Pergamon

Progress in Oceanography 50 (2001) 443–458

Progress in Oceanography

www.elsevier.com/locate/pocean

Sediment distribution, hydrolytic enzyme profiles and bacterial activities in the guts of *Oneirophanta mutabilis*, *Psychropotes longicauda* and *Pseudostichopus villosus*: what do they tell us about digestive strategies of abyssal holothurians?

D. Roberts [a,*], H.M. Moore [a], J. Berges [a], J.W. Patching [b], M.W. Carton [b], D.F. Eardly [b]

[a] *School of Biology and Biochemistry, The Queen's University, Belfast, Northern Ireland, UK*
[b] *Marine Microbiology, Ryan Institute, University College, Galway, Ireland*

Abstract

This paper describes inter-specific differences in the distribution of sediment in the gut compartments and in the enzyme and bacterial profiles along the gut of abyssal holothurian species — *Oneirophanta mutabilis*, *Psychropotes longicauda* and *Pseudostichopus villosus* sampled from a eutrophic site in the NE Atlantic at different times of the year. Proportions of sediments, relative to total gut contents, in the pharynx, oesophagus, anterior and posterior intestine differed significantly in all the inter-species comparisons, but not between inter-seasonal comparisons. Significant differences were also found between the relative proportions of sediments in both the rectum and cloaca of *Psychropotes longicauda* and *Oneirophanta mutabilis*. Nineteen enzymes were identified in either gut-tissue or gut-content samples of the holothurians studied. Concentrations of the enzymes in gut tissues and their contents were highly correlated. Greater concentrations of the enzymes were found in the gut tissues suggesting that they are the main source of the enzymes. The suites of enzymes recorded were broadly similar in each of the species sampled collected regardless of the time of the year, and they were similar to those described previously for shallow-water holothurians. Significant inter-specific differences in the gut tissue concentrations of some of the glycosidases suggest dietary differences. For example, *Psychropotes longicauda* and *Pseudostichopus villosus* contain higher levels of chitobiase than *Oneirophanta mutabilis*. There were no seasonal changes in bacterial activity profiles along the guts of *O. mutabilis* and *Pseudostichopus villosus*. In both these species bacterial activity and abundance declined between the pharynx/oesophagus and anterior intestine, but then increased along the gut and became greatest in the rectum/cloaca. Although the data sets were more limited for *Psychropotes longicauda*, bacterial activity increased from the anterior to the posterior intestine but then declined slightly to the rectum/cloaca. These changes in bacterial activity and densities probably reflect changes in the microbial environment along the guts of abyssal holothurians. Such changes suggest that there is potential for microbial breakdown of a broader range of substrates than could be otherwise be achieved by the holothurian itself.

* Corresponding author. Fax: +44-1232-236505.
E-mail address: d.roberts@qub.ac.uk (D. Roberts).

PII: S0079-6611(01)00065-9

However, the present study found no evidence for sedimentary (microbial) sources of hydrolytic enzymes.

Contents

1. Introduction

The sediments ingested from the surface by deposit-feeding holothurians include dissolved organic matter (DOM), micro-organisms, meiofauna, decaying organic debris and inorganic components. The relative proportions of these constituents determine the nutritional value of the ingested material. A problem faced by deep-sea deposit feeders is that the nutritional value of the sediments declines with distance from the source of organic material, which is generally the euphotic zone near the sea's surface. Even so, the organic input to some abyssal deep-sea sediments varies significantly with season (Billett, Lampitt, Rice & Mantoura, 1983) and is reflected in the biomasses of both bacteria (Patching & Eardly, 1997) and invertebrate megafauna (Thurston, Rice & Bett, 1998). High bacterial abundances have been recorded in the oesophageal contents of deep-sea holothurians (Ralijaona & Bianchi, 1982; Sibuet, Khripounoff, Deming, Colwell & Dinet, 1982) and there is often a proliferation of microbes in their hindguts (Deming & Colwell, 1982; Albéric, Féral & Sibuet, 1987), suggesting that sediment bacteria may play an important role in their nutrition, although the evidence supporting this view is weak (Roberts, Gebruk, Levin & Manship, 2000).

The means whereby major nutrients are assimilated by deposit feeders are not completely understood (Jumars, 1993). This question has been addressed in some deep-sea holothurians (Khripounoff & Sibuet, 1980; Sibuet et al., 1982), and these studies have suggested assimilation efficiencies of ~15% for total organic carbon (TOC) and ~22% for total nitrogen (TN) in a number of abyssal species. More recently,

Moore, Manship and Roberts (1995) reported significant declines in the organic carbon and nitrogen content of ingested sediments during their passage through the guts of *Oneirophanta mutabilis* and *Psychropotes longicauda*, but not in *Pseudostichopus villosus*. In the shallow-water species *Holothuria atra* and *Stichopus chloronotus*, assimilation efficiencies were higher, averaging 30% for TOC and 40% for TN (Moriarty, 1982). Similarly, Ginger et al. (2001) describe assimilation efficiencies for TOC ranging from 15 to 46% and for TN from 11 to 53% in various species of abyssal holothurians. However, estimation of organic matter assimilation by deep-sea holothurians is fraught with difficulties (Ginger et al., 2001).

Relationships between food quality, gut residence time and digestion reaction kinetics in deposit feeders (including holothurians), have been modelled extensively (Penry & Jumars 1986, 1987; Jumars, Mayer, Deming, Baross & Wheatcroft, 1990). Models based on chemical reactor theory include:

1. Batch reactors (BRs), in which food and enzymes (reagents) are mixed then allowed to react;
2. Plug flow reactors (PFRs), in which there is a continuous, orderly flow of reagents with no axial mixing along the tube (gut) and
3. Continuous flow stirred tank reactors (CSTRs), in which reagents undergo continuous thorough mixing.

At a simpler level, gut morphologies can be used to infer the digestive strategies of animals and can be substituted for differences in diet in analyses of resource partitioning (Penry, 1993).

Complex organic molecules must be hydrolysed before they can be absorbed and used metabolically (Boetius & Felbeck, 1995). The spectrum of hydrolytic gut enzymes is likely to have evolved to fit the normal diet of each species and so will reflect the types of organic materials that are utilised (Féral, 1989). Hydrolytic enzymes, including esterases, lipases, proteases, peptidases and saccharidases, have been investigated in the digestive tracts of both shallow-water holthurians (e.g. Féral, 1989; Manship, 1995; Mayer et al., 1997) and deep-sea holothurians (e.g. Sibuet et al., 1982; Massin, 1984; Manship, 1995). The early studies generally focused on single enzymes or small suites of enzymes. More recently the use of the apiZYM™ system (bioMerieux sa, Marey-l'Etoile, France) enables a semi-quantitative assay to be made of nineteen hydrolytic enzymes. Although this system was initially designed to assay bacterial enzyme profiles for clinical use (Gruner, von Graevenitz & Altwegg, 1992), it has facilitated the investigation of hydrolytic enzymes both in marine sediments (Poremba, 1994) and in invertebrate digestive systems (Boetius & Felbeck, 1995), and was used in this study.

This paper describes a comparative study of sediment, enzyme and bacterial profiles along the digestive tracts of *Oneirophanta mutabilis*, *Psychropotes longicauda* and *Pseudostichopus villosus* sampled at different times of year at a temperate, eutrophic site on the Porcupine Abgyssal Plain in the NE Atlantic (see Billett & Rice, 2001; Lampitt et al., 2001). The species were selected, because previous studies have shown they probably have different feeding strategies (Moore & Roberts, 1994). *Oneirophanta mutabilis* and *Psychropotes longicauda* are surface deposit feeders, whereas *Pseudostichopus villosus* probably feeds just below the sediment surface. In addition, *Psychropotes longicauda* and *Pseudostichopus villosus* may fill and empty their gut intermittently, whereas *Oneirophanta mutabilis* is a continuous, "conveyor-belt" feeder. The volumes of sediments in different parts of the gut were assessed to provide quantitative support for previous anatomical interpretations (Moore et al., 1995; Roberts et al., 1996). The apiZYM™ assays of gut tissues and contents were used to identify the sources of the hydrolytic enzymes (i.e. whether endogenous to the holothurian or derived from the sediment) and to identify any inter-specific differences in enzyme profiles which might suggest interspecific differences in diet. Bacterial profiles and activities in the gut contents were investigated to evaluate any possible bacterial contribution to holothurian nutrition.

2. Materials and methods

2.1. Samples

Samples of *Oneirophanta mutabilis*, *Psychropotes longicauda* and *Pseudostichopus villosus* were collected during RRS *Discovery* Cruises 222 (August–September 1996) and 226 (March–April 1997) at depths of ~4850 m from a eutrophic site (48°50′N, 16°30′W) on the Porcupine Abyssal Plain. For enzyme and bacterial assays, intact specimens were placed into pre-chilled seawater and dissected in a cold room within 2 h of collection. Specimens, used for investigations of the distribution of sediments along the guts, were preserved in 4% buffered formalin as soon as possible after collection.

2.2. Volumes of sediments in gut compartments

Up to 25 undamaged, preserved individuals of each species from both cruises were examined; subsamples were selected to include a representative range of the sizes present. Volumes of the contents of the pharynx, oesophagus, anterior intestine, posterior intestine, rectum and cloaca of each animal were estimated by simple displacement in water in measuring cylinders. To minimise variability resulting from the state of gut fullness (Moore & Roberts, 1994), only animals in which the gut was estimated to be >75% full were included. Statistical analyses were performed using SigmaStat, Version 1. (Jandel Scientific, Santa Clara, CA). Two-way ANOVA of ranked data revealed there was significant interaction between species and gut region, so the contents of the gut regions were compared individually by one-way ANOVA.

2.3. Enzyme assays

Samples of gut tissue and gut contents were collected from selected regions of the gut of each species. For comparison with gut contents, four replicate samples of the top centimetre of the ambient sediments were collected using a multiple corer (Barnett, Watson & Connelly, 1984). All samples were frozen and stored at −70°C.

Samples for enzyme analyses were extracted by sonication on ice, in 800 μL of cold autoclaved MilliQ-distilled water for three cycles of five seconds duration, using a 25w VibraCell sonicator (VibraCell, Danbury CT). Homogenates were centrifuged at 4°C in a microcentrifuge (5 min at 10,000*g*) and the supernatant stored in fresh tubes at −70°C prior to assay.

Semi-quantitative measures of nineteen enzymes were determined in the extracts using the apiZYM™ system (bioMerieux sa, Marey-l'Etoile, France). Samples collected during the August–September 1996 cruise were not replicated and were assayed without dilution. The sampling methodology was improved on the following cruise, in March–April 1997, by replication and protein scaling of the samples. For these samples the total protein in extracts from four individuals of each species was measured using the BCA (Bicinchoninic Acid) protein assay kit (BCA-1, Sigma, Chemical Co., St. Louis, USA) and used to scale extracts. Gut content homogenates containing <0.5mg protein ml^{-1} were not diluted, whereas those containing >0.5mg protein ml^{-1} were diluted to 0.5 mg ml^{-1}. Gut tissue homogenates were diluted to a protein concentration of 25mg protein ml^{-1}. Aliquots of 20 μl were pipetted into the 20 microtubes on the apiZYM™ strips. The incubation temperature recommended by the apiZYM™ assay protocol was 37°C, which was significantly higher than the near-bottom temperatures, so test assay strips were incubated at either 37°C, or at 4°C. Gut contents homogenates were incubated either for 4 h at 37°C, the recommended assay temperature (n=4), or for 12 h at 4°C, to simulate near-bottom temperatures and allow for the longer reaction times (n=1). Gut tissue homogenates were incubated for 2 h at 37°C (n=4) or 6 h at 4°C (n=1). Final colour intensities were scored visually on a scale from 0 to 5 corresponding to 0 to ≥40 nmol of hydrolysed substrate. The range of enzymes detected showed no differences with temperature although

activities at 37°C were higher. Consequently, all subsequent assay incubations were carried out at 37°C and for the shorter time periods. Statistical comparisons of enzyme assays involved two-way ANOVA on the untransformed data followed by pair-wise multiple comparison using Bonferroni's method (SigmaStat, Version 1).

2.4. Estimates of bacterial activity and abundance.

Bacterial activity was determined in terms of incorporation of thymidine into DNA using a modification of the method described by Moriarty (1990). Material from individual specimens was pooled as necessary to provide 5–10 ml samples of gut contents. These were placed in 50 ml centrifuge tubes, and made up to 51 ml with particle-free seawater and mixed to form a slurry. A 1 ml subsample was taken and preserved in 9 ml of 2% formaldehyde for total bacterial counts. Sufficient [methyl-3H] thymidine (NEN Life Science Products Boston, USA. Code NET-027Z. Specific activity approx. 3.5 TBq/mmol) was added to give a final concentration of 24 nM. The contents of the tubes were mixed well and 4 ml aliquots dispensed into sterile plastic Whirl-Pak sample bags (Nasco International Inc. USA). These were heat sealed and incubated at 2°C under in situ deep-sea pressure using water-filled pressure chambers. Bags were removed at intervals of up to 24 h and the incubations terminated by the addition of 8 ml of thymidine alcohol mixture (ethanol 80 ml: water 20 ml: unlabelled thymidine 10 mg). The contents of bags were transferred to 15 ml centrifuge tubes and stored at −20°C for further processing on land.

After centrifugation at 6000g for 10 min, the supernatant was discarded and 10 ml of 20% acetic acid were added to remove the carbonates. Once the bubbling had stopped, the sample was centrifuged and the sediment re-suspended in 2 ml of thymidine alcohol mixture. The sediment was filtered onto 47 mm 0.2 μm Isopore® membrane filters (Millipore, UK) and washed five times with 5% trichloroacetic acid. The filter was then placed in a 15 ml centrifuge tube, 3 ml of 0.5 M HCl were added and the tubes were heated at 95°C for 16 h. The tubes were cooled, centrifuged and finally 0.5 ml of the supernatant were transferred to a scintillation vial and 4.5 ml of scintillation cocktail (Optiphase HiSafe 3, Fisher Chemicals, Loughborough, UK) were added. The samples were counted with a liquid scintillation counter (Packard Tri-Carb™ Model 1600TR) running on a direct DPM channel to compensate for quench.

Bacteria in samples were counted according to the method of Parkes et al. (1993) except that Sybr-Gold (Molecular Probes Inc.USA) was used in place of acridine orange. An aliquot of 10 μl of preserved sample was added to 10 ml of filtered and sterilized 2% formaldehyde and vortexed vigourously for 1 min. An 8 ml subsample of the diluted sample was filtered onto black Isopore® membrane filters and 200 μl of Sybr-Gold (10X stock solution in TE buffer pH 8) was added to the remaining 2 ml and left for 5 min. The rest of the sample was then drawn onto the filter, which was mounted onto a glass slide and a minimum of mineral oil added under a coverslip. The samples were viewed using an Nikon Optiphot-2 UV microscope fitted with a 100W mercury bulb, a B-2A excitation filter for blue light, a ×100 planar objective lens and ×10 eyepieces. In most cases twenty microscope fields per filter were counted.

3. Results

3.1. Volumes of sediments in gut compartments

The proportions of sediment in the various gut compartments of *Oneirophanta mutabilis*, *Psychropotes longicauda* and *Pseudostichopus villosus* collected at different seasons are illustrated in Fig. 1. *Oneirophanta mutabilis* showed the least intersample variation for each gut compartment. Most of the ingested sediment material (>80%) was held in the anterior and posterior intestine (Fig. 1) and relatively small percentages were held in the pharynx and oesophagus (9%) and in the rectum/cloaca (8%). The intersample

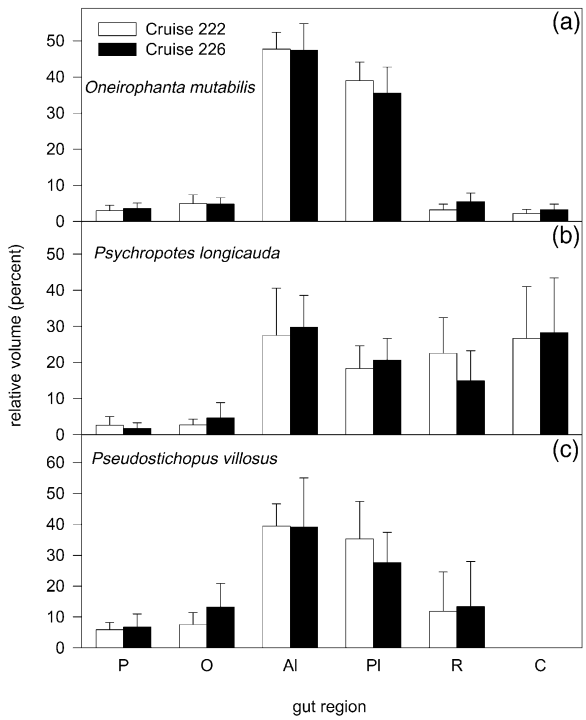


Fig. 1. Proportions of sediments (expressed as percentage of total gut volume) in different regions of the guts of: a) *Oneirophanta mutabilis*; b) *Psychropotes longicauda*; and c) *Pseudostichopus villosus* collected on RRS *Discovery* Cruises 222 (August–September 1996) [open columns] and 226 (March–April 1997) [solid columns]. AI, anterior intestine; C, cloaca; O, oesophagus; P, pharynx; PI, posterior intestine; R, rectum. For *Pseudostichopus villosus*, rectal and cloacal samples were difficult to separate and are grouped under R.

variation shown by *Pseudostichopus villosus* was slightly higher than *O. mutabilis*, and lower (<70%) proportions of the ingested sediments were held in the anterior and posterior intestine (Fig. 1) but higher proportions were held in the pharynx/oesophagus (10–15%) and rectum/cloaca (20%). In *Psychropotes longicauda*, ~10% of the total gut sediments were held in the pharynx/oesophagus, 20% in both the posterior intestine and rectum and 28% in both the anterior intestine and cloaca (Fig. 1). Two-way ANOVA of the proportions of sediment in different gut compartments showed there were no significant intra-specific differences between the different sampling seasons ($p>0.6$) and there were no significant interaction effects between cruise and species ($p>0.2$). Relative proportions of sediments in the pharynx, oesophagus, anterior and posterior intestine differed significantly for all the inter-specific comparisons ($p<0.0001$, Kruskal–Wallis one-way ANOVA on ranks; $p<0.05$ Dunn's pairwise muliple comparison). In addition, the relative proportions of sediments held in the rectum and cloaca showed significant differences between *Psychropotes longicauda* and *Oneirophanta mutabilis* ($p<0.0001$, Kruskal–Wallis one-way ANOVA on ranks; $p<0.05$ Dunn's pairwise muliple comparison). As data for rectum and cloaca samples for *Pseudostichopus villosus* were not separated, these could not be compared.

3.2. Enzyme assays

Enzyme levels in the topmost centimetre of the sediments collected at the holothurian sampling sites were all below the test's levels of detection.

Nineteen enzymes were identified either in gut-tissue or the gut-content samples of the holothurian species studied (Fig. 2 and Fig. 3). During each cruise enzyme concentrations in gut tissues and contents were positively correlated (Table 1), concentrations in the tissues were always higher than in the contents (Figs 2 and 3). The enzyme distributions for each species were broadly similar in the samples collected at different times of the year, but some differences are apparent (Figs 2 and 3). The samples from the cruise in August–September 1996, which were not diluted, had enzyme concentrations that sometimes appeared higher than those in samples from the cruise in March–April 1997.

The undiluted samples from the August–September 1996 cruise were not replicated, but the enzyme profiles in the gut tissues showed no consistent variation with gut region, but a number of enzymes showed higher levels in the gut content samples from the anterior and posterior intestines (Fig. 2A). This is best illustrated by the data for *Pseudostichopus villosus*, in which the concentrations of 14 enzymes were slightly higher in the anterior and posterior intestine than in the other gut regions (Fig. 2A). The protein-scaled samples from the March–April 1997 cruise were replicated, and the enzyme profiles in gut tissues were variable although a number of enzymes showed higher levels in tissue samples from the anterior and posterior intestine. For example, concentrations of four enzymes (1, 2, 3 and 7) were higher in the anterior and posterior intestine of *O. mutabilis* than in other gut regions of this species (Fig. 3).

Phosphoric and ester hydrolases (1–6) assayed were present in varying concentrations in gut tissue and gut sediment samples from both cruises (Figs 2 and 3) and, with the exception of acid phosphatase, showed significant differences between the contents of different gut regions (Table 2). Concentrations of alkaline phosphatase and phosphohydrolase also showed significant interspecific differences in samples from the cruise in March–April 1997 (Table 2).

The endopeptidases trypsin and chymotrypsin were either absent, or recorded in trace amounts, in material examined from both cruises (Figs 2 and 3). Exopeptidases (7–9) show similar profiles in *Oneirophanta mutabilis, Pseudostichopus villosus* and *Psychropotes longicauda* from both cruises and can be ranked in the following order of concentration: leucine aminopeptidase > valine aminopeptidase > cystine aminopeptidase. Valine aminopeptidase showed significant concentration differences between species and gut region, and leucine aminopeptidase showed significant concentration differences between gut region in both tissue and gut content samples from the cruise in March–April 1997 (Table 2).

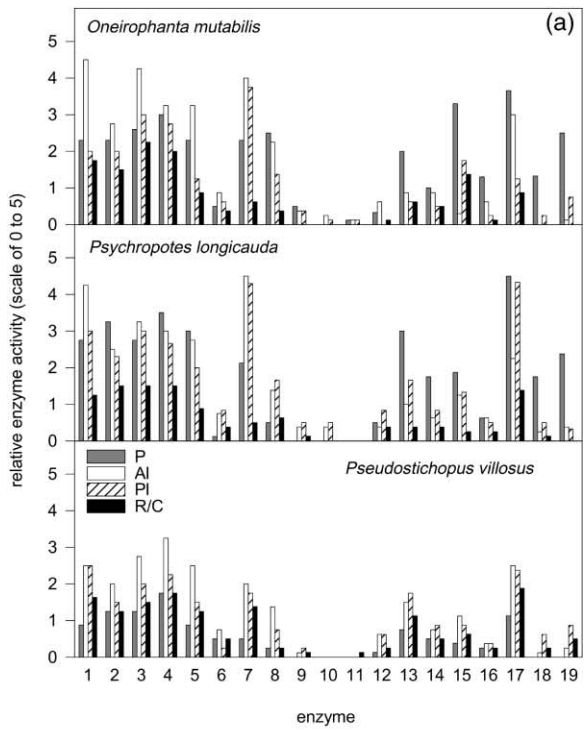
Of the major categories of enzymes assayed, glycosidases (12–19) showed greatest interspecific differences. All the glycosidases assayed were recorded in both gut and tissue samples sampled in August–September 1996; most occurred in higher concentrations in *Pseudostichopus villosus* and *Psychropotes longicauda* than in *O. mutabilis* (Fig. 2). Melibiase (α-galactosidase) and cellobiase (β-glucosidase), which were recorded at low concentrations in all samples collected in August–September 1996, they were either not detected or were at the limit of detection, in the samples collected during the later cruise (Figs 2 and 3).

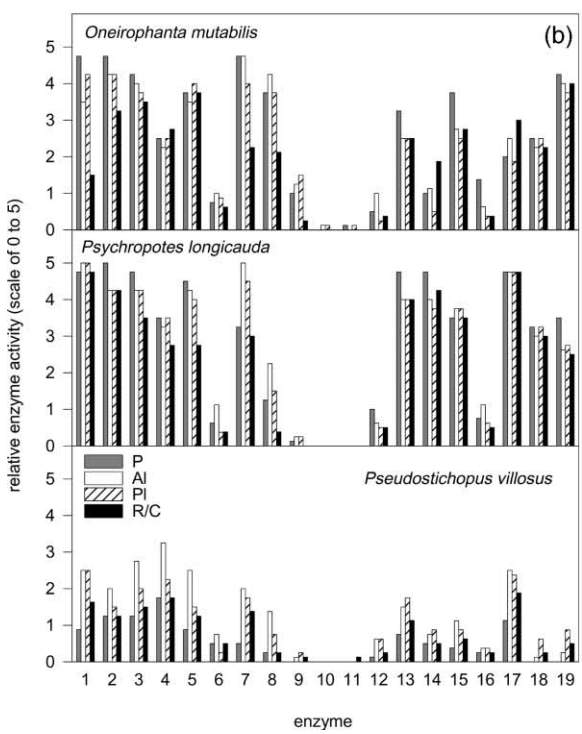
Concentrations of all other glycosidases assayed in gut contents showed significant differences between gut region and, with the exception of lactase, significant interspecific differences (Table 2). Gut tissue concentrations of glycosidases from the 1997 samples, showed there were no significant differences between gut region but there were significant interspecific differences between the majority of enzymes (Table 2).

Concentrations of chitobiase were higher in *Pseudostichopus villosus* and *Psychropotes longicauda* than in *O. mutabilis* in the samples from both cruises (Figs 2 and 3).

3.3. Bacterial activity and abundance

Although the data sets were incomplete for the two cruises, some trends were apparent. The data were most complete for *O. mutabilis*. In this species, bacterial activity profiles along the guts were similar in individuals collected during both cruises (August–September 1996 and March–April 1997) (Fig. 4a). In samples collected during both cruises bacterial activity declined moving from the pharynx/oesophagus to


(a)
Oneirophanta mutabilis
Psychropotes longicauda
Pseudostichopus villosus
P
AI
PI
R/C
relative enzyme activity (scale of 0 to 5)
enzyme
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19


(b)
Oneirophanta mutabilis
Psychropotes longicauda
Pseudostichopus villosus
P
AI
PI
R/C
relative enzyme activity (scale of 0 to 5)
enzyme
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

the anterior intestine. It then increased slightly again in the posterior intestine, but increased to its highest level in the rectum/cloaca. Bacterial abundance data showed a similar profile for the later cruise (Fig. 4b).

The data set for *Psychropotes longicauda* was more limited, and bacterial activity data were only available for specimens collected in August–September 1996 (Fig. 4c). In these samples, the bacterial activity increased from the anterior to the posterior intestine and declined slightly to the rectum/cloaca. Data on bacterial densities are only available for pharynx/oesophagus and rectum/cloaca, but were similar for both gut regions and both cruises (Fig. 4d).

In *Pseudostichopus villosus* bacterial activity in along-gut profiles were similar in individuals collected on both cruises (Fig. 4e). Bacterial activities were lower in the anterior and posterior intestine than in either the pharynx/oesophagus or the rectum/cloaca. During March–April 1997, bacterial activity was highest in the pharynx/oesophagus. Data on bacterial densities were only available for pharynx/oesophagus and rectum/cloaca samples; these did not differ between cruises, but were higher in the former (Fig. 4f).

4. Discussion

4.1. Volumes of sediments in gut compartments

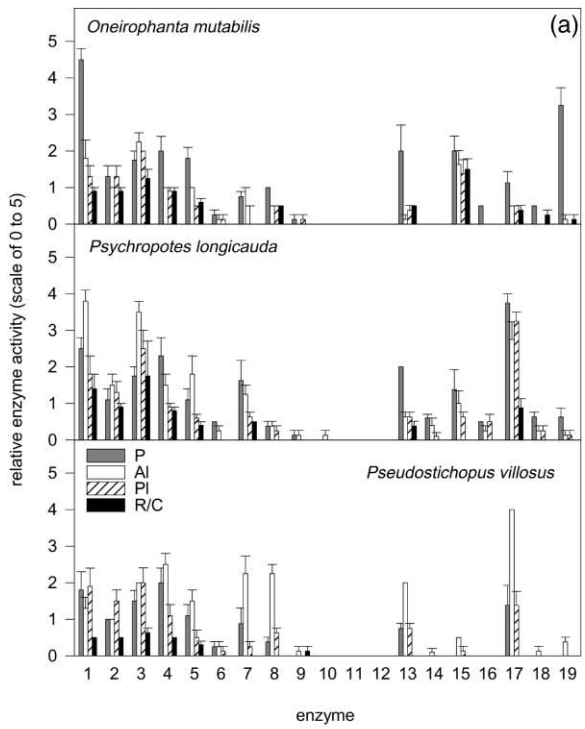
Differences reported above in the proportions of sediments in different gut regions of *Oneirophanta mutabilis*, *Psychropotes longicauda* and *Pseudostichopus villosus* support the previously reported differences in gut anatomy between these species (Moore et al., 1995). Intra-sample variations in volumes of sediments in different gut compartments were higher in *Psychropotes longicauda* and *Pseudostichopus villosus* than in *Oneirophanta mutabilis*. This, together with the relatively high intra-population variation in gut fullness in the first two species (Moore & Roberts, 1994), implies these species empty their guts periodically. There are significant interspecific differences in the proportion of sediments in the various gut compartments, but there were no intra-specific differences for different sampling periods. Thus we found no evidence for a seasonal feeding cycle in any of the species, as has been reported for shallow-water taxa such as *Parastichopus californicus* (Fankboner & Cameron, 1985).

4.2. Enzymes

The apiZYM™ test results need to be treated with some caution, because the conditions under which we ran the tests (such as pH, incubation time and temperature) may well have been sub-optimal. Also some of the enzymes may be highly substrate-specific and the test substrate may be inappropriate. Even so, they do provide valuable comparative data (see Féral, 1989; Poremba, 1994; Boetius & Felbeck, 1995). In addition, although the majority of enzymes assayed may serve a hydrolytic function in the holothurian gut, the function of some, like leucine aminopeptidase, may not primarily be digestive. The similarities in suites of hydrolytic enzymes detected in the guts of the different species, suggest there are similarities in the biochemical composition of the food resources they are exploiting, whereas the differences we observed in enzyme concentrations point to there being differences in the relative proportions of the dietary components in the food ingested.

In this study, most of the enzymes occurred in tissues from all parts of the gut. But, the evidence suggests

Fig. 2. Concentrations of enzymes found in the gut contents (A) and gut tissue (B) of abyssal holothurians sampled during RRS *Discovery* Cruise 222 (August–September 1996): Enzyme abbreviations are given in Table 2. Semi-quantitative measures of the enzymes were determined in the samples using the apiZYM™ system (bioMerieux sa, Marey-l'Etoile, France). Values of 0–5 correspond to 0 to ≥40 nmol nitrophenyl released per 20 μl homogenate. Gut regions: in the sequence: pharynx/oesophagus, anterior intestine, posterior intestine,and rectum/cloaca. Data are means of duplicated observations on individual samples.


Oneirophanta mutabilis
(a)
Psychropotes longicauda
P
AI
PI
R/C
Pseudostichopus villosus
relative enzyme activity (scale of 0 to 5)
0 1 2 3 4 5
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
enzyme

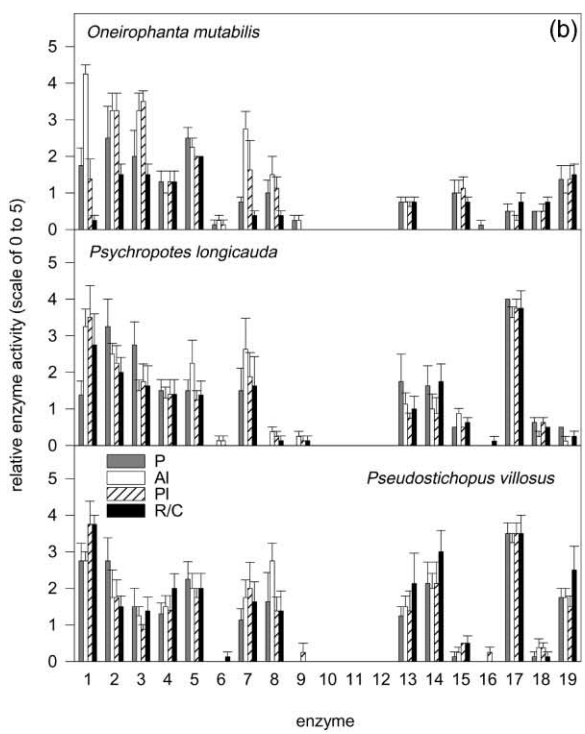

Oneirophanta mutabilis
(b)
Psychropotes longicauda
P
AI
PI
R/C
Pseudostichopus villosus
relative enzyme activity (scale of 0 to 5)
0 1 2 3 4 5
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
enzyme

Table 1
Pearson correlation values for comparisons of concentrations of enzymes in gut tissues and gut contents in abyssal holothurians from RRS *Discovery* Cruises 222 (August–September 1996) and 226 (March–April 1997) (all enzymes listed in Table 2; df =74)

	Cruise 222 (August–September 1996)		Cruise 226 (March–April 1997)	
	Pearson correlation	*P*	Pearson correlation	*P*
Oneirophanta mutabilis	0.6695	<0.0001	0.5799	<0.0001
Psychropotes longicauda	0.3157	0.0027	0.768	<0.0001
Pseudostichopus villosus	0.6127	<0.0001	0.5147	<0.0001

Table 2
Summary of two-way ANOVA on hydrolytic enzyme concentration in gut contents and gut tissues of *Oneirophanta mutabilis*, *Psychropotes longicauda* and *Pseudostichopus villosus* testing for differences among species and different regions of the gut (pharynx/oesophagus, anterior intestine, posterior intestine, and rectum/cloaca). Comparisons are for data from RRS *Discovery* Cruise 226 (March–April 1997) presented in Fig. 3. Abbreviations used for enzymes in Fig. 2 and Fig. 3 in parentheses[a]

Enzyme	Gut contents Source of variance			Gut tissue Source of variance		
	Species	Gut region	Interaction	Species	Gut region	Interaction
Phosphoric hydrolases						
alkaline phosphatase (1)	***	***	***	**	**	***
acid phosphatase (2)	ns	ns	ns	ns	ns	ns
phosphohydrolase (3)	**	***	ns	***	ns	*
Ester hydrolases						
esterase (4)	ns	***	*	ns	ns	ns
esterase lipase (5)	ns	***	ns	ns	ns	ns
lipase (6)	ns	**	ns	ns	ns	ns
Peptide hydrolases						
leucine aminopeptidase (7)	ns	***	**	ns	*	ns
valine aminopeptidase (8)	***	***	***	***	*	ns
cystine aminopeptidase (9)	ns	ns	ns	ns	ns	ns
trypsin (10)	ns	ns	ns	nd	nd	nd
chymotrypsin (11)	nd	nd	nd	nd	nd	nd
Glycosidases						
α-galactosidase [melibiase] (12)	nd	nd	nd	nd	nd	nd
β-galactosidase [lactase] (13)	ns	***	***	*	ns	ns
β-glucuronidase (14)	***	*	*	***	ns	ns
α-glucosidase [maltase] (15)	***	*	ns	***	ns	ns
β-glucosidase [cellobiase] (16)	***	***	***	ns	ns	ns
N-acetyl-β-glucosaminidase [chitobiase] (17)	***	***	***	***	ns	ns
α-mannosidase (18)	**	***	**	**	ns	ns
fucosidase (19)	***	***	***	***	ns	ns

[a] ***p <0.001; **p <0.01; *p <0.05; ns=no significant variation; nd= enzyme not detected.

Fig. 3. Concentrations (mean ± s.e.) of hydrolytic enzymes found in the gut contents (A) and gut tissue (B) of abyssal holothurians sampled during RRS *Discovery* Cruise 226 (March–April 1997): Enzyme abbreviations are given in Table 2. Semi-quantitative measures of the enzymes were determined in the samples using the apiZYM™ system (bioMerieux sa, Marey-l'Etoile, France). Values of 0–5 correspond to 0 to ≥40 nmol nitrophenyl released per 20 μl homogenate. Gut regions: in the sequence: pharynx/oesophagus, anterior intestine, posterior intestine,and rectum/cloaca.

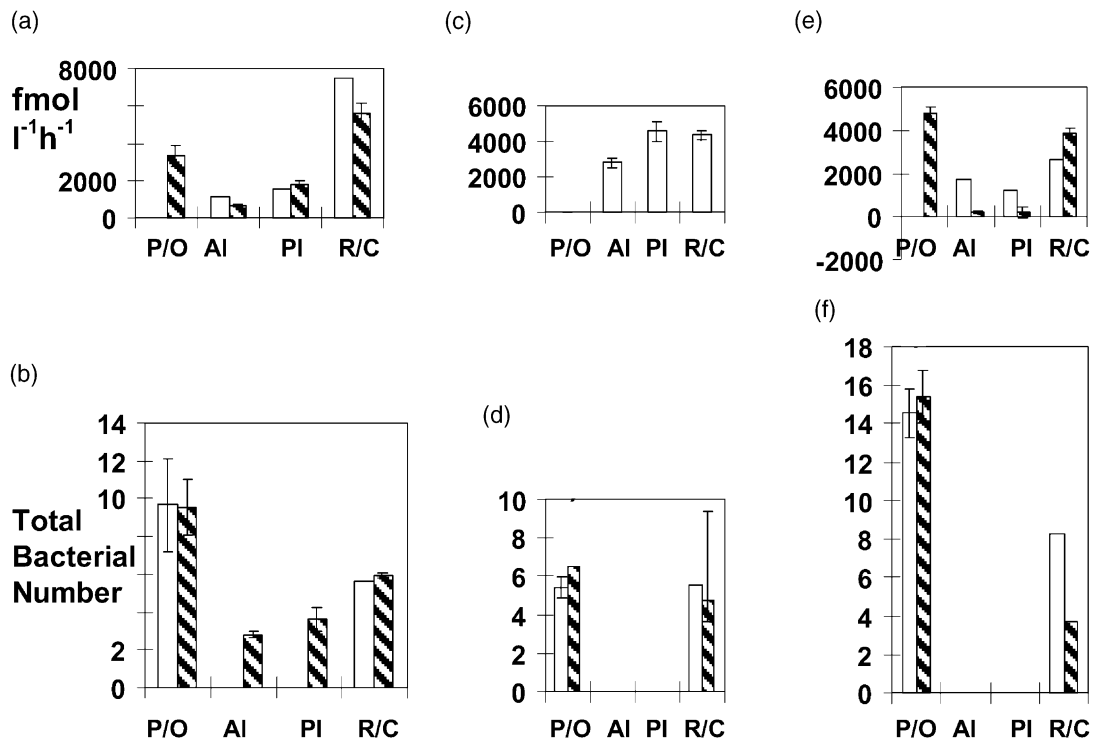


Fig. 4. Bacterial activity and abundance in the gut contents of holothurians collected on RRS *Discovery* Cruises 222 (August–September 1996) [open columns] and 226 (March–April 1997) [shaded columns]. a) *Oneirophanta mutabilis* activity; b) *Oneirophanta mutabilis* abundance; c) *Psychropotes longicauda* activity; d) *Psychropotes longicauda* abundance; e) *Pseudostichopus villosus* activity f) *Pseudostichopus villosus* abundance. Activity is expressed as fmol thymidine incorporated per hr per litre of gut content and abundance in units of 10^8 bacteria per ml of gut content. P/O, pharynx/oesophagus; AI, anterior intestine; PI, posterior intestine; R/C, rectum/cloaca. Abundances are the mean of counts carried out on duplicate specimens, with error bars indicating the maximum and minimum abundances recorded.

enzyme activity tends to be higher in the contents of the anterior intestine, thought to be the most active region for enzyme production (Féral & Massin, 1982), and in the posterior intestine of the three abyssal species we studied. The activities of phosphatases, exopeptidases and carbohydrases were generally similar to those of shallow-water deposit-feeding holothurians (Féral, 1989; Manship, 1995).

Activity of phosphatases, which hydrolyse orthophosphate monoesters and contribute to intracellular digestion, autophagy and the cycling of phosphorus (Boetius & Felbeck, 1995), was generally high in the abyssal species we examined. However, phosphatases are widely distributed, and serve many functions that are not strictly digestive. So their presence may also be the result of lysosomal rupture during the preparation of the gut tissue (Féral, 1989).

Ester hydrolases, which hydrolyse short-chain fatty acids (C4–C8), show strong activities throughout the guts of both shallow-water (Fish, 1967; Féral, 1989) and abyssal holothurians (present study). The presence of true lipases in holothurians was questioned in earlier studies (Fish, 1967; Ferguson, 1969), but more recent studies (Sibuet et al., 1982) point to efficient assimilation of lipids and relatively high lipase activities in shallow-water (Mayer et al., 1997) and abyssal taxa (present study).

Proteolytic activity is generally low in echinoderms (Lawrence, 1982; Féral, 1989). But we detected relatively high exopeptidase activity in the abyssal holothurians studied. These enzymes hydrolyse terminal peptide bonds and are important in the final stages of protein digestion. Trypsin and chymotrypsin, which

hydrolyse peptide bonds within peptide chains (endopeptidases) are also important in the initial stages of protein digestion, these were either absent or at very low levels in the holothurians suggesting that complex proteins may not figure significantly in their diets. However, secretion of both trypsin and chymotrypsin may require induction by presence of the substrate, as has been suggested for trypsin in marine copepods (Mayzaud, Roche-Mayzaud & Razouls, 1992).

Glycosidases hydrolyse glycosidic bonds to produce monosaccharides, and these will enable the holothurians to utilise disaccharides, starch and also to the hydrolysis products of cellulose and chitin (Féral, 1989). We found that the abyssal holothurians had relatively high carbohydrase activities as predicted for detritus feeders by Hylleberg Kristensen (1972). However, cellulase has not been detected in the gut tissue of shallow-water or abyssal holothurians (Lawrence, 1982; Manship, 1995). Similarly, assays by Moore and Roberts (personal communication) have failed to reveal the presence of chitinase, which hydrolyses chitin, in abyssal holothurians. Chitobiose, the oligosaccharide produced by the hydrolysis of chitin, is split by chitobiase (β-*N*-acetylglucosaminidase) (Hylleberg Kristensen, 1972). We found activity of this enzyme was higher in the digestive tracts of a *Psychropotes longicauda* and *Pseudostichopus villosus* than *Oneirophanta mutabilis*. As well as indicating the potential to digest hydrolysis products of chitin, high activities of chitobiase may also point to the digestion of bacterial membranes (Boetius & Felbeck, 1995).

The detection of low levels of melibiase (α-galactosidase) in samples of *Oneirophanta mutabilis, Psychropotes longicauda* and *Pseudostichopus villosus* collected in August/September 1996 is surprising because melibiose is primarily found in higher plants (Féral, 1989) and is unlikely to be a natural substrate in the abyssal environment. We detected cellobiase (β-glucosidase) in these three abyssal species, but only in low levels. This is an enzyme that has been reported in shallow-water holothurians (Clifford, Walsh, Reidy & Johnson, 1982; Féral, 1989), and may be associated with digestion of certain diatoms, marine fungi and bacteria (Hylleberg, 1976).

Enzyme activity in freshly assayed gut contents may either be secreted within the gut or be derived from other sources such as extracellular enzymes that have been ingested along with the sediments. Intestinal micro-organisms appear to be an important source of proteases in shallow-water holothurians (Clifford et al., 1982). However, all enzymes we identified were present in both gut tissues and gut contents, where their estimated concentrations were highly correlated. They were also higher in the gut tissues, which suggests that the tissues are the main source of the enzymes. Microbial enzymatic activity is well documented in deep-sea sediments (Boetius & Lochte, 1994; Poremba, 1995), but these enzymes were below the levels of detection of the apiZYM™ test we used in this study; an indication of the limitation of applying this test to deep-sea sediments with low enzyme activities, as pointed out by Poremba (1994).

4.3. Bacterial activity and abundance

Bacterial densities in the pharynx/oesophagus contents of *Oneirophanta mutabilis*, *Psychropotes longicauda* and *Pseudostichopu villosus* are significantly higher than those in surrounding sediments (Moore et al., 1995). This is considered to indicate that the animals are feeding either on the enriched superficial sedimentary layer or are selecting the most organic-rich components of the sediments (Akhmet'yeva, Smirnov & Bordovskiy, 1982; Sibuet et al., 1982).

In both *Oneirophanta mutabilis*, and *Pseudostichopus villosus* we found that bacterial activity declined when moving from the pharynx/oesophagus to the anterior and posterior intestine, but then increased again in the rectum/cloaca. In *Oneirophanta mutabilis* this pattern was repeated in bacterial abundances. Moore et al. (1995) also observed in the same species that bacterial densities decreased between the pharynx/oesophagus and the anterior intestine, but then found they increased in the posterior intestine and rectum/cloaca.

Our data set for *Psychropotes longicauda* was incomplete, but Moore et al. (1995) reported a decrease in bacterial densities between the pharynx/oesophagus and the anterior intestine but then an increase in

the posterior intestine and rectum/cloaca of *Psychropotes longicauda*. The profile for bacterial activities observed in August–September 1996 shows a similar trend, so it seems possible that the expanded rectum region of *Psychropotes longicauda* functions as a fermentation chamber, as was suggested by Penry and Jumars (1990) for the polychaete *Travisia foetida*. Fresh dissections of the rectal contents of *P. longicauda* reveal high bacterial abundances and a characteristic odour. However, more direct evidence is needed, such as changes in the microbial environment along the gut. Plante and Jumars (1992) used microelectrodes to monitor changes in the microbial environment along the guts of a number of deposit feeders, including holothurians, with different gut anatomies. They found oxygen concentrations, Eh and pH did not change along the guts of the bathyal holothurians *Pannychia* sp. and *Scotoplanes globosa*, which have tubular guts and probably function like "plug-flow reactors". In contrast, in the shallow-water species, *Molpadia intermedia*, which has an expanded rectum, Eh decreased between the fore- and mid-gut, but increased again in the hindgut. These differences in Eh were statistically significant and may either cause or reflect changes in the bacterial community along the gut (Plante & Jumars, 1992).

4.4. Conclusions

Differences in the proportions of sediments in different parts of the guts of *Oneirophanta mutabilis Psychropotes longicauda* and *Pseudostichopus villosus* reflect differences in gut anatomy which, according to Moore et al. (1995), suggest that these species have different digestive strategies. In *Oneirophanta mutabilis* the gut is a simple tube that may function as a Plug-Flow Reactor (PFR) processing organic-rich phytodetritus. *Psychropotes longicauda* and *Pseudostichopus villosus* have expanded oesophageal regions and, in the case of *Psychropotes longicauda*, an expanded rectum. Expanded regions of the gut may act as Continuous-flow Stirred Tank Reactors (CSTRs) that facilitate either contact of food particles with digestive enzymes (gut-content mixing) in fine sediments with low permeability or fermentation of the contents (Penry & Jumars, 1987). Gut-content mixing is probably important in deposit feeders because most are thought to utilise relatively labile organic material and to have relatively short gut-residence times (Jumars, 1993). However, the possibility of fermentation in deposit-feeder CSTRs (Penry & Jumars, 1987) remains.

Differences reported above in bacterial activity and densities in different parts of the guts of abyssal holothurians point to changes in the microbial environment along their guts. Plante and Jumars (1992) suggested that such changes could favour different populations of microbes and provide the potential for microbial breakdown of a broader range of substrates than would be possible otherwise. However, in the present study we found no evidence of sedimentary (microbial) sources of hydrolytic enzymes. Although T-RFLP analysis suggested some inter-seasonal differences in gut Eubacteria, microbial community structure in the guts of all three holothurian species was similar to that of the sediment community (Patching & Carton, personal communication). Thus digestive strategies in abyssal holothurian may simply involve exploiting sedimentary microbial processes by concentrating organic material and its microbial associates. In addition, evidence that *Psychropotes longicauda* and *Pseudostichopus villosus* have higher levels of chitobiase than *O. mutabilis,* supports the idea that these species may be utilising different components of the sediment.

Acknowledgements

This work is part of the BENGAL project (Contract No: MAS3-CT950018) funded under the EU MAST Programme.

References

Akhmet'yeva, E. A., Smirnov, B. A., & Bordovskiy, O. K. (1982). On some characteristic features of organic matter composition in the intestine contents of the bottom detritus-feeders. *Oceanology*, *22*, 755–757.

Albéric, P., Féral, J. P., & Sibuet, M. (1987). Les acides aminés libres, reflet de l'activité bactériene dans les contenuus digestifs des holothuries: différence entre zones abyssale et littorale. *Compte Rendus Hebdomadaires des Séances de l'Académie des Sciences, Paris*, *305* (3), 203–206.

Barnett, P. R. O., Watson, J., & Connelly, D. (1984). A multiple corer for taking virtually undisturbed samples from, shelf, bathyal and abyssal sediments. *Oceanologica Acta*, *7*, 399–408.

Billett, D. S. M., & Rice, A. L. (2001). The BENGAL programme: introduction and overview. *Progress in Oceanography*, *50*, 13–25.

Billett, D. S. M., Lampitt, R. S., Rice, A. L., & Mantoura, R. F. C. (1983). Seasonal sedimentation of phytoplankton to the deep-sea benthos. *Nature, London*, *302*, 520–522.

Boetius, A., & Lochte, K. (1994). Regulation of microbial enzymatic degradation of organic matter in deep-sea sediments. *Marine Ecology Progress Series*, *104*, 299–307.

Boetius, A., & Felbeck, H. (1995). Digestive enzymes in marine invertebrates from hydrothermal vents and other reducing environments. *Marine Biology*, *122*, 105–113.

Clifford, C., Walsh, J., Reidy, N., & Johnson, D. B. (1982). Digestive enzymes and sub-cellular localisation of disaccharidases in some echinoderms. *Comparative Physiology and Biochemistry*, *71*, 105–110.

Deming, J. W., & Colwell, R. S. (1982). Barophilic bacteria associated with the digestive tract of abyssal holothurians. *Applied and Environmental Microbiology*, *44*, 1222–1230.

Fankboner, P. V., & Cameron, J. L. (1985). seasonal atrophy of the visceral organs in a sea cucumber. *Canadian Journal of Zoology*, *63*, 2888–2892.

Féral, J.-P. (1989). Activity of the principal digestive enzymes in the detrivorous apodous holothuroid *Leptosynapta galliennei* and two other shallow water holothuroids. *Marine Biology*, *101*, 367–379.

Féral, J.-P., & Massin, C. (1982). Digestive systems: Holothuroidea. In M. Jangoux, & J. M. Lawrence, *Echinoderm nutrition* (pp. 191–212). Rotterdam: Balkema.

Ferguson, J. C. (1969). Feeding, digestion and nutrition in Echinodermata. In M. Flokin, & B. T. Scheers, *Chemical ecology* (Vol. 3) (pp. 71–100). New York: Academic Press.

Fish, J. D. (1967). The digestive system of the holothurian *Cucumaria elongata* II. *Distribution of the digestive enzymes. Biological Bulletin*, *132*, 345–361.

Ginger, M. L., Billett, D. S. M., Mackenzie, K. L., Kiriakoulakis, K., Neto, R. R., Boardman, D. K., Santos, V. L. C. S., Horsfall, H. M., & Wolff, G. A. (2001). Organic matter assimilation by holothurians in the deep sea: some observations and comments. *Progress in Oceanography*, *50*, 407–421.

Gruner, E., von Graevenitz, A., & Altwegg, M. (1992). The API ZYM system: a tabulated review from 1977 to date. *Journal of Microbiological Methods*, *16*, 101–118.

Hylleberg, J. (1976). Resource partitioning on the basis of hydrolytic enzymes in deposit-feeding mud snails. *Oecologia (Berlin)*, *23*, 115–125.

Hylleberg Kristensen, J. (1972). Carbohydrases of some marine invertebrates with notes on their food and on the natural occurrence of the carbohydrates studied. *Marine Biology*, *14*, 130–142.

Jumars, P. A. (1993). Gourmands of mud. In R. N. Hughes, *Diet selection: an interdisciplinary approach to foraging behaviour* (pp. 124–156). Oxford: Blackwell Scientific Publications.

Jumars, P. A., Mayer, L. M., Deming, J. W., Baross, J. A., & Wheatcroft, R. A. (1990). Deep-sea deposit feeding strategies suggested by environmental and feeding constraints. *Philosophical Transactions, Royal Society of London A*, *331*, 85–101.

Khripounoff, A., & Sibuet, M. (1980). La nutrition d'échinodermes abyssaux. *I. Alimentation des holothuries. Marine Biology*, *60*, 17–26.

Lampitt, R., Bett, B. J., Kiriakoulakis, K., Popova, E. E., Ragueneau, O., Vangriesheim, A., & Wolff, G. A. (2001). Material supply to the abyssal seafloor in the Northeast Atlantic. *Progress in Oceanography*, *50*, 27–63.

Lawrence, J. M. (1982). Digestion: post-metamorphic and larval echinoderms. In M. Jangoux, & J. M. Lawrence, *Echinoderm nutrition* (pp. 283–316). Rotterdam: Balkema.

Manship, B.A.D. (1995). *The feeding ecology of deposit-feeding holothurians*. PhD thesis, Queen's University, Belfast.

Massin, C. (1984). Structures digestives d'holothuries Elasipoda (Echinodermata): *Benthogone rosea* Koehler, 1896 et *Oneirophanta mutabilis* Theel, 1879. *Archives de Biologie (Bruxelles)*, *95*, 153–185.

Mayer, L. M., Schick, L. L., Self, R. F. L., Jumars, P. A., Findlay, R. H., Chen, Z., & Sampson, S. (1997). Digestive environments of benthic marine invertebrate guts: enzymes, surfactants and dissolved organic matter. *Journal of Marine Research*, *55*, 785–812.

Mayzaud, P., Roche-Mayzaud, O., & Razouls, S. (1992). Medium term time acclimation of feeding and digestive enzyme activity in marine copepods: influence of food concentration and copepod species. *Marine Ecology Progress Series*, *89*, 197–212.

Moore, H. M., & Roberts, D. (1994). Feeding strategies in abyssal holothurians. In B. David, A. Guille, J.-P. Féral, & M. Roux, *Echinoderms through time* (pp. 531–537). Rotterdam: Balkema.

Moore, H. M., Manship, B. A. D., & Roberts, D. (1995). Gut structure and digestive strategies in three species of abyssal holothurians. In R. H. Emson, A. B. Smith, & A. Campbell, *Echinoderm research. Proceedings of the fourth European echinoderms colloquium, London, 10–13 April 1995* (pp. 111–119). Rotterdam: Balkema.

Moriarty, D. J. W. (1982). Feeding of *Holothuria atra* and *Stichopus chloronotus* on bacteria, organic carbon and organic nitrogen in sediments of the Great Barrier Reef. *Australian Journal of Marine and Freshwater Research, 33*, 255–263.

Moriarty, D. J. W. (1990). Techniques for estimating bacterial growth rates and production of biomass in aquatic environments. *Methods in Microbiology, 22*, 211–234.

Parkes, R. J., Cragg, B., Getliff, J. M., Harvey, S. M., Fry, J. C., Lewis, C. A., & Rowland, S. J. (1993). A quantitative study of microbial decomposition of biopolymers in recent sediments from the Peru margin. *Marine Geology, 113*, 55–66.

Patching, J. W., & Eardly, D. (1997). Bacterial biomass and activity in the deep waters of the eastern Atlantic — evidence of a barophilic community. *Deep-Sea Research I, 44*, 1655–1670.

Penry, D. L. (1993). Digestive constraints on diet selection. In R. N. Hughes, *Diet selection: an interdisciplinary approach to foraging behaviour* (pp. 32–55). Oxford: Blackwell Scientific Publications.

Penry, D. L., & Jumars, P. A. (1986). Chemical reactor analysis and optimal digestion. *Bioscience, 36*, 310–315.

Penry, D. L., & Jumars, P. A. (1987). Modelling animals guts as chemical reactors. *American Naturalist, 129*, 69–96.

Penry, D. L., & Jumars, P. A. (1990). Gut architecture, digestive constraints and feeding ecology of deposit-feeding and carnivorous polychaetes. *Oecologia (Berlin), 82*, 1–11.

Plante, C. J., & Jumars, P. A. (1992). The microbial environment of marine deposit feeders guts characterised via microelectrodes. *Microbial Ecology, 23*, 257–277.

Poremba, K. (1994). Measurements of enzymatic activity in deep marine sediments using the semiquantitative apizym test system. *Acta Hydrochimica et Hydrobiologica, 22*, 166–170.

Poremba, K. (1995). Hydrolytic enzymatic activity in deep-sea sediments. *Federation of European Microbiological Societies, Microbiology Ecology, 16*, 213–222.

Ralijaona, C., & Bianchi, A. (1982). Comparaison de la structure et des potentialitiés métaboliques des communautes bactériennes du contenu du tractus digestif D'holothuries Abyssales et du sédiment environment. *Bulletin de la Centre Etudes et recherches scientifiques de Biarritz, 14*, 199–214.

Roberts, D., Moore, H. M., Manship, B. A. D., Wolff, G. A., Santos, V., Horsfall, I., Patching, J., & Eardly, D. (1996). Feeding strategies and impact of holothurians in the deep sea. In B. F. Keegan, & R. O'Connor, *Irish marine science 1995* (pp. 237–252). Galway: Galway University Press.

Roberts, D., Gebruk, A., Levin, V., & Manship, B. A. D. (2000). Feeding and digestive strategies in deposit-feeding holothurians. *Oceanography and Marine Biology: an Annual Review, 38*, 257–310.

Sibuet, M., Khripounoff, A., Deming, J., Colwell, R., & Dinet, A. (1982). Modification of the gut contents in the digestive tract of abyssal holothurians. In J. M. Lawrence, *Echinoderms: proceedings of the international conference, Tampa Bay* (pp. 421–428). Rotterdam: Balkema.

Thurston, M. H., Rice, A. L., & Bett, B. J. (1998). Latitudinal variation in invertebrate megafaunal abundance and biomass in the North Atlantic ocean abyss. *Deep-Sea Research II, 45*, 203–224.