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Interactions among Contaminants and Nutritional Lipids during Mobilization by Digestive Fluids of Marine Invertebrates

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Coastal sediments contain complex mixtures of hydrophobic compounds including organic contaminants such as polycyclic aromatic hydrocarbons and biogenic compounds such as cholesterol and phospholipids. Within the guts of benthic invertebrates, these mixtures are subjected to digestive, chemical conditions that can be rich in surfactants and proteinaceous material. Using in vitro incubations as proxy for digestive exposure, we studied the solubilization of binary mixtures of nutritional and contaminant lipids into artificial seawater and six marine invertebrate gut fluids (*Molpadia intermedia*, *Cucumaria frondosa*, *Arenicola marina*, *Arenicola brasiliensis*, *Parastichopus californicus*, and *Nereis virens*). For animals with surfactant micelles or high protein concentrations, solubilization interactions were frequent. For example, in *Arenicola marina* gut fluid, benzo(a)pyrene enhanced the solubilization of hexadecane (491% of the compound alone) and palmitic acid (130%) but hindered cholesterol (83%). Benzo(a)pyrene concentrations increased in gut fluids in the presence of cholesterol (137% of BaP alone), phenanthrene (154%), lecithin (140%), and hexadecanol (232%). In *A. marina* gut fluid, dilution with seawater indicated that these enhancements occur only when micelles are present. Sediment-water partitioning models, used to predict the bioavailability of hydrophobic organic chemicals, do not account for such interactions between solubilizates (compounds solubilized in micelles). However, for animals exposed via a digestive tract containing micelles or high protein concentrations, digestive bioavailability and perhaps bioaccumulation are likely influenced by these interactions.

Introduction

Coastal sediments are often contaminated with complex mixtures of hydrophobic organic compounds, e.g. polycyclic aromatic hydrocarbons (PAH), that can be toxic and pro-carcinogenic (1). Current mechanistic models of sediment toxicity and bioaccumulation suggest that the biological effects of a mixture of PAH can be predicted by the sum of all individual components' toxicity (the Σ PAH model (2)). Inherent in this model is the assumption that exposure to

a mixture of hydrophobic chemicals should also be additive, resulting from equilibrium partitioning between the aqueous phase and the animal. When very hydrophobic compounds are freely dissolved in water, molecules are generally thought to be too dilute to influence one another (3). However, water is not the only pathway of exposure, nor even the primary means of exposure, for some types of benthic animals.

Deposit feeders are often exposed to these contaminants primarily via the digestive tract (4–8). The gut is a complex reaction zone of hydrolytic enzymes, dissolved organic matter, and surfactants that solubilize organic compounds at many times their seawater, and equilibrium partitioning-predicted, solubility (9). In the gut fluids of the deposit-feeding polychaete *Arenicola marina*, surfactant micelles solubilize benzo(a)pyrene (BaP) at concentrations 1000 times greater than the concentration of freely dissolved molecules in seawater (9). The compounds incorporated into digestive micelles in gut fluid appear to be bioavailable (8), unlike humic-type micelles that bind hydrocarbons in an unavailable form (10). The situation is analogous to vertebrate lipid digestion, in which bile salt micelles shuttle lipids through the bulk aqueous solution to the digestive epithelium. Not only are lipids in bile salt micelles bioavailable but also solubilization into micelles is required for efficient digestive assimilation (for review, see ref 44).

Solubilizates (those compounds within the micelle (31)) are concentrated in micelles and can interact therein to influence each other's apparent solubilities. For example, bile salt micelles alone have a poor capacity for cholesterol, carrying on average less than 1 molecule per micelle (11). Solubilization of lecithin causes bile salt micelles to expand, increasing their capacity to 125 cholesterol molecules per micelle (12). By doing so, lecithin increases the in vivo digestive bioavailability of cholesterol dramatically (13). Within the guts of deposit feeders, exogenous lipids from sediment such as phospholipids, pigments, hydrocarbons, and potentially contaminants (14–17) mix with endogenous lipids such as cholesterol (Voparil, unpublished). With the multitude of compounds present in marine invertebrate guts, interactions among solubilizates are likely to influence digestion and absorption and hence influence the assimilation of nutritious lipids as well as the bioaccumulation of sedimentary contaminants.

This research tests whether solubilization into micelles leads to interactions between pairs of lipids in digestive fluids from marine polychaetes and holothuroids. We used in vitro incubations of gut fluids with pure substrates because they offer a simplified system for study. In vitro incubations compare well to in vivo deposit-feeder assimilation of very hydrophobic compounds (8, 18). Specific pairs of lipids were used to emphasize the impact of a common environmental contaminant (BaP) on the bioavailability of both nutritional lipids and other nonpolar contaminants. In this paper, the term "lipid" is used to refer to compounds that are more soluble in organic solvents than in water, following others' functional definitions (50–52). By doing so, we group a wide variety of compounds together, e.g. fatty acids, sterols, PAH, aliphatic hydrocarbons, in a way that may be unusual for some readers.

Materials and Methods

The solubilities of various lipid substrates—alone and in combination with another substrate (a binary mixture)—were measured. During binary incubations, a radiolabeled substrate was paired with an unlabeled substrate; thus only one component of the mixture was quantified. Two separate

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TABLE 1. Gut Fluids Investigated in This Study

species (location)	Taxon	feeding mode	CMD ^a	protein (g/L)	pH
<i>Molpadia intermedia</i> (WA)	holothuroid	deposit feeder	ND	0.23	6.88
<i>Cucumaria frondosa</i> (ME)	holothuroid	suspension feeder	ND	23.92	6.35
<i>Parastichopus californicus</i> (WA)	holothuroid	deposit feeder	40%	0.89	6.33
<i>Nereis virens</i> (ME)	polychaete	omnivore	29%	36.24	6.7
<i>Arenicola brasiliensis</i> (CA)	polychaete	deposit feeder	22%	40.25	6.67
<i>Arenicola marina</i> (ME)	polychaete	deposit feeder	15%	39.28	6.38
used only for intraphyletic experiment	polychaete	deposit feeder	40%	NM	6.43

^a CMD = critical micelle dilution. This is the dilution below which surfactant micelles are converted to monomers. ND indicates that micelles were not detected. Two *Arenicola marina* gut fluids were used; fluid with a CMD of 40% was used only for BaP and hexadecanol incubations with gut fluid dilutions.

incubations were therefore necessary when measuring both substrates' concentrations in the mixture. This approach avoids problems of crosstalk between counting windows when attempting to measure two different isotopes, e.g. ³H and ¹⁴C, in a single sample.

Selected solvents including artificial seawater (ASW) and gut fluids were incubated with excess substrate (about one 100-fold excess over the amount solubilized, confirmed visually at the end of the incubation) in 300 μ L glass tubes. Substrates were introduced in a carrier solvent (toluene or ethanol) which was then evaporated with N₂ gas. Most substrates were solids at experimental temperature (22 °C), except hexadecane, which was liquid. Substrates and fluid were incubated on an inverting table for 1 h, which approximates some benthic animals' gut residence times (19). Fluids were removed via glass pipet, passed through PTFE (Teflon) 0.45 μ m filters, dispensed into 10 mL of ScintiVerse BD cocktail (Fisher Scientific), and counted on a LKB Wallac 1217 RackBeta liquid scintillation counter with the detection window set at either 8–110 for ³H or 50–165 for ¹⁴C quantification. Data were quench-corrected by comparison to quench curves generated by adding different concentrations of ¹⁴C or ³H toluene scintillation standard to each of the gut fluids. As the PTFE filters were housed in plastic, some absorptive loss of these lipid substrates may have occurred during filtration; iterative filtrations indicate no more than 20% loss due to absorption. We did not correct for these possible losses. As a result, solubilization data reported may be underestimates. All incubations were performed in triplicate.

Solvents. Artificial seawater was made following the recipe of ref 20. For animals, six species of benthic marine invertebrates were collected (Table 1) that were expected to represent a range of protein and surfactant concentrations based upon previous work (21). Polychaetes were collected from intertidal regions while holothuroids were from subtidal environments. Animals were collected with care to avoid injury and dissected immediately for digestive (gut) fluids from the midgut—digestive agents are usually maximal in this section (21)—and frozen at –80 °C until use. Each gut fluid was characterized for surfactancy, protein content, and pH. Surfactant activity and micelle presence were assessed using contact angle titrations with artificial seawater (21). Total protein in gut fluids was measured using a Pierce Micro BCA Protein Assay Reagent Kit (www.piercenet.com) and compared to standard solutions of bovine serum albumin (BSA). pH was measured using an Orion Research probe (www.thermo.com).

Substrates. We chose substrates representative of different classes of lipids expected in marine invertebrates' guts, either from dietary or endogenous sources. Hexadecane is a member of the aliphatic hydrocarbons which are anthropogenic contaminants (17) and markers for terrestrial organic matter and plants (16). Hexadecanol is a free aliphatic alcohol and is a cosurfactant in some deposit-feeders' guts (Findlay,

TABLE 2. Experimental Substrate–Solvent Combinations

substrate A	substrate B	solvent ^b
hexadecane ^a	BaP	ASW, all animal gut fluids
hexadecanol ^a	BaP	ASW, all animal gut fluids
cholesterol ^a	BaP	ASW, all animal gut fluids
palmitic acid ^a	BaP	ASW, all animal gut fluids
BaP ^a	palmitic acid	ASW and <i>A. marina</i> gut fluids
BaP ^a	cholesterol	ASW and <i>A. marina</i> gut fluids
BaP ^a	phenanthrene	ASW and <i>A. marina</i> gut fluids
BaP ^a	lecithin	ASW and <i>A. marina</i> gut fluids
BaP ^a	hexadecanol	ASW and <i>A. marina</i> gut fluids
BaP ^a	hexadecanol	<i>A. marina</i> diluted with ASW

^a The measured substrate. ^b ASW = artificial seawater.

personal communication), a minor constituent of sediment lipids (17), and a product of wax ester digestion (22). Cholesterol belongs to the sterols which are found in eukaryotic cell membranes (23) and at high concentrations in deposit-feeder guts (unpublished data) likely due to sloughing of digestive cells. Palmitic acid represents the fatty acids, which are major constituents of settling particles (16) and a major product of lipase and esterase activities on more complex dietary lipids. Lecithin (phosphatidylcholine) is a phospholipid, a major component of cell membranes and often the major class of lipids in deposit-feeder guts (unpublished data). Benzo(a)pyrene (BaP) is a PAH and a common contaminant of coastal sediments (1).

Radiolabeled lipids were purchased from either American Radiolabeled Chemicals (palmitic acid [1-¹⁴C], benzo(a)-pyrene [³H(G)], and cholesterol [4-¹⁴C]; www.arc-inc.com) or Amersham (hexadecane [1-¹⁴C], hexadecanol [1-¹⁴C]; www.apbiotech.com). All had a radiopurity of at least 98%. Incubations of the substrates with DI water led to good agreement with previously reported values of aqueous solubility (24). All unlabeled lipids were purchased from Sigma (www.sigma-aldrich.com).

Substrate Combinations Tested. Various binary-substrate combinations were tested (Table 2). To determine whether interactions occur only when micelles are present, ³H-BaP and cold hexadecanol were incubated with serial dilutions of *A. marina* gut fluids and ASW. Above that gut fluid's critical micelle dilution (CMD; 40% gut fluid, 60% ASW), micelles are present. When diluted below 40%, micelles disaggregate and surfactants exist as monomers in solution. Artificial seawater and full-strength *A. marina* gut fluid were also incubated with ³H-BaP alone and with each of cholesterol, phenanthrene, palmitic acid, and lecithin to determine whether the presence of micelles leads to solubilization interactions between lipids that do not occur when ASW is the solvent. To corroborate results with *A. marina* to other species, artificial seawater and all animals' gut fluids were incubated with binary mixtures of unlabeled BaP and each of radiolabeled hexadecane, hexadecanol, cholesterol, and palmitic acid. These animal species vary widely in their

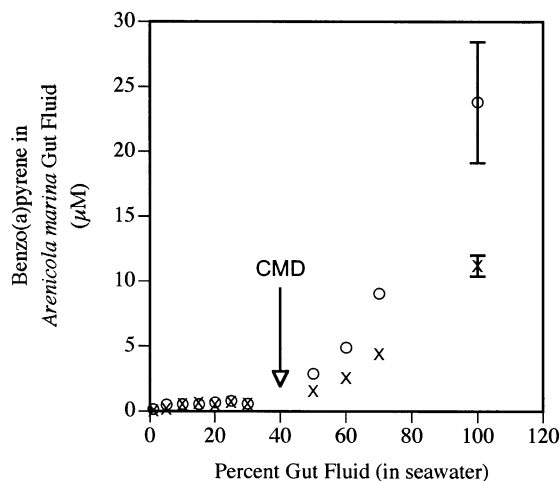


FIGURE 1. Benzo(a)pyrene solubilization with (○) and without (×) hexadecanol in *A. marina* gut fluids titrated with clean seawater. Abscissa represents the dilutions of the original gut fluid. Ordinate is the concentration of BaP solubilized. Error bars are ± 1 SD.

content of digestive agents and presence of micelles, so that this comparison served as an interphyletic test of the importance of micelles in facilitating solubilization interactions.

Results

Gut Fluid Characteristics. Gut fluid characteristics presented here (Table 1) are in general agreement with previous work (21, 25). Of the holothurroids, *Molpadia intermedia* gut fluid was most like seawater, being low in protein and devoid of surfactant micelles. Though *Cucumaria frondosa* fluid did not have micelles, protein concentrations were similar to the polychaetes' fluids. *Parastichopus* fluid was the converse of *Cucumaria*, having micelles but little protein. All polychaetes' gut fluids had both micelles and high protein contents. Differences in CMD for the two collections of *Arenicola marina* gut fluids suggests that digestive physiology is somewhat pliable and may respond to environmental and animal conditions that have not been accounted for in this study.

Mixture Solubilization. The solubilization of BaP in *A. marina* gut fluid diluted to various extent with ASW showed a biphasic concentration plot, which implies solubilization into micelles (Figure 1). The dramatic increase in BaP concentrations with increasing concentrations of gut fluid above the CMD (40% gut fluid in seawater) reflects the onset of micelles and their enhanced ability to mobilize this

compound (9). By fitting a linear regression to BaP concentration data below the CMD and extrapolating to 100% gut fluid, we calculate that nonmicellar constituents of gut fluid are responsible for 8% of the BaP solubilization—micelles are responsible for 92% of the BaP in full-strength gut fluid, in general agreement with previous work (9).

Hexadecanol had no effect on BaP solubilization without micelles in the gut fluid. With micelles, i.e. above the CMD, hexadecanol enhanced BaP solubilization, allowing full strength gut fluid to solubilize $23.8 \pm 4.4 \mu\text{M}$ BaP—more than double the concentration without hexadecanol ($11.2 \pm 0.8 \mu\text{M}$).

Some lipids enhanced BaP solubilization in full-strength *A. marina* gut fluid but had no effect in ASW (Figure 2). In the micellar gut fluid, cholesterol and phenanthrene enhanced BaP's apparent solubility by approximately the same amount as lecithin (140%). In ASW, neither of these compounds had an effect. In both gut fluid and ASW, lecithin and hexadecanol increased BaP solubilization; hexadecanol had the greatest effect, increasing relative BaP concentrations to 539% and 232% in ASW and gut fluid, respectively. The absolute enhancements were orders of magnitude higher in the gut fluid than in ASW. In *Arenicola* gut fluid, hexadecanol increased BaP solubilization $14 \mu\text{M}$. In ASW, the absolute increase of BaP due to hexadecanol was $0.02 \mu\text{M}$. Palmitic acid had no effect on BaP solubilization in either solvent.

Interphyletic Corroboration. Gut fluids generally solubilized more of each individual lipid than ASW, except for *Molpadia*'s and *Cucumaria*'s dissolution of hexadecane (Figures 3 and 4). Hexadecanol was especially enriched in all gut fluids; even *Molpadia*, with little protein and no micelles, solubilized at least 2 orders of magnitude more hexadecanol than seawater. Gut fluids with surfactant micelles were able to solubilize much greater concentrations of all of the lipids than fluids without micelles—note the change of ordinates' scales between Figures 3 and 4.

In fluids without micelles or proteins (ASW and *Molpadia* gut fluids), BaP had no observed effect on any of the other lipids' solubilities (Figure 3). However, solubilization interactions were seen between certain pairs of lipids in all other gut fluids, even *Cucumaria*. In fluids with either micelles or protein (i.e. all species except *Molpadia*), cholesterol solubilization was inhibited by the presence of BaP in the mix. The greatest relative effect was with *Cucumaria* fluid, which lost 78% of its cholesterol solubilization when BaP was present (Figure 3C); *A. marina* solubilization of cholesterol decreased the least—by only 17% (Figure 4A).

The effects of BaP on the other lipids varied among species. For example, hexadecane solubility increased to 491–844%

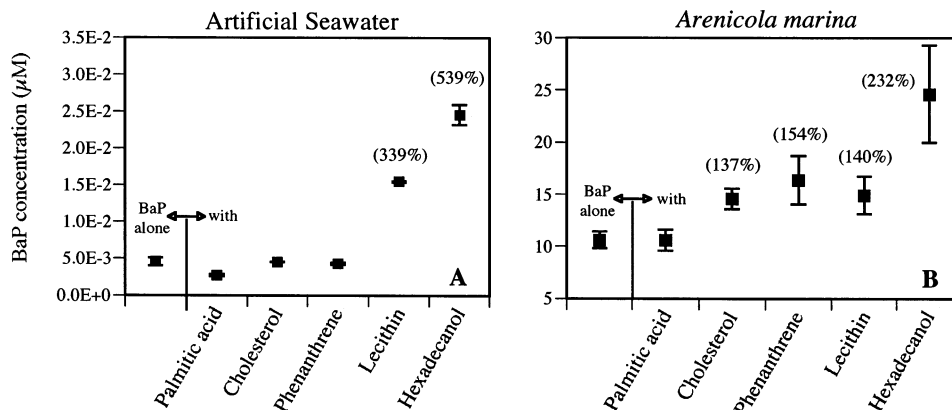


FIGURE 2. The effects of other substrates on benzo(a)pyrene concentrations in artificial seawater (A) and *A. marina* gut fluids (B). Abscissas represent the specific cosubstrates tested: palmitic acid, cholesterol, phenanthrene, lecithin, and hexadecanol. Ordinates are the concentration of BaP—notice the change of scale between A and B. Significant differences (unpaired *t*-test, $P = 0.05$) are indicated in parentheses as the percent change of BaP solubilization with cosubstrate compared to that of BaP alone. Error bars are ± 1 SD.

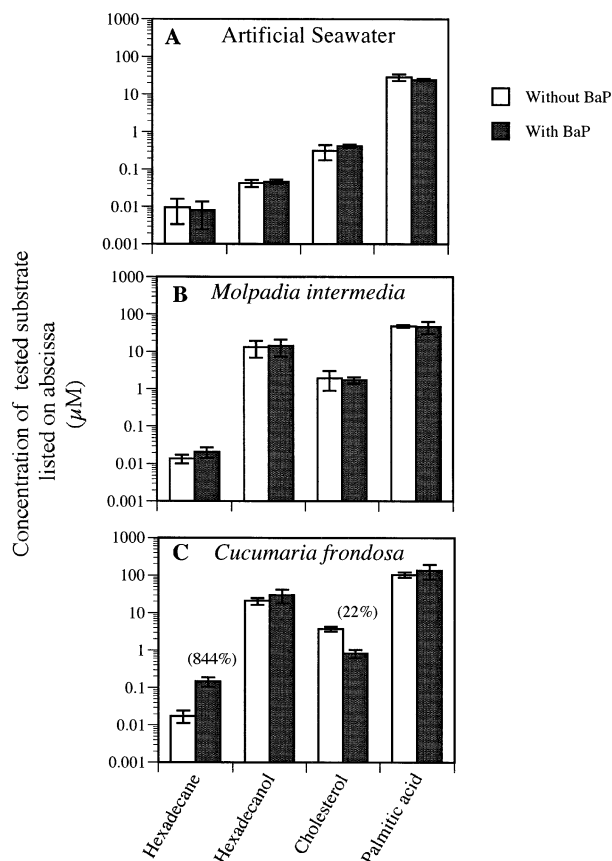


FIGURE 3. Solubilization of substrates, without (□) and with (■) BaP, in gut fluids without surfactant micelles. Abscissa is the particular substrate tested. Ordinate is the substrate's concentration. Each figure is a different fluid: (A) artificial seawater, (B) *Molpadia intermedia* gut fluid, and (C) *Cucumaria frondosa* gut fluids. Significant differences (unpaired *t*-test, $P = 0.05$) are indicated in parentheses as the percent change of the substrate's solubilization with BaP compared to that of the substrate alone. Error bars are ± 1 SD.

in *A. marina*, *A. brasiliensis*, and *Cucumaria* fluids to concentrations approaching those found in *Parastichopus* and *Nereis*, which both showed no effect of BaP. Opposite effects of BaP on palmitic acid solubilization were found in different micellar gut fluids; in both Arenicolids, BaP caused a slightly increased capacity for palmitic acid (both 130%), while the effects on *P. californicus* and *N. virens* fluids showed a decreased dissolution of palmitic acid (to 55% and 41%, respectively).

Discussion

These invertebrate gut fluids are complex blends of ingested materials in various states of digestion combined with different proportions of secreted digestive agents. The weakest gut fluid (*Molpadia*) showed no solubilization interactions, just like ASW (except for hexadecanol and lecithin see below). In these solvents, the intermolecular distance between any two lipids makes their interaction unlikely. For example, hexadecane's aqueous solubility ($0.0095 \mu\text{M}$) indicates that $\sim 6 \times 10^9$ water molecules surround each hexadecane molecule, assuming a homogeneous dispersion throughout the solution. Indeed, no interactions among binary mixtures of chlorinated organics and hydrocarbons in water have been found at aqueous solubility (26, 27) or below (28).

However, both lecithin and hexadecanol increased BaP dissolution in ASW (Figure 2A), even though hexadecanol is

only sparingly soluble (we have no data for lecithin; reported aqueous solubility is $4.6 \times 10^{-4} \mu\text{M}$ (29)). Because of their amphiphilic structure, these compounds can form polymeric aggregates in water that would not have been removed by our $0.45 \mu\text{m}$ filtration (30, 31). For example, lecithin aggregates in a number of different polymeric forms such as vesicles and liposomes that contain distinct hydrophobic regions that allow increases in the apparent solubilities of more of a hydrophobic compounds such as BaP (32).

In gut fluids with greater amounts of digestive agents, surfactant micelles and proteins were already present in solution; thus no hexadecanol or lecithin were required to form hydrophobic phases. Lipids are not only dispersed throughout these fluids as monomers but also concentrated in micelles and in the hydrophobic domains of proteins. This concentration increases the likelihood of interactions between substrates in two ways: (i) by allowing more substrate to be solubilized within the bulk aqueous phase and (ii) by concentrating hydrophobic molecules in specific regions within the fluid—the micelles or proteins. For example, hexadecanol enhanced BaP solubility in *A. marina* gut fluids only when micelles were present (at dilutions $> 40\%$; Figure 1). Within micelles, BaP and other solubilizes are in closer proximity than when freely dissolved in seawater. For example, with 98% of the BaP in micelles expanded by hexadecanol (Figure 1) and a surfactant concentration of 5mM (33), the BaP:surfactant molecule ratio is 4.5×10^{-3} in *A. marina* micelles. At aqueous solubility, the BaP:water molecule ratio is 1×10^{-14} . This ratio of solubilize molecules to solvent molecules—the molar solubilization ratio (MSR)—is commonly used to compare the relative effectiveness of micelles of different surfactants to mobilize compounds of interest (34).

The hydrophobic domains of proteins may also serve as a locus for lipid solubilization (9, 53) and interactions. Compared to micelles, these regions may not be as easily modified by the presence of other lipids because of the many different levels of organization involved in protein structure. Consequently, substrates may compete for the limited hydrophobic space. For example, BaP appeared to displace cholesterol within *C. frondosa* proteins (Figure 3). As a result, the amount of cholesterol in solution dropped toward aqueous solubility—the concentration of free monomers in solution.

Role of Solubilizes. Interactions among substrates in Arenicolids' gut fluids are qualitatively consistent with previous work using vertebrate bile salts and commercial surfactants. Solubilizes influence the size and capacity of micelles according to their interactions with water and their location within the micelle. For example, nonpolar compounds compete with one another for space in a micelle's interior. More-hydrophobic compounds are enhanced by, as well as inhibit, the solubilization of less-hydrophobic solubilizes (39–41). We found this same effect when BaP enhanced solubilization of the more hydrophobic hexadecane (Figure 3C, 4A, 4B) and was enhanced by the less hydrophobic phenanthrene (Figure 2B). Although cholesterol is not a nonpolar lipid, it partitions similarly to PAH in micellar systems (42). Being slightly less hydrophobic, cholesterol enhances BaP solubilization (Figure 2) at its own expense (Figure 4).

Polar lipids, like lecithin and hexadecanol, increase the solubility of nonpolar compounds (42, 43). Polar lipids are solubilized by micelles at the micelle–water interface and act to decrease the interfacial tension between the hydrophobic core of the micelles and the surrounding aqueous phase (39), thereby allowing the cores to swell and decreasing the surfactant concentration at which micelles form (the critical micelle concentration). These interactions within micelles suggest, rather counterintuitively, that polar nu-

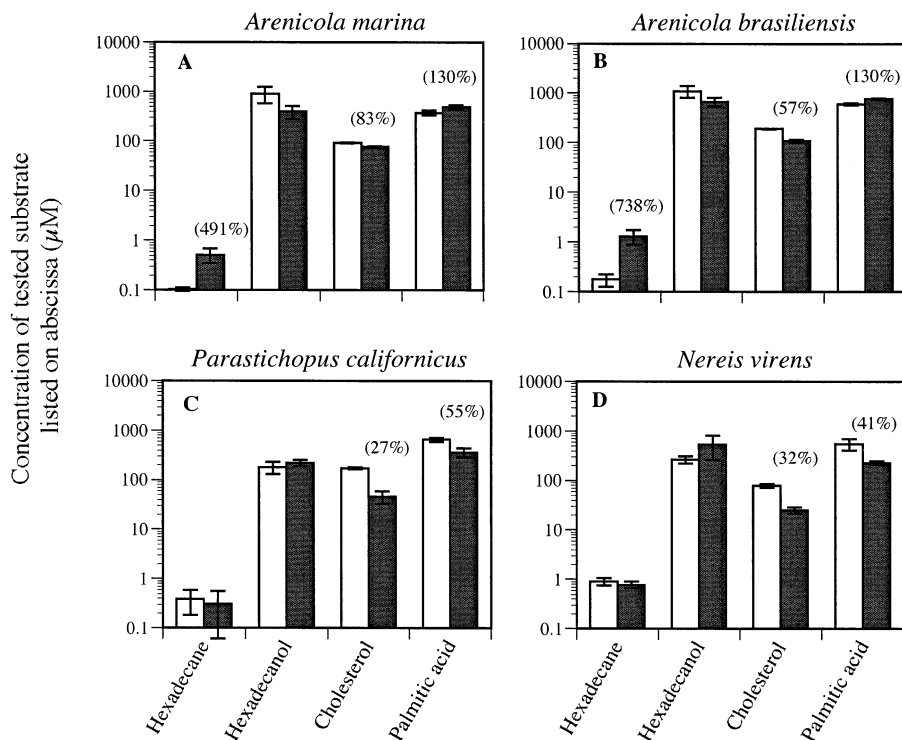


FIGURE 4. Solubilization of substrates, without (□) and with (■) BaP, in gut fluids with surfactant micelles. Abscissa is the particular substrate tested. Ordinate is the substrate's concentration. Each figure is a different gut fluid: (A) *Arenicola marina*, (B) *Arenicola brasiliensis*, (C) *Parastichopus californicus*, and (D) *Nereis virens*. Significant differences (unpaired *t*-test, $P = 0.05$) are indicated in parentheses as the percent change of the substrate's solubilization with BaP compared to that of the substrate alone. Error bars are ± 1 SD.

tritional lipids, like phospholipids or their partially digested lyso-forms, may increase the digestive solubilization of nonpolar contaminants like BaP (Figure 3B). These powerful cosurfactant effects of amphiphilic lipids during vertebrate digestion of have been widely reviewed for nutritional compounds such as fatty acids, cholesterol, and the hydrophobic vitamins (42, 44) and contaminants such as PAH (45, 46) and PCB (47). We believe our study is the first to suggest this cosurfactant effect in marine invertebrate guts.

Characterization of the surfactants and other lipids in guts of these marine invertebrates would allow clearer interpretation of the interactions found herein. Differences in the surfactants secreted by the *Arenicolids* from those of *Parastichopus californicus* and *Nereis virens* gut fluids might explain their opposing effects on palmitic acid solubilization with BaP. For example, decanol enhances aliphatic hydrocarbon solubilization by polyoxyethylene surfactants (35) but has no effect with bile salt surfactants (36). However, the surfactants in the guts of marine invertebrates have received little attention, with only Crustacean (37) and *A. marina* (33) surfactants currently identified.

In ternary and quaternary systems, substrates' solubilities often deviate from values obtained in binary systems due to additional solubilization interactions (38). A rough accounting suggests that the individual substrates solubilized in these experiments were a small fraction of the total lipid present in these gut fluids. In *A. brasiliensis*, the amount of palmitic acid solubilized ($600 \mu\text{M} \approx 0.15 \text{ g/L}$) is only 5% of the lipid already present in the fluid (2.86 g/L according to unpublished thin-layer chromatography-flame ionization detection of another sample of *A. brasiliensis* fluid). Even in *Molpadia* fluid, the radiolabeled palmitic acid ($48 \mu\text{M} = 0.0123 \text{ g/L}$) was only 21% of the total lipid ($\sim 0.06 \text{ g/L}$ according to data using another *Molpadia* fluid). Clearly, the spiked lipids are greatly outweighed by uncharacterized lipids in these fluids.

Relevance for in Vivo Contaminant Bioavailability. In this paper, we report measurements of the solubilities of

pure, lipid substrates in digestive fluids extracted from benthic invertebrates. These in vitro incubations have been found to directly relate to digestive bioavailability (18, 25, 54), though this is a somewhat contentious idea. In support of the link between gut fluid release and bioavailability, deposit-feeder's assimilation efficiency for PAH (18) and polychlorinated hydrocarbons (8) has been predicted by in vitro incubations with contaminated sediments. Lipid uptake into the epithelial cells lining the digestive tract is thought to be a passive process limited by the concentration of lipid solubilized by digestive fluids. Therefore, we suspect that interactions occurring during in vitro gut fluid incubations would reflect actual bioaccumulation in vivo; synergistic interactions between solubilization would result in enhanced bioaccumulation.

Our approach of incubating gut fluids with excess substrate probably serves to maximize interactions between two compounds, because substrates are at relatively high concentrations—their respective solubility limits. At lower concentrations, which are more representative of field conditions, solubilization interactions may be somewhat restrained. For example, naphthalene at $\sim 27\%$ of aqueous solubility in a solution of TX100 micelles enhances phenanthrene solubility, but only 61% of the enhancement that occurs when an excess of naphthalene is available (40). However, the multitude of lipids potentially present in contaminated sediments may have compounded effects on a particular substrate of interest.

Solubilization interactions in the gastrointestinal tract may help explain the current inaccuracy in predicting organic contaminant bioaccumulation using equilibrium partitioning theory (about 1 order of magnitude (48)). Interactions of BaP on hexadecane (Figures 3 and 4) and hexadecanol on BaP (Figure 2) resulted in hundreds of percent increases in gut fluid concentrations and do not bode well for expectations of increased accuracy from the current iterations of EqP-based bioaccumulation models applied to deposit-feeders. We suggest that in vitro gut fluid extractions may serve to fill

this void in sediment assessment techniques (49). Gut fluid extractions are able to provide empirical data on solubilization interactions that cannot be predicted using current theoretical models. We also developed a cocktail of commercially available chemicals that mimic *A. marina* gut fluid's solubilization of organic contaminants from sediments in hopes of allowing more widespread use of these in vitro methods (55).

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