RELATIONSHIPS BETWEEN INVERTEBRATE DETRITIVORES AND GUT BACTERIA IN MARINE SYSTEMS

by

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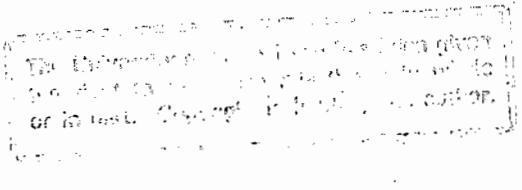

DECLARATION

This thesis reports the results of original research which I have carried out in the Marine Biology Research Institute, University of Cape Town, and has not been submitted for degree at any other university. Any technical and other assistance which I have received is fully acknowledged.

Signed by candidate

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ABSTRACT

Aspects of the feeding strategies and digestive invertebrate-microbial interactions of two saltmarsh thalassinid detritivores, the mudprawn *Upogebia africana* (Ortmann) and the sandprawn *Callianassa kraussi* Stebbing were examined. Resources available in their respective habitats were investigated together with the feeding apparatus, diet and digestive enzymes necessary for hydrolysis of refractory compounds of detritus. *U. africana* inhabits the upper reaches of Langebaan lagoon (Geelbek), while *C. kraussi* was sampled near the mouth (Oesterwal). Both species occur intertidally. Physical characteristics of sediment and water fluctuate more widely at Geelbek than at Oesterwal. Geelbek also has higher mud and clay content in the sediment, and greater particulate load in the water. The resources available in both sediment and water from Geelbek were of greater quality (assessed by proportion of living component, C:N ratio) and quantity. In terms of distribution of the resource, quality was highest in surface sediments, while quality was greatest at burrow linings. The mode of feeding, gut structure and diet of the two prawn species differ, although gut throughput rates are similar (*ca.* 6h). *U.africana* is a filter feeder which non-selectively ingests small particles which are further sorted in the modified filter-type gastric mill into larger particles which enter the midgut, and smaller particles which are channeled into the hepatopancreas. *U. africana* has a relatively large throughput gut (fore, mid, hind) allowing large meals to be taken. This may be related to its reliance on vascular plant detritus for both carbon and nitrogen requirements, as shown by stable isotope analyses. By contrast, *C. kraussi* feeds by a combination of deposit feeding and filter feeding. It is able to selectively ingest larger particles such as diatoms and protozoa as well as plant detritus. It has a typical masticating gastric mill and ingested particles are crushed in the anterior cardiac region and filtered in the posterior pyloric region; large particles are channeled to the hepatopancreas. The hepatopancreas is the main site of enzymatic digestion, and *C. kraussi* possesses both endoglucanase and C1-cellulase, and is capable of hydrolysis of refractory components of detritus. Experiments using antibiotics to prevent activity suggest that these cellulases are endogenous. *C. kraussi* has a relatively small gut which is dominated in volume by the hepatopancreas, highlighting the importance of this organ.

The presence, nature and role of gut microflora in aquatic invertebrates is synthesized from the literature, revealing the widespread occurrence of invertebrate-microbe associations. Different types of associations are reported to occur and the need to investigate the significance of different populations of bacteria in the gut is highlighted. While the taxa and physiological properties of gut

microbes of many aquatic invertebrates have been reported, very little conclusive evidence exists as to the role of bacteria in the physiology of host invertebrates. The gut microflora of *U. africana* and *C. kraussi* were examined by scanning electron microscopy and culture techniques. Both prawns hosted a diverse gut bacterial community. Of note were novel filamentous bacteria that extensively coated the midgut walls and gut contents of both species. Both prawns harboured mats of epimural rod bacteria in the hindgut, these being extensive in *U. africana*. Isolation of gut and habitat bacteria suggests that both species of prawn maintain a gut microflora distinct from the habitat microflora, in terms of both genera present and digestive capabilities. Distinction between resident gut bacteria and transient gut bacteria indicated that some habitat bacteria remain viable in the gut of *U. africana*, suggesting the potentially important phenomenon of incubation. More detailed examination of the resident bacteria observed in *U. africana* by transmission electron microscopy confirmed the presence of mats of densely-packed gram-negative epimural rod bacteria in the hindgut, and irregular shaped bacteria in the hepatopancreas. The dominant genus isolated from the hindgut was *Pseudomonas*, although members of the genus *Vibrio* were also isolated. Resident bacteria from the hindgut of *U. africana* did not exhibit chitinase, cellulase or lysozyme activity and are therefore unlikely to contribute to digestion of refractory material by the prawn. Acetylene reduction assays resulted in the detection of low rates of nitrogen fixation associated with the prawn, while relatively strong rates were found for the sediment. It was concluded that nitrogen fixation by gut microbes is insignificant in terms of the nitrogen budget of the prawn.

A comparative study on the gut microflora of an Australian mangrove crab *Sesarma messa* that feeds on fallen mangrove tree leaves (*Rhizophora stylosa*) established the presence of gut bacteria by direct counts and scanning electron microscopy. This crab feeds on nitrogen-poor food. However, acetylene reduction assays failed to implicate gut microbes with nitrogen acquisition. The widespread occurrence of epimural rod bacteria in the hindguts of decapod crustaceans was demonstrated by a SEM study of 16 species. Crustacean hindguts clearly represent suitable environments for colonization by micro-organisms despite the lack of specialized structures or modifications of the gut to facilitate this. Abundance of hindgut microflora was unrelated to the hosts taxon, habitat or geographical collection locality, but appeared to be affected by the feeding habits of the animal. The detritivore gut appears to be the most favourable habitat for microbial colonization, and this highlights the potential role of these microbes in detritivore nutrition.

GENERAL INTRODUCTION

In many marine ecosystems the detritivore and microbial food chains play important roles in trophodynamics and nutrient flux (Fenchel & Jorgensen 1977, Newell 1982, Azam *et al.* 1983, Newell *et al.* 1989). In these marine systems, almost without exception herbivores consume a comparatively small percentage (< 10%) of the primary production directly (Teal 1962, Mann 1972, Odum 1980, Lucas 1986). Most of the plant matter contributes to the organic debris pool (Newell 1982, Field 1984) or is voided as faeces by consumers with characteristically low assimilation efficiencies of < 50 % (e.g. Kofoed 1975, Zimmerman *et al.* 1979, Klumpp & Nichols 1983).

The major biotic agents of the decomposition of plant debris are microheterotrophs and invertebrate detritivores. The microheterotrophic community consists of either free-living micro-organisms that utilize and recycle nutrients largely independently of other organisms (Fenchel & Blackburn 1979, Azam *et al.* 1983), or microbes closely associated with organisms, e.g. occurring inside the guts of animals (Colwell & Liston 1962, Moriarty 1990, Plante *et al.* 1990). In many pelagic marine systems, it has been estimated that more than 50% of photo-assimilated carbon is channeled through the microbial food chain (Williams 1981, Lucas 1986). Microbial decomposition of detritus derived from macrophytes and vascular plants is well documented in coastal marine (Rice & Tenore 1981, Robertson *et al.* 1982, Tenore *et al.* 1984, Alongi & Hanson 1985, Benner & Hodson 1985, Moran & Hodson 1989, Blum & Mills 1991) and freshwater systems (Berrie 1976, Webster & Benfield 1986, Tanaka 1991). Thus a large proportion of the degradation of organic matter occurs independently of, or prior to, ingestion by organisms.

Invertebrate detritivores often dominate the faunal biomass in coastal marine systems, particularly in benthic soft-bottomed habitats (Rhoads & Young 1970, Aller & Dodge 1974, Levinton 1979). While detritivores have traditionally been considered as organisms that feed on low-quality particulate organic material (POM), this definition conceals the diversity of detritivore feeding types and the complex nature of the detrital pool. The detrital pool contains a mixture of organic sources ranging from the non-living plant-derived particles (which usually make up the bulk) to a variety of living components including meiofauna, micro-algae, protozoa and bacteria (Fenchel 1970, Zimmerman *et al.* 1979, Whitlatch 1981). The large non-living fraction of detritus is considered relatively refractory to digestion by invertebrate detritivores (Williams 1981, Tenore 1983, Bowen 1987), but may vary

in its nature depending on its source (e.g. terrestrial/marine) (Tenore *et al.* 1984). The smaller living fraction of the detrital pool is generally regarded as more labile and enriches the detritus as a food source (Findlay & Tenore 1982, Tenore *et al.* 1982). Detritivores vary in their feeding strategies both with regard to feeding mechanisms (filter-feeding, deposit-feeding, selectivity, etc.) as well as to their ingestion and utilization of the different components of the detrital pool. Faced with the problem of a plethora of feeding strategies, Jumars *et al.* (1984) redefined detritivory as "frequent feeding on material of low bulk food quality". This distinguishes between two types of feeders, i.e. macrophages, which handle individual food items, and microphages, which ingest material in bulk (Yonge 1928). Plante *et al.* (1990) suggest that macrophages, such as meiofauna, are not strictly detritivorous, being gourmets that ingest the higher quality living fraction of the detrital pool. Microphages on the other hand are true detritivores, ingesting significant amounts of particulate organic matter along with associated microbes. The relative proportions of non-living and biotic material ingested will be determined by the selectivity of the feeding mechanisms of the animal.

To assess the importance of detritivores in the recycling of nutrients, it is necessary to determine the ability of these animals to utilize detrital matter, particularly the bulk refractory components. In an attempt to tackle this problem, numerous studies have quantified the absorption efficiencies of detritivores fed on natural as well as prepared substrates (e.g. Adams & Angelovic 1970, Kofoed 1975, Yingst 1976, Lenz 1977, Foulds & Mann 1978, Stuart *et al* 1982, Hammond 1983, Klumpp & Nichols 1983, Kreeger *et al.* 1988, Langdon & Newell 1990). These studies provide insight into the ability of invertebrates to utilize organic debris and the quantitative significance of POM (e.g. Lucas & Newell 1984, Crosby *et al.* 1989). Current evidence suggests that while the POM may contribute significantly to the carbon requirements of detritivores, nitrogen must be obtained primarily from the heterotrophic components of the detrital pool (Cammen 1989). Despite this large body of literature, the role of POM in the nutrition of detritivores and the effect of this trophic group on the decay of particulate material remains an important unresolved issue, particularly due to the difficulty of relating these studies to complex natural systems (Levinton 1989).

The digestive ability of detritivores can be attributed to a number of factors. The animal may have a direct effect on degradation of POM due either to mechanical breakdown (shredding) of plant debris, or to the presence of endogenous enzymes necessary for chemical hydrolysis. A number of studies have detected the presence of enzymes capable of hydrolyzing refractory carbohydrates in the digestive systems of invertebrate detritivores (e.g. Dean 1978, Agnisola *et al.*

1981, Elyakova *et al.* 1981, Barlocher *et al.* 1989a, Langdon & Newell 1990, Chamier 1991, Walters & Smock 1991), but few have related these to digestive kinematics (gut passage time, ingestion and absorption rates) to ascertain the digestive significance to the detritivore (see Penry and Jumars 1987, Kofoed *et al.* 1989). However, the degradation of POM by the animal may be indirect due to associations with microheterotrophs.

The potential importance of digestive associations between marine detritivores and microbes has been highlighted recently in a review by Plante *et al.* (1990). In particular, the presence of a gut microflora (either ingested or resident) may be of significance to detritivores, either by digesting refractory components of detritus and making them available to the animal (Cutter & Rosenberg 1972, Martinez 1982, Musgrove 1988) or by supplying essential nutrients such as fatty acids, amino acids and vitamins (Fong & Mann 1980, Moriarty 1990). In addition, free-living microbes may precondition detritus for utilization by invertebrate detritivores (e.g. Barlocher *et al.* 1989b, Hanson & Tenore 1981). The important role of micro-organisms in the digestive physiology of some terrestrial invertebrates is well documented (Swift & Boddy 1984, Martin 1984, Breznak 1984, Bignell 1984) and it has been suggested that marine detritivores are likely to have similar close associations with gut microbiota (Martin 1984). Therefore detritivores are natural candidates to examine for gut microbe-invertebrate associations. The presence of gut micro-organisms and their interactions with invertebrate detritivores is clearly an important consideration in the overall picture of detritus degradation and utilization.

The aim of this thesis is to investigate some of the current research problems relating to detritivore feeding and detritivore-microbe associations. The bulk of the thesis consists of a series of papers addressing aspects of the feeding ecology and gut microbe associations of two different species of detritivore, the filter-feeding mudprawn *Upogebia africana* and the deposit-feeding sandprawn *Callianassa kraussi*, which inhabit disparate habitats within temperate coastal lagoons and saltmarsh ecosystems. By comparison of two different species of detritivorous prawns which have contrasting feeding and habitat characteristics, the aim is to gain insight into general principles of detritivore feeding and resource utilization. Saltmarsh ecosystems provide ideal models of detritus-dominated systems, since a major proportion of the detrital pool consists of vascular plant-derived particulate organic matter that is typically refractory to digestion by most invertebrates. The central theme throughout the thesis is the utilization of refractory plant material by detritivores and gut microbes, and the acquisition of sufficient nitrogen from a diet that is generally assumed to be low in these limiting nutrients.

The thesis is divided into two sections. The first section, consisting of three chapters, examines the feeding strategies of the two species of prawns by comparing the resources available to them in their respective habitats with their feeding characteristics. Major factors affecting the acquisition of nutrients by a detritivore include the quality and quantity of resource available, the morphology of the animal (which determines the components of the available resource that can be ingested), and the physiology of the animal (which sets limits on which of the ingested components can be assimilated).

In Chapter 1 the quality and quantity of the available resource in the respective habitats of the two detritivorous prawns are examined with regard to the relative abundance and nature of the different potential food sources. Chapter 2 examines the feeding apparatus and diet of the prawns to establish which detrital components are ingested. Stable isotope analyses are used to differentiate the relative importance of the various ingested components as food sources. In Chapter 3 the physiology of the animals is examined by a study of the capabilities of its digestive enzymes (particularly carbohydrate hydrolysis), and compared with the available resource (Chapter 1) and feeding apparatus and diet (Chapter 2) to gain an understanding of their feeding strategies. In addition, the question of endogenous versus exogenous sources of enzymes is addressed with regard to digestion of refractory components of natural detritus.

The second section of the thesis comprises five papers and examines the presence, nature and role of gut bacteria associated with both species of detritivore prawn, especially with regard to the utilization of refractory plant material and the acquisition of limiting nutrients such as nitrogen. Chapter 4 is a synthesis of the literature and highlights the important questions and processes that merit attention if the role of microheterotrophs in aquatic detritivore digestion is to elucidated. This chapter includes a comparison with terrestrial detritivore systems where microbe-invertebrate associations are well documented. Chapter 5 investigates, by scanning electron microscopy and culture techniques, the presence of gut microfloras in the two species of detritivore prawn. The taxa and digestive capabilities of habitat and gut bacteria are examined. Novel gut bacteria are reported, and the question of incubation of free-living bacteria in the guts of detritivores is addressed. Chapter 6 focuses on the potential role of prolific hindgut bacteria reported in Chapter 5, particularly with regard to symbiotic nitrogen fixation.

The focus of the thesis is widened in Chapter 7, a comparative study of the role of gut microflora in nitrogen acquisition by an Australian detritivorous mangrove crab *Sesarma messa*, while Chapter 8 investigates the presence of hindgut

microfloras in 16 species of decapod crustaceans collected world-wide to gain an understanding of the factors influencing the presence of gut microfloras.

Finally, the two sections of the thesis, i.e. the detritivore feeding studies and the investigations of gut microfloras, are integrated in the Synthesis to assess the relationships between microheterotrophs and invertebrate detritivores and their relative importance in benthic detritus-dominated systems. In addition, important questions arising from the thesis and suggested areas of future research are highlighted.

The chapters are presented in the form of separate manuscripts each dealing with a discrete section of the work, although they are linked by the common concern for elucidating aspects of detritivory and detritivore-microbial interactions. Chapters 5 and 8, as well as Appendices I and II have already been published or are in press. Consequently they differ slightly in format, complying to the requirements of the respective journals. Chapter 5 was co-authored, but I am the senior author and was responsible for the work, my co-authors playing a supervisory role. Appendices I and II report studies with which I was intimately involved, although the senior authors of each paper played the leading role. The methodological advances and results described in these papers provided supplementary information for Chapters 1 and 3, but these papers do not constitute a formal part of the thesis.

References

Adams S.M., Angelovic J.W. 1970 Assimilation of detritus and its associated bacteria by three species of estuarine animals. Chesapeake Sci 11(4):249-254

Agnisola C., Salvadore S., Scardi V. 1981 On the occurrence of cellulolytic activity in the digestive gland of some marine carnivorous molluscs. Comp Biochem Physiol 70B:521-525

Aller RC, Dodge RE 1974 Animal-sediment relations in a tropical lagoon Discovery Bay, Jamaica. J. Mar Res. 32:209-232

Alongi D.M., Hanson R.B. 1985 Effect of detritus supply on trophic relationships within experimental benthic food webs. II. Microbial responses, fate and composition of decomposing detritus. J Exp Mar Biol Ecol 88:167-182

Azam F, Fenchel T, Field JG, Gray JS, Meyer-Reil LA, Thingstad F 1983 The ecological role of water-column microbes in the sea. Mar Ecol Prog ser 10:257-263

Barlocher F, Arsuffi TL, Newell SY 1989a Digestive enzymes of the saltmarsh periwinkle *Littorina irrorata* (Mollusca, Gastropoda). Oecologia 80(1):39-43

Barlocher F, Newell SY, Arsuffi TL 1989b Digestion of *Spartina alterniflora* Loisel material with and without fungal constituents by the periwinkle *Littorina irrorata* Say (Mollusca : Gastropoda). J Exp Mar Biol Ecol 130:45-53

Benner R, Hodson RE 1985. Microbial degradation of the leachable and lignocellulosic components of leaves and wood from *Rhizophora* in a tropical mangrove swamp. Mar Ecol Prog Ser 23(3):221-230

Berrie AD 1976. Detritus, micro-organisms and animals in fresh water. In: The role of terrestrial and aquatic organisms in decomposition processes, JM Anderson & A MacFadyen (eds) pp 328-338. Oxford, Blackwells

Bignell DE 1984 The arthropod gut as an environment for microorganisms. In: Invertebrate-Microbial Interactions, Anderson JM, Raynon ADM, Walton DWA (eds.), Cambridge University Press, Cambridge, pp 205-227

Blum LK, Mills AL 1991 Microbial growth and activity during the initial stages of seagrass decomposition. Mar Ecol Prog Ser 70:73-82

Bowen S.H. 1987 Composition and nutritional value of detritus. pp 192-216. In Detritus and microbial ecology in aquaculture. Moriarty D.J.W., Pullin R.S.V. (eds.). ICLARM Conf Proc 14, 420 p. Int Cen for Living Aquatic Res Man, Manila, Philippines.

Breznak JA 1984 Biochemical aspects of symbiosis between termites and their intestinal microbiota. In: Invertebrate-Microbial Interactions, Anderson JM, Raynon ADM, Walton DWA (eds.), Cambridge University Press, Cambridge, pp 173-203

Cammen LM 1989 The relationship between ingestion rate of deposit feeders and sediment nutritional value. In: Ecology of marine deposit feeders, Lopez G, Taghon G, Levinton J (eds.), Springer-Verlag, New York, pp 201-222

Chamier AC 1991 Cellulose digestion and metabolism in the freshwater amphipod *Gammarus pseudolimnaeus* Bousfield. Freshwater Biol 25:33-40

Colwell RR, Liston J 1962 the natural bacterial flora of certain marine invertebrates. J Insect Pathology 4:23-33

Crosby MP, Langdon CJ, Newell RIE 1989 Importance of refractory plant material to the carbon budget of the oyster *Crassostrea virginica*. Mar Biol 100:343-352

Cutter, FA Rosenberg 1972 The role of cellulolytic bacteria in the digestive processes of the shipworm - requirement for the teredine borers. In: Biodeterioation of Materials, Walters AH, Hueck van der Plas EH (eds), vol. 2. Applied Science Publishers Ltd, London, pp 42-51

Dean R.C. 1978 Mechanisms of wood digestion in the shipworm *Bankia gouldi* Bartsch: Enzyme degradation of celluloses, hemicelluloses, and wood cell walls. Biol Bull 155:297-316

Elyakova L.A., Shevchenko N.M., Avaeva S.M. 1981 A comparative study of carbohydrase activities in marine invertebrates. Comp Biochem Physiol 69B:905-908

Fenchel T, Blackburn TH 1979 Bacteria in detritus food chains. In: Bacteria and mineral cycling, Academic Press, London, pp 52-78

Fenchel T. 1970 Studies on the decomposition of organic detritus derived from the turtle grass *Thalassia testudinum*. Limnol Oceanogr 15:14-20

Fenchel TM, Barker Jorgensen B 1977 Detritus food chains of aquatic ecosystems: The role of bacteria. Adv Micro Ecol 1:1-58

Field JG 1984 Flow patterns of energy and matter. In: Marine Ecology 5(2), Kinne O (ed.), Wiley & Sons, Chichester, pp 758-794

Findlay S., Tenore K. 1982 Nitrogen source for a detritivore: Detritus substrate versus associated microbes. Science 218:371-373

Fong W, Mann KH 1980 Role of gut flora in the transfer of amino acids through a marine food chain. Can J Fish Aquat Sci 37:88-96

Foulds J.B., Mann K.H. Cellulose digestion in Mysis stenolepis and its ecological implications. Limnol Oceanogr 23(4):760-766

Hammond L.S. 1983 Nutrition of deposit-feeding holothuroids and echinoids (Echinodermata) from a shallow reef lagoon, Discovery Bay, Jamaica. Mar Ecol Prog Ser 10:297-305

Hanson RB, Tenore KR 1981 Microbial metabolism and incorporation by the polychaete Capitella capitata of aerobically and anaerobically decomposed detritus. Mar Ecol Prog Ser 8:299-307

Jumars PA, Newell RC, Angel MV, Fowler SW, Poulet SA, Rowe GT, Smatacek V 1984 In: Flows of energy in marine ecosystems: theory and practice, Fasham MJ (ed.), Plenum Press, New York, pp 685-693

Klumpp D.W., Nichols P.D. 1983 Utilisation of the seagrass Posidonia australis as food by the rock crab Nectocarcinus integrifons (Latreille) (Crustacea: Decapoda: Portunidae). Mar Biol Letters 4:331-339

Kofoed L, Forbes V, Lopez G 1989 Time-dependent absorption in deposit feeders. In: Ecology of marine deposit feeders, Lopez G, Taghon G, Levinton J (eds.), Springer-Verlag, New York, pp 129-138

Kofoed L.H. 1975 The feeding biology of Hydrobia ventrosa (Montagu). I. The assimilation of different components of the food. J Exp Mar Biol Ecol 19:233-241

Kreeger D.A., Langdon C.J., Newell R.I.E. 1988 Utilization of refractory cellulosic carbon derived from *Spartina alterniflora* by the ribbed mussel *Geukensia demissa*. Mar Ecol Prog Ser 42:171-179

Langdon C.J., Newell R.I.E. 1990 Utilization of detritus and bacteria as food sources by two bivalve suspension-feeders, the oyster *Crassostrea virginica* and the mussel *Geukensia demissa*. Mar Ecol Prog Ser 58:299-310

Lenz J 1977 On detritus as a food source for pelagic filter-feeders. Mar Biol 41:39-48

Levinton JS 1979 Deposit-feeders, their resources, and the study of resource limitation. In: Ecological processes in coastal and marine systems, Livingston RJ (ed.), Plenum Press, New York, pp 117-141

Levinton JS 1989 Deposit feeding and coastal oceanography. In: Ecology of marine deposit feeders, Lopez G, Taghon G, Levinton J (eds.), Springer-Verlag, New York, pp 1-23

Lucas MI 1986. Decomposition in pelagic marine ecosystems. J Limnol Soc sth Afr 12(1/2):99-122

Lucas MI, Newell RC 1984 Utilization of saltmarsh grass detritus by two estuarine bivalves: carbohydrase activity of crystalline style enzymes of the oyster *Crassostrea virginica* (Gmelin) and the mussel *Guekensia demissa* (Dillwyn). Mar Biol Letters 5:275-290

Mann KH 1972 macrophyte production and detritus food chains in coastal waters. Mem Inst Ital Idrobiol 29(suppl.):353-383

Martin MM 1984 The role of ingested enzymes in the digestive processes of insects. In: Invertebrate-Microbial Interactions, Anderson JM, Raynon ADM, Walton DWA (eds.), Cambridge University Press, Cambridge, pp 155-177

Martinez JC 1982 The digestive microflora of *Teredo navalis* L. (Teredinidae;Bivalvia): Metabolic properties and ultimate role in digestion. Bact Mar 331:151-154

Moran TH, Hodson EB 1989. Bacterial secondary production on vascular plant detritus : relationships to detritus composition and degradation rate. Appl Env Microbiol 55(9):2178-2189

Moriarty DJW 1990 Interactions of micro-organisms and aquatic animals, particularly the nutritional role of the gut microflora. In : Microbiology in poecilotherms, Lesel R (ed), Elsevier Science Publishers.

Musgrove RJ 1988. Digestive ability of the freshwater crayfish *Paranephrops zealandicus* (White) (Parastacidae) and the role of microbial enzymes. Freshwater Biol 20:305-314

Newell RC 1982. The energetics of detritus utilisation in coastal lagoons and nearshore waters. Oceanol Acta 1982:347-355

Newell S.Y., Fallon R.D., Miller J.D. 1989 Decomposition and microbial dynamics for standing, naturally positioned leaves of the salt-marsh grass *Spartina alterniflora*. Mar Biol 101:471-481

Odum EP 1980 The status of 3 ecosystem-level hypotheses regarding saltmarsh estuaries: tidal subsidy, out-welling and detritus based food chains. In: Estuarine perspectives, Kennedy, VS (ed.), Acadenic Press, New York, pp 485-495

Penry DL, Jumars PA 1987 Modeling animal guts as chemical reactors. Am Nat 129(1):69-96

Plante CJ, Jumars PA, Baross JA 1990 Digestive associations between marine detritivores and bacteria. Annu Rev Ecol Syst 21:93-127

Rhoads DC, Young DK 1970 The influence of deposit-feeding organisms on sediment stability and community trophic structure. J Mar Res 28(2):150-178

Rice DL, Tenore KR 1981 Dynamics of carbon and nitrogen during the decomposition of detritus derived from estuarine macrophytes. Est Coast Shelf Sci 13:681-690

Robertson ML, Mills AL, Zieman JC 1982. Microbial synthesis of detritus-like particulates from dissolved organic carbon released by tropical seagrasses. Mar Ecol Prog ser 7:279-285

Stuart V., Field J.G., Newell R.C. 1982 Evidence for absorption of kelp detritus by the ribbed mussel *Aulacomya ater* using a new 51Cr-labelled microsphere technique. Mar Ecol Prog Ser 9:263-271

Swift MJ, Boddy L 1984 Animal-microbial interactions in wood decomposition. In: Invertebrate-Microbial Interactions, Anderson JM, Raynon ADM, Walton DWA (eds.), Cambridge University Press, Cambridge, pp 89-129

Tanaka Y 1991 Microbial decomposition of reed (*Phragmites communis*) leaves in a saline lake. Hydrobiologia 220:119-129

Teal JM 1962. Energy flow in the saltmarsh ecosystem of Georgia. Ecology 43(4):387-624

Tenore K.R. 1983 What controls the availability to animals of detritus derived from vascular plants: Organic nitrogen enrichment or caloric availability? Mar Ecol Prog Ser 10:307-309

Tenore K.R., Cammen L., Findlay S.E.G., Phillips N. 1982 Perspectives of research on detritus: do factors controlling the availability of detritus to macroconsumers depend on its source? J Mar Res 40(2):473-490

Tenore KR, Hanson RB, McClain J, Maccubbin AE, Hodson RE 1984 Changes in composition and nutritional value to a benthic deposit feeder of decomposing detritus pools. Bull mar Sci 35:292-311

Walters & Smock 1991 Cellulase activity of leaf litter and stream-dwelling, shredder macroinvertebrates. Hydrobiologia 220(1):29-36

Webster JR, Benfield EF 1986 Vascular plant breakdown in freshwater ecosystems. Ann Refv Ecol Syst 17:567-594

Whitlatch R.B. 1981 Animal-sediment relationships in intertidal marine benthic habitats: some determinants of deposit-feeding species diversity. J Exp Mar Biol Ecol 53:31-45

Williams P 1981 Detritus utilization by Mytilus edulis. Esturine, Coastal and Shelf Science 12:739-746

Yingst J.Y. 1976 The utilization of organic matter in shallow marine sediments by an epibenthic deposit-feeding holothurian. J Exp Mar Biol Ecol 23:55-69

Yonge CM 1928 Feeding mechanisms in invertebrates. Biol Rev 3:21-76

Zimmerman R, Gibson R, Harrington J 1979 Herbivory and detritivory among gammaridean amphipods from a Florida seagrass community. Mar Biol 54:41-47

CHAPTER 1

Resources available to two benthic detritivores in the sediments and water in a salt-marsh system

Abstract

This study examines the quality and quantity of resources available to two species of benthic thalassinid detritivore prawns, the deposit-feeding sandprawn *Callianassa kraussi* and the filter-feeding mudprawn *Upogebia africana*, in a temperate saltmarsh ecosystem, Langebaan lagoon. The physical characteristics of the sediment and water of the habitat of *U. africana* (Geelbek: upper reaches of the lagoon) fluctuated more widely than did those of the habitat of *C. kraussi* (Oesterwal: near the mouth of the lagoon). A major difference in the sediments was the higher mud content at Geelbek, which renders it a more stable environment for permanent burrow construction, thus favouring *U. africana*. The relatively unstable sands at Oesterwal facilitate deposit-feeding by *C. kraussi* but may prevent colonization by *U. africana*. The resource available in the sediment from Geelbek was of a higher quality and greater quantity than at Oesterwal. Abundance and biomass of both total resource and the biotic components of sediment detritus were greater at Geelbek, confirming higher bacterial and micro-algal productivity estimates. The proportions of biotic to non-living fractions of the detritus were greater, and the C/N ratios lower, in sediments at Geelbek than at Oesterwal, with diatoms and bacteria contributing most, respectively. In addition, it was found that at both sites, while most of the non-living POM was composed of relatively large particles (>64 μm), the biotic fraction was composed primarily of organisms of <64μm. In terms of the distribution of the resource in the sediment, quality was highest in surface sediments, while quantity was greatest at burrow linings. Surface sediments from *Zostera* beds were the richest habitats with respect to quality and quantity of detritus. The quantity of detritus, and in the water column was much greater at Geelbek compared to Oesterwal. However, quality (in terms of the proportion of biotic to non-living components of detritus) was higher at Oesterwal, despite the fact that the C/N ratio was slightly lower at Geelbek.

Introduction

Shallow marine sediments are composed of a complexity of inorganic and organic materials. The particulate inorganic fraction represents the bulk of sediment with organic matter usually making up less than 5 percent of the whole (Levinton 1989). The organic fraction generally consists of a small living component (microbiota) and a relatively large non-living component (Tenore *et al.* 1979, Whitlatch 1981), which together constitute the detrital pool (detritus) (Darnell 1967).

The contribution of different components of the organic fraction to the nutrition of deposit-feeders has been the subject of much discussion and controversy (for recent reviews see Levinton 1989, Cammen 1989). Briefly, ideas on this subject have come full circle : initially the non-living particulate organic fraction was considered to be the main source of food for marine benthos. However this hypothesis was replaced by the microbial conversion hypothesis, which argues that particulate organic matter (POM) is mainly of a refractory nature and is unavailable to detritivores as a food source. Rather, POM acts as a substrate for the growth of micro-organisms which are stripped off during its passage through the detritivore gut. It was proposed that high ingestion rates and rapid gut passage ensure sufficient sustenance from the relatively low biomass of micro-organisms associated with the non-living detritus (Newell 1965, Adams & Angelovic 1970, Fenchel 1970, Hargrave 1970). However, more recently it has been shown that the density of microbes does not always account for the energy requirements of deposit-feeders, and it seems clear that microbes alone cannot supply the carbon requirements (Baker & Bradnam 1976, Cammen 1980, Jensen & Siegismund 1980, Findlay *et al.* 1984). Therefore the microbial conversion hypothesis has now been replaced in favour of present evidence that while much of the required nitrogen may be derived from the living component of organic matter (Rice *et al.* 1986), a large proportion of the carbon is obtained directly from the non-living fraction (Klumpp & Nichols 1983, Seiderer & Newell 1985, Kemp 1986, Cammen 1989, Chamier 1991).

Reality may be more complex, however, with different scenarios holding in different systems, i.e. the relative abundance, composition and availability of the living fraction (microbiota) to local detritivores in different sediments may determine the relative importance of living and non-living organic components as food resources. Central to understanding detritivore feeding is a measure of the quantity and quality of the resource. The total quantity of resource available is usually far in excess of that utilized by the animal, being composed largely of

material refractory to digestion by most detritivores (Yingst 1976, Lopez *et al.* 1977, Williams 1981, Tenore 1983, Newell & Langdon 1986, Rice & Rhoads 1989). The quality of the resource depends on the nature of the constituents and the relative quantities of utilizable components (Tenore 1988), and the quality of a resource experienced by an animal will differ depending on its feeding mechanisms. In general terms, however, quality can be defined as the relative proportion of easily utilizable and non-utilizable components, often represented by the ratio of the labile biotic fraction to the relatively refractory non-living fraction of detritus. The quality of a resource is enhanced where nutrients that are limiting to a detritivore are present in greater abundance (Phillips 1984). Generally, the living components contain greater concentrations of limiting nutrients such as nitrogen, than does the bulk of non-living detritus which may be stripped of these components by processes such as leaching or bacterial decomposition (Gosselink *et al.* 1974, Fallon & Pfaender 1976, Suberkropp & Klug 1976, Rice & Tenore 1981, Bowen 1987). Thus a greater proportion of microbiota (and usually a lower C/N ratio) improves the quality of the detritus as a resource, e.g. protein enrichment (Tenore 1988). In addition, quality depends on the size and shape of the POM, both that of the matrix (which will influence ingestability and the cost of aquiring utilizable components), and that of the labile fraction, the availability of which will depend on its size, shape and mobility.

In terms of empirical evidence, the task of discerning among the complex mixture of organic sources in marine sediments and ascertaining their relative abundance in different sediments and their significance to different detritivores, is still in its infancy (Cammen 1989). The complex array of food sources in sediments (bacteria, protozoans, micro-algae, fresh detritus, humic substances, etc.) and their relative abundance need to be studied carefully in conjunction with feeding strategies to understand the nutrition of detritivores (Levinton 1989, Tenore 1989). Both the distribution of potential food sources in the sediment and their micro-scale features (shape, size, aggregates, attached etc.) are important considerations (Watling 1989) as they influence availability in terms of the feeding behaviour and mechanisms of the animal. Furthermore, a measure of the biologically relevant fraction, i.e. the labile component of non-living organic matter which is available to organisms, is relevant in assessing food resources recognized by detritivores (Mayer 1989). For example, nitrogen present in detritus can be in the form of non-labile humic nitrogen rather than microbial protein (Rice 1982). In addition, detritivores may be constrained by seasonal changes in POM and its components (Richardson 1991). To gain insight into general principles of detritivore feeding it has been suggested that it would be useful to compare several systems in

terms of the feeding processes of dominant detritivores (including their feeding morphology, foraging and digestive physiology) with the resources available in sediments and overlying water (Levinton 1989).

In this study, the potential food sources available to two different species of benthic detritivores, the filter-feeding mudprawn *Upogebia africana* and the deposit-feeding sandprawn *Callianassa kraussi*, which dominate the benthic biomass in different parts of a coastal saltmarsh system, Langebaan lagoon (Wynberg 1991), are examined. Previous studies have indicated that the habitats of these two detritivores differ in terms of physical characteristics of the sediment (Flemming 1977a), as well as other parameters such as chlorophyll content and diatom abundance (Fielding *et al.* 1988). The total available resource is identified and quantified and the relative abundance and biomass of the various biotic and non-living potential food sources in the sediment and overlying water column are determined as a measure of resource quality. Numerous studies have reported bioturbation effects on microbiota caused by burrowing thalassinid prawns (Ott *et al.* 1976, Suchanek 1983, Dobbs & Guckert 1988, Posey *et al.* 1991). In particular, Branch & Pringle (1987) found that the sand-prawn *C. kraussi* significantly affects density and distribution of algae, bacteria and meiofauna in the sediment. Consequently, the distribution of the resources are examined on a microscale in relation to the burrows of the prawns. In addition, the size composition of the living and total food resources are determined to provide a measure of availability to the two different detritivores in terms of their feeding mechanisms. Sampling was conducted on a seasonal basis to establish whether changes in resource supply, in terms of quality and quantity, occur.

The findings of this chapter, together with those on the feeding mechanisms and diets of the prawns (Chapter 2), and their digestive physiology (Chapter 3), contribute to the elucidation of the feeding strategies of these two detritivores. This will have a significant bearing on the likely importance of micro-organisms associated with the food ingested as well as resident microflora.

Study site

All fieldwork was done at two sites, Oesterwal and Geelbek, in Langebaan lagoon, a partially enclosed marine system situated between 33°00" to 33°15"S to 17°57" to 18°08"E, on the south-west coast of South Africa (Figure 1). Geelbek lies in the sheltered upper reaches of the lagoon and has extensive intertidal sand flats surrounded by saltmarshes. The sediment consists of fine anaerobic muddy sand

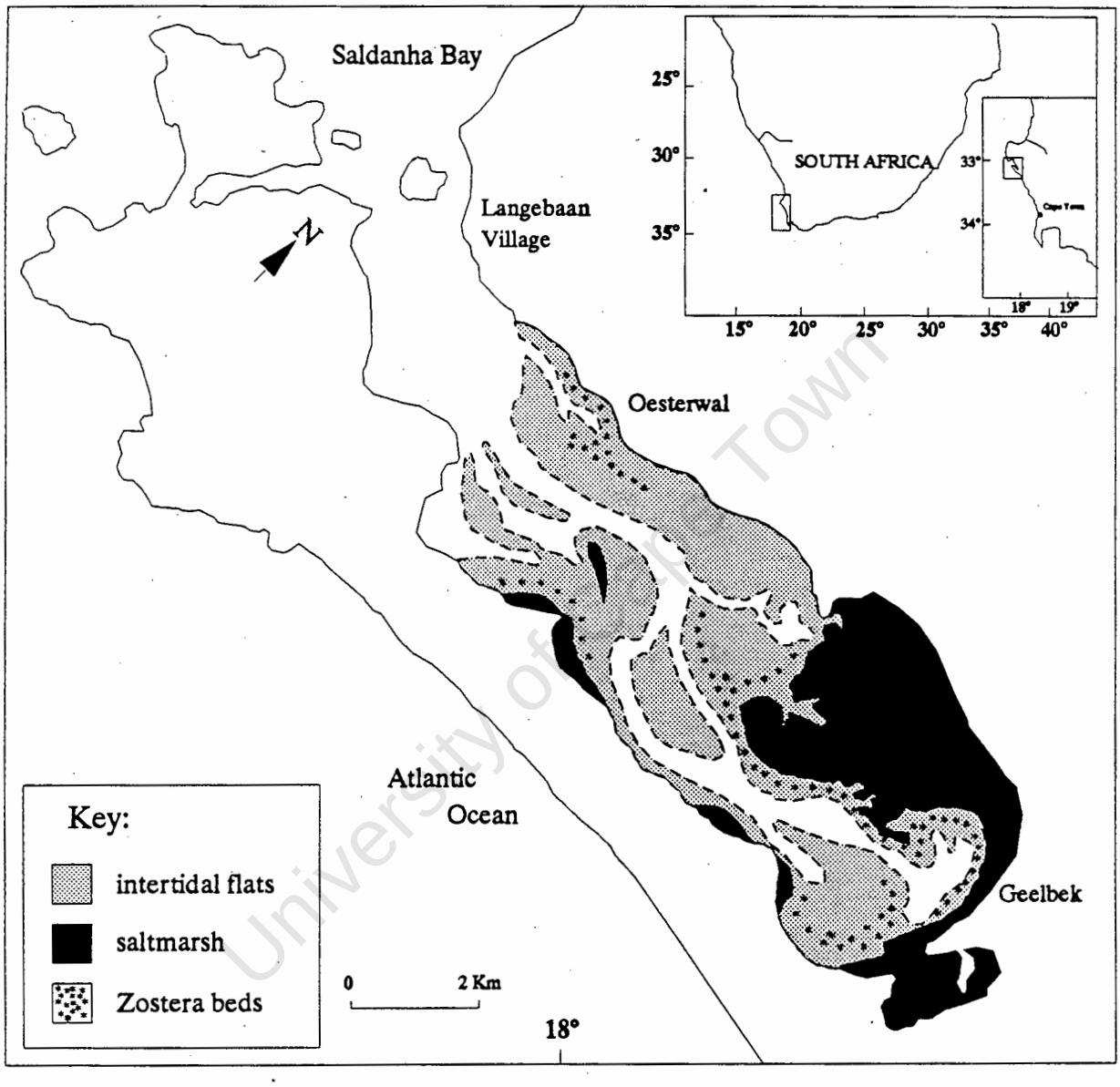

Figure 1. Langebaan lagoon, showing sampling sites, (Oesterwal and geelbek), extent of intertidal mud and sandflats, and distribution of salt marsh plants. (Ref: South Africa 1:50 000; 3317BB + 2218AA Saldanha, third edition)

(Puttick 1977) and in terms of biomass the benthic macrofauna is dominated by the filter-feeding detritivorous thalassinid mud-prawn *Upogebia africana* (approximately 110 prawns m^{-2} (Wynberg 1991)). Oesterwal is situated near the mouth of the lagoon and is subject to the effects of strong tidal currents (up to 1 m sec^{-1} (Shannon & Stander 1977)) and limited wave action. The sediment consists of fine sand (Flemming 1977b) and the intertidal benthic biomass is dominated by the deposit-feeding thalassinid sand-prawn *Callianassa kraussi* (approximately 175 prawns m^{-2} (Wynberg 1991)). Both *U. africana* and *C. kraussi* inhabit burrows in the sand and the surface of the sand at both Geelbek and Klein Oesterwal is peppered with burrow openings. Approximately half of the lagoon volume ebbs into the bay at spring low tides, leaving vast expanses of intertidal flats exposed (Shannon & Stander 1977). Saltmarsh plant production is highest at the head of the lagoon and dominant species include *Juncus kraussii*, *Spartina maritima*, *Arthrocnemum pillansii*, *Typha capensis*, *Phragmites australis*, *Sarcocornia perennis*, *Chenolea diffusa*, and *Salicornia meyerana* (for description and distribution of saltmarsh communities see Boucher & Jarman (1977) and Christie (1981)). The eelgrass *Zostera capensis* is patchily distributed around the lagoon but forms extensive mid-tide beds on the flats in the upper reaches of the lagoon (Figure 1). *Gracilaria verrucosa* is the dominant subtidal algal macrophyte occurring extensively near the mouth of the lagoon. Abrasion of leaves and die-back of saltmarsh plants are believed to be major contibuters to non-living detrital material in the sediments of Langebaan lagoon (Christie 1981). Thus a major proportion of the non-living detritus in the system is derived from vascular salt-marsh plants. Detritus derived from this source is typically refractory, low in nitrogen (Tenore 1983) and largely composed of highly complex polysaccharides (Cummins *et al.* 1973) that most macrobenthic detritivores cannot digest (Williams 1981, Tenore 1983, Bowen 1987).

Methods and materials

General sampling procedures

Sampling of intertidal sediments and the water column was conducted seasonally in summer (January 1990), autumn (April 1990), winter (July 1990), spring (October 1990), and a second summer (Jan 1991) at both study sites. Sediment sampling was always carried out during a spring low tide at mid-tide level, and subsurface water samples were collected on the same day in 0.5-1m deep water. In each season Oestewal was sampled one day prior to Geelbek. On each occasion temperature,

pH and salinity were measured and triplicate samples ($50cm^3$) of surface sediment were removed from the top 5mm between burrow openings and subjected to granulometry analyses.

Particle size analysis of triplicate samples of sediment (approximately 50g) was determined by dry (60°C for 48h) sieving into 2mm, 1mm, 500, 300, 125, 63, 38μm size classes by weight. Mean particle size (Mdø) was determined graphically and the sorting coefficient (Qdø) and skewness (Skqø) of the sediment calculated. Bulk density (g dry weight per cm^3) and organic content (after combustion for 4h at 450°C) was also determined from these samples.

The distribution and abundance of potential resources at each site was determined by sampling lagoon water and 5 sediment microhabitats on each sampling occasion. Water from 10cm below the air-water interface (sub-surface water) was collected on an incoming tide in 0.5-1m deep water. The five sediment microhabitats identified are shown in Figure 2: 1) surface sediment between prawn burrow openings; 2) surface sediment from prawn burrow opening (on the mound); 3) prawn burrow lining; 4) sediment between burrows at 10 cm depth; 5) surface sediment from within *Zostera capensis* beds (Figure 2). Results are expressed per g dry weight or per cm^3 of sediment, and volume-to-dry weight conversion ratios were determined on each sampling occasion for each microhabitat at each site.

Organic content, organic carbon and total nitrogen

Replicate (n=3) sediment cores for (1) organic content and (2) organic carbon and nitrogen analyses were taken from each microhabitat to a depth of 5 mm using, respectively, 5 and 50 ml syringes with the leur ends removed (1 and 5 cm^3 samples, respectively). Samples were transported to the laboratory on ice and frozen within 2 hours of collection. Subsequently, samples were oven-dried at 55°C for 48 h. Organic content was determined by weight loss after combustion at 450°C for 4 h. Total organic carbon was determined by the dichromate oxidation method (Walkley & Black 1934) and total nitrogen was determined, after acid digestion with sulphuric acid and hydrogen peroxide, by the phenate method (Nicholson 1984). Three seperate samples of seawater (500-2000 ml depending on particulate load) were collected from each site for determination of each of particulate organic matter (POM) and particulate organic carbon and nitrogen (POC & PON) content and filtered onto pre-ashed Whatman GF/F filters in the field. The filters were then wrapped in tin foil, placed on ice and transported to the laboratory where they were stored frozen at -20°C. Filter-papers plus particulate organic matter (POM) were later oven-dried at 60°C for 48 h. Organic content was determined by weight loss

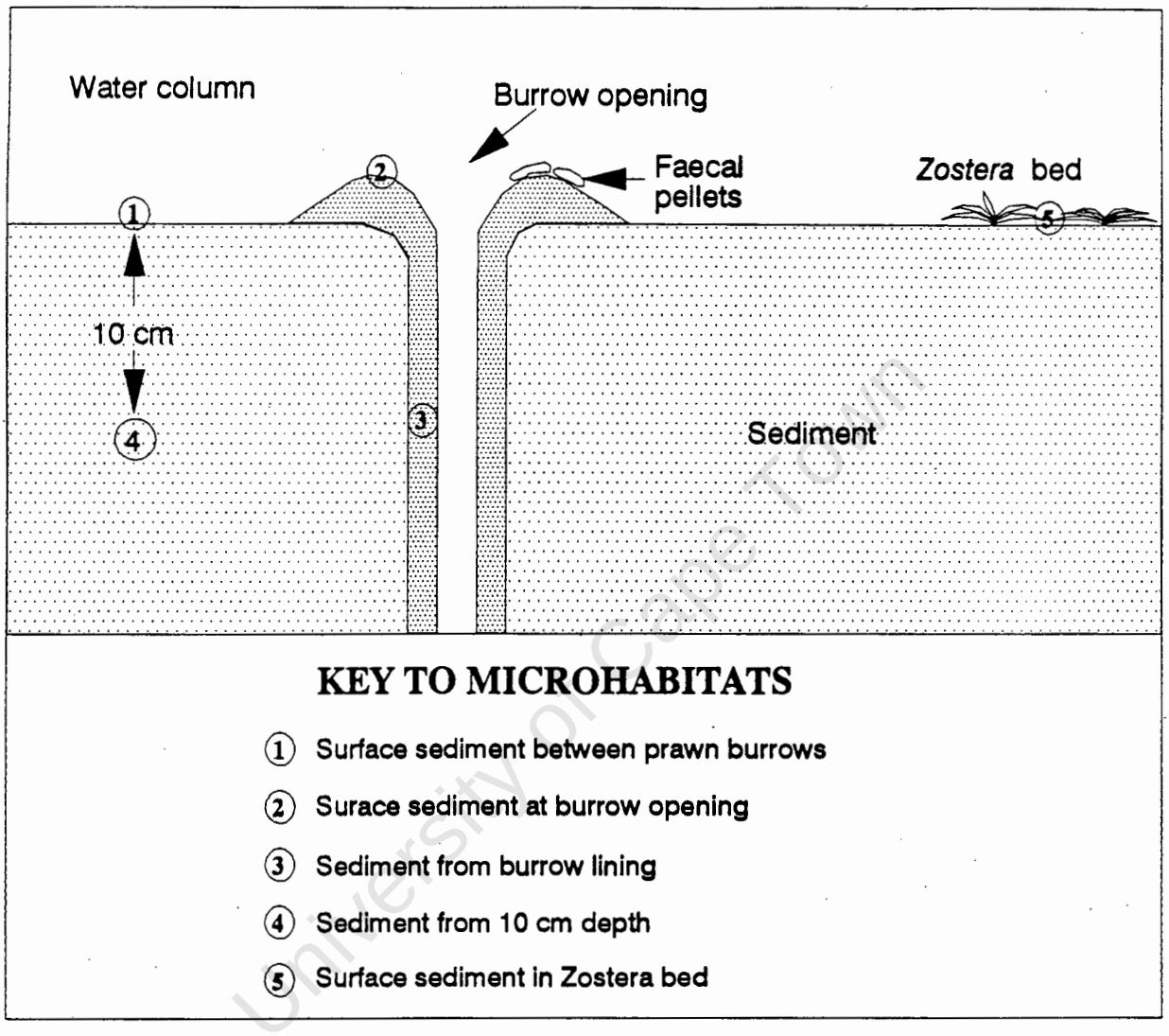

Figure 2. Diagram showing the five microhabitats sampled in the sediment at both Geelbek and Oesterwal

after combustion at 450°C for 4 h. Total organic carbon and total nitrogen were measured on a Carlo-Erba CHN Elemental Analyzer.

Density and biomass of microbiota

Bacteria. For enumeration of bacteria from each microhabitat, replicate (n=3) cores of 1 cm^3 were taken to a depth of 5mm using 5 ml syringes with the leur end removed. Samples were extruded into acid-washed vials, fixed immediately by addition of 5 ml 4 % formalin (in 0.2 μm filtered seawater) and kept in the dark at 4°C. Bacteria were enumerated by epifluorescent microscopy using the DNA stain 4'6-diamidino-2-phenylindole (DAPI) (Porter & Feig 1980). Acid-washed glass-ware was used throughout. Samples were diluted in 50 ml 0.1M tetrasodium pyrophosphate (deflocculant) and sonicated for 5 min at 40-50 kHz to extract cells and organic matter from the sediment and disperse them evenly (Ellery & Schleyer 1984, Velji & Albright 1986). Thereafter samples were further diluted in distilled water (0.2 μm filtered) to a final dilution (v/v) of 1500 and 2500 for Oesterwal and Geelbek sediments, respectively. Five ml of each diluted sample was stained with DAPI at a final concentration of 5 $\mu g\ ml^{-1}$ (Schallenberger *et al.* 1989), vortexed to ensure mixing, and incubated in the dark at 4°C. Thereafter 2 ml was filtered (in a low light environment) onto each of two irgalon black (Parsons *et al.* 1984) prestained 0.2 μm Nuclepore polycarbonate filters, which were wetted immediately prior to use with 1 % photoflow (Paterson Anti-static Wetting Agent) to facilitate even dispersion of cells on the filter. Slides were prepared using low-fluorescence microscope oil (Merck 4699). Twenty fields were counted at 1000x magnification on each of the duplicate slides for each sample using a Nikon Diaphot inverted compound microscope with a mercury light source, Nikon Neofluor 100/1.30 oil objective and Nikon DM400 filter block. A dilution series of 500, 1500, 2500, 3500, 4500, 5500, 6500, 7500, 8500, 9500, 10500, 11500 times dilution (v/v) was prepared for samples from each site at each season and counts were corrected for recovery of attached/obscured (masking) cells using the cumulative yield method of Ellery & Schleyer (1984). Average cell biovolumes were measured microscopically to be 0.26 and 0.68 μm^3 in surface sediments and *Zostera* respectively (Mazure & Branch 1979). A biovolume : carbon conversion factor of 220 fgC μm^{-3} (Bratbak & Dundas 1984, Moriarty *et al.* 1990) was used to convert biovolume to carbon biomass. Values for conversion of biovolume to carbon range from 75 to 560 fgC μm^{-3} with 215 representing the mean (see Nagata 1986, Painchauld & Therriault 1989). A value of 24.7 fgN μm^{-3} was used to convert cell volume to nitrogen (Nagata 1986).

Water samples (100 ml) for bacterial enumeration were collected in triplicate from each site, fixed immediately with concentrated formalin to a final concentration of 4 % and kept at 4°C in the dark. Enumeration followed the method of Porter & Feig (1980). Aliquots of 2 ml were stained with a final concentration of 1 μg ml^{-1} DAPI, incubated in the dark at 4°C for 5 min and slides prepared as described above for sediments. Two slides were prepared and counted per sample. Twenty fields or 400 cells were counted per slide. Counts were converted to carbon and nitrogen biomass as above for sediments using a cell biovolume of 0.26 μm^3.

Protozoa and diatoms. Sediment cores (1 cm^3, 5mm deep) were collected in triplicate from each microhabitat for enumeration of protozoa and diatoms, using a 5 ml syringe with the leur end removed. Samples were extruded into acid-washed vials, fixed immediately with 5 ml of fixative comprising 5 ml Lugol's solution (Parsons *et al.* 1984) and 20 ml concentrated formalin per litre of 0.2 μm filtered seawater, and kept at 4°C. Samples were diluted by a final factor of 50 or 200 (v/v) for Oesterwal and Geelbek samples, respectively. Thereafter the diluted sample was thoroughly shaken for 10 min and a 5 or 2 ml aliquot (Oesterwal and Geelbek, respectively) was immediately removed. Each aliquot was destained with 0.1N thiosulpate solution (1 drops per 2 ml sample), stained with rose-bengal solution (final concentration approximately 0.5 g l^{-1}) for 5 min, and finally filtered at low vacuum onto a 3 μm cellulose acetate Millipore filter moistened with photoflow (see above). Preparation of permanent slides followed the method of Crumpton (1987). Filters were placed face down on coverslips and directly impregnated with water-soluble hydropropyl methacrylate resin (Polysciences, Inc. USA)(Leduc & Holt 1965) by adding a sufficient number of drops of resin to to cover the back of the filter. After the filter was left to dissolved overnight in the resin at room temperature in a dust-free environment, the coverslip was mounted on a slide with a further drop of resin and dried at 50°C for 1 h. Diatoms and protozoans were counted at 400x magnification. Fifty or 30 fields per slide were counted for Geelbek and Oesterwal samples, respectively. This preparation and counting procedure gave a counting precision of 10% of the mean at the 95% level of confidence (Cassell 1965). Protozoa were grouped as large ciliates (50x30 μm), small ciliates (30x25 μm), large flagellates (60x40 μm) and small flagellates (30x25 μm). Biovolumes were determined assuming an oval shape. To convert numbers to biomass a carbon:biovolume conversion factor of 0.19pgC μm^{-3} (Putt & Stoecker 1989) was used for all protozoans. This value lies between those proposed by other researchers ie. 0.08 (Sherr & Sherr 1984) and 0.22 pgC μm^{-3} (Bratbak 1985). A C:N ratio of 4 (Putt & Stoecker 1989, Matthews 1991) was used to convert carbon biomass to

nitrogen biomass. Diatoms were grouped into centric, pennate, chains and other forms that did not conform to the above categories; generally < 5 % of the total. Biovolumes were calculated from measured linear dimensions assuming centric diatoms are cylindrical (large = 50x15 μm, small = 25x10 μm), pennate diatoms are ellipsoid (large = 75x50x35 μm, medium = 25x15x10 μm, small = 30x15x5 μm), and individual cells of chains are cylindrical (50-25x15 μm). Biovolumes were converted to carbon using a conversion factor of 0.11 pgC μm^{-3} (Strathman 1967) and nitrogen assuming a C:N ratio of 6 (Strickland 1960).

Triplicate 500ml water samples were collected to determine protozoan and diatom numbers and biomass, immediately fixed by addition of 2.5 ml Lugol's solution and 10 ml concentrated formalin, and kept at 4°C. Aliquots (100ml and 20 ml, for Oesterwal and Geelbek, respectively) were destained with thiosulphate solution (0.1N), stained with rose-bengal (0.5g l-1), filtered onto 3 μm Millipore filters and permanent slides were prepared as described above. Fifty and 30 fields were counted on each slide from Oestewal and Geelbek, respectively. Numbers were converted to biovolumes and carbon and nitrogen biomass as described above for sediments.

Chlorophylla. Sediment cores (1 cm^3, 5 mm deep) were collected in triplicate from each microhabitat to measure chlorophyll a content, using a 5 ml syringe with the leur end removed. Samples were extruded into vials together with a few drops of 1% $MgCl_2$ and immediately placed on ice in the dark. Triplicate water samples of 500-2000 ml (depending on particulate load) were filtered onto Whatman GF/F filters. Two drops of 1 % $MgCl_2$ were added to the filter which was then wrapped in tinfoil and placed on ice. In the laboratory, samples were kept frozen at -20°C in the dark for up to one month. Chlorophyll was extracted from sediment and water samples using 15 ml and 10 ml 90 % acetone, respectively. After the addition of acetone, samples were homogenized using a glass tissue grinder, sonicated on ice for 30 min to disrupt cells and allowed to stand overnight at 4°C. Chlorophyll content was estimated using the spectrophometric equations of Jeffrey & Humphrey (1975). A carbon : chlorophyll ratio of 40 was used to convert chlorophyll to carbon biomass (Cammen & Walker 1986, deJonge 1980). A C:N ratio of 6 (Strickland 1960) was assumed to convert carbon biomass to nitrogen.

Meiofauna. Triplicate sediment samples (approximately 15 cm^3 total) from each microhabitat were collected for the estimation of meiofauna numbers obtained using a 50 cm^3 syringe (with the leur end removed) to a depth of 5 mm. Samples were extruded into vials, placed on ice and preserved with 10 % formalin in

seawater in the field. In the laboratory, meiofauna were extracted from the sediment by repeated washings (10) onto a 45 μm mesh. Both the fraction retained on the 45 μm sieve, as well as the sediment from which meiofauna had been extracted were preserved in 10 % formalin (in seawater) containing Rose Bengal stain (0.5 $g.l^{-1}$). The fraction retained on the 45 μm sieve was halved using a plankton splitter, and the organisms were enumerated under a dissecting microscope in a counting dish lined into $1cm^2$ grids. A fraction (1/10) of the remaining sediment was examined for non-extracted meiofauna and the final count was adjusted accordingly. Non-extracted organisms accounted for less than 5 % of the total count. Meiofaunal counts were converted to biomass using mean ash-free dry weights recorded for meiofaunal taxa in a South African estuary by Dye (1978), and an ash content of 3 % (Omari 1969). Biomass was converted to carbon assuming a carbon content of 45 % (range of 16-66 (Beers 1966, Hargrave 1970, Omari 1969), and nitrogen assuming a nitrogen content of 9 % (range of 4.4-13.1 (Beers 1966, Parsons *et al.* 1984, Omari 1969)).

Statistical analyses

Analysis of variance (ANOVA) was used to compare measured parameters over season, site and microhabitat. Where significant ($p < 0.05$) differences were detected, each ANOVA was followed by a Tukey multiple range test. Homogeneity of variance of the data was determined using Bartlett's test. All statistical analyses were conducted using the Statgraphics software package (STSC Inc. 1985).

Results

Physical characteristics of sediments and water

The physical characteristics of sediments and water from Oesterwal and Geelbek are summarized in Table 1. Geelbek sediments had wider ranges in temperature, pH and salinity than those at Oesterwal, and a higher percentages of mud and organics, and a lower C/N ratio. Granulometric analysis of sediments revealed that at both sites the sediment was a fine, well-sorted sand, although the bulk density (g dry weight per cm^3) was lower at Geelbek, possibly reflecting loose packing of the more abundant organic particles at this site.

The physical characteristics of the water at the 2 sites were more dissimilar than those of the sediments. Geelbek water had higher temperatures, and

Table 1. Physical characteristics of sub-surface water and intertidal surface sediments at Oesterwal and Geelbek, Langebaan lagoon. Values represent annual means (± one standard deviation) obtained from seasonal data.

Characteristic	n	Sampling sites			
		Oesterwal	range	Geelbek	range
SEDIMENT					
Temperature (^{o}C)	7	19.53 (4.05)	11.9-24.0	19.74 (5.53)	11.2-27.0
pH	7	6.57 (0.45)	6.0-7.0	6.85 (0.32)	6.0-7.5
Salinity (^{o}/oo)	7	36.33 (1.53)	35-38	37.67 (6.43)	33-45
Median particle diameter (mm)	10	0.41 (0.02)	0.39-0.43	0.47 (0.03)	0.42-0.52
Sorting coefficient (o_1)	10	0.52 (0.05)	0.40-0.58	0.51 (0.04)	0.44-0.55
% Mud (by dry wt)	10	7.10 (2.28)	4.38-11.34	9.64 (3.06)	5.07-12.25
Bulk density (gDW. cm^{-3})	24	1.51 (0.04)	1.44-1.56	1.36 (0.05)	1.28-1.49
% organics (by dry wt)	24	0.91 (0.44)	0.38-2.18	1.48 (0.34)	0.75-2.07
C/N ratio	18	14.89 (5.42)	7.63-23.81	12.82 (6.53)	5.45-22.92
WATER					
Temperature (^{o}C)	5	17.6 (3.6)	11.2-20.0	19.4 (3.7)	13.5-24.0
pH	5	6.4 (0.4)	6.0-7.3	6.6 (0.4)	6.0-7.5
Salinity (^{o}/oo)	5	35.3 (0.6)	35-36	36.0 (3.6)	33-40
Particulate load (mg PM l^{-1})	15	11.56 (7.54)	4.93-26.15	46.75 (52.56)	11.73-160.47
% organics (by dry wt of PM)	15	31.41 (4.66)	22.17-37.01	25.06 (5.13)	15.47-30.36
C/N ratio	10	9.51 (2.11)	7.68-13.43	9.37 (1.43)	7.28-12.22

the salinity at this site fluctuated more widely. The particulate load (includes both organic and inorganic particles) of water from Geelbek was much greater than that at Oesterwal and fluctuated widely. However, the percent organics present in the particulate material was lower at Geelbek than at Oesterwal indicating suspension of relatively more inorganic particles at Geelbek. The C/N ratio of suspended particulate material was lower at Geelbek than at Oesterwal.

The size distribution of particles in the sediments from Geelbek and Oesterwal is shown in Figure 3A. It is apparant that a large proportion of the sediment at both sites comprised fine (64-250 μm) and coarse (250μm - 1 mm) sand. At both sites the highest percentage of sediment lay in the fine sand fraction. At Geelbek very little sediment was represented in the gravel fraction (>1 mm), while more sediment occurred in the mud fraction (< 64 μm) than at Oesterwal. The organic content of each sediment size fraction (Figure 3B) shows that at both Geelbek and Oesterwal the finest sediment fraction (mud) had the highest percent organics per g dry weight of sediment, especially at Geelbek (±13.5%), while a large proportion (>95%)of the sediment in the coarse and fine categories at both site was inorganic. Nevertheless, at both sites the gravel and mud fractions contributed very little (<10%) to the quantitative total organic content of the sediment (Figure 3C). At Geelbek the organic matter was spread between the coarse sand (44 %) and the fine sand (42 %), i.e. these two fractions contributed similarly to the total organic content. At Oesterwal most (78 %) of the organics present in the sediment occurred in the fine sand fraction, with much less (19.5 %) contained in the coarse sand.

Chlorophyll, total organic matter, organic carbon and total nitrogen

The concentration and distribution of chlorophyll, total organic matter, organic carbon and total nitrogen in sediment and water from Oesterwal and Geelbek are summarized in Figure 4. Statistical comparisons between sites are shown on Figure 4, and those between micro-habitats in Table 2.

The concentration of chlorophyll in sediments at Geelbek (12.7-31.2 μg cm^{-3}) was higher than that at Oestewal (7.2-21.2 μg cm^{-3}) and the differences were significant in all microhabitats except surface sediment at burrow openings (Figure 4). At Oesterwal chlorophyll content of sediment at 10cm depth was significantly lower than that at all microhabitats except burrow lining, while burrow lining did not differ significantly from any other microhabitat sampled (Table 2). At Geelbek chlorophyll content of sediment in *Zostera* beds was significantly higher than at any other microhabitat.

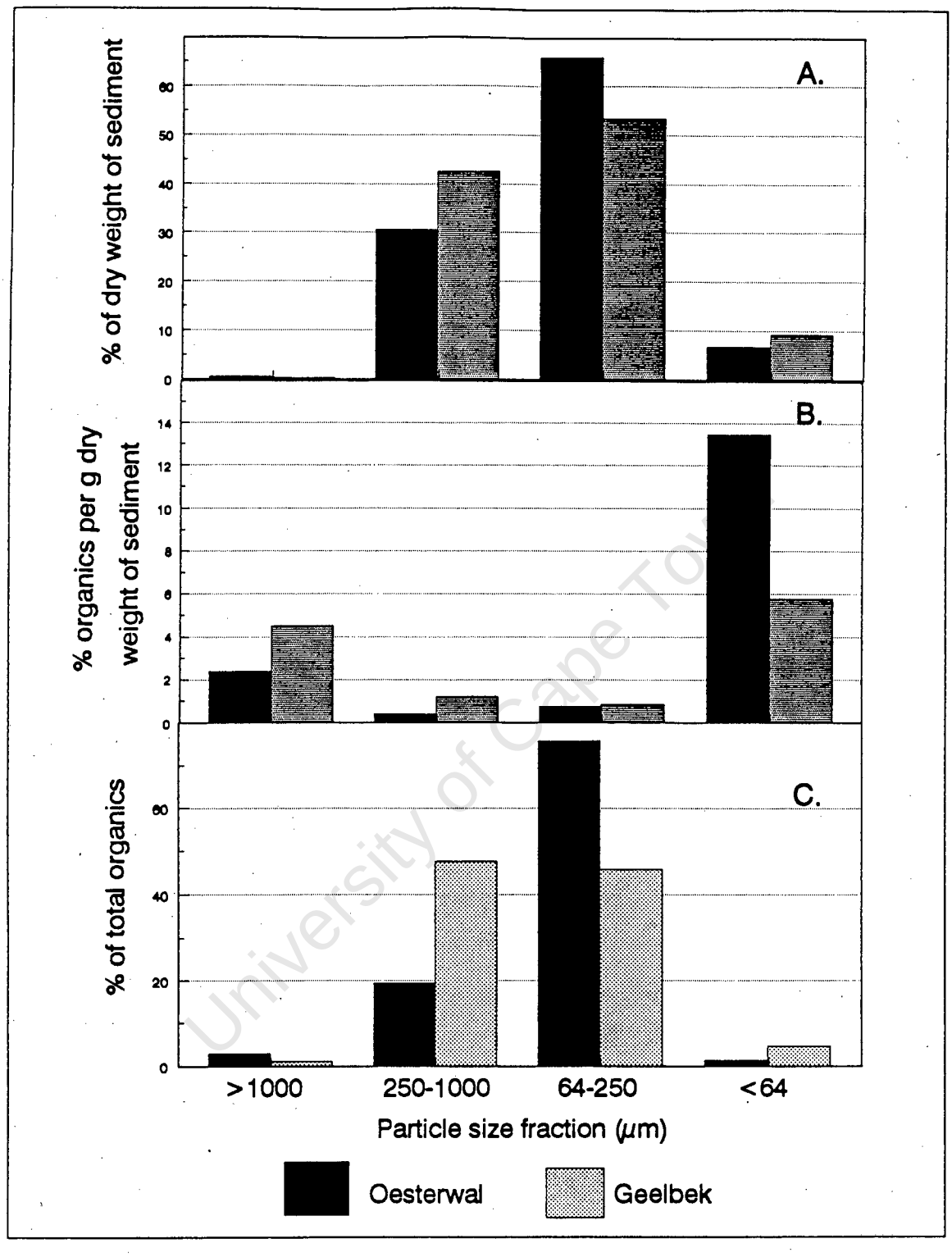

Figure 3. Distribution of organics in different size fractions of sediment at Oesterwal and Geelbek. A. Percentage of sediment represented in each size fraction. B. Organic Content (% of dry weight) of each sediment fraction. C. Percentage of total organics per g of sediment that is represented in each size fraction. Values represent annual means.

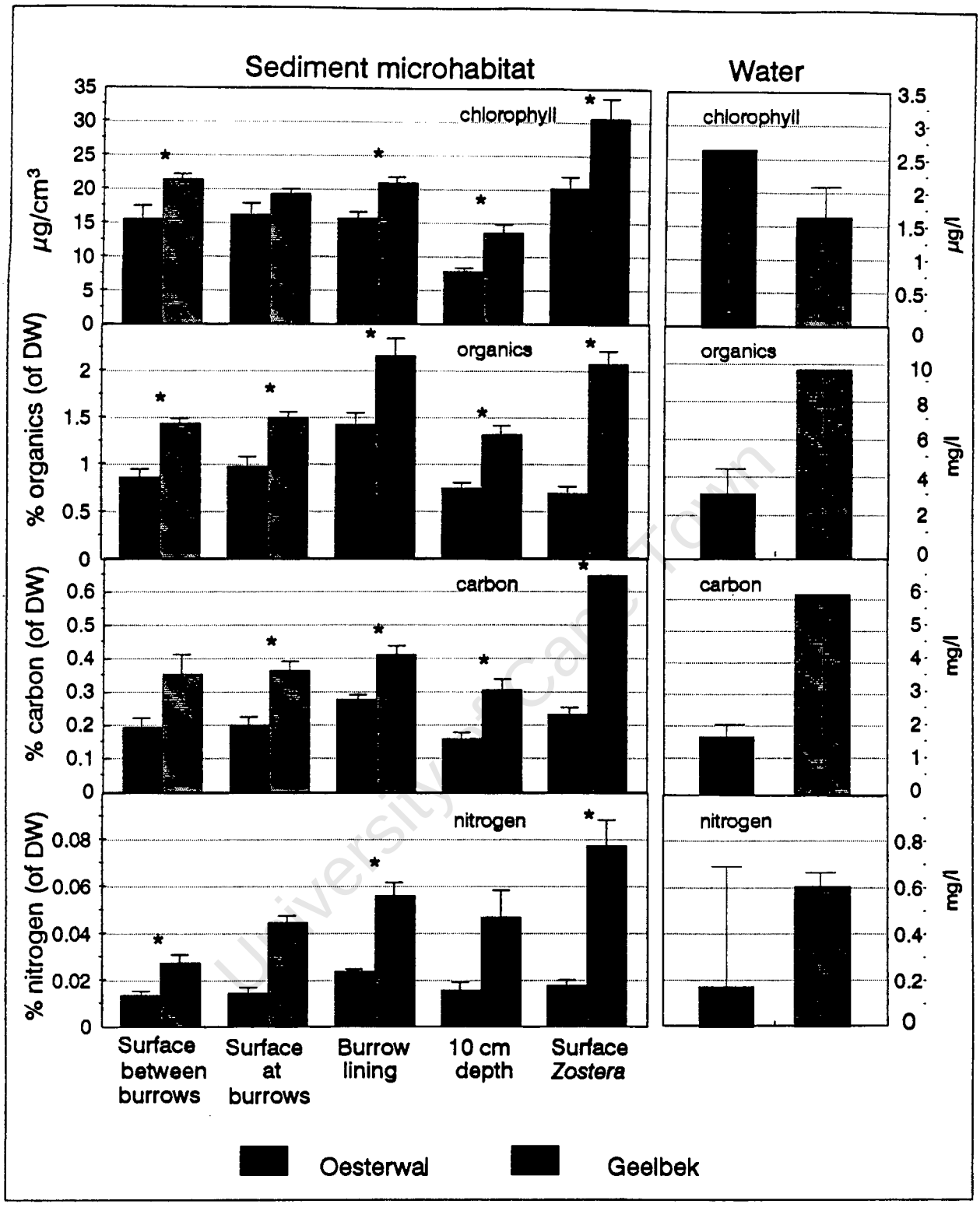

Figure 4. Mean annual chlorophyll, organics, organic carbon and total nitrogen of sediments and water from Oesterwal and Geelbek. Values represent annual means. Error bars represent one standard deviation of the mean. Where there are significant differences (ANOVA; $p<.05$; Tukey test) between sites these are indicate by an *.

Table 2. ANOVA and Tukey test results showing significant differences between sediment microhabitats in terms of percentage chlophyll, organics, carbon and nitrogen (see Figure 4). Similar letters (a,b,c) depict no significant differences ($p < 0.05$) between microhabitats. O = Oesterwaal, G = Geelbek.

Microhabitat	% Chorophyll		% Organics		% Carbon		% Nitrogen	
	O	G	O	G	O	G	O	G
Surface between burrows	b	a	a b	a b	a b	a	a	a
Surface at burrows	b	a	a b	a b	a b	a	a	a
Burrow lining	a b	a	b	c	c	a b	a	a
10 cm depth	a	a	a	a	a	a	a	a
Zostera beds	b	b	a	b c	b c	b	a	a

Table 3. ANOVA and Tukey test results showing significant differences between sediment microhabitats in terms of densities of microbiota (see Figure 5). Similar letters (a,b,c) depict no significant differences ($p < 0.05$) between microhabitats. O = Oesterwaal, G = Geelbek.

Microhabitat	Densities of Microbiota							
	Bacteria		Protozoa		Diatoms		Meiofauna	
	O	G	O	G	O	G	O	G
Surface between burrows	a	a	b	a	a b	a b	b	a b
Surface at burrows	a	a	b	a	a	a	b	a b
Burrow lining	a	a	a	a	b	b	a	a
10 cm depth	a	a	a	a	a b	a b	a	a
Zostera beds	a	a	b	a	a b	b	b	b

In all sediment microhabitats organic content at Geelbek (1.32-2.26 % of dry weight) was significantly higher than at Oesterwal (0.70-1.43 % of dry weight) (Figure 4). At Oesterwal annual means were lowest in sediments from depth and *Zostera* bed, and significantly different to that from burrow lining which had the highest organic content (Table 2). At Geelbek burrow linings had the highest organic content which were significantly higher than those of surface sediments from between burrows, at burrow openings and sediment at depth. Sediment at depth had the lowest organic content differing significantly from that at the burrow lining and in *Zostera* beds (Table 2).

Organic carbon content (% of dry weight) of sediments was higher in all microhabitats at Geelbek (0.31-0.65 %) compared to Oesterwal (0.16-0.28 %), although the difference was not significant for surface sediments between burrows (Figure 4). At both sites burrow lining and *Zostera* beds sediments contained the highest concentrations of organic carbon. At Geelbek the percent carbon in sediments from *Zostera* beds was significantly higher than in other surface sediments and from depth, but not significantly different from the burrow lining (Table 2). At Oesterwal the percent organic carbon of sediment from burrow lining was significantly higher than at all other microhabitats except *Zostera* beds, while the carbon content of sediments from *Zostera* bed was only significantly higher than that in sediment from depth (Table 2).

Although annual mean values show a trend of much higher total nitrogen content in all sediments of Geelbek (0.028-0.078 % of dry weight) compared to Oesterwal (0.014-0.024 %), nitrogen content was significantly higher (by 2 to 4.3 times) at Geelbek only in sediments from the surface between burrows, the burrow linings and *Zostera* beds (Figure 4). In addition, although annual mean values indicate that nitrogen content of sediments from the burrow linings and *Zostera* beds were higher than at other microhabitats at both sites, there were no significant differences between microhabitats at either Geelbek or Oesterwal (Table 2).

In summary, the key features that have emerged are that Geelbek sediments exhibit higher percentages of chlorophyll, organic matter, organic carbon and total nitrogen than do Oesterwal sediments. The differences between the sites are least for chlorophyll and appear most for total nitrogen, although nitrogen values were highly variable resulting in significant differences being obscured. In general, lowest values of the parameters were found in sediments from depth, while maximum values were associated with burrow lining and *Zostera* beds.

In the water column, chlorophyll content was higher at Oesterwal (2.66 $\mu g\ l^{-1}$) than at Geelbek (1.65 $\mu g\ l^{-1}$) (Figure 4), while the reverse trend was

apparant for the concentration of total organics (9.95 and 3.40 mg l^{-1}), organic carbon (6.36 and 1.84 mg l^{-1}) and total nitrogen (0.61 and 0.18 mg l^{-1}).

Density and distribution of microbiota

The density and distribution of microbiota in sediments and water from Oesterwal and Geelbek are summarized in Figure 5.

Bacteria. Bacterial densities were significantly higher by 2.2 to 2.6 times at Geelbek (6.27-9.64 x 10^9 cells cm^{-3}) than at Oesterwal (2.68-3.38 x 10^9 cells cm^{-3}) in sediments of all 5 microhabitats (Figure 5). Although annual means at Oesterwal showed slightly higher densities at the burrow lining and in *Zostera* beds, than in other microhabitats, the differences were not significant (Table 3). At Geelbek bacterial densities were highest in sediments at depth and at burrow linings, followed by *Zostera* bed.

Protozoa. Sediment protozoan densities were significantly higher by 1.8 to 5.0 times in all microhabitats at Geelbek (2.82-4.54 x 10^4 individuals cm^{-3}) compared to those at Oesterwal (0.63-1.99 x 10^4 individuals cm^{-3}) except for *Zostera* beds, where the annual mean value was higher at Geelbek, but the difference was not significant (Figure 5). A comparison of the density of protozoans in different microhabitats revealed that at Oesterwal surface sediments between and at burrow openings and in *Zostera* bed had similar protozoan densities, but significantly higher values than sediments of burrow linings and at depth (Table 3). At Geelbek, mean annual values were similarly high for surface sediments, but protozoan densities did not differ significantly between the different microhabitats.

Diatoms. Diatoms occurred in far higher densities in sediments from all microhabitats sampled at Geelbek (54.83-78.47 x 10^4 cells cm^{-3}) than those at Oesterwal (5.78-12.14 x 10^4 cells cm^{-3}) (Figure 5). Diatoms were approximately 6.5 to 9.5 times more abundant in Geelbek sediments than in Oesterwal sediments. At Oesterwal the mean annual diatom density was highest in sediment from burrow linings, but only significantly higher than that from surface sediment at the burrow opening (Table 3). At Geelbek diatom densities were greatest at the burrow lining and in *Zostera* beds, but these values were only significantly higher than that for the burrow opening (Table 3).

Meiofauna. Sediment meiofauna densities were similar at Oesterwal (7.48-171.91 individuals cm^{-3}) and Geelbek (23.47-237.18 individuals cm^{-3}) for all microhabitats except sediment at depth, where densities significantly higher at Geelbek (Figure 5). Although the annual mean value in sediment from *Zostera* beds was higher for Geelbek, this difference was not significant. At Oesterwal mean

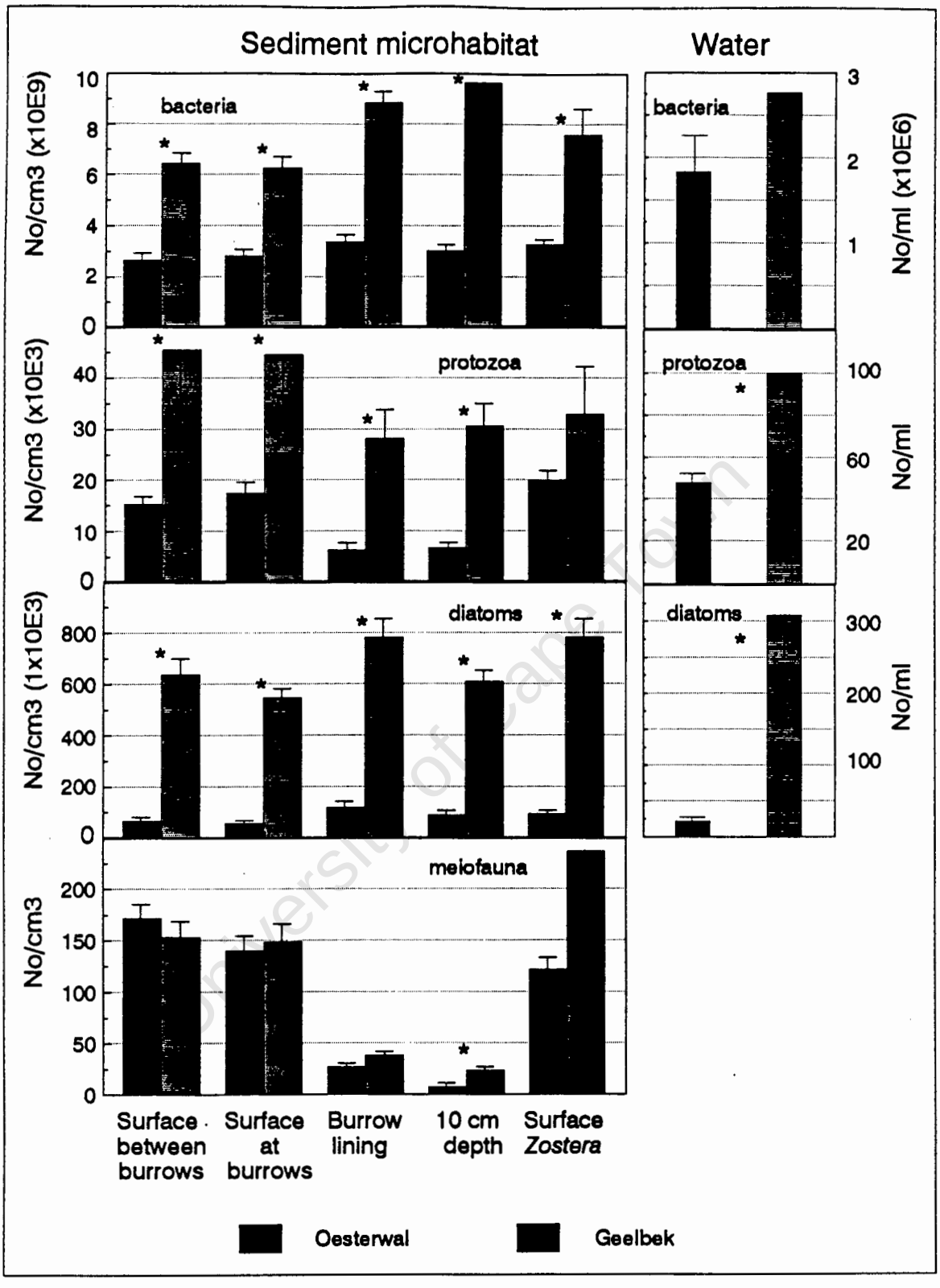

Figure 5. The density and distribution of microbiota in sediments and water from Oesterwal and Geelbek. Values represent annual means. Error bars represent one standard deviation of the mean. Where there are significant differences (ANOVA; $p < 0.05$; Tukey test) between sites these are indicated by an *.

annual densities were similar in all surface sediments, and these were significantly higher (approximately 8 times) than sediments from burrow lining and depth (Table 3). The same pattern existed at Geelbek, with meiofaunal densities being lower at the burrow lining and at depth. These two microhabitats had values that were significantly lower (approximately 8 times) than in *Zostera* beds, although not differing significantly from those of surface sediments at and between burrow openings.

Water column. In the water column, annual mean densities of bacteria (2.77×10^9 and 1.84×10^9 cells l^{-1}), protozoa (10.00×10^4 and 4.77×10^4 individuals l^{-1}) and diatoms (30.84×10^4 and 2.02×10^4 cells l^{-1}) were higher at Geelbek than at Oesterwal, respectively.

In summary, the densities of bacteria, protozoa and diatoms were invariably higher in all microhabitats at Geelbek compared to Oesterwal, while meiofaunal densities were similar at the two sites. In general, surface sediments (at and between burrow openings and in *Zostera* beds) had higher densities of protozoa and meiofauna than did sediments from depth and the burrow lining, while the highest densities of bacteria and diatoms were found in sediments from burrow linings.

Composition of protozoan, diatom and meiofaunal populations

At both Oesterwal and Geelbek the sediment protozoan communities were numerically dominated by flagellates, which were an order of magnitude more abundant than other groups, in all microhabitats (Figure 6). While flagellate densities were highest in surface sediments at and between burrows, appreciable numbers occrrred in the other 3 habitats. Ciliates were concentrated at both sites in surface sediments at and between burrow openings, with very few occurring in sediments at the other microhabitats. At both sites small ciliates and flagellates were generally far more abundant than their large counterparts. The water column protozoan community was overwhelmingly dominated (in terms of density) by small flagellates at both Oesterwal and Geelbek (Figure 6).

Sediment diatoms at Geelbek were dominated by centric diatoms (Figure 7). There were approximately 2-26 times more centric than pennate diatoms, and 14-19 times more centric than chain-forming diatoms. At this site the centric diatoms comprised mostly small cells in all sediment microhabitats. Small pennate diatoms were prominant in surface sediments at Geelbek, but in other microhabitats occurred in low densities compared to medium and large pennate cells. The densities of centric diatoms in Oesterwal sediments were similar to those

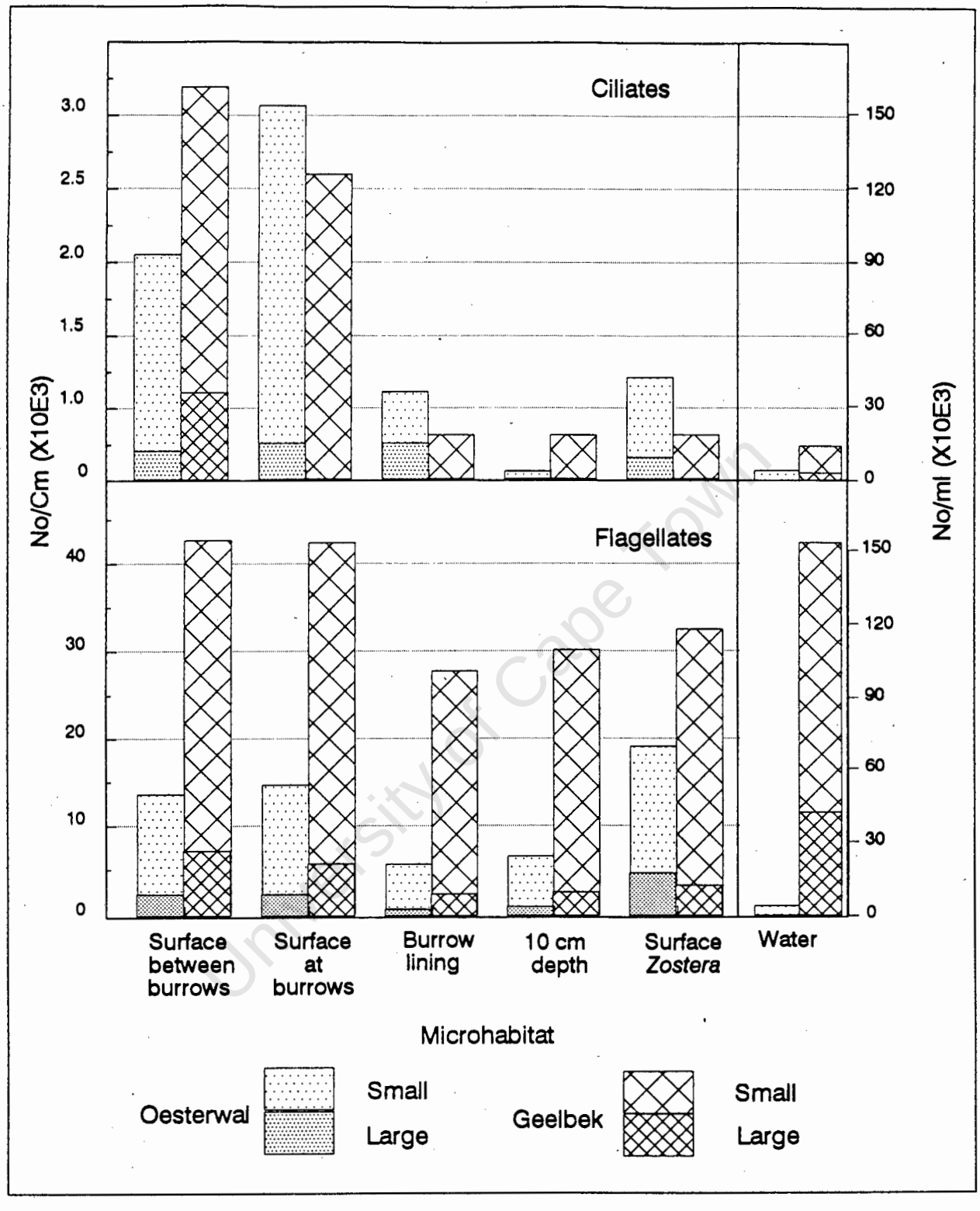

Figure 6. Size composition of ciliate and flagellate populations in sediments and water from Oesterwal and Geelbek. Values represent annual means. Size categories are as follows: large ciliates (50X30μm), small ciliates (30X25μm), large flagellates (60X40μm), small flagellates (30X25μm).

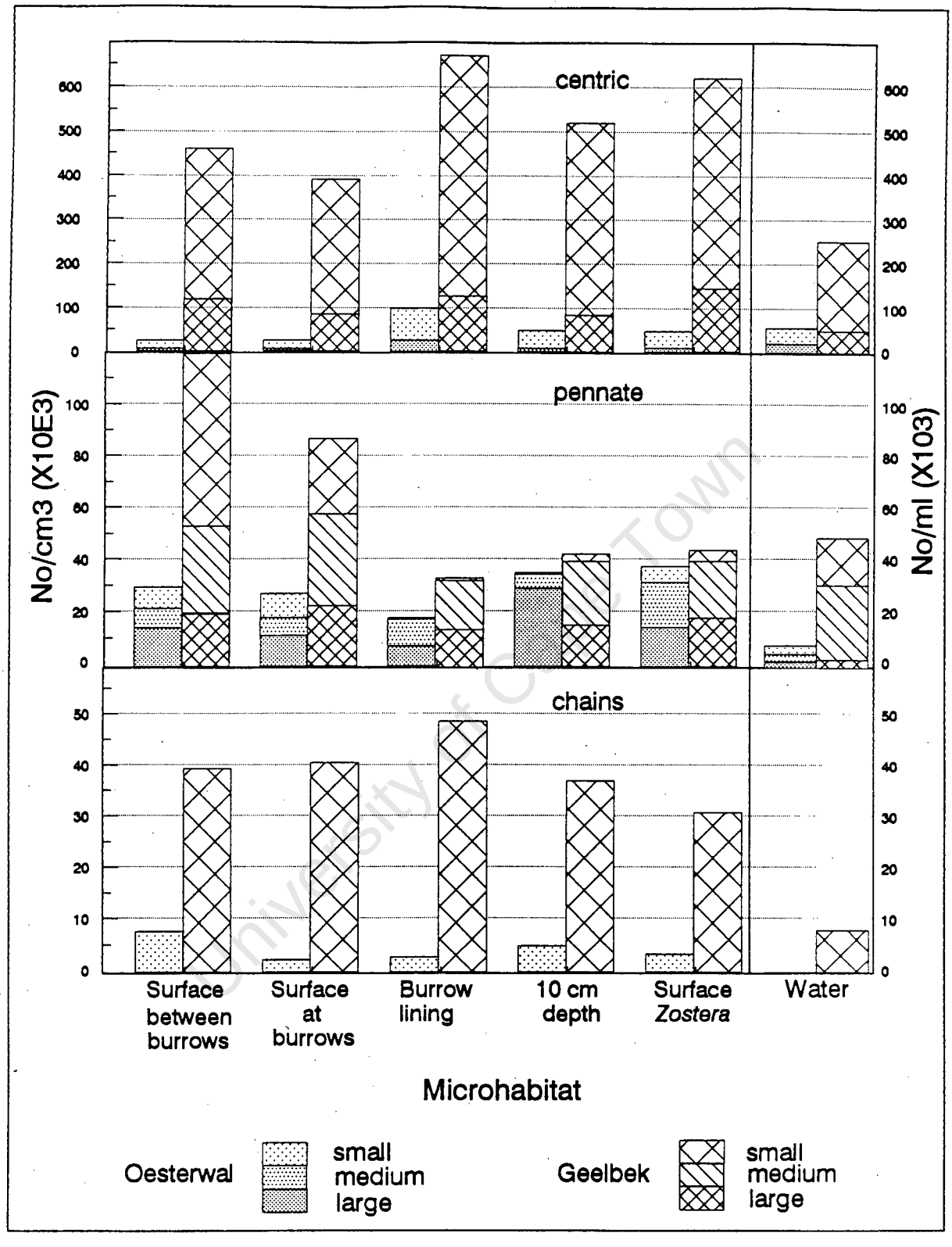

Figure 7. Size composition of diatom populations in sediments from Oesterwal and Geelbek. Values represent annual means. Size categories are as follows: large = 50X35 and 75X35μm, medium = 25X10μm, small = 25X10 and 30X5μm.

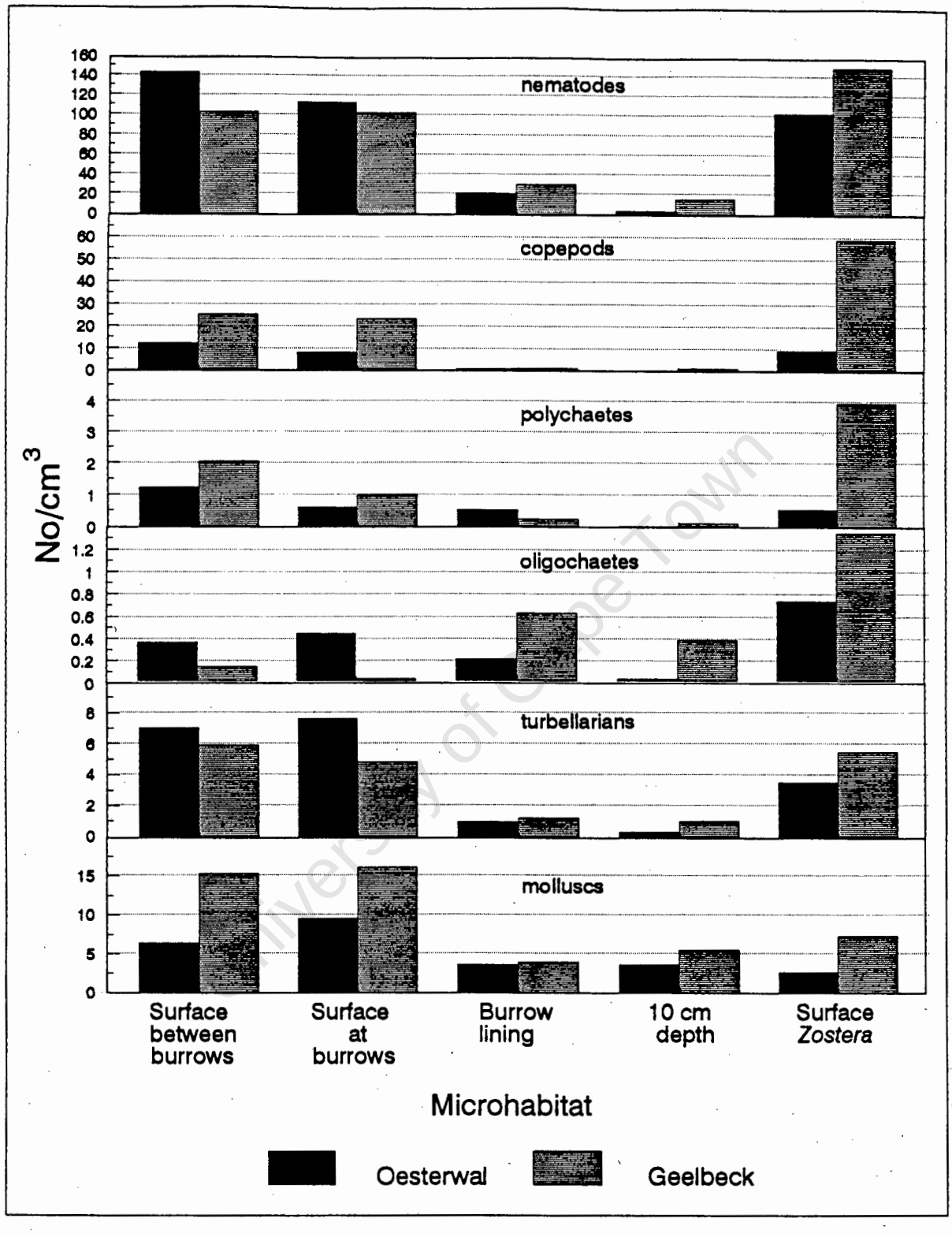

Figure 8. Composition of meiofauna in sediments from Oesterwal and Geelbek. Values represent annuals means.

of pennate diatoms. As at Geelbek, centric diatoms at this site were generally dominated by small cells, while pennate diatoms were dominated by large cells. Relatively low numbers of chain-forming cells occurred at Oesterwal compared to Geelbek. Diatoms in the water column at both sites comprised centric, pennate and chain-forming cells (Figure 7), although the latter occurred in low numbers at Oesterwal. The centric diatoms in the water at both sites were dominated by small cells, while the pennate diatoms consisted largely of medium and small cells.

Nematodes dominated the sediment meiofaunal communities in all 5 microhabitats at both sites (Figure 8). At both Oesterwal and Geelbek nematodes were present in greatest densities in surface sediments between and at burrow openings and *Zostera* beds, with very low numbers present at depth and in sediment from burrow linings. Similarly harpacticoid copepods, polychaetes and turbellarians were depressed in density in sediments at depth and at burrow linings, when compared to other microhabitats at both sites. This pattern was also apparent for oligochaetes at Oesterwal, but at Geelbek this group had its greatest densities at burrow lining, depth and especially *Zostera* bed.

Carbon and nitrogen biomass of microbiota

The source and distribution of organic carbon and nitrogen biomass in sediments and water are summarized in Figures 9 and 10.

The sediment bacterial carbon biomass was significantly higher (2.4 to 3.2 times) in all microhabitats at Geelbek (225.7-839.3 μg gDW^{-1}) compared to Oesterwal (87.8-286.5 μg gDW^{-1}) (Figure 9). Similarly bacterial nitrogen biomass in sediments of Geelbek (25.34-94.24 μg.gDW-1) was 2.4 to 3.3 times higher than those of Oesterwal (9.85-32.16) for all microhabitats (Figure 10). At both Geelbek and Oesterwal carbon and nitrogen bacterial biomass values were significantly higher in surface sediments from *Zostera* beds than in the other 4 microhabitats among which significant differences were not detected (Table 3 & 4). Carbon and nitrogen bacterial biomass was significantly higher in the water column at Geelbek (159.71 $\mu g\ l^{-1}$) than at Oesterwal (106.00 $\mu g\ l^{-1}$) (Figures 9 & 10, respectively).

Protozoan carbon biomass was 2.6-3.5 times higher in sediments from Geelbek (54.4-130.39 μg gDW^{-1}) than in those from Oesterwal (16.46-64.66 μg gDW^{-1}), although in sediments from *Zostera* beds no significant difference was detected between the two sites (Figure 9). The protozoan nitrogen biomass displayed the same pattern of differences between the two sites, with values at Geelbek (13.35-32.27 μg gDW^{-1}) 2.5 to 3.8 times higher than at Oesterwal (4.11-16.16 μg gDW^{-1}) (Figure 10). At Oesterwal annual means for both carbon and

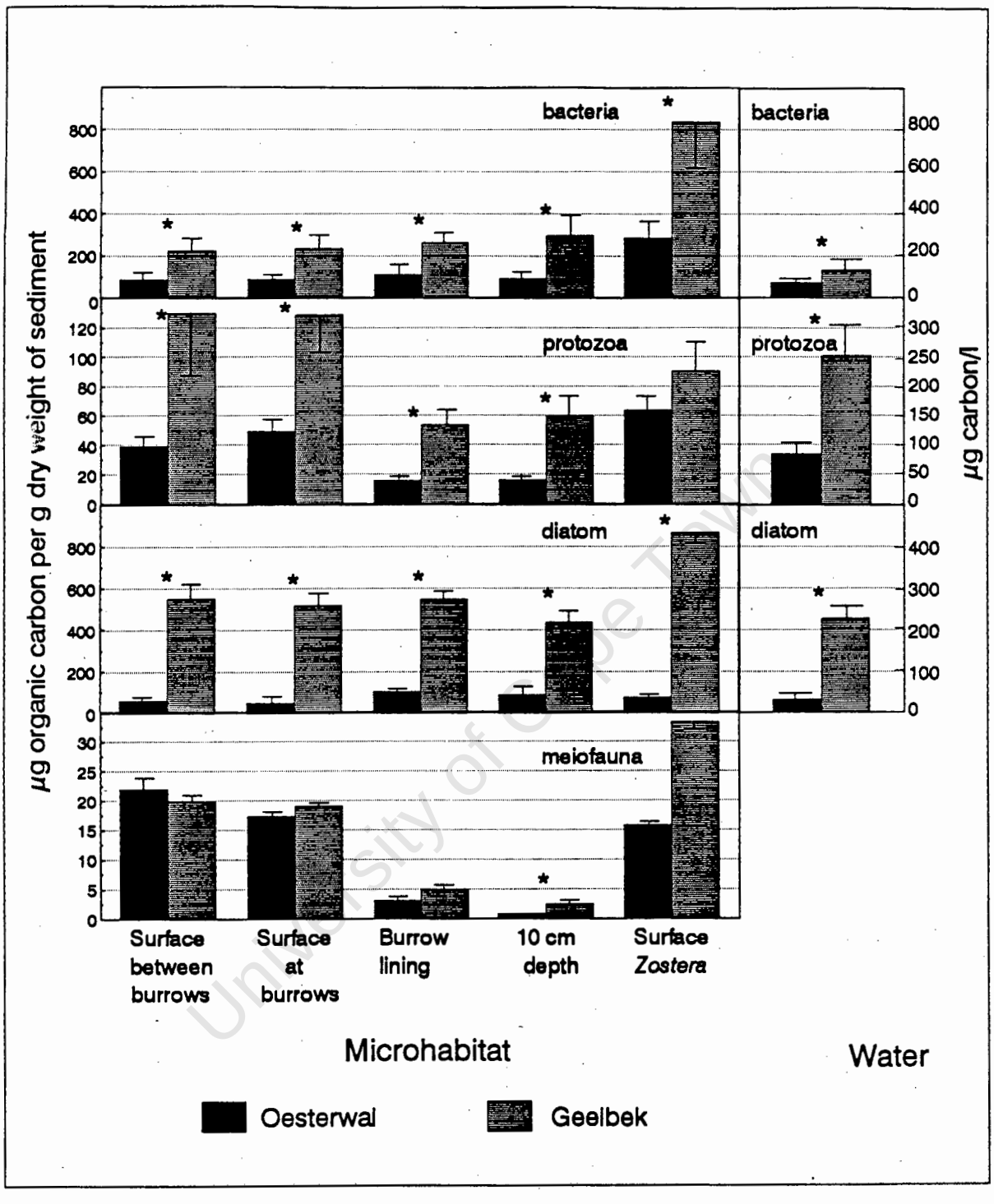

Figure 9. Carbon biomass of microbiota in sediments and water from Oesterwal and Geelbek. Values represent annual means. Error bars represent one standard deviation of the mean. Where there are significant differences (ANOVA; $p<0.05$; Tukey test) between sites these are indicated by an *.

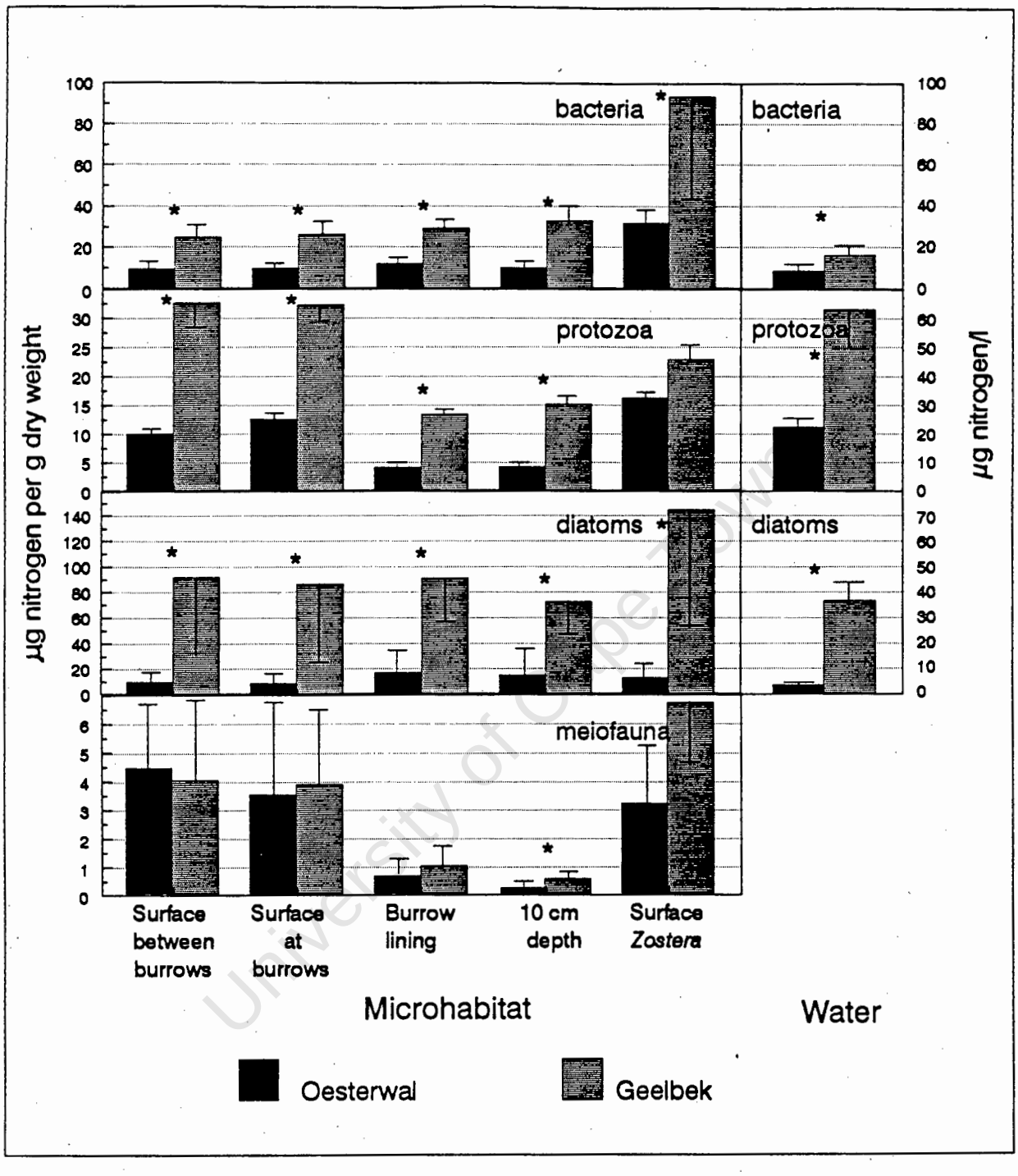

Figure 10. Nitrogen biomass of the microbiota in sediments and water from Oesterwal and Geelbek. Values represent annual means. Error bars represent one standard deviation of the mean. Where there are significant differences (ANOVA; $p < 0.05$;Tukey test) between sites these are indicated by an *.

Table 4. ANOVA and Tukey test results showing significant differences between sediment microhabitats in terms of carbon biomass contributed by different biotic components (see Figure 9). Similar letters (a,b,c) depict no significant differences ($p < 0.05$) between microhabitats. O = Oesterwaal, G = Geelbek.

Microhabitat	Organic Carbon		Bacteria		Protozoa		Diatoms		Meiofauna	
	O	G	O	G	O	G	O	G	O	G
Surface between burrows	a b	a	a	a	a b	a	a	a	b	a b
Surface at burrows	a b	a	a	a	b	a	a	a	b	a b
Burrow lining	c	a b	a	a	a	a	a	a	a	a
10 cm depth	a	a	a	a	a	a	a	a	a	a
Zostera beds	b c	b	c	c	c	a	a	b	b	b

Table 5. ANOVA and Tukey test results showing significant differences between sediment microhabitats in terms of nitrogen biomass contributed by different biotic components (see Figure 10). Similar letters (a,b,c) depict no significant differences ($p < 0.05$) between microhabitats. O = Oesterwaal, G = Geelbek.

Microhabitat	Total nitrogen		Bacteria		Protozoa		Diatoms		Meiofauna	
	O	G	O	G	O	G	O	G	O	G
Surface between burrows	a	a	a	a	a b	a	a	a	b	a b
Surface at burrows	a	a	a	a	b	a	a	a	b	a b
Burrow lining	a	a	a	a	a	a	a	a	a	a
10 cm depth	a	a	a	a	a	a	a	a	a	a
Zostera beds	a	a	c	c	c	a	a	b	b	b

nitrogen biomass of protozoa were significantly higher in surface sediments from the burrow opening and *Zostera* beds than those from the burrow lining and depth, while at Geelbek no differences were detected in protozoan biomass among the five microhabitats (Table 3 & 4). The carbon and nitrogen protozoan biomass in the water column was much higher at Geelbek (252 and 63 $\mu g\ l^{-1}$, for carbon and nitrogen respectively) than at Oesterwal (92 and 23.01 $\mu g\ l^{-1}$, respectively) (Figures 9 and 10, respectively).

Diatom carbon biomass was much higher (4.7 to 10.6 times) at Geelbek (437-871 $\mu g\ gDW^{-1}$) than Oesterwal (53-105 $\mu g\ gDW^{-1}$), in all sediment microhabitats sampled (Figure 9). Similarly, in all microhabitats diatom nitrogen biomass at Geelbek (72.85-145.09 $\mu g\ gDW^{-1}$) significantly exceeded that at Oesterwal (8.76-15.21 $\mu g\ gDW^{-1}$) by 4.7 to 10.4 times (Figure 10). At Oesterwal both carbon and nitrogen diatom biomass did not differ significantly between sediment microhabitats, while at Geelbek diatom carbon and nitrogen biomass was significantly higher in surface sediments from *Zostera* beds than in any other microhabitat (Table 3 & 4). Geelbek carbon and nitrogen diatom biomass in Geelbek water (224 and 37.9ug l^{-1}, respectively) was much higher than in Oesterwal water (18.25 and 2.93 μg l-1, respectively) (Figures 9 and 10, respectively).

Meiofaunal carbon biomass ranged between 2.6 and 33.3 $\mu g\ gDW^{-1}$ at Geelbek, and 0.5 and 22.0 $\mu g\ gDW^{-1}$ at Oesterwal (Figure 9), while nitrogen biomass ranged between 0.51-6.67 $\mu g\ gDW^{-1}$ at Geelbek and 0.1-4.4 $\mu g\ gDW^{-1}$ at Oesterwal (Figure 10). There were no significant differences between the two sites in the carbon or nitrogen biomass in any of the microhabitats, except in sediment from depth where the biomass at Geelbek was greater than at Oesterwal. At Oesterwal meiofaunal carbon and nitrogen biomass was significantly higher in surface sediments between and at burrow openings and in *Zostera* beds compared to sediment at burrow lining and at depth (Table 3 & 4). However at Geelbek only values for surface sediment in *Zostera* beds were significantly higher than those for sediment from burrow lining and depth.

In summary, the carbon and nitrogen biomass contributed by each of bacterial, protozoan and diatom populations in the sediment and water column was higher at Geelbek than at Oesterwal. Meiofaunal biomass was, however, not significantly higher at Geelbek than at Oesterwal. Protozoan and meiofaunal biomass values were highest in the surface sediments, while differences between microhabitats in terms of bacterial and diatom biomass were not as great. However, surface sediments in *Zostera* beds consistantly supported high microbial and meiofaunal biomass.

Productivity of benthic microbiota

The present study indicates that the standing stocks of bacteria and micro-algae in terms of carbon biomass in surface sediments of Geelbek were significantly higher than those of Oesterwal. Estimates of bacterial growth rates taken simultaneously with standing stock estimates (see Appendix 1) indicated that bacterial productivity was higher at Geelbek (3.52 μgC day^{-1} gDW^{-1}) than at Oesterwal (2.99 μgC day^{-1} gDW^{-1}) although the specific growth rate (production/standing crop) was higher at Oesterwal (Table 5). Similarly micro-algal productivity was higher at Geelbek (5.45 x 10^{4} μgC day^{-1} m^{-2}) than at Oesterwal (2.75 x 10^{4} μgC day^{-1} m^{-2}) (Fielding *et al.* 1988), while specific growth rate at Oesterwal was higher.

Contribution of each biotic component to total organic carbon and nitrogen

Carbon: At both sites the non-living detrital component of the total organic carbon dominated in both the sediment and water column (Figure 11). The contribution of the biotic component to total organic carbon in the sediments was much higher at Geelbek (21-28%) than at Oesterwal (8-19%) (Figure 11). At Geelbek the greatest biotic contribution to the carbon resource came from diatoms followed by bacteria and to a lesser extent protozoans. At Oesterwal the largest biotic contributors to the carbon resource were bacteria, followed by diatoms and protozoans. At both sites meiofauna represented a very small percent of the carbon. At Geelbek the proportion of the total organic carbon contributed by the biotic fraction was highest in surface sediment in *Zostera* bed (72% non-living detrital material), lowest in sediment from *U. africana* burrow lining (79% detritus), and intermediate and similar in sediments from the other microhabitats (74-75% detritus). At Oesterwal the proportion of organic carbon attributed to biota was also highest in *Zostera* bed (81% non-living detrital material), lowest in sediments associated with *C. kraussi* burrows, i.e. at burrow openings burrow linings (respectively, 90-92% detritus), and intermediate at the surface between burrow openings and at depth.

Nitrogen: The contribution by the biotic components in the sediment to total nitrogen was higher at Geelbek (26-55%) than Oesterwal (14-36%) for all microhabitats examined except surface sediments in *Zostera* bed (which were similar) (Figure 12). In general the biota contributed a greater proportion of nitrogen to the total than they did carbon. At Oesterwal, the most important contribution was made by bacteria, protozoa and diatoms, with a low but significant contribution by meiofauna in all microhabitats except burrow lining and depth. At Geelbek the most important biotic contributor to nitrogen content of the sediments

Table 6. Turnover of bacteria and micro-algae in surface sediments at Oestewal and Geelbek, Langebaan lagoon. Values represent annual means, except for autumn estimations for micro-algal production. Bacteria specific growth rate u(day^{-1}) = production/standing crop

	Sampling sites		Source
	Oestewal	Geelbek	
BACTERIA			
Standing crop ($ugC.gDW^{-1}$)	89.07	231.20	Present study
Production ($ugC.day^{-1}.gDW^{-1}$)	2.99	3.52	Tibbles *et al.* 1992
Specific growth rate ($u(day^{-1})$)	0.034	0.015	
MICRO-ALGAE			
Standing crop ($ugC.gDW^{-1}$)	56.73	536.72	Present study
Production ($ugC.day^{-1}.m^{-2}$)	$2.75x10^4$	$5.45x10^4$	Fielding *et al.* 1988

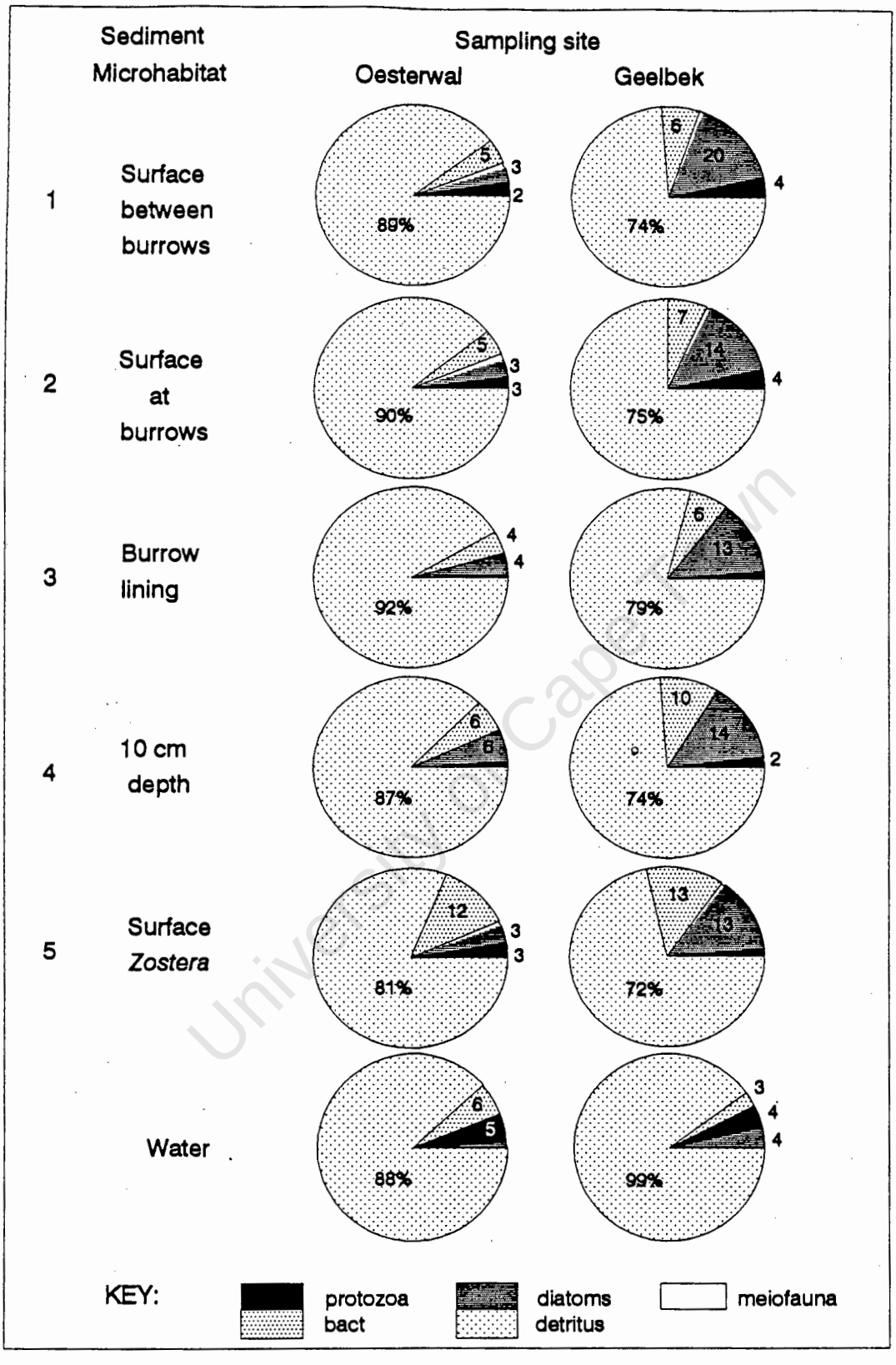

Figure 11. Percentage of total carbon in the sediment and the water contributed by each fraction at Oesterwal and Geelbek. Values represent annual means. Percentages >2 are depicted on the figure.

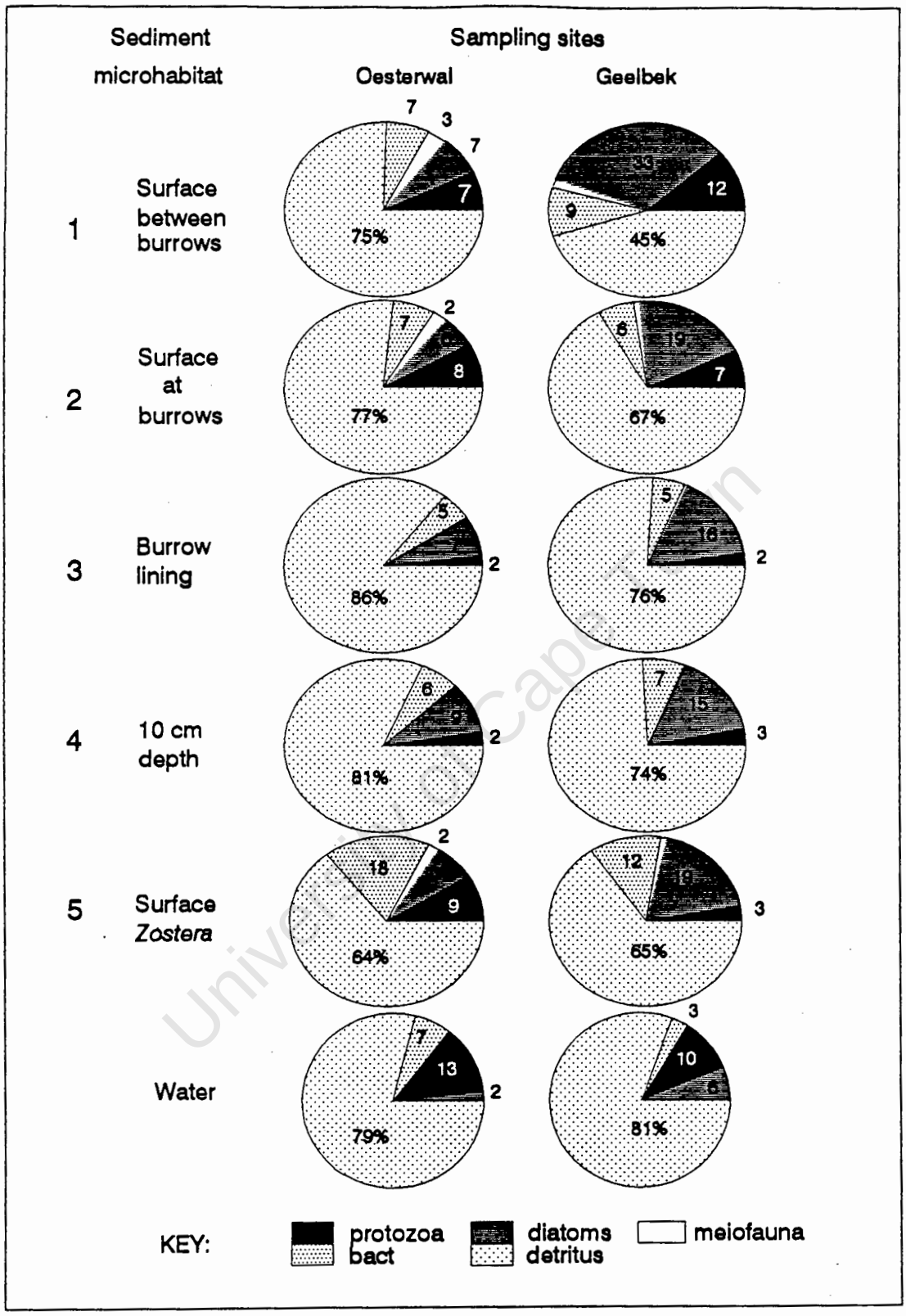

Figure 12. Percentage of total nitrogen in the sediment and the water contributed by each fraction at Oesterwal and Geelbek. Values represent annual means. Percentages >2 are depicted on the figure.

appeared to be diatoms followed by bacteria and protozoans. The contribution by meiofauna was minimal. At Oesterwal the biota formed the highest percentage of total sediment nitrogen at the surface of *Zostera* bed (only 64% was contributed by detritus), followed by surface sediments between and at burrow openings (75-77% detritus), while their contribution was lower at burrow lining and at depth (81-86% detritus). At Geelbek the biota contributed most to total nitrogen in surface sediments between burrow openings (only 45% non-living detrital material), followed by surface sediments at burrow openings and in *Zostera* bed (65-67% detritus), while lower proportions were contributed by biota at the burrow linings and at depth (74-76% detritus).

Water: In the water column the picture was somewhat different, with the contribution of the biotic fraction to the total organic carbon and nitrogen being slightly higher at Oestewal than at Geelbek (Figures 11 & 12) and, in this regard, protozoans appeared to be the prime source followed closely by bacteria and diatoms at Oesterwal and Geelbek, respectively.

Size composition of carbon and nitrogen biomass

At Oesterwal the majority of the total carbon and nitrogen biomass (bacteria, protozoa, diatoms and meiofauna) was contained in the <2 and 20-64 μm size fractions, while at Geelbek there was a greater spread of both the carbon and nitrogen biomass over the <2, 2-20 ad 20-64 μm fractions, with the highest biomass in the 20-64 μm fraction (Figures 13 & 14). At both sites very little carbon and nitrogen biomass was represented in the >64 μm size fraction (Figure 13 & 14). A similar pattern of size distribution to that found in sediments was observed for carbon and nitrogen biomass of water sorted by size (Figures 13 & 14).

Seasonality

The seasonal variations in the organic carbon and total nitrogen in the sediments and water column are shown in Figure 15. Clear patterns of seasonal differences in sediment micro-habitats were not detected for either Oesterwal or Geelbek. In terms of organic carbon, seasonal variation was greatest in the water column and in sediments from *Zostera* beds. In general seasonal variation in both carbon and nitrogen was greater at Geelbek than at Oesterwal.

Figure 16 shows the seasonal variation in the percentage of the living component of the resources available in surface sediments between burrows and water at Oesterwal and Geelbek. This provides an estimation of changes in the

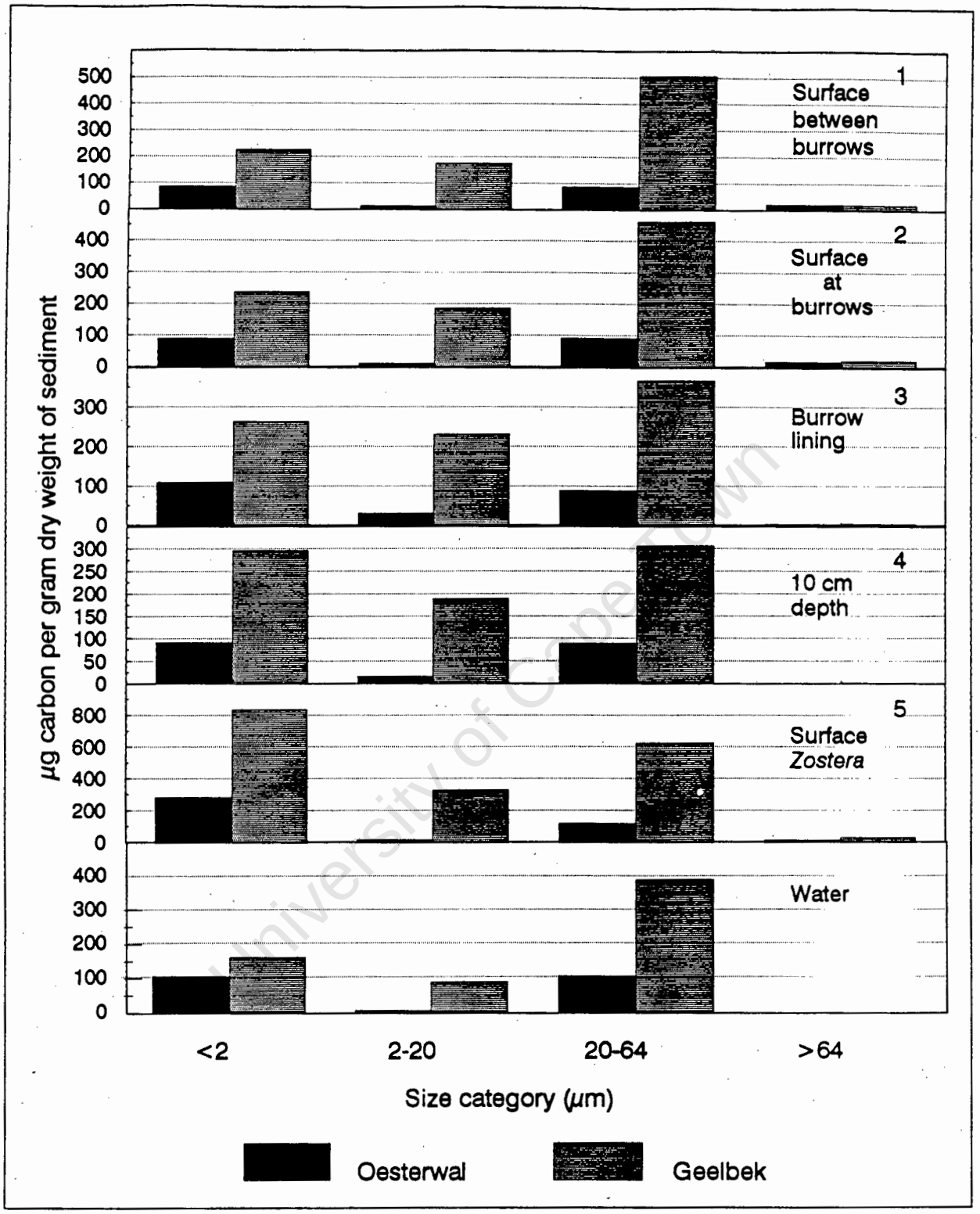

Figure 13. Amount of carbon available form different biotic size fractions in sediments and water at Oesterwal and Geelbek.

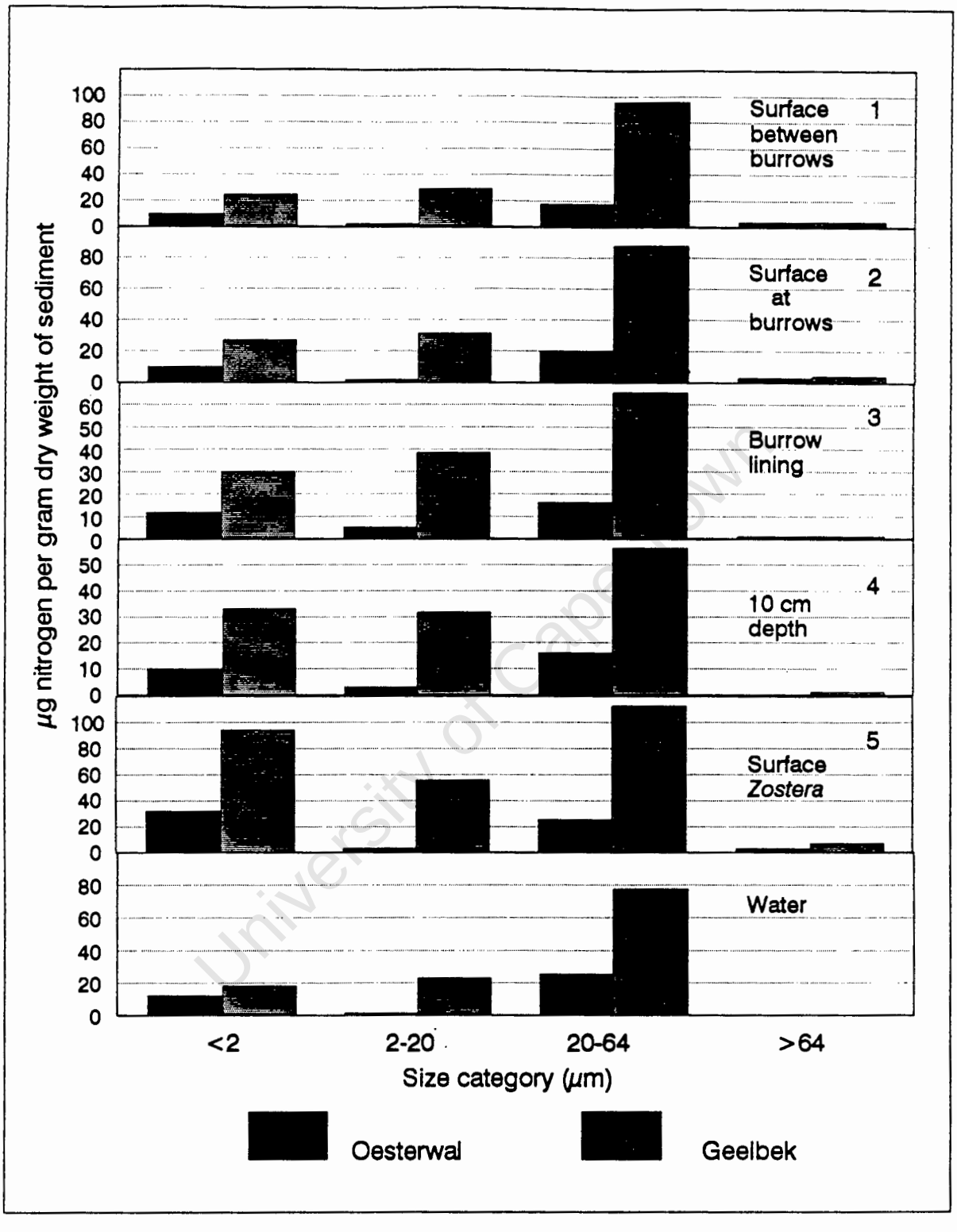

Figure 14. Amount of nitrogen available from different size fractions in sediment and water at Oesterwal and Geelbek.

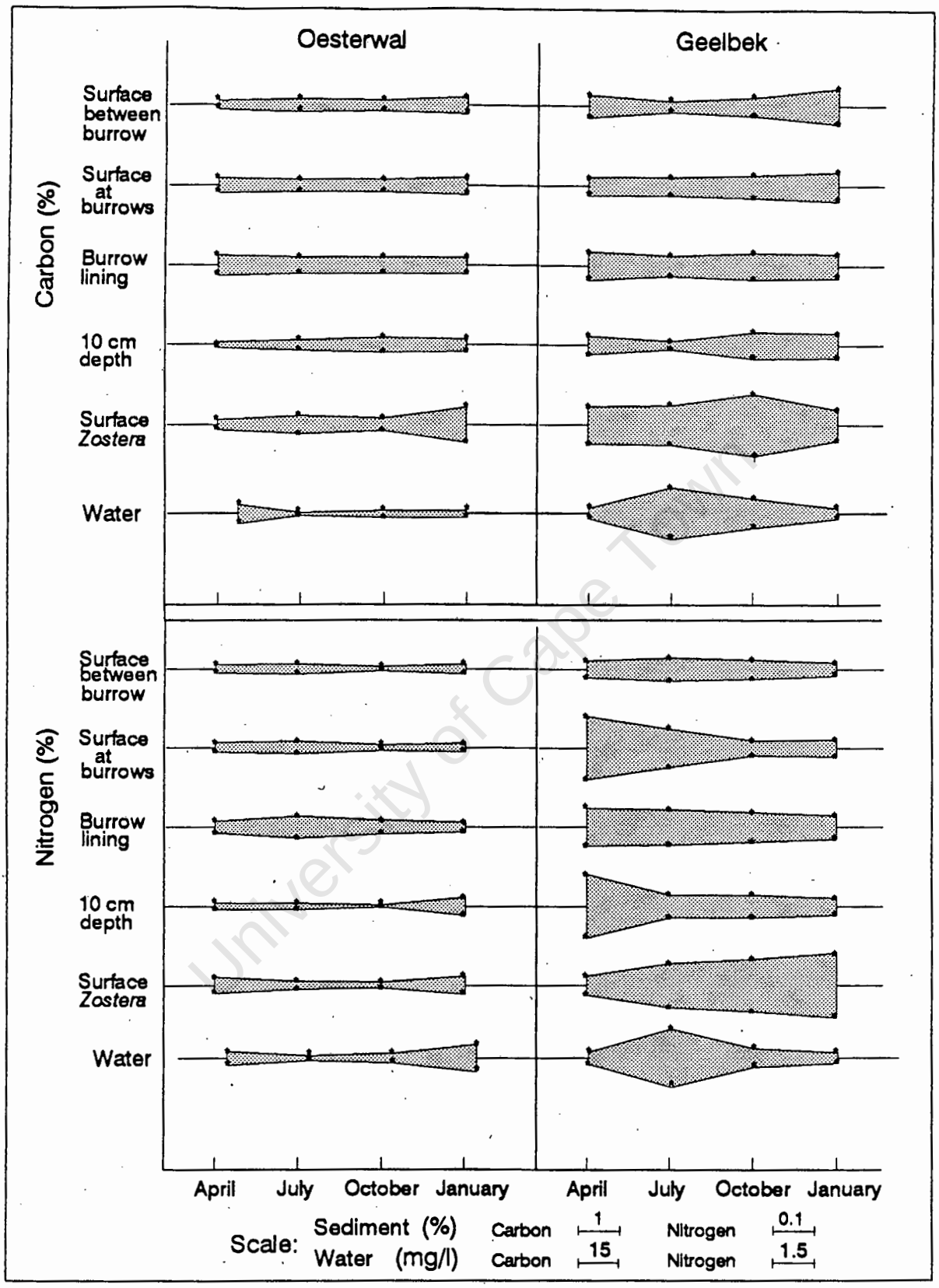

Figure 15. Seasonal variation in organic carbon and total nitrogen content of sediments from Oesterwal and Geelbek.

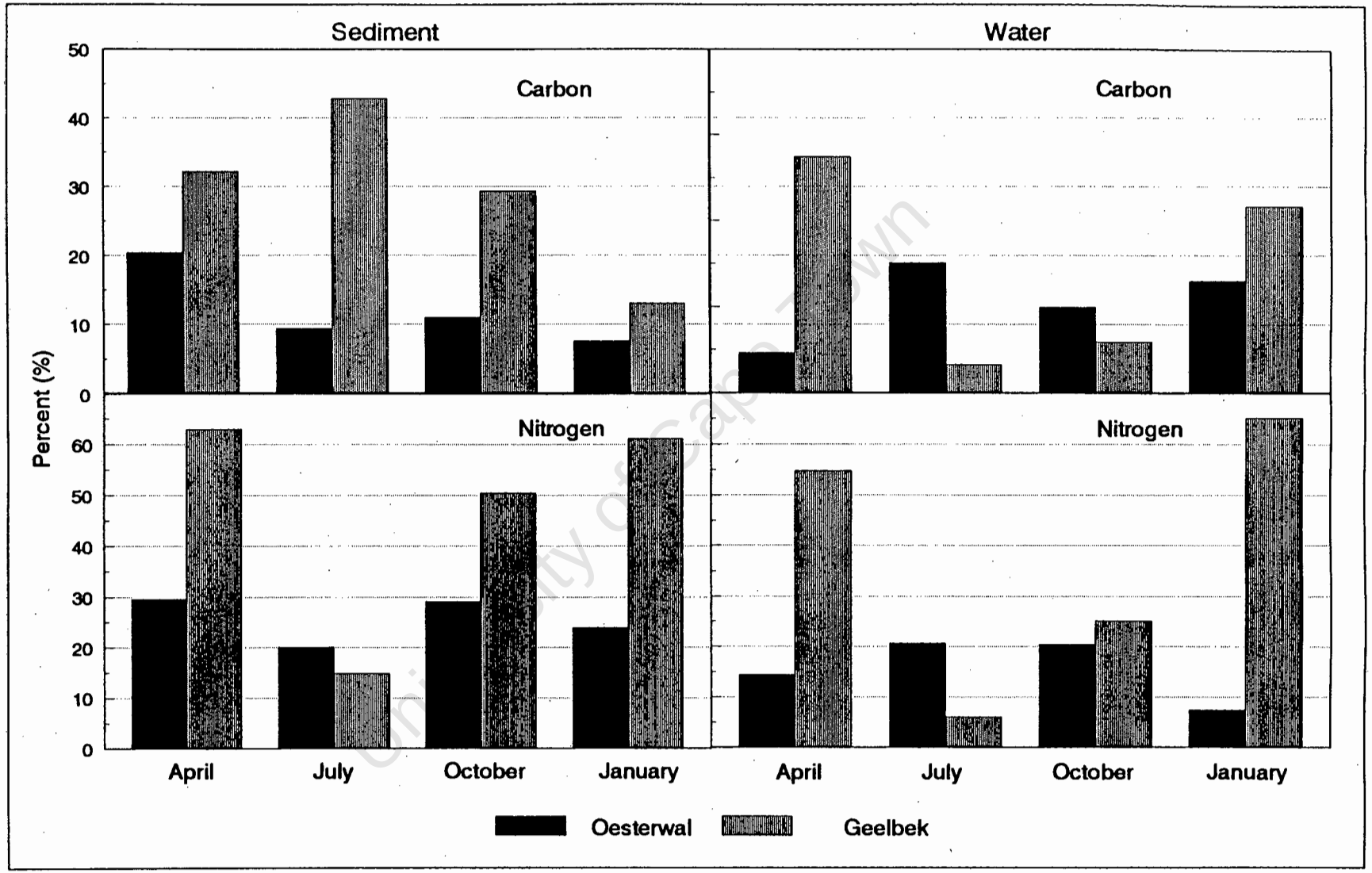

Figure 16. Percentage of the total carbon and nitrogen in the sediment (surface between burrows) and in the water at Oesterwal and Geelbek that is represented by the living (bacteria, protozoa, diatoms and meiofauna) component.

quality of the resource. No clear seasonal trends were observed with regard to the quality of carbon or nitrogen available in the water column or surface sediments at either site.

Discussion

Central to assessing the resources available to deposit feeders is a consideration of the factors that determine the ability of a given detritivore to utilize a resource, i.e. what makes a resource usable ?. The available resource can be defined as the total mix of potential sources present in the detrital pool, while food sources are the specific components that a particular animal utilizes which is a subset of the available resource. The factors that influence usability of the resource include the physical characteristics (e.g. grain size, sediment transport) of the medium in which the detritivore lives (e.g. sediment or water) which, depending on the feeding apparatus and habits of the animal, may render a substrate which is rich in potential food sources a virtual desert (Miller *et al.* 1984, Lopez *et al.* 1989). For example, sediments may be too coarse for handling or too fine for retention by the feeding appendages of the animal. The magnitude and nature of the resource are important factors, e.g. the total amount of food source (whether it above a threshold needed by a particular animal), the components (composition) of the resource (living, non-living, different types of biota), nature of the components (size, shape, density, nutrient content, motility), the sources of the components, the relative abundance of the components and the distribution of the components in the medium (patchiness) (Levinton 1980, Tenore 1981, 1983, Findlay 1982, Tenore *et al.* 1982, Phillips 1984). The quality of a resource to a particular animal depends on the nature of the resource compared to the feeding apparatus of the animal, its selective ability and digestive ability. The feeding apparatus and selective ability of the animal determine which components of the detrital pool are ingested, while the physiology of the animal (digestive ability) determines the extent to which the ingested components are utilized (assimilated). Thus resource quality must be assessed at two levels: suitability for ingestion, and availability for digestion.

To assess the importance of different food resources to *C. kraussi* and *U. africana*, both the burrow system and mode of feeding of the prawns need to be considered. The sandprawn *C. kraussi* is primarily a deposit-feeder and occurs in sandy sediments near the mouth of Langebaan lagoon (typified by the site at Oesterwal), while the mudprawn *U. africana* is a filter-feeder inhabiting the muddy upper reaches of the lagoon at Geelbek. *U. africana* inhabits stable U-shaped

dwelling burrows through which a current is drawn for filter-feeding (Hill 1967, Wynberg 1991). *C. kraussi* inhabits an irregular burrow system, the structure of which changes continuously due to excavation during deposit-feeding (Forbes 1973, Hanekom 1980). Detailed information on the feeding apparatus and diets of these two prawns appears in Chapter 2.

Physical characteristics of the habitat

If the physical characteristics of the sediments at the two sites are compared it is apparant that while the mean annual temperature and pH are similar, Geelbek sediments are a much more variable environment (Table 1). Although the mean annual salinity is higher at Geelbek, the greatest range was observed here. The low salinities observed at times may be due to seepage of fresh water from underground sources in the upper reaches of the lagoon, as well as rainfall (Flemming 1977a). The particularly high salinities at times may be due to evaporation in the relatively shallow water. Oesterwal sediments provide a more uniform environment in terms of temperature, salinity and pH, presumably because they are influenced by conditions in the sea. Similarly water at Oesterwal is more influenced by the sea. Temperature, pH and salinity are higher at Geelbek where they are also more variable. In terms of sediment granulometric characteristics the median particle size is slightly greater at Geelbek, although the mud and organic content is higher and the bulk density is lower. This may affect colonization of the prawns as the higher clay and organic content provides a more stable mud environment which allows formation of the permanent burrows of *U. africana* but may hamper the deposit-feeding action of *C. kraussi* (energetically unviable, or clogs up feeding setae). Conversely the relatively unstable sands at Oesterwal are not held together by as much mud or organics which may prevent the establishment of the permanent dwelling-burrow of *U. africana* but favour the feeding action of *C. kraussi* which involves excavation of feeding burrows. Hill (1967) found that *U. africana* was limited by substrate, requiring muddy sediments to prevent burrow collapse. In addition, once *C. kraussi* is established it may competitively exclude *U. africana* as it excavates extensively, thus disrupting *U. africana* burrows. The activities of deposit-feeders have been reported to affect the survival and colonization of other benthic fauna (Rhoads & Young 1970, Aller & Dodge 1974). It appears therefore that the differences in sediment characteriatics may in part explain the disparate distribution of *U. africana* and *C. kraussi* in the lagoon system.

Quantity of available resource

The total resource available at Geelbek is much larger than at Oesterwal as seen in the higher total organics, organic carbon and nitrogen present in both sediments and water (Figure 4). The resource at Geelbek also comprises a relatively higher abundance and biomass (carbon and nitrogen) of biotic components, i.e. bacteria, protozoa and especially diatoms (Figures 5,11 & 12). The abundance of meiofauna at the 2 sites is similar and very low. Thus the biotic standing stocks (balance between cropping and production) are greater at Geelbek and provide estimates of the amounts of the biota that would be ingested per mouthful if an animal is feeding nonselectively. However, if any particular component is selected by the animal then productivity becomes of great importance in assessing the amounts of available resource present in the water and sediment. The productivity of bacteria and diatoms at Geelbek is higher than at Oesterwal which supports the argument that Geelbek provides a richer resource in terms of quantity of both living and non-living components (Table 5).

Seasonal trends in the abundance of the total resource were not clear (Figures 15), with different patterns of organic carbon and nitrogen content for different microhabitats. However, appreciable bottlenecks in the quantity of resource available did not occur for all microhabitats and it is unlikely that they limit the distribution and feeding strategies of these two detritivores. However, resource quality, in terms of the proportion of living to non-living components varied widely, indicating that the prawns may experience periods when they must rely more heavily on the non-living components of detritus for sustenance (Figure 16). Seasonal changes in the total quantity and biomass of individual components of the detrital pool have been reported for detritus-dominated systems (Tenore 1988, Richardson 1991) and it appears that these may be related to decay cycles of dominant plants (Whitlatch 1981, Tenore 1988). In addition, responses of benthic communities to food supplementation suggests that food is seasonally limiting in some systems (Richardson 1991).

Quality of available resource

The quality of the organic matter in the sediments can be considered in a number of different ways. The proportion of living to non-living components gives a measure of the quality of the organic matter because biota are generally considered a better foodstuff to organisms that are not highly specialized feeders (Williams 1981, Findlay & Tenore 1982, Bowen 1987). This proportion is higher in Geelbek

sediments than Oesterwal sediments, but lower in Geelbek compared to Oesterwal water. There is thus a higher proportion of refractory organic detritus in Oesterwal sediments compared to Geelbek, with a reverse trend for water. Using this measure of quality, detritus in Geelbek sediments is of higher quality than that at Oesterwal, while the opposite is true for the detrital pool available in the water.

Another way to look at quality is to examine the C:N ratio of the organic matter. A lower C:N means a greater proportion of nitrogen (which is usually the limiting nutrient) per unit of foodstuff and may be caused by a high proportion of nitrogen-rich organisms (the non-living component of detritus is generally nitrogen-poor) (Tenore *et al.* 1982, Tenore 1983). In terms of this definition of quality, the Geelbek sediment offers a better quality resource than does Oesterwal as it has a lower C:N ratio. Interestingly in terms of C:N ratio the organics in the water column at Geelbek offer a marginally better quality of resource than those at Oesterwal, despite the fact that living organisms contribute a slightly smaller proportion at Geelbek.

The quality of resource perceived by a particular detritivore may also depend on the size distribution of the relevant components that represent potential food items. Where is the most abundant resource held, i.e. in which size fractions? At Oesterwal most of the total organics lie in the fine sand (64-250 μm) (Figure 3), while the majority of the biotic component in the sediments and water is contained in the 20-64 and <2 μm size-fractions with very little represented in the >64 μm fraction (Figures 13&14). At Geelbek the organic matter is spread equally between the coarse (250 μm - 1 mm) and fine sand (64-250 μm) fractions, while the biomass of the biotic components is largely contained in the 20-64 μm fraction, but also well represented in the 2 smaller size-fractions (<2 and 2-20 μm) in both the sediment and water. It therefore appears that at both sites in both water and sediment the biotic fraction is largely composed of small particles (<64 μm), while the abiotic detrital fraction is mainly composed of larger particle (>64 μm). This is of interest as many detritivores have been reported to selectively feed on smaller particles (e.g. Gremare 1988, Fenchel 1972, Taghon 1982, Self & Jumars 1988). A detritivore selecting small particles at Geelbek or Oesterwal would benefit in terms of the amount of microbiota ingested, i.e. the quality of food ingested would be higher. In addition, an inverse relationship between sediment nutritional quality and size of non-living particles has been reported (Forbes & Lopez 1989) as fine particles offer a greater surface area relative to volume which allows for increased microbial colonization (Whitlatch 1974, Nielsen & Kofoed 1982). Thus ingestion of smaller-sized non-living detrital particles should result in higher quality of food, although

the quantity of organic matter available is highest in the larger fractions of the detritus at both Oesterwal and Geelbek.

Quality also depends on which of the biotic components are most important in terms of numbers and biomass, i.e. the relative contribution of each biotic component to the total organic matter, as the need for selective feeding by a detritivore may rest with this. In both sediments and water, bacteria are most abundant followed by diatoms and then protozoa. Meiofauna occur in relatively low numbers in sediments at both sites. In terms of biomass at Geelbek the most important biotic contributer to both carbon and nitrogen is diatoms followed by bacteria and to a lesser extent protozoa, while the contribution by meiofauna is minimal. At Oesterwal, bacteria contribute most to carbon and nitrogen biomass followed by diatoms and protozoa, with a low but significant contribution by meiofauna. Thus, to exploit the small resource represented in numbers and biomass by meiofauna, an animal would have to feed selectively, but it may be practical to exploit the bacteria and diatoms by ingesting sediments and particles from the water column non-selectively, particularly at Geelbek where the biomass is high. The relatively high biomass of diatoms at Geelbek is of significance, as benthic diatoms have been identified as an important food source for many benthic animals (Jensen & Siegismund 1980, Bianchi & Levinton 1984, Sullivan & Moncreiff 1990, Quigley & Vanderploeg 1991). In addition, detritivores are reported to obtain significant portions of required nitrogen from bacteria (Cammen 1989).

Of great importance is the proportion of total organics that is "locked up" and unavailable in the non-living organic fraction (this may also affect the feasability of trying to feed selectively) (Mayer 1989). Although no measure of how much of the non-living fraction is actually bioavailable (i.e. can be assimilated by the detritivore) exists for the present study, this portion is likely to be small. Mayer (1989) reports that only a small percentage (5-30%) of the non-living organic matter present in the detrital pool is susceptable to degradation by microbial or animal enzymes. This needs to be taken into account when considering the quantity of resource available to a detritivore, as much of the organic material is bound into refractory geopolymeric materials that are of little biological relevence (Mayer *et al.* 1986).

Distribution of available resource

Of relevance to the feeding behaviour and mode of feeding of the prawns is a comparison of the two sites in terms of distribution of the resource in the sediment (i.e. within-sediment microhabitats). Do the various microhabitats around the

burrow systems differ in terms of quantity and quality? In terms of quality, surface sediments at both sites (at and between burrows and in *Zostera* beds) have a higher proportion of biota to non-living detritus than do sediments at depth and at burrow lining (Figures 11 & 12). In terms of quantity the burrow lining and *Zostera* beds have a higher total biomass of both carbon and nitrogen but the total amount of organic carbon in non-living detritus is also higher there (Figures 9 & 10). Diatom and bacteria biomass at both sites is spread evenly in the sediments with higher amounts only in *Zostera*. Protozoan and meiofaunal biomass is greatest in surface sediments and in *Zostera* beds. Thus the different biotic components are present in different abundances in different regions of the sediment. It is possible that the distribution of microbiota and organic material in the sediments is influenced by the burrowing prawns themselves. Deposit-feeders have been shown to negatively influence the density of meiofauna (Branch & Pringle 1988, Hoffman *et al.* 1984) and micro-algae (Morrisey 1988). Burrowing thalassinids are reported to affect the distribution of organics in sediments (Ott *et al.* 1976, Suchanek 1983, de Vaugelas & Buscail 1990) usually resulting in an increase in the organic content in the vicinity of a burrow, coupled with enhanced microbial activity (Aller & Yingst 1978). This effect may be partially due to the deposition of faecal pellets (which represent packages of high organic content) near the mouth of the burrow (Frankenberg *et al.* 1967).

The distribution of the total organics and the various biotic fractions in the sediment may affect the optimum feeding sites for an organism like *C. kraussi*, i.e. if it feeds below the surface it is feeding in an environment relatively depauperate in protozoa and meiofauna but rich in bacteria. On the other hand diatoms are uniformly distributed and if the animal depends on diatoms then it is not important where it feeds in the sediment. The quality of the resource at both sites is better in surface sediments, while the quantity is highest in burrow linings. *Zostera* beds represent a microhabitat with sediments of high quality and quantity. Therefore one might expect that if *C. kraussi* feeds selectively it should target surface sediments. A number of benthic detritivores are known to feed on the surface layer of the sediment (Lopez & Elmgren 1989) and vertical gradients in the abundance and composition of detritus have been reported (Whitlatch 1981, Jensen 1983). Although Hylleberg & Riis-Vestergaard (1984) report an increase in the amount of nitrogen with sediment depth (15cm) compared to surface sediments, this trend was not observed at either Geelbek or Oesterwal.

Water at Oesterwal features mostly protozoa and bacteria and is a poor resource in terms of total biomass, while Geelbek waters are relatively rich in biomass and support similar biomass values for each of diatoms, protozoa and

bacteria (Figures 9 & 10). Filter-feeding would therefore seem more profitable at Geelbek than at Oesterwal.

Conclusions

While quality and quantity of resource can be defined in general terms, the actual food sources available depend on the feeding strategy of the detritivore. Feeding strategies may be expected to optimize the utilization of the resource, and therefore information about the resource may allow tentative predictions about resource utilization by the animal. *U. africana* and *C. kraussi* occupy habitats which differ with respect to both the quality and quantity of resources available in the sediments and water column. *U. africana* lives in the muddy sediments at Geelbek where it is able to maintain permanent burrows. Large quantities of water particulates are available to support filter feeding, probably due to suspension of organic material from surface sediments. While the total POM and total biomass is higher in the water column at Geelbek compared to Oesterwal, the organics nevertheless contain a low proportion of living components with the bulk comprising non-living POM. Selective filter-feeding by this prawn would thus maximize intake of labile living components (small size fraction). If the animal feeds non-selectively it will ingest large quantities of relatively refractory material (derived from saltmarsh detritus) along with microbiota. In this case the animal may require a feeding strategy that maximizes the quantity of material ingested (e.g. increased ingestion rate and gut size) in order to increase the quantity of labile components ingested, and possibly the efficient utilization of refractory components either by endogenous enzyme activities or by association with gut microbes. However, the relatively high quality and quantity of resource available to *U. africana* suggests that this prawn may be less likely to rely on specialized feeding mechanisms or associations than is *C. kraussi*. *C. kraussi* generally occurs in more sandy, unstable sediments (typified by those at Oesterwal) where there are loose sediments which allow digging-type foraging. However, lower total organics and a poorer quality resource in terms of the availability of labile living components of detritus suggest that either selective feeding with regard to both the type of particles ingested and site of foraging, or efficient utilizion of the non-living fraction of detritus is likely to be of greater importance to *C. kraussi* than *U. africana*.

References

Adams SM, Angelovic JW 1970 Assimilation of detritus and its associated bacteria by three species of estuarine animals. Chesapeake Sci. 11(4):249-254

Aller RC, Dodge RE 1974 Animal-sediment relationship in a tropical lagoon Discovery Bay, Jamaica. J. Mar. Res. 32:209-232.

Aller RC, Yinst JY 1978 Biogeochemistry of the tube-dwellings: a study of the sedentary polychaete *Amphitrite ornata* (Leidig). J. Mar. Res. 36:201-254.

Baker JH, Bradnam LA 1976 The role of bacteria in the nutrition of aquatic detritivores. Oecologia (Berl) 24:95-104

Beers JR 1966 Studies on teh chemical composition on the major zooplankton groups in the Sargasso Sea off Bermuda. Limnol. Oceanogr. 11:520-528.

Bianchi TS, Levinton JS 1984 the importanc of microalgae, bacteria and particulate organic matter in the somatic growth of *Hydrobia totteni*. J. Mar. Res. 42:431-443.

Boucher C, Jarman ML 1977 The vegetation of the Langebaan area, South Africa.Trans. Roy. Soc. S. Afr. 42:241-272.

Bowen SH 1987 Composition and nutritional value of detritus. pp. 192-216. In Detritus and microbial ecology in aquaculture. Moriarty DJW, Pullin RSV (Eds.) ICLARM Conf. Proc. 14, 420p. Int Cen for living Aquatic Res Man, Manilla, Phillipines.

Branch GM, Pringle A 1987 The impact of the sand prawn *Callianassa kraussi* Stebbing on sediment turnover and on bacteria, meiofauna and benthic microflora. J. Exp. mar. Biol. Ecol. 107:219-235

Bratbak G 1985 Bacterial biovolume and biomass estimations. Appl. Environ. Microbiol. 49:1488-1493.

Bratbak G, Dundas I 1984 Bacterial dry matter content and biomass estimations. Appl. Environ. Microbiol. 48:755-757.

Cammen LM 1980 The significance of microbial carbon in the nutrition of the deposit feeding polychaete *Nereis succinea*. Mar. Biol. 61:9-20

Cammen LM 1989 The relationship between ingestion rate of deposit feeders and sediment nutritional value. In: Ecology of marine deposit feeders, Lopez G, Taghon G, Levinton J (eds.), Springer-Verlag, New York, pp 201-222

Cammen LM, Walker JA 1986 The relationship between bacteria and micro-algae in the sediment of a Bay of Fundy mudflat. Estuar. Coast. Shelf Sci. 22:91-99.

Cassell EA 1965 Rapid graphical method for estimating the precision of direct microscopic counting data. Appl Microbiol 13(3):293-296

Chamier AC 1991 Cellulose digestion and metabolism in the freshwater amphipod *Gammarus pseudolimnaeus* Bousfield. Freshwater Biol. 25:33-40

Christie ND 1981 Primary productivity in Langebaan Lagoon. In Esturine Ecology wqith particular reference to southern Africa. (Ed. J Day), AA Balkema, pp101-115.

Cummins KW, Petersen RC, Howard FO, Wuycheck JC, Holt VI 1973 The utlization of leaf litter by stream detritivores. Ecol. 54:336-345.

Darnell RM 1967 The organic detritus problem, pp374-375. In GH Lauff (Ed.) Estuaries; American Association for Advanced Science Publication number 83.

de Jonge VN 1980 Fluctuations in the organic carbon to chlorophyll a ratios for estuarine benthic diatom populations. Mar. Ecol. Prog. Ser. 2:345-353.

de Vaugelas J, Buscail R 1990 Organic matter distribution in the burrows of the thalassinid crustacean *Callichirus laurae*, Gulf of Aqaba (Red Sea). Hydrobiologia 207:269-277.

Dobbs FC, Guckert JB 1988 *Callianassa trilobata* (Crustacea: Thalassinidae) influences abundance of meiofauna and biomass, composition, and physiologic state of microbial communities within its burrow. Mar. Ecol. Prog. Ser. 45:69-79.

Dye AH 1978 An ecophysiological study of the meiofauna of the Swartkops estuary. I. The sampling sites: Physical and chemical features. Zool. Afr. 13:1-18.

Ellery WN, Schleyer MH 1984 Comparison of homogenization and ultrasonication as techniques in extracting attached sedimentary bacteria. Mar. Ecol. Prog. Ser. 15:247-250.

Fallon RD, Pfaender FK 1976 Carbon metabolism in model microbial systems from a temperate salt marsh. Appl. Environ. Microbiol. 3:959-968

Fenchel T 1970 Studies on the decomposition of organic detritus derived from the turtle grass *Thalassia testudinum*. Limnol. Oceanogr. 15:14-20

Fenchel T 1972 Aspects of decomposer food chains in marine benthos. Verh. dt. Zool. Ges. 65:14-23.

Fielding PJ, St J Damstra K, Branch GM 1988 Benthic diatom biomass, production and sediment chlorophyll in Langebaan lagoon, South Africa. Est. Coast Shelf Sci. 27:413-426

Findlay S 1982 Effect of detrital nutritional quality on population dynamics of a marine nematode (*Diplolaimella chitwoodi*). Mar. Biol. 68:223-227.

Findlay S, Meyer JL, Smith PJ 1984. Significance of bacterial biomass in the nutrition of a freshwater isopod (Lirceus spec.). Oecologia (Berlin) 63:38-42

Findlay S, Tenore K 1982 Nitrogen source for a detritivore: detritus substrate versus associated microbes.Science 218:371-373.

Flemming BW 1977a Distribution of recent sediments in Saldanha Bay and Langebaan Lagoon. Trans. Roy. Soc. S. Afr. 42:317-340.

Flemming BW 1977b Depositional processes in Saldanha Bay and Langebaan lagoon. Bulletin No. 8, NRIO Professional Research Series No. 2, Marine Geocsience group, Department of Geology, University of Cape Town.

Forbes AT 1973 A study of the burrowing sand prawn *Callianassa kraussi* Stebbing (Crustacea: Decapoda: Thalassinidea). Ph.D. thesis, Rhodes University, Grahamstown, South Africa. pp.1-164.

Forbes VE, Lopez GR 1989 The role of sediment particle size in the nutritional energetics of a surface deposit-feeder. I. Ingestion and absorbtion of sedimentary microalgae by *Hydrobia truncata*. J. Exp. Mar. Biol. Ecol. 126:181-192.

Frankenberg D, Coles SL, Johannes RE 1967 The potential trophic significance of *Callianassa major* fecal pellets. Limnol. Oceanogr. 12:113-120.

Gosselink JG, Kirby CJ 1974 Decomposition of salt marsh grass, *Spartina alterniflora* Loisel. Limnol. Oceanogr. 19:825-832

Gremare A 1988 Feeding, tube-building and particle size selection in te terebellid polychaete *Eupolymnia nebulosa*. Mar. Biol. 97:243-252.

Hannekom NM 1980 A study of two thalassinid prawns in the non *Spartina* regions of the Swartkops estuary. Ph.D. thesis, University of Port Elizabeth, Port Elizabeth, South Africa. pp 1-253.

Hargrave BT 1970 The utilization of benthic microflora by Hyalella azteca (Amphipoda). J. Animal Ecol. 39:427-437

Hill BJ 1967 Contributions to the ecology of the Anomuran mud prawn *Upogebia africana* (Ortmann). Ph.D. thesis, Rhodes University, Grahamstown, South Africa. pp. 1-201.

Hoffman JA, Katz J, Bertness MD 1984 Fiddler crab deposit-feeding and meiofaunal abundance in salt marsh habitats. J. Exp. Mar. Biol. Ecol. 82:161-174.

Hylleberg J, Riis-Vestergard H 1980 Ecology of marine sediments: the fate of detritus.Akademisk Forlag, Copenhagen.

Jeffery SW, Humphrey GF 1975 New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. Biochem. Physiol. Pflanz. 167:191-194.

Jensen KT, Siegismund HR 1980 The importance of diatoms and bacteria in the diet of *Hydrobia*-species. Ophelia 1:193-199

Kemp PF 1986 Direct uptake of detrital carbon by the deposit-feeding polychaete *Euzanus mucronata* (Treadwell). J. Exp. Mar. Biol. Ecol. 99:44-61

Klumpp DW, Nichols PD 1983 Utilisation of the seagrass Posidonia australis as food by the rock crab *Nectocarcinus integrifons* (Latreille) (Crustacea: Decapoda: Portunidae). Mar. Biol. Letters 4:331-339

Leduc EH , Holt SJ 1965 Hydroxypropyl methacrylate, a new water-miscible embedding medium for electron microscopy. J. Cell. Biol. 26:137-144.

Levinton JS 1980 Particle feeding by deposit-feeders: models, data and prospectus. In KR Tenore and BC Coull (Eds.) Marine Benthic Dynamics. Univ. South Carolina Press, Columbia 423-439.

Levinton JS 1989 Deposit feeding and coastal oceanography. In: Ecology of marine deposit feeders, Lopez G, Taghon G, Levinton J (eds.), Springer-Verlag, New York, pp 1-23

Lopez G, Tantichodok P, Cheng I-J 1989 Radiotracer methods for determining utlization of sedimentary organic matter by deposit feeders. In Ecology of marine deposit feeders.XII (Eds. Lopez G, Taghon G, Levinton J). pp 322.

Lopez GR, Elmgren R 1989 Feeding depths and organic absorbtion for the deposit-feeding benthic amphipods *Pontoporeia affinis* and *Pontoporeia femorata*. Limnol. Oceanogr. 34:982-991.

Lopez GR, Levinton JS, Slobodkin 1977 The effects of grazing by the detritivore Orchestia grillus on *Spartina* litter and its associated microbial community. Oecologia (Berl) 20:111-127

Matthews SG 1991 the relative role of protozoans in the flux of phytoplankton nitrogen through pelagic food webs. Unpublished MSc. thesis, University of Cape Town, Cape Town.

Mayer LM 1989 The nature and determination of non-living sedimentary organic matter as a food source for deposit feeders. In: Ecology of marine deposit feeders, Lopez G, Taghon G, Levinton J (eds.), Springer-Verlag, New York, pp 98-113

Mayer LM, Schick LL, Setchell FW 1986 Measurement of protein in nearshore marine sediments. Mar. Ecol. Prog. Ser. 30:159-165.

Mazure HGF, Branch GM 1979 A preliminary analysis of bacterial numbers and biomass in Langebaan Lagoon. Trans. Roy. Soc. S. Afr. 44:43-54.

Miller DC, Jumars PA, Nowell ARM 1984 Effects of sediment transport on deposit feeding: Scaling arguments. Limnol. Oceanogr. 29:1202-1217.

Moriarty DJW, Roberts DG, Pollard PC 1990 Primary and bacterial productivity of tropical seagrass communities in the Gulf of Carpentaria, Australia. Mar. Ecol. Prog. Ser. 61:145-157.

Morrisey DJ 1988 Differences in effects of grazing by deposit-feeders *Hydrobia ulvae* (Pennant)(Gastropoda:Prosobranchia) and *Corophium arenarium* Crawford (Amphipoda) on sediment microalgal populations. II. Quantitative effects. J. Exp. Mar. Biol. Ecol. 118:43-53.

Nagata T 1986 Carbon and nitrogen content of natural planktonic bacteria. Appl. Environ. Microbiol. 52:28-32.

Newell R (1965) The role of detritus in the nutrition of two marine deposit feeders, the Prosobranch Hydrobia ulvae and the bivalve Macoma balthica. J. Zool. 144:25-44

Newell REI, Langdon CJ 1986 Digestion and absorption of refractory carbon from the plant Spartina alterniflora (Loisel) by the oyster Crassostrea virginica (Gmelin). Mar. Ecol. Prog. Ser. 34:105-115

Nicholson G 1984 Methods of soil, plant and water analysis. FRI Bulletin No. 70. FRI Rotorua, NZ.

Nielsen MV, Kofoed LH 1982 Selective feeding and epipsammic browsing by teh deposit-feeding amphipod *Corophium volutator*. Mar. Ecol. Prog. Ser. 10:81-88.

Omori M 1969 Weight and chemical composition of some important oceanic zooplankton in the North Pacific Ocean. Mar. Biol. 3:4-10.

Ott JA, Fuchs B, Fuchs R, Malasek A 1976 Observations on the biology of *Callianassa stebbingi* Borrodaille and Upogebia litoralis Risso and their effect upon the sediment. Senckenbergiana marit 8(1/3):61-79

Painchaud J, Therriault J 1989 Relations between bacteria, phytoplankton and particulate organic carbon in the Upper St. Lawrence Estuary. Mar. Ecol. Prog. Ser. 56:301-311.

Parsons RT, Maita Y, Lalli CM 1984 A manual of chemical and biological methods for seawater analysis. Pergamon Press, London.

Phillips NW 1984 Role of different microbes and substrates as potential suppliers of specific, essential nutrients to marine detritivores. Bull. Mar. Sci. 35(3):283-298

Porter KG, Feig YS 1980 The use of DAPI for identifying and counting aquatic microflora. Limnol. Oceanogr. 25:943-948.

Posey MH, Dumbauld BR, Armstrong DA 1991 Effects of a burrowing mud shrimp, Upogebia pugettensis (Dana), on abundances of macro-infauna. J. Exp. mar. Biol. Ecol. 148:283-294

Putt M, Stoeker DK 1989 An Experimentally determined carbon:volume ratio for marine "oligotrichous" ciliates from estuaries and coastal waters. Limnol. Oceanogr. 34:1097-1103.

Puttick GM 1977 Spatial and temporal variations inter-tidalanimal distribution at Langebaan Lagoon, South Africa. Trans. Roy. Soc. S. Afr. 42:403-440.

Quigley MA, Vanderploeg HA 1991 Ingestion of live filamentous diatoms by the Great Lakes amphipod, *Diporeia* sp.: a case study of the limited value of gut contents analysis. Hydrobiologia 223:141-148.

Rhoads DC, Young DK 1970 The influence of deposit-feeding organisms on sediment stability and community trophic structure. J. Mar. Res. 28:159-178.

Rice DL 1982 the detritus nitrogen problem: new observations and perspectives from organic geochemistry. Mar. Ecol. Prog. Ser. 9:153-162

Rice DL, Bianchi TS, Roper EH 1986 Experimental studies of sediment reworking and growth of Scoloplos spp. (Orbiniidae, Polychaeta). Mar. Ecol. Prog. Ser. 30:9-19

Rice DL, Rhoads DC 1989 Early diagenesis of organic matter and the nutritional value of sediment. In: Ecology of marine deposit feeders, Lopez G, Taghon G, Levinton J (eds.), Springer-Verlag, New York, pp 59-97

Rice DL, Tenore KR 1981 Dynamics of carbon and nitrogen during the decomposition of detritus derived from estuarine macrophytes. Est. Coast. Shelf Sci. 13:681-690

Richardson JS 1991 Seasonal food limitation of detritivores in a montane stream: an experimental test. Ecology 72(3):873-887

Schallenberger M, Kalff J, Rasmussen JB 1989 Solutions to problems in enumerating sediment bacteria by direct counts. Appl. Environ. Microbiol. 55:1214-1219.

Seiderer LJ, Newell RC 1985 Relative significance of phytoplankton, bacteria and plant detritus as carbon and nitrogen resources for the kelp bed filter-feeder Choromytilus meridionalis. Mar. Ecol. Prog. Ser. 22:127-139

Self RFL, Jumars PA 1988 Cross-phyletic patterns of particle selection by deposit feeders. J. Mar. Res. 46:119-143.

Shannon LV, Stander GH Physical and Chemical charecteristicxs of water in Saldanha Bay and Langebaan Lagoon. Trans. Roy. Soc. S. Afr. 42:441-458.

Sherr BF, Sherr EB 1984 Role of heterotrophic protozoa in carbon and energy flow in aquatic ecosystems, p. 412-423. In MJ Klug and CA Reddy (Eds.), Current perspectives in microbial ecology. Am. Soc. Microbiol.

Strickland JDH 1960 Measuring the production of marine phytoplankton. Bull. Fisheries Res. Board Can. 122:172pp.

STSC Inc. 1985 Statgraphics: statistical software package. STSC Inc., Rockville, MD

Suberkropp K, Klug MJ 1976 Fungi and bacteria associated with leaves during processing in a woodland stream. Ecology 57:707-719

Suchanek TH 1983 Control of seagrass communities and sediment distribution by Callianassa (Crustacea, Thalassinidae) bioturbation. J. Mar. Res. 41:281-298

Sullivan MJ, Moncreiff CA 1990 Edaphic algae are an important component of saltmarsh food-webs: evidence from multiple stable isotope analyses. Mar. Ecol. Prog. Ser. 62:149-159.

Taghon GL 1982 Optimal foraging by deposit-feeding invertebrates: roles of particles size and organic coating. Oecologia 52:295-304.

Tenore KR 1981 Organic nitrogen and caloric content of detritus. Est., Coast. shelf Sci. 12:39-47.

Tenore KR 1983 What controls the availability to animals of detritus derived from vascular plants: Organic nitrogen enrichment or caloric availability? Mar. Ecol. Prog. Ser. 10:307-309

Tenore KR 1988 Nitrogen in benthic food chains. In: Nitrogen cycling in coastal marine environment, Blackburn TH, Sorensen J (eds.), John Wiley & Sons Ltd, pp 191-206

Tenore KR 1989 Some ecological perspectives in the study of the nutrition of deposit feeders. In: Ecology of marine deposit feeders, Lopez G, Taghon G, Levinton J (eds.), Springer-Verlag, New York, pp 309-318

Tenore KR, Cammen L, Findlay SEG, Phillips N 1982 Perspectives of research on detritus: do factors controlling the availability of detritus to macroconsumers depend on its source? J. Mar. Res. 40:473-490.

Tenore KR, Hanson RB, Dornseif BE, Weiderhold CN 1979 The effect of organic nitrogen supplement on the utilization of different sources of detritus. Limnol. Oceanogr. 24(2):350-355

Velji MI, Albright LJ 1986 Microscopic enumeration of attached marine bacteria of seawater, marine sediment, faecal matter, and kelp blades samples following pyriphosphate and ultrasound treatments. Can. J. Microbiol. 32:121-126.

Walkley A, Black IA 1934 An examination of the Degtjareff method for determining soil organic matter, and the proposed modification of the chromic acid titration method. Soil Sci. 37:29-38.

Watling L 1989 Small-scale features of marine sediments and their importance to the study of deposit feeding. In: Ecology of marine deposit feeders, Lopez G, Taghon G, Levinton J (eds.), Springer-Verlag, New York, pp 269-290

Whitlatch RB 1974 Food resource partitioning in the deposit-feeding polychaete *Pectinaria gouldi*. Biol. Bull. Mar. Biol. Lab. Woods Hole 147:227-235.

Whitlatch RB 1981 Animal-sediment relationships in intertidal marine benthic habitats: some determinants of deposit-feeding species diversity. J. Exp. Mar. Biol. Ecol. 53:31-45

Williams P 1981 Detritus utilization by *Mytilus edulis*. Est. Coast Shelf Sci. 12:739-746

Wynberg RP 1991 The ecological effects of collecting Callianassa Stebbing and Upogebia africana Ortmann for bait: Impacts on the biota of an intertidal sandflat. MSc Thesis, UCT

Yingst JY 1976 The utilization of organic matter in shallow marine sediments by an epibenthic deposit-feeding holothurian. J. Exp. Mar. Biol. Ecol. 23:55-69

CHAPTER 2

Feeding apparatus and food resources of two thalassinid detritivores, the mud-prawn *Upogebia africana* and the sand-prawn *Callianassa kraussi*

ABSTRACT

The feeding strategies of two saltmarsh detritivores, the mud-prawn *Upogebia africana* and the sand-prawn *Callianassa kraussi*, are investigated by examination of feeding apparatus, gut morphology, gut passage time and gut contents coupled with stable isotope analyses to determine the major food sources. The mode of feeding, gut structure and diet of the two prawn species differ, although gut passage rates are similar (ca. 6 h). *U. africana* is a filter-feeder which ingests small particles non-selectively. Stable isotope analyses indicate that this prawn relies on vascular plant detritus for both its carbon and nitrogen requirements. Thus it survives on a relatively poor quality diet. This may be reflected its relatively large gut which is dominated by the throughput regions (fore-, mid- and hindgut), while the hepatopancreas appears to be of lesser importance. A large gut allows ingestion of larger quantities per meal. By contrast, *C. kraussi* is a deposit-feeder equipped to feed selectively on larger particles present in the detrital pool, and has an efficient mechanism (the gastric mill) for breakdown of these components of the diet. Stable isotope analyses confirm selective feeding in that carbon is obtained from a mixture of vascular plant detritus and diatoms while nitrogen is obtained almost exclusively from the living components of detritus, i.e. diatoms and protozoa, despite the low biomass of theses detrital components. *C. kraussi* has a relatively small gut indicative of a richer quality diet, and the hepatopancreas dominates its volume effectively increasing the retention time of particles held in this region.

INTRODUCTION

Aquatic detritivores comprise a diverse group of animals occupying many habitats and ranging from filter-feeders to deposit-feeders, scavengers to specialist feeders (e.g. wood-borers). Detritivores generally have a wide variety of resources within the detrital pool available to them. The detrital pool usually comprises a small living fraction, the microbiota (bacteria, protozoa, algae, etc.) and a large non-living fraction (particulate organic matter (POM)), the bulk of which consisting of vascular plant and/or macroalgal fragments) (Tenore et al. 1979, Whitlatch 1981). The relative roles of the different resources in the nutrition of detritivores has been highlighted as one of the most important unresolved problems in their feeding ecology (Levinton 1989, Cammen 1989). It is clear that the perception of detritivores as indiscriminate feeders is not correct for many species, and it is important to compare digestive processes with food availability (Levinton 1989).

The utilization of the various available resources by a given detritivore is dependent on a number of factors. Ingestion of the different resources will depend on both the food capture mechanisms of the animal and the quality of the detritus. Food capture mechanisms are defined by the morphological and behavioural attributes of the animal (Miller 1984, Ngoc-Ho 1984, Coleman 1989, Hartmann & Kunkel 1991). Morphology, in the form of the feeding apparatus used to collect food materials, influences selectivity and can constrain feeding strategies (Whitlatch 1989), while behavioral traits should serve to maximize the gain of nutrients from detritus (Sibly 1981).

The quality of detritus to a detritivore depends on both the nature of the principle components (size, shape, mobility, nutritional composition) and their relative abundance in the detrital mixture (Phillips 1984). The nature of each component of the detrital mixture will determine its importance as a potential food source to the animal in terms of its availability and desirability with regard to the detritivore's morphological and behavioural feeding strategy, and its nutrient content and digestability to the animal (Lopez et al. 1977, Newell & Langdon 1986, Kreeger et al. 1988). It is clear that desirability for components containing nutrients that are limiting will be greater than for those that are not, providing that the animal is equipped to utilize them. The quality of detritus perceived by the animal is therefore different for each detritivore, and will depend not only on its composition per se but also on the animal's ability to utilize the components and the animal's nutrient requirements (Tenore 1989). Behavioural plasticity in foraging behaviour will probably be most important where the relative abundance of components of detritus are variable (i.e. quality changes over time and/or space), e.g. where there

are marked seasonal differences (Graf et al. 1982, Richardson 1991) or the distribution of certain important components in the detritus is patchy (Townsend & Hughes 1981, Watling 1989).

Digestability of a component by a detritivore varies with its nature and will depend on both the morphology and physiology (e.g. enzymes) of the detritivore (Phillips 1984). The nutrient gain by an animal is also dependent on ingestion rate (Cammen 1989). Ingestion rate can be confined by morphological traits e.g. gut volume can limit throughput rates. It has been suggested that digestive kinematics are expressed statically in gut morphologies (Penry & Jumars 1990). Morphology will influence the time a given component is held in the digestive tract, as well as the time of residence in different regions of the gut, and therefore digestion and absorption as these are time-dependent processes (Kofoed et al. 1989). Gut throughput time may be increased by having a longer gut (e.g. herbivores often have longer guts than do carnivores), and Sibly (1981) predicted that animals eating poorer-quality diets should have larger gut volumes. Partitioning of the gut into compartments and side pouches allows sorting of food components in the gut so that while some food components are shunted through the gut and their gut residence time equals the gut throughput time of the main food bolus, other components may be retained for much longer periods. Because nutrients are absorbed at different sites, retention time may vary depending on which nutrient is being considered. Gut throughput times and gut retention times are therefore not synonomous. Throughput time is the time from ingestion of food to defecation of undigested residue, while retention time is the time that digesta are retained in the absorptive regions of the gut (Sibly 1981). It would be advantageous to shunt less desirable components out of the gut rapidly while retaining more important ones so that more complete digestion can occur. Thus not only must a component be digestable in terms of the enzymes produced by the animal but it must be retained in the correct region of the gut long enough for efficient digestion and absorption. There is inevitably a trade-off between absorption rate and efficiency and these two factors interact to control assimilation (Boehlert & Yoklavich 1984). The chemical reactor concept developed by Penry & Jumars (1986,1987) offers predictions about how gut throughput time and the extent of digestive conversion should vary with changes in food concentration to maximize digestive efficiency.

The feeding strategies most commonly proposed and demonstrated for detritivores are optimal foraging (e.g. where the animal increases the feeding rate when food quality increases) (Taghon 1981) and "compensatory" feeding (e.g. where an animal increases its feeding rate in response to decreased food quality) (Lopez et al. 1983, Poovachiranai et al. 1986, Genoni 1991). It has been suggested

that these different scenarios represent different subsets of a continuum, along which natural systems lie (Taghon 1989). Flexibility lies in the ability to vary the gut volume or the throughput rate of food as the composition of the diet changes, i.e. a "functional ingestion response" to food supply (Cammen 1980, Taghon & Greene 1990). The optimal strategy adopted by any animal represents a trade-off (Levinton 1989, Cammen 1989) that should maximizes net energy gain (Townsend & Hughes 1981). For example, although an increased ingestion rate brings more food into the gut, it results in a decrease in the gut passage time and diminished digestion of material. This compromise sets an optimum feeding rate, which should increase with increasing food quality, i.e optimal foraging. As food quality decreases the appropriate strategy may be to increase gut residence time in order to increase digestion. However, when food quality is very poor, it may be beneficial to increase the feeding rate, thus decreasing gut passage time to expel useless food items, i.e. compensatory feeding. A more refined feeding strategy is selective feeding which may be more prevalent in detritivores than has generally been assumed (Hammond 1983, Poovachiranon et al. 1986, Cowles et al. 1988, Gremare 1988, Self & Jumars 1988, Levinton & DeWitt 1989, Lopez et al. 1989), although the costs of handling and sorting food must be considered (Taghon et al. 1978, Taghon 1988).

Thus gut morphology and configuration and digestive tactics (behavioural and physiological) together act to maximize an animal's net rate of energy and nutrient gain. Any study looking at optimal feeding strategies must consider both foraging (ingestion, morphology and behaviour) and digestion (enzymes, gut passage, gut architecture) in relation to available food resources.

This chapter compares aspects of the feeding strategies of two saltmarsh detritivores, the filter-feeding mud-prawn *Upogebia africana* and the deposit-feeding sand-prawn *Callianassa kraussi*. No previous detailed studies of the feeding appendages and gut structure of these thalassinid prawns exists, although the modes of feeding of *U. africana* (Hill 1967) and *C. kraussi* (Forbes 1973) have been described . The morphology of the feeding apparatus is investigated by scanning electron microscopy to confirm the mode of feeding and the constraints on particle capture and selection. The architectures of the digestive systems are compared with respect to relative sizes, configuration and compartmentalization, factors that affect both ingestion rates and digestive efficiency. The feeding cycles are investigated together with gut throughput times in the field. Field gut throughput times are little studied but important, together with gut structure and digestive physiology, in influencing ingestion rate and the utilization of components of detritus (Jumars & Penry 1989). Finally, the diet of the animals is investigated by gut content analyses (light microscopy and scanning electron microscopy) which provide an indication of

ingestion and selectivity, coupled with a stable isotope study to determine the relative importance of various components of available detritus as food sources.

The results of this chapter, together with studies on the digestive physiology of the animals (Chapter 3) and on the resources available in their respective habitats (Chapter 1), contribute to a comparison of the feeding strategies of, and resource utilization by, the two species of detritivorous prawn.

Methods and materials

Study site and animals

U. africana and *C. kraussi* were collected respectively from Geelbek (upper reaches) and Oesterwal (near mouth) in Langebaan lagoon (see Chapter 1 for detailed description of sampling sites). The mud-prawn *U. africana* inhabits a simple U-shaped dwelling tube through which a water current is drawn during filter-feeding (Figure 1) (Hill 1967, Schaeffer 1970, Branch & Branch 1981)), while the burrow of the sand-prawn *C. kraussi* comprises a much deeper but less uniform system of tubes with numerous short side feeding burrows excavated during the deposit-feeding action of the prawn (Figure 1) (Forbes 1973, Wynberg 1991).

Feeding apparatus

Feeding appendages (pereiopods and maxillipeds) were dissected from freshly collected, cold-anaesthetized animals and fixed in 2.5% gluteraldehyde (in 0.2μm filtered seawater) for 48h at 4°C. Samples were then processed for viewing with a Cambridge S200 scanning electron microscope (SEM) as described in Harris et al. (1991). Appendages of at least 3 individuals of each species of prawn were examined.

Gut architecture

Animals were cold-anaesthetized and fixed in 5 % formalin within 2 of collection. Total length (tip of rostrum to end of telson) and carapace length (tip of rostrum to posterior edge of carapace) were recorded. The animals were dissected to remove the entire digestive system, and the dimensions of the fore, mid and hindgut and the hindgut diverticulum were measured using a camera lucida attached to a stereo microscope (x40 magnification). Gut volume was calculated assuming an oval-

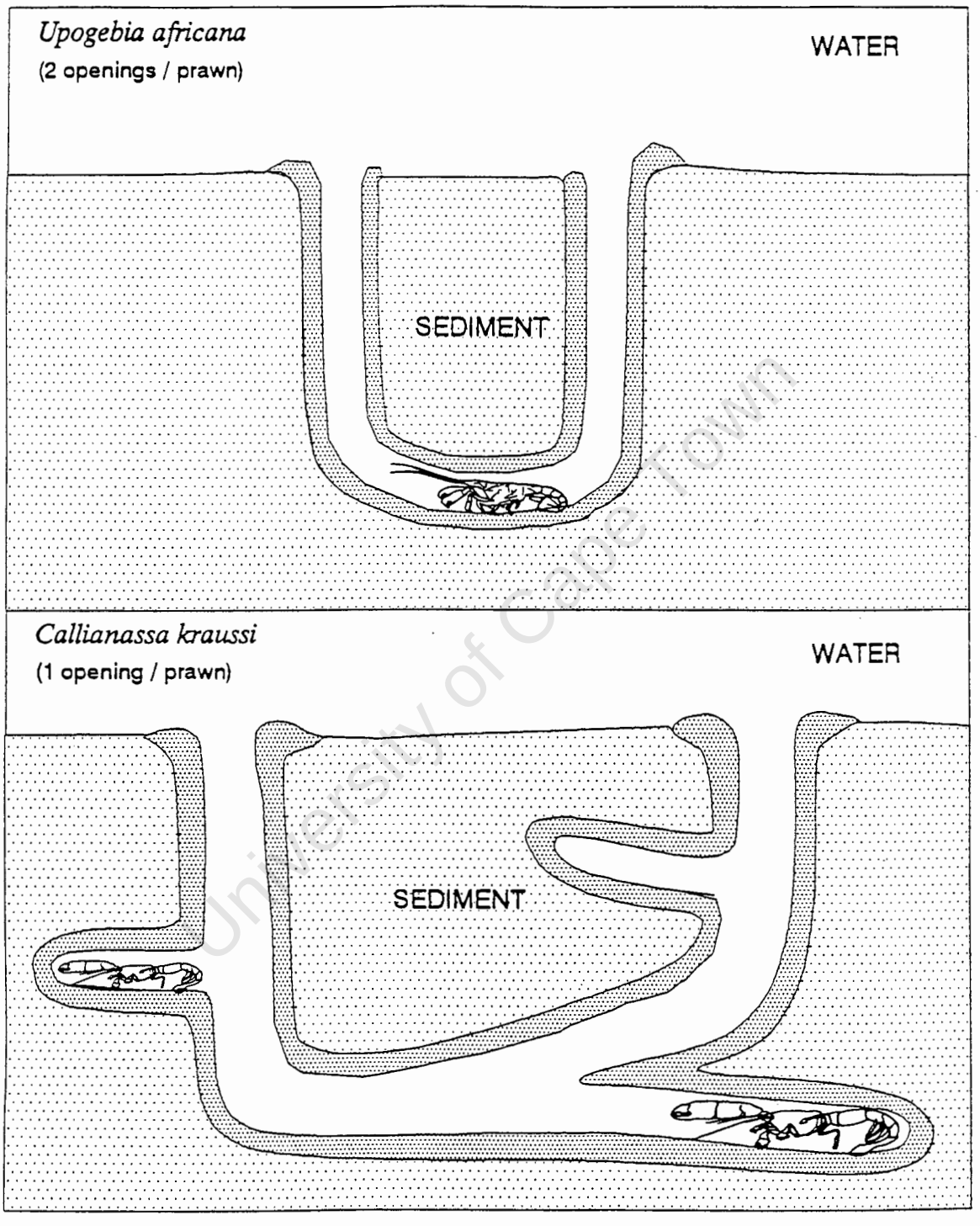

Figure 1. Diagramatic representation of simple U-shaped dwelling-burrow of *Upogebia africana* and irregular feeding-burrow of *Callianasa kraussi*

shaped foregut and cylindrical shapes for the other gut regions. The volume of the hepatopancreas was measured by displacement in water in a graduated test-tube. Dry weights of the body (minus digestive system) and the various gut regions were obtained after drying at 60°C for 48h. Results are reported in terms of ratios of body size (volume, weight and length) to gut size, relative compartmental sizes, total aspect ratios and compartmental ratios.

Feeding cycle and gut passage time

Thirty *U. africana* and 40 *C. kraussi* prawns were collected respectively from Geelbek and Oesterwal in Langebaan lagoon at 2-hourly intervals for 36h on 2 consecutive spring tides in April 1991. A random sampling grid was established at mid-tide level on the shore to prevent repeat sampling of burrows and to minimize disturbance. The 36-hour period sampled included at least 1 diurnal and 2 tidal cycles for each prawn species. Immediately on collection, prawns were examined to establish the fullness of the mid- and hindguts. This method was possible as the exoskeleton is transparent in this region allowing a direct view of the gut and its contents. Results are expressed as percentage of animals with full guts, and these were plotted for the 2 hourly sampling intervals to allow estimation of gut throughput times.

Stable isotope analyses

U. africana and *C. kraussi* were collected during September 1990, cold-anaesthetized and immediately dissected to remove muscle tissue (from the abdomen), and gut contents. On the same sampling occasion samples of sediment from Geelbek and Oesterwal, leaves of the two saltmarsh vascular plants *Zostera capensis* and *Spartina alterniflora* and a prolific epiphyte of *S. alterniflora* were collected. Samples were decalcified as follows (Sealy et al. 1987): animal and plant samples were rinsed in 1.5% HCl for 15 min, while 1.5% HCl was added to sediment samples, which were sonicated for 5 min and stirred until release of bubbles ceased. Trials with 10% HCl (see Haines & Montague 1979, Sullivan & Moncreiff 1990) on duplicate samples revealed no difference in the carbon and nitrogen standard isotope ratios. Phytoplankton samples were obtained from each site by filtering 1-2 l of lagoon water onto premuffled (450°C, 6 h) GF/F filters. All samples were dried at 50°C for 48 h.

Separate samples of 6-26mg and 15-55mg respectively of plant, muscle and gut content were analysed for $^{13}C/^{12}C$ and $^{15}N/^{14}C$ ratios. Samples of 100-

800mg of sediment and sediment organics were analysed for both $^{13}C/^{12}C$ and $^{15}N/^{14}C$ ratios. Filters plus phytoplankton and control filters were ball-milled and 40mg analyzed for $^{13}C/^{12}C$ ratios. Triplicate samples from each source were prepared for both cabon and nitrigen analyses. Each sample was loaded into a quartz breakseal tube with excess copper, copper oxide and silver foil. The tube was evacuated to 10^{-2} Torr, sealed and combusted in a furnace at 800°C for 8 h. The isotope ratios of the resultant N_2 and CO_2 gas samples were measured on a VG Micromass 602E 90° sector double-collector mass spectrometer. Stable isotope ratios are reported with reference to a standard (Fry & Sherr 1984). The difference between the sample and the standard is expressed in parts per thousand or per mil (°/oo), calculated according to the following formula:

$$sX\ (^{o}/oo) = [(R_{sample}/R_{standard})-1] \times 10^{3},$$

where X is ^{13}C or ^{15}N, R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$, and the standards for C and N are Peedee Belemnite carbonate (PDB) and atmospheric diatomic nitrogen, respectively. The measurement error was less than 0.06 °/oo.

Gut content analyses

Animals were cold-anaesthetized and dissected in the field immediately after collection. The whole guts of ten animals of both *U. africana* and *C. kraussi* were preserved separately for each of the following estimations: protozoa, diatom, meiofauna numbers and biomass, organic content, C/N ratio and chlorophyll content. Fixation and processing methods, and carbon and nitrogen biomass calculations, followed the methods described in Chapter 1 for sediments. Results are expressed per whole gut of similarly sized animals (total length ± 40 mm).

In addition, the gut contents of 5 animals of each species were collected for examination by scanning electron microscopy. These samples were prepared as described above.

Results

Feeding apparatus

U. africana is a filter-feeder which feeds by creating a current through its burrow using the pleopods, and filtering particles from the water column using a filtering basket comprising pereiopods 1 and 2 and maxilliped 3. Pereiopods 1 and 2 have a

double fringe of filtering plumose setae (Plate 1a) on the ventral margin of the appendage, and the mean distances (±s.d.) between setal filaments (setules) are 0.8 ± 0.35 um (n-15) for pereiopod 1 (Plate 1b), and 1.0 ±0.40 um (n=16) um for pereiopod 2 (Plate 1c&d). Plumose setae have two diametrically opposed rows of fine setules lining the marginal edges of a long distally tapering shaft. This suggests that *U. africana* can retain particles at least as small as 1 um, if the boundary layer affect is considered. Although *U.africana* can retain very small particles, larger mobile organisms are probably not efficiently retained by the passive filters.

C. kraussi is a burrow-dwelling deposit-feeder which uses a combination of digging and filtering to feed. Pereiopod 3 is shovel-shaped (Plate 2c) with a short squared dactyl and widely spaced clumps of coarse setae on the large rounded propodus (Plate 2d), while pereiopod 2 (Plate 2a) and maxilliped 3 have long plumose setae on the ventral margins and are therefore modified to act as filters. Pereiopod 3 is adapted to selectively capture large particles, while pereiopod 2 and maxilliped 3 are able to filter out small particles that have been suspended by the shovelling action of perieopod 3. The mean distance between the setal filaments of pereiopod 2 (filter) is 3.1 ±1.4 um (n=17) (Plate 2b) suggesting that this species may not be able to retain particles smaller than approximately 3 um, whereas the distance between setae (very few setules present) of pereiopod 3 (Plate 2d) is approximately 27 ± 9.4 um (n=19), and it appears only to be able to retain fairly large particles.

The cardiac (anterior) region of the foregut of *C. kraussi* consists of a well-developed crushing-type gastric mill typical of most decapods (see Schaefer 1970), and the denticles of the lateral tooth are reticulated to deal with crushing particles (Plate 2e&f). The filtering setae in the smaller posterior pyloric portion of the foregut sort the masticated food by size; large particles are channelled into the midgut, whereas small particles and fluids enter the digestive gland. In *U. africana* the gastric mill in the cardiac (anterior) region of the foregut is modified to act as a primary filter, squeezing rather than grinding the food (Schaefer 1970). The lateral teeth are not hardened or reticulated, and act as filters rather than as crushing teeth (Plate 1e&f). This filtering process is continued in the pyloric region where there are many setae, and this results in a seperation of large particles which are passed directly to the midgut, and fine particles which enter the digestive gland.

Gut architecture

The body length : gut length ratio of *U. africana* is slightly lower than that of *C. kraussi* indicating that the throughput gut (foregut, midgut and hindgut) of *U.*

Upogebia africana

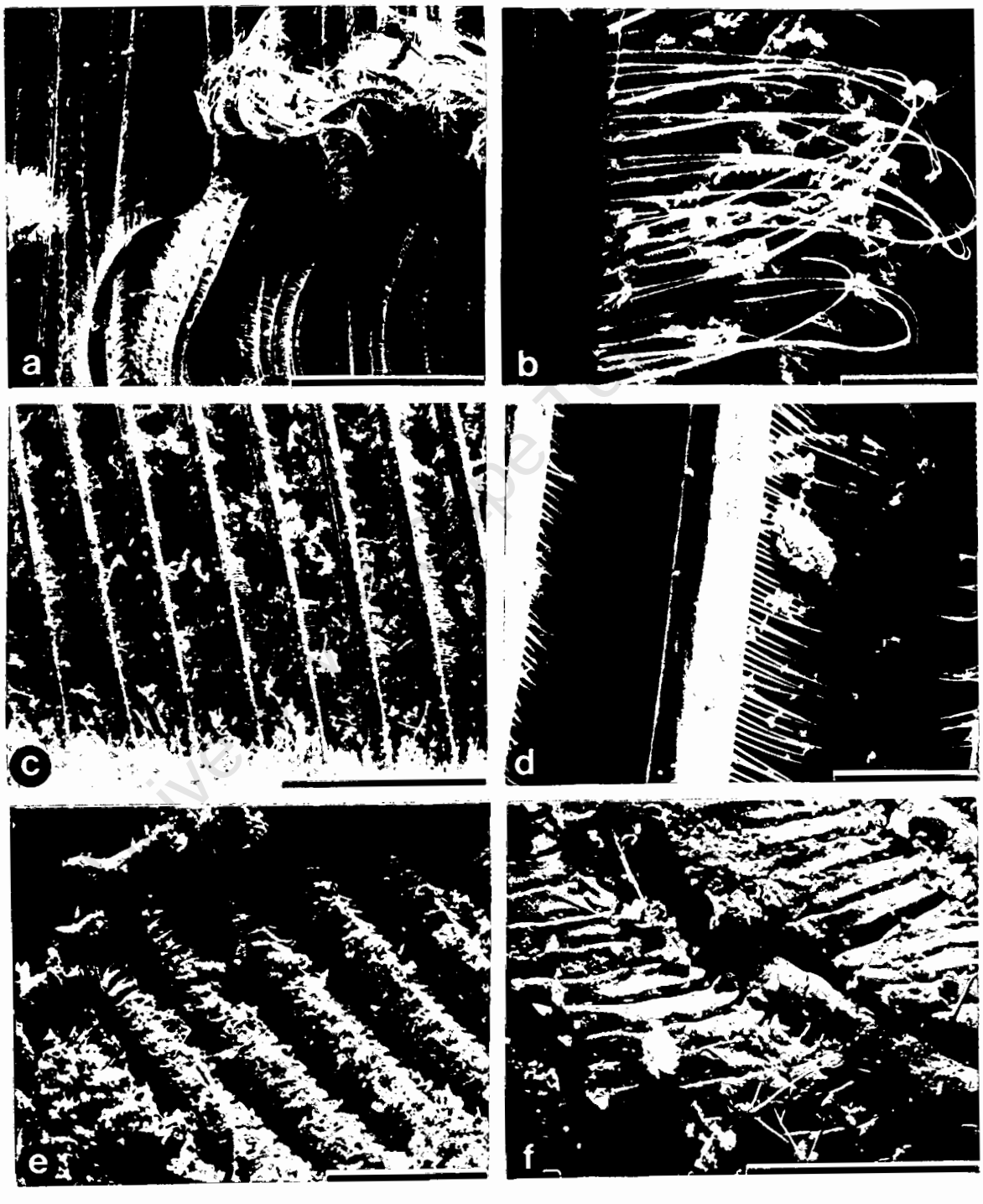

Plate 1

Plate 1. Scanning electron microscope views of feeding appendages and foregut micro-structure of *Upogebia africana*. a. Pereiopod 1, double layer of filtering setae on ventral margin, b. Pereiopod 1, Setules on filtering setae, c. Pereiopod 2, single layer of filtering setae on ventral margin, d. Pereiopod 2, diametrically opposes double row of setules on each filtering seta, e&f. Lateral tooth in foregut modified for filtration. Scale bars: a. 500μm, b. 10μm, c. 100μm, d. 20μm, e. 100μm, f 50μm

Plate 2. Scanning electron microscope views of feeding appendages and foregut micro-structure of *Callianassa kraussi*. a. Pereiopod 2, filtering setae on ventral margin, b. Pereiopod 2, diametrically opposed double row of setules on each filtering seta, c. Pereiopod 3, shovel-shaped large rounded propodus and squared dactyl, d. Pereiopod 3, clusters of setae with few setules on the propodus, e&f Lateral tooth in foregut with sharp chitinized surfaces. Scale bars: a. 100μm, b. 20μm, c. 2 mm, d. 200μm, e. 100μm, f. 30μm

Callianassa kraussi

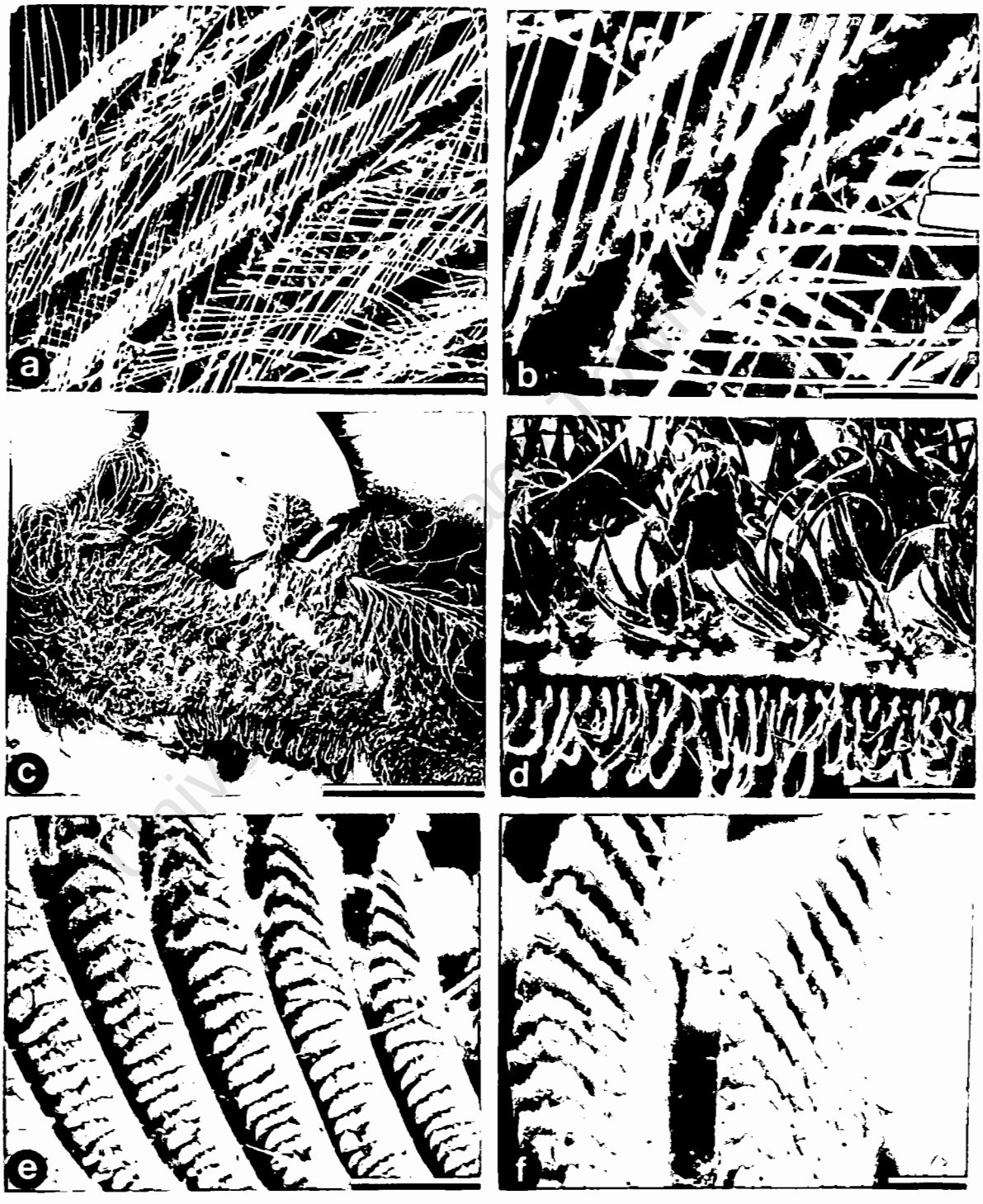

Plate 2

africana is shorter than that of *C. kraussi* (Table 1). However, the throughput gut of *U. africana* is much larger than that of *C. kraussi* in terms of both volume and weight relative to body volume and weight (Table 1). When the size of the total digestive system (including hepatopancreas) is compared to the total body size, in terms of volume and weight, that of *U. africana* is also larger than that of *C. kraussi*. The aspect ratios i.e. length/width (Table 2) of the whole gut and the various compartments are lower for *U. africana* than *C. kraussi*, indicating that the gut has a relatively larger diameter in all gut compartments of *U. africana*. Thus *U. africana*'s gut is larger, wider but shorter than that of *C. kraussi*.

The linear dimensions of the guts of the two species of prawn are shown diagrammatically in Figure 2A. The foreguts of the two species of prawn have similar lengths relative to the total gut length (Figure 2B). However, the relative midgut length of *C. kraussi* is larger than that of *U. africana*, while the hindgut length of *U. africana* is greater than that of *C. kraussi*. Thus *C. kraussi* has a longer midgut while *U. africana* has a longer hindgut. While the foregut volume of *C. kraussi* is slightly larger than that of *U. africana* relative to its body volume, the volumes of the mid and hind guts of *U. africana* are relatively larger than those of *C. kraussi* (Figure 2C). Thus different regions of the throughput gut dominate the volume in the two species of prawn. *C. kraussi* has a larger diverticulum (in terms of both length and volume) than does *U. africana*.

If the sizes of the different gut compartments are compared to the size of the total digestive system, i.e. including the hepatopancreas (Figure 3), it is clear that in terms of volume the throughput gut (fore, mid and hind) is much larger in *U. africana*, while the hepatopancreas has a much higher relative volume in *C. kraussi* than *U. africana* (Figure 3A). The relative foregut volume in particular is much higher in *U. africana* than in *C. kraussi*. In *C. kraussi* the greatest proportion of the total gut volume is made up of the hepatopancreas, while in *U. africana* the other regions, especially the foregut, contribute significantly to total volume. If relative weights (Figure 3B) are considered it is clear that the foregut of *C. kraussi* is heavier than that of *U. africana* despite the lower relative volume. This may reflect the modifications of the teeth in *U. africana* away from heavy chitinous teeth to soft filters.

Feeding periodicity and gut throughput time

Figure 4 gives the percentage of animals (*U. africana* and *C. kraussi*) with full guts at 2-hourly intervals over 2 tidal cycles. Both *U. africana* and *C. kraussi* have feeding

Table 1. Body : gut (B/G) ratios for *U. africana* and *C. kraussi*. Values represent the means (± one standard deviation) of 20 measurements.

Ratio	*U. africana*	*C. kraussi*
Body : throughput* gut		
Length	1.19 (0.15)	1.16 (0.14)
Volume	12.29 (4.05)	109.60 (31.8)
Weight	31.13 (9.75)	54.36 (21.28)
Body : Total digestive system		
Length	1.19 (0.15)	1.16 (0.14)
Volume	6.44 (1.39)	10.23 (3.83)
Weight	6.74 (1.63)	7.10 (3.01)

* foregut + midgut + hindgut

Table 2. Aspect ratios (length/width) of throughput gut (foregut, midgut and hindgut) and gut compartments of *U. africana* and *C. kraussi*. Values represent the means (± one standard deviation) of 20 measurements.

	Aspect ratio	
Gut / Compartment	*U. africana*	*C. kraussi*
Throughput gut	17.78 (1.59)	21.75 (2.03)
Foregut	1.86 (0.15)	2.07 (0.15)
Midgut	15.44 (3.55)	34.18 (4.56)
Hindgut	14.16 (3.18)	9.45 (2.08)
Diverticulum	18.53 (4.73)	36.84 (10.47)

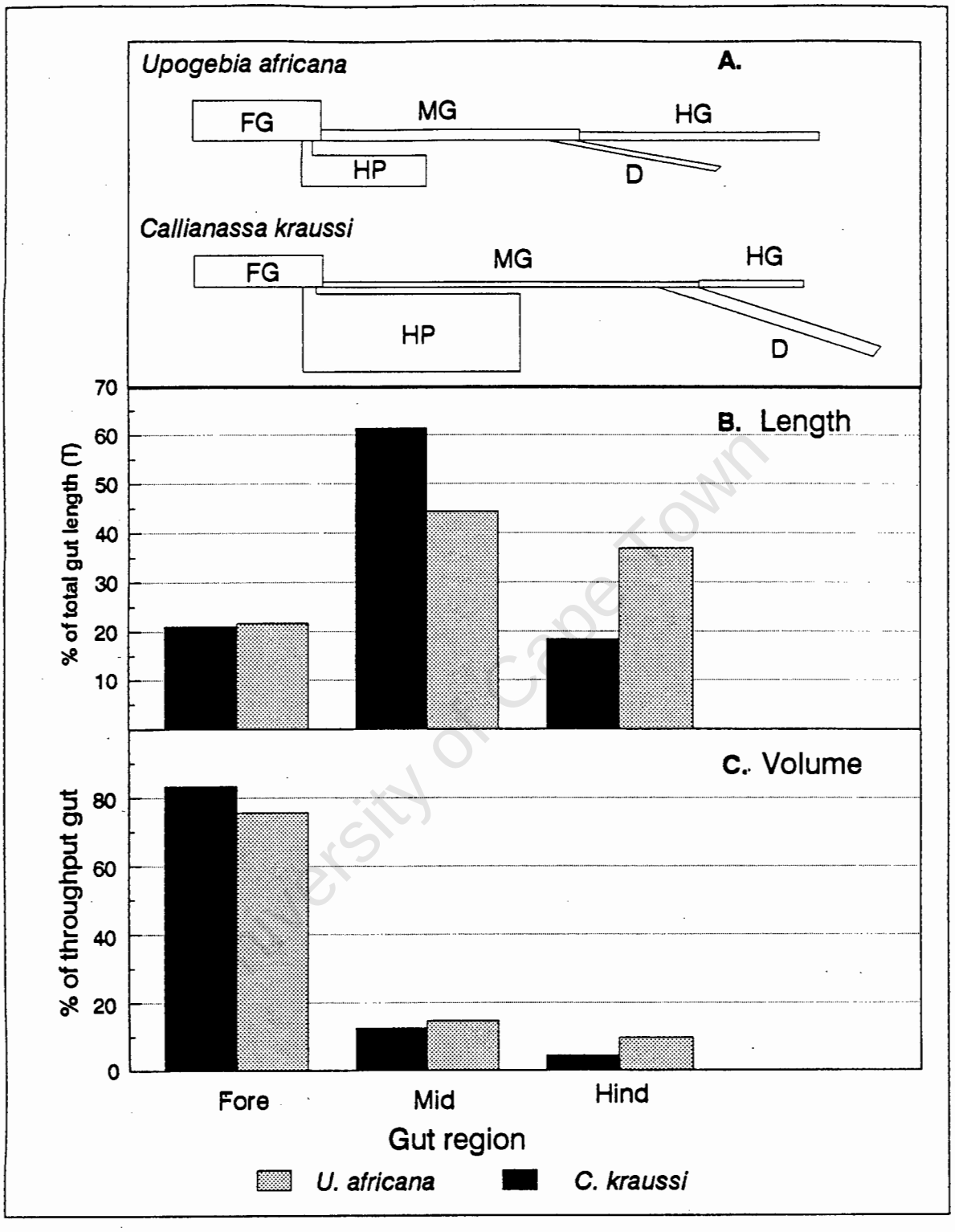

Figure 2. Digestive system configuration (A)and relative sizes (length and volume) of the compartments of the throughput gut (fore, mid & hindgut) (B & C) *of Upogebia africana* and *Callianassa kraussi.* FG = foregut; HP = hepatopancreas; MG = midgut; D = diverticulum; HG = hindgut.

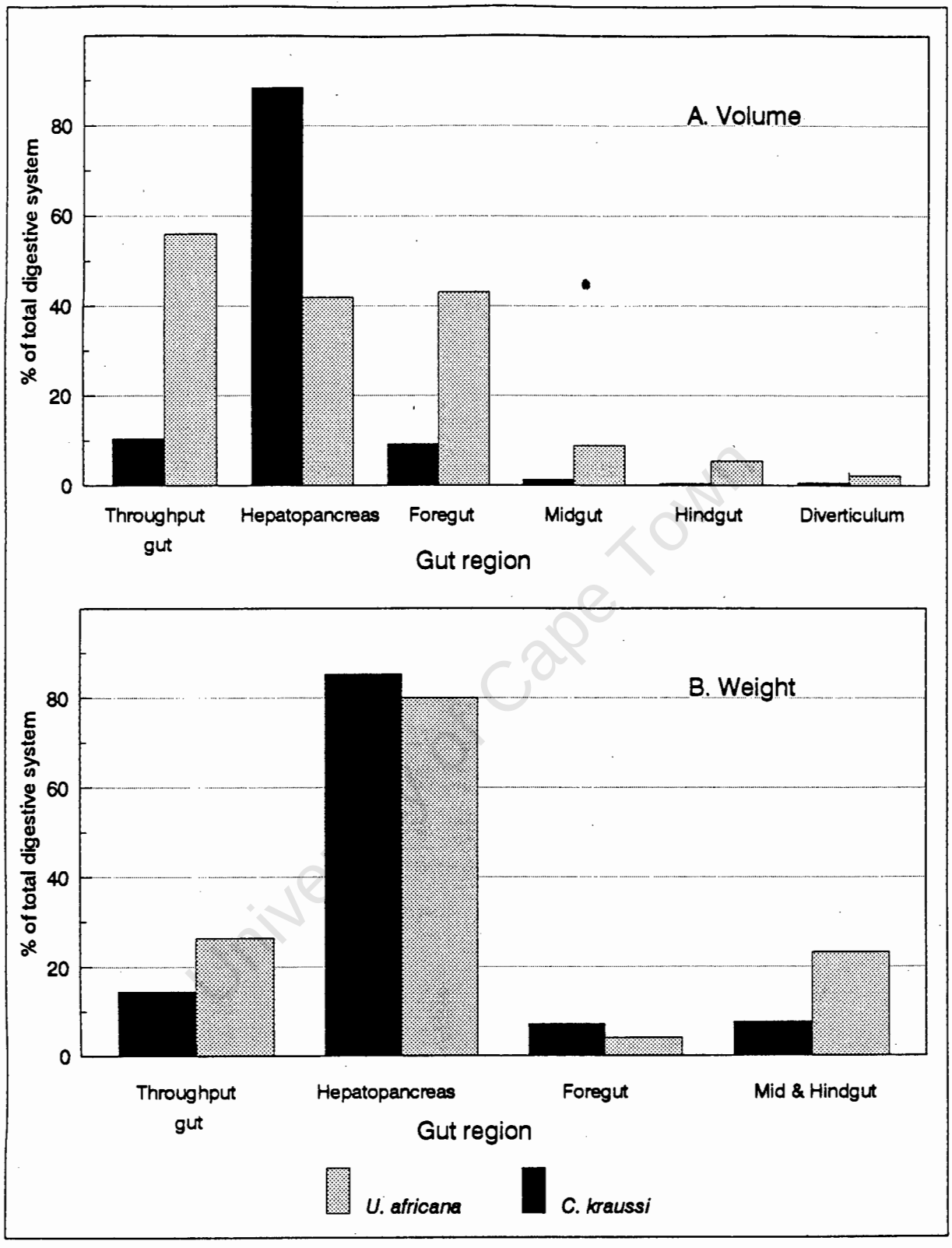

Figure 3. Proportions of the total volume and weight of the digestive system contributed by the throughput gut (fore, mid and hindgut) and the different regions of the gut.

cycles closely linked to the tidal cycle. Prawns feed on an incoming tide, while gut evacuation occurs on an outgoing tide. At high tide the number of animals with full guts is at a maximum and at low tides almost all animals have empty guts. No diurnal effect on the feeding cycle was detected, with peaks of animals with full guts occurring at high tides during the day and night in both species of prawn. The onset of feeding appears to lag the incoming tide, since the maximum peaks of fullness generally occurred approximately 2 hours after time of high tide.

Information about the prawns' feeding patterns and gut throughput times can be gained by parsimonious interpretation of the gut fullness curves (Figure 4). Both species of prawn showed individual peaks with >95% of the animals with full guts, indicating that virtually all animals had eaten a meal on those occasions. If it is assumed that all prawns fed with a constant gut throughput time over the 36 hour sampling period, then the lower peaks (<95% with full guts) indicate the proportion of the total population that had eaten a meal on those occasions, i.e. not all animals eat during each tidal cycle. The alternative explanation is that all animals do eat at every tidal cycle and that the variable height of the peaks indicates that the gut passage times of the prawns are highly variable, and that individual animals are filling and emptying their guts repeatedly during one tidal cycle. The latter scenario is highly unlikely considering that the gut passage rate of an animal would have had to change as much as 2 fold within the 36 hour sampling period to obtain peaks as low as 50% full guts as were observed (Ellis & Small 1989).

If each individual peak is considered for either species of prawn it is apparant that they are symmetrical and have a sharp apex, i.e. the filling slope is similar to the emptying slope (Figure 4). This suggests that there are no sharp cues that cause synchronized onset of feeding or of gut emptying. Assuming that the gut throughput time did not vary widely during the sampling period, then it follows that the time that any one animal has food in its gut (i.e. its gut passage time), is on average equal to half the base of the curve when extrapolated to the zero line, i.e. approximately 6 hours, because different individuals may have begun feeding at different times even although the whole population simultaneously achieved full guts at the peaks of the highest curves. For example, in the hypothetical curve shown in Figure 5 one animal may have fed at time a and emptied by time b, while a second animal fed at time c and emptied by time d, etc. The sampling bias (accuracy) with a symmetrical curve will be approximately 1 hour (half of the 2 hourly sampling interval).

Furthermore, considering the sharp peak of the curve it is possible to deduce that the feeding bout (period of active gut filling) of each animal is for a

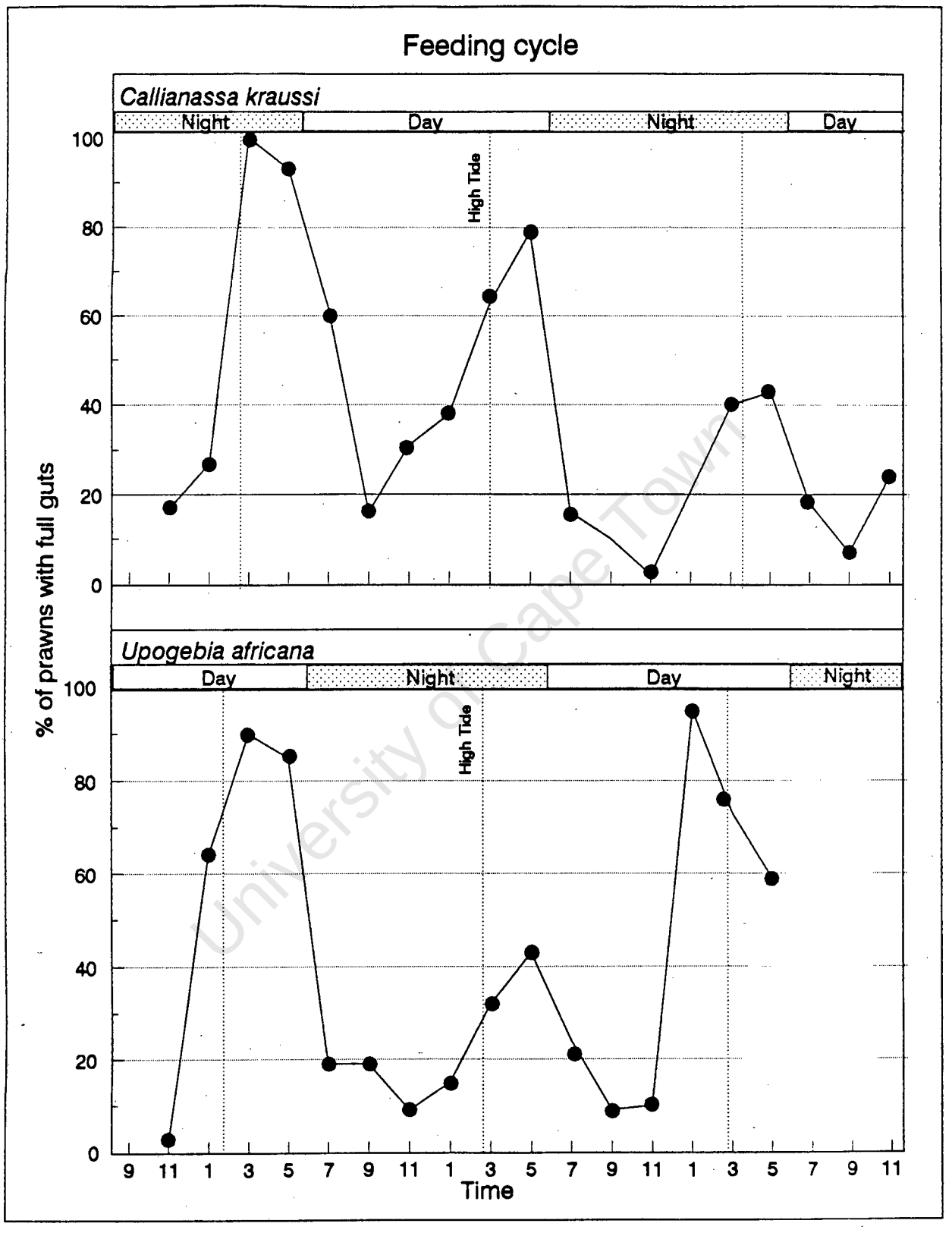

Figure 4. Feeding cycle as shown by percentage with full guts at two hourly intervals over three tidal cycles.

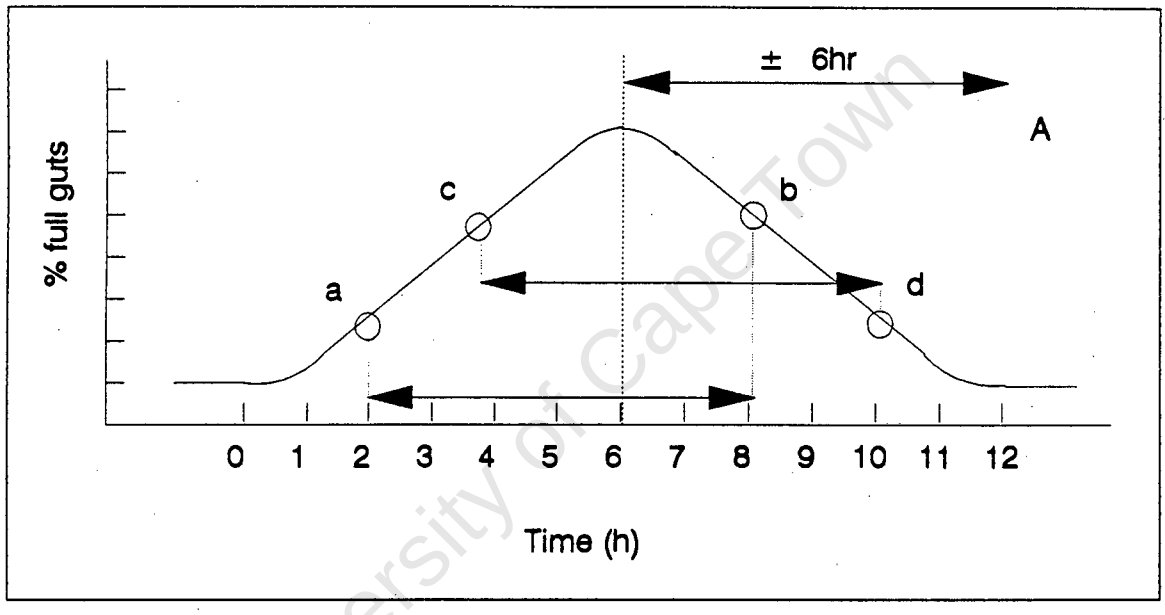

Figure 5. Schematic representation of feeding cycle and throughout time. For an explanation of a - d, see text.

maximum of 2 hours (period between sampling). Thus an animal feeds continuously for up to 2 hours. A longer feeding bout would result in a plateau in the curve (where all animals are full) with a width approximating the period of feeding, a scenario obviously not represented by the data for either *U. africana* or *C. kraussi*.

Stable isotope analyses

The stable carbon and nitrogen isotope ratios of the prawns and their potential food sources in Langebaan lagoon are given in Table 3. The ^{13}C values for *U. africana* and *C. kraussi* ranged from -11.14 to -11.16 and from -13.05 to -13.10, respectively. The stable carbon isotope ratios of *C. kraussi* are depleted by approximately 2 $^{o}/oo$ relative to those of *U. africana*. The sediment and sediment organics had similar narrow ranges of ^{13}C values at the 2 sites, i.e. ranging from -13.19 to -13.80 at Geelbek and -13.75 to -12.62 at Oesterwal. The saltmarsh vascular plants *Zostera capensis* and *Spartina alterniflora* had ^{13}C values ranging from -11.17 to -13.13, while the filamentous algal epiphyte associated with *S. alterniflora* had much more depleted ^{13}C values of -17.09 to --18.09. Another possible food source, i.e. phytoplankton in the water column, had ^{13}C values of -17.96 to -21.97. Interestingly, the gut contents of *U. africana* had values of -15.56 to -17.76 closest to phytoplankton, while that of *C. kraussi* had values closest to vascular saltmarsh plants i.e. -9.3 to -10.35.

C. kraussi was enriched with respect to its stable nitrogen isotope ratio (11.81 to 11.86) compared to *U. africana* (9.02 to 9.38). Similarly, sediment and sediment organics had higher ^{15}N values at Oesterwal (10.59-11.28) than at Geelbek (8.74 to 9.70). The two saltmarsh plants had lower narrow ranges of ^{15}N values, ranging from 5.62 to 6.57 $^{o}/oo$, and that of the epiphyte fell below this range (5.49-5.57). No ^{15}N values were obtained for gut contents and phytoplankton because too little gas was evolved from these samples.

Figure 6 shows the ^{13}C and ^{15}N values respectively for *U. africana* and its potential food sources. Considering enrichment values from food to consumer of 0-2$^{o}/oo$ and 3-5$^{o}/oo$ for carbon and nitrogen stable isotope ratios, respectively, it is clear that only *S. alterniflora* and *Z. capensis* are significant sources of carbon and nitrogen assimilated by *U. africana*. It is however possible that the epiphyte which occurs prolifically on *S. alterniflora* may contribute to nitrogen acquisition by this prawn.

It appears that *C. kraussi* obtains its carbon primarily from sediment organics which have a $^{13}C/^{12}C$ ratio slightly depleted compared to *S. alterniflora*

Table 3. Carbon (^{13}C) and nitrogen (^{15}N) isotope ratio measurements of *C. kraussi* and *U. africana* muscle tissue and potential sediment and water resources.

Source	^{13}C	^{15}N
Prawns		
C. kraussi	-13.1	11.81
	-13.08	11.86
	-13.05	11.84
U. africana	-11.14	9.14
		9.38
	-11.16	9.02
Prawn gut contents		
C. kraussi	-10.35	-
	-9.3	-
U. africana	-16.51	-
	-17.76	-
	-15.56	-
Sediment organics		
Oesterwal	-13.65	10.71
	-9.08	11.28
	-13.75	
Geelbek	-13.49	9.70
	-13.19	9.15
Sediment		
Oesterwal	-13.07	10.92
	-12.55	10.59
	-12.62	
Geelbek	-13.50	8.74
	-13.80	8.87
	-13.69	
Saltmarsh plants		
Zostera	-11.92	6.29
	-11.17	6.46
	-11.64	
Spartina	-12.83	6.57
	-12.75	5.62
	-13.13	
Water (Phytoplankton)		
Oesterwal	-17.96	-
Geelbek	-21.97	-
Algal epiphyte.		
Geelbek	-18.09	5.49
	-17.9	5.57

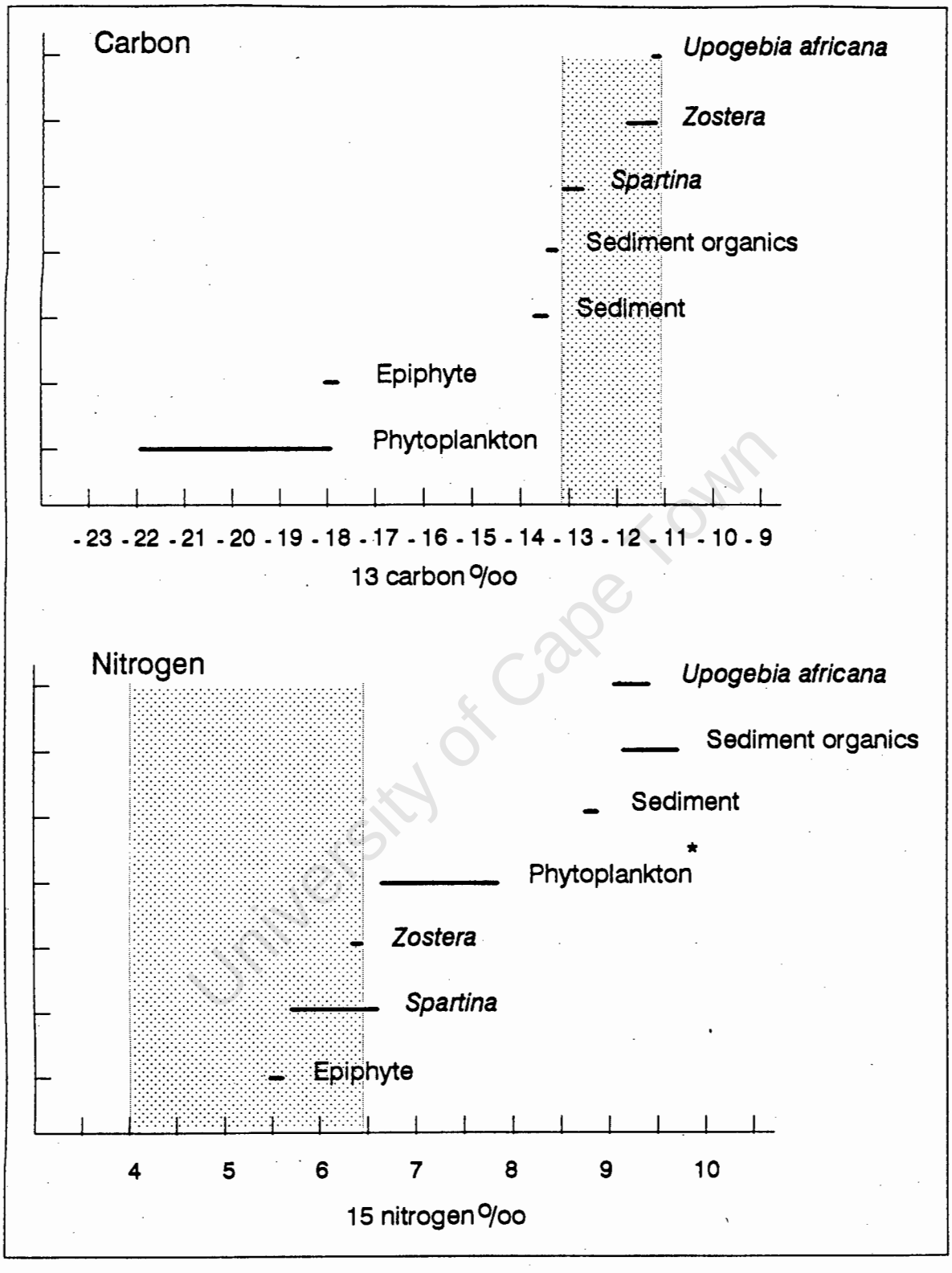

Figure 6. Stable carbon and nitrogen isotopic ratios of *Upogebia africana* and potential food sources. Shaded areas represent expected isotopic ranges of food sources. * From Sholto-Douglas *et. al.* (1991).

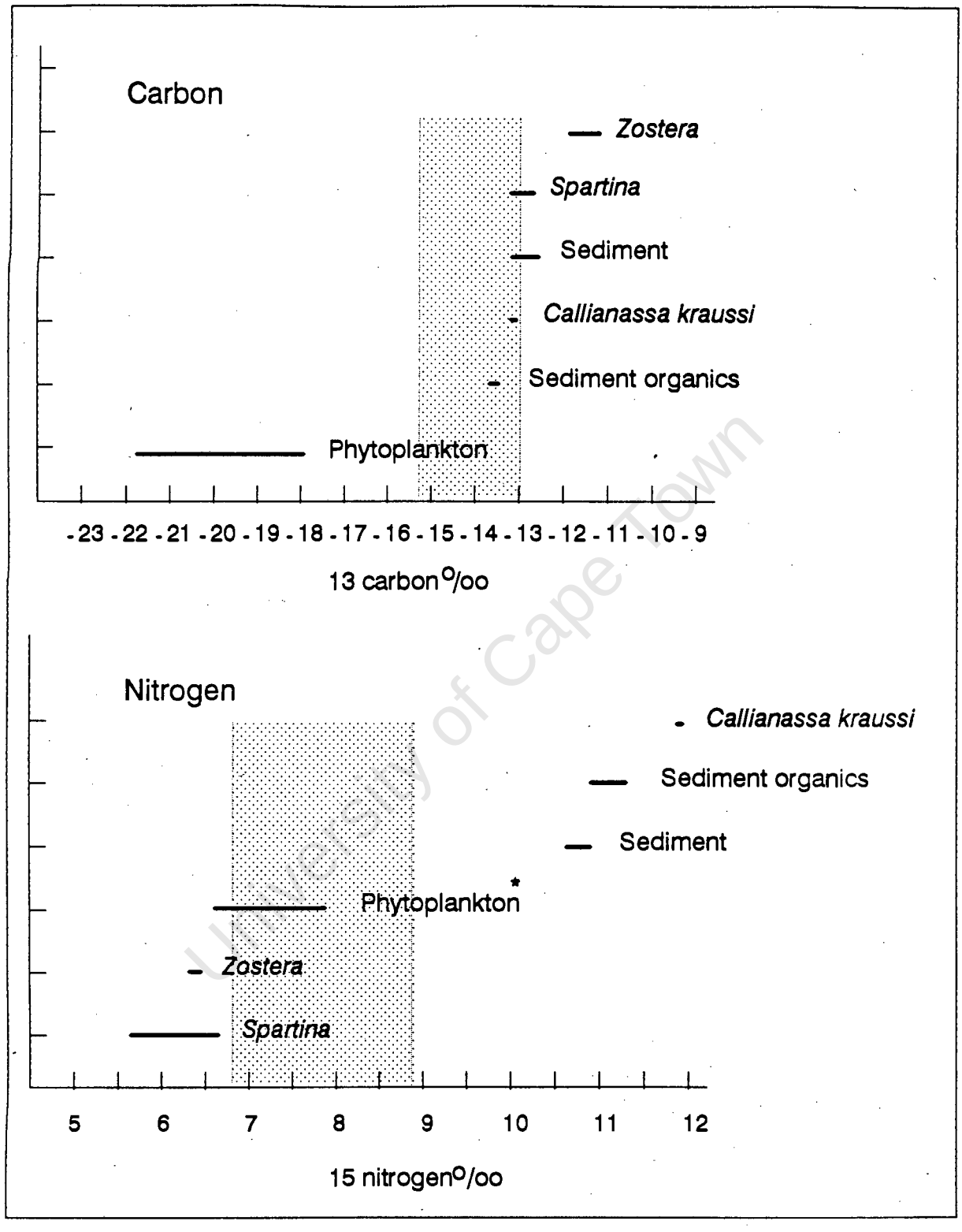

Figure 7. Stable carbon and nitrogen isotopic ratios of *Callianassa kraussi* and potential food sources. Shaded areas represent expected isotopic ranges of food sources. * From Sholto-Douglas *et. al.* (1991).

and *Z. capensis*, probably due to the presence of diatoms which have a much more depleted value (Figure 7). Thus *C. kraussi* obtains its carbon from a mixture of saltmarsh vascular plant detritus and diatoms, but the saltmarsh plants are the dominant source. In terms of nitrogen, *C. kraussi* does not appear to derive nutriment directly from any of the sources tested. However, nitogen isotope ratios for phytoplankton in the region (Sholto-Douglas et al. 1991) and those for micro-algae from other salt-marsh systems (Peterson et al. 1985), suggest that *C. kraussi* probably obtains most of its nitrogen from sediment diatoms (and possibly protozoa).

Figure 8 is a combined plot of carbon and nitrogen stable isotope ratios obtained for the two species of prawn and their potential food sources. In addition, offshore phytoplankton data (Sholto-Douglas et al. 1991) is overlayed. It is clear that *U. africana* derives both its carbon and nitrogen from the saltmarsh vascular plants, particularly *S. alterniflora*. Geelbek organics are skewed to the left (depleted) by non-plant elements i.e. diatoms which do not appear to be an important source of nutrients for *U. africana*.

The picture for *C. kraussi* is somewhat different. While *C. kraussi* appears to utilize carbon and nitrogen from phytoplankton, Oesterwal organics are strongly implicated as a carbon source (Figure 8). These organics have carbon stable isotope values that indicate a mixture of saltmarsh plants and diatoms, but are skewed towards saltmarsh plants. Therefore it is probable that *C. kraussi* uses vascular plant detritus in the sediments as its main carbon source.

Gut content analyses

Scanning electron microscope views revealed that the gut contents of both species of prawn included plant fragments, whole and fragmented diatoms, partially degraded protozoa and bacteria attached to organic matter (for further details see Harris et al. (1991), Figure 2, Chapter 5). No remnants of meiofauna were observed in the guts of either species of prawn. A distinctive feature of midgut contents, particularly those of *U. africana*, was the dense coating of the gut contents by filamentous bacteria, which were absent from hindgut contents.

Light microscopy of gut contents confirmed the presence of a bulk matrix of particulate organic matter and the presence of diatoms and protozoa in both *U. africana* and *C. kraussi* (Figure 9). Very few meiofauna were found in the gut contents of either species of prawn. The proportion of living to non-living material in the gut contents is higher in *C. kraussi* than in *U. africana*. In addition, a

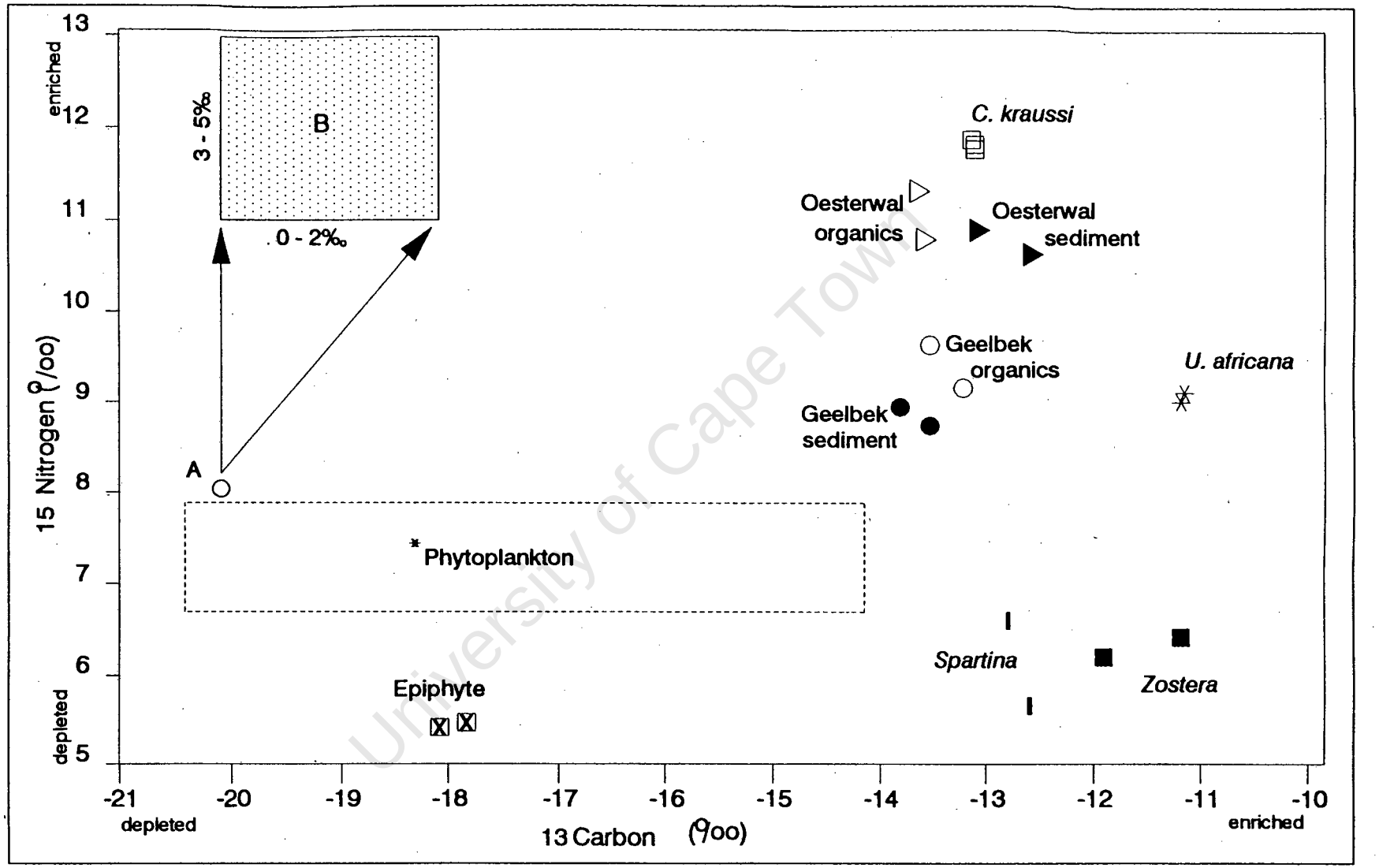

Figure 8. Nitrogen and carbon isotopic ratios of consumers and potential food sources

B represents the expected isotopic range of consumers eating a food source of isotopic carbon and nitrogen values corresponding to A.

* from Sholto-Douglas *et. al.* (1991)

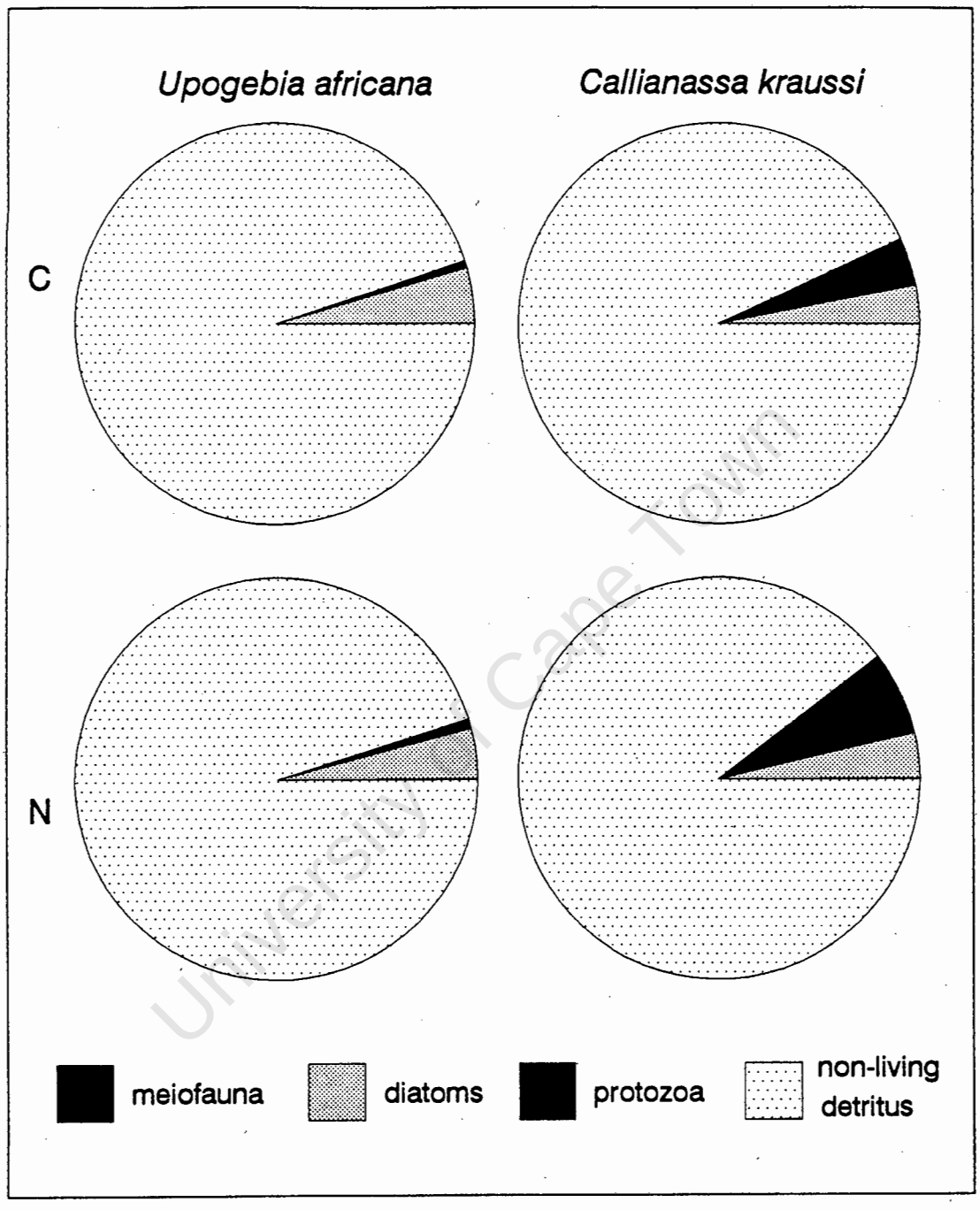

Figure 9. Relative carbon and nitrogen biomass of components of the gut contents of *Upogebia africana* and *Callianassa kraussi*.

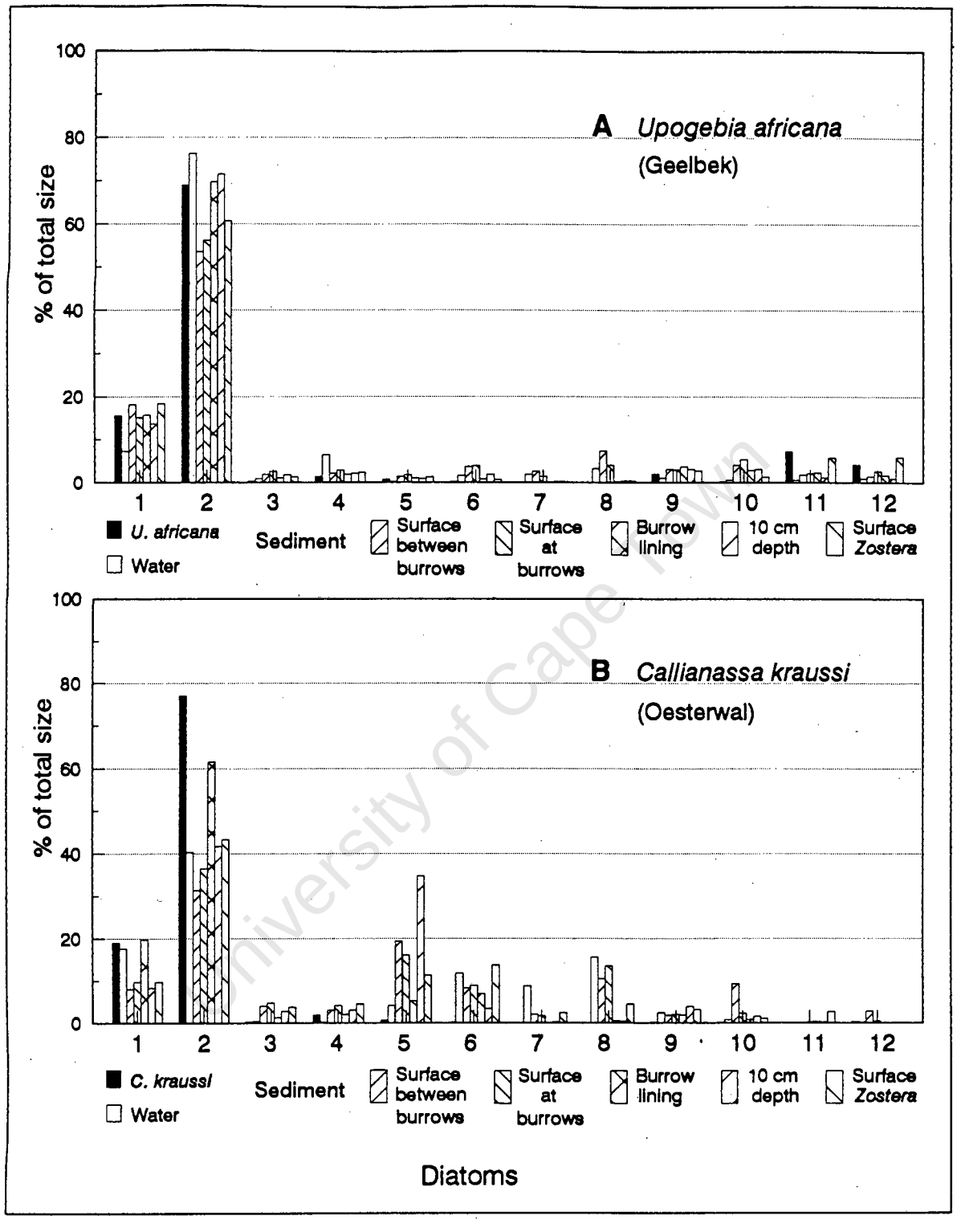

Figure 10. Relative percentages of density of different diatoms in gut contents and respective sediments and water column of *Upogebia africana* and *Callianassa kraussi*. *1 - 12 represent the different categories of* diatom counted.

much larger proportion of the carbon and nitrogen in the gut contents of *C. kraussi* are contributed by sediment protozoa than in those of *U. africana*.

If the composition and relative proportions of diatoms in the guts of the prawns are compared with those in the respective sediments and water in the habitats in which they live (Figure 10), *U. africana* appears to be less selective than is *C. kraussi*. While the composition of diatoms inside the gut of *U. africana* closely approximates that of Geelbek sediments and water column, it appears that C. kraussi does not ingest all types of diatoms, i.e. their incidence in the gut is proportionally less than in the sediment. In both species of prawn the ingested diatoms are dominated by centric diatoms, particularly those of the small size (25x10um) category. However, selection by *C. kraussi* does not seem to be size-related.

Discussion

Functional feeding morphology and diet

A number of studies have correlated the structure of feeding appendages with the diet of crustaceans (e.g. Manjulatha & Babu 1991, Mertens et al 1991, Coleman 1989, Jones 1968). Nival and Nival (1976) measured the distance between the filtering setae of a copepod, and compared this with the experimentally determined size retention efficiency. They found that the experimentally and theoretically obtained values correlated closely. Similarly, Dalley & McClatchie (1989) examined the feeding apparatus and gut contents of the euphausiid *Nyctphanus australis* by scanning electron microscopy and found that tentative predictions on the diet can be made by examination of functional feeding morphology.

Food capture mechanisms of *U. africana* and *C. kraussi* have received only superficial attention (Hill 1967, Forbes 1973) while particle size retention has not been investigated previously. Results of the present study indicate that *U. africana* is equipped to filter-feed non-selectively on suspended particles in the water column. Inter-setule distance on the filtering setae of the pereiopods indicate that this prawn is able to retain very small particles ($<0.8\mu m$) including protozoans and free-living bacteria, but is poorly equipped to capture larger mobile meiofauna (Plate 1). Most of the organic material in the sediments and water column of this prawn's habitat, Geelbek, is contained in the 20-64μm fraction, with appreciable amounts in the 2-20 and $<2\mu m$ fractions (Chapter 1). Most of the biota occur in the $<64\mu m$ fraction. Thus *U. africana* is able to exploit most of the available organic

material, as well as capture most of the biotic components present in the water column and suspended from the sediments. By contrast, *C. kraussi* is capable of selectively sorting through sediment to capture larger particles (e.g. diatoms) and possibly larger mobile prey species, as well as filter-feeding on material suspended by the shoveling action of the pereiopods (Plate 2). However, the inter-setule distances on the filtering setae of this prawn indicate that it is not able to retain very fine particles, such as unattached bacteria. Most of the organic material in the sediments at Oesterwal is contained in the 20-64 and $<2\mu$m fractions, while the biota mainly comprises organisms of $<64\mu$ (Chapter 1). *C. kraussi* is not equipped to feed selectively on particles of $<27\mu$m diameter, and cannot retain particles $<3\mu$m on its filters. Therefore the smallest size fraction of organics ($<2\mu$m) is unavailable to this prawn, but it is able to exploit the larger biota such as protozoa and diatoms.

The foregut morphologies of the 2 species of prawn are quite different. While *C. kraussi* possesses a typical gastric mill with hardened denticles for mastication of food items, *U. africana* has a foregut that is modified to act as a filter only, the cardiac region acting as a primary filter to the filtering apparatus of the pyloric region. Both the structures of the feeding appendages and foregut suggest therefore that the mode of feeding of these two prawn species differs. While *U. africana* is a filter-feeder which ingests small particles such as bacteria protozoa and diatoms together with a bulk of fine detrital material non-selectively, *C. kraussi* actively sifts through the sand selectively capturing larger higher quality particles such as diatoms, protozoa and possibly meiofauna. The filtering of ingested food continues in both the cardiac and the pyloric regions of the foregut of *U. africana* and should result in a simple separation of small and large particles so that smaller, easier to digest particles are passed into the hepatopancreas. Little physical breakdown of food material occurs in the foregut of this prawn. Food ingested by *C. kraussi* on the other hand will run the gauntlet of mastication by the hardened mandible prior to entering the foregut followed by further physical break up by the action of the chitinized gastric mill. Thereafter the masticated food is sorted into large and small particles by filtration; the small-sized fraction (cell contents, plant-derived particles) entering the hepatopancreas. The larger particles are passed directly into the midgut where they are packaged in a peritrophic membrane before being eliminated from the gut as faeces.

Numerous studies have reported diet-related differences in foregut structure of decapod crustaceans, e.g. cambarid crayfish (Caine 1976), amphipods (Coleman 1989, 1991) and euphausiids (Dalley & McClatchie 1989). In particular it appears that the size of food particles ingested may be reflected in foregut morphology. In a study which included *U. africana*, Schaeffer 1970 found that the

diets of 3 different decapods were reflected by morphological differences of the foregut. A strongly developed crushing-type gastric mill was associated with a scavenger where coarse food particles were ingested, while a filtering foregut represented a specialization to deal with very fine material. Similarly, Ngoc-Ho (1984) looked at the foregut structure of three filter-feeding crustaceans and found differences between that of the anomurans who had well developed ossicles and ingested relatively large particles, and the thalassinid *Upogebia deltaura* in which the cardiac region of the foregut is modified as a filter and which ingests much finer particles.

However, the link between foregut morphology and diet of decapods is not always so clear. For example, minor differences were found in the gastric mills of 10 euphausiid species exhibiting different feeding patterns, and the structure of the gastric mill may therefore be a conservative feature reflecting phylogenetic relationships (Suh & Nemoto 1988, Ullrich *et al.* 1991). Similarly, Growns and Richardson (1990) compared the gastric mills of 9 species of crayfish with the view to determine if the morphology varies with diet and habitat. They found that gastric mills appear to be morphologically conservative thus reflecting phylogenetic relationships, and that patterns of correlation between diet and gastric mills were not clear.

The feeding apparatus and foregut morphologies of *U. africana* and *C. kraussi* do however appear to closely reflect the diet of the prawns. Gut content analysis confirmed the ability of *C. kraussi* to concentrate richer food items (diatoms and protozoa) in its diet, compared to that of *U. africana*. The proportion of the gut contents contributed by microbiota (diatoms and protozoa) is higher in *C. kraussi*. However, neither prawn was able to efficiently capture mobile meiofauna such as nematodes and copepods as little evidence of these organisms were found in the gut contents. There is some evidence for a selective ability in *C. kraussi* feeding, as shown by the disparate proportions of some types of diatoms in the sediment and gut contents. However, this selection did not appear to be based on size and there is insufficient evidence to implicate shape, nutritive status or digestability. The proportions of different diatoms in the gut contents of *U. africana* mirrored those in the sediment and particularly the water column confirming non-selective filter-feeding.

While detritivores have traditionally been considered as non-selective feeders, it has become increasingly apparant that many different types of feeding mechanisms exist in the detritivore guild (Taghon 1989), and selective feeding has been reported for a number of species (e.g. Robertson & Newell 1982, Lopez & Cheng 1983, Miller 1984, Self & Jumars 1988, Cowles *et al.* 1988, Levinton &

& DeWitt 1989). A useful development in the quantification of the selective feeding by deposit-feeders has been radiotracer techniques (for review see Lopez *et al.* 1989). Selective feeding may explain how detritivores obtain sufficient nutriment (particularly of limiting nutrients such as nitrogen) from detrital mixtures, the bulk of which represent a poor quality food source (Levinton 1989).

In general, however, the SEM and light microscopy views of gut contents of *U. africana* and *C. kraussi* revealed similar-sized particles in the gut and the presence of the same components (i.e. vascular plant fragments, diatoms, protozoa, particle attached bacteria) albeit in different proportions. This could be explained by the fact that gut content analyses provide information about food that has already been partially processed and broken down but can give only limited information about its history. The masticating action of *C. kraussi* on larger particles probably results in gut contents of a similar appearance to those that have been obtained by *U. africana*, although by a different mechanism i.e. filtering. It is clear therefore that while the origin of the food may differ in terms of size and condition, these differences may not necessarily be apparant by gut content examination. Thus while gut content analyses can provide an indication of the proportions of food ingested, quantitive deductions about the original form of the food items (size, etc) must be made with caution. In addition, gut content analyses provide information about food ingested over a short period equal to gut passage time, but do not necessarily provide an integrated picture of the dominant food sources assimilated. Gut contents represent a mixture of both utilizable and non-utilizable components.

The analysis of stable isotope ratios of consumers and potential food sources offers a promising diagnostic approach to the differentiating among the mixture of food sources available from the detrital pool for detritivores (Levinton 1989). Stable isotopic ratios provide information about the actual absorption and assimilation of components that are ingested and are a useful tool in assessing the relative nutritive value of ingested dietary items if differential assimilation is suspected. In addition they can give an idea of the relative importance of different available and ingested sources.

Stable isotope ratios reflect a consumer's diet (Fry & Sherr 1984, Peterson & Fry 1987). Consumers are slightly more enriched in ^{13}C and ^{15}N compared to their diets, by approximately 0-2^{o}/oo for carbon (DeNiro & Epstein 1978, Montague *et al.* 1981, Macko & Estep 1984, Fry & Sherr 1984, Peterson & Fry 1987, Fenton & Ritz 1988) and 3-5^{o}/oo for nitrogen (Rau 1980, DeNiro & Epstein 1981, Minagwa & Wada 1984, Owens 1987, Peterson & Fry 1987). The use of combinations of stable isotope ratios (e.g. both carbon and nitrogen) has merit as it allows trophic relations to be traced while eliminating many ambiguities inherent in

the use of single isotope ratios as tracers (see Petersen *et al.* 1985). In addition to carbon and nitrogen, sulphur has been identified as a useful tracer in saltmarsh ecosystem trophic studies (Peterson *et al.* 1985,1986, Peterson & Howarth 1987, Sullivan & Moncreiff 1990).

In the present study carbon and nitrogen stable isotope ratios were determined for *U. africana* and *C. kraussi* and potential dietary sources from their respective habitats. The two prawns differ with regard to both their carbon and nitrogen isotopic ratios. *C. kraussi* is relatively depleted in terms of ^{13}C (by ca. $2^{o}/oo$) compared to *U. africana*, while *U. africana* is depleted in terms of ^{15}N (by greater than $2^{o}/oo$) compared to *C. kraussi*. *U. africana* appears entirely dependent on saltmarsh vascular plant detritus for both carbon and nitrogen, with little evidence of influence from diatoms and other organisms on the isotopic ratios. While *C. kraussi* obtains most of its carbon requirements from vascular plant detritus, with possible contribution by benthic micro-organisms (including diatoms) indicated by the skewing of the carbon value, a major proportion of its nitrogen must be derived from micro-organisms associated with detritus, particularly diatoms. Thus *C. kraussi* appears to have a mixed diet using saltmarsh detritus for its major carbon source and supplementing this with diatoms and protozoa to obtain sufficient nitrogen. These results are similar to those of Cranford and Grant, who found that while phytoplankton were an important component of the diet of the sea scallop *Placopecten magellanicus*, non-living detrital partocles contributed significantly to energy gain. It should be noted here that this conclusion concerning reliance on diatoms is based on carbon and nitrogen isotopic values for diatoms in other saltmarsh systems (Haines and Montague 1979, Kneid *et al.* 1980, Fry & Sherr 1984, Peterson *et al.* 1985,1986, Sullivan & Moncreiff 1990) as well as those for local offshore phytoplankton (Sholto-Douglas *et al.* 1991). This is justified by the fact that atmospheric and inorganic sources of carbon are utilized by these autotrophs, and the values obtained in the present study for sediment organics are skewed away from those of salt-marsh plant detritus towards the values obtained from the literature.

A number of studies have used stable isotope techniques to elucidate the foodwebs of saltmarsh systems (Petersen *et al.* 1986, Fry & Sherr 1984, Montague *et al.* 1981). Vascular plant detritus has been shown to be a dominant source of nutrition for some invertebrates in saltmarsh systens (e.g. Fry & Parker 1979, Haines & Montague 1979, Haines 1976, McConnaughey & McRoy 1979, Couch 1989). However, some consumers have been reported to rely on a mixed diet of saltmarsh plant detritus and benthic algae, plankton and/or demersal fauna (Peterson *et al* 1986, 1985, Deegan *et al* 1990, Couch 1989), while an increasing

number of species that rely heavily on benthic or pelagic algae have been reported (e.g. Gleason & Wellington 1988, Sullivan & Moncreiff 1990, Haines & Montague 1979, Kneib *et al.* 1980, Dauby 1989). In addition, epiphytes on vascular plants have also been identified as contributing significantly to a saltmarsh detritivores nutrition (Kitting *et al.* 1984). The reliance by detritivores on the living fraction of the detrital pool indicates the ability for selectively feeding. Thus *C. kraussi* appears to be a specialist feeder as its carbon and nitrogen isotopic values are skewed in the direction of benthic algae. These results are in agreement with the suggestion of Cammen (1989) that micro-algae offer an attractive and abundant source of available carbon and nitrogen for deposit-feeders in shallow intertidal areas, particularly as calculations suggest that the nitrogen deficit represented by feeding on detrital material cannot be met by feeding on bacteria by microbial stripping (Levinton 1989).

More elusive has been the determination, by stable isotope analyses, of the contribution of bacteria to the nutrition of saltmarsh detritivores. Experiments have indicated that bacteria have ^{13}C values within 2 $^{o}/oo$ of their growth substrates (Coffin *et al.* 1989). It is therefore difficult to differentiate between detritus and bacteria attached to it for the purpose of trophic studies. Thus indications that detritivores rely on saltmarsh vascular plant detritus for both carbon and nitrogen (as for *U. africana* (see also Hughes & Sherr 1983, Peterson 1986) do not preclude utilization of bacteria as a nitrogen source. In addition, less refractory components of detritus may supply a major source of the energy requirements of this prawn (Kreeger *et al.* 1990), particularly if microbial pre-conditioning occurs.

Caution must be exercised in extrapolating from isotope ratios of living or dead stands of seagrasses to the detrital fragments in sediment or water column, as there may be some differences. Some authors have found that the carbon ratio of marsh plants changes very minimally during decomposition by microbes (Fenton & Ritz 1988, Haines & Montague 1979), while Peterson *et al.* (1980) report that the carbon value of plant detritus differs from that of the living marsh plants due to the incorporation of atmospheric carbon by colonizing bateria, rendering the carbon ratio closer to that obtained for phytoplankton.

Not much is known about the variation in isotopic ratios of individual sources, although Gearing *et al.* 1984 report very little isotopic variation of carbon in phytoplankton, diatoms and nanoplankton, while Fogel *et al.* (1991) suggest that the carbon ration for phytoplankton may vary considerably. Another aspect to be considered is the rate of turnover of carbon and nitrogen in animal tissues. Fry & Arnold (1982) report rapid $^{13}C/^{12}C$ turnover times during the growth of a shrimp. These reflect the fast growth of the animals and the changes in the diet during

development. The point however is that the faster the turnover of the isotope, the less integrated (over time) will be the ratio and the less accurate are long term estimates of the diet.

Gut architecture and throughput time

It has been predicted that animals with poor quality diets should have larger guts than those eating higher quality foods (Silby 1981, Penry & Jumars 1990). Gut volume constrains throughput of food, thus the larger the gut the more food can be processed per unit time. Limitations on the diameter of the gut are however imposed by problems such as diffusion of converted products from the middle of the food bolus, mixing and the costs of maintainance of a large gut. Length of gut will influence gut throughput time. The longer the throughput time the greater the opportunity for digestive conversion of ingested materials. However, optimal throughput rates may differ depending on food quality (Levinton 1989, Cammen 1989), i.e. functional responses may serve to maximize energy aquisition (Taghon & Greene 1990)

U. africana has a larger gut compared to body size than does *C. kraussi*. While gut length is marginally shorter in *U. africana*, the gut volume is increased in this species by the greater gut diameter. This possibly reflects non-selective feeding by *U. africana* on poor quality small detrital particles compared to selective feeding by *C. kraussi* on larger richer components of the detrital pool. Although the guts of the 2 prawn species have similar spatial configurations, the relative sizes of the compartments differ. *U. africana* has a larger throughput gut (fore, mid, hind) while *C. kraussi* has a much larger side pouch (hepatopancreas). This suggests that the hepatopancreas is important to *C. kraussi* in increasing the retention time for digestion of particles, while *U. africana* relies more heavily on digestive processes within the foregut and particularly the relatively large hindgut. Furthermore, *U. africana* is able to take a much larger meal per unit body weight per time. The gut throughput time of the 2 species is approximately the same (ca. 6 hours) thus gut configuration may be significant in influencing gut retention time for optimum digestion of food items. It appears that they are both batch reactor types (Penry & Jumars 1986) that fill their guts for approximately 2 hours each tidal cycle. *U. africana* takes a larger meal but retains most of the ingested material in the throughput gut to be voided as faeces within 6 hours. *U. africana* has been reported to have gut bacteria in the midgut and hindgut (Harris *et al.* 1991, Chapter 5), and thus microbial involvement in digestion is a possibility considering that bacteria can

double their biomass in as little as 2 hours under favourable conditions (Plante *et al* 1990). *C. kraussi* takes a smaller meal but retains a large proportion in the hepatopancreas thus increasing the retention time of these food items beyond the 6 hour throughput time of the main food bolus, thus ensuring sufficient time for enzymatic digestion if the appropriate enzymes exist in the gut.

In polychaetes a body volume to gut volume ratio of greater than 7 is associated with a carnivorous diet (Penry & Jumars 1990), with relative gut volume decreasing with increasing diet quality. In deposit-feeders, median gut volume per unit body volume is twice that of carnivores. The body:gut volume ratio of *U.africana* is 7, while that of *C. kraussi* is aproximately 10, indicating that the diet quality (i.e. ingested material) is better for *C. kraussi*. In the case of these two species of detritivore prawn food quality, not quantity, is generally limiting and they have respectively opted to either increase gut size to process more food with the same throughput time (*U. africana*), or select quality items (*C. kraussi*). Therefore having the same gut passage time but different diets and gut morphology is not necessarily inconsistent. *C. kraussi* has higher quality food through selection but has a smaller gut and a large side pouch for digestion and absorption. *U. africana* ingests poorer quality food but has a larger gut, for processing of larger quantities.

Conclusions

In both *U. africana* and *C. kraussi* the feeding apparatus appears to match the resources ingested and utilized as food. *U. africana* is a non-selective filter-feeder with a relatively large gut, and utilizes refractory non-living suspended particulate material, primarily derived from saltmarsh vascular plants, as its dominant food source.

By contrast, *C. kraussi* uses a combination of filter-feeding and selective deposit-feeding to utilize saltmarsh plant detritus and micro-organisms (diatoms, protozoa) as carbon and nitrogen sources respectively. It has a relatively small gut, dominated by a large hepatopancreas which effectively increases retention time of food items.

The utilization of refractory saltmarsh plant detritus as dominant carbon sources suggests that cellulases, either endogenous or exogenous, should be present in the guts of both prawns. However, as *U. africana* is solely dependent on detritus, while *C. kraussi* relies partially on more labile fractions of the detrital pool, it could be predicted that *U. africana* may possess the digestive capabilities for

effective breakdown of crystalline forms of cellulose, while *C. kraussi* may rely on utilization of the more labile fractions of the saltmarsh detritus supplemented by richer items such as diatoms.

References

Boehlert GW, Yoklavich MM 1984 Carbon assimilation as a function of ingestion rate in larval pacific herring, *Clupea harengus pallasi* Valenciennes. J. Exp. Mar. Biol. Ecol. 79:251-262.

Branch GM, Branch M 1981 The living shores of southern Africa. C. Struik, Cape Town. pp.1-272.

Caine EA 1975 Feeding and masticatory structures of six species of the crayfish Procambarus (Decapoda, Astacidae). Forma. Functio. 8:49-66

Cammen LM 1980 Ingestion rate: An empirical model for aquatic deposit feeders and detritivores. Oecologia 44:303-310.

Cammen LM 1989 The relationship between ingestion rate of deposit feeders and sediment nutritional value. In: Lecture notes on coastal and estuarine studies (31): Ecology of marine deposit feeders. Springer-Verlag. New York.

Coffin RB, Fry B, Petersen BJ, Wright RT 1989. Carbon isotopic compositions of estuarine bacteria. Limnol. Oceanogr. 34(6):1305-1310

Coleman Ch.O 1989 On the nutrition of two Antarctic Acanthonotozomatidae (Crustacea: Amphipoda): Gut contents and functional morphology of mouthparts. Polar Biol. 9:287-294.

Coleman Ch.O 1991 Comparative fore gut morphology of Antarctic amphipoda (Crustacea) adapted to different food sources. Hydrobiologia 223:1-9.

Couch CA 1989 Carbon and nitrogen stable isotopes of meiobenthos and their food resources. Est. Coast. Shelf. Sci. 28(4):433-443

Cowles TJ, Olson RJ, Chisholm SW 1988 Food selection by copepods: discrimination on the basis of food quality. Mar. Biol. 100:41-49.

Cranford PJ, Grant J 1990 Particle clearance and absorbtion of phytoplankton and detritus by the sea scallop *Placopectens magellanicus* (Gmelin). J. Exp. Mar. Biol. Ecol. 137:105-121.

Dalley DD, McClatchie S 1989 Functional feeding morphology of the euphasiid *Nyctiphanes australis*. Mar. Biol. 101:195-203.

Dauby 1989 The stable carbon isotope ratios in benthic food webs of the Gulf of Calvi, Corsica. Cont. Shelf Res. 9(2):181-196

Deegan LA, Petersen BJ, Portier R 1990 Stable isotopes and cellulase activity as evidence for detritus as a food source for juvenile Gulf Menhada. Estuaries 13(1):14-19

DeNiro M.J., Epstein S. 1978 Influence of diet on the distribution of carbon isotopes in animals. Geochem. Cosmochim. 42:495-506

DeNiro M.J., Epstein S. 1981 Influence of diet on the distribution of nitrogen isotopes in animals. Geochim. Cosmochim. 45:341-351

Ellis SG, Small LF 1989 Comparison of gut-evacuation rates of feeding and non-feeding *Calanus marshallae*. Mar. Biol. 103:175-181.

Fenton GE, Ritz DA 1988 Changes in carbon and hydrogen stable isotope ratios of macroalgae and seagrass during decomposition. Est Coast Shelf Sci 26:429-436

Fogel ML, Cifuentes LA, Velinsky DJ, Sharp JH 1992 relationship of carbon availability in estuarine phytoplankton to isotopic composition. Mar. Ecol. Prog. Ser. 82:291-300.

Forbes AT 1973 A study of the burrowing sand prawn *Callianassa kraussi* Stebbing (Crastacea: Decapoda: Thalassinidea). Ph.D thesis, Rhodes University, Grahamstown, South Africa.

Fry B, Arnold C 1982. Rapid 13C/12C turnover during growth of brown shrimp (*Penaeus aztecus*) Oecologia (Berl) 54:200-204

Fry B, Sherr EB 1984 $\check{S}^{13}C$ measurements as indicators of carbon flow in marine and fresh water ecosystems. Contr. mar. Sci. 27:13-47.

Fry B, Parker PL 1979 Animal diet in Texas seagrass meadows:13C evidence for the importance of benthic plants. Estuar. Coast. Shelf Sci. 8:499-509

Gearing JN, Gearing PJ, Rudnick DT, Requejo AG, Hutchins MJ 1984 Isotopic variability of organic carbon in a phytoplankton-based, temperate estuary. Ceochimica et Cosmochimica Acta 48:1089-1098.

Genoni GP 1991 Increased burrowing by fiddler crabs *Uca rapax* (Smith) (Decapoda: Ocypodidae) in response to low food supply. J. Exp. Mar. Biol. Ecol. 147:267-285.

Gleason DF, Wellington GM 1988 Food resources of post larval brown shrimp (*Penaeus aztecus*) in a Texas salt marsh. Mar. Biol. 97:329-337.

Graf G, Bengtsson W, Diesner U, Schultz R, Theede H 1982 Bentic response to sedimentation of a spring phytoplankton bloom: process and budget. Mar. Biol. 67:201-208.

Gremare A 1988 Feeding, tube-building and particle size selection in the terrebellid polychaete *Eupolymnia nebulosa*. Mar. Biol. 97:243-252.

Grown IO, Richardson AMM 1990 A comparison of the gastric mills of nine species of parastacid crayfish from a range of habitats, using multivariate morphometrics (Decapoda: Astacoidea). Crustaceana 58:33-44.

Haines E.B., Montague C.L. 1979 Food sources of estuarine invertebrates analyzed using 13C/12C ratios. Ecology 60(1):48-56

Haines EB 1976 Relation between the stable carbon isotope composition of fiddler crabs, plants and soils in a salt marsh. Limnol. Oceanogr. 21:880-883

Hammond LS 1983 Nutrition of Deposit-feeding holothuroids and echinoids (Echinodermata) from a shallow reef lagoon, Discovery Bay, Jamaica. Mar. Ecol. Prog. Ser. 10:297-305.

Harris JM, Seiderer LJ, Lucas MI 1991 Gut microflora of two salt marsh detritivore thalassinid prawns, *Upogebia africana* and *Callianassa kraussi*. Microb. Ecol. 21:277-296.

Hartmann HJ, Kunkel DD 1991 Mechanisms of food selection in *Daphnia*. Hydrobiologia 225:129-154.

Hill BJ 1967 Contributions to the ecology of the anomuran mud prawn *Upogebia africana* (Ortmann). Ph.D thesis Rhodes University, Grahamstown, South Africa.

Hughes EH, Sherr E 1983 Subtidal food webs in a Georgia estuary: d13C analyses. J. Exp. Mar. Biol. Ecol. 67:227-242.

Jones DA 1968 The functional morphology of the digestive system in the carnivorous intertidal isopod *Eurydice*. J. Zool. Lond. 156:363-376.

Jumars PA, Penry DL 1989 Digestion theory applied to deposit feeders. In: Lecture notes on coastal and estuarine studies (31): Ecology of marine deposit feeders. Springer-Verlag. New York.

Kitting C.L., Fry B., Morgan M.D. 1984 Detection of inconspicuous epiphytic algae supporting food webs in seagrass meadows. Oecologia 62:145-149

Kneib R.T., Stiven A.E., Haines E.B. 1980 Stable carbon isotope ratios in Fundulus heteroclitus (L.) muscle tissue and gut contents from a North Carolina Spartina marsh. J. Exp. Mar. Biol. Ecol. 46:89-98

Kofoed L, Forbes V, Lopez G 1989 Time-dependent absorbtion in deposit feeders. In: Lecture notes on coastal and estuarine studies (31): Ecology of marine deposit feeders. Springer-Verlag. New York.

Kreeger DA Langdon CJ, Newell RIE 1988 Utilization of refractory cellulosic carbon derived from *Spartina alterniflora* by the ribbed mussel *Geukensia demissa* Mar. Ecol. Prog. Ser. 42:171-179.

Kreeger DA, Newell RIE, Langdon CJ 1990 Effect of tidal exposure on utilization of dietary lignocellulose by the ribbed mussel *Geukensia demissa* (Dillwyn) (Mollusca: Bivalvia). J. Exp. Mar. Biol. Ecol. 144:85-100.

Levinton JS 1989 Deposit feeding and coastal oceanography. In: Lecture notes on coastal and estuarine studies (31): Ecology of marine deposit feeders. Springer-Verlag. New York.

Levinton JS, deWitt TH 1989 Relation of particle-size spectrum and food abundance to particle selectivity and the mud snail *Hydrobia totteni* (Prosobranchia: Hydrobiidae). Mar. Biol. 100:449-454.

Lopez G, Levinton JS, Slobodkin LB 1977 the effects of grazing by the detritivore *Orchestria grillus* on *Spartina* litter and its associated microbial community. Oecologia 30:111-127.

Lopez G, Tantichodok P, Cheng I-J 1989 Radiotracer methods for determining utilization of sedimentary organic matter by deposit feeders. In: Lecture notes on coastal and estuarine studies (31): Ecology of marine deposit feeders. Springer-Verlag. New York.

Lopez GR, Cheng I-J 1983 Synoptic measurements of ingestion rate, ingestion selectivity, and absorbtion efficiency of natural foods in the deposit-feeding molluscs *Nucula annulata* (Bivalvia) and *Hydrobia totteni* (Gastropoda). Mar. Ecol. Prog. Ser. 11:55-62.

Macko SA, Estep MLF 1984 Microbial alteration of stable nitrogen and carbon isotopic compositions of organic matter. Org. Geochem. 6:787-790.

Manjulatha C, Babu DE 1991 Functional organisation of mouth parts, and filter feeding, in *Clibanarius longitarsus* (Crustacea: Anomura). Mar. Biol. 109:121-127.

McConnaughey T, McRoy CP 1979 13C label identifies eelgrass (*Zostera marina*) carbon in an Alaskan estuarine food web. Mar. Biol. 53:263-269

Mertens J, Munuswamy N, De Walsche C, Dumont HJ 1991 the filtration apparatus of anostraca ((Crustacea): species specific setulation in the genus *Streptocephalus*. Hydrobiologia 212:187-193.

Miller DC 1984 Mechanical post-capture particle selection by suspension- and deposit-feeding *Corophium*. J. Exp. mar. Biol. Ecol. 82:59-76.

Miller DC, Jumars PA, Nowell ARM 1984 Effects of sediment transport on deposit feeding: scaling arguments. Limnol. Oceanogr. 29:1202-1217.

Minagawa M, Wada E 1984. Stepwise enrichment of 15N along food chains: further evidence and the relationbetween &15N and animal age. Geochimica et Cosmochimica Acta 48:1135-1140

Montague CL, Bunker SM, Haines EB, Pace ML, Wetzel RL 1981 Aquatic macroconsumers. In The ecology of a salt marsh. LR Pomeroy and RG Weigert (Eds.) Springer Verlag, New York.

Newell RIE, Langdon CJ 1986 Digestion and absorbtion of refractory carbon from the plant *Spartina altemiflora* (Loisel) by the oyster *Crassotrea virginica* (Gmelin) Mar. Ecol. Prog. Ser. 34:105-115.

Ngoc-Ho N 1984 The functional anatomy of the foregut of *Porcellana platycheles* and a comparison with *Galathea squamifera* and *Upogebia deltaura* (Crustacea: Decapoda). J. Zool. Lond. 203:511-535.

Nival P, Nival S 1976 Particle retention efficiencies of an herbivorous copepod, *Acartia clausi* (adult and copepodite stages): Effects on grazing. Limnol. Oceangr. 21:24-37.

Owens N.J.P. 1987 Natural variations in 15N in the Marine environment. Adv. Mar. Biol. 24:390-451

Penry DL, Jumars PA 1986 Chemical reactor analysis and optimal digestion. Bioscience 36:310-315.

Penry DL, Jumars PA 1987 Modeling animal guts as chemical reactors. Am. Nat. 129:69-96.

Penry DL, Jumars PA 1990 Gut architecture, digestive constraints and feeding ecology of deposit-feeding and carnivorus polychaetes. Oecologia 82:1-11.

Peterson B.J., Fry B. 1987 Stable isotopes in ecosystem studies. Ann Rev Ecol Syst 18:293-320

Peterson B.J., Howarth R.W. 1987 Sulfur, carbon, and nitrogen isotopes used to trace organic matter flow in the salt-marsh estuaries of Sapelo Island, Georgia. Limnol. Oceanogr. 32(6):1195-1213

Peterson B.J., Howarth R.W., Garritt R.H. 1985 Multiple stable isotopes used to trace the flow of organic matter in estuarine food webs. Sci 227:1361-1363

Peterson B.J., Howarth R.W., Garritt R.H. 1986 Sulfur and carbon isotopes as tracers of salt-marsh organic matter flow. Ecology 67(4):865-874

Peterson BJ, Howarth RW, Lipshultz F, Ashendorf D 1980 Salt marsh detritus : an alternative interpretation of stable carbon isotope ratios and the fate of *Spartina alterniflora*. Oikos 34:173-177.

Phillips NW 1984 Role of different microbes and substrates as potential suppliers of specific, essential nutrients to marine detritivores. Bull. Mar. Sci. 35:283-298.

Plante CJ, Jumars PA, Baross JA 1990 Digestive associations between marine detritivores and bacteria. Ann. Rev. Ecol. Syst. 21:93-127.

Poovachiranon S, Boto K, Duke N 1986 Food preference studies and ingestion rate measurements of the mangrove amphipod *Parhyale hawaiensis* (Dana). J. Exp. Mar. Biol. Ecol. 98:129-140.

Rau G. 1980 Carbon-13/carbon-12 variation in subalpine lake aquatic insects: food source implications. Can. J. Fish. Aquatic Sci. 37:742-746.

Richardson JS 1991 Seasonal food limitation of detritivores in a montane stream: an experimental test. Ecology 72:873-887.

Robertson JR, Newell SY 1982 Experimental studies of particle ingestion by the sand fiddler *Uca pugilator* (Bosc.). J. Exp. Mar. Biol. Ecol. 59:1-21.

Schaefer N 1970 The functional morphology of the foregut of three species of decapod crustacea: *Cyclograspus punctatus* Milne-Edwards, *Diogenes brevirostris* Stimpson, and *Upogebia africana* (Ortmann). Zoologica Africana 5:309-326.

Sealy J.C., Van der Merwe N.J., Thorp J.A.L., Lanham J.L. 1987 Nitrogen isotopic ecology in southern Africa: Implications for environmental and dietary tracing. Geochim. Cosmochim. 51:2707-2717

Self RFL, Jumars PA 1988 Cross-phyletic patterns of particle selection by deposit feeders. J. Mar. Res. 46:119-143.

Sholto-Douglas AD, Field JG, James AG, van der Merwe NJ 1991 $^{13}C/^{12}C$ and $^{15}N/^{14}N$ isotope ratios in the Southern Benguela Ecosystem: indicators of food web relationships amoung different size-classes of plankton and pekagic fish; differences between fish muscle and bone collagen tissues. Mar. Ecol. Prog. Ser. 78:23-31.

Sibly RM 1981 Strategies of digestion and defecation. In Physiological Ecology: An evolutionary approach to resource use. CR Townsend and P Calow (Eds.). Blackwell Scientific Publications, London.

Suh H-L, Nemoto T 1988 Morphology of the gastric mill in ten species of euphausiids. Mar. Biol. 97:79-85.

Sullivan MJ, Moncreiff CA 1990 Edaphic algae are an important component of salt marsh food-webs: evidence from multiple stable isotope analyses. Mar. Ecol. Prog. Ser. 62:149-159.

Taghon GL 1981 Beyond selection: optimal ingestion rate as a function of food value. Am. Nat. 118:202-214.

Taghon GL 1988 The benefits and costs of deposit feeding in the polychaete *Abarenicola pacifica*. Limnol. Oceanogr. 33:1166-1175.

Taghon GL 1989 Modeling deposit feeding. In: Lecture notes on coastal and estuarine studies (31): Ecology of marine deposit feeders. Springer-Verlag. New York.

Taghon GL, Greene RR 1990 Effects of sediment-protein concentration on feeding and growth rates of *Abarenicola pacifica* Healy et Wells (Polychaeta: Arenicollidae). J. Exp. Mar. Biol. Ecol. 136:197-216.

Taghon Gl, Self RFL, Jumars PA 1978 Predicting particle selection by deposit feeders: amodel and its implications. Limnol. Oceanogr. 23:752-759.

Tenore KR 1989 Some ecological perspectives in the study of the nutrition of deposit feeders. In: Lecture notes on coastal and estuarine studies (31): Ecology of marine deposit feeders. Springer-Verlag. New York.

Tenore KR, Hanson RB, DornSeif BE, Weiderhold CN 1979 the effect of organic nitrogen supplement on the utilization of different sources of detritus. Limnol. Oceanogr. 24:350-355.

Townsend CR, Hughes RN 1981 Maximizing net energy returns from foraging. In Physiological Ecology: An evolutionary approach to resource use. CR Townsend and P Calow (Eds.). Blackwell Scientific Publications, London.

Ullrich B, Storch V, Marschall HP 1991 Microscopic anatomy, functional morphology, and ultrastructure of the stomach of *Euphausia superbia* Dana (Crustacea: Euphausiacea). Polar. Biol. 11:203-211.

Watling L 1989 Small-scale features of marine sedimentsand their importance to thestudy of deposit-feeding. In: Ecology of Deposit feeders, Lopez G, Taghon G, Levinton J (eds.), Springer Verlag, New York, pp 269-290

Whitlatch RB 1981 Animal-sediment relationships in intertidal marine benthic habitats: some determinants of deposit feeding species diversity. J. Exp. Mar. Biol. Ecol. 53:31-45.

Whitlatch RB 1989 On some mechanistic approaches to the study of deposit feeding in polychaetes. In: Lecture notes on coastal and estuarine studies (31): Ecology of marine deposit feeders. Springer-Verlag. New York.

Wynberg RP 1991 The ecological effects of collecting *Callianassa kraussi* Stebbing and *Upogebia africana* (Ortmann) for bait: Impacts on the biota of and intertidal sandflat. MSc. thesis, University of Cape Town, Cape Town.

CHAPTER 3

Cellulose digestion in two saltmarsh detritivorous thalassinid prawns, *Upogebia africana* **and** *Callianassa kraussi*

ABSTRACT

This study investigates cellulose digestion, and hydrolysis of refractory components of natural detritus, in the digestive systems of two saltmarsh detritivorous benthic prawns. The filter-feeding detritivorous mudprawn *Upogebia africana* and the deposit-feeding sandprawn *Callianassa kraussi* both possess strong amylase activities. While *C. kraussi* displayed high endoglucanase activity, *U. africana* showed very low activity. Furthermore, *C. kraussi* possesses a C1-cellulase and is capable of hydrolysis of crystalline forms of cellulose and refractory components of saltmarsh detritus. In both species of prawn the hepatopancreas was the principle site of carbohydrase activity, while activity in the midgut and hindgut was weak. This pattern of distribution of carbohydrase activity is not caused by pH conditions in the gut. Digestion in the hepatopancreas results in an effective incubation period of food with enzymes much longer than the throughput time. The differences in the digestive physiologies of the two species of prawns is discussed in relation to their feeding modes and diet, and the resources available in their respective habitats.

INTRODUCTION

Detritivorous invertebrates dominate the benthic biomass of many shallow marine systems. Frequently, for example in salt-marsh systems, the main component of detritus consists of structural carbohydrates of vascular plant origin (Tenore *et al.* 1979). The question of whether invertebrate detritivores are able themselves to effectively digest this refractory particulate organic component of detritus, or whether microbes must be relied upon for these transformations, is fundamental to the understanding of both the feeding ecology of detritivores, as well as to decomposition and nutrient cycling in the system as a whole. Early studies indicated that detritivores were unable to utilize the particulate organic fraction of detritus, but efficiently stripped off and utilized bacteria associated with it (Newell 1965, Adams & Angelovic 1970, Fenchel 1970, Hargrave 1970). These findings embody the microbial conversion hypothesis. However, it has subsequently been shown that the microbiotic component of detritus cannot alone satisfy the energy requirements of many detritivores (Baker & Bradnam 1976, Cummins & Klug 1979, Cammen 1980, Findlay *et al.* 1984). In addition, absorption efficiency experiments have demonstrated direct utilization of refractory components of detritus by some aquatic invertebrate detritivores (Adams & Angelovic 1970, Cammen 1980, Klumpp & Nichols 1983, Seiderer & Newell 1985, Sinsabaugh *et al.* 1985, Chamier 1991). A number of authors have reported the presence of gut enzymes capable of hydrolyzing complex carbohydrates in aquatic detritivores (e.g. Bjarnov 1972, Dean 1978, Elyakova *et al.* 1981, Lucas & Newell 1984, Barlocher *et al.* 1989, Brock 1989, Chamier 1991, Walters & Smock 1991). Furthermore, the classical view of the organic components of detritus may be too simplistic. The range of types of organic matter (from labile to refractory) available in the detrital pool may allow the detritivore to satisfy its energy requirements without resorting to either the utilization of the refractory components or to rapid processing of ingested material for microbial stripping (Levinton 1989, Chamier 1991). A number of recent studies highlight the potential importance of diatoms (microalgae) as a staple food source for benthic detritivores (Jensen & Siegismund 1980, Bianchi & Levinton 1984, Phillips 1984, Sullivan & Moncreiff 1990). Relatively few studies have addressed the question of whether physiological properties of invertebrates are correlated with actual food ingested and resources available (see Crosby & Reid 1971, Kristensen 1972, Gianfreda *et al.* 1979, Seiderer *et al.* 1982, Kesler 1983, Brock *et al.* 1986).

Central to the question of whether detritivores can utilize refractory components of the diet is an understanding of *in vivo* digestion processes,

particularly with regard to breakdown of refractory components such as cellulose. C1-cellulases (cellobiohydrolase) are necessary for the effective hydrolysis of complex crystalline (highly ordered) forms of cellulose (cell walls, plant fibres) and substrates such as Avicel crystalline cellulose, Whatman No. 1 filter paper and cotton linters (Ghose 1987). Cx-cellulases (endoglucanases) are active against soluble and disordered forms of cellulose, and hydrolyze substrates such as carboxymethyl cellulose (CMC) (Gilligan & Reese 1954). Amylase and glucanase enzymes act against starch and laminarin to break them down to disaccharides and monosaccharides. A further group of carbohydrases, the glycosidases, show activity in the presence of disaccharides as follows: glucosidases hydrolyze substrates such as maltose, sucrose (a-glucosidase) and cellobiose (b-glucosidase), while galactosidase is active against lactose (b-galactosidase) and mellibiose (a-galactosidase) (Bacon 1979). C1-cellulases make it possible for Cx-cellulases to act (i.e. open up complex structures), and the presence of glucosidases is important for the action of C1-cellulases as they prevent a buildup of cellobiose which negatively influences the activities of C1-cellulases (Berger 1990). A suite of carbohydrases is therefore necessary for complete digestion of complex crystalline forms of carbohydrates, and these enzymes act synergistically (Berghem & Pettersson 1974, Bacon 1979, Rouland *et al.* 1988).

Most studies of aquatic invertebrates have examined the digestion of soluble forms of cellulose, most often carboxymethylcellulose (CMC) (Horiuchi & Lane 1965, Araki & Giese 1970, Elyakova 1972,1981, Gianfreda *et al.* 1979, Kesler & Tulou 1980, Sinsabaugh *et al.* 1985, Brock *et al.* 1986, Seiderer & Newell 1988, Brock 1989, Walters & Smock 1991). Demonstration of endoglucanase (CMC-ase) activity does not necessarily imply the ability to utilize crystalline cellulose or the refractory components of naturally-occurring detritus (plant fibres, cell walls). A number of authors have considered the digestion of crystalline forms of cellulose by detritivores (Yokoe & Yasumasu 1964, Crosby & Reid 1971, Payne *et al.* 1972, Monk 1977, Dean 1978, Alexander *et al.* 1979, Scheibling 1980, Mirza & Serban 1981, Kesler 1983, Friesen *et al.* 1986, Musgrove 1988, Barlocher *et al.* 1989, Obrietan *et al.* 1991). However, C1-cellulases have generally been associated with microbial activity (Suberkropp & Klug 1976, Bacon 1979, Martin 1987, Sinsabaugh & Linkins 1990), and in most studies reporting cellulases in marine invertebrates it is not clear whether they are endogenous (animal) or exogenous (bacterial or fungal). A few authors report exogenous sources of these enzymes which are either ingested with the food (acquired enzymes) (Barlocher 1982, Sinsabaugh *et al.* 1985, Chamier & Willoughby 1986, Walters & Smock 1991) or may be produced by gut bacteria (Lasker & Giese 1954, Cutter & Rosenberg 1971, Martinez 1982,

Wainwright & Mann 1982, Musgrove 1988). However, evidence for the digestion of cellulose by marine invertebrates in the absence of micro-organisms has also been presented (Araki & Giese 1970, Payne *et al.* 1972, Monk 1977, Morton 1978, Friesen *et al.* 1986). The relative importance of endogenous and exogenous sources of cellulases in the digestive processes of aquatic invertebrates is thus poorly understood, and has not been quantified.

The ability of an invertebrate detritivore to digest refractory components of detritus is further influenced by the structure of the gut, the gut passage time, the rate of activity of enzymes and the conditions in different regions of the gut (eg pH which may influence enzyme activity) (see Townsend & Calow 1981, Penry & Jumars 1986, 1987, 1990, Kofoed *et al.* 1989). There is no point in having the enzymes if the food is not kept in the correct region of the gut for long enough for it to be hydrolysed efficiently. Reported rates of cellulase activity of invertebrate gut enzymes against refractory substrates are usually low, and are not often related to gut passage times or absorption sites. This makes it difficult to quantify the significance of digestive enzymes in the physiology of the invertebrates, and comparisons of the importance of different enzymes to animals with disparate resources available to them are limited. In fact, we are far from being able to relate measured in vitro enzyme activity rates to their quantitative relevance to the animal.

This paper compares amylase and endoglucanase activity in different regions of the guts of two saltmarsh detritivores, the mudprawn *Upogebia africana* and the sandprawn *Callianassa kraussi*, which dominate the benthic macrofaunal biomass in their respective habitats. A high proportion of the material ingested by these two prawns consists of detritus derived from vascular saltmarsh plants (Chapters 2&5), and it is of interest to determine whether these prawns have the enzymes to digest this particulate organic matter. Stable isotope analyses (Chapter 2), indicate that the filter-feeding *U. africana* relies more heavily on refractory components of detritus, than does the deposit-feeding *C. kraussi* which appears to gain a significant proportion of its sustenance from microbiota and diatoms associated with the particulate organic matter. This difference in the feeding mode and utilization of the resource may be reflected in differences in the ability to digest cellulose. Preliminary experiments, however, indicated strong endoglucanase activity for *C. kraussi*, and this prawn was further investigated for the presence of C1-cellulases, and the ability to digest the structural carbohydrate components of natural detritus. Furthermore, the question of whether the endoglucanase present in *C. kraussi* is endogenous or exogenous was examined experimentally by quantifying digestion and absorption efficiency in the presence and absence of antimicrobial agents.

METHODS AND MATERIALS

Study site and collection of material

Prawns were collected from two sites in Langebaan lagoon (33°00'S-33°13'S and 17°57'E-18°08'E), a partially enclosed shallow saltmarsh marine system situated on the southwest coast of South Africa (Figure 1, Chapter 1). The mudprawn *U. africana* and the sand prawn *C. kraussi* were collected respectively from Geelbek, in the muddy upper reaches of the lagoon, and Oesterwal, near the sandy mouth of the lagoon. Each prawn species dominates the site from which it was collected (Wynberg 1991). The saltmarsh eelgrass plant *Zostera capensis* forms extensive mid-tide beds on the mudflats at both sites (Christie 1981), while benthic diatoms are reported to contribute a significant (22%) proportion of the total primary production of carbon in the lagoon (Fielding *et al.* 1988). Ambient temperatures in the lagoon range from 14°C to 24°C (Shannon & Stander 1977).

Enzyme preparation

Prawns were transported to the laboratory and dissected over ice to remove the gut within 2 h of collection. The gut was dissected into 4 categories of pooled tissue: foregut, midgut, hindgut and hepatopancreas. Each of the pooled gut fractions was homogenized over ice with a glass tissue grinder in 10 ml phosphate buffer (pH 6.9) amended with 150 mM NaCl, centrifuged at 3000 rpm for 15 min and the supernatant decanted and suitably diluted with chilled phosphate buffer for use in subsequent enzyme assays within 24 h of preparation. Bacterial cellulase (endo-1,4-B-glucanase, Sigma C-7502) from *Aspergillus niger* was prepared in phosphate buffer (pH 6.9) at a concentration of 0.5 $mg.ml^{-1}$.

Substrate preparation

Commercial substrates were prepared in 20 mM phosphate buffer (pH 6.9) containing 150 mM NaCl at the following concentrations: soluble starch (BDH No. 10271) 1% w/v, carboxymethyl cellulose (high viscosity, BDH 27929) 1% w/v, Avicel microcrystalline cellulose (purified, particulate alpha-cellulose from fibrous

plants, FMC Corp. Prod.) 1% w/v, Whatman's No. 1 filter paper strips in 15x5mm strips.

Natural substrates: Centric diatoms (*Thalassiosira weissflogii*) were grown in culture, concentrated by centrifugation, rinsed with distilled water, dried at 60°C for 48h, ground in a mortar and pestle, and the <90 μm fraction prepared in phosphate buffer (pH 6.9) at a concentration of 3% w/v. Thereafter, the diatom solution was dialyzed against phosphate buffer for 24 h to remove free reducing sugars. Decaying *Zostera capensis* was collected from Oesterwal at Langebaan lagoon, rinsed with distilled water, dried at 60°C for 48 h, ground in a ball-mill and the <90 μm fraction harvested. A portion of this solution was prepared in phosphate buffer at a concentration of 1% w/v and dialysed against phosphate buffer for 24 h to remove free reducing sugars. The remainder of the milled *Zostera* was refluxed at 100°C in 0.05 M NaOH for 4 h, whereafter the solution was centrifuged at 3000 rpm for 10 min and the supernatant discarded. The residue was resuspended and centrifuged three times in distilled water, and the residue finally dried at 60°C for 48 h, remilled, and the <90 μm fraction prepared in phosphate buffer at a concentration of 1%. The refluxed *Zostera* was assumed to contain only the structural refractory carbohydrates of the plant.

Carbohydrase assay procedure

Preliminary assays for reducing sugar release by enzymatic action of commercial enzymes on carbohydrate substrates were conducted according to two widely used methods i.e. Bernfeld (1955) and Nelson (1944) modified by Somogyi (1952). The assay procedures and results of this study are reported in Appendix A (Fielding *et al.* 1986). It was found that quantitative assays using glucose (the most commonly used standard) for calibration purposes revealed a two to seven-fold discrepancy between the two methods, depending on the enzyme and substrates used. The Bernfeld method yielded higher reducing sugar estimates, while the Nelson-Somogyi assay was found to be some eight times more sensitive. It was concluded that the quantitative discrepancy between the two methods could be accounted for by variability in the colour response to different endproducts, and that when an appropriate standard is used the two methods are in close agreement. Glucose was found to be an inappropriate standard, and it is recommended that maltose be employed for quantitive studies of reducing sugar release by style and gut enzymes.

For further comparisons between these two methods of assaying for reducing sugars, see Brieul & Saddler (1985).

In the present study, aliquots of 250 µl enzyme extract were added to an equal volume of substrate and incubated at 20°C. Reducing sugars were measured at 660 nm using the Nelson-Somogyi method (Nelson 1944) with maltose as the standard. Absorbance values were converted using the following standard curve for maltose concentrations of 0.8 $mg.ml^{-1}$ to 0.006 mg.ml-1 was $y = 5.85x - 0.053$ ($r^2 = 1.00$, $n = 30$) where y is the absorbance at 660nm and x is the concentration of maltose in $mg.ml^{-1}$. Preliminary assays were performed to determine optimum incubation periods and enzyme extract concentrations to ensure that substrate depletion and enzyme inhibition were avoided. Enzyme activity was calculated from regression equations of the linear regions of the curves (up to 5 and 10 minutes for amylase and endoglucanase activities, respectively, and various longer incubation periods (1-48h) for complex carbohydrates and natural substrates). NaAzide was added to reaction mixtures at a final concentration of 0.2 $mg.ml^{-1}$ for assays involving long incubation periods (i.e. greater than 1 hour) to prevent bacterial activity. Preliminary trials indicated that NaAzide does not interfere with the assay reagents, and the effectiveness of this treatment was checked during enzyme assays using filter paper and refluxed *Zostera* as substrates. The results were corrected by subtracting both substrate and enzyme blanks and enzyme activities are expressed as mg maltose per mg protein of crude homogenates. The rate of enzyme activity is expressed as mg maltose released per mg protein per hour. All values reported represent the mean of three estimates. Protein contents of homogenates were assayed by the method of Lowry *et al.* (1951). The results are likely to underestimate enzyme activity of the gut as a proportion of the protein will be structural. No information is available as to the relative proportions of enzyme and structural protein in the gut tissue of these two prawn species.

Gut pH

The pH in different regions of the gut was measured by spotting samples of gut fluid from the guts of freshly collected and dissected prawns onto pH indicator paper (Spezialindikator mit Farbscala, Merck Art. 9556).

Affect of pH on C.kraussi endoglucanase activity

Endoglucanase activity of *C. kraussi* digestive gland homogenate was measured over a pH range of 5.4 to 7.4. For these assays 75 μl of enzyme extract prepared in phosphate buffer pH 6.9 was incubated with 425 μl substrate (1% CMC) made up in Sorenson's phosphate buffer of the appropriate pH. In each assay, enzyme and substrate solutions were incubated together for 5 and 10 min, and reducing sugar release after the respective time intervals measured as described above, and expressed as mg maltose released per mg protein per hour.

Affect of antibiotic treatment on endoglucanase activity and absorption efficiency of *C.kraussi*.

Prawns (*C. kraussi*) were transported to the laboratory within 1 h of collection. They were then maintained for 24 h at 20°C in glass beakers containing 0.7 μm filtered seawater that had been freshly collected from the site of prawn collection. Animals were fed with milled (<90 μm) eelgrass Z. capensis, and the water was changed after 12 h. Half of the prawns were treated with antibiotics by six-hourly additions of 100 mg/l each of Amoxil and tetracycline (broad spectrum antibiotics which include action against Vibrio, Pseudomonas and Enterobacteriacea, the dominant genera isolated from the gut of *C. kraussi* (Harris *et al.* 1991)). This combination of antibiotics was found to eliminate most bacteria in the water and the guts of the prawns in preliminary experiments. After 24 h the hepatopancreas was dissected under sterile conditions from each prawn (both those treated with antibiotics and the control animals), homogenized separately in phosphate buffer (pH 6.9), suitably diluted, and assayed for endoglucanase activity over 10 min using the methods described above. In addition, a small portion (~0.1g) of the hepatopancreas of each prawn was homogenized in 5 ml sterile sea-water, serially diluted in sterile seawater, and spread-plated onto seawater agar plates (Harris *et al.* 1991). To check the effectiveness of the antibiotic treatment colony-forming units (CFU) were counted on each plate after incubation at 20°C for 5 days, and expressed as CFU/g wet weight of tissue. In addition, a portion of each hepatopancreas was fixed with 2.5% gluteraldehyde (in 0.2 μm filtered seawater), desalinated, dehydrated to 100% ethanol, critical point dried using CO_2 as the transitional fluid, mounted on stubs, coated with gold paladium and examined under a Cambridge S200 scanning electron microscope for the presence of microbes in the lumen or on the lumen lining.

Absorption efficiencies of prawns treated with antibiotics (50 mg ampicillin and 2g streptomycin per liter of 0.2 μm filtered seawater) and fed on milled (<90 μm) *Zostera* (see above) was determined by the Conover ash-ratio method. Percentage of organics in food and faeces (dried at 60°C for 48 h) of both control (untreated) and antibiotic-treated prawns was measured by weight loss after ashing at 450°C for 4 h.

Analysis of variance tests were applied to the data to determine whether endoglucanase activities, absorption efficiencies and hepatopancreas bacterial densities differed in control and treated animals.

RESULTS

Amylase and endoglucanase activities

Extracts of the hepatopancreas displayed the highest specific amylase activities (mg maltose released per mg protein per hour) in both *C. kraussi* and *U. africana*. High amylase activity was also measured for the foreguts of both species of prawn, with lower but active rates of starch hydrolysis associated with the mid and hindguts (Table 1). Amylase activity in the hindgut homogenate of *U. africana* was however very low (Table 1). The specific amylase activities (per mg protein) of the foregut and hepatopancreas of *C. kraussi* were 4-5 times higher than those of *U. africana* (Table 1).

Specific endoglucanase activities (mg maltose per mg protein per hour) of the foregut and hepatopancreas of *C. kraussi* were appreciable and an order of magnitude higher than those of *U. africana*. However endoglucanase activities in the mid and hindgut of *C. kraussi* were low. While the hepatopancreas of *U. africana* displayed a slightly higher activity than other regions of the gut, specific endoglucanase activity of homogenates of all gut regions of *U. africana* were very low (Table 2).

If the relative sizes of the organs of the gut, the concentrations of protein per unit mass of organ and the biomass of the animals are taken into account, the contribution by each whole organ to the reducing-sugar release from each substrate per standard unit of animal tissue can be determined (Table 3). It is clear that in both species of prawn the hepatopancreas is the main site of starch hydrolysis, with the foreguts being of much lesser importance, while the contribution by the mid and hindguts are negligible. The respective contributions of the foreguts of *C. kraussi* and *U. africana* to amylase activity per unit animal tissue are similar.

Table 1. Amylase activity (reducing sugar release per unit protein per hour) of pooled homogenates of different regions of the gut of *Upogebia africana* (n = 20, carapace length = 15.50 ± 1.86 mm) and *Callianassa kraussi* (n = 10, carapace length = 16.50 ± 1.27 mm) incubated at 20^{0}C in the presence of 1% w/v starch. Values represent the means of 3 measurements.

Region of gut	Amylase activity (mg maltose. mg protein-1. h-1)	
	C. kraussi	*U. africana*
Foregut	10.55	5.83
Hepatopancreas	60.20	14.69
Midgut	3.48	1.85
Hindgut	2.10	0.13

Table 2. Endoglucanase activity (reducing sugar release per unit protein per hour) of pooled homogenates of different regions of the gut of *Upogebia africana* (n = 20, carapace length = 15.50 ± 1.86 mm) and *Callianassa kraussi* (n = 10, carapace length = 16.50 ± 1.27 mm) incubated at 20°C in the presence of 1% w/v CMC. Values represent the means of 3 measurements.

Region of gut	Endoglucanase activity (mg maltose. mg protein-1. h-1)	
	C. kraussi	*U. africana*
Foregut	4.43	0.40
Hepatopancreas	8.55	0.53
Midgut	0.90	0.40
Hindgut	0.70	0.31

Table 3. Total reducing-sugar release by amylase activity per unit dry weight of animal tissue for different organs of *Upogebia africana* (n = 20, carapace length = 15.50 ± 1.86 mm) and *Callianassa kraussi* (n = 10, carapace length = 16.50 ± 1.27 mm). Enzyme extracts were incubated at 20°C in the presence of 1% w/v starch. Values represent the means of 3 measurements.

Region of gut	Amylase activity (mg maltose. organ^{-1}. g DW prawn tissue^{-1}. h^{-1})	
	C. kraussi	*U. africana*
Foregut	40.51	51.86
Hepatopancreas	1656.31	1137.64
Midgut	1.59	3.52
Hindgut	1.14	0.19

Table 4. Total reducing-sugar release by endoglucanase activity per unit dry weight of animal tissue for different organs of *Upogebia africana* (n = 20, carapace length = 15.50 ± 1.86 mm) and *Callianassa kraussi* (n = 10, carapace length = 16.50 ± 1.27 mm). Enzyme extracts were incubated at 20°C in the presence of 1% w/v CMC. Values represent the means of 3 measurements.

Region of gut	Endoglucanase activity (mg maltose. organ-1. g DW prawn tissue-1.h-1)	
	C. kraussi	*U. africana*
Foregut	17.02	3.56
Hepatopancreas	235.16	40.67
Midgut	0.41	0.75
Hindgut	0.38	0.46

The contribution of the hepatopancreas of *C. kraussi* to starch hydrolysis is, however, much higher than that of *U. africana* per unit weight of animal.

Table 4 shows that the hepatopancreas and foregut of *C. kraussi* are capable of releasing 5-6 times more reducing sugars per unit weight of animal tissue from CMC, than those of *U. africana*, while in both species the contribution by mid and hindguts is very low. The hepatopancreas is the main site of significant hydrolysis of CMC in both *U. africana* and *C. kraussi* (Table 4).

Amylase activity (Table3) appears a more important source of reducing sugars for absorption than endoglucanase activity (Table 4) providing substrate is not limiting in the diet.

Carbohydrase activity of *C. kraussi* hepatopancreas incubated in the presence of complex carbohydrates and refractory components of naturally occurring detritus

The long incubation periods required for these assays necessitated the use of a bacterial inhibitor to prevent bacterial growth in the reaction mixture. The efficiency of the administration of NaAzide during incubation of enzyme and substrate is demonstrated in Figure 1. In the presence of NaAzide reducing sugar release from filter paper and refluxed *Zostera* by *C. kraussi* hepatopancreas and commercial endoglucanase activity continued in a linear fashion over a 20h and 12h period, respectively. In the absence of NaAzide a rapid drop in reducing sugars present per mg protein was observed after 12 and 6 h respectively. This can most likely be attributed to uptake of reducing sugars by bacteria in the reaction mixture.

The carbohydrase activity of *C. kraussi* hepatopancreas homogenate and commercially-prepared endoglucanase in the presence of various substrates are compared in Table 5. Specific carbohydrase activities (mg maltose per mg protein per hour) in the presence of CMC were similar indicating that an endoglucanase of similar activity to that of the commercial enzyme is present in the hepatopancreas. However when these two enzymes were assayed against two forms of crystalline cellulose, Avicel and Filter paper, the specific carbohydrase activity of the hepatopancreas substrate, although low compared to hydrolysis of CMC, was respectively 3.5 and 4.7 times higher than that of the commercial endoglucanase. This suggests the presence of an additional enzyme capable of digesting crystalline cellulose i.e. C1-cellulase. Furthermore the rate of reducing sugar release from dialyzed ground diatoms and dialysed ground *Zostera* by the hepatopancreas was 3.5-4 times higher than that of the commercial endoglucanase, indicating that the hepatopancreas contains enzymes capable of breakdown of naturally-occurring

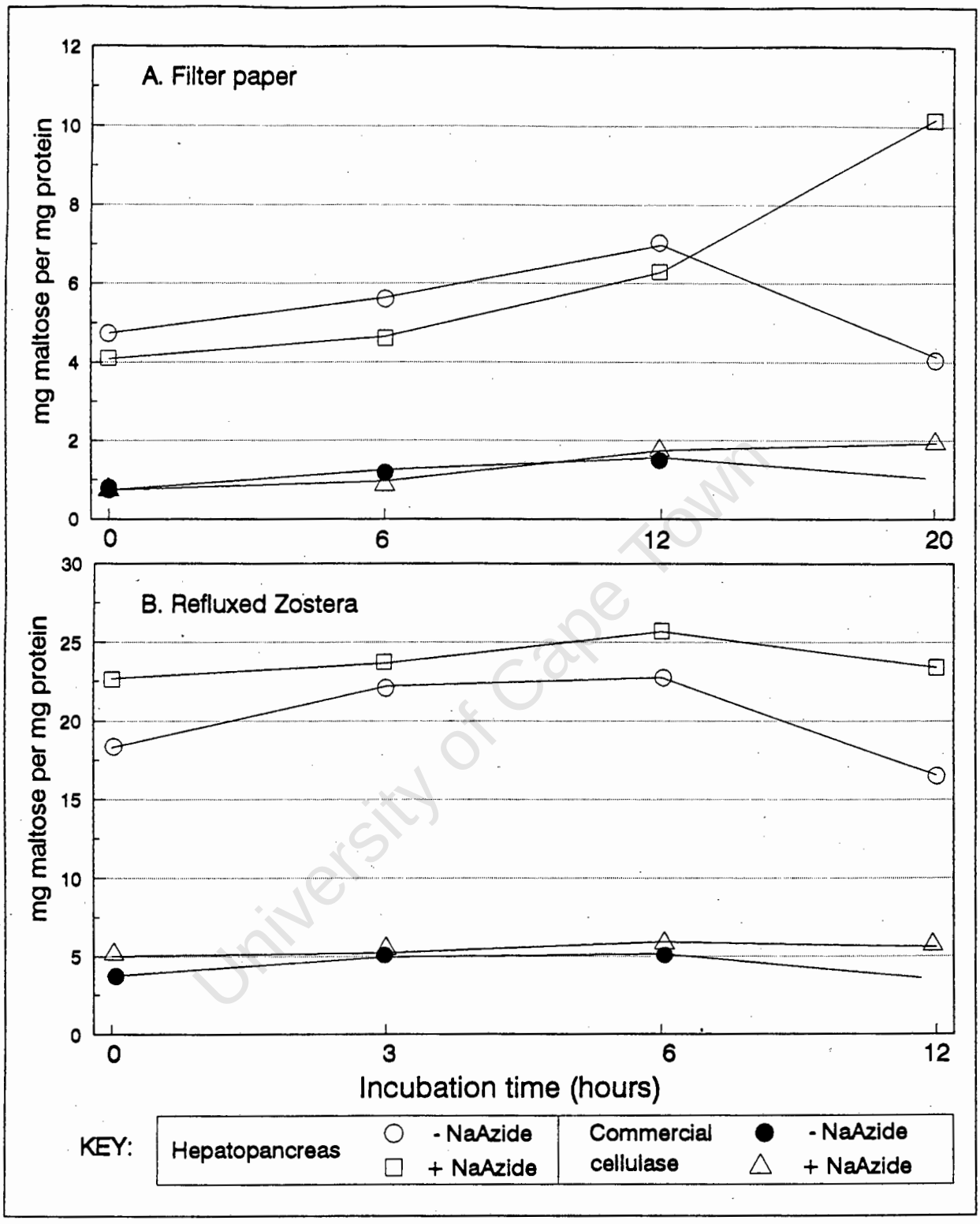

Figure 1. Affect of NaAzide addition on release of maltose from filter paper and refluxed *Zostera* by *Callianassa kraussi* hepatopancreas and bacterial cellulase.

Table 5. Carbohydrase activity (mg maltose. mg protein-1) of homogenates of hepatopancreas of *Callianassa kraussi* and commercial cellulase (endoglucanase of *Apergillus niger*) incubated at 20°C in the presence of various commercial and natural substrates. Bacterial activity was inhibited by addition of NaAzide at a final concentration of 0.2 %. Hepatopancreas homogenate and commercial cellulase contained 14.66 and 69.66 ug protein ml^{-1}, respectively.

Substrate	Reducing sugar present in substrate % w/w	Rate of reducing sugar release mg maltose. mg $protein^{-1}$. h^{-1}	
		Hepatopancreas	Comm.cellulase
CMC	0.0048	6.012	6.384
Avicel	0.0836	0.052	0.015
Filter paper	0.0044	0.308	0.065
Diatom-dialyzed	0.0268	0.749	0.190
Zostera-dialyzed	0.0562	3.490	1.091
Zostera-refluxed	0.0554	0.195	0.078

complex carbohydrates, that are not readily accessible to endoglucanase. The rate of carbohydrate hydrolysis by the hepatopancreas against refluxed *Zostera* is relatively low but nevertheless higher than that of the commercial endoglucanase suggesting that the hepatopancreas extract has capacity for hydrolysis of crystalline forms of cellulose present in the most refractory components of the naturally occurring detritus.

Figure 2 shows the curves of carbohydrase activity of *C. kraussi* and commercial endoglucanase activity when incubated in the presence of various cellulose and natural substrates. Low carbohydrase activity was observed for commercial endoglucanase activity against all of the substrates except CMC. When the hepatopancreas enzyme extract was incubated in the presence of crystalline forms of cellulose (Avicel and filter paper) a lag time of low activity was followed by an increase in the rate of reducing sugar release. A similar result was obtained when refluxed *Zostera* was used as a substrate ie initial slow release of reducing sugars from this substrate (which represents the refractory components of naturally occurring *Zostera* detritus), followed by an increase in the rate of releasing sugar release. In contrast release of reducing sugars from dialysed natural substrates (diatoms and *Zostera*) resulted in an initial relatively rapid release of reducing sugars followed by a slowing of the rate of release, and subsequent decrease in the amount of reducing sugars present. The decrease was less for the hepatopancreas extract than for the commercially prepared bacterial cellulase.

Endogenous or exogenous source of cellulase in *C. kraussi*

Specific endoglucanase activity of *C. kraussi* hepatopancreas was 2.944 (±0.289, n = 12) and 3.446 (±1.303, n = 12) mg maltose. mg protein^{-1}. h^{-1} for control and antibiotic treated animals respectively (Table 6). There was no significant differences between between the mean activities of the control and treated prawns ($p < 0.05$, n = 24). Plate counts of hepatopancreas tissue of the control (5.92 ± 4.42 CFU per mg wet weight of tissue, n = 9) and treated (0.22 ± 0.34 CFU per mg wet weight of tissue, n = 9) prawns indicated a highly significant difference in numbers of plateable bacteria ($p < 0.005$, n = 16). In addition, SEM views of treated and control hepatopancreas tissue revealed the presence of numerous microbes in the lumen of all control animals examined, while treated animals generally had far fewer. Thus the antibiotic treatment effectively depleted the bacterial population of the hepatopancreas, but did not affect endoglucanase activity. This suggests that the

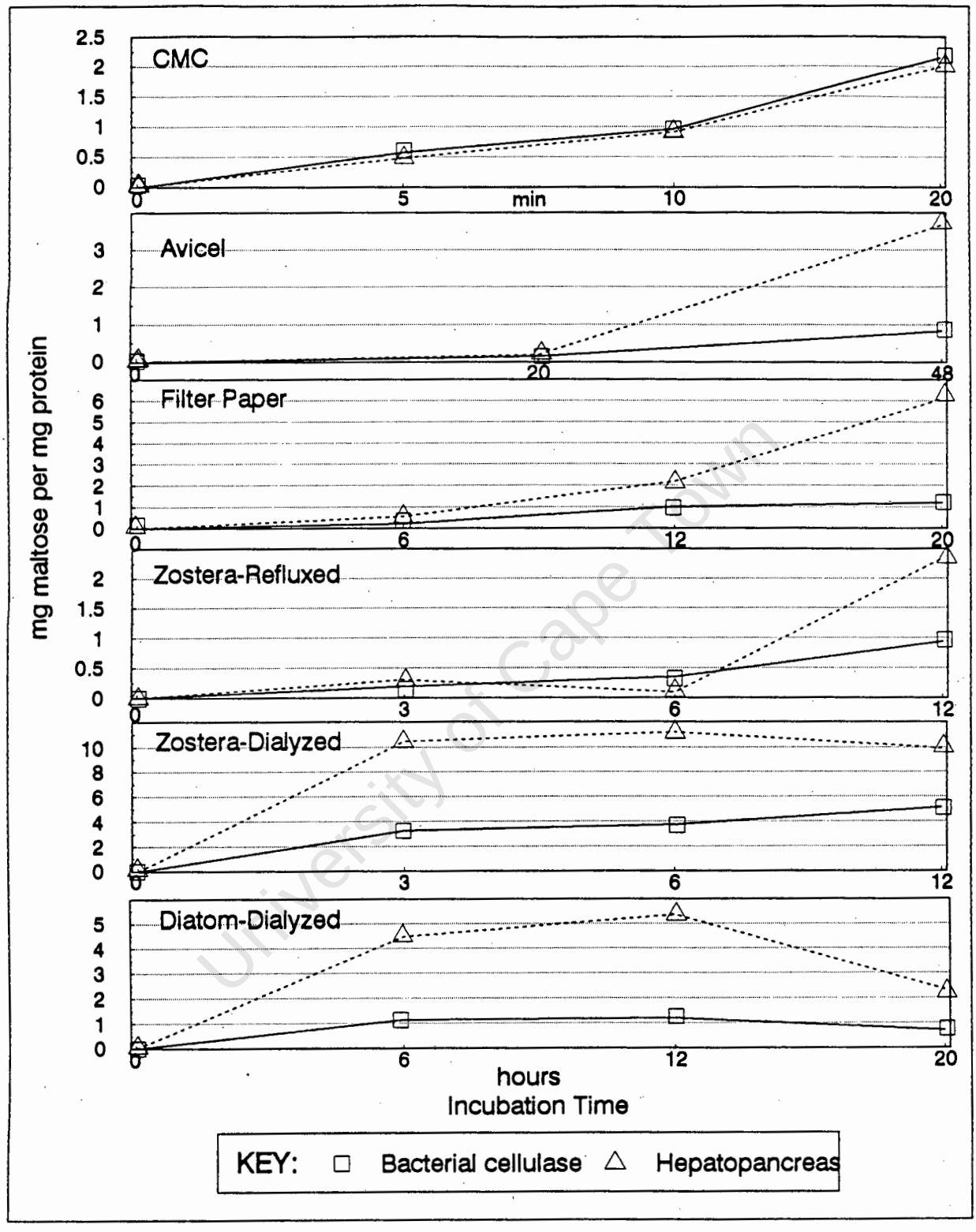

Figure 2. Curves of reducing sugar release by *Callianassa kraussi* hepatopancreas and bacterial cellulase against commercially-prepared celluloses and naturally-occurring substrates.

Table 6. Affect of antibiotic treatment on endoglucanase activity of hepatopancreas of *Callianassa kraussi* (total length 60.9 ± 6.23 mm). Enzyme extract incubated at 20oC in the presence of 1% CMC.

Prawn	Hepatopancreas endoglucanase activity mg maltose. mg protein^{-1}. h^{-1}	
	Control	Treated
1	3.978	3.252
2	4.003	3.217
3	2.130	3.529
4	1.844	3.095
5	2.713	5.341
6	3.854	3.947
7	2.104	3.166
8	3.427	2.169
9	4.432	4.097
10	3.300	2.455
11	1.906	1.147
12	1.642	5.936
Mean	2.944	3.446
S.E	0.999	1.303

Table 7. Affect of antibiotic treatment on absorption efficiency of *Callianassa kraussi* (total length 60.9 ± 6.23 mm) fed on *Zostera*, determined by the Conover ratio of organic content of food and faeces.

Prawn	Absorption efficiency (%)	
	Control	Treated
1	87.32*	83.44*
2	67.32	73.20
3	70.26	64.28
4	75.23	69.30
Mean	75.03	72.56
S.D.	8.82	8.12

* fed a mixture of *Zostera* and pelagic algae.

endoglucanase present in the hepatopancreas is of animal (endogenous) and not bacterial (exogenous) origin.

These results are corroborated by the finding that the absorption efficiency of *C. kraussi* prawns fed on ground *Zostera* is unaffected ($p < 0.05$, $n = 8$) by depletion of gut bacteria by antibiotic treatment (Table 7), indicating that the absorption of the detritus is largely if not solely due to the digestive capabilities of the prawn itself.

pH of the gut regions, and affect on *C. kraussi* hepatopancreas endoglucanase activity

In both *C. kraussi* and *U. africana* the pH is slightly acidic, but lowest in the hepatopancreas (6.39 and 6.70, respectively), close to neutral in the mid and hindguts (6.93 and 6.95, respectively), and intermediate in the foreguts (Table 8). Figure 3 shows that the rate of endoglucanase activity of *C. kraussi* hepatopancreas increases over the pH range of 5.4 to 7.4. Thus conditions in the midgut and hindgut of *C. kraussi* appear more favourable for the activity of the hepatopancreas endoglucanase than those in the more acidic foregut and hepatopancreas.

Affect of temperature on endoglucanase activity *C. kraussi*

Figure 4 gives the rate of endoglucanase activity of *C. kraussi* hepatopancreas and foregut over a temperature range of 15 to 25°C, a range that covers that experienced by the prawn in its natural habitat. The Q_{10}s of the endoglucanases present in the hepatopancreas and foregut of *C. kraussi* were 1.82 and 1.76, respectively.

DISCUSSION

Presence and strength of amylase and endoglucanase activity in *C. kraussi* and *U. africana*

The degree of utilization of ingested vascular plant detritus and associated diatoms will depend on the suite of carbohydrases possessed by the animal. Both *C. kraussi* and *U. africana* displayed appreciable amylase activities, indicating that they are

Table 8. pH of gut fluid from different regions of gut of *Upogebia africana* (n = 10, carapace length = 15.25 ± 1.86) and *Callianassa kraussi* (n = 15, carapace length = 13.4 ± 0.70).

Gut region	*U. africana*	*C. kraussi*
Foregut	6.77 (±0.20)	6.58 (±0.21)
Hepatopancreas	6.70 (±0.22)	6.39 (±0.24)
Mid&hindgut	6.95 (±0.13)	6.93 (±0.11)

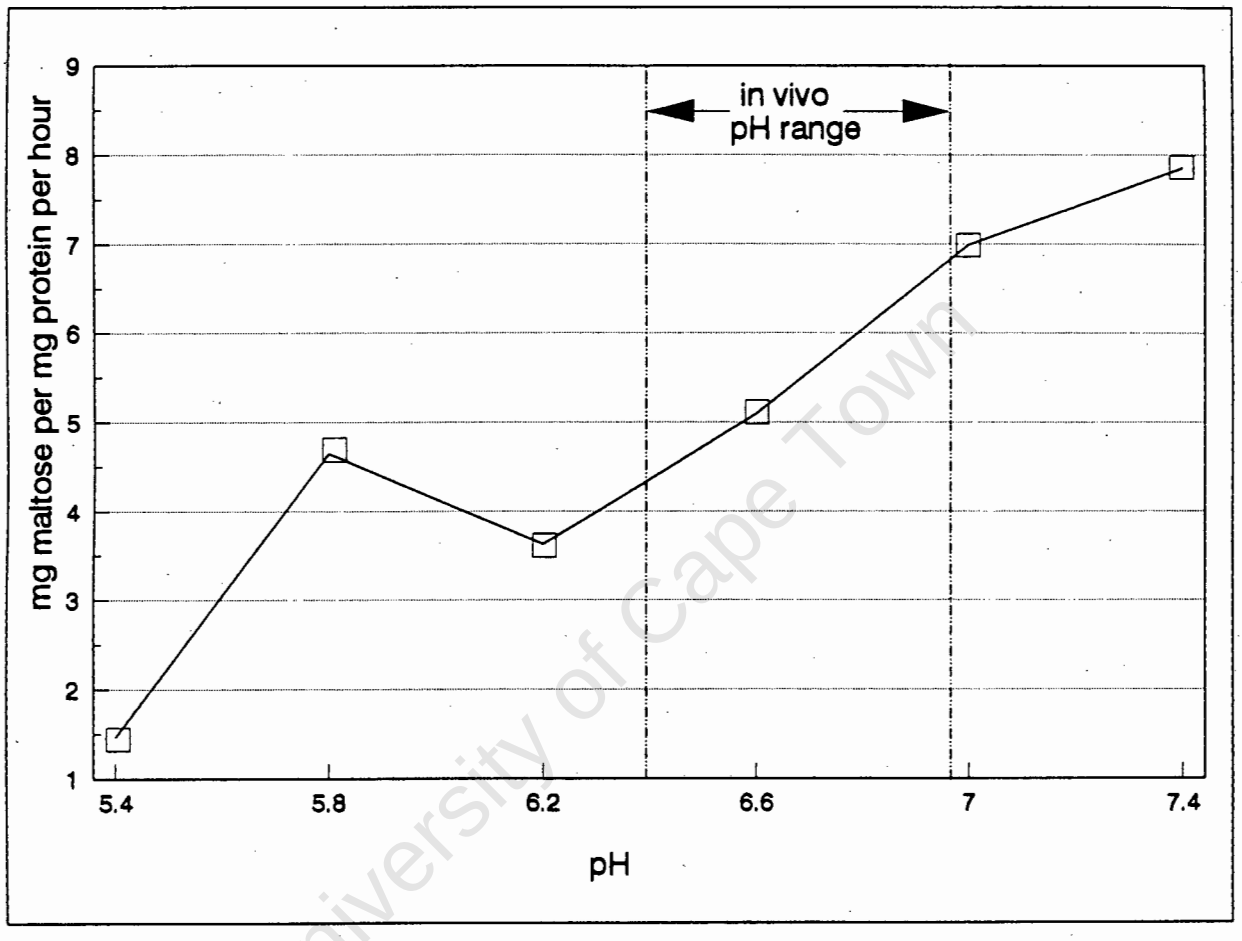

Figure 3. Affect of pH on endoglucanase activity of *Callianassa kraussi* hepatopancreas.

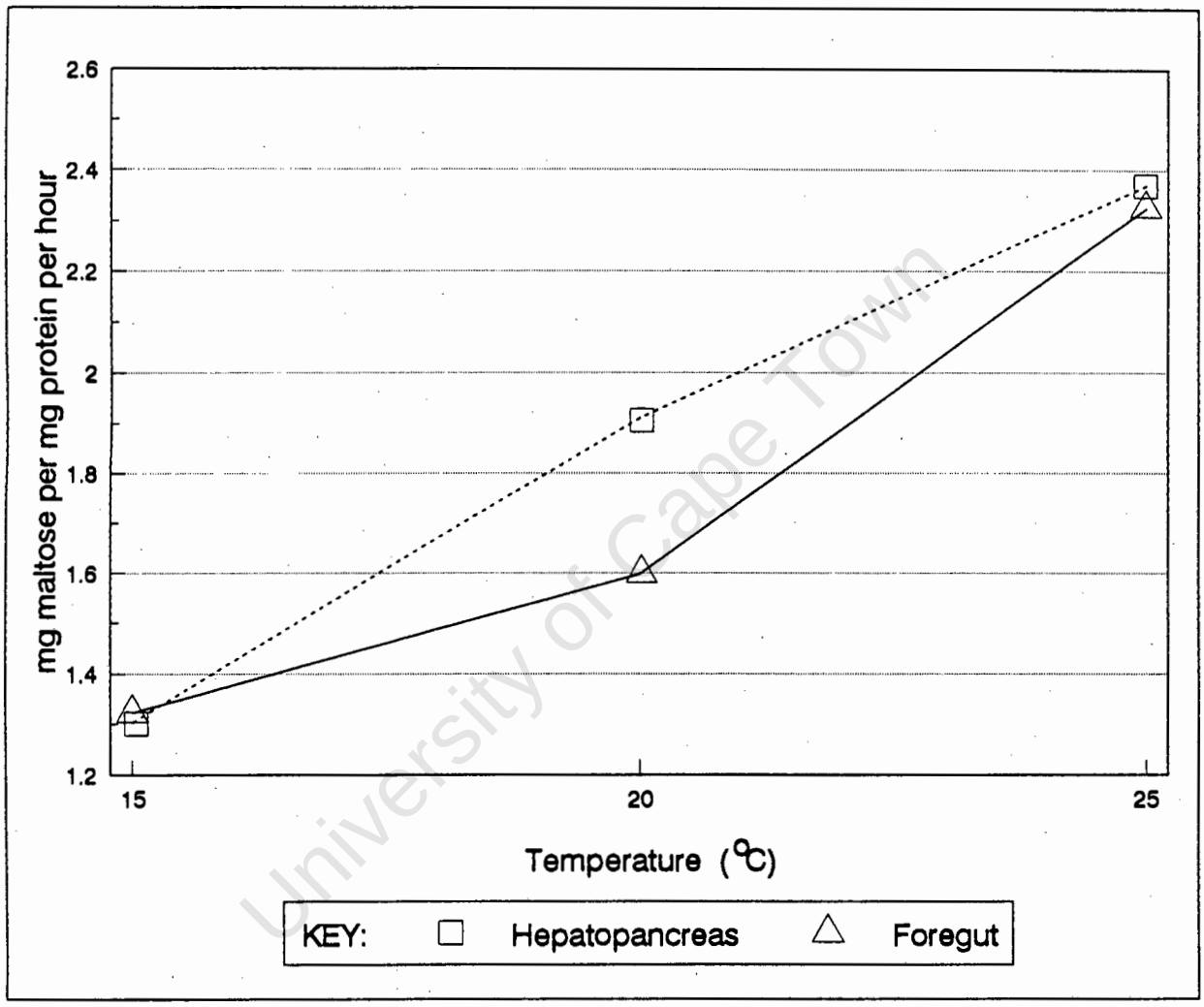

Figure 4. Affect of temperature on endoglucanase activity of *Callianassa kraussi* hepatopancreas and foregut

capable of utilization of the main storage carbohydrate (starch) present in both diatoms and vascular plant cells. However, while *C. kraussi* had high levels of endoglucanase activity, enabling the breakdown of soluble forms of cellulose, *U. africana* showed little evidence of significant activity by this enzyme. The presence of endoglucanase activity in aquatic invertebrates has been reported widely, e.g. in crustaceans (Yokoe & Yasumasu 1964, Elyakova 1972, Elyakova *et al.* 1981, Chamier & Willoughby 1986), echinoderms (Yokoe & Yasumasu 1964, Araki & Giese 1970, Elyakova *et al.* 1981), molluscs (Yokoe & Yasumasu 1964, Horiuchi & Lane 1965, Dumitru & Iordachescu 1978, Gianfreda *et al.* 1979, Kesler & Tulou 1980, Elyakova *et al.* 1981, Lucas & Newell 1984, Brock *et al.* 1986, Brock 1989), annelida (Yokoe & Yasumasu 1964, Elyakova *et al.* 1981), chordates (Yokoe & Yasumasu 1964, Seiderer & Newell 1988) and aquatic insects (Sinsabaugh *et al.* 1985, Walters & Smock 1991). It appears that most crustaceans, molluscans and annelidans studied do possess endoglucanase activity, while many echinoderms and chordates do not (Yokoe & Yasumasu 1964, Elyakova 1972, Elyakova *et al.* 1981, Brock & Kennedy 1992). It has been suggested that the presence of this enzyme may be correlated with phylogeny rather than with feeding type (Yokoe & Yasumasu 1964), although too few data exist to test this hypothesis.

Hydrolysis of crystalline cellulose and natural carbohydrates by *C. kraussi*

C. kraussi is also capable of hydrolysis in the hepatopancreas of native crystalline cellulose present in plant cell walls, as shown by reducing sugar release from substrates such as Avicel microcrystalline cellulose, filter-paper and refractory components of naturally occurring detritus i.e. refluxed *Zostera*. The hepatopancreas extract of *C. kraussi* performs much better against these native celluloses than does the endoglucanase of bacterial origin, confirming the presence of C1-cellulase in addition to endoglucanase. Thus *C. kraussi* is capable of hydrolysis of crystalline cellulose and refractory components of detritus.

The lag of reducing sugar release (i.e. an initial slow release followed by an increase in the rate of release of reducing sugars) from these three substrates by *C. kraussi* hepatopancreas extract probably reflects synergistic activity of C1- and Cx-cellulases. Presumably the initial slow activity of C1-cellulases modifies the substrate for subsequent more rapid hydrolysis by Cx-cellulases and cellobiases. Synergism between carbohydrases has been reported as important for complete hydrolysis of crystalline forms of cellulose (Berghem & Pettersson 1974, Bacon 1979, Rouland *et al.* 1988). The initial relatively rapid rate of glucogenic activity of *C. kraussi* hepatopancreas against dialysed natural substrates (diatoms and *Zostera*)

followed by a slowing of the rate, particularly compared to the commercial endoglucanase, could be explained by the impurity of these substrates compared to commercially-prepared pure crystalline carbohydrates and the refluxed *Zostera*. Initial rates probably represent Cx-cellulase and cellobiase activities on labile carbohydrates present in these substrates until depletion of these components, whereafter slow rates represent C1 activity against structural carbohydrates. These results of activity against dialysed substrates indicate that *C. kraussi* can hydrolyze a fair portion of the carbohydrates present in naturally-occurring detritus by endoglucanase activity alone, without resorting to utilization of the most refractory components. A slightly confounding result with these assays involving dialysed substrates is the result that the total reducing sugar in the assay mixtures decreased in the final time periods. This may be due to bacterial uptake after the long incubation period during these assays where the impurity of the substrate and concomitant high bacterial load may have rendered the antimicrobial agent inefficient in preventing bacterial metabolism. This affect is likely to result in an under-estimation of C1-cellulase activity, particularly as the depletion of reducing sugars was greater with the commercial enzyme than with hepatopancreas. However, the results involving dialysed natural substrates must be treated with some caution due to the uncontrolled nature of the assays.

C1-cellulase activity has been reported for a number of invertebrates, particularly Mollusca (Crosby & Reid 1971, Payne *et al.* 1972, Dean 1978, Alexander *et al.* 1979, Mirza & Serban 1981, Kesler 1983, Barlocher *et al.* 1989), with limited reports for Crustacea (Monk 1977, Chamier & Willoughby 1986, Friesen *et al.* 1986, Musgrove 1988), and Echinodermata (Scheibling 1980, Obrietan *et al.* 1991). A few workers have tested the animal enzymes against natural substrates and have also shown low but measurable rates of hydrolysis (Monk 1977, Barlocher 1982, Lucas & Newell 1984, Barlocher *et al.* 1989).

Site of endoglucanase and amylase activities

The hepatopancreas is the most important organ for carbohydrate digestion in both *C. kraussi* and *U. africana*. In both prawns, the hepatopancreas displayed the highest specific activities of amylase and endoglucanase, and made the greatest relative contribution to reducing sugar release when the size of the different digestive organs was considered. Amylase activity decreased down the length of the gut of both species of prawn. Starch hydrolysis was appreciable in the foregut, but markedly lower in the midgut and particularly the hindgut. A similar pattern of distribution of activity down the length of the gut was found for endoglucanase activity in *C.*

kraussi, i.e. significant activity in the foregut and very low activity in the mid- and hindgut. While the very low endoglucanase activity present in the gut of *U. africana* is marginally higher in the hepatopancreas than in other regions, the activity of this enzyme decreases only slightly down the gut from the foregut to the hindgut. The midguts and hindguts of both species of prawns are therefore not important sites of carbohydrate digestion. Interestingly, in *C. kraussi* the pH in the main throughput stream of the gut (fore-, mid- and hindgut) is closer to the pH optimum of the endoglucanase than is that of the hepatopancreas. While this result is somewhat curious and may reflect the pH optima of other important processes, it does indicate that pH cannot be held responsible for the low endoglucanase activity in the mid- and hindgut of *C. kraussi*. These findings confirm those of other workers who identified the hepatopancreas as an important site of cellulolytic activity in a number of crustacean species (e.g. Yokoe & Yasumasu 1964, Elyakova 1972, Elyakova *et al.* 1981, Musgrove 1988). In general, the hepatopancreas is the principle organ of production of enzymes, digestion and absorption in decapod crustaceans where the midgut is usually very short (van Weel 1970).

This pattern of distribution of carbohydrases in the gut has important implications for the utilization of ingested carbohydrates, particularly celluloses. In both *C. kraussi* and *U. africana*, fine particles from the foregut (resulting from mastication by the gastric mill or filtration by modified teeth, respectively) are channeled into the blind-ending pouches (tubes) of the hepatopancreas, while larger particles are directed into the midgut and passed out with the faeces. Digestion by enzymes and absorption is a time-dependent process (see Kofoed *et al.* 1989), and hydrolysis of refractory components of detritus requires long incubation periods . The evidence that the hepatopancreas is the main site of digestion and absorption of cellulose and starch suggests that the gut passage time of the main food bolus is not an important factor in limiting the incubation of carbohydrates for hydrolysis in the gut. While the field gut passage rate of both species of prawns is approximately 6 h, it is probable that particles are held in the hepatopancreas for much longer periods, allowing efficient hydrolysis of structural components. This is important as appreciable C1-cellulase activity against substrates such as Avicel microcrystalline cellulose, filter paper and refluxed *Zostera* was only detected after incubation periods of greater than 20, 6 and 6 h, respectively.

Endogenous or endogenous source of cellulases

The cellulases measured in *C. kraussi*, and in low concentrations in *U. africana*, may be produced by the animals themselves or be contributed by gut microbiota. The third possibility is that the enzymes are ingested with the food. The antibiotic experiments of both enzyme assays and absorption efficiencies suggest that the endoglucanase, and possibly the C1-cellulase, present in the hepatopancreas and foregut of *C. kraussi* is endogenous. *C. kraussi* can digest cellulose and absorb the refractory components of naturally-occurring detritus and purified commercially-prepared crystalline cellulose independently of bacterial activity. This result is further supported by the finding of Harris *et al.* (1991) that isolates from the gut of *C. kraussi* do not produce cellulase.

It is however possible that the low endoglucanase activity measured for the hepatopancreas and gut of *U. africana* may be attributed to microbial enzymes ingested with the food. The strength of the endoglucanase activity in different regions of the gut was not markedly different and did not decrease dramatically down the gut, suggesting that it may be associated with the ingested food (acquired) rather than animal-produced. Furthermore a study of the gut microflora of the prawn revealed ingested isolates with endoglucanase ability, and the possibility of incubation of ingested microbes in the gut (Harris *et al.* 1991). The higher activity in the hepatopancreas could represent incubation in this region where the food can be held for a long period.

The question of whether cellulases present in aquatic invertebrates is of endogenous or exogenous origin has been explored in relatively few cases, and a general pattern has not emerged. While acquired enzymes (e.g. Barlocher 1982, Chamier & Willoughby 1986) and cellulolytic gut bacteria have been reported (e.g. Cutter & Rosenberg 1971, Martinez 1982, Musgrove 1988), it appears that some invertebrates are capable of producing their own cellulases (e.g. Araki & Giese 1970, Payne *et al.* 1972, Monk 1977, Morton 1978). One reason for the lack of data concerning this issue is the difficulty in verifying the source of enzymes. Some authors have suggested that the lack of a gut microflora precludes the involvement of bacterial enzymes (e.g. Friesen *et al.* 1986), while other workers have compared cellulose digestion of animals that have been treated with antimicrobial agents with that of untreated control animals (e.g. Wainwright & Mann 1982). A number of problems with the use of antibiotics have been highlighted. It is possible that these substances may effect the gut lining of the animal and may alter the secretion of endogenous enzymes (Yerokhin 1979, Wainwright & Mann 1982). In addition, most antibiotics are active against specific types of microbes (Yetka & Wiebe 1974). It is

therefore important that broad-acting or multiple treatments are applied and that the effectiveness is verified in each case. Differentiation between acquired (ingested), gut bacterial and endogenous is important to an understanding of the role of invertebrate detritivores in decomposition processes of the system. In the case that cellulases are acquired, decomposition of cellulose in the detritus ingested will proceed independently of the invertebrates (outside the animal), and the invertebrate will at most enhance decomposition by physical shredding or as an incubator for this activity. Where detritivores produce endogenous cellulases, or host gut cellulolytic microbes, their role in decomposition of refractory cellulose components in the system is likely to be much greater. They may either act as hot spots for this activity thus modifying the detritus during its passage through the gut, or they may contribute cellulases to the system that are passed out with the faeces.

Feeding strategies: are the digestive capabilities, feeding modes, diets and resources correlated ?

C. kraussi is capable of digestion of cellulose and refractory components of ingested detritus, while *U. africana* possesses very weak cellulase activity. *U. africana* is a filter-feeding prawn able to retain small particles from the water column non-selectively, while *C. kraussi* is a deposit-feeder that selectively ingests relatively large particles which are crushed in the gastric mill. Gut content analysis of both prawns, however, indicates that while the ingestion process may differ, both have predominantly plant fragments and diatoms in the gut contents (Chapter 2). However, stable isotope analyses indicate that while *U. africana* depends exclusively on saltmarsh plant detritus for both carbon and nitrogen, *C. kraussi* obtains carbon from a mixture of saltmarsh plant detritus and diatoms, while it receives nitrogen almost entirely from diatoms and other organisms associated with detritus (Chapter 2). Why then does *C. kraussi* display strong cellulase activity while *U. africana* does not? Gut passage time can influence the digestion of ingested food by detritivores, but this is unlikely to explain differences in the physiology of these two prawns as gut throughput time was similar in both species (approximately 6 h) although food channelled into the hepatopancreas may be incubated in the presence of enzymes for a much longer period. However, due to the different actions of the foregut on ingested food in these two prawns, the material entering the hepatopancreas is likely to differ, i.e. fine refractory particles (separated from the main food bolus by filtration alone in the foregut) are likely to enter the hepatopancreas in *U. africana*, while cell contents of diatoms and larger plant fragments crushed by the foregut gastric mill are likely to be channelled into the hepatopancreas in *C. kraussi*.

However, as the density of micro-organisms in the sediment are relatively low in the habitat of *C. kraussi* (Chapter 1), cellulase ability may be important to allow carbon requirements to be met. The richer source of labile nutrients obtained by selective feeding in *C. kraussi* may obviate the necessity for this prawn to rely on the refractory components for essential nutrients such as nitrogen. Thus *C. kraussi* appears to concentrate its food by selective feeding to obtain sufficient nitrogen, and break down refractory components by endogenous enzyme activity to meet its carbon requirements, and the large hepatopancreas will increase retention time of food. Chamier (1991) suggested that the ability to digest cellulose may be of benefit to the freshwater amphipod *Gammarus pseudolimnaeus* during seasonal bottlenecks in the quality of the resource, i.e. when fine detritus is the main foodstuff. However, cellulase activity has also been suggested as important to hydrolyze cell walls of unicellular green algae efficiently (Brock 1989). It should be noted, however, that the possession of cellulase activity is not necessarily diet-related but may be vestigial and related to phylogeny (Yokoe & Yasumasu 1964). In fact a number of carnivores are reported to possess cellulases (e.g. Obrietan *et al.* 1991, Agnisola *et al.* 1981), and Kesler (1983) found that cellulase activity is not a good indicator of dietary niche separation in gastropods. However, considering the diet of *C. kraussi* and the strength of the cellulase activity associated with the hepatopancreas of this prawn, it is unlikely that the cellulase is not functional.

The utilization of vascular plant detritus by *U. africana* as its dominant food source despite the relative abundance of nutrient-rich microbiota, including diatoms, in the water column and sediments (Chapter 1) suggests a morphological constraint imposed by the filters of the feeding apparatus. This prawn is not equipped for selective feeding but may have opted for a larger throughput gut to process larger meals of fine suspended vascular plant detritus. The question of why *U. africana*, which according to stable isotope analyses relies heavily on vascular plant detritus for sustenance, does not possess appreciable cellulase activity is interesting. It may be that *U. africana* relies on conditioning of the food by microbes prior to ingestion to enable utilization of this refractory detritus. Microbial growth pre-conditions detritus, by addition of valuable microbial nutrients or by conversion of detrital polymers into digestible subunits (Barlocher 1985), which then becomes more palatable and digestible to invertebrates (Barlocher *et al.* 1989). Microbial enzymes can expose other cell-wall polymers (expose cellulose microfibrils of cell walls to attack by enzymes) to attack by animal-gut enzymes whose secretion is enhanced by these "exposed" substrates (Chamier 1991). The possible role of gut microbes in digestion of ingested detrital material and supply of nitrogen in *U. africana* is of particular interest as a few cellulose-degrading bacteria were isolated

from the gut and this prawn has spectacular mats of epimural hindgut bacteria (Harris *et al.* 1991). However the gut throughput rate is not increased over that of *C. kraussi* and side pouches (hepatopancreas) are not large. Perhaps *U. africana* relies on eating big meals (large throughput gut) of preconditioned food and using simple carbohydrases to glean enough nutrients without utilization of refractory components. Alternatively, it has been shown that low cellulase activity is not necessarily a good indication of a lack of ability to digest cellulose. For example. *Tipula abdominalis* with no detectable cellulolytic activity had a high assimilation efficiency when fed purified cellulase (Sinsabaugh *et al.* 1985). The failure to find significant cellulase activity in alimentary tracts of aquatic insects does not preclude the possibility of cellulose digestion, e.g. mammalian ruminants and some insects that rely on microbial endosymbionts for cellulose digestion exhibit little or no cellulase activity in their alimentary tracts (e.g. Hungate 1946, Bayon & Mathelin 1980). The question of how *U. africana*, which survives on a low quality diet of high C/N ratio, meets its nitrogen requirements is of interest. It seems possible that gut bacteria may be collaborators in nitrogen acquisition.

References

Adams SM, Angelovic JW 1970 Assimilation of detritus and its associated bacteria by three species of estuarine animals. Chesapeake Sci 11(4):249-254

Agnisola C, Salvadore S, Scardi V 1981 On the occurrence of cellulolytic activity in the digestive gland of some marine carnivorous molluscs. Comp Biochem Physiol 70B:521-525

Alexander CG, Cutler RL, Yellowless D 1979 Studies on the composition and enzyme content of the crystalline style of *Telescopium telescopium* (Gastropoda). Comp Biochem Physiol 64B:83-89

Araki GS, Giese AC 1970 Carbohydrases in sea stars. Physiol Zool 43:296-305

Bacon JSD 1979 Factors limiting the action of polysaccharide degrading enzymes. Chapter 11. In: Berkeley RCW, Gooday GW, Ellwood DC (eds.) 1979 Microbial polysaccharides and polysaccharases. Academic Press, New York

Baker JH, Bradnam LA 1976. The role of bacteria in the nutrition of aquatic detritivores. Oecologia (Berl) 24:95-104

Barlocher F 1982 The contribution of fungal enzymes to the digestion of leaves by *Gammarus fossarum* Koch (Amphipoda). Oecologia (Berl) 52:1-4

Barlocher F 1985 The role of fungi in the nutrition of stream invertebrates. Bot J Linn Soc 91:83-94

Barlocher F, Arsuffi TL, Newell SY 1989 Digestive enzymes of the saltmarsh periwinkle *Littorina irrorata* (Mollusca, gastropoda). Oecologia 80(1):39-43

Barlocher F, Newell SY, Arsuffi TL 1989 Digestion of *Spartina alterniflora* Loisel material with and without fungal constituents by the periwinkle *Littorina irrorata* Say (Mollusca: Gastropoda). J Exp Mar Biol Ecol 130(1):45-54

Barlocher F, Tibbo PG, Christie SH 1989 Formation of protein-phenol complexes and their use by 2 stream invertebrates. Hydrobiologia 173:243-249

Bayon C, Mathelin D 1980 carbohydrate fermentation and by-product absorption studied with labelled cellulose in *Oryctes nasicornis* larvae (Coleoptera: Scarabeidae). J Insect Physiol 26:833-840

Berger E 1990 Analysis of genes and enzymes involved in the degradation of cellulose and proteins by *Butyrivibrio fibrisolvens* H17c. PhD thesis, UCT

Berghem LER, Pettersson LG 1974 The mechanism of enzymatic cellulose degradation. Eur J Biochem 46:295-305

Bernfeld PC 1955 Amylases a and b. In: Methods in enzymology, Vol 1, Colwick SP, Kaplan NP (eds), Academic Press, New York, pp 148-150

Bianchi TS, Levinton JS 1984 The importance of microalgae, bacteria and particulate organic matter in the somatic growth of *Hydrobia totteni*. J Mar Res 42:431-443

Bjarnov N 1972 Carbohydrases in Chiromonus, Gammarus and some Trichoptera larvae. Oikos 23:261-263

Breuil C, Saddler JN 1985 Comparison of the 3,5- dinitrosalicyclic acid and Nelson-Somogyi methods of assaying for reducing sugars and determining cellulase activity. Enzyme Microb Technol 7:327-332

Brock V 1989 *Crassostrea gigas* (Thunberg) hepatopancreas-cellulase kinetics and cellulolysis of living monocellular algae with cellulose walls. J Exp Mar Biol Ecol 128(2):157-164

Brock V, Kennedy ND 1992 Quantitative analysis of crystalline style carbohydrases in 5 suspension-feeding and deposit-feeding bivalves. J exp mar biol ecol 159(1):51-58

Brock V, Kennedy VS, Brock A 1986 Temperature dependency of carbohydrase activity in the hepatopancreas of thirteen estuarine and coastal bivalve species from the North American east coast. J Exp Mar Biol Ecol 107:87-101

Cammen LM 1980 The significance of microbial carbon in the nutrition of the deposit feeding polychaete *Nereis succinea*. Mar Biol 61:9-20

Chamier A, Willoughby LG (1986) The role of fungi in the diet of the amphipod *Gammarus pulex* (L.): an enzymatic study. Freshwater Biol 16:197-208

Chamier AC 1991 Cellulose digestion and metabolism in the freshwater amphipod *Gammarus pseudolimnaeus* Bousfield. Freshwater Biol 25:33-40

Christie ND 1981 Primary production in Langebaan lagoon. In: Estuarine ecology with particular reference to Southern Africa (Day, J ed.), AA Balkema, pp 101-115

Crosby ND, Reid RGB 1971 Relationships between food, phylogeny, and cellulose digestion in the bivalvia. Can J Zool 49:617-622

Cummins KW, Klug MJ 1979. Feeding ecology of stream invertebrates. Ann Rev Ecol Syst 11:147-172

Cutter JM, Rosenberg FA 1971 The role of cellulolytic bacteria in the digestive processes of the shipworm. Material und Organismen 7/3:225-239

Dean RC 1978 Mechanisms of wood digestion in the shipworm *Bankia gouldi* Bartsch: Enzyme degradation of celluloses, hemicelluloses, and wood cell walls. Biol Bull 155:297-316

Dumitru IF, Iordachescu D 1978 Partial characterization of a cellulase preparation purified from the sea mollusc *Mya arenaria* L. Rev roum Biochim 15(4):265-271

Elyakova L.A., Shevchenko N.M., Avaeva S.M. 1981 A comparative study of carbohydrase activities in marine invertebrates. Comp Biochem Physiol 69B:905-908

Elyakova LA 1972 Distribution of cellulases and chitinases in marine invertebrates. Comp Biochem Physiol 43B:67-70

Fenchel T 1970 Studies on the decomposition of organic detritus derived from the turtle grass Thalassia testudinum. Limnol Oceanogr 15:14-20

Fielding PJ, Harris JM, Lucas MI, Cook PA 1986 Implications for the assessment of crystalline style activity in bivalves when using the Bernfeld and Nelson-Somogyi assats for reducing sugars. J Exp Mar Biol Ecol 101:269-284

Fielding PJ, St J Damstra K, Branch GM 1988 Benthic diatom biomass, production and sediment chlorophyll in Langebaan lagoon, South Africa. Est Coast Shelf Sci 27:413-426

Findlay S, Meyer JL, Smith PJ 1984. Significance of bacterial biomass in the nutrition of a freshwater isopod (*Lirceus* sp.). Oecologia (Berlin) 63:38-42

Friesen JA, Mann K.H., Novitsky J.A. 1986 *Mysis* digests cellulose in the absence of a gut microflora. Can Jour Zool 64:442-446

Ghose TK 1987 Measurement of cellulase activities. Pure & Appl Chem 59(2):257-268

Gianfreda L, Imperato A, Palescandolo R, Scardi V 1979 Distribution of B-1,4-glucanase and B-glucosidase activities among marine molluscs with different feeding habits. Comp Biochem Physiol 63B:345-348

Gilligan EB, Reese AC 1954 Evidence for multiple components in microbial cellulases. Can J Microbiol 1:90-107

Hargrave BT 1970 The utilization of benthic microflora by *Hyalella azteca* (Amphipoda). J Animal Ecol 39:427-437

Harris JM, Seiderer LJ, Lucas MI 1991. Gut microflora of two saltmarsh detritivore thalassinid prawns, *Upogebia africana* and *Callianassa kraussi*. Microb Ecol 21:63-82

Horiuchi S, Lane CE 1965 Digestive enzymes of the crystalline style of *Strombus gigas* Linne 1. Cellulase and some other carbohydrases. Bioc Bact 129:273-281

Hungate RE 1946 The symbiotic utilization of cellulose. J Elisha Mitchell Soc 62:9-24

Jensen KT, Siegismund H.R. 1980 The importance of diatoms and bacteria in the diet of *Hydrobia-species*. Ophelia 1:193-199

Kesler DH 1983 Cellulase activity in gastropods: should it be used in niche separation? Freshwat Invert Biol 2(4):173-179

Kesler DH, Tulou C.A.G. 1980 Cellulase activity in the freshwater gastropod *Amnicola limosa*. Naut 94(4):135-137

Klumpp DW, Nichols PD 1983 Utilisation of the seagrass *Posidonia australis* as food by the rock crab *Nectocarcinus integrifons* (Latreille) (Crustacea: Decapoda: Portunidae). Mar Biol Letters 4:331-339

Kofoed L, Forbes V, Lopez G 1989 Time-dependent absorption in deposit feeders. In: Lopez G, Taghon G, Levinton J (eds) Ecology of marine deposit feeders. XII, pp 322

Kristensen JH 1972 Carbohydrases of some marine invertebrates with notes on their food and on the natural occurrence of the carbohydrates studied. Mar Biol 44:130-142

Lasker R, Giese AC 1954 Nutrition of the sea urchin, *Stongylocentrotus purpuratus*. Biol Bull Woods Hole Mass 106:328-340

Levinton JS 1989 Deposit feeding and coastal oceanography. In: Ecology of marine deposit feeders, Lopez G, Taghon G, Levinton J (eds.), Springer-Verlag, New York, pp 1-23

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ 1951 Protein measurements with the folin phenol reagents. J Biol Chem 193:265-275

Lucas MI, Newell RC 1984 Utilization of saltmarsh grass detritus by two esturine bivalves: carbohydrase activity of crystalline style enzymes of the oyster *Crassostrea virginica* (Gmelin) and the Mussel *Guekensia demissa* (Dillwyn). Mar Biol Letters 5:275-290

Martin MM 1987 Invertebrate-microbial interactions: ingested fungal enzymes in Arthropod biology. Cornell University Press

Martinez JC 1982 The digestive microflora of *Teredo navalis* L. (Teredinidae; Bivalva): Metabolic properties and ultimate role in digestion. Bact Mar 331:151-154

Mirza M, Serban M. 1981 Isolation and characterization of "C" cellulase from *Mya arenaria*. Rev Roum Biochim 13(1):33-38

Monk DC 1977 The digestion of cellulose and other dietary components and the pH of the gut in the amphipod *Gammarus pulex* (L). Freshwater Biol 7:28-37

Morton B (1978) Feeding and digestion in shipworms. Oceanogr Mar Biol Ann Rev 16:107-144

Musgrove RJ 1988. Digestive ability of the freshwater crayfish *Paranephrops zealandicus* (White) (Parastacidae) and the role of microbial enzymes. Freshw Biol 20:305-314

Nelson N. 1944 A photometric adaptation of the Somogyi method for the determination of glucose. J Biol 169:375-380

Newell R 1965 The role of detritus in the nutrition of two marine deposit feeders, the prosobranch *Hydrobia ulvae* and the bivalve *Macoma balthica*. J Zool 144:25-44

Obrietan K, Drinkwine M, Williams DC 1991. Amylase, cellulase and protease activities in surface and gut tissues of *Dendraster excentricus*, *Pisaster ochraceus* and *Strongylocentrotus droebachiensis* (Echinodermata). Mar Biol 109(1):53-58

Payne DW, Thorpe NA, Donaldson EM 1972 Cellulolytic activity and a study of the bacterial population in the digestive tract of *Scrobicularia plana* (Da Costa). Proc malac Soc Lond 40:147-160

Penry DL, Jumars PA 1986 Chemical reactor analysis and optimal digestion: An optimal digestion theory can be readily derived from basic principles of chemical reactor analysis and design. Bioscience 36(5):310-315

Penry DL, Jumars PA 1987 Modeling animal guts as chemical reactors. Am Nat 129(1):69-96

Penry DL, Jumars PA 1990 Gut architecture, digestive constraints and feeding ecology of deposit-feeding and carnivorous polychaetes. Oecologia 82:1-11

Phillips NW 1984 Role of different microbes and substrates as potential suppliers of specific, essential nutrients to marine detritivores. Bull Mar Sci 35(3):283-298

Rouland C, Civas A, Renoux J, Petek F 1988 Synergistic activities of the enzymes involved in cellulose degradation purified from *Macrotermes muelleri* and from its symbiotic fungus *Termitomyces* sp. Comp Biochem Physiol B 91(3):459-466

Scheibling RE 1980 Carbohydrates of the pyloric caeca of *Oreaster reticulatis* (L). (Echinodermata: Asteroidea). Comp Biochem Physiol 67B:297-300

Seiderer LJ, Newell RC 1985 Relative significance of phytoplankton, bacteria and plant detritus as carbon and nitrogen resources for the kelp bed filter-feeder *Choromytilus meridionalis*. Mar Ecol Prog Ser 22:127-139

Seiderer LJ, Newell RC 1988 Exploitation of phytoplankton as a food resource by the kelp bed ascidian Pyura stolonifera. Mar Ecol Prog Ser 50:107-115

Seiderer LJ, Newell RC, Cook PA 1982 Quantitative significance of style enzymes from two marine mussels (*Choromytilus meridionalis* Krauss and *Perna perna* Linnaeus) in relation to diet. Mar Biol Lett 3:257-271

Shannon LV, Stander GH 1977 Physical and chemical characteristics of water in Saldanha Bay and langebaan Lagoon. Trans Roy Soc SA 42(3/4):441-460

Sinsabaugh RL, Linkins AE 1990 Enzymic and chemical analysis of particulate organic matter from a boreal river. Freshwater Biol 23:301-309

Sinsabaugh RL, Linkins AE, Benfield EF 1985 Cellulose digestion and assimilation by three leaf-shredding aquatic insects. Ecology 66:1464-1471

Somogyi M 1952 Notes on sugar determination. J Biol Chem 195:19-23

Suberkropp K, Klug MJ 1976 Fungi and bacteria associated with leaves during processing in a woodland stream. Ecology 57:707-719

Sullivan MJ, Moncreiff CA 1990 Edaphic algae are an important component of salt marsh food-webs; evidence from multiple stable isotope analyses. Mar Ecol Prog Ser 62:149-159

Tenore KR, Hanson RB, Dornseif BE, Weiderhold CN 1979 The effect of organic nitrogen supplement on the utilization of different sources of detritus. Limnol Oceanogr 24(2):350-355

Townsend CR, calow P (eds.) 1981 Physiological ecology: an evolutionary approach to resource use. Blackwell Scientific Publications, Oxford, 393pp

van Weel PB 1970 Digestion in Crustacea. In: Chemical Zoology, Florkin M, Scheer BT (eds), Vol V Arthropoda Part A, Academic Press, New York, 97-115

Wainwright PF, Mann KH 1982 Effect of antimicrobial substances on the ability of the mysid shrimp *Mysis stenolepis* to digest cellulose. Mar Ecol Prog Ser 7:309-313

Walters KH, Smock LA 1991 Cellulase activity of leaf litter and stream-dwelling, shredder macroinvertebrates. Hydrobiologia 220(1):29-36

Wynberg RP 1991 The ecological effects of collecting Callianassa kraussi Stebbing and *Upogebia africana* Ortmann for bait: Impacts on the biota of an intertidal sandflat. MSc Thesis, UCT

Yefka JE, Wiebe 1974 Ecological application of antibiotics as respiratory inhibitors of bacterial populations. Appl Microbiol 28:1033-1039

Yerokhin V Ye 1979 The use of antibiotics when studying the feeding of invertebrates on exometabolites. Hydrobiol J 14(4):77-80

Yokoe Y, Yasumasu T 1964 The distribution of cellulase in invertebrates. Comp Biochem Physiol 13:323-328

CHAPTER 4

Harris JM (submitted) The presence, nature and role of gut microflora in aquatic invertebrates: a synthesis. *Microbial Ecology*

CHAPTER 4

The presence, nature and role of gut microflora in aquatic invertebrates: a synthesis

Abstract. This paper reviews the literature concerning the gut microbiota of aquatic invertebrates, and highlights the questions and processes that merit attention if an understanding of the role of gut microbes in the physiology of host invertebrates and nutrient dynamics of aquatic systems is to be gained. A substantial number of studies report the presence of gut microbes in aquatic invertebrates. Crustacea, Mollusca and Echinodermata have received the most attention, with few studies involving other invertebrate groups. Different types of associations (e.g. ingestion, contribution of exoenzymes, incubation, parasitism) are reported to occur between gut microbes and aquatic invertebrates, and it is clear that gut bacterial communities cannot be treated as single functional entities, but that individual populations require examination. In addition, gut microbes may be either ingested transients or residents, the presence of which has different implications for the invertebrate. The most commonly reported genera of gut bacteria are *Vibrio, Pseudomonas, Flavobacterium, Micrococcus* and *Aeromonas*. Quite a number of authors report the physiological properties of gut microbes (including enzyme activities and attributes such as nitrogen fixation), while less attention has been given to consideration of the colonization sites within the digestive tract, the density and turnover of gut bacteria, and the factors affecting the presence and nature of gut microflora. In addition, very little conclusive evidence exists as to the role of bacteria in the physiology of host invertebrates, although a few studies have demonstrated a positive relationship between invertebrates and their gut microbiota, particularly with regard to nutrient gain by the invertebrate. This has resulted from a lack of process-oriented studies. The findings for aquatic gut microbes are compared to those of gut bacteria associated with terrestrial invertebrates, where gut microbes contribute significantly to nutrient gain by the host in some environments.

Introduction

In terrestrial ecosystems symbiotic associations between micro-organisms and their vertebrate and invertebrate hosts, especially those related to nutrition, are particularly well developed. A common thread in these associations is that the micro-organisms are provided with an aquatic and homeostatic medium inside the animal gut in which to flourish, while the host usually benefits from microbially-mediated digestion of ingested food or contribution of important nutrients. Free-living micro-organisms are subject to a gradation in the environment from virtually waterless (terrestrial) to totally freshwater or marine ecosystems, with a corresponding increase in their numbers as the medium becomes more aquatic. In some sense then, microbes in an aquatic environment have the choice, or at least not the dependency, of living within potential hosts, while in a terrestrial environment appreciable activity may be limited to aquatic niches such as those provided by the guts of host animals. Aquatic invertebrates may simply be able to rely on external microbial decomposition in the aquatic environment prior to ingestion of food material, thus decreasing the likelihood of symbiotic associations with gut microbes (see also Plante *et al.* (159)). Are there therefore fundamental differences in the nature and significance of gut bacteria in terrestrial and aquatic ecosystems ?

An extensive literature exists on the subject of symbiotic associations between microheterotrophs and their terrestrial animal hosts (for reviews see 24, 28, 125, 139, 176). Many of these studies have focussed on the role of the gut microflora of animals whose food quality is poor, either being refractory to digestion or low in essential nutrients such as nitrogen. For example, the gut bacteria of ruminants and termites have been shown to play an important role in cellulose digestion (e.g. 3, 18, 52, 196), and are also reported to aid in nitrogen acquisition and conservation (e.g. 27, 68, 150, 162).

By contrast, although the presence of microflora in the guts of some aquatic invertebrates has long been recognized (e.g. 20, 22, 35, 97) and endosymbiotic associations in corals and deep-sea invertebrates are known to be of nutritional importance to the host (e.g. 48, 49, 96), scant attention has been given to the significance of symbiotic relationships between gut microbes and aquatic invertebrates. Many questions concerning gut microbes of aquatic invertebrates beg consideration. For example, do the guts of aquatic invertebrates in general provide suitable habitats for microbes, and how common and widespread is the presence of gut bacteria ? What factors affect the presence and abundance of gut microbes in different aquatic invertebrates ? What kinds of associations exist between microbe

and host, and do gut bacteria play a significant role in the physiology of aquatic invertebrates ? In answering these questions and many others that can be phrased, two different approaches may justifiably be employed. One approach is that of theoretical modelling of the system under study. The merits of this approach lie in the predictive quality of such models, which can be used to highlight discrepancies between theory and data, thus uncovering areas requiring further study. This approach has been adopted for the first time for aquatic invertebrates by Plante *et al.* (159) who attempt to model detritivore-bacteria digestive associations in terms of optimal digestion theory and cost-benefit analyses. By their nature, theoretical models rely heavily on empirical evidence for both their development and subsequent testing of predictions. This necessitates the other approach, i.e. that of gathering empirical evidence. Optimally, one might expect a stepwise coupling of these two approaches, with initial gathering and collating of empirical data followed by development of models which utilize and assess the available information, and which serve to direct further empirical research by formulating predictions to be tested. It is thus clear that a thorough, synthesized understanding of existing empirical evidence is imperative if we are to efficiently identify relevant areas for future research endeavor.

This paper provides a synthesis of the literature concerning microflora associated with the digestive tracts of aquatic invertebrates. Aquatic invertebrates comprise a diverse group of animals belonging to different phyla, feeding guilds and occupying a wide variety of habitats. Probably the most important factor in linking all of these animals when investigating invertebrate-microbe interactions is the abundance of water both inside and outside the animal gut. By contrast, in most terrestrial systems the invertebrate gut represents a moist habitat in an otherwise dry environment. Within aquatic invertebrates an obvious distinction may be made between freshwater and marine animals. There are disparate salt conditions in the environment compared to the inside the gut of a freshwater invertebrate, whereas marine guts and their surrounding environments can be considered more continuous in this regard (159). Aquatic invertebrates could justifiably be further divided up into a number of sub-groups. For example, distinctions could be made according to type of excretory system, a factor which may influence the gut environment especially with regard to the availability of nitrogen to gut microbes. Animals could be divided according to habitat (eg. pelagic, benthic), the nature of which will result in the exposure of animals to different ambient microbial communities. Feeding guilds are easily identifiable, and it is probable that animals that have different diets provide disparate gut environments for bacterial colonization. Further, it has been suggested that invertebrates of some feeding guilds are more likely to benefit from

interactions with microbes than are others. For example, detritivores that are dependent on refractory organic matter may be key areas in which to look for close associations with symbiotic microbes (134, 159). In addition, the case for investigation of different phyla seperately can be argued in that evolutionary shaping of factors such as gut structure and digestive physiology might predispose animals to associations with microbes irrespective of their habitat or feeding guild. To date, only Bivalvia (Mollusca) have enjoyed a synthesis of available literature pertaining to their interactions with bacteria, possibly due to their commercial importance (168). While summaries such as these are extremely useful, the evidence currently available on aquatic invertebrate-microbe associations does not support the limitation of a synthesis to one of these subsets if the aim is to gain an understanding of the role of gut microflora in the physiology of aquatic invertebrates or in their ecosystems. It is clear that the phenomenon of gut microfloras is not confined to any of the above sub-groupings of aquatic invertebrates (e.g. phyletic or trophic types), and it is therefore important to look at similarities and differences in their occurrence among sub-groups. The pitfall of concentrating on one of these sub-groups (e.g. feeding type) alone is that cause and effect may be misinterpreted and confused with influences due to restrictions imposed by factors relating to other overlapping sub-groups (e.g. evolutionary baggage in the form of gut structure). Consideration of the gut microbe-invertebrate associations of all aquatic invertebrates allows us to distinguish more clearly between characteristics attributable to different factors such as phylum, habitat, feeding guild, etc. Limitation of the evaluation of aquatic invertebrate-gut microbe associations to one sub-group of aquatic invertebrates at this stage would impose unnecessary boundaries on our developing understanding of these associations.

In this review consideration is given to the different types of associations between gut microbes and aquatic host invertebrates, and the presence of gut bacteria in different invertebrate groups. The sites where microbes are reported to occur in the guts of these invertebrate groups and the taxa and physiological properties of gut microbes are examined in detail. Factors affecting the density and distribution of these microflora, and the role that microbes play in the physiology of host invertebrates are considered. Further this review aims to provide a framework for future research by identifying important questions, hypotheses and processes that merit attention if an understanding of the role of gut microflora in the physiology of aquatic invertebrates and nutrient flux of the ecosystem is to be gained. The review concentrates on gut bacteria because most of the available information relates to them, but where applicable, studies on gut fungi and protozoans are discussed.

Microbe-invertebrate interactions in the aquatic environment

Many interactions between micro-organisms and invertebrates are recognized as important in aquatic ecosystems. The invertebrate-microbe interaction may simply be that of predator-prey, where the invertebrate utilizes the microflora directly as a food source (e.g. 33, 38, 39, 74, 91, 106, 111, 121, 156, 227). Synergism has also been documented, e.g. where invertebrates facilitate bacterial colonization by mechanically breaking down or concentrating organic material in faecal pellets (92), while microflora may precondition food material for ingestion by invertebrates (e.g. 15, 16, 42, 43, 53, 90, 147). Activities of invertebrates (e.g. bioturbation or production of bacteriocidal substances) may enhance or inhibit growth of micro-organisms (e.g. 4, 6, 26, 145, 210) while microbes have been reported to inhibit the activities of invertebrates, e.g. as active parasites or pathogens (1, 13, 19, 31, 32, 47, 65, 69, 70, 78, 94, 99, 154, 200). In some instances, the relationships between microbes and invertebrates are of commercial significance, e.g. resulting in spoilage of shellfish and the risk of illness for the human consumer (e.g. 5, 9, 17, 36, 46, 56, 63, 76, 78, 82, 103, 104, 105, 117, 126, 185, 204).

Many types of close associations between invertebrates and microorganisms have been reported. These include external colonization, where microbes inhabit the outer surfaces of invertebrates such as sea nettles (64), crustaceans (25, 41, 56, 104, 105, 116, 219), ascidians (180) and bryozoans (222), and internal colonization where microbes either occur intracellularly or extracellularly in the tissues of the invertebrate host (e.g. 48, 49, 75, 96) or inhabit the digestive tracts of the animals.

1. Types of associations between aquatic invertebrates and their gut microflora

The most commonly reported association between aquatic invertebrates and gut microbes is that of ingestion of bacteria. Many authors document ingestion, digestion and absorption of microbes by aquatic invertebrates (e.g. 11, 33, 38, 59, 92, 106, 121, 143, 156, 165, 227), and aquatic invertebrates have been reported to possess lysozymes (181).

Lysed microbes may, however, contribute enzymes that remain active in the gut, i.e. acquired bacterial enzymes (134), and these may confer the host with additional digestive abilities. Most studies of the enzymes of aquatic invertebrates

do not attempt to separate the action of acquired enzymes from endogenous host enzymes. However, a few studies have addressed the question of exogenous enzymes. For example, Barlocher (16) reported that fungal enzymes ingested with the leaves eaten by the amphipod *Gammarus fossarum* retained their activity in the foregut and were active against structural carbohydrates, improving the amphipods ability to digest the leaves.

Other ingested microflora have been shown to survive passage through the gut and to be voided with the faeces, e.g. bacteria ingested by tubificid oligochaetes (212), the bivalve, *Mytilus edulis* (166), and the shrimp, *Penaeus setiferus* (100), and fungi ingested by the amphipod *Gammarus pulex* (14). Ingested fungi are also reported to survive in the gut of the fiddler crab, *Uca pugilator* (157), while viable ingested bacteria have been isolated from the guts of the penaeid shrimps, *Penaeus aztecus, P. setiferus* and *P. duorarum* (62, 220), the mudprawn, *Upogebia africana* (93), and the crab, *Callinectes sapidus* (103). In addition, Odintsov (151) concluded that the N_2-fixation associated with the gut of an echinoderm was a continuation of the activity of free-living bacteria ingested with the food. Differential survival of ingested free-living bacteria has been reported in the gut of the mayfly larva (11), and the number of species present is reduced as food passes through the gut of tubificid oligochaetes (212). However, this phenomenon is difficult to separate from selective ingestion without feeding experiments. Prieur (165, 166) demonstrated both selective ingestion and selective digestion of microbes by the bivalve *Mytilus edulis*. Some ingested bacteria that remain viable in the gut are, however, reported to be pathogenic and may frequently cause the deaths of their aquatic invertebrate hosts (63, 154, 170, 200, 204).

Ingested bacteria have been reported to greatly increase in numbers in the guts of some invertebrates. Plante *et al.* (158) showed rapid growth of ingested bacteria in the hindgut of the polychaete, *Abarenicola vagabunda*. Prieur (165, 166) reported that *Vibrio*-like bacteria divided a number of times in the gut of *M. edulis*. Other studies have shown greater bacterial activity and numbers in gut contents as compared to the food prior to ingestion, e.g. in the abyssal holothuroid *Psychropotes* sp. (58, 59, 60), the polychaete, *Thelepus setosus* (66), and deep-sea amphipods (221), particularly in the hindgut (59, 66). A number of studies have reported a decrease in the number of bacteria in the gut contents from the anterior to the posterior of the gut and the faecal pellets (12, 106, 212). However, in other invertebrates microbes do not show a decrease in numbers down the gut (12). However, in these studies it is not clear whether the changes are due to increased growth and proliferation of ingested microbes, the existence of symbiotic gut bacteria or colonization of gut contents by resident bacteria, and they are therefore

inconclusive. Furthermore, increases in bacterial numbers need not necessarily be beneficial to the host, which may merely be an incubator providing suitable conditions for bacterial growth. Very little is known about whether these invertebrates can utilize this bacterial biomass. This probably depends on where in the gut this proliferation occurs as certain regions of the gut may allow lysis of micribes or utilization of bacterial products, while others may not (see 159). Ingested bacteria that proliferate in the gut may contribute to digestion by preconditioning the food for utilization by the invertebrate, thus complementing the invertebrate's enzymes. However it is possible that competition for scarce resources, e.g. vitamins, may occur.

There is evidence that certain aquatic invertebrates maintain a permanent and consistant flora (e.g. 11, 44, 54, 57, 62, 123, 160, 161, 211) that is quite different from that of the habitat. Examples include the giant prawn, *Macrobrachium rosenbergii* (44), the polychaete, *Thelepus setosus* (66), the holothuroid, *Psychropotes* sp (60), the deep-sea amphipod *Lyssianassidae hirondellea* (178), the prawns, *Upogebia africana* and *Callianassa kraussi* (93), and the sea urchin, *Echinus esculentus* (201). In other species of aquatic invertebrates the presence of gut bacteria is not constant (23) nor do all individuals possess them (8, 23). This suggests that these bacteria are either ingested transient bacteria or opportunistically colonizing commensal bacteria. Some studies have reported an absence of bacteria in the guts of aquatic invertebrates (7, 25, 79, 187) although it has been shown for the latter two studies that the animals do harbor gut microbes under certain conditions (225, 226).

This leads to the question of differentiation between resident microflora and transient microbes. Resident microflora permanently occupy the gut and the majority are not voided with the faecal material, presumably because they possess attributes (such as adhesion to the gut wall) to prevent expulsion from the gut. Transient microbes are ingested with the food, survive passage through the gut (possibly proliferating in the gut) and are voided with the faeces. The term "resident" has often been used too loosely. Many authors suggest that bacteria are residents but do not provide conclusive evidence (e.g. 147, 203). Populations of gut bacteria have been interpreted as residents simply because they are comparatively stable. However a stable population may simply be due to routine differential survival of ingested bacteria in the gut. In fact, the stable gut flora of the animal may be strongly influenced by its diet and may include ingested bacteria. In addition it cannot be assumed that because microbes are attached to the gut lining they are benign residents, as some pathogens are reported to be epimural (60, 103). The

question of differentiating between residents and transients is largely unresolved, and their relative roles are rarely investigated.

In addition, a further distinction has been suggested between autochthonous gut bacteria that represent climax, natural populations which may include residents and bacteria ingested with the diet, and allochthonous bacteria which are species that do not normally establish themselves in the absence of perturbations and may often be represented by pathogens (130).

Figure 1 summarizes the types of associations that have been reported between gut bacteria and aquatic invertebrates. Ingested bacteria [1] may be lysed and absorbed [3], or lysed and contribute enzymes to the invertebrate [4], they may be pathogenic (possibly colonizing the gut lining) [5], or they may survive in the gut (transients) [6]. These survivors may simply traverse the length of the gut to be passed out unscathed with the faeces [7], they may be adversely affected in the posterior regions of the gut, or they may proliferate in some region of the gut (incubation) [8], sometimes attaching to the gut wall (epimural transients) [9]. This bacterial production, or products thereof, may be subsequently utilized by the host [10] or it may be passed out of the gut with the faecal pellets [7]. Resident bacteria (symbionts or commensals) which form permanent, relatively stable populations may inhabit pouches or crevices in the gut [11], they may colonize the gut contents or they may be epimural and attached [12]. Some of each of the categories of resident bacteria outlined above may be passed out of the gut with the excreta [7]. Depending on the survival capabilities of the ingested bacteria and the extent to which resident bacteria are voided, faeces may either be virtually bacteria-free [13], or have transient survivors only, residents only, or a mixture of survivors and residents [14]. Invertebrates must thus be seen as dynamic interfaces with the habitat, contributing to and modifying the free-living bacterial community.

It is clear therefore that gut microflora cannot always be treated as a single community, particularly as different types of association have different consequences for the animal host. Unfortunately, in the majority of cases where bacteria have been reported in the guts of aquatic invertebrates, the nature of the study does not allow one to ascertain which type of association exists between the microbes and the host. The presence of bacteria in the gut or in the gut contents is simply reported, and it is not clear whether the microbes are resident or transient. Bacteria from any region of the gut or gut contents may be a mixture of residents and ingested bacteria, and they may be free in the lumen or attached to gut structures. To gain an understanding of the importance of the different types of associations between invertebrates and gut bacteria, experiments with strict controls are needed, especially because plating techniques (which are most commonly

employed) are so selective that a relatively unimportant transient bacterium may swamp out a resident. Techniques such as radioactive labelling of specific microbes (see 37) and immunoassay procedures (1,10,172) may be of assistance in distinguishing between the disparate populations of bacteria and the different types of associations between aquatic invertebrates and gut microbes.

2. The presence and nature of gut microflora in aquatic invertebrates

This section outlines what is known about the presence of gut bacteria in different aquatic invertebrates, and the bacterial colonization sites in the digestive tracts of these host invertebrates. The densities, taxa and physiological properties of gut bacteria reported for different host species are detailed. Strengths and weaknesses in our knowledge of the presence and nature of gut microbiota of aquatic invertebrates are highlighted.

Invertebrate hosts for gut bacteria

Do aquatic invertebrate guts provide suitable habitats for microbes ? Arthropods, particularly Crustacea, have received the most attention with regard to the presence of gut microflora. Bacteria have been reported in the guts of Macrura, including prawns (44, 93), shrimps (61, 62, 63, 153, 188, 223), crayfish and lobsters (147, 189), Brachyura (56, 103, 189, 190, 206), Isopoda (112, 225, 226), Copepoda (148, 187) and Cladocera (89). Bacteria have also been reported in the guts of Amphipoda (8, 142), research being focussed on deep-sea amphipods (60, 110, 178, 192, 221). Fungi are documented from the guts of some Crustacea (14, 128, 129, 137, 157, 207). In addition, a few aquatic insect larvae are reported to have gut microflora (11, 119).

Most of the studies concerning the gut microflora of Mollusca have concentrated on the presence of gut bacteria in Bivalvia (20, 23, 31, 51, 106, 107, 123, 138, 155, 164, 165, 166, 167, 181, 193, 168). Of these, a substantial number of studies have directed their attention to the gut symbionts of teredine shipworms (40, 54, 55, 83, 84, 98, 108, 115, 136, 160, 161, 173, 211). Yeasts have also been reported in Bivalvia (120). Bacteria have also been reported in a few species of Gastropoda (113, 114, 205).

The phylum Echinodermata is the third group of aquatic invertebrates that has received a fair amount of attention with regard to the presence of a gut microflora. Bacteria have been reported in the guts of echinoids (57, 86, 87, 88, 151,

169, 191, 201), holothuroids (63, 73, 151), particularly deep-sea holothuroids (59, 60, 192), and ophuiroids (63). Fungi (208) and protozoans (131, 132) are also documented from the guts of echinoids. A few studies have reported the presence of gut bacteria in Annelida, e.g. an oligochaete (212), and various polychaetes (63, 66, 158). Microbes have also been reported associated with the digestive tract of ascidians (127, 180). In addition it is worth noting that bacteria are found closely associated with a number of species of sponges (175, 213, 214, 215, 216, 217, 218, 202, 203) and Anthozoa (95), but because they are not strictly gut bacteria they are not considered further in this review.

In conclusion, Crustacea have received the most attention, with gut bacteria being reported in a wide variety of species. Although bivalves have been frequently sampled for gut microbes, other molluscan groups require attention if it is to be ascertained whether gut microbes are a general feature of this class. Echinodermata have received far less attention than Crustacea and Mollusca, while only isolated studies concerning gut bacteria exist for other taxonomic groups of aquatic invertebrates. Thus, while the phenomenon of a gut microflora appears widespread, there is a need to investigate a wider spectrum of invertebrate taxa before general conclusions about the presence of gut microbes in aquatic invertebrates can be drawn. Although most of the invertebrates that have been studied possess a gut microflora, in a few cases absences of gut bacteria have been reported (25, 79) under certain environmental conditions (225). Different phyla of aquatic invertebrates have dissimilar gut structures, and it is possible that some may therefore be more susceptable to associations with microbes than are others. This emphasizes the need for an understanding of the factors that influence the presence of microbes in aquatic invertebrates, and the role of phylogeny.

Bacterial colonization sites in the gut

A few studies of gut bacteria are based on whole animal homogenates (8, 9, 44, 45, 51, 105, 187). Results from these studies are inevitably inconclusive as bacteria are also reported from external surfaces, coelomic fluids and other organs (e.g. 1, 25, 23, 56, 76, 116, 117, 222). Many studies have used whole gut homogenates (14, 20, 40, 60, 61, 63, 86, 87, 88, 93, 98, 151, 169, 173, 187, 192, 220, 221, 223), while others have investigated gut contents without recording the region of the gut from which samples were obtained (e.g. 8, 178, 188, 189). By their nature, these studies do not give information about the location or sites of attachment of microbiota in the gut.

Many studies do, however, report the existence of bacteria in particular regions of the gut. In Crustacea microfloras have been reported in the

oral region (mouth) (56, 104, 105), stomach (56, 153), intestine (153, 198, 206), midgut (8, 62, 93, 103, 157), hindgut (8, 62, 93, 103, 137), (207) and digestive gland (93, 147). In bivalve molluscs bacteria have been reported in the style (106, 107, 123, 133, 138, 155, 181, 193, 195), the style pouch (23), the gland of Deshayes (108, 160, 161, 211), the oesophagus (155), the stomach (23, 106, 123, 155, 166, 173, 205), the intestine (23, 113, 114, 166, 173, 194), the midgut (155), the hindgut (81, 141, 155, 165) the caecum (20, 40, 54, 155, 173), and the liver (120). In echinoderms bacteria are reported in the foregut (191), intestine (59, 131, 132, 201), hindgut (191), caecum (57). In Annelida bacteria have been recorded in the foregut (11), midgut (11, 119), hindgut (11, 89, 112, 119) and the caecum (89). A single species of ascidian has been reported to have bacteria in the endostyle, gut contents, faeces and inhalent siphon (180).

The majority of the studies summarized above each deal only with one region of the gut, and therefore do not provide information about the relative importance of different regions of the gut as colonization sites. These studies also do not give information about whether different types of microflora occur in different regions of the gut, or of the sites of colonization within a particular region, i.e. whether bacteria are epimural, free in the lumen, or attached to specific structures. Those studies that do examine a number of different regions of the gut indicate that the hindgut is an important region of bacterial colonization in Crustacea (8, 11, 62, 89, 93, 103, 119, 153). Although bacteria have been reported in the foregut, midgut and digestive gland of some crustaceans these regions of the gut are not colonized in all species investigated (8, 11, 89, 93, 147, 153). The presence of a rich and varied flora in the hindgut, and relatively poor utilization of the foregut by microorganisms has been reported for two other arthropod groups, i.e. insects and millipedes (for review see (24)).

Not as many systematic studies have been conducted for Mollusca. However, the style and Deshayes gland appear to be consistantly important as sites of bacterial colonization in most species investigated. Transmission electron microscopy has shown that bacteria may be present in the matrix of the style (123, 181), and that the Deshayes gland may be colonized by closely packed gram-negative bacteria (160, 161, 211). In addition the presence of large spirochaetes (Cristospira) in the style has been reported (e.g. 133, 168). The stomach and hindgut are also an important areas of colonization, while the midgut is less frequently inhabited by microbes (106, 123, 155, 173, 168). Too few comprehensive studies have been conducted on Echinodermata, Annelida, and Ascidiacea to draw conclusions about the relative importance of the different regions as sites of bacterial colonization (see 180, 191). The theoretical approach of Plante *et al* (159)

on detritivore-bacterial digestive associations predicts that foreguts and midguts are not likely to harbor gut microfloras, while hindguts represent more likely colonization sites. While this prediction appears to be supported by some molluscan species, the anterior region of the gut is also an important colonization site in many species.

The further distinction of micro-habitats within regions of the gut, i.e. differentiation between attached epimural bacteria, those attached to gut contents, and those free in the lumen, is relevant in terms of the functional significance of the gut bacteria to the host. Bacterial populations from different micro-sites within any region of the gut probably represent different functional groups. For example, bacterial populations attached to the gut lining are likely to be more stable as they are not as easily voided with feces. Direct nutrient flux between these microbes and the host is also more readily facilitated due to their proximity to the gut wall. On the other hand, bacteria attached to food may be more important in assisting with breakdown of ingested material and release of essential nutients from this material, but are likely to be passed out of the gut with the feces. In Crustacea electron microscopy studies have revealed the presence of epimural bacteria, both filaments and mats of bacteria, attached to the hindgut lining (59, 93, 103, 119, 191, 225, 226), while bacteria are also present attached to hindgut contents (8, 93, 119). Similarly both types of populations of microbes are found in echinoderms, i.e. those associated with gut contents (e.g. 60, 192, 208) as well as epimural bacteria (60, 192).

The approach to bacterial colonization sites in aquatic invertebrates has been somewhat unfocussed and our knowledge of the morphologies, growth patterns and methods of attachment of gut bacteria *in situ* is poor. Studies looking at whole guts or whole animals alone should be avoided as they provide limited information, while studies that investigate all the regions of the gut and combine sampling techniques (for example both direct observation by electron microscopy as well as methods for enumeration of bacteria) should prove most useful in elucidating both the most important regions of colonization in the digestive tract, as well as the in situ growth and attachment characteristics of gut microbes. Of interest is a fluorescent antibody staining technique modified for solid specimens, used by Huq *et al.* (103) to study the colonization region of *Vibrio cholerae* in the gut of the crab, *Callinectes sapidus*. Insights into micro-sites of colonization within gut regions and growth patterns of distinct populations of gut bacteria will allow a more focussed approach to the types of associations that exist and their significance.

Enumeration of gut bacteria

Quantification of the density of microbes in the guts of aquatic invertebrates has received little attention and in those studies that do enumerate the gut microflora units of density have not been standardized. For example, density has been expressed per gut, per gram, per unit volume, per length of gut or per ml of homogenate. Comparisons between species and classes of invertebrate are therefore difficult to make. Comparisons are further confounded because while most of the enumeration studies to date are based on whole guts (e.g. 60, 63, 66, 79, 153, 201, 223) or gut contents (e.g. 77, 188, 205), some authors have used whole animal homogenates (e.g. 8, 9, 184). Only a few researchers have studied the density of microbes in a number of regions of the gut (e.g. 12, 62, 79, 119).

Two methods for enumeration of gut bacteria have been widely used, i.e. plate (viable) counts (12, 60, 63, 100, 119, 138, 153, 158, 201) and direct enumeration by epifluorescent microscopy (60, 79, 119, 138). In general, the counts obtained by the latter method exceed those by the former by more than an order of magnitude, although estimates for different species have varied greatly (between 109 and 116 cells per gram dry weight) for both methods. Of these two methods the direct counting method is far more accurate and reliable, given that only a small fraction (often $< 10\%$) of bacteria present can be isolated using available culture techniques. When enumerating bacteria by epifluorescent microscopy, because gut samples contain large proportions of organic matter (comprising gut tissue and/or gut contents), it is important to take into account masking affects as proposed for enumeration of bacteria in marine sediments (eg. 67, 177). Other techniques that may prove useful in enumerating particular species of gut microbes, and their in situ colonization sites, include an enzyme-linked immunosorbant assay employed by Adams (1) to detect *V. alginolyticus* in the haemolymph of penaeid shrimps. This technique has been used more widely for the detection and identification of bacteria in fish (e.g. 10, 172). Studies utilizing radioactive labelling of microbes for trophic studies (see Carman (37)), may also be usefully adapted to distinguish quantitatively between transient and resident bacteria. A few authors have attempted gut bacterial production estimates using radiolabelled isotopes (e.g. 51, 221), and this looks to be a promising direction of research although problems with calibrating the results are acknowledged (for a recent review see 118).

There is a need for enumeration of bacteria in different invertebrate guts and particularly different regions of the gut, coupled with studies of turnover and production estimates, if an understanding of the quantitative and functional importance of gut microbes is to be gained. Bacterial populations are likely to have

varying consequences for the host invertebrate in different regions of the gut. In addition it is essential that estimates be reported in standard units (i.e. counts per gram (dry) of gut and per volume of gut). The size of the animals investigated, and the gut : animal weight and/or volume ratios should be reported to facilitate interspecific comparisons. Quantitative differentiation between transient (ingested) bacteria versus resident bacteria, and attached gut microbes versus those present in the lumen associated with the gut contents are lacking probably due to the difficulties of selectively sampling the separate populations. However achievement of this goal would provide insights into the relative importance of the respective bacterial populations and the question of incubation of ingested microbes in the guts of invertebrates.

Taxonomy of gut microbiota

The same inherent problem with available culture methods employed to estimate bacterial numbers, is encountered when determining the genera of bacteria present in a gut. Culture media are highly selective and only a small percentage of the genera present in the gut may be represented in isolate collections. However the use of similar media to investigate different sources of bacterial populations can be justified for comparative purposes. Table 1 shows that the most common genera of bacteria isolated from the guts of aquatic invertebrates are *Vibrio* and *Pseudomonas*, with *Flavobacterium, Micrococcus* and *Aeromonas* also frequently reported. Crustacea have received the most attention, with the taxa of gut microbes of representatives of Macrura, Brachyura, Copepoda and Amphipoda being reported. The study of Mollusca has covered mainly Bivalvia, and while there are relatively few studies concerning Echinodermata, representatives of four orders are represented. Other aquatic invertebrate groups have received little attention, and it is therefore difficult to draw general conclusions with regard to the genera present in the guts of aquatic invertebrates.

A number of studies report that the bacterial populations isolated from the gut differ in species composition from those isolated from the habitat or diet (e.g. 61, 62, 66, 93, 184, 201). Thus it appears that different communities of bacteria are present in the guts of aquatic invertebrates compared to their immediate habitat or their food items, a phenomenon that could be due to the presence of a resident microflora or selective ingestion and/or differential survival of ingested microbes. This has consequences for the ecosystem that the animal host lives in, as the gut may be an incubator for microbes that are not found in the

habitat but that possess characteristics that are important to nutrient cycling in the ecosystem as a whole, e.g.nitrogen fixation associated with shipworms (40).

The question of whether the various regions of the gut harbour different genera of bacteria has not been addressed. Some regions of the gut will facilitate absorption of bacterial products, while other regions may not allow use of bacterial products. Thus bacterial types with distinct physiological attributes may be of disparate relevence in different regions of the gut.

Physiological properties of gut bacteria

The majority of bacteria isolated from the guts of aquatic invertebrates have been facultative anaerobes (8, 40, 88, 93, 119, 175, 189, 190), although aerobes (e.g. 153, 188, 201, 206, 211) and occasionally obligate anaerobes (e.g. 189) have been reported. This may be partly due to the fact that not many studies attempt to isolate anaerobes. Most studies report gram-negative gut bacteria (e.g. 8, 9, 12, 20, 93, 155, 165, 169, 173, 175, 188, 189, 190, 205, 211) but some aquatic invertebrates have been shown to harbour gram-positive bacteria (e.g. 9, 166, 173, 175, 189). Both motile (8, 54, 190) and non-motile (93) bacteria are reported from the guts of aquatic invertebrates. In addition fermentation by gut microbes has been documented in a few species of bivalves (e.g. 20, 166).

Table 2 shows the most commonly reported enzyme activities of bacteria isolated from the guts of aquatic invertebrates. The largest number of studies have concentrated on Mollusca, particularly the occurrence of cellulase activity in shipworms. A large proportion of the Crustacea studied harbour bacteria with protease, lipase and chitinase enzymes, but few species appear to have bacteria that are able to digest cellulose. A few studies have been done on the echinoderms and ascidians, revealing wide spectrums of enzymes for both groups. It is important to bear in mind that the enzymes reported in these studies do not necessarily represent those that are most important but are limited to those that were specifically examined. If the functional significance of a particular isolate is to be established it follows that it is necessary to test for a wide spectrum of enzymes.

In general, there is a paucity of studies reporting the digestive capabilities of gut microbes. It is difficult to make comparisons between invertebrate taxa as different suites of enzymes have been examined by different authors. Available evidence suggests that some animals harbour bacteria that are capable of hydrolysing refractory components of detritus, but it is often not clear whether these attributes are correlated with the diet or requirements of the host, and therefore contribute significantly to nutrient gain. There is a need to investigate

particularly the gut microfloras of species of invertebrates which are most likely to benefit from exoenzymes of gut microbes, i.e. herbivores and detritivores.

In addition, because the bacterial enzymes reported represent only those produced by gut isolates obtained using selective culture techniques, failure to encounter a certain enzyme does not necessarily indicate that bacteria capable of producing the enzyme in question are not present in the natural gut microflora. The detection of bacterial exoenzymes in invertebrate gut fluids would provide a more accurate picture of the *in situ* contribution by bacteria to invertebrate digestion. It is, therefore, necessary to separate the actions of endogenous and exogenous enzymes, when investigating the digestive capabilities of aquatic invertebrates. For example, Wainwright & Mann (209) demonstrated that the ability of the shrimp, *Mysis stenolepis*, to digest cellulose was lost when the animal was treated with antibiotics, thus implying an exogenous source for the cellulase activity exhibited. This promising approach has also been adopted for the study of enzymes of some terrestrial invertebrates. For example, using antibiotic treatments Scrivener (179) demonstrated that cellulase activity of the cockroach *Panesthia cribrata* was symbiont-independent. However, the validity of such results will depend on the effectiveness of the antimicrobial agent in eliminating the gut microbes of the specific invertebrate being studied, and this must be confirmed in each case.

Nitrogen fixation has been reported for the gut microflora of only a handful of aquatic invertebrates i.e. a number of species of shipworm (40, 71, 108, 211) and sea urchins (77, 86, 87, 88). As this is a potentially important phenomenon in animals feeding on a nitrogen-low diet, and has been shown to be an important source of nitrogen for termites (21, 29, 163), it merits further investigation in a wide variety of aquatic invertebrates, particulary those whose diet has a high C/N ratio.

Physiological attributes reported for gut microbes are obviously of potential importance to the invertebrate host. However, linking of these studies of bacterial physiology to feeding studies and nutrient tracer studies are essential if it is to be established whether the host benefits from the attributes displayed by the microbes.

3. Factors affecting the presence of gut bacteria

What affects the types, numbers and colonization sites of bacteria in aquatic invertebrate guts ? Are there major differences in the presence and nature of gut bacteria from disparate phyletic and trophic groups ? Is the host a contributor to and beneficiary of the microbial ecosystem of the intestinal tract, or does it attempt

to foil bacterial colonization ? In terrestrial invertebrates many factors (e.g. gut structure, nature of gut lining, diet, condition of the host, season) are reported to influence the gut microbiota (24, 30, 124, 125).

In some aquatic invertebrate species, gut structure appears to influence the presence of gut bacteria. The presence of pouches, crevices and folds may to be important and may prevent wash-out of bacteria (130). For example, the deep cuticular bay at the beginning of the hindgut of the cladoceran, *Alona affinis*, is colonized by bacteria which seem to accumulate out of the current, and the strongly folded caecum is also inhabited by bacteria (89). The lumen of the hindgut vesicle of the parasitic isopod, *Gnathia calva*, contains bacteria (112), while the aquatic larva of the cranefly, *Tipula kotarski*, has an enlarged hindgut with epimural and loose bacteria (119). However, special adaptations for attachment of bacteria do not appear to be a prerequisite for the development of dense assemblages of gut bacteria in Crustacea (see 144), where the gut is often a simple tube without enlarged chambers or attachment structures (e.g. 93).

The nature of the gut lining may also be an important factor. In some insects bacteria are attached to spines or bristles in the hindgut (199). Bacteria are present amongst spines (brushes) in the hindgut of the prawn, *Callianassa kraussi* (93), although another thalassinid prawn, *U. africana*, supports spectacular mats of bacteria in the absence of spines or brushes on the lining of the hindgut (93). The attachment of bacteria to the hindgut lining of some Crustacea has been attributed to a requirement of certain bacteria for a chitinous substrate. *Vibrio cholerae*, for example, attach more readily to the chitinous lining of the hindgut of *Callinectes sapidus* (103) than to the non-chitinous lining of the midgut, although it is possible that conditions in the midgut preclude colonization by bacteria. The presence of a peritropic membrane in arthropods has also been suggested as a factor affecting colonization by microbes either by rendering the gut lining unstable or because it acts as a barrier to bacterial invasion (24). Zachary *et al.* (226) found that the peritropic membrane in the isopod, *Limnoria tripunctata*, may affect colonization, acting as a barrier to ingested bacteria. Dempsey *et al.* (62) suggest that the shedding of the hindgut lining accounts for variability in bacterial numbers in the penaeid shrimps, *Penaeus aztecus* and *P. setiferous*. Very little is known about the fate of gut bacteria during ecdysis and recolonization after the moult (e.g. 119). In fact, not much is known in general about colonization/inoculation by microbes and how they avoid expulsion from the gut. Imam *et al.* (108) suggest that an ability to adhere to cellulose is characteristic of gut bacteria that are symbiotic in shipworm species, and that this ability facilitates inoculation. Gut bacteria may also have to contend with the mechanical action of the gut, e.g.certain bacteria are able to resist

the grinding of the proventriculus in the shrimp, *Penaeus setiferus* (100). In addition, gut passage time coupled with gut structure have been suggested as important factors in influencing the presence and the incubation of bacteria in the gut (158). Very rapid passage rates will not provide a very stable environment for proliferation of microbes in the lumen, unless pouches outside of the mainstream of gut content flow exist. Both gut structure and gut lining are strongly coupled to feeding type while being constrained by phyletic considerations, and it is therefore possible that the presence and nature of bacteria may be different for disparate trophic and phyletic groups of aquatic invertebrates.

The diet of the host has been shown to affect species composition and activity of gut microbes. The microbial complement of the gut is reported to be plastic, changing under different diets. For instance, in the bivalve, *Unio tumidus* (183), the copepod, *Acartia tonsa* (187), and the shrimp, *P. setiferus*, the nature of the gut microflora is determined by the biological/biochemical characteristics of the ingested food (100). Guerinot (86) found that the rate of nitrogen fixation by gut bacteria of the sea urchin *Strongylocentrotus droebachiensis* is affected by diet, while Prim & Lawrence (169) reported that bacteria from the gut lumen of echinoids displayed different abilities with different host diets. In fact, even the presence or absence of a gut microflora seems to be dependent on the type of diet in some aquatic invertebrates. Mattson (137) reports that the presence of fungi is related to diet in a number of species of crab, and while Zachary *et al.* (226) and Zachary & Colwell (225) demonstrated that the, isopod *Limnoria tripunctata*, is free of gut microorganisms when it eats untreated wood, but when it inhabits creosote-treated wood, epimural creosote-resistant bacteria are present in the gut. Not much is known about whether different feeding guilds support different densities or types of gut bacteria, nor whether differences in gut bacteria related to diet are caused by the development of different resident bacteria due to differing nutrient conditions in the gut or the differential ingestion of habitat bacteria. However, different gut microfloras for different invertebrate feeding guilds would have important consequences for the digestive physiology of the host. For example, assistance by gut microbes with digestion would probably be of more significance to the fitness of detritivores (whose diet is often comprised largely of material refractory to invertebrate enzymes) than to that of carnivores.

Physical and chemical conditions inside the gut, e.g. pH, salinity, redox potential, oxygen levels, temperature, nutrient availability, toxic substances and bacteriocides, are known to affect gut bacteria in mammals and insects (for review see Bignell (24)). In aquatic invertebrates , temperature and pH are reported to affect the activity of bacteria isolated from shipworms (85, 98). Sleeter *et al.* (187)

suggest that the wood-boring isopod, *Limnoria tripunctata*, produces antibacterial agents keeping the gut relatively free of microbes. Very little is known about whether there are major differences in the gut microfloras of invertebrates inhabiting marine and freshwater habitats, although the salinity, and possibly the nitrogen concentrations (from excretion), in the gut are likely to be very different in these two groups.

The physiology of the invertebrate host can influence the gut microbiota. For example, there is a decrease in density and change in composition of gut bacteria of the prawn, *Penaeus japonica*, as the prawns progress from zoea to postlarval stages (223). The gut microbiota are also reported to change with the physiological health of the host in the prawn, *P. japonica* (223). This may be related to external environmental (ambient) conditions that stress the animal. Atlas *et al.* (8) found that the dominant bacterial taxa present in the guts of the amphipod, *Boeckosimus affinis*, changed when the animal was exposed to petroleum hydrocarbons, and that successional changes occurred when the animal was starved in captivity. Sugita *et al.* (188) reported that a change of salinity of ambient water caused a change in the gut microflora of the shrimp, *Palaemon paucidens*. In addition, infestations by pathogens have been shown to affect the natural microfloral community of some aquatic invertebrates (154, 170, 206). Presumably the changes in the physiology of the animal results in changes in the environment of the gut, and invertebrates may be more susceptible to pathogens under some ambient conditions. Another indication that ambient conditions significantly affect gut microfloras in marine invertebrates are reports that wild-caught and laboratory-reared animals of the same species may have different communities of gut bacteria (see 168). This factor must be borne in mind when attempting to assess "normal" gut microflora, and evidence of differences attributed to different environmental conditions may be of assistance in differentiating between obligate and commensal associations. Obligate associations are likely to be more robust to changes in the ambient conditions that the host is exposed to.

Physiological properties of bacteria themselves are also important in determining the types of bacteria that flourish in the gut. Adhesive ability and substrate preferences are reported to be important. For example, preferential adhesion of shipworm bacteria to cellulose (108), and the preference of some microbes for a chitinous substrate can affect the types and the locality of colonization of bacteria in the guts of different invertebrates (see Bignell (24)). Hood *et al.* (100) found that certain bacteria were able to resist low pH in the gut of the shrimp, *Penaeus setiferus*, resulting in differential survival of bacteria. Barophilic bacteria in the guts of deep-sea amphipods are more tolerant to pressure than those

from shallower waters (153, 178), and show greater growth rates under *in situ* pressure than do bacteria in the surrounding sediments (60). Because this growth enhancement is directly correlated to nutrient enhancement, it seems that deep-sea animals possess a commensal gut flora capable of responding to increased nutrient levels found in the gut.

Seasonality has also been reported to influence the presence and types of microbes found in the guts of invertebrates. Bernard (23) examined 65 species of bivalves for spirochaete bacteria of the genus *Cristospira* and found that the bacteria are not always present in those species that do at times support them, and that these differences appeared to be seasonally influenced. Similarly, Pitts & Cowley (157) found that the occurrence of yeast and other fungi in the midgut of the fiddler crab, *Uca pugilator*, is seasonal, and Shivokene *et al.* (182) reported seasonal differences in the numbers of gut bacteria in the bivalve, *Unio tumidus*. It seems likely that these seasonal differences are related to temperature as Sugita *et al.* (189) report a response of gut isolates to water temperature, and environmental temperature affects the occurrence of bacteria associated with the copepods, *Acartia tonsa* and *Eurytemora* spp. (105). These seasonal differences could merely reflect seasonal differences in the bacterial community present in the habitat or food of the invertebrates, particularly in those cases where gut bacteria are reported to reflect those in habitat (223).

At this stage not enough evidence exists to elucidate whether different habitats within the aquatic realm influence the nature of microbe-invertebrate associatons. For example, do certain aquatic habitats predispose their invertebrate occupants to gut microfloras ? Considering that ambient conditions and seasonality have been shown to alter the gut bacteria community in some aquatic invertebrates, it is probable that habitat will influence the conditions in the gut, and may therefore be an important factor influencing the presence and nature of a gut microflora.

From the examples discussed above, it is clear that many factors influence the numbers and types of gut microbes of aquatic invertebrates. Despite this, relatively few studies on aquatic invertebrates investigate the gut bacterial community of a given animal over a wide range of conditions (see Lynch & Hobbie (130)), or examine a range of invertebrates (from different feeding guilds, phyla and habitats) using comparable techniques. It is therefore currently difficult to answer any of the questions outlined at the beginning of this section with any generality for aquatic invertebrates, or to draw general conclusions about the factors that influence gut microbiota in aquatic invertebrates. It is obvious however that restricting studies to just one phylum, feeding guild or habitat may not elucidate causative factors, eg. to conclude that the presence of bacteria in detritivore guts is

related to their diet is not persuasive unless it is shown that similar animals with different diets do not have bacteria. In addition, associations between invertebrates and microbes that are not temporally and spatially stable are not likely to be of obligate significance to the physiology of host invertebrates.

4. Role of gut bacteria in physiological processes of the host invertebrate

Most studies report the presence of bacteria and their physiological properties, but either do not address the issue of the possible benefit gained by the invertebrate host from the association with microbiota, or present circumstantial evidence only. This section highlights those studies that have clearly demonstrated a relationship between the invertebrate and its gut bacteria. In addition, consideration is given to other suggested roles for gut microbes.

A few studies have attempted to separate the action of endogenous (host) enzymes from that of exogenous (bacterial) enzymes, particularly with regard to cellulase ability. The results are variable with a few authors reporting a significant contribution by bacterial cellulases (54, 122, 136, 147), others reporting endogenous cellulase activity and digestion of carbohydrates by the invertebrate alone (138, 142, 146, 155, 171, 180). Evidence for the role of gut microbes in the nutrition of aquatic invertebrates was provided by the demonstration that the ability of the mysid, *Mysis stenolopis*, to assimilate cellulose was lost when the animal was treated with antibiotics. The animals regained the ability to digest cellulose when fed ground untreated mysid guts, suggesting that the cellulose activity is associated with gut bacteria (209). In addition, by means of feeding experiments and antibiotic treatments, Vitalis *et al.* (205) showed that bacteria in the gut of the mollusc *Aplysia juliana* affect the growth rate and thus clearly play a nutritional role.

Another important phenomenon reported associated with gut bacteria is nitrogen fixation. Nitrogen fixation has only been reported in molluscan shipworms (40, 146) and in some species of echinoids (86, 87, 88, 151). Although Odinstsov (151) calculated that nitrogen fixation by gut microbes is not significant for the nitrogen balance of the sea urchin, *Strongylocentrotus intermedius*, Guerinot *et al.* (86) found that the nitrogen fixation measured in the gut of the urchin, *S. droebachiensis* could contribute 8-15 % of the host's daily nitrogen requirements. Carpenter & Culliney (37) reported that nitrogen fixation in the gut could account for a doubling of cellular nitrogen of the shipworm, *Teredo malleolus*, in 1.4 days. Demonstration of the ability of gut microbes to digest items of the host's food or synthesize nutrients, although suggesting the potential therefore, does not provide

evidence that the animal benefits nutritionally, i.e. that the nutrients are passed on to the animal. In an elegant study, Fong & Mann (77) demonstrated that amino acids produced by the gut microbes of the sea urchin, *S. droebachiensis*, were transferred to the tissues of the invertebrate. Guerinot and Patriquin (87) demonstrated that microbially fixed nitrogen is incorporated into the tissue of the sea urchin, *S. droebachiensis*. Evidence derived from the structure of Deshayes gland of the teredine shipworm, *Bankia australis*, suggests that symbiotic bacteria may be supplying the animal with essential amino acids and vitamins (160), and Gallagher *et al.* (80) found that that shipworms fed on wood of 0.2 % nitrogen content had a tissue content of 5.8% in the absence of any other nitrogen sources which suggests that gut bacteria contribute nitrogen. Furthermore, ammonia secretion by the shipworms appeared negligible and it is possible that gut bacteria were assisting in nitrogen conservation by utilizating excreted ammonia.

Gut bacteria have also been reported to enhance the host's resistance to the adverse effects of various toxins. Dempsey & Kitting (61) showed that gut bacteria of the shrimp, *Penaeus aztecus*, are resistant to phenolics and may assist in digestion of the seagrass, *Thalassia* sp.. Zachary *et al.* (226) provide evidence that creosote-resistant bacteria are important for the colonization and nutrition of the isopod, *Limnoria tripunctata*, when it inhabits creosote-treated wood. An interesting association is reported by De Ridder *et al.* (57) in which bacteria associated with nodules in the intestinal caeca of the echinoderm, *Echinocardium cordatum* appear to be important for the well-being of the echinoid as they prevent sulphate reduction of ingested sediment in the hindgut, and may aid in conditioning large particles prior to their passage through the anal aperture. Another suggested symbiotic relationship is reported for the crab, *Callinectes sapidus*: *Vibrio* attached to the hindgut wall may play a role in ion transport across the gut wall, and thus contribute to osmoregulation, although at this stage this is purely speculation (103). Another interesting hypothesis that has been raised is the contribution by gut microbes of surfactants that prevent precipitation of proteins (enzymes) by ingested tannins. A surfactant-producing bacterium has been isolated from the gut of a sawfly (152). Some aquatic invertebrates have been reported to contain surfactants in the gut fluid (e.g. 197). Considering the influence of surfactants on the ability of invertebrates to digest foods containing tannins and the widespread occurrence of bacteria in aquatic invertebrate guts, it is worth investigating whether surfactants are produced by gut microbes.

Ingested bacteria have been reported to greatly increase in numbers in the gut (59, 60, 66, 158, 178, 221). Ingested bacteria that remain viable have been reported to contribute to digestion by preconditioning the food for utilization by the

invertebrate, thus complementing the invertebrate's enzymes (16, 147, 209), and possibly enhancing secretion of endogenous enzymes by the invertebrate (147). Alternatively, the microbes may simply be cashing in on a favourable environment. For example, it has been suggested that invertebrates in the deep sea provide nutrient-rich niches for microbes (109, 221, 224), and that appreciable activity in the deep sea may be confined to the intestinal tracts of benthic animals (60). These invertebrates are thus incubators or hot spots for bacterial activity. Not much is known, however, about whether the host can utilize the bacteria. However, this association between invertebrate and microbe may enhance the bacterial numbers in the environment as the bacteria are voided with faeces, and may increase decomposition rates in the system (178). This phenomenon has been reported for invertebrate detritivores in deserts (50).

Thus only a handful of studies have demonstrated benefits in terms of nutrient gain of the host invertebrate from the gut microbes. Considering the implications of such positive interactions on the physiology of the host invertebrates, especially for those animals that are nutrient limited, further studies that investigate attributes of gut microbes as well as trace nutrient flux across the gut wall are warranted. In this regard it is worth considering techniques employed in the study of associations between aquatic invertebrates and endosymbionts, where more progress has beed made in elucidating nutrient flux. For example, examination of the carbon, nitrogen and sulphur stable isotope compositions of tissues of the bivalve, *Solemya velum*, and endosymbiotic chemoautotrophic bacteria provided evidence that the bacteria provide as much as 98 % of the carbon requirements of *S. velum*, and may also be the major source of nitrogen and sulphur acquisition (49). In addition, a number of studies discussed in this section present evidence that suggests that other potentially important associations (not directly related to nutrient gain) may exist between invertebrates and their gut microbes.

Discussion and conclusions

This section attempts to assess where we have achieved answers to the questions outlined in the introduction and to identify important areas of future research. In doing so it is useful to compare what is known about the nature and significance of gut microbes in aquatic systems with the more extensive literature available for terrestrial systems. Are there fundamental differences in the invertebrate-microbe associations in these two systems ? The guts of invertebrates provide moist havens

for bacteria in some terrestrial systems such as deserts (50), while in aquatic systems both the external environment and the gut provide niches where water is freely available. Are gut bacteria-invertebrate associations therefore more developed in terrestrial systems compared to aquatic systems? Disparities in associations found in aquatic compared to terrestrial systems should clarify whether habitat is a significant determinant of the nature of invertebrate-microbe associations, and if so, what the causative factors may be. Two other central themes may be identified as potentially important shapers of interactions between aquatic invertebrates and gut microflora. Firstly, phyletic origin of the invertebrate may be expected to define the types of associations that can take place within the gut. Secondly, because associations of bacteria with the gut are being examined, the inevitable linking of invertebrate feeding characteristics and these associations can be anticipated.

It is clear that the guts of aquatic invertebrates from different phyla, feeding guilds, and habitats do provide suitable niches for diverse and dense bacterial communities. With regard to invertebrate phyla, gut microbes have been reported most commonly in Crustacea, Mollusca and Echinoderms. However the investigation of the presence of bacteria in more taxa of invertebrates is required to establish whether the phenomenon of a gut microflora is a general feature of aquatic invertebrates or is phylogenetically restricted. Of interest is the uniformity of dominant bacterial taxa (genera) isolated from the guts of diverse species of aquatic invertebrates (Table 1). In addition microbes of similar morphology and growth characteristics have been reported in different species of aquatic invertebrate (e.g. bacteria in the hindguts of the predatory crab, *Callinectes sapidus* (103), and the detritivore prawn, *Upogebia africana* (93)), and these show a remarkable similarity to those reported in some terrestrial species (e.g.termites (30) and locusts (140)). It is possible that this is due to phyletic grouping (all three species are arthropods), which dictates that the hindgut lining is chitinous, as there is evidence that some bacteria adhere preferentially to chitin. This serves to illustrate the possibility that phylogeny may constrain consequences that other factors, such as habitat and feeding guild, may have on the presence, nature and colonization sites of gut microbes in invertebrates.

The question of whether the various invertebrate feeding types have different gut microfloras with distinct digestive capabilities requires attention. In addition, are gut bacteria more commonly associated with animals whose food quality is poor? There does appear to be a correlation between the diet and the presence of gut microflora. For example, the presence of fungi in the guts of some species of crabs is related to the diet of the animal (137), and the presence of cellulolytic bacteria has been reported for a number of wood-eating shipworms (see

211). While gut microfloras have been documented in herbivores, detritivores and carnivores alike, there is little evidence as to the relative significance of these associations in the different feeding guilds. Aquatic detritivores especially would benefit greatly from symbiotic associations like those found in ruminants and termites. Evidence of significant contributions by gut microbes to carbon utilization and nitrogen acquisition would reinforce a shift in our ideas about the sources of these nutrients to detritivores, away from the microbial conversion hypothesis, toward a view incorporating the active participation of gut microbes. The microbial conversion hypothesis suggests minimal use of refractory components of detritus (usually the organic bulk) by detritivores, and implicates stripping and direct utilization of bacteria from organic matter during passage through the gut, coupled with rapid gut throughput times (2, 72, 149). This hypothesis, however, has not consistantly accounted for acquisition of sufficient nutrients for the energy requirements of aquatic detritivores (33, 156). While much of the required nitrogen may be derived from the living component of detritus (associated microbiota), a large proportion of carbon needs to be obtained from the non-living relatively refractory fraction (34), and it is in this area that symbiotic gut microbes would be of most benefit to the host. Nitrogen limitation may, however, be experienced by specialist detritivores where the diet does not include a significant microbial compliment, e.g. wood-borers and leaf-shredders.

Habitat may, however, influence the significance of these detritivore-bacteria digestive associations. For example, terrestrial detritus is primarily derived from vascular plants and is particularly refractory to most animal enzymes. Similarly, the detritus of near-shore aquatic habitats may have a large refractory component derived from vascular plants (eg saltmashes and estuaries), and that of freshwater habitats may be primarily derived from surrounding terrestrial plants. On the other hand detritus from aquatic algal-dominated habitats is less refractory to animal digestion and one might expect invertebrate-microbe associations to be of lesser importance here. In addition, the possibility of enhanced preconditioning of organic matter prior to ingestion by invertebrates in aquatic environments compared to terrestrial environments, due to the abundance of free water in the former, may render invertebrate-microbe associations more important in the latter environment. Plante *et al* (159) adopted a theoretical approach to predict detritivore-bacteria associations. Using simple quantitative models of optimal digestion theory and cost-benefit analyses, they argue that factors such as gut passage time, bacterial doubling time inside the gut and exoenzyme production rate will influence the significance of an association. Furthermore, they suggest that cost-benefit analyses can be used to predict which regions of detritivore guts are most

suitable for bacterial colonization, and conclude that posterior regions of the gut should be more heavily colonized than are foreguts and midguts. This appears to be the case in terrestrial detritivores (Bignell 1984), but the empirical evidence to test this in aquatic detritivores is weak, with some crustacean and annelidan detritivores supporting this theory, while other taxa (eg. molluscans) appear to have varying patterns of colonization in the digestive tract. In addition, they predict that different bacterial populations will have different consequences for the host. The presence of attached and resident bacteria are more likely to represent mutualistic associations, while transient bacterial populations may represent commensalism or benign parasitism. By evaluation of the predictions of the model against empirical evidence, these authors highlight other areas of important future research on digestive associations between detritivores and gut microbes, e.g. the availability of products of animal digestion in different regions of the gut, the role of acquired enzymes, and the need for specific methods to measure parameters relating to individual populations of bacteria in the gut. In addition they suggest that it is possible to pinpoint invertebrate detritivore targets for associations, e.g. animals inhabiting saltmarshes may benefit from fermentation in the gut and a supply of inorganic nitrogen via gut microbe processes. In his work on terrestrial vertebrates, Hungate (101, 102) developed a co-operation-competition model which enabled prediction of the type of microbe-animal association likely to occur depending on the type of food ingested. The merits of theoretical approaches are that they provide a conceptual framework to marshall empirical data and direct research. I suspect that quantitative models such as these proposed to study animals ingesting food of low quality may be successfully adapted to assist in identifying fruitful areas of future research with regard to associations of microbes with aquatic invertebrates in general. The restrictions on these models at present is the fact the the empirical data currently available provides a poor base for both the detailed formulation of the models, as well as the testing of predictions.

It is clear that a number of different types of associations (e.g.incubation, contribution of enzymes and/or nutrients, parasitism) exist between aquatic invertebrates and gut microbes (Figure 1), although few studies recognize and differentiate between the different associations. Considering the different implications that diverse associations have for the host animal and the nutrient dynamics in the environment as a whole, there is a need for a more focussed approach, coupled with the pinpointing of specific colonization sites in the gut. It is not sufficient to treat all bacteria in the gut as a single entity as the populations in the gut may change in different regions of the gut, they may have different physiological properties, may be attached or in the lumen, and different types may

be significant in different regions of the gut. It is important to consider which associations are being looked at in any one study. Ascertaining the region of the gut that is colonized by microbes is of importance when considering the functional significance of gut microbes, as different regions may facilitate the utilization of bacterial products while others may not. In addition, the separation of resident and transient bacteria needs resolving. Although many authors refer to resident bacteria, few provide evidence that these bacteria represent a stable population, and fewer attempt to verify the origin of the microbes in the gut. It is probable that the action of attached resident bacteria will not be as dependent on factors such as food gut passage rate, as is that of transients which may pass through the gut too quickly for their activity to be of any significance to the invertebrate. The suggestion by Plante *et al* (159) that symbiotic associations should occur more readily when gut bacteria are residents attached to the gut lining or occur in pouches of the detritivore gut is intuitive, although evidence is at present too sparse to test this hypothesis.

Is there evidence of significant roles played by gut bacteria in the physiology of aquatic invertebrates ? While there is now a substantial literature on the gut microflora of aquatic invertebrates, for the most part the literature represents a reporting exercise of the presence of bacteria, and physiological attributes of isolated microbes, but there is usually only speculation as to their relation to the host invertebrate. However, a few studies have attempted to demonstrate a positive relationship between host invertebrates and microbes, and these studies indicate that gut microbes do play important roles in the physiological processes of some invertebrates. For example, they are implicated in the breakdown of refractory components of the diet and thus make essential nutrients available to the animals. In addition they have been shown to contribute essential compounds (e.g. by nitrogen fixation, synthesis of amino acids, contribution of vitamins) that are lacking in the diet, and there is evidence of close nutritional associations between aquatic invertebrates (such as shipworms and echinoderms) and gut microbes which are comparable to those found for termites and ruminants. This suggests that habitat type may be subordinate to other factors, such as feeding type, in influencing the strength of associations between gut microbes and invertebrate hosts, both within the aquatic environment and across the terrestrial-aquatic boundary. This is surprising as the invertebrate group that is most likely to benefit from close associations with gut microbes, i.e. detritivores (including wood-borers and leaf-eaters), might be expected to rely more heavily on microbial preconditioning of food prior to ingestion in the aquatic environment than in terrestrial environments, thus obviating the need for close microbial-invertebrate associations in aquatic systems. However, conclusions as to the relative role and quantitative significance of gut

microbes in the nutrition of aquatic and terrestrial invertebrates are premature considering the paucity of empirical data for aquatic invertebrates. Evidence for other roles of aquatic gut microbes have been suggested, e.g.facilitation of ion transport across the gut wall and resistance against adverse substances ingested with the food. Considering the potential importance of positive associations between invertebrates and gut microbes, the role of gut microbes requires special attention, and studies reporting the presence and physiological properties of gut bacteria (see Table 2) need to be followed through to their relevance to the host. In addition, studies of the digestive capabilities of aquatic invertebrates should consider the presence of gut bacteria and the relative importance of endogenous and exogenous enzymes, because evidence suggests that gut microbes may be important sources of key enzymes. Another potentially important phenomenon, i.e. that of acquired enzymes, has been reported for a crustacean species (16), and in view of the importance of acquired enzymes in some terrestrial systems (134, 135) their possible contribution should not be disregarded in studies of aquatic invertebrate enzymes.

There is evidence that some aquatic invertebrates harbour gut bacterial communities that are distinct, in terms of the taxa present and their physiological characteristics, from those found in the surrounding habitat or in the diet. Thus the gut environment provides a specific niche, and bacterial activity in the gut is not merely a continuum of that found in the environment. This has consequences for the ecosystem as a whole as the guts are providing an additional micro-habitat for microbes, and the activities and abilities of these distinct microbial communities may contribute uniquely to nutrient cycling in the system. This contribution could be significant especially where the invertebrate host is a dominant species in the system, and where the ecosystem is detritus-dominated. In terrestrial systems gut microbes are recognised as important in breaking down refractory components and contributing important nutrients such as nitrogen, especially where decomposition rates in the environment are low. In some deserts, for example, the guts of invertebrates may serve as incubators for bacterial growth (50). Are the guts of aquatic invertebrates similarly hot spots for bacterial production? The question of incubation certainly begs further consideration because of its potentially important implications. The limited evidence available hints that incubation and proliferation of bacteria does occur in the guts of aquatic invertebrates, although these findings need to be examined in conjunction with other factors such as gut passage time, utilization of the bacterial biomass and products by the host, bacterial turnover times, etc. if the significance of this phenomenon is to be ascertained.

In general there is a need for better quantification in the study of gut microbiota of aquatic invertebrates. Density, production and turnover estimates of gut microbes are very scarce, but are important. Without them it is not possible to estimate the quantitative significance of any associations or make any comparisons between microbial processes occurring inside an animal and those occurring outside in the habitat. Furthermore, because of the lack of standard units for reporting results, and a unified approach toward the enumeration of gut microbes, it is difficult to compare data that exist for different species of invertebrates. It is also clear that methods more sophisticated than general culture techniques will have to be employed to seperate individual bacterial populations quantitatively, e.g. radiolabelling and immunoassay techniques.

Finally, if an integrated understanding of the role of gut microbes in the physiology of the host invertebrates is to be gained, then the factors that influence the bacteria must be investigated over a variety of conditions (over time, localities) for a range of animals (different feeding guilds and phyla), so that their effects on the processes mediated by bacteria can be elucidated. As for their terrestrial counterparts, a number of factors are reported to significantly affect aquatic gut microflora (e.g. gut structure, host physiology, seasonality), but our knowledge of the determinants of the presence, nature and stability of gut microflora is poor. The gut microflora may change and have different physiological characteristics under different environmental conditions, and these complicating factors should not be ignored when the role of gut microbes is been investigated. In this respect two types of factors require consideration, i.e. those that have a fixed affect on associations (e.g. feeding type, phylum), and those that vary in intensity and cause gut microfloras to be plastic entities (e.g. season, health of animal).

To conclude, while a substantial literature now exists on the associations of gut microflora and aquatic invertebrates, the directions of this research have been diverse and somewhat unfocussed, and conclusive evidence for the role of gut microbes in the physiology of aquatic invertebrates has remained elusive. This condition has arisen because most studies have simply reported the presence and nature of gut bacteria without considering the implications for the host invertebrate. By contrast, the goals of studies concerning terrestrial gut microbes have centred around processes, and attributes of gut microbes have been followed through to their significance to the host invertebrate. Although the species of terrestrial invertebrates and vertebrates investigated may lack diversity compared to their aquatic counterparts, detailed studies of some groups e.g. termites and ruminants have allowed important processes to be identified and verified. The limited evidence available suggests that some aquatic gut microbes may be as

important as their terrestrial counterparts in contributing significantly to the nutritional (and other physiological) processes of their host invertebrates, as well as the nutrient dynamics of the ecosystem as a whole. There is a need for a progression towards a functional or process-oriented approach to the study of the associations between gut microflora and invertebrates in aquatic systems. In pursuing this approach, this review provides a framework for future research by identifying questions, hypotheses and processes that merit attention.

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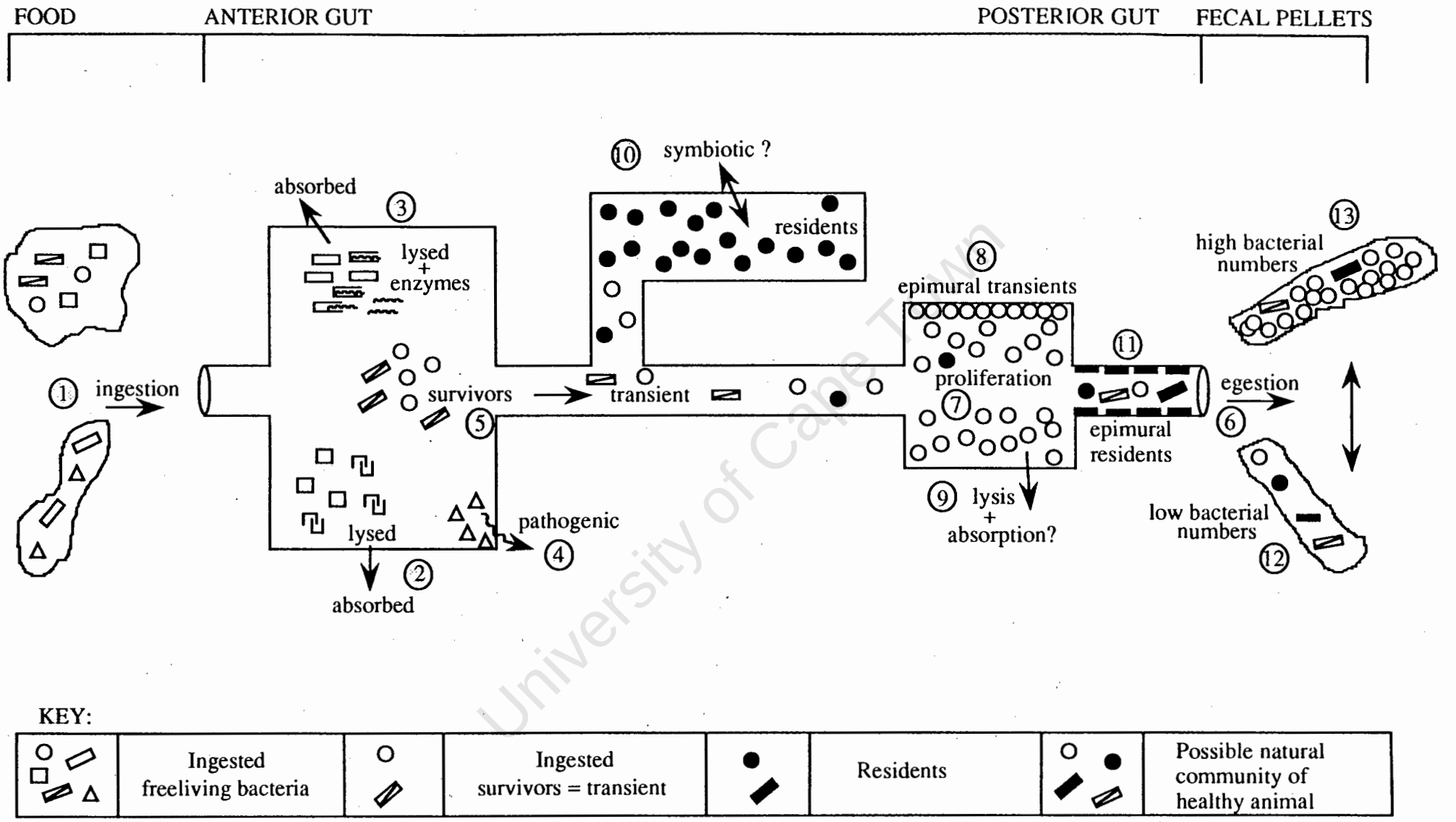

Fig. 1. Types of association reported between aquatic invertebrates and gut microbes

Table 1. Genera of bacteria (taxa)reported in the guts of aquatic invertebrates

Species of invertebrate	Genera																							Source
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	
CRUSTACEA																								
Amphipoda																								
Boeckosimus affinis	+																							(8)
amphipod sp.		+																	+	+				(153)
Macrura																								
Macrobrachium rosenbergii	+	+		+																				(44)
Penaeus spp.	+	+	+	+		+									+	+	+			+	+			(62)
Penaeus spp.			+		+							+										+	+	(220)
Penaeus aztecus	+	+	+	+		+									+	+	+			+	+			(61)
P. setiferus												+												(100)
P. japonicus	+	+	+	+				+																(223)
Upogebia africana	+	+	+											+	+			+						(93)
Callianassa kraussi	+	+	+											+	+			+						(93)
Paranephrops zealandicus				+			+																	(147)
Panulirus japonicus	+	+						+		+														(189)
Palaemon paucidens	+	+		+							+			+				+						(188)
Pleoticus robustus	+																							(63)
Plesionica sp.	+																							(63)
Munida sp.	+																							(63)
Brachyura																								
Nematocarcinus sp.	+																							(63)
Atergatis floridus	+	+			+			+																(189,190)
Schizophrys aspera	+	+	+		+									+										(189)
Tiarinia cornigera	+	+			+			+																(189)
Pachygrapsus crassipes	+	+	+		+			+																(189)
Thalamita prygmna	+	+	+		+			+																(189)
Plagusia dentipes	+	+			+			+																(189)
Callinectes sapidus	+																							(56)
Copepoda																								
Acartia tonsa	+																							(187)
Various																								
6 species	+																							(198)
MOLLUSCA																								
Bivalvia																								
Donax gouldii	+	+																						(20)
Mytilus edulis	+					+																		(165,166)
Crassostrea virginica													+											(138)
Cryptomya californica													+											(123)
Teredo furcifera									+															(173)
Nototeredo knoxi									+															(173)
Gastropoda																								
Semisulcospira libertina													+											(195)
seaslug spp.	+																							(113,114)
ECHINODERMATA																								
Echinoidea																								
Strongylocentrotus droebachiensis	+																							(88)
Tripneustes ventricosus	+																							(88)
Echinus esculentus	+	+	+	+																				(201)
Holothurea																								
Benthodytes sp.	+																							(63)
Ophuiroidea																								
Ophionema sp.	+																							(63)
ANNELIDA																								
Polychaeta																								
Thelopus setosus	+																							(66)
Nereis sp.	+																							(63)
Oligochaeta																								
Tubifex tubifex		+	+	+																				(212)
Limnodrilus hoffmeisteri		+	+	+	+							+												(212)
Peloscolex multisetosus		+		+								+												(212)
INSECTA (aquatic larvae)																								
Ephemera danica			+	+			+																	(11)

KEY TO GENERA

1	*Vibrio*	9	*Cellulomonas*	17	*Chromobacterium*
2	*Pseudomonas*	10	*Corynobacterium*	18	*Acinetobacter*
3	*Flavobacterium*	11	*Moraxella*	19	*Oceanospirillum*
4	*Aeromonas*	12	*Bacillus*	20	*Alteromonas*
5	*Micrococcus*	13	*Cristospira*	21	*Xanthomonas*
6	*Photobacterium*	14	*Enterobacterium*	22	*Bacterium*
7	*Citrobacter*	15	*Cytophaga*	23	*Achromobacter*
8	*Staphylococcus*	16	*Alcaligenes*		

Table 2. Major enzyme activities reported for gut bacteria of aquatic invertebrates.

[+ present, - absent, ? inconclusive evidence, blank = not assayed for]

Species of invertebrate	Enzymes										Source
	1	2	3	4	5	6	7	8	9	10	
CRUSTACEA											
Amphipoda											
Boeckosimus affinis							+	+	+		(8)
Macrura											
Paranephrops zealandicus			+	+				+			(147)
Penaeus aztecus	+		+				+	+	+		(61)
P. setiferus	+						+	+	+		(100)
Upogebia africana	+	+			-	+	+	+	+	+	(93)
Callianassa kraussi	+	-			+	+	+	+	+	+	(93)
Mysis stenolopis			?								(209)
Copepoda											
Acartia tonsa							+	+	+		(187)
MOLLUSCA											
Bivalvia											
Cryptomya californica	+										(123)
Bankia gouldi			+								(211)
Mytilus edulis										+	(181)
Scrobicularia plana		+	+								(155)
Unio tumidus	+		+					+			(182)
Lyrodus pedicellatus			+								(40,211)
Teredo navalis			+			+					(113,114)
T. navalis	+		+			+		+			(54)
T. navalis			+								(174,211)
T. furcifera			+								(173,211)
T. bartschi			+								(211)
Psiloteredo healdi		+									(84,85)
P. healdi								+			(84)
P. healdi			+								(211)
Gastropoda											
Aplysia juliana	+		-								(205)
ECHINODERMATA											
Echinoidea											
Strongylocentrotus purpuratus						+					(122)
S. droebachiensis	-						+	-	-		(88)
Tripneustes ventricosus	+						+	-	-		(88)
Lytechinus variegatus	+	+	+	+	+	+	+	+	+		(169)
ASCIDACEA											
Pyura stolinifera	+		-	+	+	+	+	+		+	(180)

KEY:

1	amylase	6	agarase
2	endoglucanase (CMC-ase)	7	chitinase
3	cellulase	8	protease
4	laminarinase	9	lipase
5	alginase	10	lysozyme

References

1. Adams A (1991) Detection of *Vibrio parahaemolyticus* biotype alginolyticus in penaeid shrimps using an amplified enzyme-linked immunoosorbent assay. Aquaculture 93:101-108

2. Adams SM, Angelovic JW (1970) Assimilation of detritus and its associated bacteria by three species of estuarine animals. Chesapeake Sci 11(4):249-254

3. Akin DE, Benner JB (1988) Degradation of polysaccharides and lignin by ruminal bacteria and fungi. Appl Environ Microbiol 54(5):1117-1125

4. Alongi DM (1985) Microbes, meiofauna, and bacterial productivity on tubes constructed by the polychaete *Capitella capitata*. Mar Ecol Prog Ser 23:207-208

5. Alvarez RJ (1983) Frequency and distribution of bacterial flora of penaeid shrimp. Caribbean J Sci 19:43-48

6. Amade P, Charroin C, Baby C, Vacelet J (1987) Antimicrobial activities of marine sponges from the Mediterranean Sea. Mar Biol 94:271-275

7. Araki GS, Giese AC (1970) Carbohydrases in sea stars. Physiol Zool 43:296-305

8. Atlas RM, Busdosh M, Krichevsky EJ, Kaneko T (1982) Bacterial populations associated with the Arctic amphipod *Boeckosimus affinis*. Can J Microbiol 28(1):92-99

9. Austin B, Allen DA (1982) Microbiology of laboratory-hatched brine shrimp (Artemia). Aquaculture 26:369-383

10. Austin B, Bishop I, Gray G, Watt B, Dawes J (1986) Monoclonal antibody-based enzyme-linked immunosorbent assays for rapid diagnosis of clinical cases of enteric red mouth and furunculosis in fish farms. J Fish Dis 9:469-474

11. Austin DA, Baker JA (1988). Fate of bacteria ingested by larvae of the freshwater mayfly *Ephemera danica*. Microb Ecol 15:323-332

12. Baker JH, Bradnam LA (1976) The role of bacteria in the nutrition of aquatic detritivores. Oecologia (Berl) 24:95-104

13. Barber BJ, Ford SE, Littlewood DTJ (1991) A physiological comparison of resistant and susceptable oysters *Crassostrea virginica* (Gmelin) exposed to the endoparasite *Haplosporidium nelsoni* (Haskin, Strauber and Mackin). J Exp Mar Biol Ecol 146(1):101-112

14. Barlocher F (1981) Fungi on the food and in the faeces of *Gammarus pulex*. Trans Br Mycol Soc 76:160-165

15. Barlocher F, Newell SY, Arsuffi TL (1989) Digestion of *Spartina alterniflora* Loisel material with and without fungal constituents by the periwinkle *Littorina irrorata* Say (Mollusca : Gastropoda). J Exp Mar Biol Ecol 130:45-53

16. Barlocher R (1982) The contribution of fungal enzymes to the digestion of leaves by *Gammarus fossarum* Koch. Oecologia 52:1-4

17. Baumann P, Baumann L (1977) Biology of the marine Enterobacteria: genera *Beneckea* and *Photobacterium*. Ann Rev Microbiol 31:39-61

18. Bayon C, Mathelin J (1980) Carbohydrate fermentation and by-product absorption studied with labelled cellulose in *Oryctes nasicornis* larvae (Coleoptera:Scarabaeidae). J Insect Physiol 26:833-840

19. Beattie et al (1991) Investigation of the relationship between the presence of a gram-positive bacterial infection and summer mortality of the pacific oyster *Crassostrea gigas*. Aquaculture 94(1):1-16

20. Beeson RJ, Johnson PT (1967) Natural bacterial flora of the bean clam, *Donax gouldi*. J Invert Pathol 9:104-110

21. Benemann JR (1973) Nitrogen fixation in termites. Science 64: 164-165

22. Berkeley C (1959) Some observations of *Cristospira* in the crystalline style *Saxidomus giganteus* Deshayes and in that of some other Lamellibranchiata. Can J Zool 37:53-58

23. Bernard F.R. (1970) Occurrence of the spirochaete genus *Cristispira* in Western Canadian marine bivalves. Veliger 13(1):33-36

24. Bignell DE (1984) The arthropod gut as an environment for microorganisms. In: Anderson JM, Raynon ADM, Walton DWA (eds) Invertebrate-Microbial Interactions, Cambridge University Press, Cambridge, pp 205-227

25. Boyle PJ, Mitchell R (1978) Absence of microorganisms in crustacean digestive tracts. Science 200:1157-1159

26. Branch GM, Pringle A (1987) The impact of the sand prawn *Callianassa kraussi* Stebbing on sediment turnover and on bacteria, meiofauna, and benthic microflora. J Exp Mar Biol Ecol 107:219-235

27. Breznak JA (1975) Symbiotic relationshios between termites and their intestinal microbiota. In: Symbiosis. Symposia of the Society for Experimental Biology. XXXIX:559-580. Cambridge University Press, Cambridge, pp 559-580

28. Breznak JA (1984) Biochemical aspects of symbiosis between termites and their intestinal microbiota. In: Anderson JM, Raynon ADM, Walton DWA (eds) Invertebrate-Microbial Interactions, Cambridge University Press, Cambridge, pp 173-203

29. Breznak JA, Brill WJ, Mertins JW, Coppel HC (1973) Nitrogen fixation in termites. Nature 244:577-580

30. Breznak JA, Pankratz HS (1977) In situ morphology of the gut microbiota of wood-eating termites (*Reticulitermes flavipes* (Kollar) and *Coptotermes formosanus* (Shiraki)). Appl Environ Microbiol 33(2):406-426

31. Buchanan JS (1978) Cytological studies on a new species of rickettsis found in association with a phage in the digestive gland of the marine bivalve mollusc, *Tellina tenuis* (da Costa) J Fish Diseases 1:27-43

32. Cameron DE, Garland CD, Lewis TE, Machin PJ (1988) A survey of Vibrionaceae in Tasmanian coastal waters, with special reference to bacterial species pathogenic to fish or shellfish. Aust J Mar Freshwater Res 39:145-152

33. Cammen LM (1980) The significance of microbial carbon in the nutrition of the deposit feeding polychaete *Nereis succinea*. Mar Biol 61:9-20

34. Cammen LM (1989) The relationship between ingestion rate of deposit feeders and sediment nutritional value. In: Lopez G, taghon G, Levinton J (eds) Ecology of marine deposit feeders, Springer-Verlag, New York, pp 201-222

35. Campbell LL Jr, Williams OB 1951 The bacteriology of Gulf coast shrimp. Food Technol 1951:125-126

36. Cann DC, Hobbs G, Wison BB, Horsley RW (1971) The bacteriology of "scampi" (*Nephrops norvigicus*) II. Detailed investigation of the bacterial flora of freshly caught samples. J Food Technol 6:153-161

37. Carman KR (1990) Radioactive labeling of a natural assemblage of marine sedimentary bacteria and microalgae for trophic studies: an autoradiographic study. Microb Ecol 19:279-290

38. Carman KR, Dobbs FC, Guckert JB (1989) Comparison of three techniques for administering radiolabeled substrates to sediments for trophic studies: uptake of label by harpacticoid copepods. Mar Biol 102:119-125

39. Carman KR, Thistle D (1985) Microbial food partitioning by three species of benthic copepods. Mar Biol 88:143-148

40. Carpenter JL, Culliney JL (1975) Nitrogen fixation in marine shipworms. Science 187:551-552

41. Castille FL, Lawrence AL (1979) The role of bacteria in the uptake of hexoses from seawater by postlarval penaeid shrimp. Comp Biochem Physiol 64A:41-48

42. Chamier A, Willoughby LG (1986) The role of fungi in the diet of the amphipod *Gammarus pulex* (L.): an enzymatic study. Freshwater Biol 16(2):197-208

43. Chamier AC (1991) Cellulose digestion and metabolism in the freshwater amphipod *Gammarus pseudolimnaeus* Bousfield. Freshwater Biol 25(1):33-40

44. Colorni A (1985) A study on the bacterial flora of giant prawn *Macrobrachium rosenbergii*, larvae fed with *Artemia salina* nauplii. Aquacult 49:1-10

45. Colwell RR, Liston J (1962) The natural bacterial flora of certain marine invertebrates. J Insect Pathology 4:23-33

46. Colwell RR, Liston J 1960. Microbiology of shellfish. Bacteriological study of the natural flora of Pacific oysters (*Crassostrea gigas*). Appl Microbiol 8:104-109

47. Colwell RR, West PA, Maneval D, Remmers EF, Eliot EL, Carlson NE (1984) Ecology of pathogenic vibrios in Chesapeake Bay.. In: Colwell RR (ed) Vibrios in the environment, John Wiley and Sons, New York, pp 367-387

48. Conway N, Capuzzi JM (1991) Incorporation and utilization of bacterial lipids in the *Solemya velum* symbiosis. Mar Biol 108(2):277-292

49. Conway, N, Capuzzo JM, Fry B (1989) The role of endosymbiotic bacteria in the nutrition of *Solemya velum*: evidence from stable isotope analysis of endosymbionts and host. Limnol Oceanogr 34(1):249-258

50. Crawford CS, Taylor EC (1984) Decomposition in arid environments: role of the detritivore gut. S Afri J Sci 80:170-176

51. Crosby MP, Peele ER (1987) Detection of bacterial populations in oyster tissue homogenates using direct microsopic counts and thymidine incorporation. J Exp Mar Biol Ecol 108:93-97

52. Cruden DL, Markovetz AJ (1979) Carboxymethyl cellulose decompostion by intestinal bacteria of cockcroaches. Appl Environ Microbiol 38(3):369-372

53. Cuoma V, Vanzanella F, Fresi E, Cinelli F, Mazzella L (1985) Fungal flora of *Posidonia oceanica* and its ecological significance. Trans Br mycol Soc 84(1):35-40

54. Cutter JM, Rosenberg FA (1971) The role of cellulolytic bacteria in the digestive processes of the shipworm. II. Isolation and some properties of a marine bacterial cellulase. Material und Organismen 7:225-239

55. Cutter, FA Rosenberg (1972) The role of cellulolytic bacteria in the digestive processes of the shipworm - requirement for the teredine borers. In: Walters AH, Hueck van der Plas EH (eds) Biodeterioation of Materials, vol. 2. Applied Science Publishers Ltd, London, pp 42-51

56. Davis JW, Sizemore RD (1982) Incidence of *Vibrio* species associated with blue crabs (*Callinectes sapidus*) collected from Galveston Bay, Texas. Appl Environ Microbiol 43:1092-1097

57. De Ridder C, Jangoux M, De Vos L 1985. Description and significance of a particular intradigestive symbiosis between bacteria and a deposit-feeding echinoid. J Exp Mar Biol Ecol 91:65-76

58. Deming JW, Colwell RR (1981) Barophilic bacteria associated with deep-sea animals. BioScience 31:507-511

59. Deming JW, Colwell RR (1982) Barophilic bacteria associated with digestive tracts of abyssal holothurians. Appl Environ Microbiol 44:1221-1230

60. Deming JW, Tabor PS, Colwell RR (1981) Barophilic growth of bacteria from intestinal tracts of deep-sea invertebrates. Microb Ecol 7:85-94

61. Dempsey AC, Kitting CL (1987) Characteristics of bacteria isolated from penaeid shrimp. Crustaceana 52:90-94

62. Dempsey AC, Kitting CL, Rosson RA (1989) Bacterial variability among individual penaeid shrimp digestive tracts. Crustaceana 56(3):266-278

63. Dilmore LA, Hood MA (1986) Vibrios of some deep-water invertebrates. FEMS Microb Letters 35:221-224

64. Doores S, Cook TM (1976) Occurrence of *Vibrio* and other bacteria on the sea nettle, *Chrysaora quinquecirrha*. Microb Ecol 3:31-40

65. Doubrovsky A, Paynter JL, Sambhi SK, Atherton JG, Lester RJG (1988) Observations on the ultrastructure of baculovirus in Australian *Penaeus monodon* and *Penaeus merguiensis*. Aust J Mar Freshwater Res 39:743-749

66. Duchene JC, Imbaud P, Delille (1988) Associated bacterial microflora of a subantarctic polychaete worm *Thelepus setosus*. Arch Hydrobiol 112:221-231

67. Ellery WN, Schleyer MH (1984) Comparison of homogenization and ultrasonication as techniques in extracting attached sedimentary bacteria. Mar Ecol prog Ser 15:247-250

68. Elleway RF, Sabine JR, Nicholas DJD (1971) Acetylene reduction by rumen microflora. Arch Mikrobiol 76:277-291

69. Elston R, Leibovitz L (1980) Pathogenesis of experimental vibriosis in larval American oysters (*Crassostrea virginica*). Can J Fish Aquat Sci 37:964-978

70. Elston R, Lockwood GS (1983) Pathogenesis of vibriosis in cultured juvenile red abolone, *Haliotis nifescens* Swainson. J Fish Diseases 6:111-128

71. Felbeck H, Childress JJ, Somero GN (1983) Biochemical interactions between molluscs and their algal and bacterial symbionts. In: The Mollusca Vol. 2. Environmental Biochemistry and Physiology pp 331-358

72. Fenchel T (1970) Studies on the decomposition of organic detritus derived from the turtle grass *Thalassia testudinum*. Limnol Oceanogr 51:14-20

73. Feral JP (1980) Cuticle et bacteries associees des epidermes digestif et tegamentaire de leptosynapta galliennei (Herapath) (Holothurioidea:Apoda) - premieres donnees In: Jangoux M (ed) Echinoderms: present and past, AA Balkema, Rotterdam, pp 285-290

74. Findlay S, Tenore K (1982) Nitrogen source for a detritivore: detritus substrate versus associated microbes. Science 218:371-373

75. Fischer CR, Childress JJ (1986) Translocation of fixed carbon from symbiotic bacteria to host tissues in the gutless bivalve *Solemya reidi*. Mar Biol 93:59-68

76. Fisher WS, Nilson EH, Steenbergen JF, Lightner DV (1978) Microbial diseases of cultured lobsters : a review. Aquaculture 14:115-140

77. Fong W, Mann KH ((1980)) Role of gut flora in the transfer of amino acids through a marine food chain. Can J Fish Aquat Sci 37:88-96

78. Friedman CS, Beattie JH, Elston RA, Hedrick RP (1991) Investigation of the relationship between the prescence of a gram-positive bacterial infection and summer mortality of the Pacific oyster, *Crassostrea gigas* Thunberg. Aquaculture 94:1-15

79. Friesen JA, Mann KH, Novitsky JA (1986) Mysis digests cellulose in the absence of a gut microflora. Can J Zool 64(2):442-446

80. Gallagher SM, Turner RD, Berg CJ (1981) Physiological aspects of wood composition, growth and reproduction in the shipworm *Lyrodotus pedicellatus* Quatrefagus (Bivalvia: Teredinae). J Exp Mar Biol Ecol 52:63-77

81. Garland CD, Nash GV, McMeekin TA (1982) Absence of surface-associated micro-organisms in adult oysters (*Crassostrea gigas*). Appl Environ Microbiol 44:1205-1211

82. Gjerde J (1984) Occurrence and characterisation of Aerococcus viridans from lobsters, *Homarus gammarus* L., dying in captivity. J Fish Diseases 7(5):355-362

83. Greene RV, Cotta MA, Griffin HL (1989) A novel, symbiotic bacterium isolated from marine shipworm secretes proteolytic activity. Curr Microbiol 19:353-356

84. Greene RV, Griffin HL, Freer SN (1988) Purification and charcterization of and extracellular endoglucanase from the marine shipworm bacterium. Arch Biochem Biophys 267:334-341

85. Griffin HL, Freer SN, Greene RV (1987) Extracellular endoglucanase activity by a novel bacterium isolated from marine shipworm. Biochem Biophys Res Comm 144(1):143-151

86. Guerinot ML, Fong W, Patriquin DG (1977) Nitrogen fixation (acetylene reduction) associated with sea urchins (*Strongylocentrotus droebachiensis*) feeding in seaweeds and eelgrass. J Fish Res Board Can 34:416-420

87. Guerinot ML, Patriquin DG (1981a) The association of N2-fixing bacteria with sea urchins. Mar Biol 62:197-207

88. Guerinot ML, Patriquin DG (1981b) N2-fixing vibrios isolated from the gastrointestinal tract of sea urchins. Can J Microbiol 27:311-317

89. Gunzl, H (1991) The ultrastucture of the posterior gut and caecum in *Alona affinis* (Crustacea, Cladocera). Zoomorphology 110(3):139-144

90. Hanson RB, Tenore KR (1981) Microbial metabolism and incorporation by the polychaete *Capitella capitata* of aerobically and anaerobically decomposed detritus. Mar Ecol Prog Ser 8:299-307

91. Hargrave BT (1970) The utilization of benthic microflora by *Hyalella azteca* (Amphipoda). J Animal Ecol 39:427-437

92. Hargrave BT (1976) The central role of invertebrate faeces in sediment decomposition. Brit Ecol Soc Symp 17:301-321

93. Harris JM, Seiderer LJ, Lucas MI (1991) Gut microflora of two saltmarsh detritivore Thalassinid prawns, *Upogebia africana* and *Callianassa kraussi*. Microb Ecol 21:63-82

94. Herman SS, Coull BC, Brickman LM (1971) Infestation of harpacticoid copepods (Crustacea) with ciliate protozoans. J Aquat Invert Pathol 17:141-142

95. Herndl GJ, Velimirov B (1986) Role of bacteria in the gastral cavity of Anthozoa. Deuxieme Collogue International de Bacteriologie marine, IFREMER, Actes de Colloque 3:407-414

96. Herry A, Diouris M, Le Pennec M (1989) Chemoautotrophic symbionts and translocation of fixed carbon from bacteria to host tissues in the littoral bivalve *Loripes lucinalis* (Lucinidae). Mar Biol 101:305-312

97. Hidaka T (1954) On cellulose decomposing bacteria found ijn the digestive organs of *Teredo*. Mem Fac Fisheries, Kagoshima Univ 3:149-157

98. Hidaka T, Saito K (1956) Studies on the cellulose decomposing bacteria in the digestive organs of the shipworm (*Teredo navalis*) II On the bacterial cellulase. Mem Fac Fisheries, Kagoshima Univ 5:172-177

99. Hockin DC (1984) Records of symbiotic protozoa from harpacticoid copepods of a sandy beach. Crustaceana 46(3):319-320

100. Hood MA, Meyers SP, Colmer AR (1971) Bacteria of the digestive tract of the white shrimp *Penaeus setiferus*. Bacteriological Proceedings 71:G-147

101. Hungate RE (1975) The rumen microbial ecosystem. Annu Rev Ecol Syst 6:39-66

102. Hungate RE (1976) Microbial activities related to mammalian digestion and absorption of food. In: Spillar G, Amen R (eds) Fiber in Human Nutrition, Plenum Press, New York, pp 131-149

103. Huq A, Huq SA, Grimes DJ, O'Brien M, Chu KH, Capuzzi JM, Colwell RR (1986) Colonization of the gut of the blue crab *Callinectes sapidus* by *Vibrio cholerae*. Appl Environ Microbiol 52(3):586-588

104. Huq A, Small EB, West PA, Huq I, Rahman R, Colwell RR (1983) Ecological relationships between *Vibrio cholerae* and planktonic Crustacea copepods. Appl Environ Microbiol 45:275-283

105. Huq A, West PA, Small EB, Huq MI, Colwell RR (1984) Influence of water temperature, salinity, and pH on survival and growth of toxigenic *Vibrio cholerae* serovar O1 associated with live copepods in laboratory microcosms. Appl Environ Microbiol 48:420-424

106. Hylleberg J, Gallucci VF (1975) Selectivity in feeding by the deposit-feeding bivalve *Macoma nasuta*. Mar Biol 32:167

107. Hylleberg KJ (1972) Structure and function of crystalline style of bivalves. Ophelia 10:91-108

108. Imam, Greene, Griffin (1990) Adhesive properties of a symbiotic bacterium from a wood-boring marine shipworm. Appl Env Microbiol 56(5):1317-1322

109. Jannasch HW, Wirsen CO (1973) Deep sea microorganisms: in situ response to nutrient enrichment. Science 180:641-643

110. Jannasch HW, Wirsen CO, Cutnel RL, Taylor CD (1980) An approach for in situ studies of deep-sea amphipods and their microbial gut flora. Deep-sea Res 27:867-872

111. Jensen KT, Siegismund HR (1980) The importance of diatoms and bacteria in the diet of *Hydrobia*-species. Ophelia (Suppl.) 1:193-199

112. Juilffs HB, Wagele JW (1987) Symbiontic bacteria in the gut of the blood-sucking Antarctic fish parasite *Gnathia calva* (Crustacea : Isopoda). Mar Biol 95(4):493-499

113. Kadota H (1951) Studies on the biochemical activities of marine bacteria I On agar-decomposing bacteria in the sea. Memoirs of the College of Agriculture, Kyoto Univ 59:54-67

114. Kadota H (1953) Studies on the biochemical activities of marine bacteria II On the properties of agar-digesting enzyme of *Vibrio purpureus*. Memoirs of the College of Agriculture, Kyoto Univ 66:31-38

115. Kadota H (1959) Cellulose decomposing bacteria in the sea. In: Ray DL (ed) Marine Boring and Fouling Organisms. Univ of Washington Press, Seattle, pp 332-341

116. Kaneko T., Colwell R.R. (1975) Adsorption of *Vibrio parahaemolyticus* onto chitin and copepods. Appl Microbiol 29(2):269-274

117. Kelly MD, Lukaschewsky S, Anderson CG (1978) Bacterial flora of Antarctic krill (*Euphausia superba*) and some of their enzymatic properties. J Food Sci 43:1196-1197

118. Kemp PF (1990) The fate of benthic bacterial production. Rev Aquat Sci 2(1):109-124

119. Klug MJ, Kotarski S (1980) Bacteria associated with the gut tract of larval stages of the aquatic cranefly *Tipula abdominalis* (Diptera: Tipulidae). Appl Environ Microbiol 40(2):408-416

120. Kobayashi Y, Tsubahi K, Soneda M (1953) Marine yeast isolated from little-neck clam. Bull Natl Sci Mus 33:47-52

121. Langdon CL, Newell RIE (1990) Utilization of detritus and bacteria as food sources by two bivalve suspension-feeders, the oyster *Crassostrea virginica* and the mussel *Geukensia demissa*. Mar Ecol Prog Ser 58:299-310

122. Lasker R, Giese AC (1954) Nutrition of the sea urchin, *Stongylocentrotus purpuratus*. Biol Bull Woods Hole Mass 106:328-340

123. Lawry EV (1987) *Cryptomya californica* Conrad 1837 observations on its habitat behaviour anatomy and physiology. Veliger 30(1):46-54

124. Lee A (1980) Normal flora of animal intestinal surfaces. In: Bitton G, Marshall KC (eds) Absorption of microorganisms to surfaces, John Wiley & Sons, New York, pp 145-173

125. Lee A (1985) Neglected niches. The microbial ecology of the gastrointestinal tract. Adv Microb Ecol 8:115-162

126. Lee JS, Pfiefer DK (1977) Microbiological charcteristics of Pacific shrimp (*Pandalus jordani*). Appl Environ Microbiol 33:853-859

127. Lesser MP, Stochaj WR (1990) Photoadaptation and protection against active forms of oxygen in the symbiotic procaryote *Prochlaron* sp and its Ascidian host. Appl Environ Microbiol 56(6):1530-1535

128. Lichtwardt RW (1986) The Trichomycetes. Fungal associates of arthropods. Springer-Verlag, New York, pp 1-343

129. Lichtwardt RW 1961 A stomach fungus in *Callianassa* spp. (Decapoda) from Chile. 57(6):3-10

130. Lynch JM, Hobbie JE (eds.) (1988) Micro-organisms in action: concepts and applications in Microbial Ecology, Chapter 3.3, The animal environment. Blackwell Scientific Publications, London, pp 163-192

131. Lynch JE (1929) Studies on the ciliates from the intestine of *Strongylocentrotus.* I Entorhipidium gen nov. Univ Calif Publ Zool 33:27-56

132. Lynch JE (1930) Studies on the ciliates from the intestine of *Strongylocentrotus*. II Lechriopyla mystax, Gen nov., sp. nov. Univ Calif Publ Zool 33:307-350

133. Margulis L, Nault L, Sieburth JM (1991) *Cristispira* from oyster styles - complex morphology of large symbiotic spirochaetes. Symbiosis 11(1):1-18

134. Martin MM (1984) The role of ingested enzymes in the digested processes of insects. In: Anderson JM, Raynon ADM, Walton DWA (eds) Invertebrate-Microbial Interactions. Cambridge University Press, Cambridge pp 155-177

135. Martin MM, Martin JS (1978) Cellulose digestion in the midgut of the fungus-growing termite *Macrotermes natalensis*: the role of acquired digestive enzymes. Science 199:1453-1455

136. Martinez JC (1982) The digestive microflora of *Teredo navalis* L. (Teredinidae;Bivalvia): Metabolic properties and ultimate role in digestion. Bact Mar 331:151-154

137. Mattson RA (1988) Occurrence and abundance of eccrinaceous fungi (Trichomycetes) in Brachyuran crabs from Tampa Bay, Florida. Jour Crust Biol 8(1):20-30

138. Mayasich SA, Smucker RA (1987) Role of *Cristospira* sp. and other bacteria in the chitinase and chitobiase activities of the crystalline style of *Crassostrea virginica* (Gmelin). Microb Ecol 14:157-166

139. McBee RH (1971) Significance of intestinal microflora in herbivory. Ann Rev Ecol Syst 2: 165-176

140. Mead LJ, Khachatourians GG, Jones GA (1988) Microbial ecology of the gut in laboratory stocks of the migratory grasshopper, *Melanoplus sanguinipes* (Fab.) (Orthoptera:Acrididae). Appl Environ Microbiol 54(5):1174-1181

141. Minet J, Barbosa T, Prieur D, Cormier M (1987) Mise en evidence du processus de concentration des bacteries par la moule, *Mytilus edulis* (L.). CR Acad Sci Paris Ser III 305:351-354

142. Monk DC (1977) The digestion of cellulose and other dietary components, and pH of the gut in the amphipod *Gammarus pulex* L. Freshwater Biol 7:431-440

143. Moriarty DJW (1976) Quantitative studies on bacteria and algae in the food of the mullet *Mugil cephalus* L and the prawn *Metapenaeus bennettae* (Racek and Dall). J Exp Mar Biol Ecol 22:131-143

144. Moriarty DJW (1990) Interactions of micro-organisms and aquatic animals, particularly the nutritional role of the gut microflora. In : Lesel R (ed) Microbiology in poecilotherms. Elsevier Science Publishers.

145. Moriarty DJW, Pollard PC, Hunt WG, Moriarty CM, Wassenberg TJ (1985) Productivity of bacteria and microalgae and the effect of grazing by holothurians in sediments and a coral reef flat. Mar Biol 85:293-300

146. Morton B (1978) Feeding and digestion in shipworms. Oceanogr Mar Biol Ann Rev 16:107-144

147. Musgrove RJ (1988) Digestive ability of the freshwater crayfish *Paranephrops zealandicus* (White) (Parastacidae) and the role of microbial enzymes. Freshwater Biol 20:305-314

148. Nagasawa S, Nemoto T (1988) Presence of bacteria in guts of marine crustaceans and on their fecal pellets. J Plankton Res 10:559-564

149. Newell RC (1965) The role of detritus in the nutrition of two marine deposit feeders, the prosobranch, *Hydrobia ulvae*, and the bivalve, *Macoma balthica*. J Zool 144:25-44

150. O'Brien RW, Slaytor M (1982) Role of microorganisms in the metabolism of termites. Aust J Biol Sci 35:239-262

151. Odintsov VS (1981) Nitrogen fixation (acetylene reduction) in the digestive tract of some echinoderms from Vostok Bay in the sea of Japan. Mar Biol Letters 2:259-263

152. Ohmart CP, Thomas JR, Bubela B (1988) Surfactant-producing microorganisms isolated from the gut of a Eucalyptus-feeding sawfly, *Perga affinis affinis*. Oecologia 77:140-142

153. Ohwada K, Tabor PS, Colwell RR (1980) Species composition and barotolerance of gut microflora of deep-sea benthic macrofauna collected at various depths in the Atlantic Ocean. Appl Environ Microbiol 40:746-755

154. Overstreet RM (1983) Metazoan symbionts of crustaceans. In: Bliss DE (ed) The Biology of Crustacea, Vol. 6. Pathobiology, Academic Press, London, pp 155-250

155. Payne DW, Thorpe NA, Donaldson EM (1972) Cellulolytic activity and a study of the bacterial population in the digestive tract of *Scrobicularia plana* (Da Costa). Proc Malac Soc Lond 40:147-160

156. Phillips NW (1984) Role of different microbes and substrates as potential suppliers of specific, essential nutrients to marine detritivores. Bull Mar Sci 35(3):283-298

157. Pitts GY, Cowley GT (1974) Mycoflora of the habitat and midgut of the fiddler crab *Uca pugilator*. Mycologia 66:669-675

158. Plante CJ, Jumars PA, Baross JA (1989) Rapid bacterial growth in the hindgut of a marine deposit feeder. Microb Ecol 18:29-44

159. Plante CJ, Jumars PA, Baross JA (1990) Digestive associations between marine detritivores and bacteria. Annu Rev Ecol Syst 21:93-127

160. Popham JD (1975) Further observations of the gland of Deshayes in the teredo *Bankia australis* (Bivalvia, Mollusca) Veliger 18:55-58

161. Popham JD, Dickson MR (1973) Bacterial associations in the teredo *Bankia australis* (Lamellibranchia, Mollusca) Mar Biol 19:338-340

162. Potrikus CJ, Breznak JA (1981) Gut bacteria recycle uric acid nitrogen in termites: a strategy for nutrient conservation. Proc Natl Acad Sci USA 78(7):4601-4605

163. Prestwich GD, Bentley BL, Carpenter EJ (1980) Nitrogen sources for neotropical nasute termites : fixation and selective foraging. Oecologia 46:397-401

164. Prieur D (1980) Observations de coupes histologiques en microscopie electronique a balayage : application a l'etude de microorganismes dans le tractus digestif de *Mytilus edulis*. C R Acad Sci Ser D 290:1087-1089

165. Prieur D (1981a) Experimental studies of trophic relationships between marine bacteria and bivalve molluscs. Kieler Meeresforsch, Sonderh. 5:376-383

166. Prieur D (1981b) Nouvelles donnees sur les relations entre bacteries et bivalves marins. Haliotis 11:251-260

167. Prieur D (1982) La microflore du tractus digestif des bivalves marins: etude experimentale chez la moule, *Mytilus edulis*. Malacologia 22:653-658

168. Prieur D, Mevel G, Nicolas JL, Plusquellec A, Vigneulle M (1990) Interactions between bivalve molluscs and bacteria in the marine environment. Oceanogr Mar Biol Annu Rev 28:277-352

169. Prim P, Lawrence JM (1975) Utilization of marine plants and their constituents by bacteria isolated from the gut of Echinoids (Echinodermata). Mar Biol 33:167-173

170. Provenzano AJ (1983) Pathobiology. In: Bliss DE (ed) The Biology of Crustacea, Vol. 6., Academic Press, London, 290 pp

171. Ray DL, Julian JR (1952) Occurrence of cellulase in *Limnoria*. Nature 169:32-33

172. Rose AS, Ellis AE, Adams A (1989) An assesment of routine *Aeromonas salmonicida* carrier detection by ELISA. Bull Eur Assoc Fish Pathol 9:65-67

173. Rosenberg FA, Breiter H (1969) The role of cellulolytic bacteria in the digestive processes of the shipworm. I. Isolation of some cellulolytic micro-organisms from the digestive system of Teredine borers and associated waters. Material und Organismen 4:147-159

174. Saito K, Hidaka T (1954) Studies on the cellulose decomposing bacteria in the digestive organs of the shipworm (*Teredo navalis*) III Relation between the enzyme of the digestive organs and the bacterial enzyme. Mem Fac Fisheries, Kagoshima Univ 3:50-55

175. Santavy DL, Willenz P, Colwell RR (1990) Phenotypic study of bacteria associated with the Carribean Sclerosponge *Ceratoporella nicholsoni*. Appl Env Microbiol 56(6):1750-1762

176. Savage DC (1977) Microbial ecology of the gastrointestinal tract. Ann Rev Microbiol 31:107-133

177. Schallenberg M, Kalff J, Kasmussen JB (1989) Solutions to problems in enumerating sediment bacteria by direct counts. Appl Environ Microb 55(5):1214-1219

178. Schwarz JR, Yayanos AA, Colwell RR (1976) Metabolic activities of the intestinal microflora of a deep-sea invertebrate. Appl Environ Microbiol 31(1):46-48

179. Scrivener AM, Slaytor M, Rose HA (1989) Symbiont-independent digestion of cellulose and starch in *Panesthia cribrata* saussure, an Australian wood-eating cockroach. J Insect Physiol 35(12):935-941

180. Seiderer LJ, Newell RC (1988) Exploitation of phytoplankton as a food resource by the kelp bed ascidian *Pyura stolonifera*. Mar Ecol Prog Ser 50:107-115

181. Seiderer LJ, Newell RC, Schultes K, Robb FT, Turley CM (1987) Novel bacteriolytic activity associated with the style microflora of the mussel *Mytilus edulis* (L.). J Exp Mar Biol Ecol 110:213-324

182. Shivokene Ya S, Sinyavichene DP, Shal'chyute BP (1986) Ecological and physiological features of bivalve mollusks of the superfamily Unionaceae 6. Functional activity of the microorganisms of the digestive system of *Unio tumidas* as a function of its diet. Liet Tsr Mokslu Akad Darb Ser C Biol Mokslai 6(3):28-33

183. Shivokene Ya S, Sinyavichene DP, Shal'chyute BP (1987) Ecophysiological characteristics of bivalves of the superfamily Unionacea vii. Microflora of the alimentary system of the mussel *Anodonta piscinalis* as a function of the feeding pattern. Liet Tsr Mokslu Akad Darb Ser C Biol Mokslai 7(2):48-54

184. Simidu U, Ashino K, Kaneko T (1971) Bacterial flora of phyto- and zoo-plankton in the inshore water of Japan. Can J Microbiol 17:1157-1160

185. Sizemore RK, Colwell RR, Tubiash HS, Lovelace TE (1975) Bacterial flora of the haemolymph of the blue crab, *Callinectes sapidus* : numerical taxonomy. Appl Environ Microb 29:393-399

186. Sleeter TD, Boyle PJ, Cundell AM, Mitchell R (1978) Relationships between marine micro-organisms and the wood-boring isopod *Limnoria tripunctata*. Mar Biol 45:329-336

187. Sochard MR, Wilson DF, Austin B, Colwell RR (1979) Bacteria associated with the surface and gut of marine copepods. Appl Environ Microbiol 37:570-579

188. Sugita H, Takahashi T, Kanemoto FI, Deguchi Y (1987a) Aerobic bacterial flora in the digestive tracts of freshwater shrimp *Palaemon paucidens* acclimated with seawater. Nippon Suisan Gakkaishi 53(3):511

189. Sugita H, Ueda S, Ueda R, Degushi Y (1987b) Morphology and brief characteristics of *Vibrio* sp. isolated from marine Crustacea. Bull Coll Agric Vet Med Nihon Univ 44:160-164

190. Sugita H, Ueda R, Berger LR, Deguchi Y (1987c) Microflora in the gut of Japanese coastal crustacea. Bull Jpn Soc Sci Fish 53(9):1647-1655

191. Sweijd NA, Pillay D, McQuaid CD, Bandu VH, Baecker AAW (1989) Filamentous structures associated with the gut mucosa of the sea urchin *Parechinus angulosus*. Electron Microscopy Soc S A 19:99-100

192. Tabor PS, Deming JW, Ohwada K, Colwell RR (1982) Activity and growth of miacrobial populations in pressurized deep-sea sediment and animal gut samples. Appl Environ Microbiol 44:413-422

193. Tall BD, Nauman RK (1981) Scanning electron microscopy of Cristispira species in Chesapeake Bay oysters. Appl Environ Microbiol 42:336-343

194. Tanikawa E (1937) Biological studies on organisms of coli-group isolated from the intestines of oysters. Arch Mikrobiol 8:288-306

195. Terasaki Y (1958) Studies on Cristispira in the crystalline style of the freshwater snail, *Semisulcospira libertina* (Gould) I The morphological characters and the living condition within the style. Bull Suzugamine Womens College 5:7-19

196. Thayer DW (1978) Carboxymethylcellulose produced by facultative bacteria from the hindgut of the termite Reticulitermes hesperus. J Gen Microbiol 106:13-18

197. Tugwell S, Branch GM (1991) Effects of herbivore gut surfactants on kelp polyphenol defenses. Ecology 73(1):205-215

198. Ueda R, Sugita H, Degushi Y (1988) Numerical taxonomy of vibrios isolated from coastal crustacea of Japan. Bull Coll Agric Vet Med Nihon Univ 8(45):227-235

199. Ulrich RG, Buthala DA, Klug MJ (1981) Microbiota associated with the gastrointestinal tract of the common house cricket, *Acheta domestica*. Appl Environ Microbiol 41(1):246-254

200. Unestam T (1981) Fungal diseases of freshwater and terrestrial Crustacea. In: Davidson EW (ed), Pathogenesis of Invertebrate Microbial Diseases, pp 485-507

201. Unkles SE (1977) Bacterial flora of the sea urchin Echinus esculentus. Appl Environ Microbiol 34:347-350

202. Vacelet J (1975) Etude et microscopie electronique de l'association entre bacteries et spongaires du genre *Verongia* (Dictyoceratida). J Microsc Biol Cell 23:271-288

203. Vacelet J, Donaday C (1977) Electron microscope study of the association between some sponges and bacteria. J Exp Mar Biol Ecol 30:301-314

204. Vanderzant C, Mroz, Nickleson R (1970) Microbial flora of Gulf of Mexico and pond shrimp. J Milk Food Technol 33:346-350

205. Vitalis TZ, Spence MJ, Carefoot TH (1988) The possible role of gut bacteria in nutrition and growth of the sea hare *Aplysia*. Veliger 30(4):333-341

206. Vivares BM, Baleux B (1984) Parasitism and digestive bacterial flora in decapods (Crustacea). In: Tumbay E, Yasarol S,Ozcel MA (eds) EMOP 4. The Fourth European Multicolloquium of Parasitology, pp 202-203

207. Wagner-Merner DT (1979) Observations on a trichomycete from *Uca pugilator*. Mycologia 71:669-671

208. Wagner-Merner DT, Lawrence JM (1980) Occurrence of fungi (Thraustochytriaceae) in the gut of *Lytechinus variegatus* (Lamarck) (Echinodermata : Echinoidea). Florida Sci 43(1):62-63

209. Wainwright PF, Mann KH (1982) Effect of antimicrobial substances on the ability of the mysid shrimp *Mysis stenolepis* to digest cellulose. Mar Ecol Prog Ser 7:309-313

210. Wardlaw AC, Unkles SE (1978) Bactericidal activity of coelomic fluid from the sea urchin *Echinus esculentus*. J Invert Pathol 32:25-34

211. Waterbury JB, Calloway CB, Turner RD (1983) A cellulolytic nitrogen fixing bacterium cultured from the gland of Deshayes in shipworms (Bivalvia:Teredinidae). Science 221:1401-1403

212. Wavre M, Brikhurst RO (1971) Interactions between some tubificid oligochaetes and bacteria found in the sediments of Toronto Harbour, Ontario. J Fish Res Bd Can 28(3):335-341

213. Wilkinson CR (1978a) Microbial associations in sponges I Ecology, physiology and microbial populations of coral reef sponges. Mar Biol 49:161-167

214. Wilkinson CR (1978b) Microbial associations in sponges II Numerical analysis of sponge and water bacterial populations. Mar Biol 49:169-176

215. Wilkinson CR (1978c) Microbial associations in sponges III Ultrastucture of the in situ associations in coral reef sponges. Mar Biol 49:177-185

216. Wilkinson CR, Garrone R (1980) Nutrition of marine sponges. involvement of symbiotic bacteria in the uptake of dissolved carbon In: Smith DC and Tiffon Y (eds) Nutrition in the lower metazoa, Pergamon Press Inc, Oxford, p 157-161

217. Wilkinson CR, Garrone R, Vacelot J (1984) Marine sponges discriminate between food bacteria and bacterial symbionts: electon microscope radioautography and is situ evidence. Proc R Soc London Sect B220:519-528

218. Wilkinson CR, Nowak M, Austin B, Colwell RR (1981) Specificity of bacterial symbionts in Mediteranean and Great Barrier reef sponges. Microb Ecol 7:13-21

219. Williams OB, Campbell Jr L, Rees HB (1952) The bacteriology of Gulf Coast Shrimp II Qualitative observations on the external flora. Texas J Sci 4(1):53-54

220. Williams OB, Rees HB Jr (1952) The bacteriology of Gulf Coast shrimp III The internal flora. Texas J Sci 1:55-58

221. Wirsen CO, Jannasch HW (1983) In situ studies on deep sea amphipods and their intestinal microflora. Mar Biol 78:69-73

222. Woollacot RM (1981) Association of bacteria with bryozoan larvae. Mar Biol 65(2):155-158

223. Yasuda K, Kitao T (1980) Bacterial flora in the digestive tracts of prawns, *Penaeus japonicus* Bate. Aquaculture 19(3):229-234

224. Yayanos AA, Dietz AS, vam Boxtel R (1979) Isolation of a deep-sea barophilic bacterium and some of its growth characteristics. Science 205:808-809

225. Zachary A, Colwell RR (1979) Gut-associated microflora of *Limnoria tripunctata* in marine creosote-treated wood pilings. Nature (Lond.) 282:716-717

226. Zachary A, Parrish KK, Bultman JD (1983) Possible role of marine bacteria in providing the creosote-resistance of *Limnoria tripunctata*. Mar Biol 75(1):1-8

227. Zobell CE, Feltham CB (1937) Bacteria as food for certain marine invertebrates. J Mar Res 1-2:312-327

CHAPTER 5

Harris JM, Seiderer LJ & MI Lucas (1991) Gut microflora of two saltmarsh detritivore thalassinid prawns, *Upogebia africana* and *Callianassa kraussi*. *Microb Ecol* 21:277-296

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MICROBIAL ECOLOGY

Gut Microflora of Two Saltmarsh Detritivore Thalassinid Prawns, *Upogebia africana* and *Callianassa kraussi*

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Abstract. The presence and digestive capabilities of bacteria associated with the digestive systems and habitats of two saltmarsh-burrowing detritivore thalassinid prawns (*Upogebia africana* and *Callianassa kraussi*) was examined. *U. africana* is a filter-feeding prawn inhabiting muddy deposits, whereas *C. kraussi,* a deposit feeder, inhabits coarser more sandy deposits. Scanning electron microscopy was used to examine the gut lining and associated microflora and the nature of the ingested food of both prawns. The gut contents of both prawns included plant fragments, fragmented diatoms, partially degraded protozoa, and bacteria attached to organic matter. In both *U. africana* and *C. kraussi* the midgut walls and gut contents were extensively coated by filamentous bacteria which were absent in the hindgut. The hindgut epithelium of *U. africana* was coated by mats of rod-shaped bacteria, not reported in marine invertebrates previously. The digestive glands of both species contained bacteria in the lumen. Isolation of gut and habitat bacteria suggests that both *U. africana* and *C. kraussi* maintain a gut microflora distinct from the habitat microflora. Bacteria isolated from the guts of both species of prawn differed from those isolated from their respective habitats with regards to both the genera isolated and their digestive capabilities. The dominant genera isolated from the guts of both *U. africana* and *C. kraussi* were *Vibrio* and *Pseudomonas,* with an unidentified fermenter and *Pseudomonas,* respectively dominating in the digestive glands. Bacteria of the genus *Acinetobacter* dominated the isolates from the habitats of both species of prawn. Resident gut bacteria isolated from the guts of both species of prawn exhibited lipase, protease, chitinase, and lysozyme, but not cellulase activity, and may contribute to nitrogen aquisition by the prawns. Isolates from the prawns' habitat exhibited alginase, gelatinase, and lipase activity, a few (3%) from *U. africana* habitat having cellulases. In this study a distinction between resident gut bacteria and transient gut bacteria was made. Results suggest that some habitat bacteria remain viable in the guts of *U. africana,* but not in *C. kraussi.*

Introduction

The role of microflora as food, symbiont, or competitor in the nutrition of marine invertebrate detritivores is not clearly understood. It has been pro-

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posed that the availability of nutrients derived from refractory plant detritus to invertebrate detritivores depends on decomposition and protein enrichment by microbes [28, 57], and direct utilization of microheterotrophs associated with detritus has been documented [e.g., 4, 27, 32, 33]. The microheterotrophic community does not, however, necessarily meet the requirements of animals ingesting detritus nonselectively [11]. Moreover, many marine deposit- and detritus-feeding invertebrates possess enzymes enabling the assimilation of structural components of detritus to some degree [e.g., 1, 13, 19, 22, 35, 38]. However, few studies have attempted to separate the action of endogenous enzymes from those of ingested or gut bacterial enzymes [44, 60].

While a few terrestrial invertebrate detritivores possess endogenous enzymes capable of hydrolyzing structural components of detritus (e.g., termites, for review see reference 8), both microbial symbionts and ingested bacterial enzymes are recognized to contribute significantly to detritus utilization [e.g., 15, 39, 42]. A substantial literature exists on the arthropod gut as an environment for microorganisms (for review see reference 5), and the relationship between gut microflora and host invertebrates (for reviews see references 8 and 55). The presence of gut microorganisms and their interaction with macrofauna are likely to be important considerations in the utilization of refractory detrital material in most ecosystems.

There are comparatively few studies on the presence of gut microflora, their digestive capabilities, and the relative importance of endogenous and microbial enzymes in marine detritivores. Although it has long been recognized that some marine invertebrates harbor a gut microflora [12], the presence of gut microflora in a range of aquatic invertebrate groups has only more recently been investigated. Gut microbiota have been reported in some Mollusca [e.g., 46, 59], Echinoidea [e.g., 21, 47], Annelida [e.g., 18] and Crustacea [e.g., 54, 61]. Gut bacteria capable of digesting refractory material have been reported for some aquatic detritivores [e.g., 17, 59], whereas other invertebrates possess diverse gut microflora that do not appear to facilitate the digestion of structural carbohydrates [e.g., 44, 51]. The distinction between bacterial enzymes originating from resident gut symbionts and those from acquired (ingested) bacteria incubated in the host gut has not been investigated. More information on the types and digestive capabilities of the gut bacteria of a range of invertebrate groups is required if the role of microflora in the digestive physiology of marine invertebrate detritivores is to be clarified.

We examined the nature of the gut contents and the presence of gut bacteria in two saltmarsh detritivores, the filter-feeding mud-prawn *Upogebia africana* and the deposit-feeding sand-prawn *Callianassa kraussi*. In addition, we investigated the types and enzyme capabilities of bacteria isolated from the guts and habitats of the prawns, and attempted to differentiate between gut residents, ingested environmental bacteria, and free-living environmental bacteria.

Materials and Methods

Fieldwork

Fieldwork was carried out at Langebaan lagoon (33°00′S–33°13′S and 17°57′E–18°08′E), a partially enclosed marine system situated on the southwest coast of South Africa. Samples were collected

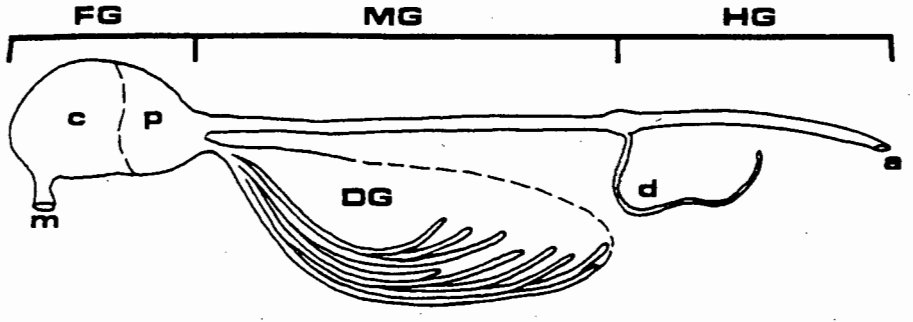

Fig. 1. Diagram of the digestive systems of *U. africana* and *C. kraussi* to show the different regions investigated, the main sites of food processing and absorption, and enzyme secretion. FG = foregut, m = mouth, c = cardiac region of foregut (*C. kraussi* = grinding mill, *U. africana* = modified as a filter), p = pyloric region of foregut (filtering setae sort food; small particles enter DG), DG = digestive gland (blind-ending tubes, enzymes secretion and food absorption, paired). MG = midgut (absorption, enzyme secretion). HG = hindgut (chitinous lining), d = diverticulum, a = anus.

at two sites: Geelbek in the muddy upper reaches of the lagoon where the mud-prawn *U. africana* predominates, and Oesterwal near the mouth of the lagoon where the substrate is sandy and the sand-prawn *C. kraussi* predominates. Ambient temperatures in the lagoon range from 14°C to 24°C [52].

Nature of Gut Contents and Microbiota

The nature of gut contents and the presence of ingested and resident gut microbiota in both *U. africana* and *C. kraussi* were investigated by scanning electron microscopy (SEM). The guts were removed within 30 min of collection, dissected into foregut, midgut, hindgut, and digestive gland, and fixed in 2.5% glutaraldehyde in 0.2 μm filtered seawater for 48 hours at 4°C. Fecal pellets were also collected for examination by SEM. Animals were transported to the laboratory within 2 hours and placed in glass beakers containing 0.2 μm filtered, autoclaved seawater at 14°C. In order to minimize the opportunity for takeover of the gut flora by ambient microbes, the water was kept at the lower end of the ambient temperature range and changed every 4 hours until all animals had cleared their guts (up to 20 hours and 35 hours, respectively, for *C. kraussi* and *U. africana*). Fecal pellets were removed from the beakers with sterile pasteur pipettes every hour to minimize microheterotrophic proliferation and colonization. All fecal pellets were fixed immediately in 2.5% glutaraldehyde in 0.2 μm filtered seawater for 48 hours at 4°C. All samples were then desalinated (100, 75, 50, 25, 0% filtered seawater in distilled water), passed through a graded alcohol series from 10% to absolute ethanol, dried in a Polaron Critical Point Dryer using liquid CO_2 as the transitional fluid, and mounted on aluminium stubs. During mounting, gut sections were split longitudinally to reveal gut contents and internal structure. Samples were then coated with gold paladium and viewed using a Cambridge S200 series Scanning Electron Microscope. Micrographs were taken of the contents and walls of foregut, midgut, hindgut, and digestive gland, and the fecal pellets (Fig. 1). The size of particulate fragments and the presence of meiofauna, diatoms, Protozoa, and bacteria was noted for each sample.

Isolation of Bacteria

Bacteria were isolated from the guts, digestive glands, and the habitats of both *U. africana* and *C. kraussi*:

A. Isolation of Gut Bacteria. An attempt was made to differentiate between the plateable bacterial community occurring in the guts of fresh animals (i.e., both ingested and resident bacteria) and that of animals which had voided all gut contents (i.e., only resident bacteria).

a) Transitory (ingested) and resident bacteria. Whole guts and digestive glands were removed with flame-sterilized instruments from five freshly collected similar-sized prawns of each species and homogenized with sterile glass tissue grinders in 5 ml autoclaved 0.2 μm filtered seawater. Three 200-μl aliquots from each homogenate were then spread onto 1% peptone seawater agar (SWA) plates [50] and incubated for 5 days at 20°C. These samples are referred to as samples from "fresh guts" hereafter in the text.

b) Resident bacteria only. Similar-sized animals were collected and brought back to the laboratory within 2 hours. They were then maintained at 14°C in beakers containing aerated (0.2 μm screened) 0.2 μm filtered seawater for a period greater than the gut clearance time. Their guts were thus emptied of ingested contents, but, resident bacteria adhering to the gut walls nevertheless remained. In order to minimize the proliferation of environmental microbes, the water in the beakers was kept at the lower end of the ambient temperature range, and water was changed approximately every 4 hours. Whole guts were then removed from the animals and processed in the same way as those from fresh animals. These samples are referred to as samples from "flushed guts" hereafter in the text.

B. Isolation of Free-Living Habitat Bacteria. Three 100-μl water samples were collected from the burrows of each prawn species on a receding tide, spread onto 1% peptone SWA plates, and incubated for 5 days at 20°C.

Thirty-two colonies were picked off a plate from each of the above sources, i.e., fresh and flushed guts, digestive glands, and the environment of each species. Bianchi and Bianchi [3] determined that 20 to 30 strains are sufficient to obtain a representative diversity of bacterial communities in marine sediment samples.

All the colonies within a sector of the plate were isolated. Pure colonies were obtained by restreaking at least three times. All 32 isolates from each source were then tested for various properties and were used for subsequent enzyme assays.

Characterization of Isolates

All isolates were tested for the following properties or activities: gram stain, motility, oxidase (oxidase reagent), catalase (3% H_2O_2), agarolytic activity (Gran's test) and fermentation of glucose (Tetrazolium indicator plates) [6], and were classified according to the following simple scheme [10, 23, 43, 53] (all isolates were gram negative):

Pseudomonas	= motile, non-fermentative
Acinetobacter	= non-motile, non-fermentative
Vibrio	= motile, fermentative, oxidase positive
Enterobacteriaceae	= fermentative, oxidase negative
Flavobacter/Cytophaga	= pigmented, no agarase
Unknown fermenter (X-ferm)	= non-motile, fermentative, oxidase positive

Degradative Enzymes of Bacterial Isolates

The utilization of a variety of substrates by bacterial isolates was determined by plate assays for amylase, cellulase, chitinase, agarase, alginase, gelatinase, protease, lipase, and lysozyme activities (Table 1). Assay plates were inoculated by toothpick from pure isolated source colonies maintained on seawater agar (SWA).

Table 1. Plate assays for degradative enzymes of bacteria isolated from the habitats and digestive systems of *U. africana* and *C. kraussi*

Enzyme	Substrate	Incubation	Visualization	Reference
Amylase	0.5% (w/v) potato starch in basal agar (BA)[a]	12–18 hours	Clear zones following flooding with Lugol's iodine	14
Cellulase	1% CMC in BA	3 days	Clear zones following flooding with congo red and dilute HCl	56
Chitinase	3% precipitated chitin in 1.5% agar in seawater overlaying BA layer	5 days	Clear halos after one week	48
Agarase	1.5% agar in nutrient-poor (1/10) seawater broth (SWB)[b]	2 days	Clear zones following flooding with Gran's iodine	29
Alginase	2.5% buffered Na alginate in 1.2% agar overlaying BA	2 days	Flood with acetone	36
Gelatinase	1% gelatine in BA	12–18 hours	Flooding with acetone	14
Protease	1% skim milk in BA	2 days	Clear zone in opaque medium	34
Lipase	1% Tween 80 in BA	3 days	Zones of opacity in clear medium	30
Lysozyme	Heat-killed target bacteria (*Pseudomonas* sp.) in 0.8% agar	3 days	Clear zone in opaque medium	50

[a] Basal agar (BA) = peptone, 1 g; yeast extract, 1 g; agar 15 g; filtered seawater 1 liter
[b] Seawater broth (SWB) = peptone, 5 g; yeast extract, 1 g; filtered seawater 1 liter

Data Analysis

Enzyme activities of the isolate collections from the different sources (digestive systems of the two different species and their habitats) were compared using correspondence analysis [58], a multivariate graphic display technique which provides a simultaneous display of rows (i.e., isolates) and columns (i.e., enzymes). This procedure enables identification of not only isolates that are similar with respect to enzyme activities, but also of enzymes that are important in determining the similarities. The 1st, 2nd, 3rd, and 4th axes were considered and were found to represent at least 75% of the information contained in the data set. In addition, chi-square analysis was used to test for differences in the digestive capabilities of bacterial isolate collections from the different sources. For this analysis it was assumed that the variables (enzymes) were independent of one another.

Results

Nature of Gut Contents

Contents of the foregut of *U. africana* (Fig. 2a) comprised large plant fragments (up to 50 μm long), whole and fragmented pennate diatoms (5–50 μm) and unidentified coccoid bodies (20–30 μm diameter) with attached bacteria (cocci, 2–3 μm) (Fig. 2b), all embedded in a matrix of very fine organic material

(particles as small as 1 μm). No remnants of meiofauna were observed. Midgut contents (Fig. 2c) consisted of large plant fragments, numerous whole diatoms and fragmented diatom frustules, partially degraded ciliated protozoa (10–15 μm diameter), and a few attached bacteria in a fine organic matrix. Large portions of the contents were coated with and penetrated by filamentous bacteria (0.5–1 μm diameter, approximately 10 μm long) (Fig. 2d). The hindgut contents (Fig. 2e) and fecal material were similar to midgut contents although the plant fragments were only up to 25 μm long, and no protozoans and very few whole diatom frustules and filamentous bacteria were evident. The fecal material was not bound in a peritrophic membrane.

Gut contents of *C. kraussi* (Fig. 2f–j) comprised large plant fragments (up to 50 μm long), diatom fragments, partially digested ciliated protozoans (8–10 μm), and variously sized coccoid cells (1–4 μm) in a matrix of fine organic material (Fig. 2f and g). Small, attached bacteria were evident on some fragments. Towards the posterior part of the midgut there was a concentration of coccoid cells (1–4 μm diameter) and filamentous bacteria coating the food (Fig. 2h). Large plant fragments and whole and fragmented diatom frustules were present in the hindgut contents (Fig. 2i). Very few filamentous bacteria were present in this region. The fecal matter was enclosed in a peritrophic membrane (Fig. 2j) and was similar in composition to the hindgut contents.

Gut Lining and Associated Resident Microflora

The foregut of *U. africana* is modified as a filter to separate large and small particles [49]. Viewed under SEM, no resident microflora were observed in this region. Similarly, examination of the foregut of *C. kraussi,* which consists of a grinding anterior gastric mill and filtering posterior region (unpublished data), revealed no resident microflora.

Examination of longitudinally opened sections of the guts by SEM (Fig. 3) revealed that the internal wall of the midgut of *U. africana* was folded longitudinally and had a microvillous lining completely coated in places by a mass of filamentous bacteria (approximately 0.5 μm diameter × 10 μm length) (Fig. 3a). The epithelium of *C. kraussi* midgut was a microvillous surface, with spherical objects of 1 to 8 μm diameter with rough or smooth surfaces commonly associated with it (Fig. 3b). Other areas of the midgut lining were smooth or covered with filamentous bacteria (very fine, 0.2 × 5 μm) (Fig. 3c and d).

The hindgut region of *U. africana* was longitudinally folded with convolutions of the smooth lining (Fig. 3e and f). Extensive single layered mats of epimural

Fig. 2. Scanning electron micrographs of the gut contents of freshly collected specimens of *U. africana* and *C. kraussi. U. africana*: **a** Foregut. Large plant fragments, diatoms, fine organic matter. **b** Foregut. Coccoid bodies and attached bacteria. **c** Midgut. Large plant fragments, diatom pieces. **d** Midgut. Filamentous bacteria coating contents. **e** Hindgut. Plant fragments, broken diatom frustules, fine organic matter. *C. kraussi*: **f** Large plant fragments in a matrix of organic matter. **g** Pennate diatoms, whole and fragmented. **h** Midgut. Filamentous bacteria coating contents. **i** Hindgut. Plant fragments, diatom frustules. **j** Faecal matter enclosed in peritropic membrane. Scale bars: **a–c, f, h** 50 μm; **d, e** 20 μm; **g** 19 μm; **i** 10 μm; **j** 200 μm.

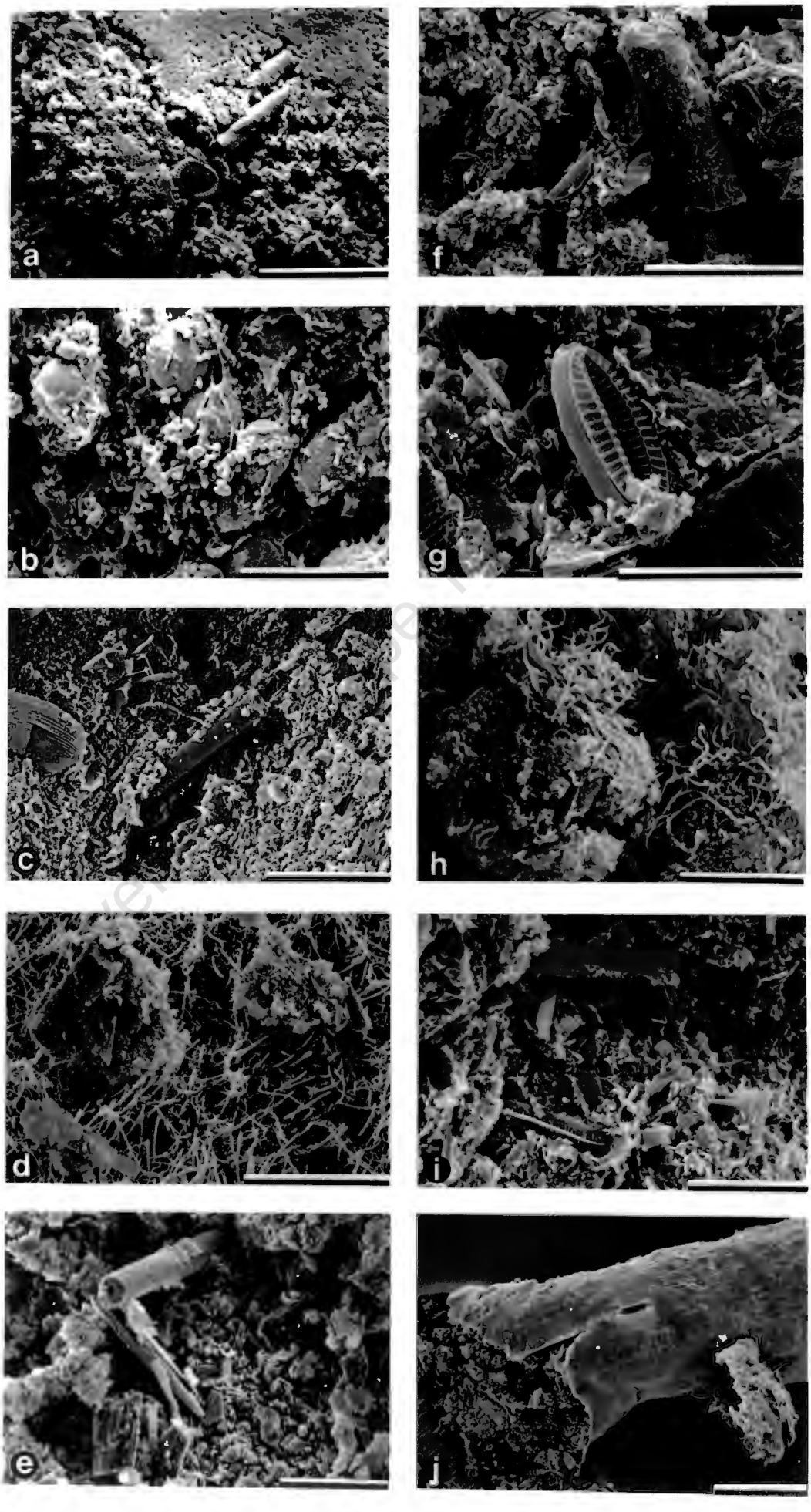

Figure 2

rod bacteria (0.5 μm diameter and ±2 μm long) were associated closely with the epithelium (Fig. 3e). These large areas of the lining, which were extensively colonized by a single bacterial morphotype, alternated with longitudinally orientated, bacteria-free panels of convoluted smooth epithelium (Fig. 3f). In addition, clumps of attached chains of large rod bacteria (approximately 1.5 μm diameter × 3 μm long) were frequently observed in the hindgut region (Fig. 3f). The hindgut wall of *C. kraussi* was also folded longitudinally and had two characteristic areas which alternated as longitudinally orientated panels, i.e., a smooth convoluted lining and a brush border lining (Fig. 3g). In some individuals epimural rod bacteria were present but were not as extensive or as closely packed as in *U. africana* (Fig. 3h).

The digestive glands of both species had compartmentalized walls in which no bacteria were apparent. The lining of the lumen was, however, colonized by numerous irregularly-shaped cells (2–3 μm) in both *U. africana* (Fig. 3i) and *C. kraussi* (Fig. 3j).

Gut and Habitat Microflora

Thirty-two colonies of bacteria were initially isolated from each source, and all isolates that remained viable were tested for a number of properties to enable classification to genera or families, and to determine enzyme activities on a variety of substrates.

Genera

Table 2 gives the number of isolates of each genus/family isolated from the digestive systems and habitats of the two species of prawn. Representatives of the genera *Vibrio, Pseudomonas, Acinetobacter, Flavobacterium,* and *Cytophaga,* the family Enterobacteriacea and the unidentified physiotype X-ferm were identified. The most common type isolated from fresh guts of *U. africana* was X-ferm, whereas members of the genus *Acinetobacter* were the most common bacteria in the collection from the fresh guts of *C. kraussi.* The resident bacterial populations isolated from *U. africana* and *C. kraussi* were dominated in the gut by strains of the genera *Vibrio* and *Pseudomonas,* whereas the digestive glands were dominated by the X-ferm physiotype (*U. africana*) and *Pseudomonas* strains (*C. kraussi*). The collections of isolates from the habitats of the two species were dominated by strains of the genus *Acinetobacter.* Bacteria

Fig. 3. Scanning electron micrographs of the midgut and hindgut linings and the digestive glands of *U. africana* and *C. kraussi.* **a** *U. africana* midgut microvillous lining coated by filamentous bacteria. **b** *C. kraussi* midgut microvillous surface and spherical objects. **c, d** *C. kraussi* midgut lining covered by filamentous bacteria and coccoid bodies. **e, f** *U. africana* hindgut convoluted smooth epithelial lining covered by extensive panels of epimural rod bacteria. Clumps of attached chains of large rod bacteria. **g, h** *C. kraussi* hindgut. Brush border lining and areas with epimural rod bacteria. **i** *U. africana.* Irregular shaped cells in lumen of digestive gland. **j** *C. kraussi.* Irregular shaped cells in lumen of digestive gland. Scale bars: **a** 100 μm; **b, c, f, g,** 20 μm; **d, e, h–j** 10 μm.

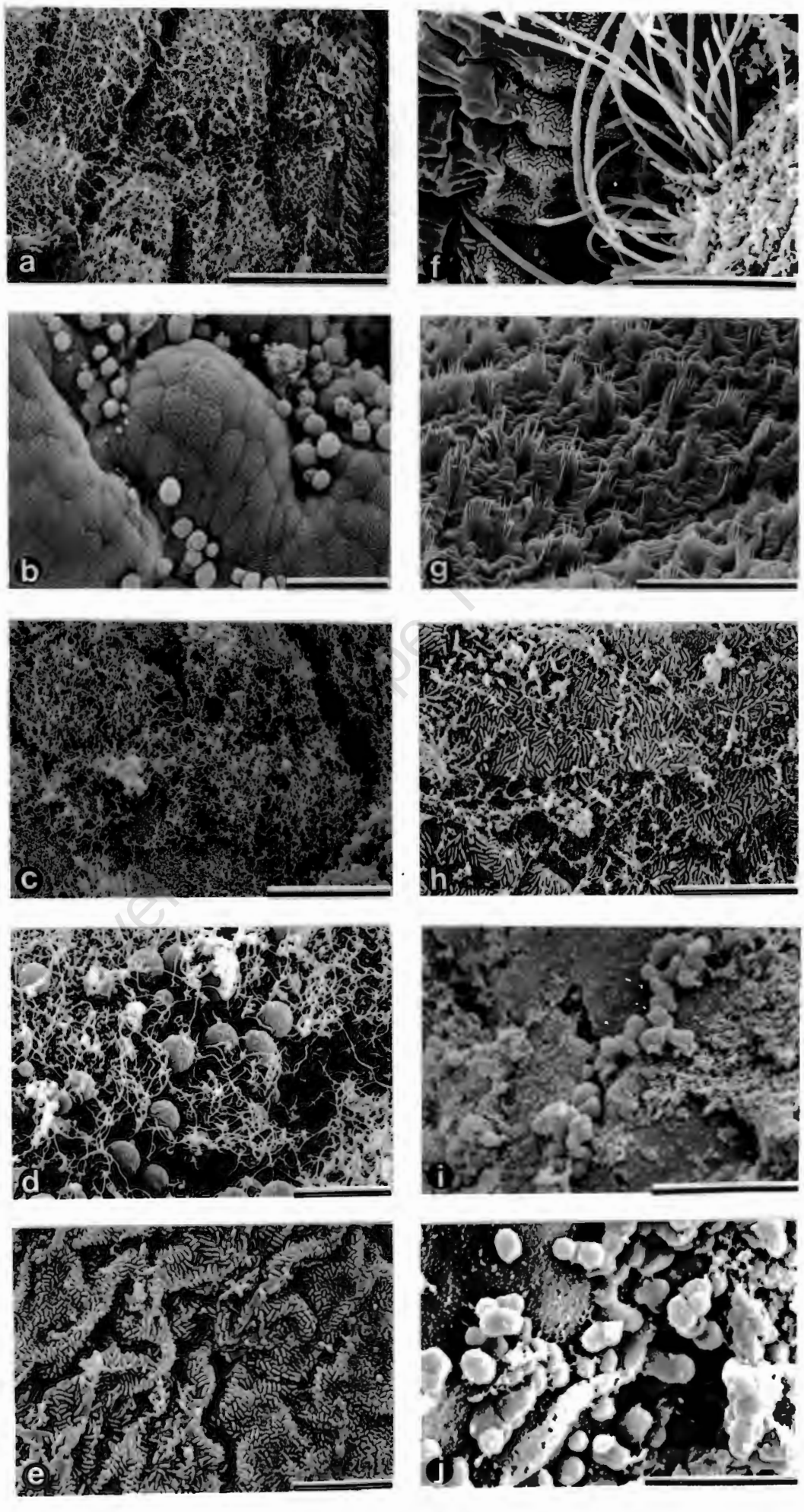

Figure 3

Table 2. Bacteria isolated from the digestive systems and habitats of *U. africana* and *C. kraussi*

	Number of isolates							
	U. africana				*C. kraussi*			
Genus/family	Fresh gut	Flushed gut	Digestive gland	Habitat Geelbek	Fresh gut	Flushed gut	Digestive gland	Habitat Oesterwal
Fermentative								
Vibrio	2	15	0	1	1	8	4	0
Enterobacteriaceae	1	0	0	2	0	0	2	0
X-ferm	8	1	32	5	4	3	1	1
Nonfermentative								
Pseudomonas	2	7	0	3	2	6	19	11
Acinetobacter	4	1	0	13	5	4	5	20
Pigmented								
Flavobacterium/ Cytophaga	4	0	0	7	2	1	1	0
Unidentified	5	11	0	2	6	8	3	0
Total no. of isolates	26	35	32	33	20	30	35	32

isolated from the habitat of *U. africana* were more diverse (including all the types found in this study) than those from the habitat of *C. kraussi* (comprising members of the genera *Acinetobacter, Pseudomonas,* and the physiotype X-ferm only).

Properties and Enzyme Activities

All isolates were gram-negative aerobes or facultative anaerobes (Table 3). A large percentage (69%) of the bacteria isolated from flushed guts (i.e., residents) of *U. africana* were motile, while isolates from fresh guts and the habitat were mostly nonmotile (31 and 15% motility, respectively). The single species isolated from the digestive gland of *U. africana* was found to be nonmotile. Bacterial collections from fresh and flushed guts, and from the habitat of *C. kraussi,* comprised fairly low percentages of motile isolates (14, 50, and 34%, respectively), whereas a large percentage (71%) of bacteria isolated from the digestive gland was motile (Table 3). Relatively small proportions (39 and 29% and 62 and 40%, respectively) of the bacteria isolated from the guts of *U. africana* and *C. kraussi* were found to be fermentative (Table 3), whereas the bacterial collections from the habitats of each prawn species had fairly high incidences of fermentative ability (58 and 94%, respectively, for *U. africana* and *C. kraussi.* A large proportion (71%) of the bacteria isolated from the digestive gland of *C. kraussi* was found to be fermentative.

A high percentage of resident bacteria isolated from flushed guts of *U. africana* and *C. kraussi* showed chitinase, protease, and lipase activity (Table 3), but there was a low incidence of alginase and gelatinase activity. Cellulase activity was not found in the collections of bacteria isolated from flushed guts of either

Table 3. The percentage of bacterial isolates from the digestive systems and habitats of *U. africana* and *C. kraussi* showing a positive response to the characterization tests and possessing the assayed digestive enzymes

	Source of isolates							
	U. africana				*C. kraussi*			
Test	Fresh gut	Flushed gut	Digestive gland	Habitat Geelbek	Fresh gut	Flushed gut	Digestive gland	Habitat Oesterwal
Gram negative	100	100	100	100	100	100	100	100
Motile	31	69	0	15	14	50	71	34
Oxidase	73	77	100	58	57	87	51	69
Catalase	54	66	100	55	62	80	17	44
Fermentation glucose	39	29	0	58	62	40	71	94
Digestive enzymes								
Amylase	15	71	0	0	48	57	31	0
Cellulase	4	0	0	3	0	0	0	0
Alginase	0	6	0	39	0	0	11	16
Agarase	27	51	0	79	10	47	26	72
Chitinase	62	77	0	9	57	90	31	13
Gelatinase	23	17	100	67	5	23	71	97
Protease	35	74	100	39	62	80	51	66
Lipase	58	74	100	70	71	77	34	97
Lysozyme	12	23	0	0	0	33	0	0
No. of isolates	26	35	32	33	21	30	35	32

species of prawn. Although the incidence of resident isolates possessing a lysozyme was fairly low, i.e., 23% for *U. africana* and 33% for *C. kraussi,* it is notable that only these two collections (flushed guts) and *U. africana* fresh gut included isolates with this ability. High proportions of the isolates from the digestive glands of the two species of prawn possessed gelatinase and protease ability.

Isolates from the habitats of both *U. africana* and *C. kraussi* showed high agarase, gelatinase, and lipase activity but no lysozyme and low chitinase activity (Table 3). Protease activity was relatively high (66%) in the collection of isolates from the habitat of *C. kraussi,* compared to that of isolates from the habitat of *U. africana* (39%). Whereas none of the isolates from *C. kraussi*'s habitat possessed a cellulase, 3% of isolates from the habitat of *U. africana* displayed cellulase activity. This finding is significant as it is only in the *U. africana* habitat and fresh gut (4%) collections that cellulase activity was found.

Isolate collections from fresh guts of the two species did not show especially high incidences of any enzyme activities (Table 3). Chitinase (77%) and lipase (74%) frequencies are high in collections from flushed guts of *U. africana,* but are only 62 and 58%, respectively, in collections from fresh guts. Similarly, the incidences of protease and lipase activities are somewhat lower in fresh (62 and 71%, respectively) than flushed (80 and 77%, respectively) bacterial gut collections in *C. kraussi.* Four percent of the isolates from the fresh gut of *U. africana* possessed cellulase ability, whereas none of those from *C. kraussi* displayed this ability.

Table 4. Chi-square values for comparison of the digestive capabilities of bacterial isolate collections from the digestive systems and habitats of *U. africana* and *C. kraussi*

Isolate collections compared	Chi-square value	d.f.	Significant difference ($P < 0.01$)
U. africana			
Fresh gut : flushed gut	40	17	+
Fresh gut : habitat	147	17	+
Flushed gut : habitat	214	17	+
C. kraussi			
Fresh gut : flushed gut	46	17	+
Fresh gut : habitat	213	17	+
Flushed gut : habitat	216	17	+
Inter-species comparisons			
Fresh guts (*U. africana* : *C. kraussi*)	61	17	+
Flushed guts (*U. africana* : *C. kraussi*)	11	17	−
Habitats (Geelbek : Oesterwal)	26	17	−

The collections of isolates from the fresh and flushed guts of *U. africana* differed significantly in terms of the incidences of enzyme activities ($\chi^2 = 40$, $P < 0.01$) (Table 4). Furthermore, both populations isolated from guts differed significantly from the habitat populations in terms of digestive capabilities. However, when fresh and flushed gut isolate collections are compared with the habitat, it is apparent that the chi-square value for flushed gut vs habitat (214) is much greater than that for fresh gut vs habitat (147) isolate collections. This suggests that collections of bacteria from flushed guts have fewer enzymes in common with the habitat bacteria than do collections of bacteria from fresh guts. Collections of bacterial isolates from fresh guts probably represent a mixture of resident gut isolates and habitat isolates that have remained viable inside the guts, although some diet selection and digestive screening has occurred.

If collections of bacteria from fresh and flushed guts and the habitat of *C. kraussi* are compared in the same way (Table 4), it can be seen that fresh and flushed gut populations are significantly different ($\chi^2 = 46$, $P < 0.01$), and when they are compared with the habitat the chi-square values obtained are large and of similar magnitudes (213 and 216). This result suggests that habitat bacteria do not survive long in the guts of the animal.

When comparisons were made interspecifically it was found that fresh guts of the two species had significantly different bacterial populations ($\chi^2 = 61$, $P < 0.01$) in terms of digestive capabilities, whereas the flushed gut and habitat populations of the two species did not differ significantly (Table 4).

The percentage of isolates possessing activity (Table 3) is an index of the bacterial collection's digestive ability as a whole. Correspondence analysis plots each individual isolate according to its complete suite of enzymes (Fig. 4). Similar isolates are plotted close together and closest to the properties that best describe their digestive capabilities. Enzymes that are correlated will be plotted close together. Figure 4 shows the clustering of the isolates according to digestive

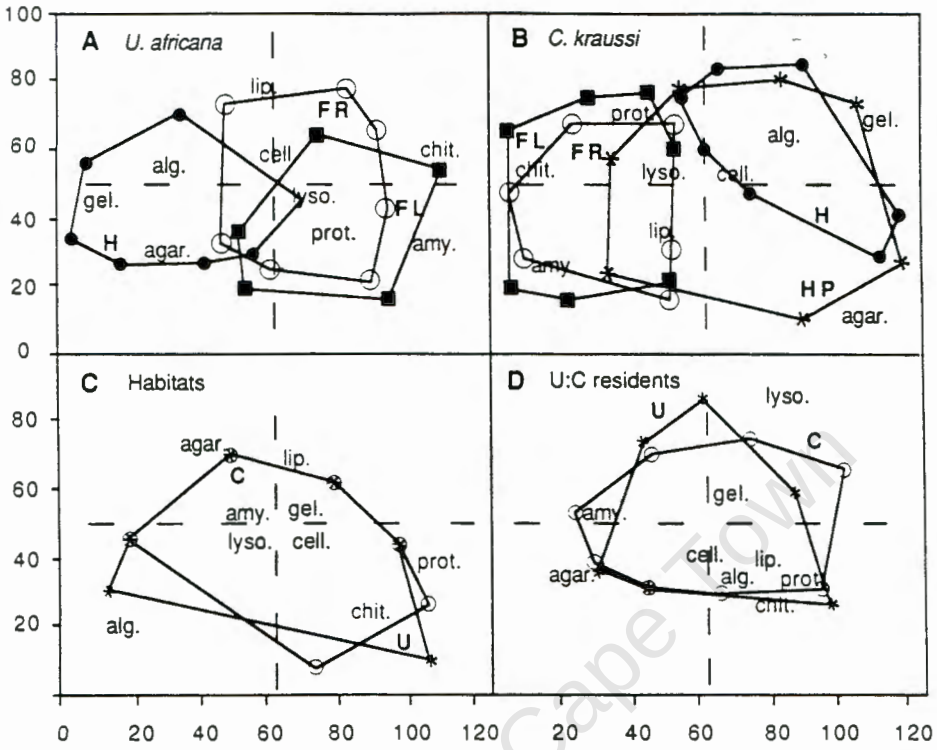

Fig. 4. Comparison by correspondence analysis of the enzyme activities of bacteria isolated from the digestive systems and habitats of *U. africana* and *C. kraussi*. Similar isolates are plotted close together, and closest to the properties (enzymes) that most define their digestive capabilities. Enzymes that frequently co-occur are plotted close together. The vertical axis represents axis 1, while the horizontal axis represents axis 2. The numbers are relative and have no units. **A** *U. africana* fresh gut (FR), flushed gut (FL), and habitat (H) isolates. **B** *C. kraussi* fresh gut (FR), flushed gut (FL), and habitat (H) and hepatopancreas (digestive gland) isolates (HP). **C** *U. africana* (U) and *C. kraussi* (C) habitat isolates. **D** *U. africana* (U) and *C. kraussi* (C) flushed gut isolates. Enzymes: agar. = agarase, amy. = amylase, alg. = alginase, lip. = lipase, lyso. = lysozyme, prot. = protease. The solid lines link perimeter data points for FR, FL, H, HP, C, and U within which isolates fall.

capabilities on axes 1 and 2. The first four axes represented 75% of the total variance in the data for Fig. 4A and 4B, 79% for Fig. 4C, and 89% for Fig. 4D.

For *U. africana* (Fig. 4A) the resident gut (flushed gut) population has a different suite of enzymes from that isolated from the habitat. Isolates from fresh guts are intermediate. The position of the resident gut population on the plot is influenced most strongly by the high incidence of chitinase (and protease) ability in this collection, whereas the distribution of the isolates from the habitat is influenced most strongly by a high incidence of gelatinase (and agarase) ability. Isolates from the digestive gland of *U. africana* were not included in this analysis as they comprised a single strain only.

Analysis of substrate utilization ability of isolates from the digestive system and habitat of *C. kraussi* shows a similar separation of isolates from the gut and habitat according to different digestive capabilities (Fig. 4B). Gut microflora cluster according to chitinase and protease ability, while habitat microflora cluster according to gelatinase (and agarase) ability. Isolates from flushed guts (i.e., residents) are, however, similar to those from fresh guts. Isolates from *C.*

kraussi digestive gland appear to have fairly diverse capabilities and overlap with both gut and habitat isolates (Fig. 4B). In the fourth axis (not shown), however, the digestive gland isolates have different enzyme profiles relative to either the gut or habitat isolates, indicating that it is a distinct flora.

A comparison of the two habitats by correspondence analysis (Fig. 4C) revealed that in terms of enzyme activities the bacterial collections are very similar, having high incidences of isolates with agarase, lipase, and gelatinase activity. Comparison of substrate utilization abilities of collections of isolates from flushed guts (i.e., gut residents) of *U. africana* and *C. kraussi* (Fig. 4D), showed that the two populations are very similar in terms of enzyme profiles. The most important similarities are high chitinase, protease, and lipase activity, low alginase and gelatinase activity, and the absence of cellulase activity in both collections.

Discussion

Both the scanning electron microscopic (SEM) examination of the guts and their contents and the isolation of gut bacteria indicate that a diverse microflora is present in the guts of both species of prawn. Bacteria were observed in and isolated from the guts of both immediately processed (fresh) animals and from those which had voided food and transient bacteria. This indicates that while bacteria are ingested with the food, there is also a permanent, resident bacterial population in both species of prawn.

Gut contents in both species of prawn comprise plant fragments, whole and fragmented diatoms, partially digested protozoa and particle-attached bacteria, all embedded in a matrix of fine organic matter. Although the gut contents of the two prawns appear similar, it is probable that they are derived differently. The foregut of *U. africana* is modified to sort small particles by filtration, whereas *C. kraussi* possesses a more typical foregut with a gastric mill to masticate larger particles. The presence of filamentous bacterial masses coating and penetrating the food in the midgut suggests that bacterial proliferation and growth occurs on the food. The absence of these filaments on hindgut contents and fecal material raises the possibility that these bacteria are lysed and utilized by the prawns as a trophic supplement, such as nitrogen. Although the presence of these filaments in the midgut may be related to the specific conditions in this region (resident filamentous bacteria coat the walls of the guts of animals that have voided all contents), the production of these bacteria inhabiting and utilizing the ingested food may be of energetic importance to the prawns. The potentially important phenomenon of very rapid growth inside the guts of detritivores has been demonstrated by Plante et al. [45] for the deposit-feeding polychaete *Abarenicola vagabunda*, although nutritive advantage to the host is treated as unlikely. In the latter study, however, bacterial proliferation was highest in the hindgut. This suggests that, in contrast to the indications for *U. africana*, the production of the bacteria incubated in the guts of the polychaete is passed out into the environment and not utilized by the animal.

Examination of the gut linings of *U. africana* and *C. kraussi* by SEM revealed the presence of novel resident gut microflora. No symbiotic gut bacteria were

observed in the foreguts of either species of prawn. Of interest is the presence of filamentous bacterial masses coating parts of the gut lining in the midguts of both *U. africana* and *C. kraussi.* This phenomenon has not been recorded previously for marine invertebrates, but similar, very thin bacterial filaments are reported to occur widely in the guts of insects and millipedes [5] and in some mammals [37].

In addition, numerous unidentified spherical objects were observed associated with the microvillous lining of the midgut of *C. kraussi.* Structures which look identical to them under SEM have been reported in previous studies, and they have been variously identified as parasitic spores [40], mucus droplets [24, 40], or partially degraded phytoplankton cells [51]. We favor the suggestion that these structures are of physiological origin as there are areas in the midgut of *C. kraussi* where outgrowths of the wall appear to be budding ovoid structures. TEM studies are needed to clarify this issue.

The spectacular mats of epimural rod bacteria in the hindguts of *U. africana* (present but much less extensive in *C. kraussi*) have also not been reported previously in marine invertebrates. However, in a TEM study, Deming and Colwell [16] observed bacteria directly associated with the hindgut lining of a deep-sea, deposit-feeding holothurian. The absence of gut bacteria in the hindguts of three species of wood-boring marine isopods, examined by SEM, has been reported [7], although the methods in this study have been questioned [5]. Interestingly, the symbiotic rod bacteria seen in the hindguts of *U. africana* closely resemble the extensive mats of symbiotic epimural bacteria in the hindgut of the lower termite *Reticulitermes flavipes* [9]. Epimural bacilli have also been reported in a SEM study of the hindgut of the grasshopper *M. sanguinipes* [40]. Our results therefore do not support the suggestion by Bignell [5] that there are major differences with regard to microbial colonization of the hindgut wall in insects and crustaceans.

The fecal material of *C. kraussi* was enclosed in an intact peritrophic membrane, whereas that of *U. africana* was not. This may be significant in explaining the relative paucity of hindgut symbionts in *C. kraussi* compared to *U. africana.* Bignell [5] suggests that the peritrophic membrane in insects may be of importance in allowing colonization of the hindgut cuticle.

Although little is known about the detailed interactions between hindgut symbionts and the insect host, they are thought to be important in nitrogen conservation and acquisition [8]. Nitrogen fixation and the conservation of nitrogen derived from excretory products by bacteria isolated from terrestrial detritivores (e.g., termites) has been documented [42]. Very little information about the contribution of resident gut bacteria to the nitrogen budget of marine detritivores exists, although nitrogen fixation and the production of essential amino acids by gut bacteria of wood-eating shipworms has been demonstrated (for review see reference 20). In addition, Guerinot and Patriquin [25] showed that the resident microflora of the sea urchin *Strongylocentrotus droebachiensis* fix nitrogen, and that this is incorporated into the animal tissue. This raises the possibility that the hindgut bacteria observed in the *U. africana* may be of some nitrogen-related nutritive advantage to the prawn.

Numerous irregular shaped bacteria were observed in the lumens of the digestive glands of both *U. africana* and *C. kraussi.* We are unaware of similar

findings in any other marine invertebrates. Bignell [5] suggests that the caeca of crustaceans and insects are inaccessible to microorganisms, and that any found in this region are likely to be specialized symbionts.

The dominant genera of bacteria isolated from inside the digestive systems of both species of prawn differed from those isolated by the same methods from their respective habitats. The isolation of different populations of bacteria from the guts of marine invertebrates and from their habitats has been previously reported, e.g., the polychaete *Thelepus setosus* [18] and the prawn *Penaeus aztecus* [17].

The dominant genera isolated from the guts of both *U. africana* and *C. kraussi* were *Vibrio* and *Pseudomonas,* with the X-ferm physiotype and *Pseudomonas* strains dominant in the digestive glands of *U. africana* and *C. kraussi,* respectively. In addition to these dominant isolates, members of the family Enterobacteriacea (rare), and the genera *Acinetobacter, Flavobacterium,* and *Cytophaga* were also isolated from the digestive systems of both prawns. The presence of gram-negative, aerobic, or facultative anaerobic bacteria, especially *Vibrio* and *Pseudomonas,* in the guts of a variety of aquatic crustaceans has been reported recently [17, 41, 54, 61]. In contrast very few gram-positive or strictly anaerobic bacteria have been isolated from the guts of marine invertebrates [54]. The present study does not investigate the presence of strict anaerobes. In view of the role of anaerobes in the nutrition of terrestrial detritivores [8], this would be a worthwhile aspect to investigate in marine detritivores.

The dominance of *Acinetobacter* in both habitats investigated in the present study is similar to the finding of Austin et al. [2], who in a comparison of two estuarine systems found that *Acinetobacter* and *Vibrio* were common in Chesapeake Bay, whereas *Acinetobacter-Moraxellela* and *Caulobacter* predominated in Tokyo Bay.

The bacteria collections isolated from inside the guts of both species of prawns in the present study have different digestive abilities relative to the collections isolated by the same techniques from the respective habitats. The bacteria from the habitats have similar digestive abilities, both with respect to the incidences of specific enzymes in the different collections, as well as with respect to the suites of enzymes possessed by isolates from different collections.

Thus both the genera and the digestive capabilities of the bacterial populations isolated from the guts and from the habitats of both species of prawn are different. This suggests that both species of prawn may maintain a resident gut microflora distinct from that of their habitats.

Our results indicate that the resident bacteria isolated from both species of prawn do not appear to contribute significantly to the degradation of refractory plant matter, but have a spectrum of enzymes better suited for exploitation of the microheterotrophic community associated with it. A high percentage of resident bacteria isolated from flushed guts of *U. africana* and *C. kraussi* possessed protease, lipase, and chitinase activity. Resident isolates showed lysozyme activity. None of the bacteria isolated from flushed guts of either species of prawn showed cellulase activity. These findings support the possibility that gut bacteria may be implicated in the nitrogen budget of marine invertebrates. It has been suggested that gut bacteria aid the animal by increasing the pool of amino acids liberated from the main food source [21], implying

a role in nitrogen acquisition. Most studies have reported that gut bacteria do not possess cellulases and chitinases, and suggest that there is little evidence of significant bacterial involvement in the decomposition of complex polysaccharides by marine invertebrates [44, 47, 51, 59]. A few researchers, however, have reported gut bacteria able to utilize cellulose [17, 31] and chitin [41]. Thus, the possibility of symbiotic gut bacterial involvement in the utilization of the structural components of detritus cannot be discounted. The presence of epimural symbionts in the gut of *U. africana* that closely resemble those occurring in the guts of some insects, particularly termites, is interesting, particularly as a number of bacteria isolated from the guts of lower termites possess cellulase activity [8]. Due to the limitations of conventional isolation techniques, the bacteria seen in the SEM study may not be represented in the isolate collections.

If the capabilities and characteristics of isolates from fresh and flushed guts of both species of prawn are compared, there is some evidence that some habitat genera may remain viable in the guts of the animals, particularly *U. africana*. Firstly, an increased incidence of the predominantly habitat genus *Acinetobacter* was recorded for fresh compared to flushed guts of *U. africana*. Secondly, while there are clear differences in the digestive capabilities of flushed gut and habitat bacterial isolates, the fresh gut isolates of *U. africana*, however, possess intermediate ability, suggesting that some bacteria from the habitat remain viable inside the guts of the animals. This observation is significant, as these bacteria may be incubated and contribute enzymes (e.g., cellulases) for the digestion of food. A few of the habitat and fresh gut bacteria of *U. africana* possessed cellulase activity, while none of the resident gut bacteria showed cellulase activity. In addition, the incidence of fermentative ability was much higher in bacterial isolates from the habitat than from the flushed gut of the prawn. There is, however, less evidence that an increase in numbers of predominantly habitat genera occur in fresh as opposed to flushed guts of *C. kraussi*. Fresh gut enzyme profiles reflect flushed guts, i.e., habitat bacteria do not survive in the gut (are lysed) or are not ingested.

Incubated enzymes contributed by ingested microbes are recognized to play an important role in the digestion of refractory matter by terrestrial detritivores [39]. Gunnarsson and Tunlid [26] showed that not all bacteria ingested by the wood-eating marine isopod *Oniscus asellus* are lysed. Plante et al. [45] fed *Vibrio* and *Pseudomonas* bacteria to the polychaete *Abarenicola vagabunda* and observed a rapid growth of the ingested bacteria in the hindgut of the animal, although these authors do not suggest significant gains by the invertebrate host. The possibility that incubated microbial enzymes may be important in the digestive physiology of marine detritivores cannot be discounted.

In conclusion, this study reports the presence of symbiotic gut bacteria, not previously documented for marine invertebrates, in two species of marine detritivore prawns. Isolation techniques coupled with multivariate data analysis indicate that the populations of bacteria isolated from inside the guts may differ from those isolated from the habitats of the two species of prawn, with respect to both the genera of bacteria isolated and their digestive capabilities. Although there is little evidence that these isolated resident gut bacteria contribute to the carbon budgets of the prawns, they may contribute to their nitrogen budgets.

This study also highlights the possibility of incubation of habitat bacteria in the guts of marine detritivores.

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References

1. Adams SM, Angelovic JW (1970) Assimilation of detritus and its associated bacteria by three species of estuarine animals. Chesapeake Sci 11:249–254
2. Austin B, Garges S, Conrad B, Harding EE, Colwell RR, Simidu U, Taga N (1979) Comparative study of the aerobic, heterotrophic bacterial flora of Chesapeake Bay, and Tokyo Bay. Appl Environ Microbiol 37:704–714
3. Bianchi MAG, Bianchi AJM (1982) Statistical sampling of bacterial strains and its use in bacterial diversity measurement. Microb Ecol 8:61–69
4. Bianchi TS, Levinton JS (1984) The importance of microalgae, bacteria and particulate organic matter in the somatic growth of *Hydrobia totteni*. J Mar Res 42:431–443
5. Bignell DE (1984) The arthropod gut as an environment for microorganisms. Joint Symposium of the British Mycological Society and the British Ecological Society, University of Exeter, Chapter 8, pp 205–227
6. Bochner BR, Savageau MA (1977) Generalised indicator plate for genetic, metabolic, and taxonomic studies with microorganisms. Appl Environ Microbiol 33:434–444
7. Boyle PJ, Mitchell R (1978) Absence of microorganisms in crustacean digestive tracts. Science 200:1157–1159
8. Breznak JA (1984) Biochemical aspects of symbiosis between termites and their intestinal microbiota. In: Jennings DH, Lee DL (eds) Symbiosis: 29th Symposium of the Society of for Experimental Biology. Cambridge University Press, Cambridge, pp 559–580
9. Breznak JA, Pankratz HS (1977) In situ morphology of the gut microbiota of wood-eating termites (*Reticulitermes flavipes* (Kollar) and *Coptotermes formosanus* Shiraki). Appl Environ Microbiol 33:406–426
10. Buchanon RE, Gibbons NE (1979) Bergey's manual of determinative bacteriology. 8th ed. Williams and Wilkins, Baltimore, Maryland, 1,268 pp
11. Cammen LM (1980) The significance of microbial carbon in the nutrition of the deposit feeding polychaete *Nereis succinea*. Mar Biol 61:9–20
12. Colwell RR, Liston J (1962) The natural bacterial flora of certain marine invertebrates. J Insect Pathol 4:23–33
13. Crosby MP, Langdon CJ, Newell RIE (1989) Importance of refractory material to the carbon budget of the oyster *Crassostrea virginica*. Mar Biol 100:343–352
14. Cowan ST, Steel KJ (1970) Manual for the identification of medical bacteria. Cambridge University Press, London, New York
15. Cruden DL, Markovetz AJ (1979) Carboxymethyl cellulose decomposition by intestinal bacteria of cockroaches. Appl Environ Microbiol 38:369–372
16. Deming JW, Colwell RR (1982) Barophilic bacteria associated with digestive tracts of abyssal holothurians. Appl Environ Microbiol 44:1221–1230
17. Dempsey AC, Kitting CL (1987) Characteristics of bacteria isolated from penaeid shrimp. Crustaceana 52:90–94
18. Duchene JC, Imbaud P, Delille E (1988) Associated bacterial microflora of subantarctic polychaete worm *Thelepus setosus*. Arch Hydrobiol 112:221–231
19. Elyakova LA, Shevchenko NM, Avaeva SM (1981) A comparative study of carbohydrase activities in marine invertebrates. Comp Biochem Physiol 69:905–908
20. Felbeck H, Childress JJ, Somero GN (1983) Biochemical interactions between molluscs and

their algal and bacterial symbionts. In: The Mollusca, vol. 2. Environmental biochemistry and physiology. Academic Press, New York
21. Fong W, Mann KH (1980) Role of gut flora in the transfer of amino acids through a marine food chain. Can J Fish Aquat Sci 37:88–96
22. Foulds JB, Mann KH (1978) Cellulose digestion in *Mysis stenolepis* and its ecological implications. Limnol Oceanogr 23:760–766
23. Fukami K, Simidu U, Taga N (1981) Fluctuation of the communities of heterotrophic bacteria during the decomposition process of phytoplankton. J Exp Mar Biol Ecol 55:171–184
24. Gremare A (1988) Feeding, tube-building and particle-size selection in the terebellid polychaete *Eupolymnia nebulosa*. Mar Biol 97:243–252
25. Guerinot ML, Patriquin DG (1981) The association of N_2-fixing bacteria with sea urchins. Mar Biol 62:197–207
26. Gunnarsson T, Tunlid A (1986) Recycling of fecal pellets in isopods: Microorganisms and nitrogen compounds as potential food for *Oniscus asellus* L. Soil Biol Biochem 18:595–600
27. Hargrave BT (1970) The utilization of benthic microflora by *Hyalella azteca* (Amphipoda). J Anim Ecol 39:427–437
28. Hargrave BT (1976) The central role of invertebrate faeces in sediment decomposition. Br Ecol Soc Symp 17:301–321
29. Hodgson DA, Chater KF (1981) A chromosomal locus controlling extracellular agarase production by *Streptomyces coelicolor* A3 (2), and its inactivation by chromosomal integration of plasmid SCP 1. J Gen Microbiol 124:339–348
30. Holding AJ, Collee JG (1971) Routine biochemical tests. In: Norris JR, Ribbons DW (eds) Methods in microbiology, vol. 6A. Academic Press, New York, pp 19–21
31. Hood MA, Meyers SP, Colmer AR (1971) Bacteria of the digestive tract of the white shrimp *Penaeus setiferus*. Bacteriol Proc 48
32. Hylleberg J (1975) Selective feeding by *Abarenicola pacifica* with notes on *Abarenicola vagabunda* and a concept of gardening in lugworms. Ophelia 14:113–137
33. Jensen KT, Siegismund HR (1980) The importance of diatoms and bacteria in the diet of *Hydrobia*-species. Ophelia, Suppl 1:193–199
34. Kaneko T, Krichevsky MI, Atlas RM (1979) Numerical taxonomy of bacteria from the Beaufort Sea. J Gen Microbiol 110:111–125
35. Klumpp DW, Nichols PD (1983) Utilisation of the seagrass *Posidonia australis* as food by the rock crab *Nectocarcinus integrifons* (Latreille) (Crustacea: Decapodia: Portunidae). Mar Biol Lett 4:331–339
36. Laycock RA (1974) The detrital food chain based on seaweeds. I. Bacteria associated with the surface of *Laminaria* fronds. Mar Biol 25:223–231
37. Lee A (1980) Normal flora of animal intestinal surfaces. In: Bitton G, Marshall KC (eds) Adsorption of Microorganisms to surfaces. Wiley, New York, pp 145–173
38. Lucas MI, Newell RC (1984) Utilization of saltmarsh grass detritus by two estuarine bivalves: Carbohydrase activity of crystalline style enzymes of the oyster *Crassostrea virginica* (Gmelin) and the Mussel *Guekensia demissa* (Dillwyn). Mar Biol Lett 5:275–290
39. Martin MM (1984) The role of ingested enzymes in the digestive processes of insects. In: Joint Symposium of the British Mycological Society and the British Ecological Society, Chapter 6. University of Exeter, pp 155–171
40. Mead LJ, Khachatourians GG, Jones GA (1988) Microbial ecology of the gut in laboratory stocks of the migratory grasshopper, *Melanoplus sanguinipes* (Fab.) (Orthoptera: Acrididae). Appl Environ Microbiol 54:1174–1181
41. Ninawe A, Banik S (1987) Chitinolytic bacteria in *Penaeus-indicus*. J Aquacult Trop 2:89–92
42. O'Brien RW, Slaytor M (1982) Role of microorganisms in the metabolism of termites. Aust J Biol Sci 35:239–262
43. Oliver JD (1982) Taxonomic scheme for the identification of marine bacteria. Deep-Sea Res 29:795–798
44. Payne DW, Thorpe NA, Donaldson EM (1972) Cellulolytic activity and a study of the bacterial population in the digestive tract of *Scrobicularia plana* (Da Costa). Proc Malac Soc Lond 40: 147–160

45. Plante CJ, Jumars PA, Baross JA (1989) Rapid bacterial growth in the hindgut of a marine deposit feeder. Microb Ecol 18:29–44
46. Prieur D (1987) A review of the relationships between bivalve molluscs and bacteria in the marine environment. First Eliat Symposium on Marine Symbiosis 4:37–50
47. Prim P, Lawrence JM (1975) Utilization of marine plants and their constituents by bacteria isolated from the gut of Echinoids (Echinodermata). Mar Biol 33:167–173
48. Reichenbach H, Dworkin M (1981) The order *Myxobacteriales*. In: Starr MP, Stolp H, Truper HG, Balows A, Schegel HC (eds) The Prokaryotes, vol. 1. Springer-Verlag, New York, pp 360–376
49. Schaefer N (1970) The functional morphology of the fore-gut of three species of decapod crustacea: *Cyclograspus punctatus* Milne-Edwards, *Diogenes brevirostris* Stimpson, and *Upogebia africana* (Ortmann). Zoologica Africana 5:309–326
50. Seiderer LJ, Davis CL, Robb FT, Newell RC (1984) Utilisation of bacteria as nitrogen resource by kelp-bed mussel *Choromytilus meridionalis*. Mar Ecol Prog Ser 15:109–116
51. Seiderer LJ, Newell RC (1988) Exploitation of Phytoplankton as a food resource by the kelp bed ascidian *Pyura stolonifera*. Mar Ecol Prog Ser 50:107–115
52. Shannon LV, Stander GH (1977) Physical and chemical characteristics of water in Saldanha Bay and Langebaan lagoon. Trans Roy S Afr 42:441–459
53. Sieburth JM (1979) Sea microbes. Oxford University Press, New York
54. Sugita H, Ueda R, Berger LR, Deguchi Y (1987c) Microflora in the gut of Japanese coastal crustacea. Nippon Suisan Gakkaishi 53:1647–1655
55. Swift MJ, Boddy L (1984) Animal-microbial interactions in wood decomposition. In: Joint Symposium of the British Mycological Society and the British Ecological Society, Chapter 4. University of Exeter, pp 89–131
56. Teather RM, Wood PJ (1982) Use of Congo red-polysaccharide interactions in enumeration and characterization of cellulolytic bacteria from the bovine rumen. Appl Environ Microbiol 43:777–780
57. Tenore KR (1983) What controls the availability to animals of detritus derived from vascular plants: Organic nitrogen enrichment or caloric availability? Mar Ecol Prog Ser 10:307–309
58. Underhill LG, Piesach M (1985) Correspondence analysis and its applications in multielemental trace analysis. J Trace and Microprobe Techniques 3:41–65
59. Vitalis TZ, Spence MJ, Carefoot TH (1988) The possible role of gut bacteria in nutrition and growth of the sea hare *Aplysia*. Veliger 30:333–341
60. Wainwright PF, Mann KH (1982) Effect of antimicrobial substances on the ability of the mysid shrimp *Mysis stenolepis* to digest cellulose. Mar Ecol Prog Ser 7:309–313
61. Yasuda K, Kitao T (1980) Bacterial flora in the digestive tracts of prawns, *Penaeus japonicus* Bate. Aquaculture 19:229–234

CHAPTER 6

Characteristics of bacteria colonizing the hindgut and hepatopancreas of the detritivorous thalassinid prawn *Upogebia africana*.

ABSTRACT

The nature and physiological properties of bacteria in the hepatopancreas and hindgut of the saltmarsh burrowing detritivore thalassinid prawn *Upogebia africana* were investigated in this study. Transmission electron microscopy revealed the presence of mats of densely-packed gram-negative epimural rod bacteria attached to the epicuticle of the hindgut by a mucous layer. Unattached clumps of irregular-shaped bacteria were present in the lumen of the hepatopancreas. Viable counts indicate that both the hindgut and hepatopancreas are important sites of colonization by resident populations of bacteria. The dominant genus present in collections of bacteria isolated from the hindgut was *Pseudomonas* (30 isolates), while members of the genus *Vibrio* were also isolated (4 isolates). An unidentified fermenter was the only strain isolated from the hepatopancreas. Resident bacteria isolated from hindguts of *U. africana* exhibited amylase, gelatinase, protease and lipase activity, but not chitinase, cellulase or lysozyme activity. They are therefore unlikely to contribute to the digestion of refractory material by the prawn. Acetylene reduction assays resulted in the detection of low rates of nitrogen fixation associated with dissected digestive tracts of the prawn, and live prawns, while relatively strong rates of nitrogen fixation were associated with sediment from the habitat of the prawns. It was concluded that nitrogen fixation by gut microbes is insignificant in terms of the nitrogen budget of the prawn.

INTRODUCTION

Gut bacteria have been documented in the digestive tracts of a number of marine invertebrates (e.g. Colwell & Liston 1960, Duchene *et al.* 1988, Dempsey *et al.* 1989, Deming *et al.* 1981), and some are reported to contribute enzymes, e.g. cellulase (Musgrove 1988, Cutter and Rosenberg 1972), that may aid in the digestion of the food. Gut bacteria have also been reported to enhance the resistance of some invertebrates to adverse effects of ingested substances, such as phenolics (Dempsey & Kitting 1987) and creosote (Zachary *et al.* 1983). In addition, gut microbes of a few marine invertebrates have been shown to fix nitrogen (Guerinot & Patriquin 1981a, Odintsov 1981) , and it has been suggested that they play a role in the nitrogen budget of some invertebrates (Carpenter & Culliney 1975, Fong & Mann 1980, Guerinot & Patriquin 1981b), especially detritivores where the diet is often poor in nitrogen.

Extensive mats of epimural rod bacteria have been reported in the hindgut of the detritivore thalassinid mudprawn *Upogebia africana* (Harris *et al.* 1991, Chapter 5). These single-layered mats of bacteria virtually form an inner skin coating up to 75% of the cuticle in the hindgut. This novel finding is particularly interesting as these mats of bacteria closely resemble those reported in the hindgut of the lower termite Reticulitermes flavipes (Breznak & Prankratz 1977) and the grasshopper Melanoplus sanguinipes (Mead *et al.* 1988). In termites, whose diet consists primarily of structural carbohydrates, gut bacterial isolates have been shown to be capable of breakdown of cellulose (Breznak 1984) and to contribute to nitrogen conservation and fixation (O'Brien & Slaytor 1982). The main food source of the prawn *U. africana* consists of detritus derived from vascular salt-marsh plants (Chapter 2) that typically have high C/N ratios and are refractory to digestion (Tenore 1983, Bowen 1987). Furthermore, *U. africana* does not appear to possess the enzymes necessary for hydrolysis of the refractory components of this food source (Chapter 3). *U. africana* would therefore benefit greatly from microbial involvement in its digestive processes and nitrogen fixation by gut microbes. Thus, the possibility of important interactions between the prawn and the gut symbionts that have been observed in it's hindgut and hepatopancreas (Harris *et al.* 1991), particularly those involving cellulose breakdown and nitrogen acquisition, is of special interest.

Whereas Chapter 5 examined the presence and the physiological and taxonomic characteristics of the whole gut microflora, this study was undertaken to investigate in greater detail the nature and physiological properties of bacteria in

the hindgut and hepatopancreas of *U. africana*. Taxa and substrate utilization abilities of gut microbes were investigated by conventional culture techniques, and the morphology and *in situ* growth characteristics of the microflora were determined by transmission electron microscopy. In addition, both live animals and *in vitro* preparations of dissected digestive organs were assayed to test for the presence of dinitrogen fixation.

MATERIALS AND METHODS

Study site. Prawns (*U. africana*) and surface sediment from between burrow openings were collected from the muddy upper reaches (Geelbek) of Langebaan lagoon, a partially enclosed marine saltmarsh system situated on the south-west coast of South Africa (33°00'S-33°13'S and 17°57'E-18°08'E).

Bacterial isolation, characterization and substrate utilization. Media, isolation techniques, and characterization and substrate utilization tests are described in detail in Chapter 5 (Harris *et al.* 1991). Prawns were collected and transported to the laboratory within 2 h. Five animals were dissected aseptically, and gut and hepatopancreas homogenates spread-plated onto seawater agar immediately. These samples are referred to as samples from "fresh guts" hereafter in the text. A further ten animals were rinsed with 0.2 μm filtered seawater and placed in 5l sterile glass beakers containing filtered (0.2 μm Nuclepore), autoclaved seawater at 14°C. In order to minimize any takeover of gut microbes by ambient microbes the water was kept at the lower end of the ambient temperature range and changed every 4 h until the animals had cleared their guts (up to 35 h). In addition faecal pellets were removed from the beakers with sterile pasteur pipettes every hour to prevent bacterial proliferation. The object of this procedure was to clear the guts of transient ingested microbes. Five of these prawns were aseptically dissected to obtain whole gut homogenates, while the remaining 5 were used to obtain homogenates of hindguts alone. These samples are referred to as "flushed guts" hereafter in the text. The homogenates were spreadplated onto seawater agar, and colony forming units (CFU) were counted on 3 replicate plates for each sample after incubation at 20°C for 5 days. Thirty-four and 32 colonies were isolated from the hindgut and hepatopancreas plates respectively (see Chapter 5 for details of the methods used).

Microscopy. Animals were transported to the laboratory within 2 h of collection, and processed in the same manner as described above to obtain "flushed guts" samples. The animals were dissected to remove the hindgut and

hepatopancreas, and these samples were then fixed immediately in 2.5 % gluteraldehyde in 0.2 μm filtered seawater for 48 h at 4°C. Thereafter, samples were rinsed twice for 10 min in cooled (4°C) 0.2M Na cacodylate-seawater buffer and post fixed in 1 % osmium tetroxide (in Na cacodylate-seawater buffer) for 1 hour at 4°C. Samples were then rinsed with distilled water and passed through a graded alcohol series from 10 % (2 changes of 10 min each) to absolute alcohol (1.5 h). Samples were then impregnated in Epon-Araldite resin (Araldite CY212 22.5g, Epon 812 24.8g, DDSA 20g, DMP30 3%v/v) by passing them through a series from propylene oxide to 75:25, 50:50, 25:75 propylene oxide:resin mixtures (2 changes of 15 min each), and finally to pure resin for 3 h. The samples were positioned in fresh resin in a mounting tray, and kept at 55°C for 36 h to set. Samples were sectioned using a diamond knife on an Ultramicrotome, and ultrathin sections (50-60 nm) were stained with uranyl acetate (2 %) and Reynold's (1963) lead citrate solution, and viewed using a Cambridge CX200 Transmission Electron microscope. Low magnification (400x) views of cross-sections of the hindgut were obtained by staining sections with toluene blue and mounting on a slide. In addition, 5 flushed hindguts were prepared for viewing by scanning electron microscopy as described in Chapter 5.

Acetylene reduction assays. Acetylene reduction assays were used to measure dinitrogen fixation activity associated with living prawns, separated (dissected) digestive tracts and hepatopancreas, and surface sediment from between burrow openings.

For estimation of nitrogen fixation associated with living prawns, acetylene reduction assays were initiated in the field. Animals and sediment samples were incubated in sterile 175 ml glass jars (with rubber septa fitted in the lids) containing 45ml of fresh filtered (0.2 μm) seawater obtained from the collection site. Animals were carefully rinsed with filtered seawater immediately after collection, and 2 animals were placed in each of 5 bottles. A further 3 bottles containing the rinsed degutted carcasses (digestive tract and hepatopancreas removed) of 2 animals each, served as controls for the possibility of nitrogen fixation associated with epibionts. Surface sediment samples (5 cm^3), collected from between burrow openings with a 5 cm^3 syringe with the leur end removed, were extruded directly from the syringes into each of an additional 3 bottles (sediment 1 in Table 4). Blanks and standard bottles contained 45 ml filtered seawater only. Acetylene (C_2H_2) at 10 kPa was added to each bottle in the field, and the samples were kept cool and transported to the laboratory within 2 h. Ethylene (C_2H_4) was added to the standard bottles to give standard gas ethylene concentrations of 0.0213 and 0.1063 $\mu g\ ml^{-1}$. All bottles were incubated at 20°C, and 100 μl gas samples were taken at 6 and 30 h after the

initiation of the experiment. Acetylene and ethylene were analysed simultaneously by gas-liquid chromotography using a Hewlett-Packard Gas Chromatograph with a Porapak N (10 ft) column. Peak area was measured and the instrument response was calibrated periodically using the standards. The relative standard deviation in replicate samples of the blank was 5.37 % of the mean. All bottles were monitored for leaks by checking the acetylene peak areas.

To estimate nitrogen fixation associated with whole dissected guts and hepatopancreas incubated in vitro, animals were returned to the laboratory and dissected under nitrogen within 1.5 hour of collection. All subsequent manipulations were carried out under nitrogen in a glove bag. Whole intact guts and hepatopancreas were aseptically removed from the animal, and the organs of 5 animals were immediately placed in each of six 5 ml serum tubes containing 3 ml of medium. Approximately 1 g of surface sediment was added to each of 6 tubes containing 3 ml of medium. Blank and standard tubes contained only media. Three different media were used in a preliminary experiment, i.e. mannitol nitrogen-deficient medium (Guerinot & Patriquin 1981a), glucose-enriched fresh filtered seawater, and fresh filtered (0.2 μm) seawater. The enriched media did not enhance nitrogen fixation rates, and therefore fresh-filtered seawater was used in all subsequent experiments. The medium was bubbled with nitrogen gas for 15 minutes immediately prior to use to drive off oxygen, and pipetted into the serum tubes which were kept under nitrogen. After addition of samples, the tubes were stoppered under nitrogen, and the headspace subsequently flushed with filter-sterilized argon gas. Acetylene (10kPa) was added to all tubes, and ethylene was added to the standards at the concentrations described above. Samples of 100 μl were analysed on the Gas Chromatograph at 0, 12 and 36 h. Rates of nitrogen fixation were calculated using a theoretical ratio of C_2H_4 mole /N2 mole = 3 (Hardy *et al.* 1973). Results are expressed per whole animal or gram wet weight of sediment.

RESULTS

Microscopy. The hindgut wall was folded longitudinally (Plate 1a,b), with extensive (up to 75% of the gut wall) panels of epimural bacteria coating the apices of the folds that protruded into the gut lumen (Plate 1a,b). Examination of the

Plate 1. Microscopy views of bacteria colonizing the hindgut and hepatopancreas of *Upogebia africana*. a. light microscopy view of hindgut showing longitudinal folding and epimural bacteria (eb) concentrated at the apices of the folds, b. SEM view of hindgut lining showing longitudinal panels of uncolonized smooth epithelium (tp) alternating with colonized panels (cp) protruding into the lumen, c-d densely-packed mats of epimural rod bacteria adhering to the hindgut lining, f-g TEM views of epimural rod bacteria showing typical gram-negative morphology, h-i irregularly-shaped microbes in the lumen of the hepatopancreas.
Magnification: a 1000x, Scale bars: b 200μm, c 20μm, d 10μm, e 1 μm.

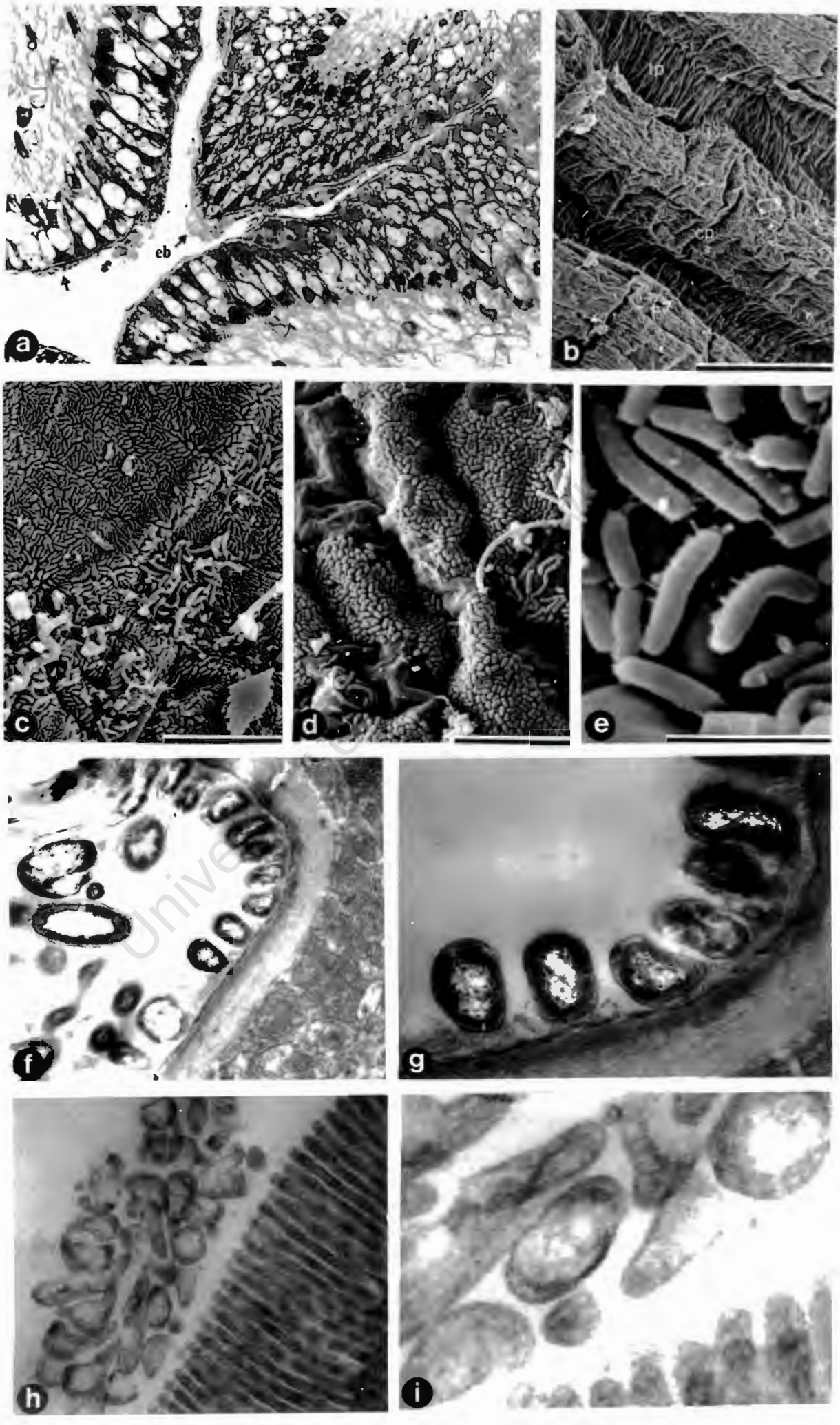


Plate 1

hindgut lining revealed the presence of densely packed rod bacteria of a single morphological type, closely applied to the cuticle (Plate 1c-e). The cuticle of the gut was found to be thick with no evidence of thinning, pitting or special modifications for attachment of the bacteria, or damage by bacteria (Plate 1f). The bacteria lining the cuticle were packed closely together, and adhered to the cuticle by a mucous layer (Plate 1g). At high magnification it could be seen that the bacteria had a cell coat characteristic of gram negative species (Plate 1g). Examination of the hepatopancreas revealed clumps of irregular-shaped bacteria (gram-negative) in the lumen (Plate 1h-i). These organisms were not attached to the lumen wall.

Viable counts. The number of culturable bacteria in fresh guts was approximately double that found in flushed guts of animals of the same size (Table 1). This suggests that while the prawn ingests bacteria with the food and/or bacterial proliferation occurs on gut contents, a resident bacterial population is present in the empty (flushed) gut. The hindgut contained approximately one-fifth of the resident bacteria present in the whole flushed gut. If the volume of the hindgut is considered relative to the total volume of the gut (see Chapter 2), it is clear that there is a concentration of the cultured bacteria in the hindgut region (278 CFU mm^{-3}) relative to the whole gut (117 CFU mm^{-3}). Colony counts from the hepatopancreas samples indicate that the hepatopancreas is an important region of bacterial colonization, contributing as many culturable bacteria per prawn as did whole fresh guts.

Taxa. The collection of bacteria isolated from the hindgut was not diverse. Only six different strains were obtained from a randomly selected collection of 34 isolates from the flushed hindgut (Table 2). Of these 34 isolates the majority (26) belonged to one strain. Thirty of the isolates were found to belong to the genus Pseudomonas, while the remaining 4 isolates were of the genus Vibrio (Table 2). Only one strain of bacteria was obtained from random selection of 32 isolates from the hepatopancreas, and this was found to be an unidentified fermenter (Table 2).

Physiological properties of resident hindgut bacteria. All bacteria isolated from the flushed hindgut of *U. africana* were gram-negative and motile (Table 3). Only one strain represented by 2 isolates was found to be fermentative. All isolates possessed proteases (milk and gelatin as substrates), while most exhibited lipase activity. None of the isolates possessed the ability to digest chitin, alginate and cellulose, and none displayed lysozyme activity. The dominant hindgut strain (strain 1, Table 3), of which there were 26 isolates, was a gram negative, motile, non-fermentative *Pseudomonad* capable of digesting proteins and lipids, but it did not exhibit cellulase, chitinase or lysozyme activity (Table 3).

Table 1. Colony counts of bacteria from the digestive tract of *Upogebia africana* (mean carapace width = 13.38 + 0.96mm).

Source	Number of CFU.whole organ^{-1}	Number of CFU.mm^{-3} gut
Fresh gut	10260	187
Flushed gut	6420	117
Hepatopancreas	12043	-
Hindgut (flushed)	1586	278

Table 2. Genera of bacteria isolated from the digestive gland and hindgut of *Upogebia africana*.

Genera/Family	Number of isolates	
	Sterile Hindgut	Hepatopancreas
Vibrio	4	-
X-ferm	-	32
Pseudomonas	30	-
Total	34	32
Number of strains	6	1

Table 3. Characteristics of and substrate utilization by isolates of bacteria from the flushed hindgut of *Upogebia africana*.

	Bacterial strains					
	1	2	3	4	5	6
Substrate/Characteristic						
Gram	-	-	-	-	-	-
Motile	+	+	+	+	+	+
Catalase	+	+	-	-	-	+
Oxidase	+	+	+	+	+	+
Fermentation (glucose)	-	+	-	-	-	-
Chitin	-	-	-	-	-	-
Starch	+	+	-	+	-	+
Gelatin	+	+	+	+	+	+
Lysozyme	-	-	-	-	-	-
CMC	-	-	-	-	-	-
Alginase	-	-	-	-	-	-
Tween80	+	+	-	+	+	+
Milk	+	+	+	+	+	+
Agar	-	-	+	-	-	-
Number of isolates	26	2	1	2	1	2

Table 4. Acetylene reducing activity and nitrogen fixation associated with the dissected digestive tract of the prawn *Upogebia africana*, live prawns and surface sediment from the habitat of the prawn.

Source	Number of replicate tubes	Number of animals per tube	Acetylene reduction nmol/animal/day nmol/ww sed/day (mean ± S.D.)	Range	Nitrogen fixation ng N2/animal/day ng N2/ww sed /day (mean ± S.D.)	Range
Dissected gut	6	5	0.03 ± 0.01	0.01-0.05	0.12 ± 0.07	0.07-0.23
Live prawns	5	2	1.16 ± 2.55	0.00-5.12	5.42 ± 11.92	0.00-23.83
Prawn carcass (gut removed)	3	2	0.23 ± 2.33	0.00-2.32	1.08 ± 10.83	0.00-10.83
Sediment 1	3	-	41.21 ± 66.61	1.52-118.10	192.32 ± 310.81	7.10-551.16
2	3	-	4.68 ± 3.07	2.84-8.20	21.83 ± 14.31	13.24-38.30

Nitrogen fixation activity. Extremely low rates of nitrogen fixation were measured for dissected guts incubated *in vitro* and for degutted carcasses of the prawn. Slightly higher rates of acetylene reduction were obtained for live animals, although zero rates were measured for some animals (Table 4). These results suggest that while very low nitrogen fixation does occur in the guts of the prawns, the amount of nitrogen acquired by nitrogen fixation (ca. 1.9 μgN year^{-1} for live animals (Table 4)), compared to that required for the growth of an animal of 10-14 mm carapace length (ca. 23.2 mgN animal^{-1} year^{-1}), assuming a mean growth rate of 270 mgDW year-*1* (Hanekom 1980) and a nitrogen content of 8.6% (Parsons *et al.* 1977), is insignificant. Nitrogen fixation associated with the surface sediment was found to be relatively strong, but very variable (means varying from 0.2033 to 192.3224 ng N g^{-1} day^{-1}).

DISCUSSION

This study shows that both the hindgut and hepatopancreas of *U. africana* are important sites of bacterial colonization. Viable counts indicate that while transient bacteria are ingested with the food, resident microbial populations that are not voided with the faeces are present in the hindgut and hepatopancreas. This result is supported by the earlier finding (Harris *et al.* 1991) that fresh whole guts of *U. africana* contain a more varied collection of isolates in terms of the genera that are represented, presumably including both ingested sediment bacteria as well as resident microbes, than do flushed whole guts, which are assumed to include only gut residents. The presence of *Pseudomonas* and *Vibrio* as the dominant genera in the flushed hindgut is consistant with findings of Harris *et al.* (1991) (Chapter 5) who found that flushed whole guts were dominated by *Vibrio* species, with *Pseudomonads* also being prevalent. In the present study the majority of bacteria in the flushed hindgut belonged to the genus *Pseudomonas*. This implies that there is a concentration of resident isolates of this genus in the hindgut relative to the rest of the gut, considering that *Vibrios* dominated in collections from the whole flushed guts (Harris *et al.* 1991). To date *Vibrio* and *Pseudomonas* are the most commonly reported genera of bacteria occurring in marine invertebrate guts, being found particularly in Crustacea (Colorni 1985, Dempsey *et al.* 1989, Dempsey & Kitting 1987, Harris *et al.* 1991, Sugita *et al.* 1987a,b,c, Sochard 1979, Ueda *et al.* 1988, Yasuda & Kitao 1980, Ohwada *et al.* 1980, Davis & Sizemore 1982, Dilmore & Hood 1986, Williams & Rees 1952), but also in some Mollusca (Colwell & Liston

1962, Kadota 1953), Echinodermata (Guerinot & Patriquin 1981a, Dilmore & Hood 1986, Unkles 1977) and Annelida (Dilmore & Hood 1986, Duchene *et al.* 1988).

Isolates of the single hepatopancreas strain were not identified to genus but were found to be fermentative (glucose) and gram-negative. Transmission electron microscopy revealed the presence of clumps of irregular shaped bacteria in the hepatopancreas. These organisms were present in the hepatopancreas lumen and did not appear to be attached to the lining. The fact that only one strain was isolated, but in high numbers, and only one morphotype seen by TEM in the lumen of the blind-ending chambers of the hepatopancreas, suggests that these microbes may represent a permanent stable population. It has been suggested that due to the inaccessible nature of the hepatopancreas, bacteria in its lumen are likely to be residents (Moriarty 1990). Very few previous studies have established the presence of bacteria in the hepatopancreas of Crustacea (e.g. Harris *et al.* 1991, Musgrove 1988), and a TEM study of the hepatopancreas of the prawn Mysis stenolepis revealed no bacteria in the lumen. (Friesen *et al.* 1986). The potential importance of bacteria in the hepatopancreas of Crustacea should not be underestimated because the absorptive surfaces in this region would facilitate the absorption of any products produced by the bacteria. This is in contrast to the relatively impermeable epithelium of the hindgut region.

The transmission electron microscopy study confirmed those of Harris *et al.* (1991) (Chapter 5) showing that the hindgut lining is extensively colonized by densely-packed epimural rod bacteria. The presence of gram negative bacteria closely applied to the epicuticle and attached by a mucous layer, was revealed. Although the rods occurred mostly in a single layer, there were some areas where the mat comprised a number of layers of bacteria. Rods of the same morphotype were also present in the lumen, and it is assumed that these had been dislodged from the gut lining. It is possible that these are resident bacteria, especially as they were present in all individuals examined (see also Harris *et al.* 1991 (Chapter 5)), and were not voided with the faeces but remained attached to the epicuticle. It has been suggested that attached gut bacteria represent permanent associations since attachment provides stability in the gut system (Savage & Blumershine 1974).

The hindgut has been identified as a site of bacterial colonization in a number of invertebrates, including insects (e.g. Klug & Kotarski 1980, Austin & Baker 1988, Bignell 1984) and crustaceans (e.g. Atlas *et al.* 1982, Dempsey *et al.* 1989), and bacteria have also been reported in the hindguts of a few molluscans (e.g. Colwell & Liston 1960, Payne *et al.* 1972) and annelidans (e.g. Gunzl 1991). An increased number of bacteria in the hindgut compared to the foregut has been reported in a holothurian (Deming & Colwell 1982), as well as proliferation of

ingested microbes in the hindgut (Plante *et al.* 1989). The presence of epimural hindgut bacteria has been reported in some invertebrates notably insects, e.g. crane fly (Klug & Kotarski 1980), mayfly (Austin & Baker 1988), termites and cockcroaches (Cruden & Markovetz 1981, see Bignell 1984 for review). A handful of studies have shown the presence of epimural bacteria directly associated with the hindgut epithelium of a few marine invertebrates, e.g. the crab Callinectes sapidus (Huq *et al.* 1986), a holothurian (Deming & Colwell 1982), and an isopod (Zachary *et al.* 1983). The evidence suggests therefore that the hindgut epithelia of a wide variety of invertebrates provide suitable sites for bacterial colonization.

In some insects where epimural hindgut bacteria occur, the epicuticle was found to be pitted (Cruden & Markovetz 1981). The existence of a thinned epicuticle or deep invaginations of the hindgut epicuticle is best developed in animals such as termites and cockcroaches where uptake of organic substances from the hindgut is of nutritional significance, and it has been suggested that such modifications increase the permeability of the cuticle (Bignell 1984) and may encourage bacterial colonization (Moriarty 1990). TEM views of the hindgut epithelium revealed that the cuticle of *U. africana* is neither thinned, nor is the epicuticle pitted nor markedly invaginated. The bacteria appear to be attached by a mucous layer alone. Evidence for facilitated transport of nutrients across the gut wall is therefore lacking. However, considering that the epimural bacteria occurred densely in all individual prawns examined, virtually forming an inner skin on the hindgut lining, it is tempting to suppose that some relationship must exist between the prawn and the microbes.

The gut bacteria isolated in this study do not possess the enzymes necessary digest ingested refractory carbohydrate-based vascular plant detritus. However, they may play a role in making amino acids and fatty acids available to the invertebrate for absorption. The collection of isolates obtained from the flushed hindgut displayed a high incidence of protease and lipase activity but no ability to digest refractory carbohydrates. The dominant strain (occurring in 26 of the 34 isolates) did not ferment glucose, and was unable to lyse target bacteria and degrade cellulose or chitin, but was able to digest proteins and lipids readily. The population isolated from the hindgut differed from that isolated from the flushed whole guts (Harris *et al.* 1991 (Chapter 5)) in that representatives of the whole gut collection were able to digest chitin (77 %) and target bacteria (23 %). This suggests that the hindgut population is distinct from that of the whole flushed gut, and supports the finding that the genera present in these two regions differ.

A few crustaceans have been reported to have isolates in the gut that exhibit cellulase activity (Musgrove 1988, Dempsey & Kitting 1987), while quite a number

of Mollusca are reported to harbour gut bacteria capable of digesting cellulose (e.g. Payne *et al.* 1972), particularly shipworms (Carpenter & Culliney 1975, Hidako & Saito 1956, Kadota 1959, Greene *et al.* 1988, Griffin *et al.* 1987, Waterbury *et al.* 1983, Rosenberg & Breiter 1969). Cellulose-degrading bacteria have also been isolated from echinoderms (Prim & Lawrence 1975). The potential importance of cellulose-degrading gut bacteria for detritivores ingesting refractory plant material is self-evident and should not be ignored.

The link between the microbes observed in the gut by electron microscopy, and those culturable on plates is tenuous due to the difficulty of isolating more than a small fraction of the bacterial community. This means that functionally insignificant microbes may dominate the isolate collections, while important microbes observed in the gut by TEM may not be represented in the isolate collection. Nevertheless isolation of microbes and examination of their physiological properties is useful to determine if they possess properties which may aid the host. However, failure to detect these properties by way of plating techniques does not exclude the possibility that the property may exist in the natural population.

To some degree, nitrogen fixation experiments with either whole gut or whole animals circumvent the problem of selective plating, as the populations remain intact. These experiments indicated that while low rates of nitrogen fixation can be detected in whole guts and live prawns, the rates are insignificant if the nitrogen requirements or dietary nitrogen intake are considered. The higher rates obtained for live animals compared to dissected guts is consistant with the finding of Guerinot *et al.* (1977) who suggest that *in vitro* preparations of guts result in sub-optimal conditions for nitrogen fixation in the gut. Although the prawns appeared to be likely candidates for the presence of nitrogen fixation in the gut, they did not display the levels of rates of acetylene reduction that are associated with the guts of shipworms and some echinoderms, and nitrogen fixation by gut microbes did not account for a significant gain of nitrogen by the host. Nitrogen fixation associated with the guts may simply be due to ongoing activity of ingested microbes as suggested by Odintsov (1981) for a sea urchin species.

Other roles have been suggested for the gut microbes of aquatic invertebrates, including ion transport across the gut wall (Huq *et al.* 1986) and protection from adverse affects of some substances such as phenolics (Dempsey & Kitting 1987). However it is also possible that gut microbes are simply commensals exploiting a favourable environment, or they could even be parasites (e.g. Barber *et al.* 1991, Cameron *et al.* 1988), although this is unlikely in the present case because they were present in all apparantly healthy individuals examined over a 3 year period. In view of the density and colonization characteristics of the hindgut

microbes of *U. africana*, further investigations are merited to test whether other significant microbe-host associations exist between this prawn and its gut microflora.

Another consideration is whether the presence of these dense epimural mats of rod bacteria in *U. africana* is an isolated occurrence or whether is it a widespread phenomenon amongst different invertebrate taxa, trophic groups and geographical localities; and, if they occur in other organisms, whether they exhibit potentially important characteristics such as nitrogen fixation.

REFERENCES

Atlas RM, Busdosh M, Krichevsky EJ, Kaneko T 1982. Bacterial populations associated with the Arctic amphipod *Boeckosimus affinis*. Can J Microbiol 28(1):92-99

Austin DA, Baker JA 1988 Fate of bacteria ingested by larvae of the freshwater mayfly *Ephemera danica*. Microbial Ecol 15:323-332

Barber BJ, Ford SE, Littlewood DTJ 1991 A physiological comparison of resistant and susceptable oysters *Crassostrea virginica* (Gmelin) exposed to the endoparasite Haplosporidium nelsoni (Haskin, Strauber & Mackin). J Exp Mar Biol Ecol 146(1):101-112

Bignell DE 1984 The arthropod gut as an environment for microorganisms. In: Anderson JM, Raynon ADM, Walton DWA (eds.) Invertebrate-microbial interactions, Cambridge University Press, Cambridge, pp 205-227

Bowen SH 1987 Composition and nutritional value of detritus. In: Detritus and microbial ecology in aquaculture. Moriarty DJW, Pullin RSV (eds.), ICLARM Conf Proc 14, Int Cen Living Aquatic Res Man, Manila, Phillipines, pp 192-216

Breznak JA 1984 Biochemical aspects of symbiosis between termites and their intestinal microbiota. In: Anderson JM, Raynon ADM, Walton DWA (eds.) Invertebrate-microbial interactions, Cambridge University Press, Cambridge, pp 173-203

Breznak JA, Prankratz HS 1977 In situ morphology of the gut microbiota of wood-eating termites (*Reticulitermes flavipes* (Koller) and *Coptotermes formosanus* (Shiraki)). Appl Environ Microbiol 33(2):406-426

Cameron DE, Garland CD, Lewis TE, Machin PJ 1988 A survey of Vibrionaceae in Tasmanian coastal waters, with special reference to bacterial species pathogenic to fish or shellfish. Aust J Mar Freshwater Res 39:145-152

Carpenter JL, Culliney JL 1975 Nitrogen fixation in marine shipworms. Science 187:551-552

Colorni A 1985 A study on the bacterial flora of giant prawn *Macrobrachium rosenbergii*, larvae fed with *Artemia salina* nauplii. Aquacult 49:1-10

Colwell RR, Liston J 1962 The natural bacterial flora of certain marine invertebrates. J Insect Pathology 4:23-33

Colwell RR, Liston J 1960 Microbiology of shellfish. Bacteriological study of the natural flora of Pacific oysters (*Crassostrea gigas*). Applied Microbiology 8:104-109

Cutter, FA Rosenberg 1972 The role of cellulolytic bacteria in the digestive processes of the shipworm - requirement for the teredine borers. In: Walters AH, Hueck van der Plas EH (Ed) : Biodeterioation of Materials Vol 2. London (Applied Science Publishers Ltd) 42-51

Davis JW, Sizemore RD 1982 Incidence of *Vibrio* species associated with blue crabs (*Callinectes sapidus*) collected from Galveston Bay, Texas. Appl Environ Microbiol 43:1092-1097

Deming JW, Colwell RR 1982 Barophilic bacteria associated with digestive tracts of abyssal holothurians. Appl Environ Microbiol 44:1221-1230

Deming JW, Tabor PS, Colwell RR 1981 Barophilic growth of bacteria from intestinal tracts of deep-sea invertebrates. Microb Ecology 7:85-94

Dempsey AC, Kitting CL 1987 Characteristics of bacteria isolated from penaeid shrimp. Crustaceana 52:90-94

Dempsey AC, Kitting CL, Rosson RA 1989 Bacterial variability among individual penaeid shrimp digestive tracts. Crustaceana 56(3):266-278

Dilmore LA, Hood MA 1986 Vibrios of some deep-water invertebrates. FEMS Microb Letters 35:221-224 Moriarty list

Duchene JC, Imbaud P, Delille 1988 Associated bacterial microflora of a subantarctic polychaete worm *Thelepus setosus*. Arch Hydrobiol 112:221-231

Fong W, Mann KH 1980 Role of gut flora in the transfer of amino acids through a marine food chain. Can J Fish Aquat Sci 37:88-96

Friesen JA, Mann KH, Novitsky JA 1986 *Mysis* digests cellulose in the absence of a gut microflora. Can J Zool 64(2):442-446

Greene RV, Griffin HL, Freer SN 1988 Purification and characterization of an extracellular endoglucanase from the marine shipworm bacterium. Arch Biochem Biophys 267:334-341

Griffin HL, Freer SN, Greene RV 1987 Extracellular endoglucanase activity by a novel bacterium isolated from marine shipworm. Biochem Biophys Res Comm 144(1):143-151

Guerinot ML, Fong W, Patriquin DG 1977 Nitrogen fixation (acetylene reduction) associated with sea urchins (*Strongylocentrotus droebachiensis*) feeding in seaweeds and eelgrass. J Fish Res Board Can 34:416-420

Guerinot ML, Patriquin DG 1981a N2-fixing vibrios isolated from the gastrointestinal tract of sea urchins. Can J Microbiol 27:311-317

Guerinot ML, Patriquin DG 1981b The association of N2-fixing bacteria with sea urchins. Mar Biol 62:197-207

Gunzl, H 1991 The ultrastucture of the posterior gut and caecum in Alona affinis (Crustacea, Cladocera). Zoomorphology 110(3):139-144

Hanekom NM 1980 A study of two thalassinid prawns in the non Spartina regions of the Swartkops estuary. PhD Thesis, Univ Port Elizabeth, South Africa

Hardy RWF, Burns RC, Holsten RD 1973 Applications of the acetylene-ethylene assay for measurement of nitrogen fixation. Soil Biol Biochem 5:47-81

Harris JM, Seiderer LJ, Lucas MI 1991 Gut microflora of two saltmarsh detritivore thalassinid prawns, *Upogebia africana* and *Callianassa kraussi*. Microb Ecol 21:63-82

Hidako T, Saito K 1956 Studies on the cellulose decomposing bacteria in the digestive organs of the shipworm (*Teredo navalis*) II On the bacterial cellulase. Mem Fac Fisheries, Kagoshima Univ 5:172-177

Huq A, Huq SA, Grimes DJ, O'Brien M, Chu KH, Capuzzi JM, Colwell RR 1986 Colonization of the gut of the blue crab *Callinectes sapidus* by *Vibrio cholerae*. Appl Environ Microbiol 52(3):586-588

Kadota H 1953 Studies on the biochemical activities of marine bacteria II On the properties of agar-digesting enzyme of *Vibrio purpureus*. Memoirs of the College of Agriculture, Kyoto Univ 66:31-38

Kadota H 1959 Cellulose decomposing bacteria in the sea. In: Marine Boring and Fouling Organisms. Ray DL (ed.), Univ of Washington Press, Seattle, pp 332-341

Klug MJ, Kotarski S 1980 Bacteria associated with the gut tract of larval stages of the aquatic cranefly *Tipula abdominalis* (Diptera: Tipulidae) Appl Environ Microbiol 40(2):408-416

Mead LJ, Khachatourians GG, Jones GA 1988 Microbial ecology of the gut in laboratory stocks of the migratory grasshopper, *Melanoplus sanguinipes* (Fab.) (Orthoptera:Acrididae). Appl Environ Microbiol 54(5):1174-1181

Moriarty DJW 1990 Interactions of micro-organisms and aquatic animals, particularly the nutritional role of the gut microflora. In : Microbiology in poecilotherms. R. Lesel (ed). Elsevier Science Publishers.

Musgrove RJ 1988 Digestive ability of the freshwater crayfish *Paranephrops zealandicus* (White) (Parastacidae) and the role of microbial enzymes. Freshw Biol 20:305-314

O'Brien RW, Slaytor M 1982 Role of microorganisms in the metabolism of termites. Aust J Biol Sci 35:239-262

Odintsov VS Nitrogen fixation (acetylene reduction) in the digestive tract of some echinoderms from Vostok Bay in the sea of Japan. Mar Biol Lett 2:259-263

Ohwada K, Tabor PS, Colwell RR 1980 Species composition and barotolerance of gut microflora of deep-sea benthic macrofauna collected at various depths in the Atlantic Ocean. Appl Environ Microbiol 40:746-755

Parsons TR, Takahasi M, Hargrave B 1977 Biological Oceanographic Processes, Pergamon Press, Oxford

Payne DW, Thorpe NA, Donaldson EM 1972 Cellulolytic activity and a study of the bacterial population in the digestive tract of *Scrobicularia plana* (Da Costa). Proc malac Soc Lond 40:147-160

Plante CJ, Jumars PA, Baross JA 1989 Rapid bacterial growth in the hindgut of a marine deposit feeder. Microb Ecol 18:29-44

Prim P, Lawrence JM 1975 Utilization of marine plants and their constituents by bacteria isolated from the gut of Echinoids (Echinodermata). Mar Biol 33:167-173

Rosenberg FA, Breiter H 1969 The role of cellulolytic bacteria in the digestive processes of the shipworm. I. Isolation of some cellulolytic micro-organisms

from the digestive system of Teredine borers and associated waters. Material und Organismen 4:147-159

Sochard MR, Wilson DF, Austin B, Colwell RR 1979 Bacteria associated with the surface and gut of marine copepods. Appl Environ Microbiol 37:570-579

Sugita H, Takahashi T, Kanemoto FI, Deguchi Y 1987a Aerobic bacterial flora in the digestive tracts of freshwater shrimp *Palaemon paucidens* acclimated with seawater. Nippon Suisan Gakkaishi (Bull Jpn Soc Sci Fish) 53(3):511

Sugita H, Ueda R, Berger LR, Deguchi Y 1987b Microflora in the gut of Japanese coastal crustacea. Bull Jpn Soc Sci Fish 53(9):1647-1655

Sugita H, Ueda S, Ueda R, Degushi Y 1987c Morphology and brief characteristics of *Vibrio* sp. isolated from marine Crustacea. Bull Coll Agric Vet Med Nihon Univ 44:160-164

Tenore KR 1983 What controls the availability to animals of detritus derived from vascular plants: organic nitrogen enrichment or caloric availability? Mar Ecol Prog Ser 10:307-309

Ueda R, Sugita H, Degushi Y 1988 Numerical taxonomy of vibrios isolated from coastal crustacea of Japan. Bull Coll Agric Vet Med Nihon Univ 0(45):227-235 ls

Unkles SE 1977 Bacterial flora of the sea urchin *Echinus esculentus*. Appl Environ Microbiol 34:347-350

Waterbury JB, Calloway CB, Turner RD 1983 A cellulolytic nitrogen fixing bacterium cultured from the gland of Deshayes in shipworms (Bivalvia:Teredinidae). Science 221:1401-1403

Williams OB, Rees HB Jr 1952 The bacteriology of Gulf Coast shrimp III The internal flora. Texas Journ Sci 1:55-58

Yasuda K, Kitao T 1980 Bacterial flora in the digestive tracts of prawns, *Penaeus japonicus* Bate. Aquaculture 19(3):229-234

Zachary A, Parrish KK, Bultmann JD 1983 Possible role of marine bacteria in providing the creosote-resistance of *Limnoria tripunctata*. Mar Biol 75(1):1-8

CHAPTER 7

DO GUT BACTERIA OF THE LEAF-EATING MANGROVE CRAB SESARMA MESSA (CAMPBELL) FIX NITROGEN ?

Abstract: Direct counts by epifluorescent microscopy established the presence and quantity of bacteria in the digestive tract of the mangrove crab *Sesarma messa* (Campbell). Bacterial densities were similar in the contents of the fore-, mid- and hindgut and in freshly-produced faecal pellets. Bacterial densities in gut contents were similar on a weight specific basis to those on aged leaves of the mangrove tree *Rhizophora stylosa*, which forms a major proportion of the diet of the crab, but significantly different to those on freshly-fallen leaves. Scanning electron microscopy detected the presence of attached epimural bacteria in the gut of *S. messa*, and dense colonization of aged *R. stylosa* leaves by bacteria especially in the vicinity of the stomata. Very low or no acetylene reduction rates were recorded in association with the gut and digestive glands of *S. messa*, while strong activity was found associated with *R. stylosa* leaves. Culture of gut bacteria revealed the presence of viable nitrogen fixing bacteria, although densities relative to colony forming units (CFU) and direct counts of the total heterotrophic bacterial flora were very low. Nitrogen fixation by gut bacteria would contribute less than 0.002 % of the nitrogen gained daily by ingestion of leaves, and less than 0.01 % of nitrogen required for growth. Nitrogen fixation by gut contents was calculated to be less than 0.3 % of that by an equivalent weight of uningested leaf material. It was concluded that while a low number of viable nitrogen-fixing bacteria are present in the gut of the crab, they do not contribute significantly to the nitrogen budget of the animal, and that mangrove crab digestive tracts are not major sites of nitrogen fixation in the mangrove system.

Key words: Gut bacteria; Mangrove crab; Nitrogen fixation

INTRODUCTION

It has been proposed that nitrogen fixation in the gut may account for a significant proportion of nitrogen acquired by many marine invertebrate herbivores and detritivores, which are often nitrogen-limited due to poor food quality (Carpenter & Culliney 1975, Guerinot *et al.* 1977). To date, nitrogen fixation has been associated with the digestive tracts of only a handful of species of marine invertebrates. Carpenter & Culliney (1975) found high rates of nitrogen fixation associated with four species of marine shipworm, and concluded that this may be an important source of nitrogen for them. A cellulolytic nitrogen-fixing bacterium has been isolated from the Deshayes gland of six species of shipworm (Waterbury *et al.* 1983). Guerinot *et al.* (1977) and Guerinot & Patriquin (1981a) reported nitrogen fixation in four species of sea urchins, and estimated that Strongylocentrotus droebachiensis could gain approximately 8-15 % of its daily nitrogen needs from this source (Guerinot *et al.* 1977). In addition, N_2-fixing vibrios have been isolated from the gastrointestinal tract of *S. droebachiensis* (Guerinot & Patriquin 1981b), and Guerinot and Patriquin (1981a) demonstrated incorporation of microbially-fixed nitrogen into the tissues of this sea urchin. Furthermore, Fong & Mann (1980) found that gut bacteria synthesize essential amino acids that are incorporated in the tissue of S. droebachiensis. In contrast, Odintsov (1981) reported that while nitrogen fixation associated with the gut of two species of urchin was much higher than that of a sea cucumber tested, it was not significant for the nitrogen balance of the echinoderms, and concluded that it was merely the continuation of the activity of ingested free-living nitrogen fixers. Considering the implications of nitrogen fixation associated with invertebrate guts, in terms of both the nitrogen budgets of marine invertebrates and nitrogen cycling in marine systems, further studies of a wider variety of marine detritivores and herbivores are needed to determine how widespread and how significant the phenomenon is.

The diet of the mangrove crab *Sesarma messa* (Campbell), which is the dominant crab in the mangrove forests of north-eastern Australia, consists to a large measure of leaves of mangrove trees, including *Rhizophora, Bruguiera, Ceriops* and *Avicennia* spp. (Robertson 1986,1988). In *Rhizophora*-dominated forests leaf-eating mangrove crabs are estimated to remove up to 80 % of the annual litter fall (Robertson 1986). The C/N ratios of these leaves is very high (ca. 110 (Bunt 1982)), and it is generally assumed that the availability of nitrogen is a limiting factor for mangrove crabs. Preliminary investigations showed that *S. messa* does harbour a gut microflora. These crabs therefore seemed to be likely candidates to display and

benefit from nitrogen fixation by gut microbes.

This study investigates the presence and density of bacteria in the digestive system of *S. messa* and on one of its major food sources, the leaves of the mangrove tree *R. stylosa*, by means of light and scanning electron microscopy. Nitrogen fixation rates associated with the digestive tract of the crab and leaves were measured to determine if nitrogen fixation may contribute significantly to the nitrogen budget of the crabs. In addition nitrogen fixers in the guts and digestive glands of the crab were enumerated and compared with plate (colony forming units) and total (direct) counts. Nitrogen acquired by nitrogen fixation was compared with estimates of nitrogen ingested with the diet and nitrogen required for growth of the crab.

APPARATUS AND METHODS

SAMPLING LOCATION

Mangrove crabs (Sesarma messa) and freshly fallen and aged leaves of the mangrove tree Rhizophora stylosa were collected intertidally from a mangrove forest, dominated by R. stylosa, at Cape Ferguson, near Townsville in northern Queensland, Australia ($19^{o}17$'S, $14^{o}03$' E), in July to September 1990.

DIRECT COUNTS

Crabs were returned to the laboratory within 1 hour of collection, cold-anaesthetized, and aseptically dissected to remove the gut and/or gut contents. Guts and contents were weighed (wet weight) and fixed in 4 % formalin (in 0.2 μm filtered seawater) for 48h at 4^{o}C. Samples were then homogenized, diluted to a standard dilution of 0.5mg/ml, and sonicated (50Hz) for 30 min at 4^{o}C to dislodge attached microbes. Samples were stained with 4',6-diamidino-phylindole (DAPI) at a final concentration of 5 μg/ml for 20 minutes at 4^{o}C in the dark, and 2 ml was then filtered onto a 0.2 μm nucleopore filter (irgalon black stained) (Porter & Feig 1980, Schallenberg *et al.* 1989). Twenty randomly-selected fields or at least 400 cells were counted at 1000x magnification on each of two slides per sample. Samples of freshly-fallen (senescent) and aged leaves (14 days in situ in a mesh bag) were

weighed and fixed in 4 % formalin (in 0.2 μm filtered seawater), and processed as described above for gut samples. A one-way ANOVA (Tukey test) was used on log-transformed data to determine if numbers of bacteria in different regions of the gut and on leaves were significantly different. Bartlett's test for homogeneity of variance was applied.

SCANNING ELECTRON MICROSCOPY

Crabs were cold-anaesthetized and dissected within 1 hour of collection. Whole guts were removed from the animals and fixed in 2.5 % gluteraldehyde (in 0.2 μm filtered seawater) for 24 hours. Samples were then passed through a desalination series (75,50,25,0 % 0.2 μm filtered seawater in distilled water) and a dehydration series (10,30,50,70,90,96,100 % ethanol), and subsequently critical point dried using CO_2 as the transitional fluid. Dried whole guts were sliced in half longitudinally using a sharp sterile scalpel, and the contents removed by gently lifting the whole food bolus out. The gut and contents were then mounted, with the inner gut lining exposed, using double-sided tape. Mounted samples were sputter-coated with gold, and viewed with a JEOL JXA-840A Electron Probe Microanalyser. The entire lengths of the guts of 5 individual crabs was viewed. Samples of freshly fallen and aged leaves were fixed, processed and viewed as described above for the gut samples.

ACETYLENE REDUCTION ASSAYS

Acetylene reduction assays were used to estimate nitrogen fixation (nitrogenase) activity associated with the digestive system of the crab *S. messa* and leaves of the mangrove tree *R. stylosa* (which had been fallen for 2-5 days). Whole intact digestive tracts and whole digestive glands were dissected from the crabs within 1 hour of collection, and were immediately placed under nitrogen gas. All further manipulations were carried out in a N_2 atmosphere in a glove bag. Three guts, three digestive glands, or approximately one gram of leaf material were respectively placed in each 8 ml sterile vacutainer tube containing 3 ml liquid medium. All media were previously bubbled with N_2 gas (0.2 μm filtered) for 30 min to drive off oxygen, pipetted into vacutainer tubes, and the open tubes equilibrated under nitrogen gas in the glove bag prior to addition of samples. Preliminary

experiments were carried out using 3 different media (glucose-enriched filtered seawater, mannitol nitrogen-deficient liquid medium (Guerinot & Patriquin 1981b) and 0.2 μm filtered seawater). The first two enriched media did not result in enhanced rates, and subsequent experiments were carried out with filtered seawater alone. Vacutainer tubes were stoppered under nitrogen, removed from the glove bag, and the nitrogen in the headspace was then replaced with argon gas. Control and standard tubes which contained media only were processed in an identical manner. Acetylene (C_2H_2) at 10 kPa (0.1 atm) was added to each tube at the start of the experiment. Ethylene (C_2H_4) was added to the standard tubes to give standard gas ethylene concentrations of 0.0125 and 0.0625 μg/ml. Acetylene and ethylene were analysed simultaneously by gas-liquid chromotography using a Hewlett Packard Model 5840A Gas Chromatograph (GC) with a Porapak N column and flame ionization detector. Peak area was measured and the instrument response was calibrated periodically using the standards. The relative standard deviation in replicate samples of the blank was 2.44 % of the mean. All tubes were monitored for leaks by checking the acetylene peak areas. Samples were incubated for a period of 24 h, with 0.2 ml sub-samples taken and the C_2H_2 and C_2H_4 peak areas read on the GC at T = 0, 7 and 24 h. Rates of fixation of nitrogen were calculate using a theoretical ratio of C_2H_4/N_2 = 3 (Hardy *et al* 1973). Results are expressed per whole organ or per wet weight of leaves. Results of nitrogen fixation activity associated with the digestive system of *S. messa* and *R. stylosa* leaves are reported in both nmol C_2H_4/h and ng N_2/day to facilitate comparisons with other studies.

ENUMERATION OF N_2 FIXING BACTERIA

Crabs were aseptically dissected within 1 hour of collection, and whole samples of digestive tracts and digestive glands separately homogenized in sterile liquid N-deficient medium (Guerinot & Colwell 1985). Samples were diluted to 3 organs per 100ml medium, and then further serially diluted (10,10-1,10-2,10-3,10-4) in N-deficient medium. Numbers of colony-forming, aerobic, heterotrophic bacteria per dilution were determined by spread-plating 100 μl of each dilution onto 3 seawater agar plates (5 g peptone, 1 g yeast extract, 15 g agar, 1 l filtered seawater). The plates were incubated at 25^{o}C for 5 days, and the total number of colony-forming units was then recorded. Total bacterial numbers were determined by direct counting. Five ml of each dilution was fixed with formalin (final concentration 4 %) at 4^{o}C for 48 h, stained with DAPI, filtered onto irgalon black stained 0.2 μm nuclepore filters, and 2 slides counted per dilution. The Most Probable Number

(MPN) of N_2 fixing bacteria was estimated with 5 tubes per dilution using the tables of Cochran (1950). Two series of the 5 dilutions with 5 tubes (each containing 10 ml) each were prepared for both the gut and digestive gland samples. One series of tubes was capped with serum stoppers and flushed with filter-sterilized N_2 gas (ie. incubated anaerobically), while the other series was cultured aerobically. All tubes were incubated at 25°C in the dark. When visible turbidity developed (3 days), acetylene was added (10kPa) to each tube. After 3-4 days 200μl gas samples were taken from each tube and analysed by gas chromatography as described for intact gut incubations. Due to time constraints, samples from only 3 tubes per dilution were read on the GC for aerobic samples. Production of C_2H_4 was scored as representing a positive MPN tube. All results are expressed as number of organisms per gram wet weight of tissue.

RESULTS

PRESENCE AND DENSITY OF GUT AND LEAF-COLONIZING BACTERIA

Direct counting demonstrated the presence of bacteria in all regions of the digestive system of *S. messa* (Table 1). There were no significant differences between the density of bacteria in the contents of the fore-, mid-, and hindgut, the freshly-produced faecal pellets, the digestive gland and the whole hindgut of the crab. Freshly-fallen leaves had significantly lower numbers of bacteria than any of the above sources (F ratio 13.06, $p < 0.001$), while aged leaves were colonized by bacteria at approximately the same density as that found per unit weight in the gut contents. The standard deviations of the means of bacterial densities in gut contents of the different regions of the gut were very high possibly reflecting different stages of gut fullness.

Scanning electron microscopy confirmed that bacteria were present both attached to gut contents as well as attached to the gut lining. Both epimural rods and cocci were observed in the gut. Epimural rods were attached both longitudinally (Plate 1a) and end-on (Plate 1b) to the gut lining. Distinct protruberant structures were present on the cell surfaces of some epimural rod bacteria (Plate 1c). Epimural cocci appeared to be coated with a mucus layer, and recessed into the epicuticle of the gut (Plate 1d), while loose cocci were observed scattered on the gut

Table 1. Bacterial densities in regions of the digestive system, the gut contents, freshly produced faecal pellets and the diet (*Rhizophora* leaves) of *Sesarma messa*. Mean size of crab investigated = carapace width of 24.1 (+ 1.28) mm.

Source	Bacteria per mg wet weight Mean(± SD) x 10^5	n
Foregut contents	4.00 (+ 4.89)	4
Midgut contents	5.20 (+ 2.75)	5
Hindgut contents	5.64 (+ 2.63)	14
Fresh faecal pellets	2.43 (+ 0.06)	4
Digestive gland	2.77 (+ 2.26)	9
Whole hindgut	6.47 (+ 6.89)	3
Rhizophora leaves (senescent)	0.08 (+ 0.03)	5
Rhizophora leaves (aged)	5.65 (+ 3.31)	5

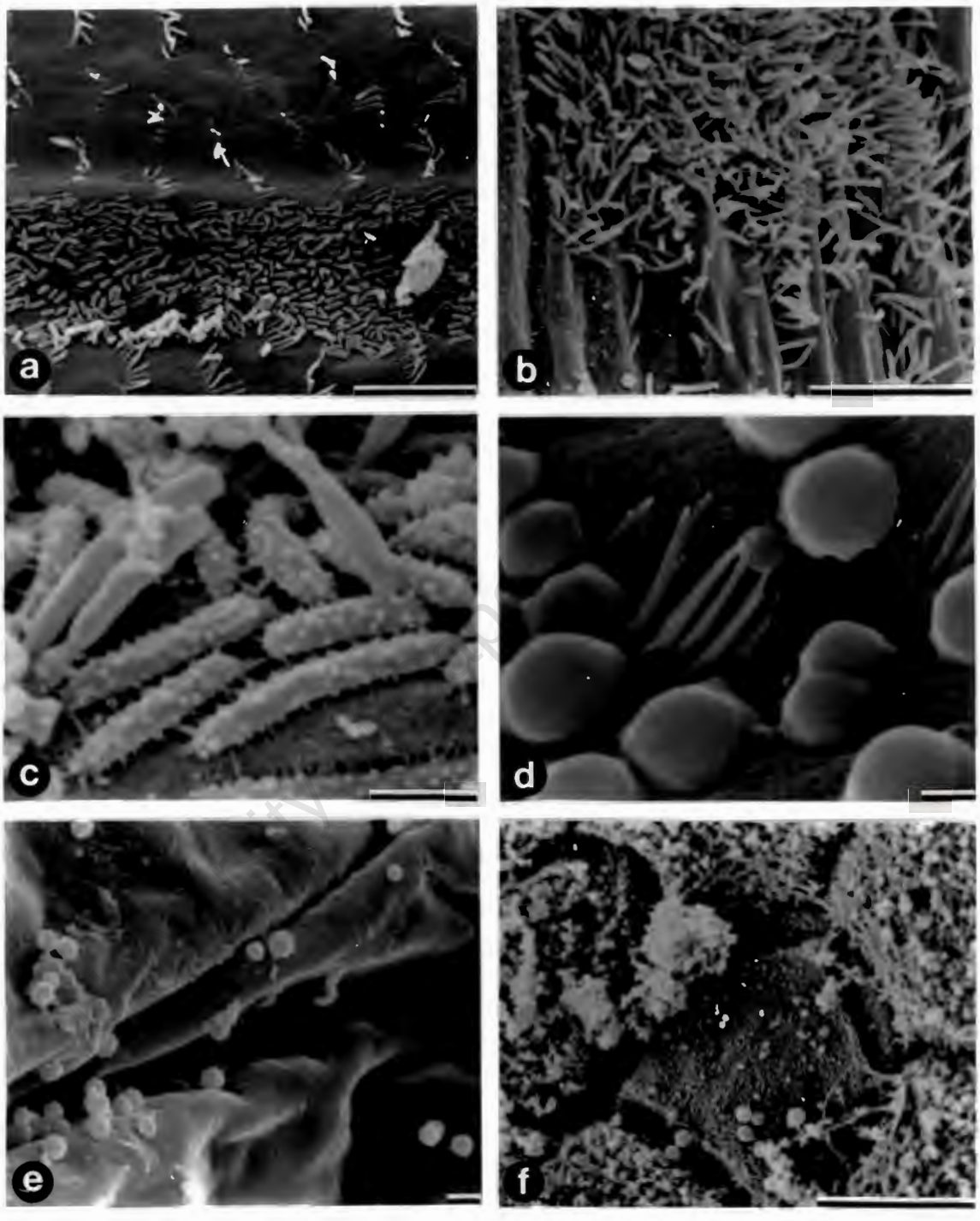


Plate 1

Plate. 1. Electron micrographs of bacteria in the gut of *S. messa*. a mats of epimural rod bacteria on hindgut brush-border lining (anterior region). b rods attached end-on to smooth epithelium in posterior hindgut. c epimural rod bacteria in hindgut with protruberances characteristic of cellulose-degrading bacteria. d epimural cocci amidst brush-borders in anterior hindgut. e loose cocci in midgut. f cocci in lumen of hepatopancreas. Scale bars: a,b,f 10 m, c,d,e, 1 m.

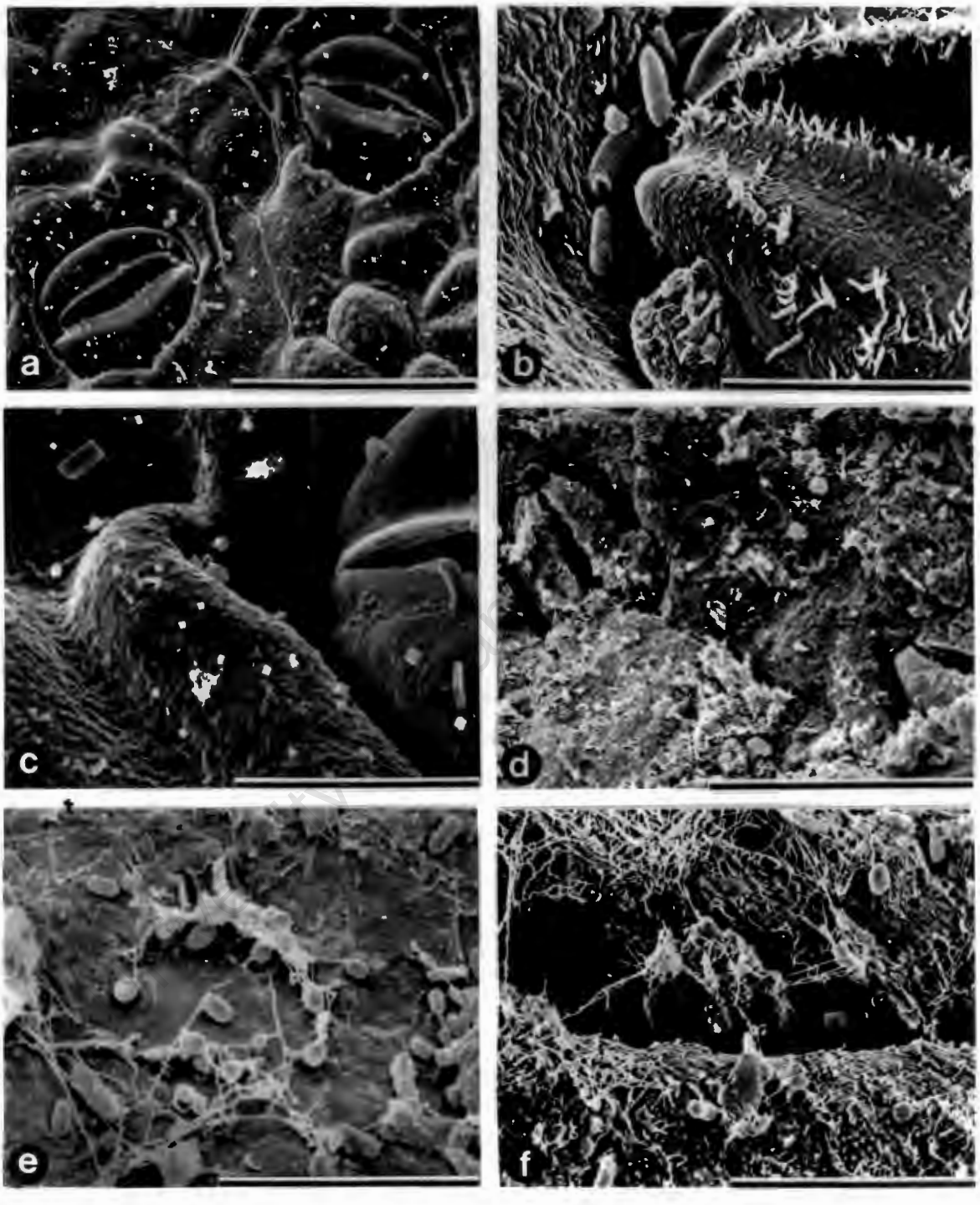


Plate 2

Plate 2. Electron micrographs of freshly-fallen and aged *Rhizophora stylosa* leaves. **a-c** surface of freshly fallen leaf. **b,c** stomata. **d-f** aged leaf. e bacteria on surface. f stomata. Scale bars: a,d 50 m, b,c,e,g 10 m.

lining (Plate 1e). Only cocci were observed in the digestive gland (Plate 1f). While low numbers of bacteria were seen attached to the surfaces of freshly-fallen leaves (plate 2a-c), aged leaves were densely colonized (Plate 2d,e) especially in the vicinity of the stomata (Plate 2f).

NITROGENASE ACTIVITY

No or very low rates of acetylene reduction were measured for composite samples (3 organs per tube) of guts or digestive glands of *S. messa* during 3 experiments to test for nitrogen fixation (Table 2). Nitrogen fixed by the gut ranged from 0 to 3.95 ng N_2/organ/day, while that of the digestive gland ranged from 0 to 10.42 ng N_2/organ/day. There was high variability in fixation rates in the different tubes of composite samples of guts and digestive glands in each experiment. Strong rates of acetylene reduction were obtained for *R. stylosa* leaves (441.00-3097.77 ng N_2/g/day) (Table 3).

ENUMERATION OF NITROGEN FIXING BACTERIA

Low numbers of viable nitrogen fixing bacteria were present in the digestive system of the crab, but these represented only a very small proportion of the culturable bacteria (colony forming units (CFU)) and total population (direct count) (Table 4). The numbers of colony forming units were an order of magnitude lower than total numbers of cells obtained by direct counting. Higher CFU and direct counts were obtained for guts compared to digestive glands, and greater numbers of both aerobically and anaerobically cultured nitrogen fixers were present in the gut (0.79×10^2 and 3.28×10^2 MPN/g, respectively) than in the digestive gland (0.54×10^2 and 1.26×10^2 MPN/g, respectively) (Table 4). For both guts and digestive glands substantially higher numbers of nitrogen fixers were present in anaerobically cultured samples than in those cultured aerobically.

Table 2. Acetylene reducing activity and nitrogen fixation associated with the digestive tracts of the crab *Sesarma messa* (carapace width 20-22mm).

Source	Experiment	Number of replicate tubes	Number of animals per tube	Acetylene reduction nmol C_2H_4/organ/h Mean (± S.D.)	Range	Nitrogen fixation ng N_2/organ/day (Mean ± S.D.)	Range
Whole gut	1	3	3	0.017 (±0.016)	0.007-0.035	1.933 (±1.752)	0.783-3.950
	2	5	3	0.003 (±0.006)	0.000-0.013	0.337 (±0.631)	0.000-1.450
	3	4	3	0.003 (±0.003)	0.000-0.007	0.308 (±0.314)	0.167-0.733
Digestive gland	1	3	3	0.064 (±0.031)	0.032-0.093	7.206 (±3.425)	3.600-10.417
	2	5	3	0.000	-	0.000	-
	3	4	3	0.000	-	-	

Table 3. Acetylene reducing activity and nitrogen fixation associated with leaves of the mangrove tree *Rhizophora stylosa* (results per gram wet weight).

Source fixation	Experiment Range	Number of replicate tubes	Acetylene reduction nmol C_2H_4/g/h Mean ± S.D.	Range	Nitrogen ng N_2/g/day Mean ± S.D.
Leaves (±876.84)	1 441.00-2035.75	3	12.94 (±7.83)	3.94-18.18	1448.96
	2	1	18.52		2074.50
(±1259.79)	3 1821.27-3097.33	3	27.56 (±11.25)	16.26-38.76	3086.46

Table 4. Enumeration of nitrogen fixing bacteria in the digestive tracts of *Sesarma messa* (mean carapace width 21.13 (+1.70) mm, Composite samples of three organs, Digestive gland mean mass 95.9 mg, Gut mean mass 49.0 mg, Counts expressed per gram wet weight of tissue)

Sample	CFU/g	Direct counts/g	MPN N-fixers/g
GUT			
Aerobic	$8.55 (\pm 3.3) \times 10^7$	$7.75 (\pm 2.62) \times 10^8$	0.79×10^2
Anaerobic			3.28×10^2
DIGESTIVE GLAND			
Aerobic	$1.49 (\pm 0.46) \times 10^6$	$8.50 (\pm 2.12) \times 10^7$	0.54×10^2
Anaerobic			1.26×10^2

DISCUSSION

The presence of a comparable density of bacteria in the fore- mid- and hindgut of the crabs, which was similar to that on aged leaves, suggests that either the crab does not lyse ingested bacteria and there are no major proliferation sites during passage of food through the gut, or that both proliferation and cropping of bacterial growth occur during passage of food through the gut. The high standard deviations can be attributed to different stages in the feeding cycle i.e. some individuals may have full guts while others may be in various stages of emptying the gut. The apparent higher numbers of bacteria recorded for whole hindgut samples (ns) suggests that attached epimural bacteria are present (especially as this procedure underestimates bacteria on the gut lining and contents because the whole gut sample weight is biased by muscle tissue). This was confirmed by the SEM study which revealed patches of dense colonization of the hindgut wall by rod bacteria. It is possible that the attached epimural flora represents a resident population of microbes. Attached resident bacteria in the hindguts of aquatic invertebrates have been reported by a few authors (Klug & Kotarski 1980, Deming & Colwell 1982, Zachary *et al.* 1983) previously. In addition to these attached microbes in the hindgut the SEM study showed that a number of different types of bacteria, both rods and cocci attached to the gut wall and free in the lumen, are present in different regions of the gut of *S. messa*.

Interestingly, patches of epimural rod bacteria with distinct protruberant structures characteristic of cellulolytic bacteria (Lamed *et al.* 1987) were observed by SEM in the gut of *S. messa*. These structures closely resemble cellulosomes (cellulose-binding, multicellulase-containing protein complexes) reported on the cell surface of *Clostridium thermocellum*, which appear to be the site of active cellulolysis (Bayer & Lamed 1986). The possibility of evidence for active cellulolytic epimural bacteria in the gut of *S. messa* is of interest considering the refractory nature of the main food source (mangrove leaves). Cellulolytic gut bacteria have been reported in a number of aquatic invertebrates (eg. Payne *et al* 1972, Prim & Lawrence 1975, Waterbury *et al* 1983, Dempsey & Kitting 1987, Musgrove 1988), although significant contributions to the digestive processes of the host are reported for only a few invertebrates (e.g. Musgrove 1988).

Viable nitrogen fixers were present in low numbers in the digestive system of the crab as shown by enumeration studies. However, nitrogen fixation rates associated with the digestive tract of the crab were extremely low. Thus, while nitrogen fixers were active in the guts of these prawns, nitrogen fixation rates were

very low. If the maximum rates of nitrogen fixation obtained for the gut and digestive gland are combined to give maximum nitrogen fixation estimates per crab (0.0144 μg N_2/crab/day), then nitrogen fixation contributes only 0.0013 and 0.0011 % of nitrogen ingested daily in leaves, assuming a daily leaf consumption of 1103 and 1349 μg/crab/day for fresh and aged leaves respectively (Fiorenzo & Robertson, unpubl. data), and a leaf nitrogen content of 0.50 % (Camilleri 1989). In addition, annual nitrogen fixation (0.0053 mg N/crab/year), as calculated for a crab of 22mm size, from maximum rates obtained in this study, would contribute only 0.0056 % of the nitrogen required for growth, assuming a growth rate of ~95 mgN/crab/year (Robertson, unpublished data). If these rates of fixation are compared with other studies, they are much lower than those obtained for three species of sea urchins by Guerinot & Patriquin (1981a), but comparable with the lowest rates measured for another urchin by Odintsov (1981). Unfortunately, earlier studies have not used standard units for nitrogen fixation, some expressing it per g of animal tissue or per whole animal, others per volume of gut. Without conversions for the sizes of animals studied and the gut:volume or gut:weight ratios it is impossible to compare the various studies. We conclude that nitrogen fixation did not contribute significantly to the nitrogen requirements of the crab *S. messa*, and that the nitrogen fixation measured is probably merely due to the continuation of nitrogenase activity of a few ingested microbes, and not the attached epimural bacteria seen in the hindgut.

If the nitrogen fixation rate of the gut contents is determined on a wet weight-specific basis (calculated by combining fixation rates of guts and digestive glands, and correcting for daily consumption rate of leaf material) is compared with that of leaves (aged and senescent combined, Table 3), then the maximum nitrogen fixation measured for gut contents of crabs (~ 5.0 mg N/day/g ww) was only 0.30 % of that of the uningested leaves (1686 mg N/day/g ww). Although this calculation may prove an underestimate for gut contents relative to leaves due to a dilution affect in the gut and the probability of higher water content in gut contents, it is clear that rates of nitrogen fixation in the gut are insignificant when compared with those associated with the leaves. It is most likely that the low rates of nitrogen fixation in the guts can be attributed to the viability of a few ingested nitrogen fixers. The high variability in fixation rates of different individuals of the same species, as seen in this study, has been reported by other authors previously (Guerinot & Patriquin 1981a, Odintsov 1981), and supports the hypothesis that the phenomenon may be attributed to ingested microbes alone. It is clear that the rate of nitrogen fixation occurring inside the gut is only a tiny fraction of that occurring externally in the habitat, and we conclude that the crab hindgut is not a significant site of

nitrogen fixation in the mangrove ecosystem.

References

Bayer, E.A. & R. Lamed, 1986. Ultrastructure of the cell surface cellulospme of *Clostridium thermocellum* and its interaction with cellulose. J. Bacteriol., Vol. 167, pp. 828-836.

Bunt J.S., 1982. Studies of mangrove litter fall in tropical Australia. Chapter 13. In: Mangrove ecosystems in Australia. Structuer, function and management. Clough B.F. (ed.), AIMS

Camilleri, J., 1989. Leaf choice by crustaceans in a mangrove forest in Queensland. Mar. Biol., Vol. 102, pp. 453-459.

Carpenter, E.J. & J.L. Culliney, 1975. Nitrogen fixation in marine shipworms. Science, Vol. 187, pp. 551-552.

Cochran, W.G., 1950. Estimation of bacterial densities by measurement of the "Most Probable Number". Biometrics, Vol. 6, pp. 105-116.

Deming, J.W. & R.R. Colwell, 1982. Barophilic bacteria associated with digestive tracts of abyssal holothurians. Appl. Environ. Microbiol., Vol. 44, pp. 1222-1230.

Dempsey, A.C. & C.L. Kitting, 1987. Characteristics of bacteria isolated from penaeid shrimp. Crustaceana, Vol. 52, pp. 90-94.

Fong, W. & K.H Mann, 1980. Role of gut flora in the transfer of amino acids through a marine food chain. Can. J. Fish. Aquat. Sci. Vol. 37, pp. 88-96.

Guerinot, M.L. & D.G. Patriquin, 1981a. The association of N2-fixing bacteria with sea urchins. Mar. Biol. Vol. 62, pp. 197-207.

Guerinot, M.L. & D.G. Patriquin, 1981b. N2-fixing vibrios isolated from the gastrointestinal tract of sea urchins. Can. J. Microbiol. Vol 27, pp. 311-317.

Guerinot, M.L. & R.R. Colwell, 1985. Enumeration, isolation, and characterization of N2-fixing bacteria from seawater. Appl. Environ. Microbiol. Vol. 50, pp. 350-355.

Guerinot, M.L., W. Fong W. & D.G. Patriquin, 1977. Nitrogen fixation (acetylene reduction) associated with sea urchins (*Strongylocentrotus droebachiensis*) feeding on seaweeds and eelgrass. J. Fish. Res. Board Can., Vol. 34, pp. 416-420.

Hardy, R.W.F., R.C. Burns & R.D. Holsten, 1973. Applications of the acetylene-ethylene assay for measurement of nitrogen fixation. Soil. Biol. Biochem. Vol. 5, pp. 47-81.

Klug, M.J. & S. Kotarski, 1980. Bacteria associated with the gut tract of larval stages of the aquatic cranefly *Tipula abdominalis* (Diptera: Tipulidae). Appl. Environ. Microbiol., Vol. 40, pp. 408-416.

Lamed, R., J. Naimark, E. Morgenstern & E.A. Bayer, 1987. Specialized cell surface structures in cellulolytic bacteria. J. Bacteriol., Vol. 169, pp. 3792-3800.

Musgrove, R.J., 1988. Digestive ability of the freshwater crayfish *Paranephrops zealandicus* (Parastacidae) and the role of microbial enzymes. Freshwater Biol., Vol. 20, pp. 305-314.

Odintsov, V.S., 1981. Nitrogen fixation (acetylene reduction) in the digestive tract of some echinoderms from Vostock Bay in the Sea of Japan. Mar. Biol. Letters, Vol. 2, pp. 259-263.

Payne, D.W., N.A. Thorpe & E.M. Donaldson, 1972. Cellulolytic activity and a study of the bacterial population in the digestive tract of *Scrobicularia plana* (Da Costa). Proc. Malac. Soc. Lond., Vol. 40, pp. 147-160.

Porter K.G., Feig Y.S. 1980 The use of DAPI for identifying and counting aquatic microflora. Limnol Oceanogr 25(5):943-948

Prim, P. & J.M. Lawrence, 1975. Utilization of marine plants and their constituents by bacteria isolated from the gut of Echinoids (Echinodermata). Mar. Biol., Vol. 33, pp. 167-173.

Robertson, A.I., 1986. Leaf-burying crabs: their influence on energy flow and export from mixed mangrove forests (*Rhizophora* spp.) in northeastern Australia. J. Exp. Mar. Biol. Ecol. Vol. 102, pp. 237-248.

Robertson, A.I., 1988. Decomposition of mangrove leaf litter in tropical Australia. J. Exp. Mar. Biol. Ecol., Vol. 116, pp. 235-247.

Schallenberg M., Kalff J., Rasmussen J.B. 1989 Solutions to problems in enumerating sediment bacteria by direct counts. Appl Env Microbiol 55(5):1214-1219

Waterbury, J.B., C.B. Calloway & R.D. Turner, 1983. A cellulolytic nitrogen-fixing bacterium cultured from the gland of Deshayes in shipworms (Bivalvia: Teredinidae). Science, Vol. 221, pp. 1401-1403.

Zachary, A., K.K. Parrish & J.D. Bultman, 1983. Possible role of marine bacteria in providing the creosote-resistance of *Limnoria tripunctata*. Mar. Biol. Vol. 75, pp.1-8.

CHAPTER 8

Harris JM (*in press*) Widespread occurrence of extensive epimural rod bacteria in the hindgut of marine Thalassinidae and Brachyura (Crustacea, Decapoda). *Marine Biology*

CHAPTER 8

Widespread occurrence of extensive epimural rod bacteria in the hindguts of marine Thalassinidae and Brachyura (Crustacea, Decapoda)

Abstract

Direct observation by scanning electron microscopy indicates that the presence of bacteria (epimural and unattached) in the hindguts of Crustacea is widespread, occurring across taxa (2 infraorders i.e. Brachyura and Thalassinidae, 9 genera, 16 species), feeding types (detritivores, scavengers and carnivores), habitats (mangroves, saltmarshes, sand/mudflats) and continents (North America, South Africa, Australia). Crustacean hindguts clearly represent suitable environments for colonization by micro-organisms, despite the lack of specialized structures or modifications of the gut to facilitate this. Mats of closely-packed epimural rods and scattered epimural rods were the most common types of bacteria observed in the guts of the Crustacea examined, although unattached rods and scattered epimural cocci occurred in some species. There were, however, taxon-dependent differences in colonization characteristics of hindgut bacteria, possibly related to differences in gut lining. Abundance of hindgut microflora was unrelated to the host's taxon, habitat or geographical locality, but appeared to be affected by the feeding habits of the animal. Mats of epimural rods were associated exclusively with detritivores, while cocci were only observed in the hindguts of scavengers and carnivores. Moreover, extensive colonization by epimural rod bacteria (covering >50% of the hindgut lining) was observed in detritivores only, while carnivores harboured few or no rod bacteria. The detritivore hindgut appears to provide a better environment for microbial habitation than does that of carnivorous crustaceans. In all cases the rod bacteria were monocultures of morphologically identical bacteria and were remarkably similar among crustacean species. The potential significance of prolific colonization of the hindguts of Crustacea by microbes is discussed.

Keywords: Crustacea, hindgut, bacteria, SEM

Introduction

Although bacteria have been reported from the digestive tracts of a number of crustaceans (e.g. Deming *et al.* 1981, Colorni 1985, Sugita *et al.* 1987a,b, Moriarty 1990), little is known about their colonization sites, morphology, growth characteristics, abundance and frequency of occurrence. Contrary to earlier hypotheses suggesting that crustacean hindguts are generally depauperate in terms of microbial colonization (Boyle and Mitchell 1978, Bignell 1984) and reports of a lack of epimural microorganisms (Sleeter *et al.* 1978, Atlas *et al.* 1982), recent evidence indicates that some crustacean hindguts may provide microhabitats for bacterial colonization of equal quality to those found in insect hindguts, where prolific colonization has been reported (e.g. Breznak and Prankratz 1977, Cruden and Markovetz 1981, Mead *et al.* 1988). Spectacular mats of epimural rod bacteria have been reported in the hindgut of the thalassinid prawn *Upogebia africana* (Harris *et al.* 1991), and epimural bacteria have also been reported in the hindguts of a few other Crustacea, e.g. the prawn *Callianassa kraussi* (Harris *et al.* 1991), the shrimps *Penaeus aztecus* and *P. setiferus* (Dempsey *et al.* 1989) and the crab *Callinectes sapidus* (Huq *et al.* 1986). In view of the important roles played by hindgut bacteria in the physiology of some insects (see Breznak 1984 for review), symbiotic associations between Crustacea and gut microbes may be of significance.

The paucity of studies to date makes it difficult to determine whether hindgut microflora occur in all Crustacea or are restricted to some taxa, trophic types or habitats. Conflicting reports as to the presence of bacteria in the digestive tracts of Crustacea have highlighted the need for an understanding of the factors that determine the presence and abundance of a gut microflora. For example, while Boyle and Mitchell (1978) and Sleeter *et al.* (1978) reported the absence of bacteria in the guts of one terrestrial and two marine isopods, and suggested that these isopods produce an anti-microbial agent that keeps the gut free of micro-organisms, Zachary and Colwell (1979) and Zachary *et al.* (1983), found that under certain conditions one of these species, *Limnoria tripunctata*, does possess a gut microflora which may be of significant benefit to the host.

This study investigates, by scanning electron microscopy, whether gut bacteria are present in the hindguts of 16 species of decapod Crustacea, representing 9 genera and 2 infraorders (Brachyura and Thalassinidae (after Abele 1982)) and comprising different feeding types, collected from a variety of habitats from 3 different continents (Africa, North America and Australia). The aim is to establish whether the hindguts of Crustacea are, in general, suitable environments for micro-organisms, and to gain an understanding about the factors that may

influence the presence, distribution and abundance of gut bacteria. In addition, the direct observations of gut microbes reported here provide information as to their *in situ* morphology and their aggregation and attachment characteristics.

Methods and materials

Table 1 lists the 16 species of Crustacea that were investigated and describes the habitats and geographical locations from which they were collected. Animals were processed within 4 h of collection. Animals were cold-anaesthetized and dissected to remove whole hindguts intact. Hindguts were fixed in 2.5 % gluteraldehyde (in 0.2 m filtered seawater) and stored at 4 ^{o}C until further processing (up to 2 mo). Samples were then passed through a desalination series (75, 50, 25 and 0 % 0.2 m filtered seawater in distilled water), through a dehydration series (10,30,50,70,90,95 and 100 % ethanol), and subsequently dried in a Polaron Critical Point Drier using CO_2 as the transitional fluid. Dried, whole hindguts were sliced in half longitudinally using a sharp, sterile scalpel, and the contents removed by gently lifting the entire food bolus out. Guts were then mounted, with the inner gut linings exposed, on aluminium stubs using a glue-graphite mixture. Mounted samples were sputtercoated with gold paladium, and viewed with a Cambridge S200 scanning electron microscope. At least 3 individual animals of each species were viewed, and in each case the gut lining of the entire length of the hindgut was viewed.

Results

Hindgut lining

The hindguts of the *Upogebia* and *Callianassa* species examined are not differentiated into distinct posterior and anterior regions (Table 2). The gut lining of the two *Upogebia* species comprises longitudinally folded convoluted smooth epithelium only (Figures 1a-d) while that of the three *Callianassa* species consists of alternating panels of smooth epithelium and brushborder (Figures 1e-h, 2a-h). Pores or pits were not observed in the hindgut lining of the *Upogebia* and *Callianassa* species investigated. In all of the brachyuran species (Figures 3-7), except *Callinectes sapidus* (Figure 7a,b), the hindgut appears to comprise two different regions, i.e. an extreme posterior region lined by smooth convoluted epithelium, and a more extensive anterior region where the gut lining consists either entirely of smooth

Figure legends :

Figs. 1-7. Electron micrographs of the hindguts of decapod Crustacea showing internal structure and bacterial colonization in the posterior and anterior regions.

Fig. 1. *Upogebia pugettensis* (a,b convoluted smooth epithelium), *Upogebia africana* (c,d convoluted smooth epithelium) and *Callianassa australiensis*, (e,f smooth convoluted epithelium, g,h brush-border lining). Scale bars: a 20 μm, b 10 μm, c 20 μm, d 5 μm, e 20 μm, f 5 μm, g 50 μm, h 10 μm.

Fig. 2. *Callianassa californiensis* (a,c,d brush-border, b smooth convoluted epithelium) and *Callianassa kraussi* (e,f smooth epithelium, g,h brush-border). Scale bars: a 50 m, b 10 μm, c 20 μm, d 10 m, e 10 μm, f 10 μm, g 20 μm, h 10 μm.

Fig. 3. *Sesarma catenata* (a,b posterior smooth epithelium, c,d anterior brush-border region) and *Sesarma cinereum* (e,f posterior smooth epithelium, g,h anterior brush-border region). Scale bars: a 20 μm, b 10 μm, c 20 μm, d 10 μm, e 20 μm, f 10 μm, g 50 μm, h 10 μm.

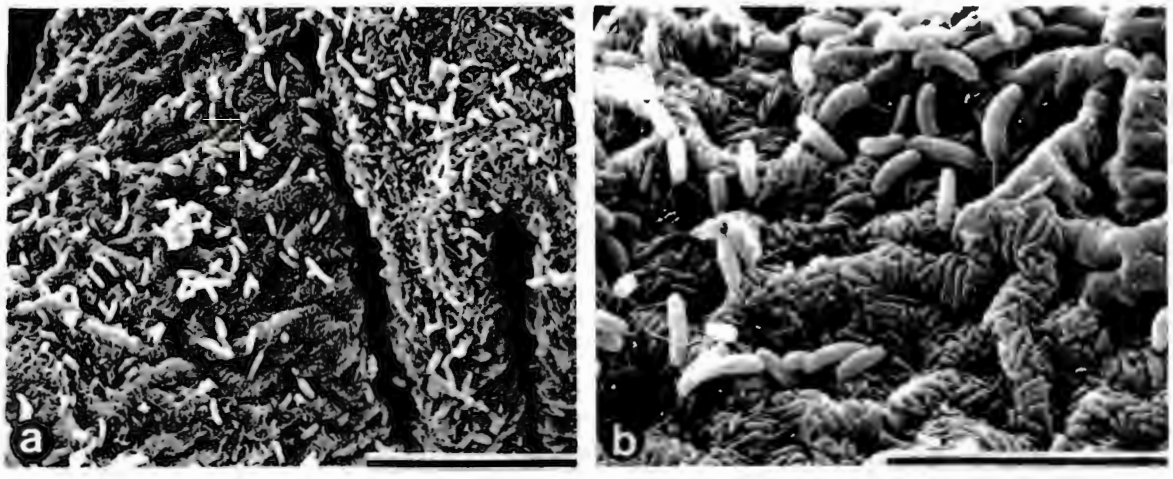
Fig. 4. *Sesarma messa* (a,b brush-border, c,d smooth convoluted epithelium) and *Uca pugnax* (e,f posterior smooth epithelium, g,h anterior brush-border region). Scale bars: a 10 μm, b 1 μm, c 10 μm, d 1 μm, e 20 μm, f 10 μm, g 20 μm, h 5 m.

Fig. 5. *Uca minax* (a,b posterior smooth convoulted epithelium, c,d anterior brush-border region) and *Uca pugilator* (e,f posterior smooth epithelium, g,h anterior brush-border region). Scale bars: a 20 μm, b 10 μm, c 20 μm, d 10 μm, e 50 μm, f 10 μm, g 20 μm, h 5 μm.

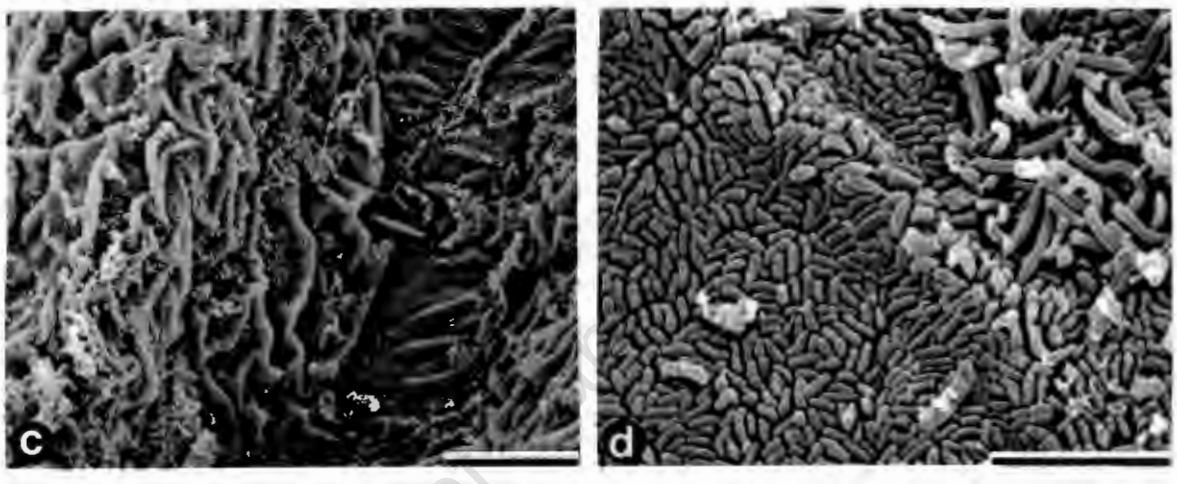
Fig. 6. *Myctirus longicarpus* (a,b posterior smooth convoluted epithelium, c,d anterior brush-border region) and *Panopeus herbstii* (e,f posterior smooth epithelium, g,h anterior brush-border region). Scale bars: a 20 μm, b 10 μm, c 20 μm, d 10 μm, e 20 μm, f 10 μm, g 20 μm, h 5 μm.

Fig. 7. *Callinectes sapidus* (a,b brush-border), *Ovalipes punctatus* (c-f brush-border) and *Scylla serrata* (g,h brush-border). Scale bars: a 20 μm, b 10 μm, c 20 μm, d 10 μm, e 20 μm, f 5 μm, g 20 μm, h 10 μm.

Upogebia pugettensis



U. africana



Callianassa australiensis

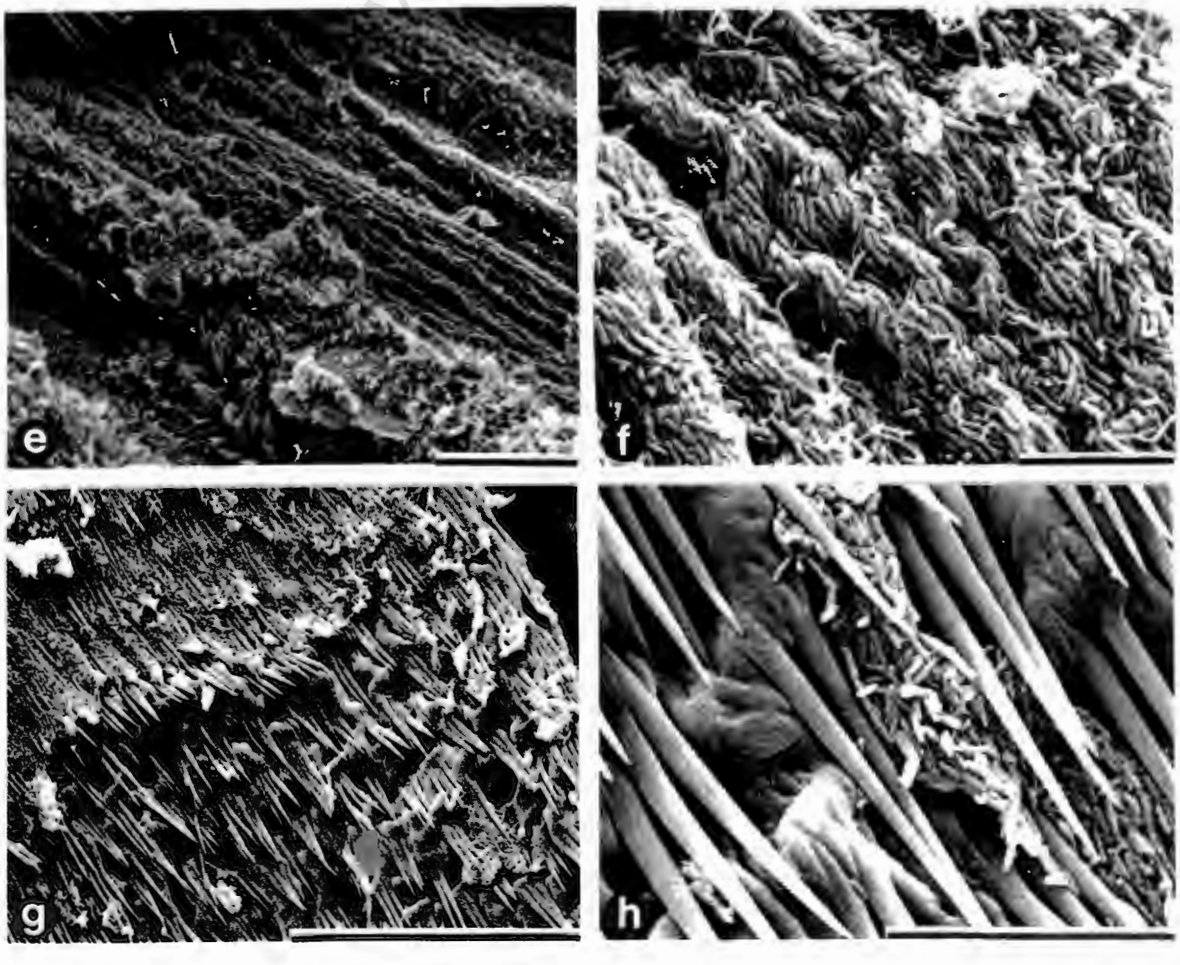
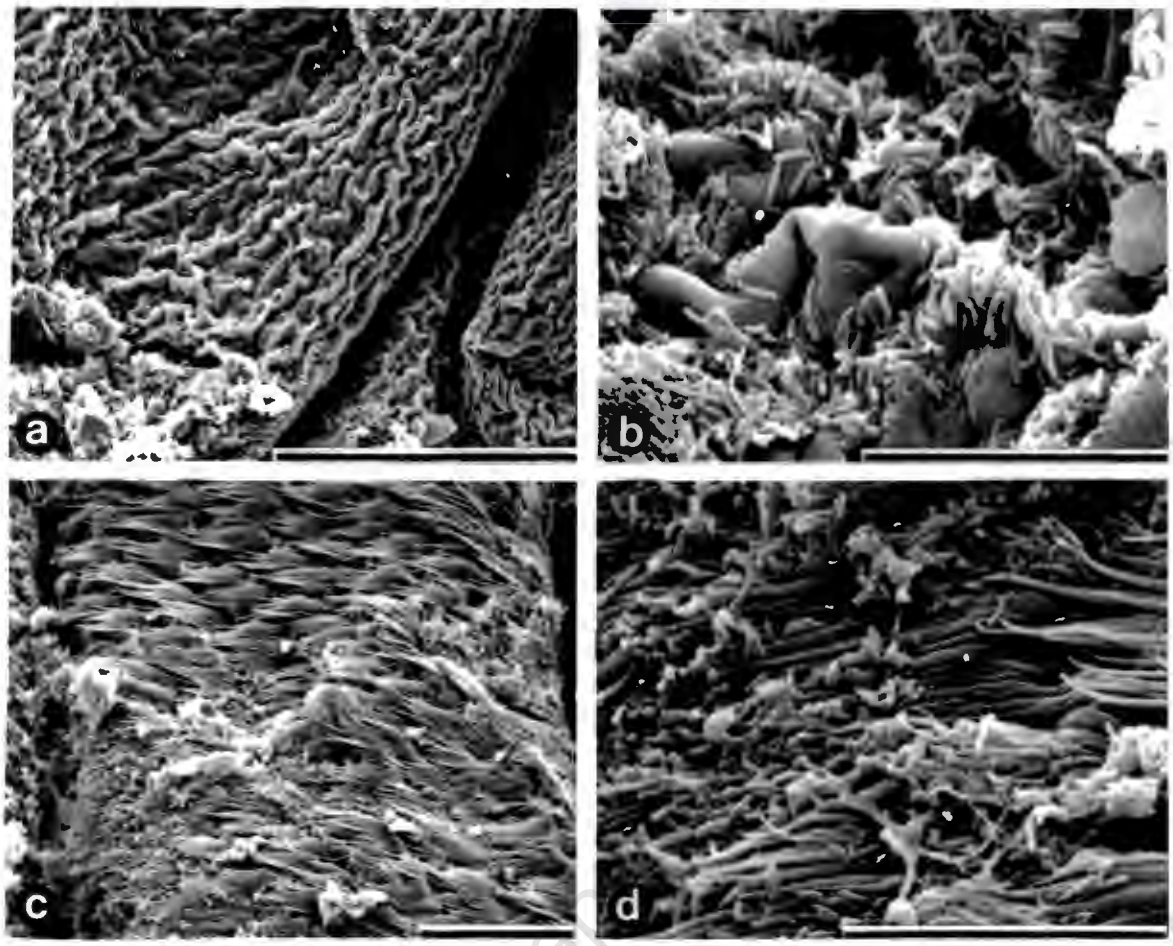


Figure 1

Callianassa californiensis



C. kraussi

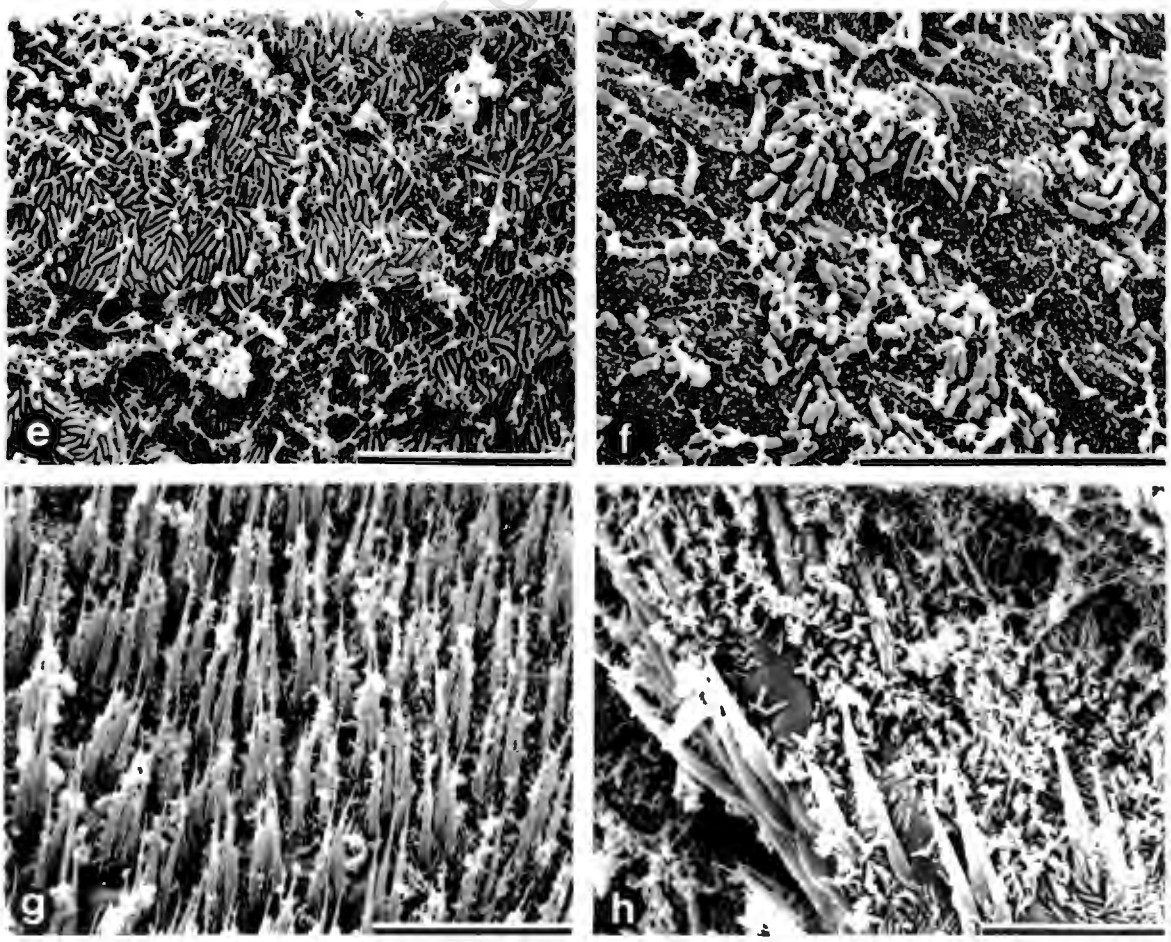
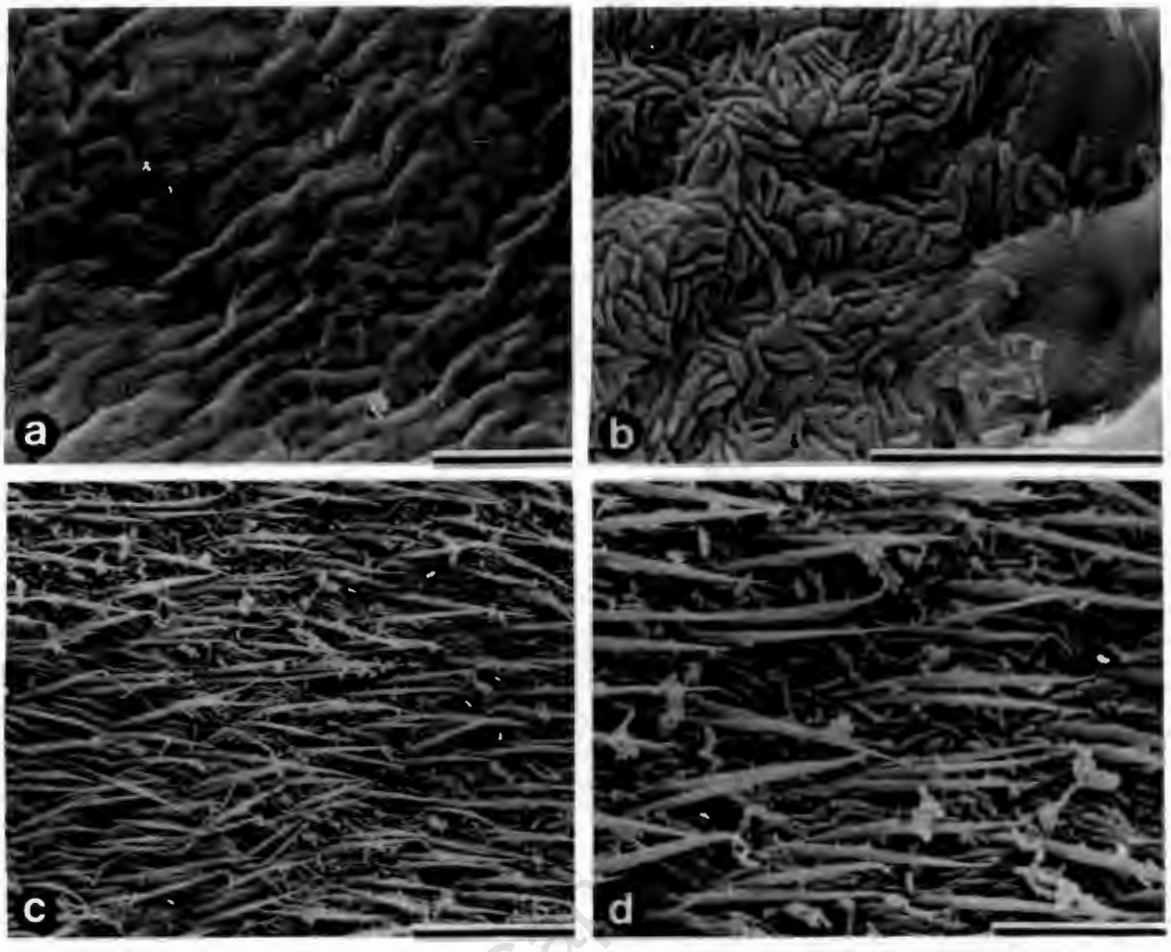


Figure 2

Sesarma catenata



S. cinereum

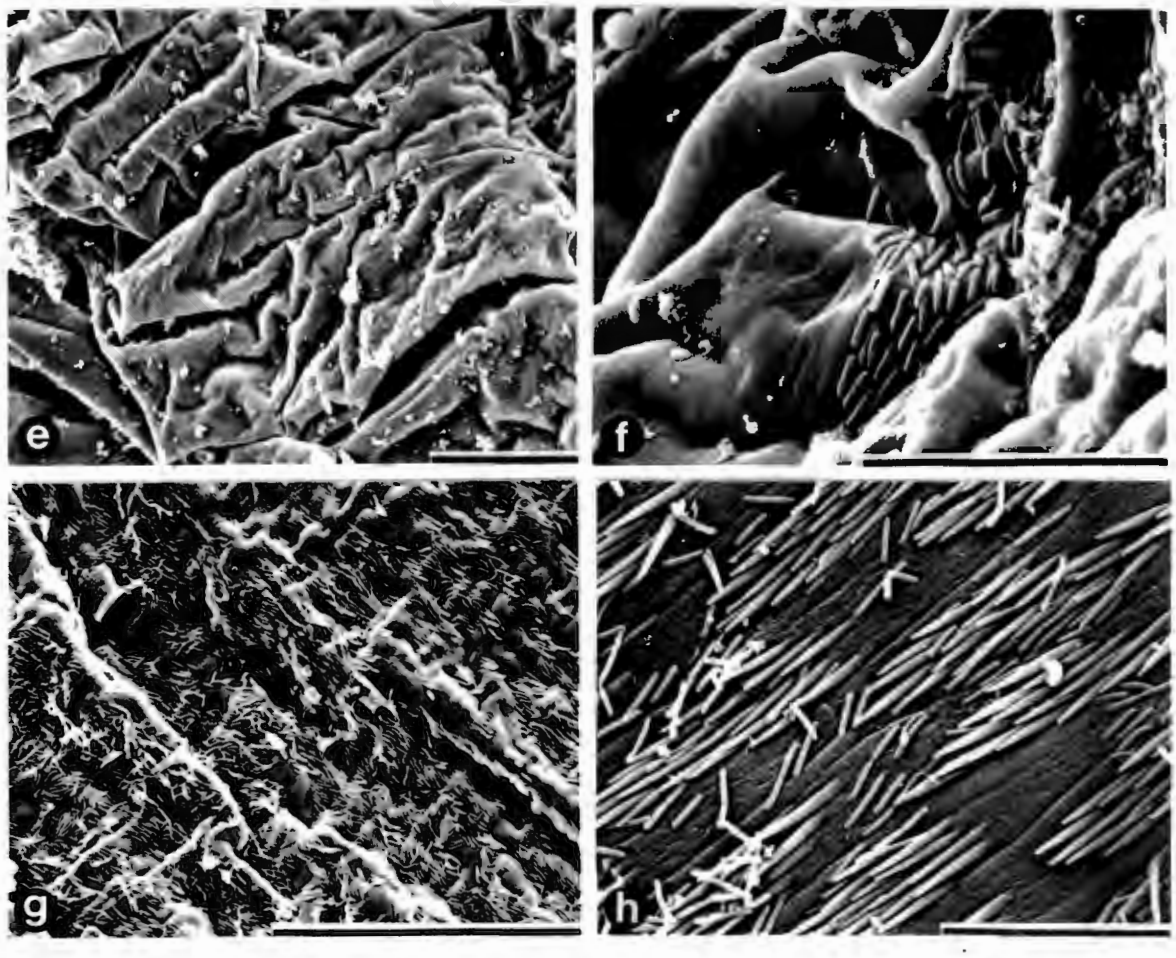


Figure 3

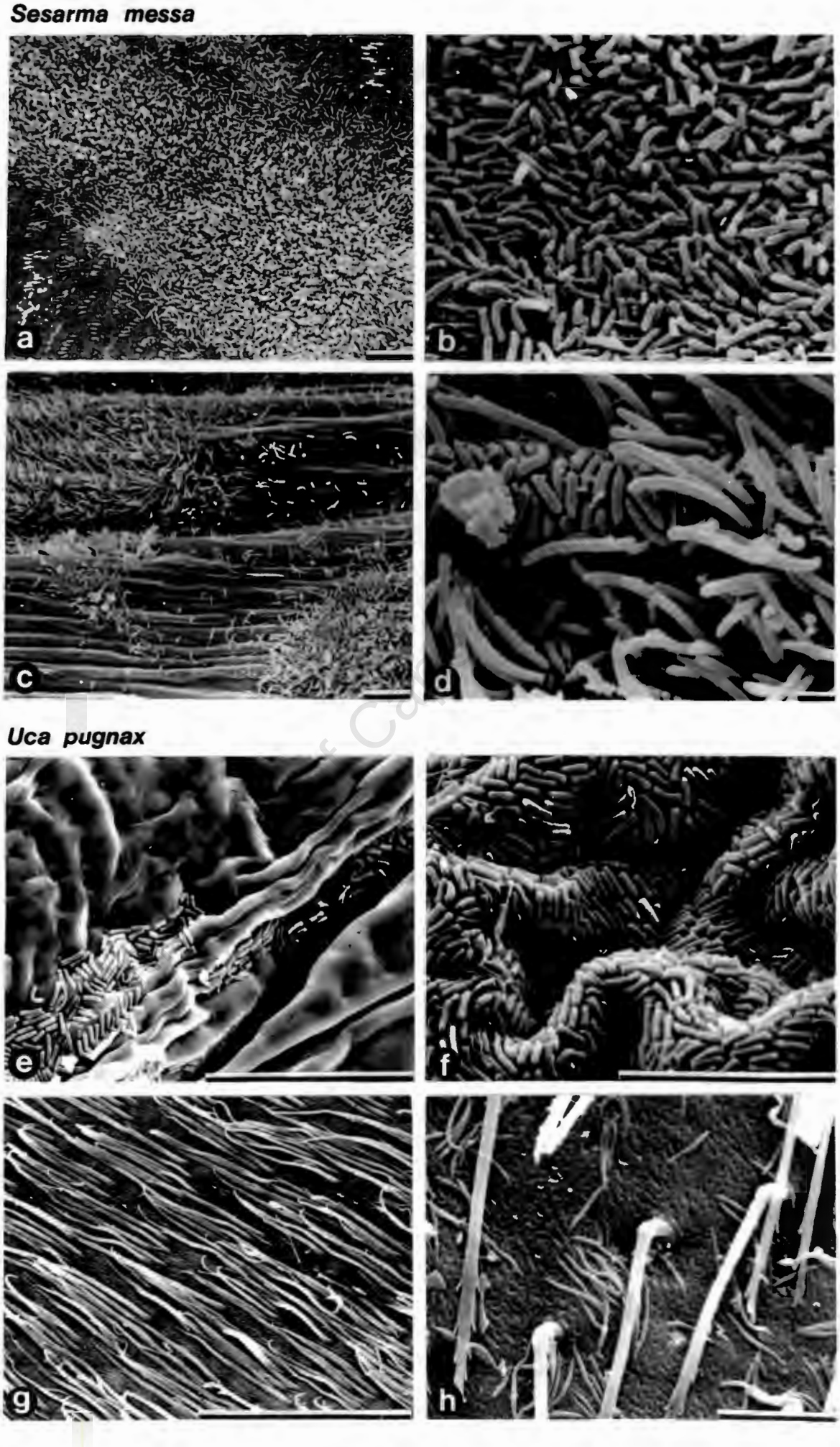
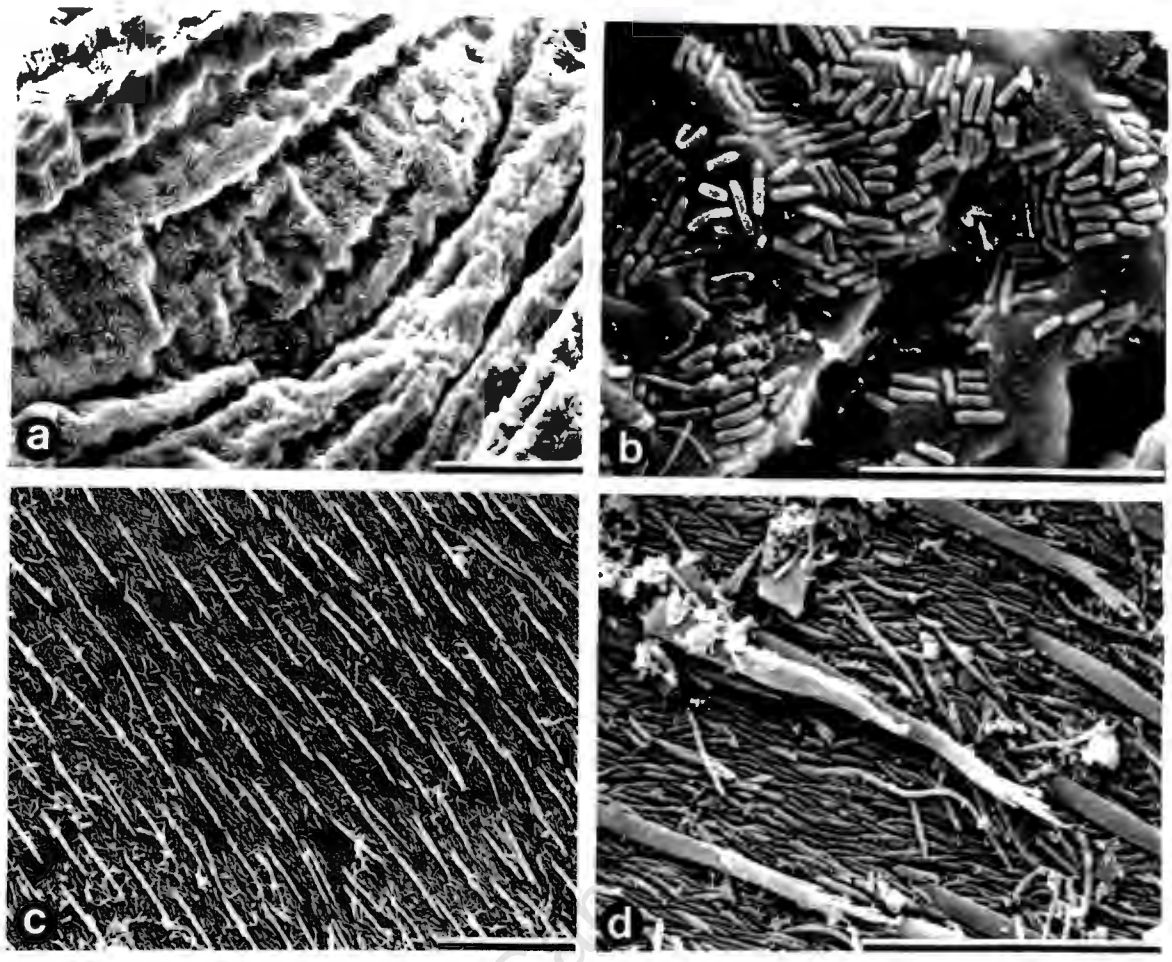


Figure 4

Uca minax



Uca pugilator

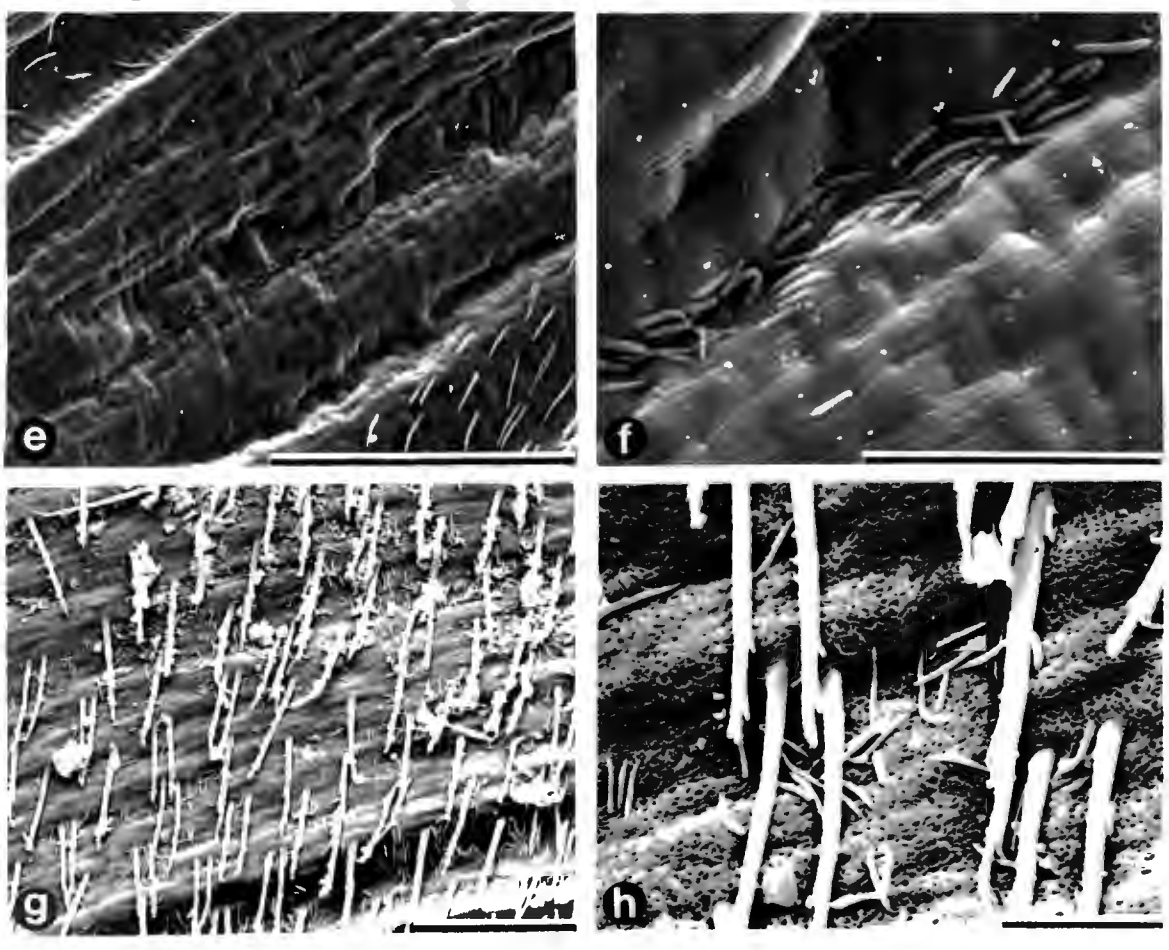
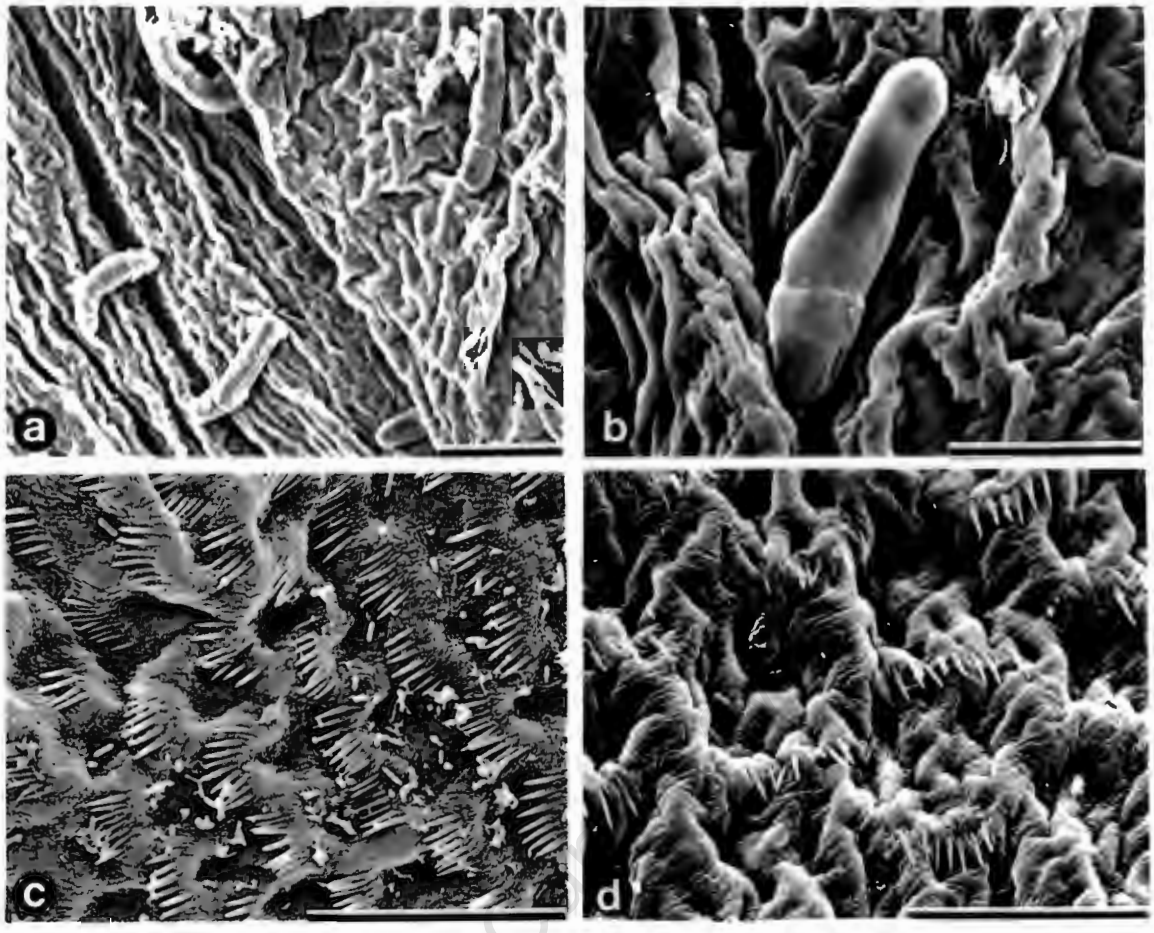


Figure 5

Mictyris longicarpus



Panopeus herbstii

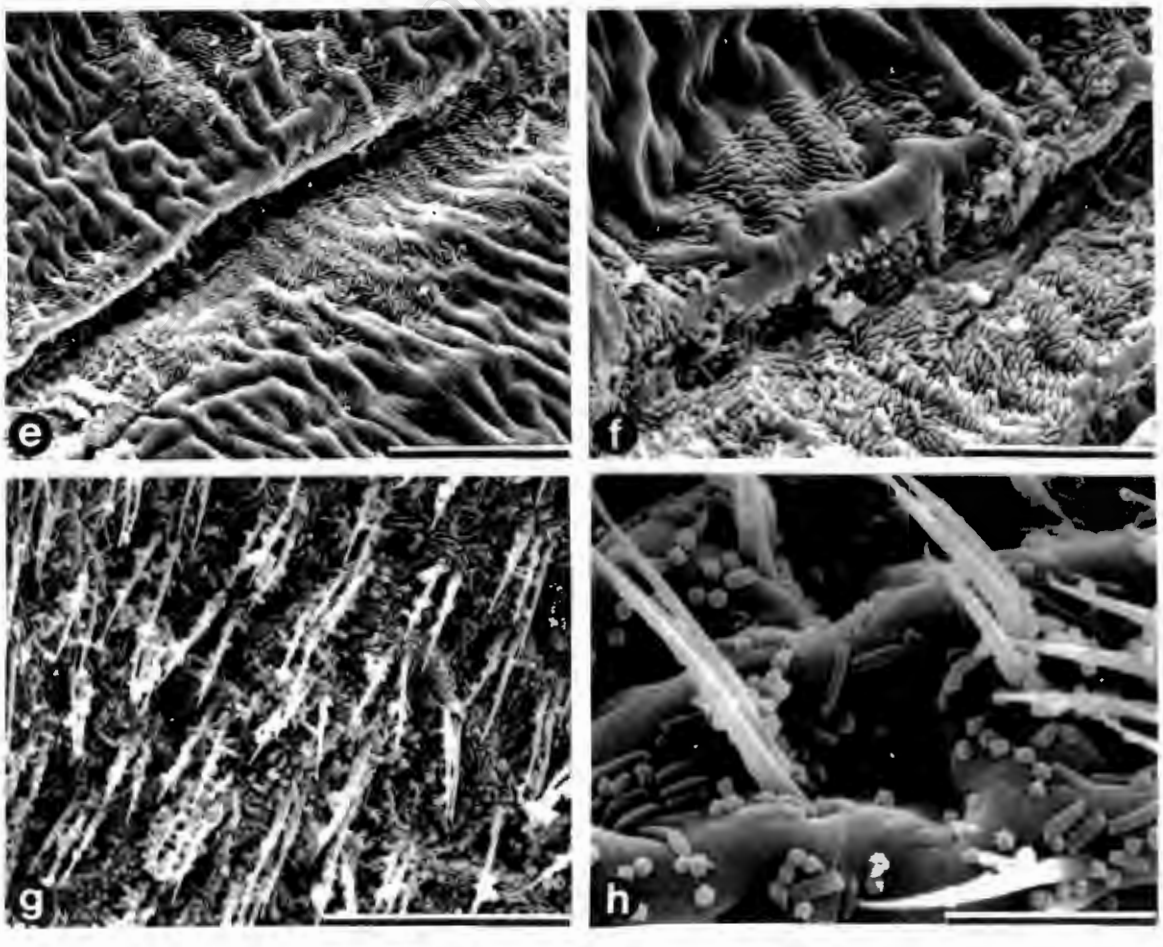
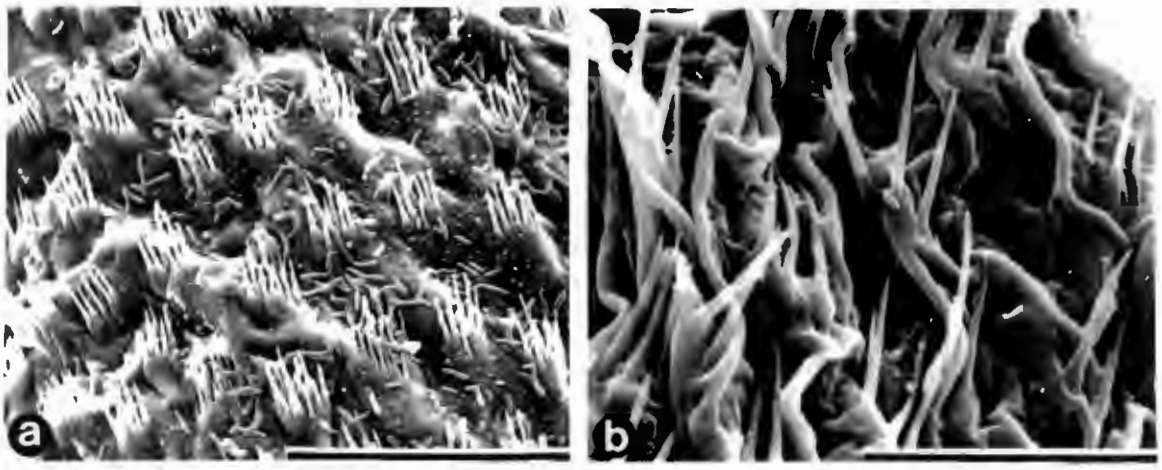
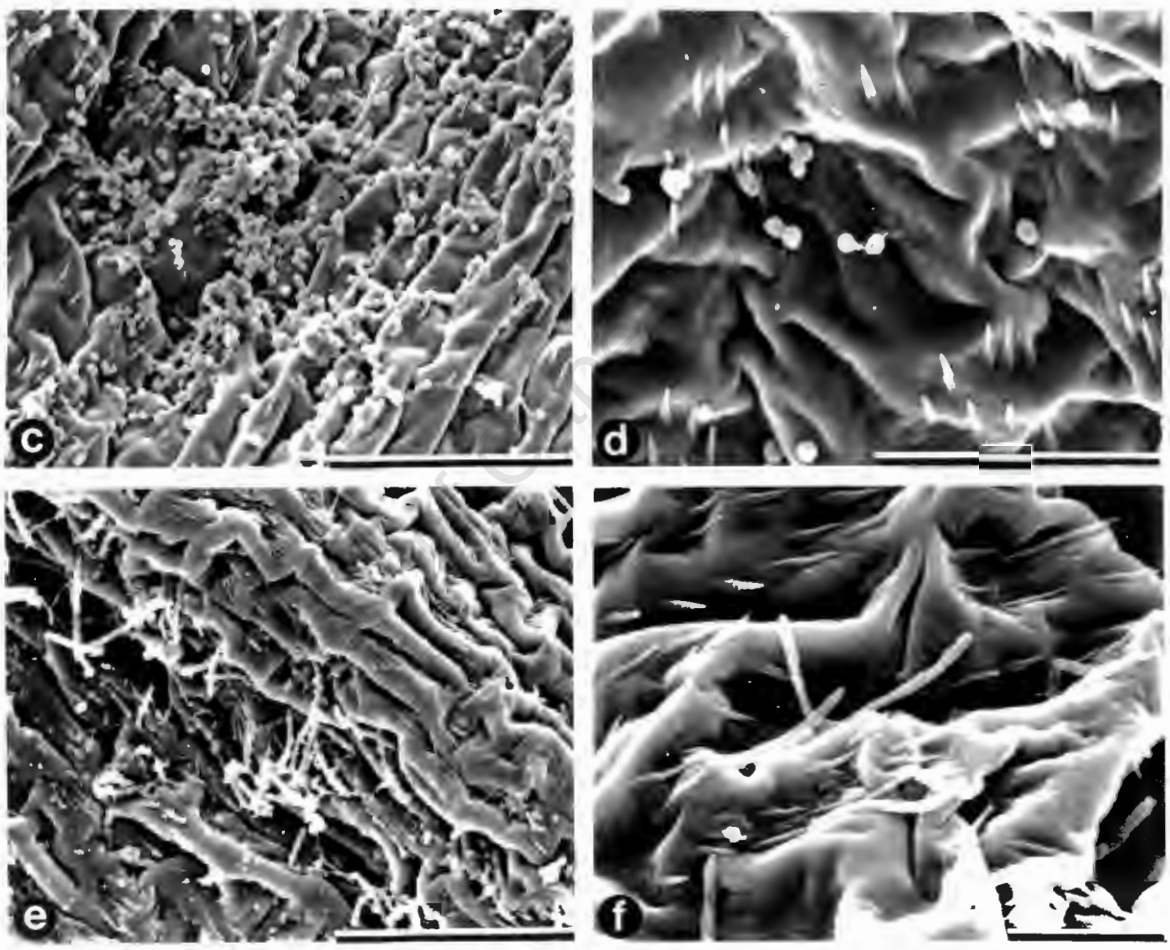


Figure 6

Callinectes sapidus



Ovalipes punctatus



Scylla serrata

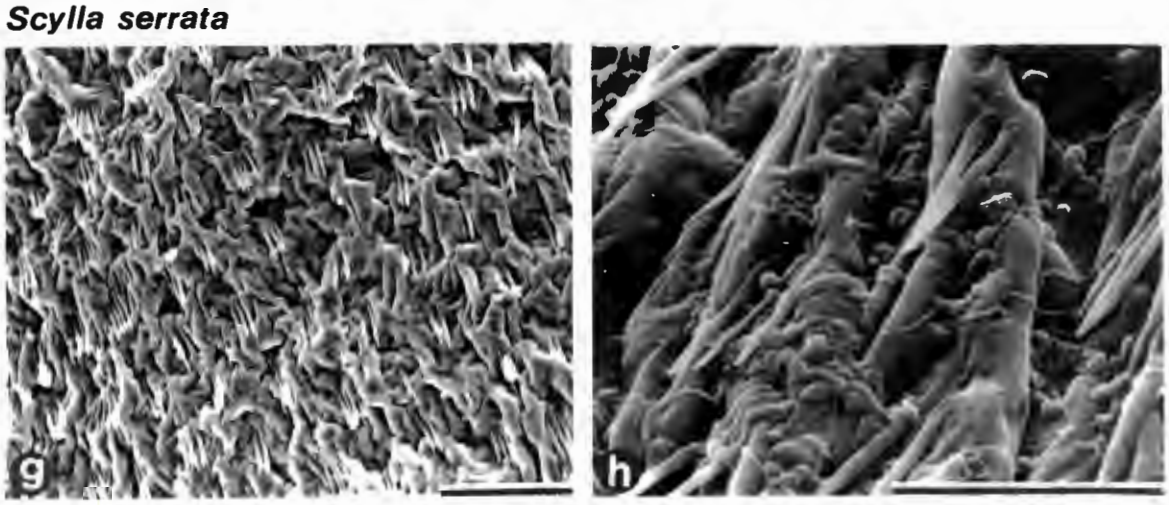


Figure 7

epithelium with numerous regularly spaced brush-like projections (= brush-border lining) (as in *Uca pugnax, U. pugilator, U. minax, Sesarma cinereum, S. catenata* and *Mictyris longicarpus*), or of longitudinally oriented panels of smooth epithelium alternating with panels of brush-border lining (as in *Panopeus herbstii, Ovalipes punctatus, S. messa* and *Scylla serrata*). In all of the *Uca* and *Sesarma* species investigated oval-shaped pores (ca. 1 m wide) are present in the posterior region (e.g. Figure 3b), while small (ca. 0.2 m diameter), closely spaced pits (pores?) are a feature throughout the hindgut lining (see Figures 4h, 5h). *Callinectes sapidus* does not appear to have a differentiated posterior region, the whole of the hindgut lining consisting of longitudinally oriented alternating panels of smooth epithelium and brush-border lining. The proportions of the anterior and posterior regions of the hindguts vary among species (Table 2).

Hindgut bacteria

Bacteria observed in the hindgut were defined as rods (cylindrical in shape) or cocci (spherical in shape). The term "rod" was used to describe cylindrical-shaped bacteria which were not obviously curved. Only markedly bowed bacteria were described as curved rods (vibrios) (Schlegel 1988). It is, however, possible that some of the rod bacteria described as straight in this study, represent curved morphotypes. This study provides views of the *in situ* morphotypes of the hindgut bacteria. It should be noted that these may not necessarily correspond to their morphologies *in vitro*, and that no conclusions can be reached as to their physiology or taxonomy. Rod bacteria were observed in the hindguts of all of the 16 species of Crustacea examined (Figures 1-7), except for the crab *Scylla serrata* in which only a few cocci were found (Figure 7g,h). Of particular interest are the extensive, single-layered mats of rod bacteria closely attached to the surface of the hindgut epithelium and present to a greater or lesser extent in either the posterior or anterior regions of 11 of the 16 species examined (Table 2). These mats of rod bacteria were found in all of the *Callianassa* and *Upogebia* species investigated, being most extensive in *Upogebia pugettensis* and *U. africana* where spectacular wall-to-wall colonisation was observed in all individuals examined (Figure 1a-d). Large mats (covering more than 50 % of the gut wall) were also found in the gut of *Callianassa australiensis*, primarily associated with the panels of smooth epithelium (Figure 1e-h), and mats of rod bacteria covering up to 50 % of the area of the hindgut lining were observed in both *C. californiensis* (Figure 2a-d) and *C. kraussi* (Figure 2e-h).

The fiddler crab *Uca minax* was the only crab species (out of 11) to have extensive mats of rod bacteria (>50% cover in 2 of the 3 individuals

Table 2. Distribution and colonization characteristics of rod bacteria in the hindguts of the Crustacea investigated in this study.

[Abundance key (percentage of total gut lining covered by bacteria) : 0 = no bacteria, 1 = 0-25 %, 2 = 25-50 %, 3 = 50-75 %, 4 = 75-100 %]

Species	Posterior hindgut (smooth convoluted lining)					Anterior hindgut (smooth convoluted and/or brush border)				
	Percent of hindgut	Bacterial abundance	Colonization			Percent of hindgut	Bacterial abundance	Colonization		
			unattached	scattered epimural	epimural mats			unattached	scattered epimural	epimural mats
Thalassinidae (prawns)										
Upogebia pugettensis	0					100	4		•	
U. africana	0					100	3			•
Callianassa australiensis	0					100	3	•		•
C. californiensis	0					100	2	•		•
C. kraussi	0					100	1	•	•	•
Brachyura (crabs)										
Sesarma catenata	50	3		•	•	50	2		•	•
S. cinereum	25	1		•	•	75	2	•	•	•
S. messa	10					90	1[a]		•	•
Uca pugnax	25	2			•	75	2		•	
U. minax	25	1			•	75	3	•	•	•
U. pugilator	25	1		•		75	1		•	
Myctiris longicarpus	10	0[b]				90	1	•		
Panopeus herbstii	20	1			•	80	3[c]	•	•	
Callinectes sapidus	0					100	1	•		
Ovalipes punctatus	5	0				95	1[a]	•	•	
Scylla serrata	25	0				75	1[d]		•	

a = cocci present
b = protozoa present
c = predominantly cocci
d = cocci only

examined) attached to the hindgut lining (Figure 5a-d). Smaller epimural mats of rod bacteria were found in the guts of the crabs *Sesarma cinereum, S. messa, S. catenata, Panopeus herbstii* and *Uca pugnax* (Table 2). The presence of scattered epimural rod bacteria, associated with the brush-border lining in particular, was characteristic of most (8 out of 11) of the crab species investigated (e.g. *Uca minax* and *U. pugilator* (Figure 5)). In crabs in general, epimural bacteria were most often found associated with brush border linings, although mats were also observed attached to smooth epithelium in the *Sesarma* and *Uca* species (Figures 3-5). Rod bacteria were also observed singly or in clumps unattached in the lumen of the hindgut in many of the species of both prawn and crab (Table 2).

The rod bacteria observed either as epimural mats or scattered loosely in the hindgut comprised monocultures of morphologically similar bacteria in all species of Crustacea examined. In addition, *U. pugettensis* possessed large curved bacteria associated with the epimural mats of smaller rods (Figure 1a,b). Coccoid bacteria were observed in only 4 out of the 16 species examined (Table 2). Scattered epimural cocci and loose cocci were observed in the hindguts of *S. messa* and *O. punctatus*. In *Panopeus herbstii* the anterior hindgut microflora was dominated by loose and epimural cocci which occurred together with scattered rods (Figure 6e-h). Very few bacteria were observed in the guts of the crab *Scylla serrata*, those seen being cocci (Figure 7g,h).

Habitat and geographical region

The presence of bacteria in the guts of Crustacea did not appear to be correlated with the geographical locality or the habitat of the animals. For example, extensive mats of bacteria were observed in the guts of both *Upogebia africana* and *U. pugettensis*, which were collected from South Africa and North America, respectively (see Tables 1 and 2). The 3 *Sesarma* species investigated all possessed mats of rod bacteria even though collected from 3 different continents and 3 different habitats (saltmarsh, mangrove, sand/mudflat) (Table 1).

Invertebrate diet

The crustacean species examined included detritivores, scavengers and carnivores (Table 3). Most of the detritivores studied possessed mats of epimural rod bacteria in their hindguts (10 out of 12 species) (see Table 2). Of these 10 species, 8 had

Table 3. Abundance of epimural hindgut rod bacteria and diet of 16 species of Crustacea.

(Abundance ie. percentage of the total area of whole hindgut lining that is colonized by bacteria : - = no bacteria, + = < 25 %, + + = 25-50 %, + + + = 50-75 %, + + + + = 75-100 %)

Species	Abundance	Feeding type (diet/gut contents)	Reference
Thalassinidae (prawns)			
Upogebia pugettensis	+ + + +	detritivore (fine organics)	Ricketts & Calvin (1962)
U. africana	+ + +	detritivore (fine organics, bacteria, diatoms)	Harris et al. (1991)
Callianassa australiensis	+ + +	detritivore (fine organics, diatoms, plant fragments)	Kenway (1981)
C. californiensis	+ +	detritivore (detritus, bacteria)	Ricketts & Calvin (1962)
C. kraussi	+	detritivore (organics, bacteria, diatoms, plant fragments)	Harris et al. (1991)
Brachyura (crabs)			
Sesarma catenata	+ + +	detritivore - scavenger (organic matter)	Day (1974)
S. cinereum	+ +	detritivore - occasionally carnivorous	Abele (1973)
S. messa	+	detritivore (leaves, organics, diatoms)	Robertson (1986), pers. obs.
Uca pugnax	+ +	detritivore (silt, diatoms, bacteria, meiofauna)	Miller (1961), Teal (1962), Hoffman et al. (1984)
U. minax	+ +	detritivore (fine organics, bacteria)	Gray (1942), Miller (1961)
U. pugilator	+	detritivore - rarely carnivorous (bacteria, diatoms, algae)	Miller (1961), Teal (1962), Robertson et al. (1981)
Myctiris longicarpus	+[a]	detritivore (organic fragments, bacteria, algae)	Cameron (1966), Quinn (1986)
Panopeus herbstii	+ +[b]	carnivore (bivalves, barnacles)	Ryan (1956), McDermott (1960), Whetstone & Everstole (1981)
Callinectes sapidus	+	predator-scavenger, rarely herbivorous	Seed (1980), Laughlin (1982)
Ovalipes punctatus	+	carnivore	Day (1974)
Scylla serrata	-[c]	carnivore (fish, few plant remains)	Day (1974), Hill (1976)

a = protozoa present

b = mixture of cocci & rods

c = cocci only

bacteria covering more than 50 % of the total hindgut lining (Table 3). The phenomenon of mats of epimural hindgut bacteria appears to be associated exclusively with detritivores. The 4 species of scavengers and carnivores did not possess mats of rods in their hindguts, although 2 species did have scattered epimural rod bacteria and 3 had scattered unattached bacteria. One of the carnivores, *Scylla serrata*, harboured only a few cocci in the hindgut. The high abundances of gut bacteria observed in detritivores were not found in carnivores (Table 3).

Discussion and conclusions

This study provides evidence that the presence of epimural rod bacteria in the hindguts of Crustacea is widespread. The presence, nature and abundance of gut bacteria are unrelated to the taxonomic group or habitat of the invertebrate host. Rod bacteria of similar morphology, attachment and aggregation growth characteristics were observed in the guts of animals of different species and genera collected from different habitats and geographical localities, and the distinctive mats of rods were found in representatives of both of the infraorders (crabs and prawns) investigated. While the *Upogebia, Callianassa, Sesarma* and *Uca* species had the highest bacterial abundance, one species of each of the latter three genera had very low bacterial cover, comparable with that of the representatives of the genera *Myctiris, Panopeus, Callinectes* and *Ovalipes* (Table 3). There were, however, taxa-related differences in the gut lining of the Crustacea investigated. The lining of the hindgut of the *Upogebia* species examined consisted entirely of smooth epithelium, while the crab species and *Calliannassa* species had either brush-border lining only, or both brush-border lining and smooth epithelium (Table 2). These taxon-related differences in gut lining appear to affect the colonization patterns of the bacteria. The presence of scattered epimural rod bacteria associated with the brush-border lining was characteriatic of most crab species, while epimural mats of rod bacteria were generally, although not exclusively, associated with smooth epithelium in prawns and crabs. However, there were exceptions to this general pattern as some species possessed mats of rod bacteria associated with the brush-border lining, while others displayed a paucity of epimural bacteria on the brush-border lining.

My study does not support the suggestion of Lynch and Hobbie (1988) and Moriarty (1990) that the anatomy of the gut must be adapted (e.g. by the possession of pouches) to allow attachment by symbiotic bacteria. While in 2

previous studies bacteria were found to be associated with gut modifications i.e. in a posterior hindgut vesicle in the isopod *Gnathia calva* (Juilffs and Wagele 1987), and in a deep cuticular bay at the beginning of the hindgut of the cladoceran *Alona affinis* (Gunzl 1991), in this study extensive colonization by gut bacteria occurred in the absence of any structural modifications of the hindgut. Although the hindgut was longitudinally folded in all species (to allow extension of the gut when full), this folding did not appear to affect colonization patterns. The nature of the gut lining did, however, appear to affect the colonization characteristics. Mats of epimural bacteria were most frequently associated with smooth epithelium, while scattered epimural rods were associated primarily with brush-border lining. The most prolific colonization was found in invertebrates whose gut lining consists of convoluted smooth epithelium throughout, and in these species the microbes appeared to prefer the nooks and crannies of the convoluted lining, which may afford the microbes with some protection against being detached and voided with faecal material. Scattered epimural bacteria in crabs were primarily associated with the brush-border lining. The spines may prevent dislodgement of the microbes. The role of the brushes on the lining is not clearly understood, but it has been suggested that they may aid in shedding the peritrophic membrane (Bignell 1984). Peritrophic membranes were not investigated in this study, but it is possible that their characteristics (permeability, intactness) influence colonization of the gut lining by bacteria. It has been suggested that they act as barriers to ingested micro-organisms in the hindgut (Bignell 1984). However, the presence of epimural bacteria outside the peritrophic membrane has been reported, indicating that mechanisms by which microbes bypass the membrane must exist in some arthropods (Zachary *et al.* 1983).

The pores present in the lining of the *Sesarma* and *Uca* crab species may be of significance as areas of increased permeability of the lining. It has been suggested for some insects that pitting of the cuticle allows transport of nutrients across the gut wall and encourages bacterial colonization (Bayon 1971, Noirot and Noirot-Timothee 1976). Interestingly, of the crab species investigated, it is only these two genera that do have pitting of the gut lining that display prolific bacterial colonization. However, the *Upogebia* and *Callianassa* species do not have pitted hindgut walls, but nevertheless have extensive microbial colonization, therefore pitting does not appear to be a general requirement for bacterial colonization in the hindguts of Crustacea. However, a common feature of all Crustacea, and therefore all species investigated in this study, is the presence of a chitinous cuticle lining the hindgut. This chitinous cuticle may render the hindgut lining of Arthropods in general a good surface for attachment by bacteria. Huq *et al.* (1986) demonstrated that *Vibrio* readily attach to the chitinous lining of *Callinectes sapidus*, and studies

have shown that the chitinous lining of the hindgut is a suitable site for bacterial attachment in insects (Bignell 1984).

Diet of the invertebrate does appear to influence the hindgut microflora. Mats of epimural rod bacteria and extensive colonization by microbes were only observed in detritivores. In a study investigating the patterns of occurrence of trichomycete fungi in the hindguts of Brachyuran crabs, Mattson (1988) found that the feeding habits of crabs determined the presence and abundance of fungi. Herbivores and detritivores harboured fungi; carnivores did not. Similar to the findings concerning bacteria in the present study, he found that *Uca* species harboured gut fungi, but *Callinectes sapidus* did not. It has been suggested that the relationship between diet and gut fungi may be due to the gut passage rate, which is generally slower in detritivores and herbivores than in carnivores (Mattson 1988). A slow gut passage rate may increase the availability of nutrients supplied by the ingested food, and may reduce the risk of dislodgement of attached microbes due to the movement of gut contents. These results support the predictions of Plante *et al.* (1990) who developed a model describing the digestive associations between bacteria and marine detritivores in terms of cost-benefit analyses. They suggest that gut microfloras are more likely to occur in detritivores than other trophic groups, and predict that hindguts should be prime sites of epimural colonization by bacteria. Other studies have found that the nature of the gut microflora of Crustacea may depend on the biological/biochemical characteristics of the ingested food (Hood *et al.* 1971, Sochard . 1979, Zachary and Colwell 1979, Zachary *et al.* 1983). This is probably because the conditions in the gut, in terms of available nutrients and physical and chemical ranges, are different under different feeding regimes, and may account for the differences observed in this study. However, at present little data concerning the physico-chemical conditions in different marine Crustacean guts, and the factors influencing these, exists.

The finding that the presence and nature of hindgut bacteria is not related to taxa or habitat but may be related to feeding type suggests that these characteristic rod bacteria are exploiting a similar niche in the guts of Crustacea in general, and that the hindgut, particularly that of detritivores, is an especially favourable environment for these microbes. This view is supported by the constancy of morphology and growth form of the rod bacteria observed in the hindguts of the diverse crustacean species. In addition, the bacterial communities in the hindguts of the Crustacea examined do not appear to be very diverse. In any one animal species there was usually a monoculture of only one morphotype (nearly always rods) and, at most, 2 different types (e.g. cocci and rods) were observed. This finding suggests

that very specific conditions exist in the hindgut that are not suitable for colonization by a plethora of different microbes, or that the bacteria observed outcompete or inhibit other types.

Considering the abundance of bacteria in the hindguts of some of the Crustacea examined in this study (where they form an inner skin of epimural bacteria covering the hindgut lining), it is of interest to consider the relationship between these microbes and their hosts. A number of studies have suggested important roles for bacteria in the guts of Crustacea. Bacteria have been reported to enhance resistance of the shrimp *Penaeus aztecus* to the adverse affects of phenolics in the diet (Dempsey and Kitting 1987), and creosote-resistant gut bacteria appear to play an important role in the nutrition the wood-boring isopod *Limnoria tripunctata* when the animal inhabits creosote-treated wood (Zachary *et al.* 1983). Cellulase activity in the guts of the freshwater crayfish *Paranephrops zealandicus* (Musgrove 1988) and the mysid *Mysis stenelopis* (Wainwright & Mann 1982) appears to be attributable to the presence of gut bacteria. It has also been suggested that epimural rod bacteria in the hindgut of the crab *Callinectes sapidus* (Huq *et al.* 1986) play a role in ion transport across the gut wall, and thus contribute to osmoregulation. However the findings of the present study do not point to an important role for hindgut symbionts in the osmoregulatory processes of Crustacea. Epimural rod bacteria were found in the guts of animals from habitats with a range of salinity (estuarine to open coast). Furthermore, neither of the predatory crabs *Scylla serrata* (an estuarine crab that must cope with wide variations in salinities) and *Ovalipes punctatus* (which occurs offshore where salinities are more constant) possessed mats of epimural rod bacteria. This suggests that hindgut symbionts are not important in assisting either species cope with salinity stresses. Another potentially important aspect of the association is that the presence of a prolific hindgut microflora may be of significance to the bacterial productivity of the environment in which the animal lives. The invertebrate may act as an incubator for bacterial growth, a phenomenon that has been reported for the polychaete *Abarenicola vagabunda* (Plante *et al.* 1989). Unattached bacteria, which were only observed in animals with epimural bacteria (and were probably detached from the gut lining) may be voided with the faeces, and may increase decomposition rates in the environment, a phenomenon which has been reported for invertebrate detritivores in deserts (Crawford and Taylor 1984). It is also possible that these bacteria are parasites. However, it is unlikely that the epimural bacteria observed in the present study are detrimental to the host considering the fact that they were observed in apparantly healthy animals that were collected from a wide range of geographical localities and habitats.

Gut bacteria have been reported to play important roles in the physiological processes of other marine invertebrates. For example, nitrogen fixation by bacteria occurs in the guts of molluscan shipworms (Carpenter & Culliney 1975, Morton 1978) and some species of echinoids (e.g. Guerinot & Patriquin 1981), while a significant contribution of bacterial cellulases is reported for shipworms (Cutter & Rosenberg 1971, Martinez 1982) and an echinoderm (Lasker & Giese 1954). It has been suggested that detritivores, whose food source is often refractory to digestion, and has a low nitrogen content, are most likely to benefit from these types of associations with gut bacteria (Plante *et al.* 1990, Martin 1984). This suggestion is of interest because the evidence of the present study indicates that among Crustacea the hindguts of detritivores are most heavily colonized by epimural bacteria.

The mats of epimural rod bacteria observed in the crustacean hindguts in this study are remarkably similar in morphology and growth characteristics to those reported in termite hindguts (Breznak and Prankratz 1977), and are thought to play an important role in the physiology of the insect host. Potrikus and Breznak (1981) reported that hindgut bacteria recycle nitogenous wastes of the termite Reticulitermes flavipes, aiding in nitrogen conservation and acquisition. Maudlin *et al.* (1978) suggest that hindgut bacteria in some termite species synthesize amino acids that are subsequently utilised by the host. Hindgut bacteria of termites have also been reported to be capable of fermentation and cellulose digestion (Breznak 1984). In view of the spectacular wall-to-wall colonization by rod bacteria of the hindgut wall of some of the Crustacea examined in the present study, it is possible that these symbionts may be of functional significance to the invertebrate host, and their potential role remains a challenge for further study.

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Literature cited:

Abele, L.G. (1973). Taxonomy, distribution, and ecology of the genus *Sesarma* (Crustacea, Decapoda, Grapsidae) in eastern North America, with special reference to Florida. Amer. Midland Naturalist 90:375-386

Abele, L.G. (1982). Systematics, the fossil record, and biogeography. Vol. 1. In: Bliss D.E. (ed.) The Biology of Crustacea. Academic Press, New York

Atlas, R.M., Busdosh, M., Krichevsky, E.J., Kaneko, T. (1982). Bacterial populations associated with the Arctic amphipod *Boeckosimus affinis*. Can. J. Microbiol. 28(1):92-99

Bayon, C. (1971). La cuticule proctodeale de la larve d'*Oryctes nasicornis* (Coleopteres Scarabeides). Etude au microscope electronique a balayage. Journal de Microscopie 11:353-370

Bignell, D.E. (1984). The arthropod gut as an environment for microorganisms. In: Anderson J.M.,Raynon, A.D.M., Walton, D.W.A. (eds.) Invertebrate-Microbial Interactions. Cambridge University Press, Cambridge, p.205-227

Boyle, P.J., Mitchell, R. (1978). Absence of microorganisms in crustacean digestive tracts. Science 200:1157-1159

Breznak, J.A., Prankratz, H.S. (1977). In situ morphology of the gut microbiota of wood-eating termites (*Reticulitermes flavipes* (Kollar) and *Coptotermes formosanus* (Shiraki). Appl. Environ. Microbiol. 33:406-426

Breznak, J.A. (1984). Biochemical aspects of symbiosis between termites and their intestinal microbiota. Chapter 7. In: Anderson J.M.,Raynon, A.D.M., Walton, D.W.A. (eds.) Invertebrate-Microbial Interactions. Cambridge University Press, Cambridge, p.205-227

Cameron, A.M. (1966). Some aspects of the behaviour of the soldier crab *Mictyris longicarpus*. Pac. Sci. 20:224-234

Carpenter, J.L., Culliney, J.L. (1975) Nitrogen fixation in marine shipworms. Science 187:551-552

Colorni, A. (1985) A study on the bacterial flora of giant prawn *Macrobrachium rosenbergii*, larvae fed with Artemia salina nauplii. Aquacult. 49:1-10

Crawford, C.S., Taylor, E.C. (1984). Decomposition in arid environments : role of the detritivore gut. S.A. Journ. Sci. 80:170-176

Cruden, D.L., Markovetz A.J. (1981). Relative numbers of selected bacterial forms in different regions of the cockcroach hindgut. Arch. Microbiol. 129;129-134

Cutter, J.M., Rosenberg, F.A. (1971) The role of cellulolytic bacteria in the digestive processes of the shipworm. II. Isolation and some properties of a marine bacterial cellulase. Material und Organismen 7:225-239

Day, J.H. (1974). A guide to marine life on South African shores. A.A Balkema, Cape Town

Deming, J.W., Tabor, P.S., Colwell, R.R. (1981). Barophilic growth of bacteria from intestinal tracts of deep-sea invertebrates. Microb. Ecol. 7:85-94

Dempsey, A.C., Kitting, C.L. (1987). Characteristics of bacteria isolated from penaeid shrimp. Crustaceana 52:90-94

Dempsey, A.C., Kitting, C.L., Rosson, R.A. (1989). Bacterial variability among individual penaeid shrimp digestive tracts. Crustaceana 56(3):266-278

Gray, E.H. (1942). Ecological and life history aspects of the red-jointed fiddler crab, *Uca minax* (Le Conte), region of Solomons Island, Maryland. Chesapeake Biol Lab Publ 51:1-20

Guerinot, M.L., Patriquin, D.G. (1981) The association of N2-fixing bacteria with sea-urchins. Mar. Biol. 62:197-207

Gunzl, H. (1991). The ultrastucture of the posterior gut and caecum in *Alona affinis* (Crustacea, Cladocera). Zoomorphology 110(3):139-144

Harris, J.M., Seiderer, L.J., Lucas, M.I. (1991). Gut microflora of two saltmarsh detritivore Thalassinid prawns, *Upogebia africana* and *Callianassa kraussi*. Microb. Ecol. 21:63-82

Hill, B.J. (1976). Natural food, foregut clearance-rate and activity of the crab *Scylla serrata*. Mar. Biol. 34:109-116

Hoffman, J.A., Katz, J., Bertness, M.D. (1984). Fiddler crab deposit-feeding and meiofaunal abundance in salt marsh habitats. J. Exp. Mar. Biol. Ecol. 82:161-174

Hood, M.A., Meyers, S.P., Colmer, A.R. (1971). Bacteria of the digestive tract of the white shrimp *Penaeus setiferus*. Bacteriol. Proc. 71:G-147

Huq, A., Small, E.B., West, P.A., Huq, I., Rahman, R., Colwell, R.R. (1983). Ecological relationships between *Vibrio cholerae* and planktonic Crustacea copepods. Appl. Environ. Microbiol. 45:275-283

Huq, A., West, P.A., Small, E.B., Huq, M.I., Colwell, R.R. (1984). Influence of water temperature, salinity, and pH on survival and growth of toxigenic *Vibrio cholerae* serovar O1 associated with live copepods in laboratory microcosms. Appl. Environ. Microbiol. 48:420-424

Huq, A., Huq, S.A., Grimes, D.J., O'Brien, M., Chu, K.H., Capuzzi, J.M., Colwell, R.R. (1986). Colonization of the gut of the blue crab *Callinectes sapidus* by *Vibrio cholerae*. Appl. Environ. Microbiol. 52(3):586-588

Juilffs, H.B., Wagele, J.W. (1987). Symbiontic bacteria in the gut of the blood-sucking Antarctic fish parasite *Gnathia calva* (Crustacea : Isopoda). Mar. Biol. 95(4):493-499

Kenway, M.J. (1981). Biological studies of *Callianassa australiensis* (Dana). MSc Thesis, James Cook University of North Queensland, Australia

Lasker, R., Giese, A.C. (1954) Nutrition of the sea urchin, *Strongylocentrotus purpuratus*. Biol. Bull. Woods Hole Mass. 106:328-340

Laughlin, R.A. (1982). Feeding habits of the blue crab *Callinectes sapidus* Rathbun, in the Apalachicola Estuary, Florida. Bull. Mar. Sci. 32:807-822

Lynch, J.M., Hobbie, J.E. (eds.) (1988). Micro-organisms in action: concepts and applications in Microbial ecology. Chapter 3.3 The animal environment. Blackwell Scientific Publications, London, p. 163-192

Martin, M.M. (1984) The role of ingested enzymes in the digested processes of insects. In: Anderson JM, Raynon ADM, Walton DWA (eds) Invertebrate-Microbial Interactions. Cambridge University Press, Cambridge pp 155-177

Martinez J.C. (1982) The digestive microflora of *Teredo navalis* L. (Teredinidae;Bivalvia): Metabolic properties and ultimate role in digestion. Bact. Mar. 331:151-154

Mattson, R.A. (1988). Occurrence and abundance of eccrinaceous fungi (Trichomycetes) in Brachyuran crabs from Tampa Bay, Florida. J. Crust. Biol. 8(1):20-30

Maudlin, J.K., Rich, N.M., Cook, D.W. (1978). Amino acidsynthesis from 14C-acetate by normally and abnormally faunated termites *Coptotermes formosanus*. Insect Biochem. 8:105-109

McDermott, J.J. (1960). The predation of oysters and barnacles by crabs of the family Xanthidae. Proceedings of the Pennysylvania Academy of Science 34:199-211

Mead, L.J., Khachatourians, G.G., Jones, G.A. (1988). Microbial ecology of the gut in laboratory stocks of the migratory grasshopper, *Melanoplus sanguinipes* (Fab.) (Orthoptera:Acrididae). Appl. Environ. Microbiol. 54(5):1174-1181

Miller, D.C. (1961). The feeding mechanism of fiddler crabs, with ecological considerations of feeding adaptations. Zoologica 46:89-100

Moriarty, D.J.W. (1990). Interactions of micro-organisms and aquatic animals, particularly the nutritional role of the gut microflora. In: Lesel, R.(ed.) Microbiology in poecilotherms. Elsevier Science Publishers.

Morton, B. (1978) Feeding and digestion in shipworms. Oceanogr. mar. Biol. Ann. Rev. 16:107-144

Musgrove, R.J. (1988) Digestive ability of the freshwater crayfish *Paranephrops zealandicus* (White) (Parastacidae) and the role of microbial enzymes. Freshwater Biol. 20:305-314

Noirot, C., Noirot-Timothee, C. (1976). Fine structure of the rectum in cockcroaches (Dictyoptera): general organization and intercellular junctions. Tissue and Cell 8:345-368

Plante, C.J., Jumars, P.A., Baross, J.A. (1989). Rapid bacterial growth in the hindgut of a marine deposit feeder. Microb. Ecol. 18:29-44

Plante, C.J., Jumars, P.A., Baross, J.A. (1990) Digestive associations between marine detritivores and bacteria. Annu. Rev. Ecol. Syst. 21:93-127

Potrikus, C.J., Breznak, J.A. (1981). Gut bacteria recycle uric acid nitrogen in termites : a startegy for nutrient conservation. Proc of the National Academy of Sciences of the USA 78:4601-5

Quinn, R.H. (1986). Experimental studies of food ingestion and assimilation of the soldier crab, *Mictyris longicarus* Latrielle (Decapoda, Mictyridae). J. Exp. Mar. Biol. Ecol. 102:167-181

Ricketts, E.F., Calvin, J. (1962). Between Pacific Tides. Stanford University Press, Stanford

Robertson, A.I. (1986). Leaf-burying crabs : their influence on energy flow and export from mixed mangrove forests (*Rhizophora* spp.) in northeastern Australia. J. Exp. Mar. Biol. Ecol. 102:237-248

Robertson, J.R., Fudge, J.A., Vermeer, G.K. (1981). Chemical and live feeding stimulants of the sand fiddler crab, *Uca pugilator* (Bosc). J. Exp. Mar. Biol. Ecol. 53:47-64

Ryan, E.P. (1956). Observations on the life histories and the distribution of the Xanthidae (mud crabs) of Chesapeake Bay. Amer. Midland Naturalist 56:138-162

Schlegel, H.G. (1988) General Microbiology. Cambridge University Press, Cambridge

Seed, R. (1980). Predator-prey relationships between the mud crab *Panopeus herbstii*, the blue crab *Callinectes sapidus* and the Atlantic ribbed mussel *Guekensia* (=*Modiolus*) *demissa*. Est. Coast. Mar. Sci. 11:445-458

Sleeter, T.D., Boyle, P.J., Cundell, A.M., Mitchell, R. (1978). Relationships between marine micro-organisms and the wood-boring isopod *Limnoria tripunctata*. Mar. Biol. 45:329-336

Sochard, M.R., Wilson, D.F., Austin B, Colwell RR (1979. Bacteria associated with the surface and gut of marine copepods. Appl. Environ. Microbiol. 37:570-579

Sugita, H., Takahashi, T., Kanemoto, F.I., Deguchi, Y. (1987a) Aerobic bacterial flora in the digestive tracts of freshwater shrimp *Palaemon paucidens* acclimated with seawater. Nippon Suisan Gakkaishi 53(3):511

Sugita, H., Ueda, R., Berger, L.R., Deguchi, Y. (1987b) Microflora in the gut of Japanese coastal crustacea. Bull. Jpn. Soc. Sci. Fish. 53(9):1647-1655

Teal, J.M. (1962). Energy flow in the salt marsh ecosystem of Georgia. Ecology 43(4):387-397

Wainwright, P.F., Mann, K.H. (1982) Effect of antimicrobial substances on the ability of the mysid shrimp *Mysis stenolepis* to digest cellulose. Mar. Ecol. Prog. Ser. 7:309-313

Whetstone, J.M., Eversole, A.G. (1981). Effects of size and temperature on mud crab, *Panopeus herbstii*, predation on hard clams, Mercenaria mercenaria. Estuaries 4:152-156

Zachary, A., Colwell, R.R. (1979). Gut-associated microflora of *Limnoria tripunctata* in marine creosote-treated wood pilings. Nature (Lond.) 282:716-717

Zachary, A., Parrish, K.K., Bultman, J.D. (1983). Possible role of marine bacteria in providing the creosote-resistance of *Limnoria tripunctata*. Mar. Biol. 75(1):1-8

Synthesis

Bacteria and other microbial organisms are recognized as important agents of decomposition of organic matter and refractory material in detritus dominated systems. In aquatic environments, the medium itself provides a suitable microbial habitat. In many terrestrial systems, and particularly in arid environments, however, invertebrates act as important incubators for microbial activity because of the lack of water in the environment. Symbiotic relationships between terrestrial detritivores and gut microbes are often well developed and finely tuned, particularly those related to the nutrition of the host organism.

In marine systems where water is freely available in the surrounding habitat one might expect that specific and close microbial-invertebrate relationships are less likely to occur or are less well developed. However detrital material, particularly that originating from vascular plants in coastal saltmarsh systems, is based largely on cellulose or other structural polymers and is poor in nitrogen. This makes such material a potentially poor food source for marine detritivores and may also be difficult to digest.

The observation that many marine invertebrates contain a bacterial community within the gut and that the isolated invertebrate digestive enzymes appear to be rather weak at detrital hydrolysis has led to the suggestion that marine detritivores could stand to gain much by establishing close microbial-detritivore interactions which parallel those observed in some terrestrial detritivores. It is therefore of interest to consider whether microbe-invertebrate associations similar to those found in terrestrial systems, form an integral part of the feeding strategies of marine detritivores.

To address these questions the feeding strategies and digestive invertebrate-microbial associations of two different benthic detritivore prawns, the mud prawn *Upogebia africana* and the sand prawn *Callianassa kraussi*, which occur in disparate habitats, are compared. *U. africana* and *C. kraussi* occupy habitats which differ in terms of both the physical characteristics of the sediments they inhabit as well as the quality and quantity of resource available to them. The resource available to *U. africana* is of a much greater quantity (total organics) and higher quality (in terms of C/N ratio and the proportion of biotic components) than is that available to *C. kraussi*.

The feeding strategies of the prawns were compared by examination of the resources available to them; their feeding mechanisms and gut structure; their digestive processes with respect to gut volume, throughput time and enzymes necessary for hydrolysis of the refractory component of detritus; and establishment

of the major food sources utilized. The presence of and physiological properties of gut microbes was also investigated.

U. africana filter feeds non-selectively on suspended material, and is capable of retaining very fine particles, including bacteria. By contrast *C. kraussi* is a selective deposit feeder capable of ingesting larger organic particles and microbiota such as protozoa and diatoms. *U. africana* has a relatively large gut, possibly reflecting non-selective ingestion of relatively poor quality material, while *C. kraussi* has a smaller gut indicating that the composition of food ingested may be of a higher quality. The hepatopancreas is, in terms of volume, a more important organ in *C. kraussi* than in *U. africana*. Both animals exhibit similar gut throughput times, although the larger hepatopancreas in *C. kraussi* may increase the retention time of a substantial proportion of the ingested food. Stable isotope analyses corroborated the reliance by *U. africana* on the lower quality fraction of the detrital pool in which most of nitrogen and carbon is obtained directly from vascular plant detritus. Although *C. kraussi* obtains most of its carbon from vascular plant detritus, it differs from *U. africana* by relying on associated microbiota to meet its nitrogen requirements. This is despite the relatively low proportion of microbiota in the sediment, and reflects the selective feeding mechanism of *C. kraussi* which ensures that higher quality items are ingested.

This difference in the resource utilization by the two prawns occurs despite the fact that the quality of the available resource is higher for *U. africana*. This probably reflects a constraint imposed on this prawn by its feeding mechanism and the physical difficulties of selective feeding in a habitat where the mud content is high. However, the results of the stable isotope ratio study do not preclude the use of bacteria associated with the detritus, which *U. africana* is equipped to retain.

The digestive processes of the two prawns differed markedly. While *C. kraussi* exhibited strong endogenous cellulase activity and the ability to hydrolyze refractory components of natural detritus, *U. africana* possessed only weak CX-cellulase activity, indicating that it is only equipped to utilize the labile components of the salt-marsh detritus. These results explain how *C. kraussi* obtains it carbon requirements from the vascular plant detritus. The question of how *U. africana* is able to satisfy its carbon requirements is less straightforward, however, since it does not appear to possess the necessary endogenous enzymes to hydrolyze the food resource on which it relies.

A number of possibilities can be offered to explain this. With its relatively large gut, *U. africana* may ingest large quantities of detritus but utilize only the more labile components (i.e. simpler carbohydrates) which are made available by the abundant microheterotrophic detrital community, thus obviating the need for

digestion of the refractory components or for selective feeding. Alternatively, *U. africana* may directly utilize the relatively large bacterial and protozoan component ingested with the detritus. However neither of these suggestions provide satisfactory explanations as the gut is not particularly large, and the animal does not ingest food continuously in a conveyor belt fashion to maximize ingestion rate, and thus may not obtain sufficient sustenance by microbial stripping of refractory material. This suggests the possibility of gut microbial involvement in the digestion of the vascular plant detritus by marine detritivores. Because of the feeding mechanisms and major food sources, *U. africana* is a much more likely candidate for important symbiotic associations with gut microbiota than is *C. kraussi*.

The second part of the thesis addresses the question of whether there are major differences in the presence, nature and role of gut microbes in the physiology of detritivores in aquatic and terrestrial systems. Of particular interest are the questions of whether the guts of marine invertebrates offer favorable habitats for micro-organisms, and whether gut microbes are of significance to the host invertebrate, or are simply occupying a favourable habitat.

Both *U. africana* and *C. kraussi* host diverse gut bacterial communities. *U. africana* harbored a far greater density of microbes than did *C. kraussi* which may reflect the greater numbers of bacteria in its habitat. Of note were novel observations of filamentous bacteria coating gut contents in the midgut of both species of prawn, as well as epimural rod bacteria in the hindguts. These resident epimural rod bacteria formed spectacular extensive single-layered mats in the hindgut of *U. africana*. In addition to resident bacteria, some ingested bacteria remained viable in *U. africana*'s gut. This highlights the probability that incubation of habitat bacteria occurs in the guts of detritivores. A few of these ingested bacteria were shown to possess cellulases, highlighting the potential significance of incubating bacteria to the breakdown refractory components. As detritivores concentrate organic material in the gut, they may represent hot spots of decomposition which could be of great significance to nutrient flux within the ecosystem as a whole, particularly as in this study gut microbial communities were shown to be different to those in the habitat in terms of both taxa and digestive capabilities. Thus organic matter may be modified in the gut and passed out as organic rich faecal packages. These results support the hypothesis that *U. africana* depends on enrichment and preconditioning of detritus by bacteria for nutrient acquisition.

The provision of nitrogen to the detritivore may be facilitated by the gut bacteria either through the recycling of ingested plant nitrogen or directly, by bacterially-mediated nitrogen fixation. Attempts to resolve this by measuring

acetylene reduction as a proxy for nitrogen fixation failed to demonstrate significant rates for this process, leading to the conclusion that nitrogen is somehow made available to the detritivore through transformation of ingested plant nitrogen. The mechanism for this remains unclear.

Two different scenarios have emerged from the comparison of the two detritivore prawns examined in this thesis; 1) low microbial biomass in the habitat coupled with independent utilization of refractory plant detritus by the invertebrate detritivore (*C. kraussi*), and 2) high microbial biomass in the habitat coupled with either microbe-dependent utilization of plant detritus, or energy requirements of the detritivore being met by microbial stripping and utilization of labile components of detritus (*U. africana*).

In an effort to find an underlying and global hypothesis linking detritivory and the involvement of microbes in marine ecosystems, the thesis was broadened to include other crustacean species collected from sites world wide. These studies showed that the phenomenon of a gut microflora is widespread in aquatic invertebrates and is not defined by phylogeny, habitat or diet of the animal. However, diet did appear to influence the abundance of gut microbes, especially in detritivore hindguts. This is an area of study that merits further investigation particularly because of the sheer densities of bacteria observed. Because of the proven significance of hindgut symbionts to terrestrial detritivores, it seems that processes such as fermentation and nitrogen recycling ought to be uncovered after carefully designed experiments have been implemented.

A common thread emerging from this study is that invertebrate-microbial interactions are ubiquitous in marine detrital systems but that the relative role of the host and bacteria seems to be dependent upon the evolutionary pressures which reward those hosts that make use of bacterial assistance in progressively more harsh nutritional environments. On the one hand, non-selective detritivory in a nutritionally poor environment may compel the detritivore to employ bacteria to upgrade the food resource (*U. africana*), while other detritivores may be able to circumvent the use of bacteria by becoming more selective. This latter approach requires the development of a specialist feeding apparatus. Nonetheless, such detritivores can still make use of microbes but in this case the association tends more toward predation than symbiosis.

Many aspects of detritivory remain unresolved, but it is clear that a detailed understanding of the resources available to marine detritivores, coupled with insights into their feeding strategies and the role of gut microbes, is invaluable in assessing the relative importance of detritivores and microbes as remineralizers of refractory organic material in detritus-dominated systems.

APPENDIX 1

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MICROBIAL ECOLOGY

Estimates of Bacterial Productivity in Marine Sediments and Water from a Temperate Saltmarsh Lagoon

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Abstract. Tritiated thymidine incorporation (TTI) into DNA was used to estimate bacterial productivity in sediment and water samples from two sites in Langebaan Lagoon, South Africa. Routine analysis of isotope dilution showed seasonal variations of approximately threefold in the thymidine precursor pool sizes for bacterial assemblages from each site. Dual label incorporation of [^{3}H]-thymidine and ^{14}C-leucine into DNA and protein, respectively, showed that pelagic but not sediment assemblages were in a balanced state of growth during TTI. This is the first report of dual label measurements of bacterial production in sediments. Sediments supported bacterial productivities that exceeded those in the water column by factors from five- to 950-fold, whereas bacterial abundance supported by sediments exceeded that in the water column by more than 3 orders of magnitude. Estimates of bacterial productivities in sediments were coincident with levels of organic content in sediments, but not with bacterial abundance. Measurements of TTI activity for 5 different benthic microhabitats at one lagoon site showed highest activity associated with seagrass beds (2.11 ± 0.84 nmol thymidine hours^{-1} g^{-1} dry weight), whereas activities decreased with depth (0.46 ± 0.21 nmol thymidine hours^{-1} g^{-1} dry weight) below sediment surface.

Introduction

Coastal marine ecosystems with saltmarsh components are highly productive communities based on high rates of primary production, frequently dominated by *Spartina* spp., *Zostera* spp., and other halophytes. Benthic diatoms may also make substantial contributions to overall primary production [21]. Owing to the refractory nature of much of this material, however, most of the primary production enters higher trophic levels through heterotrophic bacteria [21, 30]. One consequence of this is that typically nitrogen-impoverished material becomes nitrogen-enriched, with clear benefits to consumer communities. Estimates of bacterial productivity are therefore essential in quantifying biogeo-

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chemical flows, and for determining the ecological role of bacteria in the trophic dynamics of saltmarsh systems. In particular, ecologists have sought to measure bacterial production in terms of carbon or nitrogen for comparison with autotrophic production and consumer requirements.

Measurements of bacterial productivity in natural samples can be problematic. Direct observations of changes in bacterial abundance with time are difficult, particularly for sediments. However, the close association between rates of macromolecular synthesis and cell division provides a means of estimating bacterial productivity if the rate of incorporation of labeled precursors into bacterial macromolecules can be accurately measured. The use of adenine as a suitable precursor of macromolecular synthesis has been proposed [15], but results obtained from adenine incorporation are difficult to interpret as both it and ATP are involved in many different biochemical processes in all organisms [11]. Other precursors that have been proposed to measure bacterial productivity include thymidine [9] and leucine [4].

There is a direct correlation between the rates of cell division and DNA synthesis [23], which enables the technique of [*methyl*-^{3}H]thymidine (^{3}H-Tdr) incorporation (TTI) into DNA to be used to estimate the productivity of bacteria in natural systems [9]. TTI into DNA has gained widespread acceptance in microbial ecology, because it is specific to heterotrophic bacteria, and most of the label is incorporated into DNA during short incubation periods. However, several potential problems with this method have been identified, and results may be interpreted incorrectly if these potential sources of error are not recognized.

Contrary to the assumptions of earlier procedures [9, 10], the lack of specificity of ^{3}H-Tdr in the labeling of DNA and the variability of DNA content in cold trichloroacetic acid (TCA) precipitate have been demonstrated for freshwater [16, 35, 36], seawater [12, 37, 38], and sediment [3, 7, 26] bacterial assemblages. These reports emphasize the need to isolate labeled DNA from macromolecular extracts.

Isotope dilution of exogenously supplied ^{3}H-Tdr by intracellular and extracellular sources of unlabeled precursor in the final pool of ribosyl-thymine 5′-triphosphate (dTTP) may lead to underestimations of bacterial productivity. The final extent of dilution, and therefore the specific radioactivity of dTTP available for incorporation, can be measured by isotope dilution analysis [32]. If extracellular sources are insignificant, then isotope dilution can be limited by the addition of ^{3}H-Tdr at concentrations in excess of the precursor pool size measured by isotope dilution analysis [23]. However, extracellular dilution has been reported to be occasionally significant [13], and the variations of macromolecular labeling by ^{3}H-Tdr cited above suggest that isotope dilution may vary on spatial and temporal scales.

TTI measurements must represent balanced growth in bacterial assemblages if the data are to be translated into bacterial carbon production. Chin-Leo and Kirchman [4] have shown that ^{3}H-Tdr and ^{14}C-leucine (^{14}C-Leu) can be used in dual label incorporation (DLI) experiments to simultaneously measure DNA and protein syntheses. Under conditions of balanced growth, the rates of macromolecular syntheses are coupled and the ratio of Leu : Tdr incorporation should remain relatively constant over progressive periods of incubation. An-

other assumption of TTI is that all growing bacteria in the sample are capable of incorporating exogenously supplied ^{3}H-Tdr into DNA. Although most bacteria probably do possess Tdr kinase as an essential enzyme of the salvage pathway [24], the limitations of this assumption have been indicated by reports of a lack of TTI into DNA in some strains [6, 14, 32]. If a significant proportion of the bacterial assemblage is unable to transport or incorporate exogenous Tdr, then protein synthesis may exceed DNA synthesis and the ratio of Leu : Tdr may vary over time. DLI can thus also be used to establish confidence that TTI is applicable to most growing bacteria in the sample.

Bacterial productivity is linked to seagrass productivity in temperate seagrass beds [25], and is apparently also regulated primarily by phytoplankton production in pelagic systems [19, 20, 28]. Studies in a saltmarsh ecosystem in southern Africa are aimed at elucidating the pathways of carbon and nitrogen fixation and their flows into microbial and higher benthic consumers. In this study, we used slightly modified TTI methods to estimate variations in bacterial productivity in sediment and water samples from Langebaan Lagoon. Spatial differences in TTI were measured on the macroscale (two sites, Geelbek and Oesterwal) and on the microscale (different sediment microhabitats at Oesterwal). Temporal (seasonal) variations in measurements of isotope dilution and bacterial productivity were examined in both sediment and water column for each study site. Furthermore, DLI was used to assess whether bacterial assemblages were in balanced growth and to test the assumption that TTI can be used as a measure of bacterial carbon production.

Methods

Sampling Sites

Langebaan Lagoon is a partially enclosed marine system situated between 33°00′ to 30°13′S and 17°57′ to 18°08′E on the west coast of South Africa. Two dissimilar sites are located at Oesterwal, near the lagoon mouth, and at Geelbek in the southern reaches. Oesterwal is characterized by sandflats and burrow activity of the sandprawn *Callianassa kraussi*, whereas Geelbek has anaerobic mudflats with burrows mostly of the mudprawn *Upogebia africana*. Both sites have a saltmarsh component including beds of *Zostera marina*. Samples of surface sediment and water column were taken from Geelbek and Oesterwal for the seasonal study. Samples were always taken on the morning of spring tide, and the temperatures of the samples at time of collection were recorded. There was at least a 10°C difference in temperature between seasons for each study site. Temperatures of surface sediment ranged from 11.1°C in winter to 24.5°C in summer, whereas water ranged from 11.2°C in winter to 22.0°C in summer. The temperatures of samples from Geelbek were similar to those for Oesterwal for each season. Samples were transported to the laboratory in an insulated container and processed within 2 hours of collection.

Measurement of TTI in Sediments

Surface sediment samples were taken intertidally to a depth of 5 mm, away from the seagrass beds and between prawn burrow openings. Composite samples were collected over an area of approximately 5 m^2 on the sediment flats, and used to make a sediment slurry (approximately 200 g) immediately before the experiment. All experiments were carried out in triplicate. 1 μCi [*methyl-*

^{3}H]thymidine (83 Ci mmol^{-1}, Amersham Corp.) was added to 500 mg wet weight sediment slurry in microfuge tubes. Incubation at 20°C was terminated after 0 and 20 min by the addition of 1 ml extraction reagents (0.3 M NaOH, 25 mM disodium EDTA, 0.1% SDS w/v). Macromolecules were extracted and RNA hydrolyzed at 25°C for 12 hours. The sediment was pelleted by centrifugation at 8,000 × *g* for 60 sec, which was the standard centrifugation procedure used in all experiments. The sediment was washed with a further 1 ml extraction reagents. Supernatants were transferred to test tubes and neutralized with 3 N HCl before TCA was added to a final concentration of 5% (w/v). Herring sperm DNA (100 μg, Sigma Chemical Co.) was added as a carrier to aid precipitation of labeled DNA. Samples were cooled on ice for 45 min and then filtered through 1.2 μm pore size cellulose acetate filters (Millipore Corp.). The tube and filter were rinsed with 5 ml ice-cold 5% (w/v) TCA, and the filter was then washed with 5 ml phenol-chloroform (50% w/v) followed by 5 ml ice-cold 80% (v/v) ethanol. The filters were placed in 7 ml Filter-Count (Packard Instrument Co., Inc.) and disintegrations per minute (dpm) determined by Packard TriCarb and Beckman LS 5000TD liquid scintillation counters.

Sediment samples for the comparison of TTI between microhabitats at Oesterwal were taken during early summer and midsummer from five locations as follows: (1) surface sediment as for the seasonal samples; (2) sediment to a depth of 5 mm from within *Zostera* beds; (3) sediment from the rims of prawn burrow openings; (4) sediment from burrow linings; (5) sediment from a depth of 10 cm below the surface (extruded with a PVC corer and subsampled using a 10 ml syringe with the luer end removed). Two sets of midsummer samples were taken from near low and high water marks on the sandflats. For the determination of TTI in different sediment microhabitats at Oesterwal, [*methyl*-^{3}H]thymidine (28 Ci mmol^{-1}) was added at 0.15 nmol per 500 mg sediment slurry and incubated at 20°C for 20–30 min. TTI was then determined as described above.

Measurement of TTI in Water

Water column samples (500 ml) were taken from 30 cm below the surface. The method of Wicks and Robarts [40] was used with the following modifications: samples were incubated with 2 μCi [*methyl*-^{3}H]thymidine (83 Ci mmol^{-1}); extraction of macromolecules with NaOH was carried out at 4°C for 12 hours; 100 μg herring sperm DNA was added, and precipitates were collected on 0.45 μm pore size cellulose acetate filters (Millipore Corp.). Liquid scintillation counting of whole filters was performed as described above.

Determinations of Isotope Dilution, Calculations of Bacterial Productivity, and Statistical Analysis of Data

Seasonal determinations of isotope dilution were made as follows. Crystalline thymidine (Sigma Chemical Co.) was added at concentrations of 0, 0.05, 0.15, and 0.35 nmol per 500 mg sediment slurry, and of 0, 10, 30, and 70 nM for water samples. Methods for sediment and water TTI determinations as described above were then carried out. The isotope dilution plot described by Pollard and Moriarty [32] was used to determine the extent of isotope dilution in each sample. The specific radioactivity of the dTTP pool was thus corrected and bacterial productivity was then calculated for the sample using a conversion factor of 1.1×10^{18} cells produced mol^{-1} thymidine incorporated [34]. Analysis of variance (ANOVA) was performed on untransformed data sets where Bartlett's test for homogeneity of variance was greater than 0.5. Where Bartlett's test was less than 0.5, Kruskal-Wallis analysis was substituted for ANOVA.

Comparison of Tdr and Leu Incorporation Rates

Rates of TTI were compared to rates of ^{14}C-Leu incorporation using DLI methods. [*methyl*-^{3}H]thymidine (28 Ci mmol^{-1}) and ^{14}C-leucine (308 mCi mmol^{-1}; Amersham Corp.) were added

at concentrations of 0.15 and 0.30 nmol, respectively, per 500 mg sediment slurry or 10 ml water sample. Water samples were processed using the filter method described above, except that a further wash with cold 5% TCA was substituted for the phenol-chloroform wash.

Sediment samples were processed according to a modification of the dialysis method of Moriarty and Pollard [26], as this method had been previously applied to TTI studies in sediments. Sodium molybdate (20 mM) was added to selected samples to inhibit the activity of sulfate reducing bacteria [29]. Time series incubations over 3 hours were stopped by the addition of 1.5 ml ice-cold 80% (v/v) ethanol with 100 mg liter^{-1} unlabeled thymidine, and placed on ice for 1 hour. The sediment was centrifuged and washed with a further 1.5 ml ethanol. Two ml of extraction reagents were then added to extract macromolecules from the sediment and to hydrolyze RNA at 25°C for 12 hours. The sediment was pelleted by centrifugation, and the supernatant was mixed with 36 μl acetic acid and removed to dialysis tubing (12,000–14,000 daltons cut-off, Spectrum Medical Industries) using apparatus as described by Pollard [31]. The sample tubes were rinsed into the dialysis bags with 1 ml distilled water. The samples were dialysed overnight against running tap water and then transferred to scintillation vials with 10 ml Insta-gel (Packard Instrument Co., Inc.). Dual label dpm were determined by a Beckman LS 5000TD liquid scintillation counter. Energy spectrum spillover was corrected according to the method described in the manual of this instrument.

Determinations of Organic Carbon Content and Bacterial Abundance

Replicate 100 ml water samples (n = 3), collected for enumeration of bacteria, were fixed with 4% formalin (final concentration). Replicate 1 cm^3 sediment samples (n = 3) were taken using a 10 cm^3 syringe with the luer end removed, and fixed with 4% formalin (final concentration in 0.2 μm filtered seawater). All samples were stored at 4°C in the dark and subsequently stained with 4′,6-diamidino-phylindole (DAPI) for bacterial counts by the method of Porter and Feig [33]. Water samples (2 ml) were stained at a concentration of 0.1 μg ml^{-1} DAPI. Sediment samples were diluted (1:2,500 v/v for Geelbek, and 1:1,500 v/v for Oesterwal) in 0.01 M tetrasodium pyrophosphate, sonicated (30–50 mHz) for 5 min, and stained at a concentration of 5 μg ml^{-1} DAPI. Counts were corrected for sediment masking using the method of Ellery and Schleyer [8]. Randomly selected fields on each of duplicate filters per sample were counted at 1,000× magnification on a Nikon compound microscope with a Neofluar objective until 20 fields or at least 400 bacteria were counted. Bacterial numbers were corrected to dry weight (DW) sediment after determination of the water content in samples (n = 3) dried at 60°C for 48 hours.

Particulate organic content in replicate (n = 3) water samples (500–2,000 ml) was collected by filtering water onto precombusted (450°C for 6 hours) Whatmans GF/F filters. Replicate (n = 3) 1 cm^3 sediment samples for organic content were collected as described above. All samples were stored at −20°C and then dried at 60°C for 48 hours. Total organic content was determined by weight loss by dried samples when combusted at 450°C for 6 hours.

Results

Determinations of Isotope Dilution and Estimates of Bacterial Production

Time course experiments over 4 hours for both sediment and water samples showed that incubation periods of 20–30 min fell within the limits where TTI was linear with time. Isotope dilution analysis was performed on each sample to determine the variation of this parameter and to improve the accuracy of bacterial production estimates. The sizes of precursor pools in the samples

Table 1. Isotope dilution analysis of ^{3}H-Tdr in sediment and water samples from Oesterwal and Geelbek[a]

	Sediment (nmol g^{-1} dry weight)		Water (nM)	
Season	Oesterwal	Geelbek	Oesterwal	Geelbek
Autumn	0.281	0.361	9.30	7.80
Winter	0.183	0.208	7.00	3.40
Spring	0.119	0.703	0.60	6.00
Summer	0.498	0.458	0.30	1.90

[a] Values represent effective pool sizes of thymidine prior to incorporation into DNA

(Table 1) measured by isotope dilution plots [32] showed no uniform trend according to season. Table 1 shows that ^{3}H-Tdr should be added to samples from Langebaan Lagoon at concentrations above 9.3 nM for water or 0.7 nmol g^{-1} DW for sediment, and that isotope dilution determined during one season may not necessarily be applicable as estimates in other seasons. An approximate variation of threefold in isotope dilution was measured between seasons; this variation was larger in Oesterwal water (Table 1).

Estimates of bacterial productivity for sediments showed similar trends for seasonal samples from Geelbek and Oesterwal, with minima values in winter and maxima values in summer (Fig. 1). However, there was no significant difference ($P < 0.05$) in average productivities for sediment slurries from Oesterwal ($2.15 \pm 1.82 \times 10^6$ cells hour^{-1} g^{-1} DW) and Geelbek ($2.76 \pm 1.70 \times 10^6$ cells hour^{-1} g^{-1} DW). Average sediment volume to wet weight ratios were approximately 0.6 ml g^{-1} wet weight, and therefore sediments were more productive than the water column by factors from five- to 950-fold.

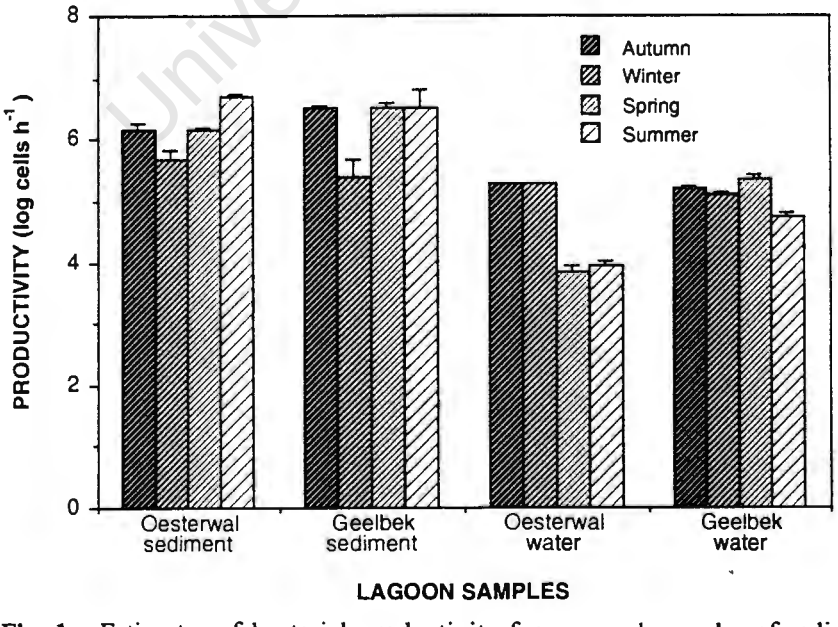

Fig. 1. Estimates of bacterial productivity for seasonal samples of sediment and water from Geelbek and Oesterwal, Langebaan Lagoon. Values represent means ± SD.

Table 2. Bacterial abundance and particulate organic content of water and sediment samples from Oesterwal and Geelbek, Langebaan Lagoon[a]

Sample	Season	Bacterial abundance[b]	Organic content[c]
Oesterwal sediment	Autumn	2.32 ± 0.10	12.54 ± 0.06
	Winter	1.34 ± 0.79	4.27 ± 0.01
	Spring	1.90 ± 0.72	6.44 ± 0.03
	Summer	1.35 ± 2.39	8.20 ± 0.01
Geelbek sediment	Autumn	4.21 ± 0.78	15.96 ± 1.05
	Winter	4.22 ± 2.06	5.52 ± 0.33
	Spring	4.40 ± 1.09	4.98 ± 0.24
	Summer	3.56 ± 1.22	12.32 ± 1.14
Oesterwal water	Autumn	2.07 ± 0.50	5.80 ± 2.12
	Winter	1.99 ± 0.08	2.07 ± 0.21
	Spring	1.40 ± 0.29	3.85 ± 0.50
	Summer	1.98 ± 0.78	5.17 ± 1.76
Geelbek water	Autumn	3.62 ± 2.16	3.27 ± 0.47
	Winter	3.96 ± 2.39	24.93 ± 5.55
	Spring	2.57 ± 1.17	17.60 ± 0.57
	Summer	2.88 ± 2.39	7.87 ± 4.46

[a] Values represent means ± SD
[b] Units in cells × 10^9 g^{-1} dry weight for sediments, and cells × 10^6 ml^{-1} for water
[c] Units in mg g^{-1} dry weight for sediments, and mg $liter^{-1}$ for water

Bacterial productivity estimates for the water column showed that productivity at Geelbek exceeded that at Oesterwal for all seasons except winter (Fig. 1). Water column productivity minima and maxima were greater for Geelbek than for Oesterwal, as was the average productivity estimate (1.40 ± 0.70 × 10^5 cells $hour^{-1}$ ml^{-1}). The average water productivity estimate was more variable for Oesterwal samples (8.26 ± 8.93 × 10^4 cells $hour^{-1}$ ml^{-1}).

Total counts of bacteria by epifluorescence microscopy showed that cells were more abundant in samples from Geelbek than in samples from Oesterwal (Table 2). Similarly, samples from Geelbek had a higher organic content than those from Oesterwal (Table 2). Sediments contained a greater percentage of organic carbon by volume, and supported larger bacterial populations, than water samples. The trends exhibited by productivity estimates for sediments (Fig. 1) were not evident in the bacterial abundance data set (Table 2). However, variations in the organic content of sediments did follow the trends of the bacterial productivity estimates.

Variability of TTI between Sediment Microhabits

Thymidine incorporation rates for 5 different sediment microhabitats at Oesterwal are shown in Fig. 2. Highest rates of incorporation occurred in *Zostera* bed sediment, whereas rates decreased significantly with depth ($P < 0.05$). TTI was not determined for surface sediment in early summer. Incorporation rates for surface sediment and burrow opening were similar, whereas TTI in burrow

Table 3. Bacterial abundance and organic carbon content of sediment microhabitats at the high tide mark at Oesterwal during midsummer[a]

Microhabitat	Bacterial abundance (cells × 10^9 g^{-1} dry weight)	Organic content (mg g^{-1} dry weight)
Surface sediment	1.35 ± 0.44	8.04 ± 0.02
Zostera bed sediment	1.79 ± 0.16	8.91 ± 0.10
Burrow opening sediment	1.22 ± 0.41	5.64 ± 0.45
Burrow lining sediment	1.75 ± 0.30	11.53 ± 0.92
10 cm depth sediment	1.73 ± 0.38	6.56 ± 0.59

[a] Values represent means ± SD

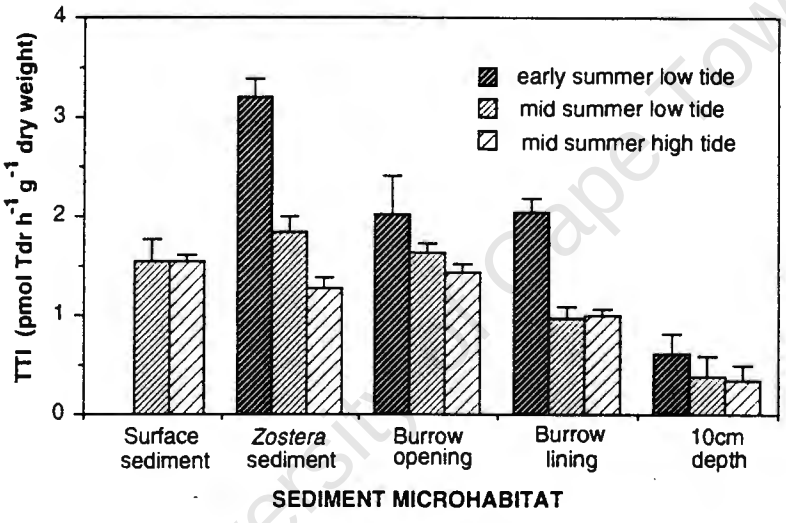

Fig. 2. TTI activities in sediment samples from different benthic microhabitats at Oesterwal. Values represent means ± SD. Average values of activity (pmol Tdr incorporated $hour^{-1}$ g^{-1} DW) for each microhabitat were 1.55 ± 0.17 in surface sediment, 2.11 ± 0.84 in *Zostera* bed sediment, 1.69 ± 0.34 in burrow opening sediment, 1.34 ± 0.52 in burrow lining, and 0.46 ± 0.21 in sediment from 10 cm below the surface.

lining was enhanced relative to that in burrow-free sediment from 10 cm depth. Bacterial abundance and sediment organic content were determined for microhabitat samples taken during midsummer from the high water mark (Table 3). Highest organic contents and bacterial densities were recorded for sediments from *Zostera* beds and burrow lining.

Comparison of Leu and Tdr Incorporation Rates

Molar ratios of Leu : Tdr incorporation were calculated for incubation periods of 30, 60, and 180 min for sediment and water samples (Fig. 3). Leu : Tdr ratios for water samples remained more constant over time of incubation relative to those for sediment samples, where the Leu : Tdr ratio tended to decrease with

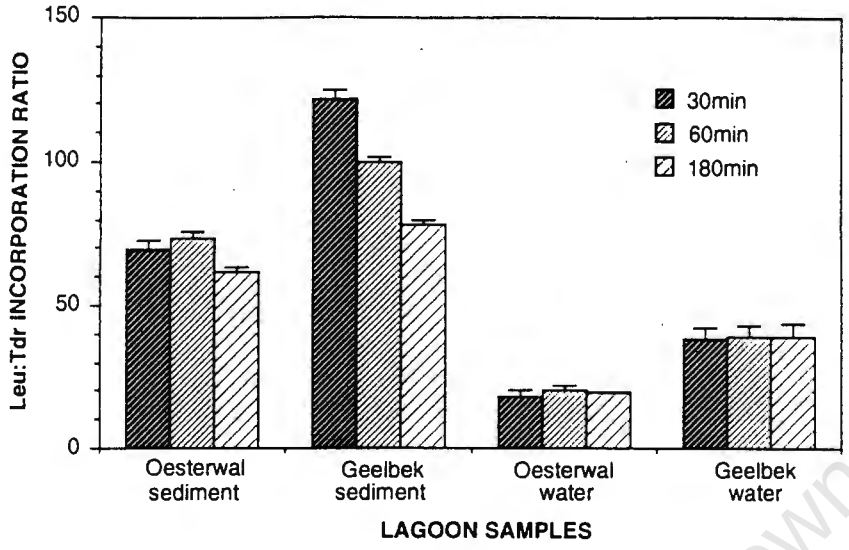

Fig. 3. Time course molar incorporation ratios of Leu : Tdr in summer samples of sediment and water from Geelbek and Oesterwal, Langebaan Lagoon. Values represent means ± SD for incubations terminated after 30, 60, and 180 min.

time. These results indicate that Tdr and Leu incorporation are measuring balanced growth in bacterial populations in water samples, but not necessarily in sediment populations.

There is an apparent relationship between the ratio of Leu : Tdr incorporation (Fig. 3) and the organic status of the sample (Table 2). This relationship is also evident in Table 4. Leu : Tdr ratios and organic carbon contents for sediments exceeded those calculated for water, whereas ratios for samples from Geelbek generally exceeded those for Oesterwal. There was little variation of Leu : Tdr incorporation ratios with sediment depth or with the addition of 20 mM sodium molybdate (Table 4). It is therefore unlikely that variations in populations of sulfate reducing bacteria were responsible for spatial variations in the Leu : Tdr ratio in this study.

Discussion

The measurement of TTI in sediments for estimates of bacterial production is more difficult than in water. We used established TTI methods for water, but modified an appropriate method for sediments to facilitate practical and economic criteria and to enable comparisons to be made. The TTI methods used here allowed the separation of DNA from general macromolecular extracts, and thus measured only label incorporated into DNA. We agree with the conclusions of Moriarty and Pollard [26] who found that filtration and dialysis methods gave similar results, although variability between samples was greater for the filtration method. Despite this variability, however, regression coefficients of our isotope dilution plots were consistently within the confidence limits required for a linear regression of 4 points.

Isotope dilution analysis showed that variations in precursor pool sizes did not follow a regular pattern according to season. Estimated pool sizes in sed-

Table 4. Dual label incorporation of ^{3}H-Tdr and ^{14}C-Leu in sediment and water samples from Langebaan Lagoon collected during early summer 1991[a]

Sample	Molybdate	Leu : Tdr ratio	Organic content[b]
0 cm[c]	−	73.0 ± 5.2	5.5 ± 0.8
0 cm[c]	+	79.7 ± 33.2	5.5 ± 0.8
5 cm[c]	−	50.7 ± 12.9	5.8 ± 0.5
5 cm[c]	+	68.7 ± 5.2	5.8 ± 0.5
10 cm[c]	−	78.0 ± 20.6	6.8 ± 1.0
10 cm[c]	+	71.4 ± 24.1	6.8 ± 1.0
20 cm[c]	−	62.9 ± 20.7	7.5 ± 2.0
20 cm[c]	+	77.2 ± 15.1	7.5 ± 2.0
OHWS[d]	−	113.2 ± 32.5	9.6 ± 1.4
OZS[e]	−	68.9 ± 11.0	10.5 ± 1.0
GHWS[f]	−	84.9 ± 28.2	24.0 ± 12.5
OW[g]	−	24.9 ± 0.1	12.5 ± 0.1
GW[h]	−	38.8 ± 3.8	48.6 ± 4.9

[a] Variations of the Leu : Tdr molar incorporation ratio were recorded with sediment depth and organic content of the sample. Samples were amended with 20 mM molybdate as indicated and incubated with ^{3}H-Tdr and ^{14}C-Leu for 30 min. Values represent means ± SD
[b] Units in mg g^{-1} dry weight for sediment and mg liter^{-1} for water
[c] Sediment from different depths below surface at low water mark, Oesterwal
[d] Surface sediment from high water mark at Oesterwal
[e] Surface sediment from *Zostera* bed at Oesterwal
[f] Surface sediment from high water mark at Geelbek
[g] Water from Oesterwal
[h] Water from Geelbek

iment and water samples from each site followed the same pattern, except for summer samples of sediment and water from Oesterwal. These data also illustrate the need to assess isotope dilution in different study sites with cognizance of its tendency for spatial and temporal variation. Our study showed that isotope dilution in the sediment could be more than 100-fold greater than that in the water column, and that approximately a threefold variation in precursor pool size occurred over an annual cycle. Determinations of isotope dilution for sediment or water in one season may not, therefore, necessarily apply to other seasons. Variability in isotope dilution may be attributed to factors such as the differential participation of bacterial aggregates in TTI, or to the participation of extracellular thiamine nucleotide pools. Jeffrey and Paul [13] differentiated intracellular from extracellular isotope dilution by inhibiting thymidylate synthase in the de novo synthesis pathway with 5-fluoro-2′-deoxyuridine. They found that extracellular isotope dilution was significant in 20% of the cases they studied. If extracellular isotope dilution is occasionally significant then the assumption, held in much of the TTI literature, that addition of thymidine at concentrations high enough to cause feedback inhibition of

thymidylate synthase, becomes problematic. The accuracy of bacterial production estimates subsequently becomes contingent on a routine analysis of isotope dilution. It is conceivable that greater variabilities in isotope dilution may be encountered in environments other than those reported here for a temperate saltmarsh.

Concurrent measurements of DNA and protein synthesis rates indicated that pelagic bacterial assemblages in summer samples from Langebaan Lagoon were in a balanced state of growth (Fig. 3). Other workers have also reported high correlations between Tdr and Leu incorporation for pelagic assemblages [4, 18]. However, the ratio of Leu : Tdr incorporation for sediments decreased with time (Fig. 3), indicating that these populations were possibly in unbalanced growth. Bacteria respond rapidly to changes in their physical and chemical environments, and protein and DNA syntheses become uncoupled when bacteria shift from one growth rate to another. Disturbance effects caused by the use of slurries for sediment TTI determinations may have caused unbalanced growth in these studies. The condition of balanced versus unbalanced growth has important implications for the calculation of bacterial carbon production from TTI, as variations in DNA concentration and cellular sizes parallel changes in rates of DNA and protein syntheses [5].

This is the first report of dual label measurements of bacterial production in sediments. Ratios of Leu : Tdr incorporation for sediments were much higher than those for water samples from Langebaan Lagoon. Saltmarsh ecosystems are known to support the growth of large populations of sulfate-reducing bacteria, which may lack the ability to incorporate exogenous Tdr into their DNA [23]. The proportion of anaerobes in a community may be expected to increase with sediment depth as oxygen levels decrease. Although incorporation of Leu and Tdr decreased with sediment depth (in contrast to bacterial abundance; Table 2), we found little variation in the Leu : Tdr ratio with respect to depth (Table 4). Moreover, the amendment of samples from different sediment depths with 20 mM sodium molybdate, to inhibit the activity of sulfate reducers [29], had little effect on the Leu : Tdr ratio (Table 4). The most likely explanation for the consistency of Leu : Tdr ratios over sediment depth is, therefore, that oxygen-sensitive anaerobes were inactivated in the sediment slurries by the disturbance of oxygen gradients.

Trends in the variation of Leu : Tdr incorporation ratios of different lagoon samples correlated with similar trends in the organic status of different lagoon samples. These patterns are also reflected in the patterns of isotopic dilution (Table 1). Variations in the Leu : Tdr incorporation ratio may be explained as follows: in contrast to leucine, thymidine is a polar molecule and may readily adsorb to organic or clay particles, effectively lowering the concentration of ^{3}H-Tdr available to bacteria for incorporation. Moreover, isotope dilution may lower specific radioactivities of the thymidine pool available for incorporation into bacterial DNA, and thus result in higher Leu : Tdr incorporation ratios.

Primary production is frequently reported to influence bacterial productivity [19, 25, 27], and maximum bacterial densities in temperate marine intertidal sediments usually correspond with increases in plant detritus in the late summer to early autumn [1]. In this study we measured TTI in seasonal samples from Langebaan Lagoon and found significant trends corresponding to expected

seasonal patterns for sediment slurries only ($P < 0.05$), where productivity maxima occurred in summer. Seasonal estimates of bacterial productivity for sediment slurries from Langebaan Lagoon were coincident with variations in the supply of organic material from primary production. Although the availability of organic material may have supported greater bacterial densities at Geelbek than at Oesterwal, bacterial abundance showed little seasonal variability with respect to particulate organic content. Average annual sediment productivity estimates were similar for Oesterwal and Geelbek, although bacterial densities were greater in the mudflats of Geelbek. However, DLI indicated the possibility that TTI did not include the growth of some populations (possibly the sulfate reducers), which may have been more abundant in the muds of Geelbek. The seasonal variability in sediment productivity was lower for Geelbek, where a larger organic pool in the mudflats probably sustained bacterial population sizes in periods of lower primary productivity.

Production estimates for water samples were more variable at Oesterwal than at Geelbek, while bacterial densities in the water column were more consistent between seasons. Oesterwal lies nearer the lagoon mouth than Geelbek and is thus more subject to the influence from tidal exchange of waters from different hydrographic origins, and therefore contains bacterioplankton assemblages that exhibit differing physiological characteristics. The water column at Geelbek was richer in particulate organic content, and supported greater bacterial densities and average annual productivity than Oesterwal. These results contrast with previous reports that bacterial concentrations in the water column were greater at Oesterwal than at Geelbek [22].

The estimates of bacterial productivity for sediments reported here are up to two orders of magnitude lower than those reported for an Australian temperate saltmarsh [24, 25]. This difference can be partly explained by the higher temperatures (up to 31°C) of the latter incubations, and the higher organic content of seagrass sediments relative to those from open flats as at Langebaan (Table 3).

We identified five different sediment microhabitats at Oesterwal (sediments from surface, *Zostera* beds, burrow heads, burrow lining, and 10 cm depth) and measured TTI in each. Our results reflect the findings of other workers. Moriarty and Pollard [25] reported that TTI in *Zostera* bed sediment was about tenfold higher than that in sediment from adjacent open flats, owing to an enrichment with organic carbon originating from the macrophytes and associated microalgae. In samples from Oesterwal, TTI was greatest in *Zostera* bed sediment, and decreased with depth.

The reduction in bacterial productivity with depth in sediment has been previously documented [17, 26, 39]. We also examined whether sediment from the burrow openings and burrow linings of the sandprawn *C. kraussi* contributed differently to overall bacterial productivity as a consequence of their exposure to possible gradients of benthic and pelagic organics and nutrients. TTI in burrow opening sediment was not significantly different ($P < 0.05$) from that in the open flats. However, TTI in burrow lining was approximately threefold greater than that in sediment away from burrows, 10 cm below the surface. Although these results support the findings of Branch and Pringle [2], who reported that more bacteria were present in the immediate vicinity of *C. kraussi*

burrows in Langebaan Lagoon than in adjacent sediments, our bacterial abundance data does not correspond with this pattern. Variation within each sediment microhabit was both temporal and spatial, as indicated by samples from early summer and midsummer, and from different locations on the sandflats. Differences in TTI between the seagrass beds, the open flats, and the subsurface sediments depend not only upon the organic content and bacterial abundance, but also upon species composition and the metabolically active proportion of the bacterial community.

The variability in TTI within and between sediment microhabitats, the differences in isotope dilution and productivity estimates from Geelbek and Oesterwal, and the variable ratios of Leu : Tdr incorporation for sediment samples emphasize the complications of converting TTI measurements into estimates of bacterial carbon production for an entire lagoon system. The calculation of bacterial carbon production from measurements of TTI assumes a constant relationship between the labeling of DNA and cellular carbon production (balanced growth). DLI measurements in pelagic assemblages showed that TTI could be used to estimate bacterial productivity in the water column, but not to obtain accurate estimates of bacterial carbon production for sediments. However, when applied to a seasonal survey, TTI indicated that bacterial production in sediment slurries from Langebaan Lagoon was coincident with variations in the supply of organic material from primary production.

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References

1. Alongi DM (1988) Bacterial productivity and microbial biomass in tropical mangrove sediments. Microb Ecol 15:59–79
2. Branch GM, Pringle A (1987) The impact of the sandprawn *Callianassa kraussi* stebbing on sediment turnover and on bacteria, meiofauna, and benthic microflora. J Exp Mar Biol Ecol 107:219–235
3. Brittain AM, Karl DM (1990) Catabolism of tritiated thymidine by aquatic microbial communities and incorporation of tritium into RNA and protein. Appl Environ Microbiol 56: 1245–1254
4. Chin-Leo G, Kirchman DL (1988) Estimating bacterial production in marine waters from the simultaneous incorporation of thymidine and leucine. Appl Environ Microbiol 54:1934–1939
5. Chin-Leo G, Kirchman DL (1990) Unbalanced growth in natural assemblages of marine bacterioplankton. Mar Ecol Prog Ser 63:1–8
6. Davis CL (1989) Uptake and incorporation of thymidine by bacterial isolates from an upwelling environment. Appl Environ Microbiol 55:1267–1272
7. Ducklow HW, Hill SM, Gardner WD (1985) Bacterial growth and the decomposition of particulate organic carbon collected in sediment traps. Continental Shelf Res 4:445–464
8. Ellery WN, Schleyer MH (1984) Comparison of homogenization and ultrasonication as techniques in extracting attached sedimentary bacteria. Mar Ecol Prog Ser 15:247–250
9. Fuhrman JA, Azam F (1980) Bacterioplankton secondary production estimates for coastal waters of British Columbia, Antarctica, and California. Appl Environ Microbiol 39:1085–1095
10. Fuhrman JA, Azam F (1982) Thymidine incorporation as a measure of heterotrophic bacterio-

plankton production in marine surface waters: Evaluation and field results. Mar Biol 66: 109–120
11. Fuhrman JA, Ducklow HW, Kirchman DL, Hudak J, McManus GB, Kramer J (1986) Does adenine incorporation into nucleic acids measure total microbial production? Limnol Oceanogr 31:627–636
12. Hollibaugh JT (1988) Limitations of the [^{3}H]thymidine method for estimating bacterial productivity due to thymidine metabolism. Mar Ecol Prog Ser 43:19–30
13. Jeffrey WH, Paul JH (1988) Effect of 5-fluoro-2′-deoxyuridine on [^{3}H]thymidine incorporation by bacterioplankton in the waters of southwest Florida. Appl Environ Microbiol 54:331–336
14. Jeffrey WH, Paul JH (1990) Thymidine uptake, thymidine incorporation, and thymidine kinase activity in marine bacterium isolates. Appl Environ Microbiol 56:1367–1372
15. Karl DM (1979) Measurement of microbial activity and growth in the ocean by rates of stable ribonucleic acid synthesis. Appl Environ Microbiol 38:850–860
16. Karl DM (1982) Selected nucleic acid precursors in studies of aquatic microbial ecology. Appl Environ Microbiol 44:891–902.
17. Karl DM, Novitsky JA (1988) Dynamics of microbial growth in surface layers of a coastal marine sediment ecosystem. Mar Ecol Prog Ser 50:169–176
18. Kirchman DL, Hoch MP (1988) Bacterial production in the Delaware Bay estuary estimated from thymidine and leucine incorporated rates. Mar Biol Prog Ser 45:169–178
19. Lovell CR, Konopka A (1985a) Primary and bacterial production in two dimictic Indiana lakes. Appl Environ Microbiol 49:485–491
20. Lovell CR, Konopka A (1985b) Seasonal bacterial production in a dimictic lake as measured by increases in cell numbers and thymidine incorporation. Appl Environ Microbiol 49:492–500
21. Mann KH (1988) Production and use of detritus in various freshwater, estuarine and coastal marine ecosystems. Limnol Oceanogr 33:910–930
22. Mazure HGF, Branch GM (1979) A preliminary analysis of bacterial numbers and biomass in Langebaan Lagoon. Trans Roy Soc S Afr 44:43–54
23. Moriarty DJW (1986) Measurement of bacterial growth rates in aquatic systems from rates of nucleic acid synthesis. Adv Microb Ecol 9:245–292
24. Moriarty DJW, Pollard PC (1981) DNA synthesis as a measure of bacterial productivity in seagrass sediments. Mar Ecol Prog Ser 5:151–156
25. Moriarty DJW, Pollard PC (1982) Diel variation of bacterial productivity in seagrass (*Zostera capricorni*) beds measured by rate of thymidine incorporation into DNA. Mar Biol 72:165–173
26. Moriarty DJW, Pollard PC (1990) Effects of radioactive labeling of macromolecules, disturbance of bacteria and adsorption of thymidine to sediment on the determination of bacterial growth rates in sediment with tritiated thymidine. J Microbiol Methods 11:127–139
27. Moriarty DJW, Roberts DG, Pollard PC (1990) Primary and bacterial productivity of tropical seagrass communities in the Gulf of Carpentaria, Australia. Mar Ecol Prog Ser 61:145–157
28. Murray RE, Hodson RE (1985) Annual cycle of bacterial secondary production in five aquatic habitats of the Okefenokee swamp ecosystem. Appl Environ Microbiol 49:650–655
29. Oremland RS, Capone DG (1988) Use of "specific" inhibitors in biogeochemistry and microbial ecology. Adv Microb Ecol 10:285–383
30. Phillips RC, McRoy CP (1980) Handbook of seagrass biology—An ecosystem perspective. Garland Press STPM, New York
31. Pollard PC (1987) Dialysis: A simple method of separating labelled bacterial DNA and tritiated thymidine from aquatic sediments. J Microbiol Methods 7:91–101
32. Pollard PC, Moriarty DJW (1984) Validity of the tritiated thymidine method for estimating bacterial growth rates: Measurement of isotope dilution during DNA synthesis. Appl Environ Microbiol 48:1076–1083
33. Porter KG, Feig YS (1980) The use of DAPI for identifying and counting aquatic microflora. Limnol Oceanogr 25:943–948
34. Riemann B, Bjornsen PK, Newell S, Fallon R (1987) Calculation of cell production of coastal marine bacteria based on measured incorporation of [^{3}H]thymidine. Limnol Oceanogr 32: 471–476
35. Robarts RD, Wicks RJ (1989) [*methyl*-^{3}H]thymidine macromolecular incorporation and lipid

labeling: Their significance to DNA labeling during measurements of aquatic bacterial growth rate. Limnol Oceanogr 34:213–222
36. Robarts RD, Wicks RJ, Sephton LM (1986) Spatial and temporal variations in bacterial macromolecule labeling with [*methyl*-^{3}H]thymidine in a hypertrophic lake. Appl Environ Microbiol 52:1368–1373
37. Servais P, Martinez J, Billen G, Vives-Rego J (1987) Determining [^{3}H]thymidine incorporation into bacterioplankton DNA: Improvement of the method by DNase treatment. Appl Environ Microbiol 53:1977–1979
38. Simon M, Azam F (1989) Protein content and protein synthesis rates of planktonic marine bacteria. Mar Ecol Prog Ser 51:201–213
39. Thorn PM, Ventullo RM (1988) Measurement of bacterial growth rates in subsurface sediments using the incorporation of tritiated thymidine into DNA. Microb Ecol 16:3–16
40. Wicks RJ, Robarts RD (1987) The extraction and purification of DNA labeled with [*methyl*-^{3}H]thymidine in aquatic bacterial production studies. J Plankton Res 9:1159–1166

APPENDIX 2

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Implications for the assessment of crystalline style activity in bivalves when using the Bernfeld and Nelson–Somogyi assays for reducing sugars

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Abstract: The crystalline style enzymes of *Choromytilus meridionalis* Krauss and other bivalves include amylase, laminarinase and cellulase which are potentially capable of liberating reducing sugars from a wide variety of structural carbohydrates. However, although colorimetric analyses of reducing sugars by the Bernfeld and Nelson–Somogyi assays give qualitative agreement, during quantitative assays using glucose for calibration purposes, there is a two to seven-fold discrepancy between the two methods, depending on the enzyme and substrate reagents used. The Bernfeld assay yields the higher reducing sugar estimate, although the Nelson–Somogyi assay is some eight times more sensitive. It seems likely that the quantitative discrepancy between the two methods can be accounted for by variability in the colour response to different end products. However, when the appropriate calibration standard is used, the two methods are in close agreement. Since these two reducing sugar assays have been previously used with an inappropriate standard (glucose) to make quantitative estimates of reducing sugar release, in an effort to calculate the energetic gain from crystalline style activity, it seems likely that such energy balance studies may be in error and these have been reevaluated.

Key words: Crystalline style; Reducing sugars; Energy budgets

Introduction

It has been reported that the crystalline style of bivalves contains digestive enzymes and therefore plays a role in extracellular digestion (Yonge, 1923). Since Coupin (1900) demonstrated the presence of α-amylase in the style of *Cardium* sp., numerous studies have supported this evidence (Lavine, 1946; Kristensen, 1972; Seiderer & Newell, 1979; Newell *et al.*, 1980). The presence of other carbohydrases such as laminarinase (Bull & Chesters, 1966; Sova *et al.*, 1970; Shallenberger *et al.*, 1974), alginate lyase (Jacober *et al.*, 1980; Marumatsu & Egawa, 1980) and cellulase (Stone & Morton, 1958; Yokoe & Yasumasu, 1964; Horiuchi & Lane, 1965, 1966; Koopmans, 1970; Elyakova, 1972) has also been reported. Lysozymes have been isolated from the styles of both *Mytilus edulis* (McHenery & Birkbeck, 1979, 1982) and *Choromytilus meridionalis* (Seiderer *et al.*, 1984).

The presence of carbohydrases suggests that bivalves are capable of utilizing structural carbohydrates, including those from detritus (Seiderer *et al.*, 1982). Attempts

have been made to relate available food to the presence of carbohydrases, and conclusions indicate that cellulolytic activity corresponds to the level of cellulose in the diet (Crosby & Reid, 1971). In a study of the carbohydrases of some marine invertebrates, Kristensen (1972) found that the highest incidence of carbohydrases occurs in detrital feeders and microherbivores. More recently quantitative estimates of the energy released from crystalline style activity have been compared with the energy requirements of a number of bivalves (Seiderer *et al.*, 1982; Lucas & Newell, 1984).

Sugars resulting from enzymatic hydrolysis of carbohydrates by crystalline style preparations have been detected by various methods. These include turbidometric determinations (Crosby & Reid, 1971; Muramatsu *et al.*, 1977), radiochemical assays (Smucker & Wright, 1984), chromatographic methods (Kristensen, 1972; Pernas *et al.*, 1981) and glucose test kits (Horiuchi & Lane, 1965; Jacober *et al.*, 1980). Viscosimetric methods of estimating enzyme activity have often been used (Yokoe & Yasumasu, 1964; Horiuchi & Lane, 1965, 1966; Okado *et al.*, 1966; Muramatsu *et al.*, 1977), but the most widely used methods are spectrophotometric (Galli & Giese, 1959; Horiuchi & Lane, 1965, 1966; Okado *et al.*, 1966; Sova *et al.*, 1970; Kristensen, 1972; Wojtowitz, 1972; Alemany & Rosell–Perez, 1973; Lindley & Shallenberger, 1976; Seiderer & Newell, 1979; Newell *et al.*, 1980; Rosiou & Iacovache, 1980; Trainer & Tillinghurst, 1982; Lucas & Newell, 1984).

Glucose oxidase–peroxidase (Horiuchi & Lane, 1966; Crosby & Reid, 1971) and KI-KIO_3 (Alemany & Rosell–Perez, 1973) reagents have been used to develop a colour in response to low molecular weight carbohydrates resulting from the action of carbohydrases on complex carbohydrates. Two of the methods most widely used in molluscan style enzyme experiments are the Nelson–Somogyi test for reducing sugars (Nelson, 1944; Somogyi, 1952) and the Bernfeld test for reducing sugars (Bernfeld, 1955; from Sumner, 1921, 1924; Sumner & Howell, 1935). The former relies on the oxidation of the free aldehyde/ketone groups of the reducing sugar, by Cu^{2+} in alkaline solution, while the latter is based on the reduction of alkaline 3,5-dinitrosalicylic acid. Both Nelson–Somogyi and Bernfeld procedures may be used to detect the presence of reducing sugars produced by the action of carbohydrases on a range of substrates, e.g. glycogen, starch, laminarin, cellulose. Therefore they are useful tools for examining and comparing enzymatic activities of homogenates of crystalline styles, stomachs and digestive glands.

Recently a number of studies have attempted to quantify the significance of style activity in terms of the energy requirements of the animal using the Nelson–Somogyi and Bernfeld methods. Seiderer *et al.* (1982) have shown that crystalline style enzymes of *C. meridionalis* and *Perna perna* can release between 4.80 and 9.63 mg glucose $\cdot$ mg^{-1} style protein $\cdot$ h^{-1} from commercial substrates and they suggest that the style enzyme may therefore release sufficient carbon from natural detritus and phytoplankton to meet the requirements of the animals. Lucas & Newell (1984), using naturally occurring detritus, obtained specific activities of the carbohydrases of the style of *Crassostrea virginica* and *Geukensia demissa* and found that style enzyme activity may account for

33 to 48% of the carbon requirements of the animals. Both of these studies used the Nelson–Somogyi and Bernfeld methods for estimating reducing sugar release by crystalline style enzymes. However, in a study of the quantitative significance of the crystalline style enzymes of *Mactra glabrata* in which both the Bernfeld and Nelson–Somogyi methods for estimating reducing sugars were employed, the Bernfeld method consistently indicated 4 to 6.7 times more reducing sugars present than the Nelson–Somogyi method using commercial enzymes, and ≈ 2.5 times more using style enzymes. Use of these assay procedures can imply that as little as 6% of the animal's energy demand is met by style enzyme hydrolysis of naturally occurring substrates (Harris, unpubl. data). Since carbon availability calculated from glucose release by style enzymes in previous studies may have been over or underestimated by a factor of ≈ 2.5 depending on the assay procedure used, these assays need to be examined with reference to the effect they may have on inferences previously made about the ecophysiology of some bivalves. Several factors can affect the reliability of the DNS assay as an accurate analytical method for evaluating reducing sugars (Miller, 1959; Farouki & Gunn, 1983; Rivers *et al.*, 1984; Breuil & Saddler, 1985), and quantitative differences between reducing sugars measured by the DNS and Nelson–Somogyi methods have been reported recently (Breuil & Saddler, 1985). In this paper the discrepancy between the two methods is investigated to illustrate the significance this has on the interpretation of results for purposes of energy budget estimations. Previously calculated energy budgets are recalculated in the light of the present data, to try and estimate the quantitative significance of style enzyme liberation of reducing sugars relative to the energetic demands of certain bivalves.

Materials and Methods

General assay

Assays for reducing sugars released by enzymatic action on a carbohydrate substrate were conducted according to Bernfeld (1955) and Nelson (1944) modified by Somogyi (1952), as detailed in Table I.

The results were corrected by subtracting both substrate and enzyme blanks and are expressed as mg glucose · mg enzyme^{-1} for commercial enzymes and as mg glucose · mg protein^{-1} for style enzymes. All values represent the mean of three estimates. Release of reducing sugars by enzymatic activity was tested over 21 min and found to be linear for at least 9 min, so all experiments employed a 9-min incubation period.

Reducing sugar release was calculated from calibration equations established using glucose as a standard. The Bernfeld calibration curve established for glucose concentrations of 0.063 to 4.0 mg · ml^{-1} was $Y = 0.01 + 0.385X$ ($r^2 = 0.98, n = 7$) where Y is the absorbance at 540 nm and X is the concentration of B – D(+) glucose in mg · ml^{-1}. The Nelson–Somogyi calibration curve for glucose concentrations of 0.0015 to 0.188 mg · ml^{-1} was $Y = -0.03 + 14.73X$ ($r^2 = 0.99, n = 7$), where Y is the absorbance at 660 nm and X is the concentration of B–(D+) glucose in mg · ml^{-1}.

TABLE I

Outline of the procedures of two methods for estimating release of reducing sugars by enzymes.

Method	Nelson (1944) modified by Somogyi (1952)	Bernfeld (1955)
Reagents	Somogyi reagent (A : B, 4 : 1)	1,3-Dinitrosalycylic acid (DNS)
Assay 16 °C for 9 min in shaking water bath	Mix 0.5 ml enzyme + 0.5 ml substrate	Mix 0.5 ml enzyme + 0.5 ml substrate
Procedure:		
(1) To halt reaction	add 1 ml Somogyi reagent and boil for 10 min Cool	add 0.5 ml DNS and boil for 5 min Cool
(2) Colour development	add 2 ml Nelson reagent and 6 ml H_2O	add 10 ml H_2O
(3) Absorbance	read at 660 nm	read at 540 nm

SUBSTRATE PREPARATION

Substrates were prepared in 20 mM phosphate buffer (pH 6.9) containing 150 mM NaCl at the following concentrations: oyster glycogen (BDH No. 38042) 1% w/v, starch (BDH No. 30264) 1% w/v, carboxymethyl cellulose (CMC, BDH) 1% w/v, laminarin (Sigma No. L9634) 0.4% w/v.

ENZYME PREPARATION

Bacterial α-amylase from *Bacillus subtilus* (Sigma, bacterial crude type 3) was prepared in phosphate buffer, pH 6.9, at a concentration of 0.25 $mg \cdot ml^{-1}$ and composite cellulase from *Aspergillus niger* (Sigma practical grade 2) was dissolved in phosphate buffer (pH 6.9) at a concentration of 0.5 $mg \cdot ml^{-1}$. Solutions of mussel crystalline style enzymes were prepared from the styles of *Choromytilus meridionalis* as described by Seiderer *et al.* (1982). Protein concentrations of the style enzyme solutions were determined according to the method of Lowry *et al.* (1951).

TEST FOR ENZYME AND/OR SUBSTRATE INTERFERENCE IN ASSAY

A series of tests were conducted to determine whether either the enzyme or the substrate was interfering with reactions taking place during Bernfeld and Nelson–Somogyi reducing sugar assays. Glucose (0.199 $mg \cdot ml^{-1}$), α-amylase (0.25 $mg \cdot ml^{-1}$) and oyster glycogen (1% w/v) were made up in phosphate buffer (pH 6.9). As the reaction equation was considered to be Enzyme + Substrate → Reaction Products, an equivalent volume of glucose solution was substituted for Enzyme + Substrate, and then for each factor in turn, and analyses of reducing sugars present were conducted by both Bernfeld and Nelson–Somogyi methods.

CALIBRATION CURVES

Standard solutions of 11 saccharides (0.4 mg · ml^{-1}) were made up in phosphate buffer (pH 6.9). The saccharides were dextrin, starch, lactose, cellobiose, maltose, galactose, mannose, fructose, xylose, arabinose and ribose. Each stock solution (0.4 mg · ml^{-1}) was serially diluted so that solutions of 0.2 mg · ml^{-1}, 0.1 mg · ml^{-1}, 0.05 mg · ml^{-1}, 0.025 mg · ml^{-1}, 0.0125 mg · ml^{-1}, and 0.0063 mg · ml^{-1} were obtained. Saccharide solutions ranging from 0.4 mg · ml^{-1} to 0.05 mg · ml^{-1} were assayed by the Bernfeld method as the lower limit for detection of reducing sugars by this method is $\approx$0.05 mg · ml^{-1}. The Nelson–Somogyi method was more sensitive and saccharide solutions ranging from 0.1 mg · ml^{-1} to 0.0063 mg · ml^{-1} were assayed by this method. Using the Nelson–Somogyi method, saccharide solutions with concentrations above 0.1 mg · ml^{-1} approached the recommended maximum limit for accurate readings on our spectrophotometer (Beckman model 25), and 0.1 mg · ml^{-1} was therefore taken as an upper limit. Phosphate buffer blanks were subtracted from experimental values and data points given in the results are the mean of triplicate readings.

RESULTS

COMPARISON OF TWO ASSAY METHODS

Bernfeld and Nelson–Somogyi assays of the reducing sugars produced by incubations of α-amylase, cellulase and mussel enzyme with glycogen, starch, CMC and laminarin are shown in Table II. Reducing sugar values measured by the Bernfeld

TABLE II

Comparison of the carbohydrase activities of α-amylase, cellulase and mussel style enzymes in the presence of starch, glycogen, CMC and laminarin by the Bernfeld and modified Nelson–Somogyi methods: values represent the release of reducing sugars after 9 min incubation at 16 °C and are expressed as mg glucose · mg enzyme^{-1} · h^{-1} for α-amylase and cellulase, and mg glucose · mg style protein^{-1} · h^{-1} for style enzymes.

Incubation	Enzyme activity		Discrepancy
	Bernfeld method	Nelson–Somogyi method	Bernfeld/Nelson–Somogyi
α-amylase + glycogen	58.9	10.29	5.72
α-amylase + starch	90.25	16.62	5.43
α-amylase + CMC	0.00	0.00	–
Cellulase + glycogen	8.25	1.24	6.65
Cellulase + starch	13.35	2.45	5.40
Cellulase + CMC	0.80	0.20	4.0
Style enzyme + glycogen	4.85	2.24	2.17
Style enzyme + starch	10.00	3.65	2.74
Style enzyme + laminarin	0.38	0.16	2.38
Style enzyme + CMC	0.00	0.05	–

method were 4 to 6.7 times higher than those measured by the Nelson–Somogyi method for commercial enzymes, and 2.2 to 2.7 times higher for style enzymes. Tests were therefore conducted to see if the enzyme or substrate was interfering in the reaction for

TABLE III

Estimation of a known quantity of reducing sugar by the Bernfeld and Nelson–Somogyi methods when substrate and enzyme are present independently: units are mg glucose · ml^{-1}.

Incubation	Reducing sugar estimate		Known sugar conc.	Discrepancy
	Bernfeld method	Nelson–Somogyi method		Bernfeld/Nelson–Somogyi
Glucose only	0.210	0.187	0.199	1.12
Enzyme + glucose	0.101	0.102	0.099	0.99
Substrate + glucose	0.116	0.098	0.099	1.18

either method. The results of substituting a glucose solution of known concentration for either the enzyme or substrate fraction of an incubation, or both, and then assaying for reducing sugars present by the Bernfeld and Nelson–Somogyi methods are shown in Table III. The measurements obtained for identical quantities of reducing sugars were comparable for the two methods when enzyme and substrate are present independently.

CALIBRATION CURVES

Linear regressions and their equations for standard curves generated by Bernfeld and Nelson–Somogyi assays of known concentrations of 11 different saccharides, are shown in Figs. 1 and 2 respectively and in Table IV. Using the Bernfeld method for the assay of various saccharides resulted in calibration curves with slopes ranging from 0.32 to 0.53 (Fig. 1). Slopes of monosaccharide pentose sugars were between 0.52 and 0.53, while high molecular weight (>600) polysaccharides produced virtually no colour reaction (slope ≈ 0). Fig. 3 shows that the slopes of the Bernfeld calibration curves increased with declining molecular weight of the saccharide ($r^2 = 0.89$). Nelson–Somogyi determinations of the same saccharides resulted in calibration curves with slopes ranging from 5.92 to 16.34 (Fig. 2). Again, a monosaccharide had the steepest slope (16.34), while high molecular weight (>600) polysaccharides produced no colour reaction.

The relationship between calibration curve slope and molecular weight of the saccharide was, however, rather better for the Bernfeld assay ($r^2 = 0.89$) than for the Nelson–Somogyi assay ($r^2 = 0.72$; see also Fig. 3).

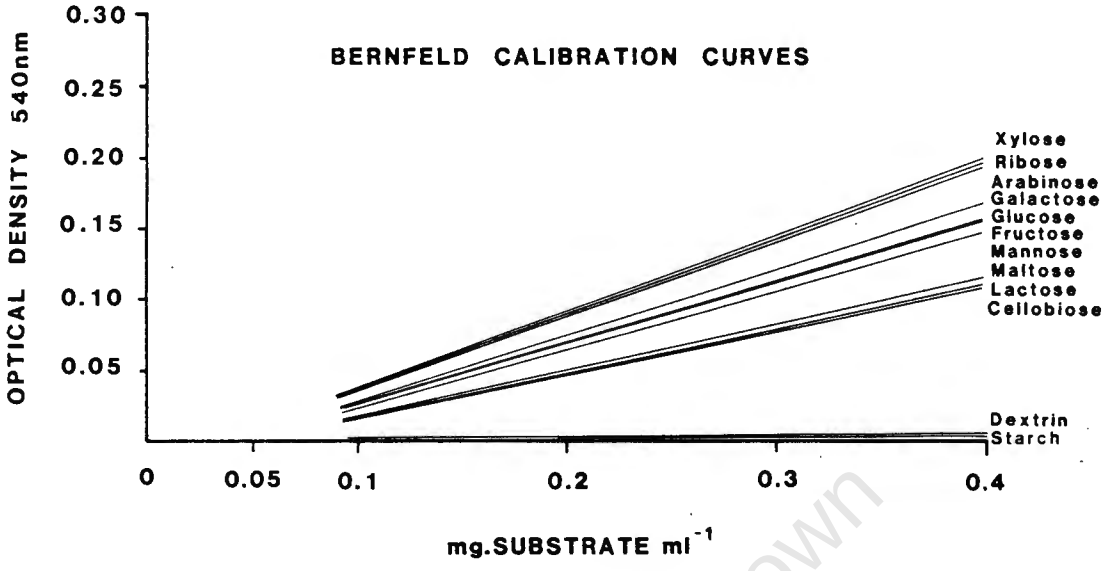

Fig. 1. Calibration curves of 12 saccharides using the Bernfeld assay for reducing sugars.

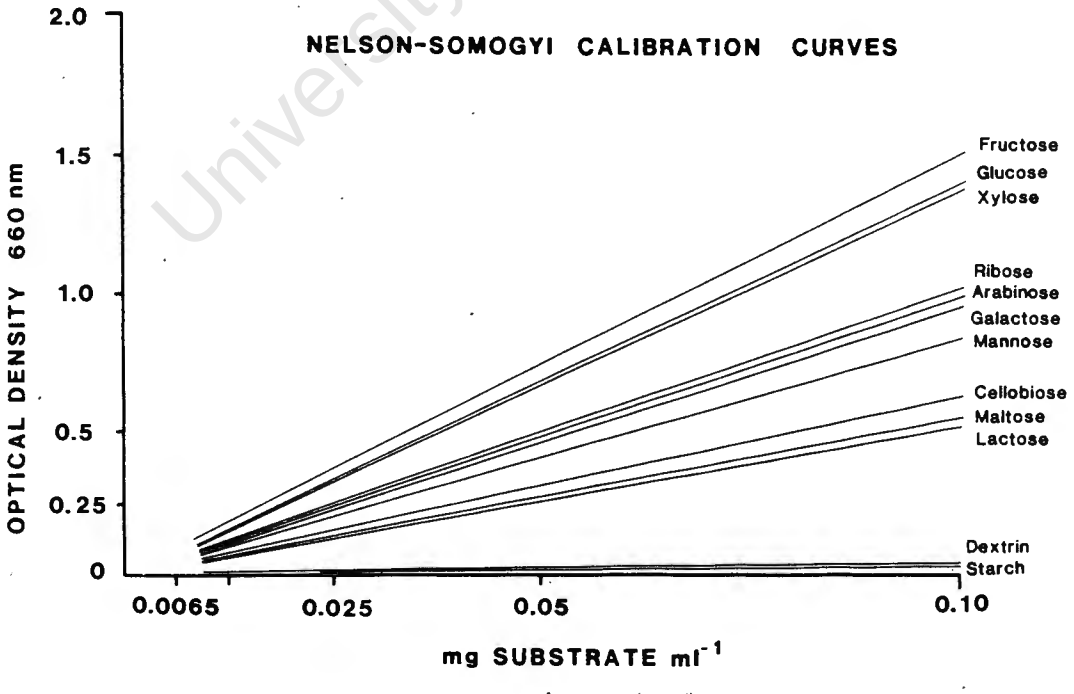

Fig. 2. Calibration curves of 12 saccharides using the Nelson–Somogyi assay for reducing sugars.

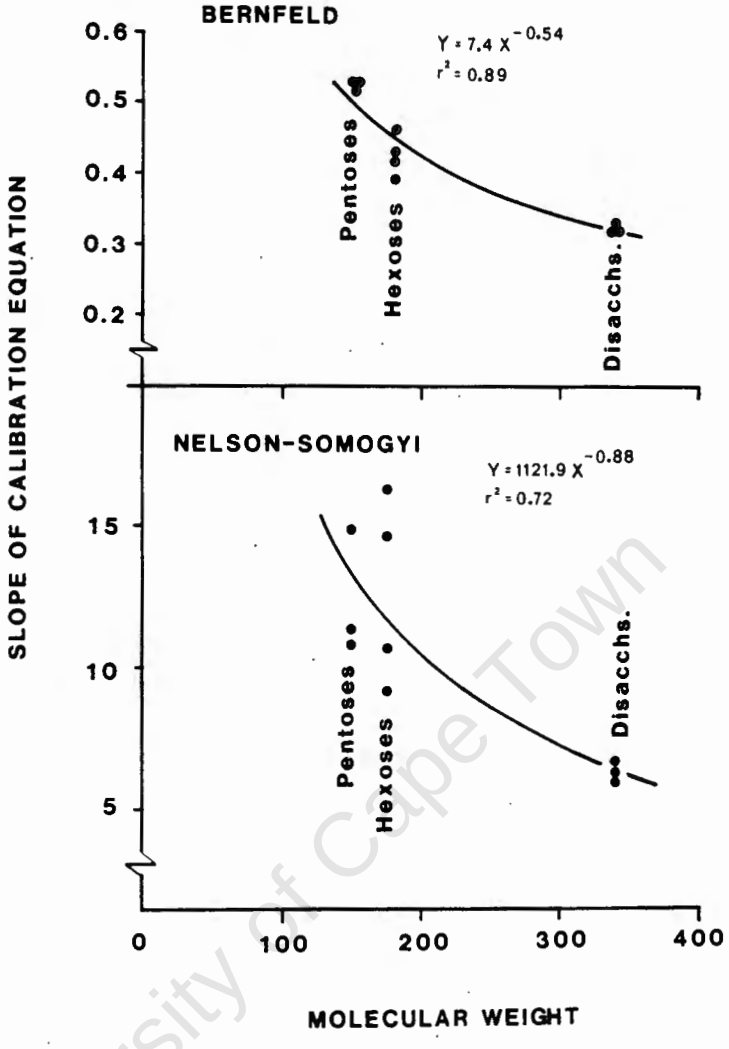

Fig. 3. A plot of calibration curve slope against saccharide molecular weights for the Bernfeld and Nelson–Somogyi assays.

TABLE IV

Standard curve regressions ($Y = a + bx$), and molecular weights of various saccharides using Bernfeld and Nelson–Somogyi assay procedures.

			Bernfeld method				Nelson–Somogyi method			
Saccharide	Type	Mol. wt.	*a*	*b*	*r*	*n*	*a*	*b*	*r*	*n*
Ribose	Pentose	150.13	−0.02	0.53	0.99	4	−0.03	11.32	0.99	5
Xylose	Pentose	150.13	−0.01	0.53	0.99	4	−0.04	14.98	0.99	5
Arabinose	Pentose	150.13	−0.01	0.52	0.99	4	−0.06	10.75	0.99	5
Fructose	Hexose	180.16	−0.02	0.43	0.99	4	−0.02	16.34	0.99	5
Mannose	Hexose	180.16	−0.02	0.42	0.99	4	−0.02	9.16	0.99	5
Galactose	Hexose	180.16	−0.02	0.46	0.99	4	−0.03	10.70	0.99	5
Glucose	Hexose	180.16	0.01	0.39	0.98	7	−0.03	14.73	0.99	7
Lactose	Disacc.	342.30	−0.01	0.32	0.99	4	−0.02	5.92	0.99	5
Cellobiose	Disacc.	342.30	−0.02	0.32	0.99	4	−0.01	6.20	0.99	5
Maltose	Disacc.	342.30	−0.02	0.33	0.99	4	−0.02	6.24	0.99	5
Dextrin	Polysacc.	+600	−0.002	0.02	0.92	4	0.00	0.50	0.94	5
Starch	Polysacc.	+10000	0.00	0.00	0.00	4	0.002	0.004	0.10	5

Discussion

RESPONSE OF THE ASSAY METHODS TO DIFFERENT SACCHARIDES

Figs. 1 and 2 show the importance of the molecular weight of saccharides in determining changes in optical density for a given concentration of reducing sugar in solution. The first derivative (dy/dx) calculated from Fig. 3 for molecular weights of 150.13, 180.16 and 342.30 is at least an order of magnitude greater for the Nelson–Somogyi method than for the Bernfeld method, indicating the greater sensitivity of the former method to the molecular weights of the saccharides assayed. The varying response of the DNS method to the saccharide assayed has been reported before (Miller, 1959; Farouki & Gunn, 1983; Breuil & Saddler, 1985). The sensitivity of both methods to the molecular weight of the saccharide assayed becomes important if energy budgets are calculated from incubations of crude enzymes and substrates which produce a number of varying molecular weight end products. From Figs. 1 and 2 it is evident that for both Bernfeld and Nelson–Somogyi methods, high molecular weight (>600) polysaccharides have regression slopes of virtually zero even though most polysaccharides have a terminal monomer present as a reducing sugar. Disaccharides have lower regression slope coefficients than monosaccharides, and of the monosaccharides, the pentose regression slope coefficients are greater than those of the hexoses for the Bernfeld method, although this is not so for the Nelson–Somogyi method. The stereochemistry of the sugar molecules may significantly affect their quantitative assay by the Nelson–Somogyi method. Using this method disaccharides have similar calibration regression slopes ranging from 5.92 to 6.72, but calibration slopes for monosaccharides range from 9.16 (mannose) to 16.34 (fructose, see Table IV). However, fructose, glucose, galactose and mannose all have a molecular weight of 180.16, so it is difficult to understand why the regression slope coefficients vary so much, particularly as glucose, mannose and galactose are stereoisomers. The fact that fructose has the steepest calibration slope may be linked to the fact that it is a ketose sugar whereas all the other sugars are aldoses. Table IV shows that using the Nelson–Somogyi method, pentose sugars also have differing calibration regression slopes (10.74 to 14.98), although all have a molecular weight of 150.13. Arabinose and ribose have similar slopes (10.74 and 11.32), but the calibration regression slope of xylose (14.98) is very similar to that of glucose (14.74). The difference in pentose calibration slope regressions may be a result of the exposed nature of the axial reducing group in xylose, whereas in arabinose and ribose the reducing group is equatorial and not quite so easily oxidised. The stereochemistry of sugar molecules does not seem to affect the Bernfeld assay procedure in any significant way (Fig. 1, Table IV).

SENSITIVITY OF ASSAY METHODS

The slopes of the mono- and disaccharide standard curves obtained for the Nelson–Somogyi method (6.24 to 14.98) were much higher than those obtained for the

Bernfeld method (0.33 to 0.53; see Table IV). Thus for the former method a large change in optical density results from a small change in reducing sugar concentration, while for the latter method, a small change in optical density is the result of a large change in reducing sugar concentration. The Nelson–Somogyi method is therefore more sensitive to changes in reducing sugar concentration than the Bernfeld method. Furthermore, the Nelson–Somogyi method is also sensitive to sugar concentrations of less than $0.05\ mg \cdot ml^{-1}$, whereas at this concentration, optical densities approach zero for the Bernfeld method (Figs. 1 and 2).

Farouki & Gunn (1983), and Breuil & Saddler (1985) also demonstrate the greater sensitivity of the Nelson–Somogyi method. In addition, the DNS method has been shown to be affected by the buffer used for enzyme dilution (Miller, 1959), the presence of Ca^{2+} ions (Robyt & Whelan, 1972), certain mineral salts (Farouki & Gunn, 1983), enzyme concentration (Breuil & Saddler, 1985), and may possibly be affected by the degree of polymerization of the residue to which the reducing group is attached (Breuil & Saddler, 1985).

QUANTITATIVE DIFFERENCE BETWEEN ASSAY PROCEDURES

When reducing sugars released from the incubation of polysaccharide substrates with commercial enzymes were estimated using both the Bernfeld and Nelson–Somogyi methods, the Bernfeld method invariably gave an estimate 4 to 6.7 times higher than the Nelson–Somogyi method if glucose was used as a standard (Table II). Furthermore, mussel crystalline style enzymes produced between 2 and 2.7 times more reducing sugars when estimated by the Bernfeld method compared with the Nelson–Somogyi method. Similarly, when investigating the action of bacterial cellulase on filter paper, Breuil & Saddler (1985) found reducing sugar estimates obtained by the dinitrosalicylic acid method were 45% higher than those obtained by the Nelson–Somogyi method. From the results presented in Table III it is evident also that neither the enzyme nor the substrate, when present independently, caused undue interference in the quantitative estimation of an analytical grade reducing sugar, and that both the Bernfeld and Nelson–Somogyi methods of assaying such pure sugars gave similar results. This suggests that it is the reaction products of polysaccharide hydrolysis which influence the estimate of free reducing sugars obtained using either method.

During the enzymatic hydrolysis of polysaccharides by crude enzyme extracts of molluscan digestive gland or crystalline style, intermediate compounds are produced which consist of di- and oligosaccharide units, which may be further hydrolysed to the constituent monosaccharides that make up the major reaction products. At the time of assay by Nelson–Somogyi or Bernfeld methods, a typical hydrolysis process is in progress, and the reducing sugars measured by the two methods probably consist of a mixture of mono-, di- and oligosaccharides. Figs. 1 and 2 show that with both methods, changes in optical density caused by changes in concentration of reducing sugar in solution are to a large extent dependent on the molecular weight of the sugar involved.

When the enzyme-substrate reaction mixture is stopped for assay before the hydrolysis is completed, the relative proportions of the oligosaccharides, disaccharides, hexoses and pentoses will therefore affect the optical density reading. If a glucose calibration curve (Table IV) is used to determine the amount of reducing sugars present in such a reaction and only disaccharides such as maltose are present in the assay mixture, any optical density reading would provide an estimate of reducing sugars present that is 15% too low by the Bernfeld method and 58% too low if the Nelson–Somogyi method is used since the maltose: glucose slope ratios calculated from Table IV are 0.85 for the Bernfeld procedure and 0.42 for the Nelson–Somogyi procedure. If only pentose sugars such as arabinose are present, the estimate of reducing sugars present would be 33% too high by the Bernfeld method and 27% too low by the Nelson–Somogyi method, since the arabinose : glucose slope ratios calculated from Table IV are 1.33 for the Bernfeld procedure and 0.73 for the Nelson–Somogyi procedure. Thus the Nelson–Somogyi method is most likely to underestimate reducing sugars present if glucose is used for calibration purposes, and if disaccharides comprise the major part of the assay mixture, the underestimation is likely to be ≈ 4 times greater than if the Bernfeld method was used.

Since mussel crystalline styles possess a suite of carbohydrases (Sova *et al.*, 1970; Kristensen, 1972; Gianfreda *et al.*, 1979; Seiderer *et al.*, 1982) a more complete hydrolysis of complex polysaccharides to component dimers or monomers is to be anticipated. This is likely to give better agreement between the two methods, as is observed when an analytically pure sugar is assayed (Table III). This would account for the reduced discrepancy between the two methods when commercial substrate hydrolysis by style enzymes rather than commercial enzymes is assayed (Table II), particularly if the latter are capable only of partial substrate hydrolysis.

If an incubation is allowed to continue until all poly-, oligo- and disaccharides are reduced to monosaccharides, then the two methods should provide similar quantitative estimates of reducing sugar when glucose is used as a standard. In one experiment, an incubation of *C. meridionalis* style enzyme and glycogen was allowed to proceed for 15 h and the reducing sugar release was measured by both methods. Using a glucose calibration curve (Table IV), reducing sugar release measured by the Bernfeld method was 0.899 mg glucose · mg protein^{-1} while that for the Nelson–Somogyi method was 0.460 mg glucose · mg protein^{-1}. Thus the Bernfeld estimation remained ≈ 2 times higher than the Nelson–Somogyi estimate, which is similar to differences (≈ 2–2.5) in reducing sugar estimates obtained during 9-min incubations of style enzymes and a glycogen substrate (Table II). Therefore, increasing the incubation time of substrate and style enzymes from 9 min to as much as 15 h does not reduce the discrepancy between the two methods. However, if maltose had been used as a standard (Table IV), reducing sugar release would be calculated as 1.088 mg maltose · mg protein^{-1} by the Bernfeld method and 1.092 mg maltose · mg protein^{-1} by the Nelson–Somogyi method. This suggests that the final reaction product was a disaccharide for which maltose was a more appropriate standard than glucose.

Glucose is therefore not necessarily the correct reducing sugar to use for calibration curves to quantify molluscan enzyme activity of α-amylase, laminarinase and cellulase. In short incubations, α-amylase hydrolyses starch and glycogen to 70–90% maltose and small amounts of glucose and dextrins (Diem & Lentner, 1970). Cellulase hydrolyses cellulose to cellobiose units (Hart & Schuetz, 1966), and laminarinase hydrolyses laminarin to laminaribiose, higher oligosaccharides and some glucose but laminaribiose is hydrolysed to glucose very slowly (Chesters and Bull, 1963; Sova *et al.*, 1970). Seiderer *et al.* (1982) show that α-amylase and laminarinase account for a very large proportion of glucogenic activity of crystalline styles of the mussels *C. meridionalis* and *Perna perna*, and other workers show that α-amylase and laminarinase are the principal bivalve carbohydrases (Horiuchi & Lane, 1966; Sova *et al.*, 1970; Kristensen, 1972). On this basis it would be more accurate to establish calibration curves using a disaccharide reducing sugar.

TABLE V

Recalculation of reducing sugar release by *Choromytilus meridionalis* style enzyme, using a maltose calibration curve from Table IV: units are mg maltose · mg protein^{-1} · h^{-1}.

Incubation	Enzyme activity		Discrepancy
	Bernfeld method	Nelson–Somogyi method	Bernfeld/Nelson–Somogyi
Style enzyme + glycogen	5.73	5.29	1.08
Style enzyme + starch	11.82	8.62	1.37
Style enzyme + laminarin	0.45	0.38	1.18
Style enzyme + CMC	0.00	0.19	–

Recalculation of reducing sugars produced by enzyme activity of mussel styles on various substrates as given in Table II, are shown in Table V using maltose rather than glucose as a standard. Reducing sugars assayed by the Bernfeld method are still higher than the Nelson–Somogyi method, but only by a factor of between 1.08 and 1.37. Clearly, an accurate and consistent measure of reducing sugar release depends upon selecting an appropriate sugar for the calibration curve. The sugar used as the standard should be representative of the dominant end products of substrate hydrolysis.

IMPLICATIONS FOR QUANTITATIVE ESTIMATES OF REDUCING SUGARS

Previous studies involving reducing sugar release by molluscan crystalline style enzymes, estimated by the Bernfeld or Nelson–Somogyi method, have often used calibration curves based on a glucose standard (Sova *et al.*, 1970; Kristensen, 1972; Lindley & Shallenberger, 1976; Gianfreda *et al.*, 1979; Seiderer & Newell, 1979; Seiderer *et al.*, 1982; Lucas & Newell, 1984). From our results it is evident that both methods can be used in this way to detect the presence of reducing sugars, although the

Nelson–Somogyi method is the more sensitive but provides a lower estimate of reducing sugars with a glucose standard, as well as being less affected by the conditions of the incubation (Miller, 1959; Farouki & Gunn, 1983; Breuil & Saddler, 1985). The selection of glucose as a standard is not unwarranted when comparative or qualitative results are sought. However, the reducing sugars present after short incubations of polysaccharide substrates with extracts of digestive gland or crystalline style enzymes are likely to be mainly disaccharides, and the molecular weight of the reducing sugar used as a standard has a marked effect on the calibration curve. It is therefore important to select an appropriate standard when making quantitative estimates of sugar release, particularly when those results are used to calculate energy budgets. For this purpose it is necessary to know what the reaction products of an enzyme-substrate incubation are likely to be before selecting a single reducing sugar as a standard for calibration purposes.

To test the significance of our findings, the energy budgets of Seiderer *et al.* (1982) and Lucas & Newell (1984) may be recalculated using more appropriate maltose and cellobiose standard regressions from Table IV, since both authors use glucose only as a standard. It makes very little difference to recalculate the Bernfeld assays of Seiderer *et al.* (1982) in terms of maltose equivalents since their glucose calibration curve ($Y = -0.02 + 0.31\,X$) is very similar to the maltose calibration equation of Table IV ($Y = 0.02 + 0.33\,X$). From Table IV it is evident, however, that sugar equivalents calculated from a maltose calibration curve will be 1.18 times greater than those calculated from a glucose calibration curve. Thus the activity of style eluates are recalculated to 5.66 mg maltose · mg total protein^{-1} relative to 4.80 mg glucose · mg total protein^{-1} for *Choromytilus meridionalis*, and 11.36 mg maltose · mg total protein^{-1} relative to 9.63 mg glucose · mg total protein^{-1} for *Perna perna* (Seiderer *et al.*, 1982). Style turnover times thus becomes 30 h rather than the original 25 h for *Choromytilus meridionalis* and 161 h relative to 136 h for *Perna perna*. Such differences may be regarded as relatively insignificant and do not alter the conclusions drawn by Seiderer *et al.* (1982) that there are major differences in total specific carbohydrase activity of the two species and that, on the basis of glucose release from commercial substrates, the carbohydrases may be able to release glucose from the phytoplankton and detrital components of the diet.

More strikingly however, recalculation of the data of Lucas & Newell (1984) using the cellobiose standard regression from Table IV shows that only 13% of the estimated carbon absorption requirements of *Crassostrea virginica* appear to be attainable by style enzyme hydrolysis of naturally occurring *Juncus* and *Spartina* detritus, compared with the original estimate of 40%. This finding raises a number of questions. Refractory detrital particles may be energetically unimportant in the diet of the animal while phytoplankton and bacteria in the water column may be far more important components of the diet. Alternatively, in vitro experiments on crystalline style enzyme activity may be a poor indicator of the animal's ability to utilize naturally occurring food particles. High assimilation efficiencies in the region of 50% have been recorded for the mussel *Aulacomya ater* fed on refractory kelp particles (Stuart *et al.*, 1982), and significant

breakdown and digestion of such particles may be a result of the action of enzymes in the stomach and digestive gland, or even gut bacteria hydrolases. Bivalves may utilize several food sources and several enzyme systems simultaneously or in sequence to acquire sufficient energy from the environment.

Lucas & Newell (1984) calculate a style turnover time of 1.7 h for *Geukensia demissa* if 40% of the carbon requirements of the animal are to be met by glucose release resulting from style enzyme hydrolysis of detrital particles. Recalculating these data using a cellobiose calibration curve shows that only 13% of the animal's carbon requirements can be met if the style turns over in 1.7 h. Alternatively style turnover time must be $\approx$30 min to meet 40% of the carbon requirements. To date, bivalve style turnover time has only been measured by Seiderer *et al.* (1982) in laboratory maintained *Choromytilus meridionalis* and *Perna perna*. Turnover times, calculated to balance the carbon budget on the basis of glucose release from easily hydrolysed commercial substrates were 25 h for *Choromytilus meridionalis* and 136 h for *Perna perna*. If refractory detrital particles are to provide a significant proportion of the daily carbon requirements of filter feeding bivalves, the style turnover times under natural conditions may well be much faster and the rhythm of style synthesis may not be linked to the period of tidal submersion, as suggested by Lucas & Newell (1984).

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References

Alemany, M. & M. Rosell-Perez, 1973. Two different amylase activities in the sea mussel, *Mytilus edulis* L. *Rev. Esp. Fisiol.*, Vol. 29, pp. 217–222.

Bernfeld, P.C., 1955. Amylases α and β. In, *Methods in enzymology*, *Vol. 1*, edited by S.P. Colwick & N.P. Kaplan, Academic Press, New York, pp. 148–150.

Breuil, C. & J.N. Saddler, 1985. Comparison of the 3,5-dinitrosalicylic acid and Nelson–Somogyi methods of assaying for reducing sugars and determining cellulase activity. *Enzyme Microb. Technol.*, Vol. 7, pp. 327–332.

Bull, A.T. & C.G.C. Chesters, 1966. The biochemistry of laminarin and the nature of laminarinase. *Adv. Enzymol.*, Vol. 28, pp. 325–364.

Chesters, C.G.C. and A.T. Bull, 1963. The enzymic degradation of laminarin. *Biochem. J.*, Vol. 86, pp. 28–46.

Coupin, H., 1900. Sur les fonctions de la tige cristalline des Acephates. *C.R. Acad. Sci. Paris*, Vol. 130, pp. 1214–1216.

Crosby, N.D. & G.B. Reid, 1971. Relationships between food, phylogeny, and cellulose digestion in the Bivalvia. *Can. J. Zool.*, Vol. 49, pp. 617–622.

DIEM, K. & C. LENTNER, Editors. 1970. *Scientific tables*, J.R. Geigy, Switzerland, seventh edition, 809 pp.
ELYAKOVA, L.A., 1972. Distribution of cellulases and chitinases in marine invertebrates. *Comp. Biochem. Physiol.*, Vol. 43B, pp. 67–70.
FAROUKI, E. & D.J. GUNN, 1983. Some effects of metal ions on the estimation of reducing sugars in biological media. *Biotechnol. Bioeng.*, Vol. 25, pp. 1905–1911.
GALLI, D.R. & A.C. GIESE, 1959. Carbohydrate digestion in a herbivorous snail, *Tegula funebralis*. *J. Exp. Zool.*, Vol. 140, pp. 415–440.
GIANFREDA, L., A. IMPERATO, R. PALESCANDOLA & V. SCARDI, 1979. Distribution of β1,4-glucanase and β-glucoside activities among marine molluscs with different feeding habits. *Comp. Biochem. Physiol.*, Vol. 63B, pp. 345–348.
HART, H. & R.D. SCHUETZ, 1966. *Organic chemistry*. Houghton Mifflin Company, Boston, third edition, 335 pp.
HORIUCHI, S. & C.E. LANE, 1965. Digestive enzymes of the crystalline style of *Strombus gigas* Linne. I. Cellulase and some other carbohydrases. *Biol. Bull.* (*Woods Hole, Mass.*), Vol. 129, pp. 273–281.
HORIUCHI, S. & C.E. LANE, 1966. Carbohydrases of the crystalline style and hepatopancreas of *Strombus gigas* Linne. *Comp. Biochem. Physiol.*, Vol. 17, pp. 1189–1197.
JACOBER, L.F., C. RICE & A.G. RAND, 1980. Characterization of the carbohydrase degrading enzymes in the surf clam crystalline style. *J. Food Sci.*, Vol. 45, pp. 381–385.
KOOPMANS, J.J.C., 1970. Cellulases in Molluscs. I. The nature of the cellulases in *Helix pomatia* and *Cardium edule*. *Neth. J. Zool.*, Vol. 20, pp. 445–463.
KRISTENSEN, J.H., 1972. Carbohydrases of some marine invertebrates with notes on their food and on the natural occurrence of the carbohydrates studied. *Mar. Biol.*, Vol. 14, pp. 130–142.
LAVINE, T.F., 1946. A study of the enzymatic and other properties of the crystalline style of clams: evidence for the presence of cellulase. *J. Cell. Comp. Physiol.*, Vol. 28, pp. 183–195.
LINDLEY, M.G. & R.S. SHALLENBERGER, 1976. Purification and characterization of a molluscan β-D-(1-3)-glucan hydrolase. *Fd. Chem.*, Vol. 1, pp. 149.
LOWRY, O.H., N.H. ROSEBROUGH, A. LAVIS FARR & R.J. RANDALL, 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, Vol. 193, pp. 265–275.
LUCAS, M.I. & R.C. NEWELL, 1984. Utilization of saltmarsh grass detritus by two estuarine bivalves: carbohydrase activity of crystalline style enzymes of the oyster *Crassostrea virginica* (Gmelin) and the mussel *Geukensia demissa* (Dillwyn). *Mar. Biol. Lett.*, Vol. 5, pp. 275–290.
MCHENERY, J.G. & T.H. BIRKBECK, 1979. Lysozyme of the mussel, *Mytilus edulis* (L). *Mar. Biol. Lett.*, Vol. 1, pp. 111–119.
MCHENERY, J.G. & T.H. BIRKBECK, 1982. Characterization of the lysosyme of *Mytilus edulis* (L.). *Comp. Biochem. Physiol.*, Vol. 71B, pp. 583–589.
MILLER, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.*, Vol. 31, pp. 426–428.
MURAMATSU, T. & K. EGAWA, 1980. Isozymes of alginate lyase in the midgut gland of *Turbo cornutus*. *Agric. Biol. Chem.*, Vol. 44, pp. 2587–2594.
MURAMATSU, T., S. HIROSE & M. KATAYOSE, 1977. Isolation and properties of alginate lyase from the mid-gut gland of wreath shell *Turbo cornutus*. *Agric. Biol. Chem.*, Vol. 44, pp. 2587–2594.
NELSON, N., 1944. A photometric adaptation of the Somogyi method for the determination of glucose. *J. Biol. Chem.*, Vol. 153, pp. 375–380.
NEWELL, R.C., I. PARKER & P.A. COOK, 1980. A possible rôle of α-amylase isoenzymes from the style of the mussel *Choromytilus meridionalis* (Krauss) following thermal acclimation. *J. Exp. Mar. Biol. Ecol.*, Vol. 47, pp. 1–8.
OKADO, G., T. NISIZAWA & K. NISIZAWA, 1966. Cellulases of a marine mollusc, *Dollabella* sp. *Biochem. J.*, Vol. 99, pp. 214–221.
PERNAS, R.V., I.R. MARTINEZ & M.F. RAMA, 1981. Purification and properties of the two major forms of β-D-galactosidase from lysosymes of *Mytilus edulis* hepatopancreas. *Comp. Biochem. Physiol.*, Vol. 69B, pp. 851–857.
RIVERS, D.B., S.J. GRACHECH, L.C. WOODFORD & G.H. EMERT, 1984. Limitations of the DNS assay for reducing sugars from saccharified lignocellulosics. *Biotechnol. Bioeng.*, Vol. 26, pp. 800–802.
ROBYT, J.F. & W.J. WHELAN, 1972. Reducing value methods for maltodextrin: chain length dependence of alkaline-3,5-dinitrosalicylate and chain length independence of alkaline copper. *Anal. Biochem.*, Vol. 45, pp. 510–516.

ROSIOU, N. & C. IACOVACHE, 1980. Partial characterization of the purified α-amylase from the soft clam *Mya arenaria* L. *Rev. Roum. Biochim.*, Vol. 17, pp. 285–290.

SEIDERER, L.J. & R.C. NEWELL, 1979. Adjustment of the activity of α-amylase extracted from the style of the black mussel *Choromytilus meridionalis* (Krauss) in response to thermal acclimation. *J. Exp. Mar. Biol. Ecol.*, Vol. 39, pp. 79–86.

SEIDERER, L.J., R.C. NEWELL & P.A. COOK, 1982. Quantitative significance of style enzymes from two marine mussels (*Choromytilus meridionalis* Krauss and *Perna perna* Linnaeus) in relation to diet. *Mar. Biol. Lett.*, Vol. 3, pp. 257–271.

SEIDERER, L.J., C.L. DAVIS, F.T. ROBB & R.C. NEWELL, 1984. Utilization of bacteria as a nitrogen resource by kelp bed mussel *Choromytilus meridionalis*. *Mar. Ecol. Prog. Ser.*, Vol. 15, pp. 109–116.

SHALLENBERGER, R.S., C. SEARLES & B.A. LEURS, 1974. Laminarinase activity in the crystalline style of the surf clam (*Spissula solidissima*). *Experientia*, Vol. 30, pp. 597–598.

SMUCKER, R.A. & D.A. WRIGHT, 1984. Chitinase activity in the crystalline style of the American oyster *Crassostrea virginica*. *Comp. Biochem. Physiol.*, Vol. 77A, pp. 239–241.

SOMOGYI, M., 1952. Notes on sugar determination. *J. Biol. Chem.*, Vol. 195, pp. 19–23.

SOVA, V.V., L.L. ELYAKOVA & V.E. VASKOVSKY, 1970. The distribution of laminarinases in marine invertebrates. *Comp. Biochem. Physiol.*, Vol. 32, pp. 459–464.

STONE, B.A. & J.E. MORTON, 1958. The distribution of cellulases and related enzymes in Mollusca. *Proc. Malac. Soc. London*, Vol. 33, pp. 127–141.

STUART, V., J.G. FIELD & R.C. NEWELL, 1982. Evidence for absorption of kelp detritus by the ribbed mussel *Aulacomya ater* using a new ^{51}Cr-labelled microsphere technique. *Mar. Ecol. Prog. Ser.*, Vol. 9, pp. 263–271.

SUMNER, J.B., 1921. Dinitrosalicylic acid: a reagent for the estimation of sugar in normal and diabetic urine. *J. Biol. Chem.*, Vol. 47, pp. 5–14.

SUMNER, J.B., 1924. The estimation of sugar in diabetic urine using dinitrosalicylic acid. *J. Biol. Chem.*, Vol. 62, pp. 287–290.

SUMNER, J.B. & S.F. HOWELL, 1935. A method for determination of saccharase activity. *J. Biol. Chem.*, Vol. 108, pp. 51–54.

TRAINER, D.G. & E.K. TILLINGHURST, 1982. Amylolytic activity of the crystalline style of *Mya arenaria* (Bivalvia, Mollusca). *Comp. Biochem. Physiol.*, Vol. 72A, pp. 99–103.

WOJTOWICZ, M.B., 1972. Carbohydrases of the digestive gland and the crystalline style of the Atlantic deep-sea scallop (*Placopecten magellanicus*, Gmelin). *Comp. Biochem. Physiol.*, Vol. 43A, pp. 131–141.

YOKOE, Y. & I. YASUMASU, 1964. The distribution of cellulases in invertebrates. *Comp. Biochem. Physiol.*, Vol. 13, pp. 323–338.

YONGE, C.M., 1923. Studies on the comparative physiology of digestion. *Br. J. Exp. Biol.*, Vol. 1, pp. 15–63.