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Food Substrates and Digestive Capabilitites of Marine Deposit Feeders

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Worked for more than 160 Hours: Yes
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Activities and Findings

Research and Education Activities:

Using chemical reactor theory as a context (Penry and Jumars 1987; Dade et al. 1990), our underlying idea was to characterize food resources used by deposit feeders through measurement of luminal contents and substrate-specific kinetics of hydrolysis and absorption. We focused on proteins initially because of their central importance in determining organism absorption efficiencies. We have:
- derived consistently, in a manner easily implemented in symbolic mathematics programs (e.g., Mathematica and Maple), reactor theory of digestion for the three kinds of ideal reactors and contrasted predictions for digestion of simple sugars versus proteins (Jumars and Martãnez del Rio 1999; Jumars 2000a);
- developed numerical models, implemented in Stella, of non-ideal reactor behaviors observed and expected in animal guts and used them to make predictions about the axial distribution of absorptive sites (Jumars 2000b);
- assessed the differences (e.g., nonspecific adsorption coefficients of hydrolytic enzymes and requirements for concentrated foods) and similarities (e.g., ratios of protease to lipase activities on similar substrates) between hydrolysis produced externally by bacterial communities and in the gut lumen by individual deposit feeders (Mayer et al. 2000);
- miniaturized luminal analyses to volumes as small as 1 A*l, extending their potential application to the majority of deposit feeders and many other small, benthic and planktonic animals;
- by manipulating the diet of an omnivore, tested and confirmed the association between deposit feeding and luminal surfactant concentrations sufficienct to form micelles (Bock and Mayer 1999);
- found severe problems with often-used (Poiseuille or pipe-flow) models of digesta flow through animals and made direct observations of mixing in the gut that challenge simple parameterizations of an 'unstirred layer' at the gut wall (Rangel and Jumars, in manuscript);
- with nonabsorbable dyes and radioactive tracers we accumulated evidence of water uptake from the lumen near the foregut-midgut junction in lugworms and indications of water inflow into the hindgut lumen;
- because of this evidence of anteriorward flow of aqueous components and underestimation of absorption efficiencies in our early reactor-theory models, began studies of hindgut function that include 16s ribosomal DNA assessment of the taxonomic affinities of commensal bacterial communities c* finding that they contain, among other taxa, strains most closely related to rumen bacteria and bivalve gill symbionts (Lau, Jumars and Armbrust 2002).

Findings:

Our theoretical modeling of deposit feeders, begun under the previous grant (OCE-9202855) and originally envisaged as a single manuscript, grew during and after lengthy review into three papers. Very thorough review of the original manuscript by Carlos Martãnez del Rio led to a collaboration rederiving reactor theory for animals that eat foods that do not require hydrolysis and to comparison of its predictions against many recent observations for nectarivores and frugivores. Our conclusion is that reactor theory under the premise of maximization of absorption rate consistently underestimates absorption efficiencies and that failure to include constraints of water uptake and excretion is a likely cause of the discrepancy (Jumars and Martãnez del Rio 1999).

The most extensive of the three papers derives reactor theory of digestion for each of the three kinds of ideal reactors (plug flow, continuously stirred tank and batch reactors) and solves for operating policies that maximize absorption rates under three different kinds of kinetics c* linear, hyperbolic and sigmoidal (Jumars 2000a). Predictions include that animals digesting proteins should show maximal ingestion rates on foods of intermediate protein concentration, whereas animals eating simpler foods like disaccharides should steadily slow ingestion rates as sugar concentrations increase. Previously, observations of compensatory feeding (faster feeding on food of lower quality) typically would have been interpreted as evidence against absorption-rate maximization. We also pointed out that the general underestimation of observed absorption efficiencies should be expected because this derivation and earlier ones neither explicitly nor implicitly include processes in the hindgut. To provide access, we derived the ideal reactor solutions in such a way as to make them easy to implement with Mathematica or other symbolic solvers.

The third paper took a very different approach to treat some of the known, non-ideal behaviors of real animal guts. It employed compartmental models, implemented in Stella, to simulate digestion in animals with tubular guts. We found the predictions of ideal reactor models to be relatively insensitive to the moderate axial mixing expecrted and observed in real guts. The implementation also permitted us to predict the optimal axial distribution of absorptive sites, explicitly connecting gut structure at the level of absorptive sites with gut function. At the ingestion rate that maximizes uptake of digestive products, absorptive sites should be concentrated near the rear of the hydrolytic-absorptive gut sections. High food quality or suboptimal ingestion rates, however, decrease the advantage of axially concentrated absorbers (Jumars 2000b).

We are also beginning to shed some new light on the questions of bacterial versus deposit-feeder roles in organic degradation and were invited to present our evolving views at the recent Baruch Symposium on Organism-Sediment Relationships. On other projects funded by ONR, we have helped to develop theory of bacteria as individual foragers (Vetter et al. 1998) and from the viewpoint of clonal fitness that appears to have some capability to explain the uniformity of benthic bacterial abundance at 10^9 cells per milliliter of pore water (Schmidt et al. 1998, 2002). Comparing the foraging of individual deposit feeders and sedimentary bacterial communities for particulate detritus via extracellular digestion has revealed some interesting similarities and differences. Bacterial communities and deposit-feeding individuals, however, are remarkably similar in lipase:protease ratios produced on a variety of foods. To succeed at returning a net profit of products to the producer,

released exoenzymes from bacteria must be tightly adsorbing, whereas digestive enzymes contained in the lumen are nonabsorptive. Bacteria succeed at low-intensity digestion of dilute and slowly hydrolyzable foods, whereas deposit feeders apply a more intense digestion to higher food concentrations of more labile foods. Containment in the gut allows deposit feeders to maintain high enzyme concentrations and surfactant concentrations above the critical concentration for micelle formation or CMC, contributing to their higher digestive intensity (Mayer et al. 2002).

A major thrust of our previous proposal was to miniaturize in vitro assays so that they could be applied to smaller, more abundant deposit feeders. We succeeded to the point where we are now able to carry out a full suite of enzyme assays on a single microliter of gut fluid. In addition to sampling protocols for smaller animals, using a micropipette, we developed enzyme assays using either a HPLC microflow cell in an HPLC fluorescence detector or a newly purchased microplate reader. The high sample handling capability of the microplate reader in fact expanded our analysis to new enzyme systems, such as alpha- and beta-glucosidase.

Our initial survey (Mayer et al. 1997) focused only on relatively large polychaetes and echinoderms, and its results confounded differences due to body size with phyletic differences. As expected if hydrolysis and absorption are to be accomplished in a shorter gut residence time, the smaller animals (polychaetes) showed higher enzyme activities. We extended our survey to about two dozen additional species from Alaska to California on the West Coast and from Maine to Florida on the East Coast, including our first carbonate-dwelling species. It included several additional phyla such as echiura, sipuncula, priapula and hemichordata. Results conformed to previous findings, with small deposit feeders generally having high enzyme activities. As before, however, this trend accounted for only a small fraction of the total variance in enzyme activity among species (Mayer et al. 2001 and in preparation).

These gut fluid collections were analyzed for trace metal contents, based on earlier suggestions of anomalously high levels of metals in deposit feeder guts. We found these high levels in animals from uncontaminated environments to carry across several phyla of benthic invertebrates (Chen et al. 2000). Experimental work subsequently showed that metals have both positive and negative impacts on digestive enzyme function, depending on concentration (Chen et al. 2002).

Deposit feeders of all sizes showed surfactant concentrations above the CMC. To test whether this correlation was a function of diet rather than taxon, we manipulated the diet of an omnivore, Nereis virens, to span the range of diets that we encountered across taxa. Ratios of lipase to protease activity, high among carnivores relative to detritivores, were elevated in Nereis fed diets of fresh cellular material c* be it animal or algal cells c* relative to a diet of unamended sediment. Elevated lipase activities presumably respond to the need to hydrolyze esterified lipids in the fresh cellular material, which constitutes a minor fraction of available food in unamended sediments. Another functional response was to elevate surfactant secretion into the gut above CMC levels if sediment was ingested (Bock and Mayer 1998).

These surfactants are able to facilitate lipid digestion. We extended previous work on contaminant lipid solubilization to study nutritional lipid digestion by deposit-feeder surfactants. We collaborated with R. Findlay (Miami U.) to identify the three major surfactants found in Arenicola gut fluid; they consist of C8 fatty acids which are amide-bonded to either glycine or leucine (Smoot et al., in preparation). We found that Arenicola surfactants are remarkably similar to vertebrate surfactants of vastly different molecular structure in terms of their capability to solubilize various nutritional lipids a* parameterized as molar solubilization ratios of nutritional lipid to surfactant. We also uncovered the possibility that some benthic invertebrates use emulsification rather than micellar solubilization to mobilize lipids for transport to the gut epithelium.

By titrating gut fluids with additional surface areas of sediment grains, we tested the hypothesis that luminal surfactants adsorb to sediment grains, making them nonabsorptive of enzymes and hydrolysis products. Results failed, however, to reject the null hypothesis of no effect (Mayer et al. 2002). These surfactants thus behave similarly to enzymes in their minimal adsorption to transiting sediment and adhere to the imperative that digestive investment in metazoans should not be lost to the environment. Interestingly, we have found that sediments highly contaminated with anthropogenic organic matter can be sorptive to surfactants.

The contrasts extracted by Mayer et al. (2002) from published results between deposit feeders and sedimentary bacteria have led to a series of experiments in which we tested hypothesized similarities and differences between animal and bacterial digestion. In particular, we need common currency in defining digestive agent activities to test our framework equation. We focused on the bacterial endmember. For example, we performed experiments with sedimentary bacterial communities that mimic the Nereis work described above to examine response of lipase:protease ratios to food substrate. We found that on natural sediments bacterial exoenzymes have similar lipase:protease ratios as detritivorous, deposit-feeding animals. Addition of fresh cellular material, in the form of algal cells, elevates lipase:protease ratios by small but significant levels, similar in direction but not in magnitude to what we found with Nereis. This finding substantiates a premise of the conceptual work c* that bacterial and deposit-feeder enzymes have considerable similarity except for the geometrical constraints of their deployment. We also found that the protease activity normalized to bacterial cell number was remarkably constant among experiments and treatments and even similar to previous data from field collections. Although there is variance in the ratio, it falls within an order of magnitude of the mean values, implying that this particular microbial digestive activity is closely tied to biomass dynamics. One striking consequence of this constancy was revealed by an experiment in which we added algal detritus to flasks with only liquid media and small bacterial inocula and also to sedimentary microcosms with bacterial densities at normal field values. In the former, protease activity followed the exponential rise and then slow falloff of bacterial biomass, while in the sediment microcosm neither bacterial biomass nor protease activity changed markedly c* in spite of similar kinetics of algal decay between the two types of incubation conditions.

Ancillary hypotheses, not driven by the animal vs. bacterial question contrast, were also addressed in these decay experiments. One result of interest is a rapid decrease in lability to enzymatic attack, as defined by our previously published EHAA method (enzymatically hydrolyzable amino acids; Mayer et al. 1995). Specifically the ratio of EHAA to acid-hydrolyzable amino acids decreases. It is not yet clear whether this

decrease in lability of amino acids is due primarily to incorporation of algal detritus into bacterial biomass or to a chemical change in the algal detritus chemistry; we have evidence for some involvement of both processes. We also observed rapid formation of dissolved humic material, especially in incubations using isolated algal membrane material. This result implies an important role for lipids in the lability of detritus, a point that we have made before in other contexts (Laursen et al. 1996).

An accurate reactor-theory model requires accurate specification not only of reaction kinetics, but also of flow patterns in and through the reactor. We have posited that most tubular guts can be approximated as a series of n mixing cells where n is approximately L/D and L and D are gut length and diameter, respectively (Jumars 2000b). We made direct observations of gut motility in a small, transparent ampharetid polychaete, Hobsonia florida. The only muscular movement observed was antiperistalsis (waves of contraction moving from rear to front), accompanied by clear mixing of particles radially and axially on length scales of approximately D. Counterintuitively, antiperistalsis is consistent with slow net drift backward of particles near the middle of the gut. Unlike peristaltic pumps, the lumen in deposit feeders is occluded only partially, and so fluid is not squeezed along in the direction of the wave. This deposit feeder has a narrower foregut followed by a broader midgut, and the two gut sections show different frequencies of antiperistaltic contractions; we hypothesized that if radial mixing is a major function of the contractions, the broader gut region should show less reduction in contraction frequency at lower temperatures, and it does (Rangel and Jumars, in manuscript). In both gut sections at all temperatures studied, contractions were frequent enough to preclude development of an 'unstirred layer' at the gut wall.

We have observed various pieces of evidence that water is taken up from the lumen in the ceca at the foregut-midgut junction and at the midgut of the lugworm Abarenicola pacifica and that water flows from the posterior of the gut toward these gut sections. Dissolved proteins reach 10 times the levels that could be produced by dissolving all the protein contained in an entire gutfull of sediment (Mayer et al. 1997). Worms placed in a solution of food coloring show similar concentration of the dye in these gut sections. Worms placed in a dilute solution of radiolabeled polyethylene glycol (PEG) show up to six-fold concentration in ceca and midguts within 10 h. In excised guts through which labeled PEG is forced by peristaltic pump, water apparently is taken up in midgut-foregut sections. The interpretation is made ambiguous, however, because our controls reveal that PEG adsorbs to the gut wall and to sediments. Osmolality of luminal fluids is consistent with flow of water out of the cecum in the ceca and midgut and into the lumen in the hindgut; lumen fluid is hyposmotic to body fluids in the ceca and midgut but is hyperosmotic to body fluids in the hindgut. Manipulating the osmolarity of luminal fluids suggests that active physiological mechanisms are involved, as apparent fluxes of water out of the lumen at the ceca and midgut and into the lumen at the hindgut appear to persist even in the presence of unfavorable osmotic gradients. Anal uptake of water has been seen in marine invertebrates, for which such a flow produces no great osmotic stress, and may also contribute to anteriorward flow. Compaction of sediments during gut passage is a less controversial mechanism for generating (potentially) anteriorward flow of the expelled fluid.

This potential pattern of water flow changes thinking about possible roles of hindgut symbionts. If substantiated, it lends credence to our earlier-suggested path of evolution from deposit feeders to vestimentiferans (Plante et al. 1990). Even if it is not substantiated, however, it is clear that a major shortcoming of reactor-theory formulations used thus far is failure to incorporate hindgut functions. As a means to begin investigating relevant microbial processes there, we ran 16s DNA analyses on the bacterial community attached to the hindgut cuticle of deposit-feeding but not suspension-feeding, thalassinid shrimp. The bacterial community is very diverse and includes strains most closely aligned with Cytophaga spp., rumen bacteria and Solemya gill symbionts. To get a better idea of potential geochemical and nutritional roles, we manipulated the diets of the shrimp and (Lau, Jumars and Armbrust 2002).

Our deposit-feeding work attracted related, more exploratory, contributions from two masterÆs students supported by separate NSF fellowships, with the NSF grant to UW providing parts of their materials and supplies needs. Micaela Parker studied deposit-feeder genetic heterogeneity. One of the chronic problems in detecting treatment effects on feeding rates of deposit feeders is the large variance among individuals within treatments, even when they are maintained for long periods under uniform conditions. Paired designs often are needed to detect treatment effects at all. We wondered whether this variability might have a genetic basis. As a very rudimentary and preliminary step in developing a system for examining deposit-feeder genetic heterogeneity, Ms. Parker assessed isozyme variability within and among Puget Sound populations of the deposit-feeding clam Macoma balthica. Although she found substantial genetic heterogeneity among individuals, there was less consistent difference among Puget Sound sampling sites than in a sympatric, suspension-feeding bivalve (Parker and Jumars, in manuscript). Results for both bivalves show some heterozygote deficiency relative to Hardy-Weinberg expectation. One explanation consistent with previous observations and these genetic results is that bivalves that feed faster or more continuously are more subject to predation, trading off energetic gain against predation risk. This hypothesis of balancing selection for and against rapid or continuous feeding may warrant more attention in the future.

Because of our accumulating evidence of liquid reflux and her interests in microbial genetics, Winnie Lau began studies of hindgut function that include16s ribosomal DNA assessment of the taxonomic affinities of commensal bacterial communities. Gut microbes cluster into three major groups, Proteobacteria related to known hydrothermal-vent endosymbionts, Cytophaga-Flavobacteria whose members are potent enzyme producers (including cellulases), and Gram-positive bacteria among which most fermenters lie (Lau, Jumars and Armbrust 2002). Her study shows that bacterial DNA extractions are feasible despite extraction difficulties from sediments and resolution problems from host DNA. Besides illuminating previous hypotheses about evolution of hindgut symbionts, it points the way toward future work on both nutritional contributions to the host and hindguts of marine animals as likely habitat sources of difficult-to-culture bacterial strains found in the ambient environment.

Training and Development:

This project supported
- part of the PhD program for Ian Voparil
- post-doctoral training for Michael Bock
- research funding for various summer intern projects related to the grant objectives

Outreach Activities:

Radio and newspaper interviews
Talks to lay groups, including the media

Journal Publications

Bock, M. and L. Mayer, "Digestive plasticity of the marine benthic omnivore Nereis viren", Journal of Experimental Marine Biology and Ecology, p. 77, vol. 240, (1999). Published

Chen, Z., L.M. Mayer, C. QuÚtel, O.F.X. Donard, R.F.L. Self, P.A. Jumars, and D.P. Weston, "High concentrations of complexed metals in the guts of deposit-feeders", Limnology and Oceanography, p. 1358, vol. 45, (2000). Published

Chen, Z. L. M. Mayer, D.P. Weston, M. J. Bock, P. A. Jumars, "Inhibition of digestive enzyme activities by copper in the guts of various marine benthic invertebrates", Environmental Toxicology and Chemistry, p. 1243, vol. 21, (2002). Published

Mayer, L.M., D.P. Weston, and M.J. Bock, "Benzo-a-pyrene and zinc solubilization by digestive fluids of benthic invertebrates - a cross-phyletic study", Environmental Toxicology and Chemistry, p. 1890, vol. 20, (2001). Published

Books or Other One-time Publications

Mayer, L.M., P.A. Jumars, M.J. Bock, Y.-A. Vetter, and J.L. Schmidt, "Two Roads to Sparagmos: Extracellular digestion of sedimentary food by bacterial inoculation vs. deposit-feeding.", (2001). Book, Published
Editor(s): J.Y. Aller, S.A. Woodin and R.A. Aller
Collection: Organism-Sediment Interactions
Bibliography: Univ. of South Carolina Press, Columbia

Web/Internet Site

URL(s):

Description:

Other Specific Products

Contributions

Contributions within Discipline:

Our findings have improved basic understanding of both food resources and digestive mechanisms of organisms living on the sea bottom.

Contributions to Other Disciplines:

These principles have application outside benthic ecology. For example, Mayer was invited to present the benthic perspective at a recent workshop focused on zooplankton nutrition. The work on bioavailability transfers well to the ecotoxicology, and has provided a basis for work on environmental contamination (discussed below).

Contributions to Human Resource Development:

In addition to the training in-house documented elsewhere, our lab has hosted visiting scientists (especially students) to learn our methods of digestive physiology and food resource measurement. Examples include L. Gulman (WHOI) and J. Judd (Berkeley). Our lab has also served as

Contributions to Resources for Research and Education:

Contributions Beyond Science and Engineering:

The principles developed in this project are now being applied in another project, funded by the Army Corps of Engineers, to develop an analytical 'cocktail' to measure the bioavailable fraction of contaminants in dredged sediments. Once completed, this new methodology should result in significant cost savings in the decision-making process leading to dredge spoils disposal.

Categories for which nothing is reported:

Any Product

Contributions: To Any Resources for Research and Education