1975. Developmental psychobiology. In Hamburg, D.A. & Brodie, H.K.H. (Eds), *American Handbook of Psychiatry*, (2nd ed.), Volume 6. New York: Basic Books.

Levins, R. 1968. *Evolution in Changing Environments: Some theoretical explorations*. Princeton University Press. 120pp.

Levitt. J. 1972. *Responses of Plants to Environmental Stresses*. Academic Press, New York 697pp.

Levitt. J. 1980. *Responses of Plants to Environmental Stresses* Vol II, (2nd ed.). Academic Press, New York 570pp.

Lewin, R. 1993. The Right Connections pp. 4-5 in J. Johnson, (Ed) Complexity. *New Scientist,* (Supplement), 13, 6 February, 8pp.

Lincoln, R.J., Boxshall, G.A. & Clark, P.F. 1985. *Dictionary of Ecology, Evolution and Systematics*. Cambridge University Press. 298pp.

Lindquist, S. 1980. Synthesis of heat shock proteins in *Drosophila* and yeast. *Developmental Biology* 77, 463-479.

Lindquist, S. 1981. Regulation of protein synthesis during heat shock. *Nature (London)* 293, 311-314, 24 September.

Lintas, C. & Seed, R. 1994. Spatial variation in the fauna associated with *Mytilus edulis* on a wave exposed rocky shore. *Journal of Molluscan Studies* 60: 165-174.

Lippold, O.C. & Winton, F.R. 1979. *Human Physiology,* 7th ed. Churchill Livingstone, Edinburgh, London & New York. 514pp.

Littlewood, D.T. & Ford, S.E. 1990. Physiological responses to acute temperature elevation in oysters, *Crassostrea virginica* (Gmelin, 1791) parasitised by *Haplosporidium nelsoni* (MSX) (Haskin, Stauber and Mackin 1966). *Journal of Shellfish Research* 9 (1) 159-163.

Livingstone, D.R., Widdows, J. & Fieth, P. 1979. Aspects of nitrogen metabolism of the common mussel *Mytilus edulis*: adaptation to abrupt and fluctuating changes in salinity. *Marine Biology* 53, 41-55.

Lombard, A.T. & Grant, W.S. 1986. Biochemical population genetics of the black mussel *Choromytilus meridionalis*. *South African Journal of Zoology* 21 2: 131-135.

Loos-Frank, B. 1968. Der entwicklungzyklus von *Psilostomum brevicolle* (Creplin, 1829) [Syn. *P. platyurum* (Mühling, 1896)] (Trematoda, Psilostomatidae). *Zeitschrift für Parasitenkunde* 31:122-131.

Loos-Frank, B. 1971. Zur Kenntnis der gymnophalliden Trematoden des Nordseeraumes IV. Ubersicht uber die gymnophalliden Larven aus Mollusken der Gezeitenzone. *Zeitschrift für Parasitenkunde* 36:206- 232.

Lowe, D.M. 1988. Alterations in cellular structure of *Mytilus edulis* resulting from exposure to environmental contaminants under field and experimental conditions. *Marine Ecology Progress Series* 46, 91-100.

Lowe, D.M. & Clarke, K.R. 1989. Contaminant-induced changes in the structure of the digestive epithelium of *Mytilus edulis*. *Aquatic Toxicology* 15, 345-358.

Lugo, A. E. 1978. Stress and ecosystems. pp.61-101 in *Energy and Environmental Stress in Aquatic Ecosystems* (Eds Thorpe, J.H. & Gibbons, J.W.). DOE

Symposium series (Conf.-771114), Oak ridge, Tenn., USA: xxii + 854pp., illustr.

Lunetta, J.E. 1969. Reproductive physiology of the mussel *Mytilus perna. Boletín de Facultade de Filosophía, Ciencieas e Letras, Universidad de Sao Paulo. Zool. Biol. Mar.* (Sao Paulo) 26: 33-11.

Lutz, R.A. & Darling-Centre, I.C. (Eds) 1980. *Mussel Culture and Harvest: A North American Perspective. Developments in Aquacultural and Fisheries Science.* Elsevir.

Machkevsky, V.K. 1989. Peculiarities of biology of the trematode *Parvatrema duboisi*, a parasite of *Mytilus galloprovincialis. Parazitologija* 23(1): 60-67.

MacPherson, J.H. & Gabriel, C.J. 1962. *Marine Molluscs of Victoria.* Melbourne: Melbourne University Press.

Madhavi, R., Lakshmibai, K.J. & Rao, N.N. 1994. Cercarial fauna of molluscs from Chilka Lake: a new bucephalid cercaria and its metacercaria. *Journal of Parasitology and Applied Animal Biology* 3(1): 79-82.

Mandler, G. 1982. Stress and Thought Processes. Chapter 7 in Goldberger, L. & Breznitz, S. (Eds) *Handbook of stress: theoretical and clinical aspects.* New York Free Press, 804pp.

Mao - Che, L., Le Campion - Alsumard T., Boury - Esnalt, N., Payri, C., Golubic S. & Bezac, C. 1996. Biodegradation of shells of the black pearl oyster, *Pinctada margaritifera var cummingii*, by microborers and sponges of French Polynesia. *Marine Biology* 126: 509-519.

Margolis, L., Esch, G.W., Holmes, J.C., Kuris, A.M. & Schad, G.A. 1982. The use of ecological terms in parasitology (report of an ad hoc committee of the American Society of Parasitologists). *Journal of Parasitology* 68(1) 131-133.

Marking, L.L. & Dawson, V.K. 1975. Method for the assessment of toxicity or efficiency of mixtures of chemicals. *Investigations in Fish Control,* US Department of Interior, Fish and Wildlife Service, Washington DC, 67, 1-7.

Marmot, M.G. & Madge, N. 1987. An Epidemiological Perspective on Stress and Health. Chapter 1 in Kasl, S.V. & Cooper, C.L. (Eds) 1987. *Stress and Health: Issues in Research Methodology.* pp. 3-26. John Wiley & Sons.

Marshall, D.J. & McQuaid, C.D. 1993. Differential physiological and behavioural responses of the intertidal mussels, *Choromytilus meridionalis* (Kr.) and *Perna perna* L. to exposure to hypoxia and air: a basis for spatial separation. *Journal of Experimental Marine Biology and Ecol*ogy 171:225-237.

Mason, J.W. 1971. A re-evaluation of the concept of "nonspecificity" in stress theory. *Journal of Psychiatric Research* 8, 323-333.

Mason, J.W. 1975a. A historical view of the stress field. *Journal of Human Stress* 1, 6-12.

Mason, J.W. 1975b. A historical view of the stress field. *Journal of Human Stress* 1, 22-35.

Mathew, P. & Menon, N.R. 1992. Toxic Responses of Bivalves to Metal Mixtures. *Bulletin of Environmental Contamination and Toxicology* 48, 185-193.

Matthews, R.A. 1973. The life-cycle of *Prosorhynchus crucibulum* (Rudolphi, 1819) Odhner, 1905, and a comparison of its cercaria with that of *Prosorhynchus squamatus* Odhner, 1905. *Parasitology* 66:133-164.

Mattiucci, S., Nascetti, G., Cianchi, R., Paggi, L., Arduino, P., Margolis, L., Brattey, J., Webb, S., D' Amelio, S., Orecchia, P. & Bullini, L. 1997. Genetic and ecological data on the *Anisakis simplex* complex, with evidence for a new species (Nematoda, Ascaridoidea, Anisakidae). *Journal of Parasitology* 83(3) 401-416.

Mayo, O. 1983. *Natural selection and its constraints*. Academic Press, London. 145pp.

Mayr, E. 1974. Teleological and Teleonomic, a New Analysis. In Cohen R. S. & Wartofsky, M. W. (Eds), *Methodological and historical essays in the Natural and Social Sciences.* Vol 14: *Boston Studies in the Philosophy of Science.* D. Reidel Publishing Company, Dordrecht Holland /Boston.

McGrath, J. E. 1976. Stress and Behaviour in Organisations, Chapter 31, pp. 1352-1395, in Dunnette, M. D. (Ed.) *Handbook of Industrial and Organisational Psychology.* Rand McNally college publishing Company, Chicago. 1740pp

McGuinnes, K.A. 1988. *The Ecology of Botany Bay and the Effects of Man's Activities: a Critical Synthesis.* Sydney: Institute of Marine Ecology, University of Sydney.

McLeese, D.W. 1956. Effects of temperature, salinity and oxygen on the survival of the American lobster. *J. Fish Res. Bd. Can.* 13, 247-272.

McLusky, D.S., Bryant, V. & Campbell, R. 1986. The effects of temperature and salinity on the toxicity of heavy metals to marine and estuarine invertebrates. *Oceanography and Marine Biology Annual Review* 24, 481-520.

Mechanic, D. 1962. *Students under stress*. New York Free Press.

Mechanic, D. 1968. *Medical Sociology, A Selective View*. New York Free Press. 504pp.

Medvedev, Z.A. 1990. An attempt at a rational classification of theories of ageing. *Biological Review* 65, 375-398.

Meier, R.L. 1972. Communications stress. *Annual Review of Ecology and Systematics* 3, 298-314.

Mendl, M.T. 1990. Developmental experience and the potential for suffering: does 'out of experience' mean 'out of mind'? *Behavioural and Brain Sciences* 13, 28-29.

Metz, J.A.J. & Diekmann, O. 1986. The dynamics of physiologically structured populations. *Lecture Notes in Biomathematics* Vol 68. Springer-Verlag, Heidelberg, 511pp.

Meyers, F.H., Jawetz, E. & Goldfien, A. 1980. *Review of Medical Pharmacology*, 7th Edition. Lange Medical Publications, Los Altos, California. 747pp.

Micallef, S. & Tyler, P.A. 1990. Effect of mercury and selenium on the gill function of *Mytilus edulis*. *Marine Pollution Bulletin* 21(6) 288-292.

Mikhail, A. 1985. Stress, a Psychophysiological Conception. pp. 30-39 in Monat, A. & Lazarus R.S. (Eds), *Stress and Coping, an Anthology*. Columbia University Press, New York.

Moller, A.P. 1996. Parasitism and developmental instability of hosts: a reveiw. *Oikos* 77(2): 189-196.

Moore, C. 1990. Unpredictability and Undecidability in Dynamical Systems. *Physical Review Letters* 64 (20) 2354-2357.

Moore, H.B. 1972. Aspects of stress in the tropical marine environment. *Advances in Marine Biology* 10, 217-269.

Moore, M. & Folt, C. 1993. Zooplankton Body Size and Community Structure: Effects of Thermal and Toxicant Stress. *Trends in Ecology and Evolution* 8, 5, 178-183.

Moore, M.N. 1991. Cellular reactions to toxic environmental contaminants in marine molluscs. pp. 157-175 in Abel, P.D. & Axiak, V. (Eds), *Ecotoxicology and the Marine Environment.* Ellis Horwood, Chichester.

Moore, M.N., Livingstone, D.R., Widdows, J., Lowe, D.M. & Pipe, R.K. 1987. Molecular, cellular and physiological effects of oil-derived hydrocarbons on molluscs and their use in impact assessment. *Philosophical Transactions of the Royal Society, London* Ser. B, 316, 603-623.

Moore, S. & Stein, N.H. 1954. A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. *Journal of Biological Chemistry* 211, 907-913.

Morris, M.R. 1976. Estados larvales de trematodes digeneos en molluscos marinos *Mytilus platensis* d'Orb. y *Brachydontes rodriguezi* d'Orb. *Revista del Museo de la Plata* 13: 65-71.

Moss, G.E. 1973. *Illness, Immunity, and Social Interaction: the dynamics of biosocial resonation.* John Wiley & Sons, New York, London, Sydney and Toronto. 281pp.

Moss, L. 1981. *Management Stress.* Addison Wesley Publishing Company. 257pp.

Munger, J.C. & Karasov, W.H. 1989. Sublethal parasites and host energy budgets: tapeworm infection in white footed mice. *Ecology* 70(4) 904-921.

Näätänen, R. 1973. The inverted U relationship between activation and performance: a critical review. In Kornblum, S. (Ed.), *Attention and Performance,* IV. New York: Academic Press, 1973.

Nagel, E. 1961. *The Structure of Science, Problems in the Logic of Scientific explanation.* Routledge & Kegan Paul. 618pp.

Nagel, E. 1979. *Teleology Revisited and Other Essays in the Philosophy and History of Science.* Columbia University Press New York. 352pp.

Naugler, C.T. & Ludman, M.D. 1996. Fluctuating Asymmetry and Disorders of Developmental Origin. *American Journal of Medical Genetics* 66:15-20.

Navarro, J.M. 1988. The effects of salinity on the physiological activity of *Choromytilus chorus* (Molina, 1782) (Bivalvia: Mytilidae). *Journal of Experimental Marine Biology and Ecology* 122: 19-33.

Nelson, R.J. & Demas, G.E. 1996. Seasonal changes in immune function. *The Quarterly Review of Biology* 71 (4): 511-548.

Newell, R.C. 1979. *Biology of Intertidal Animals*, third edition. The Rustica Press (PTY) Ltd. Wynberg, Cape.

Newkirk, G.F. 1980. Genetics of shell colour in *Mytilus edulis* L. and the association of growth rate with shell colour. *Journal of Experimental Marine Biology and Ecology* 47: 89-94.

Nisbet, R.M., Gurney, W.S.C., Murdoch, W.W. & McCauley, E. 1989. Structured population models: a tool for linking effects at individual and population level. *Biological Journal of the Linnean Society* 37, 79-99. In: (Eds) Calow, P. & Berry, R.J. *Evolution, Ecology and Environmental Stress*. Academic Press, London. 187pp.

Nixon, P.G.F. 1976. The human function curve with special reference to cardiovascular disorders: Part 1. *The Practitioner* 217, 765-770.

Nolan, C.P. 1991. Infestation of limpet shells by the conchocelis phase of an endolithic alga. *Journal of Molluscan Studies* 57: 225-238.

Odum, E.P. 1968. Energy flow in ecosystems: a historical review. *American Zoologist* 8, 11-18.

Odum, E.P. 1985. Trends Expected in Stressed Ecosystems. *BioScience* 35, 7, 41-422.

Odum, E.P., Finn, J.T. & Franz, E. 1979. Perturbation theory and the subsidy stress gradient. *BioScience* 29, 6, 349-352.

Ogawa, K. & Matsuzaki, K. 1985. Discovery of bivalve infesting pycnogonida, *Nymphonella tapetis* in a new host, *Hiatella orientalis*. *Zoological Science* 2 583-589.

Ohnishi, T., Nakamura, O., Arakaki, N. & Daikuhara Y. 1997. Effect of phosphorylated rat fetuin on the growth of hepatocytes in primary culture in the presence of human hepatocyte-growth factor: Evidence that

phosphorylated fetuin is a natural modulator of hepatocyte growth factor. *European Journal of Biochemistry* 243 (3): 753-761.

Ollason, J.G. 1991. What Is This Stuff Called Fitness? *Biology and Philosophy* 6, 81-92.

Orians, G.H. 1975. Diversity, stability and maturity in natural ecosystems. pp. 139-150 in *Unifying Concepts in Ecology* (Eds van Dobben, W. H. & Lowe-McConnell, R. H.). Junk, the Hague, the Netherlands: 302pp.

Oshima, H. 1927a. *Nymphonella tapetis* n. gen, n. sp. a pycnogon parasitic in a bivalve. *Annotationes Zoologicae Japonenses* 11:3 257-263.

Oshima, H. 1927b. Piknogono parazite vivanta en Bivalvo (Japanese with Esperanto résumé. *Bult. Sci. Fakult. Terkult. Kjusu Imp. Univ.* Vol. 2 No. 5, pp.366-378, Tab. 5 and 6.

Oshima, H. 1933a. The adult of the bivalve infesting pycnogonid, *Nymphonella tapetis* Oshima. *Annotationes Zoologicae Japonenses* 10:1 53-60.

Oshima, H. 1933b. Young pycnogonids found parasitic on nudibranchs. *Annotationes Zoologicae Japonenses* 10:1 61-66.

Oshima, H. 1935. A further note on *Nymphonella tapetis,* the egg-carrying mature male (Eurycydidae, Pantopoda). *Annotationes Zoologicae Japonenses* 15:1 95-102.

Oshima, H. 1938. Nymphonellidae, a new family of pantopoda. *Annotationes Zoologicae Japonenses* 17:3,4 229-233.

Overmier, J.B. 1996. Richard L. Solomon and learned helplessness. *Integrative Physiological and Behavioural Science* 31(4): 331-337

Overmier, J.B., Patterson, J. & Wielkiewicz, R.M. 1980. Environmental contingencies as sources of stress in animals. pp. 1-38 in Levine, S. & Ursin, H. (Eds) *Coping and Health*. Plenum Press, New York.

Page, D.S., Gilfillan, E.S., Hanson, S.A., Hotham, J.R. & Foster, J.C. 1984. Correlation of Body Burden of Heavy Metals with Physiological Stress Indicators in *Mytilus edulis* and *Mya arenaria* Exposed to Heavy Metals in the Field. In Stegman, J.J. & Heath, G.W. (Eds) *Responses of Marine Organisms to Pollutants*, *Marine Environmental Research* 14. Elsevier Applied Science Publishers, London and New York (1984). 547pp.

Paparo, A.A. 1985. The role of cerebral and visceral ganglia in ciliary activity. *Comparative Biochemistry and Physiology* 81A (3) 647-651.

Parsons, P.A. 1989. Acetaldehyde utilization in *Drosophila*: an example of hormesis. *Biological Journal of the Linnean Society* 37, 183-189. In Calow, P. & Berry R.J. (Eds) *Evolution, Ecology and Environmental Stress*. Academic Press, London. 187pp.

Parsons, P.A. 1990a. Fluctuating asymmetry: an epigenetic measure of stress. *Biological Review* 65: 131-145.

Parsons, P.A. 1990b. Risks from genetically engineered organisms: energetics and environmental stress. *Functional Ecology* 4: 265-271.

Parsons, P.A. 1996. Conservation stretegies: Adaptation to stress and the preservation of genetic diversity. *Biological Journal of the Linnean Society* 58 (4) 471-482.

Pascal, B. 1961. *The Pensees* translated by Cohen, J.M. Pensee 31, p. 37, Penguin Books, 287pp.

Pearlin, L.I. 1982. The Social Contexts of Stress. Chapter 22 in Goldberger, L. & Breznitz, S. (Eds) *Handbook of stress: theoretical and clinical aspects.* New York Free Press, 804pp.

Pekkarinen, M. 1986. Development of the cercaria of *Lacunovermis macomae* (Trematoda:Gymnophallidae) to the metacercaria in brackish-water *Macoma baltica* (Bivalvia). *Annales Zoologici Fennici* 23:237-250.

Pekkarinen, M. & Ching, H.L. 1994. Comparisons of gymnophallid digeneans from North Pacific and Baltic clams, *Macoma baltica* (Bivalvia). *Journal of Parasitology* 80(4): 630-636.

Perkins, D.V. 1982. The Assessment of Stress Using Life Events Scales. Chapter 20 in Goldberger, L. & Breznitz, S. (Eds) *Handbook of stress: theoretical and clinical aspects.* New York Free Press, 804pp.

Perkins, R.J. 1974. Stress and its relation to the ecosystem. *Int. J. Ecol. Environ. Sci.* 1, 119-127.

Peters, E.C., Gassman, N.J., Firman, J.C., Richmond, R.H. & Power E.A. 1997. Ecotoxicology of Tropical Marine Ecosystems. *Environmental Toxicology and Chemistry* 16(1):12-40.

Petraitis, P.S. 1987. Immobilization of the predatory gastropod, *Nucella lapillus*, by its prey, *Mytilus edulis*. *Biological Bulletin* 172:307-314.

Peyer, B. 1945. Uber Algen und pilze in Tierschen Hartsubstanzen. *Archiv der Julius Klaus-Stiftung fuer Vererbungs-Forschung, Sozialanthropologie und Rassenhygiene* 20 (suppl): 496-546.

Phillips, D.J.H. 1977. The use of biological indicator organisms to monitor trace metal pollution in marine and estuarine environments - a review. *Environmental Pollution* 13: 281-317.

Pickering, A.D. 1981. Introduction: the concept of biological stress. In Pickering, A.D. (ed.) *Stress and Fish.* Academic Press, London. 367pp.

Pike, A.W. & Erasmus, D.A. 1967. The formation, structure and histochemistry of the metacercarial cyst of three species of digenetic trematodes. *Parasitology* 57: 683-694.

Pipe, R.K. & Moore, M.N. 1985. Ultrastructural changes in the lysosomal-vacuolar system in digestive cells of *Mytilus edulis* as a response to increased salinity. *Marine Biology* 87, 157-163.

Pipe, R.K. & Moore, M.N. 1986. Arylsulphatase activity associated with phenanthrene induced digestive cell deletion in the marine mussel *Mytilus edulis*. *Histochemical Journal* 18, 557-564.

Piper, R.G., Mc Elwain, I.B., Orme, L.E., McCraren, J.P., Fowler, L.G. & Leonard, J.R. 1982. *Fish Hatchery Management.* United States Department of the interior, Fish and Wildlife Service, Washington D.C. 517pp.

Plato, 1983. (Tredennick H. trans.), *Phaedo* 86b, in: *The Last Days of Socrates*. Penguin Books, 199pp.

Popper, C.W., Chiueh, C.C. & Kopin, I.J. 1977. Plasma catecholamine concentrations in unanaesthetised rats during sleep, wakefulness, immobilization and decapitation. *Journal of Pharmacology and Experimental Therapeutics* 202, 144-148.

Pregenzer, C. 1983. Survey of Metazoan Symbionts of *Mytilus edulis* (Mollusca: Pelecypoda) in Southern Australia. *Australian Journal of Marine and Freshwater Research* 34: 387-396.

Prigogine, I. & Stengers, I. 1985. *Order Out of Chaos: Man's New Dialogue with Nature.* Flamingo, 349pp.

Princep, M., Bigas, M. & Durfort, M. 1996. Incidencia de *Bucephallus* (sic) *haimeanus* (Lacaze-Duthiers, 1854) (Trematoda, Digenea) en el hepatopancreas de *Ostrea edulis* Linne. *Iberus* 14(2):211-220.

Prochazka, A., Gillard, D. & Bennet, D.J. 1997a. Positive force feedback control of muscles. *Journal of Neurophysiology* 77:3226-3236.

Prochazka, A., Gillard, D. & Bennet, D.J. 1997b. Implications of positive feedback in the control of movement. *Journal of Neurophysiology* 77: 3237-3251.

Prochazka, K. & Griffiths, C.L. 1991. Factors affecting the shelf life of live cultured mussels. *Journal of Shellfish Research* 10(1) 23-28.

Prosser, C. L. (Ed.) 1993. Environmental and Metabolic Animal Physiology. In *Comparative Animal Physiology* (4th edition). Wiley Liss. 578pp.

Puppe, B. 1996. Wohlbefinden bei Nutztieren: eine verhaltensbiologische Ubersicht. *Biologisches Zentralblatt* 115: 3-15

Purdey, M. 1996. The UK Epidemic of BSE: Slow Virus or Chronic Pesticide-Initiated Modification of the Prion Protein? *Medical hypotheses* 46:429-443.

Putnam, H. 1975. Is semantics possible? In *Mind Language and Reality* Vol. 2. 7th Series of Minnesota Studies in the Philosophy of Science.

Raghukmar, C., Sharma, S. & Lande V. 1991. Distribution and biomass estimation of shell boring algae in the intertidal at Goa, India *Phycologia* 30 (3); 303-309.

Rapport, D.J. 1989. Symptoms of pathology in the Gulf of Bothnia (Baltic Sea): ecosystem response to stress from human activity. *Biological Journal of the Linnean Society* 37, 33-49. In Calow, P. & Berry R.J. (Eds) *Evolution, Ecology and Environmental Stress*. Academic Press, London. 187pp.

Rapport, D.J., Reiger, H.A. & Thorpe, C. 1981. Diagnosis, Prognosis, and Treatment of Ecosystems under stress. Chapter 19 in Barrett, G.W. & Rosenberg R. (Eds). *Stress Effects on Natural Ecosystems.* John Wiley & Sons Ltd. 305pp.

Raubenheimer, D. & Cook, P.A. 1991. Effects of exposure to wave action on allocation of resources to shell and meat growth of the subtidal mussel *Mytilus galloprovincialis*. *Journal of Shellfish Research* 9(1) 87-93.

Reason, J. 1993. *Attention: Selection Awareness and Control.* A tribute to Donald Broadbent, [Baddeley, A. & Weiskrantz, L. (Eds)]. Clarendon Press, Oxford. 436pp.

Regoli, F. 1992. Lysosomal responses as a sensitive stress index in biomonitoring of heavy metal pollution. *Marine Ecology Progress Series* 84, 63-69.

Reish, D.J. & Ayers J.L. Jr. 1968. Studies on the *Mytilus edulis* community in Alamitos Bay, California. III. The effects of reduced dissolved oxygen and chlorinity concentrations on survival and byssus thread formation. *Veliger* 10: 384-388.

Richardson, C.A. 1988. Exogenous and endogenous rhythms of band formation in the shell of the clam *Tapes philippinarum* (Adams *et* Reeve, 1850). *Journal of Experimental Marine Biology and Ecology* 122 105-126.;

Ricketts, E.F. & Calvin, J. 1968. Between Pacific Tides, 4th Ed, rev. by J.W. Hedgepeth. Stanford University press, Stanford California.

Rigler, F.H. 1975. The concept of energy flow and nutrient flow between trophic levels. In Van Dobben, W.H. & Lowe-McConnell, R.H. (Eds), *Unifying Concepts in Ecology*. The Hague, Junk. 302pp.

Roberts, D.A., Hofmann G.E. & Somero, G.N. 1997. Heat-Shock Protein Expression in *Mytilus californianus*: Acclimatisation (Seasonal and Tidal-Height Comparisons) and Acclimation Effects. *Biological Bulletin* 192:309-320.

Rodcharoen, J. & Mulla, M.S. 1997. Biological Fitness of *Culex quinquefasciatus* (Diptera: Culicidae) Susceptible and Resistant to *Bacillus sphaericus*. *Journal of Medical Entomology* 34(1): 5-10.

Roesijadi, G. & Fellingham, G.W. 1987. Influence of Cu, Cd, and Zn pre-exposure on Hg toxicity in the mussel *Mytilus edulis*. *Canadian Journal of Fisheries and Aquatic Sciences* 44, 680-684.

Rohde, K. 1993. *Ecology of Marine Parasites: an Introduction to Marine Parasitology* 2nd Edition, CAB International.

Roth, K.A., Mefford, I.M. & Barchas, J.D. 1982. Epinephrine, norepinephrine, dopamine and serotonin: differential effects of acute and chronic stress on regional brain amines. *Brain Research* 239, 417-424.

Ruff, G.E. & Korchin, S.J. 1967. "Adaptive Stress Behavior" in Appley & Trumball (Eds), *Psychological Stress*. New York: Appleton-Century-Crofts, pp. 297-323.

Ruse, M. 1973. *The Philosophy of Biology*. Hutchinson: London 231pp.

Ruse, M. 1989. Teleology in Biology: Is it a Cause for Concern? *Trends in Ecology and Evolution* 4, 2, 51-54.

Russek, L.G. & Schwarz, G.E. 1997. Perceptions of Parental Caring Predict Health Status in Midlife: A 35-Year Follow-up of the Harvard Mastery of Stress Study. *Psychosomatic Medicine* 59: 144-149

Sadok. S., Uglow, R. & Haswell, S.J. 1995. Fluxes of haemolymph ammonia and free amino acids in *Mytilus edulis* exposed to ammonia. *Marine Ecology Progress Series* 129: 177-187.

Sapolsky, R.M. 1983. Individual differences in cortisol secretory patterns in the wild baboon: role of negative feedback sensitivity. *Endocrinology* 113, 2236-2267.

Sastry, A.N. & Miller, D.C. 1981. Application of biochemical and physiological responses to water quality monitoring. pp. 265-294 in Vernberg, J., Calabrese, A. Thurberg, F.P. & Vernberg, W.P. (Eds) *Biological Monitoring of Marine Pollutants*. Academic Press New York. 559pp.

Schaffer, W.M. & Kot, M. 1986. Chaos In Ecological Systems: The Coals that Newcastle Forgot. *Trends in Ecology and Evolution* 1, 3, 58-63.

Schmidt-Nielsen, K. 1990. *Animal Physiology: Adaptation and Environment,* (4th ed.). Cambridge University Press. 602pp.

Schneiderman, N. & McCabe, P.M. 1985. Biobehavioural Responses to Stressors. Chapter 2 in Field, T.M., McCabe, P.M. & Schneiderman, N. (Eds), *Stress and Coping*. Lawrence Earlbaum Associates, Hillsdale, New Jersey. 354pp.

Scott, R. & Howard, A. 1970. "Models of Stress," in Levine & Scotch (Eds), *Social Stress*. Chicago: Aldine, pp. 259-278.

Seed, R. 1968. Factors influencing shell shape in the mussel *Mytilus edulis* L. *Journal of the Marine Biological Association of the United Kingdom* 48: 561-584.

Seed, R. 1976. Ecology, Chapter 2 p13-65. In: Bayne, B.L. (Ed.). *Marine mussels: Their Ecology and Physiology.* Cambridge, Cambridge University Press, 506pp.

Seed, R. 1978. The systematics and evolution of *Mytilus galloprovincialis* Lmk. In, *Marine Organisms*: *Genetics, Ecology and Evolution* (Beardmore, J.A. & Battaglia, B. Eds) New York; Plenum: pp. 447-466.

Seed, R. 1991. An unusually heavy infestation of pearls in the mussel *Mytilus edulis* L. *Journal of Molluscan Studies* 57, 296-297.

Selye, H. 1952. *The story of the adaptation syndrome.* Acta Inc., Montreal, Canada: 225pp.

Selye, H. 1955. Stress and disease. *Science* 122, 625-631.

Selye, H. 1956. *The Stress of Life.* New York McGraw-Hill. 324pp.

Selye, H. 1973. The Evolution of the Stress Concept. *American Scientist* 61, 692-699.

Selye, H. 1974. *Stress without distress*. The new American Library of Canada Ltd., Scarborough, Ontario, Canada: 193pp.

Selye, H. 1976. *The Stress of Life* (revised edition). Acta Inc., Montreal, Canada. 515pp.

Selye, H. 1982. History and present status of the stress concept. Chapter 2 in Goldberger, L. & Breznitz, S. (Eds) *Handbook of stress: theoretical and clinical aspects.* New York Free Press, 804pp.

Settle, T. 1993. "Fitness" and "Altruism": Traps for the Unwary Bystander and Biologist alike. *Biology and Philosophy* 8, 61-83.

Sgoifo, A. de Boer, S.F., Haller, J. & Koolhaas, J.M. 1996. Individual Differences in Plasma Catecholamine and Corticosterone Stress Responses of Wild-Type Rats: Relationship with Aggression. *Physiology & Behavior* 60 (6): 1403-1407.

Shafee, M.S. 1989. Reproduction of *Perna picta* (Mollusca: Bivalvia) from the Atlantic coast of Morocco. *Marine Ecology Progress Series* 53: 235-245.

Sibly, R. M. & Calow, P. 1989. A life cycle theory of response to stress. *Biological Journal of the Linnean Society* 37, 101-116. In Calow, P. & Berry R.J. (Eds)

Evolution, Ecology and Environmental Stress. Academic Press, London. 187pp.

Siddall, S.E. 1980. A clarification of the genus *Perna* (Mytilidae). *Bulletin of Marine Science* 30: 4 858-870.

Simkiss, K. & Mason, A.Z. 1983. Metal ions: metabolic and toxic effects, Chapter 4, pp. 101-164, in Hochachka, P.W. (Ed.) 1983. *The Mollusca* Volume 2 Environmental Biochemistry and Physiology. Academic Press, New York.

Sindermann, C.J. 1990. *Principal Diseases of Marine Fish and Shellfish Volume* 1*: Diseases of Marine Fish*, Academic Press. 521pp.

Skorupski, P. 1992. Synaptic connections between nonspiking afferent neurons and motor neurons underlying phase dependent reflexes in crayfish. *Journal of Neurophysiology* 67 (3): 664-679.

Skorupski, P., Rawat, B.M. & Bush, B.M.H. 1992. Heterogeneity and central modulation of feedback reflexes in crayfish motor pool. *Journal of Neurophysiology* 67 (3): 648-663.

Skorupski, P., Vescovi, P.J. & Bush, B.M.H. 1994. Integration of positive and negative feedback loops in a crayfish muscle. *Journal of Experimental Biology* 187:305-313.

Somerville, J. 1946. *Soviet Philosophy: A Study of Theory and Practice.* Philosophical Library, New York. 269pp.

Soot-Ryen, T. 1955. A report on the family Mytilidae (Pelecypoda) *Allan Hancock Pacific Expedition* (20) 1 175 pp.

Sprague, J.B. 1970. Measurements of pollutant toxicity to fish - II. Utilizing and applying bioassay results. *Water Research* 4, 3-32.

Sprague, J.B. 1971. Measurements of pollutant toxicity to fish - III. Sublethal effects and safe concentrations, *Water Research* 5, 245-266.

Stearns, S.C. 1992. *The Evolution of Life Histories.* Oxford University Press. 49pp.

Stephen, D. & Shetty, H.P.C. 1981. Induction of spawning in four species of bivalves of the Indian coastal waters. *Aquaculture* 25 153-159.

Sternbach, R.A. 1968. *Pain: a Psychophysiological Analysis.* Academic Press, New York and London, 185pp.

Stewart, I. 1993. A New Order, pp. 2-3 in Johnson, J. (Ed), Complexity, *New Scientist* (Supplement) 13, 6 February, 8pp.

Stock, J.H. 1959. On some South African Pycnogonida of the University of Cape Town ecological survey. *Transactions of the Royal Society of South Africa* 35: 549-567.

Strümpfer, D.J.W. 1983. Executive Dystress, Executive Eustress, and what makes the difference. *Fact and Opinion Papers No 18*, June, Faculty of Business Administration, University of the Witwatersrand, Johannesburg. 36pp.

Stunkard, H.W. 1930. An analysis of the methods used in the study of larval trematodes. *Parasitology* 22: 268-273.

Stunkard, H.W. 1932. Some larval trematodes from the coast in the region of Roscoff, Finistere. *Parasitology* 24: 321-343.

Stunkard, H.W. 1941. Specificity and host-relations in the trematode genus *Zoogonus*. *Biological Bulletin* 81: 205-214.

Stunkard, H.W. 1964. Studies on the trematode genus *Renicola:* observations on the life history, specificity, and systematic position. *Biological Bulletin* 126: 467-489.

Stunkard, H.W. 1974. The trematode family Bucephalidae - problems of morphology, development, and systematics: description of *Rudolphinus* gen. nov. *Transactions of the New York Academy of Sciences* (Series 2) 36:143-170.

Stunkard, H.W. 1983. The marine cercariae of the Woods Hole, Massachusetts region, a review and a revision. *Biological Bulletin* 164: 143-162.

Stunkard, H.W. & Uzmann, J.R. 1958. Studies on digenetic trematodes of the genera *Gymnophallus* and *Parvatrema*. *Biological Bulletin* 115(2): 276-302.

Stunkard, H.W. & Uzmann, J.R. 1959. The life cycle of the digenetic trematode, *Proctoeces maculatus* (Looss, 1901) Odhner, 1911 [syn. *P. subtenuis* (Linton, 1907) Hanson 1950], and description of *Cercaria adranocerca* n. sp. *Biological Bulletin* 116(1): 184-193.

Sudakov, K.V. 1996. Systemic integration of human functions: New approaches to diagnosing and correcting stress states. *Vestnik Rossiiskoi Akademii Meditsinskikh Nauk* (6) 15-25.

Sunila, I. 1986. Histopathological changes in the mussel *Mytilus edulis* L. at the outlet from a titanium dioxide plant in the Northern Baltic. *Annales Zoologici Fennici* 23, 61-70.

Sunila, I. & Lindstrom, R. 1985. Survival, Growth and Shell Deformities of Copper- and Cadmium-exposed Mussels (*Mytilus edulis* L.) in Brackish Water. *Estuarine, Coastal and Shelf Science* 21: 555-565.

Sutherland, J.P. 1981. The fouling community at Beaufort, North Carolina: a study in stability. *American Naturalist* 118, 449-519.

Swartz, R.C. 1987. Toxicological methods for determining the effects of contaminated sediment on marine organisms. pp. 183-198 in Dickson, K.L., Maki, A.W. & Brungs, W.A. (Eds) *Fate and Effects of Sediment Bound Chemicals in Aquatic Systems.* Pergamon, New York.

Swennen, C. 1969. Crawling tracks of trematode infested *Macoma balthica* (L.). *Netherlands Journal of Sea Research* 4, 376-379.

Swenson, R.M. & Vogel, W.H. 1983. Plasma catecholamine and corticosterone as well as brain catecholamine changes during coping in rats exposed to stressful footshock. *Pharmacology Biochemistry and Behavior* 18, 689-693.

Syme, S.L. 1967. "Implications and Future Prospects," In Syme & Reeder (Eds), *Social Stress and Cardiovascular Disease.* The Millbank Memorial Fund Quarterly, Vol. XLV, April 1967.

Tamargo, J. 1995. Cardiac cardiovascular remodelling therapy. New concepts. *Anales de la Real Academia de Farmacia* 61 (suppl.): 843-872.

Taskinen, J., Valtonen, E.T. & Gibson, D.I. 1991. Studies on bucephalid digeneans parasitising molluscs and fishes in Finland.I. Ecological data and experimental studies. *Systematic Parasitology* 19: 81-94.

Taylor, A.C. & Brand, A.R. 1975. Effects of hypoxia and body size on the oxygen consumption of the bivalve *Arctica islandica* (L.). *Journal of Experimental Marine Biology and Ecology* 19:187-196.

Tharme, R.E. 1988. *A survey of digenean trematodes in the white sand mussel* Donax serra *Röding on the Western Cape coast.* Unpublished Honours project, University of Cape Town, 85pp.

Tharme, R.E., Webb, S.C. & Brown, A.C. 1996. Organisms associated with the sandy-beach bivalve *Donax serra* Röding, with a description of *Cercaria serrae* sp. nov. (trematoda). *South African Journal of Zoology* 31(2): 86-90.

Theede, H. & Jung, C.T. 1989. Experimental studies of the effects of some environmental factors on the accumulation and elimination of cadmium by the mussel *Mytilus edulis*. pp. 615-624 in Klekowski, R.Z., Styczynska-Jurewicz, E. and Falkowski, L. (Eds), *Proceedings 21st European Marine Biolology Symposium,* Gdansk, Poland, 1988. Ossolineum, Gdansk.

Thomas, G.H. 1968. *Metalwork Technology*. John Murray London. 242pp.

Thompson, R.J., Ratcliffe, N.A. & Bayne B.L. 1974. Effects of starvation on structure and function in the digestive gland of the mussel (*Mytilus edulis* L.). *Journal of the Marine Biological Association of the United Kingdom* 54, 699-712.

Tinbergen, N. 1974. Ethology and stress diseases. *Science* 185, 20-23.

Tobias, C.A. 1970. Radiation: biological effects III. Radiation and human health, 6 Protection against external radiation. *Encyclopaedia Britannica* Vol. 18, p1030-1031.

Toman, G. 1977. Digenetic trematodes of marine teleost fishes from the Seychelles, Indian Ocean. I. Family Angiodictyidae. *Excerta Parasitologica en Memoria del Doctor Eduardo Caballero y Caballero. Insituto de Biologia, Publicaciones Especiales Universidad Nacional Autonoma de Mexico* 4: 335-341.

Toman, G. 1989. Digenetic trematodes of marine teleost fishes from the Seychelles, Indian Ocean. II. *Acta Parasitologica* 34: 235-246.

Toman, G. 1992a. Digenetic trematodes of marine teleost fishes from the Seychelles, Indian Ocean. III. *Acta Parasitologica* 37(3): 119-126.

Toman, G. 1992b. Digenetic trematodes of marine teleost fishes from the Seychelles, Indian Ocean. IV. *Acta Parasitologica* 37(3): 127-130.

Turkkan, J.S., Brady, J.V. & Harris, A.H. 1982. Animal Studies of Stressful Interactions: A Behavioural-Physiological Overview. Chapter 11 in Goldberger, L. & Breznitz, S. (Eds) *Handbook of stress: theoretical and clinical aspects*. New York Free Press, 804pp.

Ulanowicz, R.E. 1978. Modeling environmental stress. in: *Energy and Environmental Stress In Aquatic Systems* pp. 1-18 (Eds: Thorp, J.H. & Gibbons, J.W.). US Department of Energy Symposium Series 48, CONF-771114: 854pp.

Umiji, S., Lunetta, J.E. & Leonel, R.M.V. 1976. Infestation of the mussel *Perna perna* by digenetic trematodes of the Bucephalidae family, *Gen. Bucephalus. Anais Academicas Brasilieras Ciencias* 47 (Suppl.) 115-117.

Underwood, A.J. 1989. The analysis of stress in natural populations. *Biological Journal of the Linnean Society* 37, 51-78. In Calow, P. & Berry R.J. (Eds) *Evolution, Ecology and Environmental Stress*. Academic Press, London. 187pp.

Underwood, A.J., Denley, E.J. & Moran M.J. 1983. Experimental analyses of the structure and dynamics of mid-shore rocky intertidal communities in New South Wales. *Oecologia (Berlin)* 56, 202-219.

Underwood, A.J. & Peterson, C.H. 1988. Towards an ecological framework for investigating pollution. *Marine Ecology Progress Series* 46, 227-234.

Van Der Steen, W.J. 1993. Towards Disciplinary Disintegration in Biology. *Biology and Philosophy* 8, 259-275.

Van Dyne, G. 1981. Response of Shortgrass Prairie to Man-induced Stresses as Determined from Modelling Experiments. *Stress Effects on Natural Ecosystems.* Chapter 6 In Barrett, G.W. & Rosenberg R. (Eds). *Stress Effects on Natural Ecosystems.* John Wiley & Sons Ltd. 305pp.

Underwood, A.J., Denley, E.J. & Moran M.J. 1983. Experimental analyses of the structure and dynamics of mid-shore rocky intertidal communities in New South Wales. *Oecologia (Berlin)* 56, 202-219.

van Erkom Schurink, C. & Griffiths, C.L. 1990. Marine mussels of Southern Africa - their distribution patterns, standing stocks, exploitation and culture. *Journal of Shellfish Research* vol. 9 No 1 pp75-85.

van Erkom Schurink, C. & Griffiths, C.L. 1991. A comparison of reproductive cycles and reproductive output in four southern African mussel species. *Marine Ecology Progress Series* 76(2) 123-134.

van Erkom Schurink, C. & Griffiths, C.L. 1992. Physiological energetics of four South African mussel species in relation to body size, ration and temperature. *Comparative Biochemistry and Physiology* 101A (4) 779-789.

van Erkom Schurink, C. & Griffiths, C.L. 1993. Factors affecting rates of growth in four South African mussel species. *Aquaculture* 109 257-273.

Van Winkle, W. (Jr.) 1970. Effect of environmental factors on byssal thread formation. *Marine Biology* 7: 143-148.

Vélez, A. & Epifanio, C.E. 1981. The effects of temperature and ration on gametogenesis and growth in the tropical mussel *Perna perna* (L.). *Aquaculture* 22, 21-26.

Verkaar, H.J. 1986. When Does Grazing Benefit Plants? *Trends in Ecology and Evolution* 1, 6, December, 168-169.

Viljoen, C.G. 1990. *Ciliary activity of the* Donax *gill as an indicator of pollution stress.* Honours Project, Zoology Department, University of Cape Town, pp51 + appendix.

Vincent, I.C. & Leahy, R.A. 1997. Real-time Non-invasive Measurement of Heart Rate in Working Dogs: a Technique with Potential Applications in Objective Assessment of Welfare Problems. *The Veterinary Journal* 153 179-184.

Wallet, M. & Lambert, A. 1984. Caracterisation de la cercaire de *Bucephalus polymorphus* Baer, 1827 *(Trematoda, Bucephalidae)*: chetotaxie et systeme excreteur. *Annales de Parasitologie Humaine et Comparee* 59(6) 583-588.

Wallis, R.L. 1975. Thermal tolerance of *Mytilus edulis* of Eastern Australia. *Marine Biology* 30, 183-191.

Wardle, W.J. 1980. On the life cycle stages of *Proctoeces maculatus* (Digenea, Fellodistomidae) in mussels and fishes from Galveston Bay, Texas. *Bulletin of Marine Science* 30(3): 737-743.

Warwick, R.M. & Clark, K.R. 1995. New 'biodiversity' measures reveal a decrease in taxonomic distinctness with increasing stress. *Marine Ecology Progress Series* 129:301-305.

Watkins, B. & Simkiss, K. 1988. The effect of oscillating temperatures on the metal ion metabolism of *Mytilus edulis*. *Journal of the Marine Biological Association of the United Kingdom* 68: 93-100.

Watt, K.E.F. 1973. *Principles of Environmental Science.* McGraw-Hill, New York, USA, 319pp.

Webb, S.C. 1985. *A survey of helminth parasites in surfing whelks on the Western Cape coast of South Africa.* Unpublished M.Sc. thesis, University of Cape Town, 254 pp.

Webb, S.C. 1991a. *Cercaria hastata* sp. nov. (Digenea: Trematoda) in *Bullia digitalis*, a sandy beach surfing whelk from the Western Cape coast of South Africa: epidemiology and sex linked phenomena. *Journal of Natural History* 25:543-558.

Webb, S.C. 1991b. Use of acetic orcein as a fast method to fix, clear and stain cercariae for light microscopy. *Journal of Helminthology* 65:234-238.

Webb, S.C. & Korrubel, J.L. 1991. Blue green alga attacks mussels: threat to rock lobster resource? *South African Shipping News and Fishing Industry Review* 46(4): 30-31.

Webb, S.C. & Korrubel, J.L. 1994. Shell weakening in marine mytilids attributable to blue-green alga *Mastigocoleus* sp. (*Nostochopsidaceae*). *Journal of Shellfish Research* 13(1): 11-17.

Wedermeyer, G. 1981. The physiological response of fishes to the stress of intensive aquaculture in recirculating systems. In Tiews, K. (Ed) *Aquaculture in Heated Effluents and Recirculating Systems* Vol 2. Schriften der Bundesforschunganstalt fuer Fischerei, Hamburg.

Wedermeyer, G.A. & Mcleay D.J. 1981. Methods for determining the tolerance of fishes to environmental stressors. In Pickering, A.D. (Ed.) *Stress and Fish.* Academic Press, London, 367pp.

Weinstein, J.E. 1997. Flourothane-induced histological alterations in oysters, *Crassostrea virginica*: Seasonal field and laboratory studies. *Marine Environmental Research* 43(3): 201-218.

White, R.W. 1959. Motivation reconsidered: the concept of competence. *Psychological Review* 66, 297-333.

Whyard, S., Wyatt, G.R. & Walker, V.K. 1986. The heat shock response in *Locusta migratoria. Journal of Comparative Physiology* B 156, 813-817.

Whyard, S., Wyatt, G.R. & Walker, V.K. 1987. Heat shock proteins in locusta and yeast. *Cell* 48, 507-515.

Widdows, J. 1978. Physiological indices of stress in *Mytilus edulis*. *Journal of the Marine Biological Association of the United Kingdom* 58, 125-142.

Widdows, J. & Donkin, P. 1991. Role of physiological energetics in ecotoxicology. *Comparative Biochemistry and Physiology* 100C, 69-75.

Widdows, J. & Johnson, D. 1988. Physiological energetics of *Mytilus edulis*: scope for growth. *Marine Ecology Progress Series* 46: 113-121.

Wiepkema, P.R. 1987. Behavioural aspects of stress, Chapter 3. In *Biology of Stress in Farm Animals, an Integrative Approach* (Wiepkema, P.R. & van Adrichchem P.W.M. Eds.). Current Topics in Veterinary Medicine and Animal Science, Martinus Nijhoff, Dordrecht, pp. 113-183.

Williams, C.H. 1976. Some observations on the etiology of the fulminant hyperthermia-stress syndrome. *Perspectives in Biology and Medicine* 20, 1, 120-130.

Williams, H. & Jones, A. 1994. *Parasitic Worms of Fishes*. Taylor & Francis.

Williams, R.B. 1986. Hapantotypes: a Possible Solution to some Problems of Parasite Nomenclature. *Parasitology Today* 2 (11) 314-316.

Wilson, K. W. 1980. Monitoring dose-response relationships. *Rapports et Process Verbaux des Reunions Conseil International pour Exploration Scientifique de la Mer* 179, 333-338.

Windle, P.N. & Franz, E.H. 1979. The effects of insect parasitism on plant competition: greenbugs and barley. *Ecology* 60, 3, 521-529.

Wolff, H.G. 1953. *Stress and Disease.* Springfield, Ill. Charles C. Thomas.

Woodwell, G.W. 1967. Radiation and the patterns of nature. *Science* 156, 461-470.

Wright, L. 1976. *Teleological Explanations, An Etiological Analysis of Goals and Functions.* University of California Press: Berkeley. 153pp.

Wynberg, R.P., Brown, A.C. & Hole, L. 1989. Inhibition of fertilization in the sea urchin *Parechinus angulosus* by organic pollutants: correlation with molecular valence connectivity indices. *South African Journal of Marine Science* 8, 313-317.

Yerkes, R.M. & Dodson, J.D. 1908. The relation of strength of stimulus to rapidity of habit-formation. *Journal of Comparative Neurology and Psychology* 18, 459-482.

Yonge, C.M. 1962. On the primitive significance of the byssus in the Bivalvia and its effects on evolution. *Journal of the Marine Biological Association of the United Kingdom* 42:113-125.

Yonge, C.M. 1976. The "mussel" form and habit. pp. 1-12 In: Bayne, B.L. (Ed.) *Marine mussels: Their Ecology and Physiology.* Cambridge University Press, 506pp.

Young, A.J. & Lee, D.T. 1997. Aging and human cold tolerance. *Experimental Aging Research* 23(1): 45 -67.

Young, B.A. 1993. On the necessity of an archetypal concept in morphology: with special reference to the concepts of "structure" and "homology". *Biology and Philosophy* 8, 225-248.

Yousef, M.K. 1985. *Stress Physiology in Livestock*, Volume I Basic Principles. CRC Press Inc. Boca Raton, Florida. 217pp.

Yu, J.R., Chai, J.Y. & Lee S.H. 1993. *Parvatrema timondavidi* (Digenea: Gymnophallidae) transmitted by a clam, *Tapes philipinarum*, in Korea. *Korean Journal of Parasitology* 31(1): 7-12.

Zanella, A.J., Broom, D.M. & Hunter, J.C. 1992. Changes in opioid receptors in sows in relation to housing, inactivity and stereotypies, in *Animal Welfare: proceedings of the XXIV World Veterinary Congress, Rio de Janeiro 1991,* World Veterinary Association, London, pp. 159-166.

Zar, J. 1984. Biostatistical Analysis. 2nd Edition, Prentice Hall 718pp.

Zborowski, M. 1969. *People in Pain.* San Francisco: Jossey-Bass.

Zegans, L. S. 1982. Stress and the Development of Somatic Disorders. Chapter 10 in Goldberger, L. & Breznitz, S. (Eds) *Handbook of Stress: Theoretical and Clinical Aspects.* New York Free Press, 804pp.