Ecosystems (1999) 2: 215–225

ECOSYSTEMS

Aquatic Microbiology for Ecosystem Scientists: New and Recycled Paradigms in Ecological Microbiology

Jonathan J. Cole

Institute of Ecosystem Studies, Cary Arboretum, Box AB, Millbrook, New York 12545

ABSTRACT

In all ecosystems, bacteria are the most numerous organisms and through them flows a large fraction of annual primary production. In the past decade we have learned a great deal about some of the factors that regulate bacteria and their activities, and how these activities, in turn, alter ecosystem-level processes. Here I review three areas in which recent progress has been made with particular reference to pelagic ecosystems: the problem of bacterial cell dormancy; the effect of solar radiation on organic matter lability; and, the maintenance of net heterotrophy. In a system in which grazing is the major source of mortality for bacteria, bacterial cell dormancy is something of a paradox. I argue that the degree to which bacteria are grazed by flagellates (highly selective grazers) versus other grazers (cladocerans, bivalves) may explain the degree and variation in the proportion of active cells which recent evidence shows to be large. Another factor affecting bacterial activity that has come to the fore in recent years is solar radiation. Irradiation, especially in the ultra-violet range has long been thought of as simply deleterious to some bacteria. A wealth of newer evidence shows that refractory dissolved organic compounds may be converted into microbially labile compounds by solar radiation in several wavebands. This interaction between irradiation and organic matter (photolysis) may explain, in part, how dissolved organic carbon (C) may be refractory in the dark environment of the soil but become labile in the illumunated surface waters of lakes or rivers. The newer evidence shows that aquatic ecosystems, at least oligotrophic ones, are significantly subsidized by terrestrially-produced organic matter. I review here multiple lines of evidence that suggest that freshwater ecosystems are predominantly systems which respire more organic C than they produce by photosynthesis, and are therefore net heterotrophic. While net heterotrophy is an interesting exception for terrestrial ecosystems, it appears to be commonplace for aquatic systems and represents an important linkage between terrestrial and aquatic ecosystems.

Key words: microbial ecology; aquatic microbiology; review; ecosystems.

INTRODUCTION

One of the largest fluxes of C, in almost any ecosystem, is that from the pool of organic matter into microorganisms. Bacteria are responsible for a large fraction of aerobic respiration, all of the anaerobic respiration, and a large portion of the remineralization of organic nutrients. In aquatic systems, especially those receiving some allochthonous organic input, the secondary production of planktonic bacteria can be co-equal or even larger than that of the primary production of phytoplankton (Findlay and others 1991). It is understandable, then, that scientists working at the ecosystem scale

Received 25 August 1998; accepted 21 January 1999.
e-mail: colej@ecostudies.org

would be naturally drawn to the study of microorganisms. The factors that regulate the abundance, distribution, growth rate and respiration of these microorganisms (especially bacteria) are in large measure the factors that regulate some of the key functions of the ecosystem.

In the past five to ten years there have been stunning advances in the field of microbial ecology with ramifications for ecosystem scientists. Improvements in technology have allowed, in some cases, confirmation of interesting speculations from prior research. In other cases, new avenues of research have changed our concepts of microbial food webs and their interactions with other parts of the ecosystem. Focusing on planktonic bacteria, I review three of these advances and changing concepts as they pertain to aquatic ecosystems.

The review that follows is not intended as a history of aquatic microbiology. Comprehensive reviews and edited volumes exist on a number of the aspects touched on here and the reader is referred to these (Kemp and others 1993; Ford 1993). Further, there have been advances in microbial ecology that I have not touched in this review. The exciting arena, for example, of microbial genetic diversity is not included in this review and the reader is referred to Fuhrman and Davis (1997); and Tiedje and Zhou (1996) as useful technical references and to Finlay and others (1997) for some interesting perspectives on microbial diversity and ecosystem function. Instead, I have focused on a few areas that should be of particular interest to ecosystem scientists: the abundance and activity of planktonic bacteria; the biogeochemical cycles they control; and the role of planktonic bacteria in food webs.

The Active Cell Problem

As late as the 1960's, aquatic microbiologists were counting bacteria by elective cultural means. Bacteria were either plated out on media-containing agar or grown in liquid dilution culture. The bacteria that grew were counted as the 'culturable' bacteria. It was typical to register a few hundred to at most a few thousand culturable bacteria per mL of water. Improvements in both membrane filters and microscopes made it possible for many researchers to concentrate bacteria by filtration and directly count 'all' of the bacteria in a water sample. These direct counts gave values that were orders of magnitude greater than the culturable counts (Jannasch and Jones 1959). The advent of epifluorescence microscopy in the early 1970's improved greatly our ability to directly count bacteria. Fluorescent stains, such as acridine orange (AO), or 4'6-diamidino-2-phenylindole (DAPI) bind to DNA and RNA and make bacteria highly visible, increasing the number of bacteria counted (Daley and Hobbie 1975; Hobbie and others 1977; Porter and Feig 1980). The switch from tortuous-pore membrane filters to cleanly-punctured polycarbonate filters (Nuclepore) roughly doubled the amount of bacteria counted because some bacteria had been lost to the inside of the much thicker cellulose-weave (e.g. Millipore) filter (Hobbie and others 1977). By the mid-1970's, then, countable, nucleic acid-containing bacteria averaged about 10^6 mL^{-1}, among diverse ecosystems, a far cry from the 10^2 to 10^3 mL^{-1} that could be counted by elective culture methods (Jannasch and Jones 1959). A number of explanations were offered for this huge discrepancy. These explanations fell into two basic camps. One group suggested that culture techniques were too unsophisticated to allow the vast majority of planktonic bacteria to grow. The other argued that the bacteria one counted, although they contained some DNA, were either dead or moribund.

Stevenson (1978) articulated this controversy in an intriguing paper and argued that the vast majority of bacteria were neither active nor dead, but dormant. Dormancy, he argued, was a survival strategy in a fluctuating environment. This entire argument and controversy was largely eclipsed during the next decade when interest turned to the measurement of bacterial secondary production. In these techniques, one adds substrates such as ^{3}H-thymidine (Fuhrman and Azam 1980) or ^{3}H-leucine (Kirchman and others 1985; Smith and Azam 1993) to water at a concentration that saturates the uptake kinetics of the bacteria. From the V_{max} of the uptake rate into DNA (for thymidine) or protein (for leucine) one can calculate bacterial secondary production (Kirchman and Ducklow 1993). For these techniques to work, it was necessary to demonstrate that the vast majority of bacteria would utilize these substrates. Autoradiography suggested that $>$80% of countable bacteria would be labeled by ^{3}H-thymidine and other labeled substrates (Fuhrman and Azam 1982). Although it was possible that the addition of nanomolar amounts of nucleic or amino acids might cause cells to break dormancy, with the focus on measuring bacterial production (BP) and the factors that regulate it, the dormancy idea itself had a substantial period of quiescence.

Recently, interest in bacterial dormancy has been reawakened by new lines of evidence suggesting that substantial populations of inactive bacteria exist and even dominate in some environments (McFeters and others 1995). These new lines of evidence are based on technological advances and

include: more sophisticated approaches to microautoradiography (Karner and Fuhrman 1997); the use of fluorogenic compounds that can diffuse through bacterial cell walls and membranes only when the integrity of the membrane has been compromised (Jepras and others 1995); and the use of universal 16S rRNA-targeted oligonucleotide probes to identify bacterial cells which contain sufficient ribosomal-RNA to be potentially metabolically active (Karner and Fuhrman 1997). Prokaryotes do not have a nucleus but they do contain a nucleoid where DNA is concentrated. Zweifel and Hagstrom (1995) developed a destaining method for specifically staining with DAPI only the nucleoid region of *in situ* bacterial cells. This eliminates non-specific DAPI staining of other cellular components. Zweifel and Hagstrom (1995) suggested that only those cells with identifiable stained nucleoid regions should be considered to be alive or active. The non-nucleoid cells (called 'ghosts') are presumably inactive since they either contain no or little DNA. In the Baltic and Mediterranean, the proportion of ghost cells appeared to be quite large, 30 to 90% of total countable cells. Choi and others (1996), working in coastal waters off Oregon, confirmed the finding that a large portion of the epifluorescent countable cells did not have a visible nucleoid (36 to 79%). However, under conditions that stimulated cell growth, cells with visible nucleoids constituted nearly 100% of the population. The work of Choi and others (1996) suggested that the ghost cells were capable of becoming active cells. These ghost cells, which can range from at least 20 to more than 90% of the total countable cells, could be dormant cells, ready to activate when conditions become suitable.

Another approach to enumerating active and inactive bacteria has been the use of stains that change color or fluorescence upon reduction by energy intercepted along the biological electron transport system (ETS). Among the various stains used for this, tetrazolium salts have received the most attention. The tetrazolium salt 5 - cyano-2,3,-ditolyl tetrazolium chloride (CTC) becomes fluorescent upon reduction by the ETS system and can be visualized using epifluorescence microscopy (Rodriguez and others 1992) and by flow cytometry (del Giorgio and others 1996 a). The newer work suggests that only a fraction of bacterial cells have functional ETS and that this fraction varies from $<5\%$ in ultraoligotrophic regions to $>50\%$ in very productive waters (del Giorgio and Scarborough 1995). Intriguingly, the active (CTC-positive) cells are larger than the inactive (CTC negative) cells (Gasol and others 1996) and these larger cells are the most rapidly growing part of the population (del Giorgio and others 1996 b). It is not yet clear whether all CTC-negative cells are truly inactive, but recent laboratory studies indicate that diverse strains of bacteria will reduce CTC when active and that the proportion of CTC+ cells varies in proportion to activity in cultures and bacterioplankton assemblages (Sherr and others 1998).

One of the difficulties with the dormancy hypothesis is that researchers know that a large fraction of bacterial mortality is due to predation by both heterotrophic nannoflagellates (Sherr and Sherr 1988) and by some larger zooplankton such as cladocerans (Peterson and others 1978; Pace and Cole 1996; Vaque and Pace 1992). If this grazing is non-selective, dormant or very slow, growing cells can not persist as a large portion of the population. In fact, under most assumptions, slow or non-growing cells will quickly be eliminated and the population will consist nearly entirely of active cells (Figure 1). Recent grazer-elimination studies have shown the opposite effect, at least when flagellates are the dominant grazers. It has been known for some time that flagellates graze selectively on larger bacteria (e.g. Simek and Chrzanowski 1992; Chrzanowski and Simek 1990). It is also becoming clear that these flagellates selectively take the most actively growing bacteria, which are often the largest ones. Sherr and others (1992), working with coastal seawater from the southeastern United States, found that heterotrophic flagellates selectively grazed those bacterial cells undergoing active cell division. Gonzalez and others (1993), working with marine flagellates, found that motile cells were taken in preference to non-motile cells and that growing cells were taken preferentially to starved cells. By pre-filtering water from the Mediterranean Sea at several sizes, del Giorgio and others (1996b) created communities that lacked either large grazers, large and small grazers or all grazers. In the absence of grazing the fraction of active (CTC positive) cells increased dramatically. This difference could easily be seen by comparing the communities with no bacterivores (0.8-µm filtered) with communities filtered at 40-µm. The inference is that these grazers (nannoflagellates in this system) selectively crop the larger and actively growing cells leaving the slow growing or dormant cells behind (del Giorgio and others 1996b). As another test of this idea in a completely different environment, Kinner and others (1998) created fluorescently labeled bacteria in several size classes and found that groundwater nannoflagellates consumed relatively large bacteria (0.8 to 1.5-µm) faster than smaller ones. In this groundwater sys-

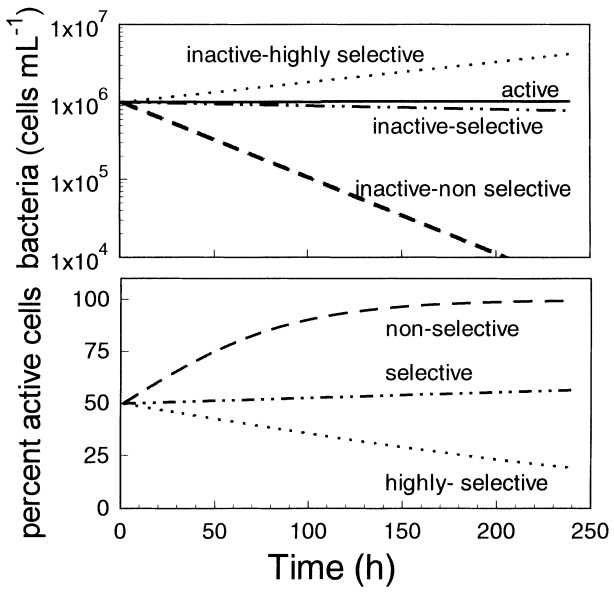

Figure 1. A simple two-population model of the dynamics of planktonic bacteria under different grazing regimes. Cells grow exponentially and experience donor-controlled mortality. The growth constant for active cells is 0.04 h^{-1} and is half this for 'inactive cells'. For active cells, the mortality constant is 0.0425 $cell^{-1}$ h^{-1}; these rates yield a near balance between gains and losses. For inactive cells, three grazing scenarios are considered. 'Non-selective' grazing – active and inactive cells are removed at the same cell-specific rate; 'selective' grazing – inactive cells are removed at half the rate as active cells; and 'highly selective' grazing – active cells are removed at one-third the rate as active cells. The initial conditions were so that the numbers of active and inactive cells were equal at 10^6 cells mL^{-1} The upper panel shows what happens to the number of active cells and inactive cells in all three scenarios. The lower panel shows what happens to the percent of active cells. Note that this model is a gross simplification of the actual situation in the field, which likely includes transfers of cells between the active and inactive pools, changes in grazing pressure, and changes in growth rates. The model shows, however, the potential for highly selective grazing (flagellates) to greatly lower the percentage of active cells. Non-selective grazing would cause the active percentage to increase.

tem, this bacterial size class was also the most rapidly growing. Cells larger than 1.5-µm and smaller than 0.8-µm both grew slower and were grazed more slowly.

There are interesting implications of this work and many questions left unanswered. First, the only feasible way that populations of both fast and slow growing bacteria could coexist (or for slow growing cells to dominate) is if grazing is selective (Figure 1), or if grazing is not a major cause of bacterial mortality. Non-selective grazing quickly results in total domination by the faster growing cells; highly selective grazing on active cells can result in domination by the slow growing cells (Figure 1). Second, microbial processes such as respiration or secondary production are crudely proportional to the total number of bacterial cells, at least on a log-log basis, with a great deal of unexplained variance (Cole and others 1988; del Giorgio and others 1997). Perhaps the variance in these plots could be reduced by basing them on the active cells. Among a set of 14 lakes in Quebec, del Giorgio and others (1996 a) found that BP was better correlated to CTC-positive cells than to total cells. Further, if flagellate grazing selectively removes the active cells, intense grazing by flagellates could conceivably slow down microbial carbon (or nitrogen or phosphorus) cycling in planktonic systems (Sterner and others 1995).

Why should ecosystem scientists pay attention to these developments? A very large pool of inactive bacteria represents a potential for a rapid change in ecosystem metabolism in response to the correct perturbation. The factors that maintain this large, inactive pool of bacteria (perhaps due to selective grazing) maintain this potential for rapid changes in metabolism. Changing bacteria at comparable density from 20% to 80% inactive might have a large effect on decomposition rates. Do non-selective feeders (cladocerans, bivalves) and flagellates have the same or opposite effect on active bacteria and therefore on ecosystem-level decomposition rates? One might imagine cladocerans or bivalves to be less actively selective than flagellates since flagellates capture individual bacteria one by one. On the other hand, the bivalve or cladoceran filtration apparatus itself may cause a very strong size selective effect. Do cells move freely between the active and inactive pools and what regulates these transitions? Do the active and inactive cells differ in their cellular stoichiometry (Chrzanowski and Kyle 1996; Chrzanowski and others 1996).

The Role of Light in Heterotrophic Activity

Solar radiation is one of the driving forces behind photosynthesis, so it clearly has a direct impact on autotrophic microorganisms. What are the effects of this radiation on heterotrophic microorganisms? Ultra-violet (UV) radiation in particular is bactericidal or at least inhibitory to many taxa (Karentz and others 1994). Few enteric pathogens, for example, survive for any length of time under exposure to even ambient levels of UV radiation (Brock 1979). We usually think of any stimulatory effect of radiation on heterotrophs as an indirect result of the production of labile organic matter through photosynthesis; more radiation (up to a point), more

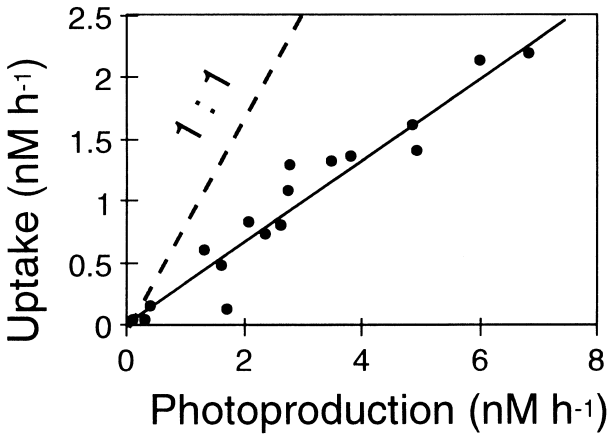

Figure 2. Photochemical production of pyruvate and the uptake of pyruvate by microorganisms in sea water. Kieber and others (1989) used ^{14}C-labelled pyruvate, along with measurements of the ambient pyruvate pool, to estimate microbial uptake of pyruvate. They measured the photochemical production of pyruvate by incubating filter-sterilized sea water (0.22 – um) in polycarbonate bottles. The line labeled 1:1 is the point at which photochemical production and microbial uptake are balanced. (redrawn from the data of Kieber and others 1989).

photosynthesis, more leaked or excreted labile compounds to support bacterial growth (Cole and others 1982; Sondergaard and Scheirup 1982; Baines and Pace 1991). It is relatively recently that new, stimulatory effects of light on heterotrophs have been discovered. In water, dissolved organic carbon (DOC) compounds are important absorbers of both visible and UV radiation (Kirk 1994; Morris and Hargreaves 1995). This radiation can alter the structure of some DOC compounds and can break large macromolecules into smaller ones (Stewart and Wetzel 1982; Geller 1985; 1986).

Interest in the increase in atmospheric UV radiation prompted researchers to more fully examine the effects of light on DOC and heterotrophic bacteria. Kieber and others (1989) showed that pyruvate, a highly labile substrate, could be produced by photochemical fragmentation of marine humic substances in seawater exposed to sunlight (Figure 2). This photochemically-produced pyruvate was rapidly consumed by bacteria (Figure 2). The photochemically-mediated pathway could be the rate-limiting step in the overall removal of DOC in the ocean (Mopper and others 1991; Moran and Zepp 1997). As UV-B radiation increases due to the decreasing of the stratospheric ozone layer, it is unclear what will happen to the rate of DOC degradation. The oceans, which are quite clear, allow UV-B to penetrate to considerable depths. This UV-B produces more labile DOC from larger refractory molecules, but it also inhibits bacterial growth. Herndl and others (1993) calculated the net effect would be net suppression of bacterioplankton activity in the upper 5 m for coastal waters and upper 10 m in the open ocean. A similar result has been found in lakes, but due to the shallower penetration of UV-B in many lakes, the inhibitory effect would be generally restricted to a thinner layer of surface water (Lindell and others 1996). Under what conditions UV-B radiation has net stimulatory or inhibitory effects is complicated by both the activity level of the bacteria and the presence of longer wavelengths of light. Kaiser and Herndl (1997) found that the presence of both UV-A and photosynthetically-active radiation (PAR) enhanced bacterial recovery from UV-B damage. Thus, UV-B exposure resulted in a peak of bacterial activity in seawater at depths between 5.5 and 10.5 m.

Many lakes have considerably more DOC than does the ocean and the stimulatory effect of light on bacteria may be greater still. Wetzel and others (1995) found that DOC leached from plants, as well as the humic and fulvic fractions of the DOC, after exposure to either UV light or sunlight, caused large and sustained increases in bacterial growth. Intriguingly, the UV or sunlight exposure caused very little in the way of detectable chemical changes in the bulk DOC. Small molecules, however (fatty acids, acetic acid, pyruvic acid, citric acid, others), were released from the DOC and rapidly metabolized by bacteria. Lindell and others (1995), in a similar type of experiment, exposed naturally occurring DOC from a humic lake to varying degrees of simulated sunlight, and found that bacterial cell abundance and biomass increased in proportion to the light dose received. Based on the observation that the stimulatory effect of sunlight on DOC degradation rates occurred below the depth of UV-light penetration, Lindell and others (1996) suggested that UV-A and PAR may play an important role in creating labile DOC. Reche and others (1998) have confirmed, in direct experiments, that exposure of lake-water DOC to ambient PAR plus UV-A (UV-B excluded) was sufficient to stimulate bacterial production, bacterial abundance and growth efficiency of the natural bacteria community. This result was foreshadowed by Geller (1986) using cultured strains of *Psuedomonas*, *Flavobacterium*, and *Erwinia*. Exposure to radiation can actually cause photooxidation of DOC to CO_2 (Graneli and others 1996; Moran and Zepp 1997). While longer wavelengths (PAR, UV-A) may play some role in this abiotic oxidation of organic matter, UV-B appears to be causing most of it (Graneli and others 1996). Thus, while UV-A and PAR may make some refractory DOC more labile to

microbes, UV-B may short-circuit some of the microbial loop altogether.

An intriguing implication of this new line of research is that DOC may leave one lightless environment (terrestrial soils, for example) as a microbially refractory compound. Upon exposure to sunlight in a relatively well-lit aquatic environment, this same DOC undergoes some photolytic degradation and becomes labile. In this sense, surface waters are actually 'windows' in an otherwise dark groundwater system. Much of the new work in this area was stimulated by the need to predict environmental effects of increased UV-B on the environment. However, some of the exciting findings have been tangential to this environmental problem. A great deal about basic photochemistry and its link to microbiology have been revealed. Moran and Zepp (1997) reviewed the role of photoreactions in the formation of biological labile compounds from DOC and created a model to calculate the role of this process at the scale of the entire ocean. They suggest that, integrated over depth, about 1 X 10^{15} g C and 0.15 X 10^{15} of N are converted into labile products by photoreactions in the ocean. These estimates, which are conservative in several respects, meet about 4% of bacterial C demand (bacterial production plus respiration) and 5% of bacterial N demand. In lakes, which are shallower and often more DOC rich, these percentages could be much higher.

Net Heterotrophy

For a long time, the organic C substrates that supported the growth of planktonic bacteria were thought to be largely of phytoplankton origin. While some scientists were keenly aware of the importance of terrestrially-derived DOC as a bacterial substrate (Wetzel and others 1972; Tranvik 1992), many of us envisioned a very tight coupling between the growth and respiration of planktonic bacteria and the release of organic C during phytoplankton photosynthesis (Larsson and Hagstrom 1979; Cole and others 1982), or phytoplankton cell lysis (Cole 1982). A good correlation is seen, among diverse aquatic ecosystems, between bacterial abundance and chlorophyll-*a* (Bird and Kalff 1984) and between bacterial production and phytoplankton net primary production (Cole and others 1988). This type of correlation works quite well at the scale of mean seasonal values (Cole and others 1988; Ducklow and Carlson 1992), but in many systems it does not predict well at all at the scale of individual sampling dates (Hobbie and Cole 1984).

Bacterial secondary production does not represent a state change in carbon; organic carbon from the environment becomes organic C in a bacterial cell. Thus, bacterial production is actually not constrained by the supply of organic C substrates to the environment (Jahnke and Craven 1995). In fact, with long enough food webs, and a high enough growth efficiency for the consumers (bacteria), one can easily construct model food webs in which bacterial secondary production exceeds the supply of organic C (Strayer 1988; Cole and Caraco 1993; Cole and Pace 1995); that is bacterial secondary production can be greater than net primary production (NPP). Unlike bacterial production, bacterial respiration is constrained and cannot exceed the supply of organic C. When either community or bacterial respiration exceeds primary production, the system is 'net heterotrophic' (see Howarth and others 1992; 1996). That is, rather than storing or exporting a net amount of organic C, these systems consume more organic C than they produce by photosynthesis. Such systems must be subsidized by an input of labile organic C from outside of the system (allochthonous organic matter). Put another way, these systems have negative net ecosystem production (NEP). Sustained negative NEP implies that organic C produced outside of the system is being respired within it.

In terrestrial ecosystems, net heterotrophy is an interesting but relatively rare exception (Polis and others 1997). Multiple lines of evidence suggest that aquatic ecosystems, at least at the oligotrophic end of the spectrum, tend towards net heterotrophy. The biomass of planktonic bacteria often exceeds that of phytoplankton in oligotrophic waters. In the Sargasso Sea, one of the most oligotrophic parts of the ocean, the biomass of heterotrophic bacteria exceeds that of phytoplankton at all depths from 0 to 2600 m. In the euphotic zone, where phytoplankton were most abundant, bacterial C and N accounted for 70% and 80% of the total biomass C and N, respectively (Fuhrman and others 1989; Cho and Azam 1990).

By itself, the dominance of bacterial biomass does not prove that these ecosystems are net heterotrophic. It could be that in oligotrophic waters the turnover time of the bacteria is much longer than that of the autotrophs. Thus the biomass pyramid is inverted (del Giorgio and Gasol 1995; Buck and others 1996), but all of the C respired by the heterotrophs could be supplied by the photosynthesis of the autotrophs. Several studies suggest that respiration also exceeds primary production in these unproductive environments. In a series of lakes, measurements of planktonic community respiration consistently exceeded that of planktonic primary production at chlorophyll-*a* levels less than about 15-µg liter^{-1} (del Giorgio and Peters 1994). A

review of the literature revealed that the respiration of bacteria (BR) alone exceeded measurements of net primary production when the latter was below about 100 µg C liter^{-1} d^{-1} (del Giorgio and others 1997). Planktonic organisms other than bacteria (zooplankton, flagellates, phytoplankton themselves) also respire. Thus, since BR is only one component of community respiration, when BR > NPP, these systems must be net heterotrophic. To date these patterns are controversial (see Geider 1997), and based on rather small data sets, and their validity, especially concerning the open ocean, continues to be debated in the literature (Williams 1998). Using a large data set of community (rather than just bacterial) R and P, Duarte and Agusti (1998) found strikingly similar patterns to those seen by del Giorgio and others (1997) for lakes, rivers, and both the coastal and open ocean: P/R ratios were less than 1 towards the oligotrophic end of the spectrum in each of the environments.

One reason the data are scant is because we have so few measurements of bacterial or community respiration. (Jahnke and Craven 1995). We have a very large number of measurements that relate BP and NPP. If we knew the relationship between BR and BP we could estimate a relationship between BR and NPP. The broad correlation between planktonic BP and NPP indicates that BP is about 20 to 40% of NPP in most environments (Cole and others 1988; Ducklow and Carlson 1992). Bacterial growth efficiency (BGE) is the conventional way to express the relationship between BP and BR. BGE is analogous to assimilation efficiency in higher organisms.

$$BGE = BP/(BP + BR)$$

Rearranging, we can solve for BR when BP and BGE are known.

$$BR = BP/BGE - BP.$$

Microbiologists are not in agreement about the magnitude of BGE nor the factors that regulate it, but this is an area of active interest (del Giorgio and Cole 1999 and references therein). Values depend in part on the type and duration of the measurement with the highest values (near 60%) based on short-term uptake and respiration of labeled substrates and the lowest values (<5%) based on long-term incubations with natural DOC. Most values fall near or below 30% (Bjornsen 1986; Daneri and others 1994; Cole and Pace 1995; Roland and Cole 1999; del Giorgio and Cole (1999). Figure 3 shows the ratio of BR to NPP as a function of both BGE and the ratio of bacterial to primary production. For most reasonable values of both BGE (10 to 30%) and the ratio of BP:NPP (0.2 to 0.4), BR exceeds NPP, implying net heterotrophy. For example, if BP were 30% of NPP, BR would exceed NPP at BGE < 23%. Thus net autotrophy would be expected only in environments in which BP were an exceptionally small fraction of NPP (the left side of the X-axis) or in cases in which BGE was exceptionally high (towards the top of the Y-axis). Until more is known about what regulates BGE, this model is only suggestive. The pattern suggests, nevertheless, that bacterial respiration of allochthonous C can exceed the input from primary production by as much as several fold given reasonable assumptions about BGE and the relationship between BP and NPP.

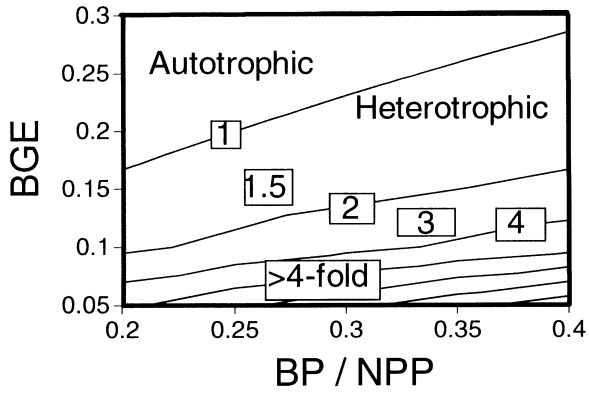

Figure 3. A modeled relationship between bacterial production (BP), bacterial respiration (BR), and phytoplankton net primary production (NPP) suggests that where NPP is low, BR will exceed NPP and the system will be net heterotrophic. The X-axis shows the ratio BP/NPP which for most systems ranges between 0.25 and 0.35 (Cole and others 1988; Ducklow and Carlson 1992). The Y-axis shows bacterial growth efficiency (BGE = BP/(BP+BR)). The isolines are the computed values of the ratio of BR/NPP for each assumed value of BP/NPP and BGE. When BR > NPP (labeled 'Heterotrophic') more organic C is respired than is produced within the system. When BR < NPP (labeled 'Autotrophic') more organic C is produced by photosynthesis than is consumed by bacteria. The only systems in which BR < NPP (Autotrophic) are those with either a particularly low ratio of BP/NPP or a particularly high value for BGE.

The extent to which the respiration of planktonic bacteria exceeds the net primary production of phytoplankton is clearly not a complete measure of metabolic balance of an aquatic ecosystem. There are populations of other heterotrophs whose respiration can be considerable. Zooplankton, for example, respire about as much C as do bacteria (Cole and others 1988; 1989). There also can be substantial inputs of autochthonous C from the primary production of benthic algae and macrophytes (Wetzel 1982), as well as respiration by microbial and

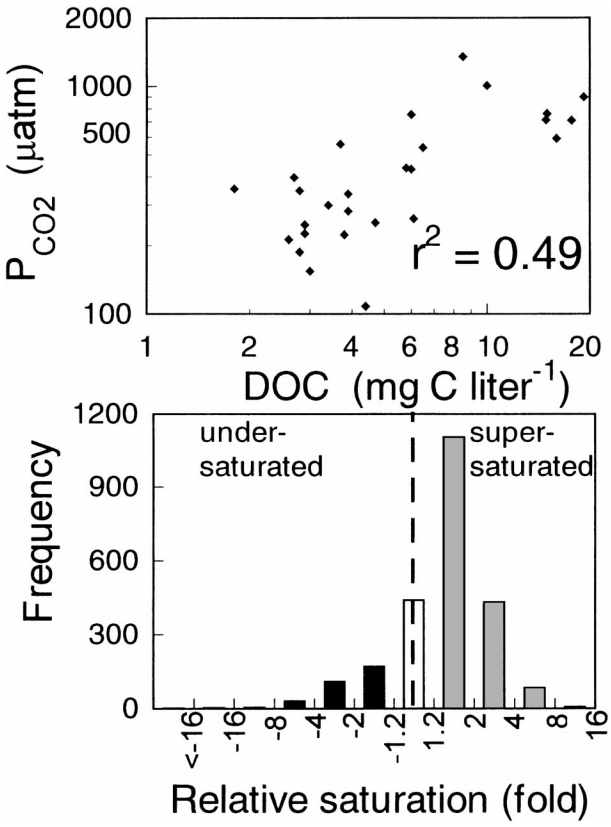

Figure 4. Upper panel – The relationship between surface water P_{CO2} and dissolved organic C (DOC) in a series of 27 lakes in northern Wisconsin. Although the variance is high, P_{CO2} and DOC are positively correlated ($r^2 = 0.49$; $p < 0.001$; redrawn from Hope and others 1996). The positive correlation may be driven by heterotrophic metabolism of allochthonous DOC. Lower panel.- Frequency plot of saturation of CO_2 for 2395 lake-dates from 69 lakes distributed around the world (data are redrawn from one of the data sets in Cole and others 1994). The Y-axis shows frequency (by samples) in each category of relative saturation. Relative saturation is defined as:

$RS = P_{CO2}$ (water)/P_{CO2} (air) for supersautration

(slashed bars) and

$RS = -P_{CO2}$ (air)/P_{CO2} (water) for undersautration

(shaded bars)

The open bar represents samples that are within ± 20% of equilibrium with the atmosphere and the dashed line represents atmospheric equilibrium. Lakes that are supersaturated in CO_2 are net sources of CO_2 to the atmosphere. This excess CO_2 can be the result of heterotrophic respiration exceeding primary production.

invertebrate benthic organisms to account for. At this point, the evidence for net heterotrophy at the ecosystem scale is very incomplete. Nevertheless, there are some patterns consistent with the idea that net heterotrophy is widespread. A number of researchers have found that the surface waters of lakes, especially oligotrophic lakes, tend to be supersaturated in carbon dioxide with respect to equilibrium with the atmosphere (Figure 4). These lakes are sources, therefore, of CO_2 to the atmosphere. Net heterotrophy at the ecosystem scale is one process that would cause a system to be a source of CO_2; net autotrophy would cause it to be a sink. However, there are several additional processes that could lead to excess CO_2 in a system including the injection of CO_2-rich groundwater (Kling and others 1991); the precipitation of dissolved carbonates (McConnaughey and others 1994); and the mixing of an in-lake source of acidity with an input of bicarbonate. Recent modeling studies suggest that the respiration in lakes of terrestrially-supplied DOC is sufficient to generate CO_2 supersaturation (Dillon and Molot 1997; Caraco and others 1998). Groundwater and emergent macrophytes may also be important additional sources CO_2 (Caraco and others 1998). Intriguingly, DOC concentration and the partial pressure of CO_2 (P_{CO2}) are positively correlated ($r^2 = 0.49$; $p<0.001$ for log-log plot) among lakes within a single region (Figure 4; upper Midwest), a point that is at least consistent with the net heterotrophy hypothesis (Hope and others 1996).

While no single piece of information conclusively proves that oligotrophic aquatic ecosystems tend towards net heterotrophy, the weight of multiple lines of evidence strongly suggests that this is the case. If true, it implies that the leakage of organic compounds from terrestrial systems subsidizes aquatic food webs. As most of this terrestrial organic C exits in the form of DOC, its utilization must be the work of bacteria. How much terrestrial DOC would it take to supply this subsidy? If we take 1000 µatm to be the typical value for P_{CO2} in a CO_2-supersaturated lake with a forested watershed (Cole and others 1994), the net efflux of C as CO_2 would be about 40 g C m^{-2} y^{-1} (assumes gas piston velocity of 0.5 m d^{-1} (Cole and Caraco 1998) and 250 ice-free days per year). If the lake has a watershed area that is 10-fold the area of the lake, the export from land needs to be only 4 g C m^{-2} (of watershed) y^{-1} to meet this demand. Given a typical forest net primary production of ~500 g C m^{-2} y^{-1} (Borman and Likens 1979), this needed export would be on the order of 1% of forest plant productivity to sustain the net heterotrophy. If this net heterotrophy respires 50% of the terrestrial DOC input to the lake (Dillon and Molot 1997), the export would need to be 2% of terrestrial primary production. This subsidy, however, can be highly significant to the lake and its food web. In a stable isotope study of a humic lake in Sweden, Meili and others (1996) found that the ^{13}C content of cladocerans (*Bosmina* and *Daphnia*)

differed substantially from that of a copepod (*Eudiaptomous*). The cladoceran's ^{13}C strongly resembled that of the surrounding terrestrial material; the copepod more closely reflected the phytoplankton material in the lake. Whereas most copepods do not consume particles as small as bacteria, cladocerans can consume bacteria directly (Peterson and others 1978). In constructing a rough C and ^{13}C budget of this system, Meili and others (1996) estimated that 40% of the cladoceran's biomass C came from bacteria that had consumed DOC of terrestrial origin.

CONCLUSIONS

Recent advances in the microbiology of aquatic ecosystems should be of keen interest to ecosystem scientists. The way a food web is structured may effect microbial processes. The activity of planktonic bacteria may be regulated by the selectivity or lack of selectivity of the bacterivores in the system. The complex ecosystem-scale factors that allow flagellates to dominate some systems, and cladocerans (or bivalves) others may have profound effects on how the microbial food web is assembled and functions. Further, DOC of terrestrial origin clearly subsidizes the metabolism of planktonic bacteria. This subsidy apparently moves up the food web to zooplankton and probably to fish. Aquatic bacteria thus link terrestrial primary production to aquatic secondary production. How labile organic matter can exit the soil system, which has an active microbial food web itself, may be a function of light energy. The soil system is dark; the surface waters of lakes are exposed to sunlight. Through photolysis, refractory DOC is converted to microbially-labile forms possibly making this exported DOC more available to the aquatic than to the terrestrial microbial community.

ACKNOWLEDGMENTS

I thank N. Caraco, P. del Giorgio, F. Roland and M. Pace for interesting discussion on all aspects of this work. Additionally, I thank M. Pace for his insightful comments on a draft of the manuscript. This is a contribution to the Institute of Ecosystem Studies, and was supported in part by the National Science Foundation.

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