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Notes and Discussions

Nitrogen Assimilation from Amorphous Detritus by Two Coastal Consumers

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The food value of recognizable pieces of dead vegetation, morphous detritus, has been the focus of many studies in coastal systems. In contrast, the nutritional quality and formation process of amorphous detritus, aggregates of dissolved organic matter (DOM), is poorly studied. We created ^{15}N-labelled aggregates from the leachate of four macrophytes, a marsh grass and three macroalgae common in New England coastal waters. We fed the labelled aggregates to two coastal consumers, the grass shrimp *Palaemonetes pugio* and the sheepshead minnow *Cyprinodon variegatus*. Fish and shrimp fed each of the labelled aggregates became labelled with ^{15}N. This study provides direct evidence for nitrogen assimilation from amorphous detritus by marine consumers. In addition, fish fed amorphous marsh grass detritus assimilated 10–40 times more nitrogen from this detritus than from morphous grass detritus. Therefore, amorphous aggregates may be higher-quality food than morphous detrital fragments for coastal consumers.

Introduction

Grass and macroalgal fragments have been the focus of many studies on detrital feeding in coastal waters (e.g. Nixon, 1980). These recognizable pieces of dead vegetation, 'morphous' detritus (*sensu* Bowen, 1984) are created during microbial and mechanical breakdown of structural plant tissue. Less well studied are 'amorphous' detrital aggregates of dissolved organic matter (DOM). Morphous fragments are usually large (about 2500–250 μm; Bowen, 1987) while amorphous particles are often smaller (250–0·5 μm), translucent, and roughly spherical (Bowen 1980, 1984, 1987; Camilleri & Ribi, 1986).

These two types of detritus may differ in food quality. In one of the few comparative studies, an aquatic consumer obtained more protein from amorphous particles than from morphous fragments (Bowen, 1984). Morphous detritus may be nutritionally inferior because it may contain refractory cellulose and lignin, secondary plant compounds, as well as humic polymers that bind proteins in older material (Valiela, 1984; Rice, 1982).

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While numerous studies show that DOM lost from marine producers may subsequently precipitate as particles (e.g. Johnson & Cooke, 1980; Biddanda, 1985), the process by which precipitation occurs, including the role of microbes, is not well understood (Bowen, 1984). Macrophytes are probably a major source of precipitated aggregates in coastal waters because DOM loss from both living and decaying macroalgae and seagrasses appears to be high. Reported DOM release rates are variable, but, for example, Hatcher *et al.* (1977) estimated DOC losses of 35–40% of C fixed by a macroalga. As another example, Marinucci *et al.* (1983) found that 66% of initial N was lost from marsh grass, *Spartina alterniflora*, within 10 days after senescence. Most of the N in the tissue of this plant is amino nitrogen (Buchsbaum *et al.*, 1991), therefore aggregates formed from *S. alterniflora* leachate are potentially nutritious for heterotrophs.

In this study we used ^{15}N as a tracer to determine whether two species of coastal consumers assimilated nitrogen from amorphous particles. We focused on N because this element appears to limit growth of marine producers and consumers (Valiela, 1984). In this study we labelled four macrophyte species with ^{15}N and created labelled amorphous aggregates from leachate of the labelled macrophytes. We then fed the labelled detritus to the consumers and determined whether the animals were labelled with ^{15}N. Presence of ^{15}N in the consumers would demonstrate that they assimilated nitrogen from the amorphous particles aggregated from DOM released by labelled producers.

Materials and methods

Labelling the producers

We used four common coastal macrophytes in this study: a brown (*Fucus vesiculosus*), a red (*Ceramium rubrum*) and a green (*Codium fragile*) macroalga and saltmarsh cord grass (*Spartina alterniflora*). The algae were collected from a pier in Woods Hole, MA, in August 1987 and the grass from Little Sippewissett marsh in Falmouth, MA, in July 1987.

To label the algae we incubated 100-g wet weight aliquots of each alga in separate jars in 2·5 l of seawater spiked with 99% enriched $^{15}NH_4Cl$ (Cambridge Isotopes, Woburn, MA). The jars were placed beneath GRO lights and aerated continuously. The temperature in the jars was maintained at about 25 °C by placing them in running seawater. Three times (days 1, 4 and 7) a week 380 mg $^{15}NH_4Cl$ was added to each jar with fresh seawater. On day 8 the algae were rinsed in seawater and frozen.

To obtain labelled *Spartina*, plants were gently removed from a sandy marsh area and placed in 10-l plastic buckets filled with sand. All leaves present above-ground were clipped. The plants were grown in a growth chamber for 6 weeks. They were watered daily with half-strength seawater that slowly drained out through a hole in the bottom of the bucket. The plants grew well under these conditions. Three times a week 50 mg of fresh $^{15}NH_4Cl$ in solution was injected into each of the several buckets with a syringe. After 6 weeks all the newly grown leaves were harvested and frozen.

Release of DOM and aggregate formation

Our general procedure for production of amorphous detrital aggregates was to leach the dead macrophytes, centrifuge the leachate to remove morphous detritus, and then to bubble the centrifuged leachate. Other studies have shown that aggregates form when seawater is bubbled (Baylor & Sutcliffe, 1963; Johnson & Cooke, 1980). Specifically, 100 g

of frozen-killed algae or grass were allowed to leach in 2·5 l of 0·22-μm filtered seawater in the dark. In pilot studies we determined the leaching time that resulted in maximum DOC production for each species. We leached *C. rubrum* and *C. fragile* for 12 h and *F. vesiculosus* and *S. alterniflora* for 6 days.

We removed morphous detritus from the leachate by centrifuging 250-ml aliquots of leachate at 4500 rpm for 10 min. The centrifuged leachate was bubbled in non-sterile jars with air pumped through airstones; the air was pre-filtered through in-line filters. The bubbling period was 4–8 days depending on the macrophyte source; previously we determined the optimum number of days for aggregate accumulation for each species. The amorphous aggregates were collected for feeding by the centrifugation method described above.

Feeding aggregates to fish and shrimp

As consumers we used the sheepshead minnow (*Cyprinodon variegatus*) and the grass shrimp (*Palaemonetes pugio*), because these species feed on detritus and algae (Johannes & Satomi, 1966; D'Avanzo & Valiela, 1990). The fish were collected in Great Sippewissett Marsh, Falmouth, MA, and the shrimp in West Falmouth Harbor, MA, in August 1987.

We fed aggregates from the four macrophyte sources to both *C. variegatus* and *P. pugio*. Animals were starved overnight and then placed in 3-l jars containing constantly aerated 22 °C seawater. Food additions were *ad libitum* with the possible exception of *C. fragile*, and the seawater was changed at each feeding. *C. variegatus* was fed for 12 h, with new food added in 5-ml aliquots every 4 h. There were nine *C. variegatus* each weighing approximately 1 g in each jar. *P. pugio* was fed for 6 h, with new food added in 5-ml aliquots every 3 h. There were 15 *P. pugio* each measuring 1·5–2·5 cm in length in each jar. Each of the four food treatments was run in triplicate for each animal.

To determine whether either shrimp or fish could become labelled by absorbing dissolved ^{15}N, we added 50 ml of each type of supernatant obtained while centrifuging aggregates to separate jars for each species and placed animals in these jars for 24 h. We assumed that most of the ^{15}N in this trial was DON rather than DIN because we used leachate. Each of these treatments was duplicated. Background ^{15}N for both species was measured in six samples of overnight-starved controls.

After feeding was completed, food was removed from the jars of both species of consumers. The seawater was changed and the animals were allowed to defaecate. To prevent the animals from eating potentially labelled faeces, we placed a screen attached to a rubber ring in the jar beneath the animals. The faecal pellets accumulated in the jar-bottom beneath the screen.

We allowed the fish to empty their guts of food for 6 h without food and then the fish were gutted. The shrimp emptied their guts for 4 h; this is eight times the duration of gut passage time for *P. pugio* (Johannes & Satomi, 1966). After the flushing period, all animals were frozen at −20 °C.

The frozen animals from each jar plus the untreated control animals were placed in a −100 °C freezer where they became brittle and were easily ground by hand with a steel mortar and pestle. Ground powders of unlabelled plus labelled and leached macrophytes were also prepared in this way. The detrital aggregates were collected by filtration onto Whatman GF/C glass fibre filter paper. All of these samples were sent to the Waikato Stable Isotope Unit in Hamilton, New Zealand, for mass spectrometry analysis of the per cent of the N present as ^{15}N.

TABLE 1. $\%_{o}$ ^{15}N in labelled and leached macrophytes and detrital aggregates that formed in the leachate. Numbers are $\bar{x} \pm$ SD of three to six replicates. Background $\%_{o}$ ^{15}N values for the macrophytes are about 0·37% ^{15}N

Macrophyte genus	Labelled and leached macrophytes	Aggregates
Spartina	26·833 ± 2·561	23·317 ± 0·227
Codium	24·732 ± 2·117	31·508 ± 0·431
Fucus	5·731 ± 0·138	11·897 ± 0·186
Ceramium	5·751 ± 1·414	9·121 ± 0·286

TABLE 2. $\%_{o}$ ^{15}N in aggregates used as food and in *P. pugio* and *C. variegatus* fed aggregates from four macrophyte sources. Numbers are $\bar{x} \pm$ SD of three replicates. Background value for both consumers is about 0·37 $\%_{o}$ ^{15}N

Aggregate source	Aggregates	*P. pugio*	*C. variegatus*
Spartina	23·317 ± 0·227	0·825 ± 0·015	0·828 ± 0·038
Codium	31·508 ± 0·431	0·560 ± 0·038	0·730 ± 0·047
Fucus	11·897 ± 0·186	0·869 ± 0·003	0·737 ± 0·013
Ceramium	9·121 ± 0·286	0·546 ± 0·016	0·694 ± 0·063

Results

Detrital aggregates formed in the centrifuged and bubbled leachate from each of the four macrophytes. Each leachate type was stained the colour of the source macrophyte on the first day of bubbling following centrifugation, but was not visibly turbid. After 1–2 days of bubbling, the leachates became cloudy and bacteria were seen in microscopic examinations of the water. By 3–4 days, a variety of flagellated protozoa and ciliates were common and aggregates were evident in all jars. Aggregates were most abundant after 2 days for *C. fragile*, 3 days for *F. vesiculosus*, 4 days for *C. rubrum*, and 5 days for *S. alterniflora*. POM collected on these peak days ranged from about 0·50 mg ml^{-1} in the *Fucus* treatments to $<0{\cdot}01$ mg ml^{-1} in the *Codium* treatments.

The $\%_{o}$ ^{15}N values of fronds and shoots of the four macrophytes that were labelled and then experimentally leached were well above background values of 0·373 ± 0·008 (Table 1). Values for *C. fragile* and *S. alterniflora* were especially high (about 25% ^{15}N). The degree of label in the aggregates was also high and different in each aggregate type; these values ranged from about 10% ^{15}N for *C. rubrum* and *F. vesiculosus* aggregates to about 30% ^{15}N for aggregates from *C. fragile*.

The $\%_{o}$ ^{15}N values of the macroalgal aggregates were several per cent higher than the values of the leached thallus (Table 1). In contrast, the degree of label in the leached *Spartina* grass and aggregates was about the same. The ^{15}N content and $\%_{o}N$ of filter blanks were very low in all experiments.

Fish and shrimp that were fed each of the labelled detrital aggregates became labelled with ^{15}N (Table 2). The ^{15}N values of both animals in each of the food treatments is well above the animals' background values. There is no clear relationship between degree of label in the food and degree of label in either consumer. If animals fed *Codium* aggregates

TABLE 3. % body N day^{-1} assimilated by *P. pugio* and *C. variegatus* from amorphous aggregates (present study) and by *C. variegatus* in three trials with morphous *S. alterniflora* (D' Avanzo & Valiela, 1990). Values are $\bar{x} \pm$ SD of animal's N contributed by food. See text for calculation equation

Aggregate source	Amorphous aggregates		Morphous detritus	
	P. pugio	*C. variegatus*	Food source	*C. variegatus*
Spartina	3·946 ± 0·162	3·952 ± 0·318	*Spartina*	0·340 ± 0·099
Codium	1·200 ± 0·202	2·280 ± 0·302	*Spartina*	0·110 ± 0·028
Fucus	8·880 ± 0·070	6·320 ± 0·214	*Spartina*	0·367 ± 0·047
Ceramium	4·266 ± 0·416	7·000 ± 1·386		

were food-limited, their ^{15}N content may be somewhat low; however, aggregates even in this treatment were not completely consumed during the experiment. The ^{15}N content of animals in the dissolved ^{15}N treatment was equivalent to background values (0·376 ± 0·003).

We calculated N assimilated by the shrimp and fish as

$$100 \times \left[\frac{\%\,^{15}N_{\text{animal final}} - \%\,^{15}N_{\text{animal initial}}}{\%\,^{15}N_{\text{labelled food}} - \%\,^{15}N_{\text{animal initial}}} \right].$$

This equation assumes mixing of N from the labelled food with N of the original animal to produce the isotopic composition of the labelled animal. Therefore, the per cent of the animals' N that is assimilated from the labelled food is a function of the change in isotopic ratio in the animal as a result of feeding relative to the degree of label in the food.

The per cent of body N assimilated by *P. pugio* and *C. variegatus* from amorphous aggregates in the present experiment is about 1–9% day^{-1} (Table 3). Fish fed amorphous *Spartina* aggregates assimilated about 4% body N day^{-1}. In a previous set of experiments with morphous *S. alterniflora* detritus, *C. variegatus* assimilated between about 0·1 to 0·4% body N day^{-1} (D' Avanzo & Valiela, 1990). Since the overall protocols of the previous experiment were similar to those of the present work, the data are comparable; *Cyprinodon* assimilated about 10–40 times more N from amorphous *Spartina* detritus than from morphous *Spartina* detritus.

Discussion

Our study provides direct evidence for N assimilation from amorphous detritus by marine consumers. The fact that both fish and shrimp assimilated N from amorphous aggregates created from four distinct sources suggests that this may be a general phenomenon. While amorphous particles are common in seawater, their food value has rarely been studied. Baylor and Sutcliffe (1963) grew *Artemia* with aggregates made by bubbling seawater. Reiswig (1971) measured POC taken up by sponges *in situ* and found that amorphous particles constituted 80% of the material. Camilleri and Ribi (1986) grew several crustaceans on aggregates made from mangrove leachate. Therefore, a variety of aquatic

organisms apparently can grow on (other studies) and assimilate N from (our study) amorphous detrital aggregates.

Nitrogen appears to limit the growth of many marine organisms, particularly those consuming abundant but low-quality detritus (Valiela, 1984). Our calculation of per cent of body N assimilated by *C. variegatus* suggests that N from the four types of amorphous aggregates, including those of *S. alterniflora*, is assimilated at a much greater rate than N from morphous *S. alterniflora* detritus (Table 3). For comparative purposes we have normalized the N exchanged on a per day basis because the duration of feeding with labelled N in the present experiment was 0·5 day, while in the previous experiment (D'Avanzo & Valiela, 1990) fish were fed for 5 days. These data corroborate Bowen's (1984) findings that proteins in morphous detritus are less digestible than proteins in amorphous detritus.

We do not know why detrital aggregates appear to be better food for consumers than some types of detrital fragments; we have not analysed the chemical composition of the aggregates used in this study other than for their $\%^{15}N$ content. We expect that highly labile compounds such as amino acids and simple sugars are released during our leaching process from both *Spartina* and macroalgae and it is these compounds that are incorporated into aggregates. In fact, in additional studies the C/N ratio of macrophyte aggregates ranged from 4·0 to 5·5 (M. Alber, unpubl.) suggesting that these aggregates would be a desirable food.

For each of the macroalgae, the $\%^{15}N$ values of the aggregates were higher than the leached macrophytes (Table 1). This is not surprising since the algae were grown with ^{15}N for a week and were probably not uniformly labelled. Perhaps the most labile fractions, containing more ^{15}N than the refractory structural compounds, leached from the algae and precipitated as aggregates. The aggregates and leached macrophyte per cent ^{15}N values were most similar in the case of *S. alterniflora*. The *Spartina* plants were initially cut to ground level and therefore the leaves collected at 6 weeks were probably more uniformly labelled.

How aggregates form from leached DOM is not known. The role of microbes in converting DOM into particles available to macroconsumers has been particularly controversial (Biddanda, 1985). In our study we could not distinguish between microbes and aggregates as N sources for consumers. We observed aggregates in microscopic examinations only after the leachate became turbid with bacteria and protozoa, suggesting that the microbes played a role in aggregate formation. Bacteria may accelerate aggregate formation through secretions of extracellular polymers (Hobbie & Lee, 1979; Paerl, 1978; Biddanda, 1986). In addition, protozoa increase particle size by feeding on smaller microbes (Heinle *et al.*, 1977). In our study, aggregate size ranged from <1 to several mm in length before centrifugation. Therefore, these particles should be available to animals with a spectrum of feeding strategies.

It is not surprising that more conspicuous and identifiable detrital particles of coastal systems—fragments of grasses and seaweeds—have been the focus of detrital foodweb studies. However, less conspicuous foods are apparently more important than previously realized in some shallow water habitats (Kneib & Stiven, 1980). The nutritional value of amorphous aggregates to coastal animals has not yet been determined; these particles are difficult to study *in situ* because their origin is usually not known and they are not easily separated from other particles. Despite these challenges, we suggest that our understanding of coastal trophic dynamics will be limited until the role of amorphous aggregates in detrital foodwebs is better understood.

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