

THE PHYSIOLOGY OF DIGESTION
IN MARINE MUSSELS:
A STUDY IN ENVIRONMENTAL ADAPTATION

by

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DECLARATION

This thesis reports the results of original research which I have carried out in the Departments of Microbiology and Zoology, University of Cape Town between 1979 and 1983. None of it has been submitted in whole or in part for any other degree and any technical assistance I have received is fully acknowledged.

Section I was carried out in collaboration with Professor R.C. Newell, Dr M.I. Lucas and Dr B. Velimirov.

Signed by candidate

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For Rick, Mont, Myra and Inez

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ABSTRACT

The black mussel Choromytilus meridionalis (Krauss) forms an important link in the food chain of the South African west coast kelp beds, transferring energy from macrophyte debris, phytoplankton and bacteria to major predators such as the rock-lobster Jasus lalandii. Although a large body of work has been carried out on the physiology, energetics and population dynamics of the black mussel, no attempt has been made to link the actual food available in the field, with the animal's digestive capabilities. This work examines the carbon and nitrogen resources available from kelp debris, phytoplankton and bacteria, and quantifies the animal's ability to utilise these resources according to its needs.

The kelps Ecklonia maxima and Laminaria pallida both undergo fragmentation from the tip and thus release structural components as particulate matter, and a dissolved fraction from the cell contents into the water column. Analysis of the dissolved fraction yielded a 93% gravimetric recovery comprising 7,2 - 7,6% sugar and polyols, 4,8 - 5,0% alginate and 4,6 - 7,2% laminarin. Sugars amounted to 6,5 - 7,1% of crude dried mucilage from both species (ca 23% of ash-free dry weight). Of these, the acyclic polyol mannitol dominated the ethanol extract accounting for approximately 77% of the free reducing sugars and polyols present in both species. Alginate accounted for 4,77% while laminarins amounted to 7,2% dry weight of the dried mucilage from E. maxima. In L. pallida, alginate accounted for 5,01% and

laminarins 4,59%. Energy released as dissolved organic components during kelp fragmentation amounts to approximately 34% of the total annual energy production of L. pallida and 17% of that of E. maxima. Total particulate energy production from both species in a small kelp bed of 700 ha is estimated as $43,25 \times 10^{10} \text{ kJ y}^{-1}$, whereas that released as dissolved organic matter amounts to $10,74 \times 10^{10} \text{ kJ y}^{-1}$.

This is equivalent to $20,24 \times 10^7 \text{ g}$ mucilage released year⁻¹ during fragmentation into the shallow water of the kelp bed, to which should be added undetermined losses of mucilage from the surface of the fronds. Studies of the concentration of dissolved organic matter in the water near a kelp bed suggest that the exudate released during fragmentation is rapidly utilised within 24 h by microheterotrophic organisms which, in turn, are available for utilisation by filter-feeders (section III). It has been shown that carbohydrates constitute approximately 53% of the total dry weight of particulate organic matter from L. pallida and, in this light, a thorough investigation of the carbohydrase capacity of the major kelp bed filter-feeders was initiated.

Carbohydrase activities in the crystalline style of the mussels Choromytilus meridionalis and Perna perna were compared. Despite the differences in relative availability of phytoplankton and kelp detrital material as potential food sources for the two mussels, both have a similar spectrum of carbohydrases, including α -amylase, cellulase, laminarinase and alginate lyase. Minor differences which exist in the specific activity of the style enzymes appear to be related to

qualitative differences in potential food supply. Major differences exist, however, in total carbohydrase activity of styles from C. meridionalis and P. perna, which are capable of liberating 9,6 mg and 4,8 mg glucose.mg⁻¹ protein.hr⁻¹ at 18°C respectively. Estimates of the style turnover time required for the carbohydrase activity of the style enzymes to meet the carbon requirements of the mussel are 25 hours in C. meridionalis and 136 hours in P. perna. The longer required turnover time of 136 hours in P. perna is associated with the higher carbohydrase activity of the style and the somewhat lower carbon requirements of the mussel. P.A. Cook (personal communication) has found that the turnover times of the styles of C. meridionalis is 18 hours and that of P. perna is 120 hours, values which correspond well with those estimated from the carbon requirements and carbohydrase activity in these mussels. In both C. meridionalis and P. perna, therefore, the spectrum of carbohydrases present in the style, and their specific activities, are well suited to release glucose from both the living phytoplankton and detrital components of the particulate matter in the water column at a sufficient rate to meet the estimated carbon requirements of the mussels.

With reference to the nitrogen requirements of the mussels which exist in a system with a relatively high C:N ratio (macrophyte debris 15,6 and phytoplankton 7), the crystalline style of Choromytilus meridionalis contains a bacteriolytic enzyme capable of lysing the majority of free-living bacteria in the adjacent water column. Estimates of the carbon to nitrogen ratio of the free-living bacteria (3,7), and of the filtration capabilities of the mussels, indicate that bacteria could meet

the nitrogen requirements of the mussels. In addition, the presence of a trypsin-like proteolytic enzyme has been established in the crystalline style, the function of which is as yet undetermined. Both the bacteriolytic and the proteolytic agents in the style are subject to considerable adaptive changes in activity, correlated with water temperature. Water temperatures of less than 13°C are associated with induction of both the bacteriolytic and proteolytic agents. During upwelling, cold water depleted in particulate matter but containing significant numbers of bacteria occurs commonly amongst the kelp beds. It is suggested that low water temperature (or an associated environmental parameter) results in the stimulation of bacteriolysis. This, in turn, permits efficient utilisation by the mussel of free-living bacteria which compensates for the depletion of phytoplankton available in the water column.

Water temperatures of greater than 13°C are associated with downwelling, during which phytoplankton-rich water is made available to the filter-feeding community. Although C. meridionalis appears to have a rather low cellulase activity, scanning electron micrographs have shown that the animal is able to digest both diatoms and flagellates within the spectrum of phytoplankton available.

It appears that despite the high primary productivity of the system from phytoplankton and macrophytes, the filter-feeding community is in fact one where intense competition for nitrogen is likely to occur. This has been substantiated by the occurrence of particularly thin mussels during the anomalous summer season of 1982/1983, during which

warm water temperatures and a corresponding lack of bacteriolysis were prevalent. It is thought that the nitrogen deficiency created during the winter season was not compensated for during the summer season as is the case in "normal" years. It is not known whether or not this effect has spread further up the food chain.

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GENERAL INTRODUCTION

There have been many studies of the potential significance of bacteria in the nutrition of marine animals (for recent reviews, see Watson 1978, and Mann, 1982). Although some filter-feeding bivalves such as the bay scallop Argopecten irradians utilise phytoplankton rather than the microbial component of plant detritus (Kirby-Smith 1976), many other filter-feeders, including both bivalves (Sorokin 1973) and sponges (Reiswig 1971), may derive a major proportion of their diet from bacteria and associated sub-particulate material. A wide range of deposit-feeding organisms, especially those exploiting detrital food resources with high C:N ratios such as occur near to saltmarshes and macroalgae, have also been shown to derive a significant proportion of their diet from the ingestion of the bacterial component of decomposing plant detritus. Such organisms include polychaetes (Tenore 1977a, b), gastropods (Newell 1965; Wetzel 1976), amphipods (Fenchel 1970), mysids (Foulds & Mann 1978), prawns (Moriarty 1976, 1977), holothurians (Yingst 1976) and fish (Moriarty 1976).

Some of these deposit-feeders may actively select smaller-sized particles from the deposits and thus enrich the bacterial component of their diet compared with non-selective deposit-feeders (for review, see Newell 1979). Prieur (1981) showed, in addition, that the gut microflora may divide several times during passage through the gut of the mussel Mytilus edulis, so that the yield from bacteria could be

considerably in excess of the standing stock or biomass of bacteria ingested with the diet. Complex interactions between the gut microflora and detritus utilisation by the host have also been implicated in studies on the nutrition of Mysis sterolepis (Foulds & Mann 1978) and Strongylocentrotus (Fong & Mann 1980). More recently, Newell and Field (1983), in a study of carbon and nitrogen flux through kelp beds, estimated that utilisation of bacteria associated with detritus could contribute as much as 69% of the nitrogen requirements of the consumer community as a whole. Both the interactions of the gut microflora with the ingested detrital diet and the utilisation of free-living bacteria ingested with particulate debris are thus potentially implicated in the nutrition of detritivores.

There have, however, been very few investigations of the digestive enzymes actually involved in the utilisation of detritus and its associated microflora by invertebrates. Kristensen (1972) and Yingst (1976) have reviewed the evidence that comparatively few deposit-feeding invertebrates possess the necessary enzymes to digest the structural carbohydrates which make up the bulk of aged plant detrital material. Stuart (1982) and Stuart et al. (1982) have, however, recently shown that the kelp bed mussel Aulacomya ater is capable of absorption of sterilised kelp detritus with an efficiency of approximately 50%. This suggests that the kelp bed mussels may be specifically adapted to utilise the particulate components of kelp debris as a carbon resource, although little is known of the relative significance of phytoplankton, particulate debris and its associated microbiota as a source of carbon and protein.

The following work was therefore undertaken to investigate the carbohydrate chemistry of kelp detritus as a nutritional resource, and its efficiency of exploitation by filter-feeding mussels. The chemistry of the dissolved and particulate components are reported in Section I of this thesis. The second section comprises an investigation of the style enzymes of the mussels Choromytilus meridionalis and Perna perna to determine to what extent these are suited to the digestion of the particulate components of detrital debris. Finally, in Section III, the relative significance of the bacteria associated with debris and phytoplankton is assessed in relation to both enzyme activity of the style and the nitrogen requirements of mussels. The thesis is concluded with a general discussion on the quantitative significance of style enzymes in meeting the nutritional requirements of mussels which utilise detrital diets under the very variable conditions associated with phases of upwelling and downwelling.

SECTION I

SOURCES OF CARBON FOR KELP-BED

FILTER-FEEDERS

INTRODUCTION

Although a good deal is now known about the chemistry of cell contents and structural components of algae (for reviews, see Von Holdt et al., 1955; Percival, 1968; Craigie, 1974; Mackie & Preston, 1974; Wood, 1974; Jensen & Stein, 1978; Stephen, 1979), there have been very few investigations of the chemistry of the particulate and 'dissolved' components of detrital material which is potentially available as a food resource for benthic suspension-feeders. Much of the dissolved component which leaches out from both living phytoplankton cells and larger macroalgae appears to be utilised by bacteria, some of which may be able to incorporate nitrogen with a high efficiency of 83-94% (Koop et al., 1982b; Stuart et al., 1982; see also Newell & Field, 1983). The particulate component may, however, have a longer residence time in the water column and could thus form a significant carbon resource for consumer communities.

It is uncertain at present whether losses over the general surface of the algae are attributable to experimental manipulation and to what

extent they occur under natural conditions. Phytoplankton cells, for example, release from 7 - 50% of their assimilated carbon as dissolved organic matter, with values of up to 38% being released at the end of a bloom when the cells may be undergoing disintegration (Hellebust, 1965; Anderson & Zeutschel, 1970; Ryther et al., 1971; Thomas, 1971; Choi, 1972; Ignatiades, 1973; Berman & Holm-Hansen, 1974; Mann, 1982). Again Moebus and Johnson (1974) showed that in

Ascophyllum nodosum and Fucus vesiculosus, excretion of dissolved organic substances were undetectable except after a period of desiccation (Moebus et al., 1974). Losses of dissolved organic matter associated with fragmentation at the tip of kelp fronds are, however, more predictable and can be related to particulate losses by analysis of the chemical composition of the mucilage and measurement of the wet weight: dry weight ratio of the tissues at the tip of the frond.

The importance of such losses of dissolved organic matter from both macrophytes and phytoplankton in the maintenance of secondary production has been widely recognised (for review, see Sieburth, 1976). Heterotrophic utilisation of dissolved organic matter may play an important part in making dissolved components available in a particulate form for organisms at higher trophic levels and, together with particulate matter released directly by fragmentation of kelp, dominates the primary production of some coastal waters (Mann, 1973; Velimirov et al., 1977; Field et al., 1980a; for reviews see Marine Ecology Volume IV; Wangersky, 1978; Conover, 1978; Sorokin, 1978; Newell, 1979). There are, however, no detailed analyses of the composition of

the dissolved organic matter released during fragmentation by kelp, nor of the quantitative significance of such material in coastal waters. The present study was therefore undertaken to analyse the major components of dissolved organic losses during fragmentation of the kelp species Ecklonia maxima and Laminaria pallida, which dominate the shallow waters on the west coast of the Cape Peninsula (South Africa), and to estimate the quantitative significance of such compounds compared with particulate matter released into the water column during the fragmentation process. These estimates thus represent minimum values for the significance of dissolved organic matter, to which undetermined losses from the surface of the frond, possibly reaching 25 - 35% of the photoassimilated carbon, require to be added.

MATERIAL AND METHODS

Collection of Samples

Samples of material from the kelps Ecklonia maxima and Laminaria pallida were removed by cutting the stipe below the meristematic zone following collection in March 1979 from a depth of approximately 0 - 5 m at Oudekraal on the west coast of the Cape Peninsula, South Africa. The material was then brought ashore and the eroding tips removed from approximately 50 fronds. A second sample of frond from below the eroding zone at the tip was removed, and a final sample was removed from the meristematic zone together with the upper part of the stipe. Material from each of the three zones was placed in a polythene bag on

ice and transported to the laboratory. The whole bag was then placed in a deep-freeze for up to 4 d prior to use.

The frozen material was cut into small pieces and placed in a covered glass funnel and the mucilage including cell contents allowed to drain into a sealed beaker overnight at 16°C. The following morning at least 10 samples of approximately 10 ml of mucilage from each of the three zones from both species were placed in preweighed clean glass vials and frozen in liquid nitrogen. They were then placed on a freeze dryer until dry, reweighed and the contents ground to a fine powder with a glass rod.

Analysis of the Dried Samples

The 60 dried samples obtained from the two kelp species were then analysed for carbohydrates, proteins and lipids. Ash-free dry weight was determined using a muffle furnace at 450°C for 12 h. The volumes and concentrations of the mucilage were then related to particulate losses from the eroding tips by measurement of the wet weight:dry weight ratio of the original kelp.

Total Carbohydrate

Total carbohydrate in the dried samples was measured using the methods of Holland and Gabbott (1971) and Holland and Hannant (1973). A weighed sample of 2 - 3 mg was homogenised in 500 μ l water. The homogenate was then split into three samples. One sample was treated

with cold 15% trichloroacetic acid to precipitate protein. Part of the supernatant was then used to determine free reducing sugars by ferricyanide reduction (Folin & Malmros, 1929); the other part after acid hydrolysis was used to determine the total carbohydrate. All results were expressed both in terms of the ash-free and crude dry weight of the powdered samples.

Component Carbohydrates

Monosaccharides and polyols

The sugars which are in the mucilage comprise a mixture of monosaccharides and more complex sugars, some of which may be strongly bound to structural components such as laminarins and alginates (for reviews see Mackie & Preston, 1974; Jensen & Stein, 1978). Monosaccharides and polyols may be extracted by reflux in 200 ml 80% ethanol for 2 - 3 h (Holligan & Drew, 1971), but long-chain polysaccharide sugars require extensive hydrolysis, in sealed glass tubes to minimise sugar oxidation, in approximately 3 ml 2N trifluoroacetic acid (TFA) for up to 24 h (Sweeley et al., 1963; Adams, 1965; Björndal et al., 1970). Alginates and laminarins were assayed separately in separate pooled dried samples of mucilage from Ecklonia maxima and Laminaria pallida.

Sugars in both the ethanol soluble extract and the TFA hydrolysate were identified by descending paper chromatography. A few drops of distilled water were added to a freeze-dried sample of each extract. A spot of the concentrated extract was then placed on Whatman No. 1

paper from a capillary tube and reference sugars made up in distilled water were run with the sample. Polyols in the ethanol soluble fraction were resolved using butanol, acetic acid and water in the ratio 2:1:1 and the chromatogram was visualised as black spots after approximately 12 h by spraying with freshly-prepared Tollen's reagent (5% ammoniacal AgNO_3 ; 5M aqueous NH_3 plus 0,1 M AgNO_3 in equal volumes) and heating for 5 - 10 min. The acid hydrolysate was run using a solvent of ethyl acetate, pyridine and water in the ratio of 8:2:1 by volume, the hexose sugar positions being identified after approximately 12 h by spraying with 3% p-anisidine hydrochloride in 1-butanol and heating at 105°C for 10 min. The sugars then gave yellow fluorescence under U/V light (see also Lewis & Smith, 1967a, b).

In order to assay the component sugars quantitatively, the ethanol soluble sample was dried on a rotary evaporator, washed to the bottom of the flask with distilled water together with an internal standard of 500 μl erythritol (100 mg per 100 ml distilled water), frozen and freeze-dried. The hydrolysate was dried on a rotary evaporator, washed in Analar methanol to form the volatile methyl ester of TFA, and redried. The internal standard of 500 μl erythritol was then added and the sample freeze-dried as in the ethanol soluble fraction above. The residue from the 24 h hydrolysis was then re-hydrolysed in sealed tubes in approximately 3 ml of 2N TFA and the hydrolysate recovered and dried with an internal standard as before.

The freeze-dried samples together with their internal standards were then derivatised prior to analysis of the component sugars with a Pye-

Unicam Series 104 Chromatograph. A sample of 850 μl anhydrous pyridine was added to each stoppered flask together with 100 μl hexamethyldisilazane (HMDS) and 50 μl trimethylchlorosilane (TMS) and the contents left to derivatise for 3 h at room temperature. The contents were then transferred into stoppered tubes and centrifuged at 800 x g for 10 min. Standards of the following known sugars were made up to 0,2, 0,4, 0,6, 0,8 and 1,0 mg ml^{-1} from stock solutions containing 100 mg ml^{-1} : fucose, xylose, galactose, mannose, glucose, mannitol, rhamnose, arabinose and erythritol (Appendix B). A sample of 500 μl of the stock erythritol was added to each of the sugars as an internal standard. These standard solutions were then freeze-dried and derivatised as described above. All samples were analysed within 12 h of derivatisation.

Samples of 10 μl were injected into the column which contained diatomite calcium as a solid support coated with 2% SE 52 as a non-polar liquid phase. The flow rate of the carrier gas (N_2) was 30 ml min^{-1} . Samples were injected at 140°C and the temperature ran isothermally for 4 min, after which it increased from 140° to 320°C at 6°C min^{-1} (see Holligan, 1971; Holligan & Drew, 1971). The relative retention time of each sugar is commonly calculated from the sugar distance (cm) relative to the position of an internal standard of erythritol. In practice, the erythritol distance is rather variable, whereas the retention time of mannitol is much more constant. Therefore the ratio between the mean of all the erythritol distances and that of mannitol has been calculated and the internal standard erythritol distance has subsequently been normalised against that of the identified mannitol

peak. The peak heights of all sugars were also corrected relative to a mean value for the peak height of the internal erythritol standard. The concentration of each sugar could then be calculated from the peak area (relative retention time x peak height) using the equations for the regressions for the sugar standard shown in Table 1.

Alginates and Laminarins

Alginates were assayed gravimetrically by a method adapted from several sources based essentially on that described by Haug (1965; see also Chapman, 1950; McDowell, 1973; Levin, 1974). A weighed sample of approximately 400 mg of dried mucilage was dissolved in approximately 10 ml 5% Na_2CO_3 to form soluble sodium alginate. 10 ml of 5% CaCl_2 was then added to the filtered solution to form a white cloud of precipitating calcium alginate and calcium carbonate. The precipitate was sedimented by centrifugation for 10 min at 300 x g. The supernatant was then removed and added to a further 10 ml 5% CaCl_2 , centrifuged and the supernatant discarded. 50 ml of 5% HCl was added to the combined sediments and left overnight. The precipitated alginic acid was then sedimented by centrifugation at 800 x g for 10 min and transferred in 2 ml 5% HCl into a preweighed 0,54 μm Sartorius cellulose acetate membrane filter and dried to constant weight at 60°C. The gravimetric recovery of a weighed sample of approximately 400 mg of alginic acid (Sigma) was found to be 92,64% using this semi-micro modification of the procedure for estimation of alginate.

Laminarins were assayed gravimetrically by a method outlined by Elyakova

TABLE 1 Values for intercept (a), slope (b) and coefficient of variation (r^2) for regression lines relating sugar concentration (μg : Y) to peak areas (height x retention time. X) for standard sugars and mannitol. Equation for the regression, $Y = a + b x$.

Sugar	Retention time (min)	a	b	r^2
a-Mannose	3,896	0,58	0,98	0,97
a-Rhamnose	4,204	0,30	1,06	0,99
Arabinose	4,276	0,27	0,86	0,99
a-Galactose	4,294	0,51	1,88	0,98
a-Glucose	4,452	1,48	0,63	0,99
B-Mannose	4,470	2,94	0,75	0,91
B-Galactose	4,550	0,94	1,03	0,98
B-Rhamnose	4,636	1,28	1,72	0,91
a-Fucose	4,674	0,48	1,48	0,98
a-Xylose	4,898	0,73	1,34	1,00
B-Fucose	4,938	1,45	0,70	0,96
B-Glucose	4,964	2,13	0,42	0,98
B-Xylose	5,264	0,67	0,98	1,00
Mannitol	5,660	0,71	0,28	0,98

and Zvyagintseva (1974). A weighed sample of 0,5 - 1,0 g of dried mucilage was washed free of pigments for 1 h with 10 ml cold 100% methanol and centrifuged at 800 x g for 10 min. The supernatant was discarded and the sediment extracted with 10 ml 0,4% HCl once at room temperature for 4 h and once at 50°C for 5 h. The extracts were precipitated with 30 ml methanol:1-butanol (3:1) with subsequent reprecipitation of the residues. The precipitate was sedimented by centrifugation at 80 x g for 10 min and dissolved in 20 ml distilled water. The aqueous solution was then treated with 1 ml 0,5 M Cetavlon (cetyltrimethyl ammonium bromide) until precipitation of the acidic polysaccharide material was complete. The laminarin in the supernatant was then precipitated with a further 30 ml methanol:1-butanol (3:1), filtered under vacuum onto preweighed 0,54 μ m Sartorius cellulose acetate paper, oven dried at 60°C and weighed.

The percentage gravimetric recovery using this method was determined by analysis of a known mixture of approximately 400 mg alginic acid (Sigma) and 400 mg laminarin (Koch-Light). It was found that the alginic acid was not completely precipitated by treatment with Cetavlon, 1,83% being left in the supernatant, whilst only 82,4% of the laminarin was precipitated by treatment of the supernatant with methanol:1-butanol. Accordingly, the figures for laminarin have been adjusted as shown on p.19 and Tables 5 and 6 to account for a loss of approximately 17% in recovery.

RESULTS

Total Carbohydrate Content of Mucilage

In the first instance it was necessary to determine the gross biochemical composition of mucilage from kelp. Such data indicated which major components required more detailed analysis and also served to verify the quantitative results obtained by other methods. The results of an analysis of a mixed sample of dried mucilage from Ecklonia maxima and Laminaria pallida are summarised in Table 2.

Total carbohydrate values were as high as 10,39% of the dried mucilage, comprising some 6,5% free reducing sugars and 3,9% polysaccharides. Each of these major components, as well as laminarins and alginates were subsequently analysed in more detail and the summed results used to calculate the energetic significance of dissolved organic matter released during fragmentation of kelp.

TABLE 2 Carbohydrate components in a mixed sample of 4,283 mg dried Ecklonia maxima and Laminaria pallida mucilage. Each value is the mean of three estimations.

Biochemical Component	Amount	
	mg	%
Total carbohydrate	0,502	10,39
Free reducing sugars	0,32	6,47
Polysaccharides	0,189	3,92

Analysis of Sugars

The component sugars in ethanol extracts of dried mucilage and following hydrolysis of the residue for up to 24 h in 2N trifluoroacetic acid were resolved by paper chromatography. The derivatised samples were subsequently assayed quantitatively by gas liquid chromatography as described on p.11. The results are summarized in Tables 3 and 4.

Total sugars and soluble carbohydrate amounted to some 6,5 - 7,1% of the crude dried mucilage from both Ecklonia maxima and Laminaria pallida (approximately 23% of the ash-free dry weight). Of these, the acyclic polyol mannitol dominated the ethanol extract, accounting for approximately 77% of the total free reducing sugars plus polyols present in both kelp species (Tables 3 & 4). Mannitol is a primary photosynthate and major translocatory carbohydrate whose presence has been recorded in many algae including Laminaria, but which is absent from the extracellular dissolved organic matter released from phytoplankton (for reviews see Von Holdt et al., 1955; Percival, 1968; Craigie, 1974; Mackie & Preston, 1974; Stephen, 1979). There were also small but variable quantities of unidentified compounds of large molecular weight which appeared in the ethanol extract and which had longer retention times than mannitol. These may represent mannitol glycosides formed from partial TMS derivatives of laminarin glycosyl units.

The sugars extracted by acid hydrolysis represent liberated components of carbohydrates of larger molecular weight and comprise principally the aldose sugars α and β galactose, together with trace amounts of

TABLE 3 *Ecklonia maxima*: analysis of sugars and mannitol in up to 4 samples of mucilage from the stem plus meristem, frond and eroded fronds. Percentage of sugars plus mannitol in mucilage expressed as g^{-1} dry weight and g^{-1} ash-free dry weight. Relative proportion of mannitol expressed as percentage of total

	Stem and meristem				Frond				Eroded frond			Mean %
Ethanol extraction ($mg\ mL^{-1}$)	4,51	11,05	18,42	10,57	4,6	11,32	8,06	12,74	3,65	14,6	6,12	
TFA extraction ($mg\ mL^{-1}$)	0,59	2,65	1,28	6,18	1,73	5,22	0,6	5,81	1,85	-	1,94	
Total identified sugars + mannitol ($mg\ mL^{-1}$)	5,1	13,7	20,24	16,75	6,33	16,54	8,66	18,55	5,5	-	8,06	
Dry weight of mucilage sample ($mg\ mL^{-1}$)	100,9	201,2	290,0	232,6	72,2	237,3	190,0	245,9	62,9	262,1	88,43	
AFDW ($mg\ mL^{-1}$)	34,50	68,81	99,18	79,56	22,02	72,38	57,95	75,01	17,23	71,82	24,22	
% Sugar + mannitol in dry mucilage	5,05	6,81	6,98	7,20	8,77	6,97	4,55	7,54	8,74	-	9,11	7,173
% of AFDW	14,78	19,91	20,40	21,05	28,74	22,85	14,94	24,73	31,92	-	33,27	23,26
Mannitol (% of total)	88,43	80,65	91,01	63,10	72,67	68,44	93,07	68,68	66,36	-	75,87	76,83

TABLE 4 *Laminaria pallida*: Analysis of sugars and mannitol in up to 4 samples of mucilage from the stem plus meristem, frond and eroded fronds. Percentage of sugars plus mannitol in mucilage expressed as g^{-1} dry weight and g^{-1} ash-free dryweight. Relative proportion of mannitol expressed as percentage of total.

	Stem & meristem			Fronds				Eroded Fronds		Mean %
Ethanol extraction ($mg\ ml^{-1}$)	6,93	10,45	11,80	6,6	13,33	9,34	2,19	8,09	9,94	
TFA extraction ($mg\ ml^{-1}$)	1,77	3,94	-	0,84	3,57	4,25	0,61	1,86	3,84	
Total identified Sugars + mannitol ($mg\ ml^{-1}$)	8,7	4,39	-	7,44	16,9	13,59	2,8	9,95	13,78	
Dry weight of mucilage sample ($mg\ ml^{-1}$)	102,2	201,3	165,1	101,3	232,9	180,0	95,7	167,1	239,9	
AFDW ($mg\ ml^{-1}$)	37,71	74,27	60,93	35,55	81,76	63,18	17,90	31,26	44,85	
% Sugar + mannitol in dry mucilage	8,51	7,15	-	7,34	7,25	7,55	3,009	5,95	5,74	6,56
% of AFDW	23,07	19,37	-	20,92	20,67	21,51	15,64	31,83	30,72	22,97
Mannitol (% of total)	79,66	72,62	-	88,71	78,87	68,73	78,21	81,31	72,13	77,53

mannose as well as larger quantities of the deoxy sugars a and B fucose. Because of the loss of sugars and mannitol from the eroded fronds of Laminaria pallida, a mean value from the stem plus meristem and frond only has been used in a calculation of the energetic contribution of these substances in the exudate whilst a mean of values from all regions has been used to express the percentage sugars and mannitol in mucilage from Ecklonia maxima.

Alginates and Laminarins

Alginate in mixed samples of dried mucilage from Ecklonia maxima amounted to 4,77% whilst that in Laminaria pallida was 5,01%. Bomb-calorimetric determination of the energetic content of the crude alginate precipitated with HCl from calcium alginate extracted from mucilage (p.12) yielded an energetic contribution of $26,06 \text{ kJ g}^{-1}$, a value which was then used to calculate the energetic significance of alginates in the mucilage released from kelp during fragmentation.

Laminarins in the dried mucilage were also assayed gravimetrically and amounted to 6,18% by weight of the dried mucilage in Ecklonia maxima and 3,92% in Laminaria pallida. These values become 7,23% in E. maxima and 4,59% in L. pallida when the 17% loss in recovery of laminarins is taken into account (p.14). Experimental determination of the energetic content of the precipitated laminarin yielded a value of $14,62 \text{ kJ g}^{-1}$ dry weight which, as in alginates, was then used to calculate the energetic contribution of laminarins to mucilage released by the kelps during fragmentation.

Energetic Contribution of Dissolved
Organic Components in Kelp Mucilage

The quantitative analyses of proteins, lipids, sugars plus mannitol, alginates and laminarins in mucilage from Ecklonia maxima and Laminaria pallida, taken from Newell et al. (1980a), allow an assessment of both the gravimetric and energetic contribution of these components to the mucilage released by kelps during the fragmentation process. The results are summarised in Tables 5 and 6, which show the percentage by weight of each component, as well as their energetic yield, the ash content, and the experimentally determined energetic content of the mucilage.

It is apparent that the analyses yielded a gravimetric recovery of as much as 93,88% in mucilage from Ecklonia maxima and 92,35% in mucilage from Laminaria pallida. The estimated energetic contribution of each of the components analysed amounts to a total of 507,52 kJ (100 g)⁻¹ dried mucilage compared with a measured energetic content of 737 kJ (100 g)⁻¹ dried mucilage in E. maxima. The corresponding mucilage contribution of the components analysed in mucilage from L. pallida amounts to 497,63 kJ (100 g)⁻¹ dried mucilage compared with a measured energetic content of 737 kJ (100 g)⁻¹ dried mucilage. The analyses of the principal components thus account for approximately 69% of the measured energetic content of mucilage from E. maxima and 68% of that from L. pallida. In the case of E. maxima the gravimetric recovery reveals a loss of 6,2% which would yield an additional 164,80 kJ if the material had an energetic content equivalent to the mean for all organic components of 26,57 kJg⁻¹. The energetic yield for 100% gravimetric recovery would

TABLE 5 Energy equivalents of major mucilage components of *Ecklonia maxima* expressed as kJ (100 g⁻¹) dry mucus. Energetic content of dry mucilage is shown as well as the percentage gravimetric and energetic recovery accounted for in the analyses. The mean energy equivalent of the components analysed is 26,57 kJ g⁻¹ and can be used to assess the energetic equivalent of gravimetric losses in the analyses.

Source	% by weight	Energy equivalent (kJ g ⁻¹)	kJ (100 g ⁻¹) dry mucilage
+ Protein	4,74	27,12	128,55
+ Fat	0,173	45,36	7,85
Sugar + mannitol	7,17	19,68	141,11
Alginate	4,77	26,06*	124,31
Laminarin	7,23	14,62*	105,70
Ash	69,8	-	-
Total	93,89	-	507,52
Energy ₁ (kJ g ⁻¹ dry mucilage)	-	7,37	737
Recovery	93,88	-	68,86

* Experimentally determined energy content of alginate and laminarin.

+ Results taken from Newell et al. (1980a).

TABLE 6 Energy equivalents of major mucilage components of Laminaria pallida expressed as kJ (100 g⁻¹) dry mucus. Energetic content of dry mucilage is shown as well as the percentage gravimetric and energetic recovery accounted for in the analyses. The mean energy equivalent of the components analysed is 26,57 kJ g⁻¹ and can be used to assess the energetic equivalent of gravimetric losses in the analyses

Source	% by weight	Energy equivalent (kJ g ⁻¹)	kJ (100 g ⁻¹) dry mucilage
+ Protein	5,27	27,12	142,92
+ Fat	0,182	45,36	8,26
Sugar + mannitol	7,56	19,68	148,78
Alginate	5,01	26,06*	130,56
Laminarin	4,59	14,62*	67,11
Ash	69,74	-	-
Total	92,35	-	497,63
Energy ₁ (kJ g ⁻¹ dry mucilage	-	7,37	737
Recovery (%)	92,35	-	67,52

+ Results taken from Newell et al. (1980a).

* Experimentally determined energy content of alginate and laminarin.

during fragmentation of kelp can be calculated from the results of the analyses of dried mucilage, the wet weight:dry weight ratio of the fronds, and from the dry mass of known volumes of mucilage.

The mean wet weight:dry weight ratio obtained for fronds of Ecklonia maxima was 6,5:1 a value similar to that of 6,657:1 obtained by N.G. Jarman (pers. comm.). Thus for 1 g particulate matter released during fragmentation, there are approximately 5,7 g mucilage. The mean weight of 10 ml of mucilage was found to be 10,289 g, thus the volume occupied by 5,7 g mucilage is 5,516 ml. The mean dry weight of 10 ml mucilage was found to be 0,662 g, so that 100 mg of the dried mucilage sample analysed (Tables 5 and 6) represents 1,511 ml of fresh mucilage. Since 1,511 ml fresh mucilage has an energetic content of 0,737 kJ, it follows that the 5,516 ml of mucilage released per dry frond contains $0,737 \times 5,516/1,511 = 2,690$ kJ. That is, for each 1 g dry weight of material eroded from the tip of the frond of E. maxima, there are 5,516 ml of mucilage which is equivalent to 2,690 kJ (or 6,675 g wet tissue contains 2,69 kJ of energy in mucilage).

N.G. Jarman (pers. comm.) has obtained a biomass:production ratio of 1:4 for E. maxima although, as seen from Tables 7 and 8, this value is subject to variability according to whether the community is a seral or climax one, and probably with depth and other factors. That is, 1 g wet mass of frond yields 4,0 g wet tissue year⁻¹. From the previous calculation 6,675 g wet frond contains 2,69 kJ mucilage; therefore 4,0 g production year⁻¹ yields 1,612 kJ year⁻¹ in mucilage.

TABLE 7 Ecklonia maxima standing crop of fronds and annual production in coastal waters off the west coast of Cape Peninsula, South Africa. Data for E. maxima based on recalculations from Mann et al. (1979) assuming a biomass:production of 1:4, and from unpublished data* kindly made available by N.G. Jarman. The calculated energetic contribution of dissolved organic matter released during fragmentation of the kelp is also shown together with its relative significance as a percentage of total energy production as growth. This figure represents the total energy production minus undetermined losses of mucilage from the general surface of the frond.

	Climax*	Seral*	Mann <u>et al.</u>
<u>Standing crop</u>			
Wet mass of frond (kg m^{-2})	12,75	7,18	5,5
<u>Production</u>			
Wet mass ($\text{kg m}^{-2} \text{ y}^{-1}$)	51,7	27,43	21,92
Dry mass ($\text{kg m}^{-2} \text{ y}^{-1}$)	7,755	4,109	3,463
Carbon ($\text{g m}^{-2} \text{ y}^{-1}$)	2 387,2	1265	1014,6
Energy ($\text{kJ m}^{-2} \text{ y}^{-1}$)	118 574	65 333	55 063
Biomass: production ratio	1 : 4,1	1 : 3,8	1 : 4
<u>Energy released in dissolved organic matter</u>			
kJ g^{-1} wet mass y^{-1}	1,612	1,531	1,612
$\text{kJ m}^{-2} \text{ y}^{-1}$	20 553,0	10 995,4	8866
% of total energy production	17,33	17,72	16,10
<u>Energy released as particulate matter</u>			
% of total production	83 - 84%		

TABLE 8 Laminaria pallida standing crop of fronds and annual production in coastal waters off the west coast of Cape Peninsula, South Africa. Data compiled from Dieckmann (1978)⁺ and recalculated from Field et al. (1977). The calculated energetic contribution of dissolved organic matter released during fragmentation of the kelp is shown together with its relative significance as a percentage of total energy production as growth. This figure represents the total energy production minus undetermined losses of mucilage from the general surface of the fronds. The combination which particulate organic matter makes is expressed as a percentage of total energy production as growth.

	8 m ⁺	13 m ⁺	Field et al
<u>Standing crop</u>			
Wet mass of frond (kg m ⁻²)	5,870	1,770	6,712
<u>Production</u>			
Wet mass (kg m ⁻² y ⁻¹)	27,589	6,177	41,346
Dry mass (kg m ⁻² y ⁻¹)	3,446	0,772	5,164
Carbon (g m ⁻² y ⁻¹)	869,1	194,6	1302,4
Energy (kJ m ⁻² y ⁻¹)	37 699	8440	56 494
Biomass: production ratio	1 : 4,7	1 : 3,5	1 : 6,2
<u>Energy released in dissolved organic matter</u>			
kJ g ⁻¹ wet mass y ⁻¹	2,186	1,581	2,883
kJ m ⁻² y ⁻¹	12 831,8	2 798,4	19 350,7
% of total energy production	34,04	33,16	34,25
Energy released as particu- late organic matter	66 - 67%		
% of total energy production			

Only the standing crop of E. maxima is needed to arrive at figures for the relative significance of dissolved and particulate losses by the kelp.

In much the same way, a mean wet weight:dry weight ratio for fronds of L. pallida of 7,928:1 was obtained, which is quite similar to that of 8,008:1 reported by Dieckmann (1978) and 8,105:1 by Field et al. (1977). The mean weight of 10 ml of mucilage was found to be 10,27 g, thus the volume occupied by 7 g of mucilage is 6,816 ml. The mean dry weight of 10 ml of mucilage was found to be 0,742 g so that 100 mg of the dried mucilage sample analysed represents 1,348 ml of fresh mucilage. An energetic content of 0,737 kJ (100 mg)⁻¹ dried mucilage equivalent to 1,348 ml of fresh mucilage was obtained. Since 1,348 ml fresh mucilage from L. pallida has an energetic content of 0,737 kJ, it follows that the 6,816 ml mucilage released g⁻¹ of dry frond contains 0,737 x 6,816/1,348 = 3,727 kJ. That is, for 1 g dry weight eroded from the tip of the frond of L. pallida there are 6,816 ml of mucilage which is equivalent to 3,727 kJ (or 8 g of wet tissue contain 3,72 kJ energy in mucilage).

Dieckmann (1978) gives a biomass:production ratio of 1:4,7 for Laminaria pallida at 8 m depth although, as seen from Table 8, this value falls to 1:3,5 at 13 m depth, whilst Field et al. (1977) obtained a production to frond biomass ratio of 1:6,2. From the previous calculation, 8 g of wet frond contains 3,72 kJ mucilage; therefore 4,7 g of production at 8 m depth yields 2,816 kJ year⁻¹ in mucilage. As in the case of Ecklonia maxima, only the standing crop is needed to arrive at figures for the annual production of energy in particulate

and dissolved components during fragmentation of both species of kelp.

Values for standing crop and production of E. maxima have been assembled in Table 7 from data supplied by N.G. Jarman (pers. comm.) and recalculated from Mann et al. (1979). In E. maxima, the dry mass of frond is 15% of the wet mass, whilst the carbon is 29,3% of the dry mass, and the energetic content in kJ is $15,9 \text{ g}^{-1}$ dry frond (Mann et al. (1979). Comparable data for L. pallida have been assembled in Table 8 from data of Dieckmann (1978) and Field et al. (1977). In this case Dieckmann (1978) has shown that the dry mass of the frond is 12,5% of the wet mass whilst carbon is 25,22% of the dry mass. The energetic content is $10,94 \text{ kJ g}^{-1}$ dry frond of L. pallida. So the main difference is that E. maxima has a greater proportion of dry mass (15% of wet weight) and more kJ g^{-1} of frond ($15,9 \text{ kJ g}^{-1}$), whereas L. pallida has a dry mass of only 12,5% of the wet weight and this has an energetic equivalent of only $10,94 \text{ kJ g}^{-1}$.

The energy released as mucilage and its significance relative to total production may be calculated as follows. Dieckmann (1978) has shown, for example, that at 8 m depth the standing crop of L. pallida is $5,870 \text{ kg wet mass m}^{-2}$ (Table 8). Since it has been shown that 1 g of wet frond yields 4,7 g of production which is equivalent to $2,186 \text{ kJ year}^{-1}$ in mucilage, a standing crop of 5870 g wet frond yields $2,186 \times 5870 = 12\,831,82 \text{ kJ m}^{-2} \text{ y}^{-1}$ as mucilage. The total energetic content of fresh frond production of L. pallida at 8 m depth is $37\,699 \text{ kJ m}^{-2} \text{ y}^{-1}$ (see Table 8 and Dieckmann, 1978). Thus the energy

released in mucilage accounts for $12\,831,82/37\,699 \times 100 = 34,04\%$ of the annual energy production of L. pallida at 8 m depth. As might be anticipated, the standing crop and production is lower at a greater depth of 13 m each 1 g of wet frond yielding $3,47 \text{ g production year}^{-1}$ or $1,581 \text{ kJ year}^{-1}$ of mucilage. The mucilage released by the standing crop of $1,770 \text{ kg wet mass}^{-2}$ is thus $1770 \times 1,581 = 2798,37 \text{ kJ m}^{-2} \text{ y}^{-1}$. The total energetic content of the fresh frond production of L. pallida at 13 m depth is $8440 \text{ kJ m}^{-2} \text{ y}^{-1}$. Thus the energy released as mucilage accounts for $2798,37/8440 \times 100 = 33,16\%$ of the annual energy production of L. pallida at 13 m depth.

Similar calculations for E. maxima are based on the standing crop and energy production of the frond. N.G. Jarman (pers. comm.), for example, has obtained a value of $12,75 \text{ kg m}^{-2}$ in a climax community of E. maxima on the west coast of the Cape Peninsula. Since it has been shown that 1 g of wet frond of L. pallida yields 4 g of wet tissue year^{-1} and that this contains $1,612 \text{ kJ year}^{-1}$ in mucilage, a standing crop of $12,75 \text{ kg wet tissue}$ yields $12\,750 \times 1,612 = 20\,553,0 \text{ kJ m}^{-2} \text{ y}^{-1}$ as mucilage. N.G. Jarman (pers. comm., Table 7) gives a total energy production in a climax community of E. maxima of $118\,574 \text{ kJ m}^{-2} \text{ y}^{-1}$. The relative significance of mucilage production is thus $20\,553/118\,574 \times 100 = 17,33\%$. The equivalent standing crop for a seral community of E. maxima is $7,18 \text{ kg}$ (Table 8) which yields $7180 \times 1,612 = 11\,574,16 \text{ kJ m}^{-2} \text{ y}^{-1}$ as mucilage. The total energy production of a seral community is $65\,333,4 \text{ kJ m}^{-2} \text{ y}^{-1}$ (Table 7) so that the relative significance of energy released as mucilage is

11 574,16/65 333,4 x 100 = 17,716% of the total energy production by the seral community.

Calculation of the significance of energy released in mucilage based on data of Mann et al. (1979) recalculated for the frond biomass of E. maxima and of Field et al. (1977) for that of L. pallida are included in Table 8 and yield similar values to those cited above. It should be emphasised, however, that both standing crop and biomass to production ratio are subject to considerable variation with both depth and community structure. Any overall estimates of energy production from the coastline as a whole must therefore be treated with a good deal of caution. Nevertheless it is possible to obtain some estimates of the amounts of dissolved and particulate organic matter which are likely to be released from a specific kelp bed of known area. N.G. Jarman (pers. comm.) has found, for example, that a kelp bed at Kommetjie on the west coast of the Cape Peninsula, has a shallow water area of 220 ha and a further 480 ha at between 8 m and 13 m depth. If the average energy production of a climax and seral community of E. maxima is taken to be $91\,953 \text{ kJ m}^{-2} \text{ y}^{-1}$ and that of L. pallida, to be $34\,211 \text{ kJ m}^{-2} \text{ y}^{-1}$ (Tables 7 & 8), it is possible to calculate the total energy production of the kelp bed, provided the relative standing stocks of E. maxima and L. pallida are known. N.G. Jarman has estimated that the standing stocks for the west coast of the Cape Peninsula south of Cape Columbine are 392 160 tons for E. maxima and 117 858 t for L. pallida. For a typical kelp bed of 700 ha, the standing stocks will be in the proportion of approximately 525 ha (or $525 \times 10^4 \text{ m}^2$) of E. maxima and 175 ha (or $175 \times 10^4 \text{ m}^2$).

of L. pallida. Total energy production for E. maxima from the kelp bed will therefore amount to $48 \times 10^{10} \text{ kJ y}^{-1}$ of which 18% or $8,64 \times 10^{10} \text{ kJ y}^{-1}$ will be in the form of dissolved organic matter. Similarly, total energy production from L. pallida in the kelp bed will amount to $5,99 \times 10^{10} \text{ kJ y}^{-1}$ of which 35% or $2,1 \times 10^{10} \text{ kJ y}^{-1}$ will be in the form of dissolved organic matter released during fragmentation by eroding kelp.

Total energy released as particulate matter from a kelp bed of 700 ha is thus $39,36 \times 10^{10}$ from E. maxima plus $3,89 \times 10^{10}$ from L. pallida = $43,25 \times 10^{10} \text{ kJ y}^{-1}$. Similarly, total energetic release in the form of dissolved organic matter from the kelp bed will be $8,64 \times 10^{10} + 2,1 \times 10^{10} = 10,74 \times 10^{10} \text{ kJ y}^{-1}$. This value represents a very large annual amount of dissolved organic matter. In E. maxima, for example, for each 1 g dry tissue eroded from the tip there are 5,516 ml which contain 2,69 kJ (p.24) whereas an L. pallida each 1 g dry tissue eroded represents 6,816 ml which contains 3,72 kJ (p.27). A mean value for both species is thus $3,21 \text{ kJ} = 6,05 \text{ ml}$ mucilage released during fragmentation. An annual dissolved energy production during fragmentation from the tips of the kelp of $10,74 \times 10^{10} \text{ kJ}$ would thus be equivalent to the release of $6,05/3,21 \times 10,74 \times 10^{10} \text{ ml} = 20,24 \times 10^7 \text{ l}$ into shallow water, to which should be added undetermined losses of mucilage from the surface of the fronds (p.7).

TABLE 9 The chemical composition of dried mucilage (DOM) and powdered frond (POM) from the kelp Laminaria pallida. Data sources are indicated at the foot of the table

COMPONENT	% of Total Dry Weight	
	DOM	POM
Protein	5,27	23,78*
Total lipid	0,18	0,58 ⁺
Carbohydrates		
Polyols (mannitol)	5,86	4,40 [#]
Sugars (fucose)	1,70	1,70 [#]
Laminarin	4,59	1,50*
Alginate	5,01	44,90*
Ash	69,74	22,94*
Total recovery	92,35	99,80
<hr/>		
% of Carbon Fixed		
Hatcher <u>et al.</u> (1977)	35	65
Johnston <u>et al.</u> (1977)	23	77
% of Energy Production	33-34	66-77

* Stuart et al. (1981)

+ Velimirov (1979)

von Holdt et al. (1955)

CONCLUSION

The results presented above show that as much as 18% of the energetic contribution of Ecklonia maxima and 34% of that of Laminaria pallida is released during fragmentation as dissolved organic matter whilst the remainder is available in a particulate form (see Tables 7 & 8). The conversion of such material by the heterotrophic community of bacteria and protozoa in kelp bed water has recently been studied in detail (see Linley et al., 1981; Linley & Newell, 1981; Lucas et al., 1981; Newell & Lucas, 1981; Stuart et al., 1981; Koop et al., 1982a, 1982b). Linley et al. (1981) and Lucas et al. (1981) showed that much of the carbon in the dissolved component is respired by the micro-heterotrophic community, only 10 - 15% being incorporated into microbial biomass. Stuart et al. (1981) and Koop et al. (1982a, b) have shown that under conditions of nitrogen enrichment this 'growth yield' or carbon conversion efficiency can reach 33%. They also showed that nitrogen, in contrast to carbon, is incorporated with an efficiency of 83 - 94% so that it seems likely that much of the protein initially released with both the particulate and 'dissolved' components will ultimately become available to consumers in the form of microbial biomass rather than non-utilised kelp protein.

The chemical composition of the dried mucilage and powdered particulate material from Laminaria pallida is summarised in Table 9 which has been compiled from various sources. It is clear that the particulate component represents a dominant source of carbohydrates and that protein in the freshly powdered fronds amounts to as much as 23,8% of

the dry weight. Such material may thus represent a principal food resource for the dense community of suspension feeders which is associated with kelp beds (see Field et al., 1977; Velimirov et al., 1977).

The following section of this thesis reports the results of an investigation of the style enzymes which are implicated in the digestion of the particulate components of kelp detritus, and of the ability of the enzymes to meet the carbon requirements of the consumer organisms. Later sections of the thesis subsequently deal with the exploitation of the microbial component as a protein resource by Choromytilus meridionalis.

SECTION II

CARBON BALANCE: QUANTITATIVE SIGNIFICANCE
OF STYLE CARBOHYDRASES
IN RELATION TO DIET

INTRODUCTION

There have been numerous studies on digestive enzymes in molluscs since the pioneer work of Coupin (1900) and Yonge (1923) established the presence of amylase (1, 4 - α - D - Glucan Glucanohydrolase) in the crystalline style of bivalves. Subsequent work has shown a wide spectrum of enzymes, notably carbohydrases, in both the crystalline style (see Yokoe & Yasumasu, 1964; Horiuchi & Lane, 1966; Kristensen, 1972; Wojtowicz, 1972; Hara et al., 1979; Jacober et al., 1980) and digestive gland (Santoro & Dain, 1981). Laminarinases (1, 3 - (1, 3; 1, 4) - β - D - Glucan 3 (4) - Glucanohydrolase) in particular, have been studied in some detail (Bull & Chesters, 1966; Sova et al., 1970a, b; Shallenberger et al., 1974) and the results suggest that many bivalves are able to digest the commoner components of macrophyte debris. More recently, studies on alginate lyase (Poly (1, 4 - β - D - Mannuronide) Lyase) (Muramatsu & Egawa, 1980), cellulases (1, 4 - (1, 3; 1, 4) - β - D - Glucan 4 - Glucanohydrolase) (Mirza & Serban, 1981) and amylases (Alemany & Rossel-Pérez, 1973; Newell et al., 1980b) have demonstrated the occurrence of several enzyme variants although their precise role is as yet unknown.

Although there is thus a good deal of information on the qualitative distribution of digestive enzymes in molluscs, there are few studies in which the hydrolytic activity of the style enzymes have been related quantitatively to the carbon requirements of bivalves. Neither, apart from the work of Crosby and Reid (1971), have there been any studies on bivalves to determine whether style enzyme activity can be related

to dietary composition. The following work was therefore undertaken to determine the role of style carbohydrase activity in the carbon balance of the mussel Choromytilus meridionalis which occurs in dense communities near to kelp beds on the west coast of the Cape Peninsula, South Africa. The results are compared with those for the mussel Perna perna which lives in plankton-dominated water on the east coast of southern Africa and which does not encounter the high particulate load from fragmented kelp debris which forms a dominant part of the material available for ingestion by C. meridionalis.

MATERIALS AND METHODS

1. Collection of Mussels and Isolation of Styles

Specimens of Choromytilus meridionalis (Krauss) from Bloubergstrand in Table Bay and Perna perna (Linnaeus) from Arniston on the south coast of South Africa were collected and transported to the laboratory. The styles from approximately 20 specimens were immediately removed, rinsed and homogenised with a glass tissue grinder in 12 ml 20 mM phosphate buffer (Appendix A) pH 7,0 containing 150 mM NaCl (see also Mathers, 1974; Langton, 1977; Seiderer & Newell, 1979). The homogenate was then centrifuged for 5 min at 15 000 x g and the supernatant used for subsequent enzyme assays.

2. Gel-Filtration

Aliquots of 5 ml style extract containing approximately 39 mg protein ml^{-1} from C. meridionalis and 12 mg ml^{-1} from P. perna were loaded onto a Biogel P60 gel filtration column (1 m x 5 cm) and eluted with approximately 300 ml 20 mM phosphate buffer pH 7,0 containing 150 mM NaCl. The eluate was collected in 2,4 ml fractions and the protein in each fraction determined using the u/v spectrophotometric method of Groves et al. (1968) for samples containing $<0,3 \text{ mg ml}^{-1}$. The absorbance of the fractions at 230 nm (1 cm light path) was determined with a Beckman Model 25 spectrophotometer. (An enzyme scan from 300 to 200 nm wavelength showed A_{230} to be a more suitable measure than A_{280} , as can be seen in Fig. 1.)

Using bovine serum albumin at concentrations between 0,02 and 0,40 mg ml^{-1} as standards, this method was found to yield a linear relationship between 1 and 350 $\mu\text{g ml}^{-1}$ ($\hat{y} = 0,01 + 5,12 x$ $r^2 = 1$) as can be seen in Fig. 2. For samples containing $<0,3 \text{ mg protein ml}^{-1}$, the method of Lowry et al. (1951) was used ($\hat{y} = 0,06 + 0,0043 x$; $r^2 = 0,99$) (Fig.3).

Of the 195 mg protein from the style of C. meridionalis added to the column, 115,9 mg were recovered; recovery of style protein from P. perna was 46,6 mg from 60 mg added to the column. The recovery of style proteins from C. meridionalis was thus 59% whilst that from P. perna was 78%.

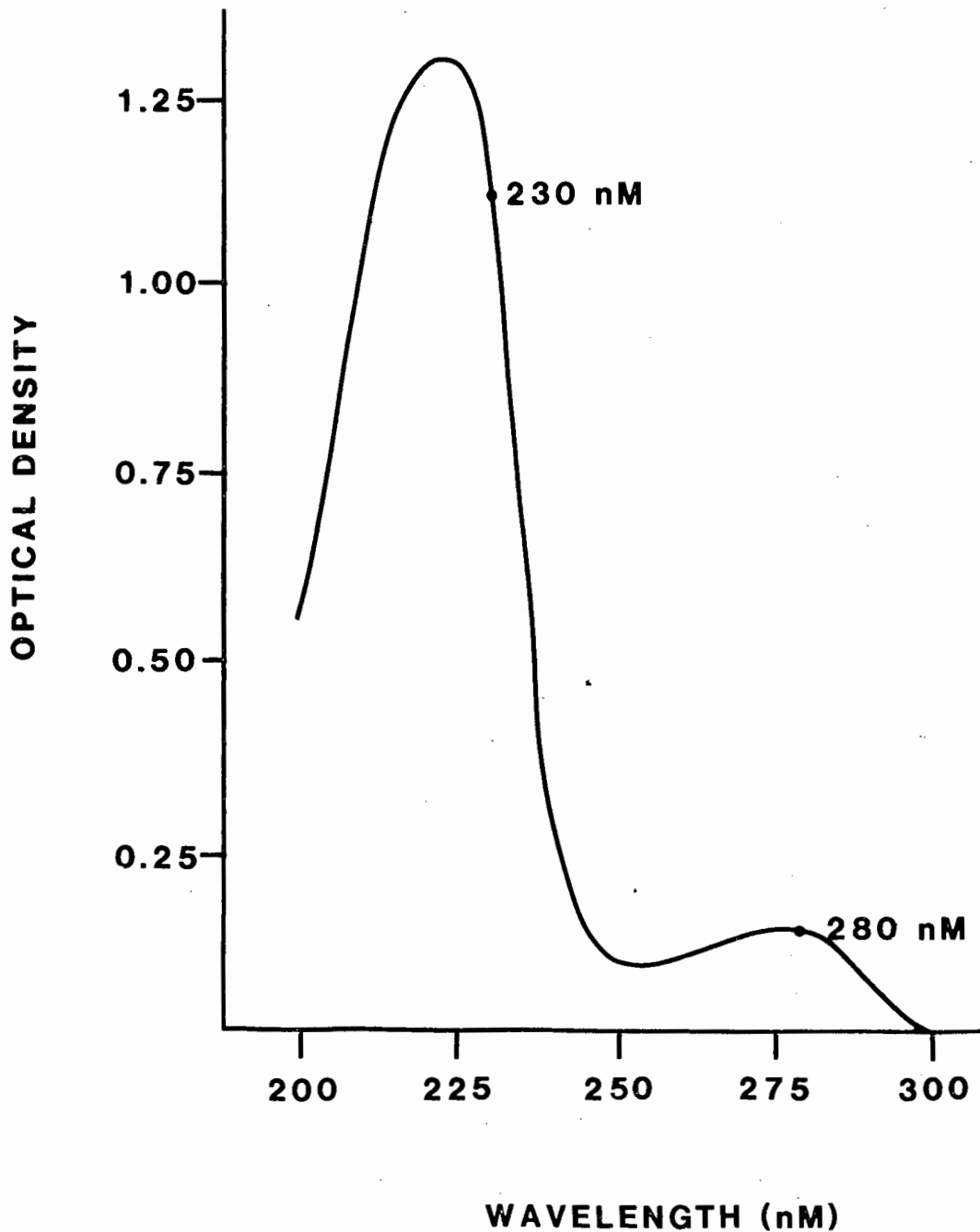


Figure 1 An ultraviolet spectrophotometric scan of the style extract of *C. Meridionalis* from a wavelength of 300nm through to 200 nm. The optical density of the extract was recorded on a chart recorder during the scan.

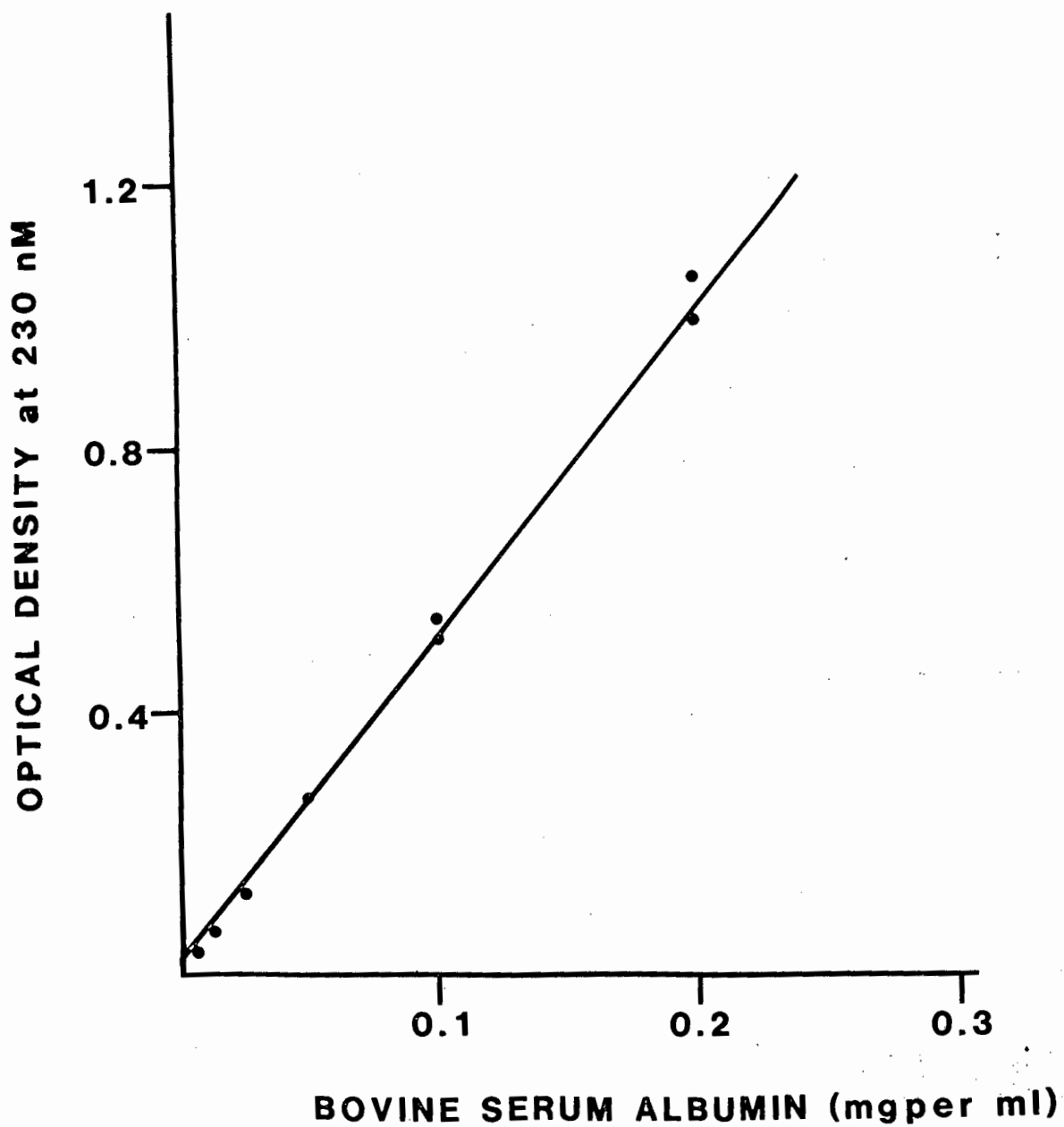


Figure 2 The optical density of standard bovine serum albumin (BSA) at 230 nM. Regression equation: ($\hat{y} = 0,01 + 5,12x$; $r = 1$; $n = 8$). Carried out according to the method of Groves et al. (1968).

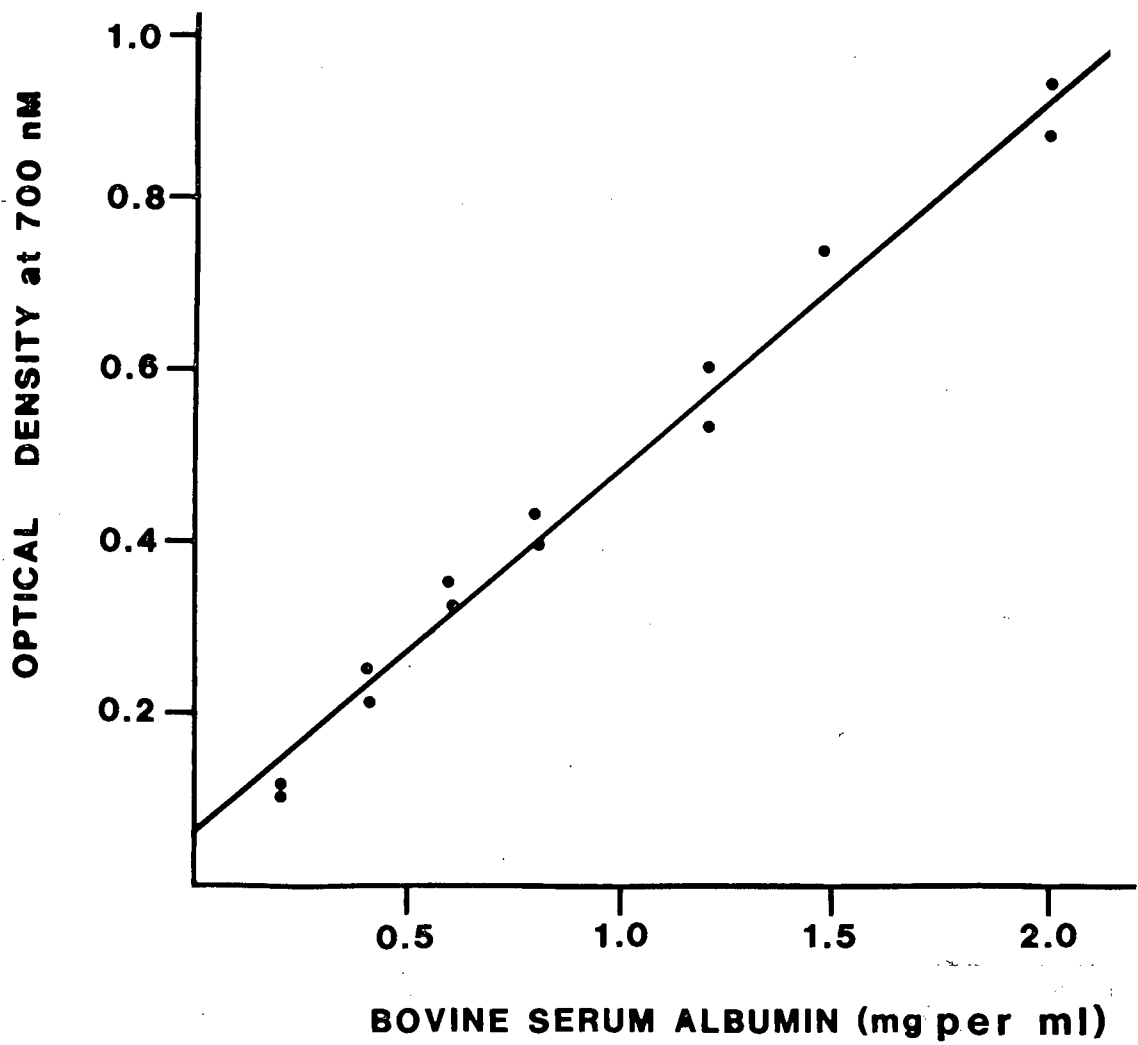


Figure 3 Calibration curve for the Folin-Lowry method of protein determination. Regression equation: ($\hat{y} = 0,06 + 0,0043 x$; $r = 1$, $n = 13$).

Alternate fraction samples were then assayed directly for α -amylase, cellulase, laminarinase and alginate lyase. The approximate molecular weight of the protein in each fraction was estimated from the elution volume using purified bovine serum albumin, ovalbumin, human growth hormone and myoglobin as standards. The molecular weights of the standards are shown in Fig. 5. Masses of 2 mg of each of the standards were eluted through the Biogel P60 gel filtration column with approximately 300 ml 20 mM phosphate buffer pH 7,0 containing 150 mM NaCl. The fractionated samples were scanned at 230 m μ , and the following molecular marker profile shown in Fig. 4 was then used to construct a calibration curve.

The calibration curve shown in Fig. 5 was drawn on a semi-log scale and was used for direct estimations of the molecular weights of the style enzymes (see p. 54). The regression equation of the calibration curve was as follows: ($\log M.W. = a + b \cdot \text{elution volume} ; \log M.W. = 410 - 1,92 x ; r = 0,98, N = 4$).

a) α -Amylase

The α -amylase activity was determined by the method of Bernfeld (1955; see also Seiderer & Newell, 1979) and the results expressed as mg glucose liberated from oyster glycogen in 8 minutes at 37°C by 200 μ l of fraction. Oyster glycogen was made up in 20 mM phosphate buffer pH 7,0 with 150 mM NaCl at a concentration of 1% w/v, such that the substrate concentration was saturated.

Each 200 μ l aliquot of eluate was incubated in a shaking water bath

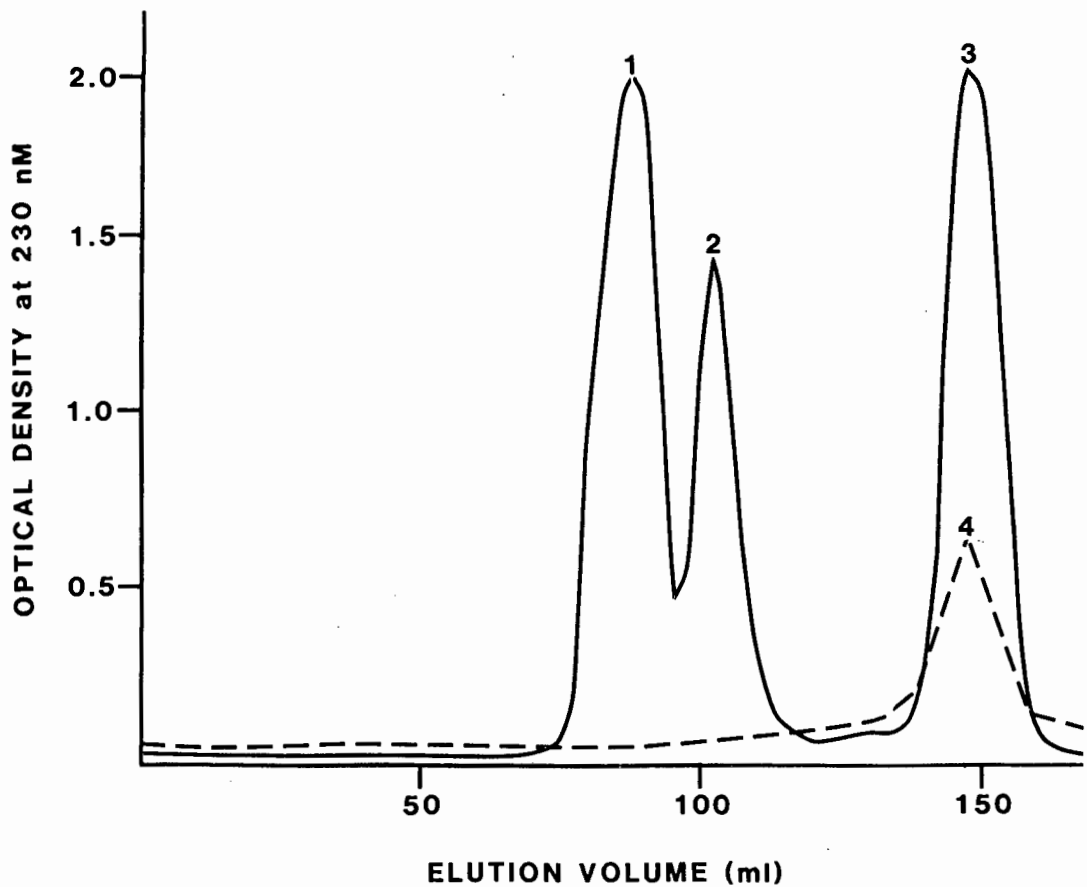


Figure 4 Trace showing the optical density at 230 nM of a series of protein standards whose molecular weights are given in Fig. 5, plotted against elution volume from a Biogel P60 gel filtration column. The protein standards are as follows: Bovine serum albumin (1); Ovalbumin (2); Myoglobin (3) and Human growth hormone (4). Note that the human growth hormone standard (--) was not run at the same time as the other three standards (—).

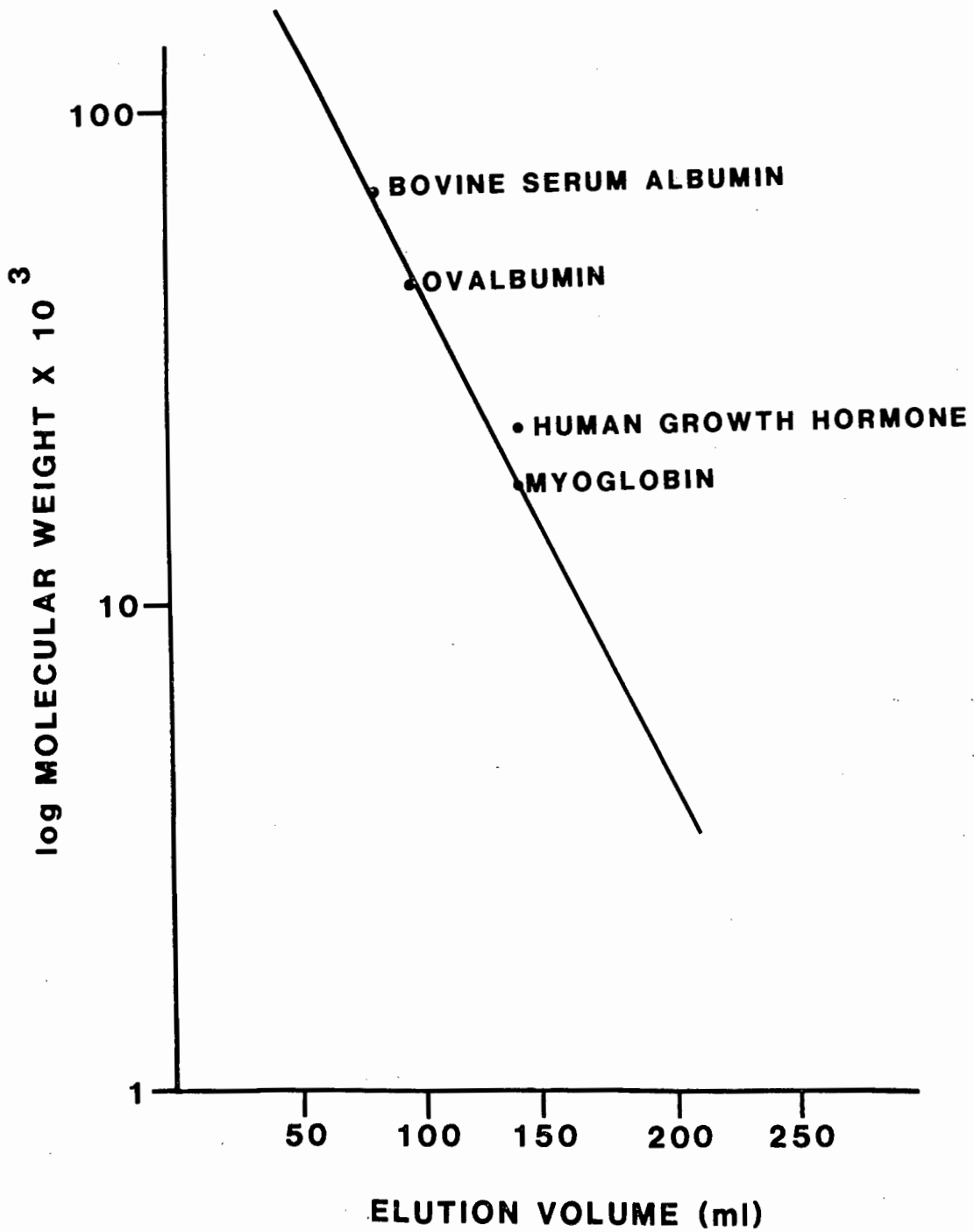


Figure 5 Graph showing the log molecular weight of a series of protein standards plotted as a function of elution volume (ml) from a Biogel P60 gel filtration column. Regression equation: ($\log \text{M.W.} = a + b \cdot \text{elution volume}$; $\log \text{MW} = 410 - 1,92x$; $r = 0,98$, $n = 4$).

for 8 minutes with 200 μl substrate solution at 37°C. The reaction was halted after 8 minutes by the addition of 400 μl dinitrosalicylate (Appendix A) reagent and the tube was heated for 5 minutes in a boiling water bath and cooled under running tap water. After the addition of 4 ml of distilled water, the absorbance of the solution containing the brown reduction product was determined spectrophotometrically at 540 nm. A blank solution without style extract was used as a control, and the corresponding glucose units were calculated from Fig. 6, a calibration curve established for glucose concentrations of 0,1 - 1,0 mg glucose ml^{-1} ($\hat{y} = -0,02 + 0,31 x$; $r = 1$, $N = 15$). Enzyme activity was then expressed as glucose evolved per mg protein in the fraction.

b) Cellulase

The cellulolytic formation of soluble reducing sugars from cellulose was determined colorimetrically using the Somogyi-Nelson reagent (Nelson, 1952) (Appendix A).

i) Calibration

A glucose calibration was carried out by adding 200 μl of glucose in concentrations of 0,01 - 0,5 mg ml^{-1} to 4,8 ml carboxymethyl cellulose. A 1 ml aliquot was removed and boiled for 10 minutes together with 1 ml of Somogyi reagent. After the tubes were cooled under running water, 2 ml Nelson reagent and 6 ml distilled water were added and the solution was allowed to stand for 20 minutes at room temperature. The resultant product was read at 660 nm on the spectrophotometer and corrected for blank (see

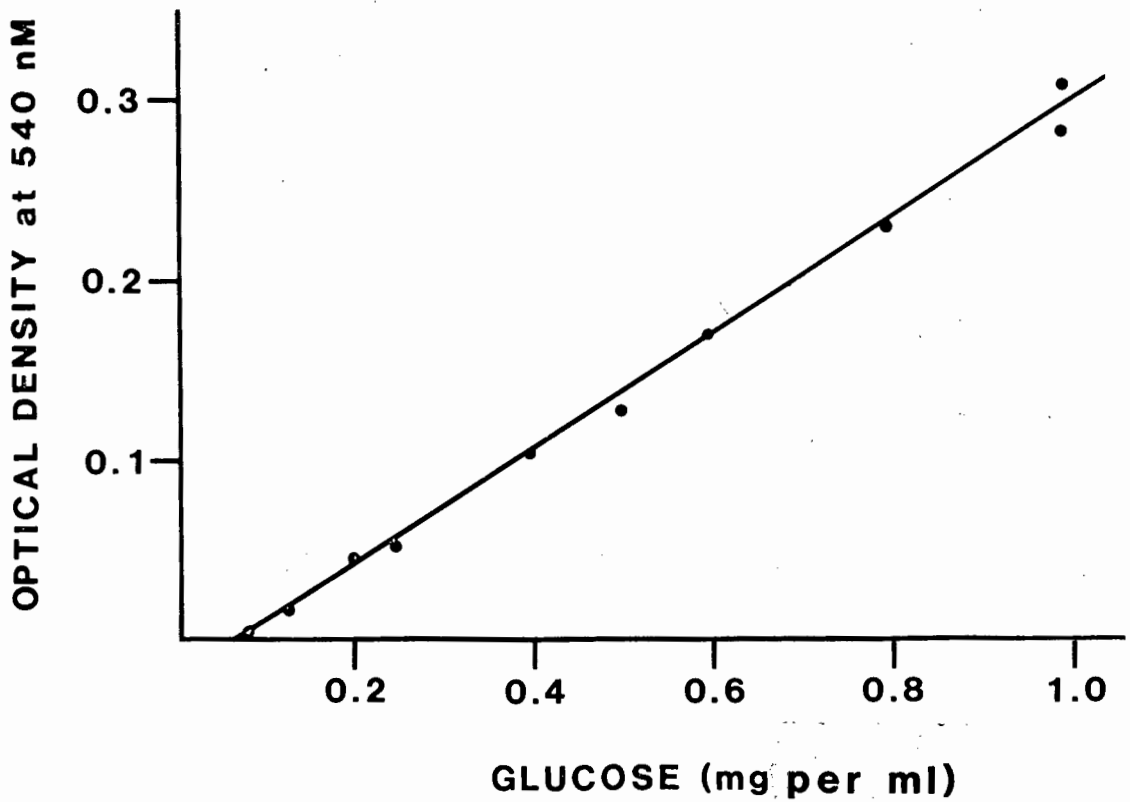


Figure 6 Glucose calibration curve established for glucose concentrations of 0,1 - 1,0 mg glucose.ml⁻¹ used as a standard in the dinitrosalicylic acid assay for α -amylase and laminarinase. Regression equation: ($\hat{y} = -0,02 + 0,31x$; $r = 1$, $n = 10$).

Fig. 7). The regression equation reads $\hat{y} = 0,01 + 0,65 x$;
 $r = 0,96$, $n = 22$.

ii) Assay

The assay was carried out in the same manner as the calibration, except that 200 μl of eluate was added to the 4,8 ml carboxymethyl cellulose in place of the 200 μl glucose. The tubes were incubated in a shaking water bath at 37°C and a time zero reading was taken after 5 minutes. This reading was subtracted from the reading taken after 24 hours to eliminate free reducing sugars in the fraction itself.

c) Laminarinase

Laminarinase activity was estimated by a modification of the methods of Lindley and Shallenberger (1976) and Jacober et al. (1980). A sample of 200 μl fraction was incubated in a shaking water bath at 37°C for 8 minutes with 0,4% (w/v) laminarin in 20 mM phosphate buffer pH 7,0 with 150 mM NaCl. The reaction was then stopped by the addition of dinitrosalicylic acid. After boiling for 5 min, 4 ml distilled water was added and the absorbance of the solution containing the brown reaction products was determined spectrophotometrically at 540 nm. The glucose equivalents were then calculated from the calibration curve for the α -amylase assay (see p. 46). The amount of glucose generated through enzymatic action was determined by subtracting the level of glucose initially present in a blank of substrate and enzyme homogenate from the glucose measured at the end of the reaction.

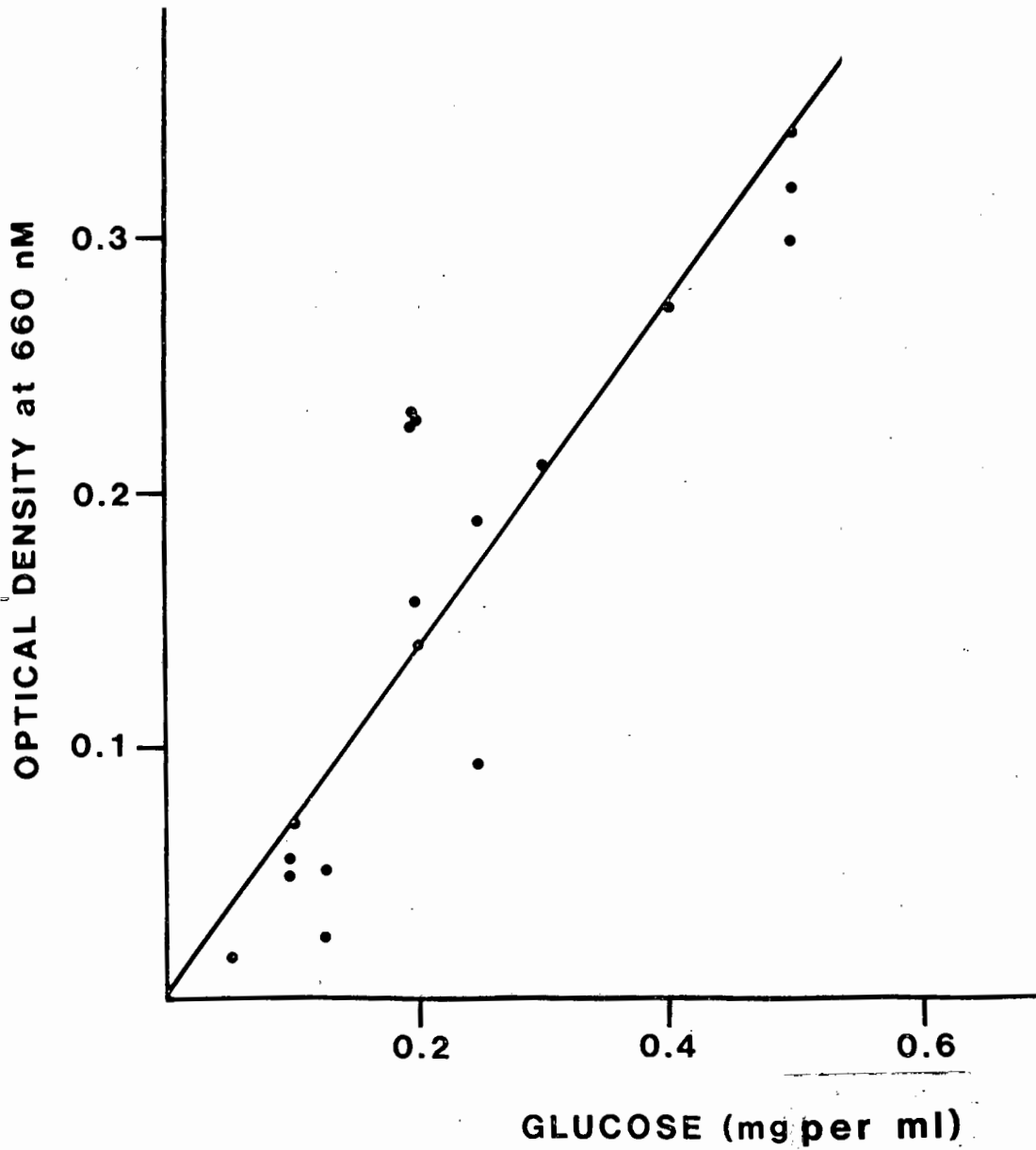


Figure 7 A glucose calibration curve established for glucose concentrations of 0,01 -0,5 mg glucose. mL^{-1} used as a standard in the modified Nelson-Somogyi assay for cellulase. Regression equation: ($\hat{y} = 0,01 + 0,65x$; $r = 0,96$, $n = 22$).

d) Alginate lyase

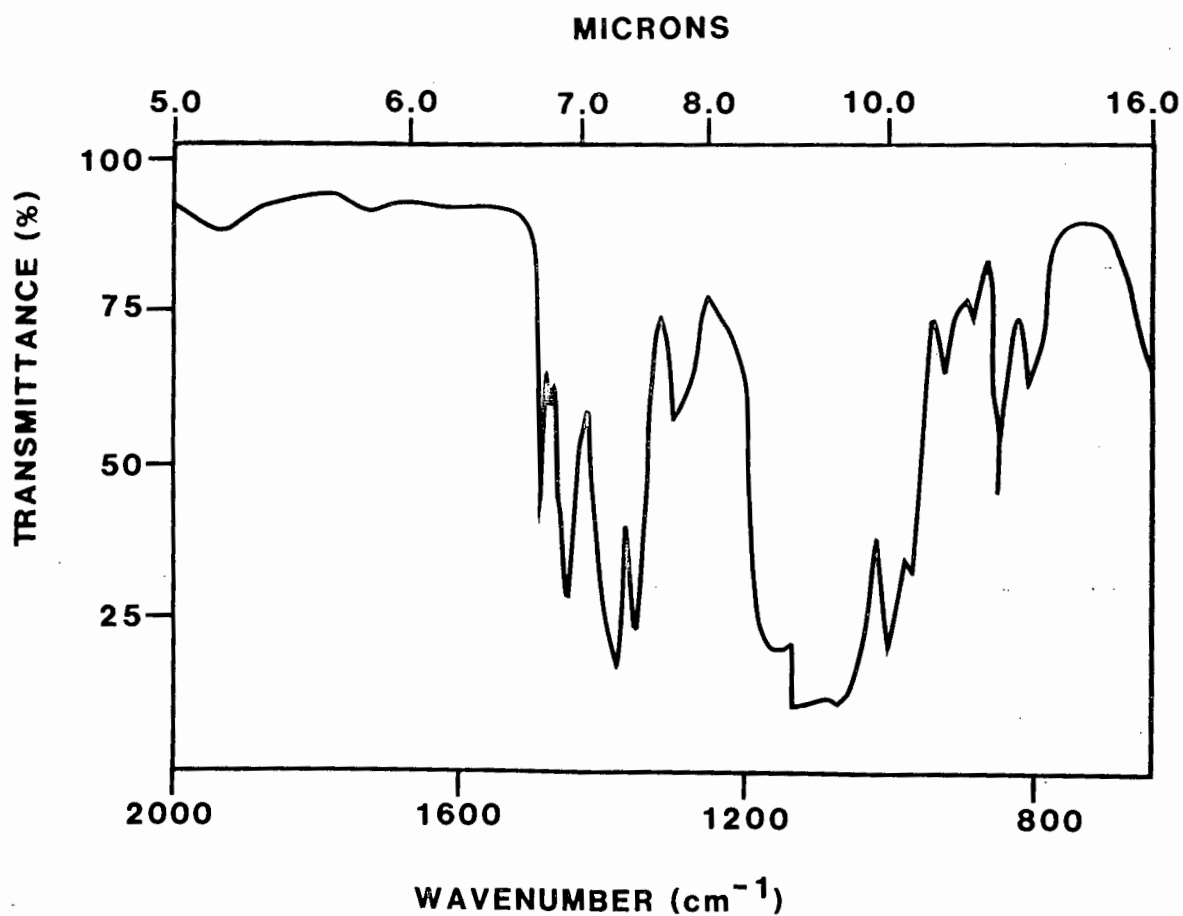
The alginate lyase activity was determined colorimetrically using the thiobarbituric acid method of Jacober et al. (1980) in which the thiobarbituric acid reacts with the aldehyde derivatives of guluronic and mannuronic acids. Reagents can be found in Appendix A.

i) Calibration

Jacober et al. (1980) used a standard curve of known 1,1,3,3,-tetraethoxypropane concentrations ranging from 0 - 25 $\mu\text{g ml}^{-1}$, which hydrolyses under acid conditions to form malondialdehyde. Since only malondialdehyde was available as a standard, an infrared scan was run to determine the comparability. In fact the scan (Fig. 8) showed malondialdehyde to be synonymous with tetraethoxypropane. Alginate degradation was therefore determined using malondialdehyde standards over the range 0,001 - 0,05 μl . Shown in Fig. 9 is a calibration curve relating optical density to volume of malondialdehyde ($\hat{y} = 0,058 + 45 x$; $r = 0,97$, $N = 50$).

ii) Assay

A 200 μl aliquot of each fraction was incubated with 600 μl sodium alginate, shaking at 37°C. At 24 hour intervals, 200 μl aliquots were removed and added to 250 μl fresh KIO_4 . The sample was allowed to stand for exactly 20 minutes, to allow periodate oxidation to take place, and then stopped by the addition of 500 μl NaAsO_4 . After 2 minutes, 2 ml thiobarbituric acid were



Sample: Tetraethoxy propane	Solvent: Nil	Scan Speed: Fast	OP.: Merrifield
	Conc: 100%	Slit: N	Date: 4.11.81.
	Cell Path: -	Remarks: OK	

Figure 8 An infrared spectrum run to determine whether or not Malondialdehyde is synonymous with 1, 1, 3, 3, - tetraethoxypropane. The report confirms their synonymy.

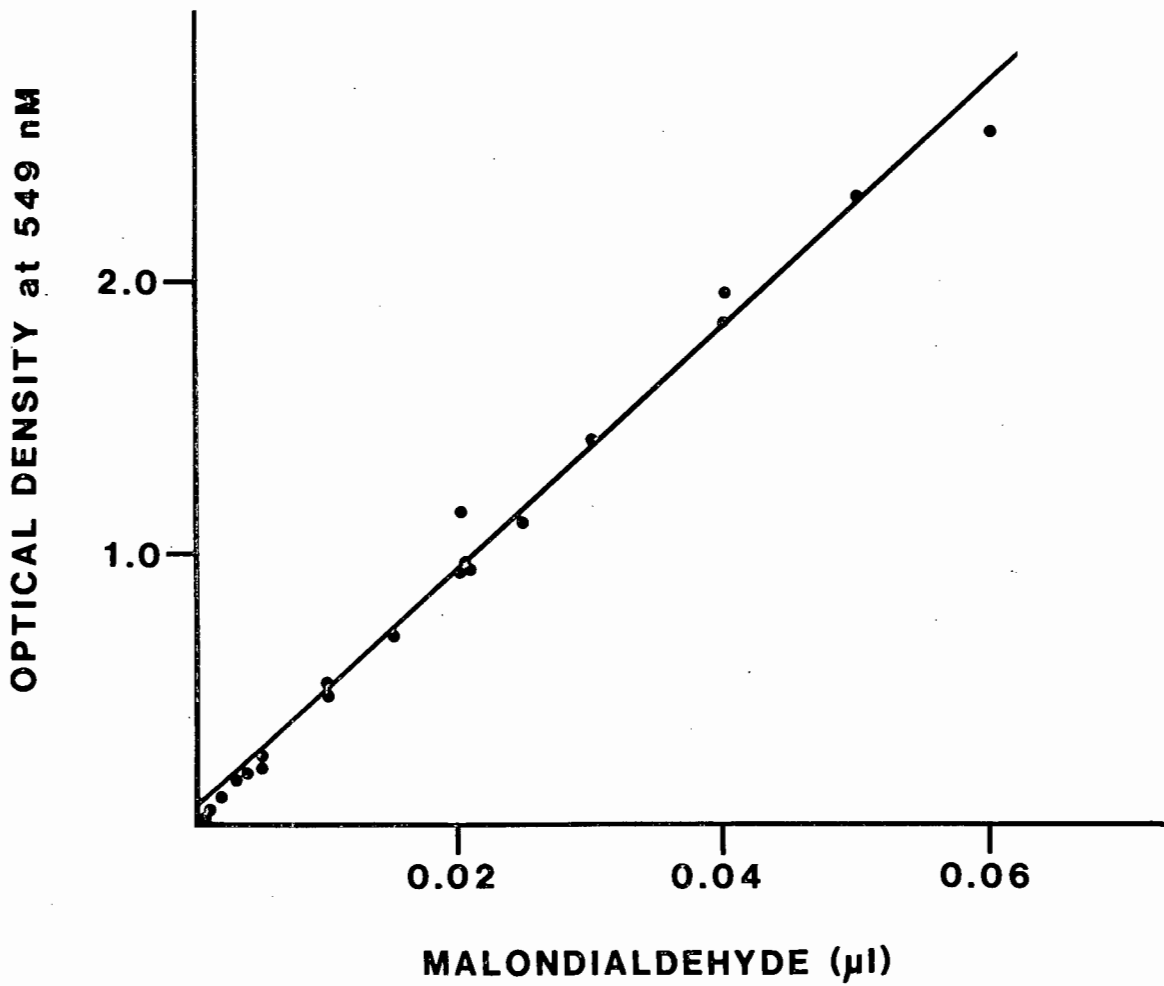


Figure 9 A malondialdehyde calibration curve established for malondialdehyde concentrations of 0,001 - 0,05 μ l used as a standard in the thiobarbituric acid assay for alginate lyase. Regression equation: ($\hat{y} = 0,058 + 45 x$; $r = 0,97$, $n = 20$).

added, and the sample was boiled for 10 minutes to allow the reaction with the aldehyde derivatives of guluronic and mannu-
ronic acids to take place. The sample was cooled rapidly and
read immediately at 549 nM on the spectrophotometer. It was
found that the thiobarbituric acid tended to precipitate out
under neutral or alkali conditions, and as such, rapid reading
of the sample was necessary.

4. SDS Polyacrylamide Gel Electrophoresis (PAGE)

In an attempt to confirm the molecular weights of the eluate fractions from the gel filtration column, an SDS-PAGE (Laemmli, 1970; O'Farrel, 1975) was run, incorporating standards of known molecular weights.

To each sample (whole crude style extract, or eluate fractions from the gel filtration column), 100 μ l of SDS buffer, and 2 μ l of Bromo-phenol blue were added and boiled for 2 minutes. The GOAT molecular marker, incorporating growth hormone (22 000), ovalbumin (43 000), human albumin (70 000) and transferrin (90 000), was treated in the same manner.

A detailed description of the setting up and running of SDS-PAGE gels follows. The glass plates (140 x 170 mm) were cleaned with alcohol, sealed with tenacious tape while incorporating the gel spacers (0,5 mm), and the edges were sealed with a 1% agarose solution. The lower

resolving gel, sufficient for 2 plates, was made up according to Appendix A and poured into the plates. In order to create a smooth lower gel interface, drops of iso-propanol were layered onto the lower gel solution, and the gel was left to set overnight. The upper stacking gel, sufficient for 2 plates, was made up according to Appendix A and poured onto the lower resolving gel after the interface had been cleaned with distilled water. The spacer comb was inserted into the upper stacking gel, and the gel left to set. The numbers and positions of wells in the stacking gel were recorded on the plate, the comb was removed, and the wells cleaned. The sealer strip of tenacious tape along the bottom of the plate was removed before electrophoresis commenced.

The plate reservoirs were filled with SDS running buffer, and aliquots of sample were injected into the wells. With the red cable attached to the bottom plug and the blue cable to the top, the power pack was switched on and run at 100 volts until the samples emerged from the bottom of the stacking gel (approximately $1\frac{1}{2}$ hours). At this point the voltage was increased to 200 volts and run for a further 2 hours, or until the front (bromophenol blue) approached the bottom of the gel. The apparatus was then dismantled, the gel carefully removed from the plate, and stained in 0,05% Coomassie blue in destain overnight. The following morning, stain was removed and destain added, and left shaking for 10 minutes. The gel was then washed in two changes of distilled water (10 minutes each) and 4 drops of glycerol were added to the final rinse. Each gel was dried separately under vacuum, using a Hoeffer gel drying apparatus.

RESULTS AND DISCUSSION

In an earlier paper on α -amylase activity in the style of C. meridionalis, it was shown that the style comprised a number of clearly recognisable protein fractions, each of which showed α -amylase activity (Newell et al., 1980b). But other carbohydrases were not assayed and it was therefore not possible to estimate whether total glucogenic activity by the style enzymes could meet the carbon requirements of the mussels. It was necessary first to establish the distribution of proteins in the styles of both C. meridionalis and P. perna, and then to assay the carbohydrase spectrum for the styles of each species.

1. Style Protein Spectrum

The distribution of style proteins in relation to elution volume is shown in Fig. 10. It is clear that in both C. meridionalis and P. perna the protein spectrum is dominated by a peak at an elution volume of 83 ml which from the protein calibration standards (see p.42), corresponds with a molecular weight of approximately 67,000, and that there is a series of smaller peaks corresponding to proteins with molecular weights of <17,000.

The results of an attempt at confirmation of the molecular weights of the eluate fractions, and further possible fractionation using SDS polyacrylamide gels, can be seen in Fig. 11. There appears to be no correlation between the estimated molecular weights of the eluate

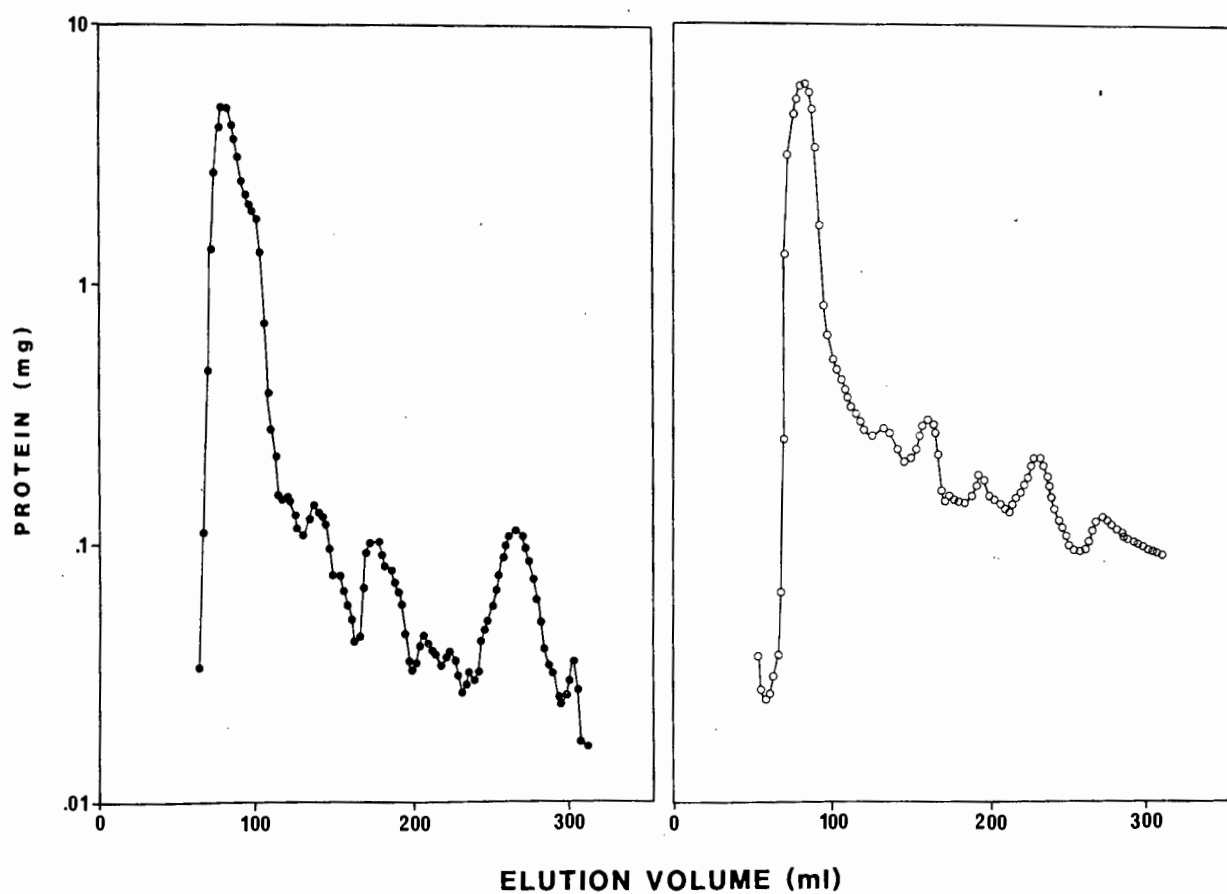


Figure 10 Protein weight (mg) as a function of elution volume in style homogenates of *Choromytilus meridionalis* (●) and *Perna perna* (○). Data expressed for an addition of 195 mg style protein to the column.

FRACTIONS

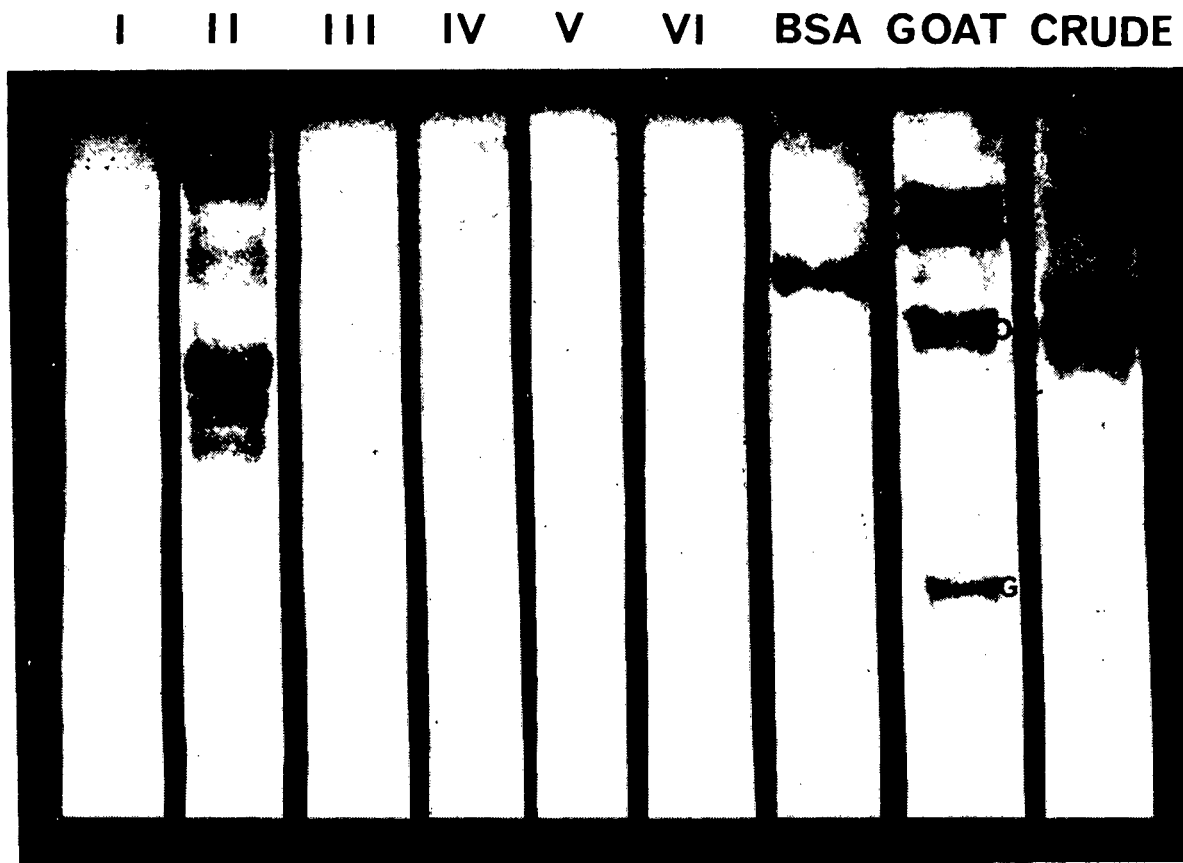


Figure 11 A sodium dodecyl sulphate polyacrylamide gel (SDS-PAGE), used in the fractionation of crystalline style extract from *C. meridionalis*. Fractions I - IV were taken from the P60 Biogel gel filtration column in the following way. Fraction I (1-82 ml); II (83-156 ml); III (157-180 ml); IV (181-202 ml); V (203-235 ml); VI (236-300 ml). The bovine serum albumin (BSA) standard was applied to the gel as a 1 mg.ml^{-1} solution. The GOAT molecular marker, incorporating growth hormone (22 000), ovalbumin (43 000), human albumin (70 000) and transferrin (90 000) was applied in a similar way. Crude extract applied directly to the gel demonstrated remarkable separation of the protein components.

fractions from the gel filtration column, and those of the SDS PAGE gel plate. A possible reason for the failure of this resolving technique may be the breaking up of component proteins into sub-units by the sodium dodecyl sulphate which is added to samples such as complex protein mixtures to facilitate their solubilisation. The appearance of these protein sub-units makes it impossible to equate these results with those of the gel filtration eluate fractions. A possible approach to this problem could be the running of native polyacrylamide gels (without SDS), although the samples may not be sufficiently soluble to enter the gel.

The gel did, however, demonstrate a number of interesting features, such as the presence of five major and one minor protein bands in fraction II, corresponding with the presence of structural proteins in Fig. 10 and four carbohydrases which have been demonstrated in Figs 12 - 15. The additional protein band, at an approximate molecular weight of 100 000, found in the crude extract reappears in fraction IV which contains only structural protein and cellulase. The purification level of the component enzymes of the crystalline style of C. meridionalis appears to be very high and it may be suggested that further preparative experimentation would yield pure forms of the component enzymes.

Using the gel filtration eluate fractions, the structural proteins could not be separated from the component enzyme proteins, and it is therefore likely that the relative enzyme activities in the style fractions reflect a variable component of structural material. For this reason,

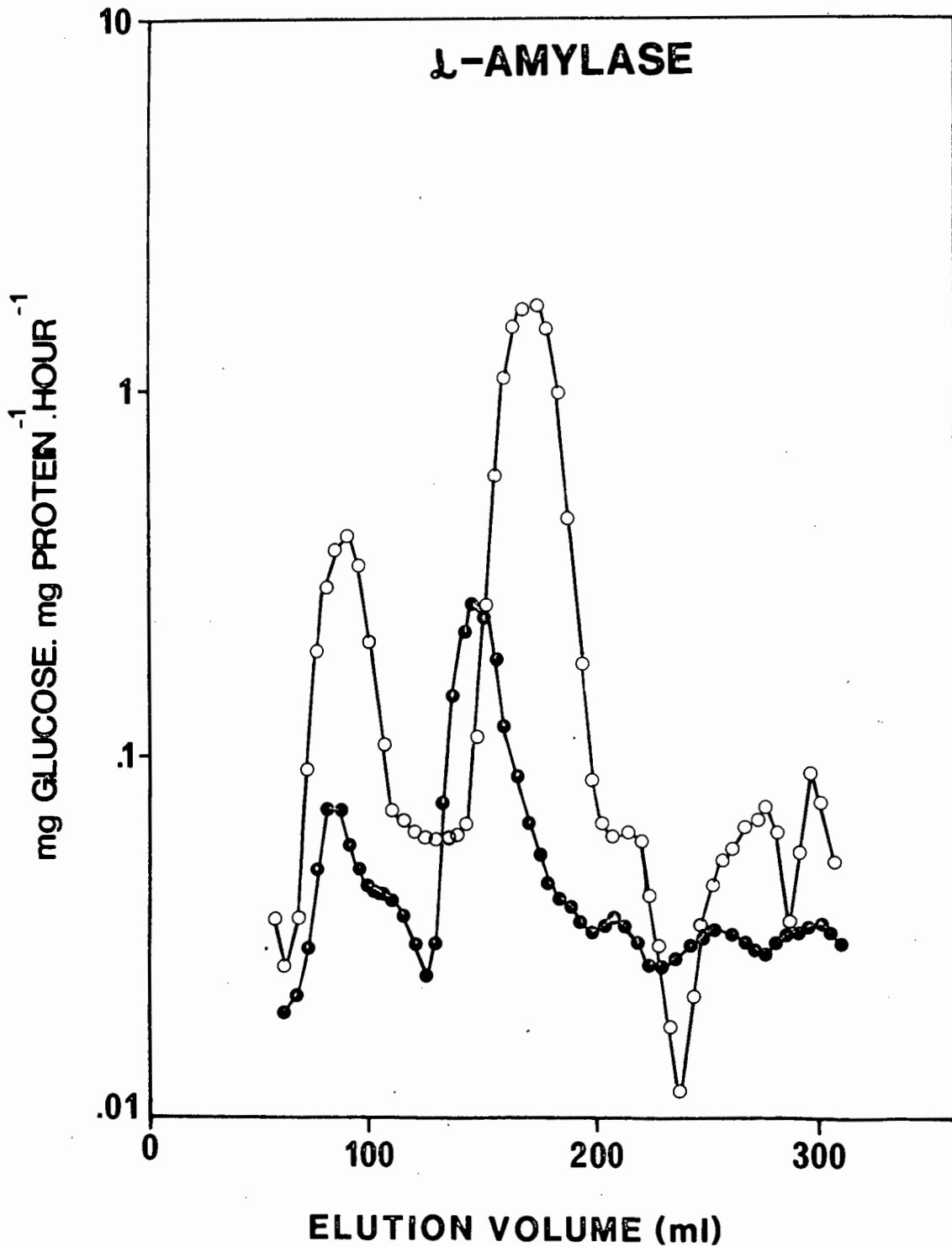


Figure 12 The glucogenic activity ($\text{mg glucose liberated} \cdot \text{mg}^{-1} \text{ protein in eluate} \cdot \text{h}^{-1}$ at 37°C) of α -amylase from the styles of *C. meridionalis* (\bullet) and *P. perna* (\circ) expressed as a function of elution volume (ml).

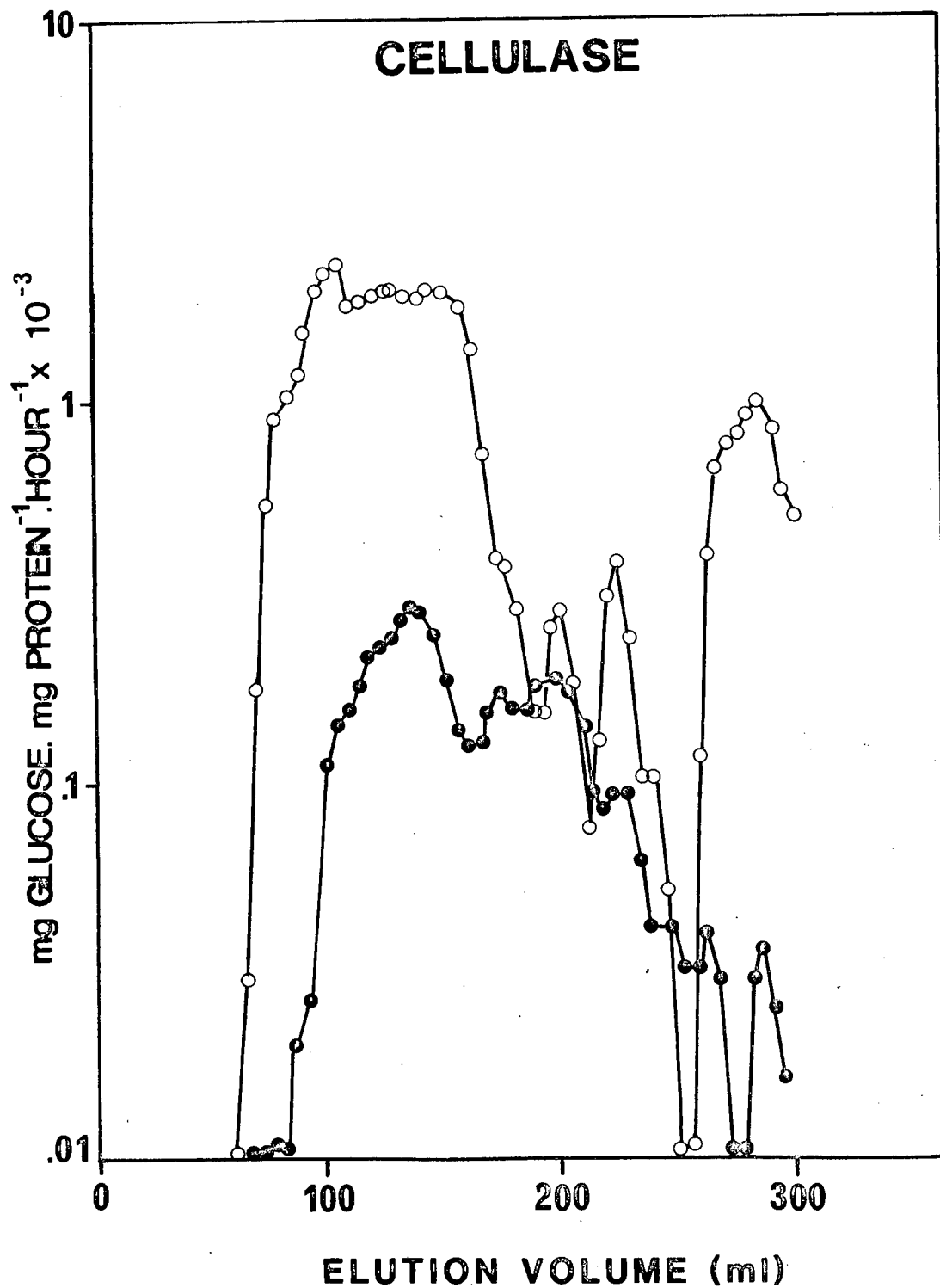


Figure 13 The glucogenic activity ($\text{mg glucose liberated} \cdot \text{mg}^{-1} \text{ protein in eluate} \cdot \text{h}^{-1}$ at 37°C) of cellulase from the styles of *C. meridionalis* (\bullet) and *P. perna* (\circ) expressed as a function of elution volume (ml).

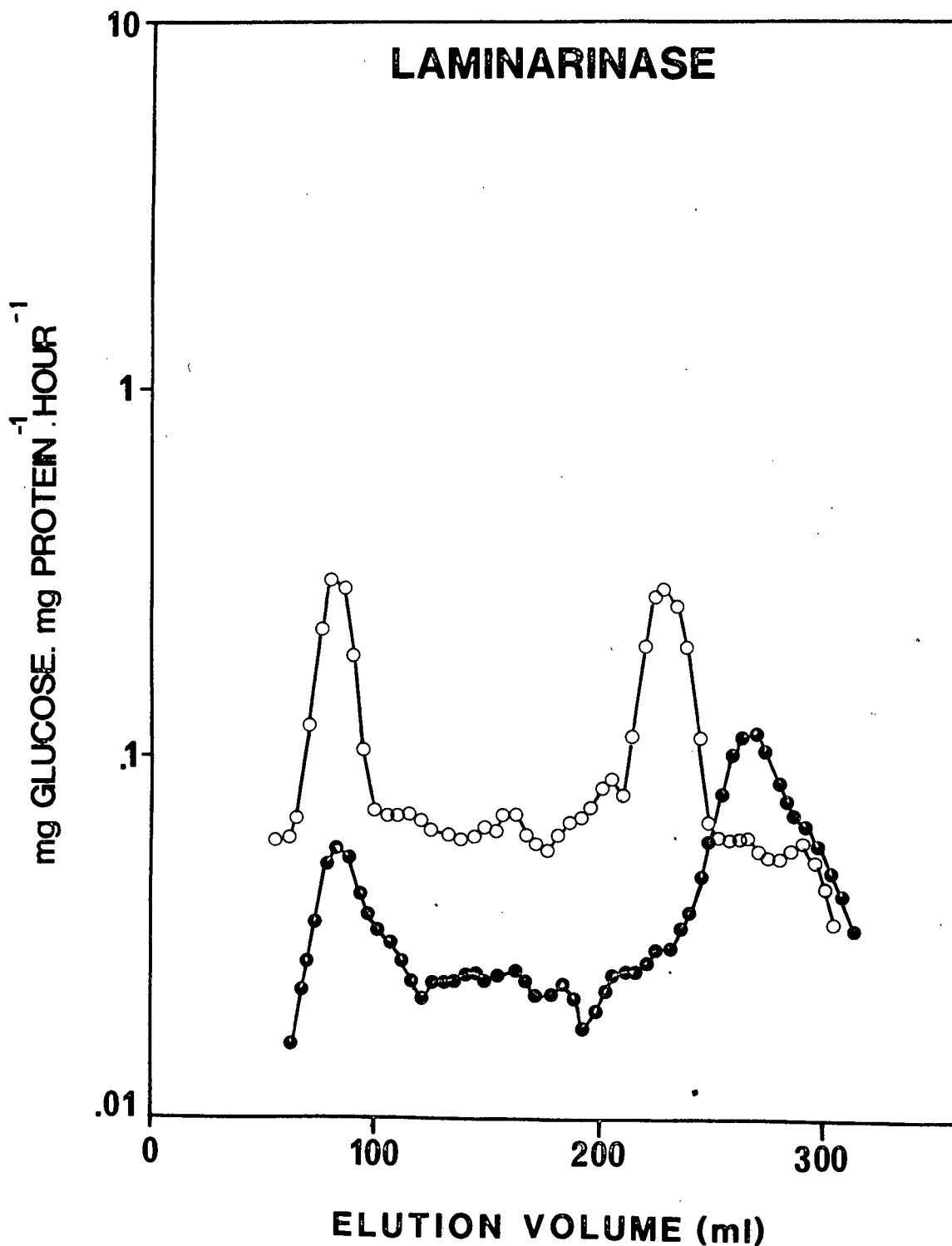


Figure 14 The glucogenic activity ($\text{mg glucose liberated} \cdot \text{mg}^{-1} \text{ protein in eluate} \cdot \text{h}^{-1}$ at 37°C) of laminarinase from the styles of *C. meridionalis* (●) and *P. perna* (o) expressed as a function of elution volume (ml).

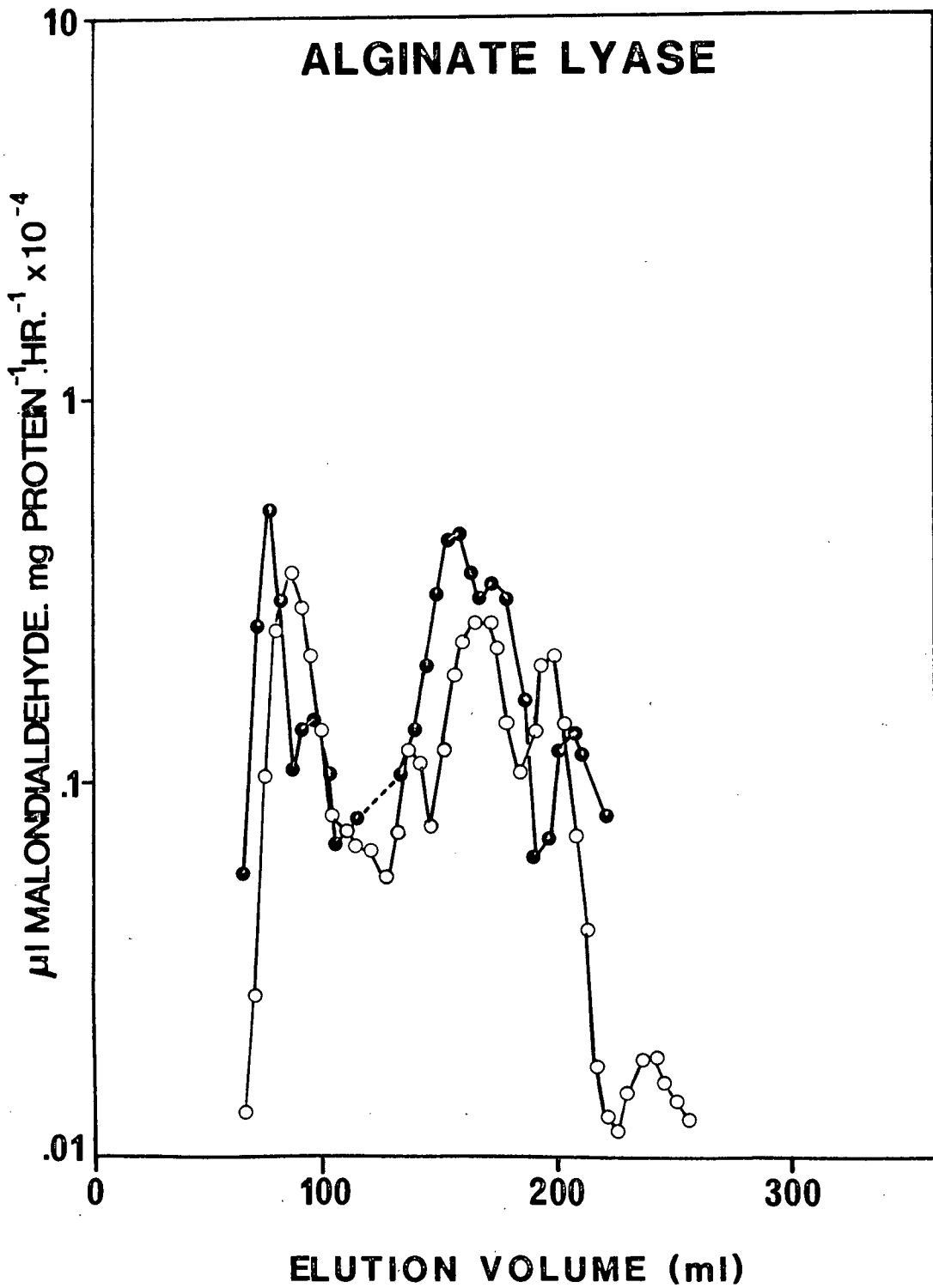


Figure 15 The activity of alginate lyase ($\mu\text{l malondialdehyde} \cdot \text{mg}^{-1}$ protein in eluate $\cdot \text{h}^{-1}$ at 37°C) from the styles of *C. meridionalis* (●) and *P. perna* (○), expressed as a function of elution volume (ml).

enzyme activities per unit protein in the larger molecular weight fractions may be relatively suppressed compared with those in the smaller molecular weight fractions whose activities probably approach the specific rates for the enzymes themselves. Estimates of total carbohydrase activity in the styles of the two mussels were therefore expressed per unit of total protein in the eluate, though the apparent 'specific activities' of the component smaller molecular weight fractions are summarised in Table 10 and Figs 12, 13, 14 and 15.

2. Carbohydrase Activity

The elution volumes for the principal style protein fractions showing carbohydrase activity are summarised in Table 10, together with the apparent 'specific enzyme activity' of the protein peaks with glucogenic activity (mg glucose liberated mg fraction protein⁻¹ h⁻¹ at 37°C) whilst the activity of α -amylase, cellulase, laminarinase and alginate lyase in the style fractions of C. meridionalis and P. perna are plotted as a function of elution volume in Figs 12 - 15. It is apparent that in each case the enzyme activity is located in at least two distinct style protein fractions and that in both C. meridionalis and P. perna glucogenic activity is dominated by α -amylase and laminarinase.

The α -amylase proteins correspond with molecular weights of approximately 67 000 and 9000 respectively, those of P. perna being of a somewhat smaller molecular weight than in C. meridionalis. Laminarinase in C. meridionalis shows one peak at an elution volume of 84 ml (67 000mw) and a stronger peak at an elution volume of 276 ml, corresponding with

TABLE 10 The elution volumes (ml) and apparent 'specific activities' of style enzymes from *Choromytilus meridionalis* and *Perna perna*. Enzyme activity expressed as mg glucose liberated at 37°C mg⁻¹ protein in the eluate fraction h⁻¹.

Enzyme	<i>Choromytilus meridionalis</i>		<i>Perna perna</i>	
	Elution volume (ml)	Activity mg glucose. mg protein h ⁻¹	Elution volume (ml)	Activity mg glucose. mg protein h ⁻¹
α -amylase	82	0,47	92	13,60
α -amylase	149	93,84	178	370,80
cellulase	142	0,05	110	0,17
cellulase	200	0,15	293	0,35
Laminarinase	84	0,35	82	8,10
Laminarinase	272	27,74	230	57,80
Alginate lyase	77	-	83	-
Alginate lyase	158	-	171	-

a lower molecular weight laminarinase and a smaller molecular weight protein of a comparable laminarinase activity at an elution volume of 228 ml. The cellulase activity in styles from both mussels is much weaker than the α -amylase and laminarinase, but also occurs in at least two different protein fractions. In both C. meridionalis and P. perna cellulase activity occurs in a protein fraction with a molecular weight of approximately 20,000 (elution volume 132 ml). In C. meridionalis there is a further zone of cellulase activity at an elution volume of 192 ml (approximately 5000 mw) but this is absent in P. perna which instead has a low molecular weight cellulase at an elution volume of 293 ml. Finally, alginate lyase from the styles of both mussels shows a peak at an elution volume of approximately 80 ml together with a major zone of activity at an elution volume of approximately 160 ml.

It can also be noted from Figs 12 - 15 that the specific enzyme activity of the style fractions from P. perna are higher than those from C. meridionalis. It is thus of some interest to relate the differing glucogenic activity of the style enzymes to the carbon requirements of each of the mussels.

3. Quantitative Significance of Style Carbohydrase Activity

The data presented above allow some estimates to be made of the total saccharogenic activity of the style & its potential ability to meet the carbon requirements of the mussels. Tables 11 and 12 show the glucogenic activity of α -amylase, cellulase and laminarinase in protein eluates from the styles of C. meridionalis (Table 11) and P. perna

TABLE 11 Total glucose liberation (mg h^{-1} at 37°C) from eluates of the style of *Choromytilus meridionalis*. Data also expressed as mg glucose liberated per mg total protein in the eluates.

Enzyme	Glucogenic activity at 37°C		% of total
	mg glucose h^{-1}	mg glucose mg total protein $^{-1} \text{h}^{-1}$	
α -amylase	372,96	3,22	67,08
cellulase	0,631	0,0054	0,11
laminarinase	182,21	1,57	32,72
TOTAL	555,80	4,80	100,0

TABLE 12 Total glucose liberation (mg h^{-1} at 37°C) from eluates of the style of Perna perna. Data also expressed as mg glucose liberated per mg total protein in the eluates.

Enzyme	Glucogenic activity at 37°C			
	mg glucose h^{-1}	mg glucose mg total protein	h^{-1}	% of total
α -amylase	323,51	6,94		72,07
Cellulase	1,03	0,022		0,23
Laminarinase	124,37	2,67		27,73
TOTAL	448,92	9,63		100,0

(Table 12), together with the rates per unit protein in the eluate. These data, which are derived from the summarised enzyme activities shown in Figs 12 - 15 and the eluate proteins in Fig. 10, show that the carbohydrase activity of both styles is dominantly controlled by α -amylase which accounts for 67,1% of glucogenesis by the style of C. meridionalis and 72,1% in P. perna. Laminarinase accounts for 32,7% in C. meridionalis and 27,7% in P. perna, whilst cellulase makes a relative contribution of only 0,1% and 0,2% respectively. The carbohydrases of both mussels thus have a similar proportional contribution to total glucogenic activity, although the absolute activity of the style protein eluate of P. perna is 9,63 mg glucose.total eluate protein⁻¹ h⁻¹ at 37°C compared with only 4,8 in C. meridionalis. These differences in absolute carbohydrase activity suggest that if the carbon requirements of C. meridionalis and P. perna are similar, C. meridionalis would require a faster style turnover to sustain its glucose requirements than P. perna (Table 12) whose style carbohydrase activity is twice that of C. meridionalis (Table 11).

The values for glucogenic activity from the style eluates can be related to the requirements of whole mussels of different sizes, provided that total style protein in relation to body size is known. A series of 20 C. meridionalis was collected from the shore and the total style protein (mg dry mass) plotted as a function of shell length (mm) (Fig. 16). This yielded the following regression equation: $Y = -3,47 + 0,16X$ ($r = 0,89$, $N = 20$) which could be used to relate the carbohydrase activity of the proteins to the carbon requirements of the mussels.

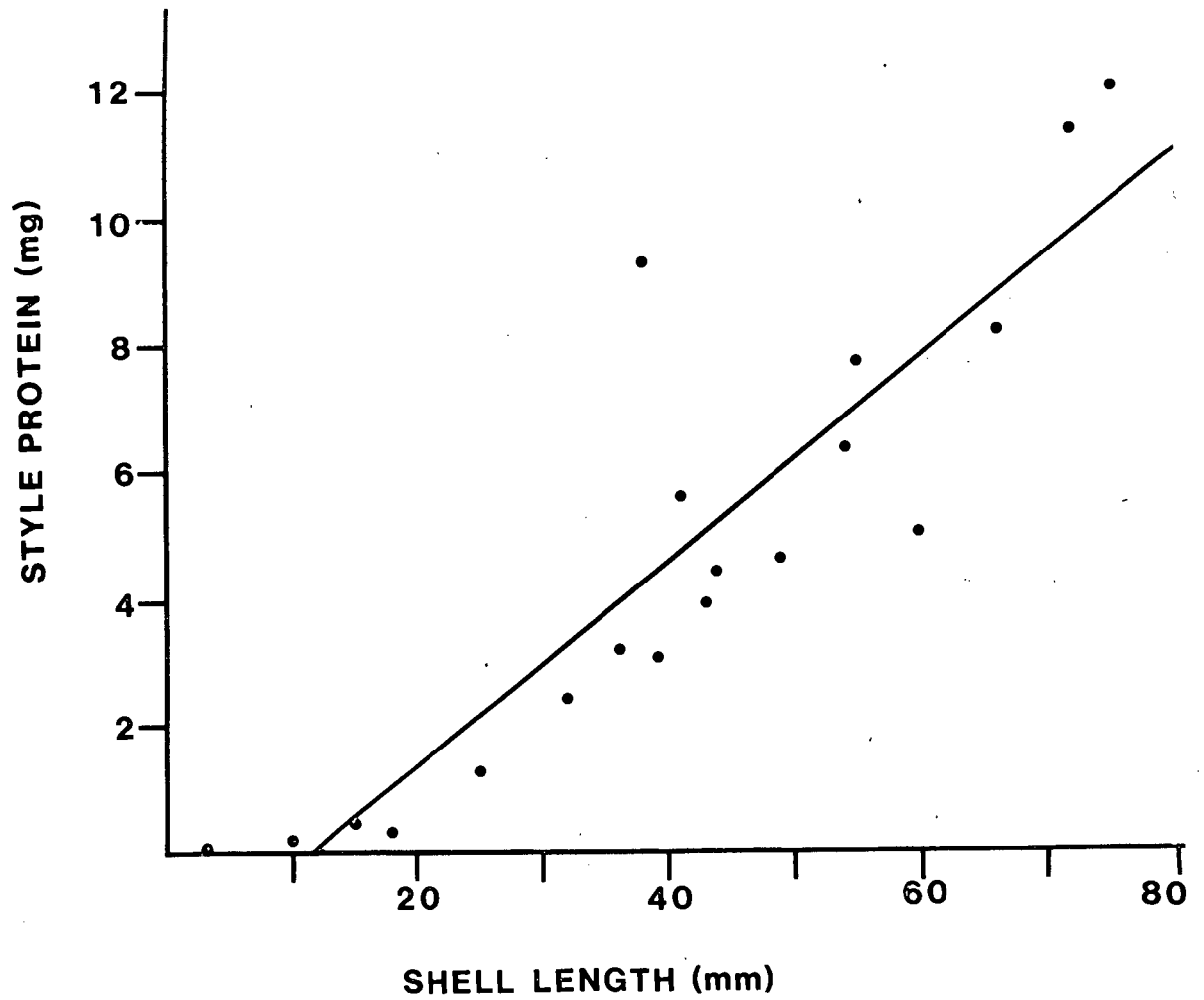


Figure 16 Total style protein (mg dry mass) of *C. meridionalis* plotted as a function of shell length (mm). Regression equation: $\hat{y} = -3,47 + 0,16 x$; $r = 0,89$, $n = 20$.

In practice, it was found to be more direct to assay the total carbohydrase activity and protein in whole styles taken from mussels of known shell length. This avoided the necessity of correcting for the recovery of proteins following elution through the gel filtration column and allowed an independent estimate of the carbohydrase activity of the whole styles. The results for C. meridionalis are summarised in Table 13 from which it is apparent that total glucogenic activity from α -amylase, cellulase and laminarinase (mg h^{-1} at 37°C) is related to shell length (mm) by the equation: $Y = -12,87 + 0,80X$. Of this, α -amylase contributes 71% of the total glucogenic activity, laminarinase 29% and cellulase only 0,1%. These values compare well with those obtained on protein eluates whose carbohydrase activity is summarised in Tables 11 and 12.

The data for whole styles of P. perna are also in general accord with the summed activities from the protein eluates shown in Table 14. In this case the summed glucogenic activity (mg glucose h^{-1} at 37°C) is related to shell length (mm) by the equation: $Y = -4,57 + 1,0X$ of which α -amylase accounts for as much as 82% of the total, laminarinase 18% and cellulase 0,13%. Although C. meridionalis experiences a high proportion of kelp debris in its natural habitat compared with P. perna which lives well away from kelp beds and where phytoplankton is a predominant item in the diet, it is clear that both mussels possess a similar array of style enzymes. The main difference, which can also be seen from the enzyme activities in the protein eluates, is that α -amylase is relatively more important and laminarinase relatively less important in the phytoplankton-feeding P. perna than in

TABLE 13 Regression equations relating whole style glucogenic activity (mg glucose h⁻¹ at 37°C, Y) from α -amylase, cellulase and laminarinase in Choromytilus meridionalis as a function of shell length (mm, X). Equations calculated from the sum of the separate enzyme activities from a series of 20 styles isolated from mussels of different shell lengths.

Enzyme	Equation of Regression	% of total glucogenic activity	N	r
α -amylase	Y= -10,5 + 0,57X	71	20	0,75
Cellulase	Y= -0,01 + 0,001X	0,1	20	0,82
Laminarinase	Y= -2,37 + 0,23X	29	20	0,72
TOTAL	Y= -12,87 + 0,80X	100	60	0,76

TABLE 14 Regression equation relating whole style glucogenic activity (mg glucose h⁻¹ at 37°C, Y) from α -amylase, cellulase and laminarinase in Perna perna as a function of shell length (mm, x). Equations calculated from the sum of the separate enzyme activities from a series of 20 styles isolated from mussels of different shell lengths.

Enzyme	Equation of regression	% of total glucogenic activity	N	r
α -amylase	Y= -7,4 + 0,82X	82	20	0,92
Cellulase	Y= 0,0006 + 0,0013X	0,13	20	0,61
Laminarinase	Y= 2,64 + 0,18X	18	20	0,84
TOTAL	Y= -4,57 + 1,0X	100	60	0,89

C. meridionalis. Whether these differences are attributable to genetic factors or reflect adaptive responses of the individuals to their major diet is, however, at present unknown.

4. Style Carbohyrase Activity in Relation to the Carbon Budget of Mussels

Griffiths (1980) has made a detailed study of the oxygen consumption of different-sized individuals of C. meridionalis also collected from Bloubergstrand, Table Bay. She found the mean value for respiration (R) was 74% of the absorbed ration (A). From this it is possible to relate the glucogenic activity of the style to the estimated carbon requirements of different-sized mussels. Similarly, Miller (Oceanographic Research Institute, Durban; personal communication) has measured the oxygen consumption of P. perna in relation to body size and his data can be used to estimate the absorbed carbon requirements for this mussel on the assumption that respiration also accounts for 74% of the absorbed ration.

Both Griffiths (1980) and Miller (personal communication) cite values for oxygen consumption at 18°C, whilst our assays for style enzyme activity were carried out at 37°C. It has previously been reported that α -amylase from C. meridionalis has a temperature coefficient which is less than 2,0 (Seiderer & Newell, 1979; Newell et al., 1980) and it was therefore necessary to establish experimentally the temperature correction for this and other component enzymes of the style rather than assume a temperature coefficient of 2,0. The results for the two

major glucogenic enzymes α -amylase and laminarinase are summarised in Table 15 which also shows the higher specific style enzyme activities for P. perna. From these data it can be calculated that summed glucogenic activity of the style enzymes of C. meridionalis at 37°C should be corrected by x0,62 to yield the rate at 18°C whilst those from P. perna should be multiplied by 0,65.

Values for the oxygen consumption and its carbon equivalent, the absorbed ration at 1,35x the respiration, and the carbon equivalent of glucogenesis from the style at 37°C (Tables 13 & 14) corrected to 18°C are summarised for C. meridionalis in Table 16 and P. perna in Table 17. From the carbon released as glucose by whole styles at 18°C, compared with the carbon requirements (A), the turnover time of the style which would be required to meet the carbon requirements of the mussels at 18°C can be calculated. These values are shown in the final columns of Tables 16 and 17.

It is immediately apparent that complete utilisation of the style would need to be achieved in 16 - 31 hours depending somewhat on body size, in order to release sufficient glucose to meet the carbon requirements of C. meridionalis. The mean value for the estimated style turnover time is 25,4 h. In contrast, the style turnover need be only 136,3 h to meet the estimated carbon requirements of P. perna at 18°C. This reflects the lower oxygen consumption requirements of the mussel compared with C. meridionalis (Tables 16 & 17) and the higher specific enzyme activity of the style in P. perna.

TABLE 15 Glucogenic activity of α -amylase and laminarinase (mg glucose released mg style protein⁻¹ h⁻¹) from the crystalline styles of Choromytilus meridionalis and Perna perna as a function of incubation temperature.

Temperature °C	<u>Choromytilus meridionalis</u>		<u>Perna perna</u>	
	α -amylase	laminarinase	α -amylase	laminarinase
10	1,029	0,646	4,186	1,210
20	1,420	0,878	6,736	2,036
30	2,455	1,263	9,799	2,663
35	2,129	1,471	10,130	2,994
40	2,064	1,649	10,609	3,525

TABLE 16 The turnover time (h) required for style carbohydrase activity to support the carbon requirements of Choromytilus meridionalis of different sizes (shell length, mm). Data for carbon requirements recalculated as mg h^{-1} from oxygen consumption ($\mu\text{l h}^{-1}$ at 18°C) and absorbed ration (A) in Griffiths (1980). Griffiths (1980) reported that the mean value of R was 74% of A. Glucogenic activity of whole styles (mg glucose h^{-1} at 37°C) calculated from Table 14 and reduced to 18°C from the data in Table 15.

	S H E L L L E N G T H (mm)					\bar{x}
	20	40	60	80	100	
Respiration (R)						
$\mu\text{l O}_2 \text{ h}^{-1}$ at 18°C	65	220	390	670	1000	-
mg C h^{-1} at 18°C	0,0348	0,1179	0,2089	0,3589	0,5357	-
Absorbed ration (A)						
mg C h^{-1} at 18°C	0,0470	0,1593	0,2823	0,4850	0,7239	-
Glucogenesis from style						
mg glucose h^{-1} at at 37°C	3,13	19,13	35,13	51,13	67,13	-
mg C h^{-1} at 37°C	1,252	7,652	14,052	20,744	26,852	-
mg C h^{-1} at 18°C	0,780	4,768	8,756	12,744	16,731	-
Style turnover						
time (h) necessary to support Carbon requirements (A)	16,60	29,93	31,02	26,28	23,11	25,39
						$+ 5,83$

TABLE 17 The turnover time (h) required for style carbohydrase activity to support the carbon requirements of *Perna perna* of different sizes (shell length, mm). Data for carbon requirements recalculated as mg h^{-1} from oxygen consumption ($\mu\text{l h}^{-1}$ at 18°C) and absorbed ration (A) in Griffiths (1980) and Miller (pers. comm.). Griffiths (1980) reported that the mean value of R was 74% of A and this value has been assumed for *P. perna*. Glucogenic activity of whole styles (mg glucose h^{-1} at 37°C) calculated from Table 14 and reduced to 18°C from the data in Table 15.

	S H E L L L E N G T H (mm)				\bar{x}
	20	40	60	80	
Respiration (R)					
$\mu\text{l O}_2 \text{ h}^{-1}$ at 18°C	22,4	93,6	215,7	390,0	-
mg C h^{-1} at 18°C	0,0120	0,0501	0,1156	0,2089	-
Absorbed ration (A)					
mg C h^{-1} at 18°C	0,0162	0,0677	0,1562	0,2823	-
Glucogenesis from style					
mg Glucose h^{-1} at 37°C	15,43	35,43	55,42	75,43	-
mg C.h^{-1} at 37°C	6,172	14,170	22,170	30,170	-
mg C.h^{-1} at 18°C	4,011	9,215	14,410	19,610	-
Style turnover time (h) necessary to support carbon requirements (A)					
	247,59	136,12	92,25	69,47	136,35
					$\pm 79,14$

CONCLUSION

The results which have been presented above show that Choromytilus meridionalis and Perna perna have a similar array of style carbohydrase enzymes despite the wide differences in the relative availability of phytoplankton and kelp detrital material as potential food resources. Alginate lyase, cellulase, α -amylase and laminarinase activity was recorded from at least two of the protein fractions in eluates from the styles of both mussels and it seems likely that the carbohydrases exist in at least two distinct molecular weight classes. Amylase 'isoenzymes' or enzyme variants have previously been recorded in the mussel Mytilus edulis (Alemany & Rosell-Pérez, 1973) as well as in the style of C. meridionalis (Newell et al., 1980b). Again, isoenzymes of alginate lyase have been recorded from the mid-gut of the gastropod Turbo cornutus by Muramatsu and Egawa (1980) and enzyme variants of cellulase have recently been recorded in Mya arenaria (Mirza & Serban, 1981) although their precise role in the digestive physiology of molluscs is uncertain. In both C. meridionalis and P. perna α -amylase is the dominant enzyme with cellulase accounting for only 0,1% of the total glucogenic activity of the style. There are, however, some differences in the specific activities of the style enzymes which appear to be related to qualitative differences in potential food supply. C. meridionalis lives on reefs adjacent to kelp beds and in this mussal laminarinase accounts for 29 - 32% of total glucogenic activity compared with only 18 - 27% in P. perna.

Despite these similarities in the carbohydrase spectrum of enzymes from the crystalline style of C. meridionalis and P. perna, there are major differences in the total specific carbohydrase activity at 18°C. Protein eluates from the style of P. perna are capable of liberating 9,63 mg glucose mg protein⁻¹ h⁻¹ compared with only 4,8 mg in C. meridionalis; these differences were also found in carbohydrase assays of whole crystalline styles from mussels of differing sizes. It can be shown that in order to liberate sufficient glucose to meet the carbon requirements of the mussels, one style would require to be utilised each 25,4 h in C. meridionalis. In P. perna, however, because of the higher carbohydrase activity of the style and the somewhat lower weight-specific carbon requirements, a utilisation of one style per 136 h would be adequate to release sufficient glucose to meet the carbon requirements of the mussel.

The turnover time estimated by P.A. Cook (pers. comm.) from the loss of ¹⁴C-label from the styles of C. meridionalis and P. perna suggest that the style utilisation is indeed related to the specific carbohydrase activity and carbon requirements of the mussels. In C. meridionalis a turnover time of 18 h was estimated from loss of activity from ¹⁴C-labelled styles whilst that of P. perna was in excess of 72 h and probably approached 120 h. In both C. meridionalis and P. perna therefore, the spectrum of carbohydrases present in the crystalline style and their specific activities are well-suited to release glucose from both the living phytoplankton and detrital components of the particulate matter in the water column at a rate which is sufficient to meet the estimated carbon requirements of the mussels.

SECTION III

UTILISATION OF BACTERIA
AND PHYTOPLANKTON AS A NITROGEN RESOURCE

INTRODUCTION

Newell et al. (1980a) have shown that the protein component of kelp debris amounts to as much as 23 - 28% of the dry weight of freshly powdered Laminaria pallida frond (see Table 9). Since microbial conversion of nitrogen from kelp detritus is achieved with an efficiency of as much as 83 - 94% (see Stuart et al., 1981; Koop et al., 1982a, 1982b), it seems likely that much of the protein originally released during fragmentation of kelp will become potentially available to consumers in the form of microbial biomass associated with the particulate fraction. The following work was therefore undertaken to investigate the potential significance of style enzymes from the mussel Choromytilus meridionalis in the utilisation of both living bacteria and phytoplankton, and the extent to which the activity of these enzymes could supply the nitrogen requirements of the mussel.

In Section II of this thesis it has been shown that the crystalline style of Choromytilus meridionalis and Perna perna contains cellulases as well as a range of other carbohydrases which are capable of liberating reducing sugars at a sufficient rate to supply the carbon requirements of the mussels on a detrital diet alone. Such enzymes are also likely to be implicated in the digestion of phytoplankton cell walls, so that the phytoplankton may represent a significant resource for the filter feeding mussel community.

Apart from the work of McHenery et al. (1979) and McHenery and Birkbeck (1979, 1982), there have been very few studies of the digestive enzymes

which are required for the utilisation of the bacterial component of such detrital diets.

The naturally occurring populations of marine bacteria are dominantly gram negative bacteria, a distinction based on their cell envelope properties. This envelope is complex, and consists of a 30-Å-wide peptidoglycan wall which in turn is covered by an 80-Å-wide outer membrane which is a mosaic of proteins, phospholipids and lipopolysaccharides (Stryer, 1981). The whole surrounds and supports the plasma membrane. Although Repaske (1958) has shown that for lysis of Escherichia coli by lysozyme, prior treatment with the chelating agent EDTA is required to destabilise the cell wall, this does not appear to be the case in bacteriolysis within the stomach of marine bivalves. McHenery et al. (1979) described the occurrence of a lysozyme-like enzyme in the bivalves Mytilus edulis, Modiolus modiolus, Chlamys opercularis and Mya arenaria and suggested that its primary role is in the utilisation of bacteria. McHenery and Birkbeck (1982) subsequently showed that the enzyme from M. edulis is a true N-acetylmuramyl-hydrolase which is capable of degrading the cell walls of a variety of bacteria including Micrococcus luteus, Escherichia coli and Bacillus subtilis.

Clearly the occurrence and activity of such lysozyme-like enzymes is of importance in estimating the significance of bacteria as a carbon, and above all, a nitrogen resource for consumer organisms which are exploiting detrital diets with a high C:N ratio. The relative resistance of the resident gut microflora to such lysozyme-like enzymes compared with free living bacteria ingested with the food may yield some insight

into the role of free-living as opposed to resident gut microflora in the nutrition of the consumer. The following section examines the occurrence and role of lysozyme-like enzymes in the utilisation of bacteria and phytoplankton as a potential resource by the kelp bed mussel Choromytilus meridionalis whose carbon balance on a detrital diet has been established in the previous section.

MATERIALS AND METHODS

1. Sampling and Preparation of Style Extract

Specimens of the black mussel Choromytilus meridionalis (Krauss) were collected from a rocky intertidal reef at Bloubergstrand, on the West Coast of South Africa, between November 1982 and February 1983 and were transferred to the laboratory for immediate extraction of the crystalline style. The styles of twenty specimens were removed, rinsed and homogenised over ice with a glass tissue grinder in 12 ml 20 mM phosphate buffer, pH 7.0, containing 150 mM NaCl (see also Langton, 1977; Seiderer & Newell, 1979; Seiderer et al., 1982b). All glassware had been sterilised immediately prior to use. The homogenate was centrifuged at 15,000 x g for 5 minutes and the supernatant diluted to 1.00 mg protein ml⁻¹ (Groves et al., 1968) with phosphate buffer before lysozyme and protease assays were performed.

2. Isolation and Culture of Bacterial Strains

In order to isolate water column bacteria, samples of seawater were taken

from Oudekraal, also on the west coast of South Africa, and incubated in the laboratory at 10°C with 0,5 g ℓ^{-1} sterilised powdered kelp detritus. This had been prepared by grinding freeze-dried tips of the fronds of Laminaria pallida, sieving to obtain particles from 43 - 63 μm diameter, followed by sterilisation for 24 hours under a UV light (see also Stuart et al., 1981). Subsamples of 1 ml were taken after 3 days of incubation and pipetted onto agar plates made of 1,5% agar in seawater growth medium (see Appendix A and Mazure, 1977). Plates were then incubated at 25 - 30°C for 24 - 48 hours. Twenty five strains were isolated and restreaked at least five times before the clones were assigned to collections and stored on slants of seawater agar at 4°C.

Bacterial isolates were also prepared from the gut contents of the mussel Choromytilus meridionalis. The guts of four mussels were removed (excluding extraneous contamination) and the contents cultured on seawater-agar plates at 20°C after treatment with 1% trypsin for 30 minutes at 30°C. Cultures were also made from homogenised gut tissues and twenty strains were isolated and stored on seawater agar at 4°C.

Each of the isolates in the collections was then incubated in an orbital shaker at 30°C for 10 hours in 2 ml seawater liquid growth medium. These were reinoculated into 50 ml growth medium and grown at 30°C for a further 12 hours before being used for preparation of experimental agarose plates.

3. Preparation and Incubation of Plates

The activity of lysozyme-like enzymes on free-living bacteria and on bacteria isolated from the gut of C. meridionalis was assayed on 0,8% agarose plates containing heat-killed target bacteria (McHenery et al., 1979). The bacterial suspensions were centrifuged at 8000 x g for 15 minutes and the pellets resuspended in 2ml sterile seawater. The suspensions were then adjusted with sterile seawater so that the final optical density in the agarose medium would be 0,122 at 600 nM, and added to 20 ml agarose medium. The media were then heat-shocked at 65°C for 10 - 15 minutes to kill the bacteria, and poured into 8,4 cm diameter petri dishes. Wells of equal diameter (2 mm) were cut into each agarose plate to receive the enzyme extract.

Eight serial ten-fold dilutions of enzyme extract (see p.82) were prepared and aliquots of 10 μ l of each of the dilutions were placed individually into the wells and incubated at 20°C for 48 hours. The intact bacteria were stained by the addition of 5 ml 0,12% crystal violet to each plate. After 2 hours, the plates were rinsed with distilled water and the diameter of the clear zones of lysis was measured. From the number of bacteria per unit surface area of the plates, the area of the clear zone could be expressed both as number of bacteria lysed per 45 hours of incubation at 20°C, and its equivalent in bacterial protein. The equations for the regressions were: Bacterial number lysed ($\times 10^5$) $\hat{y} = -0,92 + 0,11X$ (Fig. 17) and Bacterial protein (μ g) $\hat{y} = -0,36 + 0,05X$ (Fig. 18) (where X = zone diameter in mm), all calculated values.

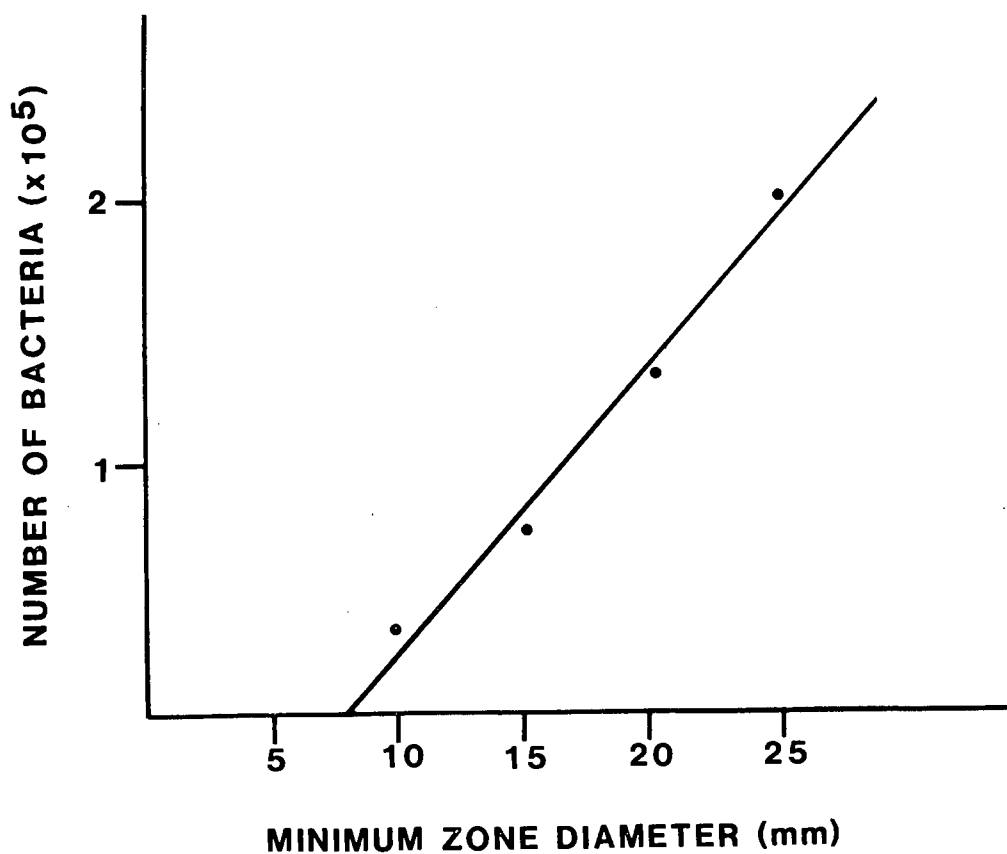


Figure 17 The area of the most dilute (smallest) zone of lysis expressed as the bacterial numbers lysed in 45 hours at 20°C in the presence of style extract from C. meridionalis. (Regression equation: $\hat{y} = -0,92 + 0,11 x$; $r = 0,99$, calculated values, fitted by the method of least squares).

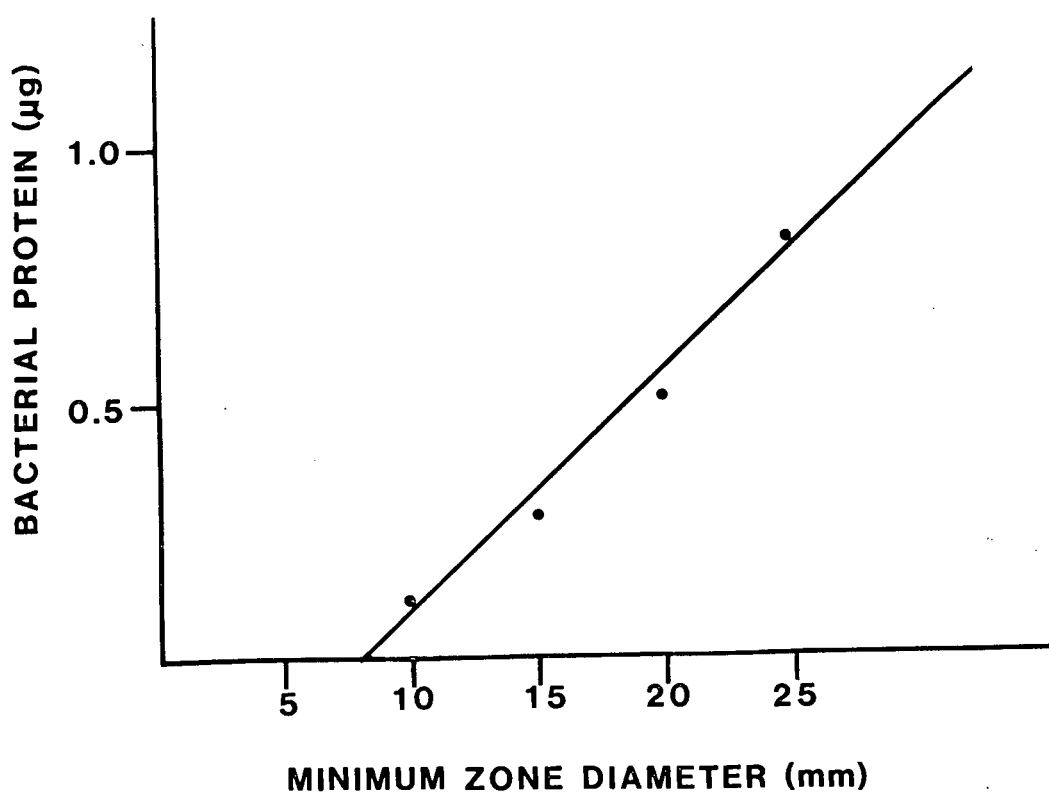


Figure 18 The area of the most dilute (smallest) zone of lysis expressed as the bacterial protein released in 45 hours of incubation at 20°C in the presence of style extract from *C. meridionalis*. (Regression equation: $\hat{y} = -0,36 + 0,05 x$; $r = 0,98$, calculated values, fitted by the method of least squares.)

4. Activity Spectrum of Crystalline Style Extract

An experiment was carried out to determine the activity spectrum of style extract of C. meridionalis, taken from mussels collected under an ambient water temperature of 11°C. A total of 43 gut, water column and kelp frond bacterial isolates were cultured as before. Lysozyme plates were poured, incorporating cultures of each bacterial isolate separately, and wells were made within each plate. Into the wells, eight 1:10 serial dilutions of style extract were pipetted, and the plates were incubated at room temperature for 45 hours. The zones of lysis were measured, and the enzyme activity was calculated as before. The plates were divided up into 19 gut isolate plates, 16 water column plates and 8 kelp frond plates. A cut-off point between susceptible bacteria and resistant bacteria was arbitrarily assigned to be 10 bacteria lysed per μl of style extract ($\times 10^5$). The percent frequencies on either side of this cut-off point were plotted against the origins of these bacterial isolates.

5. Protease Gel Assays

The potential of sodium dodecyl sulphate polyacrylamide gels (SDS-PAGE) as a general method for separating extracellular proteases was first explored by Granelli-Piperno and Reich (1978) and later refined by Heussen and Dowdle (1980). This technique exploits the fact that the inactivation of SDS of at least one specific protease-plasminogen activator is reversible, since the activity can be restored by removal of the SDS with non-ionic detergent. Heussen and Dowdle's (1980)

modification of the technique was based on the observation that if the protease substrate (gelatin and plasminogen) is copolymerised into the matrix of the SDS-PAGE at the time of casting, it is retained during electrophoresis providing in situ substrates for separated bands. The technique is effective in the separation of proteins in complex mixtures, a feature of the crystalline style of C. meridionalis. The complexity of the protein mixture has been demonstrated in the previous section by means of conventional SDS polyacrylamide gels, and gel filtration of crude style extract.

i) Method

A 1% gelatin solution was co-polymerised into the matrix of a 10% sodium dodecyl sulphate (SDS) polyacrylamide gel (Appendix A & p.52). The style extracts were incubated at 37°C for 30 minutes in 25% SDS in a ratio of 10:1 (final SDS concentration of 2,5%) plus 100 µl of glycerol and were injected into the wells of the stacking gel in 10 µl aliquots. The gels were run at a constant voltage of 100 v for 15 hours at 4°C. The plates were then dismantled and the gels incubated in 2,5% Triton x 100 for 1 hour at 30°C in an orbital shaker to remove the SDS. The gel was rinsed in water and incubated in 0,1 M glycine - NaOH pH 9,0 buffer for 4 hours at 37°C. It was then stained for a minimum of 1,5 hours in 0,2% amido black (2% in water diluted to 0,2% in destain. The stained gels were destained in 300 ml methanol, 100 ml acetic acid and 600 ml distilled water until the protease windows became apparent. The gels were rinsed and dried under vacuum on to Whatman 3 mm filter paper using a Hoeffer gel drying apparatus.

ii) Sample Treatment

The style extracts run on the protease gels were from mussels sampled on days during which the ambient sea temperature was 10, 11, 12 and 13°C for purposes of comparison. A control was run in which the style extracts were treated with Soy-bean trypsin inhibitor in an attempt to clarify the nature of the protease. The extract was incubated at 30°C for 30 minutes with a 1 mg ml^{-1} solution of trypsin inhibitor. In addition, a 1 mg ml^{-1} trypsin standard was run simultaneously, with and without inhibition.

6. Micrographs

A. Interference contrast micrographs

An isolated water column Pseudomonad was cultured in seawater growth medium (p.83) and rinsed in 5 ml 10 mM ammonium acetate buffer pH 7,0 to remove salts. To the experimental bacteria, a 1 in 5 volume of style extract which was known to have bacteriolytic activity was added. In the control tube the style extract was replaced with phosphate buffer, pH 7,0. Micrographs were taken after 30 minutes using a Zeiss Photo Microscope 3 equipped with interference contrast optics. The final magnification was 9600 x.

B. Negative stained transmission electron micrographs

Subsamples of the above cultures were taken, preserved in 0,2% glutaraldehyde, and later precipitated onto Formvar carbon-coated copper grids. These grids were negatively stained, using both

phospho tungstic acid (0,5%) and uranyl acetate (2%) separately, and viewed under a Zeiss EM 109 electron microscope at a final magnification of 40 000 X. The phospho tungstic acid was found to give a far clearer image than the uranyl acetate, and was therefore used throughout.

C. Scanning electron micrographs

The above subsamples, preserved in 0,2% glutaraldehyde were pipetted onto 10 mm diameter Nuclepore filters (0,2 μm) in aliquots of 10 μl . These filters were then taken through an alcohol series, culminating in two changes of absolute alcohol. The samples were not exposed to the air at any stage of the dehydration process. After dehydration, the samples were critical point dried, coated, and viewed under a Cambridge S 180 Scanning Electron Microscope.

7. CHN-Analysis

Particulate organic carbon and nitrogen measurements were made on seawater which had been initially filtered through a 200 μm mesh net to remove larger particulate material. A measured volume of 100-1000 mL, depending on the particulate load, was filtered under a vacuum of <12 cm Hg through 25 mm Whatman GF/C glass fibre filters which had been pre-ashed at 400°C for 6 hours. The filters were stored at -20°C and then oven-dried at 55°C prior to analysis with a Heraeus model CHN-Mikro Universal combustion analyser calibrated with cyclohexanone (Monar, 1972).

Bacterial carbon and nitrogen measurements were made on five isolates

of bacteria which had been cultured in both nutrient-rich (18 hour incubation at 30°C) and nutrient-poor (6 day incubation at 15°C) media. The nutrient-rich medium consisted of liquid seawater broth, and the nutrient-poor medium of artificial seawater (Sieburth, 1979) to which 25 µg atoms Nitrate N.mℓ⁻¹ and 6,25 mg Mannitol ℓ⁻¹ had been added. The cultured bacteria were harvested by centrifugation at 7000 x g for 10 minutes, resuspended in ammonium acetate buffer pH 7,0, and lyophilised to constant weight. These weighed samples were combusted in the Heraeus combustion analyser to obtain C:N ratios for locally isolated bacteria.

8. Lysis of Phytoplankton

Although it was demonstrated in Section II that the crystalline style extract of C. meridionalis could digest cellulose, in the form of carboxy methyl cellulose, the degree of cellulolytic activity was shown to be very low. This experiment was initiated in order to determine whether or not the extract could lyse whole phytoplankton.

i) Quantitative

Sterile pure cultures of naturally occurring phytoplankton species were obtained from the Sea Fisheries Research Institute, Sea Point Laboratory. Two species of diatom, namely Chaetoceros gracilis and Skeletonema

costatum were obtained. In addition, five species of flagellate were used, namely Pseudoisocrysis paradoxa, Tetraselmis chuii, Pyraminonas virginica, Isocrysis galbana and Chlorella sp.

Each species was centrifuged at 3000 x g for 10 minutes and resuspended in sterile, filtered seawater. This process was repeated three times to remove small marine bacteria (1 μ m) which remained suspended in the supernatant. The final suspensions of phytoplankton (10 ml), were halved, and 5 ml aliquots were pasteurised at 65°C for 10 minutes to kill any remaining bacteria. These suspensions (5 ml) of both heat killed and live phytoplankton were incorporated into a 0,8% w/v seawater agarose matrix, and 20ml aliquots were pipetted into sterile petri dishes. A control plate was made, excluding phytoplankton from the seawater agarose matrix.

Wells were made in the agarose matrix as before, and the extract of the crystalline style (sampled at 13°C ambient seawater temperature) was pipetted into the wells in serial 1:2 dilutions. These plates were incubated at 22°C for 45 hours, and stained with 10 ml 1% Congo red, which is specific for cellulose. The Congo red which had precipitated onto the agarose plate was removed with washes of 1 M NaCl.

ii) Qualitative

Sterile pure cultures of the same diatoms and flagellates as above were observed under the light microscope, using interference optics. These were photographed in their natural state, and subsamples were preserved in 1% glutaraldehyde for later Scanning Electron Microscope observations.

To the diatoms and flagellates, equal volumes of 1 gm protein ml⁻¹ extract from the style of C. meridionalis, sampled at 13°C were added, and the phytoplankton were observed intermittently under the microscope. Photographs were taken at \pm 10 minute intervals, and after 45 minutes, subsamples were preserved in glutaraldehyde for later SEM work. As a control, the phytoplankton species were diluted 1:1 in phosphate buffer pH 7.0 with 150 mM NaCl (the same buffer in which the style extract was homogenised), and were observed to maintain their shape and integrity.

RESULTS AND DISCUSSION

1. Lysis of Target Bacteria

The zones of lysis which formed the basic assay for lysozyme activity in extracts of the crystalline style from Choromytilus meridionalis are shown in Fig. 19. The assay for the activity of this factor is extremely reproducible and sensitive, due to the use of crystal violet to enhance contrast of low levels of target bacteria.

The results demonstrated in Fig. 20 show the bacterial strains isolated from the gut of C. meridionalis to be 79% resistant and 21% susceptible to lysis. A similar spectrum was found amongst the kelp-frond bacterial isolates, in which 62% were resistant and 38% were susceptible. In contrast, the bacteria isolated from the water column in the kelp bed are only 43% resistant and 57% susceptible to lysis, a feature which is incorporated into the nitrogen budget calculations in the final part of this section.

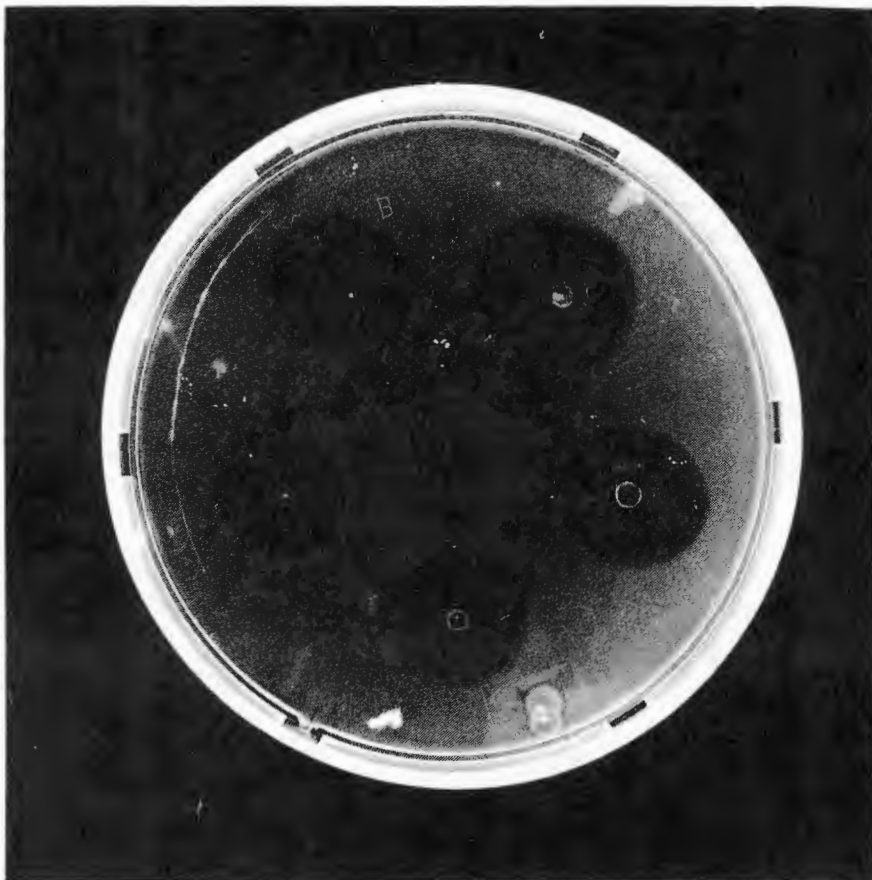


Figure 19 Plate assay for the lysozyme-like activity found in crystalline style extracts of Choromytilus meridionalis in which heat killed target bacteria suspended in seawater agarose are lysed by serial 1:10 dilutions of style extract.

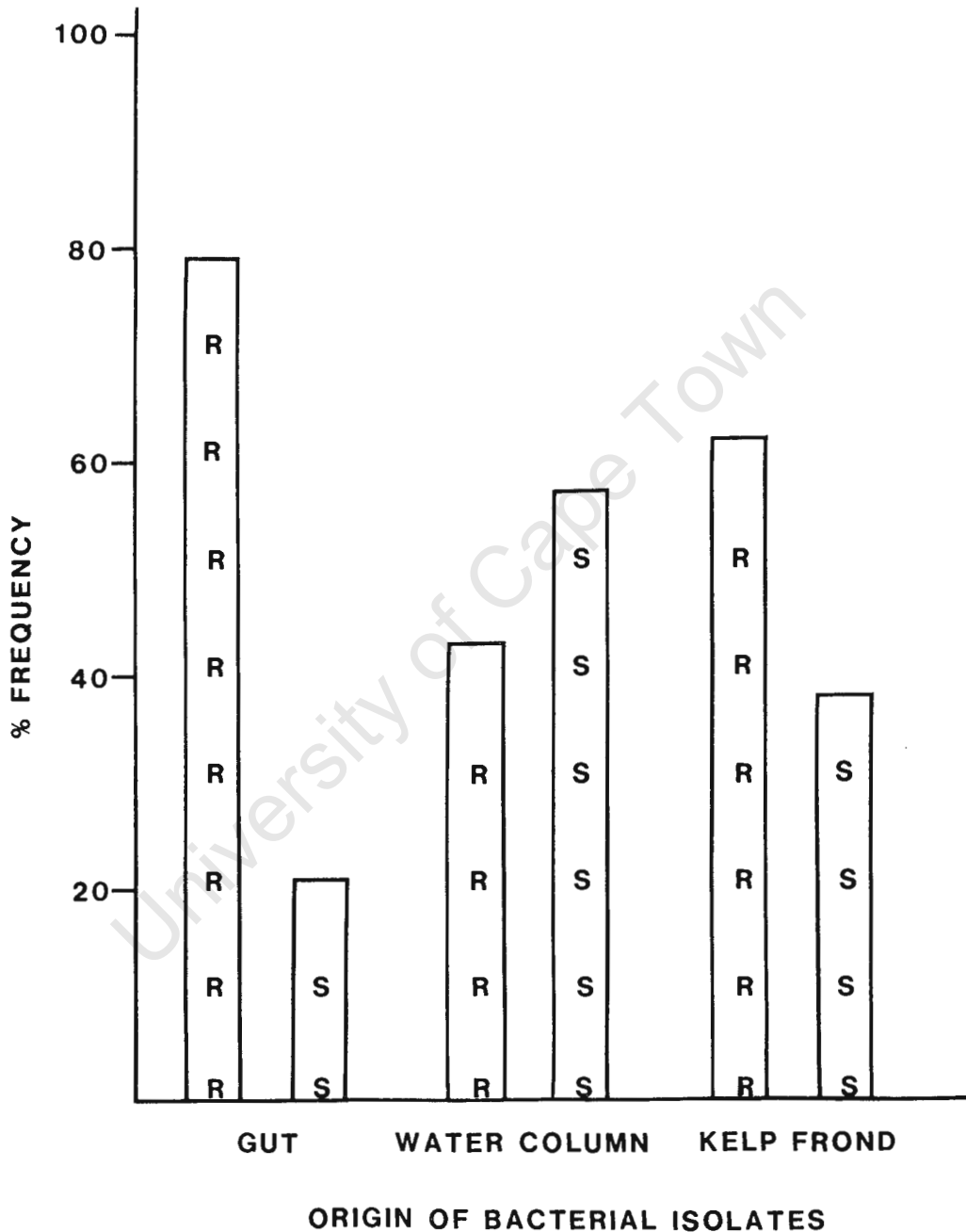


Figure 20 The activity spectrum of style extract from *C. meridionalis* on bacterial isolates from different origins. The data are expressed as percent frequency of activity of > 10 bacteria lysed per μl extract ($\times 10^5$) for susceptible bacteria (S) and < 10 bacteria lysed per μl extract ($\times 10^5$) for resistant bacteria (R).

The bacterium used as the target organism throughout most of this study was isolated from the water column near the sampling site at Oudekraal and was identified as a Pseudomonas sp. (the number assigned to this isolate was 12-01).

According to the work of McHenery and Birkbeck (1982) the lysozyme of Mytilus edulis satisfy the criteria for "true" lysozyme, proposed by Salton (1957) and Jollés (1969) viz. enzymatic activity against Micrococcus luteus cells, and by its dissolution of isolated bacterial cell walls to liberate reducing groups and N-acetylamino sugars. The bacteriolytic substance with which I am concerned does not appear to be a true lysozyme, in that it does not lyse Micrococcus lysodeikticus nor Escherichia coli, with or without the chelating agent EDTA. Like McHenery and Birkbeck (1979), I have found that the extract of the crystalline style of C. meridionalis can lyse naturally occurring populations of marine bacteria in the absence of EDTA, a feature which is probably associated with the pH conditions within the stomach. Another interesting feature of this bacteriolytic factor, is its specificity. If it were a true lysozyme, which is a specific agent in the breakdown of the bond between N-acetyl glucosamine and N-acetyl muramic acid present in all bacterial cell envelopes, it would lyse all bacterial cells equally well under similar experimental conditions. However, the selectivity which it displays, seen in Fig. 20 is remarkable.

The bacteriolytic agent of C. meridionalis appears to induce the formation of spherical protoplasts, shown in Figs 21b, 22c and 23b, and the subsequent formation of ghosts (empty cytoplasmic membranes) (shown in Fig. 23c) formed by osmotic rupture. In such a way, the cell

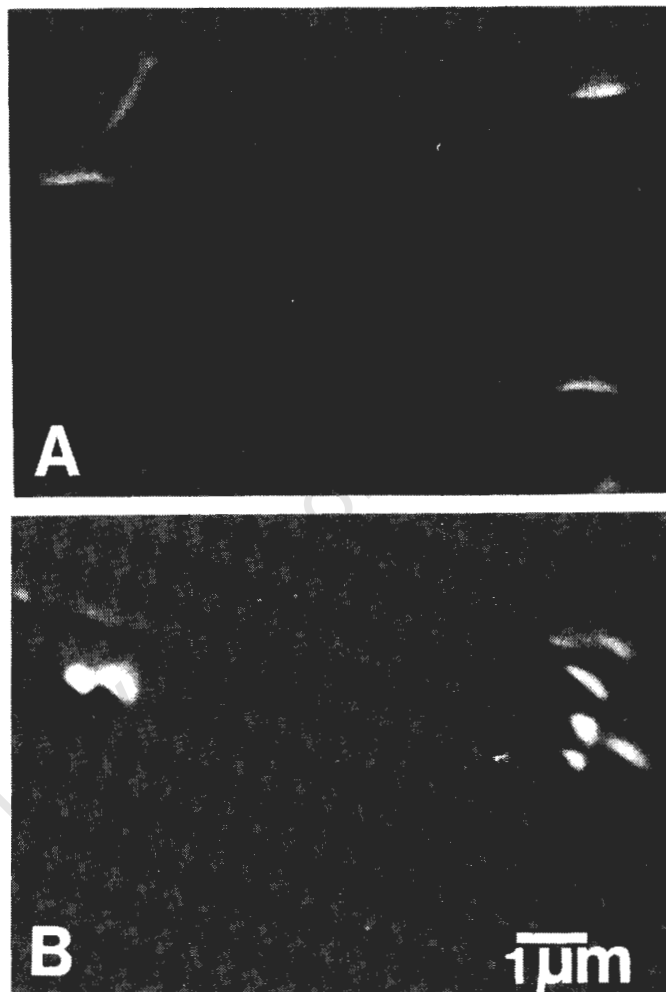


Figure 21 Interference contrast micrographs showing an intact Pseudomonad (A) and the same Pseudomonad after 30 minutes exposure to a 1 in 5 volume of style extract of C. meridionalis (B). Final magnification = 9600 x.

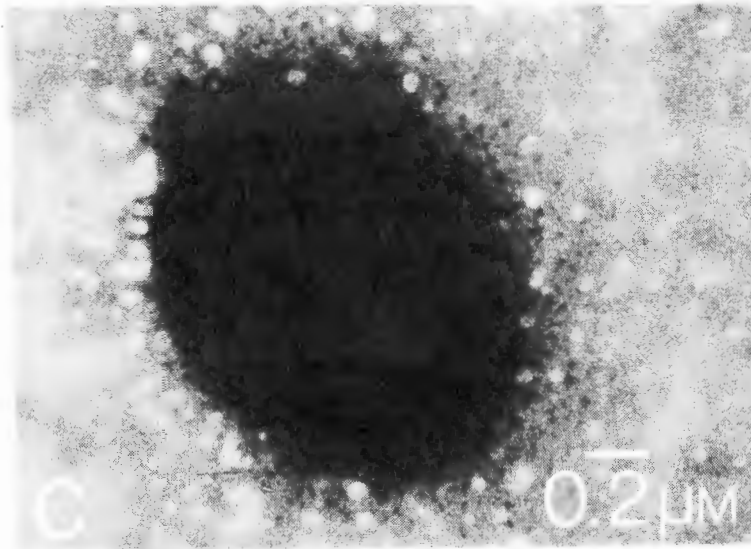
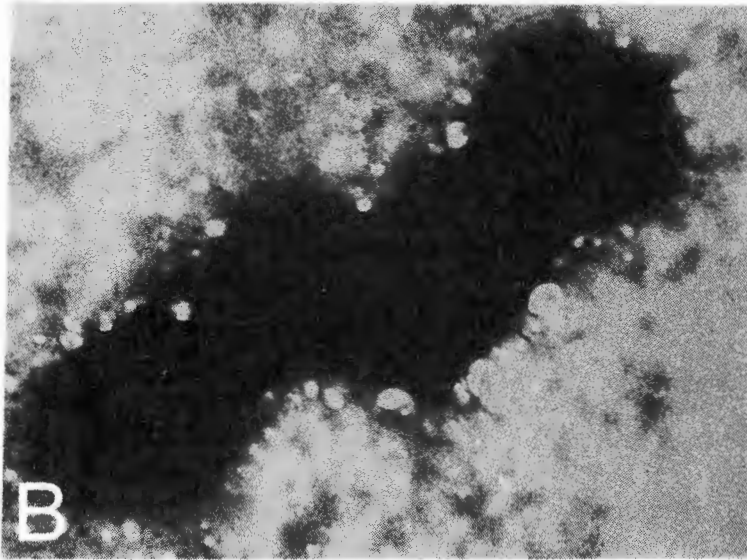
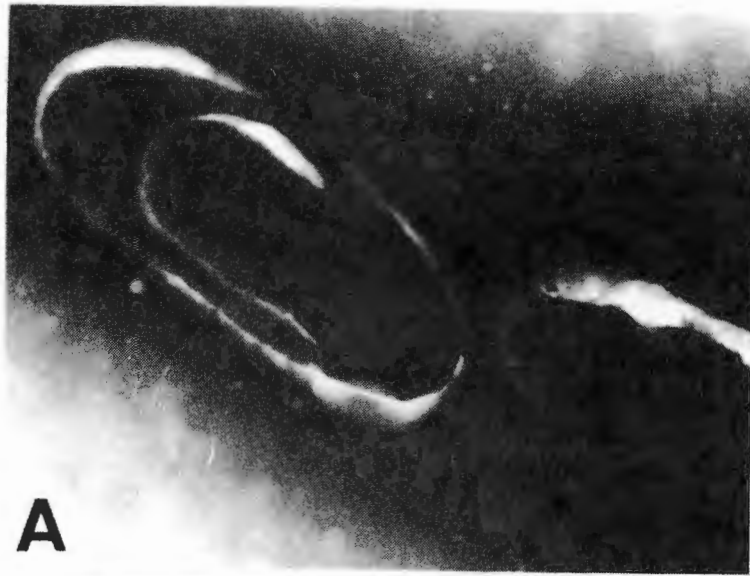


Figure 22 Negative stained transmission electron micrographs showing an intact Pseudomonad (A), the same Pseudomonad after 30 minutes of exposure to a 1 in 5 volume of style extract of C. meridionalis (B), and again after 2 hours of exposure to style extract (C). Final magnification = 40 000 x.

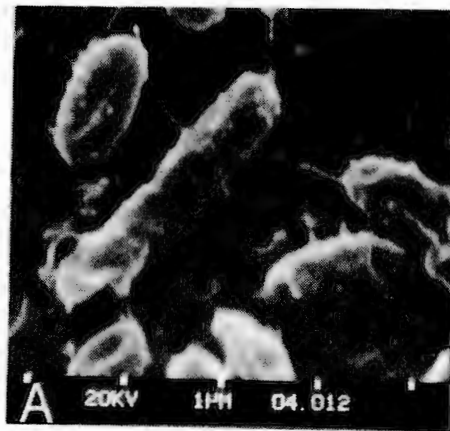
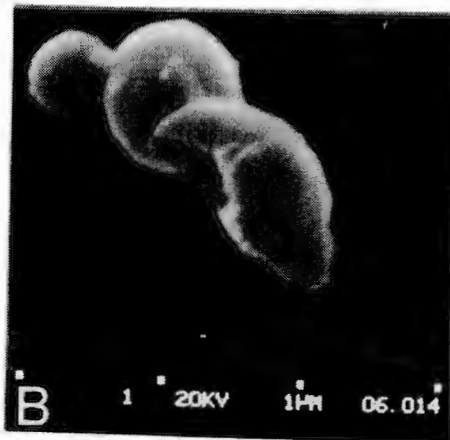
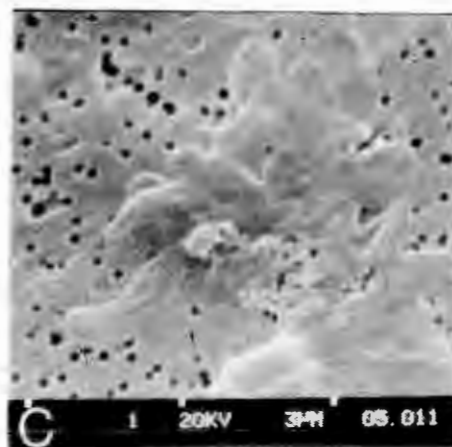
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Figure 23 Scanning electron micrographs showing an intact *Pseudomonad* (A), the same *Pseudomonad* exhibiting "bunny ears" after 30 minutes of exposure to a 1 in 5 volume of style extract of *C. meridionalis* (B), and again as "ghosts" after two hours of exposure to style extract (C). The "bunny ears" represent the first stage of plasmolysis and the "ghosts" are the empty cell envelopes which remain.

contents of the susceptible bacteria are liberated and made available for absorption by the mussel. The diameter of the zone of lysis, and hence the number of target bacteria lysed, can be calculated from the regression: Bacterial number lysed ($\times 10^5$) = $-0,92 + 0,11X$ (Fig. 17). The equivalent protein liberated from bacterial breakdown is related to the diameter of the zone of lysis by the regression: Bacterial protein (μg) = $-0,36 + 0,05X$ (where X = zone diameter in mm (Fig. 18).

2. Variations in Bacteriolytic Activity

One of the striking features of bacteriolytic activity from the crystalline style of Choromytilus meridionalis is that it is very variable, reaching a minimum during periods of onshore wind with associated downwelling of phytoplankton-rich water which is commonly from 13 - 15°C. Conversely, during offshore wind when cold upwelled water of approximately 9°C impinges on the kelp bed, there is a considerable reduction in phytoplankton available for consumption by the filter-feeders (see Field et al., 1977, 1980a,b, 1981). Turnover of style enzymes can take place within 18 hours (Seiderer et al., 1982b) and abrupt increases of lytic activity were observed within 24 hours of the onset of upwelling. These temporal variations in the activity of lysozyme-like enzymes in the style therefore appear to be actively induced by changes in environmental conditions during the upwelling-downwelling cycle.

Table 18 demonstrates the sea temperature and the bacteriolytic activity of style extracts sampled during 10 successive days in February 1983,

TABLE 18 The ambient sea temperature and the effect of bacteriolysis of extracts of the style of *C. meridionalis* sampled during 10 successive days in February 1983, on five bacterial isolates from the kelp bed water column. Bacteriolysis (number of bacteria lysed $\mu\text{l extract}^{-1} \times 10^5$).

Day	Temp °C	B A C T E R I O L Y S I S				
		M4-01	01-04	13-02	12-01	05-01
1	13,5	0	0	0	0	0
2	14,5	0	0	0	0	0
3	13,0	0,51	0,07	0,051	0,062	0,04
4	13,0	62,0	29,0	7,0	29,0	7,0
5	15,0	0,04	0,029	0,007	0,029	0,029
6	11,5	29,0	18,0	0,7	7,0	0,7
7	11,0	29×10^4	29×10^4	40×10^4	18×10^4	18×10^4
8	11,5	40,0	18,0	18,0	18,0	7,0
9	13,5	0	0	0	0	0
10	13,0	51,0	40,0	18,0	29,0	4,0

on five bacterial isolates from the kelp bed water column. These data are summarised (mean and standard deviation of bacteriolytic effect on five bacterial isolates) in Fig. 24.

The relation between the bacteriolytic activity of crystalline style extracts and the temperature of the water during the complete upwelling-downwelling cycles in February and March 1983, is shown in Fig. 25.

There is evidently an inverse correlation between seawater temperature and the activity of lysozyme-like enzymes in the style, periods of upwelling and relatively low phytoplankton abundance being associated with high bacteriolytic activity by the style. The relation between bacteriolysis and water temperature is described by the following linear regression: cells lysed by 1 μl style extract ($\times 10^5$) = $ke^{-1,24T}$ where T = seawater temperature $^{\circ}\text{C}$, $k = e^{14,88}$ cells ($r = 0,68$, $n = 67$) (Fig. 25).

3. Proteolysis

Figure 26 shows the occurrence of a high molecular weight (25 000) proteolytic enzyme, and the action of soy bean trypsin inhibitor against this protease to be coregulated by environmental water temperature. The protease activity appears to be highest at 10°C and lowest at 13°C , whereas the inhibition of this enzyme seems to be optimal at 10°C and ineffectual at 13°C .

Proteases usually split internal peptide bonds when they hydrolyse large

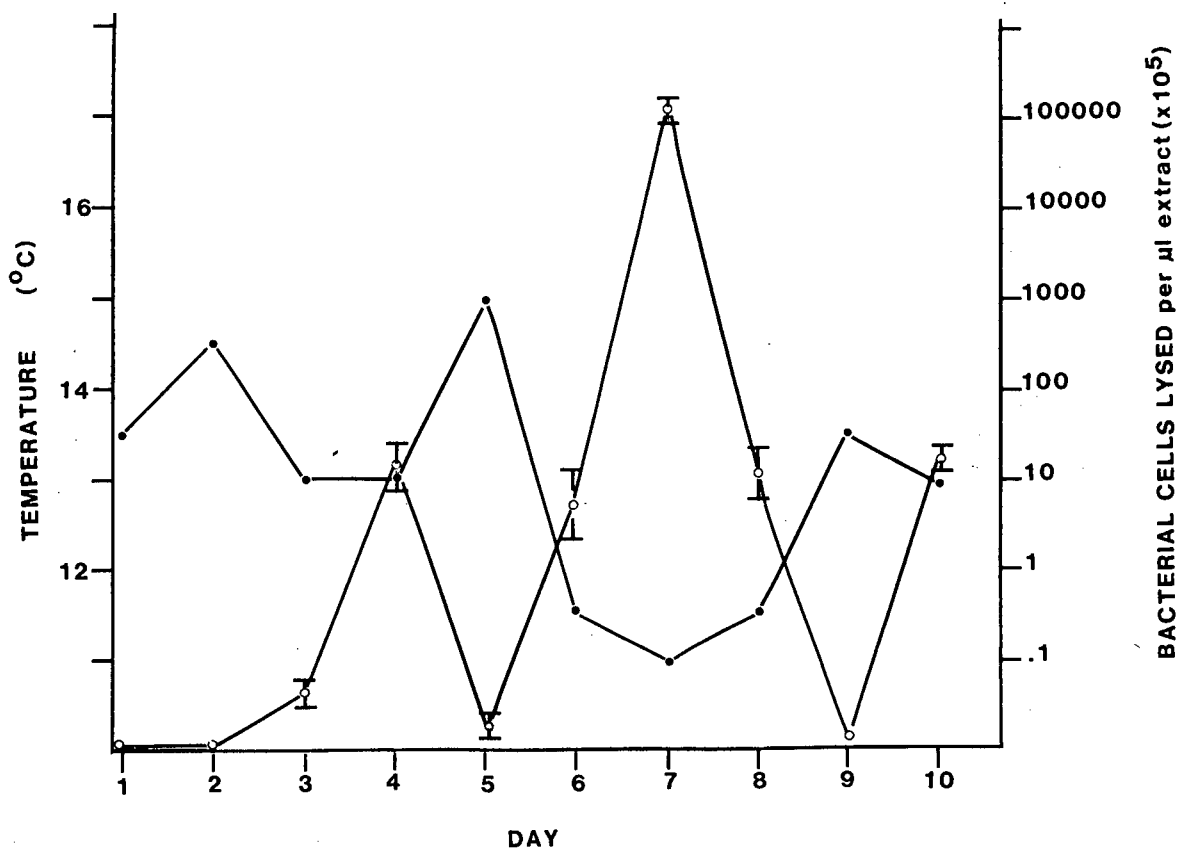


Figure 24 Ambient seawater temperatures (°C) (●) and bacteriolytic activities (○) of style extracts of *C. meridionalis* sampled during 10 successive days in February 1983. The open circles represent the means of 5 bacterial isolates tested, and the bars represent the standard deviations on those means.

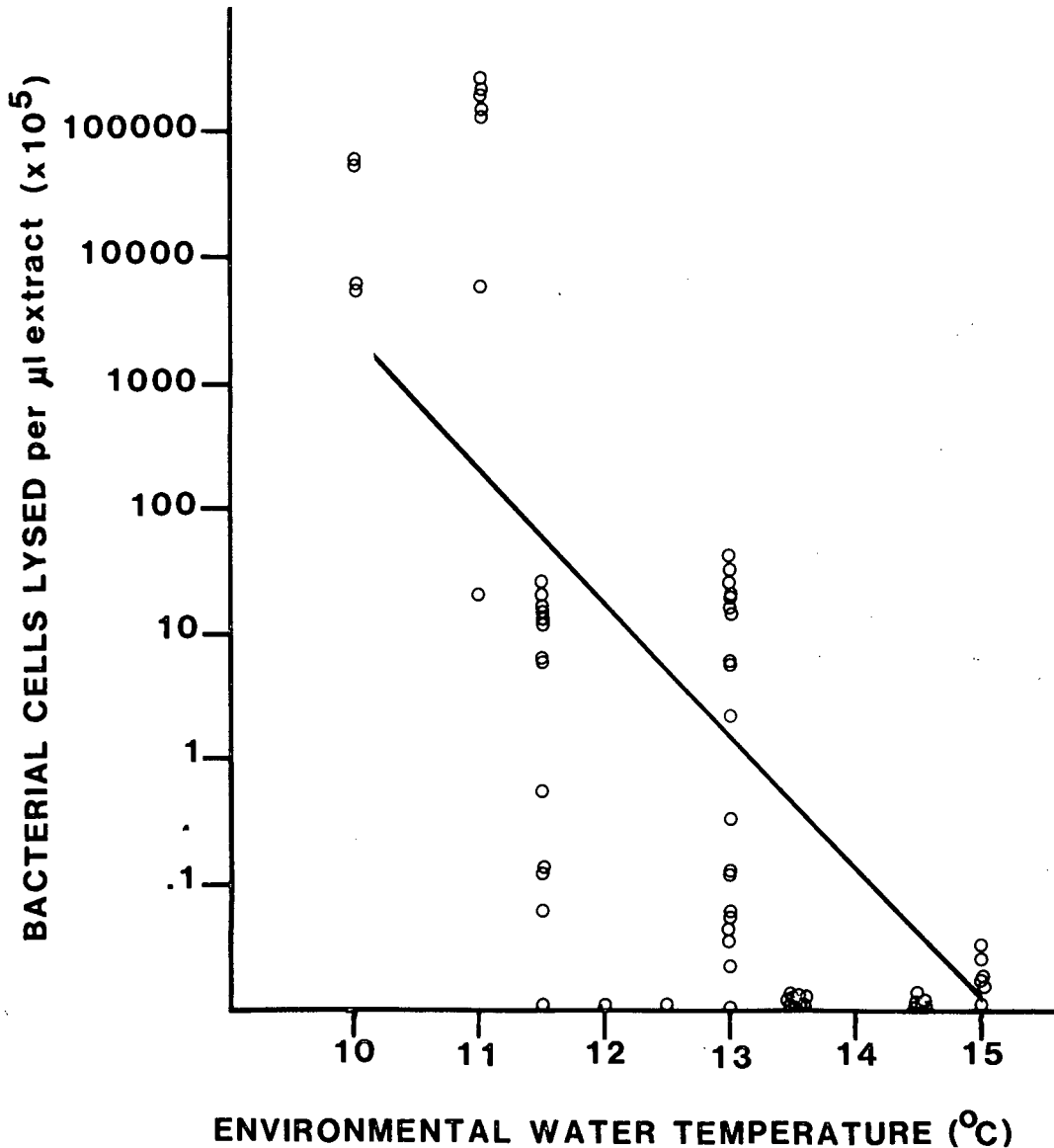


Figure 25 Numbers of bacterial cells lysed by 1 ml of *C. meridionalis* crystalline style extract, plotted as a function of environmental seawater temperature. Regression equation: (cells lysed = $Ke^{-1,124T}$ where $T = ^\circ\text{C}$; cells lysed = the number of cells lysed by 1 μl style extract ($\times 10^5$) and $k = e^{14,88}$ cells; $n = 67$, $r = 0,68$, fitted by the method of least squares). This does not necessarily imply that the phenomenon has linear kinetics.

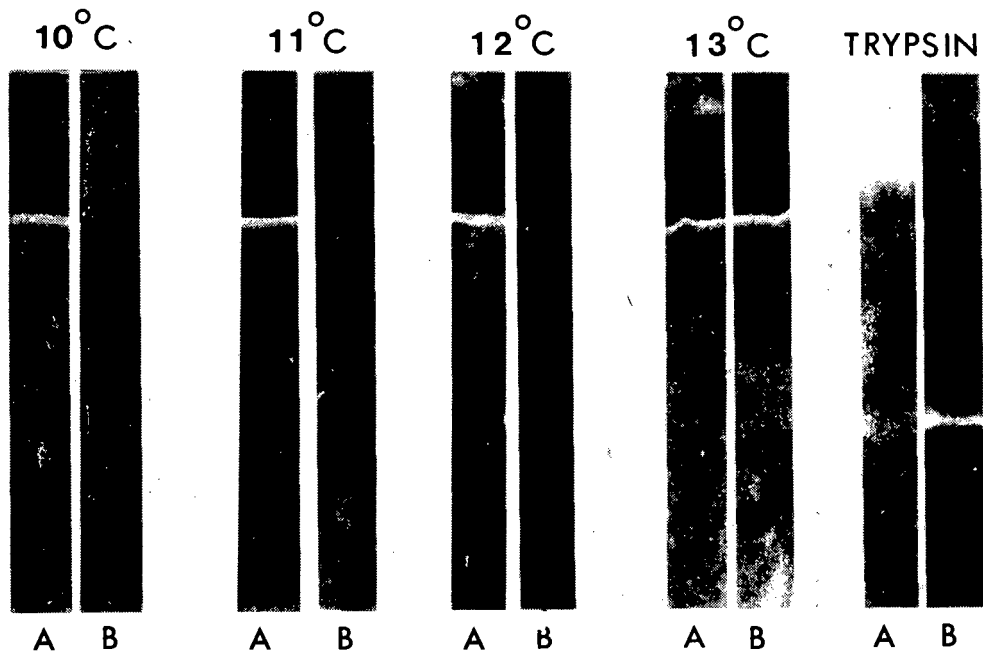


Figure 26 An SDS protease gel demonstrating the occurrence of a proteolytic enzyme in style extract of *C. meridionalis* sampled during different ambient water temperatures (10°C - 13°C). Samples A were untreated, whilst samples B were treated with soy bean trypsin inhibitor.

molecular peptides and protein substrates; the enzymes are therefore called endopeptidases (Hare, 1982). Treatment of the style extracts with soy bean trypsin inhibitor demonstrated (Fig. 26) the protease to be a trypsin-like serine protease, the most widely distributed group of proteolytic enzymes of both microbial and animal origin (Hartley, 1960). Serine proteases are generally optimally active at alkaline pH (hence the incubation of gels in buffer of pH 9,0), but they exhibit a fairly broad pH profile for hydrolysis of proteins, covering the pH range from neutrality to pH 11 (Matsubara & Feder, 1971). The serine proteases also exhibit a broad range of substrate specificities.

The mean bacterial numbers using direct microscopy were 4×10^5 cells ml^{-1} during upwelling and $2-3 \times 10^6$ cells ml^{-1} during downwelling conditions (Linley, pers. comm.).

4. C:N Ratios

The mean percent of carbon, hydrogen and nitrogen found in five isolates of bacteria grown in rich and poor nutrient growth media can be seen in Table 19. It can be seen from the total percent organic that approximately 45% is unaccounted for. Where the sample mass was sufficient, such as the five bacterial isolates grown in rich nutrient medium, samples were weighed and combusted at 450°C for 15 hours in a muffle furnace. A mean of 11,5% ash was recorded ($n = 5$, $SD = 7,92$), indicating that the remaining 34% must be water. As such, these carbon, hydrogen and nitrogen percentages should not be used as absolute values, only as ratios of one to the other.

TABLE 19 The percentage of Carbon, Hydrogen and Nitrogen found in 5 isolates of bacteria grown in rich and poor nutrient growth media. These figures should only be used as ratios of one to the other, not as absolute percentages.

Medium	Isolate	% C	% H	% N	% Total	C:N
rich	12-01	33,73	7,33	9,83	50,88	3,44
rich	T2-01	35,70	6,23	9,98	52,91	3,58
rich	M4-01	37,83	6,33	10,40	54,56	3,64
rich	B3-01	32,60	6,73	8,48	47,81	3,87
rich	16-01	32,00	7,55	10,75	50,30	2,98
poor	12-01	26,05	5,45	6,30	37,80	4,13
poor	T2-01	35,20	6,15	9,30	50,65	3,79
poor	M4-01	38,30	6,55	10,00	54,85	3,83
poor	B3-01	29,20	5,10	7,85	42,15	3,72
poor	16-01	35,75	6,25	8,80	50,80	4,06

Table 20 shows the C:N ratios of particulate material from macrophytes, phytoplankton and bacteria. From this it is clear that the bacteria which are likely to be lysed primarily during periods of upwelling of cold phytoplankton-poor water represent a relatively nitrogen-rich nutritional resource. Periods of downwelling of phytoplankton-rich water (Hutchings, 1981; Mann, 1982) are likely to be associated with a higher C:N ratio in the potential food supply in the water column.

5. Lysis of Phytoplankton

i) Quantitative

The attempt at quantification of phytoplanktolysis failed only in so far as the methodology has not been refined. Zones of lysis in the agarose plates were not visible without staining, since the contrast between turbid and clear areas was not sufficient. It appears that Congo red, as a stain, is not entirely suitable due to precipitation of the stain. The addition of 1 M NaCl served to lift the precipitate from the agarose plate, and zones of lysis were apparent, but not on a quantifiable scale. A spectrophotometric approach may yield better results in the future.

ii) Qualitative

It was found that all seven phytoplankton species with the exception of the diatom Skeletonema costatum and the flagellate Chlorella sp. were susceptible to lysis within 15 - 60 minutes of exposure to the crystalline style extract of C. meridionalis. It may be that the shapes,

TABLE 20 Comparative data on the C:N ratios of particulate or sub-particulate organic matter associated with kelp beds.

Particulate Organic Matter	C:N	Source
Macrophytes	15,6	Dieckmann, 1978; Koop <u>et al.</u> , 1982a, b.
Phytoplankton	6,6	Bailey pers. comm.
	7,3	Bishop <u>et al.</u> , 1978
Bacteria: Nutrient rich	3,56	Present study n = 9, SD = 0,28
Nutrient poor	3,91	Present study n = 5, SD = 0,18

and hence the volume:surface area ratios of the two species are responsible for this resistance to lysis. Skeletonema costatum is a slender diatom, approximately 12 μm long and 2 μm wide, and Chlorella sp. is a spherical flagellate approximately 3 μm in diameter.

Intact cells of the diatom Chaetoceros gracilis and the flagellate Tetraselmis chuii can be seen in the scanning electron micrographs of Figs 27a and 27c respectively. Figures 27b and 27d are the same two phytoplankton species after 45 minutes of exposure to style extract. The mat of material seen in Fig. 27b is proteinaceous material from the style extract. It is, however, apparent that no individuals of Chaetoceros gracilis remain intact after exposure to the enzyme extract. Figure 27d demonstrates the response of the cell membrane of Tetraselmis chuii to enzyme exposure.

The mechanism of this phytoplanktolysis may be elucidated in future work.

The extent to which the above trophic resources could meet the carbon and nitrogen requirements of the consumer mussels can be calculated from the concentration of materials in the water column, coupled with some estimates of the consumer demands based on the carbon and nitrogen budgets for individual mussels.

6. Quantitative Significance of Style Bacteriolytic Activity in Relation to the Nitrogen Budget of *Choromytilus meridionalis*

The data presented above allow some estimates to be made of the total

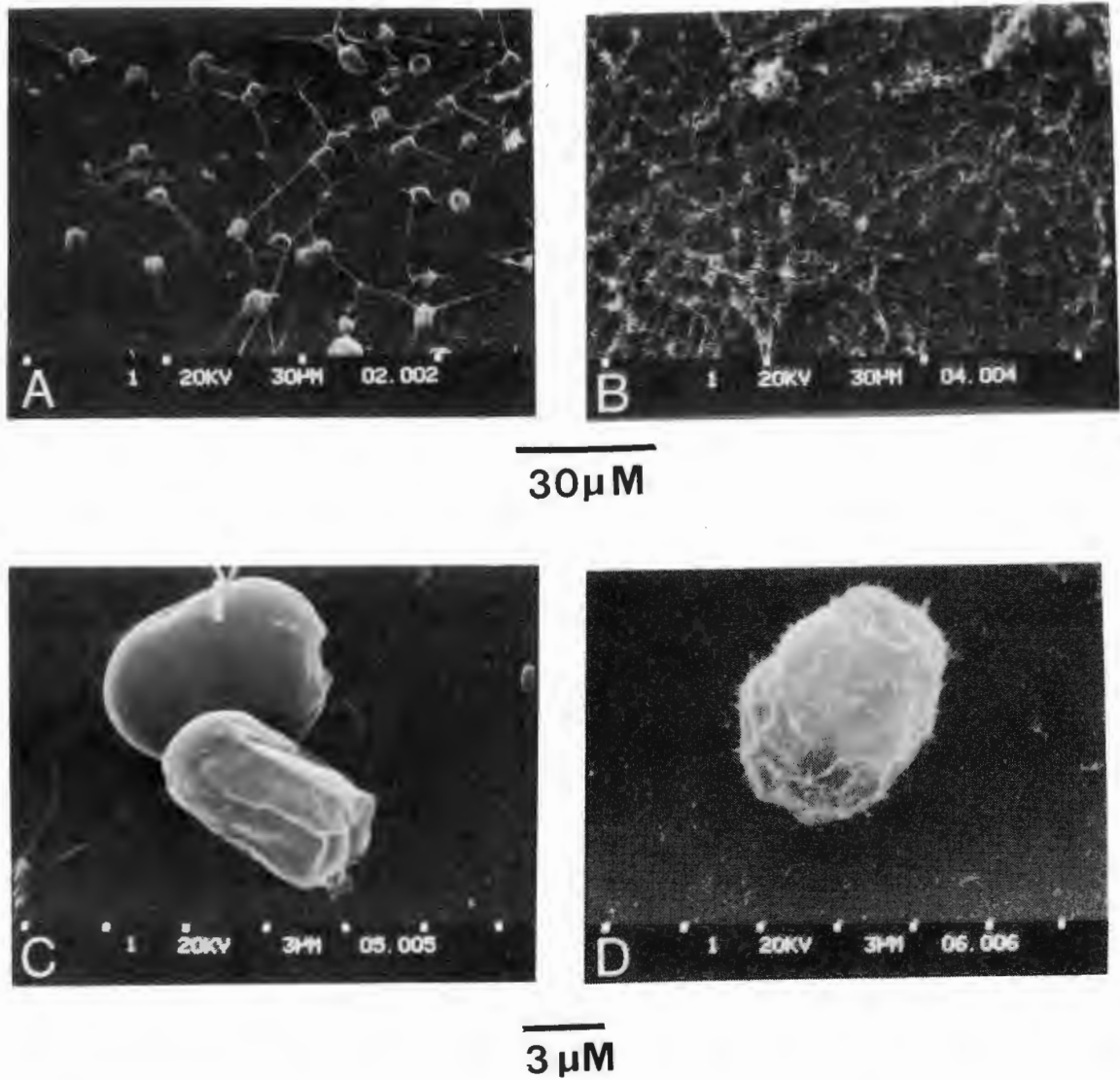


Figure 27 Scanning electron micrographs showing intact specimens of the diatom Chaetoceros gracilis (A) and the flagellate Tetraselmis chuii (C). Micrographs (B) and (D) demonstrate the effect of exposure to a 1 in 5 volume of crystalline style extract of C. meridionalis for 45 minutes. The mat of material in the background of micrograph (B) is proteinaceous material. No specimens of Chaetoceros gracilis have, however, remained intact.

bacteriolytic activity of the style, and of its potential ability to meet the nitrogen requirements of mussels of different sizes, provided that the total style protein in relation to body size is known.

The following regression (Section II) relates total style protein (mg) to C. meridionalis shell length (mm); $\hat{y} = -3,47 + 0,16x$ ($r = 0,89$, $n = 20$). This can be used to relate the bacteriolytic activity of the proteins to the nitrogen requirements of the mussels in the following way. The protein concentrations of the style extracts were standardised to $1 \mu\text{g } \mu\text{l}^{-1}$, and the cell lysis shown in the agarose plates (Fig. 19) was expressed in terms of the number of bacteria lysed per μl of extract in 48 hours. This can be converted to the amount of bacterial nitrogen made available ($N = \text{protein}/6,5$) (Newell & Field, 1983) by a single crystalline style and related to the size of the mussel. The style turnover time for C. meridionalis is approximately 18 hours (P.A. Cook, pers. comm.), hence the final figure of maximum lytic capacity of the crystalline style has been expressed as $\mu\text{g N}$ made available in 1 hour. An estimate of the nitrogen requirements of different sized mussels has been made, using the oxygen consumption data of Griffiths (1980), in which it was found that the mean value for respiration (R) was 74% of the absorbed ration (A).

Values for the oxygen consumption and its nitrogen equivalent, calculated, using C:N ratio of 4,74 (taken from Hawkins, 1983, for Mytilus edulis) and the absorbed ration at $1,35 \times$ the respiration are summarised in Table 21.

TABLE 21 The nitrogen requirements of Choromytilus meridionalis of different sizes (shell length, mm). Data for nitrogen requirements recalculated as $\mu\text{g h}^{-1}$ from oxygen consumption ($\mu\text{l h}^{-1}$) and absorbed ration (A) in Griffiths (1980) using C:N ratio of 4,74 (taken from Hawkins, 1983, for Mytilus edulis).

Shell length (mm)	Respiration (R)		Absorbed ration (A) $\mu\text{gN h}^{-1}$
	$\mu\text{lO}_2 \text{ h}^{-1}$	$\mu\text{gN h}^{-1}$	
20	65	7,342	9,91
40	220	24,873	33,58
60	390	44,072	59,50
80	670	75,717	102,22
100	1000	113,228	152,83

Footnote: Dr A.J. Hawkins (I.M.E.R., Plymouth) has pointed out that a more direct method would be to use O:N ratio. He has recently shown that in mytilus edulis this ratio varies seasonally from 21-107 in well-fed mussels, but is generally low in starved individuals. Use of the O:N ratio for M. edulis yields similar values to those which have been calculated in the above table from the C:N ratio.

Data for the filtration rate and bacterial yield from the water column for Choromytilus meridionalis of different sizes are summarised in Table 22. The filtration rates have been recalculated from Griffiths (1980) and are related to body size by the equation -
 Filtration rate (litres h⁻¹) = 6,44 x 10³ (shell length, mm)^{1,5764}.
 Since the bacterial numbers in the water column were 4 x 10⁵ cells ml⁻¹ and 2,3 x 10⁶ cells ml⁻¹ during upwelling and downwelling respectively (Linely, pers. comm.), the protein equivalents were 1,876 and 13,70 µg protein ml⁻¹ or 288,6 and 2106,9 µg N l⁻¹, respectively, using a protein:nitrogen ratio of 6,5:1. This value has been used to estimate the gross nitrogen yield available by filtration by each size class of mussel. However, as pointed out on p. 93, only 57% of the water column bacteria were found to be susceptible to lysis. This and a retention efficiency of only 10% of 0,5 µm bacteria by C. meridionalis (Stuart, 1983) were corrected for in the final calculation (Table 22).

From this it can be seen that the nitrogen yield potentially available from lysis of the susceptible component of bacteria in the water column shows a general correspondence with the estimated nitrogen requirements of each size class of mussel. In addition, the maximal lytic activity which could be achieved at high bacterial concentrations, and which is shown in the final column of Table 22, is almost four times the nitrogen required by the mussels.

It seems likely, therefore, that under upwelling conditions when the phytoplankton and macrophyte detritus loads are low, the nitrogen requirements of C. meridionalis can be met by the activity of the bacteriolytic enzymes of the style.

TABLE 22 Bacteria as a potential nitrogen resource for Choromytilus meridionalis. Filtration rates ($\ell \cdot h^{-1}$) of animals of different sizes (mm shell length) are from Griffiths (1980). Direct counts of bacterial numbers in the water column (bacteria $m\ell^{-1}$) (Linley, pers. comm.) were converted to bacterial protein ($\mu\text{g protein } m\ell^{-1}$) and thence to bacterial nitrogen ($\mu\text{gN } \ell^{-1}$). Filtration rates were used to convert the amount of bacterial nitrogen in the water column, to the amount available to the mussel, assuming that only 57% of the bacteria are susceptible to lysis (Muir et al., in prep.) and that the retention efficiency of $0,5 \mu\text{m}$ bacteria by C. meridionalis is 10% (Stuart, 1983). The maximum lytic activity ($\mu\text{g N made available } h^{-1}$) was calculated from Fig. 18.

Shell length (mm)	Filtration rate $\ell \text{ h}^{-1}$	Bacterial N from water Minimum $\mu\text{gN } h^{-1}$	Bacterial N from water Maximum $\mu\text{gN } h^{-1}$	N required by mussel $\mu\text{g N } h^{-1}$	Maximum lysis $\mu\text{g N } h^{-1}$
20	0,724	12	87	10	-
40	2,160	36	259	34	125
60	4,092	67	491	60	261
80	6,441	106	774	102	339
100	9,156	151	1099	153	535

CONCLUSION

The results which have been presented above show that the lysozyme-like enzymes present in the style of the mussel Choromytilus meridionalis are capable of lysing free-living bacteria in the water column adjacent to kelp beds. Estimates of the biomass of such bacteria through an upwelling-downwelling cycle, and of the filtration rate of different-sized mussels suggest that free-living bacteria could meet the estimated nitrogen requirements of the mussels.

The bacteriolytic and proteolytic activity of the crystalline style is, however, in a remarkable and apparently adaptive equilibrium with the food resources available for exploitation in the water column during upwelling and downwelling conditions. When upwelling occurs, the water temperature is approximately 9°C and is poor in phytoplankton. Under these conditions, lysozyme-like activity is at its maximum and bacteria predominate as a nitrogen-rich exploitable food resource with a C:N ratio of approximately 3,7. These phases of active upwelling are interspersed with an opposite flow of phytoplankton-rich warm surface water which occurs often within 24 hours following an upwelling phase (see Field et al., 1977). Under these conditions lysozyme-like activity of the crystalline style is at a minimum. Nutritional requirements are then met by the activity of the carbohydrases in the crystalline style which are capable of digesting the cell walls of phytoplankton and thus making protein available from the cell contents, as well as meeting the carbon requirements of the mussels from a detrital diet

(Seiderer et al., 1982b). Specific quantitative data on rate and region of phytoplanktolysis is, however, not yet available.

Whether these adaptive changes in the enzyme activity of the style are induced by the qualitative differences in particulate matter available in the water column, or by the abrupt temperature changes associated with the upwelling-downwelling cycle is at present unknown. The rate of turnover of the style compared with the response time for the appearance of lysozyme-like activity suggests, however, that specific enzymes may be incorporated into the style to meet relatively short-term changes in nutritional conditions in the water column near to kelp beds.

SECTION IV

SUPPLY AND DEMAND OF CARBON AND NITROGEN
IN A BENGUELA UPWELLING ECOSYSTEM

INTRODUCTION

The black mussel C. meridionalis which forms extensive beds both inter- and subtidally along the South African west coast (Griffiths, 1981), is preyed upon by a number of predators including the kelp gull Larus dominicanus, the oystercatcher Haematopus moquini, the mussel crackers Sparodon durbanensis and Cymatoceps nasutus, the rock-lobster Jasus lalandii, the gastropod Natica tecta and the starfish Marthasterias glacialis (Griffiths 1981). Although the ribbed mussel Aulacomya ater is the major dietary component of the rock-lobster J. lalandii (Pollock 1978), Griffiths and Seiderer (1980) have shown that J. lalandii exhibits a strong preference for C. meridionalis. Griffiths and Seiderer (1980) have also suggested that rock-lobster production may be severely limited by mussel availability, the effects of which have been further investigated by Seiderer et al. (1982a) using a mathematical model relating mussel and rock-lobster population dynamics. Whereas the adult Baltic blue mussel Mytilus edulis can be said to constitute a "dead end" in the food web due to very limited predation and scavenging (Kautsky 1981), the role played by C. meridionalis in the South African west coast kelp bed ecosystem appears to be of great importance (Penney & Griffiths, 1983).

It appears that 63% of the primary production in kelp beds is by macrophytes, and since less than 5% of the consumers in the community are grazers, the production is likely to pass along detritus food chains to the filter-feeders which dominate the community (Field et al. 1977; Velimirov et al. 1977).

The results presented in Section I have shown that as much as 18% of the energetic contribution of E. maxima and 34% of that of L. pallida is released during fragmentation as dissolved organic matter, whilst the remainder is available in particulate form. Stuart et al. (1981) and Koop et al. (1982a, b) have demonstrated that under conditions of nitrogen enrichment, nitrogen is incorporated into microbial biomass with an efficiency of 83 - 94%, and carbon with an efficiency of 33%. It therefore appears likely that much of the protein initially released will ultimately become available to the consumers in the form of microbial biomass. The particulate component, which comprises 24% protein and 53% carbohydrate is presumably available directly to the filter-feeding community, omitting a step in the food chain. This has become more apparent in Section II in which the ability of two filter-feeding marine mussels, sampled from very different ecosystems, to digest carbohydrates, has been demonstrated.

Possibly, owing to the variability of the relative availability of phytoplankton and kelp detrital material as potential food resources (Stuart 1982), the two mussels P. perna and C. meridionalis have a similar array of style carbohydrases. The enzymes alginate lyase, cellulase, α -amylase and laminarinase were found to be present in both species. Despite the similarities in the carbohydrase spectrum from the two species, major differences in total specific carbohydrase activity occur. For example, protein eluates from the style of P. perna are capable of liberating 9,63 mg glucose. mg protein⁻¹.h⁻¹ compared with only 4,8 mg in C. meridionalis. The time required for turnover of the crystalline style compensates for this apparent

deficiency in that the style from C. meridionalis turns over every 18 hours and P. perna styles turn over every 120 hours. In both species therefore, the carbohydrase activities are well-suited to release glucose from both the living phytoplankton and detrital components of the particulate organic matter in the water column at a rate which is sufficient to meet the estimated carbon requirements of the mussels.

The results presented in Section III have demonstrated that the bacteriolytic agent present in the style of C. meridionalis is capable of lysing approximately 57% of the free-living bacteria in the kelp bed water column. This bacteriolytic and co-regulated proteolytic activity is in an apparently adaptive equilibrium with the exploitable resources in the water column during upwelling and downwelling conditions. During upwelling conditions, the water temperature drops to below 13°C and the animal is able to fully exploit the nitrogen-rich bacterial source. Conversely, under downwelling conditions, bacteriolysis and proteolysis no longer take place, and the food source, namely phytoplankton and kelp detritus, is utilised by the carbohydrases present in the style.

Wulff and Field (1983) have divided the year up according to a recorded upwelling index. On average, summer (240 days) consists of 42% strong upwelling and 25% medium upwelling, totalling 67% upwelling; and 33% downwelling. Winter (125 days), on the other hand, consists of 10% upwelling and 90% downwelling, based on data of speed, direction and duration of winds during 1979-1981 on the west coast of the Cape

Peninsula. These features of the Benguela upwelling ecosystem have been taken into account in the calculations which link carbon and nitrogen requirements of C. meridionalis with the availability of these resources on a daily basis.

From Sections II and III, it appears that rather specific enzymes can be incorporated into the style of C. meridionalis to meet the relatively short-term changes in nutritional conditions in the kelp bed water column. To demonstrate this adaptability, a time series experiment was carried out at Bloubergstrand during March 1983, during which a number of parameters were recorded on a daily basis.

METHODS

Parameters recorded were as follows:

Water temperatures were taken routinely using a thermometer (°C).

The longshore wind component ($\text{m}\cdot\text{sec}^{-1}$) was obtained from Mark Jury, through the Meteorological Office at Koeberg, close to Bloubergstrand. This longshore wind component is calculated according to the upwelling index $V_t = U (\cos (\theta - 160))$ where U = wind speed and θ = wind direction at 10 m from the ground. These daily means were plotted on a scale of +8 to -8 with 0 representing the boundary between an upwelling (+) and a downwelling (-) phenomenon.

The chlorophyll results were obtained by Pete Fielding from water

samples collected each day. Aliquots of 800 ml seawater were filtered under vacuum through a 4,7 cm GF/F filter. The filters were subsequently folded, wrapped in aluminium foil, and frozen at -14°C until required. The filters were ground in 12 ml 90% v/v acetone with a glass rod, sonicated for 30 minutes and centrifuged at $7000 \times g$ for 15 minutes. The supernatant was carefully removed and read at 750, 664, 647 and 630 nM on the spectrophotometer against 80% v/v acetone. The results were corrected for 10 cm path length (5 cm path length cuvettes were used) and to 10 ml acetone. The reading at 750 nM was subtracted as a blank, and the calculations were carried out according to the method of Jeffrey and Humphrey (1975). The values for chlorophyll a, b and c were summed and multiplied by C/V, where C is the value (a + b + c) and V is the volume of water filtered. The results were expressed as ug chlorophyll per litre of seawater filtered.

The carbon and nitrogen data were obtained by filtering 800 ul aliquots of seawater, and treating them as on page 90 of Section III. The data were expressed as μg of nitrogen and carbon per litre, and as a ratio of C:N.

The total number of bacteria was determined by Claire Davis using the acridine orange direct count (AODC) method. Both rods and ultra-microbacteria were included in the total numbers present per ml of seawater.

The bacteriolytic activity of style extract of C. meridionalis was

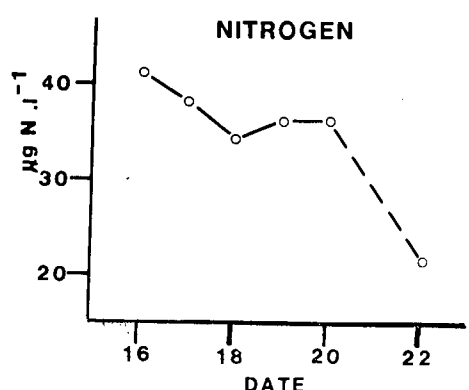
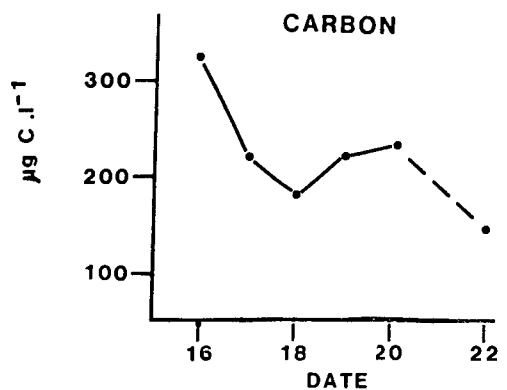
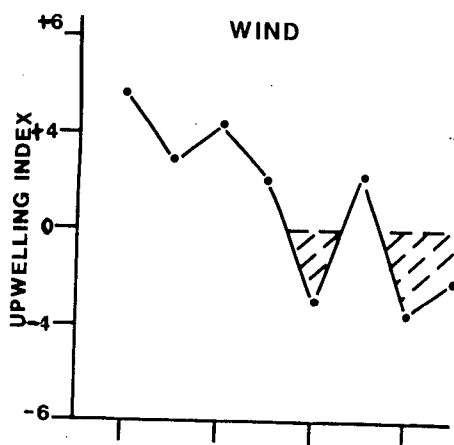
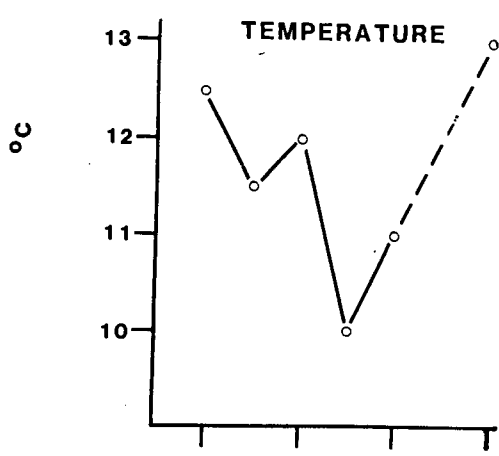
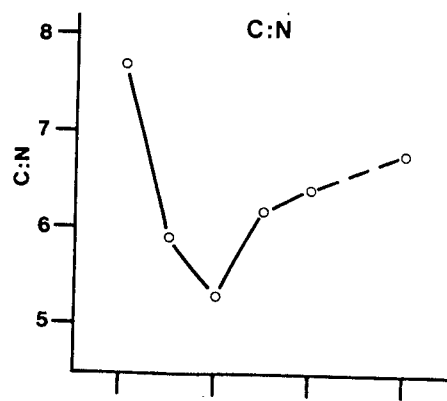
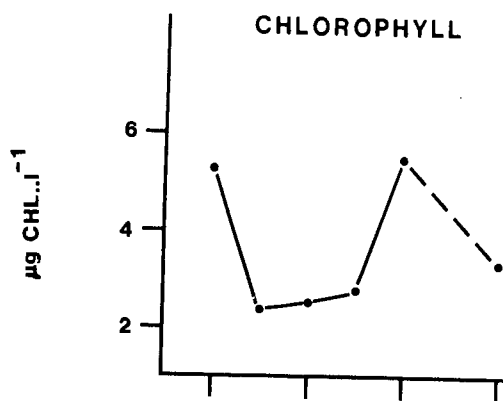
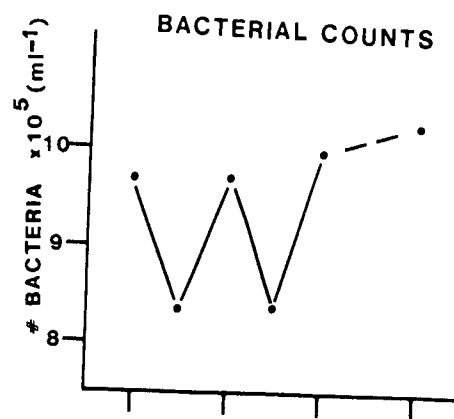
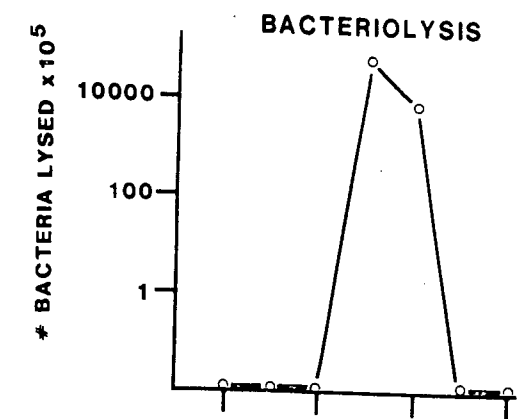
determined according to the method shown in Section III (p.84). These results were expressed in terms of the number of bacteria lysed by 1 μl of extract (1 mg protein ml^{-1}).

RESULTS, DISCUSSION AND CONCLUSION

1. Time Series Experiment

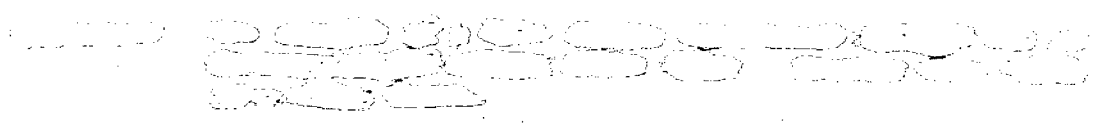
The time series experiment yielded a number of interesting preliminary results, the salient features of which can be seen in Fig. 28. The wind data in the form of upwelling indices, and the temperature data, reflect a hydrological pattern which exhibits neither classical upwelling nor downwelling characteristics. These features represent intermediate stages in the overall cycle, due to the short duration of the wind pulses. Chlorophyll concentration, total particulate carbon and total particulate nitrogen appear to follow similar trends in response to environmental conditions, with a rather low, constant concentration of chlorophyll. The C:N ratio on the other hand remains between 5,5 and 7,5, characteristic of a phytoplankton-dominated system. However, this rather low C:N ratio is thought to be an intermediate stage between the dominance of macrophyte debris (high C:N) and bacteria low (C:N), as well as reflecting the phytoplankton present in the water column.

Dr L. Hutchings (personal communication) has found a considerable range in carbon:chlorophyll "a" ratios during recent plankton dynamics cruises, but he considers a value of 60 to be representative of rapid



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phytoplankton growth, and 100 - 120 to be representative of the quiescent phase. Figure 29 demonstrates the variation in C:Chl "a" ratio within the 10 day time series, indicating that the ratio was high (90 - 130) during the 'upwelling' phase and low (60) during the 'downwelling'. This suggests that the phytoplankton are growing rapidly under inshore 'downwelling' conditions, and are quiescent during the 'upwelling phase'. A possible interpretation of this may be the dilution of phytoplankton cells which are in fact being blown offshore (Brown, 1980). The high C:Chl "a" ratio may be indicative of a low phytoplankton concentration, and a correspondingly high carbon concentration in the kelp bed water column, from other sources such as bacteria, the numbers of which appear to remain constant.

The activity of the bacteriolytic agent from the crystalline style of C. meridionalis, drops off rapidly in response to a temperature increase, perhaps indicating a drop in bacterial numbers. However, the bacterial numbers remain fairly constant throughout the time series, never changing in an order of magnitude.

It appears therefore that the response of the bacteriolytic agent cannot be linked to a change in the number of bacteria in the water column, but rather to a change in the water temperature, and possibly to the corresponding changes in particulate carbon and nitrogen.

The above results, although preliminary, demonstrate measurable responses of ecological factors to rapidly changing environmental

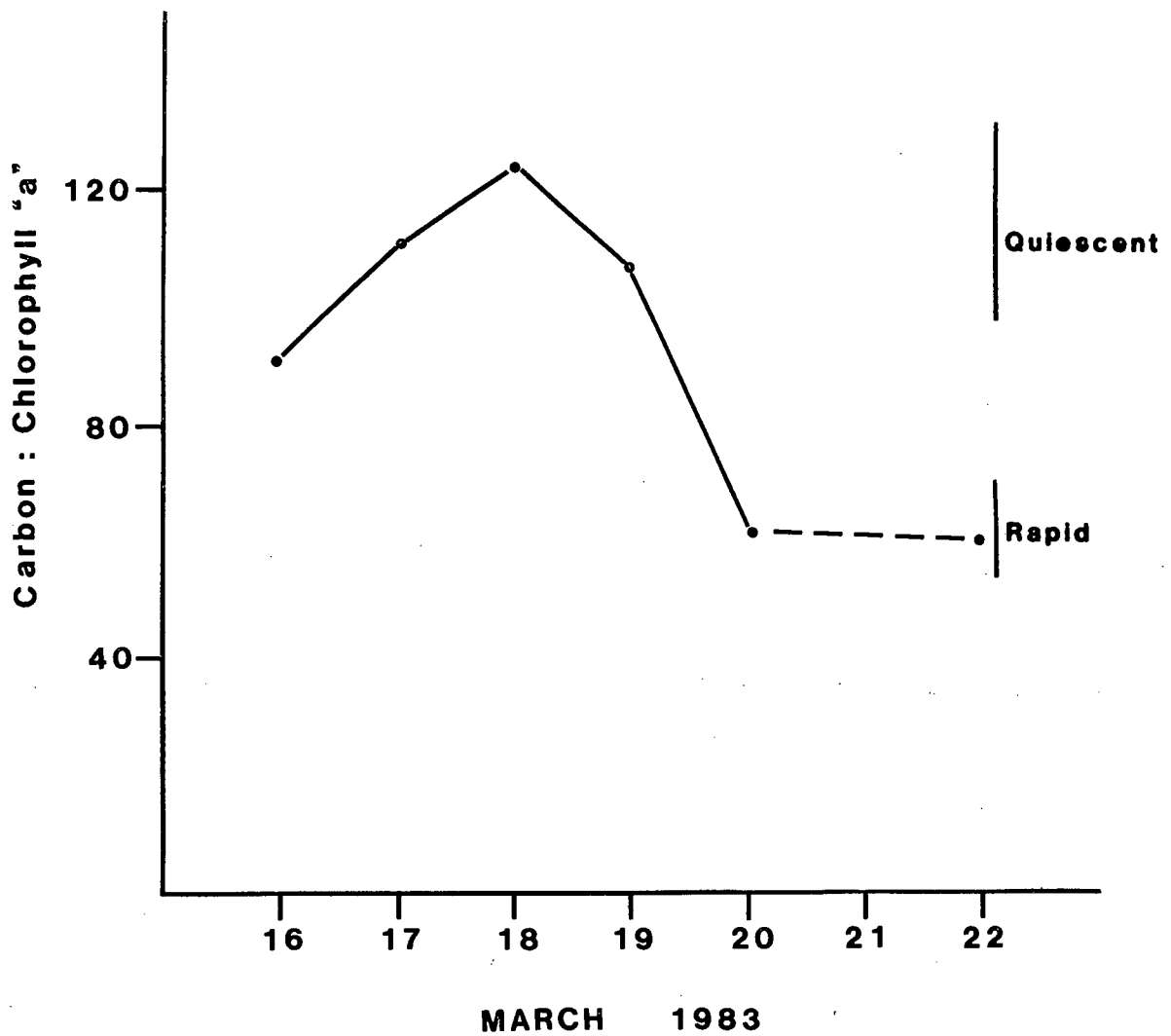


Figure 29 The variation in C:Chl "a" ratios using a 10-day time series experiment in March 1983. Dr L. Hutchings (personal communication) considers ratios above 100 to be representative of quiescent phytoplankton, and ratios below 70 to be representative of rapidly growing phytoplankton.

conditions. Wulff and Field (1983) have used a simulation model to investigate possible ecological effects of upwelling and downwelling water transport on trophic relationships in a kelp bed. Under continuous downwelling conditions, phytoplankton enters the kelp bed (Field et al. 1981; Brown 1981; Jarman & Carter 1981) and may form up to 93% of filter-feeder food. On the other hand, continuous upwelling conditions result in a consumer biomass which is limited by food availability since detritus is exported and no phytoplankton is imported (see Wulff & Field 1983). The environmental conditions during the time series experiment which was undertaken in March 1983 represent both the upwelling and downwelling phenomena, although neither of these features were very strong, with upwelling indices of +6 and -4 respectively on a scale of +8 to -8. The anomalous summer season of 1982/83 will be discussed in a subsequent section.

2. Carbon and Nitrogen Budget Satisfaction

Newell and Field (1983) have calculated the particulate component available in the water column for consumption by the dense community of filter-feeding organisms to be $599 \text{ g C m}^{-2} \text{ yr}^{-1}$ from kelp fragmentation, and $501 \text{ g C m}^{-2} \text{ yr}^{-1}$ from phytoplankton. Note that a number of assumptions have to be made in the following calculations. Parameters associated with the digestive physiology of C. meridionalis have been summarised in Table 23.

TABLE 23 A summary of parameters associated with the digestive physiology of a 60 mm black mussel Choromytilus meridionalis at 18°C.

Parameter	Value	Unit	Source
Respiration (R)	390	$\mu\text{l O}_2 \text{ h}^{-1}$	Griffiths 1980
Respiration (R)	208,9	$\mu\text{g C h}^{-1}$	} calculated from Griffiths 1980
Respiration (R)	44,07	$\mu\text{g N h}^{-1}$	
Absorbed ration (A)	282,3	$\mu\text{g C h}^{-1}$	} calculated from Griffiths 1980
Absorbed ration (A)	59,50	$\mu\text{g N h}^{-1}$	
Filtration rate	4,09	l h^{-1}	Griffiths 1980
Style glucogenesis	8,756	mg C h^{-1}	} Seiderer <u>et al.</u> 1982b
Style turnover required	31,02	hours turnover ⁻¹	
Style turnover	18	hours turnover ⁻¹	
Bacterial N from water	68	$\mu\text{g N h}^{-1}$	} Seiderer <u>et al.</u> 1983
Bacterial C from water	249	$\mu\text{g C h}^{-1}$	
Detrital N from kelp	23	$\mu\text{g N h}^{-1}$	} calculated from Newell & Field 1983 and Seiderer <u>et al.</u> 1983
Detrital C from kelp	356	$\mu\text{g C h}^{-1}$	
Detrital N from bacteria	41	$\mu\text{g N h}^{-1}$	
Detrital C from bacteria	152	$\mu\text{g C h}^{-1}$	
Phytoplankton N	42	$\mu\text{g N h}^{-1}$	}
Phytoplankton C	292	$\mu\text{g C h}^{-1}$	
Maximum bacteriolysis	261	$\mu\text{g N h}^{-1}$	} Seiderer <u>et al.</u> 1983
Maximum bacteriolysis	913,5	$\mu\text{g C h}^{-1}$	

Detrital Carbon and Nitrogen:

Assuming that the kelp bed is on average 10 m in depth, the particulate carbon produced by kelp, which has been integrated over 10 m to give a final per square metre figure, can be expressed as $59,9 \text{ g C m}^{-3} \text{ yr}^{-1}$. This is the equivalent of $59,9 \text{ mg C } \ell^{-1} \text{ yr}^{-1}$. The work of Stuart (1981) has shown the C:N ratio of kelp detritus to be 7,5, and, knowing that the C:N ratio of the kelp component is 15,6 (Dieckmann, 1978; Koop et al. 1982a, 1982b) and the C:N ratio of the bacterial component is 3,7 (Seiderer et al. 1983), the ratio of kelp carbon to bacterial

carbon was calculated to be 0,47. Assuming that kelp detritus is produced within the kelp bed throughout the year (Stuart, 1982), the kelp component of the POM comprises $0,087 \text{ mg C } \ell^{-1} \text{ day}^{-1}$. Griffiths (1980) has demonstrated the filtration rate of a 60 mm specimen of C. meridionalis to be $4,09 \text{ } \ell \text{ hr}^{-1}$, which is equal to $49,1 \text{ } \ell \text{ day}^{-1}$ assuming that the animal filters for 12 hours a day. This makes 4,27 mg carbon available to the mussel per day, and a corresponding 0,27 mg nitrogen, using a C:N ratio of 15,6 for the kelp component of detritus.

The bacterial component of the detritus, comprising up to 34% of the total bacterial biomass (Linley & Field 1982), presents a slightly different picture, since Section III has demonstrated that bacteriolysis only takes place at temperatures of less than 13°C during upwelling. Wulff and Field (1983) have used a figure of 191 days of downwelling per year, and assuming that detrital bacterial carbon is only made available to filter-feeding mussels during these periods, the amount of carbon is $0,037 \text{ mg C } \ell^{-1} \text{ day}^{-1}$. If this is filtered

by the mussel at a rate of $49,1 \text{ l day}^{-1}$, then 1,82 mg of carbon is made available to the mussel from detrital bacteria, and a corresponding 0,49 mg of nitrogen is made available, assuming a C:N ratio of 3,7.

Phytoplankton Carbon and Nitrogen:

A similar exercise can be carried out using a figure of $501 \text{ g C m}^{-2} \text{ yr}^{-1}$ released as phytoplankton production (Newell & Field 1983).

Again, assuming that the phytoplankton figures were integrated through a 10 m water column, the result can be expressed as $50,1 \text{ g C m}^{-3} \text{ yr}^{-1}$,

which is equivalent to $50,1 \text{ mg C l}^{-1} \text{ yr}^{-1}$. Phytoplankton only becomes available to kelp bed filter-feeders during periods of calm or on-shore wind and associated downwelling, which occurs during approximately 191 days of the year (Wulff & Field 1983). Taking this feature into account, the amount of carbon which would be available to the animals would be $0,071 \text{ mg C l}^{-1} \text{ day}^{-1}$. The filtration rate of $49,1 \text{ l day}^{-1}$ for C. meridionalis (Griffiths 1980), therefore makes $3,50 \text{ mg C day}^{-1}$ available to the animal assuming that it only filters for 12 hours each day. This can be converted to $0,50 \text{ mg N day}^{-1}$ using a C:N ratio of 7,0 for phytoplankton (G. Bailey, Sea Fisheries Research Institute, Cape Town, personal communication; Bishop et al. 1978).

Free Bacterial Nitrogen and Carbon:

Direct counts of bacterial numbers in the kelp bed water column were

found to be 4×10^5 cells ml^{-1} during upwelling conditions (Linley, personal communication), the protein equivalents being $1,876 \mu\text{g}$ protein ml^{-1} or $288,6 \mu\text{g N l}^{-1}$ using a protein:nitrogen ratio of 6,5 (Hawkins 1983). The filtration rate of $49,1 \text{ l day}^{-1}$ for C. meridionalis (Griffiths 1980) makes $1,42 \text{ mg N}$ available to the animal per day, assuming a retention efficiency of 10% (Stuart 1983). In addition, Section III has shown that only 57% of the water column bacteria are susceptible to lysis by the action of the crystalline style of C. meridionalis, resulting in a figure of $0,81 \text{ mg N}$ being available per day. This can be converted to $2,99 \text{ mg C day}^{-1}$ using a C:N ratio of 3,7 for bacteria (Section III).

In summary, it appears that during downwelling, an estimated $3,50$ and $2,22 \text{ mg}$ carbon are made available to the filter-feeding mussels per day by phytoplankton and the kelp component of POM respectively. This can be converted to $0,50$ and $0,14 \text{ mg N day}^{-1}$ respectively. The carbon requirements of a 60 mm specimen of C. meridionalis are $5,01 \text{ mg C day}^{-1}$ and the nitrogen requirements $1,06 \text{ mg N day}^{-1}$. In other words, during approximately half the year, 114% of the carbon requirements are met by phytoplankton and kelp POM sources alone. However, the nitrogen budget is only 61% complete, a function of the mussel's inability to digest bacteria, with a low C:N ratio of 3,7, during downwelling conditions.

During upwelling conditions, an estimated $4,81 \text{ mg C day}^{-1}$ and $1,30 \text{ mg N day}^{-1}$ are made available to the filter-feeding community by both free-living and debris-associated bacteria. A further

2,05 mg C day⁻¹ and 0,13 mg N day⁻¹ are available from the kelp component of macrophyte debris. From the above information on the carbon and nitrogen requirements of black mussels, it becomes apparent that during approximately half of the year, 96% of the carbon requirements and 123% of the nitrogen requirements are met by bacterial sources alone. A further 41% of carbon and 12% of nitrogen is supplied by the kelp component of POM, totalling 137% of the animal's carbon requirements per day, and 135% of its nitrogen requirements per day. These features have been summarised in Table 24 which relates "budget satisfaction" with the occurrence of upwelling and downwelling phenomena on a daily basis.

From Table 24, it appears that despite the high primary productivity of the system from phytoplankton and macrophytes, the filter-feeding community is in fact one where intense competition for nitrogen is likely to occur. This may be substantiated in the following sub-section in which the effect of an anomalous summer on the black mussel C. meridionalis is discussed.

3. The Anomalous Summer of 1982/1983

A recent symposium on the Benguela warm event of 1982/1983 brought to light a number of rather interesting features of the system. The work of Walker-van Heerden et al. (1983) demonstrated the north-westerly wind displacement during the entire summer period (1 November - 28 February) of 1982/1983 to be 38% less than during the summer of 1979/1980. In addition, the westerly wind displacement was 94% less than in the

TABLE 24 The relationship between carbon and nitrogen requirements of *C. meridionalis* and availability from the water column during both upwelling and downwelling conditions in a west coast kelp bed. "Budget satisfaction" represents the percentage of the animal's requirements which can be met by the available resources on a daily basis.

Hydrography	B U D G E T S A T I S F A C T I O N		
	Carbon	Nitrogen	Source
Upwelling	60%	76%	Free-living bacteria
	36%	47%	Kelp POM bacteria
	96%	123%	Total bacteria
	41%	12%	Kelp POM
	137%	135%	Total
Downwelling	0%	0%	Free-living bacteria
	0%	0%	Kelp POM bacteria
	44%	14%	Kelp POM
	70%	47%	Phytoplankton
	114%	61%	Total

corresponding summer of 1979/1980, demonstrating a virtual failure of the south east wind which drives the upwelling system operating off the south west Cape coast. It must, however, be noted that the 1979/1980 summer season was characterised by a particularly strong south easter, such that the 1982/1983 season may not have been as anomalous as it appears from the above percentages.

The work of Branch (1983) has demonstrated that the black mussels, C. meridionalis, on the west coast, have particularly low body flesh weights this year. Mussels collected by Griffiths (1981) from Bloubergstrand during 1977 exhibited the following relationships:

1. $\log (\text{shell length, mm}) = 1,5960 + 0,29 \log (\text{body weight, g})$
2. $\log (\text{shell length, mm}) = 1,9408 + 0,33 \log (\text{adductor weight, g}).$

From these two equations, any value of body weight for a given adductor weight can be calculated: e.g. 1,2 g adductor = 92 mm shell length
= 9,01 g body weight.

During January 1983, Branch (1983) found the mussels at Kreeftebaai (not far removed from Bloubergstrand) to have rather different shell length/body weight and shell length/adductor weight relationships from the previous (1977) mussels. The relationships exhibited were:

3. $\log (\text{shell length, mm}) = 1,7260 + 0,36 \log (\text{body weight, g})$
4. $\log (\text{shell length, mm}) = 1,9801 + 0,35 \log (\text{adductor weight, g})$

i.e. for 1,2 g adductor = 101 mm shell length
= 6,06 g body weight.

Note the similarities between equations 2 and 4, and the substantial differences between equations 1 and 3. These differences represent a reduction in body weight of 33%.

As pure speculation, I would like to put forward a suggestion as to the reason for this drastic reduction in body weight, and in so doing, to emphasize the importance of bacteria as a food source for the mussel, C. meridionalis.

The facts are as follows: the westerly and north westerly wind displacements during the entire summer period were 94% and 38% less than during the summer of 1979/1980; the mussel flesh weights were 33% less than during the summer of 1977/1978; the bacteriolytic agent which is present in the crystalline style of C. meridionalis is active only at temperatures of less than 13°C (Fig. 25); a calculated 63% of the animal's nitrogen requirements are supplied by bacteria alone. The animals have survived a winter period, during which 10% of the time is represented by upwelling conditions, and only 68% of the animal's nitrogen requirements can be met by the available resources. During a "normal" year, the onset of the south east winds and the subsequent upwelling of cold water would supply more nitrogen than the animal required. During the 1982/1983 summer season, however, the phenomenon was not prevalent, such that the mussels could not complete their nitrogen budgets. No data are available on the species composition of the phytoplankton prevalent inshore during the summer season of 1982/1983, but the work of Barlow (1982) has shown the dominant species of samples collected from October - March 1978/1979 to be the diatom

Skeletonema costatum, which the style extract of C. meridionalis is unable to digest. In other words, not only was bacteriolysis limited during the anomalous summer, but the phytoplankton species present may have been indigestible to the mussel, leaving only macrophyte debris to complete the carbon and nitrogen budget.

It is suggested here that the above constitutes a possible explanation for the thin mussels found this season. As a future project, it would be interesting to see how the mussels fare after the 1983 winter season, and whether or not this effect has spread further up the food chain.

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Artificial Sea Water

NaCl	} dry at 125°C, cool in a desiccator	250 g
Na ₂ SO ₄		50 g
KCl		10
A.		
NaCl	} dissolve in 7 l	205,1 g
KCl		5,8 g
MgCl ₂ ·6H ₂ O		92,8 g
CaCl ₂ ·2H ₂ O		13 g or 9,8 g anhydrous
B.		
Na ₂ SO ₄	} dissolve in 2,58 l	34,4 g
C.		
NaHCO ₃	} dissolve in 1l. Filter through 0,2 μm	16,8 g
Na ₂ HPO ₄		0,014 g
For 1 litre seawater	A	730 ml
	B	260 ml
	C	10 ml
	} autoclave	

Dinitrosalicylate Reagent

1 g of 3,5 dinitrosalicylic acid was made up in 20 ml 2N NaOH together with 30 g sodium potassium tartrate (Rochele salt) in distilled water, the solution being made up to 100 ml with distilled water. It was necessary to heat the solution while stirring to insure dissolution of the reagents.

EDTA 50 mM stock

0,1861 g 10 ml⁻¹

Folin-Lowry Protein Determination

Reagents

- A. 2% Na_2CO_3 in 0,1 M NaOH
- B. 1% CuSO_4
- C. 2% Na tartrate
- D. equal volumes B and C (1:1)
- E. 38 ml A and 2 ml D (freshly made up)
- F. Folin -Ciocalteau diluted 1:1 with distilled water

Calibration

For purposes of calibration, a stock solution of BSA was diluted to a final concentration of $0,4 \mu\text{g } \mu\text{l}^{-1}$. Volumes of 0, 50, 100, 150, 200, 300, 400, and 500 μl of the $0,4 \mu\text{g BSA } \mu\text{l}^{-1}$ solution was used in constructing the standard curve.

Method

To a 0,5 ml volume of sample (standard), add 2 ml E, and stand for 10 minutes. After exactly 10 minutes, add 200 μl of solution F, stand for a further 10 - 20 minutes, and read at 700 nM on the spectrophotometer.

Gelatin-Page Buffers and Solutions

Acrylamide-bis-acrylamide (30:1) Stock (A-bis A)

acrylamide	30 g
N,N ¹ -methylene-bis-acrylamide	1 g

Make up volume to 100 ml with distilled water. Add 5 g active charcoal and stir for 8 h, filter through Whatman's paper No. 1.

Catalysts

N N N'N' tetramethylene-ethylene diamine (TEMED)

Use undiluted.

Ammonium persulphate (10% w/v) in distilled water.

Gelatin

Dissolve 1 g of gelatin in c 80 ml distilled water by boiling, allow to cool and adjust volume to 100 ml.

Incubation buffer

Glycine (0,1 M)	15,01 g
distilled water	1000 ml

Adjust the pH to 9,0 with NaOH

Running Gel Buffer (RGB)

Tris (1,5 M)	18,17 g
SDS (10% w/v) in distilled water	4,0 ml
distilled water	96,0 ml

Adjust the pH to 8,8 with concentrated HCl

Reservoir buffer

Tris (0,025 M)	6,06 g
Glycine (0,192 M)	28,82 g
SDS (10% w/v) in distilled water	20 ml
distilled water	1980 ml

Adjust the pH to 8,5.

Stacking Gel Buffer (SGB)

Tris (0,5 M)	6,057 g
SDS (10% w/v) in distilled water	4,0 ml
distilled water	96,0 ml

adjust the pH to 6,8

Stain and Destaining Solutions

Stain:

Amido black (1% w/v) in distilled water	10 ml
destain solution	100 ml

Destain:

acetic acid	10 ml
methanol	30 ml
distilled water	60 ml

Triton X-100

Triton X-100	2,5 ml
distilled water	97,5 ml

Preparation of 10% Gelatin-gel (sufficient for 2 plates)

Solutions	Running Gel	Stacking gel
A-bis A	30 ml	0,40 ml
RGB	22,5 ml	
SGB		0,50 ml
Gelatin	9,0 ml	
distilled water	28,5 ml	3,10 ml
ammonium persulphate	0,2 ml	0,2 ml
TEMED	0,1 ml	0,1 ml

Minimal medium (using artificial seawater) (see p.

25 μg Ats N. ml^{-1}

KNO_3	} in 2 l artificial seawater	361 mg
Mannitol		50 mg

Nelson-Somogyi

1. Reagents

The substrate was 4,8 ml of prewarmed (37°C) 0,1% carboxymethyl cellulose in 20 mM phosphate buffer pH 7,0 with 150 mM NaCl.

Solution A of the Somogyi reagent consisted of 24 g Na_2CO_3 , 16 g NaHCO_3 , 12 g potassium sodium tartrate, and 144 g Na_2SO_4 in 800 ml distilled water. The reagent was boiled and sealed in a brown bottle.

Solution B of the Somogyi reagent consisted of 4 g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and 36 g Na_2SO_4 in 200 ml distilled water.

Before use, Somogyi reagents A and B were mixed in a ratio of 4:1.

The Nelson reagent consisted of 25 g $(\text{NH}_4)_6\text{MO}_7\text{O}_{24} \cdot 4 \cdot 4\text{H}_2\text{O}$ in 450 ml distilled water. The Nelson reagent was kept at 37°C for 24 hours and sealed in a brown bottle. NB: The Nelson reagent is very poisonous.

Page Buffers and Solutions

Acrylamide-bis-acrylamide stock

acrylamide	29,2 g
N,N'-methylene-bis-acrylamide	0,8 g
Make up volume to 100 ml with distilled water	

Catalysts

Refer to catalysts for gelatin-PAGE

RGB

Tris (1,5 M)	18,17 g
SDS	0,4 g
distilled water	100 ml

Adjust the pH to 8,8 with concentrated HCl

Reservoir Buffer

Tris (0,067 M)	8,13 g
Glycine (0,238 M)	35,75 g
SDS	2,5 g
distilled water	2000 l

SGB

Tris (0,5 M)	6,057 g
SDS	0,4 g
distilled water	100 ml

Adjust the pH to 6,8

Stain and Destain Solutions

Stain:

Coomassie blue	0,05 g
destain solution	100 ml

Destain:

acetic acid	270 ml
distilled water	2430 ml
propan-2-ol	900 ml

Preparation of 10% Acrylamide Gels (sufficient for 2 plates).

Solutions	Running Gel	Stacking Gel
A-bis A	12 ml	2 ml
RGB	8,2 ml	-
SGB	-	3 ml
distilled water	13,65 ml	7 ml
ammonium persulphate	160 μ l	64 μ l
TEMED	18 μ l	13 μ l

Phosphate buffer pH 7,0 with 150 mM NaCl

NaCl		43,88 g
Na ₂ HPO ₄	in 3 ℓ distilled water	10,50 g
	5 litres	
KH ₂ PO ₄	in 2 ℓ distilled water	5,44 g

Potassium periodate in 0,125 N H₂SO₄ (fresh)

H ₂ SO ₄	0,125 N	3,375 ml ℓ ⁻¹
kIO ₄	(in 10 ml H ₂ SO ₄)	0,0575 g

APPENDIX B

CHEMICALS

- Alginate - Alginic acid from kelp - Type III - Sigma, Saint Louis, Missouri.
- Arabinose - BDH England.
- Bovine Serum Albumin - fraction V - Miles
- Erythritol - Hopkin and Williams, Essex, England.
- Fucose - BDH, England.
- Galactose - BDH, England.
- Glucose - Merck.
- Growth Hormone - State vaccine Institute, Cape Town, South Africa.
- Human Albumin - Western Province Blood Transfusion Service, Cape Town, South Africa.
- Human Growth Hormone - State Vaccine Institute, Cape Town, South Africa.
- Laminarin - Koch-Light Laboratory, England.
- Mannitol - Merck.
- Mannose - Sigma, Saint Louis, Missouri.
- Myoglobin - Sigma, Saint Louis, Missouri.
- Ovalbumin - Miles.
- Peptone - Difco laboratories, Detroit, Michigan.
- Rhamnose - Merck.
- Transferrin - Sigma, Saint Louis, Missouri.
- Trypsin - bovine pancreas type III - Sigma, Saint Louis, Missouri.
- Trypsin inhibitor - Soybean type IS - Sigma, Saint Louis, Missouri.
- Xylose - Merck.
- Yeast extract - Difco Laboratories, Detroit, Michigan.

APPENDIX C
SUPPORTING PAPERS

ADJUSTMENT OF THE ACTIVITY OF α -AMYLASE EXTRACTED
FROM THE STYLE OF THE BLACK MUSSEL
CHOROMYTILUS MERIDIONALIS (Krauss) IN RESPONSE TO
THERMAL ACCLIMATION

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Abstract: The mussel *Choromytilus meridionalis* (Krauss) is a common inhabitant of the intertidal zone on the west coast of the Cape Peninsula, South Africa, and experiences temperatures of between 8 °C when immersed by the tide and at least 25 °C on exposure to air. The activity of α -amylase extracted from the crystalline style of freshly-collected mussels has a low temperature coefficient of ≈ 1.12 over much of the temperature range experienced in the natural environment. Warm acclimation results in an increase in the α -amylase activity, despite the fact that individual rate: temperature curves for extracts from mussels acclimated to 8, 15 and 22 °C have rather low temperature coefficients of 1.14-1.17 between 10 and 20 °C. The increase of activity of the α -amylase following warm acclimation may form an integral part of the improved filtration, ingestion and assimilation which is necessary to offset increased metabolic losses during the warm conditions of the summer months.

INTRODUCTION

The fact that the filtration rate of suspension-feeding bivalves generally increases with environmental temperature and thus compensates for the increased metabolic losses which are likely to occur during the summer months is now well-established, and represents one way in which such organisms are able to maintain an energetic gain from the environment (Newell, 1979; Newell & Branch, 1979). Most studies on the effects of temperature on intact molluscs, for example, have shown that filtration rate increases with exposure temperature up to an optimum, after which a relatively sharp decline occurs (Winter, 1978). The net gain from the environment by such organisms is, however, a reflection not only of a balance between filtration rate and metabolic cost or losses associated with the feeding process, but is also governed to a large extent by ingestion and assimilation efficiency. Winter (1969, 1970) has, for example, shown that in *Arctica islandica* and *Modiolus modiolus* the assimilated ration increases over the temperature range 4-20 °C and that this reflects changes not only in the filtration rate but also an increase in assimilation efficiency over this temperature range.

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For an increase in assimilated ration to occur in response to an increase in environmental temperature, both digestion and assimilation rates must thus be linked to feeding and ingestion in an integrated fashion. In the absence of a compensatory increase in digestion and assimilation, an increase in filtration and ingestion would lead merely to increased faecal losses. Rather surprisingly, there have been very few studies on the influence of environmental temperature on the activity of digestive enzymes in bivalves although Owen & Wiggs (1971), and Hofer *et al.* (1975) have studied the effect of thermal acclimation on some aspects of the digestive enzymes of vertebrate ectotherms. A good deal is now known, however, of the nature and function of digestive enzymes in bivalves (Bayne, 1976), some of which may show a clearly-defined cycle of extracellular activity which is linked with feeding rhythms associated with the ebb and flow of the tide (Morton, 1970, 1971; Langton & Gabbott, 1974; Langton, 1977).

We have used the techniques developed in such studies to investigate the effects of warm acclimation on the activity of α -amylase extracted from the crystalline style of the mussel *Choromytilus meridionalis* (Krauss) which is common on the shores of the Cape Peninsula, South Africa. The results suggest that the activity of the α -amylase can indeed be increased following warm acclimation of the mussels and that digestive activity may thus be linked with increased filtration and ingestion during the summer months.

MATERIAL AND METHODS

PREPARATION AND MATERIAL

Specimens of *Choromytilus meridionalis* of mean shell length 60 mm were collected from Bloubergstrand in Table Bay on the west coast of the Cape Peninsula, South Africa, from a situation where they encountered temperatures of from 8 °C during immersion to as much as 25 °C during exposure to air at low water. The mussels were transported to the laboratory and groups were placed in shallow tanks of aerated sea water held at 8, 15 and 22 °C for 16 days. The animals were fed twice per day with a culture of *Dunaliella* at a concentration of $\approx 4 \times 10^6$ cells·animal⁻¹·day⁻¹ which is equivalent to 0.448 mg dry wt of cells·animal⁻¹·day⁻¹. On the sixteenth day the mussels were removed from the tanks prior to feeding and the crystalline styles removed. Groups of four styles were then rinsed in distilled water and homogenized in a glass tissue grinder in 12 ml of 20 mM phosphate buffer, pH 7.0 containing 6.7 mM NaCl. Langton & Gabbott (1974) apparently homogenized in distilled water, but this led to an inactivation of α -amylase activity in our preparations, possibly due to the high pH of 8.5 or an absence of NaCl. A pH optimum of between 6.5 and 7.0 appears to be common for α -amylase activity in the style of bivalves (Mathers, 1974; Langton, 1977) and a suspension medium of pH 7.0 was used in all our experiments. The homogenates were centrifuged for 2 min at 15 000 g and the supernatant used for determination of α -amylase activity.

PROTEIN DETERMINATION

Total protein in the style extract was determined by the ultraviolet spectrophotometric method of Groves *et al.* (1968) which was also used by Langton & Gabbott (1974). A 200 μl sample of the supernatant was diluted to a final volume of 5 ml with 20 mM phosphate buffer, pH 7.0 containing 150 mM NaCl and scanned at 230 nm (1 cm light path) with a Beckman Model 25 spectrophotometer using bovine serum albumin at concentrations between 0.05 and 0.6 $\text{mg}\cdot\text{ml}^{-1}$ as standards. This method was found to yield a linear relationship between 5 and 350 $\mu\text{g}\cdot\text{ml}^{-1}$ ($\hat{Y} = -0.035 + 1.0205X$; $r^2 = 0.98$).

α -AMYLASE ACTIVITY

The α -amylase activity of the crystalline style preparations was determined by the method of Bernfeld (1955) and was expressed in terms of mg glucose liberated in 8 min by 1 ml enzyme preparation at each particular experimental temperature.

Substrate solution

Soluble starch was made up in 20 mM phosphate buffer, pH 6.9 containing 6.7 mM NaCl. The glucose liberation was then plotted as a function of starch concentration between 1 and 8 $\text{g}\cdot 100\text{ ml}^{-1}$ buffer and increased linearly up to 6.6 $\text{g}\cdot 100\text{ ml}^{-1}$ after which no further increase occurred. All subsequent assays at various exposure temperatures were then carried out at saturating substrate concentrations of 7 $\text{g}\cdot 100\text{ ml}^{-1}$ buffer, since it seems likely that crystalline style activity occurs primarily during phases of feeding when concentrations of substrate in the stomach are high (Langton & Gabbott, 1974; Langton, 1977).

Dinitrosalicylate reagent

1 g of 3,5-dinitrosalicylic acid was made up in 20 ml 2 N NaOH together with 30 g sodium potassium tartrate in distilled water, the solution being made up to 100 ml with distilled water.

Procedure

Each 1 ml sample of crystalline style extract was incubated in a shaking bath for 8 min with 1 ml substrate solution at one of a variety of experimental temperatures between 2 and 40 $^{\circ}\text{C}$, a fresh subsample being used for each incubation temperature. Up to seven replicates were used to calculate the mean activity at each incubation temperature. The reaction was halted after 8 min by the addition of 2 ml dinitrosalicylate reagent and the tube then heated for 5 min in a boiling water bath and cooled in running tapwater. After the addition of 20 ml distilled water, the absorbance of the solution containing the brown reduction product was determined spectrophotometrically at 540 nm. A blank solution without style extract was used as a control

and the corresponding glucose units calculated from a calibration curve established for glucose concentrations of 1.5 – 6 mg·ml⁻¹ ($\hat{Y} = -0.409 + 0.325X$; $r^2 = 0.96$). Enzyme activity was then expressed per mg protein in the extract.

RESULTS

THE EFFECT OF TEMPERATURE ON ENZYME ACTIVITY IN FRESHLY-COLLECTED ANIMALS

Specimens of *Choromytilus meridionalis* were collected from the shore and the α -amylase activity of extracts from groups of four crystalline styles was measured following incubation at temperatures between 5 and 35 °C. The results are shown in Fig. 1 and are summarized with other data for acclimated animals in Table I. The

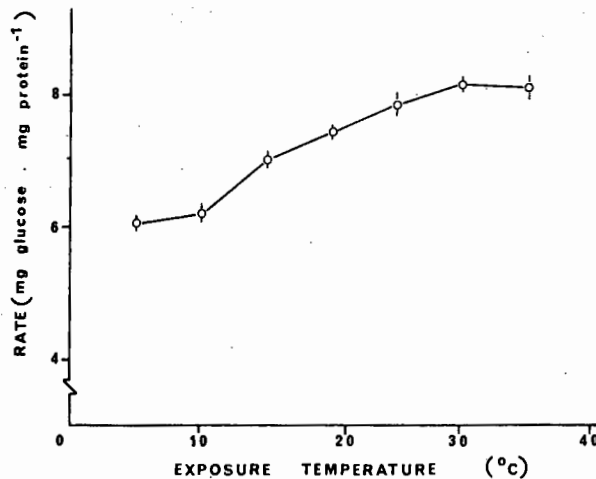


Fig. 1. Rate-temperature curve for α -amylase activity from the style of freshly-collected specimens of *Choromytilus meridionalis*. The vertical bars represent one standard error of the mean.

rate-temperature curve for α -amylase activity has evidently rather a low temperature coefficient over the temperature range studied, and is considerably less than that commonly reported for filtration and ingestion in bivalves. The Q_{10} between 10 and 20 °C, for example, is only 1.21 for α -amylase activity, whereas the value commonly exceeds 2.0 over this temperature range for filtration (for review, see Winter, 1978).

Enzyme activity in this bivalve is thus likely to proceed at a relatively uniform rate despite the major variations in temperature which the mussel experiences with the ebb and flow of the tide. There is likely to be some form of compensatory increase in α -amylase activity, however, in response to long-term seasonal changes in

TABLE I

Rates of α -amylase activity at different exposure and acclimation temperatures ($^{\circ}\text{C}$), expressed as mg glucose \cdot mg protein $^{-1}$ after 8 min incubation: \bar{Y} , mean of values; s.e., one standard error of the mean; N , number of readings.

Acclimation temperature	Exposure temperature											
	2	5	10	15	20	25	30	33	35	36	40	
Freshly collected	\bar{Y}	—	6.05	6.17	6.93	7.50	7.72	8.08	—	8.02	—	—
	s.e.	—	0.18	0.19	0.11	0.11	0.17	0.09	—	0.18	—	—
	N	—	4	4	4	4	4	4	—	4	—	—
8 $^{\circ}$	\bar{Y}	4.91	6.11	6.26	6.73	7.23	7.81	7.84	—	7.47	—	7.06
	s.e.	0	0.43	0.39	0.37	0.31	0.50	0.50	—	0.52	—	0.40
	N	1	5	5	6	5	5	6	—	6	—	5
15 $^{\circ}$	\bar{Y}	6.79	6.99	7.13	7.77	8.37	9.39	9.52	—	9.84	—	9.50
	s.e.	0.29	0.16	0.20	0.10	0.17	0.23	0.34	—	0.31	—	0.22
	N	2	7	5	7	5	5	7	—	7	—	5
22 $^{\circ}$	\bar{Y}	8.12	8.32	7.80	8.96	8.92	10.30	10.44	10.84	10.91	11.10	10.24
	s.e.	0	0.23	0.24	0.19	0.30	0.39	0.44	0	0.51	0	0.47
	N	1	7	6	7	6	6	6	1	6	1	6

environmental temperature if an increase of assimilated ration is to be linked with the increase of filtration and ingestion which is to be anticipated following warm acclimation.

THE EFFECT OF THERMAL ACCLIMATION ON ENZYME ACTIVITY

The rate-temperature curves for α -amylase activity in extracts of groups of four crystalline styles from *Choromytilus* which had been acclimated for 16 days at 8, 15 and 22 $^{\circ}\text{C}$ are shown in Fig. 2. The data are summarized together with those for freshly-collected mussels in Table I.

Each curve, like that obtained from the styles of freshly-collected animals, has a rather low temperature coefficient being between 1.14 and 1.17 over the temperature range 10-20 $^{\circ}\text{C}$ for all three acclimation groups. The level of the rate-temperature curves is, however, raised following warm acclimation and thus reflects an increase in the activity of the α -amylase from the crystalline style. The results of two-way analysis of variance are summarized in Table II which shows that variance between acclimation temperatures and between experimental temperatures was significantly greater than the residual variance (at $P < 0.001$, $F = 14.88$ for acclimation temperatures and $F = 52.12$ for exposure temperatures).

It is thus clear that whereas the shallow form of the rate-temperature curve for α -amylase activity in response to exposure temperature would preclude a major increase in digestion and assimilation following an increase in environmental

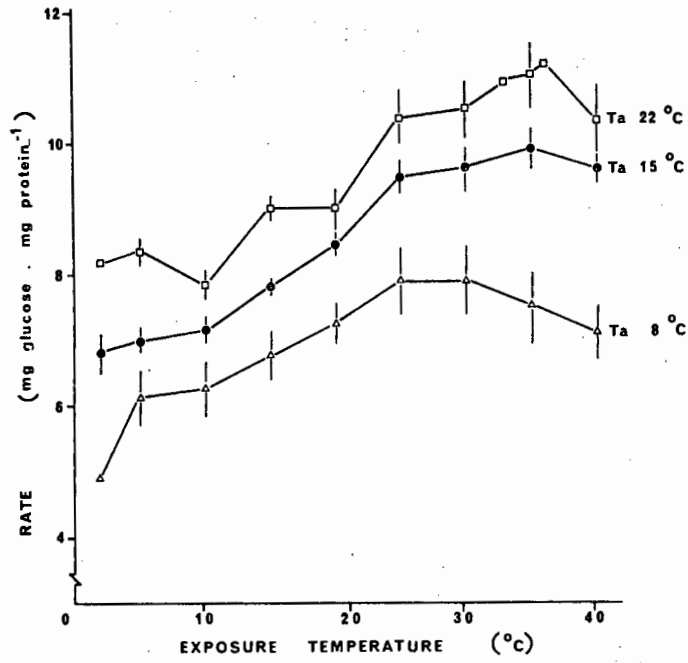


Fig. 2. Rate-temperature curves for α -amylase activity of style extracts from acclimated specimens of *Choromytilus meridionalis*. The acclimation temperature (T_a) is indicated. The vertical bars represent one standard error of the mean.

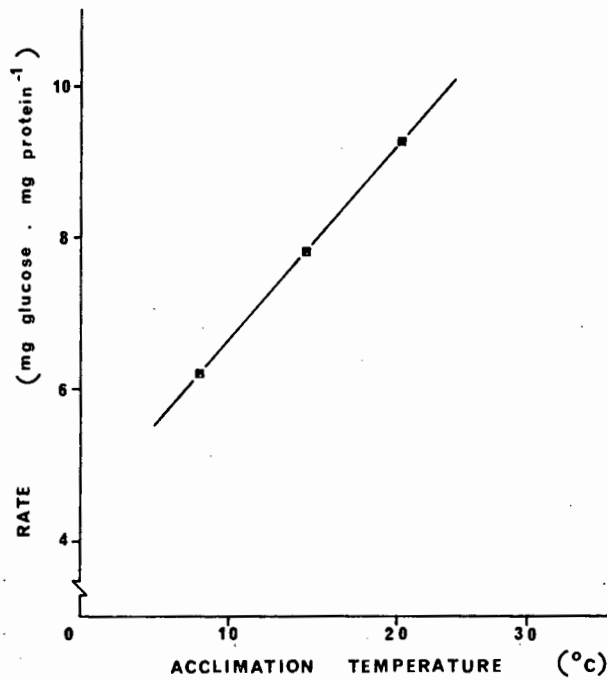


Fig. 3. Acclimated rate-temperature curve for the α -amylase activity of style extracts from *Choromytilus meridionalis*, with data points at the acclimation temperatures of 8, 15 and 22°C, respectively.

temperature during the summer months, this may be accomplished by an increase in the activity of the style enzyme. The increase in α -amylase activity is plotted as a

TABLE II

A two-way analysis of variance performed between acclimation temperatures and between exposure temperatures.

Source of variance	Sum of squares	Degrees of freedom	Mean squares
Between acclimation temperatures	115.96	19	6.10
Between exposure temperatures	64.10	3	21.37
Residual	23.57	57	0.41
Total	203.63	79	—

function of acclimation temperature in Fig. 3 and shows an increase which approaches that commonly reported for the filtration and ingestion rate in bivalves following warm acclimation (see Bayne, 1976; Winter, 1978; Newell & Branch, 1979).

CONCLUSION

The results presented above show that the activity of the α -amylase extracted from the crystalline style of the intertidal mussel, *Choromytilus meridionalis*, is increased following warm acclimation, despite the fact that the individual rate-temperature curves have a rather low temperature coefficient. Whether this is achieved by an increase of enzyme concentration in the style, by an increase in enzyme specific activity, or by the occurrence of enzyme variants or isozymes of α -amylase is at present unknown. Isozymes of amylases are, however, apparently well-known in some mammals, including man (Burdett *et al.*, 1976) and it is possible that they occur in bivalves. The increase in thermal optimum of the curves shown in Fig. 2 for α -amylase activity of animals acclimated to 15 and 22 °C compared with that for mussels acclimated to 8 °C suggests, for example, that a multiple form of α -amylase with different thermal optima may be induced in the styles of mussels following warm acclimation, much as occurs in some regulatory metabolic enzymes of ectotherms (see Hochachka & Somero, 1973; Somero & Hochachka, 1976). The increase of activity of the α -amylase following warm acclimation may then form an integral part of the improved filtration, ingestion and assimilation which is necessary to offset increased metabolic losses, and thus to maintain an energetic gain from the environment, during the warm conditions of the summer months.

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ROCK-LOBSTERS AND MUSSELS – LIMITATIONS AND PREFERENCES IN A PREDATOR-PREY INTERACTION

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Abstract: Rock-lobsters, *Jasus lalandii* (Milne Edwards), are the dominant predators in kelp-beds off the west coast of South Africa and feed mainly on mussels, two species of which (*Aulacomya ater* (Molina) and *Choromytilus meridionalis* (Kr.)) occur in the area. Rock-lobsters are limited in the maximum (critical) length of mussel they can consume, but can take larger *C. meridionalis* than *Aulacomya ater*. This is partially a function of the greater shell strength of *A. ater* although shell shape also appears to be important in limiting prey size. Measurements of attachment strength, shell failure load and flesh energy content are used to estimate yield per unit effort for different size prey of the two species. Larger individuals appear to provide a better yield, while *Choromytilus meridionalis* should be preferred up to 50 mm shell length and *Aulacomya ater* thereafter. Rock-lobsters in fact prefer small mussels – probably because they can be rapidly cracked. In choice experiments, more than twice as many *Choromytilus meridionalis* as *Aulacomya ater* are taken in these size classes – this may restrict the distribution of the former in rock-lobster grounds. Selection experiments show that although individual rock-lobsters feed on a wide size range of mussels the mean size of prey taken is an increasing function of rock-lobster length. There is a notable reluctance to take prey approaching the critical length. Feeding rates are high and are little affected by feeding regime. The intense predation pressure exerted on juvenile *A. ater* probably results in a bimodal size distribution typical of natural populations and may lead to situations in which rock-lobster productivity is severely restricted even in the presence of high standing crops of prey.

INTRODUCTION

Rock-lobsters, *Jasus lalandii* are the dominant benthic predators associated with the kelp-beds off the west coast of South Africa (Field *et al.*, 1977, in press; Velimirov *et al.*, 1977) and support an important commercial fishery. Examination of the stomach contents has shown their diet to consist mainly of mussels although sea-urchins, algae, polychaetes, and crustaceans (especially other rock-lobsters) are also taken (Heydorn, 1969; Newman & Pollock, 1974; Pollock, 1978, 1979). A number of studies have considered the relationship between density of rock-lobsters and prey availability, on the assumption that the amount of prey present is a key factor determining the productivity of the fishery. The most convenient index of prey availability is standing crop and this has been measured by Newman & Pollock (1974), Field *et al.* (1977, in press), Velimirov *et al.* (1977), and Pollock (1978, 1979).

The fact that individuals within a prey population are seldom equally liable to predation is, however, well established (Shelbourne, 1962; Ebling *et al.*, 1964; Walne & Dean, 1971; Paine, 1976; Smale, 1978; Zipser & Vermeij, 1978). Although it is recognized that similar restrictions influence the rock-lobster-mussel interaction

(Pollock, 1979) the factors determining the availability and selection of prey have not been adequately quantified.

Two species of mussel, the ribbed mussel *Aulacomya ater* (Molina) and the black mussel *Choromytilus meridionalis* (Kr.), are abundant in southern African kelp-beds. Rock-lobster grounds are usually dominated by *Aulacomya ater*, while *Choromytilus meridionalis*, although found on unsilted rocky areas and on buoys, pilings, and ropes, is generally more common on flat sandy areas, which tend to be avoided by rock-lobsters.

In this study an attempt is made to determine some of the factors limiting or influencing prey selection by rock-lobsters and to examine the effects they may have on both the structure of the prey population and predator productivity.

METHODS AND RESULTS

CRITICAL LENGTH OF PREY

In order to determine the maximum size of prey available to *Jasus lalandii* (Milne Edwards), eight pairs of size-matched rock-lobsters were held at 15 °C in separate aquarium tanks connected to a circulating sea-water system. After 24 h small mussels of known length were introduced into each tank and as these were consumed they were replaced by progressively larger individuals. This process was continued until 7 days elapsed without further mortality of mussels. The largest prey taken to this point was assumed to represent the critical prey-length for the rock-lobsters concerned.

The results of two such experiments, one using *Choromytilus meridionalis* as prey

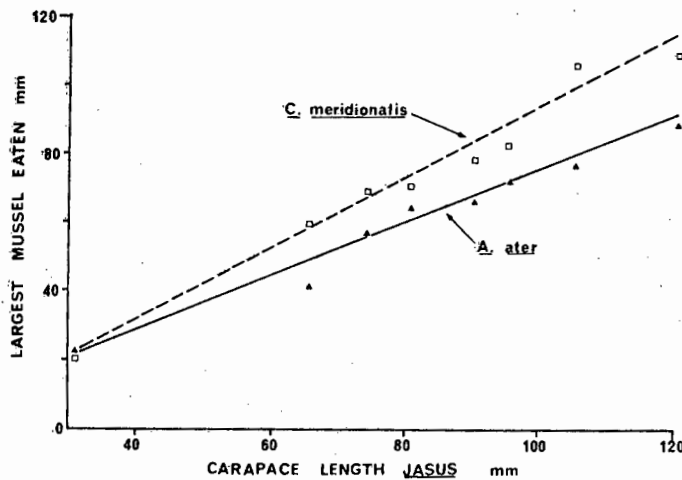


Fig. 1. Maximum sizes of mussels broken by *Jasus lalandii* of various carapace lengths: see text for regression equations.

and the other *Aulacomya ater* are given in Fig. 1. Regression lines fitted to the data give: (critical length, mm) = $-11.41 + 1.04$ (carapace length, mm); $r^2 = 0.95$, $n = 8$, for the former; and (critical length, mm) = $-4.44 + 0.79$ (carapace length, mm); $r^2 = 0.94$, $n = 8$, for the latter.

These results clearly show that rock-lobsters can break larger *Choromytilus meridionalis* than *Aulacomya ater*. Examination of broken shells showed that *Choromytilus meridionalis* were consistently fractured postero-dorsally. In *Aulacomya ater*, the point of breakage was more variable – individuals approaching the critical length being broken at the anterior apex, while smaller ones were taken at almost any point except along the ventral margin. These observations led us to investigate the rôle of shell strength in determining site of attack and critical length.

EFFECTS OF SHELL STRENGTH

Failure loads of mussel shells were measured with a Hounsfield Tensometer Compression Tester. As the geometry of crushing surfaces could affect fracture strength, rock-lobster mandibles were themselves adapted for use as crushing surfaces (*Jasus lalandii* does not have chelipeds, but crushes shells between its mandibles). This was done by mounting the mandibles in cylindrical plugs of Pratley's Quickset Epoxy Putty and inserting these into holes drilled in rubber bungs held by the tensometer. This system was used because it provided "give" and allowed the mandibles to adopt a natural posture relative to the shells as pressure was applied.

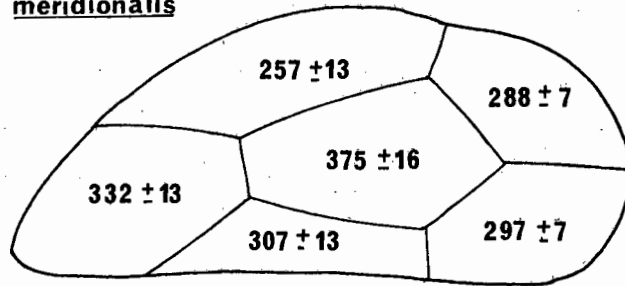
The first failure load experiment measured the relative strengths of different parts of the shells to see if these correlated with the breakage patterns. Six areas were tested on *Choromytilus meridionalis* shells, but only four on *Aulacomya ater* shells, for which less material was available. Thirty shells of 60 ± 2 mm in length were broken at the centre of each test area. The results (Fig. 2) indicate that *Choromytilus meridionalis* is significantly weaker in the postero-dorsal region (one-way analysis of variance, $P < 0.05$). The different areas of *Aulacomya ater* do not differ significantly ($P = >0.05$) although the lowest reading was for the postero-ventral region.

In *Choromytilus meridionalis* the weakest area thus corresponds with that most frequently attacked (see above). In *Aulacomya ater*, however, there are definite breakage patterns in the absence of significant variations in shell strength. These are probably determined by shell shape. Small *A. ater* are of fairly regular shape and can thus be broken at any point along the margin, but as they grow the shells flatten ventrally and this area becomes difficult to grip. In large individuals only the umbo remains narrow enough to be inserted between the mandibles and breakages are limited to this area.

On this basis the ultimate factor determining critical length would appear to be the strength of the shell postero-dorsally in *Choromytilus meridionalis* and in the umbonal region in *Aulacomya ater* and accordingly the relationship between critical length and shell strength at these points was investigated (Fig. 3). Power curves fitted

to these data give: (failure load, Newtons) = 0.38 (shell length, mm)^{1.62}; $r^2 = 0.72$, $n = 110$ for *Choromytilus meridionalis*; and (failure load, Newtons) = 1.77 (shell length, mm)^{1.33}; $r^2 = 0.73$, $n = 93$ for *Aulacomya ater*.

C. meridionalis



A. ater

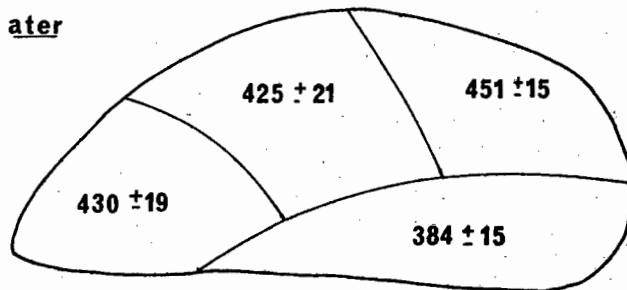


Fig. 2. Means and standard errors of force (Newtons) required to fracture various areas on the shells of *Aulacomya ater* and *Choromytilus meridionalis* of the size class $60 \text{ mm} \pm 2 \text{ mm}$: each figure represents the mean of 30 readings.

The failure loads of *A. ater* are considerably higher than those of *Choromytilus meridionalis* throughout the size range tested, although *C. meridionalis* shells show a proportionally greater increase in strength with shell length. The first of these findings agrees with the results obtained in Fig. 1, but from the second we might expect the critical length of *C. meridionalis* to have approached that of *Aulacomya ater* in the larger size classes, whereas the two lines in fact diverge.

We feel that this disparity again results from the dual effects of strength and shape, which together determine prey availability. The shape component of such a relationship is unfortunately difficult to quantify, since both absolute width and curvature affect the ability of rock-lobsters to grip and crush shells (large shells used in our feeding experiments in fact often had radiating marginal abrasions apparently caused by slippage during crushing attempts).

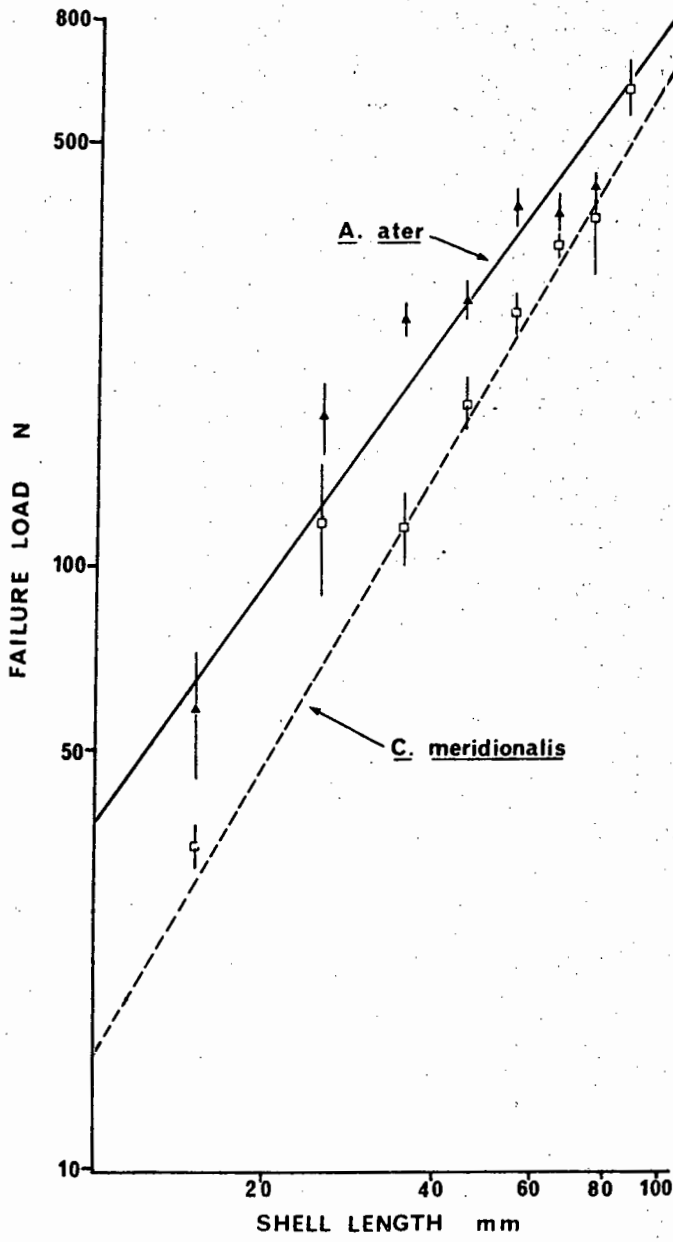


Fig. 3. Failure loads (Newtons) of mussel shells at their points of most frequent attack by rock-lobsters: each point the mean and standard error of readings within a 10-mm size class; see text for regression equations.

STRENGTH OF BYSSAL ATTACHMENT

Although shell strength is the most obvious factor restricting prey availability, rock-lobsters are normally faced with mussels attached to the substratum and hence strength of byssal attachment must also influence prey vulnerability.

The behaviour of rock-lobsters feeding on attached mussels is described by Heydorn (1969) and Pollock (1978, 1979). Heydorn states that attached mussels are manoeuvred by the anterior pereopods to incline the broad (posterior) end upwards. Small portions of shell margin are then bitten away to create an opening large enough for the first pereopods to be inserted and the shell valves pulled apart (neither the size nor species of mussel attacked in this way is stated). Pollock's observations distinguish between large and small *A. ater*. Large individuals are manoeuvred so that the narrow (anterior) end is brought between the mandibles and so must be removed from the substratum before this can be done. Small animals, which can be cracked at any point, may be consumed while attached or after being pulled free. These reports suggest that, although small mussels may be taken while attached, byssal strength influences selection of larger prey and may in fact help determine prey availability.

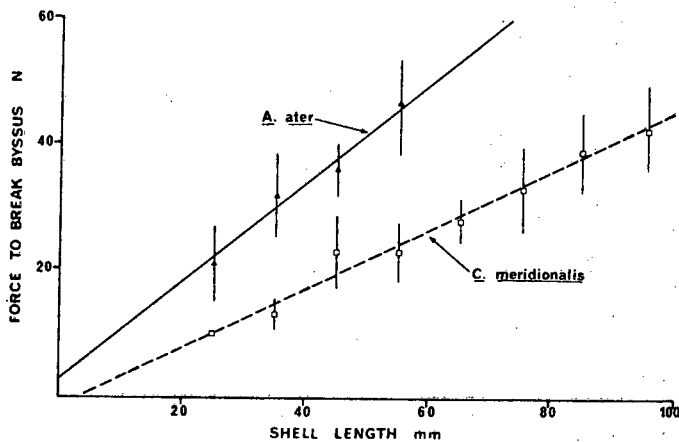


Fig. 4. Force required to break the byssal attachment of mussels of various shell lengths: mean and standard error are shown for readings within each 10-mm size class.

Byssal strength, taken as the vertical pull required to break the byssus, was thus measured, using a Salter Pocket Balance and a retort clamp to grip the mussel shells. Byssal strengths, measured in kg, have been converted to Newtons (Newtons = kg \times 9.81) and are illustrated in Fig. 4. Byssal strengths of *A. ater* were found to be consistently higher than those of *Choromytilus meridionalis* and to increase more rapidly with shell length. Regression equations relating byssal strength to shell length are: (force, Newtons) = $-1.79 + 0.47$ (shell length, mm); $r^2 = 0.98$, $n = 90$, for *C. meri-*

dionalis; and (force, Newtons) = $2.15 + 0.79$ (shell length, mm); $r^2 = 0.97$, $n = 109$ for *Aulacomya ater*.

THEORETICAL BASIS FOR PREY SELECTION

It is evident from the above results that considerably greater force is required to crush and to detach an *A. ater* than a *Choromytilus meridionalis* of equal length. The desirability of the two species and of different-sized individuals within each species may be related to the yield obtained per unit effort so expended. An appropriate way to assess yield is as energy content of the body (shell and byssus excluded). This was calculated by collecting a size range of each species, determining energy values of flesh samples by ballistic bomb calorimetry and multiplying these by dry flesh mass (3 days at 60 °C). The relationships between shell length and energy content obtained were: (flesh, kJ) = 0.00020 (shell length, mm)^{2.68}; $r^2 = 0.99$, $n = 10$ for *C. meridionalis*; and (flesh, kJ) = 0.00037 (shell length, mm)^{3.33}; $r^2 = 0.98$, $n = 10$ for *Aulacomya ater*.

The more rapid increase in body energy in *A. ater* is the result of a relatively greater increase in shell width and hence flesh mass (energy values of flesh are similar at 22.2 kJg⁻¹ in *A. ater* and 19.8 kJg⁻¹ in *Choromytilus meridionalis*).

A number of difficulties are involved in estimating the effort required to secure the prey – principally because prey of different sizes may be attacked in a variety of

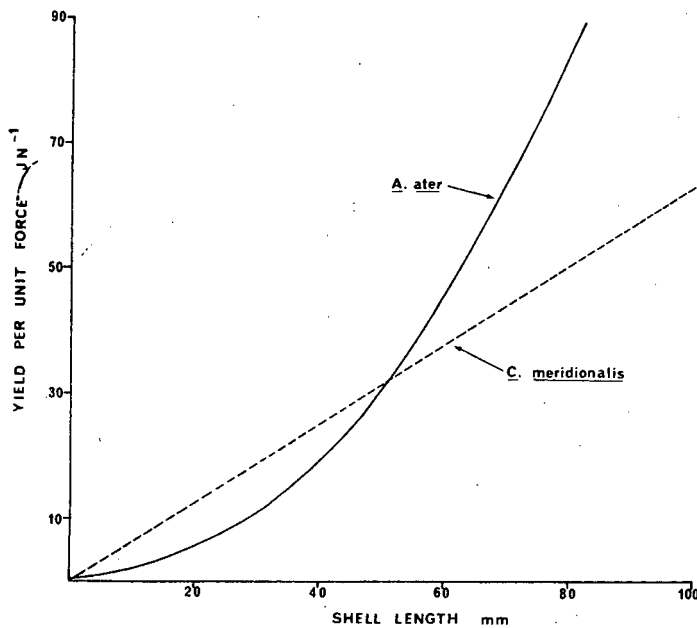


Fig. 5. Calculated yield (J) per unit force (N) required to break byssal attachment and fracture shell, as a function of shell length of mussel.

ways (Pollock 1978, 1979; Zipser & Vermeij, 1978) and considerable effort may be expended in unsuccessful attempts to dislodge or open larger individuals. For simplicity we have taken effort to be the force needed to break the byssal attachment plus the load required for compression failure of the shell. Relationships between yield per unit effort calculated on this basis and shell length are shown in Fig. 5.

The results indicate that both species provide a better yield per unit effort as shell length increases. Up to some 50 mm shell length *C. meridionalis* appears preferable to *Aulacomya ater*, but beyond this point *A. ater* becomes more profitable (a result of its rapidly increasing flesh mass). This theoretical analysis was tested by presenting individual rock-lobsters with a choice of *A. ater* and *Choromytilus meridionalis* (five individuals of each species in each 5-mm size class, 5–60 mm shell length) and recording the numbers and sizes of prey consumed daily over four days.

Of seven rock-lobsters tested only one consumed more *Aulacomya ater* than *Choromytilus meridionalis*. On Day 1 (when a full size range of both species was available) a total of 65 of the latter and 31 of the former (2.07:1) were taken. Smaller size classes tended to be selected first, although there was a noticeable trend to take larger *C. meridionalis* than *Aulacomya ater*. As a result the ratio of mussel flesh consumed was 2.35 kJ *Choromytilus* to 1 kJ *Aulacomya*. Despite a progressively increasing proportion of *A. ater* remaining in the tanks and depletion of the most favoured size classes of *Choromytilus meridionalis* the bias towards the latter was still evident after 4 days, when 1.33 *C. meridionalis* had been eaten for each *Aulacomya ater*. The fact that 1.5 times as many *A. ater* as *Choromytilus meridionalis* survived this brief experiment demonstrates the potential of *Jasus lalandii* to control prey-species composition in the natural environment.

SELECTION OF PREY WITHIN A SINGLE SPECIES

From the above it appears that rock-lobsters show a preference for prey well below the critical size and only extend their selection once these size classes become depleted. In order to illustrate this more clearly the experiment was repeated offering only *Aulacomya ater* (five per 5-mm size class, 5–60 mm). Fig. 6 gives the feeding patterns of four rock-lobsters of differing sizes maintained in this way for 7 days. It can be seen that the preferred size of prey is an increasing function of rock-lobster size, is not highly specific, and is always well below the critical length. As availability of the preferred size-classes diminishes individuals in adjoining size classes, both above and below, are taken. There is a notable reluctance to take mussels approaching the critical size while smaller individuals remain available.

Perhaps a more realistic situation, and one in which the size preferences of rock-lobsters may be more clearly demonstrated, is when the range of available prey remains constant. This may be simulated by replacing the mussels eaten each day with others of the same length class. Length-frequency distributions of prey taken by four rock-lobsters maintained in this way for 10 days are given in Fig. 7. Most of the

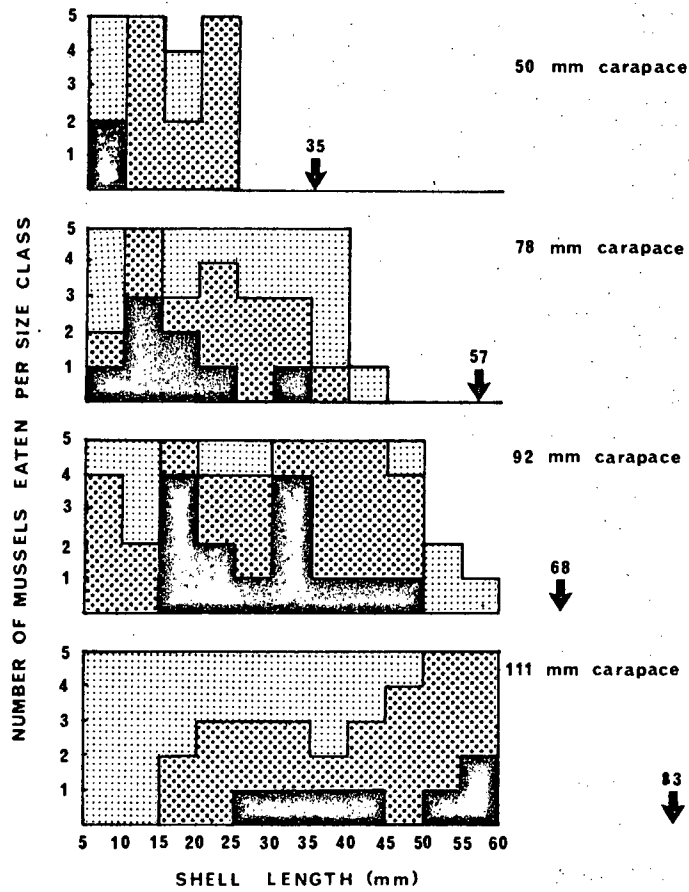


Fig. 6. Numbers and lengths of *Aulacomya ater* consumed by rock-lobsters of various sizes after 1 (black), 3 (dark stipple) and 7 (light stipple) days: each rock-lobster was initially offered five individuals each of each size class 5–60 mm; arrows indicate critical length (calculated from Fig. 1) for the rock-lobster concerned.

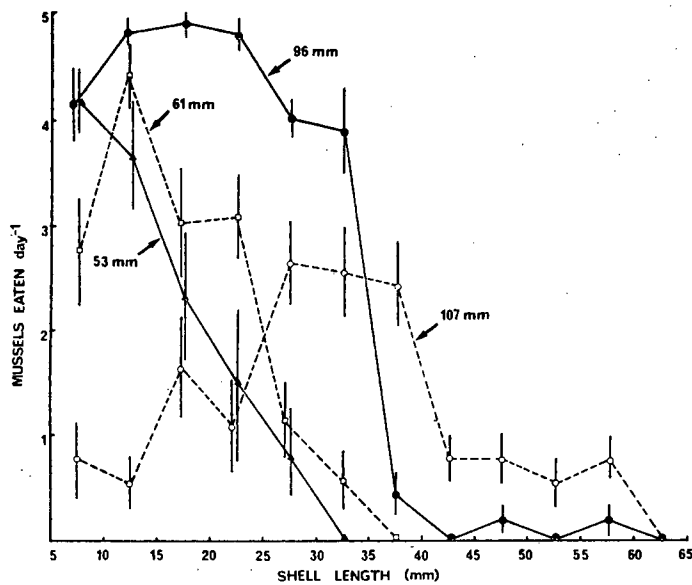


Fig. 7. Length-frequency distribution of *Aulacomya ater* eaten daily by rock-lobsters of various sizes: each reading represents the mean and standard error of 10 readings; consumed prey were replaced daily.

prey taken are of less than half the critical length. Each rock-lobster shows clear size preferences but takes some prey over a wide range of sizes. Because there is an approximate ten-fold increase in energy content with each doubling of mussel length the small number of larger prey taken account for most of the energy consumed.

FEEDING RATES

Feeding rates, in terms of kJ eaten per rock-lobster per day, may be calculated from the results on pp. 102 and 103 and are shown in Fig. 8. The results indicate that

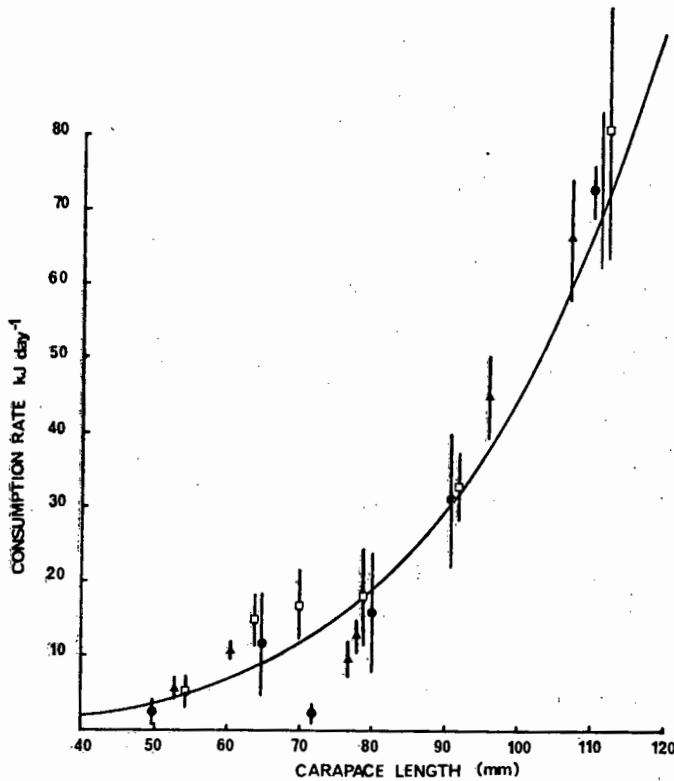


Fig. 8. Daily consumption rates of rock-lobsters of various lengths under the three different feeding regimes described on p. 102: □, choice between *Aulacomya ater* and *Choromytilus meridionalis*; ●, *A. ater* without replacement of prey consumed; ▲, *A. ater* with replacement of prey consumed.

remarkably regular ingestion rates are maintained despite changes in the species and size composition of available prey. Calorific intake is related to carapace length by the equation: (consumption, kJ day^{-1}) = $0.0000014 (\text{carapace length, mm})^{3.75}$; $r^2 = 0.78$, $n = 19$.

DISCUSSION

Our results indicate that *Choromytilus meridionalis* are considerably easier to detach and to crack than *Aulacomya ater* of equivalent size and are preferentially selected as prey when they are available. These findings parallel those of Ebling *et al.* (1964), who showed that *Nucella* from a sheltered site were more at risk to *Portunus* than stronger-shelled individuals from exposed sites. Similarly Landenberger (1968) has shown that *Pisaster* tend to prey on *Mytilus edulis* in preference to *M. californianus*, which have relatively larger adductor muscles, and are hence presumably more difficult to pull open.

It is interesting to consider whether such preferential prey selection significantly affects the distribution patterns of the two mussel species; only one of which (*Aulacomya ater*) normally dominates on rock-lobster grounds. Profound effects of predators on prey distribution patterns have been demonstrated by Paine (1974), who showed that the extension of *Mytilus californianus* into the habitat of *Pisaster* is prevented by predatory activity. The impressive ability of shore crabs to decimate mussel beds has also been described by Dare & Edwards (1976), while rapid obliteration of *Aulacomya ater* beds by *Jasus lalandii* has been observed by Pollock (1978) and by the authors. In view of such observations and the strong preference for *Choromytilus meridionalis* shown by *Jasus lalandii* it is our opinion that these voracious and abundant predators may well restrict *Choromytilus meridionalis* populations on rock-lobster grounds, particularly those where there are high densities of rock-lobsters. The effect of rock-lobsters upon individual prey populations is strongly influenced by the fact that they are strictly limited in the maximum size of mussel which they can open (see Fig. 1).

If the size composition of predator and prey populations are known we can calculate the proportion of prey available to different size classes of predator. Such size class distributions are available for Robben Island (Pollock, 1979) and are adapted and reproduced here (Figs 9, 10). Integrating these data with the critical length equations the proportions, by numbers and biomass, of the *Aulacomya ater* population available to rock-lobsters of various lengths have been calculated and are shown in Table I. These figures demonstrate that, although numerically most *A. ater* are potentially available to even the smallest rock-lobsters, a large proportion of the biomass remains inaccessible. A rock-lobster of the median size (75 mm carapace length), can in fact only feed upon 22% of the available biomass, while only 2% of rock-lobsters can crack the largest mussels in the population. This has the effect of concentrating predatory pressure on the smaller size classes in the mussel population.

Analysis of yield per unit force required to secure the prey (see Fig. 5) suggests that the optimal behaviour of rock-lobsters would be to consume prey as close as possible to the maximum size. Choice experiments, however, indicate a strong preference for prey well below the critical length (see Figs 6, 7). This disparity is

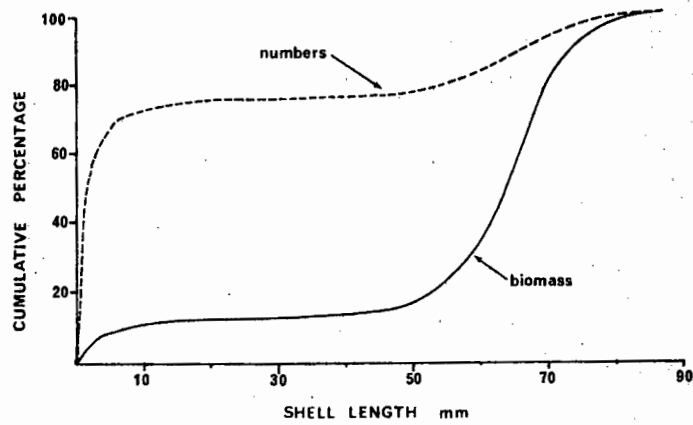


Fig. 9. Cumulative size class distribution of *Aulacomya ater* at Robben Island, by numbers and by biomass: derived from Pollock (1979).

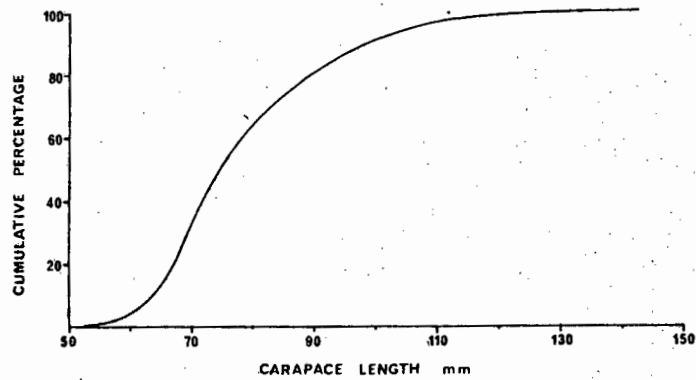


Fig. 10. Cumulative size-frequency distribution of *Jasus lalandii* at Robben Island: data derived from Pollock (1979).

TABLE I

Percentages of *Aulacomya ater* populations available to *Jasus lalandii* of various carapace lengths at Robben Island.

<i>J. lalandii</i>		<i>A. ater</i>		
Carapace length (mm)	Cumulative % population by numbers	Critical length (mm)	% available numbers	% available biomass
60	3	43.0	77	14
70	31	50.9	78	17
80	64	58.8	82	30
90	80	66.7	90	64
100	91	74.6	98	92
110	97	82.5	99	97
120	98	90.4	100	100

probably accounted for by the fact that our calculations were based on the assumption that prey can be broken by a single application of force, whereas prolonged effort is probably required to deal with prey approaching the critical size. Such a hypothesis is supported by the observations of Zipser & Vermeij (1978) on the crushing behaviour of crabs and by the more quantitative analysis of Elner & Hughes (1978). In the latter paper the time taken for a crab (*Carcinus*) to break open a mussel (*Mytilus edulis*) is shown to rise asymptotically as prey size approaches the critical length. Under such conditions prey in large size categories become sub-optimal and the behaviour pattern becomes one of taking much smaller prey – perhaps at a point approximating to the maximum which can be broken with a single application of force.

The net result of this selection process is to further concentrate predatory pressure such that almost all the rock-lobsters in the population show a preference for *Aulacomya ater* in the size range 5–45 mm (Fig. 7). The ensuing decline in these size classes gives us the strikingly uniform bimodal size-distribution of *A. ater* populations (Pollock, 1979; Griffiths, unpubl.). A similar distribution occurs in *Modiolus modiolus*, another long-lived mussel, also under conditions of intense predation pressure from the crabs *Cancer* and *Carcinus* (Seed & Brown, 1975).

The implications of rock-lobster predation on survival of *Aulacomya ater* populations are probably limited; indeed predation may have a regulatory effect, holding down the density of juveniles, which otherwise might smother the adults (cf. Berry, 1978). Reproductive output is largely a function of the density of large mussels (Griffiths & King, 1979) and is hence relatively unaffected by juvenile mortality. Replenishment of adult stocks is assured by the small proportion of juveniles which always survive between adults or in other safe habitats.

Rock-lobster production, on the other hand, may be severely restricted by scarcity of mussels in the available size classes. Pollock (1979), for example, reports a mean rock-lobster density of 0.81 individuals m^{-2} at Robben Island, where the standing stock of mussels is 5 kg (whole wet mass). As shell accounts for 65% of the mussel biomass this represents 1.75 kg m^{-2} or 5420 kJ m^{-2} of mussel flesh. The median size of the rock-lobsters is 75 mm carapace length, the optimal feeding rate at this size being 14.85 kJ day^{-1} (Fig. 8) or 4748 kJ yr^{-1} , if 1.5 months is allowed for non-feeding during moults (Pollock, 1978). The energy value of mussels in the area available to each rock-lobster (1.23 m^{-2}), at 6691 kJ, might appear to meet these demands, until it is recalled that only 22% of that biomass lies below the critical length (55 mm). The energy available is thus only 1472 kJ or 31% of the food eaten in the laboratory. Constraints such as these probably affect the majority of rock-lobsters and account for the correlation between rock-lobster growth rates and mussel biomass reported by Newman & Pollock (1974). Food restrictions are, moreover, probably most severe for smaller rock-lobsters, whose already limited food resources are competed for by larger individuals, which have recourse to larger prey in times of shortage. As only the largest rock-lobsters are capable of exploiting the

bulk of the mussel biomass these size classes should not be overfished if the conversion of mussel flesh into lobster production is to be maximized.

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ROCK-LOBSTERS, MUSSELS AND MAN: A MATHEMATICAL MODEL

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ABSTRACT

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The palinurid rock-lobster *Jasus lalandii* (*H. Milne-Edwards*), which forms the basis of an important South African fishery, feeds mainly on the mussel *Aulacomya ater* (*Molina*). Natural populations of *A. ater* exhibit a bimodal size frequency distribution, believed to be caused by intense predation pressure upon medium-sized mussels. This may lead to situations in which rock-lobster productivity is severely restricted by food availability even in the presence of a large standing crop of prey. The present rock-lobster harvesting strategy is based on a minimum size limit; however, by not catching small rock-lobsters, an imbalance may be created in the lobster size distribution, resulting in an overabundance of small rock-lobsters which have insufficient food to grow fast. The need to predict an economically and biologically optimal harvesting strategy provided motivation for the present model, in which an alternative harvesting strategy, involving decrease of the minimum size limit, was compared with the present strategy. It appears that the mussel biomass could be increased by 87% and the rock-lobster yield by 19%. Simultaneously, the rock-lobster density and egg production are seen to decrease by 24 and 35%, respectively. The model assumes that rock-lobster populations have constant recruitment; therefore, the effect of the decline in fecundity cannot be predicted. Therefore, any major policy change should be delayed until the larval biology of *J. lalandii* has been researched more fully.

INTRODUCTION

The palinurid rock-lobster *Jasus lalandii* (*H. Milne-Edwards*) forms the basis of an important and lucrative fishery on the South African west coast (Pollock, 1978). Extensive research has been directed towards the biology and population dynamics of both the rock-lobsters (Gilchrist, 1913; Von Bonde and Marchand, 1935; Heydorn, 1965, 1966; Pollock, 1973, 1978;

Newman and Pollock, 1974a, b) and their primary prey, the mussel *Aulacomya ater* (Molina) (Griffiths and King, 1979a, b), as well as the interaction between the two (Pollock, 1979; Griffiths and Seiderer, 1980), with a view to understanding the dynamics of the fishery.

Jasus lalandii is the most abundant benthic carnivore found in the kelp beds off the west coast of southern Africa (Field et al., 1977; Velimirov et al., 1977), feeding mainly upon the ribbed mussel *A. ater* which comprise the largest component of the sessile benthic fauna (Pollock, 1979). In addition, sea urchins *Parechinus angulosus*, crabs *Plagusia chabrus*, kelps *Ecklonia maxima* and *Laminaria pallida*, polychaete worms and crustaceans (particularly other rock-lobsters) are preyed on to a lesser extent (Heydorn, 1969; Newman and Pollock, 1974b; Pollock, 1978, 1979).

The present harvesting policy operates on the basis that minimum size limits will ensure a stock of rock-lobsters for harvesting in subsequent seasons. Since most of the mussel biomass is in the form of very large mussels (Pollock, 1979), which only the largest rock-lobsters can consume (Griffiths and Seiderer, 1980), the fact that small rock-lobsters are not caught could create imbalances in the size compositions of both populations. This could take the form of excess large mussels which are not preyed upon, and excess small rock-lobsters which do not have sufficient food to grow at an optimal rate. Therefore, the growth of rock-lobsters could be food-limited even in areas where mussel biomass is comparatively large (Pollock, 1979). This suggests that harvesting more rock-lobsters from the middle size classes might not be detrimental to the population. However, if too many were removed from the middle size class, too few might be left to grow into the larger size classes.

This paper describes a mathematical model relating mussel and rock-lobster population dynamics, developed to predict a biologically and economically optimal harvesting strategy.

METHODS

Mussel population structure

The mussel population structure is taken from Pollock (1979). It is interesting to compare mussel frequency per size class with mussel biomass per size class (Fig. 1). It can be seen that over 80% of the mussel biomass is composed of mussels larger than 60 mm in length, while numerically most of the population is less than 10 mm in length.

The growth rate of the ribbed mussel is slow, and is described by the Gompertz curve

$$s(t) = AB^C \quad (1)$$

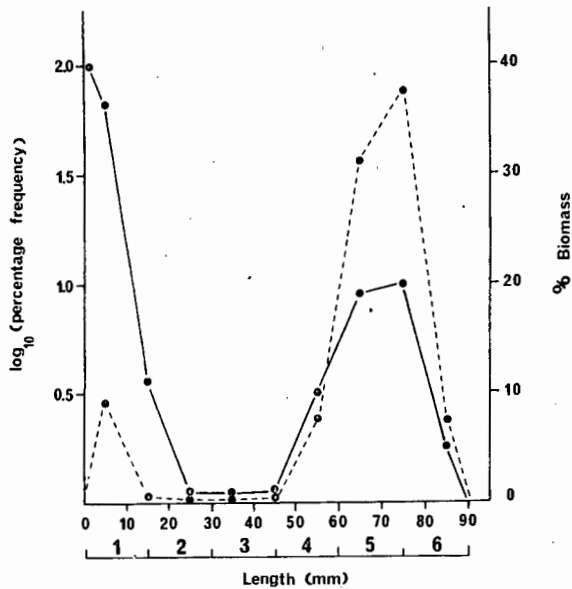


Fig. 1. *Aulacomya ater* length frequency distribution (●—●) and percent biomass per size class (●---●) of populations west of Robben Island (from Pollock, 1979): numbers 1–6 in bold type indicate the relevant size classes.

where $s(t)$ is length (mm) and t is time (years).

The coefficients A , B and C were found by the method of least squares to be 90, 0.000184 and 0.604, respectively. The standard deviations σ associated with these regression coefficients are 1.13, 0.000022 and 0.009, respectively. The size at settlement, $s(0)$, has been calculated to be 0.017 mm, and the maximum size $s(\infty)$ as 90 mm (Griffiths and King, 1979b).

The numbers of mussels in each size class are denoted by M_1, M_2, \dots, M_6 , the standard values of which are given in Table I. The size classes have an interval of 15 mm and sizes range from 0 to 90 mm shell length.

TABLE I

Standard values of variables M_i , LF_j and LM_j where M_i is the number of mussels per hectare in each 15-mm size class (starting at 1 mm), and LF_j and LM_j are the numbers of female and male rock-lobsters, respectively, per hectare, in each 20 mm size class (starting at 40 mm)

Variable	Size class					
	1	2	3	4	5	6
M_i	331×10^6	17×10^6	6.9×10^6	5.8×10^6	16×10^6	79×10^6
LF_j	2066	1790	555	0	0	0
LM_j	1489	1116	668	203	103	40

The first mussel size class is assumed to have a constant quarterly input of recruits, which does not depend on the parent population size. This is thought to be a valid assumption, since so many mussels are spawned by each individual that even if the population nears zero in all size classes, the few remaining will be able to maintain the input. Mussels are lost from each size class in three ways: (1) growth into the next class; (2) natural mortality; (3) rock-lobster predation.

In the absence of rock-lobster predation, the following equations model the mussel population:

$$\begin{aligned} dM_1/dt &= A_1 - B_1M_1 - C_1M_1 \\ dM_i/dt &= A_iM_{i-1} - B_iM_i - C_iM_i \quad (i = 2, 3, \dots, 6) \end{aligned} \quad (2)$$

where A_1 is the constant recruitment, A_i ($i = 2, 3, \dots, 6$) is the proportion of the previous class which grow into the i th class, B_i is the proportion of the i th class which grow out, and C_i is the proportion which die naturally. Clearly, $A_i = B_{i-1}$ ($i = 2, \dots, 6$).

The ribbed mussel can be divided anatomically into shell, flesh and byssus, with rock-lobsters consuming the flesh portion only. The energies (kJ) yielded by mussels of increasing sizes, taken from Griffiths and King (1979b), are given in Table II. These figures have been used in the model to estimate the optimal growth rate of rock-lobsters when the supply of mussels is known.

Rock-lobster population structure

Rock-lobster growth rate and size frequency distribution data were obtained from observations made off the coast of Robben Island, South Africa—a rock-lobster sanctuary relatively undisturbed by commercial harvesting (Pollock, 1979). The area is fringed with kelp beds and the sessile fauna is dominated by ribbed mussels.

The size frequency distributions of male and female rock-lobsters given by Pollock (1978) are shown in Fig. 2. The low frequencies in the smaller size

TABLE II
Energy yield (kJ) per mussel for mussels of increasing size

	Size-class midpoint (mm)					
	7.5	22.5	37.5	52.5	67.5	82.5
kJ available as food	0.03	1.37	7.62	23.71	55.30	108.90

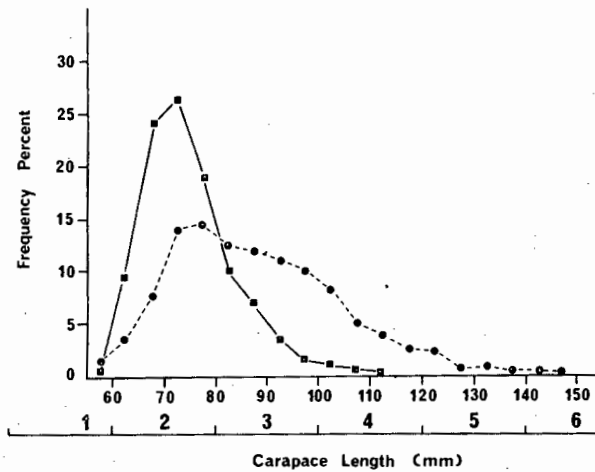


Fig. 2. *Jasus lalandii* length frequency distribution of male (●-----●) and female (■——■) populations west of Robben Island (from Pollock, 1978): numbers 1–6 in bold type indicate the relevant size classes.

classes reflect the scarcity of juveniles in the areas sampled. Pollock (1979) has suggested that recruitment of juveniles into the adult population takes place by migration from shallow to deeper waters. Although Von Bonde and Marchand (1935) stated that “At various times the entire crawfish population of a heavily populated area will go “on trek” and appear again in some other area where previously very few or no individuals were to be found”, migration has not been built into the model.

Rock-lobsters are capable of opening and eating mussels up to a certain maximum (or critical) length, depending upon their own size and strength (Pollock et al., 1979; Griffiths and Seiderer, 1980).

TABLE III

Mean numbers of mussels eaten per day by one rock-lobster (from Griffiths and Seiderer, 1980)

Mussel size-class midpoint (mm)	Rock-lobster size-class midpoint (mm)					
	50	70	90	110	130	150
7.5	5.6	5.8	7.2	1.0	0.8	0.4
22.5	3.7	5.6	9.6	4.1	2.4	1.6
37.5	0	0.2	0.8	2.4	4.0	4.0
52.5	0	0	0.2	1.0	2.0	2.8
67.5	0	0	0	0	0.2	0.4
82.5	0	0	0	0	0.1	0.4

The numbers of rock-lobsters in each size class are denoted by LM_1, LM_2, \dots, LM_6 for males, and LF_1, LF_2, \dots, LF_4 for females, the standard values of which are given in Table I. The size classes have an interval of 20 mm, and sizes range from 40 to 160 mm carapace length. The following relationship has been shown to exist between predator size and maximum prey length (Griffiths and Seiderer, 1980):

$$\text{critical length (mm)} = -4.44 + 0.79 (\text{carapace length (mm)})$$

However, experiments have shown that mussels of length considerably less than the critical are chosen as prey. The resulting bias towards prey of moderate size (5–35 mm) is believed to result in the distinctive bimodal distribution of mussel size observed in Fig. 1.

The mean numbers of mussels in various size classes eaten per day by rock-lobsters of different lengths can be seen in Table III, which is derived from Griffiths and Seiderer (1980). The numbers obtained in the laboratory were 20% higher than the numbers used in the model, but we suggest that the laboratory results overestimated the natural feeding rate by some 20% because the food was so readily available. These numbers are seen to satisfy the daily energy requirements of rock-lobsters. The kilojoule requirement per day is given (Griffiths and Seiderer, 1980) by

$$\text{kJ per day} = 0.0000014 \times (\text{carapace length})^{3.75}$$

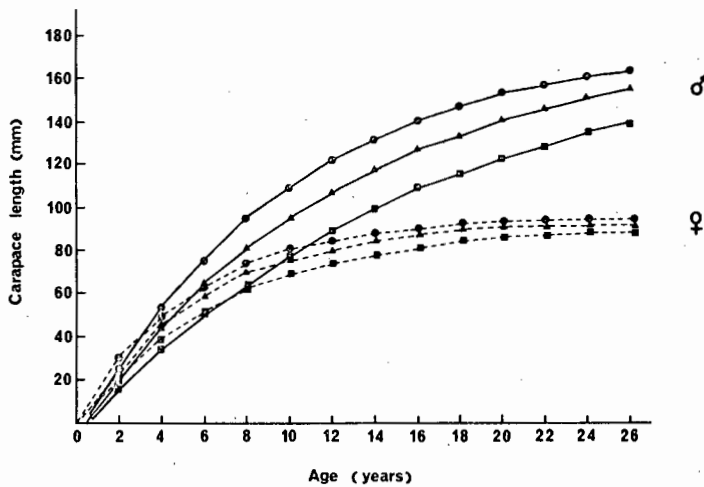


Fig. 3. Effects of varying Von Bertalanffy k value on Von Bertalanffy growth curves: *males*: ●—●, $k = 0.10$; ▲—▲, $k = 0.08$; ■—■, $k = 0.06$; *females*: ●- - -●, $k = 0.235$; ▲- - -▲, $k = 0.196$; ■- - -■, $k = 0.157$.

Pollock (1978) found that under near optimal feeding conditions in the wild, rock-lobsters grow according to the Von Bertalanffy relation

$$l(t) = l_{\infty}(1 - e^{-k(t-t_0)}) \quad (3)$$

where $l(t)$ is the carapace length at time t . The values of l_{∞} and k are calculated by regression analysis to be 177.9 mm ($\sigma = 1.32$) and 0.08 ($\sigma = 0.0014$) respectively for males, and 87.6 mm ($\sigma = 1.20$) and 0.196 ($\sigma = 0.018$) respectively for females, when time is measured in years. The male l_{∞} value is supported by Gilchrist (1913), who reported having measured male rock-lobsters of carapace length in excess of 178 mm. Different growth rates have been observed in different areas by Newman and Pollock (1977), with male Von Bertalanffy k values ranging between 0.06 and 0.10. Figure 3 shows the effect of a change in k values on the Von Bertalanffy growth curve. The growth curve for male rock-lobsters off Robben Island has a k value of 0.103 (Pollock, 1978). This is thought to be relatively high when compared with neighbouring fishing grounds (D.E. Pollock, personal communication, 1981). For this reason, an optimal k value of 0.08 for males was chosen and used in the model standard run.

Decreasing growth rate can be modelled by decreasing k , but the same maximum length will be reached eventually. Hence the rock-lobster growth rate may be made to depend on the food supply by decreasing k according to the lack of food, or according to

$$k = k_{\text{optimal}}(\text{kJ consumed/kJ required for optimal growth})$$

if food is scarce.

To calculate k , suppose that each mussel from the i th class yields K_i kJ, and that a rock-lobster from the j th size class eats optimally a total of T_j mussels ($T =$ mussels taken). The value of R_{ij} gives the proportion of mussels eaten from the i th mussel class. The total number E_i of i th class mussels eaten optimally by a j th class rock-lobster will be given by

$$E_i = R_{ij}T_j$$

which will yield $K_i R_{ij} T_j$ kJ of energy. If the total number of mussels required, E_i , exceeds the standing stock M_i , the yield from the i th mussel class is adjusted equally for all classes of rock-lobster by the ratio M_i/E_i . Thus the total energy consumed by a j th class rock-lobster is given by

$$AkJ_j = \begin{cases} \sum_{i=1}^6 K_i R_{ij} T_j M_i / E_i & (M_i < E_i) \\ \sum_{i=1}^6 K_i R_{ij} T_j & (M_i \geq E_i) \end{cases}$$

where AkJ_j is equivalent to the actual kilojoules consumed. In addition, AkJ_j is modified by a constant failure-to-eat factor F which represents the probability that a rock-lobster will open a mussel, having found one.

The factor k in eq. 3 is then computed for a j th class rock-lobster as

$$k = \begin{cases} k_{\text{optimal}}(AkJ_j/OkJ_j) & (M_i < E_i) \\ k_{\text{optimal}} & (M_i \geq E_i) \end{cases}$$

where OkJ_j is the energy required to maintain optimal growth for a j th class rock-lobster. The growth rates for each size class of rock-lobster are then found as follows. From eq. 3,

$$dl/dt = l_{\infty} - l(t)$$

The growth rate D_j out of the j th size class may then be estimated as

$$D_j = (l_{\infty} - l_j)/20$$

where l_j is the class midpoint, and 20 mm is the width of each class. The value of k_{optimal} needed to compute l_j is found by a least-squares fit.

It should be noted that a total absence of mussels will give zero growth rates for the rock-lobsters. The assumption implicit here is that the rock-lobsters can eat alternative foods which maintain them at their current size but do not supply sufficient energy for growth.

Defining LM_1, LM_2, \dots, LM_6 and LF_1, LF_2, \dots, LF_4 as the numbers of male and female rock-lobsters in each size class, the population dynamics may be modelled as follows:

$$\begin{aligned} dLM_1/dt &= D\phi M - D_1LM_1 \\ dLM_j/dt &= D_{j-1}LM_{j-1} - D_jLM_j - \mu_jLM_j \quad (j = 2, 3, \dots, 6) \\ dLF_1/dt &= D\phi F - D_1LF_1 \\ dLF_j/dt &= D_{j-1}LF_{j-1} - D_jLF_j - \mu_jLF_j \quad (j = 2, \dots, 4) \end{aligned} \quad (4)$$

where $D\phi M$ and $D\phi F$ are the male and female recruitment rates, and μ_j the natural mortality rate of each size class.

NUMERICAL METHODS AND CALIBRATION

The coupled mussel-rock-lobster system of eqs. 2 and 4 is solved numerically using the Runge-Kutta-Merson algorithm, with a time step of one month, incorporated into the DRIVER system (Furniss, 1977) to facilitate the simulations. After integrating eqs. 2 over one month, mussels eaten by rock-lobsters are removed according to the above analysis. Equations 4 are then integrated over the same period, and any rock-lobsters harvested are

removed after the integration. The number removed is $H_j L_j$, where H_j is the harvesting rate.

The objective of the model is to approach population stability while maintaining biological realism. Calibration involves the tuning of the model parameters to give the best correspondence between the model output and observed data. This is particularly important for those parameters which are difficult to measure in Nature, such as natural mortality rates. After calibrating in this way, a sensitivity analysis was carried out to select key parameters to which the model is most sensitive. Parameter values were increased and decreased by 20%, and the resulting changes in specific variables determined.

ESTIMATION OF PARAMETERS

We stress at this point that a number of assumptions have been made to simplify the model and to minimise the number of parameters. The parameter values for the standard run are shown in Table IV. They were calculated as follows.

A_j : *quarterly recruitment rate of mussels into population.* Large irregular fluctuations in recruitment appear to be a common feature in mytilid species (Griffiths, 1976).

B_i ($i = 1, 2, \dots, 6$): *proportional mussel growth rate per month.* From eq. 1, $ds/dt = \ln(C)s \ln(s/A)$

Discretising the growth rate over a period Δt ($= 1$ month), in unit time a mussel will grow by

$$\Delta s = (ds/dt)\Delta t(\text{mm})$$

The proportion of the total number of mussels which grow out of the current size class i is given by

$$B_i = \left. \frac{ds}{dt} \right|_{s_i} \frac{\Delta t}{15}$$

assuming a uniform distribution of mussels within the size class. The class interval is 15 mm and ds/dt was evaluated at s_i , the midpoint of each class. Data were taken from: Griffiths and King (1979b) and Pollock (1978).

B_6 was increased by the addition of a natural mortality rate for large mussels of 0.02 per month. This value was estimated by calibrating the model until the number of large mussels did not exceed that observed in the absence of rock-lobsters.

C_i ($i = 1, 2, \dots, 6$): *natural mussel mortalities.* These were assumed to be independent of the size of the rock-lobster population and were set by calibrating the model.

TABLE IV
Parameter values for standard run

Parameter	Size class					
	1	2	3	4	5	6
A_i	150×10^6	0.0522	0.0872	0.0922	0.0794	0.0544
B_i	0.0522	0.0872	0.0922	0.0794	0.0544	0.03
C_i	0.05	0.01	0.01	0.005	0.01	0.01
Ok_j	198	348	1964	1898	3552	6075
T_j	278	346	533	254	284	288
R_{1j}	0.6034	0.5	0.4054	0.1132	0.0847	0.0417
R_{2j}	0.3966	0.4861	0.5405	0.4811	0.2542	0.1667
R_{3j}	0	0.0139	0.0450	0.2830	0.237	0.4167
R_{4j}	0	0	0.0090	0.1226	0.2119	0.2917
R_{5j}	0	0	0	0	0.0169	0.0417
R_{6j}	0	0	0	0	0.0085	0.0417
K_i	0.03	1.369	7.62	23.71	55.3	108.9
μ_j	0.1	0.1	0.1	0.05	0.1	0.2
H_j	0	0	0.021	0.008	0.004	0.001
LM_∞	177.9					
LF_∞	87.6					
$D\phi M$	300					
$D\phi F$	300					
K_{optimal} (males)	0.08					
K_{optimal} (females)	0.196					
F	0.5					

D_j ($j = 1, 2, \dots, 6$): proportional rock-lobster growth rate per month (variable—see previous section).

OkJ_j ($j = 1, 2, \dots, 6$): food intake required by a class j rock-lobster to maintain optimal growth. This is given by

$$kJ/\text{day} = 0.0000014 \times (\text{carapace length (mm)})^{3.75}$$

and was converted from a daily experimental result to a monthly requirement for use in the model (Griffiths and Seiderer, 1980).

T_j ($j = 1, 2, \dots, 6$): total number of mussels eaten per month by a class j rock-lobster. This was also converted from the daily requirements shown in Table III (data from Griffiths and Seiderer, 1980).

R_{ij} : proportion of T_j taken from the i th mussel class. This may be calculated from the entries in Table III. Note that

$$\sum_{i=1}^6 R_{ij} = 1$$

K_i : yield (kJ) of a class i mussel. This has been shown by Griffiths and Seiderer (1980) to be

$$K_i = 0.00037 \times (\text{shell length (mm)})^{3.33}$$

l_∞ and k_{optimal} were calculated separately for males and females from a regression analysis of the Von Bertalanffy curves (eq. 3).

$D\phi M$: monthly recruitment rate of male rock-lobsters into population.

$D\phi F$: monthly recruitment rate of female rock-lobsters into population.

F : failure to eat. This was a constant.

μ_j ($j = 1, 2, \dots, 6$): natural mortality rate of rock-lobsters. See Newman (1973).

H_j ($j = 1, 2, \dots, 6$): rate of harvesting in percent per hectare per month. The standard-run value of H_3 is 25% per year, corresponding approximately to an instantaneous fishing mortality rate of 30% (D.E. Pollock, personal communication, 1980).

RESULTS AND DISCUSSION

The analyses carried out on the model were of two types: firstly, a sensitivity analysis, whereby the sensitivity of the model to changes in parameters was defined; and secondly, a predictive analysis pertaining to management policies and their effects on the population size and structure.

The sensitivity analysis (Table V) shows that the key parameters A_1 , B_i , $D\phi M$, $D\phi F$ and μ_j are the most sensitive to change. In other words, the populations are most sensitive to changes in rock-lobster and mussel recruitment rates, mussel growth rate, and rock-lobster mortality.

TABLE V
Sensitivity analysis

Parameter input	Change	Variable output (expressed as % change from standard-run values)			
		Density			Yield
Parameter		Lobsters (female)	Lobsters (male)	Mussels	
Mussel recruitment A_1	-20%	+5117	+19897	-82	+79
	+20%	0	0	+57	0
Mussel growth rate B_i ($i = 1, 2, \dots, 6$)	-20%	+1694	+4344	-79	-60
	+20%	0	0	+4	0
Male lobster recruitment $D\phi M$	-20%	0	-20	+19	-12
	+20%	+59	+120	-22	-98
Female lobster recruitment $D\phi F$	-20%	-20	0	+21	-7
	+20%	+209	+236	-37	-98
Failure-to-eat constant F	-20%	+54	+65	-17	-97
	+20%	-9	-8	+12	+8
Male Von Bertalanffy growth factor k	-20%	0	+12	-7	-5
	+20%	0	-8	+5	+8
Female Von Bertalanffy growth factor k	-20%	+14	0	-8	-11
	+20%	-9	0	+7	+5
Lobster natural mortality μ_j ($j = 1, 2, \dots, 6$)	-20%	+160	+235	-35	-98
	+20%	-7	-7	+17	-16

TABLE VI
Computed standard-run values which give a steady state, compared with observed data

Variable	Compared standard value	Observed value	Source
Total lobster density (number ha^{-1})	8034	8100	Pollock (1979)
Total lobster yield (kg $\text{ha}^{-1} \text{yr}^{-1}$)	342	140	Pollock (personal communication, 1981)
Total mussel biomass (kg ha^{-1})	67000	50000	Pollock (1979)

Calibration of the model produced a "standard run", in which the variables in Table I give rise to an approximately steady state. The standard run represents the present harvesting strategy in which the harvesting rates in each rock-lobster size class (H_j) are estimated to be 0, 0, 25, 10, 5 and 1% per year, respectively. Optimal Von Bertalanffy growth values of $k = 0.08$ and $k = 0.196$ were used for male and female rock-lobsters, respectively. To validate the model, rock-lobster standing crop, rock-lobster harvested yield and mussel biomass were computed. These are compared with observed data in Table VI.

The standard run, incorporating the present harvesting strategy, was then compared with a possible "alternative" harvesting strategy, in which the minimum size limit was reduced from 90 to 70 mm carapace length. H_j were set at 0, 25, 20, 10, 5 and 1% per year, and the optimal values of k at 0.1 and 0.216 for males and females, respectively. This alternative strategy is an example of one possible use of the model for testing management options, and was chosen in accordance with the following rationale.

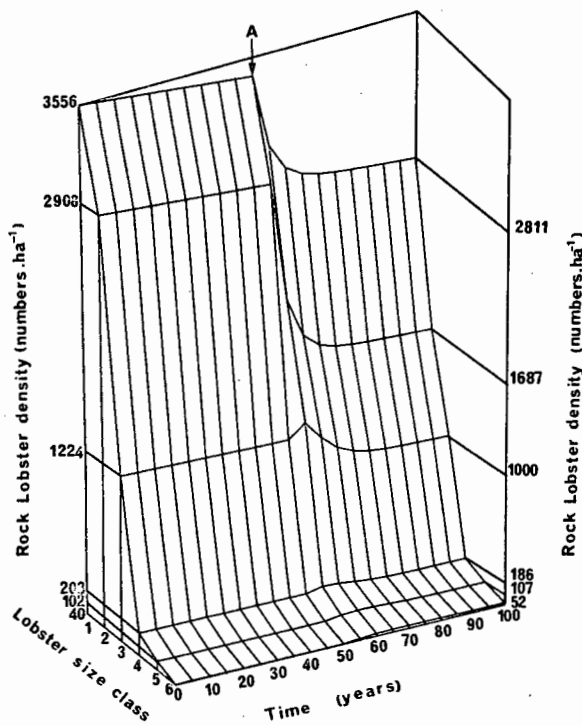


Fig. 4. Three-dimensional representation of effects of a change in harvesting policy, accompanied by corresponding change in growth rate of rock-lobsters, on population composition. Point A represents the switchover point. Axes: x_1 , time in years; x_2 , rock-lobster size classes (1-6); y , rock-lobster density (number ha^{-1}).

Newman and Pollock (1974b) have noted that the growth of rock-lobsters at Dassen Island was suboptimal in the natural state, and attributed this to lack of available food. Pollock (1979) found that 60% of the Robben Island rock-lobsters comprised individuals of carapace length smaller than 80 mm, which are restricted to feeding on mussels smaller than 60 mm in length (Griffiths and Seiderer, 1980). Figure 1 shows mussels of between 10 and 50 mm in length to be relatively scarce, suggesting that predation of this sector of the population is high. It has been hypothesised that an alternative harvesting strategy, in which smaller rock-lobsters are removed, would make more food available by reducing the predation pressure on the 10–50 mm mussels; this would enhance the growth of the remaining rock-lobsters.

Figure 4 clearly shows the predicted effect of changing to this alternative harvesting strategy (point A) on the size composition of the rock-lobster population. To illustrate the alternative policy, the model was run for 50 years with standard values, and at point A, the alternative strategy was introduced, linked with an increase in the Von Bertalanffy k value from 0.08 to 0.10 for males, and from 0.196 to 0.216 for females, to simulate the effect

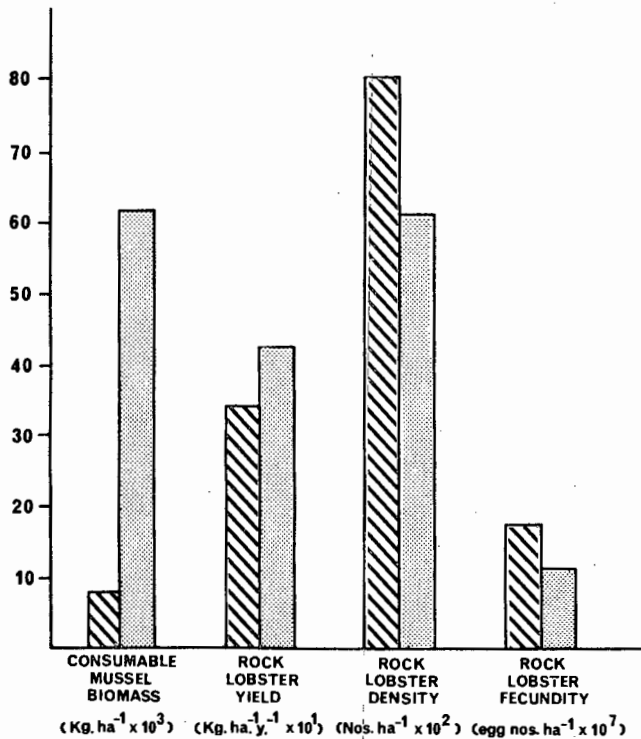


Fig. 5. Comparison between effects of "standard" and "alternative" harvesting strategies on four specific variables:

▨ standard values; ▩ alternative values.

of increased mussel availability on growth. The alternative strategy results in an immediate decline in the numbers of small rock lobsters (classes 1, 2), class 3 increases before declining, while size classes 4, 5 and 6 show slightly increased numbers. The model predicts stable numbers in all classes within 15–20 years of changing the harvesting policy.

Figure 5 shows that the consumable mussel biomass (biomass of mussels between 20 and 50 mm in length) would increase by 87% and that the rock-lobster yield (in terms of biomass) would increase by 19% if the rock-lobster harvesting strategy were changed in this way. However, the rock-lobster density is seen to decrease by 24%, and, associated with this, there is a corresponding decline in the rock-lobster population fecundity. Rock-lobster egg production has been shown to increase exponentially with rock-lobster length (Heydorn, 1965), but the marked decline in reproductive female rock-lobster numbers in the abundant size class 3 is not sufficiently compensated by the small increase in female rock-lobster numbers in class 4, in spite of the latter's greatly increased fecundity. Female rock-lobsters are never found in size classes 5 and 6. It should be noted that the modelled rock-lobster population assumes a constant recruitment rate; therefore the drop in fecundity of 35% is not fed back into the model.

The long-term advantages of a reduction in the present minimum size limit are clear, since rock-lobster production is underutilised in most areas of the west coast (Pollock, 1978). In addition, an increase of 14% could be expected in long-term rock-lobster mass yield under the alternative harvesting strategy even if growth remained unchanged, assuming that recruitment remained the same.

CONCLUSION

Kisiel (1971) stated that "The ultimate test of any model is its ability to predict, within economic and socially acceptable error bounds, future states of the system being managed". We believe that the present model has stood this test within predetermined error bounds of 20%, and that the described changes in harvesting policy could be used in management of the fishery modelled. There are, however, certain factors which must be taken into account. Firstly, a lowering of the size limit would have profound changes on the economics of the fishery, and within the first few years of such a change there would be an unprecedented boom, as hitherto unexploited resources were opened up to exploitation. Pressure for increasing the quota would be immense, yet it would be extremely unwise to allow the catch quota to rise above present-day levels of 3500–4500 tons whole body weight per annum.

Secondly, the predicted reduction in fecundity might have an important adverse effect on the population, although Lazarus (1967) has suggested that

the three large rock-lobster sanctuaries on the west coast contribute significant numbers of larval recruits to other parts of the coast. For this reason there is an urgent need for a research programme on the larval biology of *J. lalandii* to be initiated before any major policy change is introduced.

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