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Heterotrophy on ultraplankton communities is an important source of nitrogen for a sponge–rhodophyte symbiosis

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Summary

Grazing on ultraplankton by the sponge partner of an invertebrate/algal symbiotic association can provide enough particulate organic nitrogen to support the nitrogen needs of both partners. The previously unknown natural diet of the sponge in the *Haliclona–Ceratodictyon* association consists of bacteria and protozoans, which are rich sources of nitrogen. Retention of ultraplankton varied with season and time of day. During the winter there was an order of magnitude more nitrogen taken up than in summer. Time of day during each season also affected the amount of ultraplankton retained. In summer retention was higher at night whereas the opposite was true during winter. Overall, the *Haliclona–Ceratodictyon* association is able to meet its metabolic nitrogen demands through grazing on the naturally occurring water column community.

Key words: coral reef, heterotrophy, rhodophyte, sponge, symbiosis, ultraplankton, nitrogen.

Introduction

Symbioses between autotrophic and heterotrophic organisms are commonly found in environments where levels of dissolved nutrients and particulate organic matter are low. Coral reefs are notoriously oligotrophic yet they harbour a suite of invertebrate/phototroph associations (Muscatine and Porter, 1977). Deep-sea equivalent symbioses can be found at hydrothermal vents and cold seeps where invertebrate/chemoautotroph associations are common (Fisher, 1990; Childress and Fisher, 1992). In both of these environments the energy source for the autotroph, be it light or chemical, is not limiting, but amounts of particulate matter (i.e. food) for the heterotroph are low (Lewis, 1977; Hatcher, 1988, 1990; Sorokin, 1990). Most of the organic carbon requirements (including energy needs), of many symbiotic associations can be met by the autotrophic partner (Fisher, 1990; Childress and Fisher, 1992; Muscatine et al., 1984). But other nutrients are needed for maintenance of such associations and these may be acquired through heterotrophy by the invertebrate host (Pile and Young, 1999).

After carbon, nitrogen is the next most important nutritional requirement for both autotrophs and heterotrophs, as it is essential for the synthesis of amino acids. Marine autotrophs take up dissolved inorganic nitrogen (DIN) in the forms of ammonium, nitrate or nitrite, whereas heterotrophs obtain their nitrogen as particulate organic nitrogen (PON), as part of their diet, and in some cases may also use dissolved organic nitrogen (DON). Cnidarian/algal associations are amongst the most frequently studied symbiotic associations. Cnidarian hosts are well-documented carnivores and/or omnivorous suspension feeders, whose feeding supplies the nitrogen to maintain both partners of the association (Cook et al., 1992; Wang and Douglas, 1998). However, not all invertebrate hosts have the capacity to feed. The vestimentiferan worms common to hydrothermal vents and cold seeps obtain nitrogen by taking up DIN from the overlying water column (Fisher, 1990; Childress and Fisher, 1992). Other cold seep and hydrothermal vent organisms such as bivalves can meet their metabolic carbon demand through their chemoautotrophic symbionts while the invertebrate host supplies a portion of the nitrogen by grazing on ultraplankton (Pile and Young, 1999).

Symbioses between sponges and algae are common on coral reefs. We have been examining the nutritional relationship the symbiotic association between the sponge *Haliclona cymaeformis* (Esper, 1794) (Demospongiae, Haplosclerida; formerly *Sigmadocia symbiotica*) and the red macroalga *Ceratodictyon spongiosum* (Zanardini, 1878) (Rhodymeniales, Rhodophyta). For the *Haliclona–Ceratodictyon* association most or all of the carbon required by the alga is derived from photosynthesis, but very little photosynthate is translocated to the sponge (less than 1.3% of total photosynthetically fixed carbon; A. Grant, unpublished data). However, nitrogen stable isotope values of +4.88±0.28‰ and +2.33±0.18‰ for the sponge and alga, respectively, indicate that nitrogen for both is most likely to be derived from heterotrophic sources (Davy

et al., 2002). As sponges are known to graze primarily on ultraplankton (Pile et al., 1996; Pile, 1997), it is the most likely source of nitrogen to the association and carbon to the sponge. Ultimately, the *Haliclona–Ceratodictyon* association would need to consume 0.275 mg N day^{-1} g^{-1} in order to maintain its nitrogen balance/content (Davy et al., 2002). In the light of these findings we conducted a series of feeding experiments to determine the natural diet of the sponge and its feeding ecology, in order to ascertain if it is possible for the sponge to consume enough PON to sustain the relationship. Since it is highly likely that both food availability and water processing by the sponge would vary with season and time of day, we used flow cytometry to measure feeding rates in summer and winter, with time of day (day *vs* night) nested within season.

Materials and methods

Seawater and branches of the *Haliclona–Ceratodictyon* association were collected from the lagoon of One Tree Reef (23°30′S, 152°06′E), southern Great Barrier Reef; both the association (Price et al., 1984; van Soest, 1990) and location (Trautman et al., 2000) have been described. Winter trials were conducted between 20th and 21st July, 2000 and summer trials between 8th and 9th January, 2001. Daytime trials were conducted between 13:00 h and 15:00 h and night time trials between 19:00 h and 23:00 h, and water was collected 30 min prior to the start of trials. All sponges were kept submerged in the lagoon until they were needed. Owing to the small size of the sponge oscula, direct measurements of the exhalant current could not be made, so indirect measurements of feeding were used. Incubations were conducted in 2 liter beakers, containing 1 liter of seawater, vigorously aerated using two airstones. During the daytime experiments, a constant irradiance of 132 μE was provided by a 12 V, 50 W quartz halogen lamp (Precis, Cleveland, OH, USA) positioned 70 mm from the beaker, while at night the beakers were shielded from ambient light by a wooden screen covered with a black cloth. The irradiance at the edge of the beaker was measured at 0 μE using a Licor photometer (LI-189; Lincoln, NB, USA). Temperature was monitored throughout the incubations; it did not change over the course of either of the trials by more than ±1°C, but did vary between seasons. In winter the temperature ranged from 21–22°C and in summer from 28–30°C.

Water samples for analysis of ultraplankton by flow cytometry were collected with a Gilson (Middleton, WI, USA) pipettor from beakers that contained either the association or seawater only (N=3). Triplicate 1 ml samples were collected at different points within the beaker at the beginning of each trial and after 15 min. Water samples were preserved for flow cytometry following standard protocols (Pile et al., 1996), transported to Flinders University on dry ice, and maintained at –80°C until analysis. An additional 10 ml of water from each beaker was filtered onto a 0.02 μm black polycarbonate filter and frozen for subsequent visual confirmation of ultraplankton populations.

Treatment beakers contained one sponge branch, which was returned to the lagoon at the end of the trial. At the conclusion of each experiment, each sponge was blotted dry with a paper towel and weighed to the nearest 0.01 g. The sponges were then returned to the lagoon. The relationship between wet mass and dry mass was quantified by linear regression analysis from paired samples (Sokal and Rohlf, 1981). Fresh sponge branches (N=58) were collected and their blotted wet mass determined. These branches were then dried at 60°C and reweighed. The flux rate of association was then normalized to the association dry mass to meet scientific conventions for the reporting of fluxes.

Ultraplankton populations were quantified using a FACscan (Becton Dickson, San Jose, California, USA) at Flinders University, South Australia following the techniques of Marie et al. (1997). Orange fluorescence (from phycoerythrin), red fluorescence (from chlorophyll) and green fluorescence (from DNA stained with SYBR Green) were collected through band-pass interference filters at 650, 585 and 530 nm, respectively. The five measured parameters, forward- and right-angle light scatter (FALS and RALS), and orange, red and green fluorescence were recorded on 3-decade logarithmic scales, sorted in list mode, and analysed with custom-designed software (Vaulot, 1989). Ultraplankton populations were identified as general cell types of bacteria (Bac), *Prochlorococcus* sp. (Pro), *Synechococcus*-type cyanobacteria (Syn), and protozoans (Proto). Cell types were visually confirmed, and mean cell diameter measured (N=50) using epifluorescence microscopy.

Depletion rates of ultraplankton were calculated assuming an exponential growth and clearance of prey following the methods of Ribes et al. (1998). In summary, prey growth rate k (h^{-1}) is computed as:

$$k = \frac{\ln(C_f/C_o)}{t_f - t_o} , \quad (1)$$

where C_o and C_f are the prey concentrations in the beaker at the initial time t_0 and final time t_f. The clearance rate F (volume processed by g^{-1} wet mass sponge $time^{-1}$) is computed as:

$$F = V(g/w) , \quad (2)$$

where V is the volume water in the beaker, w is the wet mass, and g is the grazing coefficient (h^{-1}), which is computed as:

$$g = k_c - k_g , \quad (3)$$

where k_c is the prey growth rate in the control beakers and k_g is the apparent growth in the grazing beakers. The ingestion rate I (prey ingested g^{-1} wet mass $time^{-1}$) is:

$$I = FC , \quad (4)$$

where C is the average prey concentration during the trials, calculated as:

$$C = \frac{C_o(e^{(k-g)(t_f - t_o)} - 1)}{(k-g)(t_f - t_o)} . \quad (5)$$

A conservative estimate of daily carbon (C) and nitrogen (N) availability was calculated empirically by converting the

ingestion rate (I) to an equivalent in g C and N. Computations assumed a 12 h:12 h light:dark cycle in winter and a 14 h:10 h light:dark cycle in summer. For heterotrophic bacteria we employed cell conversion factors of 20 fg C per cell with a C:N ratio of 3.5 (Wheeler and Kirchman, 1986). Available C and N from phytoplankton and protozoans was determined as a function of biovolume, using epifluorescence microscopy (Ribes et al., 1998, and references therein). We are aware of the limitations of such calculations and sufficient data are presented so that if better cell-to-carbon and cell-to-nitrogen conversions become available, fluxes can be recalculated.

Results

As expected, there is a strong linear relationship (F=590.42; P<0.001; r^2=0.91) between dry mass and wet mass of this sponge (Fig. 1). As sponges can regenerate from fragments that are returned to the natural habitat, the excellent fit of this relationship avoids the need to sacrifice any further individuals to determine the dry mass of the *Haliclona–Ceratodictyon* association used in future experiments. This finding will help to preserve the species.

Heterotrophic bacteria and two autotrophic prokaryotes, *Prochlorococcus* sp. and *Synechococcus*-type cyanobacteria, comprised over 90% the ultraplankton community in One Tree Lagoon regardless of season (Table 1). The only abundant eukaryotic organisms were a variety of heterotrophic ciliates that are grouped here as protozoans. No autotrophic eukaryotes were found during any of the sample periods. The C:N ratio of the ultraplankton community of 3.1–4 reflects the dominance of heterotrophic organisms found within the lagoon of One Tree Island (Table 1).

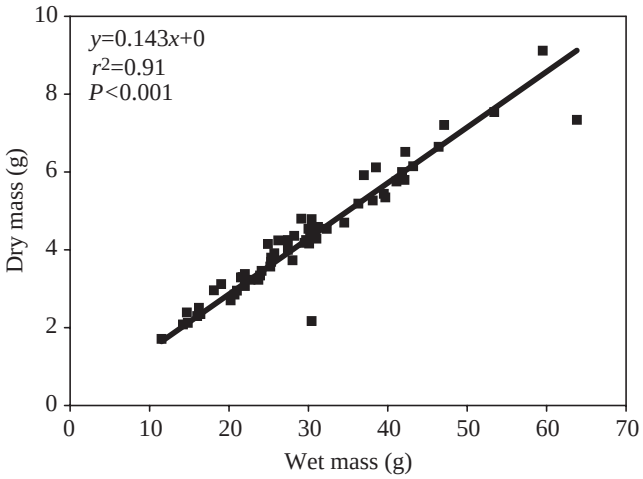

Fig. 1. Relationship between *Haliclona–Ceratodictyon* association wet and dry mass.

Ultraplankton communities varied slightly with both the time of day and season. During the summer sampling period we recorded more than an order of magnitude increase in *Synechococcus sp.* at night. All other types of ultraplankton have variations of less than 20% both within and between seasons (Table 1).

The presence of the *Haliclona–Ceratodictyon* association significantly reduced the growth rates of heterotrophic bacteria and protozoans. The net effect, combined day and night growth rates, of the *Haliclona–Ceratodictyon* association on the growth rate of heterotrophic bacteria showed no difference by season. However, during winter the *Haliclona–Ceratodictyon* association had a greater negative effect on growth during the day, while in summer there was

Table 1. *Calculated availability of living particulate organic carbon (POC) and living particulate organic nitrogen (PON) as a function of time of day and season at One Tree Island lagoon*

	Winter				Summer			
Type of ultraplankton	Day	%	Night	%	Day	%	Night	%
Carbon (μg C l^{-1})								
Heterotrophic bacteria	6.39±1.11	88	5.42±0.92	86	5.42±1.48	90	5.19±0.13	59
Prochlorococcus	0.34±0.09	5	0.50±0.09	8	0.28±0.09	5	0.35±0.19	4
Synechococcus	0.45±0.21	6	0.33±0.24	5	0.18±0.08	3	3.11±2.71	35
Protozoans	0.08±0.01	1	0.09±0.15	1	0.15±0.05	2	0.11±0.03	1
Total	7.26		6.34		6.03		8.76	
Nitrogen (μg N l^{-1})								
Heterotrophic bacteria	1.82±0.32	88	1.55±0.26	86	1.55±0.42	85	1.48±0.37	68
Prochlorococcus	0.05±0.05	2	0.08±0.01	4	0.04±0.01	2	0.06±0.03)	2
Synechococcus	0.07±0.07	3	0.05±0.04	3	0.03±0.01	2	0.49±0.05	23
Protozoans	0.12±0.02	6	0.12±0.01	7	0.20±0.07	11	0.16±0.04	7
Total	2.06		1.80		1.82		2.19	
C:N	3.5		3.5		3.3		4.0	

Values are means ± S.D. (N=3).
Haliclona cymiformis is very common in the area where the water samples were collected.

Table 2. *Differences in numbers/particulate organic carbon (POC) contributed by each type of ultraplankton*

Source	d.f.	SS	*F*
(A) Heterotrophic bacteria			
Treatment (T)	1	1.665	3.909*
Time of day (TOD)	1	0.089	0.211^{ns}
Season (S)	1	0.253	0.595^{ns}
T×TOD	1	0.067	0.159^{ns}
T×S	1	0.596	1.398^{ns}
L×S	1	1.951	4.580*
T×TOD×S	1	0.730	1.713^{ns}
Error	16	6.814	
(B) *Prochlorococcus*			
Treatment (T)	1	0.129	0.200^{ns}
Time of day (TOD)	1	0.623	0.963^{ns}
Season (S)	1	1.264	1.952^{ns}
T×TOD	1	0.577	0.892^{ns}
T×S	1	0.157	0.242^{ns}
L×S	1	1.801	2.782^{ns}
T×TOD×S	1	0.307	0.475^{ns}
Error	16	10.357	
(C) *Synechococcus*			
Treatment (T)	1	0.760	0.533^{ns}
Time of day (TOD)	1	2.693	1.890^{ns}
Season (S)	1	3.611	2.534^{ns}
T×TOD	1	4.939	3.466^{ns}
T×S	1	3.687	2.587^{ns}
L×S	1	3.922	2.752^{ns}
T×TOD×S	1	8.534	5.989*
Error	16	1.425	
(D) Protozoans			
Treatment (T)	1	28.258	62.714***
Time of day (TOD)	1	0.423	0.939^{ns}
Season (S)	1	2.485	5.514*
T×TOD	1	0.380	0.844^{ns}
T×S	1	1.257	2.789^{ns}
L×S	1	9.290	20.618***
T×TOD×S	1	6.669	14.800**
Error	16	0.451	

Repeated-measures analysis of variance (ANOVA) for each type of ultraplankton. Time of day (day *vs* night) is nested within season (winter *vs* summer). *$P<0.05$; **$P<0.01$; ***$P<0.001$; $^{ns}P>0.05$.

a negative effect only at night (Table 2, Fig. 2). For protozoans, there was a seasonal difference, with more negative growth rates during the winter than summer and the same diel pattern as seen in heterotrophic bacteria was found (Table 2, Fig. 2). The *Haliclona–Ceratodictyon* association did not appear to have a statistically significant effect on *Prochlorococcus* sp. and *Synechococcus*-type cyanobacteria (Table 2, Fig. 2).

The *Haliclona–Ceratodictyon* association removed more particulate material from the water column community in winter than summer (Table 3). Both the carbon and nitrogen fluxes were an order of magnitude higher in the winter than the summer. During the winter most of the carbon and nitrogen flux occurred during the night. During the summer there was only a slight difference between the day- and night-time fluxes (Table 3).

Discussion

The ability of any organism to meet its nutritional needs is dependent upon both ecosystem and organismal processes. For suspension-feeding invertebrates, the composition of the water column community dictates the available diet. The water column community within the lagoon of One Tree Island is dominated by ultraplankton and skewed towards heterotrophic species (Table 1). This is not unexpected, as on other Pacific coral reefs there are shifts in the structure of the water column community in relation to position on the reef. Charpy and Blanchot (1998) have demonstrated that water column communities in atoll lagoons have a much greater prokaryotic ultraplankton biomass than nearby oceanic waters (2–10 times higher) and lack autotrophic eukaryotes. Also, as water traverses a coral reef, as much as 90% of the ultraplankton can be removed from the water (Ayukai, 1995). The primary factor driving this change in community structure in the water column is most likely to be grazing by benthic invertebrates (Ayukai, 1995; Charpy and Blanchot, 1998). Ultimately, the food that is available for organisms to eat within the One Tree Lagoon is characteristic of Pacific coral reef lagoons and can be described as minute, nitrogen-rich particles.

As in other Pacific coral reef water column communities, there was no discernible seasonal variation in ultraplankton community structure (Furnas and Mitchell, 1987; Charpy, 1996). *Synechococcus* was the only genus to show a diel variation, with greater abundance at night than during the day (Table 1). There could be two sources for this increase. First, the doubling of *Synechococcus* cells, which occurs synchronously at night in natural populations (Campbell and Carpenter, 1986; Jacquet et al., 1998), will result in new cells. This characteristic of ultraplankton communities means that for a certain window of time, there is an increase in the amount of both C and N available for capture by suspension feeders. Second, there may be a diel migration of benthic cyanobacteria into the water column, which will significantly increase the number of cells. It remains to be seen if invertebrate grazers can take advantage pulsed increases in food availability by changing their grazing activities.

The *Haliclona–Ceratodictyon* association is capable of retaining heterotrophic bacteria and protozoans but is apparently unable to retain *Prochlorococcus* and *Synechococcus* (Fig. 2). While this may suggest that *H. cymiformis* grazes selectively on heterotrophic organisms, this is highly unlikely as sponges have no known mechanism for particle selection. Rather, it is more likely that the low abundance of *Prochlorococcus* and *Synechococcus* in One Tree Island Lagoon as compared to other coral reefs (Ayukai, 1995; Charpy, 1996; Charpy and Blanchot, 1998) prevents

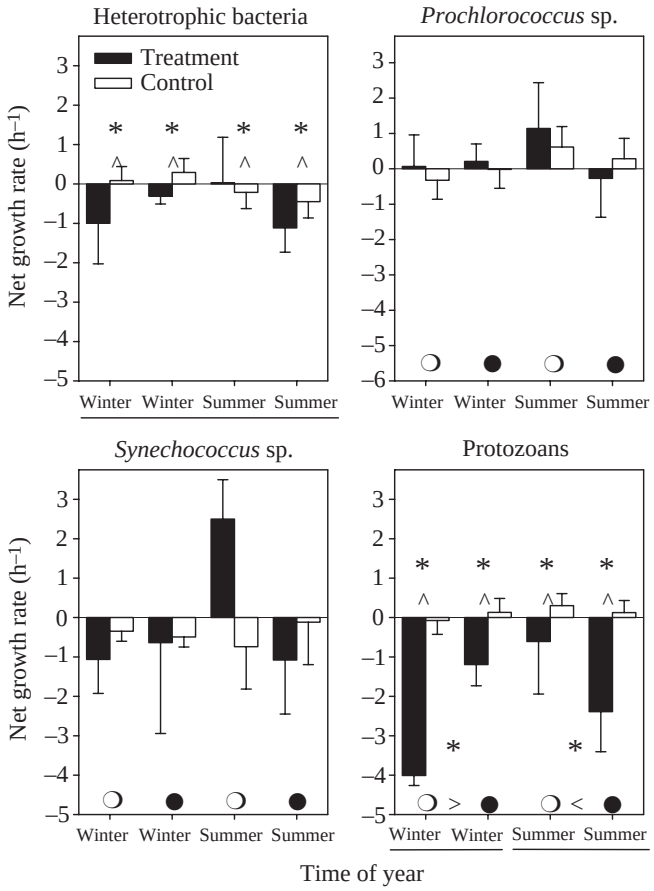

Fig. 2. Net growth rate of ultraplankton in the presence (Treatment, solid bars) and absence (Control, open bars) of the *Haliclona–Ceratodictyon* association. Values are means ± S.D. *Significant differences between treatments and controls and time of day (^), day (open circles) *vs* night (closed circles) (P<0.05). Significant seasonal differences (P<0.05) are present if treatments (winter *vs* summer) do not share an underline.

their capture by the choanocytes as seen in Caribbean sponges (Pile, 1999). Regardless of the mechanism, the natural diet of *H. cymiformis* is nitrogen-rich with a C:N ratio ranging from 3.3 to 4 (Table 1).

Surprisingly the *Haliclona–Ceratodictyon* association removed nearly an order of magnitude more PON and POC from the water column in winter than summer (Table 3). As there was no discernible difference between the ultraplankton communities, the greater flux is probably due to an increase in the amount of water processed by the sponge. It has been demonstrated in a temperate sponge that water processing and temperature are positively correlated (Riisgård et al., 1993). *H. cymiformis*, however, appears to be increasing water processing rates as the temperature decreases from summer to winter. This result, while unexpected, may be linked to the unique association between the sponge and algal partners. It may be that, in summer, higher rates of photosynthesis (Trautman, 1996) supply more oxygen and organic carbon to the sponge partner than in winter, allowing it to process less water and still meet its metabolic needs.

Grazing on ultraplankton appears to be an excellent source of carbon and nitrogen to the sponge partner in the association. Unlike most symbioses with either photo- or chemotrophic partners (Muscatine et al., 1984; Fisher, 1990; Childress and Fisher, 1992), in this association there appears to be little transfer of organic carbon from the autotrophic partner to the heterotrophic partner (A. Grant, unpublished data). Grazing by the heterotrophic partner is required as we have no evidence of significant nutritional support to it from the algal partner. This lack of nutrient transfer may be a result of the extracellular nature of the association; in associations in which significant transfer of nutrients has been reported from algal symbionts, the algae have been intracellular, facilitating the exchange of nutrients (Trautman and Hinde, 2001).

We conclude that nitrogen obtained from grazing on ultraplankton supports the nitrogen metabolism of both partners in this sponge–algal association. The sponge can

Table 3. *Mean daily uptake of carbon and nitrogen by the sponge* Haliclona cymiformis *from the plankton community*

	Winter			Summer		
Type of ultraplankton	Day	Night	Total	Day	Night	Total
Carbon (mg C g^{-1} dry mass day^{-1})						
Heterotrophic bacteria	2.75	3.88	6.63	ns	ns	ns
Synechococcus	0.01	0.06	0.07		0.09	0.09
Protozoans	0.06	0.08	0.14	0.12	0.07	0.19
Total			6.84			0.28
Nitrogen (mg N g^{-1} dry mass day^{-1})						
Heterotrophic bacteria	0.79	1.11	1.90	ns	ns	ns
Synechococcus		0.01	0.01		0.01	0.01
Protozoans	0.08	0.10	0.18	0.16	0.10	0.26
Total			2.09			0.27

The natural light:dark cycle is assumed to be 12 h:12 h for winter and 14 h:10 h for summer.
Fluxes that are less than 0.01 mg are indicated as not significant (ns).

retain enough PON to meet the projected nitrogen budget of the association. The sponge partner would need to take up 0.275 mg N g^{-1} dry mass day^{-1} to meet the nutritional needs of both partners (Davy et al., 2002). Thus, uptake in summer is sufficient to maintain the association, while in winter the fluxes are an order of magnitude greater than required to supply the association's nitrogen requirements for maintenance. The surplus of nitrogen obtained during the winter could only be allocated to growth if the association's other metabolic requirements were met through the overall greater uptake of particulate material.

Research on the nutritional interactions in many invertebrate/autotrophic symbiotic associations has focused on the flux of carbon between the partners. However, recent advances in understanding the feeding ecology of invertebrate partners other than the Cnidarians are revealing that grazing on ultraplankton is an important nutritional source of nitrogen for these associations (Pile and Young, 1999). Ultraplankton is the most common food source in the world's oceans (Stockner and Antia, 1986; Stockner, 1988; Sherr and Sherr, 1991) and its role in structuring benthic communities is only beginning to be elucidated (Gili and Coma, 1998). The role that grazing on ultraplankton plays in maintaining complex biological communities where symbiotic associations are prevalent, such as coral reefs and hydrothermal vents, remains to be explored.

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