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# Plankton availability and retention efficiencies of cold-seep symbiotic mussels

*Abstract*—Mussels from deep-sea methane/sulfide seeps in the Gulf of Mexico supplement their symbiotically acquired nitrogen by feeding selectively on nitrogen-rich bacterioplankton. The previously unknown natural diet of the mussels consists of bacteria, *Synechococcus*-type cyanobacteria, and protozoans. Overall retention increased with increasing mussel size, though the largest mussels did not retain bacteria. Mussels can obtain as much as 0.12 $\mu$mol N $g^{-1}$ $h^{-1}$ by filter feeding on natural water-column communities. Previous calculations indicate that nitrogen acquired through the symbionts is inadequate for maximal growth, but our conservative estimates suggest that nitrogen obtained by filter feeding is similar to that acquired by symbionts and may be an important component in the nutritional requirements of seep mussels. Additionally, we conducted a series of in situ measurements of flow and food availability over an extensive mussel bed located at the Brine Pool. Our measurements indicate that biogenic flow due to mussel pumping generates near-bottom turbulence that prevents the development of a food-depleted layer over the mussel bed.

Deep-sea hydrothermal vents and methane/sulfide cold seeps support a variety of benthic animals that obtain most or all of their nutritional needs from endosymbiotic chemosynthetic bacteria (Corliss et al. 1979; Paull et al. 1984; Kennicutt et al. 1985; Kulm et al. 1986; Brooks et al. 1987; MacDonald et al. 1990*b*). The nutritional requirements and energy budgets of some wholly chemoautotrophic forms have been well characterized (Fisher 1990; Childress and Fisher 1992), but the nutritional role of heterotrophy in mixotrophic species is poorly understood. Various clams and mussels are among the most common organisms inhabiting vents and seeps (Paull et al. 1984; MacDonald et al. 1990*a,b*). Carbon isotope ratios, $\delta^{13}C$, for these bivalves are often similar to values for methane (Rau 1981; Kulm et al. 1986; Brooks et al. 1987; Kennicutt et al. 1992; Fisher et al. 1994), indicating that carbon for growth is obtained via translocation from the symbionts. Nitrogen isotope ratios, $\delta^{15}N$, are much more variable, indicating a broad range of nitrogen sources. Very negative values in some populations are indicative of symbiotically assimilated dissolved inorganic nitrogen (DIN), whereas positive values in other populations suggests that at least some of the nitrogen is obtained heterotrophically (Brooks et al. 1987; Kennicutt et al. 1992; Fisher et al. 1994). However, the feeding ecology of vent and seep bivalves is unknown, probably because available foods in these systems, mostly particles $<5$ $\mu$m, and grazing on them by macroinvertebrates were extraordinarily difficult to study prior to the application of laser-based technologies (Gili and Coma 1998).

The cold seep mussel *Bathymodiolus childressi,* and pre-

viously known as Seep Mytilid IA (Gustafson et al. 1998), is a conspicuous and abundant member of the cold seep communities in the Gulf of Mexico (Kennicutt et al. 1985; Brooks et al. 1987; MacDonald et al. 1990*a,b*). Endosymbiotic chemoautotrophic bacteria residing in the gills oxidize sufficient methane to support all of the reduced carbon requirements for mussel growth (Childress et al. 1986). However nitrogen, phosphorus, and other nutrients are required for growth as well. DIN is taken up, assimilated by the symbionts, and translocated to the host at rates that are significantly lower than the rates of methane uptake (Lee et al. 1992; Lee and Childress 1995, 1996). On the basis of this rate difference, it has been suggested that mussel growth is nitrogen limited.

The potential role of nitrogen acquired by heterotrophy in the growth of mussels is unknown. *B. childressi* can suspension feed, and it has been suggested that the role of suspension feeding is to provide nutritional supplements necessary for growth (Page et al. 1990). However, the nutritional importance of heterotrophy is unknown, as food availability and the feeding ecology of the mussels remains to be quantified. Hence, we have characterized the natural diet, quantified retention efficiencies, and estimated carbon and nitrogen fluxes through feeding for six size classes of *B. childressi.* Our data suggest that ultraplankton obtained heterotrophically are an essential source of the nitrogen required for growth.

Dense beds of *B. childressi* surround the Brine Pool (27°43′24″N, 91°16′30″W), a well-studied collapsed salt diapir at a depth of 650 m on the Louisiana Slope (MacDonald et al. 1990*b*). Here, mussels of all sizes from spat to 130 mm shell length may be found in abundance. Size–frequency analysis revealed shell-length modes at 10, 30, 60, and 100 mm (MacDonald et al. 1990*b*). We examined feeding across this entire size range by using mussels with mean shell lengths of 10.41, 27.46, 45.55, 60.96, 84.47, and 116.38 mm.

Diets and retention efficiencies were quantified by allowing mussels to feed on natural seawater in a small (1.17-liter) flow-through flume, gravity fed from 20-liter containers of seawater. The seawater was collected at the Brine Pool by positioning a piece of tubing attached to the end of the retractable arm within 20 cm of the sea floor and filling containers in the rear compartment of the sub. Water was keep in the dark at 6°C for no more than 12 h prior to the experiments. Flow rates within the flume were between 3 and 5 cm/s, sufficient to exchange the entire volume of the flume every minute. All measurements were conducted in a cold room (6°C) onboard the ship within 24 h of collecting the mussels that had been held in oxygenated tanks in the cold room. Mussels ($n = 5$) from each size class ($n = 6$) were individually placed in the flume and allowed to acclimate to the conditions of the flume for at least 15 but not more than 30 min. When placed in the flowing water, mussels generally oriented toward the flow, gaped their shells, and began filtering water (as visualized with fluorescein dye) within 1 min. Water samples of 1 ml were collected using syringes from the exhalent siphon and adjacent to the inhalant siphon and preserved for flow cytometry following standard protocols (Pile et al. 1996).

Ultraplankton populations were quantified using an Epic Elite flow cytometer (Coulter Electronics Corporation, Hialeah, Florida) at Harbor Branch Oceanographic Institution following the techniques of Marie et al. (1996). Orange fluorescence (from phycoerythrin), red fluorescence (from chlorophyll), and green fluorescence (from DNA stained with SYBR Green) were collected through bandpass 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 green fluorescence were recorded on three-decade logarithmic scales, sorted in list mode, and analyzed by custom-designed software (CYTOWIN; Vaulot 1989). Ultraplankton populations were identified to general cell types of bacteria (Bac), *Synechococcus*-type cyanobacteria (Syn), and protozoans, visually confirmed, and mean cell diameter measured ($n = 50$) using epifluorescence microscopy. Photosynthetic detritus was determined from an additional 500 ml of water and was analyzed for chlorophyll *a* (Chl *a*) using acetone extraction (Strickland and Parsons 1972).

The mean retention efficiency for each size class was calculated as ([mean cell count ambient − mean cell count exhalent]/mean cell count ambient) × 100 for each type of ultraplankton. Student *t*-tests were used to determine if the retention efficiency for each type of ultraplankton was significantly >0 employing a Bonferroni transformed experimentwise error of $\alpha = 0.0001$. If more than one type of ultraplankton was retained, analysis of variance (ANOVA) models were used to determine significant differences between retention efficiencies (Zar 1984).

Mussel-mediated carbon and nitrogen flux were conservatively estimated using a modified version of the general model for organism-mediated flux (Pile et al. 1996):

$$\text{organism} - \text{mediated flux} = \frac{\Delta\text{water} - \text{column property}}{\text{vol. processed}} \times \frac{\left(\dfrac{\text{vol. processed}}{\text{pumping unit}}\right)}{\text{time}} \quad (1)$$

where the water-column property is the cell count of ultraplankton, volume processed is liters, and pumping unit is one *B. childressi.* The mean number of ultraplankton cells removed was calculated as the retention efficiency times ambient levels reported in Table 1, and this was converted to an equivalent g C and g N. For heterotrophic bacteria a cell-to-carbon conversion factor of 20 fg C cell$^{-1}$ with a C/N ratio of 3.5 (Wheeler and Kirchman 1986) was employed. *Synechococcus*-type cyanobacteria utilized a carbon-to-cell conversion factor of 470 fg C cell$^{-1}$, and cell-to-nitrogen was determined as a function of biovolume (=3.05 $\mu$m$^3$) multiplied by 0.26. We selected these factors as they are for cells with mean diameters that are equal to or greater than those found during this study (Ducklow et al. 1993). For protozoans, fg C and N were determined as a function of biovolume (=16.75 $\mu$m$^3$) with the conversion factors of 1.9 for C and 0.26 for N (Putt and Stoecker 1989; DeBiase et al. 1990). Chl *a* was converted to carbon employing a conversion factor of 30 (Ayukai 1995) and to nitrogen assuming a Redfield C/N ratio of 6.625 (Fasham et al. 1990). The carbon or nitrogen attributed to *Synechococcus*-type cyanobacteria

Table 1. Composition of near-bottom water column community. All types of ultraplankton were determined using flow cytometry and were confirmed visually with epifluorescent microscopy. Standard cell-to-carbon conversion factors were used to empirically calculate carbon and nitrogen values (Putt and Stoecker 1989; DeBiase et al. 1990; Ducklow et al. 1993). Values in parentheses represent the percentage of the total available. NA, not applicable.

| | Bacteria | *Synechococcus* type cyanobacteria | Protozoans | Chl *a* | Total |
|---|---|---|---|---|---|
| Quantity of organism ($10^3$ cells $ml^{-1}$) | 359 (97) | 6.34 (2) | 3.11 (1) | NA | 368 |
| Carbon ($\mu g\ L^{-1}$) | 7.18 (58) | 2.98 (24) | 2.07 (17) | 0.020 (0.2) | 12.3 |
| Nitrogen ($\mu g\ L^{-1}$) | 2.05 (73) | 0.27 (10) | 0.50 (17) | 0.003 (0.1) | 2.82 |

was subtracted from the total attributed to Chl *a* to determine the contribution of the photosynthetic eukaryotes >10 $\mu$m. Assimilation of nitrogen and carbon from heterotrophic sources was calculated as organism-mediated flux times assimilation efficiency. *B. childressi* has a conservative assimilation efficiency for carbon of 51.2% (Page et al. 1990). There are no published rates of nitrogen assimilation efficiency through heterotrophy for *B. childressi.* We assumed that there is no difference between C and N assimilation efficiency as there is no difference in other mussels (Hawkins and Bayne 1985). In order to compare estimated C and N assimilation from heterotrophy with the estimated C and N assimilated via chemoautotrophy, we have estimated assimilated C and N from heterotrophy using a model *B. childressi* with a shell length of 59.1 mm, as it is the mean shell length of mussels used in other studies (Page et al. 1990; Lee et al. 1992; Lee and Childress 1995). For comparisons we have employed the organism-mediated flux for the mean size of 60.96 mm (Table 2) and a volume processing rate of 1.81 liters $g^{-1}\ h^{-1}$ (Clausen and Riisgård 1996) because there is no published rate of volume processed for *B. childressi.* This rate is for *Mytlius edilus,* a species that has previously been compared to *B. childressi* that is of similar size and quantified under similar temperature ranges. We understand the inherent risks with such calculations, but the data are presented in such a way that should better cell-to-C or -N conversions, assimilation efficiencies, or volume processing rates become available, fluxes may be recalculated.

A single cross-bed transect was conducted on the northern edge of the pool from the DSRV *Johnson SeaLink II.* Starting at the upstream edge of the bed with additional stations at 5, 12, and 18 m (the far edge of the bed), we measured velocity of the water and collected water samples along a vertical profile. A Marsh McBirney 2000-01 flow probe was positioned using the arm of the sub at 1, 5, and 30 cm above the mussel bed and 10, 10-s mean measurements were recorded. Immediately following this, four 20-ml water samples from each height were collected through tubing on the end of the arm. Water samples were preserved for flow cytometry and analyzed as previously stated.

Ultraplankton (plankton <5 $\mu$m; Murphy and Haugen 1985) dominated the water column community over the Brine Pool (Table 1). Bacteria that are an exceptionally rich source of nitrogen were numerical dominants and also com-

Table 2. Estimated *Bathymodiolus childressi*–mediated carbon (C) and nitrogen (N) fluxes of ultraplankton ($\mu$g [L of water processed]$^{-1}$ individual$^{-1}$). Assimilated C and N are presented in parentheses and were calculated by employing the conservative assimilation efficiency of 51.2% (Page et al. 1990). Mussel sizes represent the mean (± SD) of a size class.

| | | C and N fluxes of ultraplankton ($\mu$g [L water processed]$^{-1}$ individual$^{-1}$) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Size of mussel | Element | Bacteria | T | Cyanobacteria | % | Protozoans | % | Total |
| 10.41 (±2.61) | C | | | 0.5 (0.3) | 100 | | | 0.5 (0.3) |
| | N | | | 0.1 (0.1) | 100 | | | 0.1 (0.1) |
| 27.46 (±3.24) | C | 1.4 (0.7) | 57 | | | 1.1 (0.6) | 43 | 2.5 (1.3) |
| | N | 0.4 (0.2) | 73 | | | 0.2 (0.1) | 26 | 0.6 (0.3) |
| 45.55 (±3.78) | C | 1.4 (0.7) | 40 | | | 2.2 (1.1) | 60 | 3.6 (1.8) |
| | N | 0.4 (0.2) | 58 | | | 0.3 (0.2) | 42 | 0.7 (0.4) |
| 60.96 (±6.31) | C | 3.9 (2.0) | 42 | | | 5.4 (2.8) | 58 | 9.3 (4.8) |
| | N | 1.1 (0.6) | 60 | | | 0.7 (0.4) | 40 | 1.8 (1.0) |
| 84.47 (±2.55) | C | 5.8 (3.0) | 50 | | | 5.9 (3.0) | 50 | 12.0 (6.0) |
| | N | 1.7 (0.9) | 67 | | | 0.8 (0.4) | 33 | 2.5 (1.3) |
| 116.38 (±3.53) | C | | | | | 5.9 (3.0) | 100 | 5.9 (3.0) |
| | N | | | | | 0.7 (0.4) | 100 | 0.7 (4.0) |

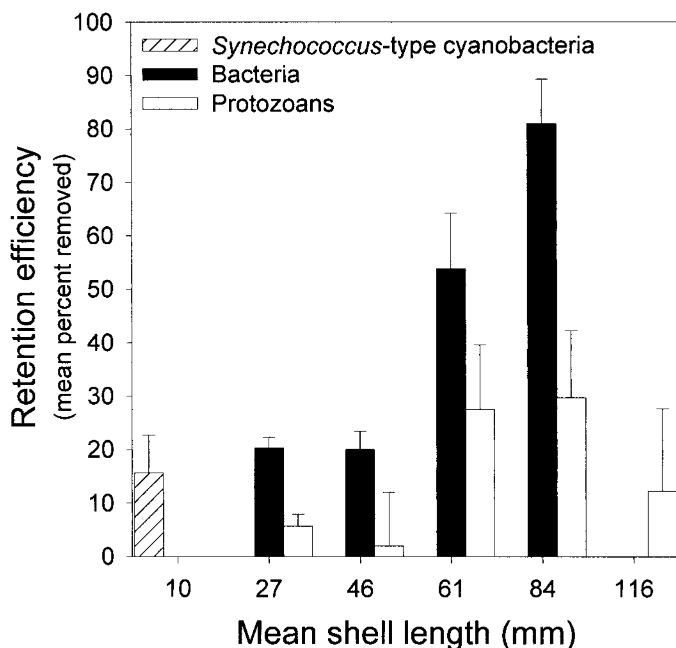


Fig. 1. Retention efficiencies ($\bar{x}$ ± standard deviation [SD], $n$ = 5) for *Synechococcus*-type cyanobacteria, bacteria, and protozoans for six sizes of *B. childressi*. All retention efficiencies were significantly greater than 0 ($t$-test, Bonferroni transformed experimentwise $\alpha < 0.0001$). For mussels ranging in size from 27 to 84 mm retention efficiency of bacterial cells was always significantly greater than retention efficiency of protozoans.

prised the largest carbon source. Algal cells >10 $\mu$m, as quantified by Chl *a* content of the water, were an insignificant source of carbon and nitrogen (<1%) to this benthic community.

The natural diet of the mussels changed with the size of the mussels (Fig. 1). All of the size classes tested retained significant amounts of ultraplankton. However, only the smallest mussels retained *Synechococcus*-type cyanobacteria (17%; Student's $t$-test $t_4 = 9.32$). The next four size classes had a mixed diet of bacteria and protozoans, with bacteria always being selectively retained over larger protozoans (two-way ANOVA, $F_{3,39} = 4.32$, $P = 0.013$). The largest mussels retained only protozoans (25%; Student's $t$-test $t_4 = 8.77$). For the intermediary size classes retention efficiencies for bacteria (27.46 mm = 19%, Student's $t$-test $t_4 = 9.04$; 45.55 mm = 20%, Student's $t$-test $t_4 = 9.86$; 60.96 mm = 53%, Student's $t$-test $t_4 = 11.32$; and 84.47 mm = 80%, Student's $t$-test $t_4 = 14.59$) and protozoans (27.46 = 5%, Student's $t$-test $t_4 = 8.70$; 45.55 = 11%, Student's $t$-test $t_4 = 8.94$; 60.96 = 27%, Student's $t$-test $t_4 = 10.01$; and 84.47 = 30%, Student's $t$-test $t_4 = 9.88$) increased with increasing mussel size up to the largest size class, where retention of both kinds of organisms decreased markedly. Mussels obtain significant amounts of both carbon and nitrogen through feeding (Table 2). Overall, bacteria supplied a majority (58–73%) of the N to mussels that grazed on more than one type of ultraplankton. In contrast, C flux was more balanced between the various food groups when the diet consisted of more than one food type.

Flow conditions over the mussel bed at the Brine Pool are indicative of biogenic flow created by the exhalent currents of the mussels (Fig. 2). A near-bottom boundary layer is present near the upstream edge of the mussel bed, but it quickly breaks down as water flows across the bed. Twelve meters from the edge, current velocity in the turbulent bottom layer is actually greater than the velocity 1 m above the bed. Overall, concentrations of bacteria and protozoans do not change over the bed as water moves across the bed.

There is abundant plankton at the Brine Pool that could support heterotrophic feeding by suspension-feeding macroinvertebrates. Total carbon content of the water overlying the Brine Pool was 12.3 $\mu$g liter$^{-1}$, a value that is higher than that typically found on Pacific coral reefs, another ecosystem dominated by mixotrophic benthic macroinvertebrates (Ayukai 1995). Ultraplankton was the dominant food source, and surprisingly *Synechococcus*-type cyanobacteria were a component of the plankton community. It is likely that these autotrophs were transported into deep water by deposition of fecal pellets from the overlying pelagic community (Lochte and Turley 1988; Graf 1989; Pfannkuche and Lochte 1993). In addition, sedimenting cyanobacteria are believed to contribute a substantial amount of nitrogen, rather than carbon, to the deep sea as phycoerythrin is a net store of nitrogen (Lochte and Turley 1988).

Previous studies have shown that cold-seep mussels and hydrothermal vent clams can feed on and assimilate cultured bacteria and algae (Page et al. 1990, 1991) and suggested that a primary role of heterotrophy is to obtain nutritional supplements necessary for growth. This is the first study to demonstrate that *B. childressi* is capable of grazing on the natural water column community and quantify their diet. The natural diet of mussels clearly has an ontogenetic shift from *Synechococcus*-type cyanobacteria to heterotrophic bacteria and protozoans and finally, as maximum size is reached, only protozoans. As is typical of bivalves, *B. childressi* grazed selectively on the ultraplankton community (Hawkins et al. 1996; Ward et al. 1997, 1998) as opposed to other active suspension feeders that graze unselectively on ultraplankton (Pile et al. 1996, 1997). Retention of plankton does not necessarily reflect digestion in bivalves as particles may subsequently be rejected in pseudofeces. But, a variety of bivalves retain and digest unarmored plankton, such as protozoans, over armored plankton, such as diatoms, that are retained but rejected in pseudofeces (Shumway et al. 1985). We did not observe the production of pseudofeces during the course of our study and for the purposes of this study will assume that the protozoans retained were digested. Clearly, future investigations should determine not only the assimilation efficiency of the various types of ultraplankton but also the water-processing rates for cold-seep and hydrothermal vent bivalves in order to quantify the actual fluxes of carbon and nitrogen.

When the diet of the mussels consisted of more than one component of ultraplankton, bacteria were always retained at a higher efficiency than protozoans. Because bacteria are significantly smaller than protozoans it is unlikely that the preference is due to mechanical constraints of the filtering mech-

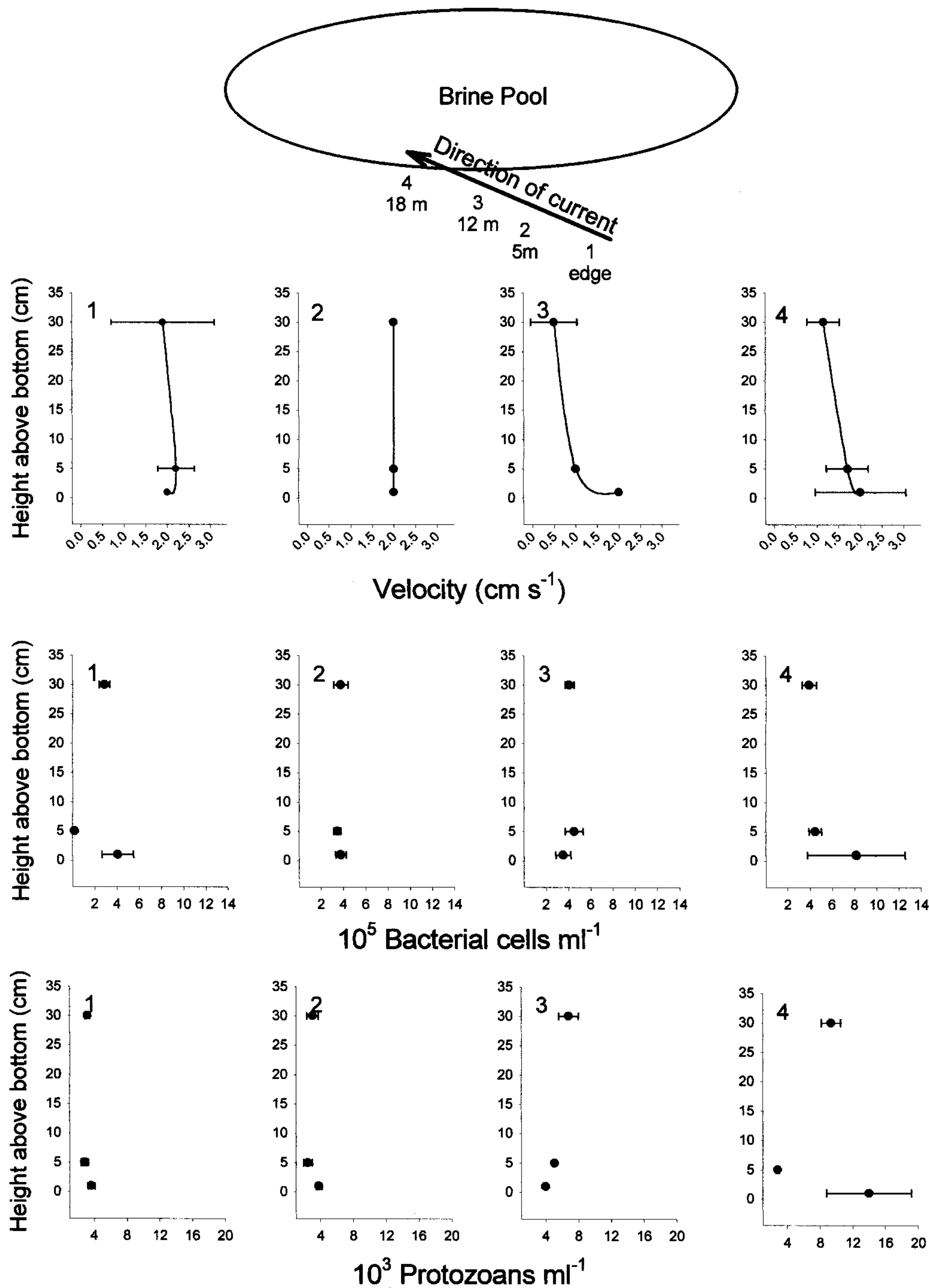


Fig. 2. Near-bottom velocity ($\bar{x} \pm$ SD, $n = 10$), bacterial ($\bar{x} \pm$ standard error [SE], $n = 4$) and protozoan ($\bar{x} \pm$ SE, $n = 4$) profiles for a transect across a portion of the mussel bed located at the Brine Pool. Mixing of near-bottom water occurs due to biogenic flow created by the suspension feeding activity of the mussels. Correspondingly, near-bottom ultraplankton does not become depleted despite the fact that some individuals can retain as much as 80% of the bacterial cells from water that they process. Note that frequently the error bars are eclipsed by the size of the points.

anism (Rubenstein and Koehl 1977). Rather, selective sorting at the palps of the mussels is occurring, reflecting either some type of nutritional benefit of bacteria over protozoans or a feeding deterrent by some protozoans (Ward and Targett 1989; Hawkins et al. 1996; Ward et al. 1997, 1998).

It has been calculated that incorporation of DIN by a mussel of average size is limited by physiological processes to a maximum rate of 0.08 $\mu$mol N $g^{-1}$ $h^{-1}$ (Lee and Childress 1995), whereas a similar-sized mussel can conservatively obtain 0.12 $\mu$mol N $g^{-1}$ $h^{-1}$ by filter feeding. Thus, feeding

may yield 50% more N than can be obtained from symbionts. By combining heterotrophic and autotrophic feeding mechanisms, mussels avoid nitrogen limitation in growth. It is noteworthy that selective feeding on bacteria ceases when the mussels approach an asymptotic shell length, indicative of a cessation of growth.

Stable isotope values for mussel tissues do not indicate a major contribution of carbon from heterotrophic sources, suggesting that carbon acquired through feeding may be quickly respired to support basal metabolism or released as dissolved organic carbon (DOC) as seen in other mixatrophic organisms such as corals (Falkowski et al. 1993) and sponges (Borowitzka et al. 1988). Stable nitrogen isotope ratios are more variable, most likely because DIN varies spatially and/or temporally; nevertheless, >80% of the mussels evaluated from the Gulf of Mexico have positive $\delta^{15}N$ values (Brooks et al. 1987; Kennicutt et al. 1992), some as high as +5. These very positive $\delta^{15}N$ values probably reflect dietary nitrogen incorporated into tissues. By contrast, $\delta^{15}N$ values of hydrothermal vent mussels vary through a smaller range (−8 to +8) with no clear pattern (Kennicutt et al. 1992; Fisher et al. 1994). Such variability likely mirrors small-scale patchiness of both DIN and ultraplankton and suggests that mechanisms of nitrogen acquisition are plastic, the relative importance of feeding and DIN uptake shifting with changing environmental conditions.

In some instances, near-bottom plankton communities are depleted as much as an order of magnitude due to the grazing activity of extensive suspension-feeding communities (Buss and Jackson 1981; Peterson and Black 1987; Pile et al. 1997). If this occurred at the Brine Pool, mussels on the down-stream end of the bed could be nitrogen limited. In this system, however, the benthic boundary layer is not depleted because of the near-bottom turbulence created by the pumping mussels themselves (O'Riordan et al. 1995). Thus, biogenic turbulence prevents the critical supply of bottom plankton from becoming depleted.

Heterotrophically obtained nitrogen from grazing on ultraplankton is an essential nutritional supplement for growth in these cold-seep, symbiotic mussels. While only the smallest mussels retained autotrophic plankton, seasonal pulses of sedimenting surface blooms are associated with bacterial and protozoan blooms within the benthic boundary layer in the deep sea (Lochte and Turley 1988; Graf 1989). Consequently, seasonal variation in all components of the plankton community may play a hitherto unsuspected role in the nutrition, growth, and reproduction of hydrothermal vent and cold-seep organisms. More importantly, these biological communities are maintained through a complex balance of heterotrophy and autotrophy that remains to be explored.

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