

Utilization of algal and bacterial extracellular polymeric secretions (EPS) by the deposit-feeding brittlestar *Amphipholis gracillima* (Echinodermata)

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ABSTRACT: Like many deposit-feeding organisms, the burrowing brittlestar *Amphipholis gracillima* feeds on particulate organic matter in surface sediments. Microbial exopolymeric secretions (EPS) are carbohydrate-enriched polymers produced by microalgae and bacteria that bind aggregates and form dense biofilms near the sediment-water interface. EPS are assimilable by some benthic infauna and may be utilized as a significant carbon source. EPS are absorbed by some deposit-feeders, including a holothurian, and may be supplemental sources of nutrition. The burrowing brittlestar *A. gracillima* is a deposit-feeder that was used in a mass balance approach to model the incorporation of radiolabeled EPS by bottom feeders. Brittlestars were fed ¹⁴C-labeled, laboratory-cultured EPS from the marine bacterium *Pseudoalteromonas atlantica* and a benthic diatom (*Nitzschia* sp.) via sediment-bound and aqueous exposures. Comparison of absorption efficiencies (AE) showed that both polymer types are highly absorbed by *A. gracillima* (AE = 83 to 99%). Absorption of sediment-bound bacterial and algal EPS was similar (92.2 and 90.1%), but bacterial EPS absorption was significantly ($p < 0.05$) higher in sediment-bound (92.2%) than aqueous (83.3%) exposures. Algal EPS absorption was significantly higher in aqueous (99.9%) exposures. These findings suggest that EPS may represent a significant energy source for this deposit-feeding ophiuroid and other organisms with similar feeding habits. Additionally, *A. gracillima* appears to be especially adept at utilizing EPS resources from benthic diatom communities.

KEY WORDS: Echinoderm · Extracellular polymeric secretions · Brittlestar · Deposit-feeding · EPS

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INTRODUCTION

Extracellular polymeric secretions (EPS or exopolymers) are a class of high molecular weight exudates produced by bacteria and microalgae. For the microbes that produce them, these polysaccharide-like polymers facilitate surface attachment, help trap passing nutrients, exchange genetic material, stabilize sediments, and buffer cells against environmental stressors (Sutherland 1990, Freeman & Locke 1995, Jahn & Nielsen 1995, Decho 2000, De Brouwer & Stal 2001). EPS form a structuring matrix for ubiquitous-occurring aquatic biofilms, whose biomass can far exceed that of bacterial cells (Barlocher & Murdoch 1989, Lappin-

Scott & Costerton 1995). Several marine bacteria produce EPS, including planktonic (Corpe 1970, Berkeley et al. 1980) and deep-sea species (Vincent et al. 1994).

This has forced us to expand modern definitions of bacterial and algal biomass to include the mass of EPS that are closely associated with cells (Goto et al. 1999). Deposit-feeding animals in marine habitats are exposed to EPS in surface films, coated particles, and marine aggregates like transparent exopolymer particles (TEP). TEP are formed from biotic (namely phytoplankton in origin) and abiotic processes like chemical precipitation (Alldrege et al. 1993, Passow 2000) and settle rapidly as marine snow (Passow et al. 1994).

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As a food source, microbial EPS have a potential impact on our understanding of benthic nutrition. Because EPS are ubiquitous in marine systems, seasonal changes in microbial productivity and population change the type of sedimentary carbohydrates available for benthic consumption (Decho 2000). For example, diatom EPS (made mostly of laminarin) on a mudflat can contain approximately 6.04 mg of EPS g^{-1} of surface sediment (Underwood et al. 1995). In the low saltmarsh these concentrations can be as high as 18.37 mg g^{-1} . When bacteria dominate the microbial community, EPS present are often more heteropolysaccharidic but are poorly characterized (Decho 2000, Stal 2000). Even in moderate abundances, however, EPS may have a trophic impact on smaller benthic organisms with low metabolisms. Polychaetes (Jennings & Gelder 1976, Decho & Lopez 1993), copepods (Decho & Moriarty 1990), and amphipods (White & Findlay 1988) are among the benthic macrofauna that absorb microbial EPS. EPS also bind metals (Bitton & Friehofer 1978, Whitfield 1988, Ford & Mitchell 1992) and displace essential metals of biological use, like calcium, in the process (Decho 1997). As pollutants become bound to biofilms, the same charge-related processes traditionally used in wastewater treatment to concentrate toxins in wastewater sludges could also cause sediment films to become pollutant-laden in sediments where benthic species live and feed.

Brittlestars are excellent representatives of the infaunal deposit-feeding community that are exposed to microbial exopolymers, although their economic and ecological importance is not easily recognized. They are scavengers and act as disposers of life and death 'by-products' and contribute to sediment turnover (Fell 1966). Through sub-lethal predation, brittlestars provide nutritional input to shrimp, crabs, and flatfish and could be an important link transferring EPS energy to higher trophic levels (Pape-Lindstrom et al. 1997). *Amphipholis gracillima* (Stimpson) (also *Microphiopholis gracillima*, Turner) belongs to the *Amphiuridae* family, and occurs in South Carolina (Fox & Ruppert 1985), along the eastern shore of the United States, in the Virgin Islands, and Brazil (Thomas 1962). Using its arms and tube feet, it burrows 10 to 15 cm deep in subtidal sediments and feeds at the surface and below (Gielazyn et al. 1999). *A. gracillima* is capable of degrading some algal EPS and probably has enzymes that can hydrolyze other types of EPS found in biofilms (Hoskins et al. 2001). In this study, brittlestars were offered radiolabeled exopolymers cultured from algae and bacteria to determine whether and how efficiently the deposit-feeding ophiuroid could absorb sediment-bound or aqueous exopolymers of microbial origin.

MATERIALS AND METHODS

Brittlestars were collected from Debidue Creek, a pristine mudflat in North Inlet, South Carolina (37° 20' N, 79° 10' W). Individuals were hand-collected from sieved sediment and transported in coolers to the laboratory. Individuals were held in aquaria for approximately 1 mo at the field conditions under which they were collected (25°C, salinity = 32 ppt). Ground Tetramin[®] fish food (Tetrawerke, Germany) was fed to the brittlestars biweekly (approximately 0.03 mg animal⁻¹) and stopped 4 wk prior to the feeding experiment.

Exopolymer isolation. Axenic cultures of *Pseudoalteromonas atlantica* (*Pseudomonas atlantica*; ATCC #43666) were purchased from the American Type Culture Collection and grown for 4 d in Difco-enriched marine medium (25 ppt, pH 8) at 25°C and at 100 rpm on a shaker table. The log-phase culture was radiolabeled with 2.5 mCi uniformly labeled ¹⁴C D-glucose ml⁻¹ culture and allowed to grow a further 6 d. On Day 10, the cultures were fixed with formalin (0.5% final concentration) and centrifuged at 10 000 rpm $\times g$ for 20 min. The pelleted cells were discarded. The exopolymer dissolved in the supernatant was precipitated in ethanol and re-suspended in deionized water after each precipitation (Decho 1993). Precipitation was repeated 3 times, after which the polymer was redissolved and dialyzed in deionized water. Any remaining formalin residue was eliminated during the precipitation and dialysis processes. Dialysis water was changed 3 times at 8 to 12 h increments. Dialyzed polymer was freeze-dried and stored at -70°C until use.

Non-radiolabeled *Pseudoalteromonas atlantica* cultures were extracted as above and sent to the Complex Carbohydrate Research Center (CCRC) at the University of Georgia (USA) for compositional analysis of EPS and to the laboratory of John Hedges in the School of Oceanography at the University of Washington (USA) for comparative analysis. Analyses were performed to determine the major sugar subunits. At the CCRC, samples were hydrolyzed using freshly prepared 1 M methanolic-HCl for 16 h at 80°C. Each sample was N-acetylated using methanol, pyridine and acetic anhydride at room temperature for 6 h. The released sugars were derivatized with Tri-Sil (Pierce Chemical) and the samples were run on a gas chromatograph using a Supelco column. Forty μg of Myo-inositol (#15125, Sigma-Aldrich) was added as an internal standard. Dr. John Hedges performed analyses of *P. atlantica* polymer using high-pressure liquid chromatography to identify individual sugar monomers.

In order to test absorption of microalgal EPS by *Amphipholis gracillima*, axenic cultures of a *Nitzschia* species of diatom were grown for 21 d at 25°C in

enriched F/2 media. At 3 wk, cultures were labeled with 50 mCi uniformly labeled ^{14}C sodium bicarbonate, allowed to grow up to 60 d, then harvested as above. Algal EPS samples were not available at the time compositional analysis was performed. However, literature supports that diatoms produce monosaccharidic EPS in the form of laminarin. Laminarin is a type β -glucuronan composed of glucuronic acid and mannose.

Absorption experiments. Treatments were: bacterial exopolymer bound to sediment (sediment-bound), bacterial exopolymer dissolved in seawater (aqueous), algal exopolymer bound to sediment, and algal exopolymer dissolved in seawater. Brittlestars were offered 0.5 g of sediment food mixtures containing radiolabeled bacterial or algal EPS bound to untreated sediment from the brittlestar collection site and Tetramin[®] fish food (3.086 mg animal⁻¹). Fish food was added to increase palatability. Brittlestars in aqueous treatments received 100 μl of food solution prepared by dissolving bacterial or algal EPS in filtered, autoclaved seawater. Before food mixtures were prepared, the activity of a subsample of each polymer type was measured using a Packard Tri Carb liquid scintillation counter (LSC, Hewlett Packard, model #2100TR/2300TR) in disintegrations per minute (dpm). Algal polymer had an activity of 5.8×10^5 dpm mg^{-1} EPS; bacterial polymer was 5.43×10^3 dpm mg^{-1} EPS. Food mixtures for treatments using sediment-bound algal polymer were mixed with sediment to a final activity of 2.917×10^5 dpm g^{-1} food mixture. Sediment-bound bacterial EPS treatments had a final concentration of 2.07×10^4 dpm g^{-1} food mixture. Final aqueous activities were 2.54×10^{-4} and 1.4×10^{-3} dpm ml^{-1} for the algal and bacterial exposures, respectively. Individual brittlestars were randomly assigned to each treatment ($n = 10$) using a standard table of random digits (Moore & McCabe 1989). Each was placed in a 6.35 cm diameter culture dish (Carolina Biological Supply, #BA-74-1000) filled with 10 ml seawater and allowed to acclimate in the dark for 12 h. All seawater used in this study was filtered on a 0.45 micron Supor-450 membrane (Gelman Sciences, P/N 60173) and a Whatman GF/C filter (VWR Scientific Products #28497-685) and then autoclaved to reduce bacterial activity. Approximately 0.5 g of food mixture was placed in each dish for the sediment-bound EPS treatments. For dissolved treatments, the sediment was replaced with a 1 ml aliquot of EPS solution containing the appropriate exopolymer. All animals were fed at the same time and in darkness for 12 h. After feeding, brittlestars were removed from dishes, rinsed with seawater and placed in 10 ml vials with fresh seawater for 24 h. This 24 h 'cold' incubation (with no food mixture) allowed animals to digest or egest the food they had ingested and represented

the gut evacuation period. Afterwards, animals were rinsed again and the disc cover was removed. The stomach was flushed with seawater to insure that undigested food was not included in tissue activity measurements. Material expelled during the cold evacuation period and this gut rinse was included in the measure of egesta as gut contents. Brittlestars were dissected into 2 body fractions: the disc cover and the oral frame and arms. Each fraction was suspended in 1 ml seawater and digested separately with 2 ml Scintigest (Fisher Scientific, #SO-X-10) at 100°C until tissue was completely solubilized. All samples were suspended in Scintisafe Econo I liquid scintillation cocktail (#SX20-5, Fisher Scientific Products). Activity (in dpm) was measured with the LSC.

Respiration measurements. The amount of ^{14}C lost due to respiration was estimated by feeding 4 brittlestars radiolabeled EPS of each polymer type in the same manner as in the sediment-bound treatment. The cold incubation technique was modified to measure CO_2 release. After feeding, these brittlestars were placed in a 500 ml syringe bottle in 10 ml seawater. A suspended basket was fashioned from the bulb of a transfer pipette and nested with a folded piece of filter paper. After 24 h, 2 ml of H_2SO_4 was added to kill the brittlestar and release dissolved carbon dioxide from the water. The filter paper was saturated with 1% w/v KOH and the bottle was agitated at 50 rpm for 2 h so the released $^{14}\text{CO}_2$ would absorb into the filter paper. The paper was then suspended in cocktail and read in the LSC. The respiration activity (in dpm) was included in measurements of loss in absorption efficiency calculations. Respiration activities were also converted to micrograms of carbon (from CO_2) based on the activity of the polymer used for each treatment. This calculation was performed for each individual and represented carbon lost via $^{14}\text{CO}_2$ by each brittlestar over 24 h. Respiration rates were calculated and expressed in units of $\text{mg C ind.}^{-1} \text{ h}^{-1}$.

Absorption calculations. *Amphipholis gracillima* has a mouth that leads into an esophagus and a blind stomach (Pentreath 1969). Because of its incomplete digestive tract, typical mass balance equations that gauge uptake by fecal evacuation of label could not be used for this brittlestar. Unutilized food is expelled as egesta, making it necessary to modify a tissue/feces-based equation to accommodate the waste produced by this deposit-feeder. Absorption efficiency was defined as the percentage of radioactive label absorbed by the brittlestar divided by the total uptake. It was calculated based upon the amount of radiolabel absorbed and egested by the animal (Decho 1993):

$$\% \text{ absorption efficiency (AE)} = [1 - ({}^{14}\text{C egestion} : {}^{14}\text{C ingestion})] \times 100 \quad (1)$$

The values obtained from the cold incubation and the subsequent rinse were combined to represent gut contents expelled by the brittlestar (egestion). During dissection, the gut contents were flushed before brittlestars were read in the LSC. Though very little material was obtained, it was also viewed as unprocessed food and treated as egesta. Total ingestion was estimated by summing the egestion and the radioactivity of each brittlestar at the end of the experiment. The absorption efficiency reflected uptake of radiolabeled carbon that had been incorporated into tissues. It was corrected for background radioactivity values measured in animals fed unlabeled food, and for unused food in the gut. Background values were attributed to natural fluorescence and not radiolabel. The imposed 12 h feeding period affected the absorption efficiency calculation by making it time-dependent since feeding was artificially ceased and not naturally ended by the brittlestar. However, no feeding activity was observed at 12 h. This time-dependent absorption efficiency was the final datum of interest and was used for statistical comparisons between treatment groups. Absorption efficiencies were assessed and compared using normality tests and an ANOVA using polymer type and exposure as single factors. Two-factor analysis was eliminated due to interaction between the factors. All statistical analyses were performed using Statistical Analysis System software (SAS Institute).

Partitioning between the disc cover, oral frame and arms. Because the majority of the metabolically active tissue in *Amphipholis gracillima* is in the oral frame and arms, comparisons made between the incorporation of EPS into the 2 body sections were made proportional by adjusting the radioactivity measurements in each section by its respective tissue mass. The radioactivities of disc tissues and those in the arms and oral frame were divided by the mean ash-free dry weight (AFDW) of the tissue (disc = 2.33 mg mean AFDW; oral frame and arms = 35.38 mg mean AFDW; Dobson et al. 1991).

RESULTS

Analyses by the CCRC indicated that the exopolymer of *Pseudoalteromonas atlantica* consisted of 70 mol% glucose (Table 1) and 72% total carbohydrate. The remaining sugars (in rank order) were mannose, galactose, galacturonic acid and glucuronic acid. Independent analyses by Dr. John Hedges at the University of Washington showed similar composition, 74% glucose. Mannose composition was higher (14%) and galactose only slightly lower (9%). Hedges' analyses did not include uronic acids or total carbohydrate percentage, but trace analyses for arabinose, fucose, ribose, and rhamnose revealed <1% of each monomer.

Amphipholis gracillima absorbed both algal and bacterial polymer with high efficiency, with very little carbon loss due to respiration (Table 2). Loss of ^{14}C ranged 0.02 to 0.08 $\mu\text{g } ^{14}\text{C ind.}^{-1}$ over 24 h (Table 3). These losses were equivalent to less than 1/10 of a percent of the total body carbon of animals fed algal EPS and less than 1/1000 that of brittlestars fed bacterial EPS. In aqueous- or sediment-bound form EPS was taken up by brittlestars with 83% or higher efficiency (Table 2). Analysis of the \log_{10} -transformed absorption efficiencies showed no evidence against normality via a Wilk-Shapiro test ($p = 0.1704$; Shapiro & Wilk 1965). Normality plots were linear and residual plots were homoscedastic. When brittlestars fed bacterial exopolymer were compared across exposures, animals that received sediment-bound polymer utilized it more efficiently (92.2%) than those exposed to the same polymer in aqueous form (83.3%, 2-sided $p = 0.0364$; Table 2). While sediment-bound algal polymer was absorbed with an efficiency of (90.1%) and was similar to that of sediment-bound bacterial polymer (92.2%), the aqueous algal polymer was absorbed much more effectively than the bound form (>99.9%, 2-sided $p = 0.0005$; Table 2).

Calculations evaluating the total ^{14}C incorporated into the disc cover versus the oral frame and arms showed that brittlestars given EPS in sediment form absorbed almost all the EPS into the disc cover (Fig. 1). In both bacterial aqueous and sediment-bound treatments the radiolabel accumulated in the disc was an order of magnitude greater than that in the oral frame and arms. This was also true for the aqueous algal treatment. Disc concentra-

Table 1. Carbohydrate composition of *Pseudoalteromonas atlantica* exopolymer determined by 2 independent analytical laboratories and methods. CCRC = Complex Carbohydrate Research Center at the University of Georgia (USA). UW = Dr. John Hedges, University of Washington School of Oceanography. -: analysis was not performed

Sugar	Mole %	
	CCRC	UW
Mannose	9.8	14.28
Galactose	9.1	8.81
Glucose	70.3	74.17
Galacturonic acid	6.5	-
Glucuronic acid	4.0	-
Arabinose	-	0.28
Ribose	-	0.70
Xylose	-	0.33
Fucose	-	1.21
Rhamnose	-	0.73
Total accounted for	99.7	100.51
% carbohydrate	72%	

Table 2. Absorption efficiencies (AE) of *Amphipholis gracillima* fed bacterial and algal exopolymer in aqueous and particulate form (mean \pm SE). Statistical differences are indicated by asterisks ($\alpha = 0.05$). * = EPS AE differed between exposures; ** = AE of dissolved EPS differed between polymer types

Exposure	Polymer type	
	<i>Pseudoalteromonas atlantica</i> EPS*	<i>Nitzschia</i> EPS*
Sediment-bound	92.2 + 1.86	90.1 + 1.15
Dissolved**	83.3 + 3.49	>99.9 + 3.62

tions of ^{14}C in brittlestars fed sediment-bound algal EPS were much higher than oral frame and arm levels.

DISCUSSION

EPS availability

Amphipholis gracillima clearly has the ability to absorb secreted algal and bacterial extracellular polymeric secretions. Compositional analyses of bacterial polymers demonstrated that they represent an energy source that can be efficiently used by this animal. Ophiuroids have seasonal and opportunistic feeding habits—they may scavenge, feed on particles or be carnivorous (Dearborn & Edwards 1982, Hendler 1982). In South Carolina, their feeding activity is highest between February and November, when temperatures are rising and organic production increases. Although *A. gracillima* has been described primarily as an indiscriminant surface deposit-feeder, omnivore, and scavenger (Singletary 1970), high algal exopolymer absorption efficiencies are appropriate given the ecology of the mudflat this species lives in. Such high absorption efficiencies also make a plausible connection between high seasonal benthic diatom production and the feeding behavior of the brittlestar. Through its ability to absorb both algal and bacterial EPS, *A. gracillima* could capitalize on each carbon source during its time of abundance. Cammen (1980) observed a similar seasonal dynamic when he calculated a partial monthly carbon budget for the deposit-feeding polychaete *Nereis succinea*. In the fall and winter months, bacteria contributed more to the carbon requirement of the polychaete (7 to 10%) than microalgae (2 to 8%). In the warmer months, the algal contribution increased to 7 to 10% as well, diminishing to lower values with the end of the warm season. In other environments, microalgal biomass can be an order of magnitude higher than bacterial biomass in surface sediment

Table 3. Carbon loss by respiration of *Amphipholis gracillima* fed sediment-bound bacterial and algal EPS. Values are expressed as micrograms of carbon respired as carbon dioxide by brittlestars over 24 h. Carbon was converted from ^{14}C measured in disintegrations per minute (dpm)

Individual	Bacterial EPS	Algal EPS
1	0.0537	14.64
2	0.774	3.82
3	0.0198	1.99
4	lost	18.45
Mean	0.025 \pm 0.01	4.86 \pm 2.02

(Ruble 1982) and can account for over 70% of the organic carbon in some size fractions of the sediment (Cammen 1980). Such an opportunistic absorption strategy would make sense for other members of the benthos like bivalves (Decho & Luoma 1996) and polychaetes that may also need flexible dietary strategies in order to survive.

In comparison with the abundance of EPS found in natural biofilms, EPS additions provided in this study are conservative and much lower than the levels benthic invertebrates may typically encounter in their natural habitat. Using extraction methods similar to those in this study, Underwood et al. (1995) analyzed marine intertidal sediments for soluble (hydrophilic, labile) and capsular (EDTA-extractable) EPS. They found 4.78 to 10.94 mg total EPS g^{-1} sediment in samples from different areas of the saltmarsh. Total diatom EPS abundance was approximately 6.04 mg g^{-1} sediment in mudflat samples where the microbial community was dominated by diatom films. Soluble diatom EPS like that used in this study accounted for 1.64 \pm 0.9 mg g^{-1} sediment in these films. Upper saltmarsh samples in which filamentous bacteria made up 50% of the microbial community had 10.94 mg g^{-1} sediment. Samples from a cyanobacterial mat yielded roughly half that amount. Relative to the sediment abundance of EPS measured by Underwood et al. (1995), *Amphipholis gracillima* may be exposed to 0.82 mg g^{-1} sediment diatom EPS in the natural environment, more than the 0.255 to 0.577 mg algal EPS that brittlestars received in this study. Therefore, it is possible that *A. gracillima* and other deposit-feeders have twice the EPS abundance available to them in natural sediments than was present in this study. In some environments, EPS abundance may be 3 to 6 times as high. If volumes of this magnitude are coupled with absorption efficiencies greater than 90%, there is great potential for substantial microbe-macrofauna energy transfer and more evidence that microbial constituents may significantly support some sediment communities. Although bacteria coated with capsule polymers are differentially

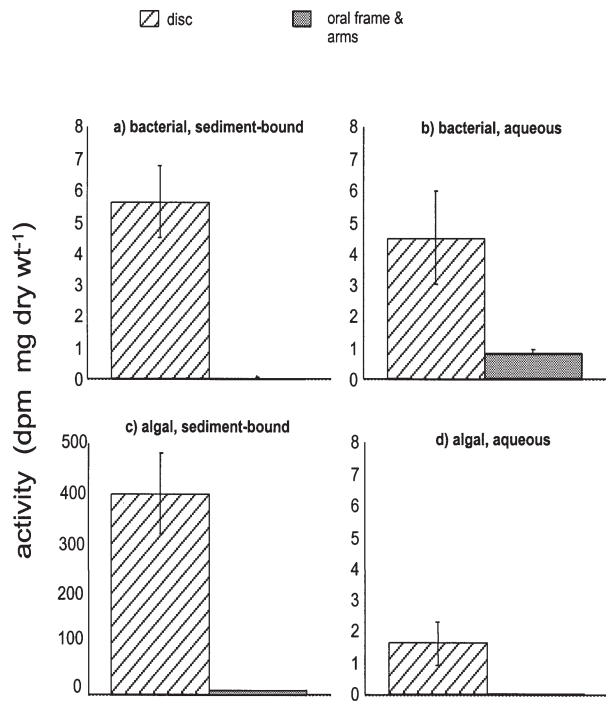


Fig. 1. Partitioning of ^{14}C -labeled exopolymer in the disc, oral frame and arm tissues of *Amphipholis gracillima*. Values represent true absorption in each body section (mean dpm \pm SE, $n = 11$). Absorption values were divided by the mean tissue mass (disc = 2.33 mg, oral frame and arms = 35.38 mg) from Dobson et al. (1991)

digested by deposit-feeders and some EPS are more degradable by gut enzymes than others (Plante & Shriver 1998, Plante 2000), this research supports the idea that some sediment communities may be significantly supported by their microbial constituents (Plante et al. 1990).

Energy gain

The carbon needs of *Amphipholis gracillima* may be largely satisfied by the cellular and polymeric components of microbial films because, unlike other deposit-feeders with greater energy requirements, brittlestars have less metabolically active tissue compared to other benthic invertebrates of similar size. The typical echinoderm skeleton forms more than 50% of the dry weight of the organism and is greater than 71% for most ophiuroids (Emson 1984). Since the skeleton of *A. gracillima* comprises 58 to 71% of its total weight (Dobson et al. 1991, Stancyk et al. 1994) it should have reduced energetic costs compared to organisms with a greater amount of metabolically active tissue (Lawrence 1987, Clements et al. 1988). Ophiuroid oxygen

uptake rates are lowest of the 4 echinoderm classes. Carbon loss measured in this investigation was low, as are literature values for oxygen use in this species (Clements et al. 1988). If we use respiration as a gauge for energetic demand, our uptake data suggest that brittlestars are able to effectively exploit EPS, which, from the energetic perspective of the brittlestar, are a rich resource.

Brittlestars absorbed 83 to 99% of the 0.127 to 2.295 mg polymer they received in the sediment food mixture. *Amphipholis gracillima* received approximately 0.988 to 24.18 μg carbon from EPS in 12 h and respired 0.0198 to 18.45 μg carbon in the form of $^{14}\text{CO}_2$ over the 24 h cold incubation period. These outcomes indicate that brittlestars are able to utilize microbial EPS efficiently, regardless of type or form.

Although energy budgets have not been constructed for *Amphipholis gracillima*, the mean individual has an ash-free dry weight of 52.47 mg and an estimated caloric content of 0.93 kcal g^{-1} dry weight (Dobson et al. 1991). Based on these values, the average specimen of *A. gracillima* has a total body energy content of 355.2 J. Numerical densities of amphiurids in the Mediterranean Sea (*Amphiura angulari* and *Amphiura antarctica*) and in the South Indian Sea (*Amphiura chiajei* and *Amphiura filiformis*) represent 15.9 to 46.8 kJ m^{-2} (Lawrence & Guille 1982). Thirty *A. gracillima* in a square meter in North Inlet, South Carolina, have a standing crop of approximately 10.66 kJ. Since amphiurids are regularly exposed to repeated sub-lethal predation (Pape-Lindstrom et al. 1997) and rapidly regrow lost tissue (Stancyk et al. 1994), they represent a renewable energy source for their predators, and one that is probably supported by sedimentary carbon sources like microbial EPS. In the SE United States, *A. gracillima* is abundant in muddy environments and is commonly preyed upon by shrimps and crabs. Our data indicate that brittlestars could be important not only to nutrient cycling, but also to energy transfer to commercially important species.

Role of EPS exposure

Amphipholis gracillima accumulated more of the carbon label in the disc cover than in the oral frame and arms most likely because digestion and absorption across the gut wall was still taking place. Baird & Thistle (1986) found a similar short-term concentration of radiolabeled *Pseudoalteromonas atlantica* polymer in the hemal vessels associated with the intestine of a holothurian.

The form of exposure affected both bacterial and algal polymer utilization. Brittlestars absorbed aque-

ous algal polymer much better than aqueous bacterial polymer, with the highest absorption efficiency of all treatments (>99.9%, Table 2). Utilizing non-particulate EPS is important for an animal with regenerative capabilities like *Amphipholis gracillima*. When threatened, brittlestars may 'autotomize' or cast away the arms or central disk cover (including the gut). For regenerating brittlestars that have lost their disc cover or for other soft-bodied invertebrates that regenerate, resorption and dissolved uptake are the primary form of sustenance. Fielman et al. (1991) showed that regenerating brittlestars directly store and consume energy to replacement tissues until the animal develops to what is considered its 'minimal functional configuration'—3 arms and a disc with a gut capable of processing food. It takes 8 to 14 d for *A. gracillima* to regenerate a functioning gut (Dobson 1986). Until then, the brittlestar takes up dissolved nutrients such as amino acids or resorbs stored nutrients, using these resources to regenerate lost tissues (Clements et al. 1988). High absorption efficiencies in the aqueous uptake treatments of both polymers support the contention that brittlestars are probably effective consumers of dissolved organics, especially during regeneration.

The ubiquity of bacterial biofilms in marine sediments makes it likely that brittlestars are exposed to particles with EPS bound to them. A typical biofilm in muddy sediment is most distinct on the surface, where diatoms and other phototrophs generate EPS (Decho 2000). Deposit-feeders foraging at the sediment-water interface disrupt microbial films as they burrow and feed. As an indiscriminate particle feeder, *Amphipholis gracillima* most likely consumes sediment-bound EPS in this manner and in amounts greater than those provided to the animals in this study.

Mechanisms of dissolved material uptake

Symbiotic bacteria often aid animals in the acquisition of nutrients and are normally considered as being associated with the gut. However, several species of echinoderms, including 10 ophiuroids, have subcuticular bacteria living under the epidermis (Kelly & McKenzie 1995). Although no ecological role has been identified for these bacteria, their presence has implications for feeding across developmental stages, uptake of dissolved organic material and recognition of antigens (Kelly & McKenzie 1995). A portion of the dissolved EPS accumulated by *Amphipholis gracillima* could be attributable to endosymbionts. However, in experiments conducted to measure the contribution of subcuticular bacteria to dissolved nutrient uptake, Lesser & Walker (1992) showed that only 1% of the total dissolved amino acids taken up by the ophiuroid

Amphipholis squamata could be attributed to its subcuticular bacteria. We considered the role of subcuticular and gut bacteria to be minimal, so no effort was made in this study to separate their uptake of radiolabel from that of the brittlestar.

This is the second study to demonstrate the utilization of microbial EPS by an echinoderm since EPS uptake was demonstrated in a holothurian (Baird & Thistle 1986). *Amphipholis gracillima* is the first ophiuroid shown to incorporate microbial EPS into its tissues. These results support the hypothesis that microbial EPS may contribute significantly to the energetic needs of infaunal ophiuroids. Additionally, the pronounced absorption of algal and bacterial EPS shown here illustrates a short pathway for microbial exudates to be utilized by higher trophic levels and indirectly by their predators. The results suggest that microbial exopolymers may represent an important nutritional component in benthic ecosystems.

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