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## The role of microbial food webs in benthic-pelagic coupling in freshwater and marine ecosystems

Adele J. Pile

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**THE ROLE OF MICROBIAL FOOD WEBS IN BENTHIC-PELAGIC  
COUPLING IN FRESHWATER AND MARINE ECOSYSTEMS**

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A Dissertation

Presented to

The Faculty of the School of Marine Science

The College of William and Mary in Virginia

In Partial Fulfillment

of the Requirements for the Degree of

Doctor of Philosophy

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by

Adele Jean Pile

1996

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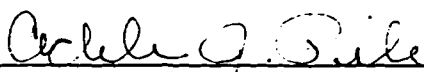
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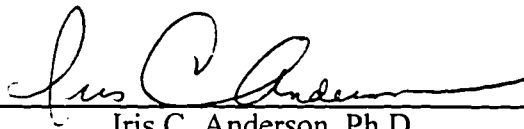
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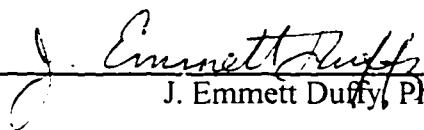
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## Dedication

This dissertation is lovingly dedicated to my mother,  
Patricia Ann Charney Pile Coe,  
in appreciation of her endless encouragement, support, and love.  
But, most of all because she gave me a chemistry set when I wanted more Barbies  
with the foresight to see that the Barbie I had could do chemistry.

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# THE ROLE OF MICROBIAL FOOD WEBS IN BENTHIC-PELAGIC COUPLING IN FRESHWATER AND MARINE ECOSYSTEMS

## ABSTRACT

A majority of carbon in freshwater and marine ecosystems is in the form of ultraplankton, heterotrophic and autotrophic plankton  $< 5 \mu\text{m}$  including heterotrophic bacteria, *Prochlorococcus*, cyanobacteria, and autotrophic eucaryotes. However, ultraplankton and subsequently microbial food webs have yet to be incorporated into models of benthic-pelagic coupling despite the preponderance of macroinvertebrates with the capacity to feed on ultraplankton. I have examine the role of microbial food webs in benthic-pelagic coupling in three ecosystems: Lake Baikal, Siberia, Russia; Gulf of Maine, Northwest Atlantic Ocean; and Conch Reef, Florida Keys, USA. Using sponges as a model organism and *in situ* measurements, I have quantified (1) suspension feeding on ultraplankton and (2) release of dissolved inorganic nitrogen (DIN) and phosphorus (DIP) resulting in direct evidence that benthic macroinvertebrates do occupy the level of primary consumer within the microbial food web.

Dual-beam flow cytometry was employed to quantified sponge suspension feeding on five types of ultraplankton: heterotrophic bacteria, *Synechococcus*-type cyanobacteria, autotrophic picoplankton  $< 3 \mu\text{m}$ , autotrophic eucaryotes  $3\text{-}10 \mu\text{m}$ , and in marine ecosystems *Prochlorococcus*. Grazing by the freshwater sponges *Baikalospongia intermedia* and *B. bacillifera* and the boreal marine sponge, *Mycale lingua*, was unselective for all types of ultraplankton with efficiencies ranging from 63-99%. This is the first time that grazing on *Synechococcus*-type cyanobacteria and *Prochlorococcus* by macroinvertebrates has been quantified in freshwater and marine ecosystems. Conversely, the coral reef sponges *Ircinia felix* and *I. strobilina* release significant amounts of DIN and DIP as a result of grazing on procaryotic plankton. Using a general model for organism-mediated fluxes, it is conservatively estimate that through active suspension feeding sponges in Lake Baikal retain  $1.97 \text{ g C day}^{-1} \text{ m}^{-2}$  and *M. lingua* retains  $29 \text{ mg C day}^{-1} \text{ m}^{-2}$  while at Conch Reef sponges released  $204 \mu\text{mol DIN day}^{-1} \text{ m}^{-2}$  and  $48 \mu\text{mol DIP day}^{-1} \text{ m}^{-2}$ . A majority of the carbon retain at all three locations was from procaryotic cell types suggesting that ultraplankton are an important overlooked component of benthic-pelagic coupling.

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**THE ROLE OF MICROBLAL FOOD WEBS IN BENTHIC-PELAGIC  
COUPLING IN FRESHWATER AND MARINE ECOSYSTEMS**

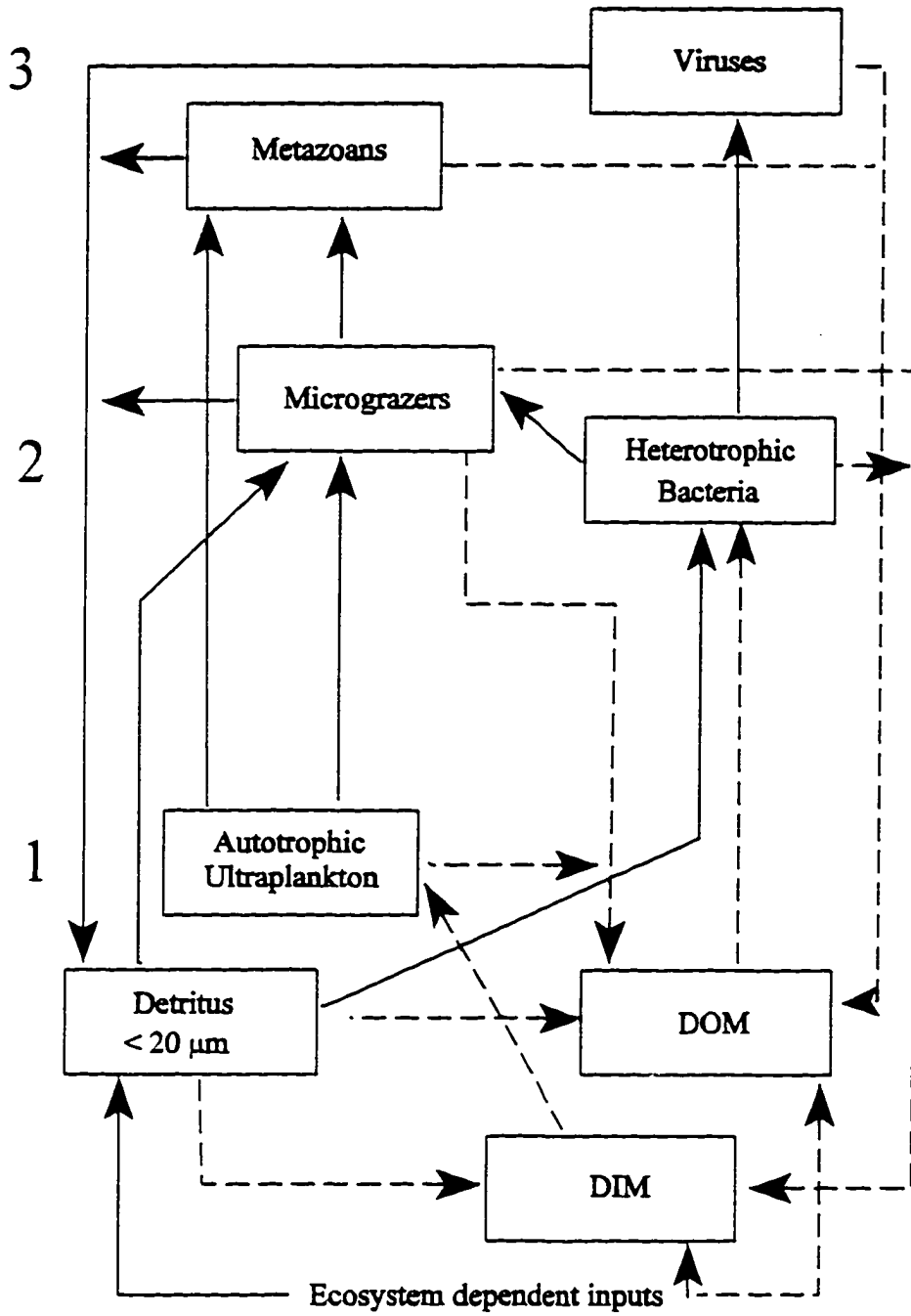


## Chapter I

### General Introduction

During the past decade research, on the water-column communities of heterotrophic bacteria and autotrophic prokaryotes and eucaryotes less than 5  $\mu\text{m}$  (ultraplankton Murphy and Haugen 1985) has discovered a complex network of trophic interactions between ultraplankton, heterotrophic nanoflagellate and ciliates, and metazoans; or the microbial food web (Azam et al. 1983, Sherr, E. B. and Sherr 1991). The theory concerning this discovery posits that in the euphotic zone of the water column the microbial food web shunts a significant fraction of the primary production away from the traditional linear food chain into a microbial food web that incorporates multiple trophic links between ultraplankton, small non-pigmented flagellates and ciliates (micrograzers), and larger protozoans (Figure 1). The microbial food web is in part supported by pools of dissolved organic material (DOM) and inorganic material (DIM) which nutritionally support primary production by autotrophic ultraplankton and secondary production by heterotrophic bacteria. The guild of primary consumers consisting of organisms that graze on ultraplankton, and is currently reserved for micrograzers, heterotrophic nanoflagellates and ciliates. The secondary consumers feed on the first trophic level and the larger fraction of the autotrophic ultraplankton. The feeding by both the primary and secondary consumers results in the release of DOM and DIM which can support production of autotrophic ultraplankton and heterotrophic bacteria. There are other additional important sources of DOM and DIM, such as leakage of photosynthate from phytoplankton and up welling of nutrients, and their contributions to the pools is ecosystem specific. More recently the incorporation of viruses (Murray and Eldridge 1994) and

Figure 1. Schematic diagram depicting the flow, as indicated by the direction of the arrows, of particulate (solid lines) and dissolved (dashed lines) material through a microbial food web in the euphotic zone. Adapted from Azam et al. (1983) and Sherr and Sherr (1991). Relative trophic level is indicated by vertical position.



microscopic detritus (Posch and Arndt 1996) into microbial food webs has added additional levels of complexity to the food web. An organism can be considered a primary consumer in the microbial food web if it grazes primarily on ultraplankton. Current models of microbial food webs have excluded macroinvertebrates from the guild of primary consumers due to a lack of direct evidence that macroinvertebrates feed on ultraplankton (Azam et al. 1983, Sherr, E. B. and Sherr 1991).

There is strong evidence to hypothesize that ultraplankton is an important food source for benthic macroinvertebrates. In theory, there are a variety of organisms that have the capability to remove ultraplankton from the water that they process (Rubenstein and Koehl 1977, Shimeta and Jumars 1991). Reiswig (1971a) found that a majority of the particulate organic carbon retained by Caribbean coral reef sponges was unresolvable using the techniques available at the time. He hypothesized that the unresolvable particulate organic carbon (URPOC) was plankton  $< 2 \mu\text{m}$  (picoplankton). More recently, Ayukai (1995) found net retention of heterotrophic bacteria and *Synechococcus*-type cyanobacteria, up to 90%, at Davies Reef, the Great Barrier Reef, Australia. The resultant net flux of carbon to the benthos was equal to the primary production estimates of the benthos for the same reef. He hypothesized that this flux was the result of grazing by the benthic community, and suggests that in some shallow communities microbial plankton can be an important source of exogenous carbon. This evidence is contrary to the hypothesized minimal effect of benthic bacterioplankton grazing at Davies Reef, which was based on a limited number of organisms with relatively low, typically  $< 5\%$ , retention efficiencies of heterotrophic bacteria (Sorkin 1973, Ducklow 1990). Other researchers have found that the growth rates of heterotrophic

bacteria are higher than those necessary to balance the grazing pressures of micrograzers on coral reefs (Moriarty 1979, Moriarty et al. 1985, Linley and Koop 1986) and in salt marshes (Sherr, B. F. et al. 1986, 1989) and theorized that grazing of bacterioplankton by the benthic community is a major contributing factor in plankton community dynamics. While pelagic macroinvertebrates such as salps and tunicates, are known to graze on ultraplankton, the feeding rates have yet to be accurately quantified due to the difficulty of accurately quantify feeding on ultraplankton (Alldredge and Madin 1982). The unexplored role of macroinvertebrates grazing on ultraplankton at the organismal to ecosystem level has further consequences since investigators have ignored the ultraplankton to macroinvertebrate trophic link and excluded macroinvertebrates from the theory of the microbial food web (Azam et al. 1983, Sherr, E. B. and Sherr 1991).

The missing piece of evidence that has precluded inclusion of macroinvertebrates in the microbial food web is knowledge of their feeding ecology. Quantifying suspension feeding on ultraplankton is difficult. However, recent advances that simplify the techniques used to quantify microbial plankton and their application to the feeding ecology of macroinvertebrates promise to provide new insights.

#### *Methods employed during these studies*

This research employed novel techniques to quantify the feeding ecology of sponges in freshwater and marine ecosystems. Suspension feeding was directly measured *in situ* as the retention of ultraplankton and coupled with concurrent measurements of sponge pumping activity. Ultraplankton was quantified using dual-beam flow cytometry. Since this is the

first time that these techniques have been used in combination to quantify the feeding ecology of any organism on ultraplankton their choice merits some discussion.

### Indirect vs Direct Measurements of Microbial Retention

Feeding by suspension feeders can be quantified using direct or indirect measurements. Traditionally, particle retention was measured indirectly by following cell concentrations over a fixed period of time in a closed system. This measure is commonly known as a clearance rate. Clearance rates have many variants but can generally be calculated after Jørgenson (1943):

$$\ln C_t = -rt/V + \ln C_0 \quad (1)$$

where  $r$  is clearance rate,  $t$  is time,  $V$  the volume of water in the experimental container,  $C_0$  the concentration of cells at  $t = 0$ , and  $C_t$  the concentration of cells at a designated time. Linear regression is then employed to determine  $r$ . Clearance rates of ultraplankton have been conducted on bivalves with natural bacterioplankton (e. g., Wright et al. 1982, Werner and Hollibaugh 1993) and with cultured bacteria and autotrophic eucaryotes  $< 5 \mu\text{m}$  (e. g., Stuart and Klumpp 1984, Lesser et al. 1992). Clearance rates are typically determined in beakers ranging in volume from 0.5-1.5 l containing multiple feeding individuals, with samples collected every 10-30 min over experimental periods of 1 -1.5 h (Wright et al. 1982, Werner and Hollibaugh 1993). Removal of water samples from the experimental containers typically results in a greater than 10% decrease in the volume of the water over the experimental period which can effect pumping rates of bivalves (e. g., Wright et al. 1982,

Werner and Hollibaugh 1993). The calculation of clearance rates makes the assumption that the rate of particle capture is independent of particle concentration and volume processed. In other words, organisms are presumed to capture particles with the same efficiency regardless of concentration of particles, and the volume flow rate of water processed by the organisms is assumed constant during the experiment.

The effect of abundance and quality of food on the suspension-feeding behavior of bivalve molluscs has been well studied (e.g., Bayne 1993). Ultimately this body of research has found that the rate at which water is processed by active suspension feeders is inversely proportional to food availability with a threshold concentration of food at which the change in pumping rate occurs. Thus, during measurements of clearance rates as the concentration of food decreases the volume of water that the organism processes per unit of time increases. During the course of incubations for determining clearance rates the rate at which water is being processed changes due to the decrease in particle concentration as a result of feeding by the organism. Although this phenomenon has yet to be observed in suspension feeders that graze on ultraplankton, it is not unreasonable to believe that these types of suspension feeding behaviors would be found.

Clearance rates have also been used to estimate the amount of water processed by organisms. The best example of why this should not be done comes from a study by Riisgård et al. (1993). They directly measured pumping activity in the sponge *Haliclona urceolus* during incubations that measured clearance rates. The directly measured pumping rates were double those that were empirically calculated from the clearance rates. They do not suggest a mechanism for the differences, but conclude that pumping rates calculated from

clearance rates are gross underestimates. An accurate measurement of the pumping activity of suspension feeders is essential for determining their feeding ecology. The amount of water processed by active suspension feeders can be easily quantified using a simple heated microthermistor flow probe designed by LaBarbera and Vogel (1976) either *in situ* or under laboratory conditions.

The calculation of clearance rates assume that the environmental changes occurring in the container over the study period do not affect the volume of water processed by the experimental organism. Suspension feeders pump at their highest rates when maintained under fully aerobic conditions, yet there has been no measure of oxygen consumption during any of the experiments that have determined ultraplankton clearance rates (Harbison and Gilmer 1976, Alldredge 1981, Stuart and Klumpp 1984, Kemp et al. 1990, Lesser et al. 1992, Riisgård et al. 1993, Werner and Hollibaugh 1993) and only three investigators aerated their containers during experiments (Wright et al. 1982, Lesser et al. 1992, Riisgård et al. 1993). Macroinvertebrates also release large quantities of ammonium from remineralization of organic material which can reach toxic levels in experimental containers in a short period of time. As an example, consider 3-5 mussels, *Geukensia demissa* (2.8 g dry weight), placed in a 1 l container for a 90 min incubation (after Wright et al. 1982). *Geukensia demissa* has an oxygen consumption of 0.06 ml O<sub>2</sub> g<sup>-1</sup> dry weight min<sup>-1</sup> (Booth and Mangum 1978) and an ammonium release rate of 0.30 µg g<sup>-1</sup> dry weight min<sup>-1</sup> (Jordan and Valiela 1982). If the experiment was begun with oxygen at 8 ml l<sup>-1</sup> and 20 µMol ammonium (after Jordan and Valiela, 1982), it would take 30 min for the level of oxygen to reach hypoxic levels (< 3 ml O<sub>2</sub> l<sup>-1</sup>) and ammonium concentrations to become toxic. Since most organisms are placed in



containers for a 15 min acclimation period, adverse conditions would be attained before the observations began if the chamber volume is too small.

More direct measurements of retention of particles can be conducted (1) at the organismal level where the concentrations of prey cells in the water being filtered by the feeding organism are compared to the concentration found in the exhalent current or (2) at the community level by following the concentration of plankton as it flows over a benthic community. At the organismal level retention efficiency can be computed following Palmer and Williams (1980):

$$R = 1 - \left(\frac{C_e}{C_a}\right) \quad (2)$$

where R is percent retained or released.  $C_e$  is concentration of cells in exhalent current.  $C_a$  is concentration of cells in ambient water. Positive values represent net uptake and negative values net release. Direct measurements have been performed *in situ* (Reiswig 1971a) and in the laboratory with cultured cells (Stuart and Klumpp 1984) and local water (Randløv and Riisgård 1979, Jørgensen et al. 1984).

The technique requires the isolation of the exhalent current which may be difficult in some organisms. Samples larger than 2 ml require passive collection whereas small volumes,  $\leq 2$  ml, can be collected by hand (Stuart and Klumpp 1984). Samples can be easily contaminated by ambient water if collected faster than the velocity of the exhalent current, by using an apparatus for collection that has a cross sectional area larger than the size of the exhalent jet, or if the exhalent current is not well defined due to turbulence in the overlying water.

More recently, benthic boundary layer theory has been applied to quantify directly the retention of plankton by benthic communities (Fréchette et al. 1989, Koseff et al. 1993, Butman et al. 1994, Savarese et al. 1996). The theory postulates that in some benthic habitats boundary layers develop where the sum of input of plankton from the overlying water column due to turbulent mixing and settling of plankton is less than that retained by the benthos. Relative to the benthos, the thickness of the boundary layer is constrained by the physical parameters of shear velocity, bottom roughness, and the organismal component of the ability of the excurrent jets to penetrate out of the boundary layer (O'Riordan et al. 1995). Thickness of the boundary layer is related inversely to shear velocity and directly to bottom roughness. Ultimately, the benthic community only has access to, and can only affect, the water column within the boundary layer. Theoretically, changes in concentrations of any measured parameter within the boundary layer have to be the result of the benthos. So, simply measuring concentration gradients coupled with simultaneous measurements of the boundary layer will yield a benthic community-mediated flux.

All of the aforementioned parameters can be empirically calculated, yet the theory has not specifically been tested in the field or with ultraplankton. Boundary layer theory has been used to examine feeding by the blue mussel, *Mytilus edulis*. Depletion of phytoplankton within the boundary layer correlated with the results of a model of grazing within experiments performed in a flume (Fréchette et al. 1989, Butman et al. 1994). Also, *M. edulis* depleted phytoplankton concentrations, while not affecting bacterioplankton concentrations in field studies of water flow over mussel beds (Wright et al. 1982). The lack of a feeding effect by *M. edulis* on bacterioplankton was expected, because the mussels did

not retain bacterioplankton in concurrent laboratory studies (Wright et al. 1982). However, these studies do demonstrate that under some conditions benthic communities have limited vertical access to water column communities, and demonstrate that horizontal flow, as opposed to vertical mixing, is essential for benthic communities to meet their nutritional requirements.

In summary, at the organismal level, direct measurements of feeding are more reliable for determining retention of ultraplankton while community level fluxes are best directly quantified using boundary layer theory. Direct measurements of feeding place less stress on organisms as they are easily conducted *in situ* and, if coupled with the power of dual-beam flow cytometry, the small water samples can be easily collected. However, some organisms do not lend themselves to direct measurements of feeding since they do not have defined exhalent currents or they are passive suspension feeders. For such instances community level fluxes, either *in situ* or in flumes, are the preferable measurements. Clearance rates should only be used as a last resort and only if environmental parameters are monitored carefully during the experiments by employing recirculating metabolism chambers (e. g., Patterson et al. 1991).

#### Quantification of ultraplankton

Currently, there are two methods that can accurately identify and enumerate ultraplankton: epifluorescence microscopy and flow cytometry. The techniques are similar in that they rely on the autofluorescent properties of photopigments and DNA stains to distinguish between the different cell types. Flow cytometry has been shown to be 40%

more accurate than epifluorescence microscopy, as it removes the human error of visually identifying and counting cells (Karl 1994). Further, *Prochlorococcus*, an autotrophic prokaryote ubiquitous to the world's oceans (Chisholm et al. 1988, Olson et al. 1990, Li et al. 1992, Campbell et al. 1994), and an integral component of coastal water column communities, theoretically can be retained by any organism that can graze on bacterioplankton. *Prochlorococcus* spp. has only been identified using flow cytometry (Chisholm et al. 1988) as the autofluorescence of chlorophyll a in *Prochlorococcus* spp. can not be seen with the human eye. More recently, epifluorescence microscopy coupled with the power of computer imaging systems has been used to identify and quantify *Prochlorococcus* spp. (H. Ducklow personal communication). If traditional epifluorescence microscopy techniques are utilized, *Prochlorococcus* spp. are incorrectly identified as heterotrophic bacteria (Campbell et al. 1994). *Prochlorococcus* spp. can be identified using either single-beam (one laser) or dual-beam (two lasers) flow cytometry. However, with the use of DNA stains, dual-beam flow cytometry can be used to quantify heterotrophic bacteria and autotrophic prokaryotes simultaneously (Monger and Landry 1993) whereas researchers who have utilized single-beam flow cytometry to quantify suspension feeding in macroinvertebrates have employed the erroneous assumption that all nonautofluorescing particles are detritus (Cucci et al. 1985, Shumway et al. 1985, Lesser et al. 1992). In the euphotic zone the most accurate way to identify and enumerate microbial communities is either dual-beam flow cytometry or epifluorescence microscopy with computer imaging, followed by the combination of single-beam flow cytometry with epifluorescence microscopy (sensu Li et al. 1992). The use only of epifluorescence microscopy or single-

beam flow cytometry should be avoided as neither technique can identify all types of ultraplankton.

In general, flow cytometry is more suited for studies using macroinvertebrates as it only requires small samples, 1 ml (Campbell et al. 1994), as opposed to 4-50 ml necessary for epifluorescence microscopy (Wright et al. 1982, Werner and Hollibaugh 1993). Small samples facilitate the use of direct measurements to quantify the retention of microbial plankton as small samples reduce the possibility of contamination by ambient water (Stuart and Klumpp 1984). Also, flow cytometry is much faster, taking only 5 min to analyze a sample as opposed to 70 min with epifluorescent microscopy (H. Quinby, personal communication) allowing for the large number of samples necessary for statistical analysis. Ultimately, for the quantification of the diet and retention efficiencies of macroinvertebrates the ease of sample collection and superior accuracy makes dual-beam flow cytometry a better technique than epifluorescence microscopy for identifying ultraplankton.

#### *Organism of choice*

Sponges are the predominant suspension feeding organisms in many marine and fresh water communities and appear to feed primarily on ultraplankton. Sponges are second to corals in abundance in reef communities (e. g., Pichon and Morrissey 1981, Wilkinson 1987, Wilkinson and Cheshire 1990), and can cover 21-90% of the available habitat in temperate marine communities (Witman and Sebens 1990). Sponges can dominate macroinvertebrate biomass in some freshwater ecosystems (Bailey et al. 1995). Although active suspension feeders, sponges can passively filter water via induced flow by currents (Vogel 1974, 1977)

and in coral reef systems have been estimated to filter the entire water column over their communities every 24–48 hours (Reiswig 1974). Sponge pumping rates vary with changes in temperature and salinity in laboratory studies (Fell et al. 1989, Riisgård et al. 1993) yet diel and seasonal variations in *in situ* sponge pumping generally remain uninvestigated (Reiswig 1971b).

All sponges are heterotrophs but sponges with endosymbiotic cyanobacteria or algae have a phototrophic component to their nutrition. Translocation of carbon compounds from endosymbionts in sponges is similar to that of corals and is a convenient analog (Wilkinson 1979, Falkowski et al. 1993). The translocation of glycerol from symbionts can account for 50% of the nutritional needs of some sponge species common to the Great Barrier Reef, but remains generally uninvestigated for Caribbean species (Wilkinson 1979, Wilkinson and Cheshire 1990). However, carbon in a similar form, translocated from the endosymbionts of corals, is quickly respired rather than incorporated into new biomass and is considered "junk food" (Falkowski et al. 1993). Additionally, new evidence suggests that the translocation of glycerol-derived, small organic phosphates translocated by the symbiont to the sponge host (Wilkinson 1979) may be used by the host sponge as electron donors in oxidation-reduction reactions, similar to the 3-phosphate shuttle between chloroplast and cytoplasm in plants (Arillo et al. 1993).

Sponges consume a variety of plankton, generally bacterioplankton (Reiswig 1971a, 1975) which may supply compounds necessary for the synthesis of animal proteins or nucleic acids that are required for growth and reproduction (Falkowski et al. 1993). The techniques utilized by previous researchers to quantify suspension feed in sponges included

radiolabeling of plankton, direct counts, and plating of bacteria, and accounted for 17-100% of the metabolic requirements of the sponges (Reiswig 1971a, 1975, van de Vyver et al. 1990). The investigators suggested that sponges may be using dissolved organic carbon (DOC) or picoplankton (e. g., heterotrophic bacteria, prochlorophytes, *Synechococcus*-type cyanobacteria) that could not be resolved with conventional methods (Reiswig 1971a).

Two studies suggest that as a consequence of sponge heterotrophic feeding and or endosymbiotic exudates, sponges are a source of dissolved inorganic nitrogen (DIN) and phosphorous (DIP) to coral reef communities (Corredor et al. 1988, Schubauer 1988). These fluxes ultimately depend on sponge pumping, which fluctuates with photoperiod and temperature (Reiswig 1971b), and nutritional mode (heterotrophy vs. autotrophy). Consequently, the magnitude of DIN flux may change on a diel to seasonal scale. The few estimates of sponge contributions to local DIN standing stock suggest that dense assemblages of the endosymbiotic sponge, *Chondrilla nucula*, can supply in excess of 100% of the local coral reef community daily DIN requirements (Corredor et al. 1988) and that sponges function as the dominant DIN recycling taxa and are second to nitrogen fixing reef algae as an overall source of DIN to coral reefs (Schubauer 1988).

Overall, sponges are an ideal organism for studying the role of microbial food webs in benthic-pelagic coupling. They are hypothesized to feed primarily on microbial plankton and in return release DOM and DIM which can support production of heterotrophic bacteria and autotrophic ultraplankton. They are active suspension feeders with well defined exhalent currents that can easily be sampled. Sponges can be easily studied *in situ* using modern SCUBA techniques. Globally, sponges can be a biomass dominant in freshwater and marine

ecosystems. These observations on sponges suggest that they are an important, under studied, component of benthic communities.

#### *Scope of the dissertation*

This dissertation addresses the fundamental question 'Are macroinvertebrates members of the guild of primary consumers within microbial food webs?' Chapters 2 and 3 are devoted to describing the diet, retention efficiencies, and distribution of two species of freshwater sponge and one species of marine sponge. The fourth chapter details the release of dissolved inorganic nitrogen and phosphorus by two species of coral reef sponges at the organismal and ecosystem level. Chapter 5 provides a general overview of how microbial food webs can be incorporated in benthic pelagic coupling.



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## Chapter II

Trophic effects of sponge feeding within Lake Baikal's littoral zone. 2.

Sponge abundance, diet, feeding efficiency, and carbon flux

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## ABSTRACT

Endemic freshwater demosponges dominate the benthic biomass in the littoral zone of Lake Baikal, Siberia, Russia. We measured *in situ* sponge abundance and grazing and calculated sponge-mediated fluxes of picoplankton (plankton  $< 2 \mu\text{m}$ ) for two common species, *Baikalospongia intermedia* and *B. bacillifera*. The encrusting sponge *B. intermedia* covers 38% of the available surface area, while the globose sponge *B. bacillifera* covers only 2%. Using dual-beam flow cytometry we quantified sponge suspension feeding on four types of picoplankton: heterotrophic bacteria, *Synechococcus*-type cyanobacteria, autotrophic picoplankton with one chloroplast (APP I), and autotrophic picoplankton with two chloroplasts (APP II). *B. intermedia* was an unexpected net source for APP I and APP II, with exhalant cell concentrations 37 and 12 times above ambient levels respectively, while heterotrophic bacteria and *Synechococcus*-type cyanobacteria exhalant concentrations were decreased by 71 and 58% respectively. Feeding efficiencies for *B. bacillifera* were significantly higher than those of *B. intermedia* for all types of plankton except *Synechococcus*-type cyanobacteria, which was not statistically different (84% for heterotrophic bacteria, 66% for *Synechococcus*-type cyanobacteria, 99% for APP I, and 81% for APP II). Using a general model for organism-mediated fluxes, we conservatively estimate that through active suspension feeding sponges are a sink for  $1.97 \text{ g C d}^{-1} \text{ m}^{-2}$ , mostly from procaryotes, and a net source of  $0.85 \text{ g C d}^{-1} \text{ m}^{-2}$  in the form of picoeucaryotic cells. Further, grazing by these extensive sponge communities can create a layer of picoplankton depleted water overlying the benthic community in this unique lake.

## INTRODUCTION

Planktonic cells less than 5  $\mu\text{m}$  in size, ultraplankton, are responsible for a large share of the primary and secondary production in freshwater and marine ecosystems (Stockner and Antia 1986, Hobbie 1988, Stockner 1988) yet the role of ultraplankton in benthic-pelagic coupling remains uninvestigated. Many benthic invertebrates from a variety of phyla have the capability to feed on this component of the water column community (Rubenstein and Koehl 1977, Jørgensen 1983, Jørgensen et al. 1984). The most conspicuous component of freshwater and marine benthic communities that has previously been shown to feed primarily on ultraplankton are the sponges.

Sponges are the most common benthic invertebrates in some freshwater lakes (Bailey et al. 1995) and ponds (Frost et al. 1982), second to corals in abundance in reef communities (e. g., Wilkinson 1987), and can cover 21-90% of the available habitat in temperate marine communities (Pomponi and Meritt 1990, Witman and Sebens 1990). All sponges are heterotrophs but sponges with endosymbiotic cyanobacteria or algae have a phototrophic component to their nutrition (Wilkinson 1983). Sponges consume a variety of plankton, generally bacterioplankton and autotrophic plankton < 2  $\mu\text{m}$  (picoplankton) (Reiswig 1971b, 1975, Wilkinson 1978, Huysecom et al. 1988, van de Vyver et al. 1990). Although active suspension feeders, sponges can passively filter water via induced flow by currents (Vogel 1974, 1977), and have been estimated to filter the water over their communities every 24-48 hours (Reiswig 1971a, Savarese et al. 1996).

Lake Baikal is the world's deepest (1637 m maximum depth), largest by volume (23,000  $\text{km}^3$ ), and oldest (ca. 25 million years) body of freshwater (Zhadin and Gerd 1963).

Unlike most lakes, the littoral zone has extensive sponge communities and three species dominate the rocky substrate: *Baikalospongia bacillifera*, *B. intermedia*, and *Lubomirskia baicalensis* which have globose, encrusting, and branching forms respectively. All three species are brilliant green due to their endosymbiotic relationship with zoochlorellae. Surprisingly, sponge abundance, diet, and the impact of sponges on water column communities has never been assessed.

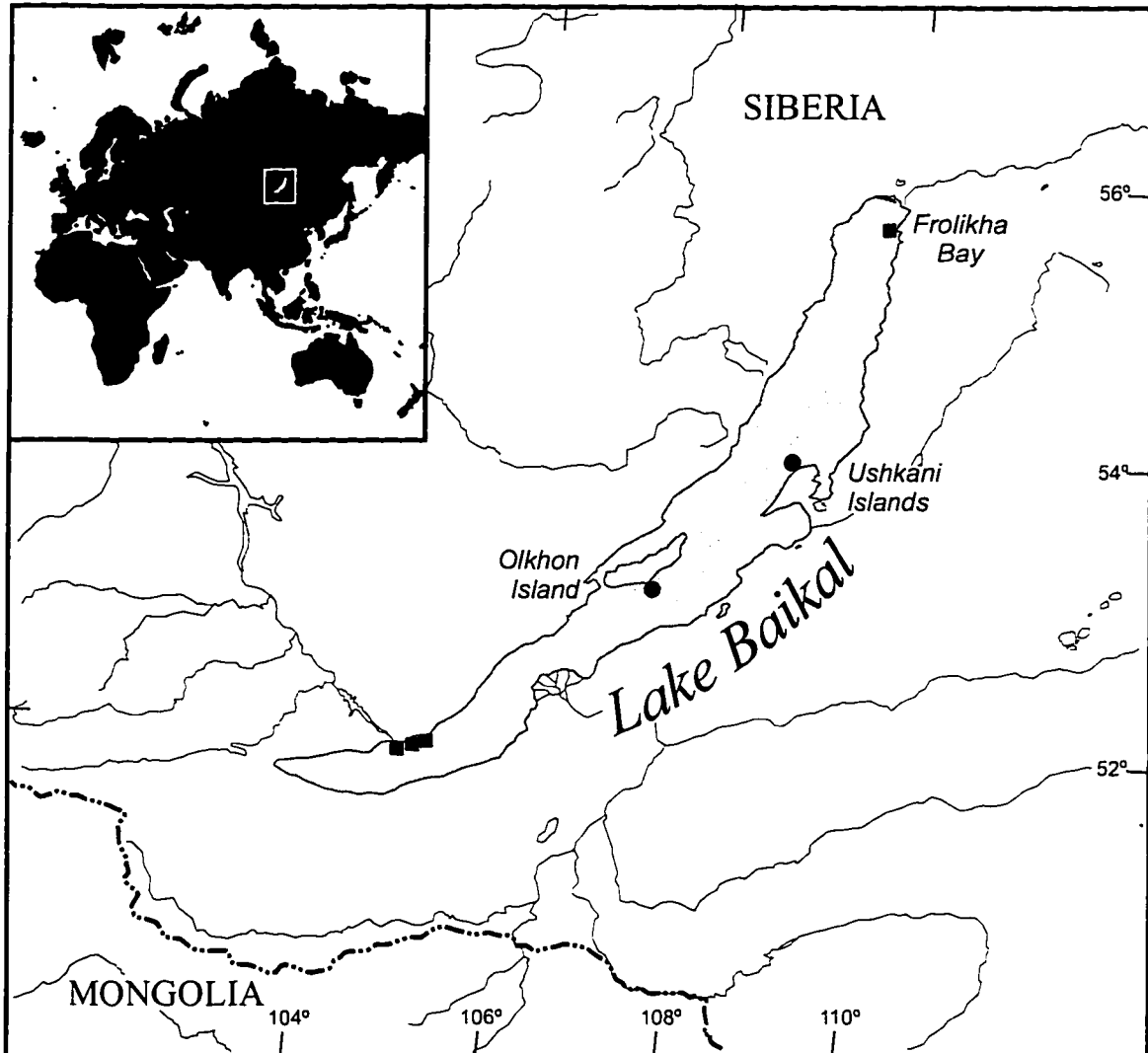
## METHODS

During August 1993, we measured sponge-mediated fluxes of picoplankton at two locations in Lake Baikal (Figure 2) using an integrated set of *in situ* measurements. Sponge-mediated fluxes of picoplankton were calculated from empirical measurements using a generalized model for active suspension feeders that incorporates organismal and community measurements and can be stated verbally as:

$$\text{organism-mediated flux} = \frac{\Delta \text{water column property}}{\text{volume processed}} \times \frac{\frac{\text{volume processed}}{\text{pumping unit}}}{\text{time}} \times \frac{\text{number of pumping units}}{\text{benthic surface area}} \quad (3)$$

In this case, the water column property is picoplankton concentration and the pumping units are sponge oscula. Sponge pumping was determined using *in situ* measurements and is reported in part 1 by Savarese et al. (1996). This paper encompasses the remaining two components, sponge diet and abundance and examines the sponge-mediated fluxes of heterotrophic and autotrophic picoplankton by two species, *B. bacillifera* and *B. intermedia*.

Figure 2. Map Lake Baikal, Siberia, Russia showing the locations of the video transects (■) and the feeding studies (●).



Previous researchers have had difficulty accurately quantifying suspension feeding on ultraplankton using techniques such as radiolabeling of plankton, direct counts, and colony counts (Reiswig 1971b, 1975, Wilkinson 1978, Huysecom et al. 1988, van de Vyver et al. 1990). They accounted for 17-100% of the metabolic requirements of the sponges (Reiswig 1971b, 1975, Wilkinson 1978, van de Vyver et al. 1990) and Reiswig (1971b) suggested that sponges may be using dissolved organic carbon (DOC) or plankton that could not be resolved with conventional methods. Recent advances in laser based technologies have resulted in more accurate methods for the quantification of ultraplankton. Single-beam flow cytometry has been used to quantify suspension feeding by macroinvertebrates on particles larger than 3  $\mu\text{m}$  (Cucci et al. 1985, Shumway et al. 1985, Lesser et al. 1992) however, the application of dual-beam flow cytometry to quantify both heterotrophic and autotrophic picoplankton (e. g., Monger and Landry 1993, Campbell et al. 1994) has yet to be utilized to quantify suspension feeding in organisms that are known to feed primarily on ultraplankton. Using dual-beam flow cytometry, we elected to quantify suspension feeding by sponges on heterotrophic and autotrophic picoplankton since (1) it is the component of the water column community that sponges are most likely to feed on and (2) all previous research on freshwater and marine ecosystems indicates that a majority of the water column productivity is within this size range. We do not suggest that this is the *only* component of plankton within the water column community or the *only* component that sponges may be affecting.

To quantify sponge suspension feeding, 1 ml water samples were collected by



at a depth of 12 m. Five samples were taken from water adjacent to a sponge and five from the exhalent current of a sponge osculum. Additionally, five 1 ml were collected at the depths of 0, 0.5, and 1 m from the bottom, as well as 1 and 5 m from the surface by SCUBA divers to quantify water column picoplankton at each location. Picoplankton samples were preserved for flow cytometry using standard protocols (Campbell et al. 1993) and held in either liquid nitrogen, dry ice, or at  $-80^{\circ}$  C till processing.

Plankton  $< 3 \mu\text{m}$  were quantified at the University of Hawai'i Flow Cytometry Facility using an EPICS 753 flow cytometer (Coulter Electronics Corporation, Hialeah, Florida). Samples were quick thawed, spiked with  $0.57 \mu\text{m}$  Polysciences Fluoresbrite standard beads, diluted 1:9 with filtered deionized water, and stained with Hoechst 33342 following Monger and Landry (1993).  $50 \mu\text{l}$  of sample were illuminated with 1 W, of the 488 nm line of a 5 W argon laser, and a 225 mW UV laser focused through confocal optics. Orange fluorescence (from phycoerythrin), red fluorescence (from chlorophyll a), and blue fluorescence (from DNA stained with Hoechst 33342) were collected through band pass interference filters at 575, 680, and 450 nm, respectively. The five measured parameters, forward- and right-angle light scatter (FALS and RALS), orange, red, and blue fluorescence were recorded on 3-decade logarithmic scales, sorted in list mode, and analyzed with a custom-designed software (CYTOPC, authored by Daniel Vaultot, CNRS and UPMC, Station Biologique, Roscoff, France). Picoplankton populations were identified to general cell type, heterotrophic bacteria, *Synechococcus*-type cyanobacteria, autotrophic picoplankton with one chloroplast (APP I), and autotrophic picoplankton with two chloroplasts (APP II), and visually confirmed using epifluorescence microscopy.

Differences between cell counts from ambient and exhalent current water of each type of picoplankton were analyzed using paired *t*-tests for each sponge with a Bonferroni transformed experimentwise  $\alpha$  of 0.00625 to determine the effects of sponges on picoplankton (Sokal and Rohlf 1981). The mean feeding efficiency for each sponge was calculated as  $((\text{mean cell count ambient} - \text{mean cell count exhalent}) / \text{mean cell count ambient}) \times 100$  for each type of picoplankton and analyzed as a function of sponge species (*B. bacillifera* vs *B. intermedia*) using paired *t*-tests with a Bonferroni transformed experimentwise  $\alpha$  of 0.0125 (Sokal and Rohlf 1981).

Total sponge percent cover and mean number of sponge oscula for *B. intermedia* and *B. bacillifera* were determined from underwater video transects (n=12). Three 8 m. haphazardly selected transects at a depth of 12 m were videotaped at three locations (Figure 2) by SCUBA divers using a Panasonic V-99 video camera in an Ikelite underwater housing. At the fourth location three 8 m transects were videotaped using a remotely operated vehicle. Twenty randomly selected 1 m<sup>2</sup> quadrants from each transect were analyzed for percent cover of the bottom by the sponges *B. intermedia*, *L. baicalensis*, and *B. bacillifera*, a red, filamentous alga, rock, and uninhabitable substrate (sand). Mean number of sponge oscula m<sup>-2</sup> for *B. intermedia* and *B. bacillifera* was determined by directly counting oscula within the randomly selected quadrats thereby eliminating the effects of size of individuals as well as the three dimensional nature of hard bottom communities in the flux equation.

To determine sponge-mediated fluxes of "living carbon" mean number of picoplankton cells removed or expelled by an osculum was converted to g C using the per cell conversion factors of 20 fg for heterotrophic bacteria and 470 fg for *Synechococcus*-type

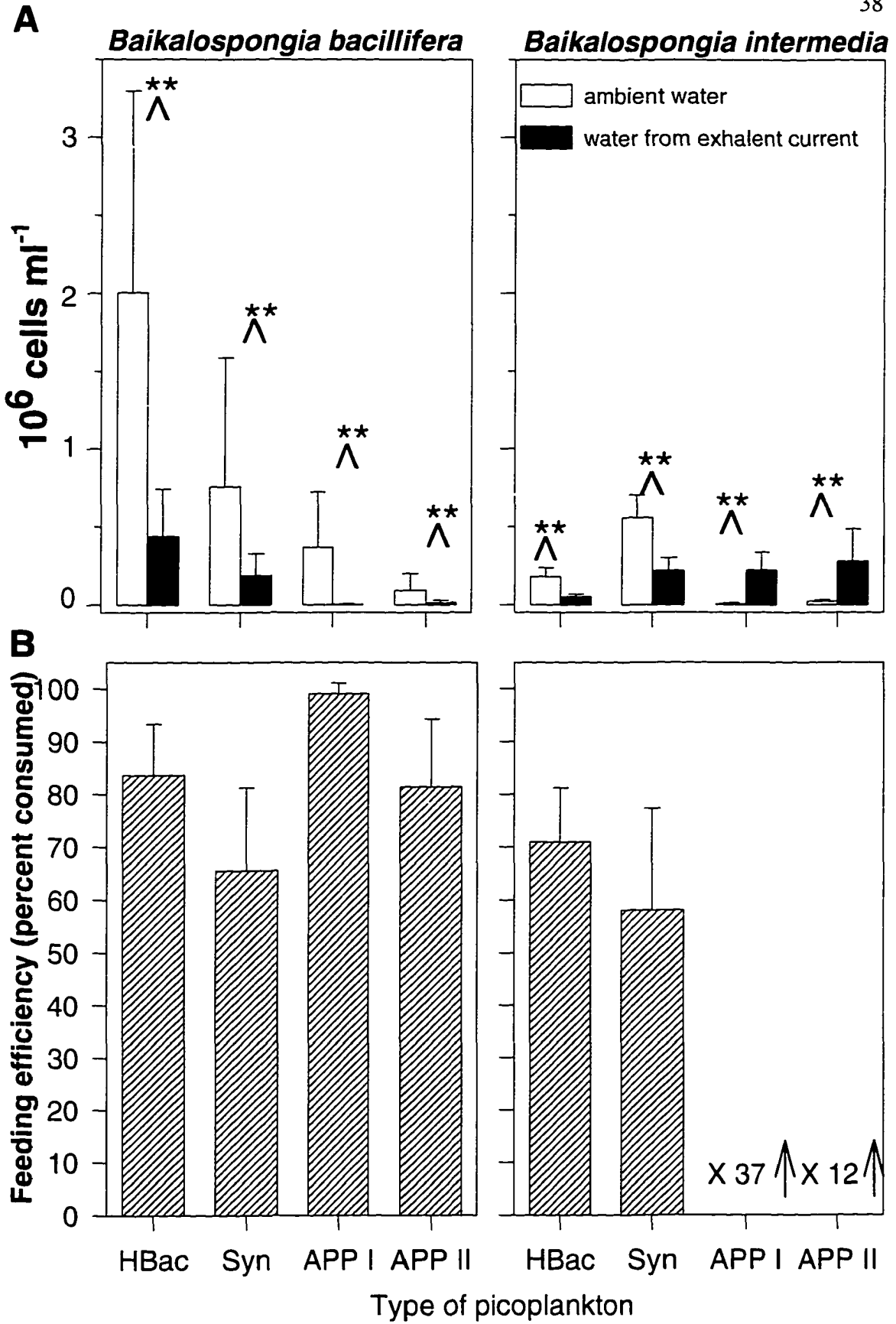
cyanobacteria. These conversion factors were selected as they are for cells with mean diameters that are equal or greater than those found during this study. Carbon in the form of the eucaryotic cells of APP I and APP II were determined using the formula  $fg\ C = 433 \times (\text{biovolume } (\mu\text{m}^3))^{0.366}$  with APP I and APP II having biovolumes of 0.35 and 0.50  $\mu\text{m}^3$  respectively as determined from epifluorescence microscopy (Campbell et al. 1994 and references therein).

Instantaneous and diel pumping rates were determined for *B. bacillifera* by Savarese et al. (1996) and are comparable to that of other sponges (Reiswig 1971a, 1974, Savarese et al. 1996). Additional pumping measurements were conducted on *B. intermedia* and although comparable to *B. bacillifera*, small sample sizes were not adequate for statistical comparison. Hence, we utilized the mean pumping rate of *B. bacillifera*, 0.13 ml sec<sup>-1</sup> oscula<sup>-1</sup> (plug flow model), in flux calculations for both species (Savarese et al. 1996). In contrast to tropical marine sponges (Reiswig 1971a), these sponges demonstrated no diel variation in pumping during the period of this study (Savarese et al. 1996). However, we used the assumption that all the oscula pumped actively for 12 hrs each d to obtain a *conservative* estimate of sponge activity.

## RESULTS

Both sponges were highly efficient at removing picoplankton by active suspension feeding with efficiencies ranging from 58-99%. *B. bacillifera* significantly reduced concentrations of all types of picoplankton (Figure 3A). In contrast, *B. intermedia* significantly reduced heterotrophic bacteria and *Synechococcus*-type cyanobacteria from

Figure 3. (A) Concentration of each type of picoplankton in the ambient water and water from the exhalent currents of the globose sponge *Baikalospongia bacillifera* and the encrusting sponge *B. intermedia* and (B) feeding efficiencies ( $\bar{x} \pm s$ , n=10). HBac= heterotrophic bacteria, Syn= *Synechococcus*-type cyanobacteria, APP I= autotrophic picoplankton with one chloroplast, and APP II= autotrophic picoplankton with two chloroplasts. (A) White bars denote ambient water, and black bars, water from the exhalent current ( $\bar{x} \pm s$ , n=50). All cell concentrations between ambient water and exhalent current water within cell types were significantly different as determined using paired *t*-tests with a Bonferonni transformed experimentwise experimental  $\alpha=0.00625$  (\*\*).



ambient levels while both APP I and APP II were significantly increased by 37 and 12 times above ambient levels, respectively (Figure 3A). The feeding efficiency for *B. bacillifera* on heterotrophic bacteria was 84% and significantly higher than the feeding efficiency of 71% for *B. intermedia* (*t*-test,  $t_{17}=-2.82$ ,  $P=0.011$ ). The mean feeding efficiencies of 66% and 58% respectively on *Synechococcus*-type cyanobacteria were not significantly different (*t*-test,  $t_{17}=-0.95$ ,  $P=0.36$ ). The contribution of APP I and APP II by *B. intermedia* was significantly different than the respective removal efficiencies of 99% (*t*-test,  $t_{17}=-40.3$ ,  $P < 0.001$ ) and 81% (*t*-test,  $t_{17}=-33.7$ ,  $P < 0.001$ ) by *B. bacillifera* (Figure 3B).

Uninhabited substrate, or rocks, was the most common component of the benthos, comprising 45% of the surface area whereas sand only accounted for 11%. *B. intermedia* was the most abundant sponge, covering 36%, followed by *L. baicalensis* with 6%, and *B. bacillifera* covered only 2% of the benthos. The only other noncryptic component of the benthic community was a filamentous red algae and it covered 2% of the benthos (Figure 4). None of the three noncryptic sponges were found living on any portion of the bottom not covered by hard substrate, thus sediment is considered an area uninhabitable by sponges. When percent cover was recalculated to that of habitable benthic surface area, total sponge cover rose to 47%. Mean oscula  $m^{-2}$  for *B. intermedia* was 154.9 and for *B. bacillifera* 21.4.

Both *B. bacillifera* and *B. intermedia* obtained a majority of the carbon in their diet, an integrated removal of  $1.87 \text{ g C d}^{-1} \text{ m}^{-2}$ , from procaryotic cell types (Table 1). Although eucaryotes were removed by *B. bacillifera*, the addition of APP I and APP II to the water column by *B. intermedia* resulted in a net production of  $0.75 \text{ g C d}^{-1} \text{ m}^{-2}$ . During this study we were unable to determine whether the APP I and APP II coming from *B. intermedia* were

Figure 4. Mean percent cover at 12 m of the sponges *Baikalospongia intermedia*, *B. bacillifera*, *Lubomirskia baicalensis*, a red filamentous alga, rock, and sand.

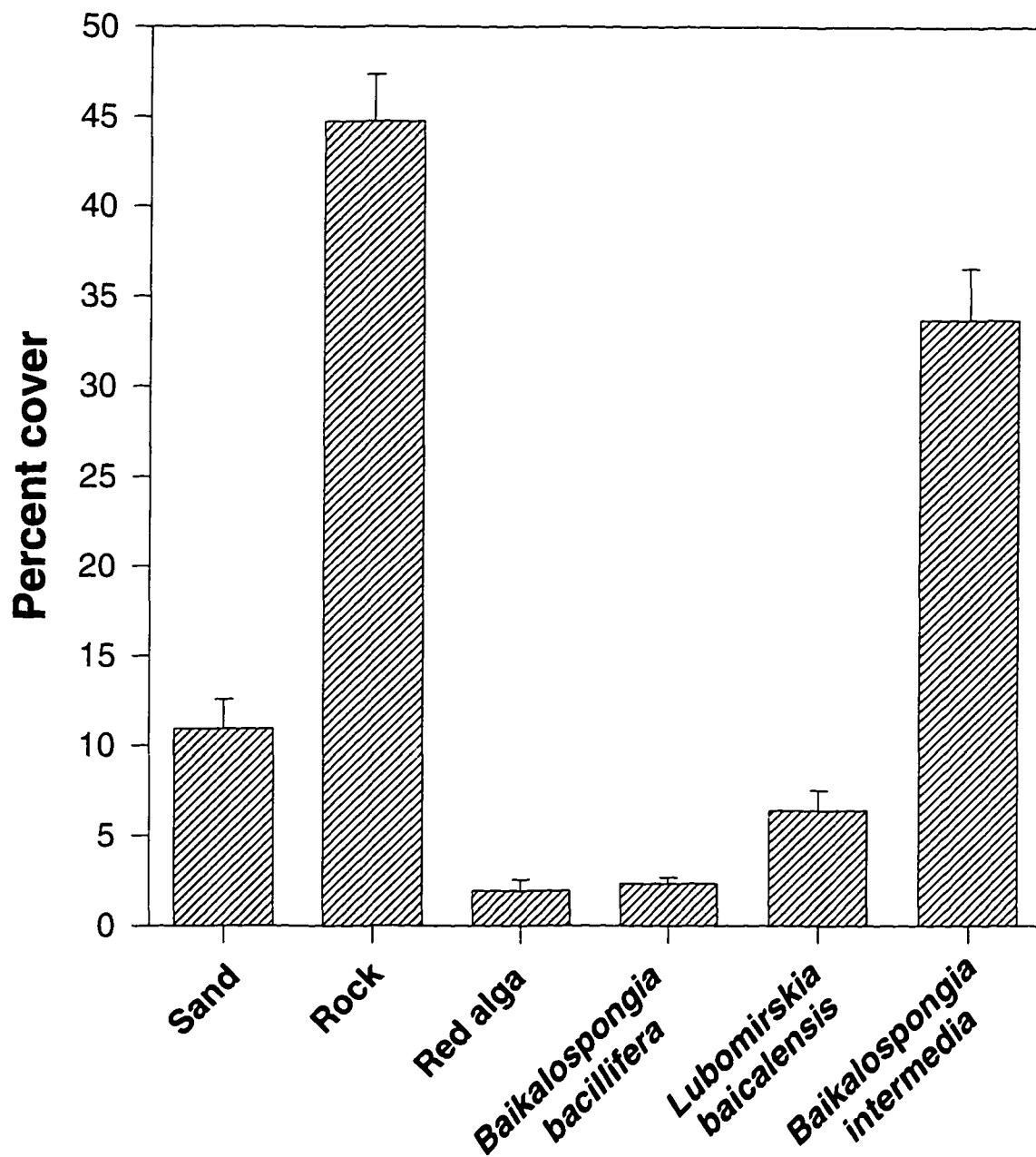




Table 1. Estimated mean daily g C removed from (-) or added to (+) the picoplankton community by the globose sponge *Baikalospongia bacillifera* and the encrusting sponge *B. intermedia* occupying 1 m<sup>2</sup> of the benthos in Lake Baikal's littoral zone at naturally occurring densities. Estimates were computed assuming that sponges were actively pumping for 12 hrs each day. Integrated effect is the net effect of *B. bacillifera* and *B. intermedia* on the picoplankton community. na-not applicable

Picoplankton component	Sponge-mediated flux (g C d <sup>-1</sup> m <sup>-2</sup> )				Integrated effect
	<i>Baikalospongia bacillifera</i>	% diet	<i>B. intermedia</i>	% diet	
<b>Procaryotes</b>					
Heterotrophic bacteria	-0.04	8	-0.03	2	-0.07
<i>Synechococcus</i> -type cyanobacteria	-0.37	72	-1.43	98	-1.80
Total procaryotes	-0.41	80	-1.46	100	-1.87
<b>Eucaryotes</b>					
Autotrophic picoplankton with 1 chloroplast	-0.08	15	+0.32	n/a	+0.24
Autotrophic picoplankton with 2 chloroplasts	-0.02	5	+0.53	n/a	+0.51
Total eucaryotes	-0.10	20	+0.85	n/a	+0.75
Total of all cell types	-0.51	100	-0.61	100	-1.12

viable, or nonviable organisms that would contribute to the detrital biomass. Our results suggest that relative to the water column, sponges are a carbon sink for procaryotes, but a carbon source for eucaryotes.

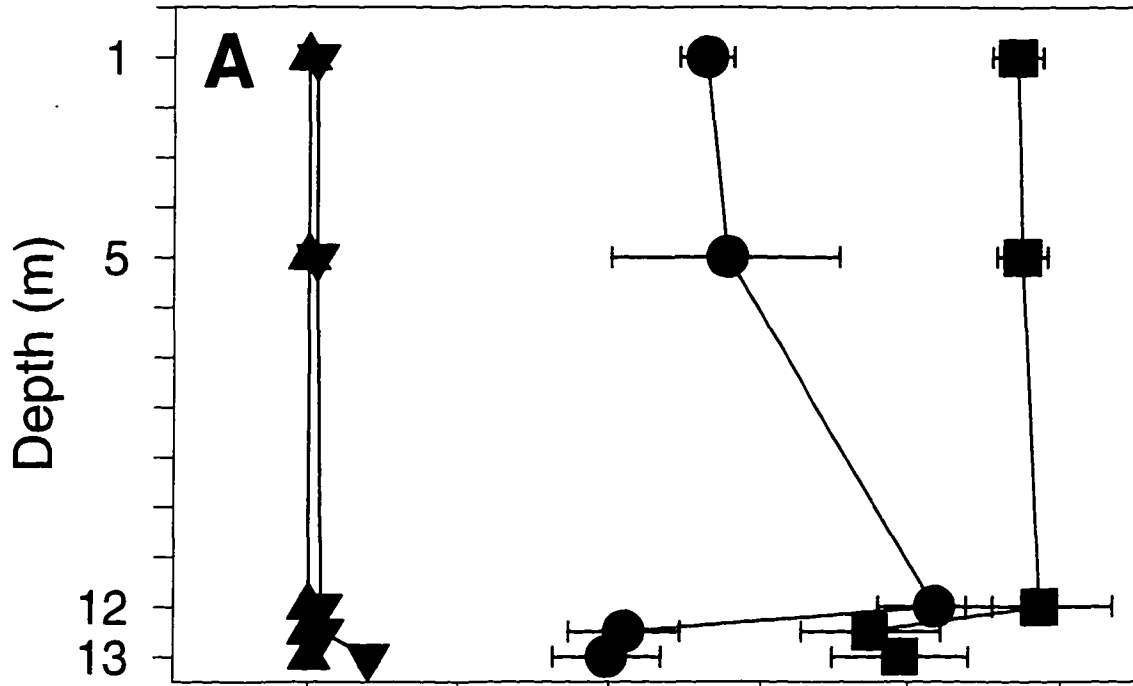
Water column profiles at the two study sites reflect the net decrease and increase in cell types (Figure 5) creating food-depleted (or enhanced) layers overlying these extensive suspension feeding communities. Both heterotrophic bacteria and *Synechococcus*-type cyanobacteria were depleted within 1 m of the benthos at both locations despite the dramatically different topography of the benthic communities. Olkhon Island has a gentle sloping littoral zone while Ushkani Island is a steep, almost vertical wall. Further, the Olkhon Island location was dominated by *B. intermedia* (Pile and Patterson, personal observation) and there is a resultant increase in APP II near the bottom.

## DISCUSSION

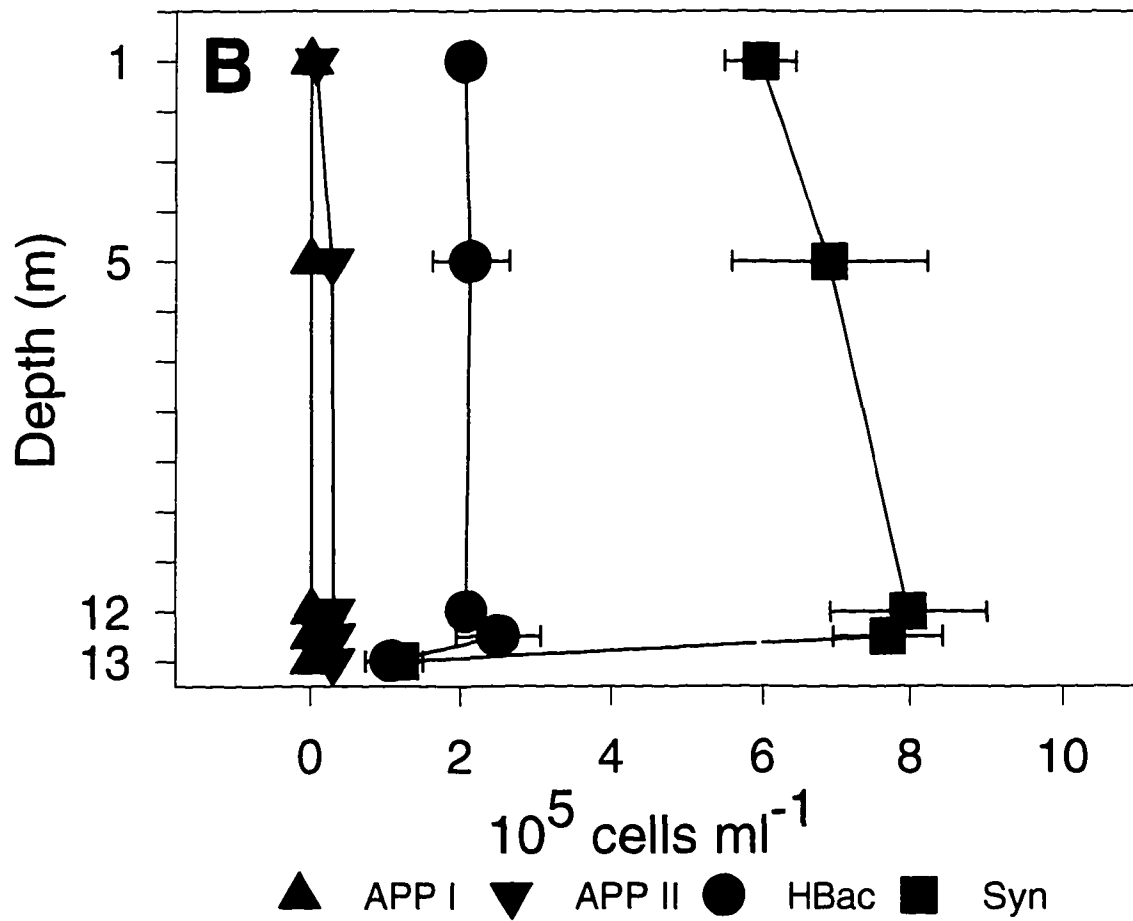
The application of dual beam flow cytometry to quantify picoplankton proved to be a powerful new tool for the quantification of suspension feeding by macroinvertebrates on heterotrophic and autotrophic picoplankton. This is the first record of grazing by freshwater macroinvertebrates on *Synechococcus*-type cyanobacteria. Both *B. bacillifera* and *B. intermedia* were highly effective at grazing on picoplankton with efficiencies ranging between 58-99%. These efficiencies are comparable to that of other freshwater and marine

Figure 5. Water column profiles for two locations overlying sponge communities. HBac (●)= heterotrophic bacteria. Syn (■)= *Synechococcus*-type cyanobacteria, APP I (▲) = autotrophic picoplankton with one chloroplast, and APP II (▼) = autotrophic picoplankton with two chloroplasts ( $\bar{x} \pm s$ , n=5). Benthic topography at Olkhon Island (53° 3.99' N, 107° 18.99' E)(Figure 2A), located on the western shore, is characterized by a gentle slope away from shore; Ushkani Island (53° 52.46' N, 109° 00.94' E) (Figure 2B), on the eastern shore, is a wall. Both locations have a decrease in HBac and Syn within 1 m of the benthic community. At Olkhon Island (B), a site dominated by *B. intermedia*, there is an increase in APP II within 0.5 m of the benthic community.

# Olkhon Island



# Ushkani Island



source (Huyssecom et al. 1988, van de Vyver et al. 1990, Riisgård et al. 1993). The *in situ* techniques we employed are preferable to laboratory experiments using artificial food sources as they accurately reflect the organisms' ability to graze on natural assemblages of picoplankton.

*B. intermedia* was an unexpected source for two types of picoeucaryotes, APP I and APP II. While pelagic organisms have been found to be a source of bacterial plankton (Nealson et al. 1984, Lee and Ruby 1994) this is the first evidence that a benthic macroinvertebrate is a source of autotrophic plankton to the water column. Nealson et al. (1984) found that release of symbiotic luminescent bacteria by shallow-water species of monocentrid and anomalopid fishes had an irregular pattern while Lee and Ray (1994) found that release of episymbiotic luminescent bacteria from the light organs of squid occurred at dawn when they were no longer needed by their hosts. Since the doubling times of bacteria in the light organs of the host can be one half of that in seawater they suggest that expulsion is a form of population regulation. Additionally, Lee and Ray (1994) found that the luminescent bacteria, *Vibrio fischeri*, were a component of bacteria plankton communities only in areas with populations on the host squid.

Like many sponges *B. intermedia* is covered by a thick mucus coating that can either inhibit or enhance the growth of episymbionts (Becerro et al. 1994). Given the magnitude of the increases above ambient levels, we suggest that APP I and APP II may be expelled by the sponge after living within the mucus layer on the exterior or within the aquiferous system of the sponge via the exhalent currents, similar to a mechanism described by Ducklow and Mitchell (1979a, b) for bacteria and coral. They demonstrated that bacteria living within the

external mucus coating of corals utilize the mucus as a source of nutrients and can be released from the corals to the water column community. Unfortunately, it is impossible to tell from this study if the APP I and APP II expelled from *B. intermedia* are living cells that would enter the carbon pool associated with the water column or dead cells that would contribute to the detrital biomass or if there is any periodicity in production. However, we recently found two species of sponge common to coral reefs that are also a net source of autotrophic eucaryotic picoplankton and neither exhibited any diel variation in expulsion (Pile 1996). This suggests that expulsion of episympiotic autotrophs is most likely mediated by the pumping activity of the sponge.

Sponges dominated the littoral zone of Lake Baikal, covering 47% of the available surfaces. A percent cover of 47% for sponges is unusual for a freshwater ecosystem and difficult to compare to reported sponge biomass and occurrence for freshwater ecosystems (Frost et al. 1982, Bailey et al. 1995). However, it is comparable to some coral reef (Wilkinson 1987), temperate marine (Witman and Sebens 1990), and near shore Antarctic benthic communities (Dayton et al. 1974).

Our estimates of sponge-mediated fluxes of picoplankton, which incorporated *in situ* measurements of sponge grazing, sponge abundance, and sponge pumping, found that sponges in Lake Baikal's littoral zone are a net sink for procaryotic cells types while a net source for eucaryotic cell types. Sponges removed  $1.97 \text{ g C d}^{-1} \text{ m}^{-2}$  from the water column, mostly in the form of procaryotic cell types. Although all types of picoplankton contributed to the daily sponge-mediated flux, *Synechococcus*-type cyanobacteria was by far the largest component, contributing 91% of the total daily flux. Previous estimates for tropical marine

sponges found that  $0.80\text{--}1.80 \text{ g C d}^{-1} \text{ m}^{-2}$  were necessary to meet metabolic carbon demands (Reiswig 1971b). Further, he found that 80% of the carbon flux by marine sponges was from "unresolvable particulate organic carbon", most likely heterotrophic and autotrophic plankton which could not be identified using methods available at the time. By using dual-beam flow cytometry to accurately quantify picoplankton and converting direct counts of cells to carbon using standard conversion factors, we estimated a slightly higher sponge-mediated flux of  $1.97 \text{ g C d}^{-1} \text{ m}^{-2}$ . Baikal sponges are grazing on a water column dominated by procaryotic cell types, similar to that of coral reef ecosystems (Stockner and Antia 1986, Hobbie 1988, Stockner 1988, Ayukai 1995), but they comprise more of the benthos than sponges on coral reefs (Reiswig 1971b) and thus they have a higher estimated sponge-mediated flux.

The grazing rates of these sponges were so high that food-depleted layers developed over the benthos. Food-depleted layers have been previously identified in a variety of other communities dominated by suspension feeders (Glynn 1973, Buss and Jackson 1981, Peterson and Black 1987, Fr chet te et al. 1989, Butman et al. 1994), yet the ability of organisms that feed primarily on picoplankton to create food-depleted or enhanced layers had been undocumented. Food-depleted layers develop when removal of plankton by suspension feeders exceeds input of plankton from higher in the water column from turbulent vertical diffusivity and sinking (Fr chet te et al. 1989, Butman et al. 1994, Savarese et al. 1996). We found picoplankton depleted and enhanced layers overlying both communities where feeding studies were conducted. Both days were calm, with little wind mixing of the water column, suggesting that horizontal flow of picoplankton rich water over the community is necessary to provide a heterotrophic food source for the sponges.

The extensive sponge communities in Lake Baikal's littoral zone significantly impact local picoplankton communities through active suspension feeding. Due to the large volume of water in the lake it is unlikely that the sponge communities will affect the total picoplankton community. More importantly, in this study we have demonstrated that extensive macrobenthic communities can be supported by heterotrophic and autotrophic picoplankton. We recommend *in situ* measurements coupled with the power of dual-beam flow cytometry as a new tool for quantifying the grazing by macroinvertebrates on picoplankton. This technique promises a better understanding of the flow of carbon within closely coupled benthic-pelagic ecosystems such as those in shallow, near shore communities in marine and freshwater ecosystems.



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## Chapter III

*In situ* grazing on plankton < 10  $\mu\text{m}$  by the boreal sponge *Mycale lingua*

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## ABSTRACT

Ultraplankton, heterotrophic and autotrophic plankton  $< 5 \mu\text{m}$ , are the most abundant food source in the world's oceans, yet their role as a food source for macroinvertebrates is largely unexamined. We quantified *in situ* feeding on heterotrophic and autotrophic plankton  $< 10 \mu\text{m}$  by the boreal sponge *Mycale lingua* using measurements that quantified sponge feeding efficiencies, pumping rates, and abundance to determine the contribution of plankton  $< 10 \mu\text{m}$  to sponge carbon intake. Using dual-beam flow cytometry we identified 5 populations of plankton  $< 10 \mu\text{m}$ : heterotrophic bacteria, *Prochlorococcus*, *Synechococcus*-type cyanobacteria, autotrophic eucaryotes  $< 3 \mu\text{m}$ , and autotrophic eucaryotes  $3\text{-}10 \mu\text{m}$ . *Mycale lingua* nonselectively grazed on all types of plankton  $< 10 \mu\text{m}$ . *Prochlorococcus* was filtered with the highest efficiency (93%), followed by *Synechococcus*-type cyanobacteria (89%), autotrophic eucaryotes  $3\text{-}10 \mu\text{m}$  (86%), heterotrophic bacteria (74%), and autotrophic eucaryotes  $< 3 \mu\text{m}$  (72%). We conservatively estimate that *M. lingua* at naturally occurring densities can obtain  $29 \text{ mg C day}^{-1} \text{ m}^{-2}$  feeding on plankton  $< 10 \mu\text{m}$ , with 74% resulting from ultraplankton, suggesting that ultraplankton are an important overlooked component of benthic-pelagic coupling.



## INTRODUCTION

Planktonic cells less than 5  $\mu\text{m}$  in size, ultraplankton, are responsible for a large share of the primary and secondary production in marine ecosystems (Stockner and Antia 1986, Hobbie 1988) yet the role of ultraplankton in benthic-pelagic coupling remains uninvestigated. Although there are a variety of macroinvertebrates that have the capability to feed on ultraplankton (Rubenstein and Koehl 1977, Jørgensen 1983, Jørgensen et al. 1984), the most conspicuous component of marine benthic communities that has previously been shown to feed primarily on ultraplankton are the sponges (Reiswig 1971b).

Sponges are ubiquitous to both freshwater and marine ecosystems, constituting the dominant active suspension feeding macroinvertebrate in many communities from freshwater streams to the world's oldest, deepest lake (Lake Baikal)(e. g., Frost and Williamson 1980, Sand-Jensen and Pedersen 1994, Pile et al. 1996), tropical to Arctic waters (e. g., Reiswig 1973, Dayton et al. 1974, Wilkinson 1987), and from the deep sea to estuaries (e. g., Koltun 1970, Pomponi and Meritt 1990, Vacelet et al. 1994). The unique ability of these organisms to adapt to all ecosystems by utilizing a variety of food sources ranging from dissolved organic material (DOM: Reiswig 1990) to small crustaceans (Vacelet and Boury-Esnault 1995) suggested that they may be able to exploit ultraplankton as a primary food source.

Globally sponges feed primarily on picoplankton (plankton < 2  $\mu\text{m}$ ) with efficiencies as high as 99% (Reiswig 1971b, 1990, Huysecc m et al. 1988, van de Vyver et al. 1990, Pile et al. 1996). Yet, we are aware of only three studies that utilized *in situ* techniques to determine the natural diet of sponges (Reiswig 1971b, 1990, Pile et al. 1996), one of which was conducted in temperate marine communities (Reiswig 1990). Accurately quantifying

ultraplankton make such studies difficult. These difficulties have been recently overcome with the application of laser-based technologies, such as dual-beam flow cytometry, to accurately identify and enumerate heterotrophic and autotrophic ultraplankton simultaneously (Campbell et al. 1994). Single-beam flow cytometry has been employed to quantify suspension feeding in bivalves, tunicates, and gastropods on autotrophic plankton greater than 3  $\mu\text{m}$  in laboratory studies (Cucci et al. 1985, Shumway et al. 1985, Lesser et al. 1992). Yet, many macroinvertebrates in a variety of taxa have the capability to remove particles much smaller than 3  $\mu\text{m}$  and dual-beam flow cytometry is a more effective tool to accurately identify and quantify suspension feeding on both heterotrophic and autotrophic plankton in all size classes (Campbell et al. 1994, Pile et al. 1996).

Considering that freshwater sponges have removal efficiencies of ultraplankton up to 99% (Pile et al. 1996) coupled with sponges' ability to process copious volumes of water (Reiswig 1971a, Gerrodette and Flechsig 1979, Riisgård et al. 1993, Savarese et al. 1996) suggests that under some conditions sponges can substantially reduce the ultraplankton components of the water column community. We investigated the grazing of the boreal sponge *Mycale lingua* on plankton < 10  $\mu\text{m}$  using a series of *in situ* measurements coupled with the power of dual-beam flow cytometry to identify plankton < 10  $\mu\text{m}$  and found that this macroinvertebrate is extremely efficient at removing ultraplankton during active suspension feeding.

## METHODS

Sponge-mediated "living" carbon flux was calculated from empirical measurements employing the following model that can be used to determine organism-mediated fluxes for active suspension feeders and stated verbally as:

$$\text{organism-mediated flux} = \frac{\Delta \text{water column property}}{\text{volume processed}} \times \frac{\text{volume processed}}{\text{pumping unit} \times \text{time}} \times \frac{\text{number of pumping units}}{\text{benthic surface area}} \quad (3)$$

where  $\Delta$  water column property is the change in cell number as a unit volume is filtered by the organism and the pumping unit is one osculum. We conducted *in situ* measurements on six *Mycale lingua* at Ammen Rock Pinnacle, in the Gulf of Maine (northwest Atlantic Ocean: 42°51'25" N, 68°57'11" W), from September 15-19, 1994 that quantified (1) sponge feeding on plankton <10  $\mu\text{m}$  using dual beam flow cytometry, (2) instantaneous sponge pumping rate using a heated microthermistor flowmeter, and (3) sponge abundance using photo quadrats.

*Mycale lingua* is a yellowish-white sponge with a pillow like shape that is common on rock walls at Ammen Rock Pinnacle (Witman and Sebens 1990). Individuals are multioscular and, when fully expanded, oscula have diameters ranging from 13-23 mm. Observations of sponges indicate that individual oscula respond negatively to touch by closing. Care was taken during all sampling to avoid touching the sponges with the experimental apparatus. During this study we observed periods when all the oscula of an individual closed and water transport through the sponge, visualized with fluorescein dye, was at a minimum indicating periods of pumping inactivity. These events were rare and asynchronous between individuals and locations.

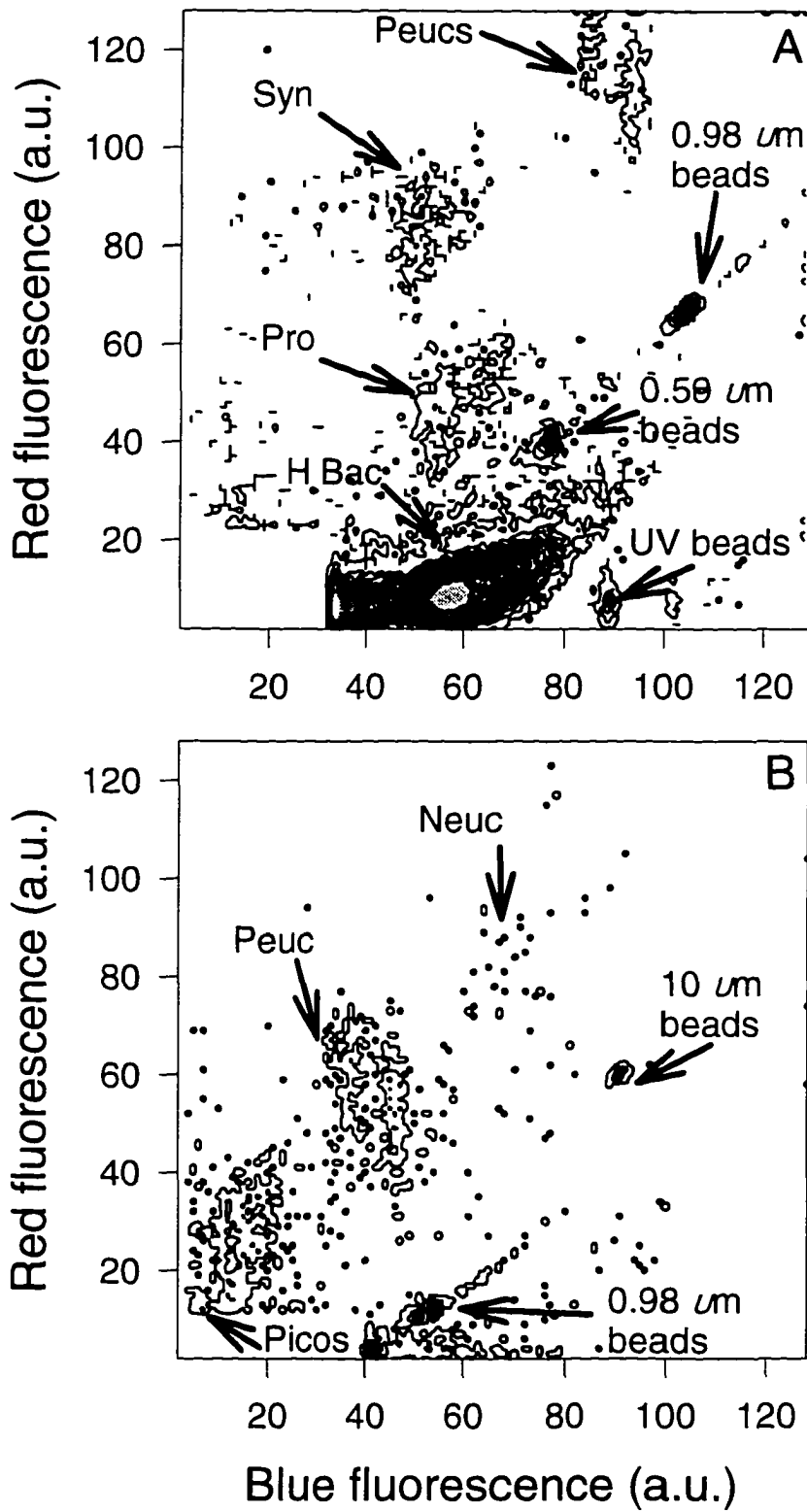
Sponge feeding on plankton  $<10 \mu\text{m}$  was quantified using dual-beam flow cytometry at the University of Hawai'i Flow Cytometry Facility with an EPICS 753 flow cytometer (Coulter Electronics Corporation, Hialeah, Florida). 1 ml water samples collected by SCUBA divers with 1 cc tuberculin syringes from 6 *Mycale lingua* at a depth of 30 m at two locations at Ammen Rock Pinnacle. Five samples were taken from the exhalent current of different oscula within a sponge ( $n=28$ ) and five from ambient water at 0 m and 0.25 m ( $n=20$ ) from the bottom at each location with the average at these two depths comprising the ambient water concentrations and preserved for flow cytometry using standard protocols (Campbell et al. 1994). Samples were spiked with 0.59 and 0.98  $\mu\text{m}$  polystyrene beads and 50  $\mu\text{l}$  of sample illuminated with 1 W. of the 488 nm line of a 6 W argon laser, and a 225 mW UV laser focused through confocal optics. Orange fluorescence (from phycoerythrin), red fluorescence (from chlorophyll a), and blue fluorescence (from DNA stained with Hoechst 33342) (Monger and Landry 1993) were collected through band pass interference filters at 575, 680, and 450 nm, respectively. Samples were then spiked with 10  $\mu\text{m}$  polystyrene beads and the discriminators reset to include the 10  $\mu\text{m}$  beads, and another 50  $\mu\text{l}$  of sample processed as previously described so that larger plankton could be quantified. The five measured parameters, forward- and right-angle light scatter (FALS and RALS), orange, red, and blue fluorescence were recorded on 3-decade logarithmic scales, and sorted in list mode. Plankton populations were analyzed and enumerated with custom-designed software (CYTOPC, Vaulot 1989). Plankton populations were identified to the general cell types of heterotrophic bacteria, *Prochlorococcus*, *Synechococcus*-type cyanobacteria, picoeucaryotes (autotrophic eucaryotes 2-3  $\mu\text{m}$  diameter), and nanoeucaryotes (autotrophic

eucaryotes 3-10  $\mu\text{m}$  diameter) (Figure 6) because there is limited information on the identification of picoplankton using dual-beam flow cytometry from the Gulf of Maine. Cell types were visually confirmed, except for *Prochlorococcus*, and cell diameters measured for picoeucaryotes and nanoeucaryotes using epifluorescence microscopy.

Differences between cell counts from ambient and exhalent current water of each type of picoplankton were analyzed using two sample t-tests with a Bonferroni transformed experimentwise experimental  $\alpha$  of 0.01 to determine the effects of sponges on picoplankton (Sokal and Rohlf 1981). The mean feeding efficiency for each sponge was calculated as  $[(\text{mean cell count ambient water} - \text{mean cell count exhalent current water}) / \text{mean cell count ambient water}] \times 100$  for each type of picoplankton and analyzed as a function of type of plankton using one-way analysis of variance (ANOVA) models and Ryans Q-Test employed to determine differences between means (Underwood 1981, Day and Quinn 1989). The assumption of homogeneity of variance was tested with Bartlett's test. In order to maintain homogeneity of variance for the test of the effect of sponges on picoplankton, all cell counts for ambient and exhalent water were  $\log(x + 1)$  and data back transformed for graphical representation. In all other instances, either the variances were homogeneous, or the hypotheses were rejected at  $\alpha$  values lower than the  $P$ -values of the test for homogeneity of variance when homogeneity of variance could not be achieved using any type of transformation (Underwood 1981).

Instantaneous sponge pumping was quantified using a heated microthermistor flowmeter (modified from LaBarbera and Vogel 1976). 45 second records were obtained by placing the microthermistor within the exhalent current perpendicular to the flow after

Figure 6. Contours of cell abundance in ambient water from Ammen Rock Pinnacle in 50  $\mu$ l samples. Red fluorescence results from chlorophyll a excitation and blue fluorescence from DNA stained with Hoechst 33342. A. Picoplankton with HBac = heterotrophic bacteria, Pro = *Prochlorococcus*, Syn = *Synechococcus*-type cyanobacteria, Peuc = autotrophic eucaryotes 2-3  $\mu$ m cell diameter. B. Pico- and nanoeucaryotes with Peuc = autotrophic eucaryotes 2-3  $\mu$ m cell diameter, Neuc = autotrophic eucaryotes 3-10  $\mu$ m cell diameter and Picos = all types of picoplankton from panel A. Note that in panel B the blue and red fluorescence have relative higher settings, shifting the relative position of the 0.98  $\mu$ m beads and clustering all of the types of picoplankton together. a.u. arbitrary units.



water samples were collected for the feeding study (n=28). The output of the flowmeter was encoded on a tape recorder using a frequency to voltage converter. Recordings were later converted to voltage and sampled at 10 Hz using a GW Instruments Model 411 A/D converter connected to an Apple Macintosh Plus. Mean velocity for 10 second segments was determined from the recordings using a program written in the software *Mathematica* (Wolfram Research, Inc. Champaign, Illinois, USA). Sponge oscula were videotaped immediately following the use of the heated microthermistor flowmeter and oscular area determined from digitized images using NIH Image 1.52. Volume processed per unit time was calculated using  $Q = uA$  where  $Q$  is volume flow ( $\text{ml sec}^{-1}$ ),  $u$  is velocity ( $\text{cm sec}^{-1}$ ), and  $A$  is the oscular area ( $\text{cm}^2$ ). This estimate of volume processed assumes a model of plug flow, or that the velocity profile of exhalent current is rectangular, rather than laminar pipe flow in which the velocity profile is parabolic. This assumption is supported by the shape of exhalent currents in ascidians (Fiala-Medioni 1973, 1978) and it is most likely true for sponges (Savarese et al. 1996).

Sponge percent coverage and mean number of sponge oscula  $\text{m}^2$  were determined from 4 permanent transects consisting of 92,  $0.25 \text{ m}^2$  photo quadrats at 30-35 m depth. The percent cover of sponges in the photo quadrats was determined by projecting each photographed quadrat onto a grid of 200 random dots (2 mm diameter). Sponges with dots falling on them were identified, summed per quadrat, and expressed as percent of 200 dots. Oscula of *Mycale lingua* were enumerated within each quadrat. Mean percent cover and number of sponge oscula  $\text{m}^2$  were then calculated as the average of 92 quadrats.

To obtain a conservative estimate of "living carbon" fed upon by sponges, mean



number of cells removed  $\text{ml}^{-1}$ , as determined using flow cytometry, was converted to  $\text{mg C}$  for each of the 5 types of plankton  $<10 \mu\text{m}$  using standard cell conversions. Cellular conversions to carbon were  $20 \text{ fg C cell}^{-1}$  for heterotrophic bacteria (Ducklow et al. 1993),  $53 \text{ fg C cell}^{-1}$  for *Prochlorococcus* (Morel et al. 1993), and  $470 \text{ fg C cell}^{-1}$  for *Synechococcus* (Campbell et al. 1994). These conversion factors were selected as they are for cells with diameters less than those found during this study thereby most likely underestimating the carbon available in the forms of heterotrophic bacteria, *Prochlorococcus*, and *Synechococcus*-type cyanobacteria. Values for eucaryotes were computed using  $\text{pg C} = 0.433 \times (\text{biovolume})^{0.866}$  (Verity et al. 1992) with the mean biovolumes of  $10.3$  and  $82.4 \mu\text{m}^3$  respectively as determined from measurements of the cells using epifluorescence microscopy. Further, we assumed that *Mycale lingua* was actively pumping at the mean instantaneous rate for 12 h a day. Some tropical marine sponges have a diel periodicity in pumping activity that results in  $18 \text{ h day}^{-1}$  of active pumping while some freshwater and marine sponges do not demonstrate any periodicity in pumping activity (Reiswig 1971a, 1974, Gerrodette and Flechsig 1979, Riisgård et al. 1993, Savarese et al. 1996). Further, by assuming a 12 h pumping period we are most likely under estimating the volume processed daily by *Mycale lingua*.

It is of interest to determine if *Mycale lingua* is selectively grazing on any of the components of the plankton community  $< 10 \mu\text{m}$ . Selectivity indices require the probability that a particle in a given size category will be retained by the filtering apparatus and ingested (Vanderploeg and Scavia 1979). This is generally empirically calculated from microscopic measurements of the filtering apparatus and is unavailable for *M. lingua*. Therefore, to

determine if *M. lingua* is grazing selectively on any proportion of the plankton community < 10  $\mu\text{m}$  the percent of carbon in the diet of sponges was compared to the percent of carbon in the plankton component using a Kolmogorov-Smirnov two sample test (Sokal and Rohlf 1981).

## RESULTS

Heterotrophic bacteria were the most abundant food available followed by *Prochlorococcus*, *Synechococcus*-type cyanobacteria, picoeucaryotes, and nanoeucaryotes (Table 2). *Mycale lingua* significantly decreased all 5 types of plankton <10  $\mu\text{m}$  (Table 2, Figure 7A) from ambient concentrations at feeding efficiencies ranging from 72-93% (Figure 7B). *M. lingua* was most efficient (93%) feeding on *Prochlorococcus*, *Synechococcus*-type cyanobacteria (89%), and nanoeucaryotes (87%). Although not statistically different from each other, feeding efficiencies on *Prochlorococcus*, *Synechococcus*-type cyanobacteria, and nanoeucaryotes were significantly higher than the feeding efficiencies on heterotrophic bacteria (74%) and picoeucaryotes (72%) which were not different from each other (ANOVA  $F_{4,25} = 14.27$ ,  $P < 0.0001$ ).

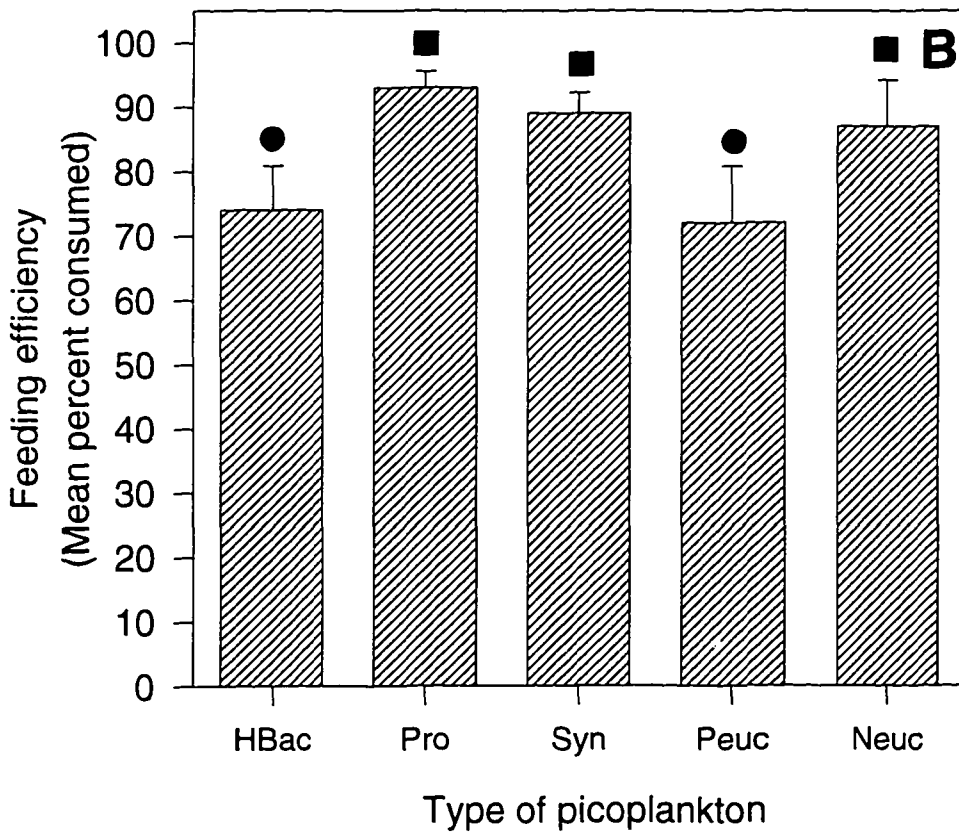
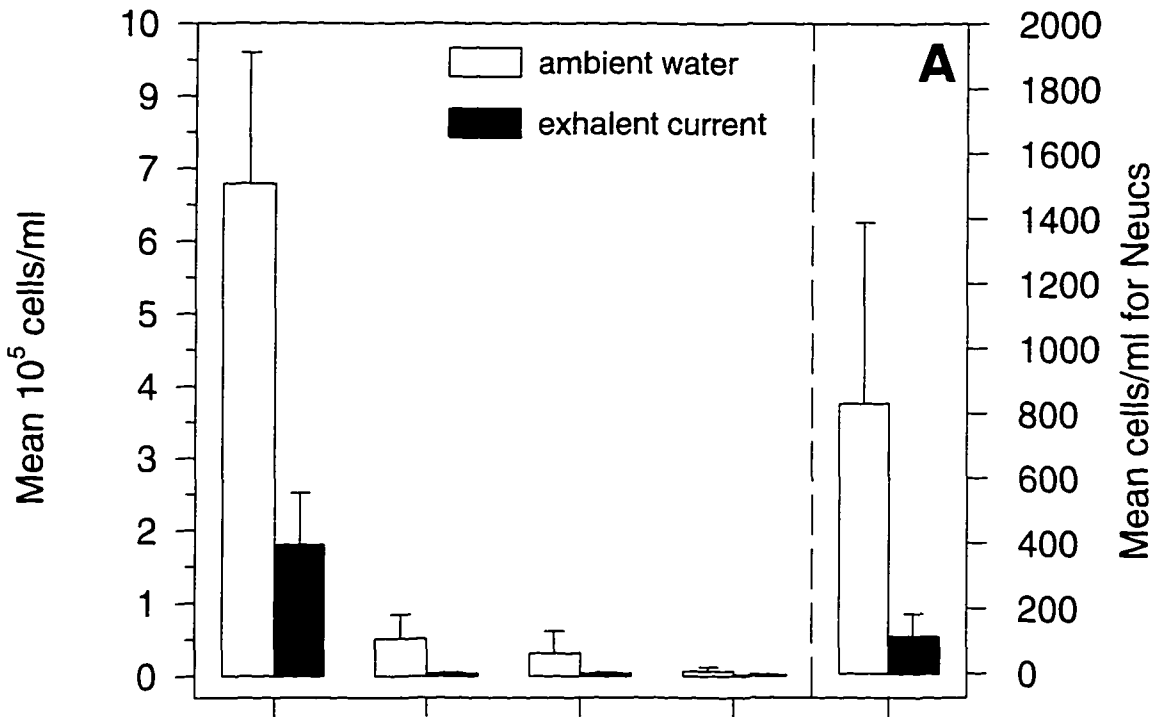
The mean velocity of the exhalent currents was 14.0  $\text{cm sec}^{-1}$  ( $s = 9.7 \text{ cm sec}^{-1}$ ) with a mean oscular diameter of 0.12  $\text{cm}^2$  ( $s = 0.07 \text{ cm}^2$ ) resulting in a mean sponge pumping rate of 1.6  $\text{ml sec}^{-1} \text{ oscula}^{-1}$  ( $s = 1.4 \text{ ml sec}^{-1}$ ). Sponges on rock walls at 30-35 m depth at Ammen Rock Pinnacle cover 21% of the available benthic surface area with *Mycale lingua* covering only 8.9% of the benthic surface area, resulting in 7.6  $\text{oscula m}^{-2}$  ( $s = 33.6 \text{ oscula m}^{-2}$ ).

Table 2. Summary of the effect of individual *Mycale lingua* on the 5 types of plankton < 10  $\mu\text{m}$ . Mean cells  $\text{ml}^{-1}$  (std) in the ambient water and the exhalent current and t-values from two sample t-tests employing a Bonferroni transformed experimentwise  $\alpha = 0.01$ .

Type of plankton	Ambient	Exhalent	t
Heterotrophic bacteria	$6.79 \times 10^5$ ( $1.81 \times 10^5$ )	$1.79 \times 10^5$ ( $0.72 \times 10^5$ )	9.22****
<i>Prochlorococcus</i>	$5.16 \times 10^4$ ( $3.28 \times 10^4$ )	$0.34 \times 10^4$ ( $0.15 \times 10^4$ )	5.09***
<i>Synechococcus</i> -type cyanobacteria	$3.15 \times 10^4$ ( $3.00 \times 10^4$ )	$0.33 \times 10^4$ ( $0.16 \times 10^4$ )	3.24**
Autotrophic eucaryotes < 3 $\mu\text{m}$	$6.68 \times 10^3$ ( $5.82 \times 10^3$ )	$1.86 \times 10^3$ ( $0.77 \times 10^3$ )	2.85**
Autotrophic eucaryotes 3-10 $\mu\text{m}$	832 (557)	113 (71)	4.45****

\*\*  $P < 0.01$ . \*\*\*  $P < 0.001$ . \*\*\*\*  $P < 0.0001$ .

Figure 7. The effect of *Mycale lingua* on plankton <10  $\mu\text{m}$ . (A) concentration of each type of picoplankton in the ambient water and water from the exhalent currents of the sponge *M. lingua* and (B) feeding efficiencies. The abscissa is the same for both graphs with HBac = heterotrophic bacteria. Pro = *Prochlorococcus*. Syn = *Synechococcus*-type cyanobacteria. Peuc = autotrophic eucaryotes 2-3  $\mu\text{m}$  cell diameter. Neuc = autotrophic eucaryotes 3-10  $\mu\text{m}$  cell diameter. A. Pooled cell concentrations of ambient water and water from the exhalent current. Sponges significantly reduced concentrations of all types of plankton < 10  $\mu\text{m}$ . White bars denote ambient water ( $\bar{x} \pm s$ , n=20) and black bars denote water from the exhalent current ( $\bar{x} \pm s$ , n=28). Y-axis for Neuc in 1a is on the right. B. Back transformed mean feeding efficiencies ( $\bar{x} \pm s$ , n=6) of *M. lingua* on plankton < 10  $\mu\text{m}$ . Bars sharing a symbol are not significantly different.



Employing the model for organism mediated fluxes (1) and the carbon equivalent of the mean number of cells eaten  $\text{ml}^{-1}$ , mean sponge pumping rate per oscula, and mean number of oscula per  $\text{m}^2$ , we conservatively estimate that for this benthic environment,  $29 \text{ mg C d}^{-1} \text{ m}^{-2}$  is captured by *Mycale lingua* through active suspension feeding. Carbon acquisition is evenly distributed between procaryotic and eucaryotic plankton  $<10 \mu\text{m}$  (Table 3) and not statistically different from those of the water column community (Kolmogorov-Smirnov two sample test,  $D_{5,5} = 36$ ,  $P < 0.01$ ). We are aware of the limitations of making such calculations and the data are presented in such a manner that should better cell conversions become available sponge-mediated fluxes can be recalculated.

## DISCUSSION

*Mycale lingua* is highly efficient at grazing on heterotrophic and autotrophic plankton  $< 10 \mu\text{m}$  with feeding efficiencies comparable to those of other marine (Reiswig 1971b, 1975, Stuart and Klumpp 1984) and freshwater demosponges (Huysecom et al. 1988, van de Vyver et al. 1990, Pile et al. 1996). More importantly, the use of dual-beam flow cytometry has allowed us to accurately quantify the diet of *M. lingua*, including the previously undocumented feeding of any macroorganism on *Prochlorococcus*.

*Prochlorococcus* are photoautotrophic, procaryotic picoplankton typically  $< 0.8 \mu\text{m}$  in diameter that can be easily and accurately distinguished from heterotrophic bacteria using flow cytometry (Chisholm et al. 1988, Olson et al. 1990, Li et al. 1992, Veldhuis and Kraay 1993, Campbell et al. 1994). Although they are extremely abundant (c.  $10^5 \text{ ml}^{-1}$ ), contributing up to 35% of the total biomass of plankton  $<20 \mu\text{m}$ , and found in all of the

Table 3. Estimated mean mg C removed from the picoplankton community daily by the sponge *M. lingua* occupying 1 m<sup>2</sup> of the benthos at Ammen Rock Pinnacle at naturally occurring densities. Estimates were computed assuming that sponges were actively pumping for 12 hrs each day. % diet was calculated assuming all cells removed were consumed and % plankton component was calculated as the proportion of total "living carbon" of plankton < 10 μm in the ambient water.

Picoplankton component	Sponge-mediated flux		
	mg C d <sup>-1</sup> m <sup>-2</sup>	% diet <sup>1</sup>	% plankton component <sup>1</sup>
Procarvates			
Heterotrophic bacteria	5	18	20
<i>Prochlorococcus</i>	1	5	5
<i>Synechococcus</i> -type cyanobacteria	6	22	19
Total procarvates	13	45	44
Eucaryotes			
picoeucaryotes (2-3 μm)	8	29	32
nanoeucaryotes (3-10 μm)	8	26	24
Total eucaryotes	16	55	56
Total of all cell types	29	100	100

<sup>1</sup>Kolmogorov-Smirnov two-sample test indicates distributions are not significantly different ( $P < 0.01$ ).

world's oceans, their abundance and distribution is highly variable (Chisholm et al. 1988, Olson et al. 1990, Li et al. 1992, Veldhuis and Kraay 1993, Campbell et al. 1994). They appear to increase in abundance at lower latitudes and with distance from land margins (Chisholm et al. 1988, Olson et al. 1990, Li et al. 1992, Veldhuis and Kraay 1993, Campbell et al. 1994).

*Prochlorococcus* is an integral component of the picoplankton in coral reefs (Pile 1996) and other ecosystems (Chisholm et al. 1988, Olson et al. 1990, Li et al. 1992, Veldhuis and Kraay 1993, Campbell et al. 1994) dominated by pelagic and benthic macroorganisms with filters designed to capture particles 0.8  $\mu\text{m}$  or smaller (Rubenstein and Koehl 1977, Jørgensen 1983, Jørgensen et al. 1984). The diet of these taxa may have been incorrectly identified using conventional methods and total carbon-flux underestimated using traditional methods to identify plankton. Grazing on *Prochlorococcus* by both micro- and macroorganisms needs to be further quantified using dual-beam flow cytometry to resolve the flow of carbon in marine ecosystems.

Instantaneous pumping rates for *Mycale lingua* are on the higher end of the range of those of other sponges (Reiswig 1971a, Gerrodette and Flechsig 1979, Riisgård et al. 1993, Savarese et al. 1996). Previous researchers have estimated the affect of sponges on water column communities by determining the time for a community of sponges to process the entire overlying water column (turnover rate) (Reiswig 1974, Gerrodette and Flechsig 1979, Savarese et al. 1996). They have extrapolated the pumping rates with the fictitious assumption of a well mixed water column. Despite the inherent problems with these types of calculations we estimate that at Ammen Rock Pinnacle *M. lingua* at naturally occurring



densities processes a column of water 0.532 m high each day, taking 56.2 days to turnover the entire 30 m water column. A turnover rate of 56.2 is much higher than previously determined near daily turnover times for other shallower sponge dominated communities (Reiswig 1974, Savarese et al. 1996). However, it is highly unlikely that sponges can affect the water column community more than 1 m from the substrate and that shear velocity, bottom roughness, and the strength of horizontal flow plays an important role in providing unfiltered water to the benthic community.

The extensive sponge community of Lake Baikal's littoral zone can create picoplankton depleted layer of water within 1 m of the benthos (Pile et al. 1996) while other benthic communities dominated by suspension feeding macroinvertebrates result in similar food depleted layers (Glynn 1973, Buss and Jackson 1981, Peterson and Black 1987, Fr chet te et al. 1989, Butman et al. 1994). Mean flow speeds during this study period were  $0.20 \text{ m sec}^{-1}$  (Witman and Patterson unpublished data) which were 3 times as high as those found in Lake Baikal (Savarese et al. 1996) and would most likely preclude the development of a food depleted boundary layer over Ammen Rock Pinnacle. To understand the effect of sponges on ultraplankton communities a more accurate descriptor is to determine the percentage of water that passes over the Ammen Rock Pinnacle daily that can be filtered by *Mycale lingua*. Ammen Rock Pinnacle has benthic surface area of  $160 \text{ m}^2$  (Witman unpublished data), and a volume of  $1730 \text{ m}^3 \text{ day}^{-1}$  passes over the pinnacle that is available to sponges. Using the assumption that the sponges are only actively pumping for  $12 \text{ h day}^{-1}$ , *Mycale lingua* can conservatively process 5% of the water that passes over Ammen Rock Pinnacle. The integrated feeding efficiency on ultraplankton (all procaryotes +

picoeucaryotes) is 76%. *Mycale lingua* has a gross daily grazing effect of removing 4% of the ultraplankton from the near bottom water. The incorporation of the remaining suite of benthic invertebrates that feed primarily on ultraplankton that are common at Ammen Rock Pinnacle (Witman and Sebens 1988), such as the remaining sponges, ascidians (Riisgård et al. 1980, Stuart and Klumpp 1984), juvenile and adult bivalves (Riisgård et al. 1980, Stuart and Klumpp 1984), and bryozoans (Winston 1978), will substantially increase the percentage of water that is grazed by suspension and filter feeders and most likely significantly impact near bottom, water column communities. More importantly, in shallower near shore ecosystems the effect of grazing by benthic invertebrates on water column communities will be greater.

Previous estimates of tropical sponge, daily carbon metabolic requirements range between 80 and 1800 mg C d<sup>-1</sup> m<sup>-2</sup>, which was met by a diet that consisted of bacteria and unresolvable particulate organic carbon (most likely other types of picoplankton) (Reiswig 1971b). Sponges in Lake Baikal's littoral zone can obtain 1970 mg C d<sup>-1</sup> m<sup>-2</sup> through active suspension feeding on both heterotrophic and autotrophic plankton < 3 μm (Pile et al. 1996). Our estimates of areal carbon flux of 29 mg C d<sup>-1</sup> m<sup>-2</sup> for *Mycale lingua* are lower than that previously described and this sponge is less abundant by an order of magnitude (Reiswig 1971b, Pile et al. 1996). Considering this, *M. lingua* is obtaining carbon from plankton <10 μm at rates similar to other sponges.

*Mycale lingua* obtained carbon in nearly equal proportions from procaryotic and eucaryotic plankton < 10 μm. This is in contrast to the freshwater sponges in Lake Baikal's littoral zone where a majority of carbon was obtained from *Synechococcus*-type

cyanobacteria (Pile et al. 1996) and tropical marine sponges where 80% of captured carbon was obtained from bacterioplankton and unresolvable particulate organic carbon (Reiswig 1971b). None of the sponges examined with flow cytometry were selectively feeding on any component of the plankton community (Pile et al. 1996, this study) suggesting that the composition of the plankton community is an important factor in a sponge's ability to meet its metabolic carbon requirements. Ultimately, variability in water column community composition at many temporal scales (e. g., seasonal, diurnal, and short term physical events such as internal waves and storms) can affect sponge nutrition.

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## Chapter IV

The coral reef sponges *Ircinia felix* and *I. strobilina* as a source of dissolved inorganic nitrogen and phosphorus:  
Implications for the role of the microbial food web  
in benthic-pelagic coupling

To be submitted to Limnology and Oceanography

### ABSTRACT

Sponges are an abundant, ecologically important member of Caribbean coral reefs yet their function in the cycling of dissolved inorganic nitrogen (DIN) and phosphorus (DIP) is largely unexplored. Employing short, *in situ* incubations in recirculating metabolism chambers, I found that the tropical sponges *Ircinia felix* and *I. strobilina* are a significant source of ammonium, nitrate, and soluble reactive phosphate. Release rates of all species of DIN and DIP was higher for *I. felix* than *I. strobilina*. Total DIN and DIP release were calculated using *in situ* measurements of sponge pumping rate (volume flux) and abundance. At naturally occurring densities for Conch Reef, Florida Keys, USA *I. felix* released 186  $\mu\text{mol DIN d}^{-1} \text{m}^{-2}$  and *I. strobilina* released 18  $\mu\text{mol DIN d}^{-1} \text{m}^{-2}$ . DIP release rates were 43 and 5  $\mu\text{mol d}^{-1} \text{m}^{-2}$  respectively. These release rates are an order of magnitude greater than those reported for coral reef sediments suggesting that regeneration of macronutrients by sponges may be an important component in the cycling of nutrients in coral reefs.

## INTRODUCTION

Coral reef ecosystems are characterized by extremely low concentrations of dissolved inorganic nutrients in the overlying waters (Webb et al. 1975, Andrews and Müller 1983, Entsch et al. 1983). Water that flows over coral reefs generally shows a net increase in dissolved inorganic nitrogen (DIN) and no net change in dissolved inorganic phosphorus (DIP) (see review by D'Elia and Wiebe 1990) despite some of the highest levels of gross primary production in the world (Lewis 1977). Researchers have explained the paradox of extremely high productivity within oligotrophic waters as due in part to the efficient recycling of limiting macronutrients, such as nitrogen and phosphorus, by the benthos. Current theory postulates that a complex suite of interactions between microbial and reef macrofaunal communities results in areas of the reef that are net sources or sinks of DIN and DIP (e. g., Pomeroy et al. 1973, Capone and Carpenter 1982, Atkinson 1987, Boucher et al. 1994, Atkinson et al. 1995).

The highly productive benthic communities of coral reefs are supported by endogenous and exogenous sources of dissolved and particulate organic material. Endogenous sources include the fixation of carbon via primary production and of nitrogen via microbial processes. These are important sources of carbon and nitrogen in biologically available forms to the reef community (e. g., Lewis 1977, Capone and Carpenter 1982). Primary production by symbiotic zooxanthellae support scleractinian corals that provide the carbonate framework for the reef. However, for coral reef organisms that do not have an autotrophic component to their diet an exogenous source of carbon and nitrogen is required for growth.

There is strong evidence to hypothesize that ultraplankton (heterotrophic and autotrophic prokaryotes and eucaryotes  $< 5 \mu\text{m}$ ) is an important exogenous source of particulate organic material for coral reefs. In the late 1960's Reiswig (1971) found that a majority of the particulate organic carbon retained by Caribbean coral reef sponges was unresolvable using light microscopy, the technique available at the time. He hypothesized that the unresolvable particulate organic carbon (URPOC) was heterotrophic and autotrophic plankton less than  $2 \mu\text{m}$  (picoplankton). More recently, Ayukai (1995) found that as water traverses two Pacific coral reefs up to 90% of the ultraplankton was retained by the reefs. He hypothesized that this was the result of grazing by the benthic community. The resultant net flux of carbon from the water column was equal to the benthic primary production estimates for the same reef. The role of the microbial food web in benthic-pelagic coupling may be more important in the Caribbean, where sponges are second to corals in abundance and are primarily heterotrophic (Wilkinson 1987, Wilkinson and Cheshire 1990).

Coral reef sponges graze primarily on ultraplankton through active suspension feeding (Reiswig 1971, Pile 1996). Sponge communities can process copious amounts of water, with estimated turnover times of the water column on some Caribbean reefs as short as 1 day (Reiswig 1974). Sponges have complex associations with epi- and endosymbiotic micro- and macroorganisms (Rützler 1990, Vicente 1990, Duffy 1992, 1996, Shieh and Lin 1994, Duarte and Nalesso 1996) that make it difficult to differentiate between processes mediated by the host and the symbiont. Activity of photosynthetic symbionts of sponges can result in net primary production in some species (Wilkinson 1983). Processes of the microbial symbionts of sponges, such as nitrification (Corredor et al. 1988) and

nitrogen fixation (Wilkinson and Fay 1979, Shieh and Lin 1994), result in sponges being a net source of DIN within the complex reef community. Therefore, for the purpose of this study sponges and their associated assemblages are considered a single entity and function as net sources or sinks of material. The remineralization of organic nitrogen to ammonium and its subsequent export or processing by sponges and their microbial symbionts has yet to be investigated.

Previous research on the flow of macronutrients within coral reefs has focused on the contribution of various microbial processes within coral reefs to DIN pools (Webb and Wiebe 1975, Webb et al. 1975, Capone 1977, Capone et al. 1992). Yet the few studies available on the release of DIN and DIP by macroorganisms, such as fish (Meyer and Schultz 1985a) and sponges (Corredor et al. 1988), suggests that the benthic macrofaunal community can be a significant source of DIN and DIP. It can be hypothesized that the extensive biomass of sponges on Caribbean reefs, combined with their ability to graze on microbial plankton, and process copious amounts of water gives sponges the potential to contribute significantly to the overall flow of nitrogen through coral reef ecosystems.

I investigated rate of release of DIN and DIP in two sponges, *Ircina felix* and *I. strobilina*, common to Conch Reef, Florida Keys, USA. Using short, 30 min, *in situ* incubations in recirculating metabolism chambers both sponges were found to be a significant source of ammonium, nitrate, and soluble reactive phosphate. Sponge-mediated fluxes of DIN and DIP were empirically calculated at the organismal and community level to estimate the contribution of these sponges to the coral reef nitrogen budget. Further, sponge-mediated fluxes of DIN and particulate organic nitrogen (PON) are developed into



a conceptual model to show that microbial food webs are an integral component of benthic-pelagic coupling.

## METHODS

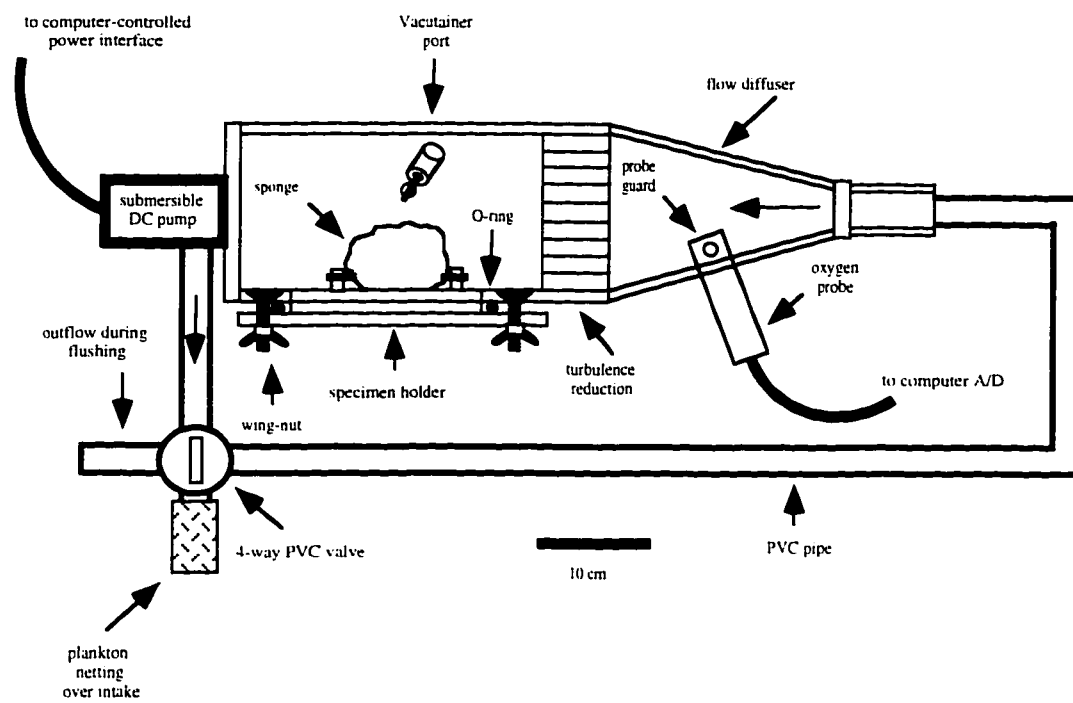
This study was conducted at Conch Reef, Florida Keys, USA (24°57' N and 80°27' W). Conch Reef has been the location of the NOAA *Aquarius* underwater habitat described by Leichter et al. (Leichter et al. 1996) for 5 years, and has served as a study site for many coral reef investigations. *Ircinia felix* and *I. strobilina* are common sponges at this location comprising 0.73 and 0.12% of the benthic surface area respectively, whereas total cover of noncryptic sponges is 7% of the benthic surface area (Pile 1996). They are both black in color and have an endosymbiotic relationship with the photosynthetic cyanobacterium *Aphanocapsa feldmanni* (Vicente 1990). Both sponges are multioscular; *I. felix* has a pillow like morphology whereas *I. strobilina* has a globose shape with the oscula located at the top of the sponge. Sponges were carefully removed from the substrate by SCUBA divers and held near the *Aquarius* habitat in mesh bags 12-24 h prior to use. Before incubations sponge health was visually assessed by divers and only healthy sponges, as indicated by an intact sponge with fully expanded oscula, were used.

Incubations were conducted in recirculating flow chambers (Patterson et al. 1991) which were deployed *in situ*. *In situ* studies are favored over laboratory studies since there is less stress on the organism and environmental parameters, such as light and temperature, closely simulate those in nature. Additionally, this study employed a recirculating system that mimics horizontal flow commonly found in nature. Horizontal flow is very important

to sponges as it can induce flow through the sponge (Vogel 1974, 1977). While it is not known if *Ircinia felix* or *I. strobilina* can take advantage of induced flow, the system employed mimicked to some extent the flow environment of the reef and replicated the thermal and light environment very closely. During this study the chambers were deployed on the sand near *Aquarius* at a depth of 18 m to utilize the power supply from the habitat. Dives were made from the saturation habitat to monitor the chambers and take samples. Recirculating flow chambers were slightly modified from the original design to accommodate this study (Figure 8). Chambers were fitted with a 500 GPH submersible bilge pump, resulting in a horizontal flow speed of c. 5 cm sec<sup>-1</sup>. Horizontal flow speed of near bottom water was measured daily during the study near sponges on Conch Reef and daily means ranged from 3 to 5 cm sec<sup>-1</sup>. Each chamber has a volume of 7 l and was fitted with a YSI polarographic oxygen sensor. A 4π Li-Cor irradiance meter was set up on one of the chambers and connected to a Li-Cor LI-1000 sensor datalogger in the habitat.

Changes in DIN and DIP were measured over 30 min in 6 recirculating metabolism chambers with the haphazardly assigned treatments of 1 *Ircinia felix*, 1 *I. strobilina*, or no sponge (water control). Incubations were conducted on 26, 27, and 29 May 1995 resulting in six replicates for each treatment. Sponges were placed in the chambers, the base plate was secured, and the horizontal flow was initiated. 60 ml water samples were taken by SCUBA divers using sterile syringes with stopcocks at 0, 5, 10, 15, and 30 min. Samples were sent to the surface and 30 ml of each were immediately filter-sterilized (Gelman, Supor Acrodisc, 0.2 μm) into a 50 ml sterile centrifuge tube and held on ice until returning to the lab. Samples were then frozen until analysis for ammonium, nitrate, nitrate + nitrite, and soluble

Figure 8. Diagram of recirculating metabolism chamber as designed by Patterson et al. (1991) and modified for this study.



reactive phosphate at Florida International University, Miami, FL using an Alpkem 4 channel RFA 300 Rapid Flow Analyzer as described by Leichter et al. (1996).

After completion of each experiment the sponges were removed from the metabolism chambers, frozen, and returned to the Virginia Institute of Marine Science, Gloucester Point, VA for combustion. Sponges were repeatedly rinsed in freshwater to remove any salts and meticulously dissected to remove any associated macrofauna. One *Ircinia felix* had 3 brittle stars associated with it. Release rates of DIN and DIP from this sponge were within the 95% confidence interval of the mean of the 5 other individuals. Thus, it was deemed appropriate to include the results for statistical analysis. Sponges and the 3 brittle stars were dried at 100 °C until attaining equilibrium, which was defined as no change in weight over a 12 h interval. They were then combusted at 550 °C for 4.5 h to obtain ash free dry weight (AFDW). Prior to dissection, the number of oscula for each sponge was enumerated.

Release of ammonium, nitrate, nitrite, and soluble reactive phosphate were normalized to both g AFDW of sponge and number of oscula:

$$\frac{R}{N} = V_c(C_t - C_c) \quad (4)$$

where R = amount released,  $V_c$  = volume of the chamber,  $C_t$  = concentration of the treatment chamber at time = t,  $C_c$  = concentration of the control chamber at time = t, and N = normalizing parameter (g AFDW or number of oscula per individual). Release in control chambers was normalized using  $C_t$  = concentration at time = t,  $C_c$  = concentration at  $T_0$ , and N = 1. Release during the incubation was analyzed as a function of sponge species (*Ircinia felix* vs. *I. strobilina* vs. control) using analysis of covariance with time as the covariate

(ANCOVA, Sokal and Rohlf 1981). Simple linear regression was employed to determine the rate of release (Sokal and Rohlf 1981). Concentrations of nutrients in one of the control chambers were greater than all of the other control chambers by three orders of magnitude due to an unknown error. Rather than have these values bias statistical analysis the mean control values were substituted, the error degrees of freedom were reduced, and  $F$  values adjusted (Sokal and Rohlf 1981). Variances were found to be homogeneous using Cochran's  $C$  test (Sokal and Rohlf 1981). Residuals were visually examined and appeared randomly distributed.

Sponge-mediated fluxes of all species of DIN and DIP were empirically calculated employing the following model of organism-mediated fluxes that can be verbally stated as:

$$\text{organism-mediated flux} = \frac{\Delta \text{water column property}}{\text{volume processed}} \times \frac{\text{volume processed}}{\text{pumping unit} \times \text{time}} \times \frac{\text{number of pumping units}}{\text{benthic surface area}} \quad (3)$$

where the  $\Delta$  water column property is the net rate of ammonium, nitrate, nitrite, and soluble reactive phosphate released per osculum, volume processed is 1 ml, time is 1 day, pumping unit is 1 osculum, and benthic surface area is 1 m<sup>2</sup>. This will result in a sponge mediated flux in  $\mu\text{mol d}^{-1} \text{m}^{-2}$ . Additional *in situ* measurements of sponge pumping rate, using heated microthermistor flowmeters, and sponge abundance, using video transects, were obtained during the study (Pile 1996). Mean pumping rates for *Ircinia felix* during the period of the study were  $6.4 \times 10^4 \text{ ml osculum}^{-1} \text{d}^{-1}$  and  $4.9 \times 10^4 \text{ ml osculum}^{-1} \text{d}^{-1}$  for *I. strobilina* (Pile 1996). At Conch Reef there is a mean of 5.9 oscula m<sup>-2</sup> for *I. felix* and a mean of 2.7 oscula

$\text{m}^{-2}$  for *I. strobilina* (Pile 1996).

A conceptual model of sponge-mediated fluxes of DIN and PON was developed using additional data on sponge feeding from a concurrent study (Pile 1996). Ambient concentrations of DIN were determined from the control chambers for the entire 18 m water column overlying a  $\text{m}^2$  benthos, 18,000 l. Concentrations,  $\mu\text{mol}$ , were converted to mg N using atomic ratios of all species of DIN. Fluxes of particulate nitrogen were calculated from sponge-mediated fluxes of ultraplankton. Sponge-mediated carbon fluxes (Pile 1996, Table 3) were converted to mg N  $\text{m}^{-2} \text{day}^{-1}$  using a C:N ratio of 4:1 for heterotrophic bacteria; 6:1 for *Prochlorococcus*, *Synechococcus*-type cyanobacteria, and picoeucaryotes; and 8:1 for nanoeucaryotes (Wheeler and Kirchman 1986). Standing stocks of PON in the form of ultraplankton were calculated from cell concentrations in the ambient water at Conch Reef for an integrated water column of 18 m (Pile 1996, Table 1).

This study has employed the most conservative values so that any errors will result in an underestimate of the reported fluxes. It is most likely that the cell to carbon and nitrogen conversion factors will change as the techniques to estimate cellular carbon and nitrogen become better and the data have been presented in such a manner that should more accurate conversion factors be reported the fluxes may be recalculated. Further, the estimated fluxes of DIN and PON utilize only the data collected during this study, late May of 1995, and will most likely vary within and between years.

## RESULTS

*Ircina felix* released significantly greater amounts of ammonium, nitrate, nitrite, and soluble reactive phosphate than did water controls during the 30 min incubation in the metabolism chambers (Figures 9-10). *I. strobilina* released significant amounts of ammonium, nitrate, and soluble reactive phosphate while not affecting nitrite (Figures 9-10). As a function of both AFDW of sponge (Table 4) and number of oscula (Table 5) rates of release by *I. felix* were significantly higher than those of *I. strobilina* for all species of DIN and DIP. Release rates for *I. felix* were nearly four times those of *I. strobilina* for ammonium and nearly double those of *I. strobilina* for nitrate and soluble reactive phosphate when normalized to either g AFDW of sponge or osculum (Table 6). 73% of the DIN released by *I. felix* was in the form of ammonium. In contrast, the DIN released by *I. strobilina* was evenly divided between ammonium and nitrate.

Environmental conditions were constant during the 30 min incubations. Dissolved oxygen levels varied < 5%. Irradance did not vary within or between incubations. Temperature, collected by an S4 InterOcean current meter deployed near the *Aquarius* habitat recording 1 min averages, did not indicate any significant temperature changes ( $\pm 0.2^{\circ}$  C) during the incubations.

## DISCUSSION

Although it is most desirable to measure the release rate of DIN and DIP directly from water collected from the exhalent current of the sponge, current analytical techniques require too large of a sample to be accurately collected. Instead, short incubations within



Figure 9. Time course of release of ammonium, nitrate, nitrite, and soluble reactive phosphate normalized to AFDW ( $\bar{x} \pm \text{std.}$ ,  $n=6$ ) by *Ircinia felix* ( $\blacktriangle$ ), *I. strobilina* ( $\blacksquare$ ) compared to empty chamber controls ( $\bullet$ ) during the 30 min incubations. Lines represent linear regressions, which were all significant at  $\alpha < 0.05$ , and  $r^2$  values are adjacent to each regression.

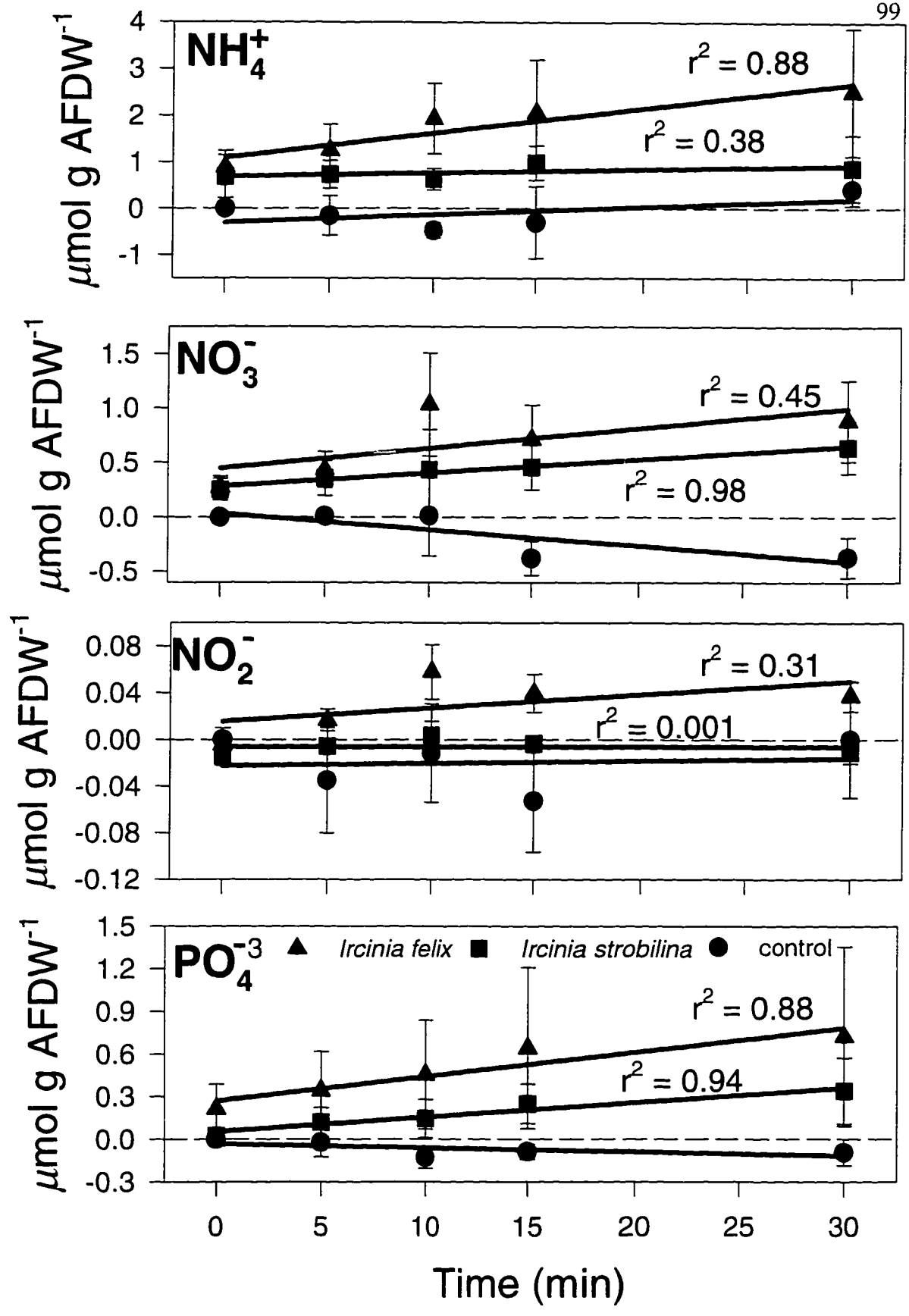


Figure 10. Time course of release of ammonium, nitrate, nitrite, and phosphorus normalized to number of osculum ( $\bar{x} \pm \text{std.}$ ,  $n=6$ ) by *Ircinia felix* ( $\blacktriangle$ ), *I. strobilina* ( $\blacksquare$ ) compared to empty chamber controls ( $\bullet$ ) during the 30 min incubations. Lines represent linear regressions, which were all significant at  $\alpha < 0.05$ , and  $r^2$  values are adjacent to each regression.

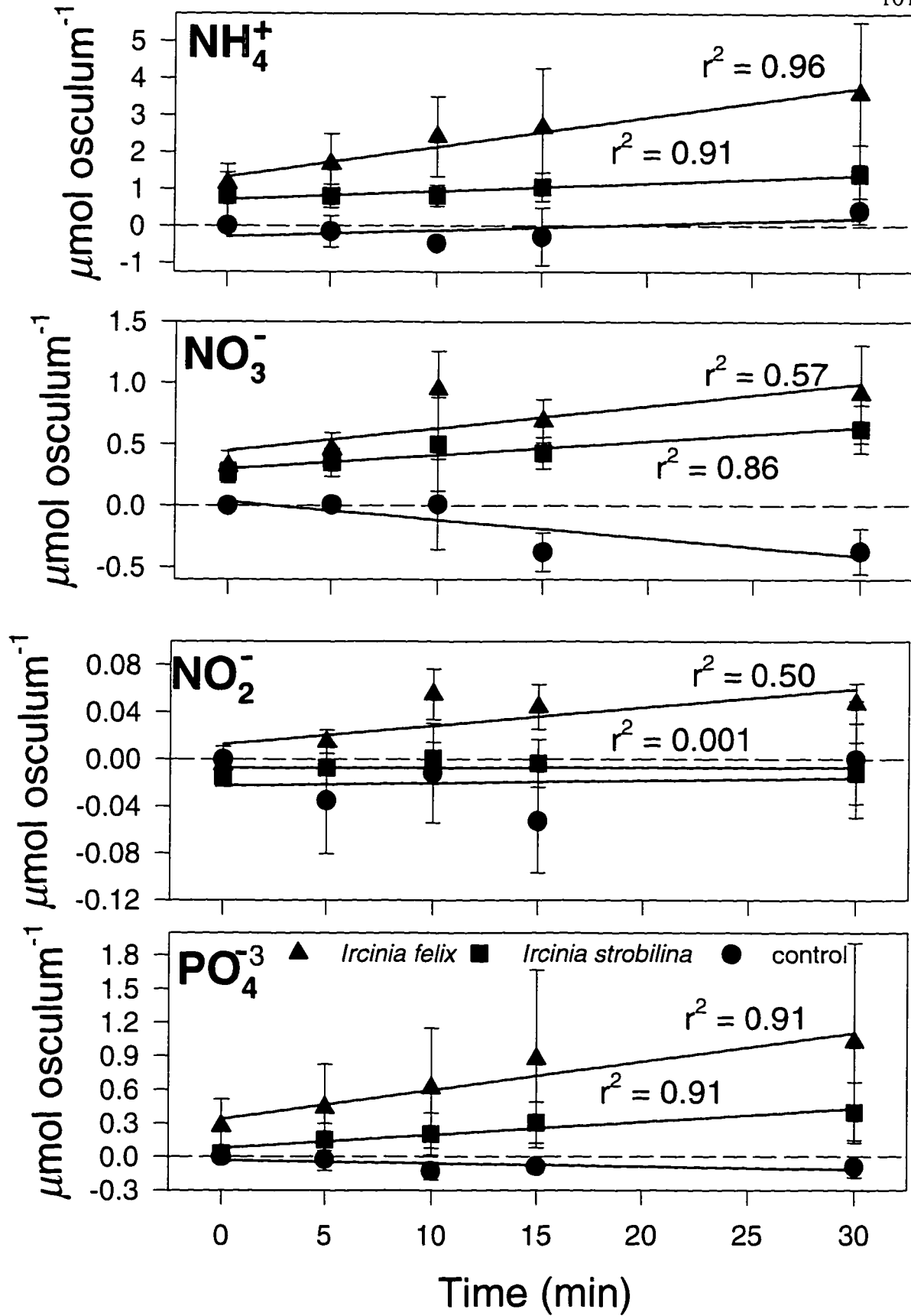


Table 4. Results of analysis of covariance for ammonium, nitrate, nitrite, and soluble reactive phosphate release  $\text{g}^{-1}$  AFDW as dependent on species of sponge (*Ircinia felix* vs *I. strobilina* vs controls).

Nutrient	Source of variation	df	MS	F
Ammonium	Covariate (time)	1	8.964	4.10 <sup>ns</sup>
	Species of Sponge	2	19.293	8.83 <sup>****</sup>
	Error	74	2.185	
Nitrate	Covariate (time)	1	0.520	1.46 <sup>ns</sup>
	Species of Sponge	2	3.985	11.19 <sup>****</sup>
	Error	74	0.356	
Nitrite	Covariate (time)	1	0.002	0.95 <sup>ns</sup>
	Species of Sponge	2	0.017	7.98 <sup>**</sup>
	Error	74	0.002	
Phosphate	Covariate (time)	1	0.819	1.79 <sup>ns</sup>
	Species of Sponge	2	1.647	3.60 <sup>*</sup>
	Error	74	0.458	

\*  $p < 0.05$ . \*\*  $p < 0.01$ . \*\*\*\*  $p < 0.0001$ . <sup>ns</sup> not significant

Table 5. Results of analysis of covariance for ammonium, nitrate, nitrite, and soluble reactive phosphate release osculum<sup>-1</sup> as dependent on species of sponge (*Ircinia felix* vs *I. strobilina* vs controls).

Nutrient	Source of variation	df	MS	F
Ammonium	Covariate (time)	1	15.740	3.73 <sup>ns</sup>
	Species of Sponge	2	37.642	8.92 <sup>****</sup>
	Error	74	4.222	
Nitrate	Covariate (time)	1	0.352	1.60 <sup>ns</sup>
	Species of Sponge	2	4.611	21.02 <sup>****</sup>
	Error	74	0.219	
Nitrite	Covariate (time)	1	0.002	0.77 <sup>ns</sup>
	Species of Sponge	2	0.024	9.31 <sup>****</sup>
	Error	74	0.003	
Phosphate	Covariate (time)	1	1.555	1.77 <sup>ns</sup>
	Species of Sponge	2	3.015	3.44 <sup>*</sup>
	Error	74	0.877	

\*  $p < 0.05$ . \*\*\*\*  $p < 0.0001$ . <sup>ns</sup> not significant

Table 6. Mean *Ircinia felix*- and *I. strobilina*-mediated fluxes of dissolved inorganic nitrogen and soluble reactive phosphate. Values in parentheses are the percent of the total DIN flux. Rates individual<sup>-1</sup> were calculated using the mean number of oscula individual<sup>-1</sup> from video transects taken at Conch Reef (Pile 1996) and mean pumping rates. nc - no change.

Sponge	Ammonium	Nitrate	Nitrite	Phosphate
<i>Ircinia felix</i>				
$\mu\text{mol g}^{-1}$ AFDW d <sup>-1</sup>	14.7 (73)	5.3 (26)	0.3 (1)	4.4
$\mu\text{mol osculum}^{-1}$ ml <sup>-1</sup>	$3.7 \times 10^{-4}$	$9.1 \times 10^{-5}$	$6.4 \times 10^{-6}$	$1.1 \times 10^{-4}$
$\mu\text{mol individual}^{-1}$ d <sup>-1</sup>	147	37	2.6	46
<i>I. strobilina</i>				
$\mu\text{mol g}^{-1}$ AFDW d <sup>-1</sup>	3.6 (54)	3.1 (46)	nc	2.0
$\mu\text{mol osculum}^{-1}$ ml <sup>-1</sup>	$8.5 \times 10^{-5}$	$4.0 \times 10^{-5}$	nc	$3.3 \times 10^{-5}$
$\mu\text{mol individual}^{-1}$ d <sup>-1</sup>	49	23	nc	19

recirculating metabolism chambers provide a satisfactory alternative to measuring release rates. Previous researchers who employed metabolism chambers to measure DIN or DIP release in sponges had exceptionally long incubations of 4 h (Corredor et al. 1988, Schubauer 1988) compared to this study with incubations of 0.5 h. D'Elia (1977) points out many of the advantages of using short incubation periods for studies with organisms. Ultimately, he suggests that incubations should be ended as soon as a measurable change in the substance is detected.

One must remember that the "organism" responsible for nitrogen transformation in this study might be better considered a community or consortium of sponge and its microbial symbionts. This sponge community is likely to be highly sensitive to changes in supply of particulate organic material and oxygen. *In situ* pumping rates of these sponges indicated that they would process the 7 l volume of the chamber in c. 30 min. At the time of the study we did not know the filtration efficiencies of these two sponges, but some sponges have filtration efficiencies as high as 99% on natural ultraplankton communities (Pile et al. 1996a, b). The creation of a food-depleted environment within the metabolism chamber, and the cessation of sponge grazing, could affect DIN and DIP release in two ways. At the organismal level, reduction in the food supply would reduce the rate of remineralization of organic nitrogen and phosphorus to ammonium and soluble reactive phosphate and concurrently affect sponge pumping rates. Second, reducing the supply of dissolved organic carbon to the associated microbial community might affect processes such as heterotrophic nitrogen fixation or denitrification which require a labile carbon source. Another potential source of error can result from a change in the oxygen levels within the chamber. Nitrogen



cycling processes in microorganisms are extremely sensitive to dissolved oxygen levels and the pathways of nitrogen regeneration will change with decreased oxygen levels, ultimately changing the species of nitrogen released. During the 30 min incubations there were negligible changes in oxygen levels within the metabolism chambers. More importantly, it was possible to measure significant increases of DIN and DIP and compute a mean rate of release for all species of DIN and DIP during a 30 min incubation.

At the organismal level *Ircinia felix* and *I. strobilina* were significant sources of DIN and DIP regardless of the normalizing biomass parameter. Statistically, either way of normalizing the release of DIN and DIP to biomass resulted in the same interpretation. Researchers typically normalize release of macronutrients to AFDW, which is impossible to extrapolate to an ecological scale without destroying the habitat. By normalizing the release rates of DIN and DIP to the number of oscula (i.e., pumping units) a nondestructive and ecologically relevant rate of release is easily obtained. Number of oscula on a sponge can easily and reliably be obtained using nondestructive methods, such as video or photographic surveys. Furthermore, by presenting rates of release normalized to AFDW and osculum it is easier to compare to other fluxes from a variety of sources. However,  $r^2$  values for the rates of release  $\text{osculum}^{-1}$  for all species of DIN and DIP were higher than  $r^2$  values for the rates of release  $\text{g}^{-1}$  AFDW in *I. felix*, which suggests that the number of oscula may be a better predictor of release rates. Values of  $r^2$  for *I. strobilina* were better for ammonium when normalized to oscula and for nitrate and soluble reactive phosphate when normalized to  $\text{g}$  AFDW, which suggests different microbial processes in *I. strobilina* than *I. felix*. Further evidence that the microbial processes are different between these sponges is found

in the composition of DIN release. *Ircinia strobilina* released proportionately more DIN as nitrate than *I. felix*. These trends are consistent with the hypothesis that *I. strobilina* hosts nitrifying bacteria which were responsible for the conversion of ammonium to nitrate.

Although it was not within the scope of this study to determine the pathways of nitrogen release, it appears that the source of the net release of DIN and DIP from *Ircinia felix* and *I. strobilina* results from remineralization of organic matter by the sponge and subsequent transformations by microbial symbionts. Both of these sponges graze selectively on ultraplankton, feeding on heterotrophic bacteria, *Prochlorococcus*, *Synechococcus*-type cyanobacteria, and autotrophic eucaryotes < 10  $\mu\text{m}$ , obtaining an average of 10 and 9 mg C  $\text{d}^{-1} \text{m}^{-2}$  respectively during this study period (Pile 1996). Ammonium and soluble reactive phosphate are most likely remineralized from these organic sources and released by the sponge as metabolic byproducts. In turn, the ammonium released is less than that taken up by the cyanobacterial and heterotrophic bacterial symbionts and that by nitrifying bacteria to be used for nitrification. The release of nitrate and nitrite by *I. felix* most likely results from nitrification, as this sponge does not appear to be fixing nitrogen (I. Anderson, unpublished acetylene reduction data). The same is most likely true for *I. strobilina*.

Release of ammonium by *Ircinia felix* and *I. strobilina* contrasts with studies done on the sponges *Chondrilla nucula* and *Anthosigmella varians*, which showed that both species were a source of nitrate and nitrite and a sink for ammonium (Corredor et al. 1988). Rates of nitrate and nitrite release  $\text{g}^{-1}$  AFDW for *I. felix* and *I. strobilina* are one fifth of those of *C. nucula* and an order of magnitude greater than those of *A. varians* (Corredor et al. 1988). In addition, Schubauer (1988) determined that 5 species of coral reef sponges

were sources of DIN with some release of DIP; however, he did not normalize his rates of release to biomass and thus comparisons are impossible. Since coral reefs are generally found to have a high diversity of sponges (Wilkinson and Cheshire 1990), and there is no detectable pattern between the four species that have been examined in their function as net sinks or sources, further studies are merited to examine how the total sponge community may be affecting DIN and DIP over the reef.

Organism-mediated fluxes of DIN and DIP have been examined for other reef organisms demonstrating that the reef community is comprised of organisms functioning as net sinks and sources. During daylight, corals with symbiotic zooxanthellae are net sinks for ammonium and nitrate while symbiotic corals at night and aposymbiotic corals are net sources (D'Elia and Webb 1977, Muscatine and D'Elia 1978). A similar trend is true for phosphorus, with symbiotic corals being a sink and aposymbiotic corals a source (D'Elia 1977, Atkinson 1987, Sorokin 1992). Aposymbiotic giant clam (*Tridacna gigas*) larvae and newly settled recruits release ammonium until acquisition of their symbionts when they then become a sink for DIN (Fitt et al. 1993). Haemulid fishes also release ammonium and phosphorus over the reef (Meyer and Schultz 1985a).

Locally, individuals release large amounts of DIN and DIP (Table 6) and may create a microenvironment within the reef of increased levels of macronutrients. Meyer and Schultz (1985b) found that grunts that school over corals at night released DIN and DIP which enhanced coral growth. This seems unusual, since coral uptake of ammonium and phosphate decreases during dark conditions (D'Elia 1977, Muscatine and D'Elia 1978). In contrast, sponges are releasing large amounts of nutrients as long as they are pumping.

*Ircinia strobilina* does not demonstrate a diel pattern in pumping rate, but *I. felix* decreases pumping activity at night (Pile unpublished data). The fact that these sponges are releasing macronutrients at rates comparable to those of the grunts, but during the day when uptake by organisms with phototrophic symbionts is greatest, is testimony to the importance of these processes to the coral reef ecosystem.

At the community level these two sponges are also a significant source of DIN and DIP to Conch Reef (Table 7). Release rates of nitrate+nitrite are 25% of those reported for *Chondrilla nucula* (Corredor et al. 1988), but this is due to the greater biomass of *C. nucula*, which is 14 times greater than that of *Ircinia felix* and *I. strobilina* at Conch Reef. This clearly demonstrates the importance of community structure in the types and amount of DIN released over the reef. Release rates of ammonium by *I. strobilina* are comparable to those resulting from microbial transformations in the unconsolidated sediments within and surrounding coral reefs in the Caribbean and the Great Barrier Reef (Corredor and Morell 1985, Williams et al. 1985, Capone et al. 1992). Release rates for *I. felix* are comparable to those found in the sediments associated with the coral reefs of New Caledonia (Boucher et al. 1994) which are an order of magnitude greater than those of the Caribbean and Great Barrier Reef. This is remarkable considering that these two sponges comprise < 1% of the benthic surface area whereas sediment estimates are for 100% coverage. Sponges release ammonium at a rate one order of magnitude greater than release rates for zooplankton and microheterotrophs, the major sources of ammonium within the water column over coral reefs (Hopkinson et al. 1987, Bishop and Greenwood 1994).

Net rates of community release of DIN and DIP have been measured at Gray's Reef.

Table 7. Summary of fluxes of inorganic nitrogen and phosphorus  $\mu\text{mol day}^{-1} \text{m}^{-2}$  by coral reef organisms, reef sediments, and reef communities. Positive values represent release and negative values uptake relative to the water column.

Source	$\text{NH}_4^+$	$\text{NO}_3^-$	$\text{NO}_2^-$	$\text{NO}_3^- + \text{NO}_2^-$	Total DIN	$\text{PO}_4^{3-}$	Reference
<b>Organism</b>							
<b>Sponges</b>							
<i>Ircinia felix</i>	138	37	2	39	186	43	This study
<i>I. strobilina</i>	12	6	0	6	18	5	This study
<i>Chondrilla nucula</i>	-269			11520	11251		(Corredor et al. 1988)
<i>Anthosigmella varians</i>	-366			48	-318		(Corredor et al. 1988)
Haemulid fishes, <i>Porites</i> <sup>a</sup>					3845	246	(Meyer and Schultz 1985a)
<i>Acropora palamata</i> <sup>a</sup>					9584	630	(Meyer and Schultz 1985a)
Zooplankton	12						(Bishop and Greenwood)
Microheterotrophs	41						(Hopkinson et al. 1987)
<b>Sediment-Water Column</b>							
Mona Island, Puerto Rico <sup>b</sup>	20						(Corredor and Morell 1985)
Tague Bay, St. Croix	72						(Williams et al. 1985)
New Caledonia, mud	-176						(Boucher et al. 1994)
grey-sand	276						(Boucher et al. 1994)
white-sand	360						(Boucher et al. 1994)
Great Barrier Reef <sup>c</sup>	55						(Capone et al. 1992)

Reef Community						
Gray's Reef, low density	526		240	766	215	(Hopkinson et al. 1991)
medium density (day)	13700		4800	18500	1600	(Hopkinson et al. 1991)
medium density (night)	21500		6400	27900	2000	(Hopkinson et al. 1991)
Eniwetok Atoll, Transect II	2592	1590				(Webb et al. 1975)
Eniwetok Atoll, Transect III	-415	-708				(Webb et al. 1975)
Temperate estuaries, sediment <sup>d</sup>	22000	730	90	820	22820	-21000 (Fisher et al. 1982)

<sup>a</sup> Studies were conducted on fish schooling over *Porites* or *A. palamata*.

<sup>b</sup> Mean of 4 locations with a range of 0.7-0.96  $\mu\text{mol m}^{-2} \text{day}^{-1}$ .

<sup>c</sup> Mean of 4 locations with a range of 26-93  $\mu\text{mol m}^{-2} \text{day}^{-1}$ .

<sup>d</sup> Mean of 8 locations.

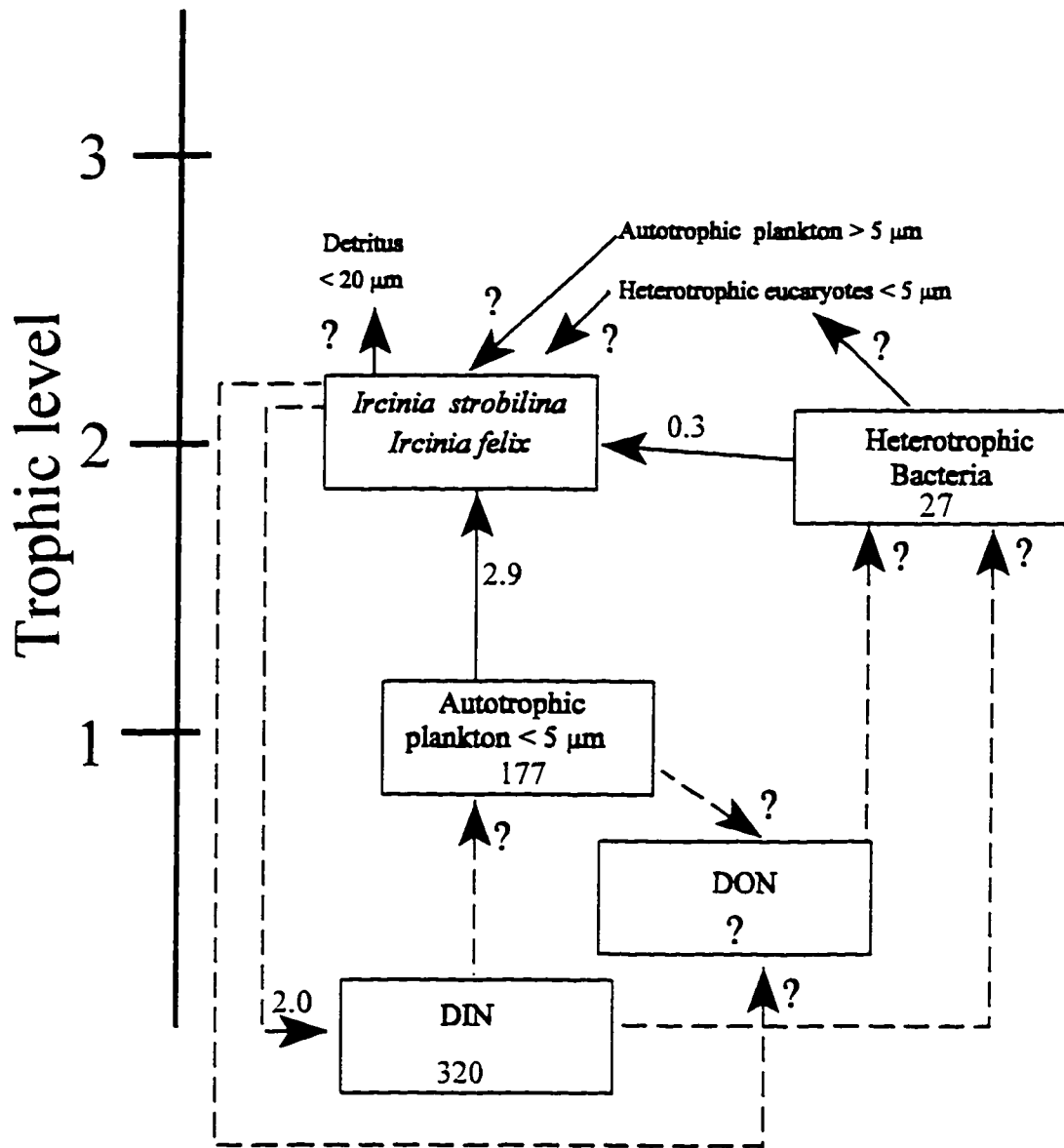
Georgia, USA (Hopkinson et al. 1991) and Enewetok Atoll (Webb et al. 1975) (Table 7); a proportion of this release probably resulted from sponges. Hopkinson et al. (1991) conducted *in situ* incubations by enclosing portions of the reef. They found a net release of DIN and DIP from the community comprised of algae, soft corals, sponges, other macroinvertebrates, and adjacent sediment. Sponge cover in the low density treatment at Gray's Reef was 0.8% and it was comparable to the sponge cover of *Ircinia felix* and *I. strobilina* at Conch Reef. If the total sponge community at Gray's Reef is releasing DIN and DIP at comparable rates to *I. felix* and *I. strobilina*, 70% of the net release could have resulted from sponges.

Grazing by *Ircinia felix* and *I. strobilina* on heterotrophic bacteria and autotrophic procaryotes and eucaryotes  $< 5 \mu\text{m}$  can support the rates of release of DIN (Figure 11). Sponges are an estimated net sink for  $3.2 \text{ mg N m}^{-2} \text{ day}^{-1}$  in the form of PON, 2% of the standing stock, while releasing  $2 \text{ mg N m}^{-2} \text{ day}^{-1}$  in the form of DIN. Therefore, ca. 30% of the total PON retained by the sponges from ultraplankton can be incorporated into growth or released as dissolved organic nitrogen (DON) and detritus. The assimilation efficiency of ultraplankton PON by sponges is not known. However, bivalves maintained on a diet of bacterioplankton have assimilation efficiencies near 50% (Langdon and Newell 1990, Werner and Hollibaugh 1993). These efficiencies are higher than suggested here, but all of the potential nitrogen sources have not been accounted for in this study and for the order of magnitude type of calculations employed this is a reasonable assimilation efficiency.

It is interesting to note that on a daily basis, the amount of DIN released by *Ircinia felix* and *I. strobilina*,  $2 \text{ mg N m}^{-2} \text{ day}^{-1}$ , is 1% of the DIN,  $320 \text{ mg N m}^{-2}$ , in the overlying

Figure 11. Schematic diagram of the daily flow of nitrogen between the water column and the sponges *Ircinia felix* and *I. strobilina* at Conch Reef, Florida Keys, USA in May of 1995. Arrows indicate the direction of the flow, with solid arrows representing particulate nitrogen and dashed arrows dissolved nitrogen. Values,  $\text{mg N m}^{-2}$ , are adjacent to each line and standing stocks are indicated in boxes for an integrated water column of 18 m. Ambient levels of DIN are for 18,000 l, the volume of water overlying 1  $\text{m}^2$  of benthos. Question marks are for a hypothesized flow that has not been quantified. Position in the vertical indicates trophic level. Note that sponges are a sink for heterotrophic and autotrophic ultraplankton and a source of DIN which can support production by heterotrophic and autotrophic ultraplankton. *Ircinia felix* and *I. strobilina* have a mean trophic level of 2.08 indicating that they are members of the guild of primary consumers of this microbial food web.





water column at Conch Reef. It is unrealistic to expect the benthos to affect the entire 18 m water column, and employing an integrated water column of 1 m, or near bottom water, release of DIN by *I. felix* and *I. strobilina* is 15% of the DIN available. An alternative way of looking at how the amount of DIN being released could effect the benthos is to estimate the concentrations of DIN above the benthos. A useful concept for making these estimates is fill time (Dinnel and Wiseman 1986).

Fill time is the amount of time it takes to change a water column property in a volume of water. They are useful for understanding how inputs maybe affecting an ecosystem and can provide perspective on the magnitude of inputs. However, they are limited since they assume that there is no loss of the material entering the volume of water and homogenous mixing of the volume of water. Fill time,  $T$ , for DIN at Conch Reef was determined after Dinnel and Wiseman (1986) where:

$$V(t) = \int_{t-T(t)}^t (R + P - E) dt \quad (5)$$

where  $V(t)$  is the ambient concentration of DIN,  $R$  is the sponge-mediated flux of DIN,  $P$  is precipitation, and  $E$  is evaporation. Precipitation and evaporation are zero so the equation can be rewritten as:

$$V(t) = \int_{t-T(t)}^t R dt \quad (6)$$

and solved to determine the height of a volume of water, with a base of one m<sup>2</sup>, at which DIN concentrations were doubled. Fill time of DIN estimates that water column DIN levels could be doubled within 10 cm of the bottom as a result of *I. felix*- and *I. strobilina*-mediated fluxes. It may be hypothesized that the benthic community is blanketed by a water mass that is dramatically different than the remainder of the water column. Ultimately mixing near the benthos will determine if the DIN and DIP released is utilized by the benthic or water column community.

More importantly, there is a direct transfer of PON in the form of heterotrophic bacteria, *Prochlorococcus*, *Synechococcus*-type cyanobacteria, and picoeucaryotes from the water column to the benthos and a subsequent remineralization and release of PON to DIN.

These two sponges are clearly members of the guild of primary consumers within a microbial food web since a majority of their diet is autotrophic plankton < 5 µm (Azam et al. 1983, Sherr and Sherr 1991). A good descriptor an organisms trophic position within a microbial food web is the mean trophic level. Mean trophic levels for *I. felix* and *I. strobilina* were determined following Baird and Ulanowicz (1989) with values from Figure 11 and are 2.07 and 2.08 respectively. Further, the grazing activity of these two sponges results in the release of dissolved inorganic and organic material that can support production of heterotrophic and autotrophic ultraplankton thereby helping to close the microbial loop (Azam et al. 1983).

The cycling of DIN and DIP within coral reefs involves a complex suite of processes from the organismal to the community level. These processes will vary from reef to reef and are dependent on the community structure and physical environment of the reef. Flow over

a coral reef can greatly affect the rates of metabolism and photosynthesis of corals (Patterson et al. 1991) and photosynthesis in algae (Carpenter et al. 1991) with concurrent effects on the uptake and release of macronutrients by the benthic community (Carpenter et al. 1991, Atkinson and Bilger 1992, Bilger and Atkinson 1995). Additionally, physical events such as internal waves (Wolanski and Delesalle 1995), tidal bores (Leichter et al. 1996), and geothermal endo-upwelling (Rougerie et al. 1992) can enhance DIN and DIP levels over reefs and the role that these events play in the cycling of nutrients is only beginning to be examined. Clearly the microbial food web is an important source of exogenous nitrogen to Conch Reef and most likely other coral reefs. Further studies are required to determine the full extent of the role of the microbial food webs as sources of exogenous materials via benthic-pelagic coupling not only on coral reefs but in other aquatic environments.

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## Chapter V

The incorporation of macroinvertebrates  
into the microbial food web paradigm

### ABSTRACT

The current paradigm of microbial food webs postulates that a majority of the primary production in freshwater and marine ecosystems is shunted away from traditional linear food chains through a complex web that incorporates multiple trophic links between heterotrophic and autotrophic plankton less than 5  $\mu\text{m}$ , small non-pigmented flagellates and ciliates, and large protozoans. There is compelling new evidence that benthic and pelagic macroinvertebrates can be primary consumers within microbial food webs. Some freshwater and marine macroinvertebrates, that can dominate the benthic biomass in some systems, feed primarily on plankton less than 5  $\mu\text{m}$  and in return release dissolved organic and inorganic material which supports primary production by heterotrophic and autotrophic plankton less than 5  $\mu\text{m}$ . This often over looked microbial-macroinvertebrate trophic link is responsible for a significant transfer of carbon from the water-column communities to the macrobenthos in some shallow water ecosystems.

## INTRODUCTION: SCOPE OF THE REVIEW AND DEFINITIONS

Research on benthic-pelagic coupling has concentrated on the interactions between organisms that generally feed on plankton greater than 10  $\mu\text{m}$  (Graf 1992). This has resulted in an incomplete view since, except for periods when diatom blooms are present, a majority of the pelagic primary and secondary production in freshwater and marine ecosystems is from autotrophic and heterotrophic plankton less than 5  $\mu\text{m}$  (ultraplankton) (Stockner and Antia 1986, Stockner 1988). It is unlikely that some suspension-feeding macroinvertebrates would not utilize the most abundant food source in aquatic ecosystems and while the direct evidence is limited, in theory there are a variety of organisms that have the capability to remove ultraplankton from the water that they process (Rubenstein and Koehl 1977, Shimeta and Jumars 1991).

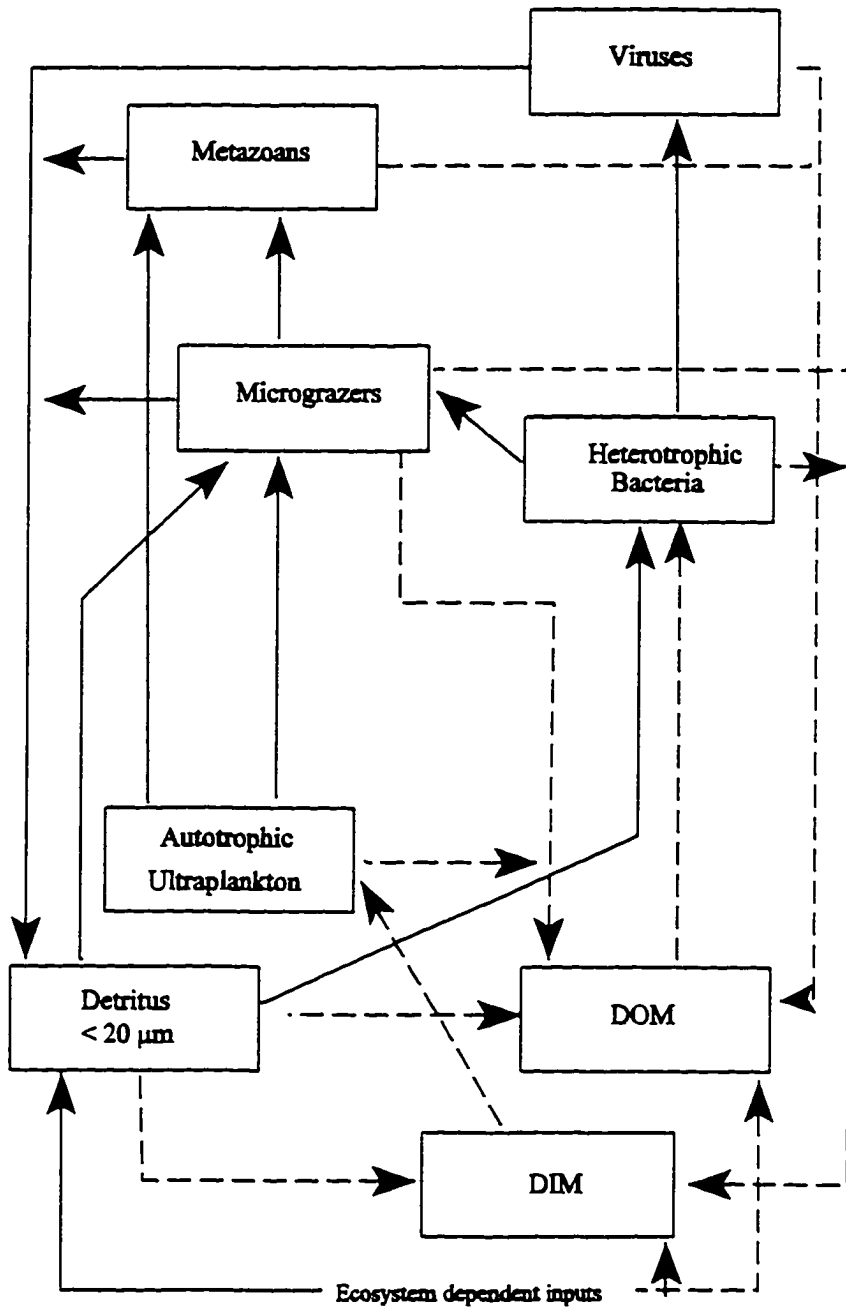
There is strong evidence to suggest that ultraplankton is an important food source for benthic macroinvertebrates. Reiswig (1971) found that a majority of the particulate organic carbon retained by Caribbean coral reef sponges was unresolvable using the light microscopic techniques available at the time. He hypothesized that the unresolvable particulate organic carbon (URPOC) was plankton that could not be quantified using the techniques available at the time of the study. More recently, Ayukai (1995) found up to 90% retention of microbial plankton by Pacific coral reefs and hypothesized that this was the result of grazing by the benthic community. Other researchers have found that growth rates of heterotrophic bacteria are higher than necessary to meet the grazing pressures of micrograzers (heterotrophic nanoflagellates and ciliates less than 20  $\mu\text{m}$ ) on coral reefs (Moriarty 1979, Moriarty et al. 1985, Linley and Koop 1986) and in salt marshes (Sherr, B.

F. et al. 1986, 1989) and speculated that grazing of bacterioplankton by the benthic community is a major contributing factor to plankton community dynamics. The largely unexplored role of macroinvertebrate grazing on ultraplankton has further consequences as the microbial to macroinvertebrate trophic link has been excluded from microbial food web paradigm (Azam et al. 1983, Sherr, E. B. and Sherr 1991).

During the past decade research on the water-column community of ultraplankton has resulted in an understanding of a complex network of trophic interactions between procaryotic and small eucaryotic plankton, the microbial food web (Azam et al. 1983, Sherr, E. B. and Sherr 1991). In the euphotic zone of the water column, the microbial food web theoretically shunts a significant fraction of the primary production away from the traditional linear food chain, where each consumer occupies a single trophic level, into a microbial food web that incorporates multiple trophic links between heterotrophic and autotrophic ultraplankton, small non-pigmented flagellates and ciliates, and larger protozoans (Figure 12). The microbial food web is based on pools of dissolved organic material (DOM) and inorganic material (DIM) that nutritionally support primary production by autotrophic ultraplankton (photosynthetic procaryotes and eucaryotes less than 5  $\mu\text{m}$ ) and secondary production by heterotrophic bacteria. The primary consumers of the microbial food web graze on ultraplankton and heterotrophic bacteria which results in a release of DOM and DIM. Primary consumers may be thought of as a functional group of micrograzers that is currently considered to consist of heterotrophic nanoflagellates and ciliates. The secondary consumers of a microbial food web, currently consisting of metazoans, typically feed primarily on the primary consumers and the larger size fraction of autotrophic ultraplankton



Figure 12. Schematic diagram depicting the flow, as indicated by the direction of the arrows, of particulate (solid lines) and dissolved (dashed lines) materials through a microbial food web in the euphotic zone. Adapted from Azam et al. (1983) and Sherr & Sherr (1991). Relative trophic level is indicated by position in the vertical.



which results in the release of DOM and DIM which can support production of ultraplankton. More recently researchers have incorporated viruses (Murray and Eldridge 1994) and microscopic detritus (Posch and Arndt 1996) into microbial food webs has added additional levels of complexity to the food web. Pelagic and benthic macroinvertebrates have not been incorporated into the current models of microbial food webs due to a lack of direct evidence as to their function and position.

As previously described, an organism belongs to the guild of primary consumers in a microbial food web if it grazes primarily on ultraplankton. The subsequent release of DOM and DIN that results from grazing activity is important as it serves to help close the microbial loop by supporting production of ultraplankton. Current models of microbial food webs have excluded macroinvertebrates from the guild of primary consumers. There is compelling new evidence from freshwater and marine ecosystems that certain macroinvertebrates do occupy this niche. This chapter presents a brief overview of the evidence that in some ecosystems the microbial food web is an overlooked exogenous source of carbon for the benthic community. In such communities, grazing by the benthos can significantly reduce ultraplankton communities. Further, the inclusion of benthic grazing on bacterioplankton into models of the flow of materials within shallow ecosystems can balance the production of bacterioplankton with community grazing demands.

#### *Definition of terms*

The terminology used to describe plankton, suspension feeding, and trophic levels within microbial food webs can be confusing. Researchers have employed the same terms

interchangeably to describe plankton or the same term for different processes in suspension feeding. It is beyond the scope of this review to redefine plankton, the suspension feeding process, or trophic levels within microbial food webs but it is desirable to present the information using a consistent language. Therefore, conventional terms from the most recent literature will be employed and care has been taken to summarize carefully the studies reported using these standardized terms.

Microbial plankton includes free living heterotrophic bacteria, autotrophic prokaryotes, heterotrophic microflagellates, ciliates, and autotrophic eucaryotes  $< 20 \mu\text{m}$ . Populations of autotrophic eucaryotes ranging in size from  $0.2$  to  $5 \mu\text{m}$  will be referred to as picoeucaryotes, unless the species of plankton has been identified (Stockner and Antia 1986). Ultraplankton is defined as organisms less than  $5 \mu\text{m}$  and consists of heterotrophic bacteria, the phototrophic prokaryotes (*Prochlorococcus* sp. and cyanobacteria), and picoeucaryotes (Murphy and Haugen 1985).

Suspension feeding is a broad term used to describe the process of grazing on suspended material, usually plankton but sometimes including detritus. It is frequently used interchangeably with filter feeding which is capture of particles with a filtering mechanism. Filter feeding can be passive, in that the filtering apparatus is exposed to the environment for particle capture, or active, in which the organism creates a current to draw water across a filtering mechanism. In this review, the broad term suspension feeding will be employed and further distinguished as either active or passive. Active suspension feeders usually employ pumping by the organism and the volume processed is a function of pumping rate which is expressed as volume per unit time (e. g.,  $\text{ml sec}^{-1}$ ).

Trophic position within a food web can be determined as a function of the trophic levels at which an organism feeds. Trophic positions are easily identified in linear chains; primary producers occupy the first trophic level, herbivores the second, the third trophic level are the carnivores, etc. (Lindeman 1942). But most heterotrophic species are omnivores, and simultaneously occupy multiple trophic levels. Hence, a mean trophic level is a useful descriptor of the position of an omnivorous heterotrophic organism within a food web. The mean trophic level is calculated as the sum of the proportion of the diet at each trophic level (Levine 1980). For example, if 60% of an organism's diet comes from the second trophic level ( $0.6 \times 2$ ), and 40% from the third trophic level ( $0.4 \times 3$ ) then the organism's trophic level is 2.4. These noninteger values are more representative of the feeding modes of organisms within food webs (Baird and Ulanowicz 1989). However, they make the use of conventional terminology such as primary and secondary consumer more difficult.

It can be argued that the aforementioned hypothetical organism with a trophic level of 2.4 is a primary consumer. Consider that noninteger trophic levels  $\pm 0.5$  of a whole number trophic level represent feeding a majority of the time at the integer value. Therefore, within food webs organisms that have trophic levels ranging from 1.5-2.49 are members of the guild of primary consumers. Secondary consumers have trophic levels ranging from 2.5-3.49. The primary producers within microbial food webs (trophic level=1) are autotrophic ultraplankton less than 5  $\mu\text{m}$ . Heterotrophic bacteria are at the second trophic level. Clearly, in most ecosystems food webs extend farther than primary and secondary consumers but since the focus of this chapter is to demonstrate that macroinvertebrates occupy the level of primary consumer and that microbial food webs are an important source

of exogenous material to benthic communities conceptualized flows of materials are limited to the components of the microbial food web.

### **TECHNIQUES USED TO QUANTIFY SUSPENSION FEEDING IN MACROINVERTEBRATES**

Researchers have had limited success in identifying the diet of macroinvertebrates that feed on ultraplankton. Concurrently, the quantification of the grazing pressure of macroinvertebrates on ultraplankton has generally been limited to indirect evidence or hypotheses. This is because the techniques that allow researchers to quantify the feeding ecology of macroinvertebrates on ultraplankton directly have only recently become available.

Currently there are two methods that can identify all types of ultraplankton, dual-beam flow cytometry and epifluorescence microscopy coupled with computer image analysis. Flow cytometry has been used by biological oceanographers to quantify heterotrophic and autotrophic ultraplankton for the past 10 years (Campbell et al. 1994). The more traditional technique of epifluorescence microscopy can also quantify all types of ultraplankton if coupled with the power of computer image analysis (H. Ducklow personal communication). Since flow cytometry requires only 1 ml of sample and is more accurate and faster than all other techniques currently used to quantify ultraplankton (Karl 1994) it is better suited for the study of macroinvertebrate grazing of ultraplankton. Dual-beam flow cytometry has been successfully employed to quantify grazing on ultraplankton by both freshwater and marine macroinvertebrates and was instrumental in the first quantification of the feeding ecology of freshwater and marine macroinvertebrates that graze on

*Synechococcus*-type cyanobacteria and *Prochlorococcus* (Pile et al. 1996a, b). It is a powerful tool that has only recently been utilized by benthic ecologists and further application will undoubtedly provide additional insight into the dynamics of carbon flow in aquatic ecosystems.

Suspension feeding by macroinvertebrates has traditionally been quantified at the organismal level in the laboratory with indirect measurements of food depletion in closed volumes (i.e. clearance rates), as opposed to direct measurements that compare cell concentrations of ambient water to water from the exhalent current (Stuart and Klumpp 1984). There have been numerous studies on a variety of organisms that have measured indirectly the retention of ultraplankton both *in situ* and in the laboratory. Typically, researchers measured the concentrations of monocultures of bacteria and algae (e.g., Stuart and Klumpp 1984), radiolabeled bacteria (Sorkin 1973), or natural ultraplankton communities (Harbison and Gilmer 1976, Alldredge 1981, Wright et al. 1982, Kemp et al. 1990, Lesser et al. 1992, Werner and Hollibaugh 1993) over time, and clearance rates were calculated as a function of the change in cell concentration in the container over time. These types of studies were very important as they suggested that macroinvertebrates, such as salps (Alldredge and Madin 1982, Michaels and Silver 1983), could substantially reduce ultraplankton communities in the water that they processed and inspired researchers to develop methods that could directly quantify suspension feeding of ultraplankton by macroinvertebrates.

Direct measurements of suspension feeding compare the concentrations of ultraplankton in ambient water to those in water processed by the organism, with that being

retained considered eaten. Water processed is easily collected in active suspension feeders from the exhalent current. Direct measurements of feeding by passive suspension feeders is quantified by comparing cell concentrations in water that is collected before and after traversing the organism or community. Direct measurements of suspension feeding by macroinvertebrates on ultraplankton are much less common (Reiswig 1971, 1975, Stuart and Klumpp 1984, Fabricius et al. 1995, Pile 1996, Pile et al. 1996a, b) and have been conducted *in situ* as well as in the laboratory. Direct measurements of suspension feeding are preferred since, unlike indirect measurements of feeding, direct measurements of grazing can be combined with direct measurements of the amount of water processed by suspension feeders and abundance to calculate suspension feeder community-mediated fluxes of ultraplankton (Pile et al. 1996a). Also, community-mediated fluxes can be determined by comparing concentrations of ultraplankton in water as it flows past a community of active or passive suspension feeders (Wright et al. 1982). There is only one study that examined suspension feeding on bacterioplankton with this method and found no retention of heterotrophic bacteria by a bed of blue mussels, *Mytilus edulis* (Wright et al. 1982). Quantification of macroinvertebrate-mediated fluxes of ultraplankton are essential for determining the role and function of macroinvertebrates within microbial food webs and have only just begun to be examined (Stuart and Klumpp 1984, Pile et al. 1996a).

### **MACROINVERTEBRATES AS SINKS FOR ULTRAPLANKTON**

Surprisingly little information exists describing feeding by benthic macroinvertebrates on ultraplankton. The first direct measurements of macroinvertebrates



grazing on ultraplankton were *in situ* studies performed by Reiswig in the late 1960's on three species of tropical sponges. He found that ultraplankton could meet the metabolic requirements of these sponges (Reiswig 1971). Since this pioneering work, a majority of the research that has directly quantified retention of ultraplankton has been done in the laboratory with particles or pure cultures, followed by laboratory studies with natural plankton assemblages, and then *in situ* studies. Organisms from many phyla, including Porifera, Cnidaria, Mollusca, and Annelida, retain ultraplankton and are often common, dominant species in benthic communities (Table 8).

There are numerous studies where the retention of ultraplankton by macroinvertebrates was measured indirectly under carefully monitored environmental conditions. The Atlantic ribbed mussel *Geukensia demissa*, a ubiquitous component of the salt marshes of the east coast of the United States, readily removes natural bacterioplankton from the water that it filters (Wright et al. 1982). An additional study provides evidence that ribbed mussels can retain *Synechococcus*-type cyanobacteria (Kemp et al. 1990). Unfortunately the lack of replication during the study (n=1) prevents the inclusion of *Synechococcus*-type cyanobacteria in the known diet of Atlantic ribbed mussels until more studies are completed. Pelagic macroinvertebrates have also been found to retain ultraplankton. The salps *Cyclosalpa floridana*, *C. affinis*, and *C. polae* all retain ultraplankton (Harbison and McAlister 1979). A majority of the particles found on the filtering apparatus of the appendicularians *Oikopleura dioica* and *Stegasoma magnum* are bacterioplankton and ultraplankton (Alldredge 1981) and the pelagic tunicate *Pegae confederata* can retain *Synechococcus*-type cyanobacteria (Harbison and Gilmer 1976).

Table 8. Summary of retention efficiencies of benthic macroinvertebrates on ultraplankton from freshwater (F) or marine (M) ecosystems (E). Direct measurements were conducted (C) either *in situ* (I) or in the laboratory (L). Negative values indicate retention and positive values are release of cells.

Organism	E	C	Heterotrophic bacteria	<i>Prochlorococcus</i>	Cyanobacteria	Picoeucaryotes	Ultraplankton	Source
<b>Sponges</b>								
<i>Mycale sp.</i>	M	I					-98% <sup>a</sup>	Reiswig 1971
<i>Yerongia gigantea</i>	M	I					-98% <sup>a</sup>	Reiswig 1971
<i>Tethya crypta</i>	M	I					-98% <sup>a</sup>	Reiswig 1971
<i>Haliclona permollis</i>	M	L	-77%					Reiswig 1975
<i>H. anonyma</i>	M	L	-85%				-99% <sup>b</sup>	Stuart and Klumpp 1984
<i>Baikalospongia bacillifera</i>	F	I	-84%	n/a	-66%		-99%	Pile et al. 1996a
<i>B. intermedia</i>	F	I	-71%	n/a	-58%		280%	Pile et al. 1996a
<i>Mycale lingua</i>	M	I	-74%	-93%	-89%		-72%	Pile et al. 1996b
<i>Ircinia felix</i>	M	I	-30%	-26%	-48%		91%	Pile 1996
<i>I. strobilina</i>	M	I	-56%	-52%	-53%		38%	Pile 1996
<b>Soft Corals</b>								
<i>Dendronephthya hemprichii</i>	M	I		retained			-1.7-4.5% <sup>c</sup>	Fabricius et al. 1995

<b>Polychaetes</b>						
<i>Sabella penicillus</i>	M	1.			> -90% <sup>d</sup>	Jørgensen et al. 1984
<i>Chaetopterus variopedatus</i>	M	1.	-30%		> -90% <sup>d</sup>	Jørgensen et al. 1984
<b>Bivalves</b>						
<i>Monia squama</i>	M	1.			> -90% <sup>d</sup>	Jørgensen et al. 1984
<i>Cardium glaucum</i>	M	1.			> -90% <sup>d</sup>	Jørgensen et al. 1984
<i>Petricola phaeoladiformis</i>	M	1.			> -90% <sup>d</sup>	Jørgensen et al. 1984
<i>Dreissema polymorpha</i>	F	1.			> -90% <sup>d</sup>	Jørgensen et al. 1984
<i>Unio pictarum</i>	F	1.			> -90% <sup>d</sup>	Jørgensen et al. 1984
<i>Anodota cygnea</i>	F	1.			> -90% <sup>d</sup>	Jørgensen et al. 1984
<i>Choromytilus meridionalis</i>	M	1.	-42%	-94% <sup>b</sup>		Stuart and Klumpp 1984
<b>Ascidians</b>						
<i>Ascidella aspersa</i>	M	1.			-70-99% <sup>c</sup>	Randløv and Riisgård 1979
<i>Molgula manhattensis</i>	M	1.			-70-99% <sup>c</sup>	Randløv and Riisgård 1979
<i>Clavelina lepadiformis</i>	M	1.			-100% <sup>c</sup>	Randløv and Riisgård 1979
<i>Ciona intestinalis</i>	M	1.			-100% <sup>c</sup>	Randløv and Riisgård 1979
<i>Ascidia virginia</i>	M	1.			> -90% <sup>d</sup>	Jørgensen et al. 1984

<i>A. obliqua</i>	M	L.		> -90% <sup>d</sup>	Jørgensen et al. 1984
<i>A. metula</i>	M	L.		> -90% <sup>d</sup>	Jørgensen et al. 1984
<i>Stylela clava</i>	M	L.		> -90% <sup>d</sup>	Jørgensen et al. 1984
<i>Pyura stolonifera</i>	M	L.	-94%	-99% <sup>b</sup>	Stuart and Klumpp 1984

n/a = not applicable, *Prochlorococcus* has not been found in freshwater ecosystems.

<sup>a</sup>Studies were conducted prior to the discovery of cyanobacteria and prochlorophytes and ultraplankton is the combination of Reisswig's categories of bacteria, unarmored cells, and unresolvable particulate organic carbon (URPOC).

<sup>b</sup>Measurements employed monocultures of bacteria and picoeucaryotes of different sizes.

<sup>c</sup>Measurements determined plankton retention. A majority of the plankton retained determined by examining gut contents as picoeucaryotes 1-3 μm while cyanobacteria was retained it was not quantified. Retention is flow dependent.

<sup>d</sup>Measurements were conducted using natural plankton assemblages to quantify the size of particles at which a 90% retention efficiency was attained and did not quantify the composition of the plankton community.

<sup>e</sup>Measurements were conducted using natural plankton assemblages and the size of the particles retained, not the type of plankton, determined.

Pelagic larvae of macroinvertebrates also consume ultraplankton. Larvae of the Northern quahog, *Mercenaria mercenaria* (Gallager et al. 1994) can graze on and assimilate *Synechococcus*-type cyanobacteria and the pelagic larvae of crown of thorns starfish, *Acanthaster planci*, readily utilize *Synechococcus*-type cyanobacteria and picoeucaryotes (Ayukai 1994). Pelagic larvae and newly settled juveniles of the blue mussel, *Mytilus edulis*, retain ultraplankton with the highest efficiencies as compared to other plankton components in natural water (Riisgard et al. 1980). In freshwater ecosystems, the larvae of the mosquito, *Anopheles quadrimaculatus*, preferentially consume ultraplankton (Merritt et al. 1992).

Retention does not prove that assimilation occurs in these suspension feeders, but retention does remove the ultraplankton from the water column community and consequently it is no longer available for consumption by other grazers. A majority of the carbon consumed by macroinvertebrates is quickly respired and released as inorganic carbon from the benthos. Therefore, the flow of carbon from the water column to the benthic community may constitute an important flux in providing metabolic maintenance of benthic communities dominated by macroinvertebrates that graze on ultraplankton.

#### **SUSPENSION FEEDING MACROINVERTEBRATES AS SOURCES OF DISSOLVED ORGANIC AND INORGANIC MATERIAL**

Originally, the closure of the microbial loop relied primarily on the leakage of dissolved organic carbon (DOC) from phytoplankton cells and the release of dissolved organic and inorganic nitrogen (DON and DIN respectively) by micrograzers (Azam et al. 1983). DOC leakage rates from picoeucaryotes are typically between 10 and 25% of primary

production (Wood et al. 1992). More recently, Jumars et al. (Jumars et al. 1989) concluded that DOC released as a result of incomplete ingestion, digestion, and absorption by animals can theoretically contribute significantly to bacterial nutrition. However, this route of assimilation has yet to be quantified as it is most likely occurring over very short time scales (less than 5 min). Until recently, the technical difficulty of quantifying DOC in natural water samples has prevented the measurement of DOC release by macroinvertebrates. However, one study found that bacterial doubling times in the laboratory are significantly shorter when cultured in a medium enriched with the dissolved materials (DOC, DON, and DIN) released by the blue mussel, *Mytilus edulis* (Tupas and Koike 1990). This suggests that DIM and DOM released by macroinvertebrates is in a form that is readily usable by bacterioplankton.

DIN and DON are also required by ultraplankton and necessary for closure of the microbial loop. In marine ecosystems, nitrogen is considered often to be the limiting nutrient and heterotrophic bacteria and phototrophic ultraplankton may compete intensively for DIN and DON (e. g., Wheeler and Kirchman 1986, Gilbert 1993, Kirchman 1994). DIN (ammonium, nitrate, and nitrite) and DON (usually amino acids) are released by macroinvertebrates as metabolic byproducts (e. g., Hammen 1968). At the organismal level, measurements of DIN and DON release by macroinvertebrates have been conducted indirectly as current analytical techniques require too large of a sample to be accurately collected from exhalent currents for direct measurements. Of the organisms that have been found to graze on ultraplankton through direct measurements (Table 8), only five have also had DIN or DON release quantified and all have been found to release significant amounts of DIN into the environment (Table 9). Release of DON by the Atlantic ribbed mussel,

Table 9. The rates of release of dissolved inorganic nitrogen (DIN) by macroinvertebrates that feed primarily on ultraplankton. DIN can result from remineralization (R) or nitrification by endosymbionts (N).

Organism	DIN	Process	Source
Sponges			
<i>Ircinia felix</i> <sup>a</sup>	15 $\mu\text{g DIN hr}^{-1}$	R, N	Pile in preparation
<i>I. strobilina</i> <sup>a</sup>	1 $\mu\text{g DIN hr}^{-1}$	R, N	Pile in preparation
Bivalves			
<i>Geukensia demissa</i> <sup>b</sup>	42 $\mu\text{g NH}_4\text{-N hr}^{-1}$	R	Jordan and Valiela 1982
Ascidians			
<i>Ciona intestinalis</i> <sup>b</sup>	192 $\mu\text{g NH}_4\text{-N hr}^{-1}$	R	Markus and Lambert 1983
<i>Stylela clava</i> <sup>b</sup>	64 $\mu\text{g NH}_4\text{-N hr}^{-1}$	R	Markus and Lambert 1983

<sup>a</sup>  $\text{g}^{-1}$  AFDW.

<sup>b</sup>  $\text{g}^{-1}$  dry weight.

*Geukensia demissa*, was not detected during three hour *in situ* incubations (Jordan and Valiela 1982) but was found during longer laboratory incubations (Hammen 1968). The lack of detection during the *in situ* incubation could have been due to the immediate uptake of DON by water column communities or epibionts of the mussels. Ultimately, turbulent mixing near the benthos will determine if the DIN is utilized by benthic community or available to the water column community for closure of the microbial loop.

The contribution of benthic community mediated fluxes of DIN and DON have been empirically calculated from measurements made at the organismal level and indirectly measured with *in situ* enclosures of the benthos. Coral reef sponges have been found to be a significant source of ammonium and nitrate, with sponge-mediated fluxes being an order of magnitude greater than those of unconsolidated sediments (Corredor et al. 1988, Capone et al. 1992, Pile in preparation). A temperate reef community composed of sponges, soft corals, and other macroinvertebrates which can graze on ultraplankton released significant amounts of DIN and DON that resulted in their net export from the reef (Hopkinson et al. 1991). The Atlantic ribbed mussel, a biomass dominant in salt marshes along the eastern United States, is responsible for 31% of the ammonium flux in a salt marsh (Jordan and Valiela 1982). The net exports of organism-mediated fluxes of DIN and DON from benthic communities make the DIN and DON available to the water column community and thus help close the microbial loop.

Dissolved inorganic phosphorus (DIP) is considered the primary limiting nutrient for ultraplankton in freshwater ecosystems and parts of some estuaries (Kirchman 1994). Releases of DIN, DON, DIP and dissolved organic phosphorus (DOP) by freshwater



macroinvertebrates have yet to be quantified but if they are comparable to the fluxes found in marine macroinvertebrates they may contribute significantly to the nutrition of the ultraplankton community. Marine macroinvertebrates that graze on ultraplankton release significant amounts of DIP as a result of remineralization of organic matter (Pile in preparation). Benthic macroinvertebrate-mediated fluxes of DOP and DIP can result in the net export of DOP and DIP from a temperate reef (Hopkinson et al. 1991). Overall, the contribution of macroinvertebrate-mediated fluxes of DOM and DIM to the nutrition of ultraplankton is unresolved and should be a research priority.

#### **EXAMPLES OF MICROBIAL FOOD WEBS IN BENTHIC-PELAGIC COUPLING**

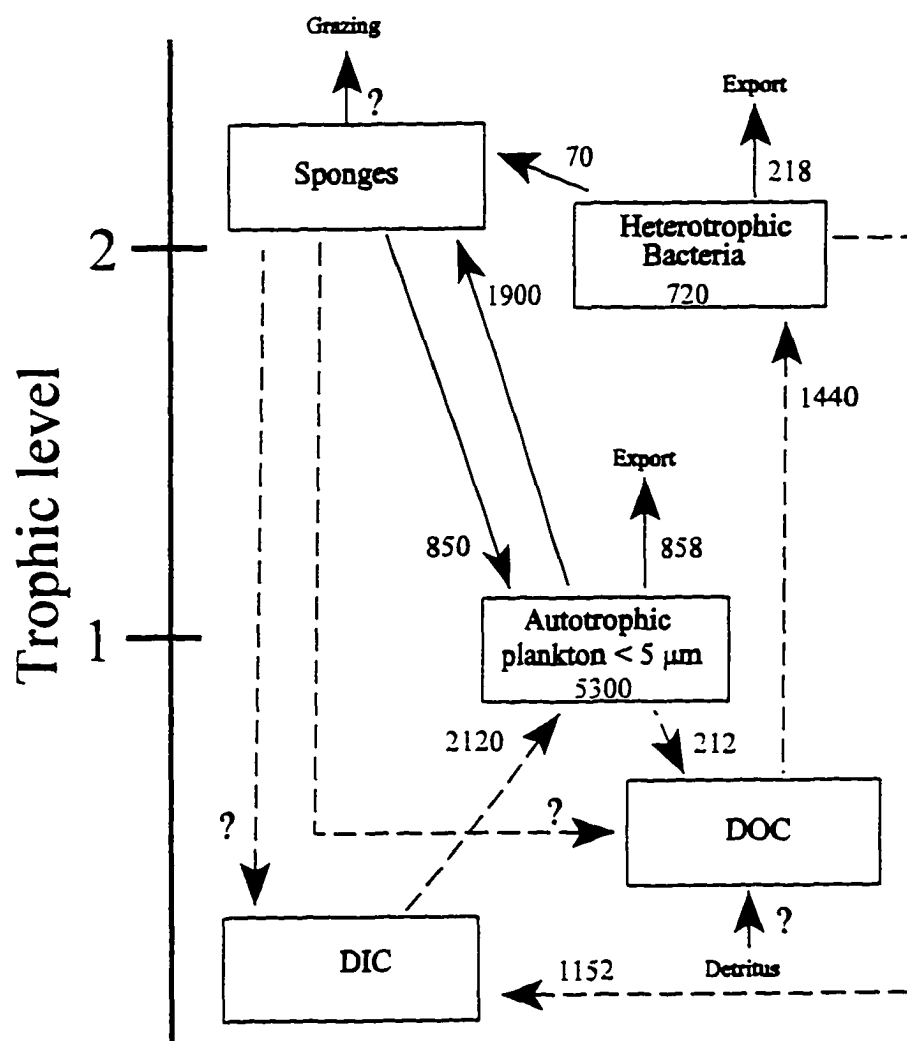
Many benthic communities are dominated by macroinvertebrates that are potential members of the guild of primary consumers within the microbial food web. However, the role of the microbial food web in benthic-pelagic coupling has historically been overlooked because the role of microbial food webs as a source of carbon for the macrobenthos and the concurrent impact of the benthos has been considered minimal in marine ecosystems (Azam et al. 1983, Ducklow 1990). More recently direct evidence from two ecosystems: Lake Baikal, Siberia, Russia, and the salt marshes surrounding Sapelo Island, Georgia, USA, indicate that the incorporation of microbial food webs in models of benthic-pelagic coupling results in the transfer of a significant amount of microbial biomass through the benthos.

### *Lake Baikal*

Lake Baikal is the world's oldest lake (ca. 25 million years) and contains over 1000 endemic species. The benthos of the shallow areas of Lake Baikal (mean depth 12 m) is dominated by three species of sponge, *Baikalospongia intermedia*, *B. bacillifera*, and *Lubomirskia baicalensis*, that cover over 54% of the benthic surface area (Pile et al. 1996a). As is typical of most lakes, the plankton community of Lake Baikal is dominated by ultraplankton; including heterotrophic bacteria; *Synechococcus*-type cyanobacteria and autotrophic picoeucaryotes, which contribute 80% of the total primary production within the water column (Nagata et al. 1994). The homogenous composition of a benthic community dominated by three sponge species that feed primarily on the highly abundant ultraplankton makes the littoral zone of Lake Baikal an excellent location for the *in situ* study of the role of microbial food webs in benthic-pelagic coupling.

Active suspension feeding by the extensive sponge community in Lake Baikal can significantly reduce ultraplankton near the bottom. Measurements taken at both the organismal and community level resulted in the first *in situ* evidence that freshwater macroinvertebrates graze on *Synechococcus*-type cyanobacteria as well as heterotrophic bacteria and picoeucaryotes (Pile et al. 1996a). At the organismal level retention efficiencies ranged between 58-99% of the ultraplankton  $\text{ml}^{-1}$  processed (Table 8) resulting in a trophic level of 2.01 (calculated from Figure 13). Therefore, sponges in the littoral zone of Lake Baikal are members of the guild of primary consumers. The suspension feeding activity of the benthos of the littoral zone is further evident by the formation of a food depleted layer ca. 1 m thick overlying the benthos. The water column within 1 m of the benthos had cell

Figure 13. Conceptualized daily flow of carbon between the water column and the benthos in the littoral zone of Lake Baikal, Siberia, Russia. Arrows indicate the direction of flow with dashed lines representing dissolved carbon and solid lines particulate carbon. Values,  $\text{mg C m}^{-2}$ , in boxes are standing stocks and near arrows are fluxes. Water column values represent an integrated water column of 12 m. Trophic level within the food web, as indicated by position in the vertical, was determined following Baird and Ulanowicz (Baird and Ulanowicz 1989). Unknown fluxes are indicated by ? next to an arrow.



concentrations nearly an order of magnitude less than the remainder of the water column (Pile et al. 1996a). Development of the food depleted layer is supported empirically by the boundary layer conditions present when the measurements were made (Savarese et al. 1996).

The flow of carbon between the benthos and water column can be conservatively estimated for the littoral zone of the lake (Figure 13). Calculations of carbon flow employed some general assumptions of ultraplankton dynamics when actual values for Lake Baikal were unavailable: carbon assimilation by heterotrophic bacteria is 20% (Hobbie and Crawford 1969) and DOC leakage from phytoplankton is 10% (Wood et al. 1992). Sponges serve as a sink for  $70 \text{ mg C day}^{-1} \text{ m}^{-2}$  in heterotrophic bacteria and  $1900 \text{ mg C day}^{-1} \text{ m}^{-2}$  in autotrophic ultraplankton (Pile et al. 1996a). The sponges removed nearly 25% of the production by bacteria,  $288 \text{ mg C m}^{-2}$  (growth rate  $\times$  standing stock; Nagata et al. 1994, Pile et al. 1996a), that occurs in an integrated water column 12 m deep. More importantly, they consume 10% of the standing stock of  $70 \text{ mg C day}^{-1} \text{ m}^{-2}$  out of  $720 \text{ mg C m}^{-2}$  available, which is equivalent to a water column ca 1 m high or the thickness of the food depleted layer. Sponges in the lake function as a net sink for autotrophic ultraplankton.  $1900 \text{ mg C day}^{-1} \text{ m}^{-2}$  are retained by sponges which is 90% of the production within the water column during a day. A majority of this is *Synechococcus*-type cyanobacteria (Nagata et al. 1994, Pile et al. 1996a). If we consider the carbon production of photosynthetic ultraplankton to be the sum of that from the water column and the benthos ( $2120 + 850$ ) then the sponges are consuming only 60% of the production per day in the shallow part of the lake.

Productivity of heterotrophic bacteria requires  $1440 \text{ mg C day}^{-1} \text{ m}^{-2}$  in the form of DOC. A small portion, 15%, is leakage of DOC from primary producers, and the remainder

most likely results from grazing activity and leaching of DOC from detrital material. Release of DOC by sponges has not been quantified but is highly likely. The contribution of sponges to the DOC and DIC pools is further complicated since the assimilation efficiency of carbon, as well as the release of DIC through respiration, is unknown for these sponges. Unfortunately this lack of information makes it impossible to estimate the flow of carbon through the sponges. However, it does not prevent closure of the microbial loop.

Closure of the microbial loop is unique in this system as the sponges release picoeucaryotes at a rate that actually results in the benthos being a net source of carbon in the form of picoeucaryotes (Pile et al. 1996a), the primary producers of the food web. It is not known whether the picoeucaryotes being released by the sponges are viable organisms that would contribute to the biomass of the water column or dead organisms that would contribute to the detrital biomass. If the cells are living, then the sponges are contributing directly to the biomass of the primary producers, who in turn leak DOC. If the cells are dead, DOC will leach from them and they will most likely be colonized by bacteria to form aggregates. Whether the cells are living or not is unimportant since the overall affect is the same: these cells contribute to the DOC pool that supports production of heterotrophic bacteria and close the microbial loop. Ultimately, the microbial food web is an important exogenous source of carbon to the benthos of the littoral zone of Lake Baikal.

### *Salt Marshes*

Sapelo Island, Georgia, is located off the mid-Atlantic coast of the United States. The marshes of the region have been well studied during the past 15 years (e. g., Pomeroy

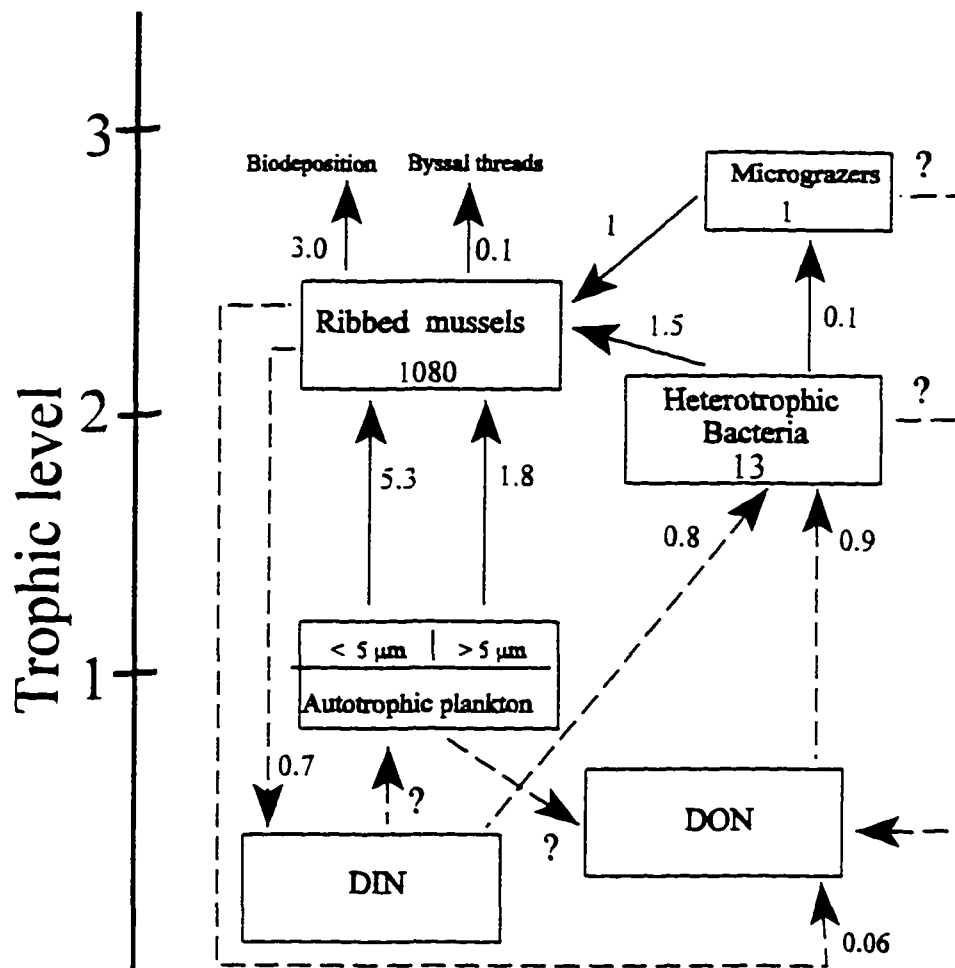
and Wiegert 1981). Grazing within the water column by flagellates and ciliates does not balance bacterial production (Sherr, B. F. et al. 1986, 1989). This leads to a general hypothesis that grazing by the benthos can significantly impact the water column community and that the microbial food web is an important exogenous source of material to the benthic community. I must reiterate that it is only a hypothesis as the data on grazing by the benthic community lacks experimental replication and some assumptions on the rates of processes were assumed from literature values from other ecosystems.

As is typical of the east coast of the United States, the intertidal *Spartina alterniflora* marshes of Sapelo Island have extensive communities of the Atlantic ribbed mussel (*Geukensia demissa*) (Kemp et al. 1990). Atlantic ribbed mussels can retain ultraplankton (Wright et al. 1982) and *in situ* organism mediated fluxes have been estimated in the marsh but lack replication (n=1) (Kemp et al. 1990). However, if these fluxes are combined with estimates of the release of nitrogen by the mussels (Hammen 1968, Jordan and Valiela 1982) and the utilization of nitrogen by bacterioplankton (Wheeler and Kirchman 1986) and autotrophic ultraplankton, a flow of nitrogen between the water column and the benthos can be hypothesized (Figure 14). Fluxes were calculated using literature values for the late summer.

Atlantic ribbed mussels retain 1.5 and 5.2 mg N day<sup>-1</sup> m<sup>-2</sup> from heterotrophic bacteria and autotrophic ultraplankton, respectively, through active suspension feeding (Kemp et al. 1990). These mussels also significantly reduce populations of micrograzers. Thus, Atlantic ribbed mussels feed at three trophic levels within this microbial food web. The mean trophic

Figure 14. Conceptualized daily flow of nitrogen between the water column and the benthos in the salt marshes of Sapelo Island, Georgia, USA. Arrows indicate the direction of flow with dashed lines representing dissolved nitrogen and solid lines particulate nitrogen. Values,  $\text{mg N m}^{-2}$ , in boxes are standing stocks and near arrow are fluxes. Water column standing stocks are for an integrated water column of 0.2 m and ambient concentrations of DIN are for 200 l, the overlying water column. Trophic level within the food web, as indicated by position in the vertical, was determined following Baird and Ulanowicz (Baird and Ulanowicz 1989). Unknown fluxes are indicated by ? next to an arrow.





level of 2.38 (computed from Figure 14) places Atlantic ribbed mussels within the guild of primary consumers of this ecosystem.

Mussels will release  $0.06 \text{ mg N day}^{-1} \text{ m}^{-2}$  in the form DON (Hammen 1968),  $0.7 \text{ mg N day}^{-1} \text{ m}^{-2}$  in ammonium which can be used to support production of autotrophic ultraplankton and heterotrophic bacteria. Heterotrophic bacteria incorporate  $1.7 \text{ mg N day}^{-1} \text{ m}^{-2}$  (Sherr, E. B. et al. 1986, Wheeler and Kirchman 1986), equally partitioned between DON and DIN during the summer (Wheeler and Kirchman 1986). Atlantic ribbed mussels can supply 66% of the DON requirements of heterotrophic bacteria. Heterotrophic bacteria and autotrophic ultraplankton would compete for the DIN fraction which is supplemented by fluxes from the sediments and detritus. Also, Atlantic ribbed mussels biodeposit  $3 \text{ mg N day}^{-1} \text{ m}^{-2}$  and secrete  $0.1 \text{ mg N day}^{-1} \text{ m}^{-2}$  in byssal threads (Jordan and Valiela 1982). Thus, ca. 60% of the nitrogen is assimilated by the mussels and this assimilation efficiency is comparable to those of other bivalves fed a diet of bacterioplankton (Langdon and Newell 1990).

Atlantic ribbed mussels consume 88% of the bacterial production each day. This is reasonable if one considers that the population of mussels also grazes on the micrograzers (Kemp et al. 1990), that are the other major consumers of heterotrophic bacteria. Atlantic ribbed mussels can remove 90% of the micrograzers from the overlying water column in 1 hour (Kemp et al. 1990). Considering this, only  $0.01 \text{ mg N day}^{-1} \text{ m}^{-2}$  in heterotrophic bacteria is consumed each day by micrograzers in water overlying mussel communities (Sherr, E. B. et al. 1986, Sherr, B. F. et al. 1989). Combined, grazing of heterotrophic bacteria by water column and benthic communities can account for 94% of the heterotrophic

bacterial production. The inability of grazing by water column communities to equal bacterial production (Sherr, B. F. et al. 1989) may be accounted for if grazing of heterotrophic bacteria by the benthic community is incorporated into models of material fluxes. More importantly, it clearly demonstrates that the microbial food web is an important source of exogenous nitrogen to the benthic community of the marshes of Sapelo Island.

### SUMMARY

Overall, macroinvertebrates from a variety of ecosystems have been shown to be primary consumers within the microbial food web as they are a sink for ultraplankton and in return are a source of DOM and DIM that nutritionally support the microbial food web. The microbial food web is an important source of exogenous carbon to benthic communities dominated by macroinvertebrates that are capable of utilizing ultraplankton. Ecologically, we tend to bias our interpretation of the flow of matter through ecosystems in terms of energy, which in the photic zone is expressed as carbon. Most likely, a majority of the carbon from the microbial food web is quickly respired, hence the link vs sink controversy (Ducklow et al. 1986, Sherr, E. B. and Sherr 1987), and this carbon is used for metabolic maintenance by macroinvertebrates as it is an abundant, stable energy source. Heterotrophic bacteria can be an exceptional source of nutritionally important nitrogen, which is either assimilated for new production or remineralized and released as DON and DIN to be utilized by the water column community. Heterotrophic bacteria in near shore communities have a C:N ratio of 3-4 while the C:N ratio of autotrophic ultraplankton ranges from 6-8 (Wheeler and Kirchman 1986). In general, the transfer of nitrogen from ultraplankton to higher trophic

levels is conserved, in that it is not lost as  $N_2$  and may be easier to follow making it a better predictor of the importance of microbial food webs in benthic-pelagic coupling.

Macroinvertebrates that have the capacity to graze on ultraplankton are globally distributed in benthic and pelagic habitats. We have only begun to understand the role that these organisms play in the cycling of carbon and other nutrients within aquatic environments and much remains to be learned. Particularly important will be to (1) expand our base of knowledge on the feeding ecology of ultraplankton grazers from larval to adult life-history stages; (2) understand better the fate of carbon and nitrogen from ultraplankton to macroinvertebrates by establishing the assimilation efficiencies; and (3) resolve the role of the microbial food web as an exogenous source of carbon and other nutrients to some of the most highly productive communities on the globe.

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