A new sabellid that infests the shells of molluscs and its implications for abalone mariculture

Thesis submitted in fulfilment of the
requirements for Masters Degree in Science

by

Kevin Ruck

April 2000

Declaration

A new sabellid that infests the shells of molluscs and its implications for abalone mariculture

I hereby

a) grant the University of Cape Town free licence to reproduce the above thesis in whole or in part, for the purpose of research

b) declare that

(i) the above thesis is my own unaided work, both in concept and execution, and apart from the normal guidance from my supervisor, I have received no assistance except as stated below:

The cross-infection experiment in Chapter 3 was completed in collaboration with Dr. R.W. Day (Department of Zoology, University of Melbourne)
The gelatin microcapsule preparations in Chapter 7 was made according to my specifications by the Polymers Programme, MATTEK, CSIR.
A portion of the liposome work in Chapter 7 was done in collaboration with Mr Kurt Sales of the Department of Biochemistry, University of Cape Town.

(ii) neither the substance nor any part of the above thesis has been submitted by myself in the past, or is being, or is to be submitted for a degree in the University or any other university.

Signature removed
K.R. Ruck

30 April 2000
Date

Acknowledgements

Thanks to my supervisor Peter Cook for advice and guidance through the many years I have spent in his laboratory. It has been more than just the attaining of a degree, but a learning curve for life.

I wish to thank the Abalone Farmers Association of South Africa (AFASA) for funding much of the research, and also making a bursary available towards my study costs.
The FRD also provided a student bursary for 2 years.

Rob Day for the interest and enthusiasm he has for natural science. Some of it has rubbed off I hope...

Carrie Culver and Armand Kuris for sharing their ideas and discoveries by Internet initially and later through friendship.

Patience Xulu and Heidi Rolfes of the CSIR, MATTEK for supplying the microcapsules timeously and for being willing to adapt as needed.

Lastly, to the residents of 13 Frankfort Road for sampling assistance on that epic field trip of 1997.

CONTENTS

ABSTRACT

Infestations of sabellid polychaetes were found in South African farmed abalone in 1994. Growth experiments confirmed that infested abalone had reduced growth rates. Surveys of both the intertidal and subtidal region at various locations around the South African coastline revealed that the sabellid was endemic to the region. It occurred in a range of mollusc species, but some species were more susceptible than others. The fact that some molluscan species became infested with worms only when exposed to them in the laboratory suggests that environmental factors may play a role in controlling natural levels of infestation. Different host selection on the East Coast of South Africa suggests that there may be more than one species of worm.

Larvae disperse by crawling and settle at the growing edge of the shell underneath the mantle. Although the larvae are benthic, there is limited transfer of larvae through the water column, which can result in export of larvae from infested tanks. The risks of dispersal are discussed in a farm management context. Abalone kept in more hygienic laboratory conditions tended to grow faster than in farm tanks and had lower levels of infestation by sabellid larvae. This was attributed to the sabellids being less fecund under these conditions. This was probably caused by lack of food as evidenced in a separate starvation experiment.

Based on experimental observations and farm experience it was possible to make management recommendations to limit the effect of the sabellid. Quantitative measurement tools to assess the impact and productivity of sabellid infestations were developed. Management of infestations on a farm requires a combination of inhibiting transfer and productivity of the worm and also promoting growth of the abalone. However, the constant risk of exposure to natural populations of sabellids necessitates the development of a treatment to eradicate sabellid infestations.

The use of microcapsules as a drug delivery mechanism was explored and holds promise. Gelatin microcapsules, oil immulsions and liposomes were experimented with. All formulations were successfully produced in the desired size range of 2-30

μm. In all cases the sabellids readily ingested the capsules. Various toxins were successfully added to the formulations, but none of the treatments were able to harm the sabellids. The problem appeared to be the short passage time within the guts and the inability of the sabellids to digest the outer encapsulating layers to expose the toxins. More research is required to find the correct combination of toxin and delivery mechanism.

CHAPTER 1 INTRODUCTION

On rocky shores, space is often the limiting factor when it comes to distribution of species (Dayton 1971). This is particularly true for sessile species and strong competition occurs between organisms from taxonomically diverse backgrounds. Species have different adaptations, which allow them to dominate under different specific conditions. For example primary colonisers like some algal species (Sousa 1979) or barnacles (Foster 1987) are adapted to rapidly colonise freshly disturbed areas. These colonisers are generally short-lived species with rapid dispersal abilities and high reproductive rates and such organisms often rely on physical disturbances to create space for them to colonise (Thistle 1981). These species have been termed opportunists according to the disturbance model developed by Grassle and Sanders (1973). On the other hand, species that are able to displace these primary colonisers are part of the climax community and tend to be slower growing and longer lived (Grassle & Sanders 1973). These species are more able to withstand the rigors of the physical environment and to resist predation and competition (Sousa 1979). Certain species have evolved extraordinary physiological and structural adaptations to exploit extreme niches of the intertidal habitat. These specialised species thus avoid many of the competitive interactions which more generalised species experience.

Other options available are whether to be sessile or motile. The environment often predisposes what kind of organism can survive. Rough waters pounded by surf are ideal for sessile animals like tunicates, barnacles and mussels. These animals are reliant on food being supplied to them by water currents. Motile organisms such as polychaetes and isopods generally have to rely on shelter by other sessile organisms and thus colonise secondary space. The disadvantage of this is outweighed by the fact that they can actively search for food, move away when conditions become unfavourable and also escape predators. Semi-motile organisms such as limpets are also well adapted to rough conditions but the advantages of tenacity are offset by loss of motility again (Branch & Branch 1981).

Another interesting facet of secondary space, which is often overlooked, is that created by the shells of primary space colonisers such as mussels, limpets and abalone. There are numerous species that are secondary colonisers of these created spaces and are termed epibionts (e.g. Rützler 1970, Stebbing 1973, Vance 1978). Some have even taken this to the extreme by becoming obligatory epibionts. Indeed the considerations which apply to primary surface area colonisation are also true here and are, in fact, more complex due to an added variable, being the interaction between host and epibiont. There may be advantages and disadvantages to the host in having epibionts (e.g. Ruck 1990). Camouflage is the most obvious advantage (e.g. sponge crabs)(Barnes 1980) and friction or drag is the most obvious disadvantage (e.g. mussels dislodged). Animals therefore may either encourage or deter others from settling depending on the effect that will be created. Lastly, the environment the animal inhabits may preclude settling of larvae because, for example, it may be exposed to high turbulence or severe desiccation. Besides the advantage of space acquisition, the epibiont also benefits in other ways. It may benefit through protection by the host (Osman & Haugsness 1981) or food provision (sponge crab). A more subtle benefit to sessile epibionts is that they may avoid adverse conditions, which they otherwise would have succumbed to, because their motile hosts move away from harmful microhabitats.

This study focuses on a newly discovered species and genus of the family Sabellidae, which occupies the shells of various molluscs in a novel way. It has taken the colonisation of secondary space to the extreme, in that it has developed the ability to colonise the shell at the same time as the host animal deposits it. This type of early space acquisition is unusual, although similar behaviour is recorded for bryozoan and serpulid larvae encrusting the youngest portions of the fronds of *Laminaria,* thus ensuring the future availability of space to grow into (Stebbing 1972).

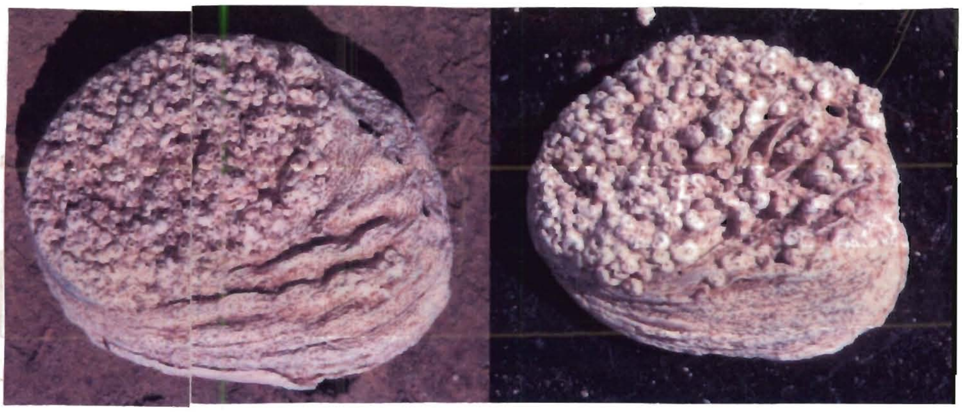

Figure 1: Heavy *Spirorbis* infestations end abruptly after the onset of sabellid infestations. The sabellids inhibit spirorbid settlement in some manner.

After settlement the sabellid can protect this space against the settling of other larvae by mechanical interference or even ingestion of larvae (Figure 1)(see also Woodin 1976). There is a distinct advantage of acquiring this space as it is afforded protection against predation and the environment and is ideally positioned for acquiring food. Only species such as sponges, which spread laterally, have been found successful at displacing the sabellid.

In the natural environment the sabellid can be found on a number of mollusc species. Most individuals of the abalone *Haliotis midae* are infested in small to moderate proportions without any noticeable effect on the host. However, in abalone farms, the worm can reach epidemic proportions, and is clearly harmful to the host as evidenced by reduced growth rates, weakened shells and, in severe cases, mortalities. In the natural environment, one may be tempted to regard the sabellid as a commensal, but following the original interpretation of the word as "one who eats at another's table" or "one who lives at another's expense" (Noble & Noble 1982), the worm clearly has characteristics of a parasite, especially since the worm appears to not have a free-living form. This parasite is of great concern to the abalone farmers who desire a means of eradicating what has become a serious pest.

CHAPTER 2 DESCRIPTION

2.1 HISTORY

While conducting growth trials at an abalone farm on the west coast of South Africa in 1994, it was noticed that one group of animals exhibited virtually zero growth, and experienced higher mortalities than other groups. A closer examination revealed that the shells of these animals were exceptionally weak and prone to breakage, which presumably lead to the mortalities. The shells also had slightly abnormal shapes, the characteristic fluting of the shell being absent. The weakening of the shell was associated with numerous tiny holes, occupied by a polychaete worm (Figures 2 & 3). Tiny, larval worms were also found infesting the growing edge of the shell (Figure 4). Initially it was thought that this was caused by *Polydora*, a documented shell borer in abalone (Blake & Evans 1973, Sato-Okoshi & Okoshi 1996), but closer examination of the worm suggested that it was the same animal that had been recently discovered in Californian abalone farms (Oakes & Fields 1996).

The worm is a sabellid polychaete, which has been named *Terebrasabella heterouncinata* and occupies a new genus in the family Sabellidae (Fitzhugh & Rouse 1999). It has caused severe damage to the abalone industry in California, and there has been some release of the sabellid into the environment around abalone farms (Culver & Kuris in prep.). A project was initiated in California to study the threat posed both to farms and the natural environment (Culver *et al* 1997), since the sabellid is not native to California (Lafferty & Kuris 1996).

A similar project was initiated in South Africa with the backing of the Abalone Farmer's Association of South Africa (AFASA) to assess the risk to abalone farms and to determine whether the polychaete is endemic to South Africa, and if so, its distribution in wild stocks.

The polychaete, with its branchial feeding crown of bipinnate radioles (commonly known as the fan), is quite clearly from the family Sabellidae. The only other family with bipinnate radioles are the Serpulidae, but they have a calcareous tube with a stalked operculum often present (Day 1967). The sabellid has a number of unusual features, which make it unique enough to warrant the introduction of a new genus (Fitzhugh & Rouse 1999). Even though there are other sabellid species that are associated with mollusc shells (Fitzhugh & Rouse 1999) and even one, *Caobangia,* which burrows within the shell (Jones 1969) the behaviour of this sabellid is still unprecedented. The way it has been able to exploit a totally new habitat niche in a highly effective way is probably the feature that makes it stand out most as being different. This subtle shift in mode of life has opened up many habitat possibilities for the species but, at the same time, placed it in danger of being limited by a high degree of specialization. This chapter will serve as a broad characterisation of the species by focusing on aspects of the anatomy of the worm and also outlining the extraordinary life cycle.

2.2 LIFE CYCLE

Like many other sabellids *Terebrasabella heterouncinata* is a simultaneous hermaphrodite, producing both eggs and sperm at the same time (pers obs, Fitzugh 1996). Outcross fertilisation is regarded as the rule for many simultaneously hermaphroditic animals (Heath 1977, Ghiselin 1969), to prevent the phenomenon of inbreeding depression of which there are many examples (Beaumont & Budd 1983, Charlesworth & Charlesworth 1987). However, inbreeding depression is not always found and there have been arguments defending the advantages of self-fertilisation under certain conditions (Jain 1976). Hsieh (1997) has argued the case for self-fertilisation for another species of sabellid polychaete, *Laonome albicingillum.* That *T. heterouncinata* is able to self-fertilise and produce viable reproductive offspring has been established recently (Finley *et al* 2000). The extent that this occurs in the wild or whether cross-fertilisation is preferred, and the methods of ensuring this, are unclear at present.

The adults brood a small number of large eggs at the base of their tubes until they have developed in this protected environment into viable larvae (Figure 5). This probably ensures a high survival of offspring and would place the sabellid amoung k-selected organisms (MacArthur & Wilson 1967). This strategy may be related to its small body size, since it is limited in the number of eggs it can produce and must thus ensure a high rate of survival of the offspring rather than release them into the plankton (Sella 1991). Brooding is, in fact frequently associated with hermaphroditism (Ghiselin 1969) and for this species, which inhabits a protective tube, it is logically the favourable strategy. The sexual reproductive modes available in the Sabellidae, range from broadcast spawning to intratubular brooding to ovoviviparity, with the only consistency being that all have lecithotrophic larvae (Rouse & Fitzhugh 1994). The egg production and development of larvae appear to be rapid. During an experiment it was observed that the turnaround time from larval settlement to the time that the resulting adults were able to produce larvae themselves was in the region of four months. Finley *et al* (2000) have since shown that the generation time is highly temperature dependent with faster development at higher temperatures.

The larvae emerge from the tube when they are ready to settle and are capable crawlers that move fairly rapidly over the substrate, presumably as an adaptation to avoid being dislodged by strong water currents (Figure 5 - #4). The larvae locomote in a gliding fashion using a band of cilia on the ventral side, which extends down their entire length (the neurotroch). They settle under the mantle of the growing edge of the shell of a suitable host species. Larvae seem to have the ability to detect suitable areas to settle, which is consistent with larval behaviour in other species (Wilson *et al* 1968, Morse *et al* 1979, Crisp *et al* 1984, Hadfield 1986). This is discussed in more detail in Chapter 3.

Settlement of large numbers of larvae on an abalone shell is illustrated in Figure 4. Once the larvae have inserted themselves beneath the mantle, they secrete a membranous tube and maintain an opening to the outside. This tube is then coated with freshly deposited shell of the host. Once settled, the larvae begin metamorphosing into juveniles. They lose their eyespots and sensory tentacles and produce a feeding crown. As they grow they develop more setigers and there is elongation of the body. There is also evidence that they enlarge the size of the tubes as they grow.

2.3 ADULT MORPHOLOGY

2.3.1 Light microscopy

Much of the structural anatomy was learnt by observing the worms under both dissecting and compound light microscopes. The technique used to observe them while still alive was as follows.

By crushing the shell, live worms were liberated and placed on a microscope slide along with a drop of seawater. The weight of a coverslip flattened the worm without killing it. Internal organs were clearly visible, including the contents of the gut. An added advantage was the ability to observe activities of the worm such as setal motion, food digestion and blood flow.

2.3.2 Preparation of electron microscope sections (see Cross 1987)

2.3.2.1 Scanning electron microscopy

Individual worms that were removed from the shell or small fragments of shell were used in preparations. Primary fixation of 24 hours was allowed in 0.2M gluteraldehyde in seawater buffer. The samples were rinsed in fresh buffer before being fixed for 10 minutes in 1 % buffered osmiumtetraoxide in seawater buffer. A seawater-freshwater dilution and ethanol dehydration series followed. The SEM samples were critical point dried and vacuum coated with gold paladium at the Electron Microscope Unit at the University of Cape Town. The specimens were viewed on a Cambridge S200 scanning electron microscope.

2.3.2.2 Transmission electron microscope

Individual worms were used for TEM preparations. The same procedure was followed as for the SEM sections except that the sections were fixed for an hour in 1 % buffered osmiumtetraoxide. After the ethanol series, the worms were transferred to 100 % acetone for an hour. The fixed specimens were then impregnated with resin (SPURRS) by increasing the concentration in stages. This was carried out over at least 48 hours. The resin blocks were then incubated at 60°C for 24 hours. The blocks were sectioned with a glass ultramicrotome and thick sections were viewed before cutting the thin sections. The sections were placed on wire grids, which were then stained with uranyl acetate and lead citrate. The specimens were viewed on an Etoshi H600 transmission electron microscope.

2.3.3 Observations

Adult worms occupy the shells of various gastropod species as described in Chapter 3. In certain circumstances the worms may reach extremely high densities in a single host shell, such as in abalone farms. In these situations the shell may become riddled with holes, each occupied by a single worm. Figures 2 and 3 show such a badly infested abalone shell. The sabellid feeding-crown can be seen emerging from its burrow in Figure 6. When particles are present in the water, as in this photograph, then water motion created by the feeding crown can be observed. The worms seem to have efficient particle filtration and selection ability. Studies on clusters of the sabellid *Eudistylia vancouveri* demonstrated up to 75 % reduction in particles in a single pass of water over the cluster (Merz 1984). Most of the particles taken were in the small size classes (3-6 μm). Dale (1957), however, concluded from his studies of a variety of sabellids in still water that they are less efficient than other suspension feeding invertebrates in terms of volume and kinds of particles retained. That study ignored the advantage gained by the ambient flow of water past the crown, which in nature is probably the norm, and the crown seems to be designed more as a trapping network than a current generator. Indeed the crown is orientated differently when there is an ambient current rather than in still water (Merz 1984). Compare this to a mussel that is forced to create a current to process-particle laden water, regardless of the presence of ambient currents or not.

The adult worms are themselves extremely small (1-2 mm length), but are more elongate with a greater number of setigers than the juveniles. When worms are removed from their burrows they have a characteristic curvature at their distal ends (see Figures 7 - 9), which presumably reflects the direction that their burrows have to follow to allow for growth, since the shell thickness becomes a limitation.

The worms are hermaphroditic, possessing both eggs and sperm simultaneously. The eggs at 200µm are fairly large relative to the adults (Figure 8) and each worm may harbour a number of eggs at various stages of development at the base of the tube.

In live worms, the blood can be observed coursing through the major blood vessels, which are visible through the skin of the worm. A large dorsal and ventral blood vessel can be seen in Figure 9. In addition, a ring of blood is present around the base of the feeding crown (see Figure 8), which appears to join the blood of the dorsal and ventral vessels. The branchiole is also the region for gaseous exchange and indeed the blood vessels can be seen in the branches of the radioles (Figure 11&13)

The feeding crown consists of two branchial lobes (Figure 9), which have two palps in the centre which are presumably involved in food selection (Figure 10). The feeding tentacles are covered by cilia, which work together in waves (Figure 13). When dead, the above is not apparent but it is clear from Figure 12 that the cilia extend outwards in either direction from a central groove in the middle of the tentacle. These cilia create strong feeding currents, which draw the particle-laden water into the centre of the crown. Many particles are also rejected and can be seen shooting out from the centre of the crown. Some of these particles are not taken into the mouth but diverted to the side of the worm and deposited around the entrance of the tube, where they would be combined with mucus to form an extension to the existing burrow. These extensions are only seen in abalone that are kept individually, since the movement of other individuals dislodges the tubes. The only function of the extended tubes may be to provide space for growth but this appears unnecessary and may be a behavioural remnant from its evolutionary history, since this function is vital in other tube worm species.

Food particles, of the correct size-range, pass into the mouth and then move rapidly down the oesophagous and become concentrated in the gut (see Figure 9). The digestive tract is lined with cilia (see Figures 14 - 17) responsible for moving food particles around during the digestive process. It has also been observed that the worms are able to void the gut rapidly of any noxious particles in the same manner. The presence of microvilli for nutrient absorption can be observed in Figure 17.

Often present within the gut of most individuals are tiny unicellular organisms, which can be seen clearly at high power using an oil immersion lens. The organisms have a striated (ribbed) body wall with a large nucleus visible through the body wall and are only capable of writhing movements. They have been identified as probably being acephalin gregarines (A. Kuris, pers comm., Barnes 1980). There may be as many as 7-9 within the gut of a single worm, but do not seem to pose a serious threat to their host. An individual is visible within the gut in Figure 18 and a TEM section of the same in Figure 19.

The worm has a number of pairs of setae which are present on some of the segments of the body (Figures 7 & 20-25). The first 5 pairs are present from an early development stage as they can also be seen in the larvae (see Figure 26). These are the largest setae and are possibly used for rapid retraction into the burrow. Each setal group consists of a number of setae of various sizes (see Figure 22). The shape of the notosetae on the dorsal side are elongated and tapered (see Figure 20), whereas the neurosetae on the ventral side are shorter and have a toothlike projection at the end (Figure 21). These setae are termed uncuni, defined as being sharp and claw-liked, often bearing teeth (Day 1967). Posteriorly there are setigers bearing more uncuni which have a different shape and have many teeth (Figures 24 & 25). With a fluorescence microscope, they fluoresce bright yellow, which makes viewing much easier (Figure 25). The presence of different types of uncuni (acicular anteriorly and avicular posteriorly) within the same body region is peculiar to this species, hence the species name - *heterouncinata* (Fitzhugh & Rouse 1999). As these teeth face forward, their function could be to prevent the worm being dislodged from its burrow. The importance of the orientation of hooked setae in resisting removal has been demonstrated across a number of tube dwelling taxa (Woodin & Merz 1987). The lightweight honeycomb pattern of chitin within the setae provides rigidity and is visible in cross-section (Figure 23). At the basal region of the worm there are also a number of smaller setae which are perhaps used for grip at the base of the tube or for manipulation of the stored eggs. A faecal groove, which is lined with a large band of cilia, runs the length of the worm on the dorsal side. It is used to transport faeces away from the anus to the exterior of the burrow, a process that is essential considering the brooding habit.

2.4 LARVAL MORPHOLOGY

The eggs and larvae are orange in colour and are large (200µm and 500µm respectively) relative to the adults (1200-1500µm). Various stages of development can be seen in Figures 5 & 26. The motile sperm was observed when the body wall was broken and the sperm ejected. A TEM section that appears to be through the region of sperm storage is shown in Figure 27. The final stage larvae before emerging from the burrow are approximately 500 µm in length with 5 pairs of setae on the sides of the body and two dark eyespots (Figure 28). A closer view of the head region shows the eyespots and also numerous "whiskers" in the front which are presumably important for sensory purposes and enables the larvae to target the correct area for settlement (Figure 29). The neurotroch is a broad cilial band on the ventral side of the body, which is used for locomotion (Figure 26). The larva has a large reserve of nutrients, which can be seen in Figure 28. This energy is probably needed during the search for a suitable settlement area, but also in addition for metamorphosis and development of the feeding crown that is required for access of food for further growth and development.

2.5 SHELL AND BURROW MORPHOLOGY

Newly settled larvae are quickly covered by shell, deposited by the host. This can be seen in Figure 30. Day *et al* (2000) showed that in *Haliotis rufescens* the shell that covers the larvae was typically nacreous with thick layers of aragonite laid down at a much faster rate than normal synthesis. There were also blocks of calcite laid down at the edges of the larval tubes. This process is disruptive to normal shell extension, which follows a typical sequence of aragonite deposition (the nacreous layers) laid down on a protein-calcite matrix (the prismatic layers) (Hawkes *et al* 1996, Fritz *et al* 1994, Erasmus *et al* 1994). Thus, the nacreous layers normally follows behind a prismatic front at the growing edge, which serves as a template. Thus, the presence of thick nacre at the growing edge is unusual and premature and disruptive to normal growth. That a similar situation exists for *H. midae* is supported by these photographs, where the nacreous layers are clear compared to opaque carbonate layers. The insides of worm burrows are lined with a proteinaceous sheath, which can be observed in SEM preparations. It appears as if the sabellid alters the direction of the burrow expansion to avoid the mantle and other burrows. The shell of a severely infested host becomes riddled with holes as seen in Figure 32 C. The perforations severely weaken the integrity of the shell.

Figures 33 and 34 show evidence of burrow enlargement: firstly the extension of a burrow laterally to break through to a neighbouring burrow and secondly the extension of the burrow vertically to break through to the mantle cavity of the abalone. In this case there is a more typical wound response by the abalone of an initial patch of conchiolin, which lays the basis for further aragonite deposition (Hawkes *et al* 1996, Fritz *et al* 1994).

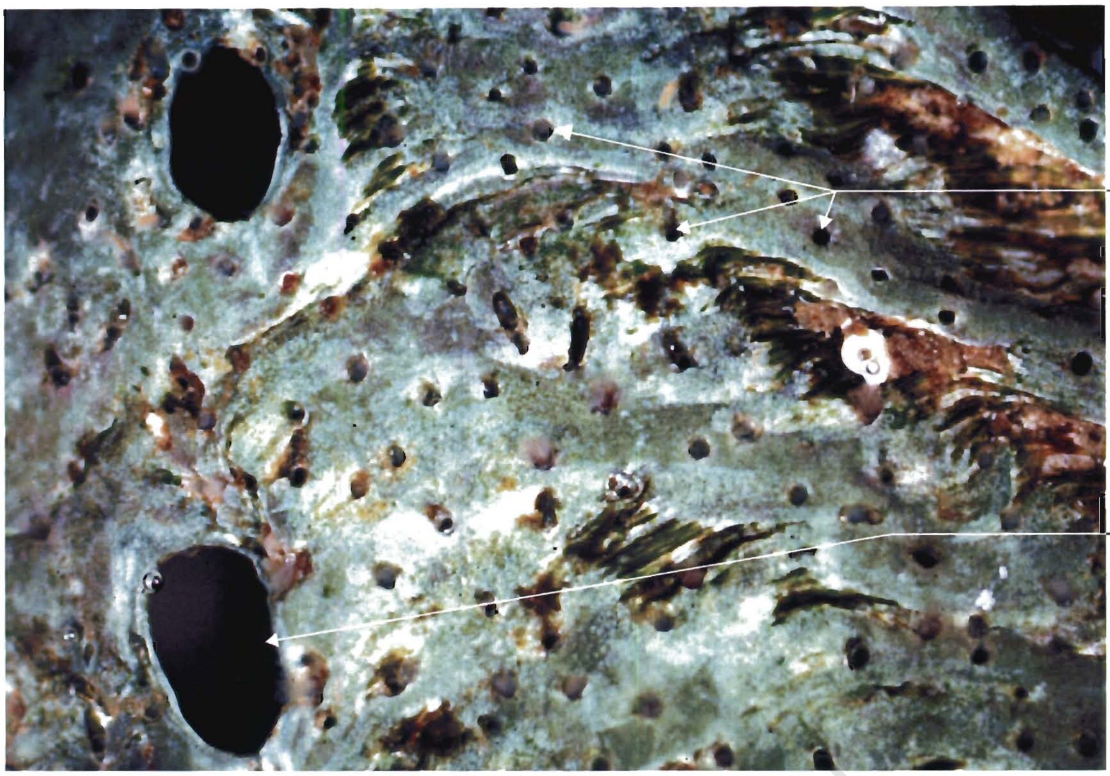

Figure 2: View of the outside of a shucked abalone shell (with respiratory pores) to show the numerous holes caused by the sabellids.

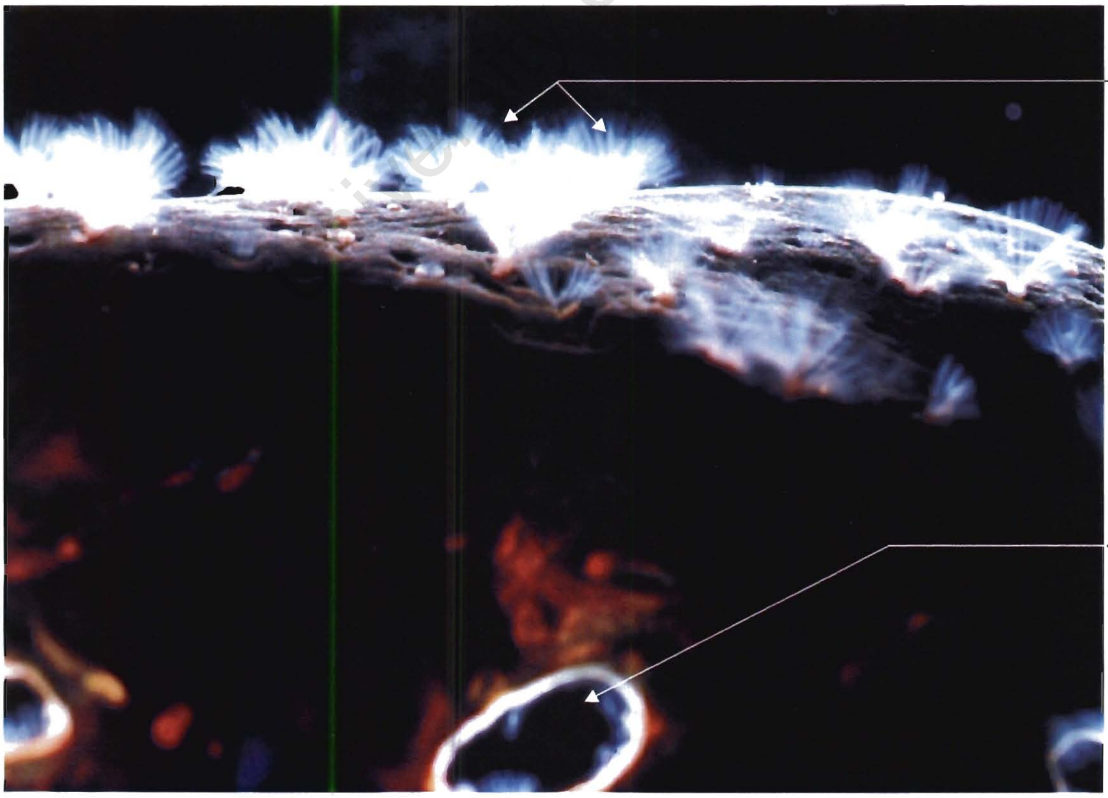

Figure 3: Same view of abalone shell as above with sabellid feeding crowns emerged in feeding posture. (MAG 40x)

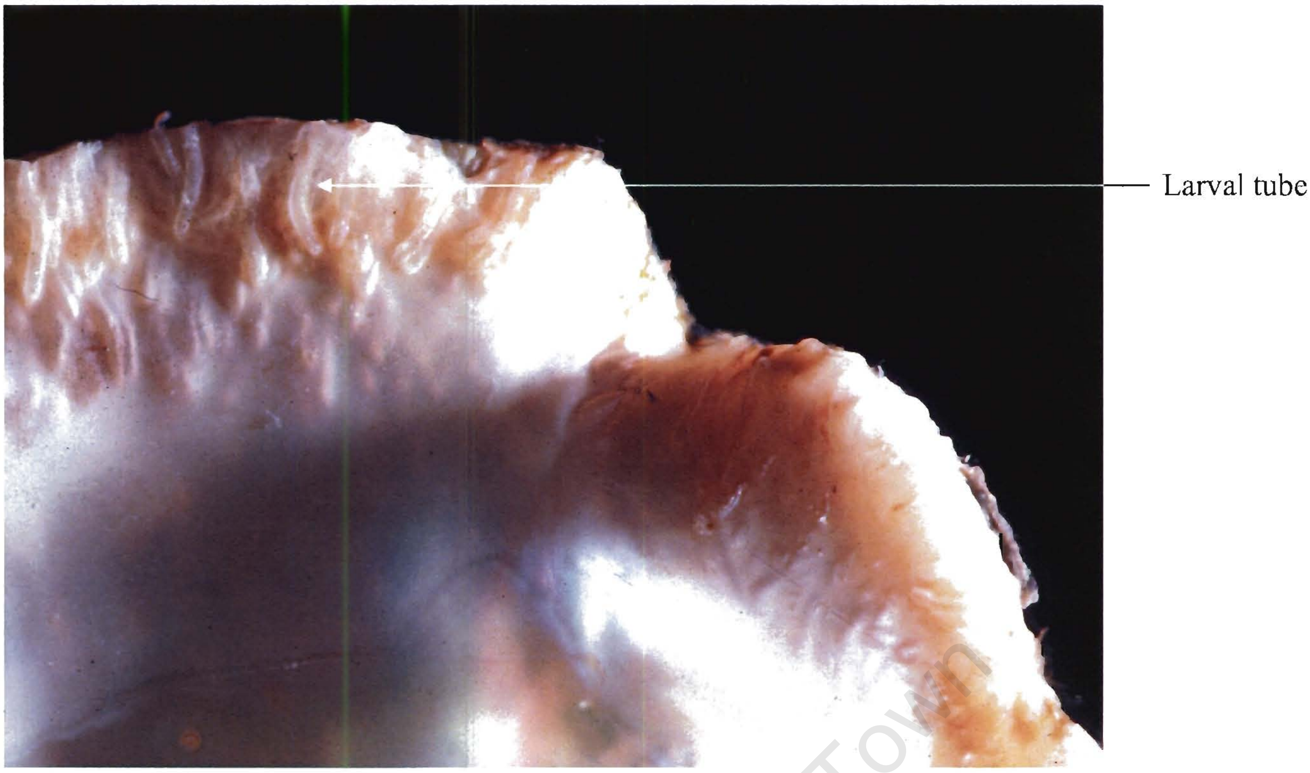

Figure 4: View of the leading edge of a shucked abalone shell, to show the freshly settled sabellid larvae, which are present in extremely high densities. The weakening and abnormal growth of the shell is apparent.

Figure 5: Stages of the development of sabellid larvae from 1 - egg to 4 - motile pre-settlement larva.

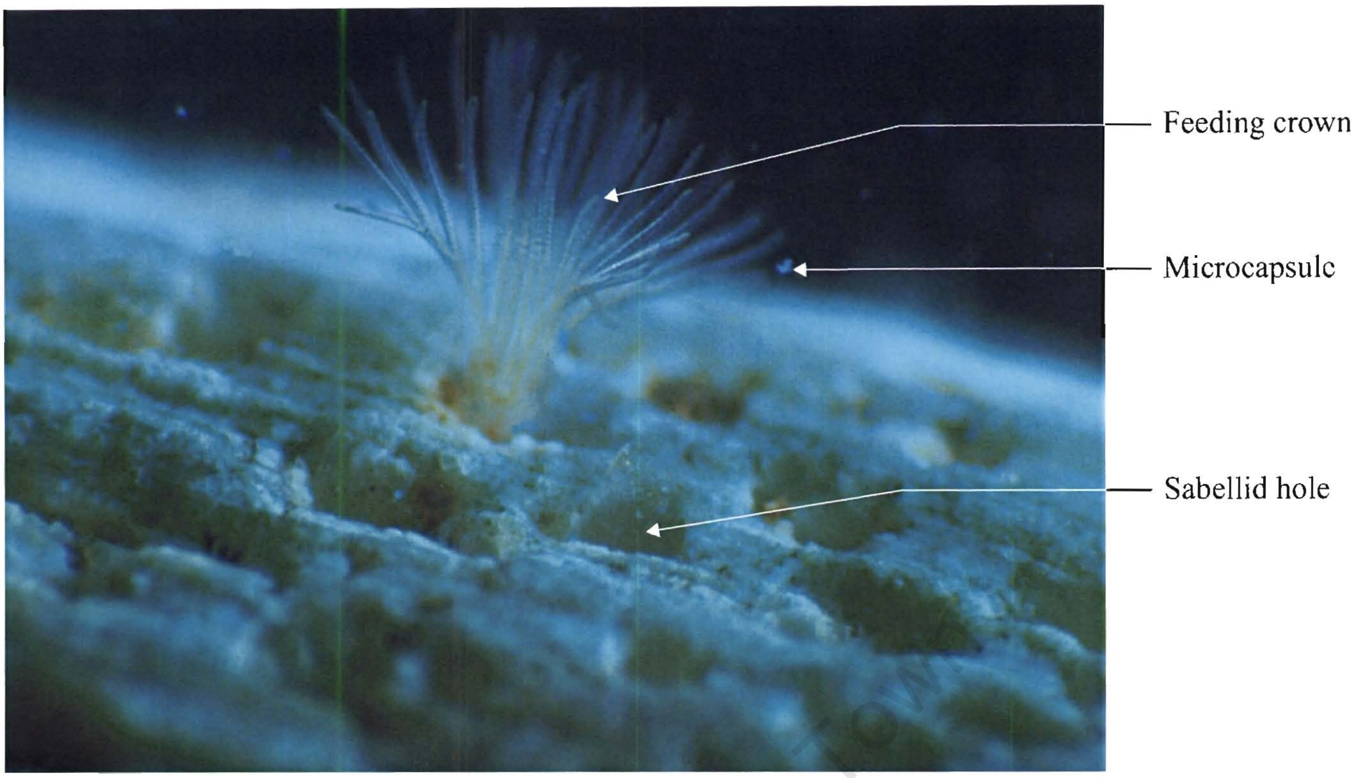

Figure 6: Magnified view of a single sabellid with crown emerging out of its hole in the abalone shell. (MAG. 100 x)

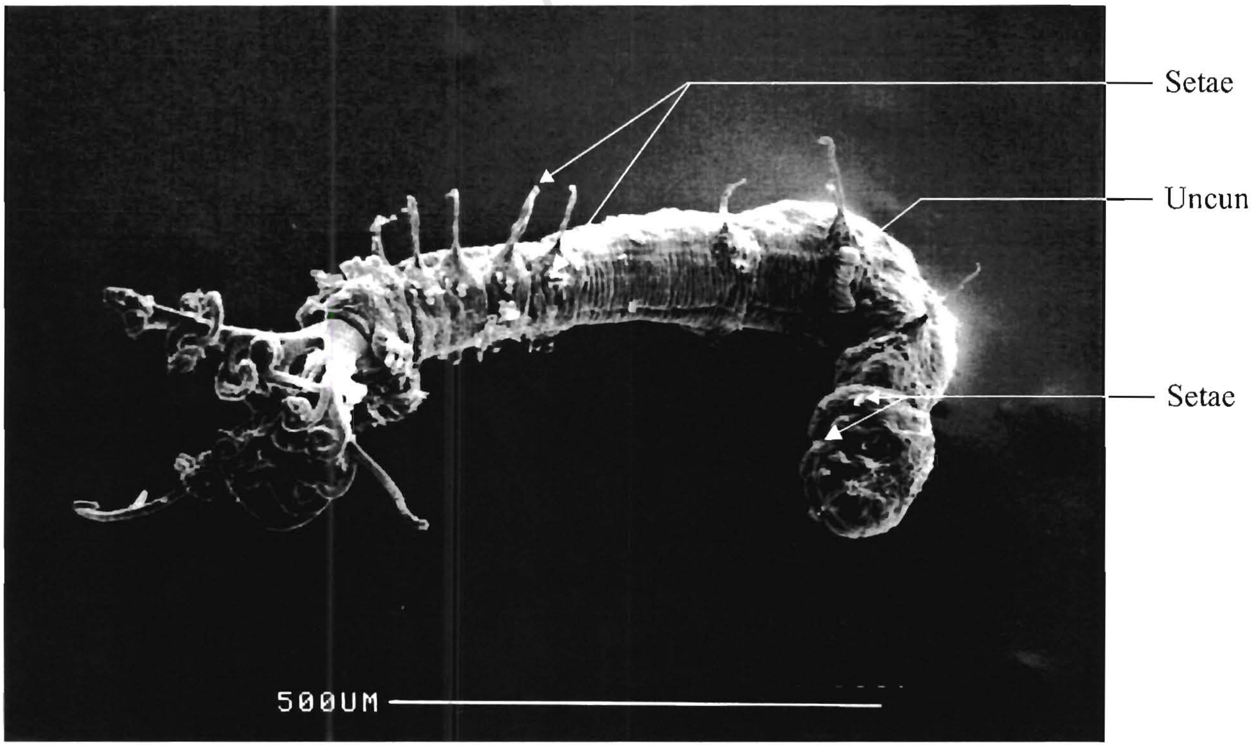

Figure 7: Scanning electron microgram of an adult sabellid worm. Note that during the SEM preparation process some shrinkage occurs.

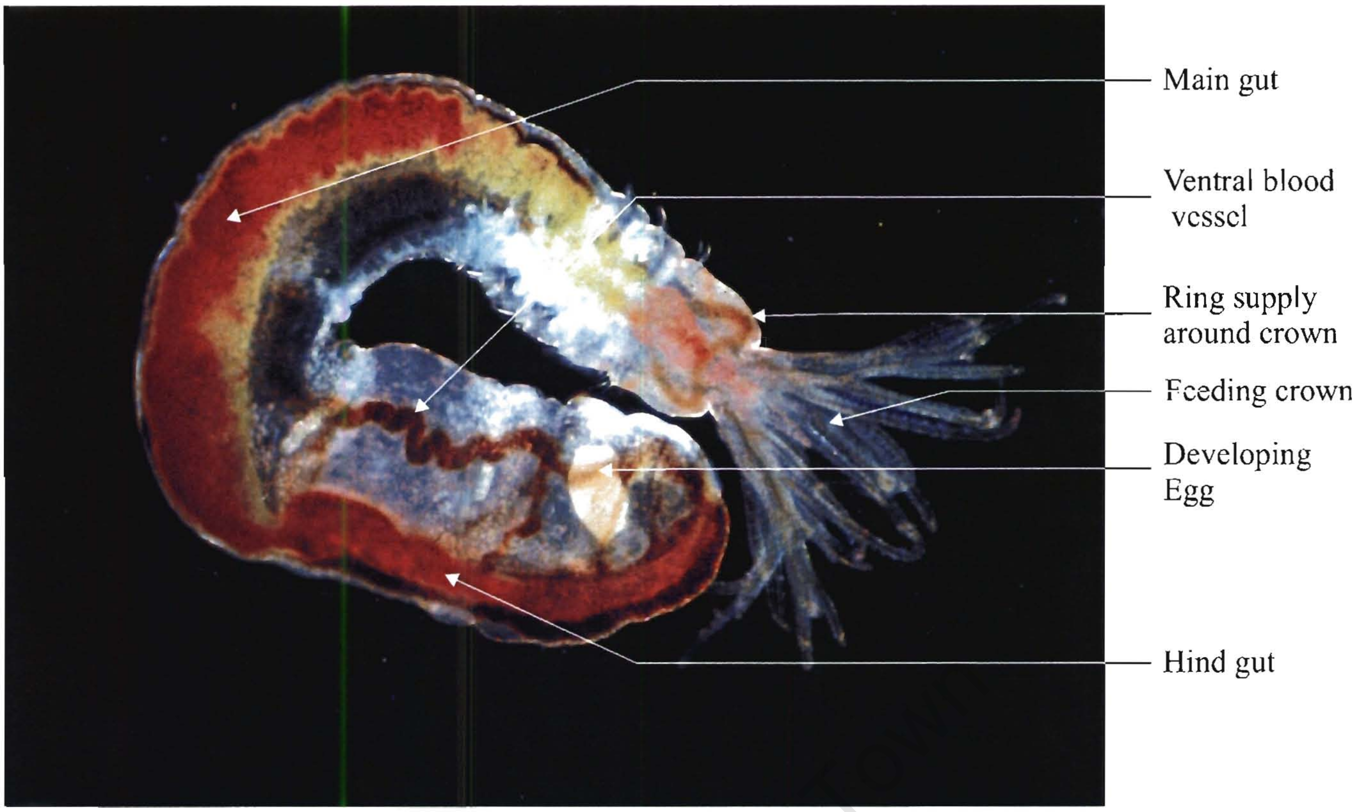

Figure 8: Whole mount of an adult sabellid viewed with compound microscope with dark field. The gut has been injected with a red dye solution. (MAG 100x)

Figure 9: Whole mount of an adult sabellid viewed with compound microscope using bright field. The gut contains blue pigmented microcapsules. (MAG 100x)

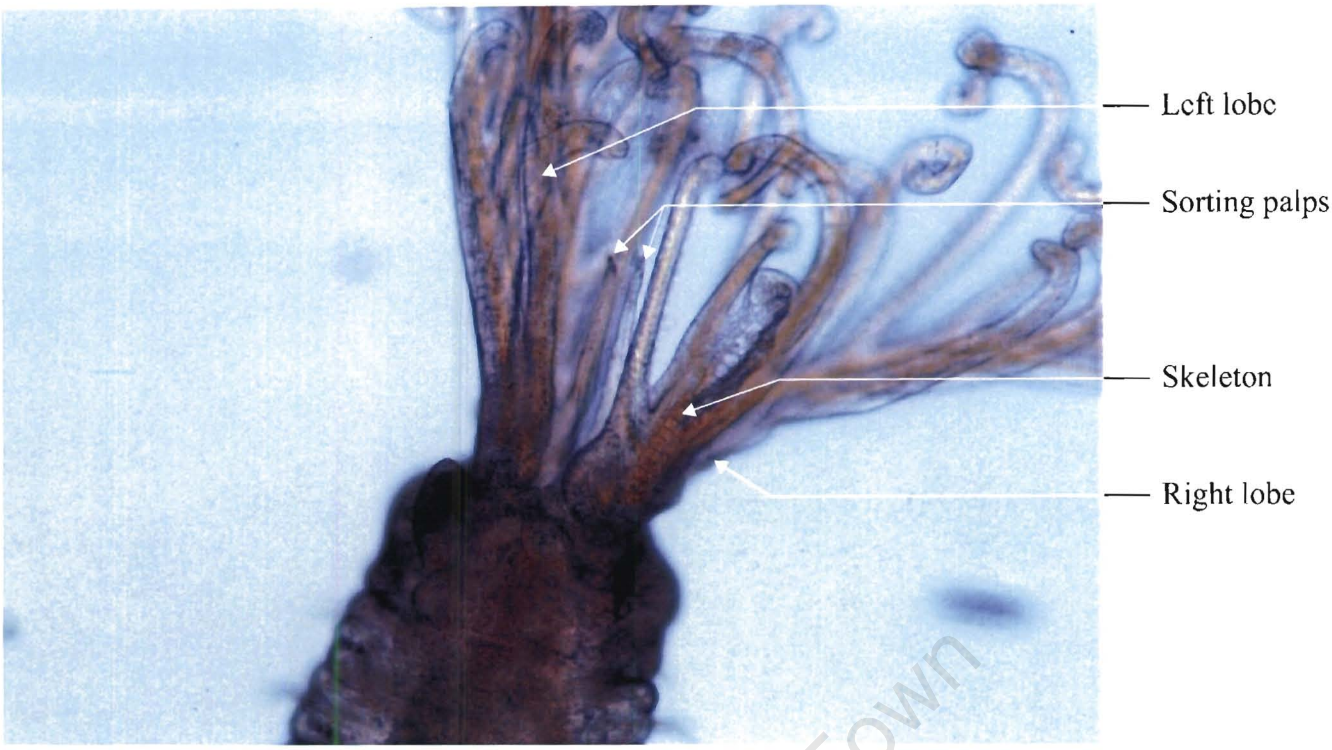

Figure 10: Close up of the feeding crown region of an adult sabellid. The crown is separated into two lobes with two central sorting palps. (MAG 250x)

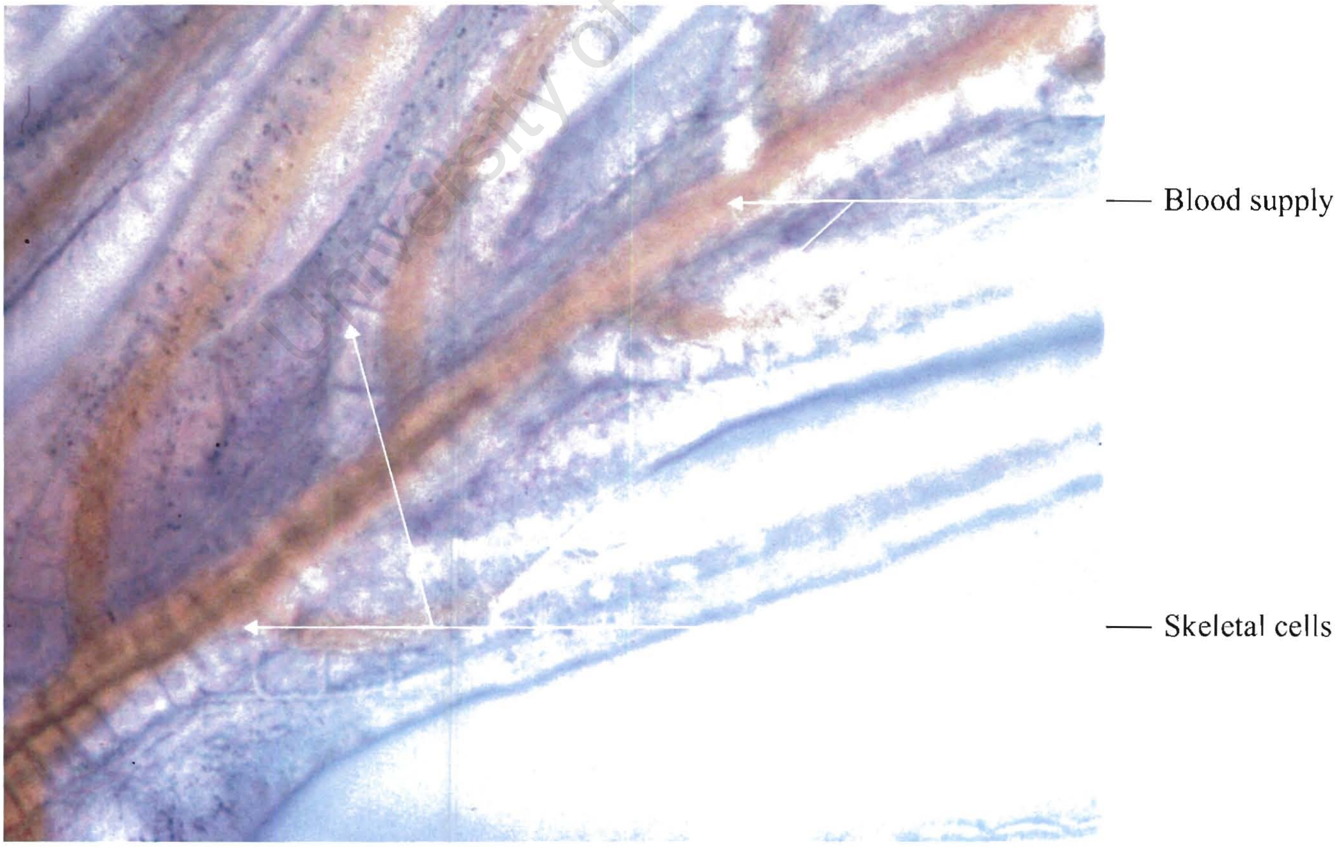

Figure 11: Further magnification of the feeding crown to show tentacles with their blood supply and cartilagenous skeletal structure. (MAG 400x)

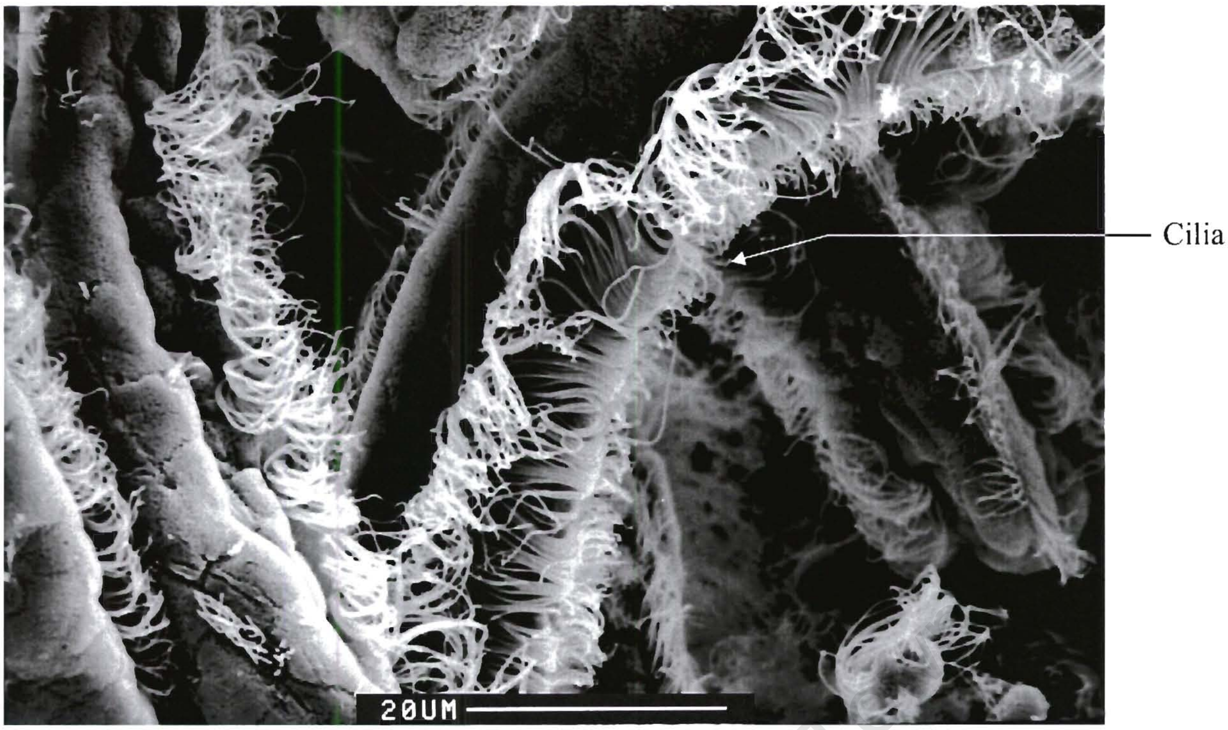

Figure 12: Scanning electron micrograph of the feeding crown of an adult sabellid to show the individual cilia.

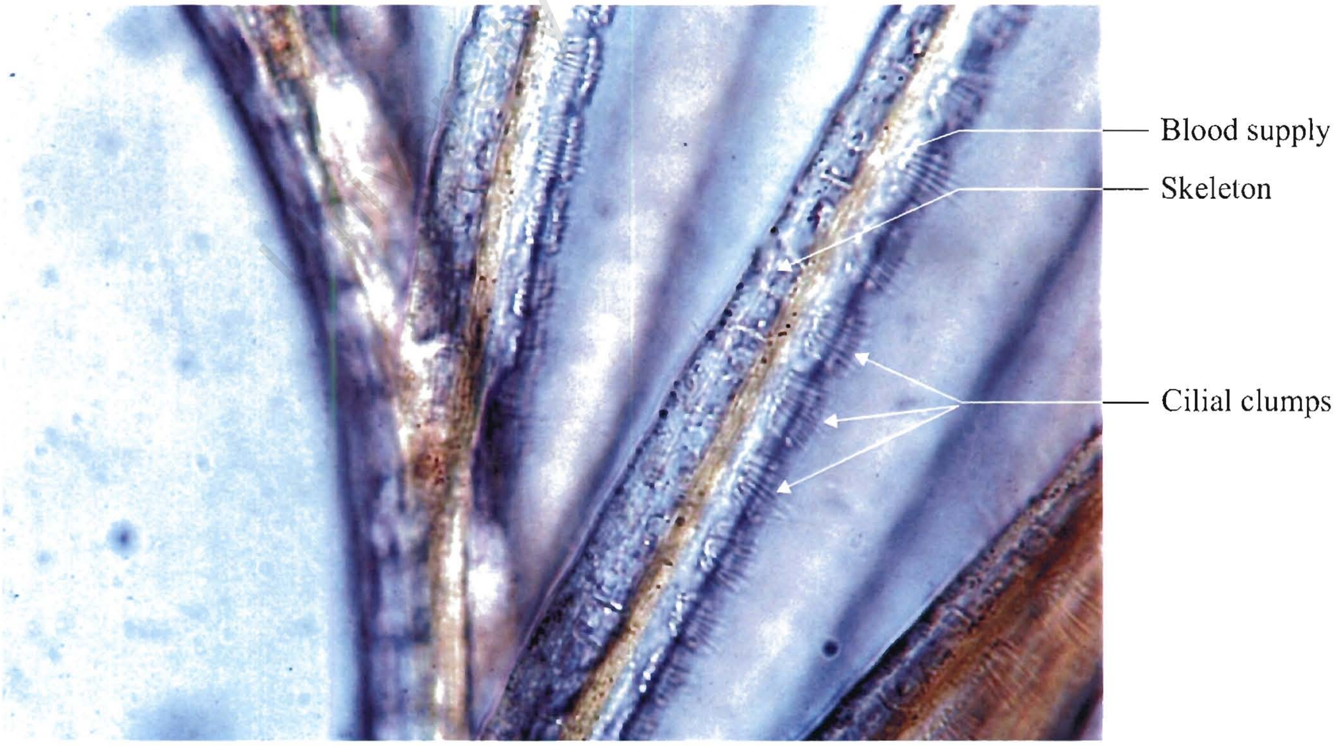

Figure 13: Further magnification of the feeding crown to show individual tentacles and cilia which are work together in a wave motion. (MAG 400x)

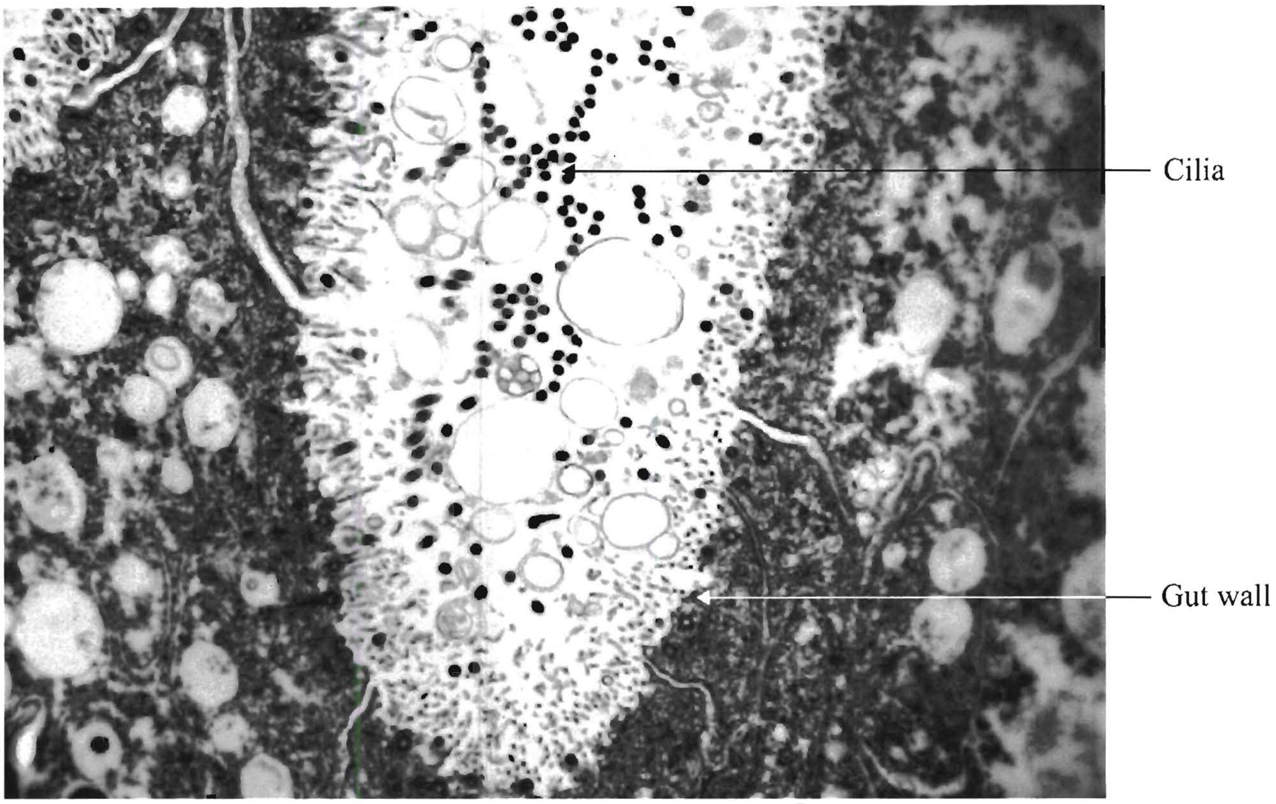

Figure 14: Transmission electron microgram of a section through a branch of the gut of an adult sabellid. The sabellid had been fed liposomes which are visible in the gut. (MAG. 5000 x)

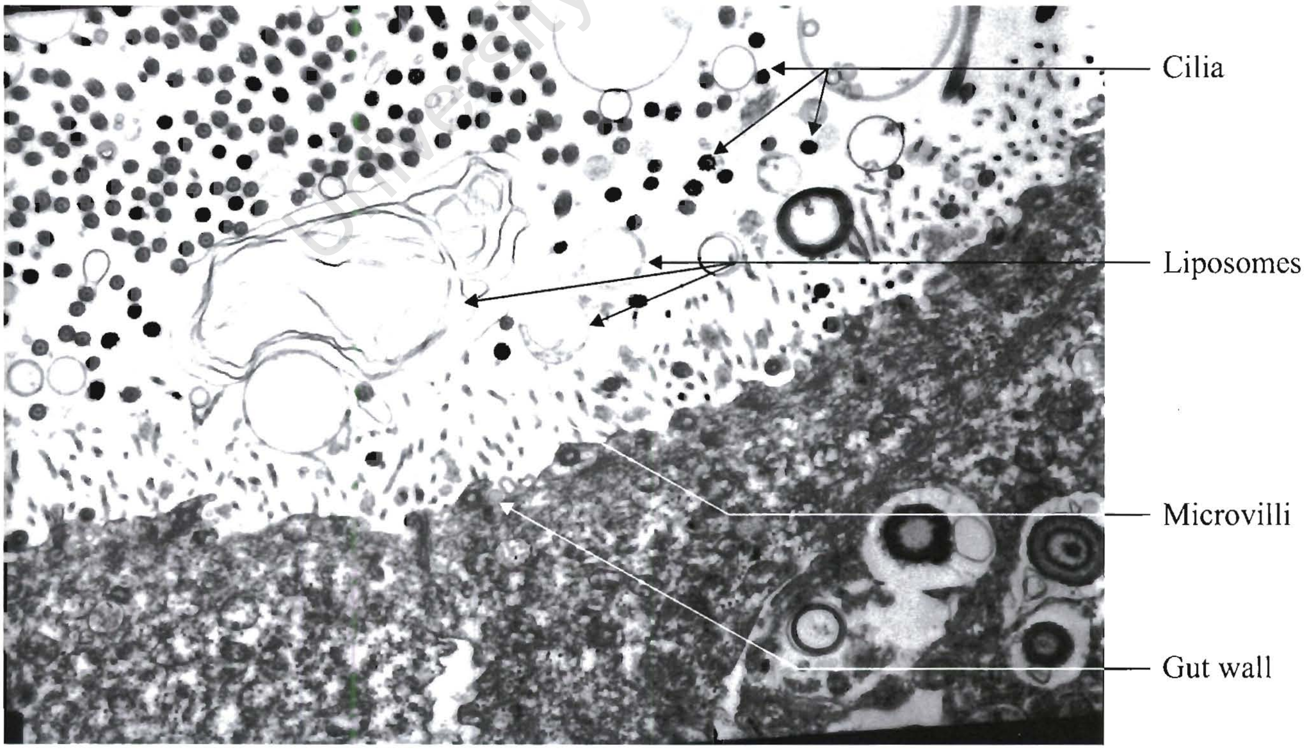

Figure 15: Transmission electron microgram of the gut wall region of an adult sabellid. Numerous cilia and liposomes are visible. (MAG 7300 x)

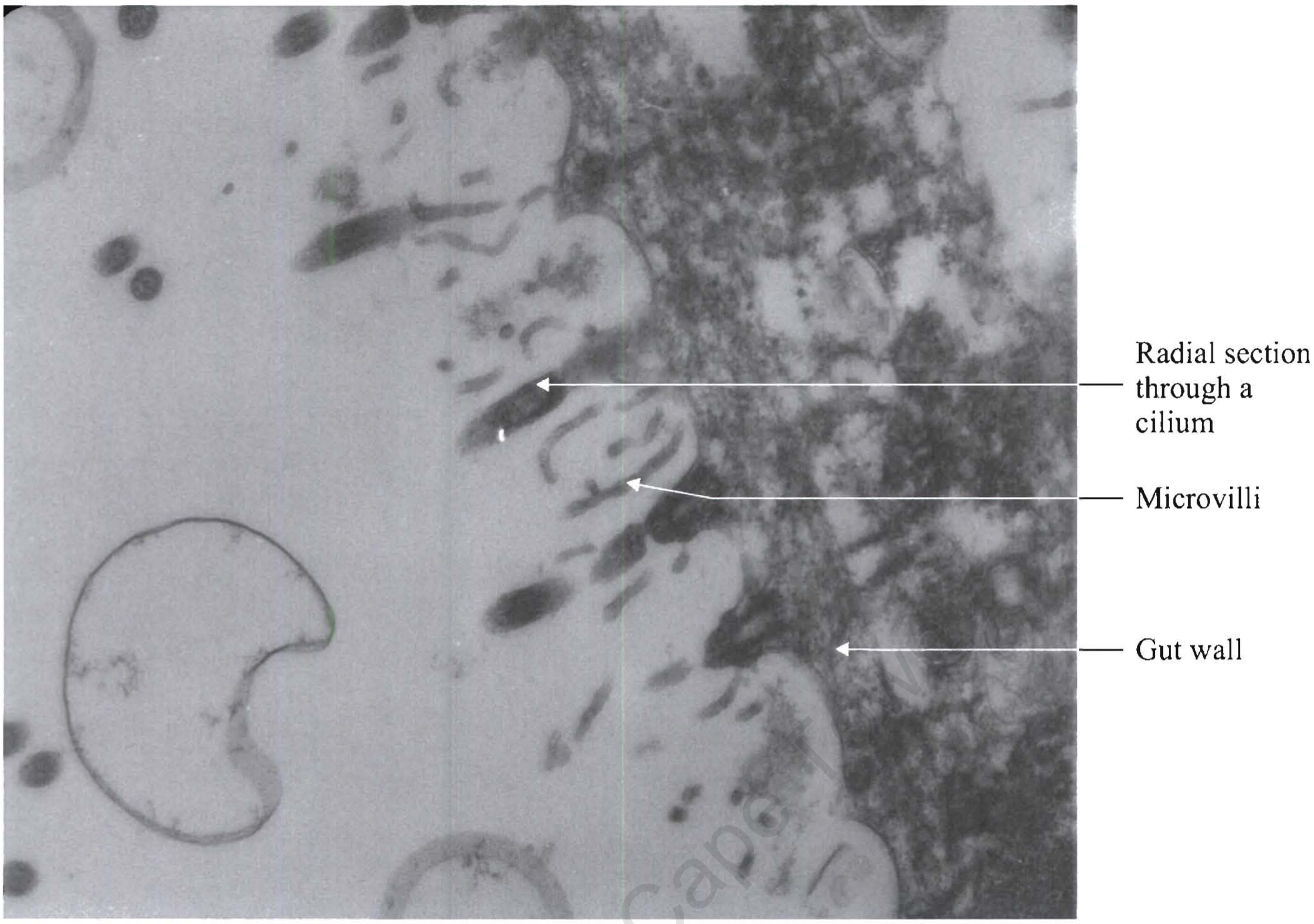

Figure 16: Higher magnification of the gut wall of an adult sabellid. The bases of cilia are visible and digestive microvilli are present between the cilia. (MAG 30 000 x)

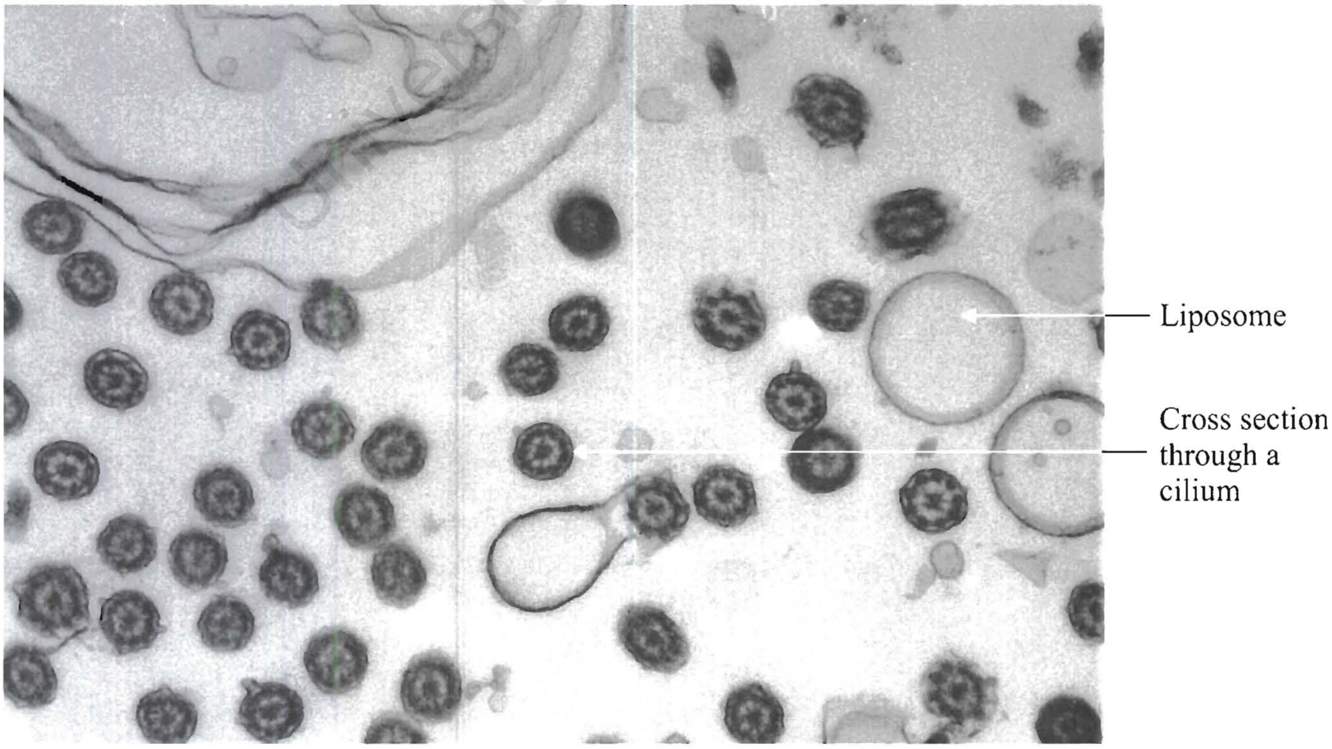

Figure 17: Transmission electron microgram of the gut region of an adult sabellid. The cross sections of the cilia show the characteristic 2 central surrounded by 9 outer fibrils. (MAG 30 000 x)

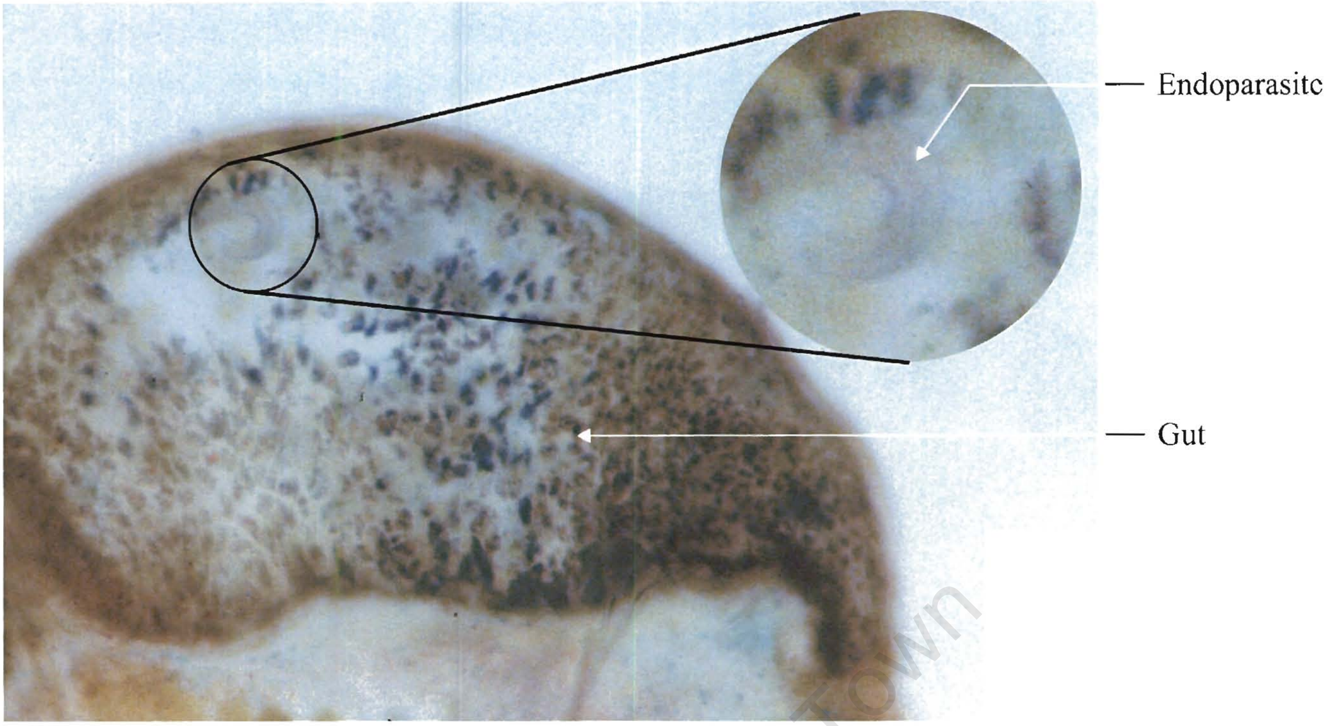

Figure 18: Portion of the gut of a sabellid worm to show the presence of a gut endoparasite. The parasite is shown magnified in top right of photograph. (MAG 250 x)

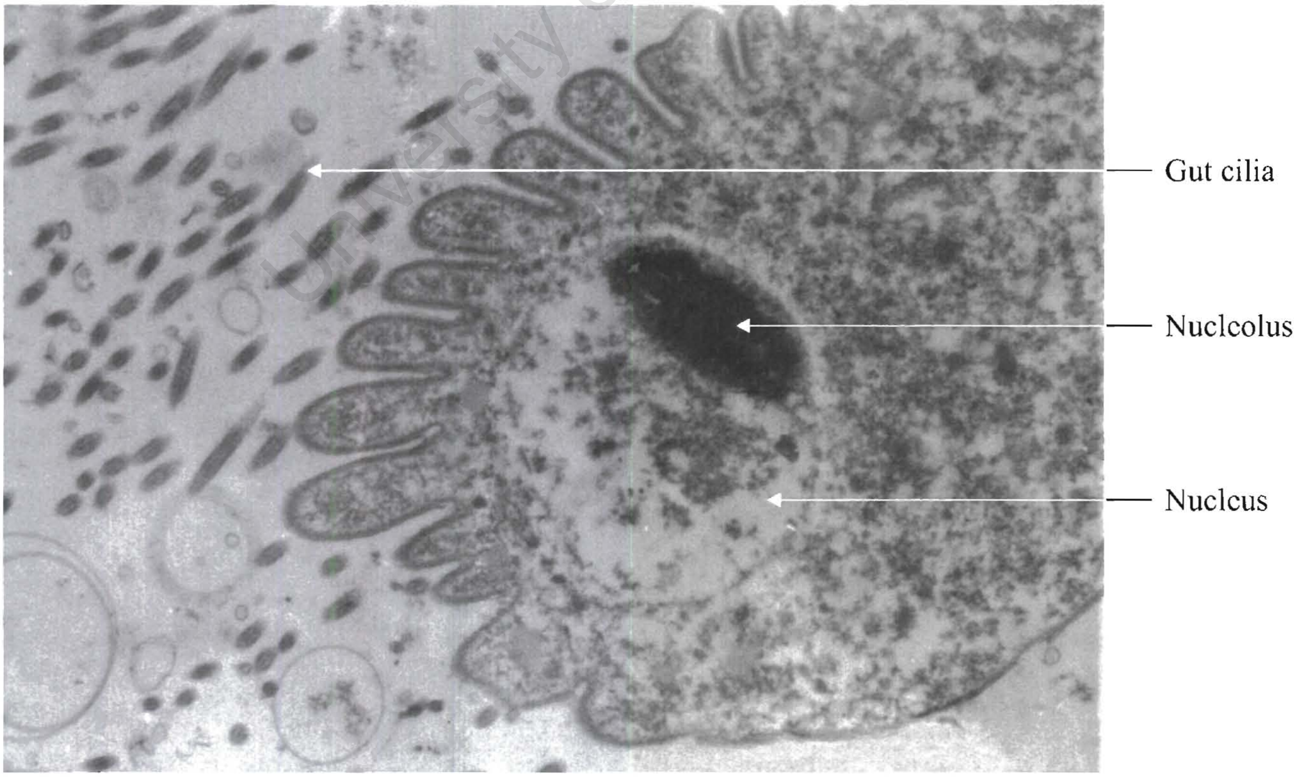

Figure 19: Transmission electron microgram of the gut of a sabellid to show a section through an endoparasitic, encephalin gregarine. (MAG 5000 x)

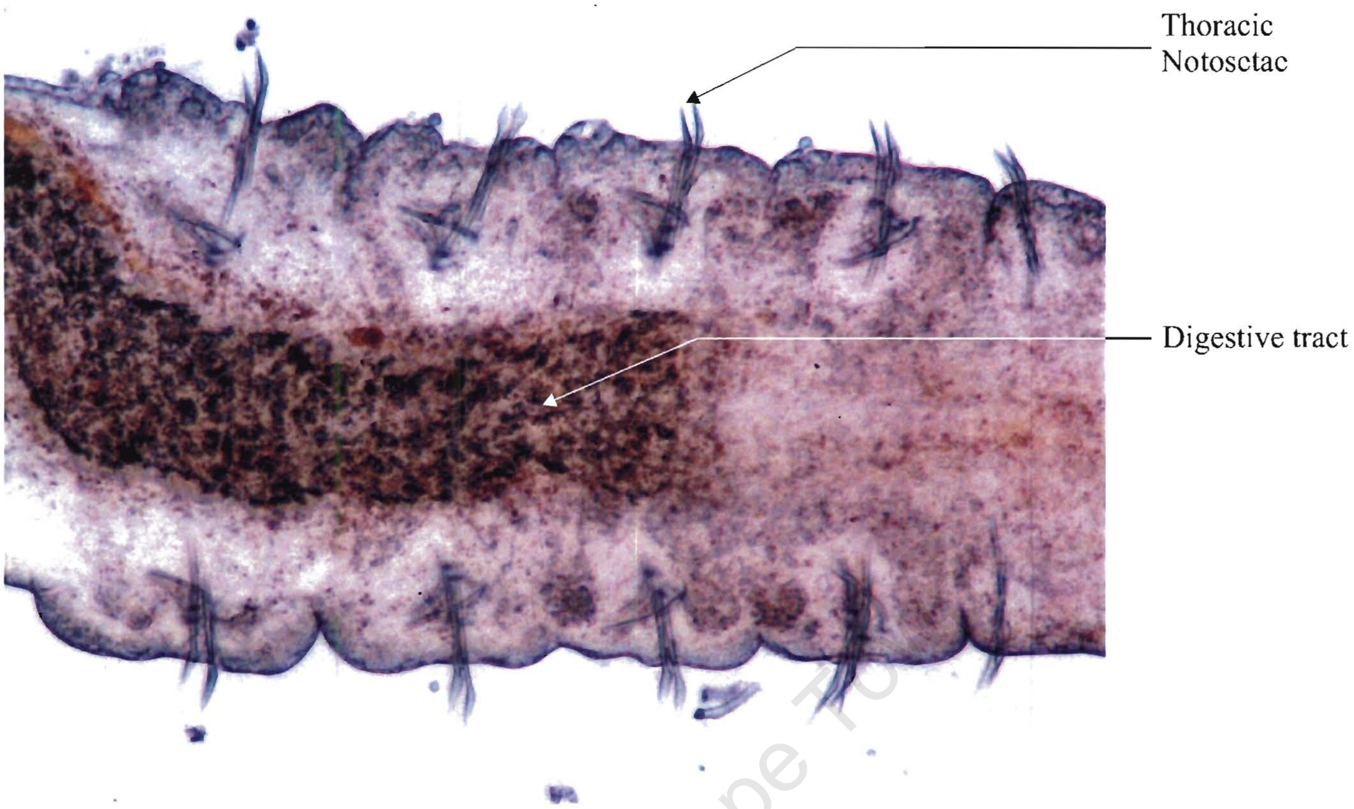

Figure 20: Dorsal view of the anterior five segments of adult sabellid to show the position and relative size of the setae. (MAG. 250 x)

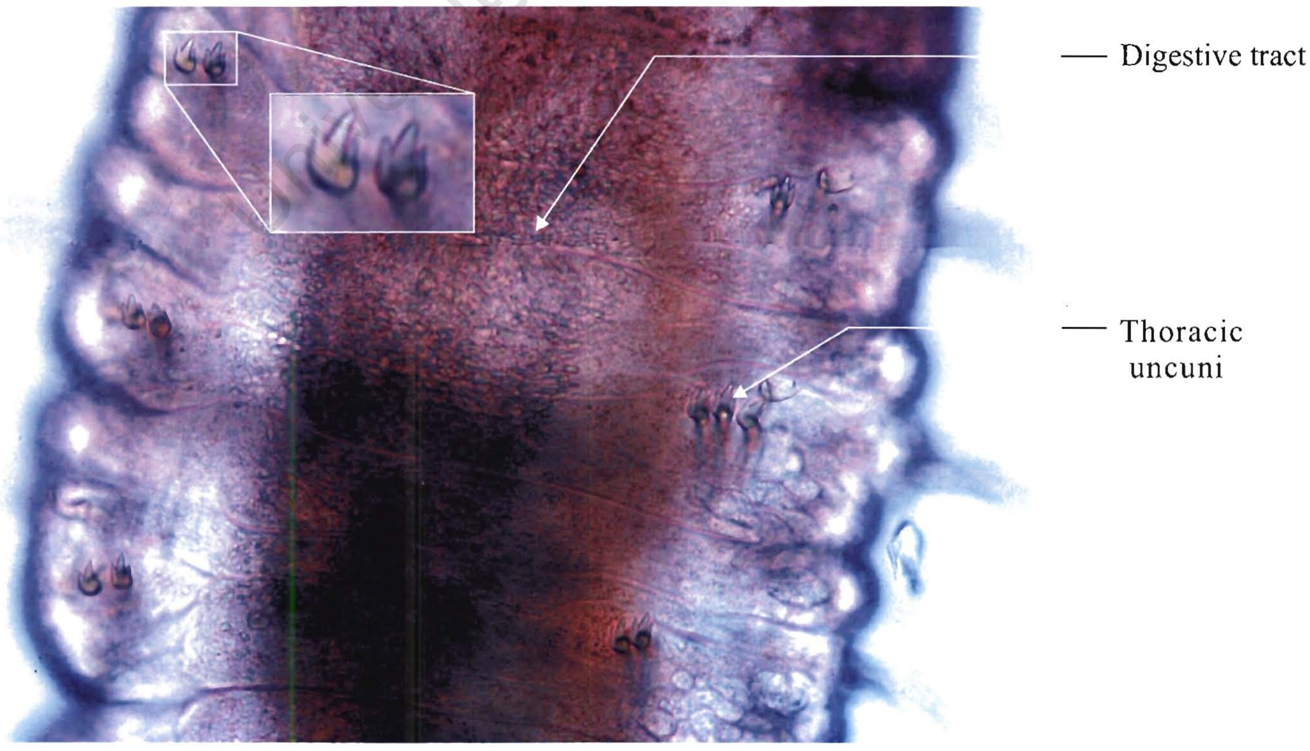

Figure 21: Ventral view of the anterior five segments of adult sabellid to show the position and relative size of the setae. A magnified inset of the hooked uncuni is included. (MAG. 250 x)

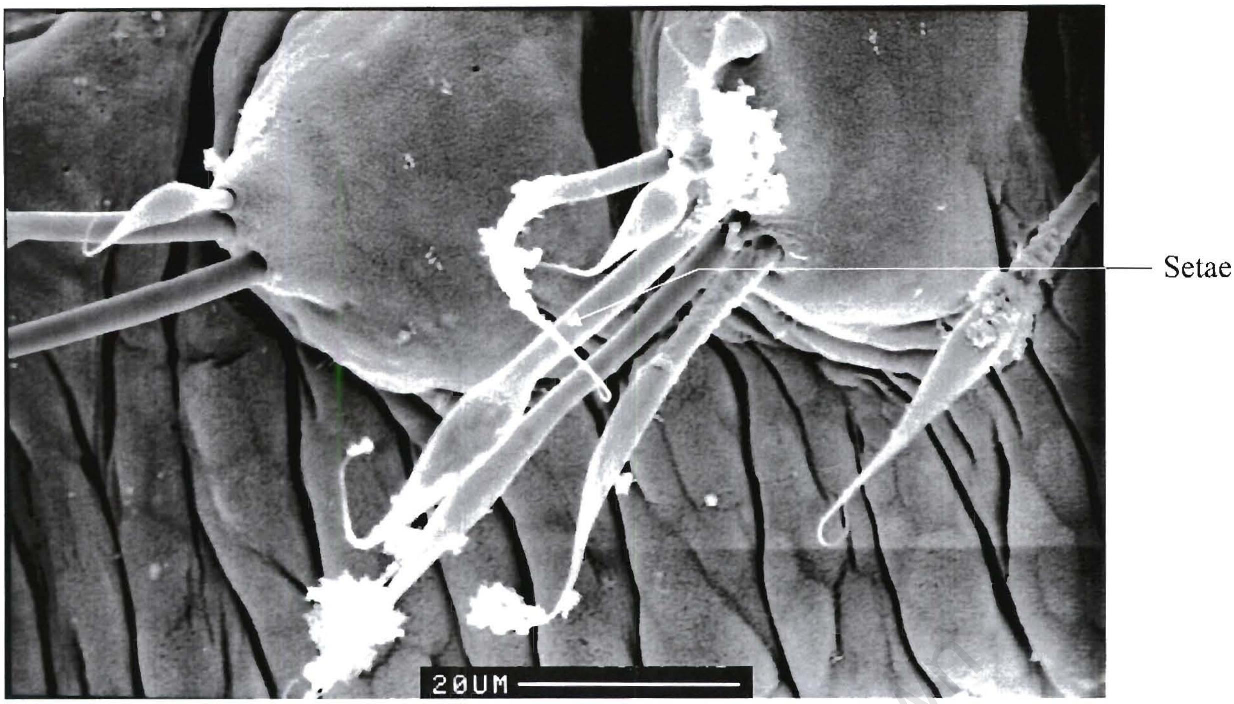

Figure 22: A scanning electron microgram of thoracic setigerous lobes from the anterior segments of the worm..

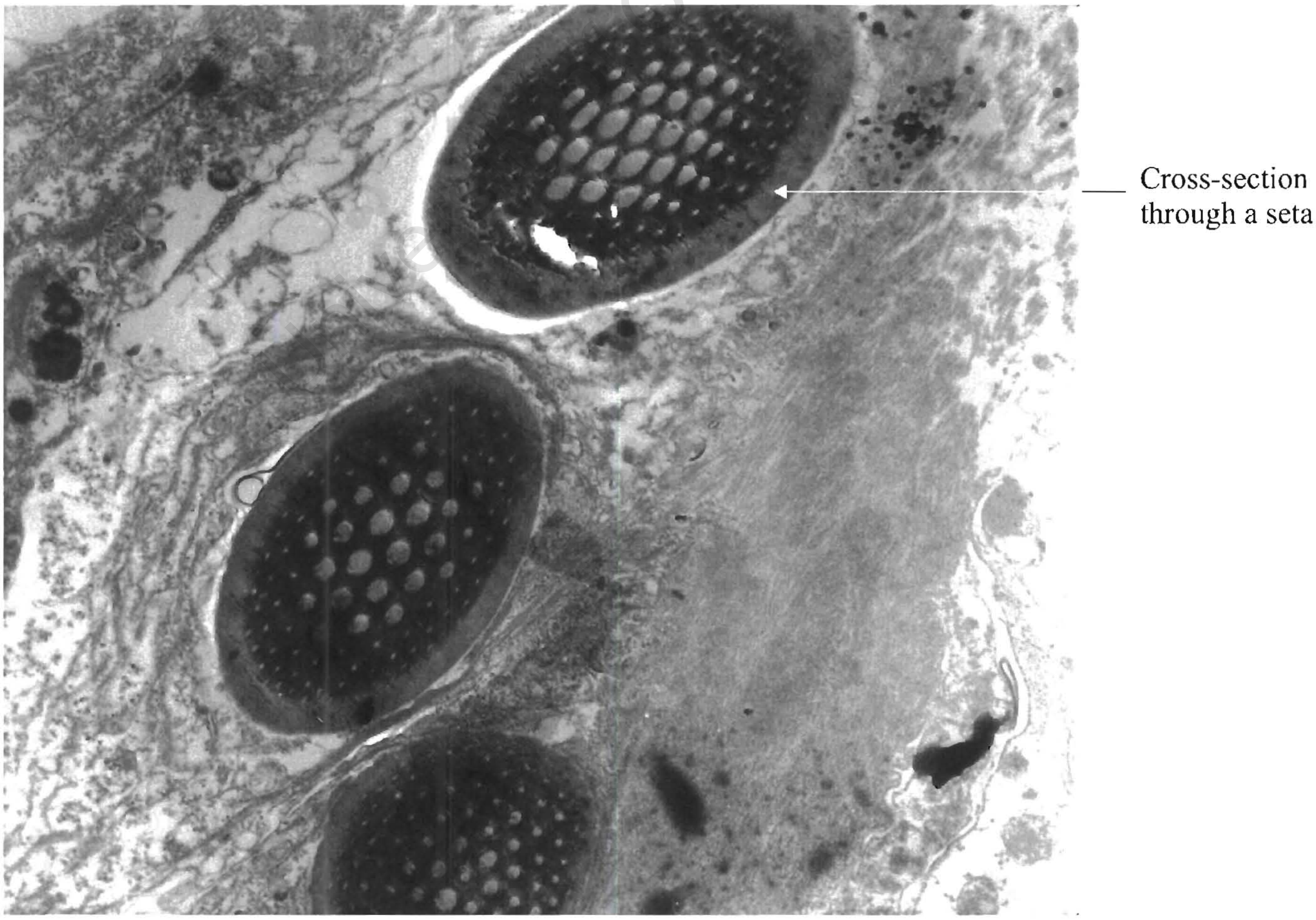

Figure 23: Transmission electron microgram of a section through three setae. A honeycomb structure provides strength while maintaining light weight. (MAG. 5500 ×)

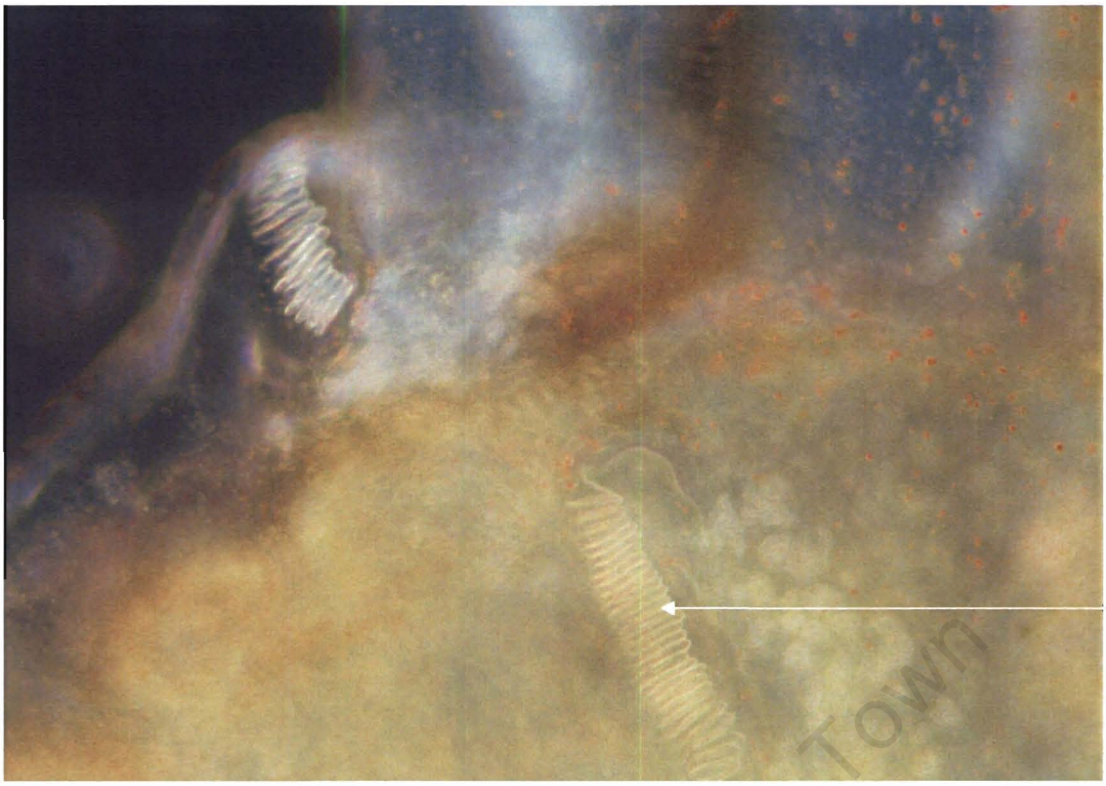

Figure 24: Magnified view of the uncuni on a posterior setiger of an adult sabellid. Note the flecks of pigmentation in the skin too. (MAG 400x)

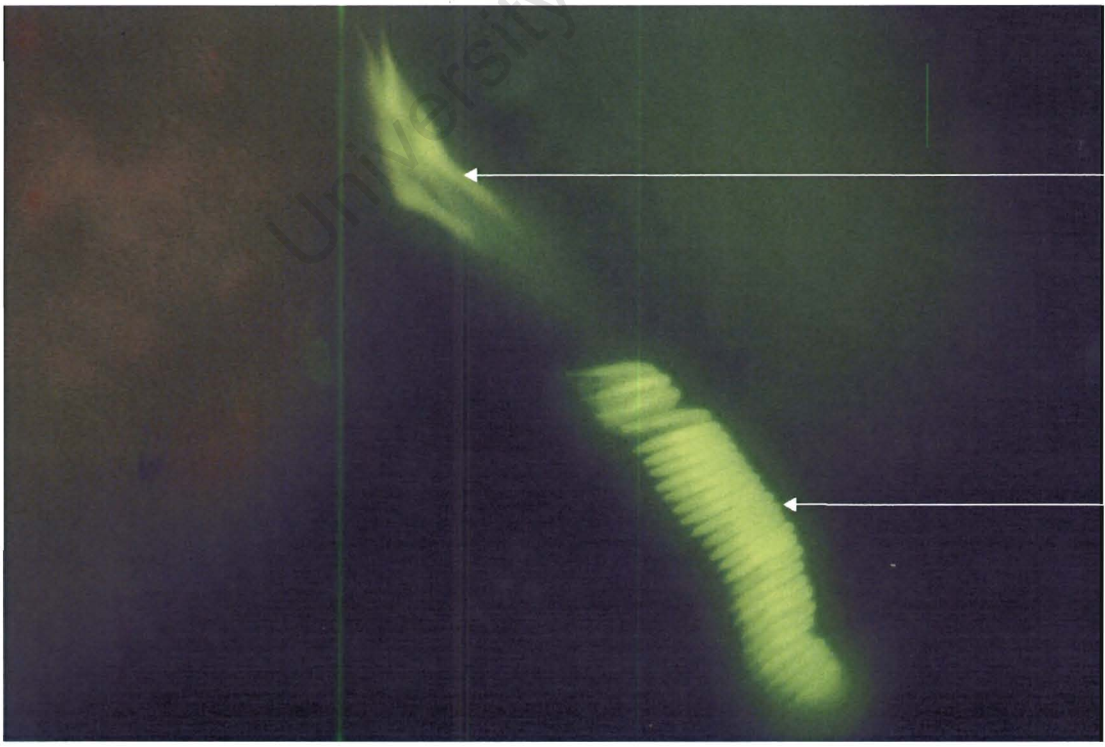

Figure 25: View of a sabellid worm through a fluorescent microscope. The setae fluoresce brightly against the background. Here the posterior uncuni and its associated setae are visible. (MAG 400x)

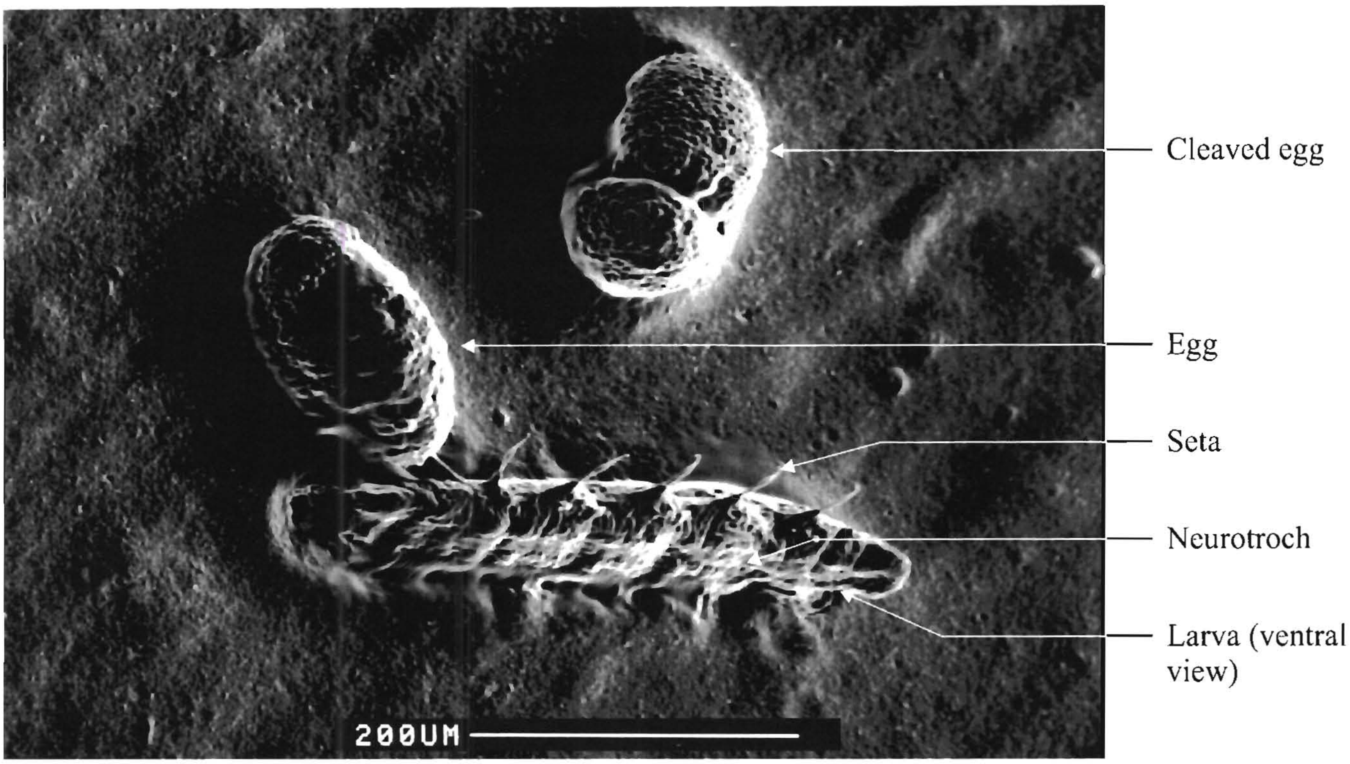

Figure 26: Scanning electron microgram of sabellid eggs and larva.

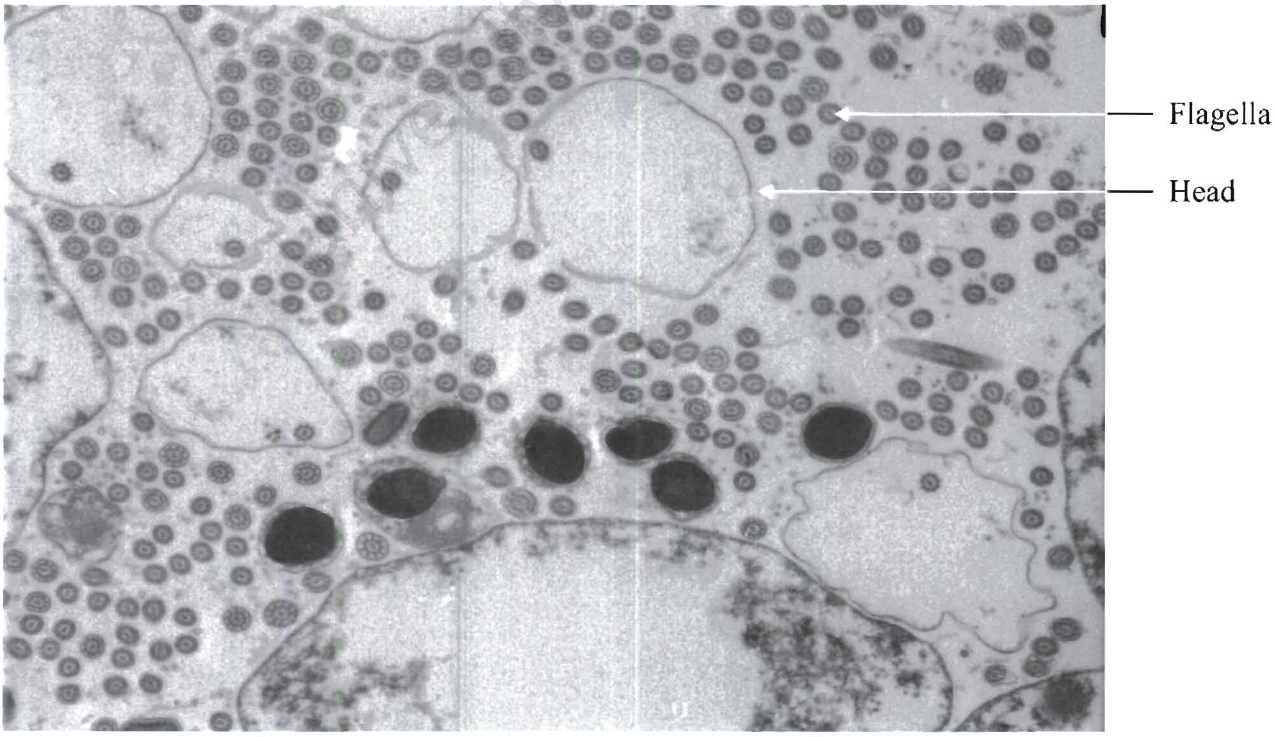

Figure 27: Cross-section through sperm of sabellids. (MAG- 10 000 x)

Figure 28: Magnified view of the dorsal a sabellid larva.

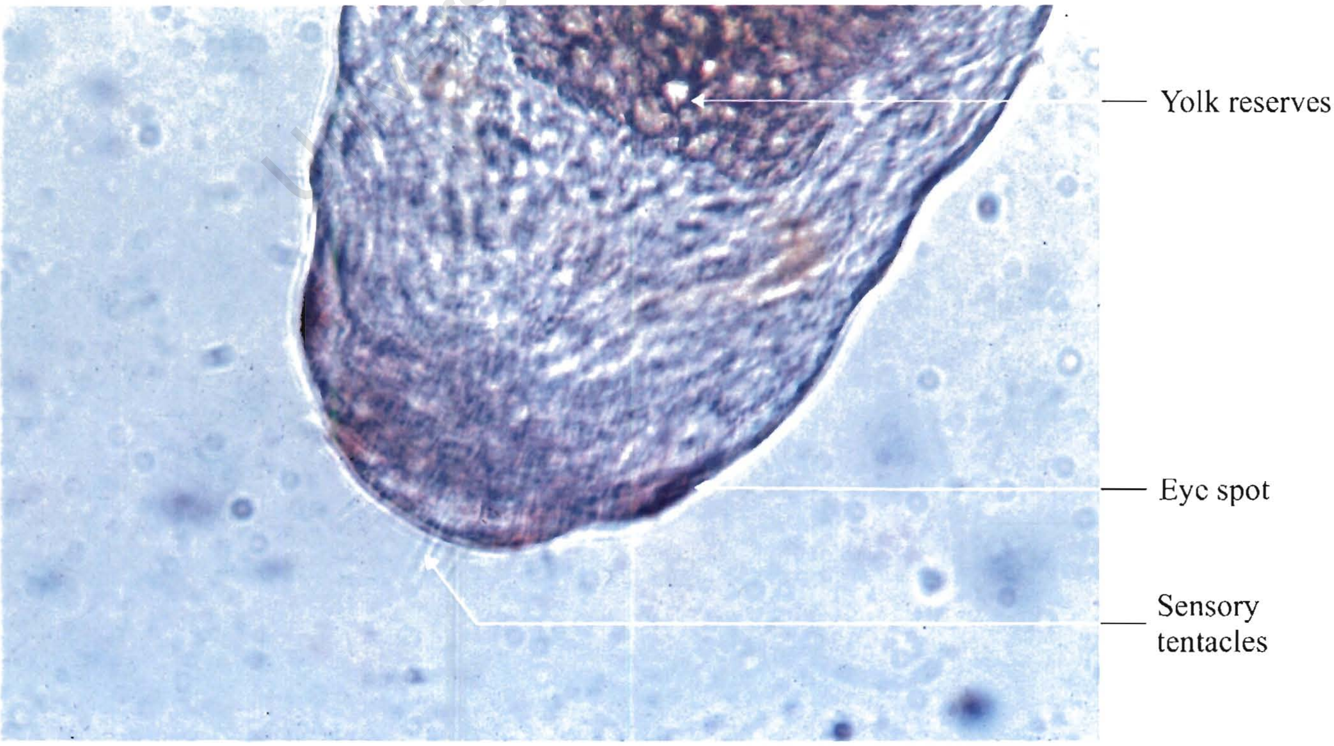

Figure 29: Magnified view of the head region of a sabellid larva (1000 xmagn.)

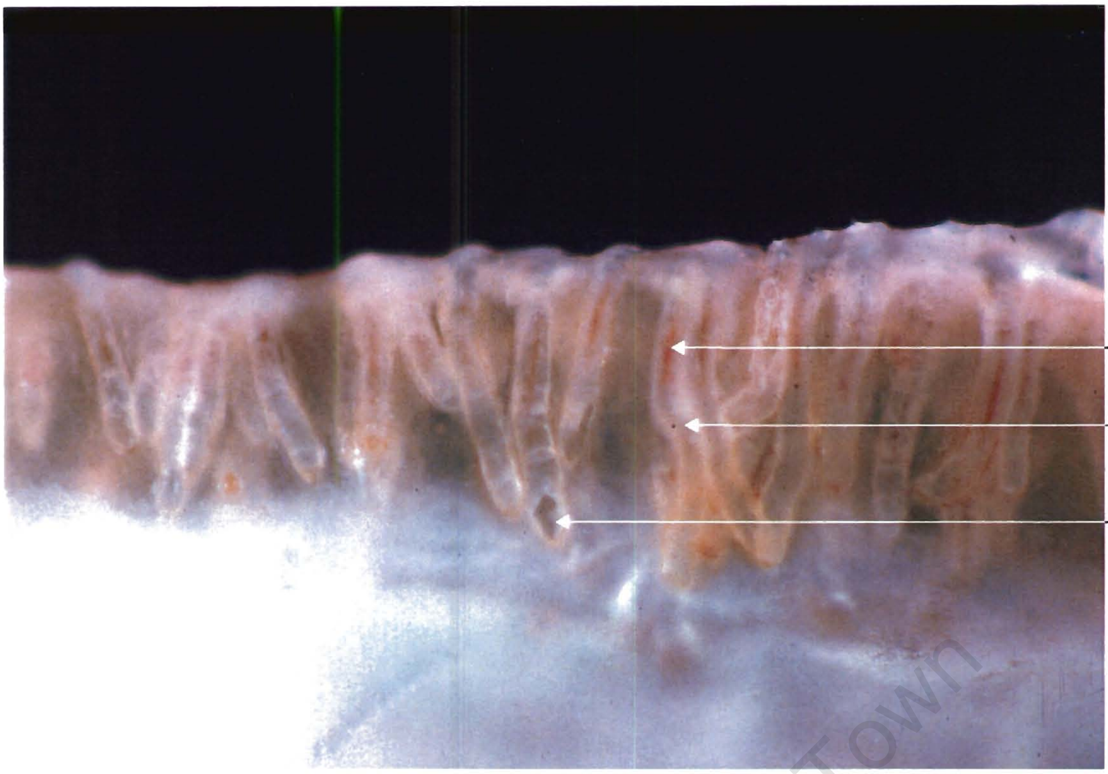

Figure 30: Freshly settled sabellid larvae can be seen through the thin nacreous shell deposited over them by the abalone. (MAG. 250 x)

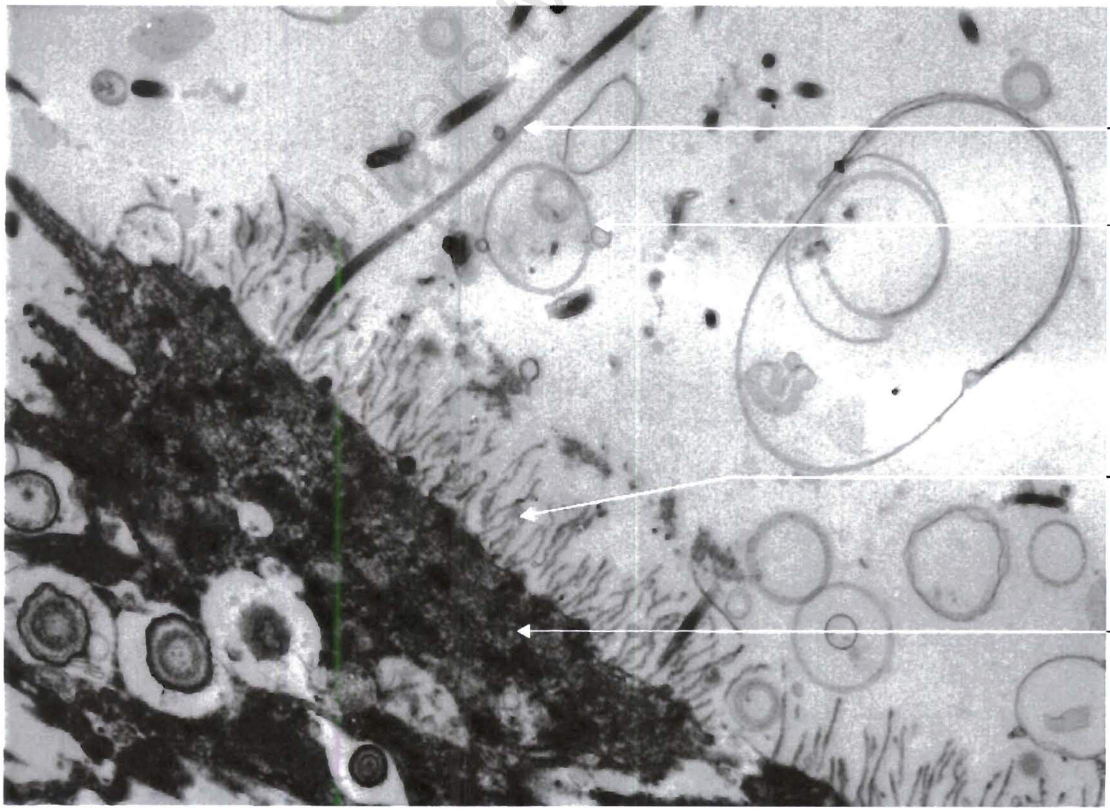

Figure 31: Transmission electron microgram of the gut region of an adult sabellid. The sabellid had been fed liposomes which are visible in the gut. (MAG. 7700 x)

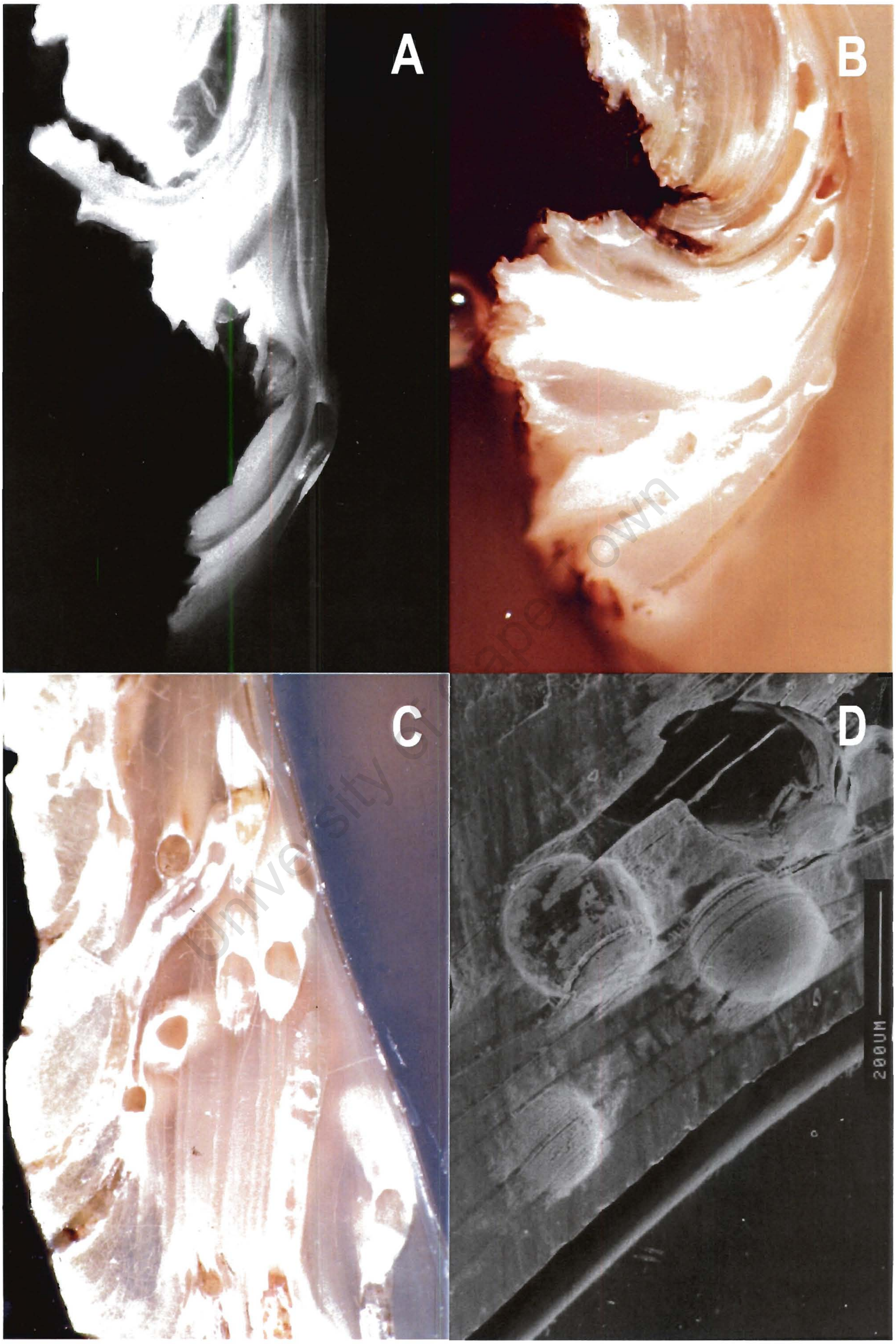

Figure 32: Composite to show the effect that sabellids have on the shell of the abalone *H. midae*. Very newly established burrows can be seen in A & B. The extent that the burrows perforate the shell is clear in C and an SEM image in D shows how the burrows erode away into the nacrous layers.

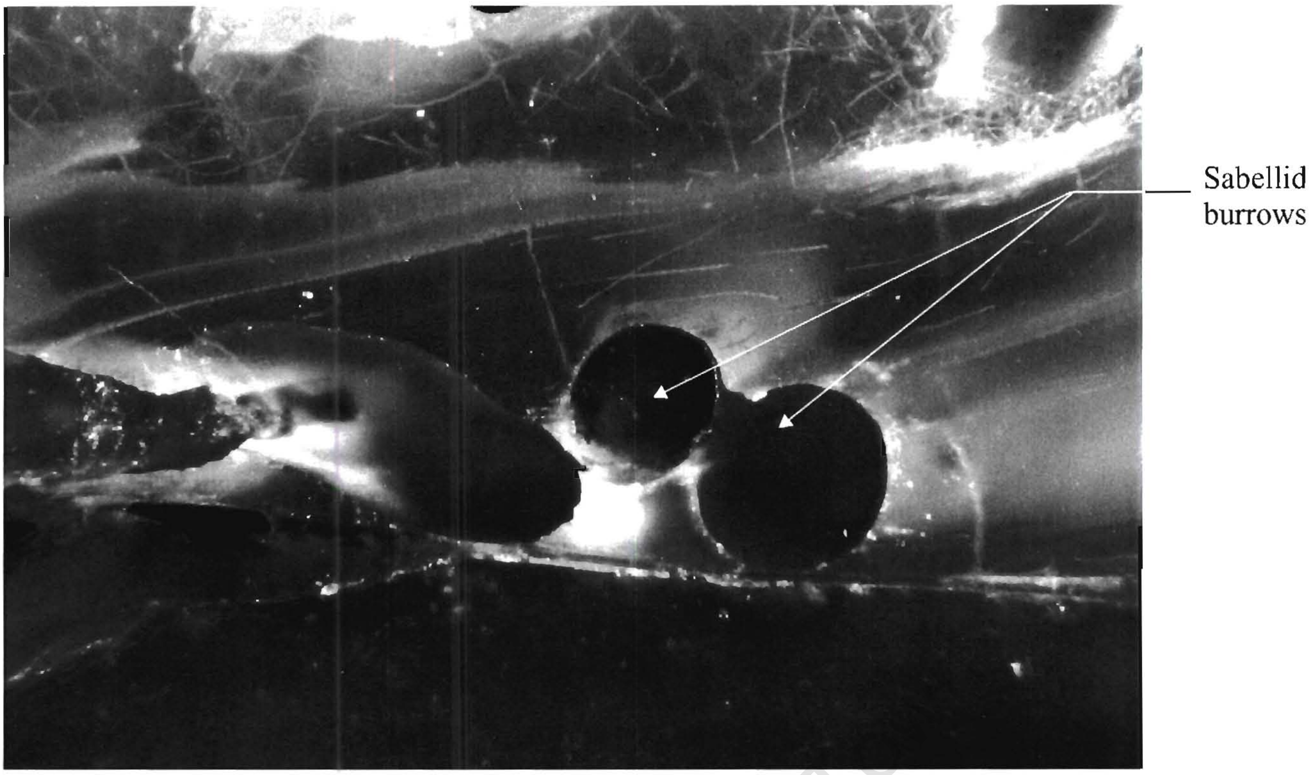

Figure 33: Cross-section through the shell of *H. midae* to show evidence of burrow enlargement by the sabellid. Here it is clear where two burrows have expanded to the point where they have broken through into the other.(MAG 100x)

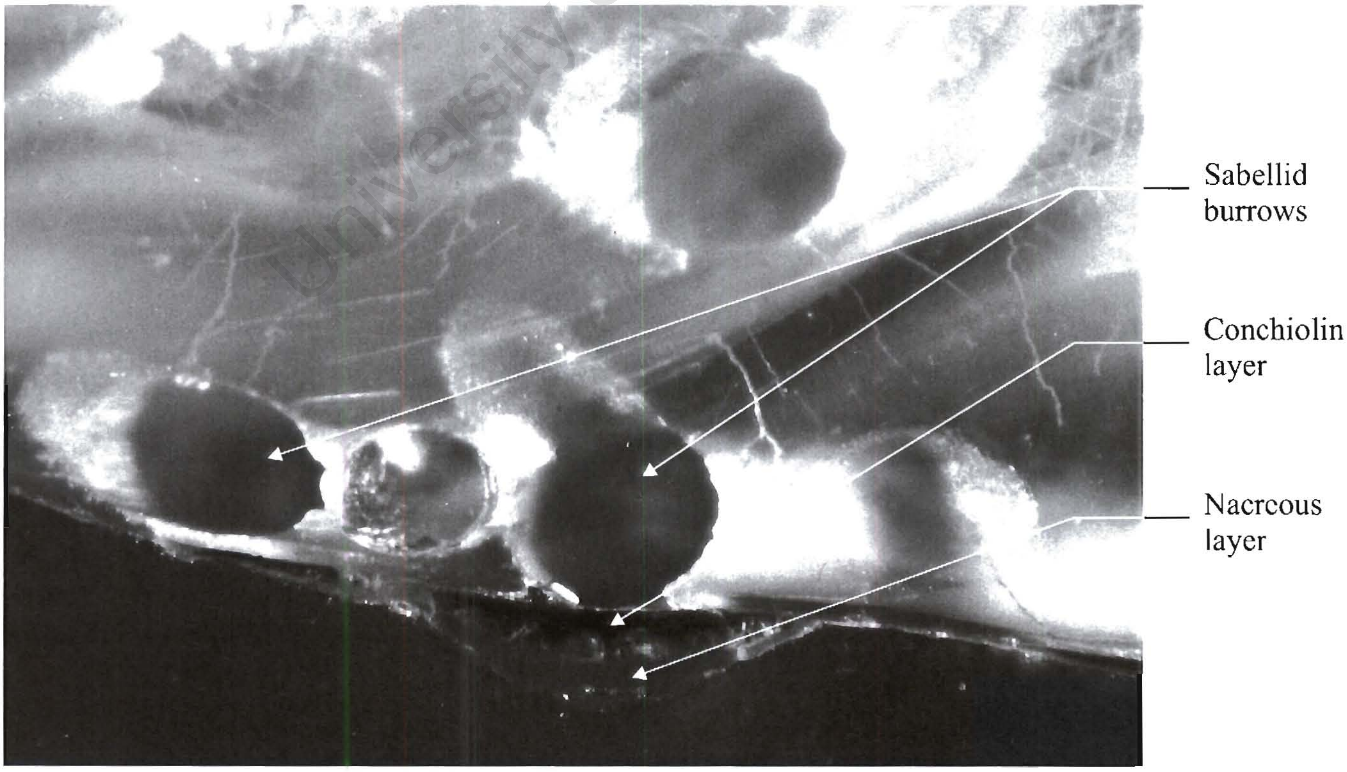

Figure 34: Section through a shell of *H.midae* to show the way that a sabellid burrow had broken through into the mantle cavity of the abalone, and caused the abalone to respond by covering the invasion with conchiolin and nacre. (MAG 100x)

CHAPTER 3 DISTRIBUTION

3.1 INTRODUCTION

The discovery of a sabellid worm, which adversely affects growth, in the shells of cultured abalone in California in 1993 (Oakes & Fields 1996, Kuris & Culver 1999) and South Africa in 1994 (Ruck & Cook 1998), prompted the search for as much information as possible regarding the worm to fully understand the threat it posed. There is sparse record of shell boring behaviour by sabellids (Jones 1968) and in California the worm was originally only associated with abalone farms (Oakes & Fields 1996, Kuris & Culver 1999). In contrast the sabellid was discovered to be widespread in South Africa and infested a range of host gastropods, which lead Ruck & Cook (1998) to conclude that the worm was endemic to South Africa. This suggests that the worm was accidentally introduced to California by abalone farmers intending to experiment with South African abalone, *H. midae* (Kuris & Culver 1999). Subsequently much has been learnt about the biology of the worm (Culver *et al* 1997, Ruck & Cook 1998, Kuris & Culver 1999) and its taxonomy (Fitzhugh & Rouse 1999). Preliminary work on the natural distribution of the worm showed that it occupies a broad geographical range in South Africa and that an array of gastropod species from a number of genera are utilised as hosts by the sabellid (Ruck & Cook 1998). From the results an attempt has been made to explain the patterns observed and to determine the major factors that play a role in the natural distribution of *T. heterouncinata*.

3.2 METHODS

3.2.1 Dive surveys: large-scale patterns

Samples of molluscs were collected in the intertidal and subtidal regions from various locations around South Africa (see Figure 35).

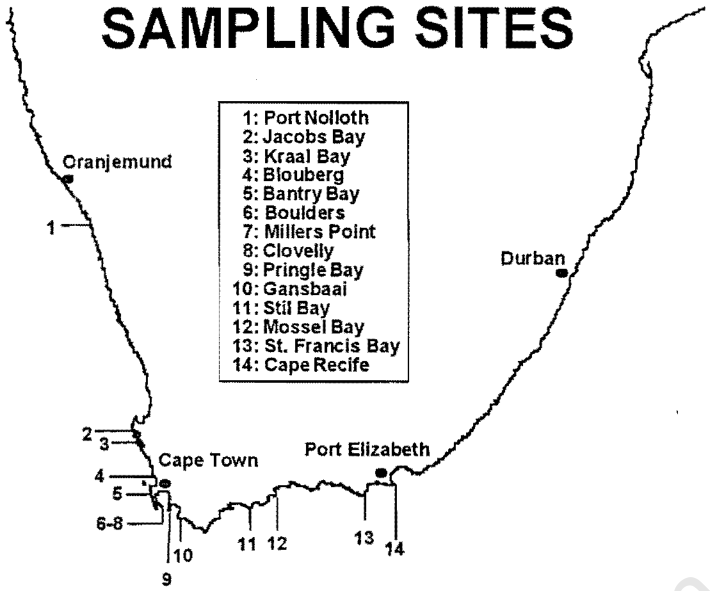

Figure 35: Map of South Africa to show the relative positions of the sampling sites.

At each location numerous samples of molluscs were collected and the abundance of each species in the sample reflects the abundance of that particular species in the location sampled, since no particular species were targeted. When dive samples were collected, areas where abalone occurred, such as rocky points or outcrops, were targeted. For this reason juveniles of the various species tended to be under-represented since in many species the juveniles find shelter under boulders and in crevices in the intertidal. This behaviour made them more difficult to notice without specifically targeting them. The samples collected were examined for the presence of sabellids. The larvae at the leading (growing) edge of the shell were counted for each specimen as shown in Figure 36. This provided a snapshot of the current rate of infestation of each host, but did not reveal the history of the infestation of hosts.

The shell length of the host was also recorded. To allow comparisons between different species and individual sizes, a larval count per cm of shell growing edge was calculated. To accomplish this, linear regression equations relating shell length to growing edge length were formulated for each species that had sabellids present. These measurements were obtained using a video camera linked to a computer with digital imaging software (JL genius Megascan ver.3.01) on representative batches of animals from different species.

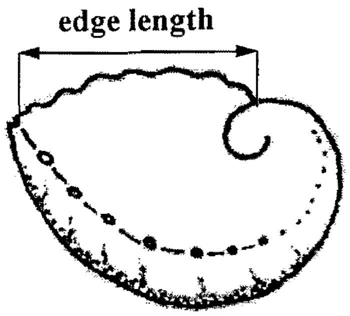

Figure 36: A diagram of an abalone shell to illustrate the region sampled for larval counts.

3.2.2 Dive surveys: small-scale patterns.

Since variables such as water turbulence, intertidal height and sources of larvae may play a role in infestation rates, a special sample of four common, co-occurring species (*Turbo cidaris, Turbo sarmaticus, H. midae* and *Oxystele sinensis*) was collected from a single location in Gansbaai, which would exclude these variables. All species were exposed to the same conditions outlined above since they occupied the same habitat and are often present side by side. In addition they are very mobile grazers which would ensure a high level of physical contact between individuals. The area sampled in this instance was a few kilometres away from the abalone farm mentioned below. It was an area of the subtidal less than 25m^2, 3m depth and was sampled intensively by removing as many individuals as were found of each of the four species. Differences in infestation rates would thus reflect larval choice or host resistance.

3.2.3 Special cases

From the results obtained in the general sampling it was discovered that certain species appeared to be resistant to infestation. To determine whether this was indeed so or merely a sampling artefact, special samples were collected. In addition, laboratory experiments were set up in aquaria where different species were exposed to sabellid larvae via heavily infested abalone.

3.2.3.1 Crepidula porcellana

C. porcellana is a common and abundant filter-feeding limpet species in South Africa. It lives on the shells of other molluscs and is thus small (15 mm SL). From a location (Millers Point) where the gastropod snail *T. cidaris* was found to be heavily infested, *C. porcellana* individuals, which were epibionts on heavily infested *T. cidaris* individuals were examined for sabellid larvae.

3.2.3.2 Oxystele sinensis and Oxystele tigrina

These two winkle species occur over a wide geographic range from Port Nolloth on the west coast to Transkei on the east coast of southern Africa (Branch *et al* 1994). They are abundant grazers of the sub and intertidal at many locations. Large samples of both species were collected from the lower intertidal at Gansbaai, a location where both the sabellid and the snails were common. Sizes ranged from 24-31 mm shell length in *O. tigrina* and 29-37 mm in *O. sinensis.*

3.2.3.3 Mussels

In addition to the mussels sampled during dive surveys (data not included), as an extra test, 26 individuals of *Mytilus galloprovincialis* ranging in size from 24-40 mm, were taken from a basket at an abalone farm, which also contained many heavily infested abalone. The mussels were also dissected to expose the mantle and shell growth edge.

3.2.3.4 Forced exposure in the laboratory

Once the fieldwork was completed, laboratory experiments were undertaken to assess the susceptibility of different molluscan species to infestation. A range of species was kept in aquaria together with heavily infested abalone. Three replicate tanks were used; each with 4-6 individuals of each species, kept together with six heavily infested abalone. The introduced animals were measured and their sabellid infestations estimated at the start of the experiment, and again after two weeks, by counting the number of sabellid larvae per linear cm of shell. The experiment was conducted twice.

3.2.4 Export of larvae from an abalone farm

In California the sabellid was discovered in the intertidal region around the outflows of abalone farms (Culver & Kuris in prep.). This was attributed to the discharge of larvae directly via the water column, but also due to the export of shell debris in the effluent which would carry viable sabellid populations with them (Culver & Kuris in prep.). Samples were collected at the outfall of an abalone farm with known sabellid infestations in Gansbaai to assess the effect on intertidal populations of molluscs.

3.2.5 *O. sinensis* cross-infection experiment

Since heavily infested individuals of *O. sinensis* were found on the east coast of South Africa in contrast to other regions, an experiment was conducted to compare the infectivity of *O. sinensis* based on the geographical origin of the potential hosts – "victims" and the infestors. Heavily infested *O. sinensis* infestors (25 mm SL.) were collected from Port Elizabeth. Eight individuals were included in each treatment tank. Heavily infested *H. midae* infestors (40 mm SL.) originated from an abalone farm in Gansbaai. Three live and two shucked individuals were included in each treatment tank. Victims were represented by uninfested *O. sinensis* (15 mm SL.) from Port Elizabeth and Cape Town and uninfested *H. midae* (15 mm SL.) from a Gansbaai abalone farm. Two victims in each case were included in each treatment tank. Each treatment had 4 replicates for a total of 24 aquaria (2 l). Combinations of treatments are represented in Table I.

Table I: Summary of treatments used in cross-infection experiment.

	Victims		**Infestors**	
Treatment	**Species**	**Origin**	**Species**	**Origin**
1	*O.sinensis*	Port Elizabeth	*O.sinensis*	Port Elizabeth
2	*O.sinensis*	Port Elizabeth	*H. midae*	Gansbaai
3	*O.sinensis*	Cape Town	*O.sinensis*	Port Elizabeth
4	*O.sinensis*	Cape Town	*H. midae*	Gansbaai
5	*H. midae*	Gansbaai	*O.sinensis*	Port Elizabeth
6	*H. midae*	Gansbaai	*H. midae*	Gansbaai

3.3 RESULTS

3.3.1 Dive surveys: large scale patterns

The dive data for all sites and the more common species are presented graphically in Figure 37. The sabellid is widespread and was found in varying degrees of abundance at each location sampled. It is clear from the figure that the sabellid was not limited to abalone but infested various unrelated genera. In fact, the majority of molluscan species sampled were not very susceptible to infestation by the sabellid in the wild (gray bars in Figure 37). The common hosts were seen to be the scavenging whelks, *Burnupena papyracea*, other *Burnupena* spp, the abalone *H. midae*, and the large turban shells *T. cidaris* and *T. sarmaticus*. The many limpets present (*Patella* spp) and the winkles (*O. sinensis* and *O. tigrina*) were not infested at most locations even though they were found in high abundances. An exception to this is Cape St. Francis, which had more species infested than any other site and clearly the sabellid is behaving differently there than at other locations. Locations with few infested hosts were Bantry Bay, which appeared to lack suitable host species and Mossel Bay, which was unusual in that it had high numbers of typical hosts, *H. midae* and *T. sarmaticus* both of which were sabellid-free. Limpets that are found high up on the shoreline such as *Patella granularis* were unsurprisingly free from infestations and this was attributed to the limitations imposed on larval distribution by desiccation. Limpets inhabiting turbulant zones such as *Patella cochlear*, and *Patella argenvilla* were also not infested.

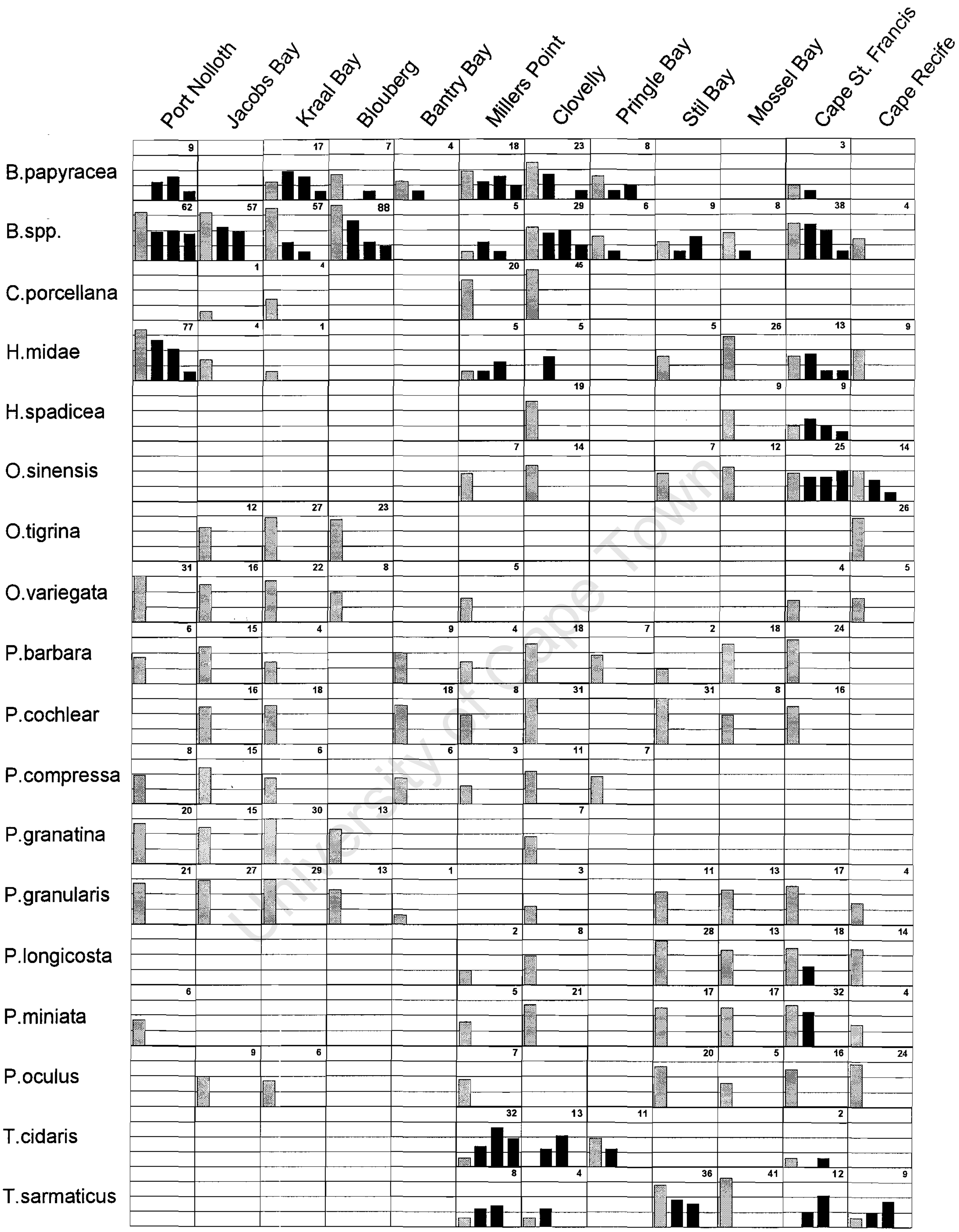

Figure 37: Summary of distribution data for the more common species by location. The x-axis is a log scale of counts/cm (0, 0-0.5, 0.5-1, 1-2) = (none, light, med, heavy). The y-axis represents the log of frequency in each count/cm class (0-0.5, 0.5-1, 1-1.5, 1.5-2). The number within each grid is the sample size. All zero values are represented by gray bars the rest by black bars.

3.3.1.1 Host size dependence

The relation between infestation and host size was investigated for common host species. One would expect larger, presumably older, individuals to be more infested, as successful infestation would often persist due to subsequent self-infestation.

H. midae

Small abalone had significantly lower counts than medium abalone (p=0.001)(ANOVA, Newman-Keuls test). The larger size classes of abalone had the lowest sabellid counts again significantly different from medium abalone (p=0.00002) and small abalone (p=0.03)(ANOVA, Newman-Keuls test)(Figure 38). The very low counts in larger abalone could be due to resistance or due to the fact that they occupy different habitat as they grow older. Larger *H. midae* occupy offshore reefs in powerful surge conditions, perhaps providing less opportunity for sabellid larvae to colonise the shell. But larger abalone tend to be less prone to heavy infestation in the farm situation too (pers obs), which may mean they are more able to resist infestation.

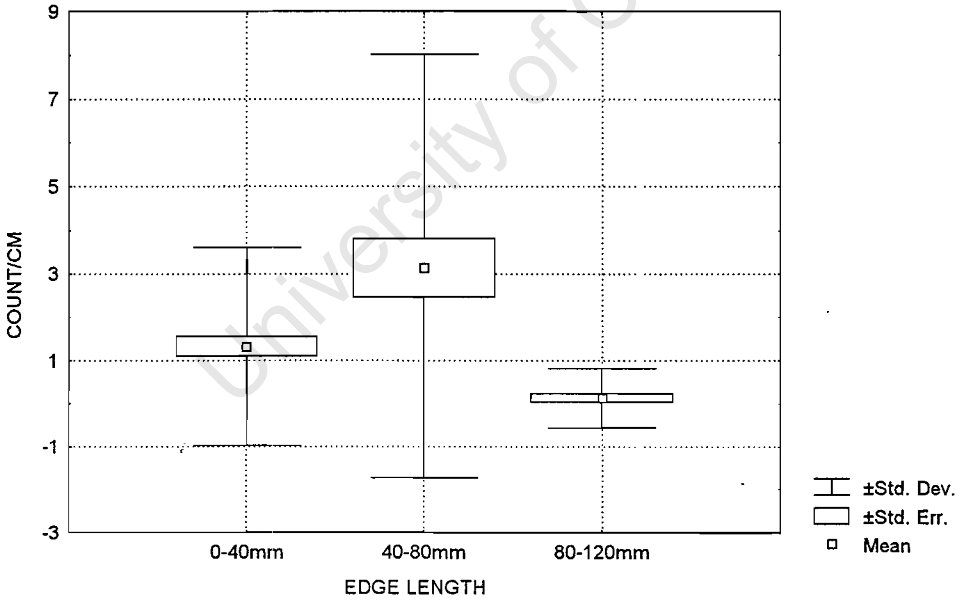

Figure 38: Sabellid larval counts per cm of shell growth edge as related to the total edge length for *H. midae*. The data are combined from all locations.

Burnupena spp.

Figure 39 shows a skewed distribution which may suggest that smaller *Burnupena* are less infested than larger individuals as may be expected if self-infection is common after initial infestation. The small size class is significantly different from both the medium (p=0.00002) and large size classes (p=0.04) (ANOVA, Tukeys HSD comparisons).

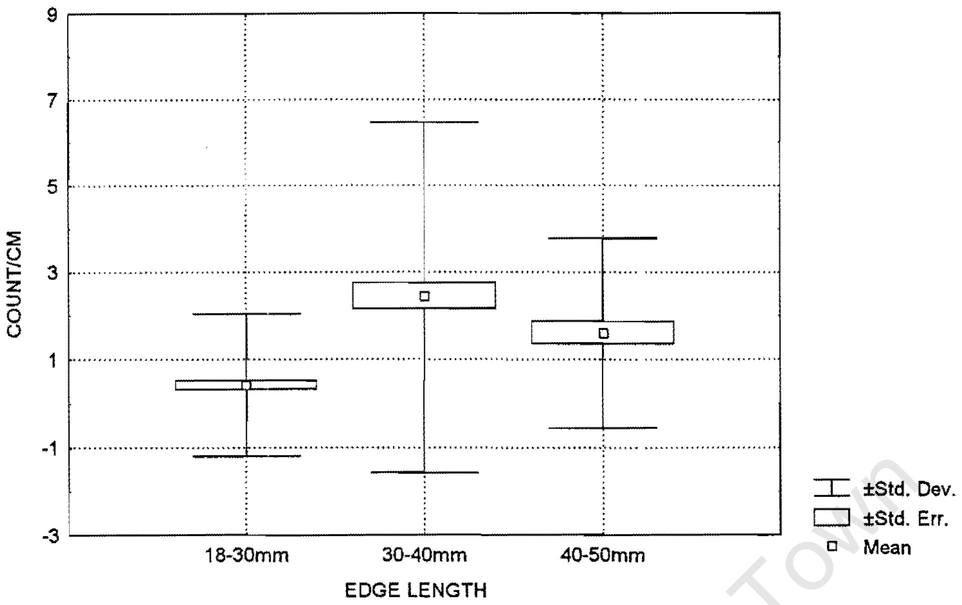

Figure 39: Sabellid larval counts per cm of shell growth edge as related to the total edge length for *Burnupena.* spp. The data are combined from all locations.

However, more sampling in the smaller size classes would be needed to strengthen this result. A better sampling strategy would be to sample in one particular location and to include individuals over a broad size range to provide a clearer picture. In our particular sampling strategy the aims were to include a broad range of species in each dive and thus the small size classes were often overlooked, as they are difficult to locate unless they are specifically targeted.

3.3.2 Dive surveys: small-scale patterns

The special sample of co-occurring hosts showed that the sabellids are more likely to infest certain hosts than others (Figure 40). With the exception of *T sarmaticus* there were equivalent densities of the selected species, yet the sabellid only infested three of the hosts. Also, these three hosts had very few individuals that were free of sabellid larvae at the growing edge.

Even though the abundance of *T. sarmaticus* was low, the animals sampled were heavily infested or not at all. This suggests that the sabellid is perhaps not reliant on transfer from host to host within the same species, but can also transfer between species. Since this did not occur between the other hosts and *O.sinensis*, this species appears to have a resistance to infestation. It is also possible that the sabellid larvae avoided *O.sinensis* in the presence of other more favourable species. Both frequencies and log frequencies are included in the graph to allow comparisons with Figure 37.

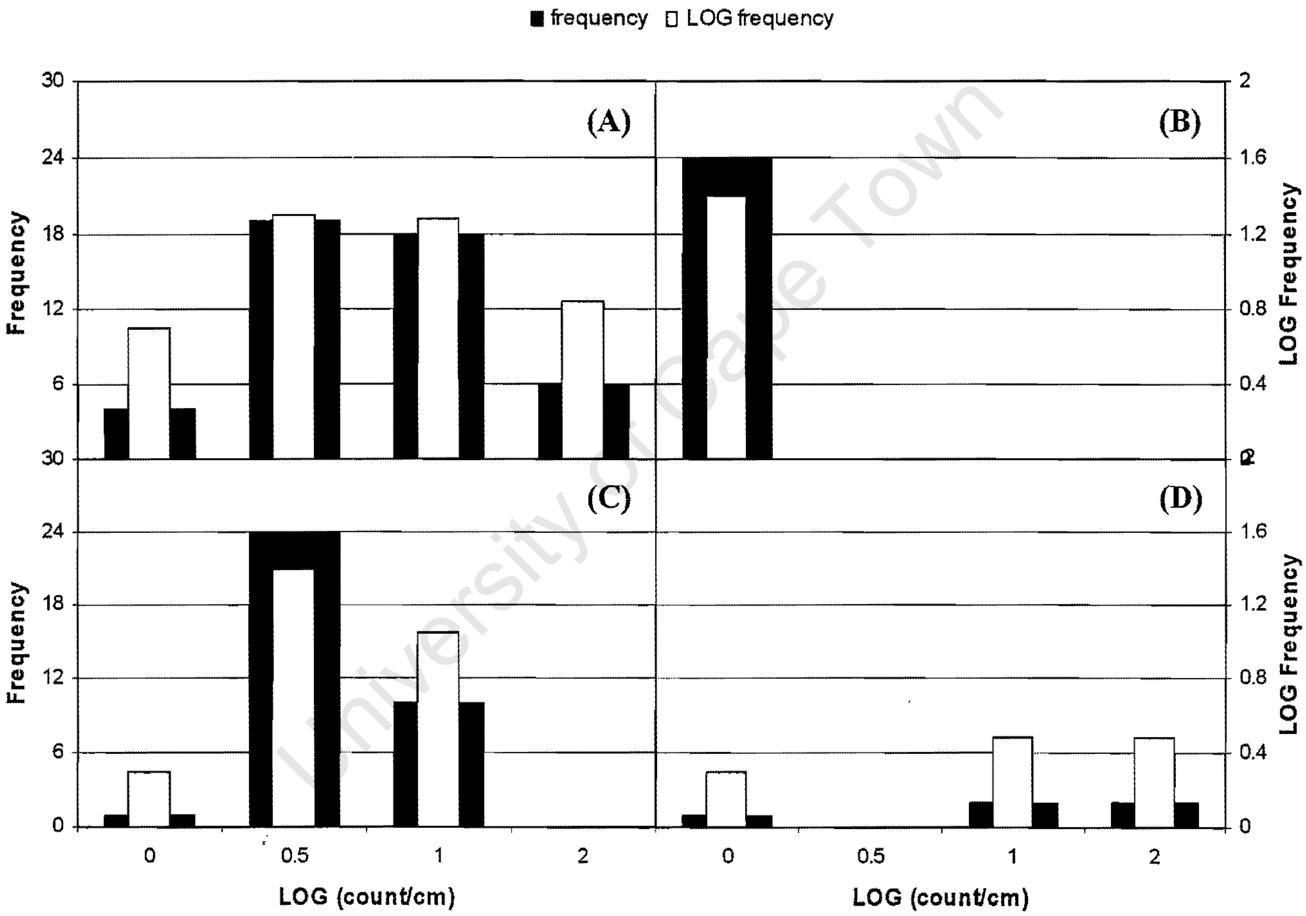

Figure 40: Sabellid larval counts at the leading edge of a special sample of co-occurring species collected at the same subtidal location from Gansbaai. (A=*H. midae*, B=*O. sinensis*, C=*T. cidaris*, D=*T. sarmaticus*).

3.3.3 Special cases

3.3.3.1 C. porcellana

None of the *C. porcellana* taken from the heavily infested *T. cidaris* were infested at all (n=10, shell length 22-29 mm). The leading edges were examined as well as the rest of the shell for sabellid tubes. The absence of infestation suggests a resistance in this species, since it would most definitely have been exposed to larvae. The most likely explanation for resistance would be mechanical as *C. porcellana* clamps very firmly down to the surface and only allows a small aperture for filter-feeding. Their small absolute size could also be a factor since sabellid larvae are fairly large (Ruck & Cook 1998, Culver *et al* 1997). Kuris and Culver (1999) have also remarked on the lack of infestations in *Crepidula* spp. in California.

3.3.3.2 Mussels

Towards the start of the investigation, the bivalves *Aulacomya ater* and *Mytilus galloprovincialis* were never found with any sabellid infestations. This was also true for the special sample collected at the abalone farm. This presumed resistance was attributed to the method of shell deposition in these species, specifically the position of the mantle with respect to the shell. The following illustration will show how the larvae are effectively precluded from the growing edge of the shell in mussels, in contrast to abalone and limpets (Figure 41). They are physically unable to crawl between the mantle and the shell, because the mantle lies within an impenetrable sleeve of shell, which it secretes.

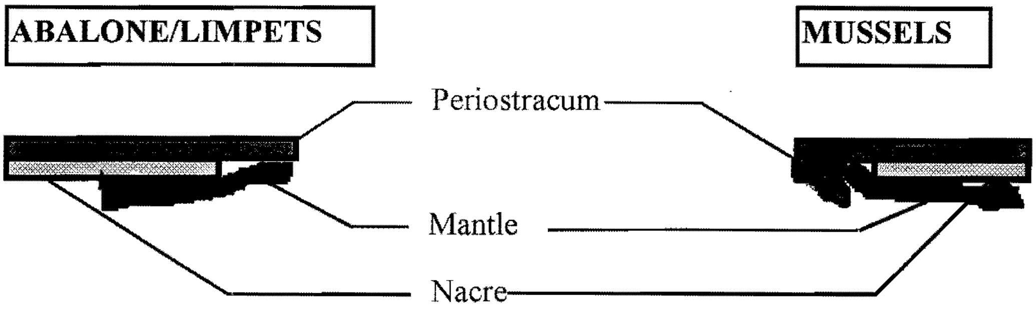

Figure 41: Diagram to illustrate the structural difference between mussels (*Mytilus* and *Aulacomya*) as compared to patellid limpets and abalone in the positioning of the mantle in relation to the shell it forms.

3.3.3.3 Oxystele resistance

The species that was found to have the lowest percentage of infestation even under the laboratory conditions of forced exposure was the winkle *O. sinensis.*

The larger sample taken at Gansbaai showed that both *Oxystele* species can be infested by sabellid larvae (see Figure 42). *O. tigrina* was, however more susceptible than *O. sinensis*, which had an incidence of only one individual, which carried a single fresh uncoated larva. It is possible that this larva may not have been able to establish itself for the long term. Gansbaai is also an anomalous site since the sample area was at the outflow area of an abalone farm, which had confirmed sabellid infestations in the abalone.

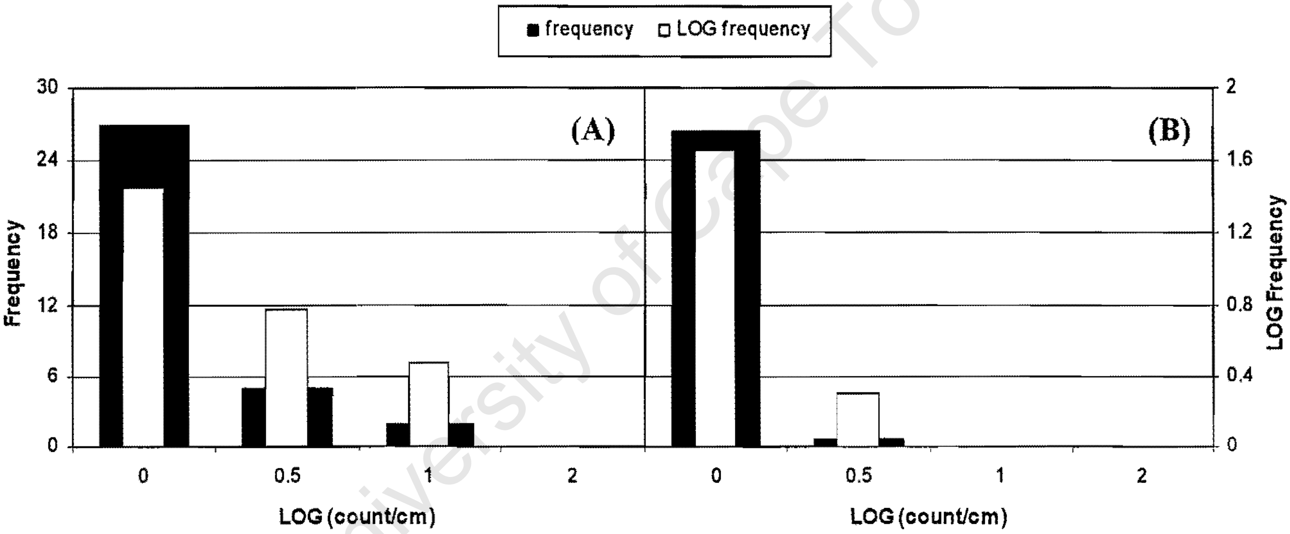

Figure 42: Larval sabellid counts at the growing edge of *O. tigrina* (A) and *O. sinensis* (B). Frequencies of animals within each count class are represented as a normal and a log function.

3.3.3.4 Forced exposure in the laboratory

There may be various reasons why some species are more prone to infestation than others and the laboratory experiment was designed to investigate some of the possible reasons. The results are shown in Figure 43, which records the density of sabellid larvae at the growing edge of the shell, a measure of the intensity of infestation.

Almost all species that exhibited very low or zero infestation in the wild became infested when exposed to larvae in the laboratory. The levels of larval colonisation, however, were still lower than those of the host species commonly infested in the wild. Whether this was due to larval preference or host resistance is unclear. An exceptional case was the winkle *O sinensis,* which never acquired any infestations in the experiments. Some of the high-shore species, such as *P. granularis* and *Oxystele variegata,* spent a large proportion of the time above the water line in the aquaria, reducing their exposure to the larvae.

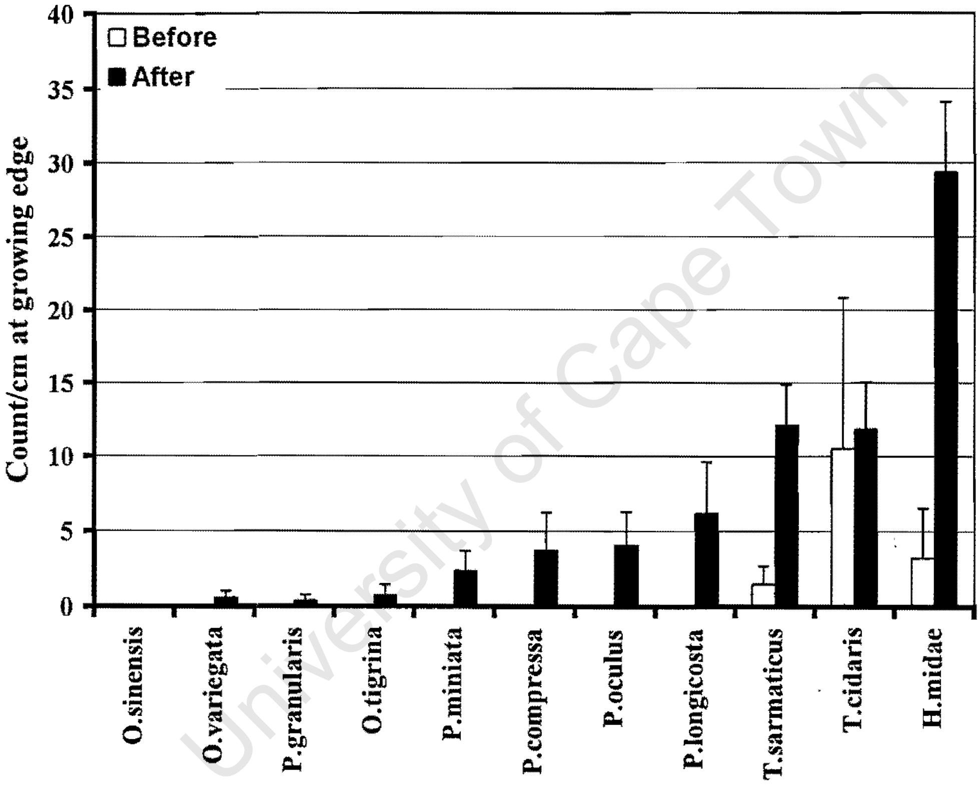

Figure 43: Results of the mixed species experiments. Bars indicate the density of sabellid larvae at the growing edge of the shell before and after being exposed to heavily infested abalone. Time period was 14 days with n=6(4-6). Error bars are one standard deviation of the mean.

Had they been forced to remain submersed, it is possible that the counts at the end of the experiment may have been higher. Even though *T. sarmaticus* and *H. midae* exhibited high counts at the start of the experiment (as would be expected from the results in Figure 37), the counts increased markedly when they were exposed to the infested, farm-grown abalone in the aquarium. This suggests that the source abalone from the farm were more severely infested than wild abalone. In a separate experiment the susceptibility of *Haliotis spadicea* to infestation was tested in the laboratory. Sabellid larvae readily infested individuals placed in tanks with infested *H. midae* within a few days.

3.3.4 Export of larvae from an abalone farm

Prevalence is defined as the number of individuals of a host species infected, divided by the number of hosts examined (Margolis *et al* 1982). The prevalance of sabellids in the various mollusc species were all higher at the Gansbaai site than elsewhere (Table II). In other areas the limpet species were generally free of sabellids, whereas at this site the percentage of infested individuals were fairly high (especially *Patella longicosta).* This site was near the outfall of an abalone farm containing infested abalone. It is possible that the abundant export of sabellid larvae from the farm creates a situation atypical of that which normally occurs in the wild and allowed these species to become more infested than is normally case. In effect this was a large-scale replication of the forced exposure experiment above.

Table II: The prevalence of sabellids in the more common species at the Gansbaai site compared to the rest of the sites 1-10 combined.

Species	Total excluding Gansbaai			Gansbaai site		
	Sample Size	With Sabellids	Prevalence (%)	Sample Size	With Sabellids	Prevalence (%)
Abalone						
H. midae	140	84	60	33	30	91
Limpets						
Patella miniata	32	0	0	39	3	8
Patella longicosta	15	0	0	39	10	26
Patella oculus	22	1	5	36	7	19
Patella barbara	64	0	0	18	0	0
Patella compressa	56	0	0	36	1	3
Patella granularis	81	0	0	21	0	0
Whelks						
Burnupena spp.	231	83	36	18	14	78
Winkles						
Oxystele tigrina	39	0	0	70	8	11

Oxystele sinensis	45	0	0	81	1	1
Oxystele variegata	74	0	0	36	0	0
Turban shell						
Turbo sarmaticus	17	14	82	24	21	88
Turbo cidaris	91	81	89	33	32	97

3.3.5 O. sinensis cross-infection experiment

Victim	O.sin.(PE)	O.sin.(PE)	O.sin.(CT)	O.sin.(CT)	H.mid.(G)	H.mid.(G)
Infestor	O.sin (PE)	H.mid(G)	O.sin(PE)	H.mid(G)	O.sin(PE)	H.mid(G)

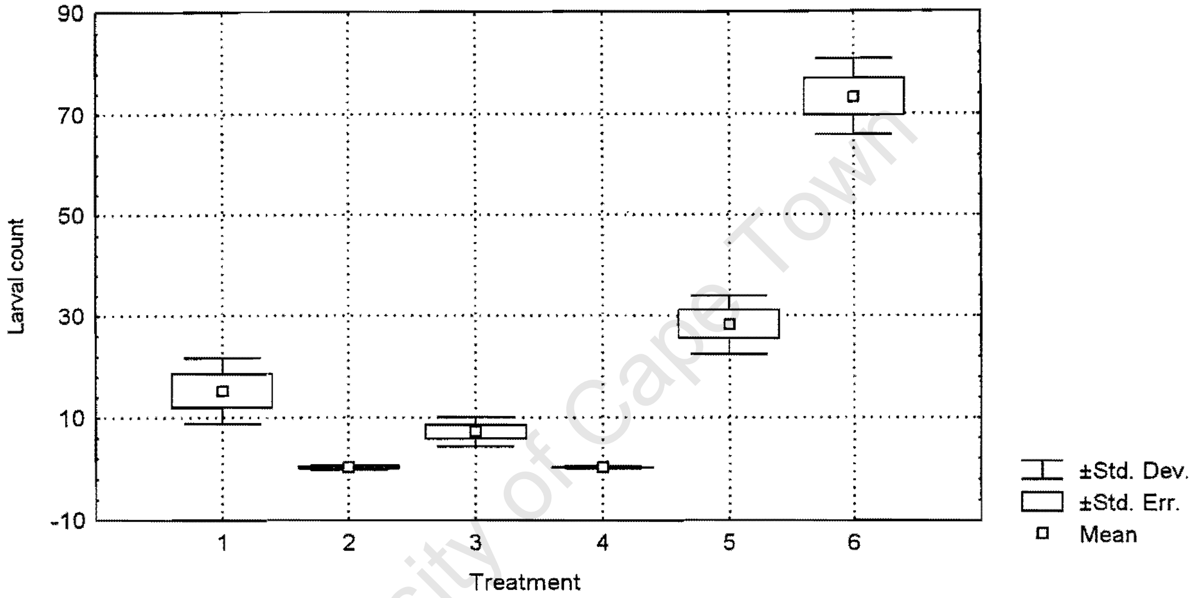

Figure 44: Total larval counts at the leading edges of "victims" in each treatment of the cross-infection experiment. For details of each treatment see Table I.

The results from the various treatments are represented graphically in Figure 44. Statistical differences among treatments were tested using 1-way Tukeys HSD multiple comparisons on the logged data. The adjusted Bonferroni probability levels were calculated to be 0.00851.

In both treatments 2 and 4 there were very few larvae at the growing edge. In addition it was noted that the counts recorded here were in each case larvae that had freshly settled and had not been coated by shell yet. It is possible that they may not have been able to establish themselves. Thus, sabellid larvae sourced from Gansbaai abalone were largely unsuccessful at colonising *O. sinensis* from both Cape Town (CT) and from Port Elizabeth (PE).

The counts in treatment 3 were significantly greater than treatment 4 ($p<0.000$). This was a surprising and interesting result since it shows that in contrast to the above, *O. sinensis* from CT can in fact be infested by sabellid larvae sourced from PE *O. sinensis*. Similarly counts of treatment 1 were greater than treatment 2 ($p<0.000$) showing that O.sinensis from PE were infested by worms from PE conspecifics.

Treatment 3 counts were significantly less than counts in treatment 5, which indicates that *H. midae* from Gansbaai is more susceptible than *O. sinensis* from CT ($p<0.000$)

Treatment 1 was less than treatment 5 and greater than treatment 3. However in both cases the results were marginally not significant ($p = 0.009$, see Bonferroni level above). Although the results are only marginally not significant, they support the contention that there is a gradient of susceptibility to sabellid larvae when all are faced with the same source of larvae. Thus, counts from treatment 5 were greater than treatment 1 which were greater than treatment 3. In other words, *H. midae* was most susceptible followed by *O.sinensis* from PE followed by *O. sinensis* from CT, when infested by *O. sinensis* from PE.

Lastly *H. midae* was much more infested by worms from *H. midae* than by worms from *O. sinensis* (treatments 5 & 6; $p<0.000$) although a possible confounding factor was that the source locations were different. This last result probably had more to do with the differences in densities of larvae from the infestors than the difference in victims' susceptibilities, since this could not be controlled. If that was the case, then it makes the lack of settlement in treatments 2 and 4 even more striking.

3.4 DISCUSSION

The distribution data showed a few interesting patterns but also produced some contradictory results. From the array of species sampled, only a handful of species is commonly infested in the wild. These species are abundant and have similar distribution patterns. They often occur together, side by side, and it appears as if the sabellid transfers from one species to the other. Also of note is that these species are motile and gregarious and their close proximity is probably a benefit for effective transfer of sabellid larvae. Limpets, such as *P. miniata* and *P. barbara,* which are found in the same vicinity, had low levels of infestation. This may be partly due to their solitary nature.

There are two factors, which control the distribution of the worms. Physical, environmental factors play an important role as was shown by the forced exposure experiments. From this it was clear that many of the species, which are rarely or never infested in the field were susceptible to forced exposure. From this it was deduced that the physical stresses that the larvae would be exposed to in the field (such as turbulence and desiccation) may play a role in restricting larval dispersal. Thus, high-shore species and species inhabiting turbulent zones were rarely found to be infested.

The other controlling factor is natural resistance of potential hosts. Species such as the mussels *A. ater*, *M. galloprovincialis* and the slipper limpet *C. porcellana* appear to be immune to infestation and the mechanism of resistance appears to be physical. Other species also appeared to be resistant as evidenced in the forced exposure experiment, where they were much less infested than the normal hosts. Of these species *O. sinensis* was the most marked example. Unfortunately it is not clear from this experiment whether the observed pattern is due to host resistance or larval choice, since the experiment was run with all species at the same time. From the cross-infection experiment it appears as if there is a gradient of susceptibility depending on host species.

Contradictory results for *O. sinensis* came from the dive surveys on the East Coast. Whatever resistance may have been present elsewhere was clearly not playing a role at these locations. The cross-infection experiment indicated that the worms were indeed behaving differently when they originated from Port Elizabeth. It is even possible that the worms may be a different species. In fact, it is certainly highly likely that individual populations are not interbreeding, given the large areas of sandy beach coastline between some of the rocky regions (more so on the East Coast, Bally *et al.* 1984) and the fact that the larvae are lecithotrophic and disperse by crawling.

The lack of interbreeding is particularly true when East and West Coast populations are considered, since two distinct oceanic water currents come into play. The East Coast receives the warm Agulhas current that flows rapidly southwestward along the southern African coast and moves offshore, southward along the Agulhas bank south of Cape Agulhas. The West Coast receives the cold Benguela current that drifts north-westward and results in wind-induced upwelling of nutrient rich water, supporting the extensive kelp beds (Shannon & Nelson 1996). These two currents are not only opposing in direction, but are also separated by differential temperature and density (Shannon & Nelson 1996). There is oceanographic evidence that there is very little exchange of water between the eastern and western sides of Cape Agulhas (Boyd *et al* 1992, Boyd & Oberholster 1994). So transfer of short lifespan span, lecithotropic larvae across this zone should be limited. Sweijd (1999) found genetic evidence of this for *H. midae*. This region also marks the border of the large kelp beds (Stegenga *et al.* 1997), which may play a role in limiting the export of larvae from localised regions (Jackson & Winant 1983, Eckman *et al* 1989). Thus, in each geographical region, the worms may have developed different behaviours based on the make-up of potential hosts. A recent discovery of a new species of *Terebrasabella* in Australia shows that the genus may not be as restricted as first thought (Murray & Rouse 1998).

The results from the Gansbaai site demonstrate what impact an abalone farm can have on the environment. This site was anomalous in that more species were infested and the intensities of infestation were higher. This effect may have been very localised, however, in that the samples were taken very close to the farm outfall. Similar export of larvae from Californian farms was cause for environmental concern since the worm was not endemic and resulted in an extremely labour intensive cleanup operation to remove all the potential carrier species from the area surrounding the farms outfalls (Kuris & Culver 1999, Culver & Kuris in prep).

It was interesting that other polychaete worms were also fairly commonly encountered in certain limpets particularly *Patella barbara* and *Patella miniata*. The worms were all spionids, unidentified polydorans with characteristic U-shaped burrows.

CHAPTER 4 DISPERSAL

4.1 INTRODUCTION

Strategies for larval dispersal can take two extremes. One of these is the production of large quantities of small eggs with no parental care, whilst the other option is to produce a few large eggs that are nutrient rich and have a greater chance for survival. To further ensure survival, the eggs may be brooded and protected. These two strategies have become known as r-selected and K-selected respectively, based on the equations from the population growth models developed by MacArthur and Wilson (1967). The sabellid in this study has opted for the latter method to ensure reproductive success. Larvae only emerge from the parental tube when they are large and well developed and they are assured of a place to settle on the same shell as their parents. They are lecithotrophic, crawling larvae and do not have the ability to swim, although larvae have been observed suspended at the surface of the water, held there by surface tension. Obviously, in an abalone farm situation, it is essential to know the dispersal abilities of the larvae in order to contain the problem.

4.2 METHODS

4.2.1 Transfer of larvae

An experiment was conducted to test the dispersal abilities of the worms in a laboratory situation. Twenty uninfested abalone in the 15-23 mm size range, and 10 heavily infested abalone in the 50-60 mm size range, were used for experiments. Three scenarios were created in aquaria kept at a constant temperature of 17°C with filtered, flow-through seawater. Firstly, uninfested abalone were allowed to mix with the infested abalone on the base of the aquarium (mixed treatment). Secondly, uninfested abalone were completely separated from infested abalone by a suspended basket system (suspended basket treatment). The baskets were not touching each other or the sides of the aquarium so the only way for larvae to reach the other basket was through the water column. In the third scenario, the uninfested abalone were again separated from the infested animals by baskets, but in this case the baskets rested on the base of the aquarium so that larvae were free to crawl along the base of the aquarium between baskets (base basket treatment). The entire experiment was conducted twice with the same baskets. The uninfested abalone were examined at approximately 20-day intervals. Shell lengths of abalone were recorded and a subsample of 10 abalone was used for larval counts as described in Chapter 3 above. At the end of the experiment, the newly infested abalone from the mixed treatment were separated and further monitored (growth and larval counts) for a period of two months.

Possible export of larvae from culture tanks was tested by filtering water from the outflows of culture raceways that contained infested stocks of abalone through a series of sieves of different mesh sizes. Sieves with pore diameters of 100 μm, 58 μm, 45 μm and 32 μm were used in sequence. Firstly, water was allowed to flow freely for a period of a few hours, but in a second attempt to quantify the results a sample of 500 *l* was passed through the sieves and examined under a dissecting microscope for the presence of larvae.

4.2.2 Sabellid fecundity

It was noticed that when abalone were moved from raceways to experimental tanks within the laboratory (lower stocking densities and cleaner tanks) they grew much faster. At the same time, the worm count at the growing edge dropped. Thus, it was decided to compare the condition of the sabellid worms as follows. Six heavily infested abalone from the second dispersal experiment were sacrificed and the entire shell was crushed using a pair of pliers. Three abalone from their counterpart stocks in the culture raceways from which they were taken, were also crushed. Prior to crushing, the larval count per cm of shell edge was determined as described in Chapter 3. The crushed fragments were placed in seawater in a petri dish, marked with 0.5cm gridlines. A large enough area of the petri-dish was examined to obtain a representative ratio of eggs and larvae relative to adults (defined as those worms of any size possessing feeding crowns). The experiment was repeated at another farm where animals had also been separated and kept in laboratory conditions.

4.2.3 Starvation

In addition the effect of starvation on fecundity was tested as follows. Infested abalone were held in 4 aquaria with two replicate baskets within each aquarium. Aquarium A received 1 µm cartridge-filtered seawater and no feed for the abalone. Aquarium B received 18 µm drum-filtered seawater and no feed. Aquaria C and D received the same water as B but the abalone were fed artificial feed and kelp respectively. All tanks were aerated with an airstone. At regular intervals abalone were removed from each basket, crushed and assessed as described above.

4.2.4 Larval settlement

A preliminary experiment was designed to test the ability of the larvae to target suitable settlement surfaces. Competent larvae were collected by crushing infested abalone shell and placed in a petri dish containing seawater and equally sized pieces of abalone shell, foot muscle and mantle tissue. The pieces of tissue ($0.5cm^2$) were each weighted down by a block of glass. The petri dish was kept in the dark overnight and examined thereafter to determine larval distribution. A blank was also run i.e. larvae placed in a petri-dish with only seawater to test if larval settlement would occur without any stimulus and whether this would be random.

4.3 RESULTS

4.3.1 Larval transfer

Even though the two dispersal experiments were not run concurrently, a similar pattern emerged. Results are presented in Figures 45 and 46. Abalone in the mixed tank acquired infestations very quickly. At the first measurement (which occurred between 18-22 days after initiation) there were already substantial numbers of larvae at the growing edge. In the first dispersal experiment there were high counts of sabellid larvae and the levels remained high. The growth rate of the mixed animals was lower than those from the other treatments (Figure 45). In the second dispersal experiment, the initial counts were much lower and declined further towards the end of the experiment. Thus, even though the growth rate appeared slower initially, it increased towards the end of the experiment to become similar to that of the other two treatments. In both experiments, there were no noticeable differences between the suspended basket treatment and the base basket treatment. This was true for both counts and growth rates. It was concluded from this that larvae were not effective at crossing the short distance separating the two baskets. There was a build-up of detritus on the base of the aquarium, which may have restricted the benthic movement of larvae. It is interesting that there was transfer of larvae from infested to uninfested animals in the suspended basket treatment, implying that the larvae passed through the water column, presumably dislodged by the water motion created by the aeration in the tank. The levels of transfer were very low, however, and would be unlikely to influence the growth rates in the short term.

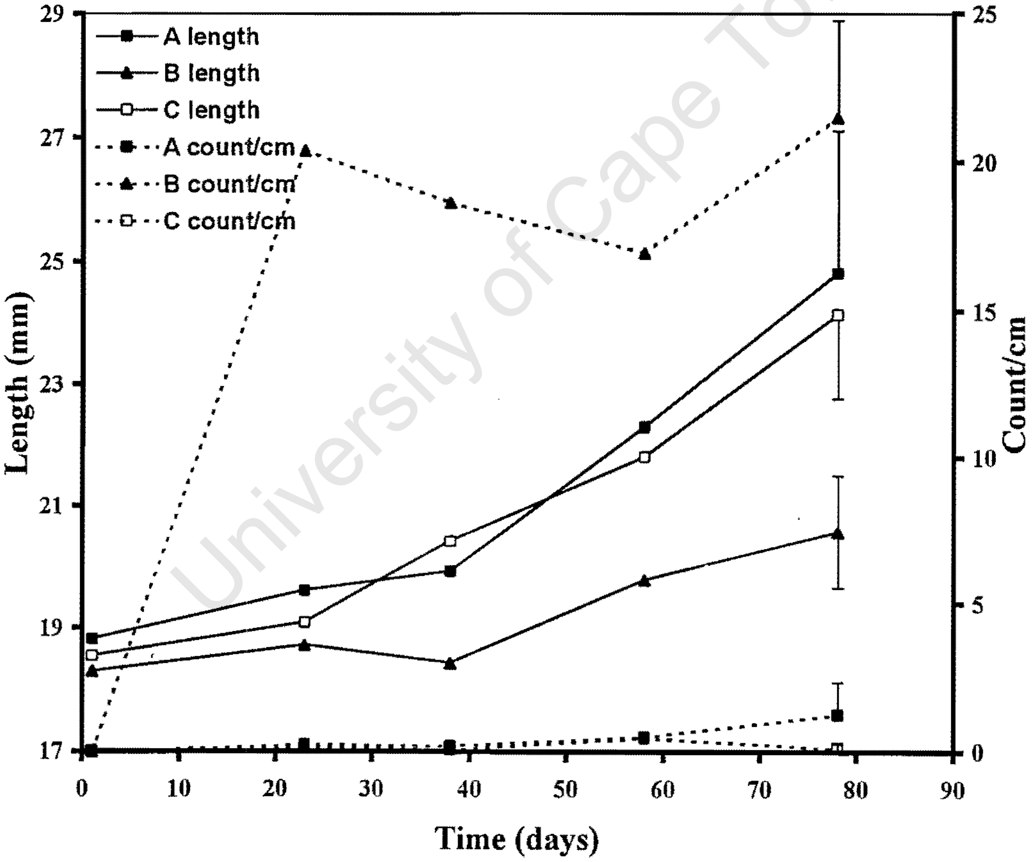

Figure 45: Dispersal experiment one. Growth rates of abalone and larval counts at the growing edge of the shell from the three dispersal treatments. Treatment A = suspended baskets, Treatment B = mixed animals, Treatment C = base baskets.

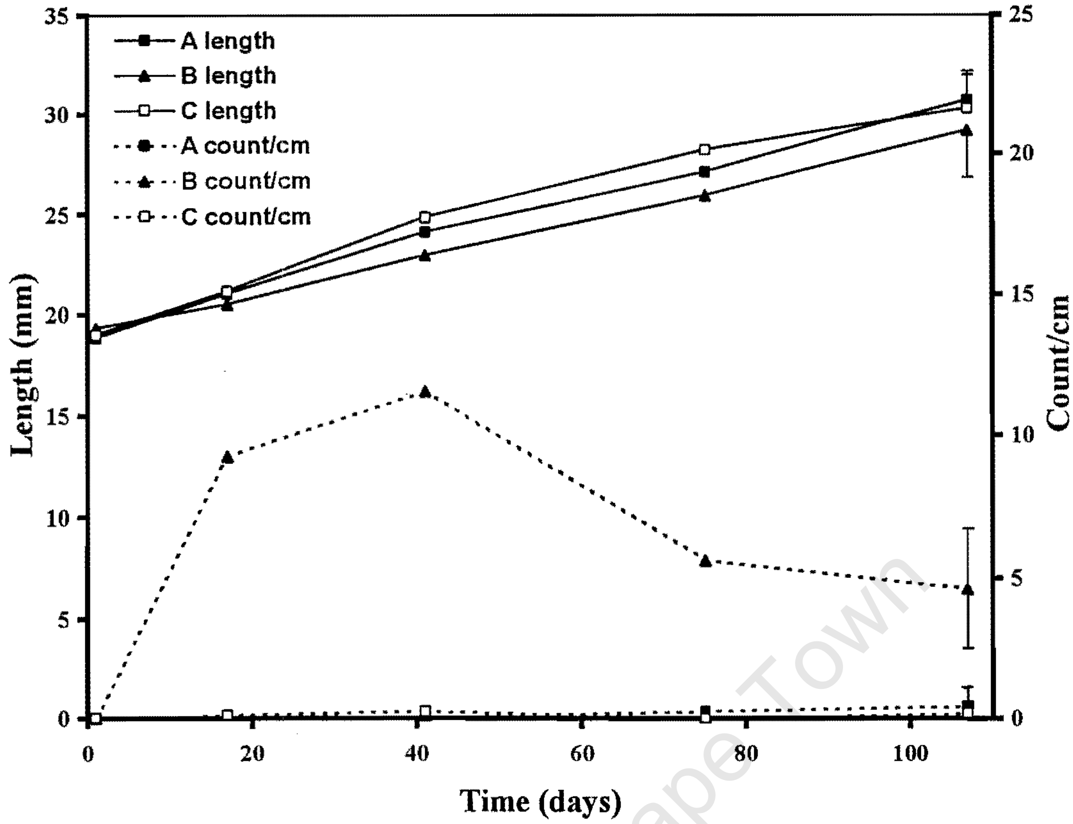

Figure 46: Dispersal experiment two. See Figure 45 for details.

Figure 47 illustrates the progress of the abalone from the mixed treatment in the first dispersal experiment after they were removed from the source of infestation. There was a sudden disappearance of larvae at the growing edge of the shell, coupled with an acceleration in abalone growth. Only after day 150 did larvae appear at the growing edge of the shell, originating from these new infestations. These levels were sustained for some time and the abalone growth rate decreased. From these data it was possible to estimate the time taken for the sabellid population to become reproductive from the larval stage. Assuming the first sabellids (which colonised the shell during days 1-10) were the first ones to produce larvae sometime after day 120, then it took approximately 4 months to complete the cycle at 17°C.

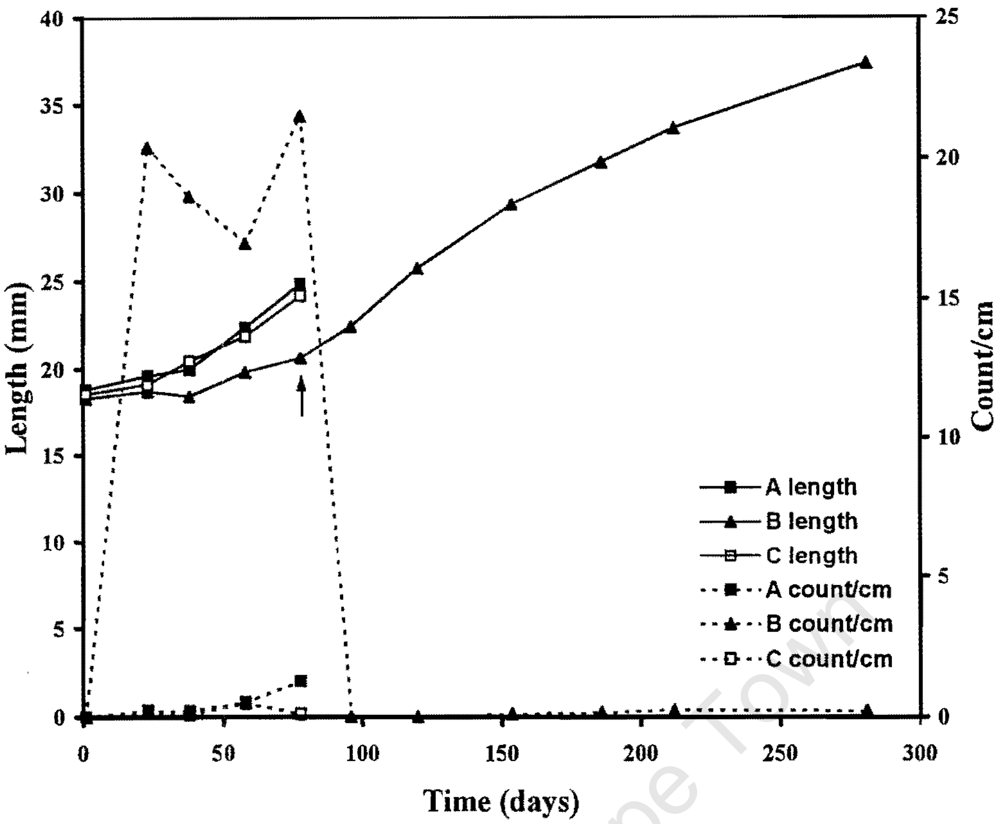

Figure 47: Progress of newly infested animals from treatment B in dispersal experiment one following termination of experiment (indicated by arrowhead).

Table III presents the results of the filtering experiment. A fairly high degree of export of larvae from infested raceway tanks occurred. The tanks contained in the region of 1000 heavily infested abalone in the 50-60 mm size class. Most larvae were caught in the 58 μm mesh.

Table III: Larval counts from filters placed at the outflows of engrossing raceway tanks containing infested abalone.

	Larval count			
Pore size	**100 μm**	**58 μm**	**45 μm**	**32 μm**
Timed sample (Farm 1)	0	22	0	0
500 l sample (Farm 2)	0	3	1	0

4.3.2 Sabellid fecundity

Figure 48 compares animals from the laboratory with their counterparts in typical high-density, raceway culture conditions. The count of larvae per cm at the growing edge is shown above the bars. It is clear that the animals in the laboratory had lower infestation rates than the animals from the raceway, presumably because the worms become less reproductive in the laboratory. This suggestion is supported by the observation that in the raceway there were far more larvae per adult, and eggs outnumbered adults.

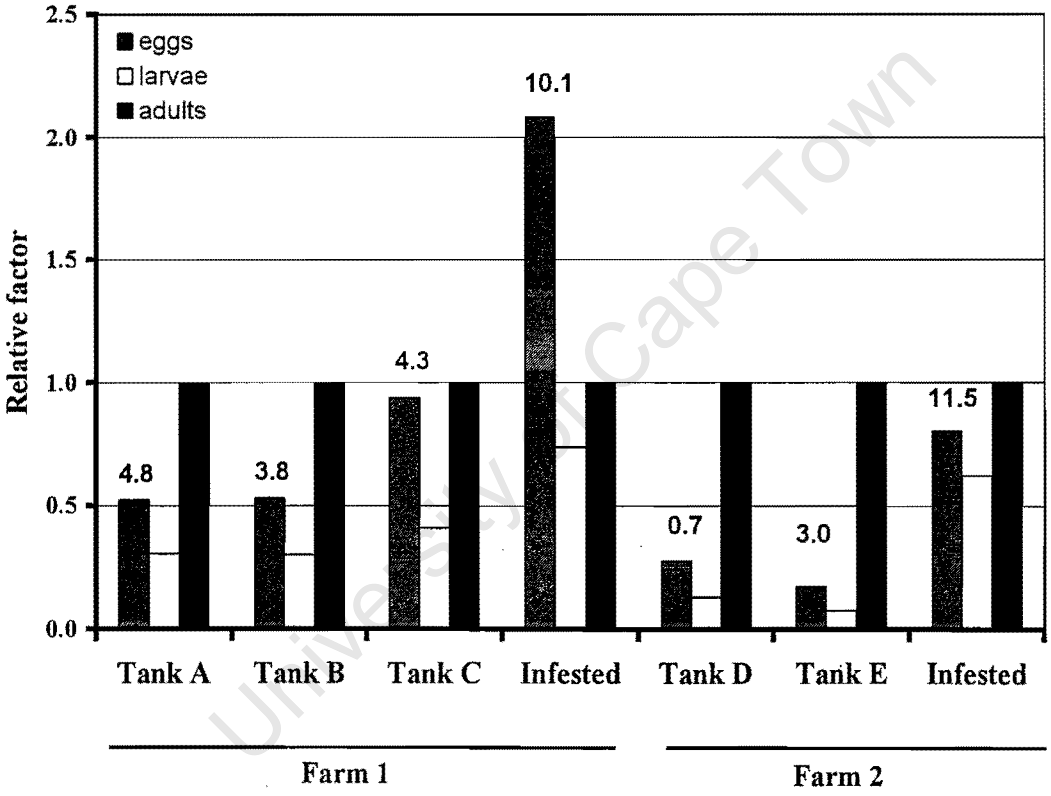

Figure 48: The ratio of eggs and larvae relative to adults are recorded for abalone that had been used as sabellid source animals in dispersal experiment two (Tank A, B & C). These are compared with animals of the same cohort, which had remained in the culture raceways. Similar data are recorded from another farm where some animals had been moved into laboratory conditions. Values above the bars indicate the larval counts/cm.

4.3.3 Starvation

From the starvation experiment it is clear that starvation had an effect on sabellid fecundity (Figure 49). The most rapid effect occurred in treatments A & B where no feed for the abalone was included into the tank. The difference in the filtration of the water had no significant effect. Treatments C and D were also similar to each other but there was also a reduction in fecundity and this may be due to the fact that the particulate matter was not very high in the tanks even with feeding. Indeed, these tanks are comparable to the above "laboratory conditions" (Figure 48). The differences between all the treatments became less over time and all seemed to approach a level where very few eggs and larvae were produced. This low production led to a low number of larvae settling at the shell edge (see count/cm values within the bars, Figure 49). The anomaly of treatments A and B having higher counts/cm than the treatments with higher egg productions is explained by the fact that the abalone were not growing due to lack of feed. At the end of the experiment the average weights of the abalone from treatments A - D were 16 g, 15 g, 26 g and 20 g respectively. Thus, the counts at the edge of the shell included past settlements and remained high in A and B, whereas new shell growth covered previous settlements in C and D and only current settlements were counted.

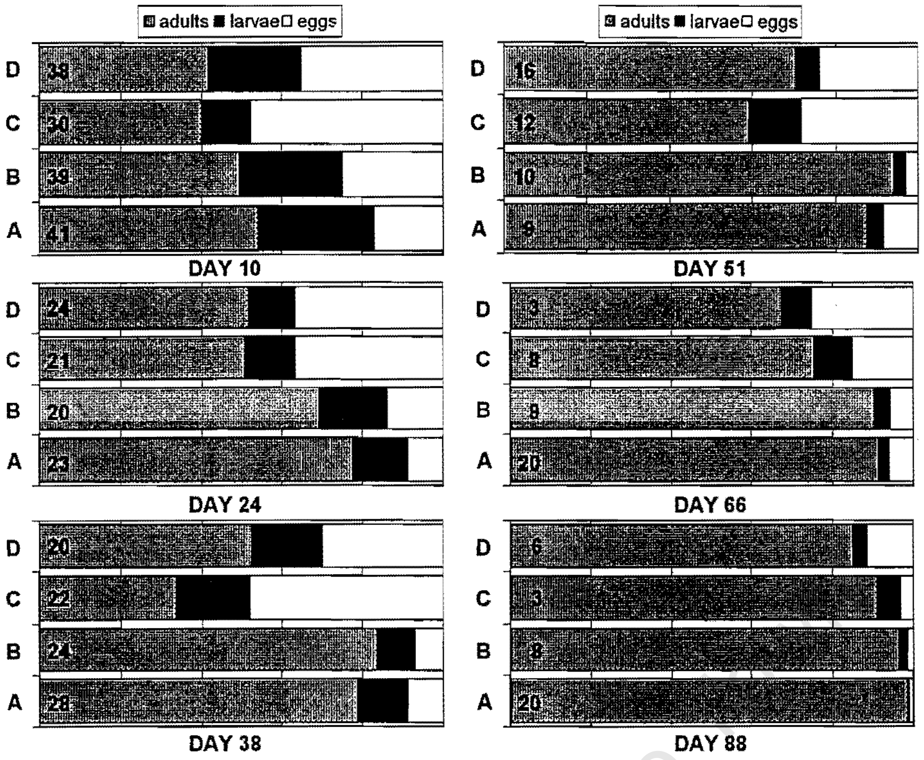

Figure 49: Results from the starvation experiment show the ratio of adult sabellids to larvae and eggs. Included within the bars are figures for the larval count/cm at the shell edge. Treatment A = no feed, 1 μm water; treatment B = no feed, 18 μm water; treatment C = artificial feed, 18 μm water; treatment D = kelp, 18 μm water.

4.3.4 Larval choice

Larvae observed in petri dishes were found to be very active, with a rapid crawling motion of about 1.5 body lengths per second. They resisted being dislodged by water currents by adhering to the substratum on a string of mucus. They possess two eyespots and what seem to be sensory tentacles at the anterior end (Chapter 2, Figure 29). It appeared from this preliminary experiment that the larvae preferentially settled under pieces of abalone tissue as opposed to dead shell, but when this was not available they settled on the glass of a petri dish, produced a tube and even metamorphosed to produce a feeding crown. Further development in the absence of a host was not studied.

4.4 DISCUSSION

The dispersal of larvae is an important consideration for assessing the risks to abalone farmers. However, it may also provide some insights into the observed patterns in the natural distribution of the worms.

It is possible that the easiest place for a larva to settle would be on the same host shell as its parent worm. This would be easily attainable since the larvae would simply have to crawl a distance of a few centimetres and locate the mantle. There are however potential disadvantages to this. Competition with other recruits from the same shell would result and then eventually the reproductive limitation of only having genetically similar individuals to mate with.

There would be obvious evolutionary advantages to the successful colonisation of a new, uninfested host. Although the larvae are large and crawl from the tubes to the settling site, they were also observed to be carried in the water column. This mechanism was not as successful at producing successful colonisation of distant hosts. Whether this larval movement was accidental or intentional is unclear. However, the benefits of locating a new host may outweigh the costs of losing larvae in the water column and thus larger scale dispersal behaviour by the larvae could be selected for. Genetic studies to determine the degree of similarity between individuals on a common host, and also between hosts, would be enlightening. Over a broader geographical range, this would also be extremely interesting given the anomalous patterns observed in the distribution of the sabellid.

The fecundity of the sabellids appears to be linked to the amount of suspended material available to them. The exploited food may be kelp detritus or abalone faeces or a combination of both. The importance of kelp on growth of suspension feeders has been demonstrated (Duggins & Eckman 1994, Duggins *et al* 1989, Eckman & Duggins 1991). Under laboratory conditions, suspended material becomes limited due to a combination of factors such as filtering of water and also less faecal production at lower stocking densities. This leads to an extreme reduction in the production of eggs and larvae. This fact is an important consideration for abalone farmers since a reduction in suspended solids within culture tanks should reduce the impact of the sabellid.

In a similar experiment, studying the effect of food quantity on fecundity of the polychaete *Capitella,* Qian (1994) found that when food was abundant, more eggs were produced, but the eggs were smaller and had lower energy content. He hypothesised that there is a trade-off between fecundity and egg quality so that when food-limited, fewer eggs are produced with higher quality offspring, which would favour their survival. This is a different response from that found in *Mytilus edulis* (Bayne *et al* 1978) where eggs not only fewer in number but also containing lower energy levels were produced in nutrient stressed adults. Although size and quality of eggs were not examined in the present study, the only difference that was noticed (but was subjective) was that the eggs appeared to be paler than normal. This may reflect their nutrient levels. The degree of nutrient stress suffered may be important too and the difference between starvation versus nutrient limitation may induce different responses.

An interesting observation on the effect of the sabellid on abalone growth arises from the dispersal experiments. As soon as abalone were exposed to sabellid larvae they experienced a reduction in shell growth. These same abalone, when removed from the source of larvae, experienced a surge in growth again. This implies that there was a direct physical interference of larvae on growth. The abalone appear to halt normal shell growth and are forced to cover up the larval invasion. The evidence for this is the presence of aragonite around the sabellid tubes (Day *et al* 2000). There may be a chemical interference in the shell deposition process since the crystallisation of shell follows a distinct pattern (Hawkes *et al* 1996, Fritz *et al* 1994, Erasmus *et al* 1994).

Dispersal of larvae within abalone farms is clearly a danger that needs to be understood. Larvae may enter the inflow pumped-ashore water although the numbers should be small given that the larvae are predominantly benthic. However, export of larvae from abalone tanks is significant and thus this water should be treated accordingly. The water-borne transport of larvae is limited however and thus compartmentalisation of larvae in baskets within tank systems provides a means of limiting the rapid dispersal of larvae, in the event of accidental introductions.

Since larvae were observed to settle and metamorphose on the glass of a petri-dish it is conceivable that the sabellids may be able to exist free of a host. Thus tanks should be properly cleaned when new abalone are introduced into a tank that has been infested previously.

CHAPTER 5 IMPLICATIONS FOR ABALONE MARICULTURE

5.1 INFLUENCE ON GROWTH RATES

To make abalone farming profitable, certain growth rate requirements have to be met. In this regard, factors that slow growth, have to be addressed. Preliminary data suggested that the sabellid infestations radically reduced growth rates. An experiment was therefore set up to determine how growth rates are influenced.

5.1.1 Methods

The worm was first noticed in South Africa during a growth experiment involving three cohorts of abalone. Three replicate baskets of each cohort: A (11-13 mm), B (20-25 mm) and C (35-41 mm) were held in three separate aquaria. Only one cohort (C) was found to be heavily infested with sabellid worms. The baskets were staggered in each aquarium to eliminate tank effects. Each basket contained 20 abalone, which were measured and weighed each month.

A second growth experiment was conducted to confirm the effect of the sabellids on growth. In this case, animals of the same size were used and either mixed together with five heavily infested abalone (sabellid effect) or kept isolated (sabellid free). This was a relative measure since it was not possible to find animals that were totally sabellid free. Three replicates, containing 10 abalone each were used in each case. Animals were weighed and measured at monthly intervals for 3.5 months. Larval counts were performed on a subsample of five animals at 17 days and then at each sampling interval. For this, the abalone was held upside down under a dissecting microscope and the mantle was carefully pushed aside to expose the growing edge of the shell. Larvae at the growing edge, between the spire and the last respiratory pore, were counted (see Figure 36 - Chapter 3). The length of this edge was measured using a vernier caliper. The number of larvae per cm of shell edge was then calculated. Control animals were not sabellid free, but had low levels of infestation, and thus it was necessary to include sabellid counts for control animals. In all treatments the abalone were maintained on a diet of the kelp, *Ecklonia maxima*.

5.1.2 Results

Growth rates of abalone from the initial growth experiment are presented in Figure 50. To allow comparisons between abalone of different sizes, growth was expressed as percentage length increments. During the first six weeks of the experiment, the animals in size class C showed virtually no growth. In fact, there was even negative growth in some aquaria, presumably due to shell breakage. The shells became very fragile and prone to breakage when infested. After day 75, the animals in size class C started to grow faster. This may have been an effect of laboratory conditions as discussed in Chapter 4.

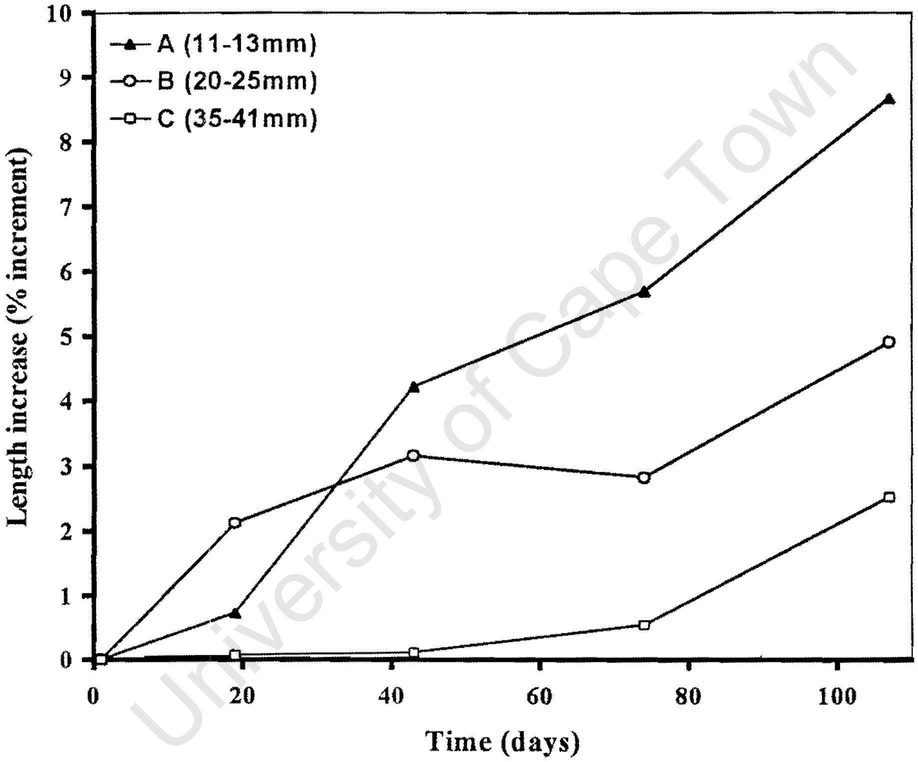

Figure 50: Growth increments over time of three cohorts of abalone in a growth study. Cohort C was found to be heavily infested by sabellids.

The results of the second growth experiment, in which animals of the same starting size were used, are summarised in Figure 51. Both length and weight data are given, along with larval counts at the growing edge of the shell. Since the control animals were found to have low levels of infestation, their larval counts are included with the last two measurements. The experiment must therefore be considered as a comparison in growth between moderately and lightly infested abalone. Significant differences in growth for both weight and length (t-test of means $p < 0.05$, df = 4) were recorded. Higher counts of larvae do occur in culture situations (up to 25/cm, see Figure 45 – Chapter 4) and these abalone are likely to exhibit even slower growth than represented here.

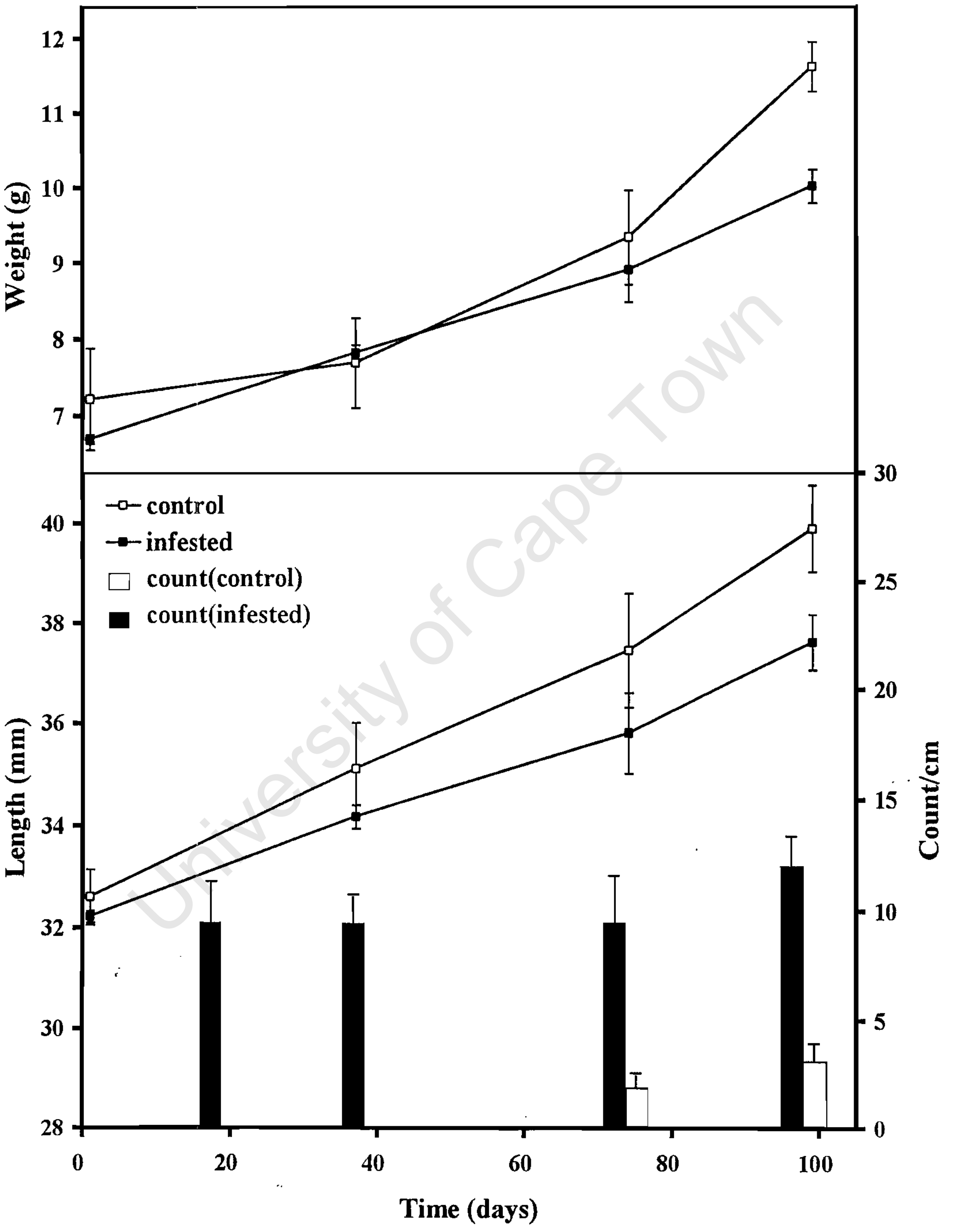

Figure 51: Growth trial to determine the effect of sabellids on growth. The histogram indicates the numbers of larvae at the growing edge of the shell. The control groups were found to have low levels of infestation which are included as clear bars. Error bars are one standard deviation of the mean.

5.2 OTHER IMPLICATIONS

In addition to a reduction in growth rates when the sabellid infestation is severe, farmed abalone may also suffer more severe damages. The response to large numbers of sabellid larvae settling under the mantle causes the shell deposition to alter. Thus the shell may take on a domed appearance due to downward growth instead of the normal lateral growth with fewer respiratory pores. This effect is particularly pronounced in the Californian species *H. rufescens* (Culver *et al* 1997)

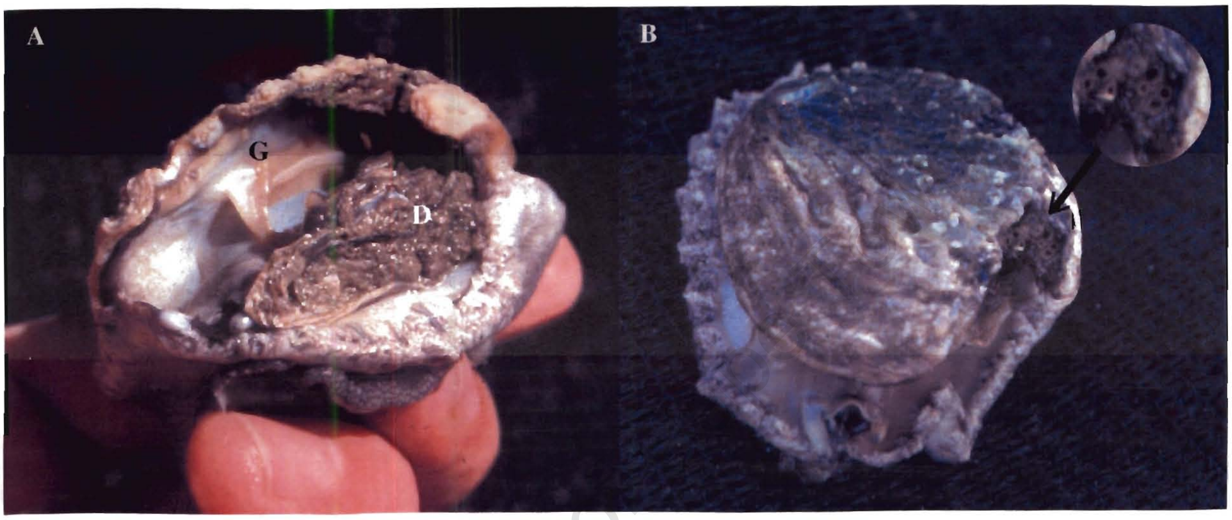

Figure 52: Photographs of sabellid infested abalone with secondary infestations of polydora in the mantle cavity. In both abalone the entire section of shell has broken away along the respiratory pores. A) View from below into the gill (G) cavity. Note the detrital material accumulated by the worms (D). B) View from above with an enlargement of the polydora burrow openings.

High levels of infestation also cause the shell to become perforated by larval burrows, which makes the shell extremely weak and prone to breakage. Under farm conditions abalone tend to push against each other and badly infested individuals typically exhibit broken shell edges particularly in the region of the tremata (Figure 52). Once the shell is broken here there may be a reduction in the respiratory performance of the abalone. Shell growth is also disrupted since that portion of the mantle will be attached to the broken piece of shell and unlikely to re-establish the normal structure.

Another negative effect is secondary infestation by other worms such as *Polydora* spp. These boring species are able to utilise the burrows created by the sabellid. In addition they easily invade the region described above where the shell is broken near the gills. Large mud blisters form in this region and it is extremely difficult for the abalone to recover from this sort of infestation. When an abalone has reached this stage of deterioration then the chances of mortality are extremely high. Even if survival is possible, the appearance of the abalone is extremely unpleasant and not really suitable for the desired whole-live market. See Figure 52.

5.3 DISCUSSION

The effect of the sabellid on abalone only becomes apparent at high densities. At low to moderate numbers, infested abalone do not appear to suffer any noticeable negative effects. Hence the reticence to term the worm a parasite. However, the effect of even one worm settling under the mantle induces the abalone to react in a way that is disruptive to its normal functioning. When high numbers of worms are present then the abalone is seriously compromised even to such an extreme that it dies. The two impacts described in this chapter are related to two different aspects of the worm. The first influence, reduction in growth, is caused by larval worms settling under the mantle. This may have no relation to the number of adult sabellids currently in the shell. For instance, a previously uninfested abalone, when placed along with heavily infested abalone, could experience a sudden reduction in growth. For the same reason a heavily infested abalone could experience normal growth if all the sabellids were to suddenly become infertile.

In contrast the weakening of the shell and breakage resulting therefrom is due to the adult sabellid loading within the shell. The larval production has no direct bearing on this. When an abalone has suffered from high densities of sabellid infestations with shell breakages and secondary infestation by polydorans then it often reaches a point of no return and gradually experiences a decline in condition. There is a loss in weight and almost zero shell growth of poor quality. These animals eventually die and it would be better to cull them at an earlier stage.

The impact of sabellid infestations on abalone mariculture is serious in various parts of the world. In California, where it was introduced it cost farmers thousands of dollars in lost stock and poor growth and directly or indirectly led to the closure of some installations. It has since spread to facilities in both Mexico and Chile, where farmers are experiencing similar problems. In South Africa, where the worm is native, there is the constant threat of new infestations. Infested stocks that are slow-growing and deformed occupy valuable tank space for long periods of time with poor conversion rates and yields. Many farmers here regard it as currently being the most serious threat to successful abalone faming.

CHAPTER 6 MANAGEMENT

6.1 INTRODUCTION

Information gained during the project provides the opportunity for guidelines to be set with respect to controlling the sabellid problem on abalone farms. By following these guidelines it should be possible to avoid the extreme effects that a sabellid epidemic may cause. Indeed, in California, some abalone farmers have been effective in eradicating the worms from their facilities through strict quarantine and culling procedures (Ray Fields *pers comm*). Release of sabellids into the wild at the outfalls of abalone farms also appears to have been contained and eradicated. Again this has been through an aggressive culling campaign, removing any shell debris containing worms and also all the susceptible host species in the area (Culver & Kuris *in prep*). This exercise has been successful because the sabellid is not endemic to California. Thus, once the worm has been removed from a system, there is little risk of reintroduction. In South Africa, on the other hand, the worm is abundant and widespread in the intertidal and subtidal all around the coastline. Thus, there is the constant threat of new infestations being introduced into a farm from the wild. Therefore, suitable management practices need to be in place continuously to prevent the introduction of the worm and also to limit the effect of the worm within facilities, by limiting its dispersal and its production. This chapter will discuss the risks faced by South African abalone farms and also provide information to assist farm managers in containing the sabellid problem.

6.2 EXPOSURE

All abalone that are produced in hatcheries are sabellid free. The abalone are initially simply too small for the relatively large sabellid larvae to establish themselves. The smallest abalone observed with a sabellid infestation were 9 mm (pers obs) and 2 mm (Culver *et al* 1997). Thus the aim of the abalone farmer should be to minimise the risk of contamination of these abalone for as long as possible during the growout process.

6.2.1 Sources of infestation.

6.2.1.1 Incoming water

Since sabellid have benthic, crawling larvae, the chance of them entering the water column could be expected to be very limited. However the action of water currents could place larvae in suspension and thus the risk of larvae entering farms via inlet water is very real. The results from Chapter 3 show that within a farm the larvae are transferred within the water column and there is, in fact, quite a high degree of export of suspended larvae from infested tanks. The cost of treating all the incoming water of a production-scale abalone farm would generally outweigh the benefits of negating what is estimated to be a small source of sabellid. One option available is filtration of the water, since 52µm sieves were effective in trapping larvae in these experiments (Chapter 3). A settlement trap could also be effective in settling out larvae before they enter culture tanks. Other methods of treating the water to kill larvae (e.g. chlorinating) are impractical on this scale.

6.2.1.2 Other gastropods introduced with feed (kelp)

Some abalone farms feed abalone macroalgae harvested from the sea. In South Africa the kelp, *Ecklonia maxima* is the bulk of the feed. There are species of gastropods associated with this alga and they are invariably brought into the farms along with the kelp. The species most commonly found is the kelp limpet *P. compressa*, which, although it is rarely infested in the wild, is susceptible in laboratory experiments and has been seen to be heavily infested when the kelp was collected from shallow intertidal region (pers obs). The other species commonly introduced with cast up kelp is *O. variegata*. Again this is rarely infested in the wild and thus must be considered a low risk. The potential of sabellid larvae being present on the kelp itself is unknown as larvae have not been observed when not in association with a host outside of the laboratory. To minimise these risks, the kelp should be cleared of all gastropods and dipping the kelp in freshwater for a period, 20 minutes at least, can even be considered. Cast up kelp should be avoided. Farmers who use artificial feeds do not suffer from these risks.

6.2.1.3 Infested abalone

Exposure of uninfested abalone to infested gastropods is the most effective way of transferring sabellids. Even the introduction of an infested abalone into a tank for a short time can lead to the entire tank being badly infested within a few months. Infested abalone can be introduced from the wild (e.g. broodstock) or from other abalone farms. Provided they are held separate from the uninfested stock all is well, however there are numerous ways that infestation can spread within a farm as discussed below.

6.2.2 Transfer within a farm

With the above in mind it should be taken for granted that portions of the abalone stock will always be infested. Generally this will be larger abalone purely because of the longer time of potential exposure. These abalone should be effectively managed to prevent or limit the risk of transfer to uninfested stocks. This transfer may take many forms.

Within tanks limiting the degree of physical contact between abalone can slow the spread of infestation and an effective way of doing this is by compartmentalisation. Baskets raised off the tank floor are effective in this regard, as the larvae do not effectively transfer between baskets and larvae also rain down from the baskets (Culver *et al* 1997).

Transfer between tanks is possible through larvae carried in the effluent water and tanks should thus each receive their own water supply and not be downstream from others. The potential of escapees from tanks crawling into adjacent tanks or being placed into uninfested tanks by staff needs to be addressed. Kelp feed should not be transferred between tanks as it may carry larvae. When tanks are emptied and used to house different abalone, they should be adequately cleaned to ensure that no larvae remain. Chlorine or freshwater can be used for this purpose. Infested abalone should be segregated as far as possible from uninfested stock to limit these effects. Housing them in a section of their own with dedicated staff is most desirable.

Grading of abalone is another risky operation particularly when grading machinery is used. Ideally, grading should follow a sequence beginning with abalone that are infestation-free and moving on to heavily infested abalone. Either that or the equipment should be sterilised between each batch of abalone.

6.3 MANAGING EXISTING INFESTATIONS

Once it has been established that a tank is infested with the sabellid and it has been properly isolated to prevent further spread of the infestation, it is necessary to minimise the effect that the worm may have on their growth. This may take two forms. The first aspect is to provide the best conditions possible for abalone growth. When abalone are growing faster the effect of larvae is reduced since the abalone can cope with the interference much better. Presumably this is because they are producing enough shell to cover any larvae that have settled. The second is to limit those conditions that favour worm productivity. There may be an interaction between factors, which affect both of these variables.

6.3.1 Stocking densities

Abalone farmers are forced to push stocking densities to the upper limit due to the high cost of providing the necessary infrastructure. But, the lower the stocking density the greater the growth rates of abalone will be. At lower stocking densities there will also be less physical contact between animals and there will be a lower transmission of larvae. This would limit the effect of larvae on growth too.

6.3.2 Water temperature

Higher water temperatures increase growth rates of abalone up to a point where they become detrimental to growth. For farmed *H. midae* temperatures typically range from 11-19°C. Temperatures around 18-19°C provide better growth rates, whereas when temperatures go above 22-24°C then the abalone become stressed and there is a reduction in growth. The effect that temperature has on the worm is unclear as limited work has been done. Finley *et al* (2000) suggest that the production of the sabellid is temperature dependant. Larval development was quicker at higher temperatures. Leighton (1998) has recorded that temperatures of 28°C for a period of 24 hours are sufficient to kill all stages of the worm. For *H. midae* these temperatures are intolerable and are thus not applicable. Farm experience has shown that the best results are achieved at optimum temperature for abalone growth notwithstanding that this may encourage faster worm production.

6.3.3 Particulate matter

Since the worms are filter-feeders that rely on suspended organic matter for their nutrition, the aim should be to limit the amount of food available. Cleanliness of tanks is important in this regard. When abalone were housed in the laboratory they always showed improved condition and the fecundity of the worms dropped, presumably due to lack of food.

6.4 MONITORING INFESTATIONS

Abalone farmers should regularly monitor their stock so that they can detect new infestations, locate abalone that are experiencing growth reductions and stress due to sabellid infestations and target areas in farm design, which encourage worm productivity. The following methods are intended as rapid assays for carrying out these tasks and should be comparable between farms too, as they are fairly robust despite a degree of subjectivity.

6.4.1 Level of infestation

This is an assessment of the current problem, with which the abalone are faced; a snapshot view of the condition of the abalone with respect to sabellid infestations.
Basically it is a standardised sabellid larval count as related to the growing edge of the abalone shell.

6.4.1.1 Method:

A one or two centimetre length is marked off in the centre of the growing edge of the shell. The numbers of sabellid larvae are then counted along this length (see Figure 53). The larvae can be observed easily by holding the abalone upside down between thumb and forefinger and gently pushing the mantle aside with a blunt knife or similar object. A dissection microscope is also needed for the counting. Since the shell of the abalone covers the larvae after a short time it is possible to see freshly settled as well as larvae that were deposited some time previously by virtue of the fact that the shell nacre is transparent (see Figure 53 B). All larvae that are seen should be counted (within limits) no matter if they are deeper in shell or freshly deposited provided they are still clearly visible. By this method a count of 19 larvae would be reached from Figure 53 B. The counts are then standardised by converting to a count per cm.

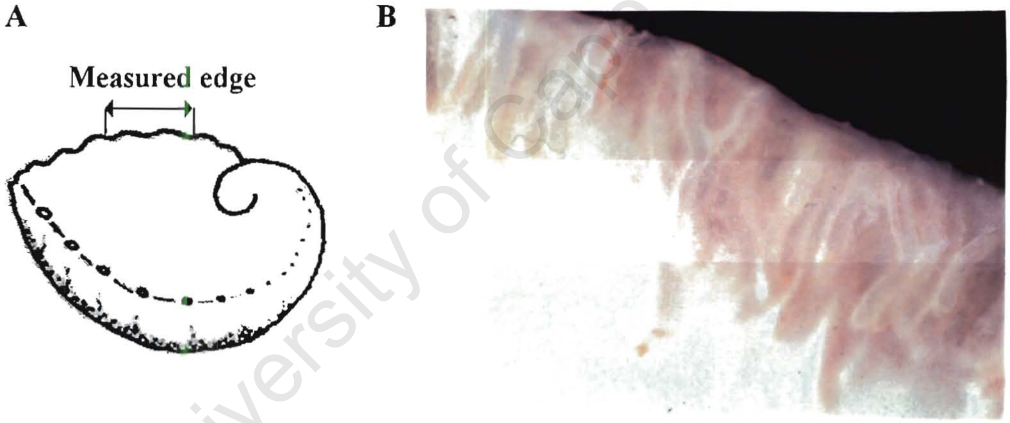

Figure 53: Diagram (A) to illustrate the method of counting larvae. A known length is marked in the centre of the growth edge using marker pen and then all larvae counted within that area. A typical view of a length of about 1cm is shown in (B) where the larval count would range between 17 and 21 depending on subjectivity.

6.4.1.2 Results:

Low to moderate counts (1-5 larvae/cm) are an indication that the abalone are not too adversely affected and shell growth is not too impaired. In contrast high counts (>10 larvae/cm) would be cause for concern as abalone under these conditions are likely to be slow growing and also stressed. A low count/cm can indicate two things. Firstly that there is little source of sabellid larvae or conditions are not good for sabellid reproduction. Secondly it is possible that the larvae are just as abundant but the abalone are growing at such a rate that the number of larvae relative to clean shell are low. Obviously the opposite is also true and a high count/cm may be from an old spate and the abalone have not grown beyond that, even though the sabellid stocks may not be very productive. This is why a separate measure of sabellid productivity is needed.

6.4.2 Sabellid reproductive index:

This is a measure of the productivity of the sabellid worms that have previously infested the abalone shells i.e. the source of future infestations. This measure is important in providing information about what conditions favour abalone growth in the presence of sabellid worms.

6.4.2.1 Method:

An infested abalone shell is shucked and the shell crushed into small pieces using a pair of side-cutters or pliers. The broken shell is placed in a petri dish in seawater and any large pieces of shell removed. A counting grid of 0.5 –1 cm blocks should be placed beneath the dish. Then, the area should be systematically covered using a dissection microscope, using the grids as a location guide, and the numbers of larvae, eggs and adults counted. The ratio of eggs and larvae relative to adults can then be calculated.

This method also has a subjective component in the definition of the various categories. Sabellid eggs develop through a few stages into crawling larvae. They start as round orange eggs, which divide and cleave into orange, worm-like, half-moon shaped larvae and then eventually into a crawling larvae with eyespots and setae. Thus, larvae have been defined here as all the motile stages (even without eyespots) and eggs as all the inert looking stages including the half-moons (see Figure 5 in Chapter 2).

Adults have been classified as all worms that possess a feeding crown even though this definition would include juvenile worms, which are unlikely to be reproductive. But to distinguish between the two would be too difficult for quick sampling. Typically 2-3 abalone from a particular treatment are sufficient replication.

6.4.2.2 Results

The results can be used to assess the effectiveness of methods to control the worm productivity. Typical results are presented in Table IV.

Table IV: Examples of high and low sabellid productivity counts

		Count	
Low fecundity example	**Adults**	**Larvae**	**Eggs**
Numbers	88	14	23
Ratio	88/88	14/88	23/88
% relative to adults	100	16	26
High fecundity example	**Adults**	**Larvae**	**Eggs**
Number	88	67	124
Ratio	88/88	67/88	124/88
% relative to adults	100	76	141

CHAPTER 7 TREATMENT

7.1 INTRODUCTION

Although management recommendations as discussed in Chapter 6 have proved to be useful in containing infestations and allowing reasonable growth of abalone, there is a serious demand for a treatment to contain or eradicate the worm. Only extreme enforcement of management practices would be effective in containing the problem. With the constant threat of introduction of sabellids from the natural habitat through pumped seawater or harvested macroalgal feeds, it is conceivable that it would be impossible to eliminate the sabellid completely from stocks. There would thus be a constant threat of it reaching epidemic proportions within sectors of a farm. Thus, the development of a treatment that would be able to reduce sabellid intensities to low levels or limit larval production, would be extremely beneficial.

There has been much work done along these lines, with limited success unfortunately. Trevelyan *et al* (1994) explored a number of chemical agents and found nothing that could harm the worm without adversely affecting the abalone. The fact that the worm is able to withdraw into its burrow provides it with a safe microenvironment that affords it protection against the extremes of the external water environment. A number of preliminary trials conducted in the present study showed that the worm was extremely resistant to a number of treatments such as salinity changes, vermicides, dehydration and anoxia. This line of experimentation was not pursued, based on others' experience and the fact that the abalone in each case were found to be more sensitive.

The idea of a potential predator, such as an isopod to be used as a biocontrol was considered. But this was not likely to be effective, as sabellids show remarkable powers of regeneration, after transections at almost any level of their bodies (Hill *et al* 1993, 1994). Replacement of an entire head with branchial crown, collar, mouth and neural ganglia and connectives is possible after an anterior cut (Hill *et al* 1993). There is a possibility that predators could be found which would nip away the exposed branchial crown. This would act to reduce the time for feeding and thus decrease production. This would be a limited control since it would be non-lethal but inhibit growth and reproduction. In two spionid species the loss of one or two palps caused the worms to increase their exposure time, presumably as a response to the reduction in feeding efficiency. There was a trade off between the risk of predation and the gain of energy to replace lost parts (Lindsay & Woodin 1992). Kuris and Culver (1999) performed quite extensive trials using predators as potential control agents without success.

The one success to date involves a physical method of control. Trevelyan *et al* (1994) developed a method utilising a low melting temperature wax dip, which was effective at coating the abalone shell and smothering the worm burrows. Although sabellids were killed, the method had many shortcomings in that it was extremely labour intensive, and caused relatively high abalone mortalities.

Another successful method, which is not available to *H. midae* farmers was reported by Leighton (1998), who showed that all stages of sabellids in red abalone, *Haliotis rufescens,* were killed if maintained at water temperatures of 28.5°C for 48 hours. These temperatures were near the thermal tolerance of the abalone tested and a few mortalities of abalone occurred. Naturally these temperatures are way above *H. midae*'s tolerance.

It was decided that the only way to achieve success would be to target differences in the two species and exploit these to develop a successful treatment. A major difference that was apparent was that the worms are filter feeders whereas the abalone are grazers. Thus it was decided to try and introduce an encapsulated toxin in microparticle form, which would target the worm only, by virtue of the fact that it would be the only one able to ingest significant amounts.

7.2 METHODS

7.2.1 Preparation of gelatin microcapsules

Encapsulation by complex coacervation was carried out by the CSIR Polymer Research Group in Pretoria. The capsules were made in the size range of 1-30 µm.

The basic method involved the emulsification of oil in a gelatin solution. The addition of a second polymer solution (gum arabic) and adjustment of the pH resulted in the formation of coarcevate droplets, around the oil. The hardening of the capsules was achieved by cooling, which resulted in the gelling of the coarcevate around the oil droplet. Further hardening was achieved by adding formalin to chemically crosslink the gelatin. To complete the chemical hardening reaction, the temperature of the system was increased to 50°C. The microcapsules were then rinsed to remove excess formalin (Kondo 1979). Various compounds were added to the oil phase for experimental trials. A dark blue pigment was used as a visual indicator and oil soluble toxins (moxidectin, trichlorfon, phenanthrene, ivermectin) were added at saturation concentrations to assess toxicity.

7.2.2 Oil Emulsions

The method used was based on prior work done by Heras *et al* (1994) and Robinson (1992). Both of these papers describe the use of lipid microspheres in the enrichment of the diets of oysters.

Fish oil (purified anchovy oil), soy lecithin (Sigma Aldrich) and vegetable oil (Sunflower oil) were mixed in the ratio 50:30:20 (% w/w) by sonicating with a sonic probe at 40°C (Branson, sonifier – cell disruptor, B-30). Six grams of this mixture was then sonicated with 40 ml of a 2 % solution of polyvinyl alcohol (PVA) in filtered seawater preheated to 40°C. The size distribution of the spheres depended on the time period and power of the sonication. Less than 5 minutes was sufficient to produce beads that were smaller than 30 µm. Sudan III was used as a stain to monitor the passage of the beads. Toxins were incorporated in the lipid phase (ivermectin, phenanthrene, copper sulphate) at high saturation doses. In the case of copper sulphate, which was not oil soluble, the crystals were ground into a fine powder and then mixed into the oil by sonication and formed a precipitate. The idea was that upon digestion within the worms gut, the copper ions would be released and end up in solution within the gut.

7.2.3 Application of beads to sabellids

The beads were typically rinsed to remove unencapsulated toxins either by repeat centrifugation (5000 rpm for 15 min) and dilution with filtered seawater or by use of a dialysis membrane. The beads were delivered to sabellids within shucked or live abalone in 250 ml deep-walled petri dishes. The feeding activity of the worms and the behaviour of the abalone could be monitored by placing the dishes under a dissecting microscope. At various intervals after the feeding period the shells were crushed and the worms were examined under the compound microscope as described in Chapter 2.3.1.

7.2.4 Liposomes

The above methods were restrictive in that they only permitted oil soluble toxins to be utilized and required that the worms digested the oil to gain access to the toxins. A liposome method of bead formation, which allowed the encapsulation of an aqueous phase within a thin lipid phase, was chosen to solve some of these problems. Liposomes have been used as delivery tools of nutrients or therapeutants in the aquaculture industry before (Touraki *et al* 1995, Hontoria *et al* 1994, Ozkizilcik & Chu 1994). There is extensive literature on liposome production, properties and their use as carriers of drugs (review in Gregoriadis 1984).

In short, liposomes are artificial spheres consisting of a bilayer of lipid encapsulating a toxin or other material to be investigated. Phospholipids are the main constituents of liposomes and precipitate into monolayers in aqueous solutions. The amphiphilic nature of these phospholipids maximises the interactions with water by minimising the contact of the hydrophobic tails with water thereby forming a micelle. Liposomes can be made from a variety of lipids. Generally they are composed of phosphatidylcholine and cholesterol from egg yolk or soya bean in a ratio of 2:1 or 1:1 (phosphatidylcholine:cholesterol). The cholesterol intercalates between adjacent phosphatidylcholine molecules and attenuates the diffusion of solutes through the bilayer. There are three main types of liposomes based on the diameter of the beads. Large multilamellar liposomes (ca 20-200 μm in diameter) unilamellar liposomes (ca. 20-50 μm in diameter) and small unilamellar liposomes (ca. 20-50 nm in diameter) (Woodle & Papahadjopoulos 1989). Large unilamellar liposomes are the liposomes of choice as they have high encapsulation efficiency (greater than 14 %) and are reported to be more stable (Shek *et al* 1983). There are various mechanisms for preparing liposomes, however all utilise a lipid phase and an aqueous phase to produce the liposomes.

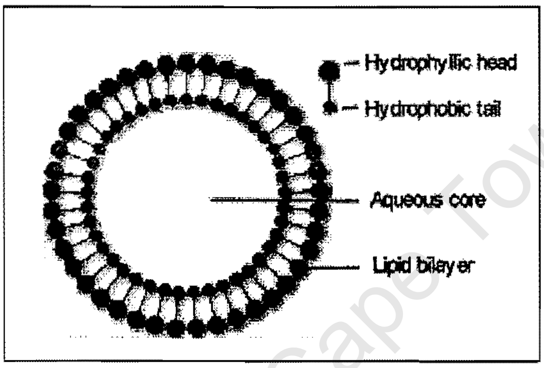

Figure 54 A: Diagram to illustrate the structure of a unilamellar liposome.

Lipid molecules are amphiphiles (hydrophobic on one side and hydrophilic on the other) and thus form bilayers as shown in Figure 54 A. The lipids in these bilayers are in the same configuration as those forming cell membranes in humans and other higher animals. Agitation of the lipid preparation by ether vortexing or sonication results in conversion from a large multilamellar (LMV) onion-type vesicle to one that is unilamellar containing a single membrane encapsulating the aqueous phase. Two methods were utilised for liposome preparation:

7.2.4.1 Large multilamellar vesicles (LMV)

The lipid (Phosphatidylcholine extracted from soya) was dissolved in chloroform. The solution was evaporated at 25°C in a rotary evaporator (Buchi). The preparation was kept above the phase transition temperature of the lipid (-7°C to –15 °C) using a water bath. The resultant lipid film was then hydrated with the aqueous phase consisting of a fluorescent dye, malachite green in phosphate buffered saline or seawater. The organic phase was removed under agitation using glass beads under continuous rotary evaporation. The resultant LMV's were subsequently cooled and placed in a dialysis membrane at 4°C to remove the unencapsulated aqueous phase. The vesicles were identified by fluorescent microscopy as vesicles with average diameters of 5 - 100 μm.

7.2.4.2 Reverse phase evaporation vesicles (REV)

A method for creating large unilamellar vesicles was utilised as described by Szoka and Papahadjopoulos (1978). Phosphatidylcholine was extracted from egg-yolk by the method of Singleton *et al.* (1965) using differential solvent extraction over aluminium oxide. The lipid was dissolved in chloroform and cholesterol was added to stabilise the membrane. A molar ratio of phospholipid:cholesterol of 50:50 was used. The lipid-cholesterol mixture was added to a reaction flask and the organic phase removed under reduced pressure by rotory evaporation at 25°C. Diethyl-ether was added to the lipid film along with the aqueous phase (consisting of 20 mM malachite green in sea water or phosphate buffered saline) and sonicated in an ice bath at 4°C. The diethyl ether was removed at 40°C in the rotary evaporator to remove the organic solvent. At this stage the multilamellar liposomes were formed by reverse phase evaporation. Buffer (either phosphate buffered saline (PBS) or seawater) was added and the mixture, which was sonicated at 4°C to convert the liposomes to the unilamellar, form. The suspension was immediately extruded through a 0.2 μm polycarbonate membrane. This served to decrease the heterogeneity of the preparation. Any unentrapped material was separated from the liposomes by means of Ficoll centrifugation based on the method of Fraley *et al.* (1981). Liposomes were mixed with an equal volume 20 % Ficoll in a centrifuge tube and overlaid with 2.0 ml of 5 % Ficoll and 0.5 ml of Phosphate buffered saline (PBS). The preparation was centrifuged for 20 minutes. Liposomes banded at the 5 % Ficoll interface and could be aspirated in a volume of 150 μl. This method successfully removed 95 % of the unentrapped material. Excess Ficoll and malachite green was removed by dialysis for 18 hours against the same buffer (seawater or phosphate buffered saline). The vesicles were identified by negative staining electron microscopy. Carbon-coated copper grids were floated on a 20 μl sample of freshly dialysed liposomes for 10 minutes. The grids were washed with distilled water prior to flotation for 10 minutes each on 2 % uranyl acetate and 1 % lead citrate. Samples were viewed and photographed in a Ziess EM109 transmission electron microscope.

7.2.4.3 Ingestion and digestion of the liposomes by the sabellids

A water soluble fluor (malachite green) in phosphate buffered saline or sea-water was used as the aqueous phase to be encapsulated within the liposomes. The fluor enabled visualization of individual liposomes under the fluorescent microscope. As previous microcapsule preparations investigated failed to be digested by the sabellids, it was decided to ascertain whether liposomes were digested or lysed in the gut prior to continuing with the next step of incorporating a toxin. Two methods were highlighted as having the potential to reveal whether the liposomes were digested. These were:

1: FLUORESCENCE MICROSCOPY AND FLUORIMETRY.

By using a fluorescent marker within the liposomes it was possible to test the encapsulation efficiency of these liposomes (as this would be an important factor when encapsulating a toxin) and also whether the liposomes were lysed to release the contents. The mechanism of fluorescence relies on a principle called autoquenching. A molecule fluoresces due to the excitation of its electrons, which then emits light. The molecule absorbs energy from a particular wavelength and emits light at another wavelength. The fluor is autoquenching at high concentrations as the excitation and emission spectra overlap. As a result of this overlap, the transfer of electrons (fluorescence energy transfer), which is not detectable by fluorimetry, occurs between adjacent fluorescent molecules. Membrane damage of liposomes by detergent or other means results in the release of the fluor and its dispersal into surrounding buffer thereby diluting it. Whereas the distance between each fluorescent molecule inside the liposome was small, that in solution is much greater and the fluorescent energy transfer, which is inversely proportional to the sixth power of the distance between donor and acceptor molecules, decreases. Thus, intact liposomes are essentially non-fluorescent but once damage has occurred, enhanced fluorescence is observed (Weinstein *et al* 1984). The fluorescent marker used in this case was malachite green and the detergent Triton X-100 was used for disruption of membranes at a final concentration of 0.02 %. An Aminco SPF 500 spectrofluorimeter was used to measure the intensity and wavelength of the fluorescence.

An increase in fluorescence of the voided material after addition of detergent would indicate that the liposomes traversed the sabellid gut undamaged, and that total digestion had not taken place. This technique would not give quantitative data, as it would be difficult to assess how much the sabellid had eaten. Two parallel experiments were carried out:

Each experiment contained one unfed sabellid in seawater, and four fed sabellids (a total of 10 sabellids). Liposomes were prepared as described previously. Approximately 100 μmoles of liposome was added to a volume of 250 ml containing sabellid worms in shell. These worms were pre-starved for 48 hours and allowed to feed for 10 minutes (experiment 1) and 20 minutes (experiment 2) respectively. After feeding, the sabellids were removed from their shells and rinsed with seawater. Each sabellid was transferred to a reaction vial containing 1 ml of seawater. Fluorescence was determined periodically and after 3hours, Triton X-100 was added to a final concentration of 0.01 %. Fluorescence was determined at 217 nm.

2. ELECTRON MICROSCOPY

Since the liposomes were too small (less than 50 μm in diameter) to visually assess under a compound microscope, disruption of the membrane by the sabellids was in question. It was thus necessary to determine this using a transmission electron microscope (TEM).

Firstly, the liposomes used for the feeding experiments were themselves examined by electron microscopy. Liposomes were prepared as previously described and immobilised in 2 % agarose. The preparation was sectioned into 1cm rounds and fixed with 2.5 % glutaraldehyde in PBS. Post fixation was in 1 % osmium tetroxide for 90 minutes after which the agarose was subjected to ethanolic and acetone dehydration followed by five changes of epoxy resin. The epoxy resin was hardened at 60°C after the sixth change prior to sectioning using a Reichert Ultracut-S ultramicrotome.

Sections of approximately 90 nm were cut and floated on copper grids prior to staining for 10 mins each with 2 % uranyl acetate and 1 % lead citrate.

Secondly, sabellid worms fed with liposomes after starvation for 5 days were viewed under the fluorescent microscope. This was done to ensure that ingestion had taken place. These sabellids were then immobilised in cacodylate buffer containing 2.5 % glutaraldehyde after 30 minutes and 2.5 hours respectively. Post fixation was in 1 % osmium tetroxide for 90 minutes followed by ethanolic and acetone dehydration followed by five changes of epoxy resin. After the sixth change the resin was hardened at 60°C prior to sectioning. Various portions of the gut were sectioned to 90 nm using a Reichert Ultracut-S ultramicrotome. These sections were treated as before. Visualisation and photography was in a Zeiss EM109 or 200CX transmission electron microscope.

Use of gold colloids as (TEM) markers

A method specific for the electron microscope was also investigated to assess digestion. The aim was to encapsulate inert electron-dense colloidal gold within the liposomes. Disruption of these liposomes by digestion or other means would result in these microparticles dispersed throughout the gut. Aggregations of the particles would indicate that these liposomes remained intact and that digestion had not occurred. The advantage of this method was that it did not rely on negative staining techniques since the particles would be clearly identifiable by TEM. Colloidal gold (atomic no. 79) was prepared by reduction by sodium citrate (Hyatt 1986, Vardell & Polak 1987). A solution of 50 ml of 0.01 % $HAuCl_4$ was heated to boiling and 1.75 ml of 1 % sodium citrate added. Boiling was continued until a lavender colour resulted. This colour change was indicative of gold colloid with a diameter of 12 nm. The resultant suspension was used as the aqueous phase for the liposomal preparation.

7.2.4.4 Toxin test

It was decided to investigate whether a toxin could be incorporated into the liposomes and fed to the sabellids. Since copper sulphate is a known potent toxin and was readily available, it was decided that this should be used for the preliminary studies. Copper sulphate was used in the aqueous phase during the formation of LMV's as described above. Copper sulphate proved to be problematic in that it interfered with the formation of the membrane bilayer due to the highly charged ions formed. Free radicals are generally problematic in liposomal systems since they cause peroxidation of the lipid bilayer (Konings 1984). Thus, the choice of toxin is critical because of these chemical interactions. Chemicals can be bound to the lipid phase or the aqueous phase depending on their properties with varying degrees of efficacy (Defrise-Quertain *et al* 1984).

7.3 RESULTS

7.3.1 Gelatin capsules

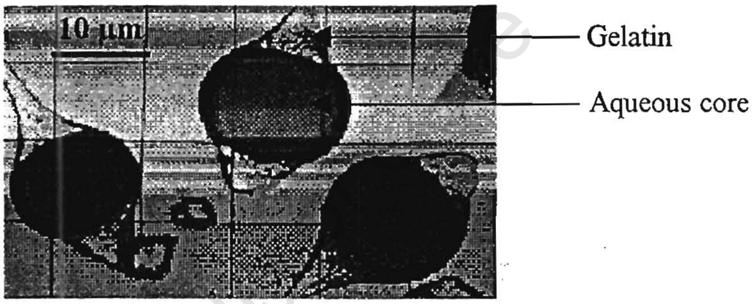

Figure 54 B: Microcapsules formed by the process of complex coacervation. Picture courtesy of P. Xulu, Mattek, CSIR.

The gelatin coated coacervate microcapsules were made in the size range 2-40 µm (Figure 54 B). Initially they contained a blue pigment. Sabellid worms that were exposed to the capsules in aerated seawater readily ingested the capsules. After a 1-2 hour exposure most individuals had large volumes of capsules concentrated within the main gut (Figure 55). The largest particle size that was measured within the gut was 35 µm in diameter. Most particles were in the size range of 3-15 µm. After another 2-3 hours the capsules had been transported to the hind-gut where they appeared to still be intact (Figure 56). This observation was based on a visual assessment since there was no dispersion of the blue pigment .

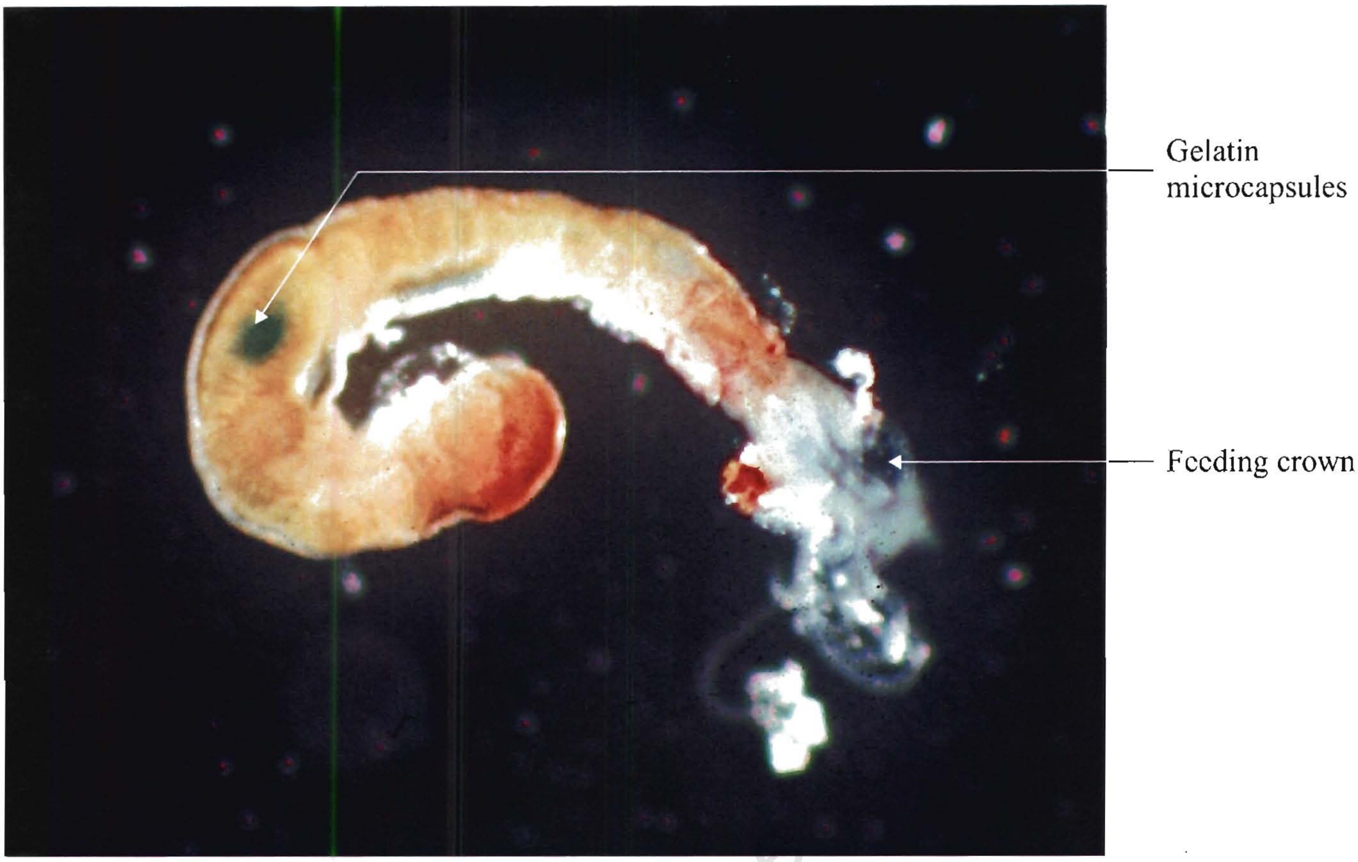

Figure 55: An adult sabellid removed from an abalone shell after feeding on blue-stained gelatin microcapsules, viewed under a dissection microscope. (40 x MAG)

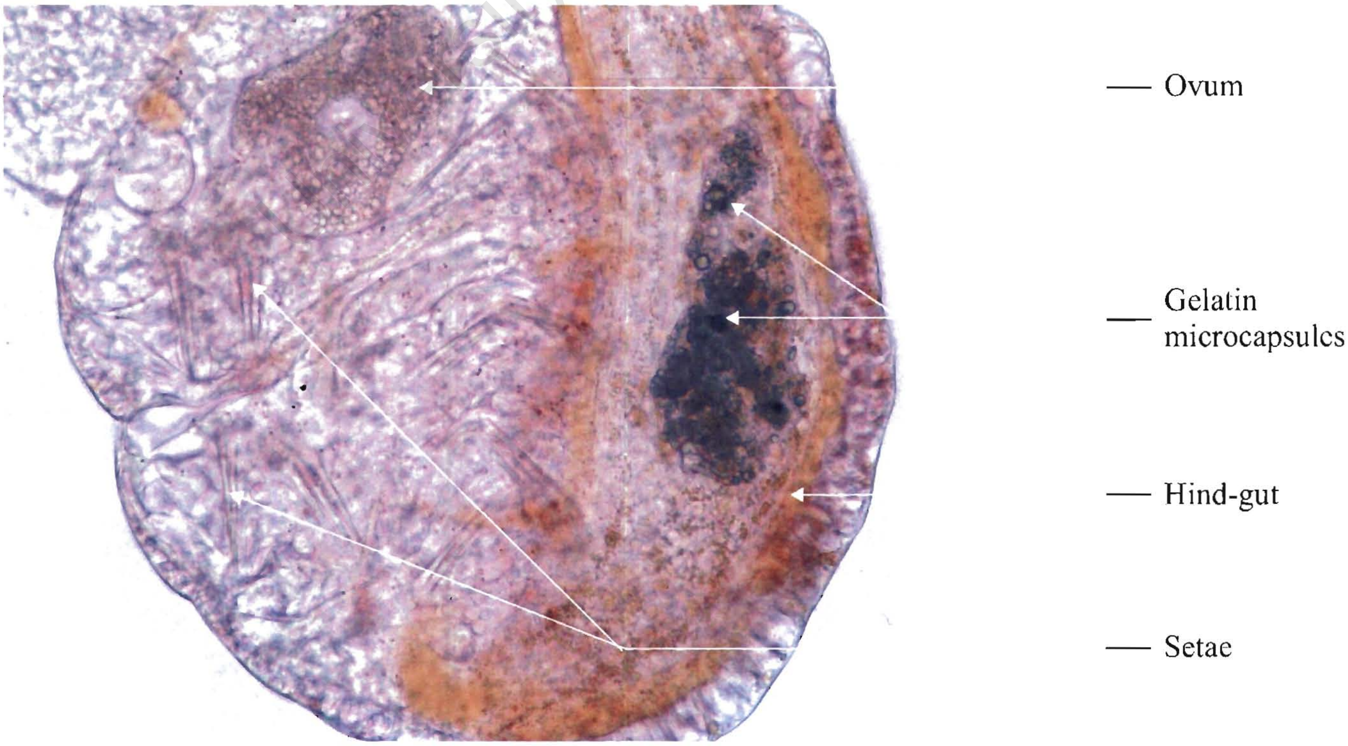

Figure 56: An adult sabellid removed from an abalone shell after feeding on blue stained gelatin microcapsules, viewed under a compound microscope (400 X magn.). The individual capsules are visible in the hindgut and appear totally undigested.

The toxins (moxidectin, trichlorfon, phenanthrene, ivermectin) incorporated in the capsules supported this observation, since the worms showed no adverse reaction to these capsules either.

7.3.2 Oil emulsions

If the sabellids were unable to digest the gelatin in the above formulation then the oil emulsions would be a way to avoid this problem. The oil beads formed were of a suitable size range (Figure 57) and the pigment again enabled visual assessments. The sabellids ingested the formulations readily as before. The beads within the gut coalesced into larger spheres which indicated that some alteration occurred (Figure 58). However, again there was no dispersion of pigment. The large, resultant oil vesicles were voided within hours after ingestion. When phenanthrene was included in the oil there was no noticeable effect on the worms after ingestion. When copper sulphate crystals were included in the formulation then the worms showed signs of stress, withdrew into their burrows and would not feed. However, when the beads were rinsed overnight in a dialysis membrane, the worms ingested the beads readily once more. The explanation for this could be that there were copper ions bound to the outside of the beads that the worms detected. After rinsing, these were removed. There should still have been copper ions within the beads, however and the lack of response by the sabellids after eating the rinsed beads indicates again that digestion was either too limited or not occurring at all.

7.3.3 Liposomes

7.3.3.1 Investigation of the encapsulation efficiency of liposomes.

The REV liposomes were characterised using the TEM as having average diameters of 50 – 100 nm. Liposomes containing malachite green were assayed in an Aminco SPF 500 spectrofluorometer at 217 nm. Malachite green was found to have a excitation wavelength peak at 217 nm and an emission wavelength peak at 316 nm.

The relative background fluorescence at a dilution of 1 % was determined to be 0.022. Addition of Triton X-100 (a detergent) to a final concentration of 0.01 % resulted in

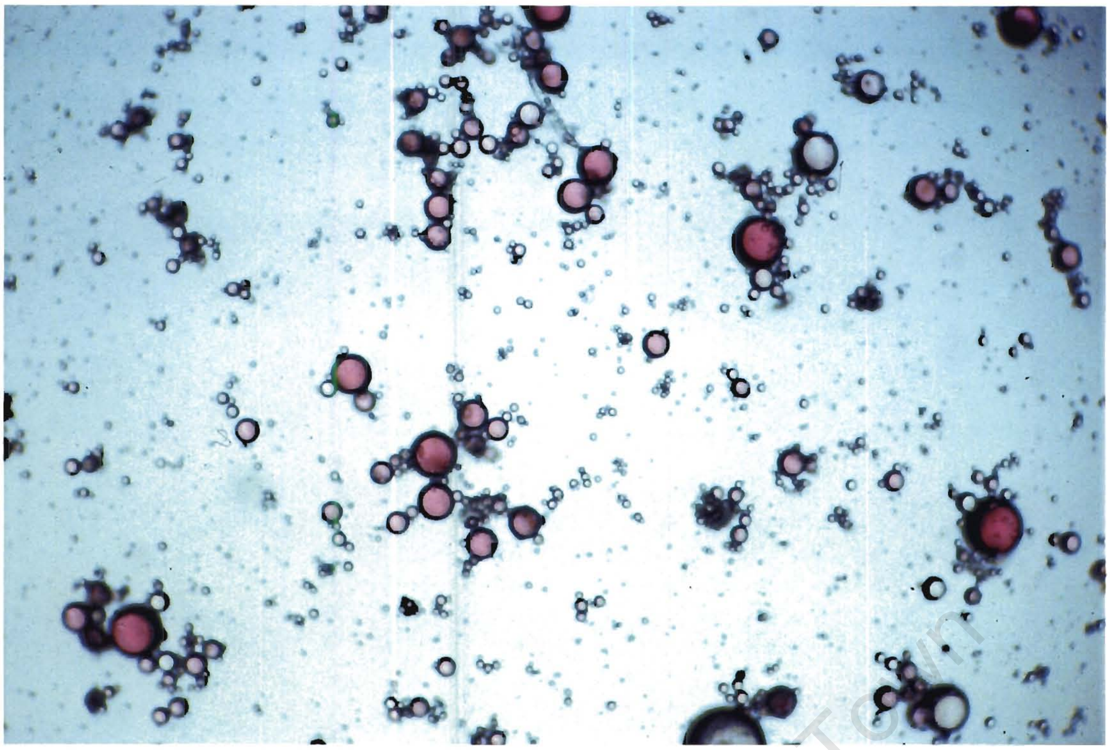

Figure 57: Oil ~~im~~emulsion droplets viewed under the compound microscope at high power (400 x magn.). Stained with Sudan black.

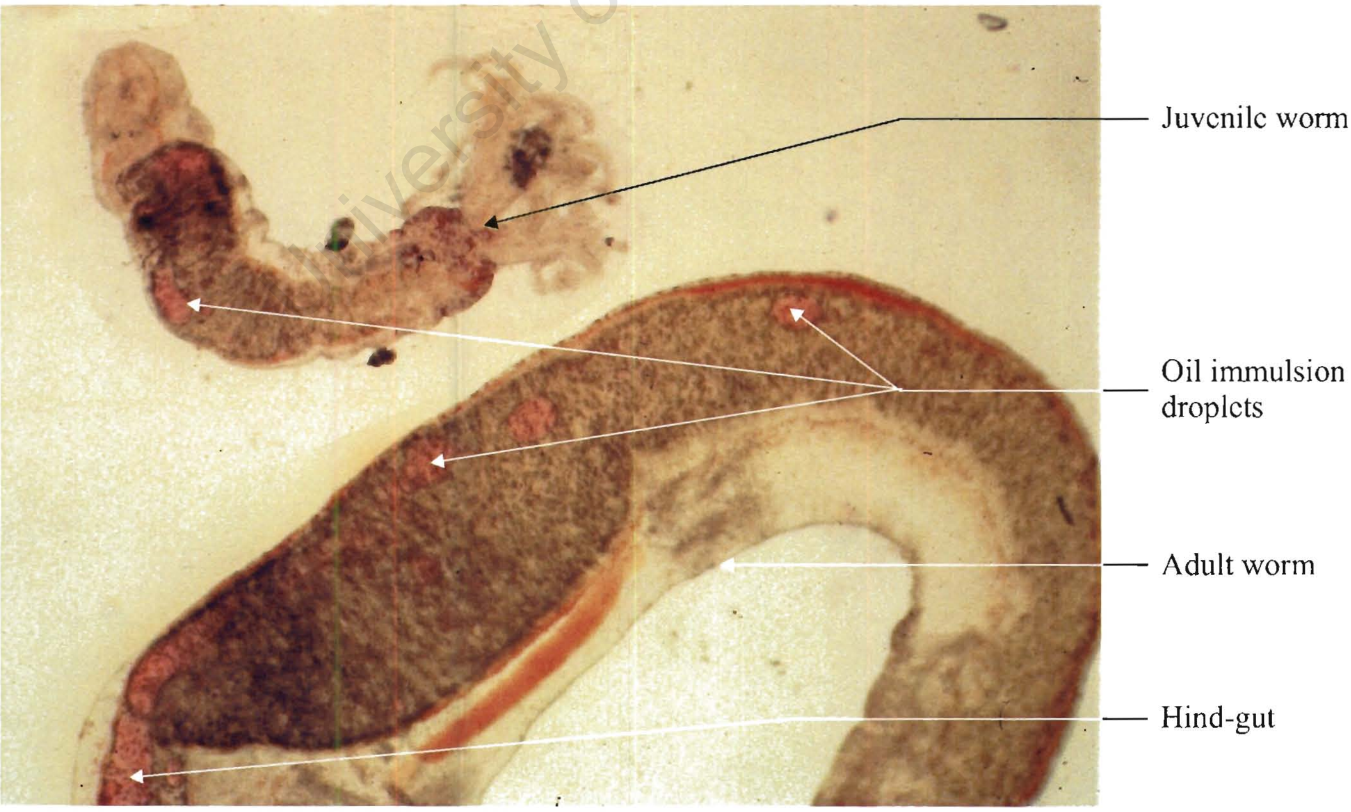

Figure 58: A juvenile and adult sabellid worm removed from the abalone shell after feeding on the oil ~~im~~emulsion above. The droplets have coalesced into larger droplets which are passing into the hindgut of the adult wormas one large vesicle. (MAG 400×)

the fluorescence increasing markedly to 0.570. This indicated encapsulation of malachite green within the liposomes. Moreover, an encapsulation efficiency of 11.5 % was determined using the formulae of Guiot and Baudhuin (1984). These authors reported an encapsulation efficiency of 14 % for liposomes with an average diameter of 100 nm.

7.3.3.2 Ingestion and digestion of liposomes

1: FLUORESCENCE MICROSCOPY AND FLUORIMETRY

The same liposome preparation used for electron microscopy was fed to the sabellids and ingestion confirmed under a fluorescent microscope. An increase in fluorescence indicative of ingestion was observed at various intervals after feeding. Sabellids were examined 5 minutes after ingestion then at intervals of 1 hour later and finally 2.5 hours after feeding. Soon after ingestion, the liposomes appear to have been packaged into a large clump within the foregut (Figure 59). No fluourescence could be detected in either the foregut or the hindgut of the control samples (sabellids that had been starved for 5 days). After 2.5 hours from the onset of first feeding, liposomes could be seen as clumps of fluorescent spheres, which had shifted to the hindgut of the sabellids (Figure 59). Several parallel sabellid samples had voided the liposomes after 2.5 hours. These voided vesicles appeared to still be intact, but a visual assessment was unreliable. This experiment failed to indicate digestion since the liposomes themselves fluoresced immediately after ingestion, and at two hours after ingestion were not noticeably brighter than those immediately after feeding.

The relative background fluorescence of liposomes only at a dilution of 1 % was determined to be 0.015. Addition of Triton X-100 (a detergent) to a final concentration of 0.01 % resulted in the fluorescence increasing to 0.200. The data for this experiment are shown in Table V. The mean ± standard deviation for experiment 1 before and after addition of the detergent was 0.118 ± 0.08 and 0.26 ± 0.107. The mean ± standard deviation for experiment 2 before and after addition of the detergent was 0.2 ± 0.08 and 0.246 ± 0.071.

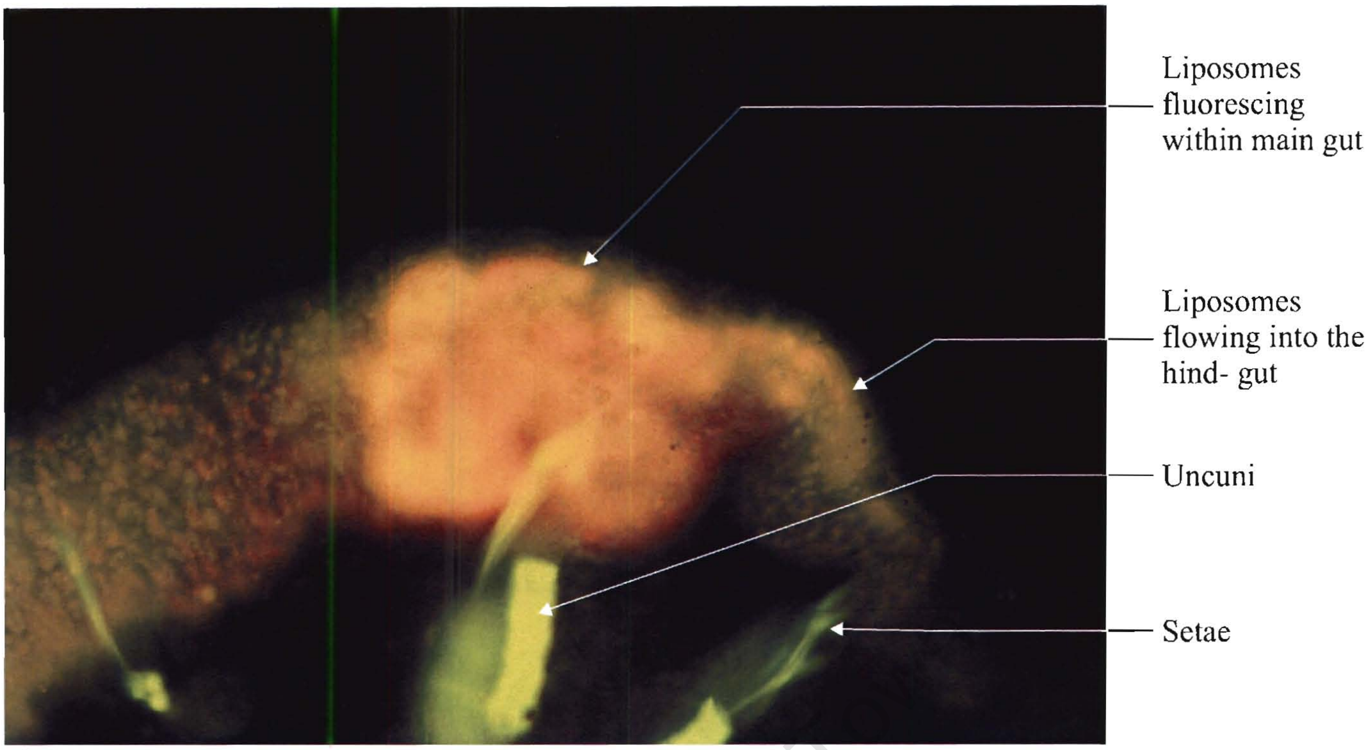

Figure 59: An adult sabellid worm viewed under a fluorescence microscope. The worm had been fed liposomes with encapsulated malachite green. The setigers fluoresce bright yellow. (MAG 100 x)

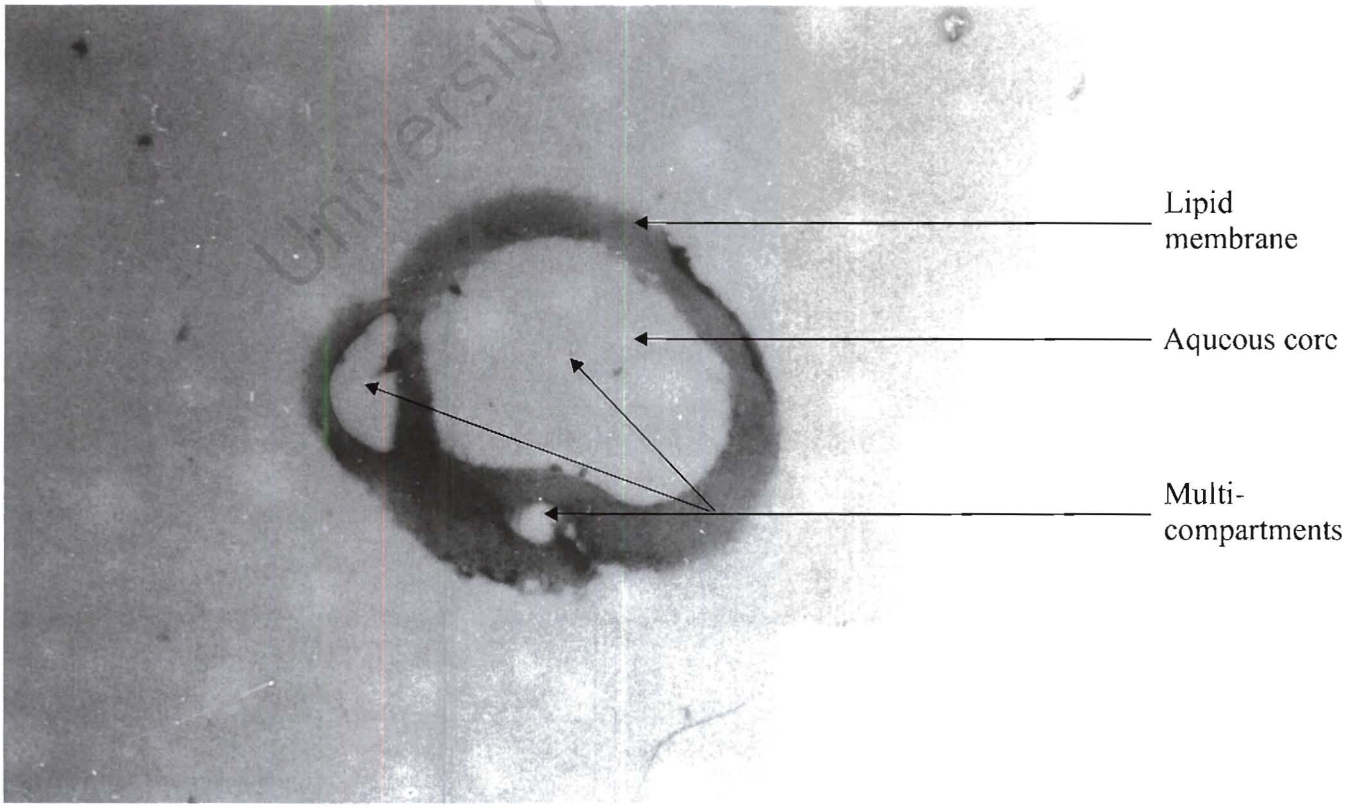

Figure 60: Transmission electron microgram of a large multilamellar vesicles (LMV) imbedded in an agarose section. (MAG. 20 000 x)

Table V: Fluorescence of liposomes ingested and voided after 3 hours.

	Before Triton	*After Triton*
Experiment 1		
Control	0	0
Sabellid 1	0.100	0.103
Sabellid 2	0.050	0.400
Sabellid 3	0.075	0.300
Sabellid 4	0.250	0.250
Experiment 2		
Control	0	0
Sabellid 1	0.200	0.205
Sabellid 2	0.105	0.150
Sabellid 3	0.302	0.304
Sabellid 4	0.250	0.325

In each experiment, two out four of the sabellids appeared to have digested the liposomes as there was no significant increase in fluorescence. The other two showed a significant increase. Despite the fact that there was an increase upon addition of detergent of two out of four for each experiment, the background fluorescence of these was greater, indicating that they perhaps ate more. It is possible that there is a finite amount of liposome that each sabellid can digest in a given time. A more definitive result could be achieved if one knew exactly how much each sabellid consumed.

2. ELECTRON MICROSCOPY

Liposomes (LMV) concentrated and immobilised in agarose gel as described previously were treated and sectioned for viewing in a TEM. The resultant images (Figure 60) provided a control of intact undigested liposomes sectioned to 90 nm slices. The electron microgram of these liposomes shows intact membranes. These electron dense lipids stained with uranyl acetate appear donut-like.

Figure 61 and 62 show electron micrograms of sections of the hindgut of a sabellid 2.5 hours after feeding. Intact liposomes can be seen in these thin sections similar to those in Figure 60. These liposomes appeared undigested after 2.5 hours. The multilayered "onion ring" effect of LMV's could be seen in some of the micrographs (Figure 62). Other thin sections (data not shown) showed similar liposomes adjacent to the cilia in

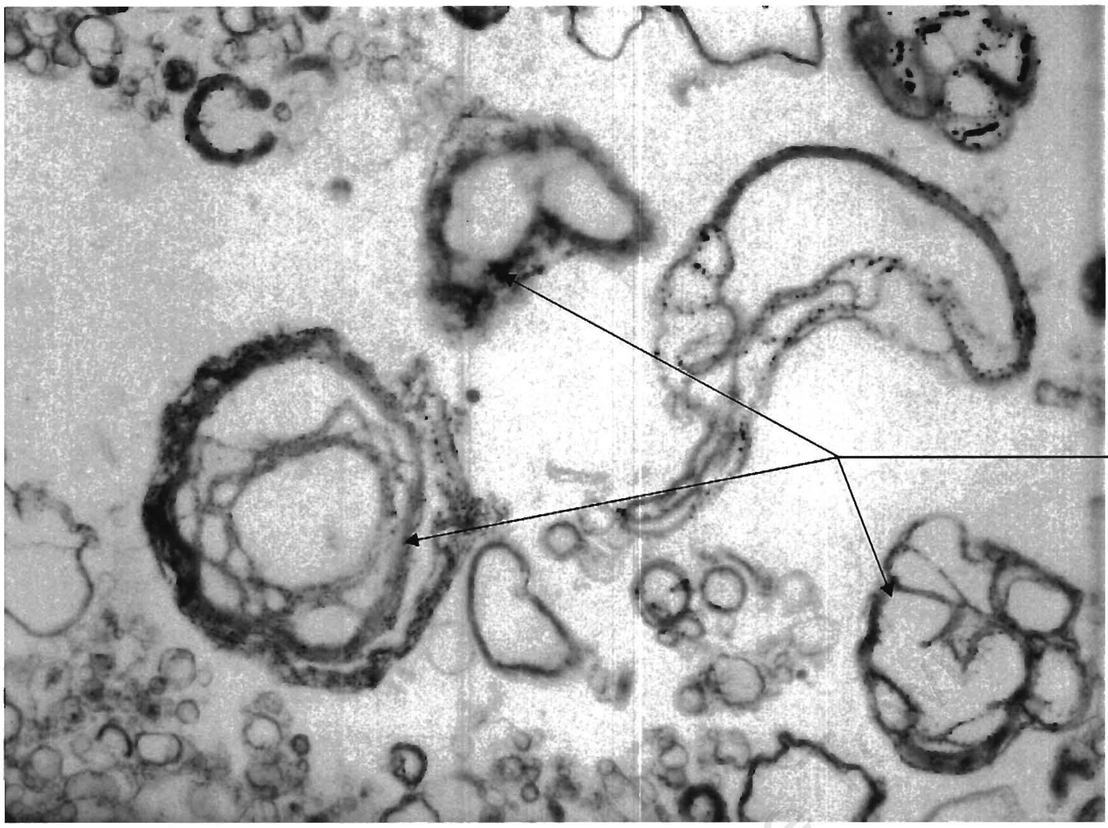

Figure 61: Transmission electron microgram of a section of the hindgut of a sabellid worm 2.5 hours after feeding on liposomes. The vesicles appear to be undigested. (MAG 20 000 x)

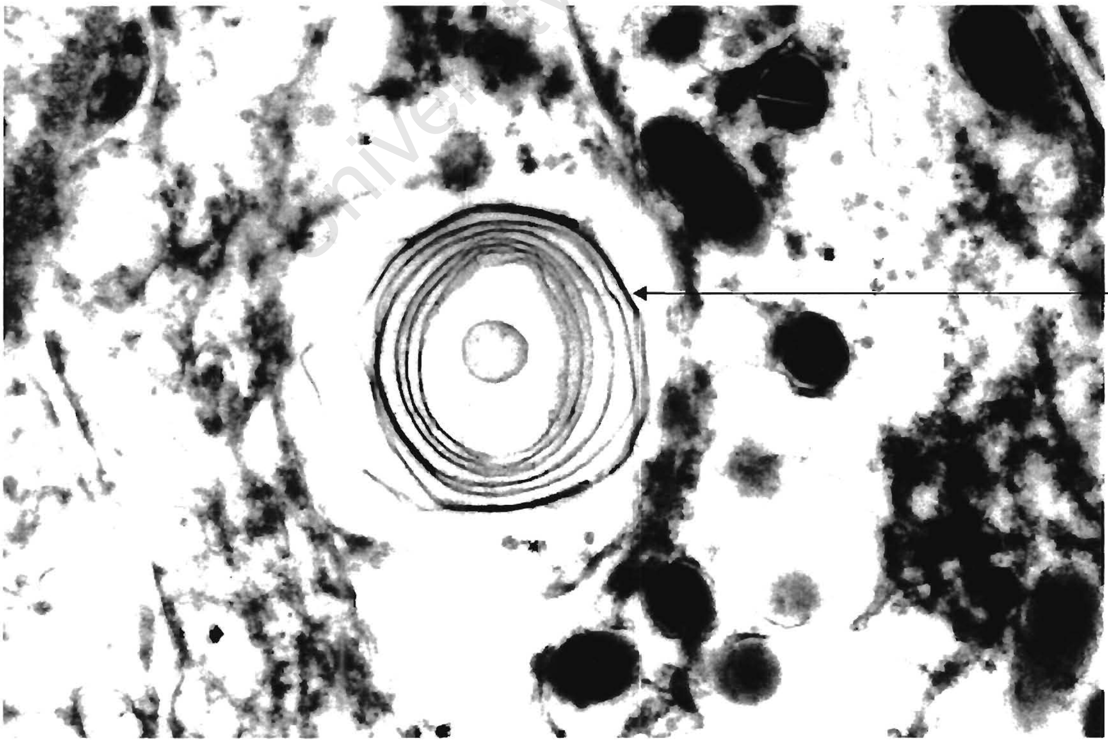

Figure 62: Transmission electron microgram of an intact large multilamellar vesicle (LMV) in a section of the gut of a sabellid worm. (MAG. 30 000 x)

the gut. As liposomes appeared unbroken and hence undigested after 2.5 hours, it was decided not to section the sabellids fixed after only 30 minutes.

There were similar vesicles in control sabellids that had not been fed liposomes, however these appeared to occur in all sabellids (fed and unfed) and there were no liposomes or structures similar to that observed in the fed sabellids, indicating that what had been observed were liposomes. Many of the sabellids that were sectioned appeared to have nothing in their guts. Sabellids had specifically been chosen with fluor visible inside them. Thus, it is possible that the negative staining technique, which is vital for visualisation under TEM did not penetrate deep enough or perhaps the sectioning was not in the correct region of the gut. As the liposomes appeared intact in the gut after 2.5 hours, digestion of these liposomes was inconclusive.

Use of gold colloidal markers

The formation of the gold colloid mixture in the desired size range was achieved easily. However, this formulation interfered with the liposome preparation in much the same way as the copper sulphate solution did before. It was possible that the sodium citrate, which was still present in solution, may have been the problematic agent. An attempt to solve this by concentrating the inert gold particles by dialysis was unsuccessful, since the gold colloid appeared to adhere to the dialysis membrane. The method may still have merit if liposomes can effectively be formed; perhaps the use of a differential Ficoll gradient to separate out the gold colloid could be used.

7.4 DISCUSSION

Chemical treatments and extremes of the physical environment were not effective against the sabellid, since they were more resistant to changes than were the abalone. The ability of the worms to withdraw into their burrows may be the reason why these treatments were ineffective. The burrow opening is very small and there would be very little interchange of water between the inside of the burrow and the external environment. This provided the worm with a buffer against poor water quality. The abalone on the other hand with their exposed gills and glandular skin are very sensitive to water quality. Thus, treatments developed against mudworms (*Polydora*) in oysters such as brine dips and air exposure are not an option for abalone since they are not able to clamp shut as the bivalves are. The wax dips developed by Trevelyan *et al* (1994) would be more effective if this were the case. It is known that abalone can withstand long periods of exposure (pers obs, Tegner & Butler 1989). An indication of the sabellids resilience is that when this was explored as a control mechanism, they were able to recover after a period of 24 hr of total anoxia (N_2 aeration in seawater).

Of all the treatments experimented with, microencapsulation technology was the most attractive. This technology is not new to aquaculture and has been used effectively to enhance the nutrition of larvae, (e.g. Hontoria *et al* 1994, Ozkizilcik & Chu 1994, Chu *et al* 1982, Kanazawa *et al* 1982) and adult bivalves (Heras *et al* 1994), in the application of antibiotics (Touraki *et al* 1995) and as a tool to study assimilation (Kreeger *et al* 1996). It is a way of delivering toxins in a highly specific way. Since the worms concentrate the capsules within their gut, the toxins can be accumulated in high, localised concentrations whereas overall doses are kept low. In this way any residual toxins after treatment would not be a threat to the abalone or the environment.

Of the three delivery mechanisms used, liposomes appeared to be best suited. Both the oil in gelatin and oil immulsion treatments was highly effective at carrying oil soluble drugs, but their downfall was the apparent inability of the worms to digest the products. The natural diet of the worm is still in question and thus it is unclear what digestive enzymes are present within the gut. Since the worms are so small it proved difficult to establish these factors by traditional methods. The worms appear to ingest particles unselectively from the water column except based on size. In abalone tanks these particles would mostly consist of abalone faeces and uneaten food debris. Perhaps they do not digest the particles ingested directly, but benefit from bacterial populations that are present in the detritus. Whatever the case, the liposome formulations, particular REV liposomes, would have the best chance of digestibility due to the thin walled structure.

The results in this study were inconclusive, but there was some evidence that the liposomes may have been partly digested by the sabellids. The research did not continue beyond this model system approach. Further tests using different markers, such as radio-labels (Kreeger *et al* 1996) or refining the gold colloid method may provide more definitive answers about the ability of the sabellids to digest the liposome membranes and thereby release the aqueous core. In a parallel study by Shields *et al* (1998) sabellids were fed lipid walled microcapsules (LWMs). Gut passage was extremely quick in some worms (15-30 min) and there was evidence of digestion of the lipid. Copper sulphate was also chosen as a toxin in this study, but the results were negative due to leaching of the low MW toxin from the core. Thus the option of incorporating various compatible toxins within the liposomes, though premature, may be successful.

The one factor that may prove to be a downfall to the above strategies is that the worm may have a defence mechanism in place for avoiding harmful particles. The first evidence of this was noted when oil immulsion beads containing copper sulphate was introduced. The worms could detect the toxin in beads that were not properly dialysed and did not feed on them at all. The other behaviour that was observed, was that the sabellids are able to void their gut contents very rapidly. Gut contractions in combination with the action of the numerous cilia, which line the gut, are able to clear the entire contents of the gut rapidly (pers. obs.). Successful encapsulation of a toxin may be short-circuited by this behaviour once the toxins are released within the gut.

A final untested technique may avoid this problem. Synthetic polymer gels or hydrogels are relatively new to the field of controlled drug release (Bae *et al* 1992, Kim *et al* 1994)). By careful engineering of the polymer it is possible to design gels, which shrink or expand by many orders of magnitude depending on the external conditions (Zhang et al 1992, Matsuo & Tanaka 1992, Annaka & Tanaka 1992). Factors such as temperature, pH or salinity can produce these changes. The gels can be made in any size from 0.3 µm upwards (T. Tanaka, pers. comm.) and are thus suitable for sabellid feeding. There is the potential that these polymers can be used to rupture the gut or block it indefinitely if the difference in conditions internally versus externally can be suitably manipulated and harnessed in the design of the polymer. The advantage of this method is that no toxins are released into the environment and the worms would be unable to develop a resistance to the treatment since it is mechanical.

Failing this it is possible to include certain drugs within the polymer (even Cu^{++} ions), which would be released upon shrinkage or swelling.

It was noticed during the feeding trials that other filterfeeding polychaetes, spirorbids and spionids, also ingested various forms of the microcapsules. The successful development of one of the above microcapsule treatments may thus prove beneficial over a broader field than abalone mariculture alone.

REFERENCES

Anderson, R.M. 1993. Epidemiology. In: F.E.G. Cox (ed.). *Modern Parasitology. A textbook of parasitology. Second edition.*. Blackwell Scientific Publications, Oxford.

Annaka, M. & Tanaka, T. 1992. Multiple phases of polymer gels. *Nature* 355: 430-432.

Bae, Y.H., Okano, T. & Kim, S.W. 1992. Hydrogels, swelling and drug loading and release. *Pharma. Res.* 9: 283-289.

Bally, R., McQuaid, C.D. & Brown, A.C. 1984. Shores of mixed sand and rock: and unexplored marine ecosystem. *S. Afr. J. Mar. Sci.* 80: 500-503.

Barnes, R.D. 1980. *Invertebrate Biology, 4th ed.* International edition. Holt-Saunders, Japan Ltd.

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. J.Mar.Biol.Ass.U.K.* 58: 825-841.

Beaumont, A.R. & Budd, M.D. 1983. Effects of self-fertilisation and other factors on the early development of the scallop *Pecten maximus. Mar Biol.* 76: 285-289.

Blake,J.A. & J.W. Evans. 1973. Polydora and related genera as borers in mollusk shells and other calcareous substrates (Polychaeta:Spionidae). *The Veliger* 15(3):235-249.

Branch G.M. & Branch M. 1981. *The living shores of Southern Africa.* Struik Publishers, Cape Town.

Branch, G.M., Griffiths, C.L., Branch. M.L. & Becklay. L.E. 1994. *Two Oceans - A Guide to the Marine Life of Southern Africa.* David Phillip, Cape Town. 360pp.

Boyd, A.J. & Oberholster, G.P.J. 1994. Currents off the west and south coasts of South Africa. *SA Shipping News and Fishing Industry Review. September/October, 1994*: 26-28.

Boyd, A.J., Taunton-Clarke, J. & Oberholster, G.P.J. 1992. Spatial features of the near-surface and midwater circulation patterns off western and southern South Africa and their role in the life histories of various commercially fished species. *S. Afr. J. Mar. Sci.* 12: 189-206.

Charlesworth, D. & Charlesworth, B. 1987. Inbreeding depression and its evolutionary consequences. *Ann.Rev.Ecol.Syst.* 18: 237-268.

Chu, F-L., Webb, K.L., Hepworth, D. & Roberts, M. 1982. The acceptability and digestibility of microcapsules by larvae of Crassostrea virginica. *J. Shellfish. Res.* 2: 29-34.

Crisp, D.J., Walker, G., Young, G.A. & Yule, A.B. 1984. Adhesion and substrate choice in mussels and barnacles. *Journal of Colloid and Interface Science.* 104(1): 40-50.

Cross, R.H.M. 1987. *A Handbook on the Preparation of Biological Material for Electron Microscopy.* Electron Microscope Unit. Rhodes University. Grahamstown.

Culver, C.S. & A.M. Kuris (in prep). Eradication of a newly introduced species: mission possible.

Culver, C.S., A.M. Kuris & B. Beede. 1997. *Identification and management of exotic sabellid pests in California abalone aquaculture*. California Sea Grant College Program. Publication No. T-041. ISBN 1-888691-05-1.

Dales, R.P. 1957. Some quantitative aspects of feeding in sabellid and serpulid fan worms. *J.Mar.Biol.Assoc.U.K.* 36: 309-316.

Day, J.H. 1967. A monograph of *the polychaeta of Southern Africa*. British Museum of Natural History, London. 1817pp.

Day, R.W., Culver, C., Kuris, A., Belcher, A. & Morse, D. 2000. The parasite *Terebrasabella heterouncinata* (Polychaeta) manipulates shell synthesis in *Haliotis rufescens*. *Proceedings of the 4th International Abalone Symposium, Cape Town.*

Dayton, P.K. 1971. Competition, disturbance and community organisation: the provision and subsequent utilisation of space in a rocky intertidal community. *Ecol.Monogr.* 41: 351-389.

Duggins, D.O., Simenstad, C.A. & Estes, J.A. 1989. Magnification of secondary production by kelp detritus in coastal marine ecosystems. *Science* 245: 170-173.

Duggins, D.O. & Eckman, J.E. 1994. The role of kelp detritus in the growth of benthic suspension feeders in an understory kelp forest. *J.Exp.Mar.Biol.Ecol.* 176: 53-68.

Eckman, J.E., Duggins, D.O. & Sewell, A.T. 1989. Ecology of understory kelp environments. I. Effects of kelp on flow and particle transport near the bottom. *J. Expt. Mar. Biol. Ecol.* 129: 173-187.

Eckman, J.E. & Duggins, D.O. 1991. Life and death beneath macrophyte canopies: effects of understory kelps on growth rates and survival of marine, benthic suspension feeders. *Oecologia* 87: 473-487.

Erasmus, J., Cook, P.A. & Sweijd, N. 1994. Internal structure and growth lines in the shell structure of the abalone, *Haliotis midae*. *J. Shellfish Res.* 13: 493-501.

Defrise-Quertain, F, Chatelain, P., Delmelle, M. & Ruysschaert, J. 1984. Model studies for drug entrapment and liposome stability. In: Gregoriadis (ed.). *Liposome Technology - Volume II.* CRC Press, Boca Raton, Florida.

Finley, C.A., Friedman, C.S. & Mulligan, T.J. 2000. Life history of an exotic sabellid polychaete, *Terebrasabella heterouncinata*: the influence of temperature on fertilization strategy. *Proceedings of the 4th International Abalone Symposium, Cape Town.* University of Cape Town, Zoology Department.

Fitzhugh, K. 1996. A polychaete threatens California's abalone culture industry. *Terra* 33(4): 4-5.

Fitzhugh, K. & Rouse, G.W. 1999. A remarkable new genus and species of fan worm (Polychaeta: Sabellidae: Sabellinae) associated with some marine gastropods. *Invertebrate Biology* 118(4): 357-390.

Foster, B.A. 1987. Barnacle ecology and adaptation. In: A.J. Southward. (ed.) *Barnacle Biology.* A.A.Balkema. Rotterdam.

Fraley, R., Straubinger, R., Rule, G., Springer, L., Papahadjopoulos, D. 1981. Liposome mediated delivery of DNA to cells: enhanced efficiency of delivery by changes in lipid composition and incubation conditions. *Biochemistry* 20: 6978-6987.

Fritz, M., Belcher, A.M., Radmacher, M., Walters, D.A., Hansma, P.K., Stucky, G.D., Morse, D.E. & Mann, S. 1994. Flat pearls from biofabrication of organized composites on inorganic substrates. *Nature* 371: 49-51.

Ghiselin M.T. 1969. The evolution of hermaphroditism among animals. *Q.Rev.Biol.* 44: 189-208.

Grassle, J.F. & Sanders, H.L. 1973. Life histories and the role of disturbance. *Deep Sea Res.* 20:643-659.

Gregoriadis (ed.) 1984. *Liposome Technology. Vols. 1-3.* CRC Press, Boca Raton, Florida.

Guiot, P. & Baudhuin, P. 1984. Size distribution analysis of liposomes by electron microscopy. Gregoriadis, G. (Ed). *Liposome Technology*. CRC Press USA. pp. 163-178.

Hadfield, M.G. 1986. Settlement and recruitement of marine invertebrates: a perspective and some proposals. *Bull.Mar.Sci.* 39: 418-425.

Hawkes, G.P., Day, R.W., Wallace, M.W., Nugent, K.W., Bettiol, A.A., Jamieson, D.N. & Williams, M.C. 1996. Analyzing the growth and form of mollusc shell layers, in situ, by cathodoluminescence microscopy and raman spectroscopy. *J. Shellfish Res.* 15(3): 659-666.

Hyatt, A.D. 1986. Immunogold labelling techniques. In: M.A. Hayat (ed.). *Basic techniques for Transmission Electron Microscopy.* Academic Press, Inc.

Heath, D.J. 1977. Simultaneous hermaphroditism: cost and benefit. *J. Theor. Biol.* 64: 363-373.

Heras, H., Howie-Kean,J. & Ackman, R.G. 1994. The potential use of lipid microspheres as nutritional supplements for adult *Ostrea edulis*. *Aquaculture* 123: 309-322.

Hill, S.D., Shipley, A.M & Smith, P.J.S. 1993. Endogenous currents during wound healing and regeneration in sabellid fanworms. *Biol. Bull* 187: 253-254.

Hill, S.D., Shipley, A.M & Smith, P.J.S. 1994. Ionic fluxes during wound healing following segment amputation in sabellid fanworms. *Biol. Bull* 185: 303-304.

Hontoria, F., Crowe, J.H., Crowe, L.M. & Amat, F. 1994. Potential use of liposomes in larviculture as a delivery system through *Artemia* nauplii. *Aquaculture* 127(2-3): 255-264.

Hsieh, H.L. 1997. Self-fertilisation: a potential fertilisation mode in an estuarine sabellid polychaete. *Mar.Ecol.Prog.Ser* 147: 143-148.

Jackson, G.A. & Winant, C.D. 1983. Effects of a 'kelp' forest on coastal currents. *Contl. Shelf Res.* 2: 75-80.

Jain, S.K. 1976. The evolution of inbreeding in plants. *Ann.Rev.Ecol.Syst.* 7: 469-495.

Jones ME 1969. Boring of shell by *Caobangia* in freshwater snails of southeast Asia. *Amer. Zool.* 9: 829-835.

Kanazawa, A., Teshima, S, Sasada, H. & Rahman, S. 1982. Culture of the prawn larvae with micro-particulate diets. *Bull. Jap. Soc. Sci. Fish.* 48: 195-199.

Kim, Y.H., Bae, Y.H. & Kim, S.W. 1994. pH/Temperature-sensitive polymers for macromolecular drug loading and release. *J. Contr. Rel.* 28: 143-150.

Kreeger, D.A., Hawkins, A.J.S. & Bayne, B.L. 1996. Use of dual-labeled microcapsules to discern the physiological fates of assimilated carbohydrate, protein carbon, and protein nitrogen in suspension-feeding organisms. *Limnol. Oceanogr.* 41(2): 208-215.

Kondo, A. 1979. *Microcapsule Processing and Technology.* Marcel Dekker, Inc., New York.

Konings, A.W.T. 1984. Lipid peroxidation in liposomes. In: Gregoriadis (ed.). *Liposome Technology - Volume I.* CRC Press, Boca Raton, Florida.

Kuris, A.M. & Culver, C.S 1999. An introduced sabellid polychaete pest infesting cultured abalones and its potential spread to other California gastropods. *Invertebrate Biology* 118(4): 391-403.

Lafferty, K.D. & A.M. Kuris. 1996. Biological control of marine pests. *Ecology* 77(7):1989-2000.

Leighton, D.L. 1998. Control of sabellid infestations in green and pink abalones *Haliotis fulgens* and *H. corrugata* by exposure to elevated water temperatures. *J. Shellfish Res.*17(3): 701-707.

Lindsay, S.M. & Woodin, S.A. 1992. The effect of palp loss on feeding behaviour of two spionid polychaetes: changes in exposure. *Biol. Bull.* 183: 440-447.

MacArthur, R.H. & Wilson, E.O. 1967. *The Theory of Island Biogeography.* Princeton, NJ: Princeton Univ. Press.

Margolis, L., G.W. Esch, J.C. Holmes, A.M. Kuris, & G.A. Schad. 1982. The use of ecological terms in parisitology (report of an ad hoc committee of the American Society of Parisitologists). *J.Parasitol.*, 68(1):131-133.

Matsuo, E.S. & Tanaka, T. 1992. Patterns in shrinking gels. *Nature* 358: 482-485.

Merz, R.A. 1984. Self-generated versus environmentally produced feeding currents: A comparison for the sabellid polychaete *Eudistylia vancouveri*. *Biol. Bull.* 167: 200-209.

Morse, D.E., Hooker, N., Duncan, H. & Jensen, L. 1979. γ-Aminobutyric acid, a neurtransmitter, induces planktonic abalone larvae to settle and begin metamorphosis. *Science* 204:407-410.

Noble, E.R. & Noble, G.A. 1982. *Parasitology: the Biology of Animal Parasites*. Lea and Febiger, Philadelphia.

Oakes, F.R. and R.C. Fields. 1994. Infestation of *Haliotis rufescens* shells by a sabellid polychaete. *Aquaculture* 140:139-143.

Osman, R.W. & Haugsness, J.A. 1981. Mutualism among sessile invertebrates: a mediator of competition and predation. *Science* 211: 846-848.

Ozkizilcik, S. & Chu, F.L.E 1994. Uptake and metabolism of liposomes by Artemia larvae. *Aquaculture* 128(1-2): 131-141.

Qian, P.Y. 1994. Effect of food quantity on growth and reproductive characteristics of *Capitella* sp. (Annelida: Polychaeta). *Invertebrate Reproduction and Development* 26(3): 175-185.

Murray, A. & Rouse, G. W. 1998. New cryptic species of Terebrasabella (Polychaeta: Sabellidae) from Australia. *6th International Polychaete Conference,* Brazil.

Robinson, A. 1992. Dietary supplements for reproductive conditioning of *Crassostrea gigas* Kumamoto (Thunberg). I. Effects on gonadal development, quality of ova and larvae through metamorphosis. *J. Shellfish Res.*11(2): 437-441.

Ruck, K.R. 1990. Aspects of the interaction between the mussel, Mytilus Galloprovincialis and the barnacle *Notomegabalanus algicola.* Honours project, University of Cape Town.

Ruck, K.R. and Cook, P.A. 1998. Sabellid infestations in the shells of South African molluscs: Implications for abalone mariculture. *J. Shellfish Res.*17(3):693-700.

Rützler, K. 1970. Spatial competition among porifera: solution by epizoism. *Oecologia* 5: 85-95.

Sato-Okoshi, W. & Okoshi, K. 1997. Survey of the genera *Polydora*, *Boccardiella* and *Boccardia* (Polychaeta, Spionidae) in Barkley Sound (Vancouver Island, Canada), with special reference to boring activity. *Bulletin of Marine Science* 60(2): 482-493.

Sella, G. 1991. Evolution of biparental care in the hermaphroditic polychaete worm *Ophryotrocha diadema. Evolution* 45(1): 63-68.

Shannon, L.V. and Nelson, G. 1996. The Benguela: Large scale features and processes and variability. In: Wefer, G. Berger, W.H., Seidler, G. and Webb, D.G. (eds). *The South Atlantic. Present and post circulation.* Spriger, Berlin. 644pp.

Shek, P.N., Yung, B.Y.K. & Stanacev, N.Z. 1983. Comparison between multilamellar and unilamellar liposomes in enhancing antibody formation. *Immunology* 49: 37-44.

Shields, J.D., Buchal, M.A. & Friedman, C.S. 1998. Microencapsulation as a potential control technique against sabellid worms in abalone culture. *J. Shellfish Res.* 17(1): 79-83.

Singleton, W.S., Gray, M.S., Brown, M.L. & White, J.L. 1965. Chromatographically homogenous lecithin from egg phospholipids. *Journal of the American Oil Chemists Society* 42: 53-56.

Sousa, W.P. 1979. Experimental investigations of disturbance and ecological succession in a rocky intertidal algal community. *Ecological monographs* 49(3): 27-254.

Stebbing, A.R.D. 1972. Preferential settlement of a bryazoan and serpulid larvae on the younger parts of laminaria fronds. *J.Mar.Biol.Ass.U.K.* 52: 765-772.

Stebbing, A.R.D. 1973. Competition for space between the epiphytes of *Fucus serratus* L. *J.Mar.Biol.Ass.U.K.* 53: 247-261.

Stegenga, H., Bolton, J.J & Anderson, R.J. 1997. *Seaweeds of the South African west coast.* In: Hall, A.V. (ed). Contributions from the Bolus Herbarium, Number 18. Bolus Herbarium, University of Cape Town. 655pp.

Sweijd, N. 1999. Molecular markers and abalone reseeding as tools for the conservation and management of the South African abalone (perlemoen), *Haliotis midae* Linn., resource. Phd thesis, University of Cape Town.

Szoka, F. & Papahadjopoulos, D. 1978. Procedure for preparation of liposomes with large internal aqueous space and high capture by reverse phase evaporation. *Proc. Natl. Acad. Sci. USA*. 75(9): 4194-4198.

Tegner, M.J. & Butler, R.A. 1989. Abalone seeding. In: Hahn, K. (ed.) *Handbook of culture of abalone and other marine gastropods.* CRC Press Inc. Florida. 348pp.

Thistle, D. 1981. Natural physical disturbances and communities of marine soft bottoms. *Mar.Ecol.Prog.Ser.* 6: 223-228.

Touraki, M., Rigas, P. & Kastritsis, C. 1995. Liposome mediated delivery of water soluble antibiotics to the larvae of aquatic animals. *Aquaculture* 136: 1-10.

Trevelyan, G., Fields, R.C., Arthur, P.F. & Oakes, F.R. 1994. The use of wax to control the shell parasites of red abalone, *Haliotis rufescens.* Intra-industry paper.

Vance, R.R. 1978. A mutualistic interaction between a sessile marine clam and its epibionts. *Ecology* 59: 679-685.

Vardell, I.M & Polak, J.M. (1987). EM. Immunolabelling. In: Sommerville, J. & Scheer, U. (Ed.). *Electron Microscopy in Molecular Biology: A practical approach.* IRL Press Ltd.

Weinstein, J.N., Ralston, E., Leserman, L.D., Klausner, R.D., Dragsten, P., Henkart, P. & Blumenthal, R. 1984. Self-quenching of carboxyfluorescein fluorescence: uses in studying liposome stability and liposome-cell interaction. In: Gregoriadis (ed.). *Liposome Technology - Volume III.* CRC Press, Boca Raton, Florida.

Wilson, D.P. 1968. The settlement behaviour of the larvae of *Sabellaria alveolata* (L.). *J.Mar.Biol.Ass.U.K.* 48:387-435.

Woodin, S.A. 1976. Adult-larval interactions in dense infaunal assemblages: patterns of abundance. *Journal of Marine Research* 34(1): 25-37.

Woodin, S.A. & Merz, R.A. 1987. Holding on by their hooks: Anchors for worms. *Evolution* 41(2): 427-432.

Woodle, M.C. & Papahadjopoulos, D. 1989. *Liposome preparation and size characterisation. Methods in Enzymology Vol 171.* Academic Press Inc.

Zhang, Y-Q., Tanaka, T. & Shibayama, M. 1992. Super-absorbency and phase transition of gels in physiological salt solutions. *Nature* 360: 142-144.