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Emulsions versus micelles in the digestion of lipids by benthic invertebrates

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Abstract

Lipids can be valuable sources of energy and nutrition for organisms, but their assimilation into organisms is hampered by the difficulty of transporting these hydrophobic compounds through water. In the digestive tract of metazoans, extracellular digestion of lipids requires transport of insoluble materials from the zone of digestion to the site of absorption, which is facilitated by formation of lipid aggregates in the form of micelles or emulsions. We examined the use of these two transport modes in benthic invertebrate animals via experiments and cross-phyletic characteristics of gut fluids. We examined seven benthic species for emulsions versus micelles. An obligate carnivore used emulsions (most droplet diameters were 1–20 μm), whereas deposit feeders used much smaller micelles, and a suspension feeder used neither. We tested two possible forcing factors for this trend. First, we found emulsions but not micelles to be subject to straining by sediment—a filtration process that reduces permeability to colloidal materials. Second, we varied the ratio of gut fluid to food concentration. High ratios, found with dilute food conditions characteristic of deposit feeding, favored micelles, and low ratios favored emulsions. Both of these findings are consistent with the cross-phyletic results.

Nutritional lipids are rich sources of energy; they contain more calories per unit weight than do proteins or carbohydrates. Many lipids are also essential nutrients. Heterotrophic organisms have difficulty digesting and assimilating these compounds, however, because lipids dissolve poorly in water. For marine heterotrophs, dissolved salts further decrease the solubility of most lipids (the ''salting-out'' effect; Setschenow 1889).

Mobilization of lipids into the aqueous phase can be enhanced by surfactants because they have both hydrophobic and hydrophilic regions that stabilize the lipid–water interface and they can form aggregates with a hydrophilic exterior and hydrophobic interior. These aggregates can in turn solubilize other lipids (which are then called ''solubilizates''; Carey and Small 1970). Bacteria use this strategy sparingly in aquatic systems because secretion of surfactants can involve net loss of carbon and nitrogen in dilute media. Metazoans, on the other hand, more frequently use surfactants that mobilize ingested particulate lipids for fluid-phase transport across the aqueous gut lumen to the gut wall for assimilation (Van den Oord et al. 1965; Vonk and Western 1984; Mayer et al. 1997). Their advantage lies in enclosure of their extrasomatic gut environment, which permits recovery of the secreted surfactants along with digested lipids (Mayer et al. 2001*a*). The transfer of hydrophobic solubilizates into aggregates increases their mobility in the gut by orders of magnitude (Voparil and Mayer 2000).

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Aggregates vary in size, depending on the particular surfactant and solubilizate constituents and their relative concentrations. The smallest aggregates are surfactant micelles (hydrodynamic radii $\leq$ 4 nm for human bile salt micelles ex vivo; Hernell et al. 1990), which form without additional solubilizates. Much larger aggregates, such as vesicles and emulsion droplets, form when specific types of solubilizates surpass threshold concentrations in the fluid phases (Staggers et al. 1990). Particularly effective emulsifiers include ionized fatty acids, monoglycerides, and phospholipids (Carey et al. 1983). The largest aggregates are emulsion droplets, defined as two immiscible liquid phases, one dispersed in the other in the form of droplets (Barnes 1994), that can be large enough to be visible to the naked eye.

Micelles require greater investment in chemical capital (surfactant molecules) per solubilizate molecule transported, and, therefore, they might be disadvantageous for use by organisms that are food limited. The return on this investment can be parameterized by the molar ratio of solubilizates to surfactants (MSR) in aggregates. This ratio scales according to volume : surface area, assuming that surfactants dominate the surface and solubilizates dominate the interior. This scaling ($[4\pi r^3/3]/[4\pi r^2] = r/3$; r = radius) leads to MSR values for micellar systems that are orders of magnitude smaller than values for emulsions (Carey and Small 1970).

Micelles and emulsion droplets can behave like suspensions of solid colloids or particles. For example, aggregates passing through tortuous particulate media can be captured by constrictions of smaller sizes. This retention, called ''straining,'' should affect larger aggregates more than smaller ones, and it is a common problem in the extraction of oil from rocks. When drilling fluids emulsify, recovery of hydrocarbons can decrease by 55% (Castro-Dantas et al. 2001). Surfactant injection is a common strategy for clearing clogged oil wells. Greater concentrations of surfactant relative to solubilizate disperse larger emulsion droplets into micelles, which can flow more easily through

Table 1. Marine invertebrate gut fluids investigated.

Species (location)	Taxon	Feeding mode	CMD*	Emulsion†
Cucumaria frondosa (ME)	Holothuroid	Suspension feeder	ND	No
Molpadia intermedia (WA)	Holothuroid	Deposit feeder	ND	No
Parastichopus californicus (WA)	Holothuroid	Deposit feeder	40%	No
Arenicola brasiliensis (CA)	Polychaete	Deposit feeder	22%	No
Arenicola marina (ME)	Polychaete	Deposit feeder	15%	No
Nereis virens (ME) - sediment diet	Polychaete	Omnivore	26% (avg.)	No
Nereis virens (ME) - mussel diet	Polychaete	Omnivore	22% (avg.)	Yes
Glycera dibranchiata (ME)	Polychaete	Carnivore	ND‡	Yes

* CMD, critical micelle dilution; the dilution below which surfactant micelles are converted to monomers. ND indicates that micelles were not detected.
† Via Nile Red visualization.
‡ This gut fluid was too viscous for accurate CMD measurement via dilution with seawater.

the constrictions of the medium (Angle 2001; Castro-Dantas et al. 2001).

Within the gut of a deposit feeder, which is analogous to the situation in an oil well, fluidized material needs to migrate through a complex of mineral grains if absorption is to occur. Deposit feeders may have to deal with straining when mobilizing ingested lipids within their digestive tracts. In some deposit feeders, sediment traversing the digestive tract is not radially mixed (Penry 1989), requiring solubilized material to pass through the sediment to reach absorptive cells lining the gut for assimilation. Sediment straining may thus decrease the efficiency of lipid assimilation by deposit feeders. Straining should have greater effect on larger aggregates, such as emulsion droplets, and provide impetus for deposit feeders to favor micellization rather than emulsification to transport lipids in the gut.

In this paper, we examined tendencies for emulsification versus micellization as lipid transport mechanisms in benthic invertebrates. We focused on the potential for straining by determining the permeability through sediment of micelles and emulsion droplets suspended in a deposit feeder's gut fluid. We analyzed these two types of aggregates in an interphyletic collection of animals coupled with a within-species study of the omnivore *Nereis virens*, which were fed foods of different compositions. Finally, we tested the mode of aggregation (micelles vs. emulsion droplets) under varying in vitro food lipid and digestive fluid conditions.

Materials and methods

Interphyletic survey—Seven species of benthic animals were collected from subtidal and intertidal sites in Washington, California, and Maine (Table 1). They were deposit feeders, except for one suspension feeder (*Cucumaria frondosa*) and an obligate carnivore (*Glycera dibranchiata*). Uninjured animals were dissected immediately for collection of fluid from the digestive tract. Gut fluids were removed by carefully cutting open the body wall and inserting a pipette tip directly into the midgut. Most individuals had sediment in the gut, likely indicating active feeding at the time of collection, but the presence or absence of sediment was not used as a criterion for keeping the fluids from an individual. The midgut usually has maximal enzyme activities and surfactant concentrations (Mayer et al. 1997). Fluids from individuals of each species were pooled, centrifuged (1,200 g for 10 min) to remove mineral particles, and stored at −80°C until surfactancy and emulsion droplet size were measured. Experience indicated that such treatment did not influence the presence or absence of micelles and emulsion droplets. These gut fluids were not filtered, which would have removed emulsion droplets.

Surfactancy measurements—We measured the change in the contact angle of gut fluid during titration with artificial seawater (ASW; Parsons et al. 1984) on Parafilm following the methods of Mayer et al. (1997) to determine the presence of surfactant micelles in the gut fluids. If micelles are present, contact angles remain constant during initial dilution. Once diluted below the surfactants' critical micelle concentrations (CMC), contact angles increase to become more like those of pure water. This inflection point is termed the "critical micelle dilution" (CMD); greater concentrations of a particular surfactant cause lower CMDs. A CMC value is specific for a particular surfactant; thus, we cannot use CMD values to compare concentrations of surfactants in different species' gut fluids because the identities of the surfactants used by these different animals are unknown.

Emulsion droplet size analysis—In order to visualize lipid aggregates, 200 μL of each animal's gut fluids were incubated with 10 μg of the fluorescent, lipophilic dye Nile Red (Invitrogen; www.probes.com). Nile Red in chloroform was dispensed into glass test tubes, and the chloroform was evaporated under N_2 gas for 15 min. Gut fluids were added to the tubes and incubated with Nile Red for 60 min on a rotary shaker (30 revolutions per minute [rpm]) in the dark. After incubation, Nile Red fluorescence in the fluids was visualized using a Zeiss Universal microscope with epifluorescence attachment. Filters used for fluorescence were a 480 ± 60 nm filter for excitation, a 515-nm dichroic filter, and a 530-nm long-pass filter for emission (Chroma; www.chroma.com). Digital images were captured with an Apogee KX85 Charge Coupled Device camera with a Cambridge Research and Instrumentation, Inc. tunable RGB (red–green–blue) filter for full-color images (www.apogee-ccd.com). Camera controls were adjusted at the onset for realistic color and intensity and standardized for all subsequent images. Because this

camera setup captures red, green, and blue images in succession and then digitally combines them to form a full-color image, any movement of the droplets results in some blurring of the final images. Under the microscope, emulsion droplets appeared spherical with sharply defined borders, whereas micelles appeared as an unresolved background haze.

Digital images of the fluids were analyzed using the "measure/count" function of Image-Pro Plus software (Media Cybernetics; www.mediacy.com). Light intensity required to differentiate an emulsion droplet from the background was manually adjusted for each image to ensure recognition of all visible droplets. For images of mussel tissue and gut fluid (see following), emulsion droplets were distinguished from tissue particles by eye. Each droplet's radius was calculated by Image Pro Plus after the software was calibrated with an external scale.

Feeding experiment—Individuals of the omnivorous polychaete, *Nereis virens*, were fed three different diets to determine the plasticity of their strategies for mobilizing lipids in the diet, i.e., whether the sizes of lipid aggregates in the gut are influenced by the composition of ingested material. Worms and the surface sediments surrounding their burrows were collected from the intertidal mud flat in Lowes Cove, Walpole, Maine, U.S.A. Worms were assigned to a dietary treatment (8 worms per treatment) and housed individually in $7 \times 7 \times 10$ cm buckets with screens placed over all openings to prevent escape. Buckets were placed randomly in a large, flowing-seawater table with sufficient seawater flow to prevent anoxia.

Dietary treatments for this experiment were (1) freeze-dried mussel tissue (*Mytilus edulis*), (2) organic matter–free sediment amended with freeze-dried mussel tissue, and (3) natural sediment. These treatments represented, respectively, a carnivorous diet (very high lipid, no mineral), a lipid- and protein-enriched deposit feeder's diet (high lipid, high mineral), and a normal deposit feeder's diet (low lipid, high mineral). Mussels were collected from the Darling Marine Center dock, and their tissues were homogenized in a blender. The resulting mussel paste was freeze dried in ice-cube trays. For the organic matter–free sediment + mussel treatment, Lowes Cove sediments were muffled at 400°C for 12 h to oxidize organic matter and physically homogenized by mortar and pestle. Mussel tissue (the same as used in the first treatment) was added to sediment at 1% by weight. This fraction of mussel tissue was chosen to match the organic carbon content of typical sediment. For the regular deposit-feeder diet, sediment was rinsed repeatedly with clean seawater to remove debris, passed through a 1-mm sieve, and freeze dried in ice-cube trays. The food treatments were measured for organic carbon and nitrogen on a Perkin Elmer 2400B elemental analyzer following vapor-phase acidification to remove carbonate minerals. Food treatments were cleaned out and replaced with fresh material every 72 h; there was always excess food for the animals. On the ninth day, the animals' gut fluids were removed for surfactancy measurements and emulsion droplet size analysis.

Straining of lipid aggregates—*Arenicola marina* gut fluid (containing micelles) and an aliquot of this fluid amended to include emulsion droplets were passed through a column of sediment under conditions designed to mimic this animal's gut. Individuals of *A. marina* (a deposit-feeding polychaete) and sediment from their feeding funnels were collected from Lubec, Maine, U.S.A. Animals were stored in seawater up to 4 h, and gut fluids were removed by carefully cutting open the body wall and inserting a pipette tip directly into the stomach. Individuals' fluids were pooled, passed through a 0.45-μm PTFE (Teflon) membrane filter, decanted into plastic containers, stored on ice until returned to the laboratory, and then stored at -80°C until use.

Freshly collected *Arenicola* gut fluids contained micelles, as indicated by contact angle changes of gut fluid during titrations with artificial seawater (see following); 0.5 g L^{-1} of Self Emulsified Liquid Concentrate (SELCO; INVE, www.inve.be) was added, and the mixture was vortexed (30 s) to create an emulsion. SELCO is a proprietary mixture of lipids and proteins formulated to emulsify in seawater and commonly used to increase the delivery of nutritional lipids to aquacultured animals. Both fluids were spiked with the highly hydrophobic ^{3}H-benzo(a)pyrene (BaP) at $\sim 5.0 \times 10^{-3}$ μmol L^{-1} to serve as a tracer for the lipid aggregates suspended in solution. This concentration is well below the micellar solubilization capacity for BaP in this gut fluid (Voparil and Mayer 2000), thereby ensuring incorporation of BaP into the aggregates rather than formation of a solid precipitate.

The gut environment was mimicked with sediment from *Arenicola*'s feeding pits. Both fluids were passed once through columns of freeze-dried Lubec sediment supported by a 20-μm Nitex mesh (Sefar Solutions, www.sefar.com). Sediments were packed to a height of 3 mm (the radius of the midgut), and overlying, labeled fluid was drawn through under slight vacuum ($\sim$650 torr) to decrease the time required for fluid permeation. This vacuum may have deformed larger droplets, allowing them to squeeze through restrictions that would be too narrow under less energetic, in vivo conditions, but such squeezing may be characteristic of the transition from less compacted midgut to more compacted hindgut (Kermack 1955). Both fluids were passed through Nitex mesh alone to test and adjust for possible BaP adsorption to this support. Polycarbonate membrane filters of 3.0-, 1.0-, and 0.45-μm pore diameter (Nuclepore) were also used to compare sediment filtration to filtration with a system of monodisperse pore sizes. Permeability was calculated from measurements of ^{3}H-BaP activity of the gut fluid before and after filtration (corrected for sorption to Nitex). Radioisotopic activity was counted in ScintiVerse BD cocktail (Fisher Scientific, www.fishersci.com) on an LKB Wallac 1217 RackBeta liquid scintillation counter.

Controls of emulsification—We attempted to create an emulsion in *A. marina* gut fluids to test whether the paucity of lipids in the normal deposit-feeder diet might preclude emulsion droplet formation. Gut fluid was incubated with freeze-dried mussel tissue (the same as used previously) at

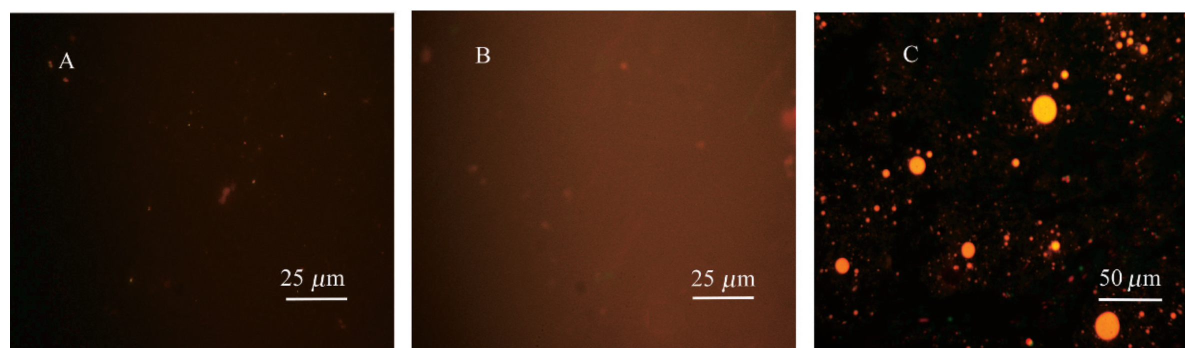

Fig. 1. Nile Red solubilization by eight different animals' gut fluids indicates three different conditions: almost no lipid solubilization, micelles, and emulsions. (A) *Cucumaria frondosa* gut fluids are dark, with specks of fluorescence that do not appear to be spherical, thus indicating solid particles. (B) *Arenicola marina* gut fluids have a diffuse orange glow in the background that indicates micellar solubilization of Nile Red. This appearance is characteristic of all deposit feeders on their natural diets in this study (except *Molpadia*). (C) *Glycera dibranchiata* gut fluids show spherical lipid droplets (emulsion) ranging in diameter up to 25 μm. The golden tint of the droplets suggests a relatively more hydrophilic environment, for example, an emulsion of phospholipids. The background color is almost perfectly black. No gut fluids showed red or orange autofluorescence.

solid : fluid ratios from 5 to 180 (g mussel [L gut fluid]$^{-1}$). Mussel was considered an unusual, but potentially realistic food substrate, the ingestion of which might lead to formation of large droplets of lipid. The solid : fluid ratio was varied to determine the threshold amount of mussel flesh (and, by proxy, constituent lipids) required for onset of emulsification. Aggregates were stained with Nile Red and measured following the same procedure outlined already. Incubations were performed once, but three different images of each resultant fluid were taken for analysis.

Results

Interphyletic survey—Emulsion droplets were found only in the gut fluid of the carnivore *Glycera dibranchiata* (Table 1; Fig. 1C). All deposit feeders except *Molpadia* had micelles in their gut fluids, according to contact-angle titrations and Nile Red incubations. Incubations with Nile Red yielded a diffuse, background glow indicative of solubilization into micelles, which are individually too small to resolve by light microscopy. Gut fluids from animals eating natural sediment also contained amorphous material that seemed to be detritus. These particles were neither spherical nor did they fluoresce strongly after incubation with Nile Red, as would be characteristic of lipid aggregates (Fig. 1B).

The deposit feeder *Molpadia* and the suspension feeder *Cucumaria* lacked micelles in their gut fluids. These two species solubilize a variety of lipids including hexadecane, hexadecanol, palmitic acid, cholesterol, and BaP to an extent no greater than does seawater (Mayer et al. 2001*b*; Voparil unpubl. data). *Cucumaria* and *Molpadia* fluids exhibited little Nile Red fluorescence and were almost completely dark (Fig. 1A), supporting CMD data that indicated no surfactant micelles. Instead of using extracellular digestion, these species may rely on the intracellular digestion of dietary solids. Echinoderms are thought to be capable of intracellular digestion (Rosati 1968; Filimonova and Tonkin 1980; Lawrence 1982), which circumvents the dissolved-phase transport step by advecting particles through the gut lumen and then engulfing them into the cells lining the digestive tract (phagocytosis).

We were unable to determine the presence of micelles in the gut fluid from *Glycera dibranchiata*. This fluid was too viscous to transfer accurately with an air displacement pipette and did not mix well with artificial seawater during contact-angle titrations. In fact, the viscosity of this fluid was the original impetus for our attention to the differences between emulsions and micellar fluids in their abilities to flow.

Feeding experiment—Within the *Nereis virens* diet experiment, emulsion droplets were found only in the guts of those individuals that ate pure mussel tissue (Fig. 2). These droplets had a mean diameter of 7.7 $\pm$ 6.2 μm (mean $\pm$ standard deviation [SD]). Individuals eating natural sediment or the organic matter–free, sediment + mussel tissue contained micelles but no emulsion droplets in their guts. Mussel tissue still has cellular structure, such as cell walls, which would not yield spherical particles. Emulsion droplets contain lipids only in a fluid state, so they will minimize interfacial tension and be spherical. Whether emulsions were present or not, feeding treatments had no effect on the CMD of *Nereis virens* gut fluids: all of the worms feeding on natural sediment, and all but one individual for each other treatment, had surfactant micelles in the gut as indicated by contact-angle titrations and Nile Red incubations.

Food treatments varied in their contents of organic carbon and nitrogen (Table 2). The mussel diet had much more (>100 times) organic carbon than the diets containing sediment and had lower C : N ratios than the natural sediment. Total levels of organic carbon and nitrogen in the mussel-amended sediment were similar to natural Lowes Cove sediments (Table 2). Organic carbon and nitrogen in mussel tissue is likely in more labile forms, however, e.g., phospholipids and proteins, which would emulsify in the gut. Previous analysis of this sediment and another batch of freeze-dried mussel tissue indicated that both samples had ~25% of total organic material as lipids (Bock and Mayer

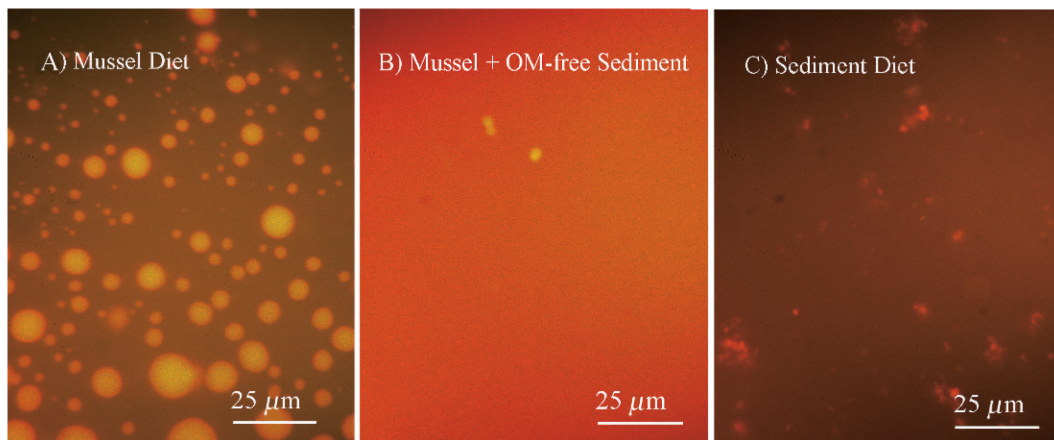

Fig. 2. Nile Red fluorescence images from *Nereis virens* gut fluids from individuals with different diets: (A) Worms on a mussel diet; emulsion droplets are distinct within the gut fluid; (B) Mussel and organic matter–free sediment; (C) sediment diet; worms ingesting sediment have few or no visible droplets. All fluids have a fluorescent "haze" in the background indicating presence of micelles.

1999). Although this number seems high for the sediment, the low total organic carbon (TOC) values imply a coarse sand with organic matter that is often relatively labile (Dauwe et al. 1999; Mayer et al. 2002).

The three fluids containing emulsion droplets (unadulterated fluid from the carnivorous *Glycera dibranchiata*, gut fluid from *N. virens* fed mussel tissue, and *A. marina* gut fluid amended with SELCO) had similar maximum droplet sizes (Fig. 3). The minimum values of ~1 μm represent the lower detection limit for our analytical approach (resolution limit with red light), but contact-angle titrations and red haze after incubation with Nile Red confirmed that smaller aggregates, i.e., micelles, were present. *Glycera* and modified *Arenicola* gut fluids had similar dispersions of emulsion droplets, whereas *N. virens* gut fluids tended to have more droplets of intermediate and larger size (>4 μm).

"Straining" effects on aggregates of different sizes—Micelles passed through sediment columns and polycarbonate filters more efficiently than did larger emulsion droplets (Fig. 4). BaP in micelles was unaffected by even the smallest polycarbonate membrane filter (0.45 μm); there was no significant difference between the initial micellar fluid's BaP concentration and the concentration after passage through any of the polycarbonate membranes (*t*-test for comparison of means). There was no evidence that BaP absorbed to any of the materials in these filters. In contrast, emulsion droplets were retained by all of the filters, and as expected, smaller pore sizes of the filter increased retention of emulsified BaP.

With sediment as a filter, both micelles and emulsion droplets were strained from solution, but micelles were less so. Roughly 90% of micelles passed through the sediment column, whereas 74% of emulsion droplets did. These retention figures are consistent with literature indicating that straining becomes important when the ratio of colloid to sediment particle diameter reaches the 0.005–0.05 range (reviewed in Bradford et al. 2005).

Gut fluid : food substrate ratio—Mussel tissue readily formed an emulsion when mixed with deposit-feeder gut fluid above a solid : fluid ratio of ~80 g L^{-1} (Fig. 5). This experiment supports the concept of a threshold in the relative concentrations of solubilizates and surfactant before emulsification occurs. Paucity of lipids in the gut may, therefore, explain the lack of emulsions in deposit-feeder guts.

Discussion

Our findings of straining and the effect of the food : gut fluid ratio provide some explanation for the distribution of emulsions versus micelles among most of the species studied here. If food substrates vary along a continuum ranging from biomass without sediment (i.e., carnivory or herbivory) to normal bulk sediments with bioavailable food concentrations of mg (g sediment)$^{-1}$ (as implied by, say, digestible protein concentrations; Mayer et al. 1995), then these two influences should each favor emulsion in the former and micelles in the latter. In other words, incorporation of sediment into the diet both dilutes the food dosage and causes straining. Changes in the nature of the organic matter component (biomass vs. largely humified organic matter) along this continuum likely also contribute to the nature of lipid aggregates formed. More careful study of cases that occupy the central parts of this continuum might help shed light on genotypic versus

Table 2. Total organic material associated with *Nereis virens* feeding treatments.

Sample	Organic carbon (mg g^{-1})	Nitrogen (mg g^{-1})	C : N ratio
Natural sediment	3.42	0.37	9.24
Organic matter–free sediment	0.37	0.03	12.33
Organic matter–free sediment + mussel	3.85	0.74	5.20
Mussel tissue	401.58	82.79	4.85

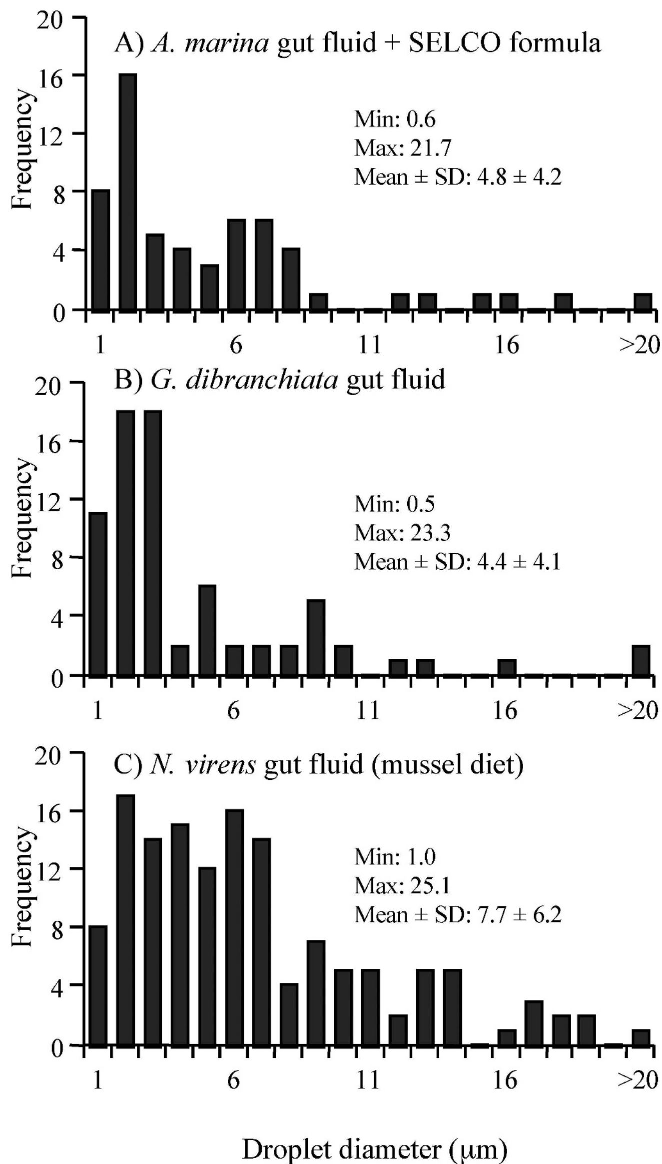

Fig. 3. Droplet diameter frequency distributions for three gut fluids containing Emulsions: (A) *A. marina*, (B) *G. dibranchiata*, and (C) *N. virens*. Numbers on the x-axis represent the bins for droplets of specific sizes; a value of 2 on the x-axis represents droplets of size $= 1 < x < 2$.

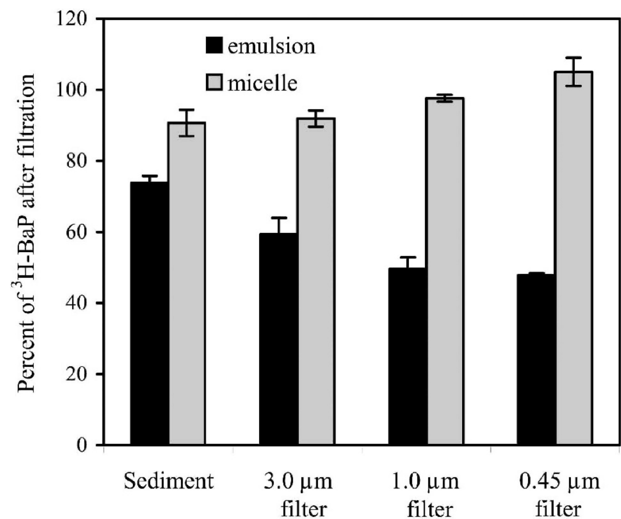

Fig. 4. Straining of ^{3}H-BaP-labeled lipoidal aggregates by sediment and polycarbonate membrane filters. Labels on the x-axis indicate materials through which the fluids containing either emulsion droplets or micelles were passed. Ordinates are the percent of the ^{3}H-BaP spike in each fluid after filtration. Data for fluids containing emulsion droplets are in black; fluids containing micelles are in gray.

phenotypic (including behavioral) controls on the use of these two types of lipid aggregates in benthic animals. Last, there are other possible influences that might lead animals to adopt one or the other mode of lipid aggregate, e.g., small micelles are more diffusible than larger emulsion droplets, but our work shows a cross-phyletic trend that is consistent with roles for both of these processes.

Our results show that straining may be a significant impediment to lipid transport via emulsions and hence assimilation in marine deposit feeders, even though emulsions require less investment in biochemical capital than surfactant micelles for each solubilizate molecule transported. The factors governing emulsion filtration have been well studied because of its importance to many industrial processes. Passage through a porous medium can trap emulsion droplets as well as decrease the permeability by the bulk fluid as pores become clogged and fluid is forced to flow through a reduced number of pores and more circuitous routes (Soo and Radke 1984). Capture increases dramatically as the ratio of droplet size approaches pore size and becomes significant when

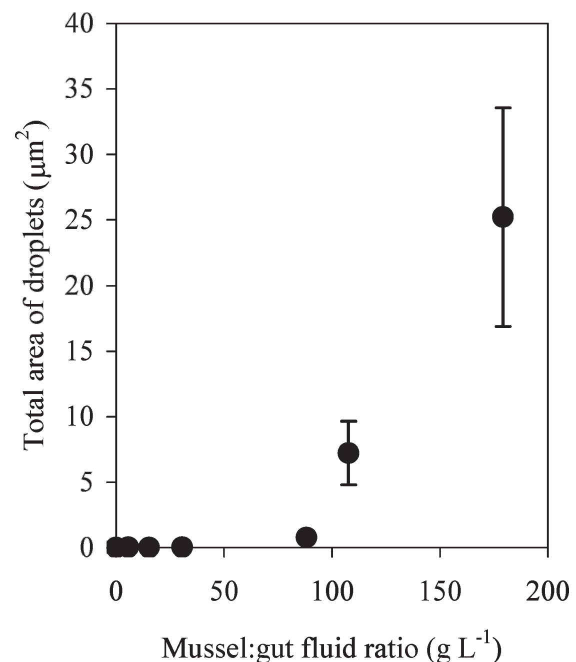

Fig. 5. The area of fluorescence increases dramatically as emulsion droplets form when mussel meat is mixed with *A. marina* gut fluid above a solid : fluid ratio of ~80 g L^{-1}. Error bars are ±1 SD.

emulsion droplets have sizes of at least a few percent of the particle size of a granular particle matrix. Permeability to lipoidal aggregates is positively related to solid-particle size, smoothness of grain surfaces, and the sorting and porosity of the medium (Yan et al. 1991; Vidrine et al. 2000). Electrostatic interactions between the charges on the surface of the aggregates and on the surface of the solid phase can also affect straining (Hofman and Stein 1991). For example, opposite charges can immobilize droplets on the solid surface, followed by aggregation and formation of larger emulsion particles (Vidrine et al. 2000). These factors suggest that emulsion droplets of 1–20 μm size, such as those found in our study, should be quite susceptible to straining by fine sands and smaller grains. Straining should become more significant as sorting of the sediment decreases, i.e., as fine particles are included in the sediment.

The ratio of food substrate to gut fluid also emerges as an important influence on whether micelles or emulsions will be favored. The very well-studied digestive systems of *Homo sapiens* have had micelle-emulsion transition mapped very carefully as a function of varying contents of food items and secreted compounds (Carey et al. 1983; Staggers et al. 1990). These systems are well described, as are the identities and concentrations of the important constituents, e.g., the concentrations of particular bile salts present in the gut and the concentrations of emulsifying lipids, are already known. Identification of the specific surfactants and emulsifying lipids present would be required to create similar phase diagrams for the conditions in deposit-feeder guts, but very little is known about these biochemicals in deposit feeders.

Our finding that emulsion droplets appear at a ratio of ~80 g mussel tissue (L gut fluid)$^{-1}$ applies only to the tested combination of *A. marina* gut fluid and mussel tissue, but it provides some basis on which to consider realistic constraints on emulsions versus micelles in benthic animals. This concentration, if provided by ingested sandy sediment with porosity of 0.5 (v/v), would imply a concentration of digestible organic matter of about 10% of the sediment by weight. Such a high concentration is out of the range of bulk, unpolluted sediments, which generally have concentrations of at least an order of magnitude less than this value. Thus, in normal habitat for deposit feeders, and in the absence of particle sorting in the gut that elevates this concentration, the ratio of food substrate to gut fluid should favor micelle formation. It is very conceivable, however, that patch or particle selection (e.g., sloppy carnivory, or ingestion of cached algal materials; Jumars et al. 1990) could yield such concentrations in ingested material, and we suggest that, in such cases, there could arise a sufficient food : gut fluid ratio to induce emulsions in a matrix in which micelles would have been otherwise preferable. An animal, in this event, might secrete extra surfactant to disperse emulsion droplets into smaller micelles—a strategy employed in oil extraction and environmental remediation. Moreover, early in the evolution of deposit feeding, sand deposits may have been much richer (Jumars et al. 2007). Likewise, it is possible that the mixture of secreted surfactants could be altered to manipulate lipid aggregates. Smoot et al. (2003) found three different surfactant structures in *A. marina*, and we have found the concentration ratio among these three compounds to vary among individuals (Voparil unpubl. data).

Such plasticity might help to explain a discrepancy between our results and those of Bock and Mayer (1999). The experimental conditions for mussel tissue fed to *N. virens* were identical between the two studies, but we found micelles present in the animals in this study, whereas Bock and Mayer (1999) found none. We have no explanation for this discrepancy other than possible physiological variations among individuals between the two experiments that might have led to greater surfactant secretion in our experiments. Alternatively, it is possible that the mussel tissue used in the Bock and Mayer (1999) experiment had a higher lipid concentration that led to emulsification by hydrolyzed food lipids and obviated surfactant secretion.

In conclusion, the difficulties of mobilizing dietary lipids through an aqueous medium can be overcome by a number of different solutions. Rigorous selection of nutritious material before ingestion, such as during carnivory, allows the use of the most efficient transport vesicles (emulsions) for extracellular mobilization, while the presence of nondigestible material in the gut complicates matters. Animals were shown here to exhibit different solutions to this problem, adjusting surfactant : food matrix ratios to either optimize return on investment in chemical capital or reduce straining by sediment. These considerations shed light on the fundamental advantage in lipid digestive approaches allowed to animals with enclosed digestive organs relative to bacteria that must release digestive enzymes and surfactants to open systems (Mayer et al. 2001), and perhaps they point to the reason why the former can rely on heterotrophy for essential lipids more so than the latter.

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