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MINIREVIEW

Small Players, Large Role: Microbial Influence on Biogeochemical Processes in Pelagic Aquatic Ecosystems

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ABSTRACT

Although prokaryotes are small in size, they are a significant biomass component in aquatic planktonic ecosystems and play a major role in biogeochemical processes. A review of the recent literature shows that the relative importance of prokaryotes to material and energy fluxes is maximized in low-productivity (oligotrophic) ecosystems and decreases in high-productivity (eutrophic) ecosystems. We conclude that competition with eukaryotic autotrophs for dissolved nutrients and competition with phagotrophic heterotrophs and physical processes (sinking, photooxidation) for organic carbon (C) play important roles in determining the relative abundance and impact of prokaryotes in aquatic systems. Oligotrophic systems have low nutrient concentrations, with high proportions of dissolved nutrients in organic form, which favors prokaryotic heterotrophs over phytoplankton. Furthermore, a high proportion of the available organic C is dissolved rather than particulate, which favors prokaryotic heterotrophs over phagotrophic heterotrophs. In eutrophic systems, increased relative concentrations and loading of inorganic nutrients and increased relative concentrations of particulate organic C select for phytoplankton and phagotrophic heterotrophs over prokaryotic heterotrophs. Increased particle sinking fluxes and/or decreased excretion of organic carbon (EOC) may also decrease the relative importance of prokaryotic heterotrophs in eutrophic systems. In oligotrophic systems, interactions between autotrophs and heterotrophs are tightly coupled because the dominant heterotrophs are similar in size and growth rates, as well as having similar nutrient composition to the dominant autotrophs, small phytoplankton. In eutrophic systems, increased productivity passes through zooplankton that are larger and have slower growth rates than the autotrophs, leading to a greater potential for decoupled auto- and heterotrophic production and increased export production.

Key words: bacteria; biogeochemistry; prokaryotic heterotroph; autotroph; oligotrophic ecosystems; eutrophic ecosystems; phosphorus; respiration.

INTRODUCTION

In the past few hundred years, our understanding of the role that microbes play in ecological and biogeochemical processes has grown at a phenomenal rate. Von Leeuwenhoek, who invented the microscope in the 17th century, was the first to observe microbes in lake water, but progress in microbiology came slowly, and the field of microbial ecology did not come into existence until the middle of the 20th century. Early on, Vernadsky

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(1945) recognized the importance of microbes to global processes, and many scientists in the early 20th century acknowledged that bacteria and other microbes were an important but unquantifiable component of ecosystems. For example, although Lindeman (1942) put the microbial "ooze" at the center of his diagram depicting trophic dynamics in lakes, he had little understanding of the influence of microbes on the carbon, energy, and nutrient fluxes that he studied in Cedar Bog Lake, MN, USA. Riley (1951) recognized their importance in ocean geochemistry but lamented that appropriate methods for studying them were not available.

We are now able to quantify the microbial influence on biogeochemical processes, mainly as a result of huge methodological advances in environmental chemistry, epifluorescence microscopy, image analysis and the myriad of stains that enable visualization of the microbial world. Initially, fluorescent stains (Francisco and others 1973; Hobbie and others 1977; Porter and Feig 1980) were used primarily to quantify bacterial abundance and biomass, but nowadays there are stains available that enable the quantification of many aspects of microbial metabolism (respiration, degradation enzymes) and the visualization of previously unobservable components, such as viruses (Suttle 1993; Noble and Fuhrman 1998). When coupled with image analysis (Psenner 1990), stains can be used, not only to count bacteria but also to determine their biomass. Radioisotopes (Fuhrman and Azam 1982; Simon and Azam 1989) and fluorescent stains (Tranvik 1997; Cotner and others 2001) have allowed the measurement of bacterial growth rates independent of other food web components. Advances in environmental chemistry (stable isotopes, ultafiltration, dissolved organic carbon [DOC], high performance liquid chromatography (HPLC), dissolved inorganic carbon [DIC], and O_2) have also furthered our ability to study the influence of microbes on biogeochemical processes.

Furthermore, molecular methods have led to refinements in our understanding of the prokaryotic components in the planktonic food web. We know that a small but relatively constant proportion of prokaryotic microbes growing in the water column are Archaea (1%–10%) (Massana and others 1997; Aravalli and others 1998), whereas the remainder are Bacteria. In this review, we focus primarily on the effects of heterotrophic prokaryotes (comprised of members from Archaea and Bacteria) on biogeochemical processes. We use the terms "prokaryotic heterotroph" and "bacteria" (no italics) interchangeably. Because many of these organisms have multiple trophic roles, it is becoming more and more difficult to discriminate between autotrophy and heterotrophy.

The purpose of this review is to describe current perspectives on the role of prokaryotic heterotrophs in pelagic ecosystem processes. To accomplish this, we use a cross-system continuum, similar to that of Legendre and Rassoulzadegan (1995), from low-nutrient-availability oligotrophic systems to high-nutrient eutrophic systems, because great insight can be gained by looking at the differences in these systems and the biomass and activity of the associated microbes. We have included studies from both freshwater and marine systems. Although many advances in aquatic microbial ecology have come from work performed in marine systems, insights can also be gleaned from the freshwater literature that either confirm or challenge work done in marine systems. This review is an attempt to bring together important cross-system observations from both limnetic and marine microbial ecology.

Our thesis is that the microbial influence on pelagic processes in aerobic water columns is maximized in oligotrophic systems. As systems increase in productivity, so does the "relative" influence of particle-feeding heterotrophs, such as protozoans, zooplankton, and fish, and the relative influence of osmotrophic heterotrophs is somewhat diminished. We are not, however, suggesting that microbes do not play important roles in the biogeochemistry and food webs of eutrophic and hypereutrophic systems. In fact, in absolute terms, both microbial abundance and activity are routinely higher in eutrophic than in oligotrophic waters. Further, many anaerobic biogeochemical processes that are entirely mediated by microbes, such as methanogenesis and sulfate reduction, are accentuated in eutrophic systems, but that is not the focus of this review.

The Microbial Loop in an Ecosystem Context

Perhaps the most important conceptual advance in this area was the recognition that Lindeman's "bacterial ooze" was really a food web in and of itself. The idea of a microbial food web was first proposed by Pomeroy in the mid-1970s (Pomeroy 1974) and developed further in a key paper published in the early 1980s (Azam and others 1983). Both of these papers explored the idea that there are multiple trophic levels (bacteria, autotrophic picoplankton, flagellates, and ciliates) contained within the microscopic world and outlined the important implications for food webs and biogeochemical fluxes. This microbial trophic structure was referred to as the "microbial loop" (Azam and others 1983). Subse-

quent to the elucidation of the microbial loop concept, there was considerable discussion of the implications of these organisms to ecosystem function (Ducklow and others 1986). The focus of these discussions has been on the impact of the microbial loop on the cycling of matter—that is, nutrients—and the dissipation of energy (respiration).

The idea that the microbial loop manifests its largest relative impact in oligotrophic pelagic ecosystems was discussed by Legendre and Rassoulzadegan (1995). They argued that pelagic food webs vary from dominance by herbivorous consumers and their predators to dominance by microbial consumers and their predators—that is, the microbial loop—with intermediate mixes of these end members forming a sort of trophic continuum. The end-member regions (complete dominance by herbivores and/or the microbial loop) are unstable. Therefore, pelagic systems tend toward "multivorous," more stable systems (Legendre and Rassoulzadegan 1995). In this review, we will explore the factors that push these food webs toward one end member or the other.

One approach is to simply examine how the biomass at the base of the microbial loop—that is, bacterial biomass—varies from the most oligotrophic to the most productive systems. These comparisons indicate that prokaryotic heterotroph abundance generally varies by less than two orders of magnitude from oligotrophic to eutrophic systems (Cole and others 1988; Cole and Caraco 1993), varying from 0.5 to 1×10^6 cells ml^{-1} in oligotrophic systems and 1 to 10×10^6 cells ml^{-1} in eutrophic systems. At 20–30 fg carbon (C) per cell (Lee and Fuhrman 1987), this translates to 10^{-5} to 10^{-4} g C L^{-1}. This contrasts with the herbivorous food web, where the biomass of different components varies quite a bit more. Along a trophic gradient, phytoplankton biomass can vary from 10^{-7} to 10^{-1} g C L^{-1} and zooplankton can vary from 10^{-3} to 10^{-1} g C L^{-1} (McCauley and Kalff 1981; Ducklow and Carlson 1992). Gasol and Duarte (2000) used published studies to show that a log-log plot of prokaryotic heterotroph abundance against chlorophyll had a slope of less than 0.5 and used this and other relationships to conclude that the relative importance of bacteria in pelagic food webs decreases with increased chlorophyll concentrations.

It should be noted, however, that most studies that have made comparisons such as these have primarily estimated prokaryotic heterotroph biomass by using abundance and then applying a constant estimate of carbon content per cell. More recently, it has been shown that per cell estimates of 20–30 fg C $cell^{-1}$ are probably too high, with most cells being closer 10–20 fg C $cell^{-1}$, especially in oligotrophic systems (Loferer-Krößbacher and others 1998; Cotner and others 2001). Because of their small size and the fact that volume varies as a cube of the cell radius, small differences in size have a large effect on biomass. Therefore, the apparent dampened changes in prokaryotic heterotroph biomass across trophic gradients may be partially an artifact of not having accurate biomass measurements in most studies.

Low Bacterial Biomass Relative to Phytoplankton in Eutrophic Systems: Importance of Bacterivory and Viral Mortality

The relatively low variation in bacterial biomass across trophic gradients could be the outcome of something that dampens bacterial biomass relative to other food web components at the high end or increased biomass at the low end of this gradient. This paradox has perplexed many microbial ecologists. It has been argued that bacterial biomass is dampened in eutrophic systems by increased protozoan grazing (Sanders and others 1992) or increased viral mortality (Weinbauer and others 1993; Weinbauer and Peduzzi 1995). It has also been proposed that organic carbon is less available to bacterioplankton in eutrophic systems due to reduced relative phytoplankton exudation and/or more sedimentation (Gasol and Duarte 2000).

Despite the fact that prokaryotic heterotroph abundance is constrained relative to other pelagic food web components, productivity varies by about five orders of magnitude in different marine systems (from 0.02 to more than 2000 μg C L^{-1} d^{-1}) (Ducklow and Carlson 1992), implying that mortality must increase in proportion to abundance to compensate. Sanders and others (1992) argued that prokaryotic heterotroph biomass is constrained by nutrient availability in oligotrophic systems and that grazers (primarily flagellates) consume bacterial biomass at increasing rates as systems become more productive. Other researchers have demonstrated that protozoan bacterivores select the largest, fastest-growing cells (Sherr and others 1992). Therefore, in eutrophic systems with a higher frequency of large, rapidly growing cells, protozoan bacterivory would increase as well.

Alternatively, others have proposed that viral lysis is an increasingly likely fate for bacteria in eutrophic systems. Weinbauer and Peduzzi (1995) showed that viral abundance increased proportionately with prokaryotic heterotrophs across spatial

(trophic) gradients in the Adriatic Sea. The frequency of viral-infected cells increased across these same gradients. Because heterotrophic nanoflagellates did not vary as strongly as viruses with prokaryotic heterotroph abundances, they suggested that viral-mediated mortality is the most important constraint in eutrophic systems and that heterotrophic nanoflagellate-mediated mortality is the most important prokaryotic heterotroph constraint in less productive systems. This pattern was supported by Gasol and Duarte (2000), who showed a stronger correlation between bacteria and viruses than between bacteria and nanoflagellates. Enhanced rates of flagellate grazing by ciliates and/or larger zooplankton in eutrophic ecosystems may decrease their role in these systems.

Decreased Availability of Primary Production as a Constraint

Another possible explanation for decreased bacterial influence in eutrophic systems is that organic C is somehow less available to bacteria in productive ecosystems. At first, this idea seems counterintuitive because growth rates and growth efficiencies tend to be high in productive ecosystems (del Giorgio and Cole 1998; Biddanda and others 2001). Nonetheless, DOC often accumulates in freshwater and marine systems during phytoplankton blooms, suggesting either that heterotrophic microbial metabolism cannot keep up with phytoplankton production, or that DOC produced under bloom conditions is less susceptible to microbial attack (Sherr and Sherr 1996). In the Mississippi River plume, DOC accumulated in the region of maximal phytoplankton production and was dominated by high-molecular-weight material, mostly carbohydrates. Although this material was abundant, it was not readily consumable by prokaryotic heterotrophs (Amon and Benner 1996; Gardner and others 1996), suggesting that microbes were not able to decompose the material as fast as it was being produced.

If there were systematic differences in the proportion of primary production that is excreted by phytoplankton, this too, could make DOC less available to bacterioplankton in eutrophic systems than in oligotrophic systems. When Baines and Pace (1991) examined this issue using data extracted from studies in both marine and freshwater systems, they concluded that extracellular release of organic C increased as a constant proportion of primary production; thus, they were unable to confirm the hypothesis that excreted DOC (EOC) is less available to prokaryotic heterotrophs in eutrophic systems. However, when they examined freshwater lakes independent of marine systems, they found that EOC release was relatively constant in eutrophic systems, suggesting that, at least in lakes, a decreased proportion of primary productivity was available to prokaryotic heterotrophs in eutrophic systems. Furthermore, several studies in marine systems have shown that EOC as a proportion of primary production decreases with increasing trophic state (Anderson and Zeutschel 1970; Thomas 1971; Fogg 1983).

Two other factors that could make organic C less available to bacterioplankton in eutrophic systems relative to oligotrophic systems are sinking and herbivory. If sinking fluxes are a greater proportion of primary production in eutrophic systems than in oligotrophic systems, then a smaller fraction of the organic matter produced would be available for prokaryotic heterotroph decomposition, all other factors being equal, in eutrophic systems. Productive oceanographic regions where there is significant new production are regions of high export of organic matter from the euphotic zone (Peinert and others 1989). Baines and others (1994) examined this issue in oceans and lakes and found that export ratios (the fraction of primary production lost to sinking) increased with productivity in oceans and decreased slightly in lakes. Therefore, one would expect that lakes would have a higher proportion of primary production available for heterotrophic growth in eutrophic lakes than in comparable eutrophic regions of the ocean. Consistent with this observation, a cross-system comparison showed that the ratio of bacterial C to phytoplankton C was higher in lakes than in oceans, but the trend did not show an increasing deviation in the most productive systems (Simon and others 1992).

There have been relatively few cross-system comparisons of herbivory impact on primary production, and their conclusions are somewhat ambiguous. Many experimental studies in freshwater lakes have suggested that herbivory (primarily by zooplankton) is most important in oligo- to mesotrophic lakes, and a smaller proportion of primary production is grazed in eutrophic systems (McQueen and others 1986; Elser and Goldman 1991). However, an intercomparison of many systems of differing trophic status did not show a decreased effect of herbivory in eutrophic systems (Cyr and Pace 1993), and another study showed that herbivory increased with increasing nutrient content of autotrophs (decreasing C:nutrient ratios) (Cebrián and others 1998). More nutrient-rich autotrophs were observed in the most productive ecosystems, implying that herbivory should be an increasingly

important loss for primary production in eutrophic systems.

High Bacterial Biomass Relative to Phytoplankton in Oligotrophic Systems

Alternatively, dampened changes in bacterial biomass across a trophic gradient may be interpreted as increased prokaryotic heterotrophic biomass relative to other biological components in oligotrophic ecosystems—that is, overrepresentation of these organisms at the low end of the trophic gradient. It is becoming clear that in oligotrophic systems, both marine and freshwater, small prokaryotic organisms dominate the microbial biomass. Fuhrman and others (1989) showed that prokaryotic heterotrophs were a dominant biomass component in the North Atlantic, comprising 70%–80% of the microbial C and N in the euphotic zone. Estimates of the size of the bacterial biomass component in the Sargasso Sea decreased with the recognition that another prokaryote, *Prochlorococcus sp.*, a bacterial-sized photoautotroph, was likely counted as a heterotrophic bacterium using epifluorescence methods. Revised measurements using a cooled CCD camera (Olson and others 1993) or the now more commonly used flow cytometric methods (Olson and others 1990) show that prokaryotic heterotrophs are still a very large component of the microbial biomass and that the other two major components in the oligotrophic oceans are the prokaryotic photoautotrophs, *Prochlorococcus* and *Synechococcus* (Karl 1999). In the North Pacific, approximately half of the sestonic particulate organic carbon (POC) is bacterial organic C (Cho and Azam 1988), and a similar relationship has been observed in the Sargasso Sea (Cotner 2000). Similarly, in the oligotrophic Laurentian Great Lakes, prokaryotic heterotrophs and *Synechococcus* are large components of the microbial biomass (Fahnenstiel and others 1998). In a survey of the marine literature, Ducklow and Carlson (1992) showed that biomass of prokaryotic heterotrophs can exceed algal biomass in systems where chlorophyll levels are less than 0.05–1 $\mu g\ L^{-1}$. Our observations across a trophic gradient in Minnesota lakes showed that prokaryotic heterotrophs represented a variable percentage of the seston C, nitrogen (N), and phosphorus (P), with highest relative composition in the most oligotrophic system, Lake Superior (Figure 1) (Biddanda and others 2001) and decreasing percentages of all nutrients as total seston concentrations increased.

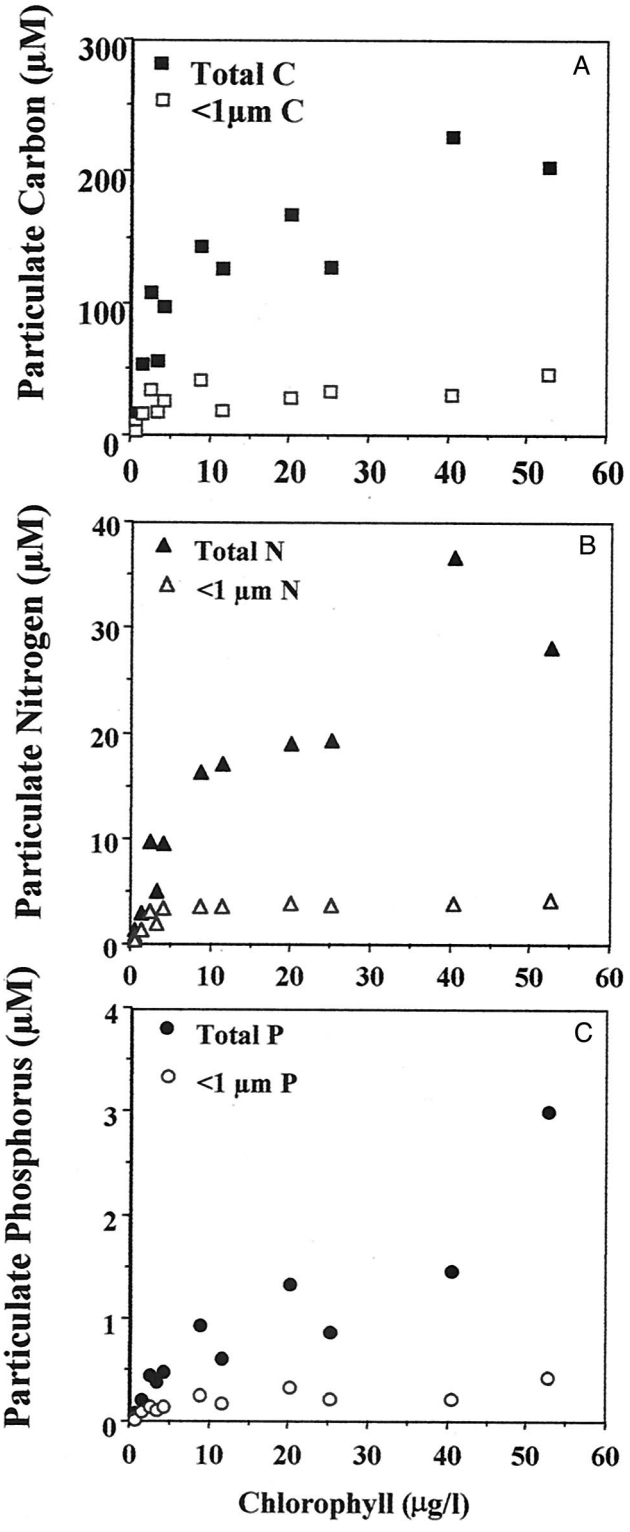

Figure 1. Total and less than 1 µm particulate organic (**A**) carbon, (**B**) nitrogen, and (**C**) phosphorus along a trophic gradient in Minnesota lakes. Microscopic analysis demonstrated that the less than 1 µm size-fraction is primarily composed of prokaryotic heterotrophs. Chlorophyll concentrations along the x-axis were used to indicate the trophic state of these systems.

Several reasons have been proposed to explain why prokaryotic heterotroph biomass is highest relative to phytoplankton biomass in oligotrophic systems, but the predominant explanations relate to allochthonous C subsidies being important to bacterial production in oligotrophic systems, decreased bacterivory, and/or prokaryotic heterotroph access to nutrients that either are not available to phytoplankton, or just not available to them at the low ambient concentrations in oligotrophic systems.

Allochthonous C Subsidies

An increased relative abundance of dissolved organic nutrients could contribute to an increased prokaryotic heterotroph and microbial food web role in oligotrophic systems. In the central gyres of the North Atlantic (Michaels and others 1996) and North Pacific (Karl 1999), almost all of the N and P in these systems is dissolved and organic. However, much of the organic matter is old and therefore slow to degrade, given a mean age of deep-water oceanic DOC of approximately 5000 years (Williams and Druffel 1987).

Nevertheless, a variable fraction of aquatic DOC is available, and it supports a relatively high biomass of bacteria. The fact that most of the nutrients in oligotrophic systems are associated with dissolved organic molecules may benefit prokaryotic heterotrophs in competition with phytoplankton for nutrients and in competition with other heterotrophs for organic C. Typical particulate P concentrations in the oligotrophic oceanic gyres are 10 nM with dissolved organic P concentrations nearly 10 or more times this value. In our survey along a trophic gradient in freshwater lakes, the ratio of dissolved organic matter to dissolved inorganic nutrients was higher and more variable in oligotrophic systems and low—that is, relatively more inorganic nutrients—in eutrophic systems (Figure 2). In freshwater lakes, prokaryotic heterotrophs consumed organic phosphomonoesters at higher rates than phytoplankton at ambient concentrations (Cotner and Wetzel 1992). A bacterially produced enzyme (5′-nucleotidase) was shown to be important to both organic C and P uptake in marine (Ammerman and Azam 1985) as well as freshwater systems (Cotner and Wetzel 1991). Similarly, almost all amino acid uptake in marine systems was by the prokaryotic heterotroph size-fraction (Wheeler and Kirchman 1986).

Recent evidence of net heterotrophy (when respiration exceeds primary production) suggests that organic matter inputs from other ecosystems may supplement bacterial metabolism in lakes. We recently showed that allochthonous DOC accumulates in Lake Michigan in the winter and is subsequently consumed by prokaryotic heterotrophs in the summer (Cotner and others 2000; B. A. Biddanda and J. B. Cotner forthcoming). As much as 10% of the microbial carbon demand may be met by organic carbon imported from rivers over this period (Biddanda and Cotner forthcoming). In a survey of lakes of varying trophic status, low-productivity systems were the ones most consistently net heterotrophic (del Giorgio and Peters 1994). Many lakes are supersaturated with carbon dioxide (CO_2), further suggesting net heterotrophic production (Cole and others 1994). A comparison of both freshwater and marine systems similarly showed that the most unproductive ecosystems require organic C subsidies from other regions within the system or from terrestrial habitats to maintain net heterotrophic conditions (Duarte and Agustí 1998).

The idea that oligotrophic systems are net heterotrophic is controversial. Pelagic studies often ignore production in the littoral (macrophytes and periphyton), which can be a significant component, especially in shallow lakes. There may be further methodological problems associated with observations of net heterotrophy. Recently, Carignan and

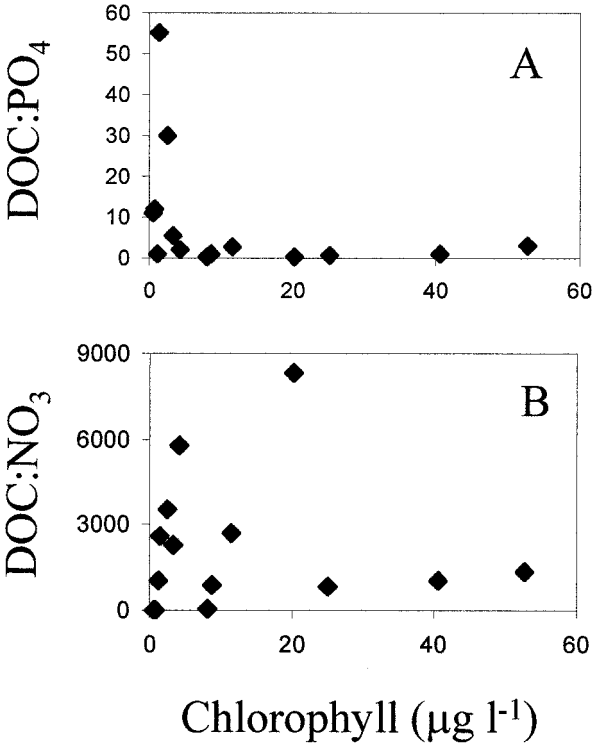

Figure 2. Ratios of (**A**) DOC:PO_4 (SRP mol:mmol) and (**B**) DOC:NO_3, (mol:mol) in a survey of Minnesota lakes. Ratios are plotted against chlorophyll concentrations to indicate the lake's trophic state.

others (2000) argued that several studies inferring net heterotrophy using the ^{14}C method for primary production measurements have underestimated production because they "missed" EOC production by phytoplankton. Furthermore, as we have discussed, EOC is likely to represent a larger fraction of primary productivity in oligotrophic waters, perhaps biasing these measures to net heterotrophy in these systems. Clearly, more studies need to be performed addressing the importance of allochthonous C on an ecosystem scale to aquatic systems.

Access to Nutrients

It is not clear at this time what enables prokaryotes to dominate in oligotrophic systems, but it may have to do with lower energetic costs associated with their simple biomass composition (Neidhardt and others 1990) or their high affinity for inorganic nutrients (Button 1986), which is also related to their small size. Small prokaryotic cells may be better adapted to low-nutrient environments simply because they have less internal machinery; consequently, their cells can be smaller than eukaryotes. A survey of marine and limnetic systems recently showed that picophytoplankton increase as a proportion of the total phytoplankton community in oligotrophic systems (Bell and Kalff 2001), suggesting that the same advantages that small prokaryotic heterotrophs have in oligotrophic systems are also advantageous to autotrophs as well. In spherical cells, as the cell gets smaller in diameter, the surface to volume ratio increases; therefore, there are fewer internal demands for nutrients and an increased relative capacity to supply those nutrients.

Furthermore, as growth rates increase, prokaryotic heterotroph cells increase in size (Ammerman and others 1984), but the internal density decreases (Simon and Azam 1989), decreasing internal viscosity and increasing diffusion rates. In eutrophic systems, external nutrient concentrations are higher, so cells can be larger and adopt more varied life history strategies. Because of increased complexity in their life history and their increased size, they require more intracellular machinery (endoplasmic reticulum, Golgi apparatus, and so on) to move materials around inside of the cell. Under these conditions, diffusion and movement of materials within the cell are important constraints on single-celled organisms (Koch 1996).

There are several ecological strategies that can ensure cell survival under conditions of low nutrient availability. One strategy is to reduce the cell's requirements for a given nutrient; another is to acquire the nutrient more effectively than other organisms. One way to reduce the requirement for nutrients is to grow more slowly, but a population of cells runs the risk of extinction if competing organisms grow faster (assuming losses are greater than zero). Alternatively, if a cell grows but also uses nutrients more efficiently, it could gain an ecological advantage. One way to use nutrients more efficiently is to decrease the requirement for a nutrient, either by decreasing in size or by reducing the relative quantity of a particular nutrient in the cell. Organisms that use the latter strategy should have lower nutrient biomass composition at growth rates similar to other organisms. In fact, what has been observed is that prokaryotic heterotrophs are generally richer in nutrients, such as N and P, than other sestonic components (Bratbak 1985; Nagata 1986; Vadstein and others 1988). Carbon–phosphorus (C:P) ratios of bacterial biomass measured under varying conditions can be as high as 200–500:1 (Tezuka 1990; Elser and others 1995a) or as low as 10:1 (Bratbak 1985). However, there seems to be a growing consensus that prokaryotic heterotrophs have lower C:P ratios than phytoplankton (that is, they are richer in P). Phytoplankton biomass in oligotrophic oceans is typically near the Redfield ratio (C:P 106:1) (Redfield 1958). Freshwater seston ratios in oligotrophic systems are typically higher than this value and more variable than marine systems (Hecky and others 1993; Sterner and others 1997).

There is some evidence to suggest that relatively slow growth is part of an adaptive strategy for prokaryotic heterotrophs in oligotrophic systems. In the oligotrophic subarctic Pacific, bacterial biomass turned over in 17–21 days, whereas phytoplankton biomass turned over in 1.0–2.1 days (Kirchman and others 1993). Our measurements in Lake Superior indicate a similarly slow prokaryotic heterotroph growth rate in this ultraoligotrophic lake. In the oligotrophic Sargasso Sea, bacterioplankton biomass turns over once every 5–15 days and phytoplankton biomass turns over every 0.5–2 days (Fuhrman and others 1989; Carlson and others 1996). However, in all of these studies, prokaryotic heterotroph turnover times are overestimated because many of the cells may not be active (Zweifel and Hagstrom 1995). Nonetheless, typically prokaryotic heterotroph biomass is turning over two to three times slower than phytoplankton in low-nutrient systems.

Prokaryotes apparently are able to acquire nutrients more effectively than other planktonic components in oligotrophic systems and compensate for their high nutrient content. Rhee (1972) showed that *Pseudomonas*, supplemented with glucose, could severely limit the acquisition of P by *Scenedes-*

mus in culture. Most of the field studies that have looked at "interdomain" competition between *Eucarya* and Bacteria/Archaea have examined relative uptake of P and N. These studies generally show that prokaryotic heterotrophs acquire these inorganic nutrients very effectively at low nutrient concentrations in nature; whereas, as concentrations increase, an increasing proportion is acquired by phytoplankton (osmotrophs larger than 1 μm) (Currie and Kalff 1984; Suttle and Harrison 1988; Cotner and Wetzel 1992). Because of their small size, prokaryotic heterotrophs have a high affinity for dissolved nutrients (low relative K_t) (Button 1986), but consequently they are not able to take up nutrients as effectively at high nutrient concentrations (low relative V_{max}) (Fenchel and others 1998). There is a great deal of variation in the K_t's and V_{max}'s of aquatic prokaryotic heterotrophs; nevertheless, these generalities suggest that these organisms should acquire nutrients more effectively than their phytoplankton competitors when ambient concentrations are low. In oligo- and mesotrophic freshwater lakes, a significant proportion of ambient P is consumed by prokaryotic heterotrophs (Rigler 1956; Bentzen and others 1992; Cotner and Wetzel 1992) and this can constrain autotrophic production. In eutrophic systems where inorganic loading rates are high, phytoplankton are able to sequester an increased fraction of the available nutrients because of their larger size (Suttle and others 1990; Cotner and Wetzel 1992). Because nutrients are often delivered to lakes and the coastal ocean episodically, there is strong selection for large cells that can maintain uptake of a potentially limiting nutrient and store it internally for subsequent growth (Suttle and others 1987).

A further consequence of prokaryotic heterotroph small size is that there is little room in the cell to store nutrients. Some diatoms are able to store enough P as polyphosphate to support nearly 100 subsequent generations (Horne and Goldman 1994). Although prokaryotic heterotrophs are also able to store polyphosphate, this strategy is probably not employed in oligotrophic systems given their small size; however, this topic has not been studied extensively.

Surprisingly, there is considerable evidence indicating that prokaryotic heterotroph growth is often limited by inorganic nutrients (especially P) in many different kinds of ecosystems (Toolan and others 1991; Coveney and Wetzel 1992; Elser and others 1995b; Pomeroy and others 1995; Cotner and others 1997), even relatively productive ones. This is consistent with the relatively low C:P that has been measured in these organisms. Previously, most researchers assumed that prokaryotic heterotroph growth was limited by the organic C supply, most of which came from phytoplankton productivity. Interestingly, one of the important ideas proposed by Azam and others (1983) in their microbial loop paper was that the primary mineralizers of inorganic nutrients in aquatic ecosystems are not prokaryotic heterotrophs but their grazers, consistent with the idea that prokaryotic heterotroph growth could be nutrient-limited.

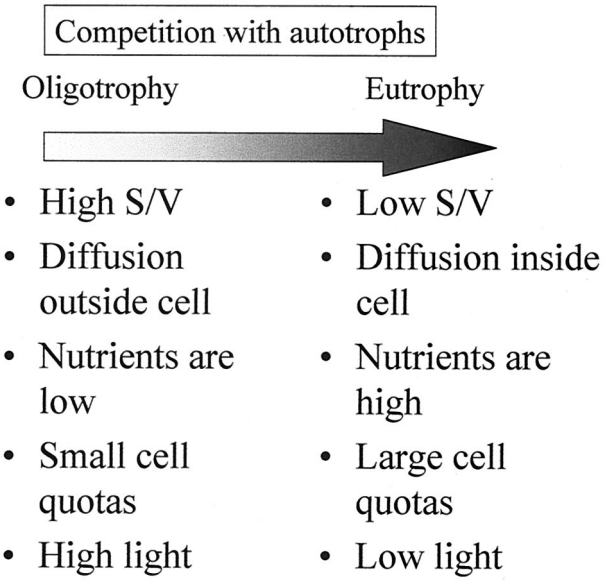

Figure 3. Hypothesized factors affecting cell size and nutrient competition among autotrophs and heterotrophs in oligotrophic and eutrophic systems. Competition with prokaryotic heterotrophs is an important constraint to phytoplankton biomass production, especially in oligotrophic systems. S/V refers to the surface-to-volume ratio.

We summarize changes in nutrient competition along a trophic gradient in Figure 3. As we have discussed, low nutrient availability selects for small prokaryotic heterotrophs in oligotrophic systems because of their small size, high surface-to-volume ratios, and small cell quotas. High ambient nutrient availability in eutrophic systems selects for larger osmotrophs, primarily filamentous and colonial cyanobacterial and eukaryotic photoautotrophs, facilitated internal diffusion (in eukaryotes) and internal nutrient storage abilities.

Interactions of Solar Radiation, DOC, and Nutrients

Nutrients and DOC may select for small prokaryotic organisms in oligotrophic systems, but solar radia-

tion may also play a role. There has been a plethora of studies on the effects of ultraviolet (UV) radiation on aquatic organisms, but few community to ecosystems scale experiments have been performed. In one study performed in the St. Lawrence Estuary, UV radiation was manipulated and the effects on mixed communities were observed. These experiments showed that the relative abundance of diatoms decreased and flagellated autotrophs and heterotrophs increased when UV radiation was increased artificially (Mostajir and others 1999). Concomitant with changes in autotrophs, there was a shift in the planktonic heterotroph community structure from herbivorous zooplankton to a microbially dominated food web, suggesting that UV radiation can redirect organic matter fluxes from the herbivorous to the microbial food web in ecosystems.

One mechanism whereby UV radiation can affect the quantity of organic C passing through the microbial loop is through direct photochemical alteration of the bioavailability of dissolved organic matter (DOM). An interesting study in the Gulf of Mexico looked specifically at the effects of UV radiation exposure on the availability of DOC to bacteria that were not exposed to UV radiation (Benner and Biddanda 1998). DOC from the mixed layer became more recalcitrant to prokaryotic heterotroph degradation with UV exposure. Alternatively, deep-water DOC became more labile to prokaryotic heterotroph degradation, and growth rates increased after organic matter was exposed to UV radiation. We recently confirmed these observations in Lake Superior during stratification (B. A. Biddanda and J. B. Cotner unpublished).

Many studies have shown that UV radiation can enhance prokaryotic heterotroph activity in humic-rich waters (Lindell and others 1995; Wetzel and others 1995; Miller and Moran 1997; Reitner and others 1997). Humic substances absorb strongly in the UV range and therefore are some of the most photoreactive compounds in lake and seawater (Bertilsson and Tranvik 2000). Furthermore, several studies have shown that UV radiation can release labile P (Francko and Heath 1982; Cotner and Heath 1990) and N (Bushaw-Newton and Moran 1999; Kieber 2000) at low concentrations from dissolved humic substances. Similarly, organic matter from freshwater macrophytes was more readily consumed by prokaryotic heterotrophs after exposure to UV radiation (Wetzel and others 1995). These experiments demonstrate that DOC in different regions of the water column or from different source material can be variably available to bacterioplankton. The degree of previous exposure to UV radiation may constrain how much organic matter prokaryotic heterotrophs can extract. Furthermore, these experiments, and others like them, suggest that prokaryotic heterotrophs may "compete" with abiotic factors, such as UV radiation for labile DOC, at least in some environments, such as the mixed layer in high-light ecosystems (Figure 3).

Other portions of the solar spectrum, such as photosynthetically active radiation (PAR) may be equally important in regulating the flow of organic matter into the microbial loop. If phytoplankton nutrient uptake is light-dependent (Lean and Nalewajko 1976; E. Litchman personal communication) and prokaryotic heterotroph uptake is not, then it would explain the increased ratio of phytoplankton to prokaryotic heterotroph biomass observed in the Sargasso Sea when the mixed layer is shallow (Cotner 2000). As mean light levels increase—that is shallower mixed layer—autotrophic picoplankton may compete more effectively for inorganic nutrients. In Third Sister Lake, Michigan, we observed an increased flux of inorganic P into phytoplankton biomass as the lake stratified in the summer (Cotner and Wetzel 1992).

In oligotrophic marine systems, DOC often accumulates in the mixed layer when these systems become more strongly stratified (Carlson and others 1994; Murray and others 1994; Williams 1995). As stratification increases, the physical separation between the mixed layer and nutricline increases; and as phytoplankton compete more effectively for inorganic nutrients, nutrients become more limiting to productivity. DOC may accumulate under these conditions either because it does not limit prokaryotic heterotroph productivity—that is, they are limited by inorganic nutrients (Toolan and others 1991; Coveney and Wetzel 1992; Pomeroy and others 1995; Cotner and others 1997; Zohary and Robarts 1998)—and/or perhaps DOC accumulates as it becomes more unavailable due to longer UV radiation exposure (Benner and Biddanda 1998). If phytoplankton are more competitive for inorganic nutrients as the mixed layer decreases, it would increase the likelihood that prokaryotic heterotrophs are limited by nutrients rather than organic C.

A recent model of the microbial food web along a trophic gradient has been used to explain DOC accumulation in oligotrophic lakes and oceans (Thingstad and others 1997). In this model, prokaryotic heterotroph growth was shown to be controlled by DOC availability in low-P, ultraoligotrophic systems, by bacterivores at moderate P levels, and—interestingly—by P concentrations again at the highest P-loading rate (Thingstad and others

1997). This apparent paradox arises in the model because, at high P-loading rates, phytoplankton consume an increased proportion of available P due to their increased biomass. It is in this latter range where DOC accumulates. Although the model represents a trophic gradient, all of the nutrient concentrations were in a range that would qualify these systems as oligo- to mesotrophic. In support of this conclusion, Sondergaard and Middleboe (1995) showed that the affinity of prokaryotic heterotrophs for DOC decreases along a trophic gradient—that is, DOC in eutrophic systems is less susceptible to microbial attack—but this could also be due to several other factors, such as increased bacterial grazing, decreased EOC release, or increased bacterial growth efficiency (BGE).

In the Thingstad model, DOC accumulates in the euphotic zone of lakes and oceans in moderately productive systems because of competition with phytoplankton for a limiting nutrient (P). An alternative view, which is consistent with the idea of nutrient-limited microbial growth in oligotrophic systems and bacterivore or viral control in more productive systems, is that in oligotrophic systems, prokaryotic heterotroph growth is P-limited, BGEs are low, and DOC does not accumulate. As P loading increases, microbial biomass can accumulate sufficiently to stimulate bacterivore or viral biomass; these predators then control prokaryotic heterotroph biomass. At that point, DOC concentrations can accumulate in the euphotic zone as a consequence of increased predator control of biomass and increased BGEs. This view is supported by the fact that prokaryotic heterotroph biomass does not increase to the same extent as other food web components in eutrophic systems. Furthermore, the increased BGEs found in eutrophic systems (del Giorgio and Cole 1998; Biddanda and others 2001) suggest that microbes are able to use organic C more efficiently in these systems.

Biogeochemical Implications

The dominance of prokaryotic heterotrophs in oligotrophic systems becomes even more apparent when we look at metabolic processes, such as growth rates and respiration. Maximal potential growth rates and metabolic processes scale proportionately to organism size ($\text{volume}^{-0.25}$) (Fenchel and others 1998). Consequently, small organisms such as prokaryotes are able to dominate metabolic processes in ecosystems where they are abundant relative to other components—for instance, in oligotrophic lakes and oceans. Prokaryotic heterotrophs are the most significant component of total community respiration in oligotrophic systems, whereas their relative contribution decreases in eutrophic systems (Biddanda and others 1994, 2001; del Giorgio and others 1997). In our trophic survey in Minnesota lakes, and including some previous measurements from the Gulf of Mexico, the prokaryotic heterotroph size-fraction generated as much as 90% of the total community oxygen demand in the most oligotrophic systems and as little as 10% in the most eutrophic lakes (Biddanda and others 2001).

Part of the explanation for the important role played by prokaryotic heterotrophs in oxygen and carbon fluxes in oligotrophic systems has to do with their high proportional biomass, but changes in BGE also occur along this gradient and impact the relationship. BGEs typically increase from oligotrophic to eutrophic systems (del Giorgio and Cole 1998). A simple mechanistic explanation for this observation is that prokaryotic heterotroph growth is more likely to be nutrient-limited in oligotrophic systems; therefore, organic C is used less efficiently. del Giorgio and Cole (1998) argued that the maintenance costs for aquatic bacteria increase in low-productivity systems. In our lake survey, BGEs increased from as low as 5% in our most oligotrophic systems to as high as 40% in the most eutrophic systems (Biddanda and others 2001). Off the southeast coast of the United States, Griffith and others (1990) and Hopkinson and others (1989) showed an increasing near-shore to offshore gradient in the contribution of the less than 1 μm size-fraction to community respiration. Modeling and experimental evidence indicated that BGEs increased inversely with the C:N ratio of their substrates, implicating substrate stoichiometry and nutrient limitation as factors responsible for low BGE.

An important implication of such high quantities of organic C passing through prokaryotic heterotrophs in oligotrophic systems with most of it being respired is that there is little left over to support productivity at higher trophic levels (Ducklow and others 1986; Legendre and Rassooulzadegan 1995). Most of the C and nutrients that pass into the microbial loop pass out as inorganic nutrients and CO_2. Broad support for this observation can be found by examining the relative proportions of dissolved and particulate nutrients across a trophic gradient. In oligotrophic systems, dissolved components predominate over particulate nutrients, and the particulate components increase in eutrophic systems (Wetzel 1984). In lakes and streams, DOC:POC ratios of 6–10:1 are typical (Wetzel 1995). In our survey of Minnesota lakes, the DOC:POC ratio varied from 3 to 15:1, with the highest values in

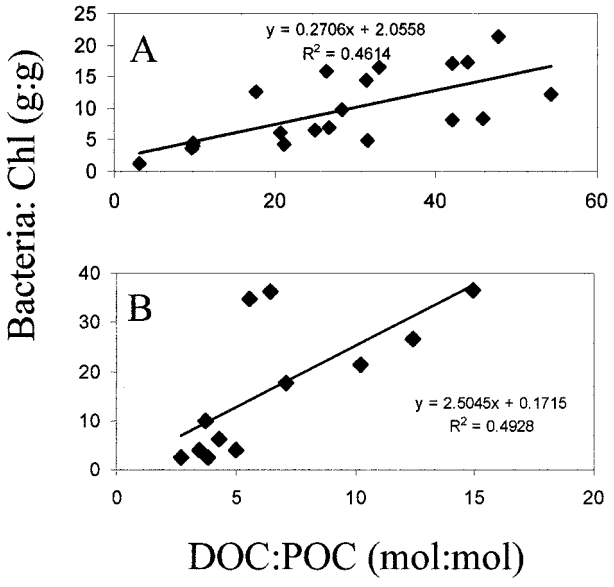

Figure 4. The relationship between the DOC:POC ratio and prokaryotic heterotroph (bacteria) biomass normalized to phytoplankton biomass. Chl = chlorophyll. (**A**) is based on data from del Giorgio and Peters (1992); (**B**) is derived from data collected by J. B. C. and B. A. B. in Minnesota lakes.

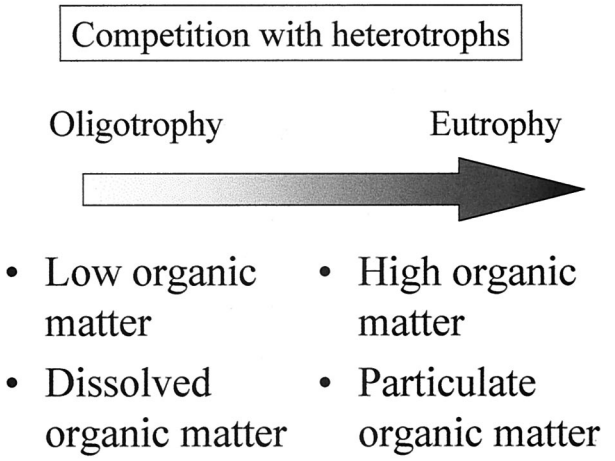

Figure 5. Hypothesized factors affecting prokaryotic heterotroph interactions with eukaryotic, phagotrophic heterotrophs. In oligotrophic systems, high relative DOC:POC ratios select for prokaryotic heterotrophs over phagotrophs because of the limited ability of phagotrophs to consume dissolved organic matter. In eutrophic systems, an increased proportion of available organic matter is particulate, thus selecting for phagotrophs.

oligotrophic systems (Biddanda and others 2001). In this survey, prokaryotic heterotroph biomass increased relative to phytoplankton biomass (chlorophyll) with an increase in the DOC:POC ratio (Figure 4). We found a similar increase in bacterial biomass relative to phytoplankton biomass in published data from the 1994 survey by del Giorgio and Peters of 20 southern Quebec lakes (Figure 4). These observations suggest that prokaryotic heterotrophs are most abundant relative to phytoplankton and other planktonic components when dissolved nutrients are high relative to particulate nutrient concentrations.

It has been argued that prokaryotic heterotrophs and metazoans compete for detrital resources in aquatic systems (Pomeroy and Wiebe 1988). Therefore, when most organic matter is dissolved rather than particulate, prokaryotic heterotrophs dominate; phagotrophic organisms and phytoplankton increase in relative abundance as particulate matter increases across the trophic gradient (Figure 5). Therefore, an important distinction between oligotrophic and eutrophic systems has to do with the dominant heterotrophs at the base of the food web. In herbivorous food webs, the dominant heterotrophs have mouths, whereas in microbial food webs they do not (Laws 1993).

Bacterial Metabolism and Export Production

The extent to which organic matter passes through the microbial loop is likely to affect the amount of organic matter subsequently available for higher trophic levels and also for export out of the euphotic zone. Systems in which a high proportion of organic matter passes through microbial heterotrophs and the microbial loop are likely to support less production in higher trophic levels because of the high number of trophic transfers and the low growth efficiency of prokaryotic heterotrophs and protozoans in natural systems (Legendre and Rassooulzadegan 1995). Respiration measurements and other studies comparing C flow have shown that prokaryotic heterotroph production can be more than 100% of primary production at any given point in time (Scavia and Laird 1987; Ducklow and Carlson 1992). Bacterial production is not necessarily constrained to be less than primary production because of spatial or temporal disequilibria between primary and secondary productivity (Scavia and Laird 1987), allochthonous C inputs, or alternatively because bacterial carbon production is not "lost" from an ecosystem until it is respired (Strayer 1988). The ecosystems in which prokaryotic heterotroph production represents high proportions of primary productivity tend to be ones

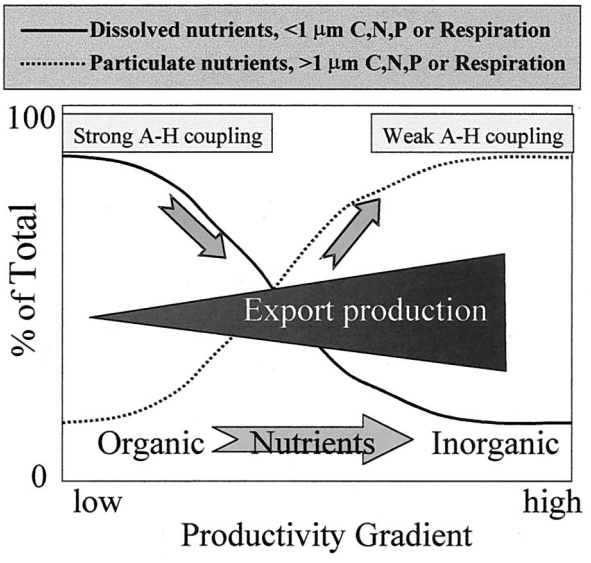

Figure 6. Changes in nutrient characteristics across a productivity gradient. In low-productivity systems, nutrients are primarily organic and dissolved, and autotrophic–heterotrophic coupling (A–H coupling) is strong, —that is, prokaryotic heterotroph respiration is equal to or greater than primary production, with little organic matter remaining for export to the sediments. In eutrophic systems, nutrients are primarily inorganic and particulate, and A–H coupling is weak, —that is, high export production.

where productivity and presumably export are relatively low (Ducklow and Carlson 1992) (Figure 6).

Another factor that may affect C flux through the microbial loop is temperature, with decreased relative fluxes occurring at low (near 0°C) temperatures (Pomeroy and Deibel 1986; Pomeroy and others 1991). These authors argued that during the spring algal bloom in cold North Atlantic waters, a decreased proportion of organic matter is dissipated through the microbial loop relative to warmer systems. Although net primary productivity is typically maximized in summer when insolation rates are highest, slight differences in autotrophic and heterotrophic production through the winter and spring could generate high spring biomass if the differences occur over a long enough period. Wetzel (1995) argued that winter productivity is an important part of ecosystem metabolism in small lakes. If bacterial production and respiration are more temperature-sensitive than photosynthesis, bacterial production and/or respiration should decrease more at low temperatures than does photoautotrophy. In a survey of the oceanographic literature, there was no difference in prokaryotic heterotroph production in cold and temperate oceans at their respective temperatures (Rivkin and others 1996), but subsequent work showed that BGEs decrease with increasing temperatures (Rivkin and Legendre 2001). We recently documented a similar relationship in Lake Michigan (Biddanda and Cotner forthcoming). The majority of the C flux through bacterioplankton is through respiration, typically representing 70%–100% of the bacterial C demand.

Observations of high relative prokaryotic heterotroph biomass, low BGEs, and high microbial respiration rates in oligotrophic systems suggest that nutrient regeneration efficiencies should be higher in oligotrophic systems than in eutrophic systems. However, a recent paper showed that phosphorus cycling efficiencies did not vary across a trophic gradient (Hudson and others 1999). The authors argued that regeneration was proportional to biomass and that regeneration efficiencies (the proportion of net production that is regenerated) are not higher in oligotrophic systems. An important aspect of this debate is whether particle sinking fluxes increase with productivity—that is, sinking fluxes increase disproportionately to productivity across a trophic gradient. Baines and others (1994) showed that sinking fluxes do increase more than productivity in the ocean but not in lakes, supporting Hudson and others' argument for freshwater systems. However, the data set for the relationship between productivity and sinking fluxes in lakes was limited to a set of small Canadian lakes that may or may not be representative of lakes as a whole. Sinking fluxes in small lakes measured with sediment traps (as was done in the 1991 study by Baines and Pace) are likely to overestimate particle export from the euphotic zone because of sediment resuspension and slumping. Even in large lakes such as Lake Michigan, resuspension can be the major mechanism for particle and C transport through the water column on an annual basis (Eadie and others 1984). Although resuspension is maximized in the unstratified period, organic matter and nutrients that are resuspended and incorporated into the food web (Cotner and others 2000) could potentially impact fluxes after the lake stratifies. The potential to overestimate this flux is particularly high in small lakes, where resuspension likely represents a major proportion of the particles raining down on the lake floor.

Conclusions

We have outlined some of the important changes that occur in microbial biogeochemical processes along a trophic gradient in lakes and oceans. The relationships we have discussed are summarized in

the "hourglass figure" (Figure 6), which describes the transformations in the material processing by microbes along a theoretical trophic gradient. We argue that prokaryotic heterotrophs mediate biogeochemical processes to a disproportionately high level in the most oligotrophic ecosystems, primarily because they can cope with low nutrient availability and consume dissolved organic nutrients (Figure 6). Because prokaryotic heterotrophs and the phytoplankton that are dominant in oligotrophic systems, primarily small cyanobacteria, are similar in size and have similar growth rates and nutritional modes (osmotrophic), euphotic zone autotrophic and heterotrophic processes are tightly coupled in these systems. This means that there is little net export of organic matter from the euphotic zone. Increased inorganic nutrient loading along the trophic gradient increases the mean growth rates and size of phytoplankton, as well as the total suspended particle concentrations. Increased phytoplankton size and particle concentrations shift the planktonic community from domination by osmotrophic heterotrophic microbes to domination by phagotrophic zooplankton. Consequently, the role of the microbial web is maximized in oligotrophic systems, and the herbivorous, zooplankton-dominated web is maximized in eutrophic systems (Legendre and Rassooulzadegan 1995). Because of this shift in the dominant heterotrophs, material fluxes are dominated by particles in more eutrophic systems. The maximum growth rates of most zooplankton are less than the maximum growth rates of most phytoplankton, and autotrophic and heterotrophic processes are more likely to be decoupled in the herbivorous web, with increased export production.

The processes discussed here are relevant to the human impact on productivity in aquatic ecosystems, both freshwater and marine. Because productivity and decomposition are nearly balanced in oligotrophic ecosystems, they are particularly vulnerable to small increases in nutrient loading. Nutrient increases in oligotrophic lakes and oceanic gyres can have strong feedbacks to productivity. There are many documented cases of cultural eutrophication in lakes and coastal ocean regions. However, increased use of fertilizers and the potential for long-range transport of nutrients that volatilize (N) or that are moved great distances on dust particles (P, Iron) may change the characteristics of biogeochemical processes in remote regions of the ocean (Hansell and Carlson 1998; Pahlow and Riebesell 2000), such as the central gyres, and seemingly remote lakes, such as Lake Superior (Bennett 1986). Furthermore, climate change (increased UV radiation, lower DOC) may compromise the ability of aquatic systems to respond to the increased loading of nutrients.

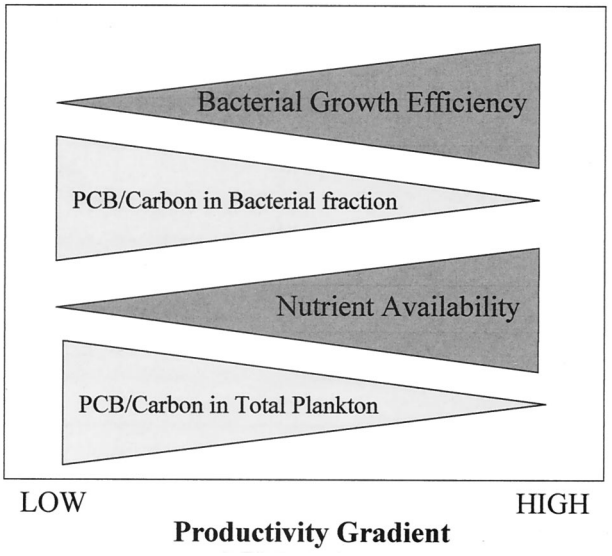

Figure 7. Hypothesized relationships between microbial metabolism and contaminant accumulation across a productivity gradient. BGE increases with increasing nutrient availability and productivity. PCB per unit of bacterial carbon is highest in the least productive waters and lowest in the most productive waters. A similar PCB–carbon relationship is expected to occur in higher plankton as well.

Another feedback for human-impacted systems relates to contaminants in aquatic food webs. Because oligotrophic systems are by definition nutrient-poor and biomass is dominated by prokaryotic heterotrophs and the microbial food web, there is great potential for bioconcentration of contaminants to occur through these organisms. The same factors that enable scavenging nutrients at low concentrations (small size, rapid metabolism, and so on) also facilitate contaminant accumulation in their biomass for subsequent transfer to other components in the food web. Furthermore, the low prokaryotic heterotroph growth efficiencies typically observed in oligotrophic systems increase the number of C transfers through the microbial food web and further increase the potential for bioconcentration (Figure 7). In fact, low growth efficiencies have been shown to increase contaminant levels in metazoans (Meili 1997), and prokaryotic heterotrophs have been shown to accumulate PCBs more efficiently than phytoplankton in a marine system (Axelman and others 1997). These observations suggest that prokaryotic heterotrophs may be a critical component of the food web that can pro-

mote our understanding of the behavior of contaminants in aquatic systems. Moreover, they demonstrate the relevance of considering microbial activity across trophic gradients beyond a purely academic perspective.

If contaminant behavior and the microbial food web are coupled in the manner that we have suggested, it may explain why some of the most contaminant-laden systems are also the most oligotrophic. For example, in a scenario where the loading of contaminants is similar in oligotrophic and eutrophic lakes, the dominance of the microbial food web in the former may facilitate increased trophic transfer and maintenance of materials in the water column, whereas in eutrophic systems there are fewer trophic levels and an increased burial of organic contaminants in the sediments.

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