

Rapid nitrogen uptake by marine bacteria

Abstract—In order to test the rapid nitrogen uptake capabilities of marine bacteria, we first grew natural populations in NH_4^+ and glutamate-limited continuous cultures over a range of steady-state dilution rates, to establish different physiological states. We then pulsed the cultures with saturating concentrations of ^{15}N -labeled NH_4^+ or glutamate and found that rapid uptake, measured as the ratio $V_M' : \mu_M$ (V_M' is the time-dependent maximum specific uptake rate of nitrogen, and μ_M is the maximum growth rate), decreased with time of incubation from ≈ 3 –4 at 40 s to ≤ 1 by 5–15 min. Relative growth rate $D : \mu_M$ (D is the steady-state dilution rate) had no systematic effect on this relationship. Although enhanced nitrogen uptake is apparent in marine bacteria, it is far less dramatic than what we and others have observed previously in some marine diatoms: e.g., $V_M' : \mu_M > 5$ –50 has been measured in several diatoms during similar 1-min incubations, and values > 1 have been observed during 2-h incubations. These results point toward fundamental differences in the temporal and spatial scales that control the exposure to and ability to exploit ephemeral nutrient patches of marine bacteria and phytoplankton. Bacteria probably are sustained by more frequent and relatively short contacts with smaller nutrient patches both in size and concentration than those experienced by phytoplankton. The latter must overcome greater diffusion constraints because of their larger size. Evidence from recent studies, suggestive that bacteria respond to point sources of nutrients surrounding larger microbes through motility and chemotaxis, is consistent with our finding of moderately enhanced nitrogen uptake by these organisms.

Marine phytoplankton have the ability to take up NH_4^+ for short periods of time (seconds to minutes) at rates far in excess of that required to balance growth (McCarthy and Goldman 1979; Glibert and Goldman 1981; Goldman and Glibert 1982; Parslow et al. 1984, 1985; Zehr et al. 1988). This ability is greatly enhanced at low relative growth rates but is a feature of some species even when they are growing at rates close to maximal (Goldman and Glibert 1982). The possession of rapid nitrogen uptake capabilities has been suggested to be a mechanism by which marine phytoplankton can obtain their ration of nutrients by episodic contacts with microenvironments containing elevated concentration of nutrients. Microenvironments such as marine snow particles and fecal pellets (Alldredge and Cohen 1987; Alldredge and Silver 1988) and zones surrounding smaller particles, including individual phytoplankton cells (Azam and Ammerman 1984), are major sites where nutrient concentrations are elevated substantially over those in the bulk fluid. Bulk fluid concentrations in oligotrophic waters typically are below the limits of detection, so any micro source of nutrient enrichment can be important to microbial survival (McCarthy and Goldman 1979). Thus marine phytoplankton in the open ocean probably live a “feast or famine” existence, in which the ability to sense and move from one site of nutrient enrichment to another is a key to survival (Goldman 1984).

Marine bacteria most likely face similar challenges in obtaining nutrients. In fact, from a number of contemporary studies, there is an abundance of evidence that motility and chemotactic behavior lead to clustering of bacteria in micro-patches surrounding point sources of nutrients (Mitchell et al. 1996; Blackburn et al. 1998; Blackburn and Fenchel 1999). Although the emphasis of these studies has been on physicochemical and hydrodynamic factors involved in clustering, the implications have been that clustering is nonrandom and is a way for bacteria to exploit, with rapid uptake capability, ephemeral nutrient patches. To date, however, little quantitative information exists on how bacteria respond to such patches.

In this study, we address the question of rapid nitrogen uptake by bacteria in a series of experiments similar to those we performed earlier with phytoplankton (Goldman and Glibert 1982). We first grew natural populations of marine bacteria to steady state in nitrogen-limited continuous cultures, as described elsewhere (Goldman and Dennett 2000). In summary, the inoculum was a natural assemblage of marine bacteria obtained by filtering water from Vineyard Sound, MA, through a sterile 0.6- μm membrane filter; it was then used directly in establishing steady state in the continuous cultures, which usually occurred within a few days. As was stated elsewhere (Goldman and Dennett 1991), our choice in using natural populations over isolates (the process approach—Hobbie 1988) was because we wanted the maximum response to the different growth conditions. We did not attempt to characterize the species comprising the assemblages. The growth medium had a C:N ratio of 30:1 by atoms either with combinations of NH_4^+ and glucose or glutamate and glucose, to ensure that nitrogen would be the limiting nutrient. In all cases, we maintained a fixed concentration of 500 $\mu\text{mol L}^{-1}$ of nitrogen in the medium, and we added sufficient PO_4^{3-} (150 $\mu\text{mol L}^{-1}$ P) to ensure that nitrogen was limiting growth. We attained a range of steady-state dilution rates from 0.02 to 0.60 h^{-1} , mostly for measurements of particulate organic carbon, particulate organic nitrogen, residual NH_4^+ , glutamate, and glucose, which were used in the companion study (Goldman and Dennett 2000), and three dilution rates ($D = 0.02, 0.20, \text{ and } 0.60 \text{ h}^{-1}$) for the rapid nitrogen uptake experiments reported in this study. In performing the rapid uptake experiments, we first added 1 ml of a 12.5 mmol N L^{-1} stock solution of ^{15}N -labeled NH_4^+ or glutamate (99% enriched preparation), followed by 125 ml of culture from the continuous culture to a 150-ml water-jacketed incubation vessel to give a final ^{15}N concentration of 100 $\mu\text{mol L}^{-1}$ (steady-state concentrations of NH_4^+ and glutamate were below the levels of detection). We assumed that this concentration of nitrogen was sufficient to saturate uptake. We kept the temperature in both the continuous cultures and incubation vessels at 24°C and vigorously stirred the vessels with magnetic stirring bars. We sampled at 20, 40, and 80 s and at 5, 15, and 30 min after time zero

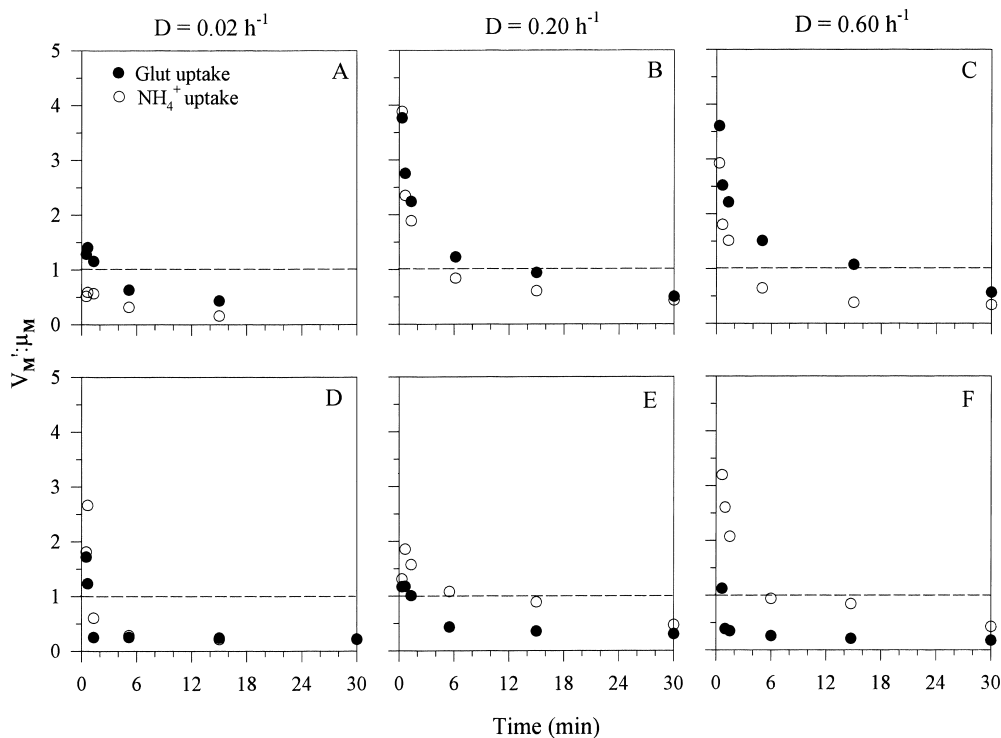


Fig. 1. Time course of changes in $V_M' : \mu_M$ by natural assemblages of bacteria first grown to steady state at dilution rates D (A,D) 0.02 h^{-1} , (B,E) 0.20 h^{-1} , and (C,F) 0.60 h^{-1} on (A–C) glutamate or (D–E) NH_4^+ and then pulsed with ^{15}N -labeled glutamate or NH_4^+ . Dashed lines represent $V_M' : \mu_M = 1$, where V_M' is the time-dependent maximum specific uptake rate and μ_M is the maximum specific growth rate.

by rapidly withdrawing 10 ml of sample by automatic pipette and filtering it through a double-glass fiber filter (Whatman GF/F), followed by a 50-ml rinse with filtered seawater. We were careful to ensure that the filters were not sucked dry before the rinse water was added, so as to avoid hypotonic osmotic shock to the filtered cells. We then dried the filters and assayed for ^{15}N incorporation with a Jasco NA-1 atomic emission spectrometer. By assaying control samples containing cells that were killed with a few drops of HgCl_2 , we found that only biological uptake of ^{15}N -labeled nitrogen was occurring. The ^{15}N data are reported as V_M' , the maximum specific nitrogen uptake rate in h^{-1} (V_M' is used to denote that the rate is variable; see Goldman and Glibert 1983), in order to calculate the ratio $V_M' : \mu_M$ in which μ_M is the maximum specific growth rate in h^{-1} . On the basis of data from the previously described batch and continuous culture studies (Goldman and Dennett 2000), we estimated μ_M to be $\sim 0.67 \text{ h}^{-1}$.

We observed a trend of decreasing $V_M' : \mu_M$ with time of incubation through 30 min both for cultures grown on glutamate plus glucose (Fig. 1A–C) and NH_4^+ plus glucose (Fig. 1D–F), regardless of steady-state D , represented as the relative growth rate $D : \mu_M$ (Fig. 2). During the first 4–6 min of incubation, there was a rapid decrease in $V_M' : \mu_M$ from values as high as 3–4 during the first sampling period (20 s) to 1–1.5 and less by 5–15 min, followed by a more gradual decrease over the remaining incubation period to values of ~ 0.3 – 0.5 at 30 min (Fig. 1). We also found a trend of

higher $V_M' : \mu_M$ over the incubation periods when both the growth and uptake nitrogen sources were the same than those when they were different. For example, glutamate uptake was higher than NH_4^+ uptake when the cultures were grown on glutamate (Figs. 1A–C, 2), and just the opposite occurred when NH_4^+ was the substrate for the steady-state cultures (Figs. 1D–F, 2).

Our choice in using the parameters $V_M' : \mu_M$ and $D : \mu_M$ provides us with a relatively simple and convenient way to judge the uptake capabilities of the bacterial populations under a wide range of physiological states and also allows us to see on a comparative scale the potential for rapid uptake rates of bacteria with our previously established values for phytoplankton. For example, it is clear from Fig. 2 that any rate of uptake above the dotted line, which describes a 1 : 1 relationship between $V_M' : \mu_M$ and $D : \mu_M$, represents uptake in excess of that necessary to maintain μ_M . Thus we see clearly that the bacterial populations in our experiments had an enhanced nitrogen uptake capability at all relative growth rates when exposed to a pulse of nitrogen (Fig. 2) that could be maintained for 5–15 min (Fig. 1). Given our limited data set, however, we cannot discount the possibility that different bacterial populations were selected for over the range of dilution rates tested (Harder and Kuenen 1977) and were responding with different uptake capabilities. As we discussed elsewhere (Goldman and Dennett 2000), if species selection occurred, then we would have expected the maximum response for a given relative dilution rate, not unlike what

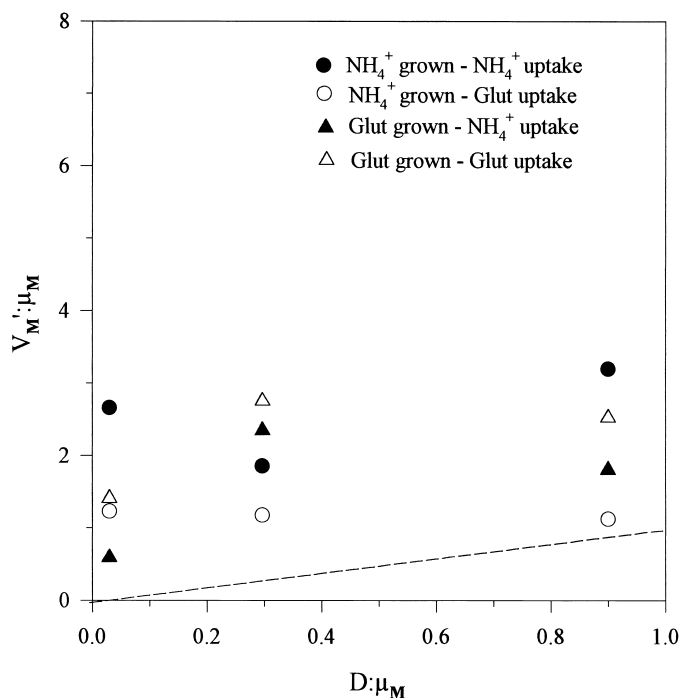


Fig. 2. Effect of $D:\mu_M$ on $V_M':\mu_M$ for different combinations of steady-state and pulsed N sources and after a 40-s incubation with a pulsed N source. The dashed line represents a 1:1 relationship between $D:\mu_M$ and $V_M':\mu_M$.

would be expected under natural conditions. Because the range of relative dilution rates we used in the current study spans the range from extreme nitrogen to non-nutrient limitation, it would appear that marine bacteria can respond with surge uptake to nitrogen pulsing over a wide range of nutrient conditions. In fact, we have preliminary evidence (Goldman and Dennett unpubl. data) that indicate surge uptake of glutamate under carbon-limiting conditions when glutamate was the sole carbon and nitrogen source (medium C:N ratio of 5:1).

Two important points emerge from these experiments. First, although it is evident that marine bacteria have an enhanced NH_4^+ and glutamate uptake capability, on a relative scale it seems that the magnitude of this uptake potential is far less than what some phytoplankton species possess (Goldman and Glibert 1982). For example, several diatoms displayed extraordinary NH_4^+ uptake potential in ^{15}N assays, similar to the current ones: during 1-min incubations $V_M':\mu_M$ varied from 5 to 53 for *Phaeodactylum tricorutum* and from 9 to 13 for *Chaetoceros simplex* over a range of decreasing $D:\mu_M$ from 0.6 to 0.2 (Goldman and Glibert 1982). During even longer (5 min) incubations, $V_M':\mu_M$ remained ≥ 4 over the full range of $D:\mu_M$, both for these species as well as for another diatom, *Thalassiosira weissflogii*. Parslow et al. (1985) and Zehr et al. (1988), in showing similarly large uncoupling between NH_4^+ uptake and growth in the diatom *Thalassiosira pseudonana* that decreased over short (minutes) time periods, suggested that this phenomenon involved, as a first step, the filling of internal NH_4^+ pools. Intuitively, we would expect pool size to be a function of cell size, although Dortch et al. (1984) showed that this

pattern among phytoplankton is not always followed. However, given that bacteria as a group are so much smaller than most phytoplankton species, it seems reasonable to suggest that there is a difference, not only in pool size among the two microbial groups, but also in the number of transporters (which control utilization of the nutrient pool and subsequent growth) located in the cytoplasmic membrane. Cell size then could provide a simple explanation for the large difference in $V_M':\mu_M$ between phytoplankton and bacteria that we observed. This conclusion is consistent with the suggestion of Jumars et al. (1993) that phytoplankton are more capable of exploiting infrequent pulses of nutrients than are bacteria.

This explanation leads to the second important conclusion from our results. Phytoplankton, particularly nonmotile diatoms, probably live a more nomadic existence than bacteria in nutrient-impooverished waters and are exposed to micro patches of elevated nutrients levels more infrequently. Possessing the ability to store nutrients rapidly and in relatively large amounts under such conditions would give larger phytoplankton the ability to survive in a "feast or famine" environment. Bacteria, in contrast, are probably exposed to nutrient patches more frequently and thus are not required to reside within and exploit an individual patch for periods as long as those imposed on large phytoplankton, thereby allowing for lower storage capacity. Such a conclusion is consistent with our previous observation that some phytoplankton species could sustain enhanced NH_4^+ uptake ($V_M':\mu_M > 1$) for 2 h (Goldman and Glibert 1982), compared with only ~ 15 m maximum for bacteria (Fig. 1).

Bacteria probably can exploit lower nutrient levels within patches for enhanced uptake better than larger phytoplankton simply because of their lower diffusion constraints (Karp-Boss et al. 1996). From recent evidence (Blackburn et al. 1998; Blackburn and Fenchel 1999), it appears that bacteria, through motility and chemotactic behavior, can reside for periods on the order of minutes within even slightly elevated nutrient patches arising from grazing and cell lysis. Such activity is a common feature of the microbial loop and is consistent with the lowered storage requirements of bacteria that we observed. Conceivably, bacteria are in much closer proximity to point sources of nutrients than are larger phytoplankton—the clustering hypothesis of Azam and Ammerman (1984)—and thus respond on different spatial and temporal microscales than phytoplankton to ephemeral nutrient pulses. The lowered but still enhanced uptake capabilities of these microbes most likely represents an evolutionary adaptation to life in such environments.

It is important to recognize, however, that although bacteria use a wide array of carbon and nitrogen sources, simple compounds such as NH_4^+ , free amino acids, and monosugars often are major substrates for growth (Keil and Kirchman 1991; Skoog and Benner 1997). Although little is known about the uncoupling of uptake from subsequent metabolism of complex molecules, the basic mechanisms of surge uptake and storage seem to be a common response of bacteria under a wide variety of growth conditions (Koch 1979). Thus, although it is true that bacteria and phytoplankton are not always competing for the same nutrients and are using very different carbon sources, our comparison of surge uptake of simple nitrogen compounds by these microbes on the basis

of size differences seems reasonable. Further research on this important topic clearly is warranted. Finally, one important point that comes from this and our previous studies on pulsed NH_4^+ uptake is that kinetic models of uptake and growth such as the Monod, Droop, and Janusian models (Droop 1973; Goldman 1977; Button 1985) that are premised on steady-state conditions (that is, the specific uptake rate $V =$ the specific growth rate μ) do not adequately describe this phenomenon. Simply, V_M' and K_S (the half-saturation coefficient for uptake) are time-dependent variables over short time periods, whereas μ and K_U (the half-saturation coefficient for growth) on the same temporal scale are relatively constant (Goldman and Glibert 1983).

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