

## **Digestive environments of benthic macroinvertebrate guts: Enzymes, surfactants and dissolved organic matter**

by Lawrence M. Mayer<sup>1</sup>, Linda L. Schick<sup>1</sup>, Robert F. L. Self<sup>2</sup>, Peter A. Jumars<sup>2</sup>,  
Robert H. Findlay<sup>3</sup>, Zhen Chen<sup>1</sup> and Stephen Sampson<sup>1</sup>

### ABSTRACT

Hydrolytic enzyme activity, surfactancy, and dissolved organic matter in the digestive lumens of 19 benthic echinoderm and polychaete species were examined, using consistent and quantifiable methods. Enzyme activities were compared with those of extracellular enzymes from ambient sediments. Enzyme activities ranged over five orders of magnitude, with averages decreasing in the order polychaetes > echinoderms > sediment. Highest activities in animals were usually associated with the fluid phase in midgut sections, with posteriorward decreases indicating little export to the external environment. At some phyletic levels, activity correlated inversely with animal size. Hydrolase patterns reflected food type; for example, high lipase:protease ratios in carnivores reflected esterified lipids in their diets. High surfactant activity was found in gut sections having high enzyme activity. Deposit feeders had the most intense surfactancy, including evidence for micelles. While enzymes reflected the biochemical nature of the digestible food substrate regardless of feeding mode (e.g., deposit vs. suspension feeder), surfactants reflected dilution of this digestible substrate with mineral grains. Dissolved organic matter levels were high, with amino acids reaching levels > 1M and lipids commonly 1 g L<sup>-1</sup>. Among polychaete deposit-feeders, low molecular weight amino acids reflected the composition of the food substrate, but were present at much higher concentrations than could be explained by sediment present in the gut—suggesting longer residence times for fluid than for transiting sediment particles. Deposit feeder digestive fluids are better able to solubilize sedimentary food substrates than are sedimentary extracellular enzymes, owing to either more powerful solubilizing agents or to their deployment in freely diffusing, dissolved form. Gut environments may lead to chemical condensation as well as solubilization reactions.

### 1. Introduction

Animal guts represent a reaction zone in which animals must quickly solubilize food into an absorbable form and extract it from indigestible residue. This rapid solubilization results from biogeochemical conditions quite different than those encountered in sediments, providing a temporary, albeit anomalous, environment for transiting sedimentary particles. These particles can range from the mineral-dominated sediment, ingested by

1. Darling Marine Center, University of Maine, Walpole, Maine, 04573, U.S.A.

2. School of Oceanography, University of Washington, Seattle, Washington, 98195, U.S.A.

3. Department of Microbiology, Miami University, Oxford, Ohio, 45046, U.S.A.

deposit-feeders, to isolated particles of organic matter, ingested by carnivores, herbivores, and highly selective detritivores.

The study of organic matter cycling in sediments has benefited from study of digestive agents such as sedimentary extracellular enzymes (e.g., Mayer and Rice, 1992). Because sedimentary particles frequently transit through animal guts (Aller, 1982; Thayer, 1983), particle exposure to their digestive capabilities also needs examination. This paper examines digestive systems of benthic macrofauna as environments to which sedimentary particles are exposed, considering surfactancy, enzyme activity and dissolved organic matter. We address organic matter absorption and dissolved trace metals in companion papers.

Animals are presumed to solubilize food primarily via enzymatic cleavage of polymers to soluble forms that are diffusible to the gut epithelium, where they can be either hydrolyzed further or absorbed. Enzymes in benthic invertebrates have been studied for over a century (reviews in Jeuniaux, 1969; Vonk and Western, 1984). However, from a perspective of the environment experienced by transiting particles this literature is limited by methodology. The vast preponderance of work has examined homogenates of gut tissues (e.g., Vonk and Western, 1984; Féral, 1989; references therein), an approach which releases enzymes from the enzyme-rich secretory cells lining the gut wall. While this approach provides relatively large amounts of enzymes, useful when analytical sensitivity is lacking, it does not reproduce the conditions experienced by particles passing through the animal's lumen. Very little work has (1) examined enzymes contained within the gut lumen (exceptions include DeVillez and Reid, 1971; Kermack, 1955) or (2) quantified enzyme activity, especially in a cross-phyletic or environmental framework. Our work examines rates of bond hydrolysis by luminal fluids across gut sections and species, and compares them with sedimentary hydrolytic capability.

In our early work on enzyme systems, we noticed intense surfactancy in the luminal fluids of some invertebrates. Surfactancy was detected in invertebrates early in this century (cf., Vonk, 1962 and Vonk and Western, 1984), but no broad survey has been conducted to check for its extent. We survey surface tension-lowering compounds, using contact angle as a reconnaissance tool. We also examine micellization of these surfactants, following the lead of Tugwell and Branch's (1992) study of various herbivores.

Luminal fluids are often intensely colored, indicating high dissolved levels of chromophoric materials. We have analyzed amino acids and lipids as indicators of dissolved organic matter and important classes of nutritional compounds.

We focus on animals that have relatively simple, tubular guts, with primarily extracellular digestion. The bulk of sediments ingested by deposit feeders follows this extracellular digestive route, and so it is reasonable to examine them first for geochemical significance. Tubular guts are relatively easy to interpret in terms of particle transit (*sensu* reactor models of Penry and Jumars, 1986, 1987). Extracellular digestion is more easily quantitated, for the same reason and because digestive fluids are more available than from

Table 1. Species investigated in this study, along with location (ME = coastal Maine and WA = Puget Sound, Washington), water depth sampled (I = intertidal), taxon (P = polychaete, H = holothuroid, E = echinoid), and feeding mode (SS = subsurface, SFC = surface, DPF = deposit feeder, FF = suspension feeder, C = carnivore, O = omnivore).

Species (location)	Depth (m)	Taxon	Feeding mode
<i>Abarenicola vagabunda</i> (WA)	I	P	SS-DPF
<i>Arenicola marina</i> (ME)	I	P	SS-DPF
<i>Amphitrite johnsoni</i> (ME)	I	P	SFC-DPF
<i>Brisaster latifrons</i> (WA)	200	E	SFC-DPF
<i>Brisopsis lyra</i> (ME)	100	E	SFC-DPF
<i>Cucumaria miniata</i> (WA)	I	H	FF
<i>Cucumaria frondosa</i> (ME)	10–20	H	FF
<i>Eupolymnia heterobranchia</i> (WA)	I	P	SFC-DPF
<i>Glycera dibranchiata</i> (ME)	I	P	C
<i>Leptosynapta clarki</i> (WA)	I	H	SFC-DPF
<i>Molpadia intermedia</i> (WA)	200	H	SS-DPF
<i>Nereis virens</i> (ME)	I	P	O
<i>Nephtys caeca</i> (WA)	I	P	C
<i>Nephtys caecoides</i> (WA)	I	P	C
<i>Parastichopus californicus</i> (WA)	10	H	SFC-DPF
<i>Schizobranchia insignis</i> (WA)	I	P	FF
<i>Strongylocentrotus droebachiensis</i> (ME)		E	H
<i>Thelepus crispus</i> (WA)	I	P	SFC-DPF
<i>Travisia foetida</i> (WA)	200	P	SS-DPF

intracellular digestive vesicles. Accordingly, we present data for polychaetes and echinoderms, leaving the more complex case of intracellular digestion for later analysis.

## 2. Methods and materials

*a. Animals.* Individuals of 19 benthic species were collected from Puget Sound (Washington) and the coast of Maine, in both subtidal and intertidal environments (Table 1). Animals were collected with care to avoid injury and either dissected immediately or kept alive in mud from their habitat in flowing seawater tables until dissection. Most animals were dissected within days of collection, with the exception of *Brisaster latifrons* and *Molpadia intermedia*, which were maintained for ca. 6 mo in a flowing seawater tank in sediment from their native habitat.

We usually examined ten replicate individuals of each species. Dissection and removal of digestive fluids was performed by opening the body wall and extracting fluid by one of two methods. For animals from Washington we inserted a hypodermic syringe into an intact gut and slowly extracted the fluid, centrifuging to clarify when necessary. For Maine animals we opened the gut wall, with care to restrict opening cells that might leak enzymes, spooned out the sediment slurry into a centrifuge tube, and weighed. No systematic

differences in enzyme activity were found between these two approaches. Phosphate buffer (1 mL, pH 8) was added and the slurry centrifuged to separate the fluid and sediment, followed by drying the sediment to obtain dry weight. Digestive fluids were frozen immediately in an ultracold freezer ( $-80^{\circ}\text{C}$ ) and kept frozen until analysis. Experiments showed that negligible enzyme activity was lost by this storage, but that  $-20^{\circ}\text{C}$  freezing can result in loss of significant activity.

*b. Enzymes.* Enzyme activity was measured using substrate monomers attached to fluorophores via the appropriate linkage. For glucosidase we used glucose attached by  $\beta$ -glucosidase bonds to methylumbelliferone (MUF). For esterase and lipase activities we used butyrate and palmitate, respectively, esterified to MUF. For protease we used alanine attached via peptide bond to methylcoumarinyl amide (MCA). These MCA-MUF substrates have the advantages of great sensitivity and wide dynamic range. Methods were similar to those reported previously (Mayer, 1989), except that digestive fluids rather than sediment slurries were the norm. Digestive fluid was diluted with 15 mL of pH 8 phosphate buffer, and 1 mL of this solution was placed in a fluorescence cuvette; 0.1 mL of enzyme substrate (100  $\mu\text{M}$ ) was added, vortexed, and the ensuing hydrolysis reaction was monitored by measuring the fluorescence ( $\lambda_{\text{ex}} = 355 \text{ nm}$ ,  $\lambda_{\text{em}} = 440 \text{ nm}$ ) of the free MCA or MUF as it was cleaved from the conjugate. Slopes of the plots of fluorophore release over time were converted to molar hydrolysis rates after correcting for fluorescence quenching by measuring the fluorescence of 1  $\mu\text{M}$  solutions of unbound MCA or MUF in the same diluted solutions.

Digestive fluids were diluted to varying extents into the pH 8 buffer, depending on the amount and activity of available digestive fluid. We found some effect of dilution in separate experiments; the largest impact was a 40% increase in protease activity upon 10-fold dilution. Variable dilution thus increases uncertainty, but likely less than 50%, a minor amount relative to the range of values we obtained.

Our choice of pH 8 provides a reasonable estimate of *in vivo* activity, because larger deposit feeder guts typically have pH of 7–8 (Féral, 1989; Plante and Jumars, 1992). Our sensitivity to the presence of glucosidases is reduced at this pH (these enzymes have optima and hence are more easily detected at pH 6—Féral, 1989). However, we believe that our results more closely approximate *in vivo* conditions. Because reported pH values vary among species, there are no doubt variations in activity *in vivo* that are not detected in our use of pH 8 assay conditions.

We compared the MCA-amino acid conjugates to other substrates in order to test for their relevance to solubilization of large proteins. Azoalbumen and hide powder azure, substrates with chromophoric groups attached to large proteins, were incubated with digestive fluids and the release of dissolved chromophores was monitored using a UV-VIS spectrophotometer. This comparison showed reasonable ordinal correspondence between the two approaches (e.g. Alanine-MCA vs. azoalbumen after log transformation was positively correlated with  $p < 0.05$ ), indicating that the fluorophoric substrates represent

overall proteolytic activity. The chromophoric substrates provide less sensitivity in routine analysis but are probably more indicative of overall proteolytic activity. They may be more indicative of endoprotease activity (enzymes that cleave nonterminal peptide bonds) while the fluorophoric, single amino acid substrates may be more indicative of exoproteases (enzymes that cleave terminal peptide bonds).

To test if alanine-MCA provided a representative peptide bond, we compared its activity patterns among gut sections to those of phenylalanine- and lysine-MCA. This experiment showed reasonable correspondence among these substrates (data not shown), validating our routine use of alanine-MCA.

We examined relative enzyme activities of fluid and sediment in the lumen. The wet sediment plug from various gut sections of *Parastichopus* and *Thelepus* were removed, separated by centrifugation, and the fluid and solid separates then added to buffer and assayed for enzyme activity.

*c. Surfactant activity.* We assessed surfactant activity by measuring the static contact angle between a 2  $\mu\text{L}$  droplet of digestive fluid and a hydrophobic surface (Parafilm). Droplets were placed on Parafilm and the sideview image of the droplet entered into an image analyzer. The tangent was drawn at the droplet-Parafilm intersection, and the angle determined between this tangent and the horizontal Parafilm surface. We also measured another indicator of surface tension—the droplet height-to-radius ratio, from the same image.

Surfactants can form micelles—colloidal aggregates that can act as a separate phase in solution. Micellization is often detected by the ability of a surfactant-rich solution to maintain a low surface tension (i.e., contact angle) in spite of dilution. This buffering results from an equilibrium between the concentration of surfactant monomers in solution and the pool of surfactant held in micellar aggregates. Monomer concentration will be roughly constant as long as some micelles are present. This constancy will be maintained during dilution until micelles disappear at a concentration termed the critical micelle concentration (CMC). Further dilution will lead to an increase in surface tension as the monomer concentration is lowered. The degree of dilution necessary to convert all of the micelles to monomers (i.e., to reach the CMC) in a given sample, is termed the Critical Micellar Dilution factor (CMD). The CMC cannot be determined from our data, and would require molecular-level concentration data for the surfactant(s). Surfactant micelles in digestive fluids were detected by titrating 2- $\mu\text{L}$  droplets with artificial seawater, by injecting with a micropipettor and monitoring the contact angle. Titrations of sodium laurate solutions at concentrations above and below its well-known CMC established the validity of this approach.

*d. Dissolved amino acids.* Digestive fluids were analyzed for dissolved amino acids in high- and low-molecular weight forms by taking two subsamples of the diluted digestive fluid and bringing one to 10% v/v trichloroacetic acid (TCA, see Mayer *et al.*, 1995). After

centrifugation, the TCA-treated subsample represents the low molecular weight fraction, and the high molecular weight fraction was calculated as the difference between the treated and untreated subsamples. After 6N HCl hydrolysis for 22–24 h at 110°C in a dry bath, the samples were analyzed for individual amino acids, by HPLC, or for total amino acids, by orthophthaldialdehyde (OPA) fluorimetry (Mayer *et al.*, 1995).

*e. Dissolved lipids.* Digestive fluids were analyzed for total dissolved lipids by extracting a subsample of the diluted digestive fluid using a modified one-phase dichloromethane:methanol extraction (Findlay, 1996). Lipids were recovered after phase separation in the organic (dichloromethane) phase and passed through a Na<sub>2</sub>SO<sub>4</sub> column to remove water and particulate matter. Lipids were dried under a stream of N<sub>2</sub>. The residue was dissolved in a minimum volume of chloroform (100–500 µL) and transferred to a pre-extracted, tared, tin weigh boat. The solvent was removed under a stream of N<sub>2</sub> and total lipid determined by weighing with a Cahn microbalance.

### 3. Results

*a. Enzyme Activity.* Luminal proteolytic enzyme activity was dominated by dissolved rather than adsorbed enzymes in the one echinoderm species, *Parastichopus*, and the one polychaete species, *Thelepus*, tested (Fig. 1). A significant fraction of the total gut activity was associated with the sediment in *Parastichopus*. However, the activity associated with the sediment was of the same magnitude as has been observed in sediments (e.g., Mayer, 1989, with similar incubation conditions), so it is unclear if the solid-phase activity represents native sedimentary enzymes or adsorbed enzymes derived from the animal. In the case of *Thelepus* the dissolved phase activities (ca. 60–80 nmol g<sup>-1</sup> min<sup>-1</sup>) were two orders of magnitude higher than those associated with the solid phase. In neither case, therefore, is there evidence for significant adsorption of the animal's enzymes.

Variance in the data was high, with ranges of an order of magnitude common for replicate ( $n = 10$ ) enzyme activities. Standard deviations often equaled means. Enzyme activities among individuals for one gut section or summed along the gut were normally distributed in about half of the cases. Often there was insufficient digestive fluid from certain gut sections for all analyses, so that replication was less than the ten individuals typically sampled.

Enzyme activities among species ranged over five orders of magnitude (Table 2). Protease and esterase activities were generally the most intense, averaging 10<sup>2</sup>–10<sup>3</sup> µM min<sup>-1</sup> in the detritivorous polychaetes (e.g., *Amphitrite*, *Schizobranchia*). We observed a maximum value of over 5000 µM min<sup>-1</sup> for protease in *Eupolyornia*. Lower activities were found in carnivorous polychaetes and all echinoderms. Activities of <0.02 µM min<sup>-1</sup> (our typical detection limit) were found for various enzymes in some echinoderms.

Lipase activities were generally intermediate in strength. The highest values, of 10<sup>2</sup>–10<sup>3</sup> µM min<sup>-1</sup>, were found with *Glycera*, a carnivorous polychaete. The lowest values were found in echinoderms.

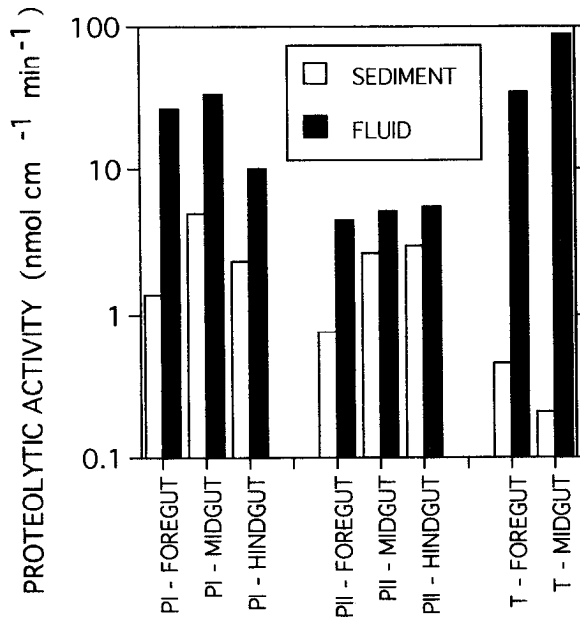


Figure 1. Distribution of luminal protease activity in digestive fluid vs. gut sediment for gut sections of two individuals of *Parastichopus californicus* (PI and PII) and one individual of *Thelepus crispus* (T). Activities (nmol-MCA min<sup>-1</sup>) are normalized per cm of length of gut section from which fluids and sediment were drawn.

$\beta$ -glucosidase activities were usually the lowest among these enzymes. These low levels are likely due in part to the lower pH optimum for glucosidases—typically around pH 6 (Clifford *et al.*, 1982), relative to our assay pH of 8. We tested for  $\alpha$ -glucosidase in several holothuroids but found negligible activity (data not shown).

The central gut segments generally contain the highest activities. Because we sampled morphologically distinct gut segments, and morphology is more complex anteriorly (Penry, 1989), these maxima typically occurred toward the anterior portion of the gut. There were occasional exceptions to this trend—e.g., *Cucumaria frondosa* had a maximum in protease activity in the most posterior section, as was observed for *Cucumaria elongata* previously (Fish, 1967). Caecae, found in *Arenicola* and *Brisaster*, had high activities.

We performed one special sampling of *Arenicola* to expand the size range of individuals sampled within a species, and hence assess variability due to size and/or developmental state. A strong inverse relationship between size and activity of protease and esterase resulted (Fig. 2). No such inverse relationships were found among individuals within other species, which is likely due to our typical use of similarly sized animals.

The relative activities of these four enzyme systems for ambient intertidal sediments were similar as those found in the detritivorous polychaetes (Table 3), with high protease and esterase and low lipase and glucosidase. Extracellular enzyme activities of intertidal sediments increased markedly with dilution of sediment into substrate-containing buffer, in

Table 2. Mean values for digestive fluids: enzyme activities, contact angles, dissolved low- and high-molecular weight amino acids (LO-MW and HI-MW), and dissolved total lipids among replicates for all species. Units: enzyme activities ( $\mu\text{M}$ -substrate hydrolyzed  $\text{min}^{-1}$ ), contact angles (degrees), amino acids (mM), and lipids ( $\text{g L}^{-1}$ ). Numbers in parentheses after "Contact angle" refer to the number of individuals found with evidence of micelles in any gut section, divided by the total number of individuals examined by titration. Abbreviations: A = Anterior, M = Mid, P = Posterior, S = Small, L = Large. Dashes indicate no data, data in italics refer to singletons; replication otherwise ranges from 2–14.

<i>Abarenicola vagabunda</i>					
	Foregut	Caecum	Midgut	Hindgut	
PROTEASE	312	71.0	607	5.5	
ESTERASE	183	79.0	258	9.0	
GLUCOSIDASE	13.0	6.3	18.0	1.8	
<i>Arenicola marina</i>					
	Esophagus	Caecum	Stomach	A. Intestine	Rectum
PROTEASE	6.5	80	111	27.5	1.9
ESTERASE	14.3	125	141	10.2	2.9
LIPASE	2.7	25.8	18.8	3.1	0.8
GLUCOSIDASE	0.3	1.3	2.0	6.7	0.0
CONTACT ANGLE (4/6)	67.9	42.8	38.0	—	80.6
HI-MW AMINO ACIDS	59.9	143	233	8.5	22.0
LO-MW AMINO ACIDS	57.6	167	215	114	5.0
<i>Amphitrite johnstoni</i>					
	Esophagus	A. Stomach	P. Stomach	A. Intestine	P. Intestine
PROTEASE	107	850	988	649	38.2
ESTERASE	602	1095	1214	730	45.2
LIPASE	20.6	18.8	19.3	12.4	6.0
GLUCOSIDASE	6.1	7.2	6.6	6.7	1.4
CONTACT ANGLE (2/6)	—	31.7	32.8	33.9	68.6
HI-MW AMINO ACIDS	88.8	201	160	122	69.2
LO-MW AMINO ACIDS	280	302	324	286	67.9
<i>Brisaster latifrons</i>					
	Foregut	Midgut	Fore Caecum	Hind Caecum	Hindgut
PROTEASE	0.1	0.2	0.9	2.1	0.0
ESTERASE	0.8	5.1	36.9	5.3	0.2
LIPASE	0.7	0.8	3.1	1.2	0.2
GLUCOSIDASE	0.0	0.1	0.1	0.0	0.0
CONTACT ANGLE (4/4)	64.3	32.5	36.5	74.0	83.6
HI-MW AMINO ACIDS	1	3	7	23	1
LO-MW AMINO ACIDS	4	11	13	22	1
LIPIDS	0.1	—	—	—	—

Table 2. (Continued)

<u><i>Brisopsis lyra</i></u>					
	Foregut				
PROTEASE	0.4				
ESTERASE	10.4				
GLUCOSIDASE	0.6				
<u><i>Cucumaria frondosa</i></u>					
	Esophagus	Stomach	A. Intestine	P. Intestine	
PROTEASE	2.3	2.8	2.3	5.4	
ESTERASE	13.5	18.3	45.3	17.7	
LIPASE	19.1	27.8	24.7	17.8	
GLUCOSIDASE	6.3	1.3	23.6	12.6	
CONTACT ANGLE (0/4)	73.9	67.9	52.7	63.3	
HI-MW AMINO ACIDS	—	82.9	338.3	193.2	
LO-MW AMINO ACIDS	61.3	27.1	272.5	45.7	
<u><i>Cucumaria miniata</i></u>					
	Foregut	Midgut	Mid/Hind	Hindgut	
PROTEASE	8.5	3.1	1.7	0.8	
ESTERASE	11.8	11.2	9.6	9.8	
LIPASE	1.8	4.1	0.3	0.2	
GLUCOSIDASE	21.7	5.6	0.6	0.6	
CONTACT ANGLE (0/4)	52.7	57.1	61.7	72.2	
<u><i>Eupolyornia heterobranchia</i></u>					
	Foregut	Midgut	Mid/Hind	Hindgut	
PROTEASE	2262	1081	322	12.6	
ESTERASE	151	54.5	22.7	1.5	
LIPASE	13.4	4.4	2.1	2.3	
GLUCOSIDASE	120	48.5	14.9	0.3	
CONTACT ANGLE (1/3)	38.0	49.6	62.2	76.2	
HI-MW AMINO ACIDS	321	127	26.0	10.0	
LO-MW AMINO ACIDS	117	52	16.0	22.0	
LIPIDS	2.1	0.3	1.5	0.01	
<u><i>Glycera dibranchiata</i></u>					
	Pro- boscis	Esophagus	A. Intestine	M. Intestine	P. Intestine
PROTEASE	17.8	56.1	17.1	56.8	78.8
ESTERASE	43.3	24.5	36.2	67.6	66.7
LIPASE	11.0	462	110	46.6	61.8
GLUCOSIDASE	23.5	33.4	0.8	38.0	10.8
CONTACT ANGLE (0/5)	68.3	72.5	60.0	59.4	62.3
HI-MW AMINO ACIDS	229	237	378	431	301
LO-MW AMINO ACIDS	239	216	126	279	339

Table 2. (Continued)

<u><i>Leptosynapta clarki</i></u>				
	Foregut	Midgut	Mid/Hind	Hindgut
PROTEASE	0.7	1.0	1.2	0.1
ESTERASE	4.5	9.3	3.4	0.6
LIPASE	1.5	2.0	2.2	0.3
GLUCOSIDASE	0.15	0.42	0.01	0.00
CONTACT ANGLE (3/4)	68.4	63.5	63.0	84.0
HI-MW AMINO ACIDS	23	15	15	0
LO-MW AMINO ACIDS	11	18	11	3
LIPIDS	2.1	0.7	1.7	—

<u><i>Molpadia intermedia</i></u>				
	Foregut	A. Midgut	P. Midgut	Hindgut
PROTEASE	0.01	0.05	0.07	0.04
ESTERASE	0.83	2.07	0.64	0.06
LIPASE	0.08	0.96	0.36	0.02
GLUCOSIDASE	0.006	0.050	0.013	0.003
CONTACT ANGLE (2/3)	81.5	33.4	54.8	82.3
HI-MW AMINO ACIDS	—	5	—	2
LO-MW AMINO ACIDS	1	1	11	1

<u><i>Nephtys caeca</i></u>				
	Foregut	Midgut	Mid/Hind	Hindgut
PROTEASE	1.3	0.3	0.7	1.0
ESTERASE	141	48.7	97.8	79.5
LIPASE	40.7	12.4	16.1	14.5
GLUCOSIDASE	2.6	0.4	1.0	1.0
CONTACT ANGLE (0/3)	55.7	58.9	55.0	52.8

<u><i>Nephtys caecoides</i></u>				
	Foregut	Midgut	Mid/Hind	Hindgut
PROTEASE	1.7	1.1	1.2	2.0
ESTERASE	45.1	81.8	77.3	38.8
LIPASE	15.9	28.2	25.2	10.4
GLUCOSIDASE	0.6	0.6	0.9	0.5
CONTACT ANGLE (0/4)	65.3	62.4	57.2	68.0
HI-MW AMINO ACIDS	6.0	6	10.0	19
LO-MW AMINO ACIDS	16	16	31	25
LIPIDS	0.07	0.7	1.3	0.6

contrast to the relatively small effect of dilution for digestive fluids. Under dilution conditions similar to those of the digestive fluids, sediment enzyme activities were of similar magnitude to luminal fluids in the echinoderms in this study. Sedimentary protease activities were similar to our previous findings (Mayer, 1989) under similar dilution conditions.

*b. Surfactants.* Contact angles are inversely related to the ability of a solution to wet a surface, and positively related to the solution's surface tension. Surfactants increase the

Table 2. (Continued)

<u><i>Nereis virens</i></u>						
	A. Esophagus	P. Esophagus	A. Intestine	M. Intestine	P. Intestine	Rectum
PROTEASE	24.3	31.2	40.1	23	8.8	—
ESTERASE	64.4	250	438	281	74.4	—
LIPASE	39.4	74.7	82.7	38.6	21.9	—
GLUCOSIDASE	0.1	1.3	2.1	2.8	0.3	—
CONTACT ANGLE (6/6)	72.5	66.5	44.3	42.5	61	84.1
HI-MW AMINO ACIDS	664	104	30.2	44.4	16.8	—
LO-MW AMINO ACIDS	18.9	152	76	75	30.7	—
<u><i>Parastichopus californicus</i></u>						
	Esophagus	Foregut	Midgut	Mid-Hind	Hindgut	
PROTEASE	0.20	0.39	0.19	0.16	0.12	
ESTERASE	0.8	10.3	4.2	1.5	1.4	
LIPASE	2.4	0.5	0.2	0.5	0.3	
GLUCOSIDASE	0.02	7.8	5.7	1.3	1.2	
CONTACT ANGLE (5/5)	76.1	49.7	53.6	86.4	90.3	
HI-MW AMINO ACIDS	—	2.3	1.7	—	0.4	
LO-MW AMINO ACIDS	—	13.4	8.8	—	1.2	
LIPIDS	—	—	—	—	—	
<u><i>Schizobranchia insignis</i></u>						
	Foregut	Midgut	Mid/Hind	Hindgut		
PROTEASE	58.3	319	339	261		
ESTERASE	13.5	69.1	76.4	55.8		
LIPASE	0.6	2.7	2.9	1.8		
GLUCOSIDASE	3.4	16.8	21.5	13.0		
CONTACT ANGLE (0/4)	72.6	53.9	51.4	64.4		
HI-MW AMINO ACIDS	12	21	24	18		
LO-MW AMINO ACIDS	11.0	22	21	11		
LIPIDS	0.48	0.4	0.3	0.91		
<u><i>Strongylocentrotus droebachiensis</i></u>						
	Esophagus	A.S. Intestine	P.S. Intestine	A.L. Intestine	P.L. Intestine	Rectum
PROTEASE	0.6	1.9	7.3	1.8	0.5	0.4
ESTERASE	5.9	74.1	331.1	75.9	3.7	5.4
LIPASE	1.6	1.3	1.1	1.2	0.3	0.6
GLUCOSIDASE	0.3	1.1	3.7	1.5	0.7	0.2
CONTACT ANGLE (2/6)	71.7	54.9	42.3	59.8	67.1	72.4
HI-MW AMINO ACIDS	2.1	15.7	0.7	20.4	8.5	7.3
LO-MW AMINO ACIDS	21.4	51.8	137.2	39.0	13.5	12.7

ability of aqueous solutions to wet hydrophobic surfaces; lower contact angles indicate greater spreading of a droplet and hence higher surfactant activity. Surface tension can be calculated from contact angle for well-constrained systems, but we have not made this conversion because certain assumptions in the relationships between these two parameters are not met properly in our system. The droplet height:width ratio, another indicator of

Table 2. (Continued)

<i>Thelepus crispus</i>			
	Foregut	Midgut	Hindgut
PROTEASE	883	363	16.0
ESTERASE	501	65.0	7.2
LIPASE	33.0	1.0	0.4
GLUCOSIDASE	107	6.1	0.2
CONTACT ANGLE (3/6)	44.0	61.0	64.0
HI-MW AMINO ACIDS	24.0	29.0	12.0
LO-MW AMINO ACIDS	26.0	25.0	13.0
LIPIDS	—	0.4	0.3

<i>Travisia foetida</i>			
	Foregut	Midgut	Hindgut
PROTEASE	3.0	13.0	8.3
ESTERASE	5.2	262.0	24.0
GLUCOSIDASE	0.0	0.2	0.2

surface tension, was tightly correlated with contact angle, further corroborating the relationship between our measurements and surface tension. The contact angle of pure water was  $>100^\circ$ , while solutions with high concentrations of sodium laurate, a strong surfactant, had angles of  $20\text{--}30^\circ$ .

Virtually all digestive fluids had contact angles lower than seawater (Table 2; Fig. 3), indicating the ubiquity of surface tension-lowering compounds. Lowest contact angles were usually found toward the middle of the digestive systems, coinciding with the highest enzyme activities. Aggregating all contact angle data from all species and gut sections

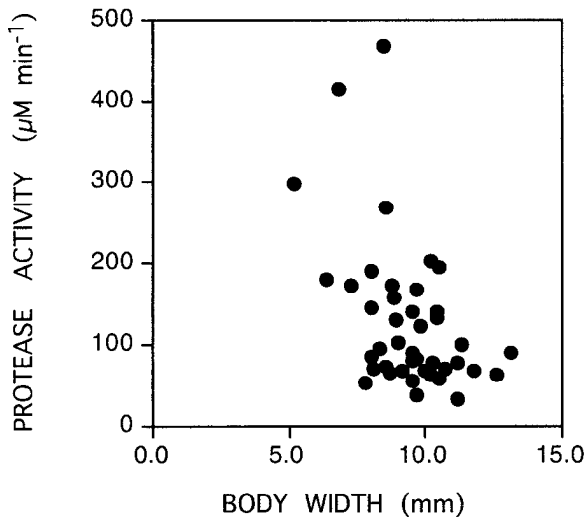


Figure 2. Protease activities in combined foregut and midgut of individuals of *Arenicola marina*, vs. the body width of the individual. Inverse correlation significant at  $p < 0.001$ .

Table 3. Activity of extracellular enzymes in intertidal sediment, as nmol [mL-pore water]<sup>-1</sup> min<sup>-1</sup> (in parentheses as nmol [gdw-sediment]<sup>-1</sup> min<sup>-1</sup>). Each column represents a different dilution of sediment with buffer solution, with the column headings indicating the final concentration of solids in the slurry (mg-dry weight mL<sup>-1</sup>). The rightmost column represents conditions most similar to those of the digestive fluid enzyme measurements, in terms of dilution of original fluids by buffer solution, while the leftmost column represents the conditions typically used for sediment enzyme activity determinations in Mayer (1989). "n.m." means not measured, while "b.d." refers to activity below detection limit.

	247	98	20	3.2
PROTEASE	0.14 (0.31)	0.36 (0.76)	1.59 (3.37)	2.55 (5.44)
ESTERASE	n.m.	n.m.	5.2 (11.0)	11.3 (24)
LIPASE	b.d.	b.d.	b.d.	b.d.
GLUCOSIDASE	0.056 (0.12)	0.1 (0.21)	0.259 (0.55)	0.188 (0.4)

reveals a polymodal distribution, with peaks at 35–40, 50–55, 70–75, and 95–100° (Fig. 3). Species that ingest large amounts of sediment, such as *Arenicola*, *Molpadia*, and the terebellid polychaetes, had the lowest contact angles—25–40°, similar to sodium laurate. Higher contact angles (>40°) were found in animals that ingest very little sediment, such as carnivores and suspension feeders, and one species that ingests coarse-grained sediment (*Leptosynapta*).

Most titrations of digestive fluid with clean seawater resulted in plots that were concave upward. Little or no increase in contact angle was observed upon initial dilution, followed by more pronounced increases as dilution proceeded. Tugwell and Branch (1992) examined similar titration curves, fitting a two-component equation to their data and calculating CMD as the point of maximum curvature. To establish micellization, we applied a somewhat more stringent criterion of requiring two linear segments of different slope in titration plots after log transformation of the concentration axis (Shinoda, 1963). This approach showed that many plots with concave-upward shape did not exhibit two-phase behavior after log transformation, and hence did not indicate micelle presence.

We found frequent evidence for the presence of micelles in the digestive fluids of individuals of several species, in the form of titration plots with an obvious break-point (Fig. 4). The clearest examples were found with samples that began with especially low contact angles before dilution, which supports the interpretation of presence of micelle-forming surfactants. All deposit-feeding species showed evidence of micelles, while no suspension feeders did (Table 2). *Nereis virens*, an omnivore that is often carnivorous but ingests mostly sediment (Olivier *et al.*, 1993), had strong micellization. No other carnivore did. Micelles were not as ubiquitous as low contact angles; only three species (*Parastichopus*, *Brisaster*, *Nereis*) showed micelles in every individual examined.

In those digestive fluids with clear break-points, roughly an order of magnitude dilution was required to reach the CMD (Fig. 4). Absence of evidence for micelles does not necessarily imply that the surfactants in the digestive fluids are incapable of micelle formation; their concentrations may simply have been below the CMC. We occasionally

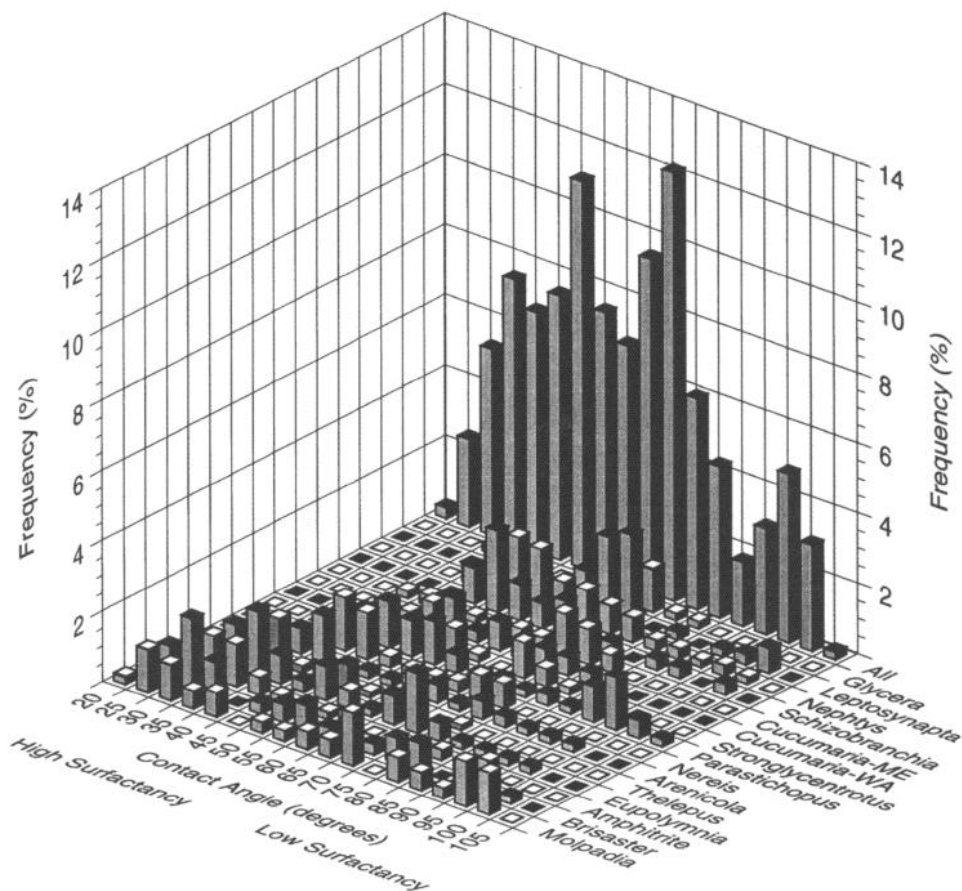


Figure 3. Frequency distribution of contact angle data. The frequency of each contact angle is collected into  $5^\circ$  bins; for each species the frequency data are expressed as percent of the entire data set for all species. Each  $5^\circ$  bin is then summed across species in the row marked "All." Lower contact angles indicate higher surfactant activity. *Cucumaria WA* = *Cucumaria miniata*, while *Cucumaria ME* = *Cucumaria frondosa*.

observed minima in the contact angle near breaks in slope, which result from mixtures of surfactants (Shedlovsky *et al.*, 1949). As the CMC of a dominant surfactant is approached, other surfactants previously solubilized within the micelles are released; if these released minor components are more effective at surface-tension lowering than the dominant compound then a minimum in contact angle ensues. This complexity might arise from either multiple surfactants explicitly secreted by the animal or surfactants derived from hydrolysis of the food substrate (e.g., monoglycerides).

*c. Dissolved organic matter.* Total amino acid concentrations in digestive fluids ranged from 2 to 1300 mM (Table 2). As with enzyme activities, variance was high, with ranges of

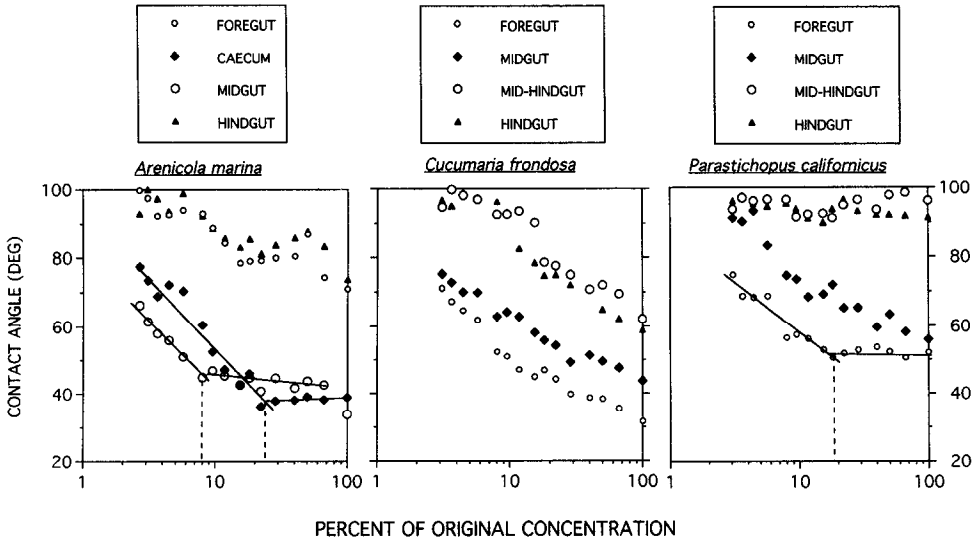


Figure 4. Representative plots of contact angles during seawater titration of digestive fluids for one individual each of *Arenicola marina*, *Cucumaria frondosa*, and *Parastichopus californicus*. Abscissa represents the dilution of the original digestive fluid (100% is pure digestive fluid), and ordinate represents the angle between the droplet and Parafilm substrate. Solid lines represent visual best fits, drawn only for those gut sections with evident micellization. Vertical dashed lines indicate the CMD (e.g., an intersection of 10% on the abscissa indicates that the digestive fluid must be diluted tenfold to eliminate micelles).

an order of magnitude common. Higher concentrations tended to be associated with polychaetes, though the holothuroids *Parastichopus* and *Cucumaria frondosa* had levels comparable with many of the polychaetes. Amino acids divided roughly evenly between the low- and high-molecular weight fractions, as separated by TCA precipitation. Midgut sections usually showed the highest values.

Amino acid composition was determined on samples from *Parastichopus*, *Brisaster*, *Abarenicola*, *Thelepus*, *Molpadia*, *Glycera*, *Strongylocentrotus*, *Eupolymnia*, and *Nereis*. Compositions of the total amino acid pool in all species (e.g., *Parastichopus* data shown in Fig. 5) were similar to typical biological tissues (Cowie and Hedges, 1992). Although molecular weight fractions did not differ greatly, the low-molecular weight fractions in many deposit feeders were often lacking in methionine, in contrast to the 1–2% methionine in the high-molecular weight fractions which are normally found in biological tissue. This methionine deficiency is consistent with the lack of methionine in the enzymatically hydrolyzable amino acids (EHAA) found in sediments (Mayer et al., 1995) and high active uptake of methionine by *Parastichopus* gut walls (Self et al., 1995). In the lower molecular weight fractions we also frequently observed relatively high concentrations of glycine and taurine, which are common osmolytes in marine invertebrates and have been observed previously in starfishes (Ferguson, 1975).

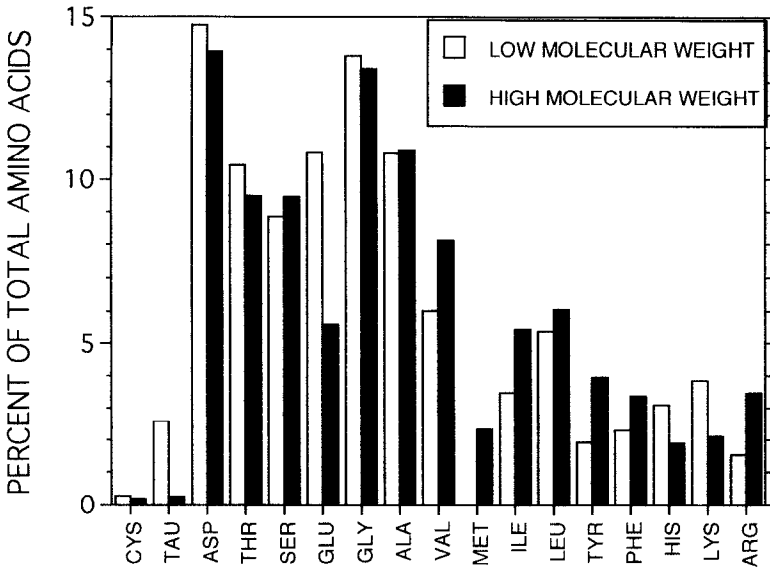


Figure 5. Amino acid compositions (as percent of total analyzed amino acids) of high and low molecular weight fractions of digestive fluid from the foregut of a specimen of *Parastichopus californicus*. The concentrations of total amino acids were 2.6 and 3.3 mM for the low and high molecular weight fractions, respectively. CYS refers to cysteic acid, which represents a mixture of cysteic acid originally present in the gut and that formed by hydrolysis of cysteine during the analysis, likely dominated by the latter. The low molecular weight fraction is notable by its high taurine (TAU) and missing methionine (MET).

Pairwise correlation analysis showed that among gut sections within an individual and between individuals, the high molecular weight amino acid fractions were closer in composition to one another than they were to low molecular weight fractions within the same gut section (e.g., *Abarenicola* data in Fig. 6). This pattern is consistent with the high and low molecular weight pools being somewhat decoupled, with the high molecular weight pools representing the digestive agents (i.e., enzymes) derived from the animal and the low molecular weight pools representing hydrolyzed food derived from the sediment.

The total lipid concentrations of selected species were not as high as amino acids, being all below  $2.5 \text{ mg L}^{-1}$  (Table 2). There were no clear relationships between concentration and either gut section or phyletic position.

#### 4. Discussion

In this paper we focus on the environment of an invertebrate gut, as experienced by a transiting particle. These particles necessarily include nutritionally available organic matter, the target of ingestion, but may range in their degree of dilution with inert mineral particles from essentially zero (e.g., live prey) to as much as three orders of magnitude.

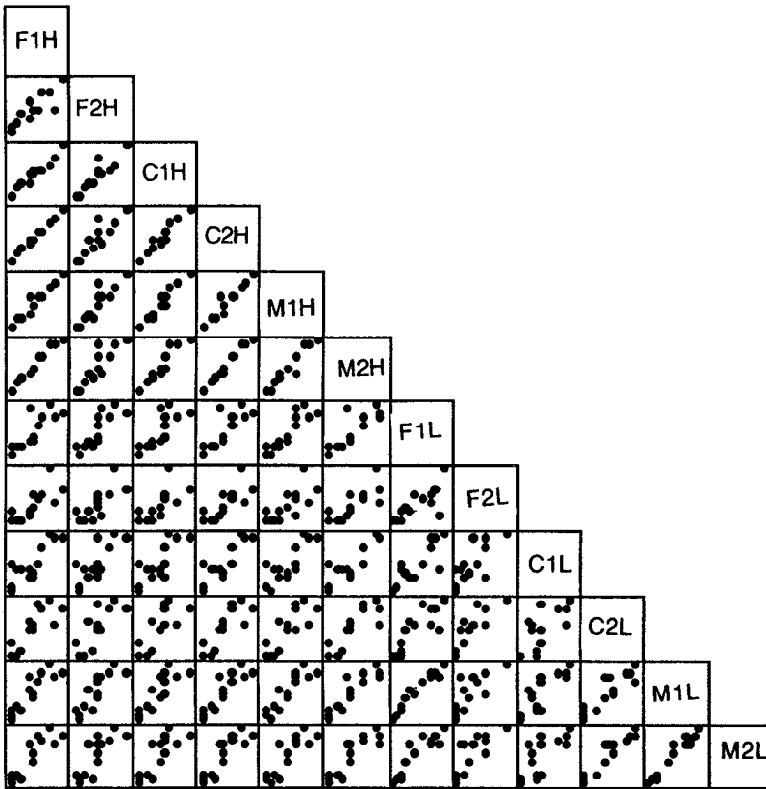


Figure 6. Scatter plot matrix of amino acid concentrations in digestive fluids from two individuals of *Abarenicola vagabunda*. Ordinates and abscissas represent individual amino acid concentrations of amino acids hydrolyzed from the low- and high-molecular weight separates. Absolute values vary among plots and are not shown for clarity. Codes refer, in order, to gut section (F = foregut, C = caecum, M = midgut), number of individual (1, 2), and molecular weight (L = low and H = high). Correlations are grouped according to low- and high-molecular weight compositions, showing that high molecular weight fractions show relatively high correlations between gut sections of an individual and between individuals and hence are under stronger control by the animal. The low-molecular weight fractions are less well correlated with the high-molecular weight fractions and with one another, except between the midgut sections of the two individuals. Hindgut compositions showed weaker correlations than the other sections and are omitted for clarity.

How do gut microenvironments vary in response to the different food assemblages ingested or with the species doing the ingesting?

*a. Enzymes.* The intensity of enzyme activity varied enormously among the species studied. The animals examined here represent two large taxonomic groups—holothuroids and polychaetes. The polychaetes had generally higher enzyme activities and usually smaller gut volumes than the holothuroids (Fig. 7). Thus size is a potential correlate at the

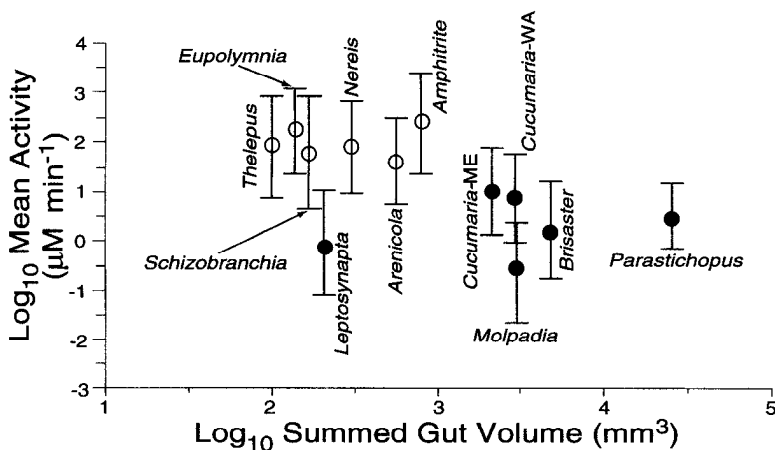


Figure 7. Enzyme activities (summed along all gut sections measured) of each species vs. animal size as indicated by gut volume. Echinoderms are plotted with filled circles and polychaetes plotted with open circles. Error bars are standard deviations.

phylum level. Size was also important among individuals of a polychaete species (Fig. 2). However, there was no significant size effect among species within a phylum (Fig. 7). Because size may correlate with many other biotic variables, it is premature to speculate as to the causes of these variations.

The ratios among the enzyme activities also showed considerable variation, which may reflect adaptation to food type. Enzymatic responses to food substrate have been occasionally observed (e.g., Stuart *et al.*, 1985), but not using quantitative measurements with luminal fluids.

Lipase activities were generally higher in carnivorous relative to detritivorous polychaetes, while the reverse was true for protease activities (Fig. 8). These trends were especially pronounced in the digestively active gut sections. Low lipase activity in deposit-feeding polychaetes has been suggested before based on tissue homogenates (Kay, 1974; Michel *et al.*, 1984).

The lipase:protease ratio may be indicative of the food source among the polychaetes. Lipases are esterases that function well only at a lipid-water interface. If the major function of digestive lipases is hydrolysis of esters found in hydrophobic lipid aggregates such as lipid bilayers and storage triglycerides, then a high lipase:protease ratio may signal a focus on cellular lipids. These highly esterified lipids are in greatest relative abundance in living biomass or relatively fresh cellular detritus. For carnivorous polychaetes the lipase:protease ratio is generally  $\gg 1$ , while for detritivorous polychaetes (e.g., *Arenicola*, the terebellids, the sabellid) the ratio is generally  $< 0.04$  (Fig. 9). Sedimentary extracellular lipase was undetectable, so that the lipase:protease ratio is zero and hence most similar to detritivorous polychaetes. We suggest that this trend results from the higher relative abundance of esterified and nonpolar lipid aggregates in biomass, relative to more polar,

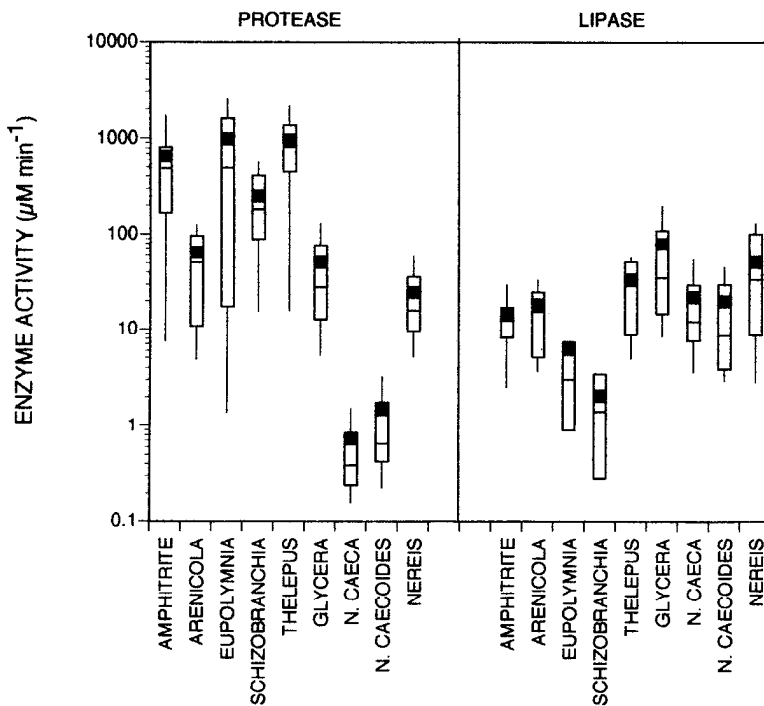


Figure 8. Protease and lipase activities of polychaetes. First five species (*Amphitrite*, *Arenicola*, *Eupolyornia*, *Schizobranchia*, and *Thelepus*) are detritivores (D), while following four species are primarily carnivorous (C). Top, middle, and bottom horizontal lines of boxes represent 75th, 50th, and 25th quantiles, respectively; whiskers extend to 90th quantiles and the solid squares are means of the data. Note trend for detritivores to have higher protease and lower lipase activities than carnivores.

de-esterified, degradation products such as monoglycerides in detritus. Such progression in lipid types can be seen in the transition from spring bloom biomass peaks to post-bloom detrital lipids (Parrish, 1987; Parrish *et al.*, 1995). The more polar degradation products are presumably less aggregated into hydrophobic phases and can be attacked with non-lipase esterases.

The lack of lipase in the extracellular enzymes in sediment might reflect a general inability of sedimentary bacteria to attack and digest live cells. Low lipase:protease ratios have also been observed for extracellular enzymes in deep sea sediments (Boetius, 1995). Hence bacteria might be exhibiting the same qualitative enzyme response to detritus as detritivorous polychaetes that perhaps feed on the same substrate.

The intense proteolytic activity of the detritivorous polychaetes is notable, with different possible explanations. First, detrital proteinaceous material is relatively difficult to hydrolyze, showing slower hydrolysis kinetics than biomass proteins (Mayer *et al.*, 1995; Laursen *et al.*, 1996). Relatively short gut residence times of deposit-feeding polychaetes

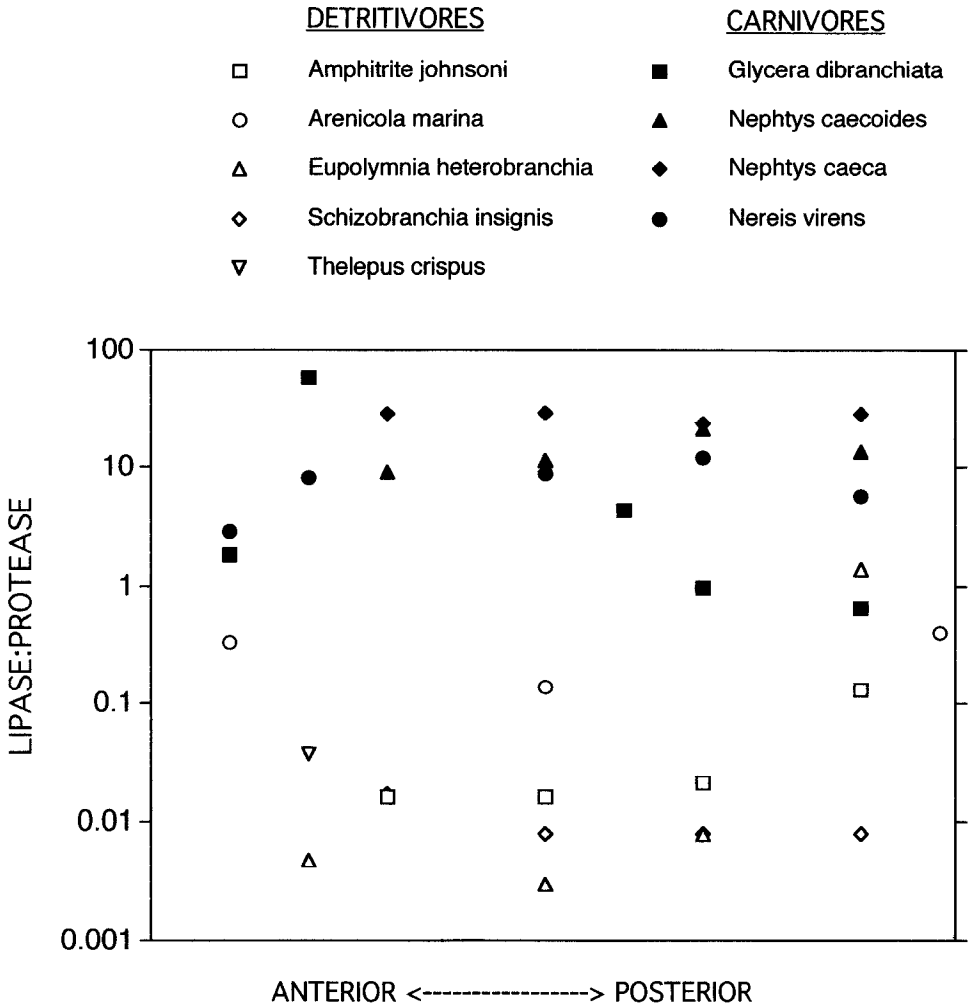


Figure 9. Lipase:protease activity ratios (dimensionless) for detritivorous polychaetes (open symbols) and carnivorous polychaetes (closed symbols). Values are medians of individual animal ratios when at least three replicates were present. Relative positions along gut are approximate and intended more to provide ordinal rather than absolute position between mouth and anus. For the digestively intense middle sections, the carnivores have ratios  $\gg 1$  while the detritivores have ratios  $\ll 1$ .

may require a faster hydrolysis, accomplished with higher proteolytic activities. Second, nitrogen limitation of sedimentary detritivores may induce greater investment in proteolytic activity.

The holothuroids sampled all have lipase:protease activity ratios more similar to the carnivorous than the detritivorous polychaetes, albeit with much lower activities. Thus the trends observed among the polychaetes and sedimentary extracellular enzymes do not

appear to apply to this phyletic group. Low protease activity has been noted for echinoderms (Johnson *et al.*, 1980; Féral, 1989; Lawrence, 1982). Echinoderms have higher assimilation efficiencies for lipids than other compound classes (Lane and Lawrence, 1982; Sibuet *et al.*, 1982; DeRidder *et al.*, 1985), consistent with these relatively high lipase activities.

Among the echinoderms, those that feed primarily on fresh planktonic detritus (*Parastichopus*, the two *Cucumaria*) had relatively high  $\beta$ -glucosidase activities. *Parastichopus* is primarily a deposit feeder, while the two *Cucumaria* species are primarily suspension feeders. Féral (1989) noted similar enzyme suites between deposit- and suspension-feeding holothuroids, and suggested that the two groups differ only in their mode of ingestion but not in the food material that they digest. Our data support his suggestion.

We found no patterns among species for esterase activity, other than its frequently high magnitude. This high activity has been noted before in benthic invertebrates, though its role is not understood (Vonk and Western, 1984).

Our results using consistent enzyme substrates and luminal fluids show that holothuroids have distinctly different enzyme approaches than polychaetes, contrary to the similarity between these two groups inferred by Féral (1989). Detritivorous polychaetes clearly have higher enzyme activities, and a greater focus on proteolysis, than detritivorous holothuroids. Echinoderms are notably slow moving and often comprise tissues with low metabolic demands (Lawrence, 1987). This condition is compatible with lower luminal enzyme activities and rates of nutritional uptake.

*b. Surfactants.* The experimental method by which we have detected surfactants in these digestive fluids must be interpreted with caution. Surfactancy is not a simple property with clear implications for the digestive process. Different surface tension-lowering compounds vary in their implications for the many chemical processes that occur during digestion, as well as their response to the measurement techniques we used. We observed, for example, that *Parastichopus* and *Cucumaria frondosa*, two animals with similar enzyme profiles, have similar contact angles with undiluted digestive fluid. Yet titration of *Parastichopus* digestive fluids showed greater evidence for micelles (Fig. 4), and a greater frothiness during sample handling. Clearly its digestive fluid has different surfactant properties than those of *Cucumaria*, properties not reflected in a single contact angle measurement. The undiluted contact angle reflects ability to wet a hydrophobic surface, while the presence of micelles may give clues regarding the ability to hold lipids in solution. Vonk (1962) noted discrepancies between surface tension measurements and the ability of digestive fluids to solubilize lipids.

Only deposit feeders had luminal fluids with contact angles consistently as low as commercial surfactants and showed evidence for micelles. This trend cut across phyla. The carnivorous and suspension-feeding polychaetes had higher contact angles and no evidence for micelles, in contrast with the deposit-feeding polychaetes. Likewise, the two suspension-feeding *Cucumaria* species showed no micelle presence, in contrast with the

deposit-feeding holothuroids (*Parastichopus*, *Brisaster*, *Molpadia*). The herbivorous *Strogylacentrotus* showed some evidence for micelles, but shares with deposit feeders the habit of ingesting abundant material of low food value (Jumars *et al.*, 1984; Lopez and Levinton, 1987).

Surfactants may provide different functions in these digestive systems. First, dissolution of food particles may be enhanced by surfactant molecules, either by lifting adsorbed food polymers from sediment surfaces or disaggregation of lipid matrices. Second, surfactants can serve as activating or deactivating agents for digestive enzymes, as do bile salts in vertebrates. Third, surfactant micelles may hold lipids in solution beyond their aqueous solubility, facilitating further release from sediment by keeping free monomer concentrations low and enabling movement between sediment particle and gut epithelium. Fourth, surfactants may prevent adsorptive loss of secreted digestive agents onto transiting sediment (see below). The finding of lowest contact angles in deposit feeders such as *Thelepus* is consistent with the very small fraction of its luminal enzyme activity found associated with the sediment particles. Fifth, these compounds may provide lubrication to modify transport behavior of the viscous slurry. Last, the surfactant activity may not result from secreted compounds but instead be a product of digestion; partially hydrolyzed food lipids, oligopeptides, or humic materials dissolved from the sediment can all lower surface tension.

We have, as yet, no molecular identification of the surfactants in these animals. Several lines of evidence indicate that proteins are not responsible for the intense surfactant activity of the deposit feeders, although they may be involved in the higher contact angle modes (Fig. 3).

*c. DOM.* Dissolved amino acid concentrations in polychaetes often exceed the amount of food substrate that could have been obtained from the sediment present in the gut. As an example, the average concentrations of total amino acids in midgut sections of *Arenicola* and *Amphitrite* are about 400 mM. Using typical water:sediment ratios for these midgut sections (Plante and Mayer, 1994), coupled to a potential enzymatically hydrolyzable amino acid (EHAA) concentration for bulk sediments of 2 mg g<sup>-1</sup> (Mayer *et al.*, 1995), the sediment present in the gut could yield only 1–4% of the observed dissolved concentrations. It seems unlikely that these animals select for particles enriched in EHAA by 25- to 100-fold.

The dissolved amino acids are roughly divided evenly between low- and high-molecular weight fractions. If the high molecular weight fraction is dominated by secreted enzymes from the animal, then the animal invests much more in digestive enzymes for a given sediment mass than can be obtained in nutrition. Adsorptive loss of even a minor fraction of these enzymes to transiting sediment must be a significant risk, and the enzymes must be protected against adsorption. Surfactants (see above) or extensive surface glycosylation of enzyme proteins may be solutions to this problem.

If the low molecular weight amino acids consist primarily of hydrolyzates from

sediment, then these high accumulations of hydrolyzate imply that digestive fluids have longer gut residence times than particles transiting through. Though net particle movement is clearly toward the posterior, it is not clear that fluid movement strictly follows particles. For example, anteriorward, anti-peristaltic movement has been noted in a number of polychaetes (Hanson, 1948; Dales, 1955), which may represent a selective retention of gut fluids in mid-gut sections while sediment is moved toward the rear. These high accumulations of apparent hydrolysates appear only in the polychaete deposit-feeders; the echinoderm concentrations are of similar order as would be expected from the sediment EHAA levels.

Accumulation of food hydrolysates would enhance active or passive uptake (Self *et al.*, 1995) via increased concentration gradients. Jumars (1993) suggested that animals feeding on dilute substrates can increase their absorption rates by this reflux mechanism. Reflux has been demonstrated in birds (Place, 1992), in connection with fat uptake.

*d. Overall digestive capability: functional vs. phyletic grouping.* Our data suggest that deposit feeding is associated with intense surfactancy, as evidenced by very low contact angles and micellization. This trend carries across the polychaete-holothuroid phyletic boundary. Within polychaetes there is a clear enzyme profile response to the digestion of live cells vs. detritus; the former leads to high lipase activity while the latter leads to relatively more intense proteolytic activity. This relationship seems to hold across mode of ingestion—viz. the detritivorous, suspension-feeding sabellid is similar to the detritivorous, deposit-feeding terebellids but different than the carnivorous polychaetes. A profile similar to those of detritivores is seen with the sedimentary extracellular enzymes, which presumably also operate on detrital material. However, these detritivorous enzyme profiles do not extend to the detritivorous holothuroids, whose relative enzyme activities more closely resemble carnivorous polychaetes. There may be, therefore, an overriding phyletic control on enzyme profiles at some phyletic level.

Functional grouping of sedimentary heterotrophs should therefore consider not only mode of ingestion (e.g., suspension vs. deposit feeders), but could also benefit from separate consideration of the biochemical nature of the food digested. The enzymatic similarities between suspension and deposit feeding polychaetes (*Schizobranchia* vs. terebellids) and holothuroids (*Cucumaria* vs. *Parastichopus*) indicate similar biochemical food substrate in spite of different modes of ingestion. These physiological data support behavioral work showing single species to switch between suspension and deposit feeding depending on physical conditions (e.g., Taghon *et al.*, 1980). Presumably there is no difference in the nature of the food substrate—there is only a difference in the means by which it is procured and in its degree of dilution with inert sedimentary material. This similar biochemical nature induces similar enzymatic response in the diverse animals feeding upon it. However, surfactancy depends on whether they procure this substrate from the water column or sediment bed. Thus enzyme suites may be more indicative of the biochemical nature of the substrate to be digested while surfactancy reflects the extent of

inert, perhaps adsorptive, diluent of this substrate. *Nereis* is interesting in that its enzyme profile reflects a biomass substrate while its surfactant activity is consistent with deposit feeding, a result of ingestion of sediment during its feeding. This profile supports its classification as an omnivore.

Similar species from the east and west coasts of the U.S. had similar suites of digestive agents. Examples include *Cucumaria frondosa* vs. *Cucumaria miniata*, *Abarenicola vagabunda* vs. *Arenicola marina*, and *Brisaster latifrons* vs. *Brisopsis lyra*.

*e. Implications for sedimentary organic matter.* Are the intense activities of these digestive agents exported into the surrounding sediment? The decreases in enzyme activities and increases in contact angle from the midgut sections toward the anterior and posterior ends of these animals suggest that these agents are largely conserved. The animals' digestive agents are apparently reabsorbed before mixing of gut fluids with the exterior environment, to avoid a net nutritional deficit. Enhanced residence time for fluids relative to sediment, coupled with partitioning of digestive agents toward the fluid phase, is consistent with retention of digestive agents. Even without gut fluid retention, however, the long hind-gut sections of deposit-feeders (Penry and Jumars, 1990) may facilitate the reabsorption of digestive agents.

Can comparison of enzyme activities in animal digestive fluids to those of extracellular enzymes in sediments allow estimation of their relative impact on sedimentary substrates? In general, we found that polychaetes have much higher enzyme activities than the extracellular sedimentary enzymes. For example, polychaete protease activities, normalized to the weight of sediment in the gut, exceeded those in ambient sediment by one to three orders of magnitude. Echinoderms, however, did not have much higher activities than sediment, at least for protease and esterase. Some echinoderms had considerably higher glucosidase activities, and all had higher lipase activities.

However, these enzyme activities should not be applied in a simple, scalar fashion to the kinetics of organic matter hydrolysis in sediments. While developing an analytical method for measuring enzymatically hydrolyzable amino acids (EHAA, Mayer et al., 1995), we compared the ability of *Parastichopus* digestive fluids and sedimentary extracellular enzymes to hydrolyze native sedimentary substrate. Some of the data are reported in Figures 2 and 3 of that paper. Along with other unpublished data, our results showed that digestive fluids hydrolyzed more EHAA than did the sedimentary enzymes. However, the protease activity of *Parastichopus* digestive fluid measured by the MCA substrates is, on average, lower than that of sedimentary enzymes (compare Tables 2 and 3 of this paper under similar dilution conditions).

Why are these metazoan digestive fluids more effective than the sedimentary extracellular enzymes? We suggest two possibilities. First, the suite of digestively active compounds—enzymes, surfactants, and perhaps other agents—are chemically better able to solubilize protective matrices, such as cell membranes that protect proteins (Laursen et al., 1996).

Second, animal luminal enzymes are deployed in a more mobile, dissolved form against

sedimentary substrates, as compared to sedimentary extracellular enzymes which are largely adsorbed (Mayer, 1989). The latter are most efficient if focused on local substrate close to a bacterial cell (Vetter *et al.*, in manuscript). Our *in vitro* enzyme assay imposes agitated slurry conditions on each system, with a mobile, dissolved substrate rather than a particulate one, so that both metazoan and sedimentary enzymes have similar access to analytical substrate (dissolved fluorophores). However, nutrition *in vivo* is produced from a particulate substrate, to which the metazoan enzymes have greater access because they are dissolved and more mobile. Thus the measured activity of sedimentary enzymes will appear to be higher than their access to natural substrate, relative to that of digestive fluids.

Enclosed digestive systems provide an advantage to metazoans by allowing high levels of secreted enzymes to work on dispersed substrate. Bacteria cannot risk protracted loss of exuded enzymes to the environment in amounts greater than their nutritional gain. Recent modeling suggests that sedimentary bacteria effectively utilize substrate only within about five cell radii of their location (Vetter *et al.*, in manuscript), due to net diffusive loss of more distant materials. A metazoan's enclosed gut allows greater recovery of its invested enzymes and nutritional gain by efficient absorption. If the high molecular weight amino acid concentrations measured in this study represent primarily secreted proteins from the animal, then many species (especially polychaetes) secrete more protein than could be obtained from a given volume of sediment. This high investment in digestive agents is profitable only if the investment can be reabsorbed efficiently, which is difficult for sedimentary bacteria exuding cell-free enzymes. Bacterial populations would find it advantageous to use this strategy only in monoculture conditions or in enclosures.

Different enzyme activities among animal species may be compensated, to some extent, by differing gut residence times. A longer gut residence time can increase the net integrated enzyme exposure of a transiting particle. Inadequate gut residence time data are available to calculate trends for the species discussed in this paper.

The variations in digestive capability presented here indicate that the bioavailable fraction of nutritional material in sediments varies according to the nature of the heterotroph feeding upon it. Bioavailability is a property of an organism-particle interaction, and is not an inherent property of the substrate alone. Whether different bioavailability of sedimentary substrates to metazoans vs. bacteria results from chemical or spatiotemporal aspects of this interaction requires further attention.

*f. Collateral reactions.* Material solubilized from sediments during gut passage should be primarily nutritional compounds for absorption, assuming accuracy in the evolutionary "design" of solubilizing agents, but may include incidental solubilization of other compounds. The digestive capability of animals thus has implications for mobilization of non-nutritional materials such as sedimentary contaminants. Accordingly, we find much greater amounts of toxic metals and polycyclic aromatic hydrocarbons to be solubilized by animal digestive fluids than by seawater (Mayer *et al.*, 1996), due to the high dissolved

organic ligands and surfactants. Other candidates for inadvertent solubilization include minerals and nutritionally useless organic matter such as humified substances.

Although we have focused on the solubilizing properties of digestive fluids, their high enzyme activities and dissolved organic matter levels set the stage for reactions in the opposite direction. We also find very high dissolved trace metal concentrations in these fluids (Chen and Mayer, unpublished). Guts may be sites of chemical condensations among these potential reactants. Many hydrolytic enzymes can form bonds as well as break them, high organic matter concentrations enhance condensations such as melanoidin formation, and trace metals can promote intermolecular associations by multi-ligand complexation. A possible example of such a reaction is the esterification of sterols and chlorins during passage of phytoplankton cells through zooplankton guts (Harradine *et al.*, 1996), a reaction perhaps inadvertently catalyzed by digestive esterases.

Benthic invertebrate digestive systems are thus chemically intense environments. While not unusual in Eh/pH (Plante and Jumars, 1992), they do represent zones in which sedimentary organic matter should undergo accelerated hydrolysis, solubilization and perhaps recombination.

*Acknowledgments.* We thank K. Hardy, T. Miller, M. Thiel, J. Smoot, C. Thomasson, C. Wood and L. Watling for help in lab and field, and the Director of the Friday Harbor Laboratory. This work was supported by NSF and ONR. Contribution #301 from the Darling Marine Center.

#### REFERENCES

- Aller, R. C. 1982. The effects of macrobenthos on chemical properties of marine sediment and overlying water, *in* Animal-Sediment Relations, P. L. McCall and M. J. S. Tevesz, eds., Plenum Press, 53–102.
- Boetius, A. 1995. Microbial hydrolytic enzyme activities in deep-sea sediments. *Helgol. Meeresunters.* 49, 177–187.
- Clifford, C. J. Walsh, N. Reidy and D. B. Johnson. 1982. Digestive enzymes and subcellular localization of disaccharidases in some echinoderms. *Comp. Biochem. Physiol.*, 71B, 105–110.
- Cowie, G. L. and J. I. Hedges., 1992. Sources and reactivities of amino acids in a coastal marine environment. *Limnol. Oceanogr.*, 37, 703–724.
- Dales, R. P. 1955. Feeding and digestion in terebellid polychaetes. *J. Mar. Biol. Assoc. U.K.*, 34, 55–79.
- DeRidder, C. M. Jangoux and E. van Impe. 1985. Food selection and absorption efficiency in the spatangoid echinoid, *Echinocardium cordatum* (Echinodermata), *in* International Echinoderm Conference, B. F. Keegan and B. D. S. O'Connor, eds., Balkema, 245–251.
- DeVillez, E. J. and R. M. Reid. 1971. Comparative properties of polychaete trypsin. *Comp. Biochem. Physiol.*, 38B, 235–238.
- Eberhardt, J. 1992. Isolation and characterization of five serine proteases with trypsin-, chymotrypsin- and elastase-like characteristics from the gut of the lugworm *Arenicola marina* (L.) (Polychaeta). *J. Comp. Physiol.*, B 162, 159–167.
- Fauchald, K. and P. A. Jumars. 1979. The diet of worms: A study of polychaete feeding guilds. *Oceanogr. Mar. Biol. Annu. Rev.*, 17, 193–284.
- Féral, J.-P. 1989. Activity of the principal digestive enzymes in the detritivorous apodous holothuroid *Leptosynapta galliennei* and two other shallow-water holothuroids. *Mar. Biol.*, 101, 367–379.

- Ferguson, J. C. 1975. The role of free amino acids in nitrogen storage during the annual cycle of a starfish. *Comp. Biochem. Physiol.*, 51A, 341–350.
- Findlay, R. H. 1996. The use of phospholipid fatty acids to determine microbial community structure, in *Molecular Microbial Ecology Manual*, A. D. L. Akkermans, J. D. van Elsas and F. J. de Bruijn, eds., Kluwer Academic Publishers, 1–17.
- Fish, J. D. 1967. The digestive system of the holothurian, *Cucumaria elongata*. II. Distribution of the digestive enzymes. *Biol. Bull.*, 132, 354–361.
- Hanson, J. 1948. Transport of food through the alimentary canals of aquatic annelids. *Quart. J. Micr. Sci.*, 89, 47–51.
- Harradine, P. J., P. G. Harris, R. N. Head, R. P. Harris and J. R. Maxwell. 1996. Steryl chlorin esters are formed by zooplankton herbivory. *Geochim. Cosmochim. Acta*, 60, 2265–2270.
- Jeuniaux, C. 1969. Nutrition and Digestion, in *Chemical Zoology*, M. Florkin and B. R. Scheer, eds., Academic Press, 4, 69–91.
- Johnson, D. B., B. Rushe, B. Glynn, M. Canning and T. Smith. 1980. Hydrolases in the digestive tracts of some echinoderms, in *Echinoderms: Present and Past*, Jangoux, M., ed., Balkema, 313–317.
- Jumars, P. A. 1993. Gourmands of mud: Diet selection in marine deposit feeders, in *Mechanisms of Diet Choice*, R. N. Hughes, ed., Blackwell Scientific Publishers, 124–156.
- Jumars, P. A., R. C. Newell, M. V. Angel, S. W. Fowler, S. A. Poulet, G. T. Rowe and V. Smetacek. 1984. Detritivory, in *Flows of Energy and Materials in Marine Ecosystems: Theory and Practice*, M. J. Fasham, ed., Plenum Press, 583–593.
- Kay, D. G. 1974. The distribution of the digestive enzymes in the gut of the polychaete *Neanthes virens*. *Comp. Biochem. Physiol.*, 47A, 573–582.
- Kermack, D. M. 1955. The anatomy and physiology of the gut of the polychaete *Arenicola marina* (L.). *Proc. Zool Soc. Lond.*, 125, 347–381.
- Lane, J. M. and J. M. Lawrence. 1982. Food, feeding and absorption efficiencies of the sand dollar, *Mellita quinquesperforata* (Leske). *Estuar. Coastal Shelf Sci.*, 14, 421–431.
- Laursen, A. K., L. M. Mayer and D. W. Townsend. 1996. The lability of proteinaceous material in estuarine seston and subcellular fractions of phytoplankton, *Mar. Ecol. Prog. Ser.*, 136, 227–234.
- Lawrence, J. M. 1982. Digestion, in *Echinoderm Nutrition*, M. Jangoux and J. M. Lawrence, eds., Balkema, 283–329.
- 1987. *A Functional Ecology of Echinoderms*. Croom Helm, London, 340 pp.
- Lopez, G. R. and J. S. Levinton. 1987. Ecology of deposit-feeding animals in marine sediments. *Quart. Rev. Biol.*, 62, 235–260.
- Mayer, L. M. 1989. Extracellular proteolytic activity in the sediments of an intertidal mudflat. *Limnol. Oceanogr.*, 34, 973–981.
- Mayer, L. M. and D. L. Rice. 1992. Early diagenesis of protein: A seasonal study, *Limnol. Oceanogr.*, 37, 280–295.
- Mayer, L. M., L. L. Schick, T. Sawyer, C. Plante, P. A. Jumars and R. L. Self. 1995. Bioavailable amino acids in sediments: A biomimetic, kinetics-based approach, *Limnol. Oceanogr.*, 40, 511–520.
- Mayer, L., Z. Chen, R. Findlay, J. Fang, S. Sampson, L. Self, P. Jumars, C. Quet el and O. Donard. 1996. Bioavailability of sedimentary contaminants subject to deposit-feeder digestion, *Env. Sci. Technol.*, 30, 2641–2645.
- Michel, C., M. Bhaud, P. Boumati and S. Halpern. 1984. Physiology of the digestive tract of the sedentary polychaete *Terebellides stroemi*. *Mar. Biol.*, 83, 17–31.
- Olivier, M., G. Desrosiers, C. Retiere, and J.-C. Brethes. 1993. Variations spatio-temporelles de l'alimentation du polychete *Nereis virens* en zone intertidale (estuaire maritime du Saint-Laurent,

- Québec). *Vie Milieu*, 43, 1–12.
- Parrish, C. C. 1987. Time series of particulate and dissolved lipid classes during spring phytoplankton blooms in Bedford Basin, a marine inlet. *Mar. Ecol. Prog. Ser.*, 35, 129–139.
- Parrish, C. C., C. J. McKenzie, B. A. MacDonald and E. A. Hatfield. 1995. Seasonal studies of seston lipids in relation to microplankton species composition and scallop growth in South Broad Cove, Newfoundland. *Mar. Ecol. Prog. Ser.*, 129, 151–164.
- Penry, D. L. 1989. Tests of kinematic models for deposit-feeders' guts: patterns of sediment processing by *Parastichopus californicus* (Stimpson) (Holothuroidea) and *Amphicteis scaphobranchiata* Moore (Polychaeta). *J. Exp. Mar. Biol. Ecol.*, 128, 127–146.
- Penry, D. L. and P. A. Jumars. 1986. Chemical reactor analysis and optimal digestion. *BioScience*, 36, 310–315.
- Penry, D. L. and P. A. Jumars. 1987. Modelling animal guts as chemical reactors. *Am. Nat.*, 129, 69–96.
- Penry, D. L. and P. A. Jumars. 1990. Gut architecture, digestive constraints and feeding ecology of deposit-feeding and carnivorous polychaetes. *Oecologia*, 82, 1–11.
- Place, A. R. 1992. Comparative aspects of lipid digestion and absorption: physiological correlates of wax ester digestion. *Am. J. Physiol.*, 263, R464–R471.
- Plante, C. and P. Jumars. 1992. The microbial environment of marine deposit-feeder guts characterized via microelectrodes. *Microb. Ecol.*, 23, 257–277.
- Plante, C. J. and L. M. Mayer. 1994. Distribution and efficiency of bacteriolysis in the gut of *Arenicola marina* and three additional deposit feeders. *Mar. Ecol. Prog. Ser.*, 109, 183–194.
- Self, R. F. L., P. A. Jumars and L. M. Mayer. 1995. In vitro amino acid and glucose uptake rates across the gut wall of a surface deposit feeder. *J. Exp. Mar. Biol. Ecol.*, 192, 289–318.
- Shedlovsky, L., J. Ross and C. W. Jakob. 1949. The effect of certain impurities on the surface and interfacial tension of aqueous solutions of sodium dodecyl sulfonate. *J. Coll. Sci.*, 4, 25–33.
- Shinoda, K. 1963. The formation of micelles, in *Colloidal Surfactants: Some Physicochemical Properties*, K. Shinoda, T. Nakagawa, B. Tamamushi, and T. Isemura, eds., Academic Press, 1–96.
- Sibuet, M., A. Khripounoff, J. Deming, R. Colwell and A. Dinet. 1982. Modification of the gut contents in the digestive tract of abyssal holothurians. in *International Echinoderm Conference*, J. M. Lawrence, ed., Balkema, 421–428.
- Stuart, V., E. J. H. Head, and K. H. Mann. 1985. Seasonal changes in the digestive enzyme levels of the amphipod *Corophium volutator* (Pallas) in relation to diet. *J. Exp. Mar. Biol. Ecol.*, 88, 243–256.
- Taghon, G. L., A. R. M. Nowell and P. A. Jumars. 1980. Induction of suspension feeding in spionid polychaetes by high particulate fluxes. *Science*, 210, 562–564.
- Thayer, C. W. 1983. Sediment-mediated biological disturbance, in *Biotic Interactions in Recent and Fossil Communities*, M. J. S. Tevesz and P. L. McCall, eds, Plenum Press, 479–625.
- Tugwell, S. and G. M. Branch. 1992. Effect of herbivore gut surfactants on kelp polyphenol defenses. *Ecol.*, 73, 205–215.
- Vonk, H. J. 1962. Emulgators in the digestive fluids of invertebrates. *Arch. Int. Phys. Bioch.*, 70, 67–85.
- Vonk, H. J. and J. R. H. Western. 1984. *Comparative Biochemistry and Physiology of Enzymatic Digestion*, Academic, London, 490 pp.