

The Microbial Environment of Marine Deposit-Feeder Guts Characterized via Microelectrodes

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Abstract. Microbial viability and growth in animal guts are dependent upon conditions influenced by both the physiological activities of the animal and the activities of the microbes themselves. To examine the relative contribution of these influences, the guts of *Molpadia intermedia* (a subtidal holothuroid) and a variety of other marine deposit feeders from diverse habitats were probed with mini- or microelectrodes to measure oxygen, Eh, and pH. In general, bulk oxygen and pH conditions of the gut mimicked those of ambient sediments, revealing nearly neutral pH and zero oxygen in sub- and intertidal animals, with more oxygen in bathyal animals ingesting oxygenated sediments. Eh in guts of subsurface deposit feeders that likely subduct and aerate sediments before ingestion did not mimic sediments. Axial Eh profiles, in contrast to those of pH and oxygen, revealed significant changes along the gut. In most deposit feeders, values decreased from mouth to midgut, suggesting high rates of microbial metabolism within the gut. Increases in Eh were observed in the most distal portion of guts, however, likely due to anal intake of aerated water, and throughout the guts of terebellid polychaetes that feed on highly reducing sediments. This addition of a strong oxidant by the animal may be necessary to avoid sulfide poisoning and may provide access to organic products by stimulating chemoautotrophy. Radial profiles of the gut revealed sharp gradients of Eh and oxygen. In general, steep redox gradients stimulate bacterial metabolism and may lead to exceptionally high respiratory rates. Radial diffusion calculations made using oxygen profiles surrounding the gut reveal that, as predicted by digestion theory, oxygen consumption rates are rapid and are higher in the hindgut, where the digestive products of the animal are available to microbes, than in the foregut.

Introduction

Ingestion by deposit-feeding invertebrates strongly influences types and rates of microbial metabolic processes [23] as well as microbial community com-

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position [e.g., 59]. Recent findings suggest a general trend of initial decrease in number of transient bacteria in the foregut and anterior midgut with gut passage. In contrast, growth appears to be accelerated and numbers again increase in the hindgut and feces [15, 42]. Species differ in response to gut passage, leading to changes in community composition [16, 50, 59].

Environmental conditions ultimately control microbial community structure and metabolism. Bacterial population abundances and processes have been correlated with specific physicochemical conditions, e.g., pO_2 , Eh, pH, temperature, light, and osmolarity [55]. In terrestrial and freshwater environments, marked changes in pO_2 , Eh, and pH between foods and the guts of soil- and litter-feeding detritivores have been demonstrated [2, 4], and speculation is that the correspondingly unique gut environments are populated primarily by indigenous commensals [3]. In the marine environment, dominant taxa of detritivore communities are strikingly different. Polychaetes and echinoderms are abundant deposit feeders in the oceans but are of little or no importance in freshwater and terrestrial systems. The water-balance reasons for sealing internal environments off from external influence in terrestrial and freshwater settings are largely absent in the marine realm. Thus we have hypothesized [43] that the gut environment will not depart as radically from ambient conditions in marine organisms as it often does elsewhere. Rapid food throughputs of deposit feeders [e.g., 56] further constrain the extent to which gut conditions can differ from those of the food before ingestion. Rhoads' [51] classic review suggests only mild acidity in marine deposit feeders' guts. Previous studies, however, appear to be limited to single measurements per individual and thus cannot resolve internal microenvironments for microbes or changes during gut passage.

There are reasons to believe that the gut environments of deposit feeders will show greatly enhanced geochemical activity. They are sites of unparalleled microbial growth on sediments, as judged from total direct counts [18, 42, 53], capabilities of isolates [15], and population growth estimates of marked strains in transit [42]. If this growth is aerobic, it must generate considerable oxygen demand that should be reflected in either the flux of oxygen into the gut or longitudinal decrease in oxygen concentration with gut passage. Moreover, we have been unsuccessful in applying more direct radiotracer methods (i.e., 3H -thymidine) to obtain confirmation of the rapid microbial growth observed as changes in bacterial number [42]; even the introduction of the radiotracer is a problem because deposit feeders will not in general ingest food from a given location upon demand. Thus the distribution of pH, Eh and, in particular, O_2 along and across the gut, when coupled with an appropriate flux model, may serve to provide independent constraints on microbial growth. Even if little change is found along the gut, however, the transport may have significant influence upon surviving bacteria. Subsurface deposit feeders in particular usually defecate in a depth stratum shallower than the one in which they feed, sending attached bacteria to a new redox environment, often with remarkable frequency [61].

To determine the magnitude of environmental change in gut transit, in this study we measured both axial and radial trends of pO_2 , pH, and Eh through

the guts of various marine deposit feeders via micro- and mini-electrodes. We concentrated our efforts on the subsurface deposit-feeding holothuroid, *Molpadia intermedia*, due to its large size, simple gut morphology, ease of collection, and gut-wall characteristics conducive to this type of study. We supplemented these observations with a broader taxonomic and environmental array of much sparser measurements to assess the generality of the results. Other physico-chemical parameters important to microbial growth, such as light and temperature, were not examined but should be similar in sedimentary and gut environments. Oxygen level is an important determinant of rates and modes of decomposition and, of course, influences the composition of microbial communities. Further, if changes in oxygen levels can be recorded, biological oxygen demand (BOD) of these environments can be estimated. In the absence of O₂, Eh values can indicate, on a gross level, which types of anaerobic metabolic processes dominate [20, 58]. As with O₂ for aerobic processes, Eh levels set thermodynamic limits on feasibility of conversions. Eh change can reveal anaerobic decomposition or input of oxidants. Concomitant measurement of pH is essential, since Eh is pH dependent. High or low pH can indicate a selective or general antibacterial strategy, possibly an adaptation for harboring specific symbionts [4]. Changes in pH can also reveal qualitative aspects of decomposition, e.g., whether proteins or carbohydrates are being decomposed. Additionally, intestinal redox potential as well as pH can strongly influence enzymatic activity.

Methods

Specimen and Sediment Collection

Eight species of deposit feeders were collected from five sites. The mobile, surface deposit-feeding holothuroids, *Pannychia* sp. (Holothuroidea: Laetmogonidae) and *Scotoplanes globosa* (Holothuroidea: Elipidiidae), were collected from site 1. The apodous holothuroid, *Molpadia intermedia* (Holothuroidea: Molpadiidae), the burrowing urchin, *Brisaster latifrons* (Echinoidea: Schizasteridae), and *Travisia foetida* (Polychaeta: Opheliidae) were collected at site 2. Each of these animals feeds primarily below the sediment surface. We collected the tentaculate polychaetes, *Thelepus crispus* (Polychaeta: Terebellidae) and *Eupolymnia heterobranchia* (Polychaeta: Terebellidae) from sites 3 and 4, respectively, and the common funnel feeder, *Abarenicola pacifica* (Polychaeta: Arenicolidae), from sites 4 and 5. Site 1, at a depth of 1,200 m, was located in Santa Catalina Basin off the coast of southern California. Bottom temperature is constant at 2°C. Specimens were collected either via otter trawl (*Scotoplanes*) or in cores (*Pannychia*) taken with the manipulator arm of the deep-sea research submersible ALVIN. From site 2, a subtidal site of 200 m depth located in central Puget Sound (Washington), animals were collected in box cores. Temperatures of the muddy sediment at this site ranged from 9–10°C among numerous samplings. At site 3 located in Garrison Bay (San Juan Island, WA), a region of organically rich, poorly sorted mud (12°C at collection), terebellids were dug with a shovel at low tide, as were animals at site 4, located in False Bay (San Juan Island, WA), where medium sands ranged from 13–17°C, and at site 5, a medium to coarse sand on Alki Beach (Seattle, WA). Sediment temperatures were between 13 and 15°C at various collection times at this site. Sediments from the depths of the organisms' feeding structures were collected in all cases and will be referred to as "ambient sediment." The need to sample guts rapidly, however, prevented us from being able to sample ambient sediments with high precision from presumed burrow sites of ingestion.

Electrodes

Oxygen electrodes were constructed following the method of Reimers et al. [45]. Tip diameters ranged from 50 to 200 μm , depending on the gut-wall characteristics of the intended animal of study. The lower limit of tip diameter was set by the sturdiness needed to puncture gut walls of the various animals. Electrodes were polarized at -0.75 V using a Diamond General (Ann Arbor, MI) model 1231 microsensor. Electrodes were prepoisoned by insertion into sulfide-rich sediments, so as to avoid changes in sensitivity once measurements had started. Probes were recalibrated between measurements whenever necessary. Each electrode was tested for variability between stagnant and stirred conditions; electrodes were discarded if the change was $> 5\%$. Electrodes were calibrated using seawater that had been saturated with O_2 by air bubbling and either anoxic sediments or argon-flushed seawater. Excessive drift (defined as $> 10\%$) was detected by comparison of calibrations before and after sample measurements. Limits of detection at 22°C were about 1% of saturation, or 2 μM . The 90% response time was ~ 2 sec, so profiles on the emersed animals could be completed with little change from ambient temperatures; animals were kept at in situ temperatures prior to O_2 measurement. For logistical reasons, in a few instances calibration solutions and experimental samples (sediments or guts) were at dissimilar temperatures. To correct for differential solubility and diffusion rates at different temperatures, experiments with oxygenated seawater in the laboratory were performed. A correction factor of $2.29\% (\text{C}^\circ)^{-1}$ decrease was used in those measurements in which guts and calibration solutions were at dissimilar temperatures. The current measured for the sample of interest was corrected by this factor to give the current that would have been measured at the temperature of standards (22°C). This correction factor corresponds well with the $2.1\% (\text{C}^\circ)^{-1}$ decrease in diffusion coefficient with cooling for oxygen in seawater [6]. The ratio of this corrected current to that of the standard was then used to convert to oxygen concentration by employing solubilities appropriate for each temperature.

Eh electrodes were constructed as were oxygen electrodes except that they did not receive gold plating or the DePex membrane. A salt bridge of 1 N KCl, 1% agar was used [3] with a standard half cell of 1 N KCl solution. The potential of the half cell at room temperature is $+235$ mV; this value is added to the E.M.F. registered by the potentiometer to give Eh, the potential of the redox system. Dibasic potassium phosphate buffer (0.66 M) of pH 7.0, which gives an Eh of $+630$ mV, was used as a standard. Stabilization time was variable and often long; a reading was considered stable and recorded when $\Delta\text{mV} (\text{min})^{-1}$ was < 1 . Temperature had little effect, and corrections proved unnecessary. Reproducibility in phosphate buffer was good (± 5 mV) but much worse in sediments or guts, at times ± 100 mV, at least in part due to natural, fine-scale spatial variability.

The pH electrodes were purchased from World Precision Instruments (New Haven, CT). Each consisted of a glass pH electrode mounted in a 25-gauge hypodermic needle. Tip diameter was 500 μm , so these instruments must be considered mini- rather than microelectrodes [cf. 47]. The same reference and salt bridge construction as for Eh was used except 3.5 N KCl was employed for greater stability. Phosphate buffers of pH 4.4, 7.0, and 9.6 (and 3% salinity) were used as standards.

Measuring Equipment

Current output was read from the chemical microsensor (Diamond General) display of O_2 partial pressure and was later converted to O_2 concentration. Digital readouts of potential, in mV, for both Eh and pH were registered on the same display. A Faraday cage was employed to reduce electrical noise when necessary (e.g., shipboard).

Axial Profiles

Deposit feeders were dissected rapidly to expose intact digestive tracts (and their guts divided into principal regions; cf. Fig. 1). Axial and longitudinal profiles of deposit feeders were taken by

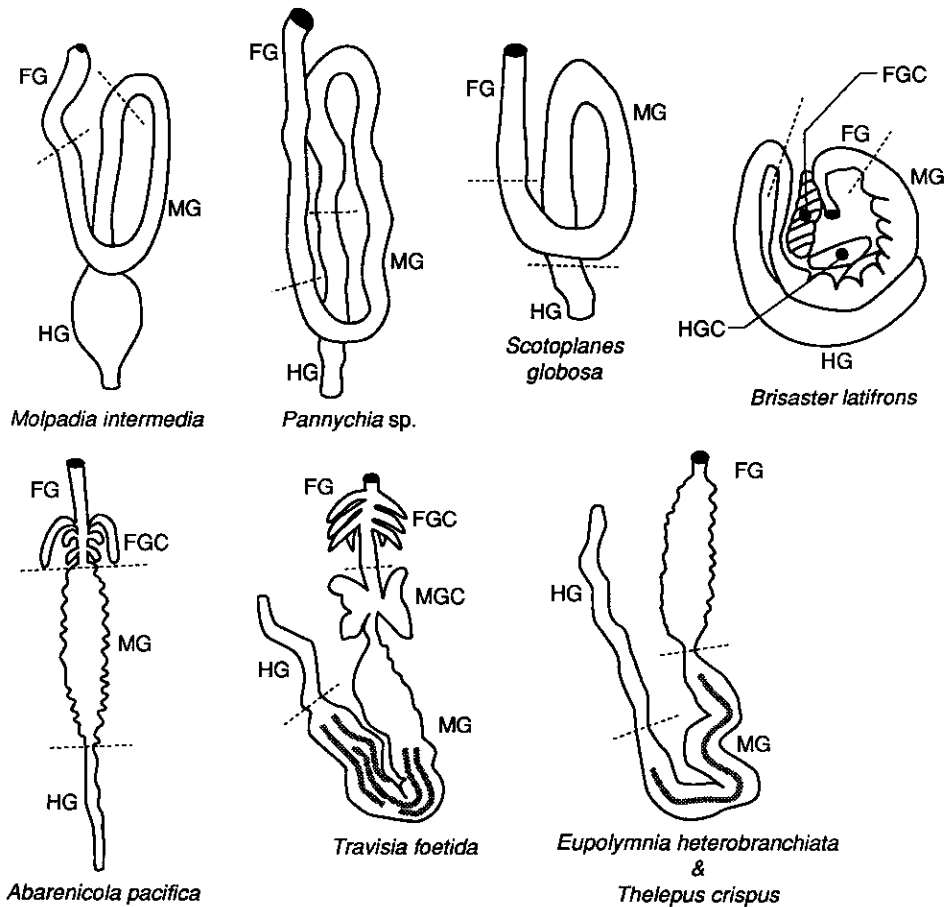


Fig. 1. Schematic of the probed deposit-feeder guts, with foregut (FG), midgut (MG), and hindgut (HG) divisions indicated (not to scale among species). Measurements were made as near to the center of each region as possible. FGC, MGC, and HGC refer to fore-, mid-, and hindgut ceca, respectively.

puncturing the gut wall with electrodes and lowering the probe to the center of the gut lumen with a micromanipulator. Gut sections probed were foregut, midgut, and hindgut (Fig. 1). Gut diverticula also were probed when present.

Radial Profiles

Radial profiles for a subset of the above deposit feeders were taken by puncturing the gut walls, then moving the probe through 0.5-mm (for most) or finer increments using a micromanipulator. Gut walls were thin, usually $<100\ \mu\text{m}$ but occasionally near $200\ \mu\text{m}$ thick in the midgut of the polychaetes, so that measurements within the gut wall were not possible; measurements taken to be at the "gut wall" were those readings when the probe contacted the coelomic (serosal) side of the wall. One caveat is that when the gut was punctured with the probe, some deformation occurred, with rebounding to near its initial location. Therefore proximity to gut wall is taken as $\pm 0.25\ \text{mm}$, but measurements are accurate relative to one another. That is, 0.5-mm depth is $\pm 0.25\ \text{mm}$, but

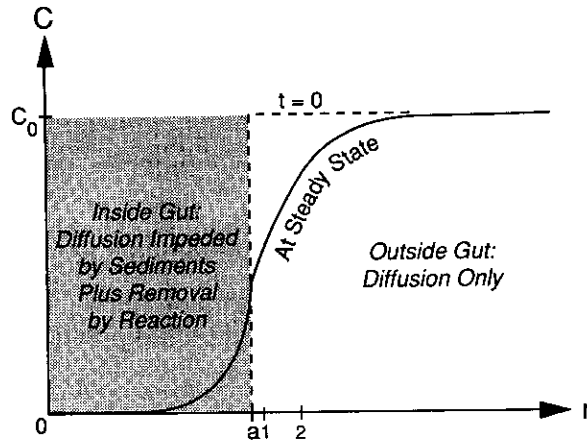


Fig. 2. Schematic oxygen profiles at time zero and at steady state for excised gut segments placed in sterile seawater. Within the gut, oxygen concentration is altered by the impedance of grains (tortuosity) as well as by oxygen consumption, so measurements for calculation of steady-state fluxes were taken outside the gut of radius a , in the linear portion of the steady-state profile (at r_1 and r_2). For further details, see the text.

1.0 mm depth is $0.5 \text{ mm} \pm 5 \mu\text{m}$ (as micromanipulator increments are calibrated to $10 \mu\text{m}$) deeper than the 0.5-mm measurement.

Models of Radial Diffusion

Initial radial profiles suggested that oxygen was being consumed by the gut contents. We had hoped to calculate inward oxygen flux, but measurement of oxygen in coelomic fluid proved difficult. Readings showed high spatial variability within the animal and were not reproducible. Therefore, sections of the hindgut of *Molpadia intermedia* were doubly ligated at each end, severed, removed and placed in petri dishes of sterile, unstirred seawater. Oxygen gradients surrounding and within these gut sections were then recorded at 2, 5, and 24 hours. For comparison, gut sections fixed with either 0.1 M HgCl_2 or 5% formalin were monitored concurrently. Oxygen flux into ligated foregut sections was also measured at 5 and 24 hours for comparison to rates in the hindgut. All experiments of this type were done in a coldroom at 10°C . Oxygen levels up to and into the gut were profiled.

To model O_2 diffusion into gut sections we treated the gut as an infinite cylinder, assumed an initial concentration of zero within the cylinder, and a uniform, oxygen-saturated environment outside the cylinder (Fig. 2). Upon immersion of the gut sections in saturated, sterile seawater, an initially large gradient drives diffusion of O_2 into the gut. The rate of diffusion and the concentration profile change rapidly with time, i.e., the system is in unsteady state. To model the expected O_2 profiles, we employed a modification of Crank's equation [12, eq. 3.10] for unsteady diffusion out of a cylinder:

$$C(r) = \frac{C_0}{2Dt} e^{(-r^2/4Dt)} \int_0^a e^{(-r'^2/4Dt)} I_0\left(\frac{\Gamma r'}{2Dt}\right) r' dr' \quad (1)$$

where I_0 is the modified Bessel function of the first kind of order zero, C is concentration ($\mu\text{mol liter}^{-1}$), a is cylinder radius (cm), r is radial distance (cm) from cylinder center, r' is the integration variable, t is time (sec), and D is the apparent diffusion coefficient in water. At 10°C , D was taken to be $1.57 \times 10^{-5} \text{ cm}^2 \text{ sec}^{-1}$ [6], and a cylinder radius (a) of 0.3 cm was used in all calculations.

This equation was formulated for diffusion of a solute within a cylinder to a surrounding medium of concentration zero, whereas we wished to model the diffusion of O_2 from a saturated, exterior environment into a cylinder of initial concentration zero. We therefore needed the complement of Crank's equation. This requirement is made clear by defining $C' = C_0 - C(r)$, so that the equation for unsteady diffusion into our gut cylinder can be written:

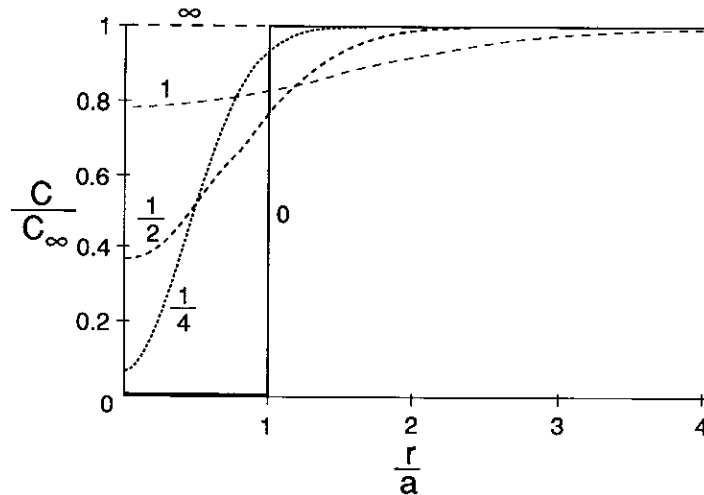


Fig. 3. Predicted solute distributions for a cylindrical sink of initial concentration zero as a function of (nondimensional) time. A uniform diffusion coefficient holds inside and outside the sink (see text). Numbers on curves are values of $(Dt/a^2)^{0.5}$.

$$C' = C_0 - \frac{C_0}{2Dt} e^{(-r^2/4Dt)} \int_0^a e^{(-r'^2/4Dt)} I_0\left(\frac{rr'}{2Dt}\right) r' dr' \quad (2)$$

Solving eq. 2, the solute gradient is seen to diminish, initially rapidly, as t increases (Fig. 3). Integrating the area between the resulting curves and that when $t = \infty$ (Fig. 3), we calculate the time needed to eliminate a given percentage of the initial concentration difference. Provided the time to remove most, say 90%, of the gradient is small relative to the interval of our measurements, we can then assume that the remaining O_2 flux is due to consumption. That is, the system has reached steady state, and diffusion rate into the cylinder is equal to and driven by consumption rate in the cylinder so that

$$\frac{\partial C}{\partial t} = \frac{1}{r} D \frac{\partial}{\partial r} \left(r \frac{\partial C}{\partial r} \right) - R = 0 \quad (3)$$

where we calculate R as oxygen consumption in $\mu\text{mol cm}^{-3} \text{d}^{-1}$.

Integrating and solving for the boundary conditions we get the equation:

$$R = \frac{4D(C_{r_2} - C_{r_1})}{(r_2^2 - r_1^2)} \quad (4)$$

where r_1 and r_2 are distances from the center of the gut, falling within the region where $\partial C/\partial r$ is roughly linear, and C_{r_1} and C_{r_2} are the associated O_2 concentrations. We chose to use a portion of the O_2 profile outside of the gut rather than inside to avoid the need for porosity and diffusion coefficient values for the luminal contents. Locations of r_1 and r_2 were selected so as to fall within that portion of the sigmoidal curve which approximated a straight line (Fig. 2). The reading at the gut wall is r_1 , and r_2 refers to the farthest distance out from the wall before the R^2 value for a linear, least-squares regression falls below 0.98 or, in the cases of the HgCl_2 -poisoned gut, at 5 and 24 hours, the point at which the highest R^2 (0.94 for both) value is reached. Gut radii were calculated from circumferences of opened gut sections measured at the conclusion of profiling. Summing gut radii and distances of O_2 concentration measurements from the gut wall then gave values for r_1 and r_2 .

Results

Axial Profiles

O₂, Eh, and pH profiles for a typical *Molpadia intermedia* reveal that, in general, physicochemical conditions of material in the deposit-feeder guts examined mimicked the conditions of ambient bulk sediments for pO₂ and pH, whereas Eh values appeared to reflect selectivity in feeding or rapid change not apparent in the other measurements (Fig. 4). Strong pH shifts and reducing conditions, characteristic of many terrestrial and freshwater sediment- and detritus-consuming invertebrates [3], were not observed (Table 1). Spatial and temporal variability of measured parameters is high in sediments and was therefore high among the guts probed as well. In all cases, except surface deposit feeders from bathyal depths, anoxia prevailed within the main gut tract. Eh, however, was rarely reducing (i.e., <0); pH remained nearly neutral and did not appear to change significantly with gut passage. Although extreme conditions of Eh (i.e., reducing conditions) were not frequent, values did appear to decline from foregut to midgut in subtidal animals (*Molpadia*, *Brisaster*, and *Travisia*) and in *Abarenicola*. Values then increased from midgut to hindgut (Fig. 4 and Table 1). In the two terebellids, which ingest reduced sediments, redox potential becomes more positive throughout the gut. In the bathyal holothuroids, Eh was constant throughout the gut. Because spatial and temporal variability was high, a paired statistical test was needed. The Wilcoxon signed-rank test was used; this test, however, requires a minimum sample size of 6 to show significance. Therefore only values for *Molpadia* could be tested for significant differences with this robust procedure. Decrease from foregut to midgut ($P < 0.01$) and increase from midgut to hindgut ($P < 0.02$) were both significant. Paired-sample *t* tests were used for $n < 6$. Although no significant trends were found in these samples (Table 1), small sample size made achievement of high significance unlikely.

Oxygen was present in the diverticula of some of these animals (Table 2). The absence of sediment in all diverticula examined would seem to allow conditions more characteristic of the animal's organs and coelom rather than of the sediment ingested. Nearly neutral pH, positive Eh, and low O₂ values were found in all diverticula examined, with the exception of the midgut caecum of *Travisia foetida*, which was highly reducing. Unfortunately, due to difficulty in collecting this species (the animal is rare and deeply burrowing) and the fragility of its gut, only one cecum was successfully probed for Eh.

Radial Profiles

Radial profiles of field-collected animals revealed oxygen and Eh gradients between the gut wall and the center of the gut lumen. These gradients were observed in all species examined: *Abarenicola pacifica*, *Molpadia intermedia*, *Brisaster latifrons* (Fig. 5), and *Thelepus crispus*. Radial profiles of pH were performed in one deposit feeder, *Molpadia intermedia*, but gradients were not

Table 1. Physicochemical conditions of marine deposit-feeder guts^a

Animal	Ingested sediment ^b	Foregut	Midgut	Hindgut
Echinodermata				
<i>Molpadia intermedia</i>				
Eh ^c	+99 ± 151 (5)	+340 ± 162 (9)	+122 ± 198 (9)	+259 ± 156 (9)
pH	7.2 ± 0.3 (6)	7.5 ± 0.2 (8)	7.5 ± 0.2 (8)	7.5 ± 0.3 (8)
O ₂ ^d	0 ^e (6)	0 (9)	0 (9)	0 (9)
<i>Brisaster latifrons</i>				
Eh	+378 ± 69 (3)	+305 ± 148 (4)	+249 ± 120 (4)	+269 ± 134 (4)
pH	7.5 ± 0.1 (2)	7.3 ± 0 (2)	7.4 ± 0 (2)	7.4 ± 0.1 (2)
O ₂	0 (4)	0 (4)	0 (4)	0 (4)
<i>Pannychia</i> sp.				
Eh	+466 (1)	+359 ± 113 (3)	+386 ± 73 (3)	+404 ± 34 (3)
pH	—	—	—	—
O ₂	49 (1)	114 ± 79 (3)	102 ± 85 (3)	115 ± 62 (3)
<i>Scotoplanes globosa</i>				
Eh	+466 (1)	+360 ± 102 (4)	+375 ± 60 (4)	+377 ± 94 (3)
pH	—	—	—	—
O ₂	49 (1)	13 ± 6 (4)	14 ± 5 (4)	26 ± 19 (3)
Annelida				
<i>Abarenicola pacifica</i>				
Eh	+76 ± 241 (3)	+295 ± 140 (3)	+143 ± 98 (3)	+189 ± 84 (3)
pH	7.2 ± 0.3 (3)	7.0 ± 0.5 (3)	7.3 ± 0.8 (3)	7.0 ± 0.7 (3)
O ₂	0 (4)	0 (4)	0 (4)	0 (4)
<i>Travisia foetida</i>				
Eh	+86 ± 100 (2)	+65 ± 71 (2)	+36 ± 167 (2)	+90 ± 202 (2)
pH	7.7 ± 0.6 (3)	6.9 ± 0.4 (3)	7.2 ± 0.9 (3)	7.7 ± 0.5 (3)
O ₂	0 (2)	0 (2)	0 (2)	0 (2)
<i>Eupolyornia heterobranchia</i>				
Eh	-109 ± 83 (2)	-144 ± 33 (2)	-37 ± 127 (2)	-7 ± 255 (2)
pH	6.9 (1)	6.8 (1)	7.6 (1)	7.2 (1)
O ₂	0 (1)	0 (1)	0 (1)	0 (1)
<i>Thelepus crispus</i>				
Eh	-181 ± 2 (2)	-37 ± 55 (4)	+4 ± 140 (4)	+12 ± 117 (4)
pH	7.2 ± 0.1 (2)	7.6 ± 1.3 (2)	7.5 ± 0.4 (2)	7.0 ± 0.5 (2)
O ₂	0 (4)	0 (4)	0 (4)	0 (4)

^a Mean values with ±1 SD; sample size in parentheses^b Sediment collected from depth of anterior end of deposit feeder; values of actually ingested sediments may be different, especially in feeding pockets associated with subsurface feeders^c Values in mV^d Values in μM^e Below detection limit (ca. 1% saturation)

observed. Given the size of the pH electrodes, however, gradients on the scale of those for Eh and oxygen would be difficult to detect.

Integrating from curves like those in Fig. 3, we calculate that the time at which 90% of the initial gradient should disappear in the absence of further

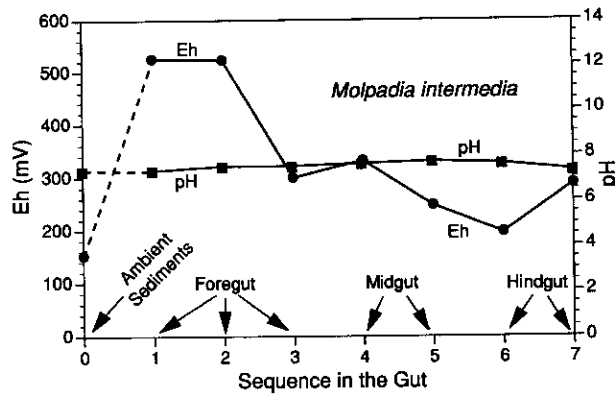


Fig. 4. Typical trends of pH and Eh through the gut of *Molpadia intermedia* and other subtidal deposit feeders. Oxygen was undetectable in all gut sections.

Table 2. Physicochemical conditions of gut diverticula

Deposit feeder	Diverticula		
	Foregut	Midgut	Hindgut
<i>Abarenicola pacifica</i>			
Eh	+429 ± 37 (2)	—	—
pH	6.5 ± 1.1 (2)	—	—
O ₂	50 ± 51 (3)	—	—
<i>Travisia foetida</i>			
Eh	—	-149 (1)	—
pH	—	7.3 ± 0.3 (2)	—
O ₂	—	0 (1)	—
<i>Brisaster latifrons</i>			
Eh	+380 ± 37 (2)	—	+337 ± 81 (3)
pH	7.8 ± 0.8 (3)	—	7.7 ± 0.6 (3)
O ₂	21 ± 29 (2)	—	2 ± 3 (2)

oxygen uptake is ~4 hours. The assumption of steady state, therefore, holds at 5 and 24 hours but is slightly less appropriate at 2 hours when roughly 80% of the gradient, in theory, has been dissipated. These times should be slight overestimates, as the initial gradient is not as large as assumed; i.e., initial O₂ concentration just beneath the gut wall is >0. Results of experimental hindgut O₂ profiles reveal steep gradients (Fig. 6), indicating high initial oxygen flux at 2 and 5 hours, with some decrease by 24 hours (Table 3) along with increased penetration of oxygen into the sediment of the gut. Flux into fixed gut sections was always lower than that for unfixed sections but was significant at short intervals, decreasing to negligible levels by 24 hours (Table 3). The results of two similar experiments using formalin-fixed gut sections gave qualitatively similar results. Flux was greater in hindgut than in foregut sections at both 5 and 24 hours (Fig. 7 and Table 3). At both times, oxygen flux into the foregut was near 40% of that into the hindgut. For comparison with sedimentary oxygen consumption values, which are expressed on an areal basis, our values in units of $\mu\text{mol cm}^{-3} \text{ day}^{-1}$ were converted to $\mu\text{mol cm}^{-2} \text{ day}^{-1}$ by multiplying by $\pi r^2/l$

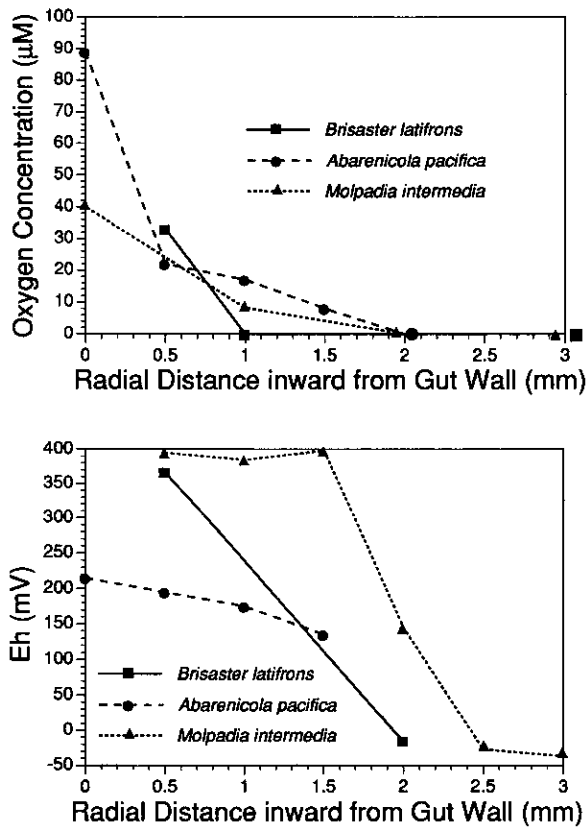


Fig. 5. Characteristic radial profiles of oxygen (top) and Eh (bottom) from the wall toward the center of the guts of various deposit feeders.

Table 3. Oxygen consumption in *Molpadia* foregut, hindgut, and poisoned hindgut^a

Gut section	O ₂ consumption (µmol cm ⁻³ day ⁻¹)		
	2 hours	5 hours	24 hours
Hindgut	10.59 <i>1.75</i>	14.63 <i>2.41</i>	6.91 <i>1.14</i>
Hindgut (poisoned)	7.15 <i>1.18</i>	1.74 <i>0.29</i>	-0.38 <i>-0.06</i>
Foregut	—	6.52 <i>0.98</i>	2.83 <i>0.42</i>

^a Values converted to units of µmol cm⁻² day⁻¹ indicated in italics

$2\pi rl$ (where l is the length of the cylinder) or $r/2$ (Table 3). This calculation in terms of outer area of the gut cylinder clearly biases the comparison in the direction of making gut fluxes appear less important. Gut radii were 3.0, 3.3, and 3.3 mm for FG, HG, and HG + HgCl₂, respectively.

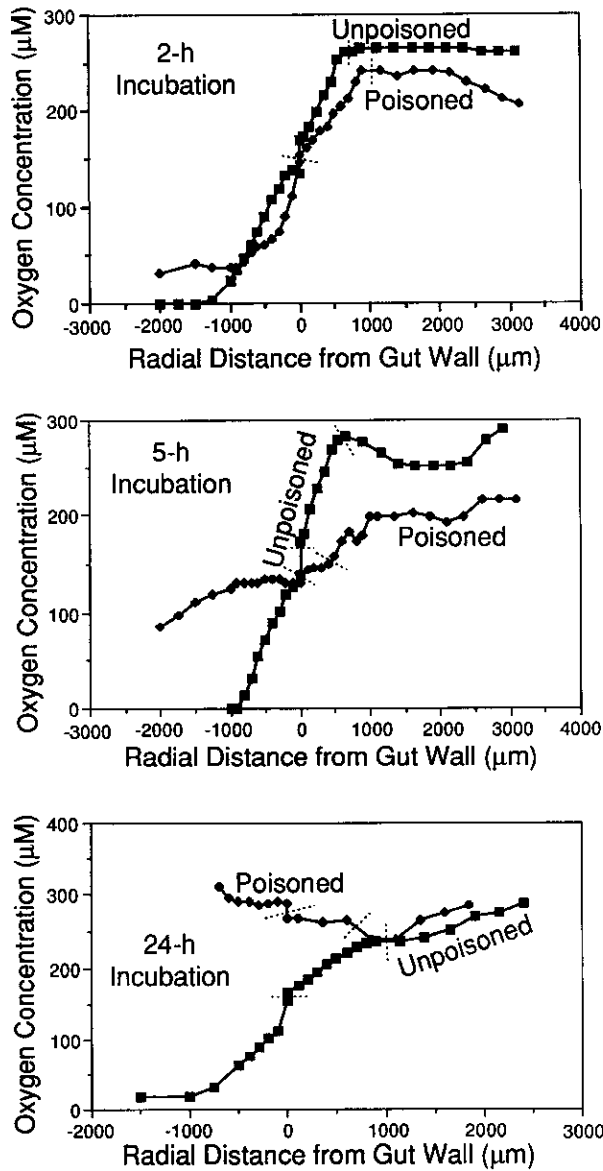


Fig. 6. Oxygen profiles near and into the hindgut of *Molpadia intermedia*. Guts were doubly ligated, removed from the animal, and placed in either sterile seawater or a 0.1 M HgCl₂-sterile seawater solution. Oxygen profiles were recorded at 2 (top), 5 (center), and 24 (bottom) hours. Negative values of "z" are those within the gut lumen. Dashed lines denote that portion of the profile used to calculate oxygen consumption.

Discussion

Axial profiles of oxygen revealed that gut values mimic those of the ambient sediment, but the caveat that physicochemical conditions of ingested sediment may differ from ambient sediment due to selective ingestion and burrow microenvironments must be kept in mind. Values were below detection in all but the deep-sea animals. Ingested materials in bathyal animals are likely to be oxygenated; in their environment oxic sediments can extend to depths of cen-

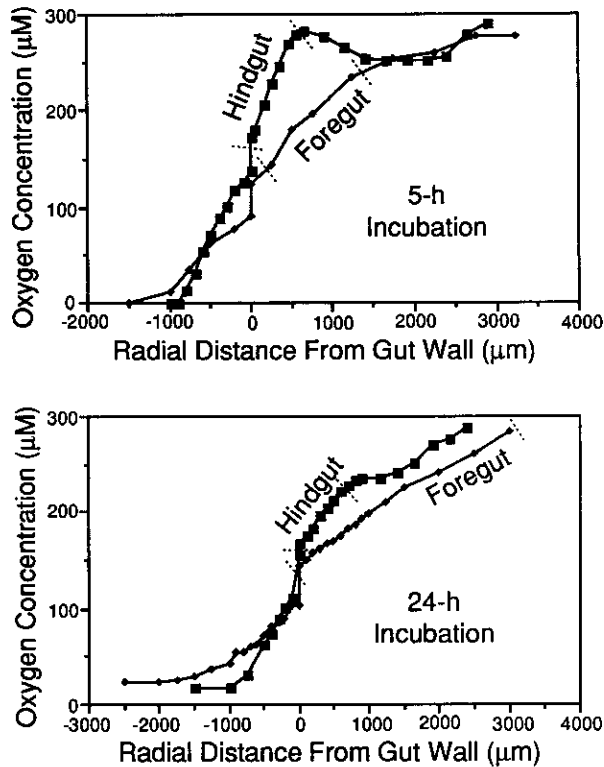


Fig. 7. Oxygen profiles near and into the foregut (top) and hindgut (bottom) of *Molpadia intermedia*.

timeters. We must caution, however, that the time between removal from seabed and measurement for these animals was 4–8 hours. The bulk of microbial metabolism in shallow-water deposit-feeder guts appears likely to be anaerobic. This observation is particularly interesting given the high growth rates of gut bacteria recorded in the hindguts of these animals [42]. Growth of facultative anaerobes would appear to be favored. High rates of formation of products of anaerobic respiration and fermentation thus are likely. The fates of these products remain unknown, but the possibility of benefit to the animal is intriguing.

The prevalence of low O_2 in the guts of these animals is somewhat surprising. Surface deposit feeders should ingest oxygenated sediments, whereas subsurface deposit feeders mix and irrigate sediments prior to ingestion. Our data suggest that, if oxygenation occurs due to pumping of water by subsurface feeders, biological or chemical processes in the surrounding anoxic environment or in the foregut quickly consume added oxygen. Thus, oxygen cannot be measured in ingested sediments, but the oxidation due to irrigation should be reflected in Eh measurements. Both bathyal holothuroids and the terebellids employed in our study are considered surface feeders. Our data, however, support the contention of Nowell et al. [35] that *Eupolyornia heterobranchia* feeds below the surface. Our observation is that these animals are oriented head-down when collected at low tide. Because these tentaculate feeders irrigate the sediment

prior to ingestion, we would expect material to be oxygenated then ingested without further exposure to surrounding reducing sediments. Our specimens, however, were collected at low tide when both the burrow and pore waters are likely O_2 depleted [34], so that irrigation may not provide oxygen. Terebellids and other infauna living in organically rich sediments must endure such conditions of anaerobiosis at low tide [34].

With the exception of *Travisia*, the observed Eh profiles revealed none of the extremely low values in main gut tracts or diverticula that are characteristic of many terrestrial detritus feeders [2, 3, 5], but rather tend to roughly resemble those values in the sediment. We have proposed that the absence of negative Eh values is due to the "openness" of marine animals relative to terrestrial and freshwater organisms [43]. The differing osmotic challenges faced by animals in each of these environments determines their permeability to their surroundings. Marine organisms are highly open to their environments; thus isolated niches where physicochemical conditions can be radically altered are rare. Relatively short gut residence times in deposit feeders may also make such changes more unlikely. In terrestrial animals conditions of unusual chemistry are thought to favor association with a specific and small community or single population of microbes adapted to the unique conditions, whereas other microbes, potential competitors, are inhibited. Strongly reducing conditions and extremely high pH are examples of this type of strategy.

By analogy with terrestrial systems, the electropositive Eh and nearly neutral pH observed in the marine deposit-feeder guts examined suggest that exclusive environments for symbionts are not prevalent and that associations with microbes may be "looser" (facultative rather than obligate) than in many terrestrial detritivores. It therefore appears that foods are readily digested by the animal without the intervention of specific symbionts. The one exception in *Travisia foetida* may indicate a symbiotic association. The elaborate and unusual gut of this species (suggestive of a foregut fermentation chamber), along with the characteristic "rotting garlic" odor for which the species was named, have led to previous speculation that microbial fermentation may be important to the nutrition of this animal [41], but more direct evidence is required. Harsh chemistry, extremely acidic conditions for example, can be used by an animal to nonspecifically eliminate competitive or pathogenic microorganisms [17]. Although microbial numbers have been shown to decrease sharply in the guts of marine detritivores, the use of adverse pH as a means of killing microbes remains undocumented. Other mechanisms, probably enzymatic digestion, must be responsible for their removal.

The profiles do, however, exhibit some changes in redox potential along the gut. In *Molpadia*, *Brisaster*, *Abarenicola*, and *Travisia* an initial decrease in Eh between the foregut and midgut is followed by an increase in the hindgut. The possibility that the latter is due to relatively higher diffusive flux of oxygen through the posterior region of the gut (or decreased consumption) cannot be discounted, but this trend is more probably due to infusion of oxygen to the gut lumen via the anus. Many holothuroids, including *Molpadia*, have respiratory structures attached to the rectum or cloaca. Polychaetes have been shown to exhibit "anal drinking" [22; R.F.L. Self, personal communication]. The polychaetes in our study appear to take in oxygen using this behavior. Mech-

anisms to oxidize this portion of the gut may be necessary to combat S^{2-} toxicity in this highly active anaerobic environment. Alternatively, the organic products of chemoautotrophy in the hindgut could be greatly enhanced with the provision of strong oxidants. This idea is especially intriguing in the case of the terebellids, which contain reducing sediments. Eh continues to rise throughout the gut in these animals. How these animals oxidize gut contents during low tide is unclear. Water may be less oxygen depleted near the tail, closest to the surface, than near the mouth, or oxygen stores in the worm may diffuse across the gut wall enough to alter gut Eh.

Neither significant Eh increase nor decrease was apparent in the guts of the bathyal holothuroids. Relatively lower microbial activities associated with the low concentrations of labile organic matter and low temperatures did not cause noticeable reduction of oxygen or Eh during gut passage. Drop in redox potential between the foregut and hindgut in the shallower subtidal animals examined was most likely attributed to microbial activity. Animal-produced anaerobiosis is possible [52] but not shown to be common, and no advantage for the animal in this case is apparent. A crude estimate of rate of Eh change can be calculated using the Eh profiles and gut residence time estimates. Using dry weight of gut contents and ingestion rate calculations based on Cammen's [9] empirical relationship between ingestion rate and body dry weight, residence time for *Molpadia* from our site was estimated to be 10 hours. Assuming half this time for transit between foregut and midgut, a rough estimate of $\Delta 50 \text{ mV hour}^{-1}$ is found. Not surprisingly, this rate is much lower than the highest rates observed in laboratory pure cultures, which can exceed 500 mV hour^{-1} [25]. The few data for rates of Eh change in environmental samples, however, suggest that our gut rates also are high. For instance, values of $<2 \text{ mV hour}^{-1}$ in aquatic sediments [33] and $\sim 17 \text{ mV hour}^{-1}$ for decomposing algae in seawater [62] are low relative to ours. These data and the rate of 38 mV hour^{-1} in sewage [25] support the idea that activity is high in these gut environments.

Translation of rates of Eh change into decomposition rates is impossible without knowing the specific redox reactions contributing to the overall Eh of the system and the degree of poisoning in the system. What is clear is that as redox potential changes along the gut, different populations of microbes must find conditions for growth most favorable. Factors affecting residence time, such as food quality, gut length, and temperature, are likely to determine dominant microbes in the guts and fecal materials of these animals. Bacterial growth in at least some guts is rapid relative to surrounding sediments. Doubling times on the order of hours have been observed in guts [15, 42] compared to several days to weeks in most sediments [14, 31]. Coupled with high frequencies of ingestion [30, 57, 61], such growth rates have the potential to alter microbial community dominance patterns of the bulk sediment. Drastic changes in the relative numbers of bacterial types between ingested material and feces have been observed in both freshwater [59] and marine [16, 18] systems. Differential growth during gut passage could be due either to differing growth kinetics, e.g., different half-saturation constants (K_m) for digestive products in the gut, or to differing tolerances to physicochemical conditions. The revelation of anaerobic conditions in this study and the results from Duchene et al. [18], in which a transition from an aerobic *Pseudomonas* population to a *Vibrio*-dominated

population was observed with passage through the gut of *Thelepus setosus* (Polychaeta: Terebellidae), support the latter. In addition, recent in situ studies using immunofluorescence reveal that pseudomonads are digested by *Abarenicola pacifica* (Polychaeta: Arenicolidae) but show no regrowth [Plante, unpublished data], in contrast to the total bacterial community, a vibrio, and an aeromonad, all of which demonstrate a strong rebound in numbers in the hindgut [42].

Radial profiles of both Eh and O₂ indicate diffusion of oxygen into the gut lumen from the coelomic fluid through the gut wall or from vascularized gut tissue, resulting in sharp Eh and O₂ gradients. Similar but even sharper gradients exist at the gut walls in termites and ruminants [26]. The significance of these sharp gradients is the potential for greatly accelerated microbial metabolism that requires redox coupling. The gradients found over centimeters in sediments are here reduced to millimeters. To maintain these gradients the supply of oxygen must be depleted rapidly by microbial utilization, as has been demonstrated even in very small (radius = 200 μm) pelagic fecal pellets [1]. It is unclear whether this high activity is a result of the provision of oxygen to chemoautotrophs or whether digestive products of the animal fuel heterotrophic activity that concurrently depletes the oxygen. Using radioisotopes, the extent of chemosynthetic activity could be estimated from CO₂ uptake.

We had hoped to calculate oxygen consumption rates across the gut wall within the animal from electrode data. Measurements of oxygen concentration within the coelomic fluid, however, were inconsistent and not reproducible. High concentrations of dissolved organic matter likely adversely affect electrode performance. Additionally, sharp gradients within the small volume of fluid in close proximity to internal organs probably preclude the maintenance of stable bulk-phase concentrations. Uncertainties surrounding gut residence times for field-collected animals also complicated our effort to calculate O₂ consumption. Instead, we resorted to experiments in which oxygen gradients were measured around gut sections placed in sterile seawater. The most important assumption in the calculations of consumption is that of steady state ($\partial C/\partial t = 0$). The calculations made using eq. 2 and Fig. 3 indicate that the concentration changes over the long intervals at which measurements were made were mild enough to have little influence on our calculated consumption values. A much more complicated model reflecting the composite nature of our system, however, could be used to give more accurate calculations of the time course of diffusive oxygen flux into our experimental gut. For example, the equations for conduction of heat in composite cylinders (eqs. 13.8.7 and 13.8.8 [10]) could be expressed in terms of mass, and the complement taken as we did for Crank's [12] equation. In our situation the differences in diffusion coefficients are not great ($\sim 1 \times 10^{-5}$ for similar sediments [27, 48, 49] compared to 1.57×10^{-5} for seawater used in our original calculations), however, so our estimations using the much simpler eq. 2 should suffice. For comparison, repeating our calculations using the diffusion coefficient of 1×10^{-5} shows that at 5 hours $\sim 90\%$ of the initial gradient will have been dissipated, compared with 92% with the seawater diffusion coefficient, and by 24 hours, $\sim 98\%$ is gone with either of the coefficients. Use of the composite model would result in figures between those found when using either the sediment or seawater diffusion

coefficient. The other reason that we opted for the simpler solution of Fig. 2 is that we felt unsafe assuming a spatial pattern (or uniformity) of the reaction rate inside the gut, aiming our experimental design instead at measuring the purely diffusive gradient outside the gut.

Distinguishing biological from chemical consumption is difficult even with the use of poisons. Addition of formalin or HgCl_2 should eliminate biological activity, but inhibition will not be instantaneous. Additionally, the initial oxygen gradient remains, and unsteady flux will continue. Once the parallel poisoned gut becomes as oxygenated as the surrounding water, we can assume that all microbial activity has been eliminated and that "excess" diffusion due to the initial gradient has ceased, so that all flux in the unpoisoned treatment should be due to microbial activity. Thus, we can estimate a biological consumption of $1\text{--}2 \mu\text{mol cm}^{-2} \text{ day}^{-1}$ at 5 and 24 hours. That the oxygen concentration of the coelomic fluid could not be measured and is likely lower than the saturation level used in this experiment does not seriously endanger our use of this consumption rate for animals in the field, as the rate of bacterial respiration is independent of oxygen concentration at all but the lowest levels [20, 48, 63].

We cannot rule out the possibility that the gut itself may be consuming oxygen, but the nature of the concentration gradients on the luminal side of the wall (Figs. 6 and 7) indicates that the gut contents are indeed a sink. That measured concentration profiles qualitatively resemble those predicted (Fig. 2) and that calculations of consumption using portions of the profile on either side of the gut wall (employing best estimates for the apparent diffusion coefficients of sediments in the gut) are similar (data not shown) suggest that the wall is unlikely to be a significant sink. The sharp drop in concentration at the gut wall (Figs. 6 and 7) may be an artifact caused by the deformation of the wall when punctured, or the wall may act as a barrier to diffusion (Fig. 3.7 in [12]; Fig. 5 in [32]).

Comparison of our consumption estimates with those in sediments is difficult for at least three reasons: bulk-phase oxygen concentrations in our experimental system are dissimilar to those where in situ sediment fluxes have been measured, most oxygen consumption estimates have been made with respirometers that include oxygen consumption due to animal activities, and the few existing consumption data from profiles are from either deep-sea or intertidal environments. The respirometer data of Pamatmat [37] from essentially the same location from which *Molpadia* were collected are the most appropriate for comparison. In situ oxygen concentrations here range from 60–100% of saturation [36]. Additionally, since biological oxygen consumption, a major portion of total consumption, should be concentration-independent, these data can be compared fairly confidently. Pamatmat's sediment consumption rates of $0.86\text{--}1.29 \mu\text{mol cm}^{-2} \text{ day}^{-1}$ are slightly lower than our hindgut values of $1.14\text{--}2.41 \mu\text{mol cm}^{-2} \text{ day}^{-1}$, even though respirometer data typically produce rates 2–4 times those obtained from oxygen profiles in nearshore environments [46]. Respirometer data from other locations of comparable depth and temperatures give similar results ranging from $0.24\text{--}1.19 \mu\text{mol cm}^{-2} \text{ day}^{-1}$ [11, 19, 38]. We know of no comparable data obtained using microelectrodes. Not surprisingly, deep-sea values obtained using microelectrodes are lower by 1–3 orders of

magnitude [44–46]. Rates are comparable, i.e., $2.66 \mu\text{mol cm}^{-2} \text{ day}^{-1}$ at an organically rich (6–7%), shallower (20 m), and warmer (16°C) environment, but are considerably higher within *Fucus* fragments ($10.8 \mu\text{mol cm}^{-2} \text{ day}^{-1}$), and *Beggiatoa* mats ($5.3\text{--}9.6 \mu\text{mol cm}^{-2} \text{ day}^{-1}$) [29]. Oxygen consumption within the hindgut of *Molpadia* appears to be stimulated so that values more closely resemble those from shallower, warmer locations than those of surrounding sediments. Fecal material deposited on the sediment surface will also have a high BOD. We strongly suspect that in some environments pellets produced by deposit feeders may account for a significant fraction of O_2 uptake measured with respirometers.

Comparisons of fluxes into foregut and hindgut sections suggest that oxygen consumption is greater in the hindgut. Direct measurement of microbial growth in these environments is difficult, but previous results using numerical differences suggest that the hindgut is the site for the most rapid growth of bacteria [15, 42]. Tenuous calculations made using our O_2 consumption data and the cell yield conversion factors for various laboratory strains of bacteria [7, 8] or the equation of Sorokin and Kadota [54] relating production to respiration, combined with the estimated abundance of 3×10^9 bacteria (dry g) $^{-1}$ (Plante, unpublished data), suggest generation times on the order of 2–4 hours. Such a growth rate would be very high for sediments, especially at 200 m and 10°C , but we cannot place much reliance on this type of calculation, as the relationship between respiration and growth varies widely among bacterial types and physiological states. Rapid hindgut growth is predicted based on theoretical arguments of digestive product distribution [43]. Optimal digestion theory predicts that animals operating to maximize their own rates of absorptive gain will exhibit high microbial availability of digestive products in their hindguts and feces. Although quantitative estimates of growth rates are not yet possible, our results support these predictions.

Our results are sufficient, however, to reject some of the simplifying assumptions of reactor models thus far employed in optimal digestion theory [13, 40, 43]. The deposit feeders in this study would be modeled as plug-flow reactors. Perfect radial mixing and continuous, orderly flow are assumed in this reactor type. Digestive reactions are considered to be radially homogeneous. The observed gradients reveal that radial mixing is not perfect, supporting the X-radiographic observations of Penry [39] for the holothuroid *Parastichopus californicus*. Additionally, increases in posterior Eh levels suggest reversed flow, at least in the fluid portion of gut contents. The degree to which the utility of these models is compromised by these unmet assumptions is unclear without explicit evaluation of these violations.

Despite demonstration of O_2 flux into the gut, both radial and longitudinal profiles show that the bulk of gut contents remains anaerobic. Peristaltic motion nonetheless will mix ingested material to some extent during gut passage. The guts of deposit feeders, then, provide a unique environment where both mechanical agitation and anaerobiosis are found; outside the gut, sediments that experience mixing due to the movement of animals or water are oxygenated concurrently. Mixing, rather than oxygen per se, likely accounts for field observations that aerobic decomposition of organic matter sometimes is faster than anaerobic decomposition [e.g., 27, 28]. In the laboratory, where mixing

and oxygenation can be separated, aerobic and anaerobic rates of decomposition of labile organic matter are comparable [21, 60]. Agitated anaerobic environments should exhibit greatly stimulated bacterial activity and decomposition relative to stagnant ones. Biogeochemical conversion in deposit feeder guts may rival conversion rates in oxic zones yet will exhibit the characteristics of anaerobic degradation, i.e., end-products of anaerobic respiration such as NH_3 , H_2 , H_2S , and CH_4 , incomplete mineralization, and free fermentation products.

That some O_2 is supplied to the gut via the gut wall and anus should further stimulate conversion, as inhibitory end products of anaerobic metabolism will be removed when oxygenated. Additionally, anoxic decomposition of fresh organic matter may be faster or slower than oxic decomposition, depending on the nature of the substrate [24]. An environment of fluctuating oxygen status, then, may show both faster decomposition rates as well as greater extent of conversion. Furthermore, many deposit feeders, especially those feeding below the interface, feed and defecate at different depths, accentuating changes in redox conditions. Deposit feeding, including events before ingestion and after defecation, subjects organic matter to fluctuating oxygen status. The effect of such a condition on decomposition warrants further exploration.

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