

- calcification and photosynthesis in the coccolithophore *Emiliania huxleyi* under steady-state light-limited growth. *Mar. Ecol. Prog. Ser.* **142**: 87–97.
- BRAND, L. E., W. G. SUNDA, AND R. R. L. GUILLARD. 1983. Limitation of marine phytoplankton reproductive rates by zinc, manganese, and iron. *Limnol. Oceanogr.* **28**: 1182–1198.
- CAVENDER-BARES, K. K., E. L. MANN, S. W. CHISHOLM, M. E. ONDRUSEK, AND R. R. BIDIGARE. 1999. Differential response of equatorial Pacific phytoplankton to iron fertilization. *Limnol. Oceanogr.* **44**: 237–246.
- COALE, K. H., AND OTHERS. 1996. A massive phytoplankton bloom induced by an ecosystem-scale iron fertilization experiment in the equatorial Pacific Ocean. *Nature* **383**: 496–501.
- HUTCHINS, D. A., G. R. DiTULLIO, Y. ZHANG, AND K. W. BRULAND. 1998. An iron limitation mosaic in the California upwelling regime. *Limnol. Oceanogr.* **43**: 1037–1054.
- MARTIN, J. H. 1990. Glacial–Interglacial CO<sub>2</sub> change: The iron hypothesis. *Paleoceanography* **5**: 1–13.
- , R. M. GORDON, S. E. FITZWATER, AND W. W. BROENKOW. 1989. VERTEX: Phytoplankton/iron studies in the Gulf of Alaska. *Deep-Sea Res.* **36**: 649–680.
- MUGGLI, D. L., AND P. J. HARRISON. 1997. Effects of iron on two oceanic phytoplankters grown in natural NE Subarctic Pacific seawater with no artificial chelators present. *J. Exp. Mar. Biol. Ecol.* **212**: 225–237.
- PAASCHE, E., AND S. BRUBAK. 1994. Enhanced calcification in the coccolithophorid *Emiliania huxleyi* (Haptophyceae) under phosphorus limitation. *Phycologia* **33**: 325–330.
- PARSONS, T., Y. MAITA, AND C. M. LALLI. 1984. A manual of chemical and biological methods for seawater analysis. Pergamon.
- PRICE, N. M., B. A. AHNER, AND F. M. M. MOREL. 1994. The Equatorial Pacific-ocean—grazer-controlled phytoplankton populations in an iron-limited ecosystem. *Limnol. Oceanogr.* **39**: 520–534.
- RIEBESELL, U., I. ZONDERVAN, B. ROST, P. D. TORTELL, R. E. ZEEBE, AND F. M. M. MOREL. 2000. Reduced calcification of marine plankton in response to increased atmospheric CO<sub>2</sub>. *Nature* **407**: 364–367.
- SIGMAN, D. M., D. C. MCCORKLE, AND W. R. MARTIN. 1998. The calcite lysocline as a constraint on glacial/interglacial low-latitude production changes. *Glob. Biogeochem. Cycles* **12**: 409–427.

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## Evidence for tight coupling between active bacteria and particulate organic carbon during seasonal stratification of Lake Michigan

**Abstract**—Suspended particulate organic matter (POM) plays a critical role in the planktonic ecology of Lake Michigan during seasonal thermal stratification. We show, based on stable isotope and ribosomal RNA (rRNA) data, that the relationship between suspended POM and active biomass changes as thermal stratification persists. Stable isotope data indicated that sources of suspended POM change between July and October, moving from primary production at a deep chlorophyll layer to recycling-based production in surface waters. Concomitant change in the distribution of active bacterial and eukaryotic biomass was observed as indicated by rRNA abundances. Active bacterial and eukaryotic biomass were highly correlated throughout the year. However, the correlation between suspended POM and active bacterial biomass varied seasonally and reflected the transitions in planktonic ecology. Suspended POM from depths >60 m was primarily of sedimentary origin. The combined application of stable isotope and rRNA analysis of suspended POM indicated a dynamic relationship between the bulk POM reservoir and living planktonic biomass.

The release of bioactive elements via the cycling of suspended particulate organic matter (POM) is a fundamental control on photosynthesis and, hence, the planktonic ecology of thermally stratified lakes. However, suspended POM is not a passive reservoir composed entirely of detrital material, but encompasses most of the active plankton including phytoplankton, zooplankton, and bacteria. Few studies have specifically addressed the relative contribution and ecologi-

cal role of active particulate biomass to the cycling of POM as a whole.

Phytoplankton, via the photosynthetic fixation of carbon and nutrients, represents a source of suspended POM on which zooplankton feed. Another source of POM is heterotrophic bacteria; they consume dissolved organic matter (DOM) of planktonic origin and convert it to particulate biomass. Protozoa and larger zooplankton, in turn, consume phytoplankton, bacteria, and other POM and excrete bioactive elements metabolized by phytoplankton and bacteria. Thus, phytoplankton, zooplankton, and heterotrophic bacteria are not only the active biological components of suspended POM, they are also the primary agents of POM cycling.

The role of heterotrophic bacteria in the cycling of POM is a function of the abundance and quality of organic carbon and the trophic structure of the lake. These factors may vary seasonally. Pace and Cole (1994) have developed a model to predict the response of heterotrophic bacteria to resource availability as inferred from heterotrophic bacterial biomass or production (Fig. 1). When bacterial mortality by grazing is low, a tight relationship is expected between bacteria and available resources. Alternatively, when grazing is dominant, only a weak relationship is expected. This model provides a conceptual framework with which to evaluate possible linkages between bacteria and POM.

In Lake Michigan, there is evidence that heterotrophic bacterial growth may be limited by DOM availability (Gard-

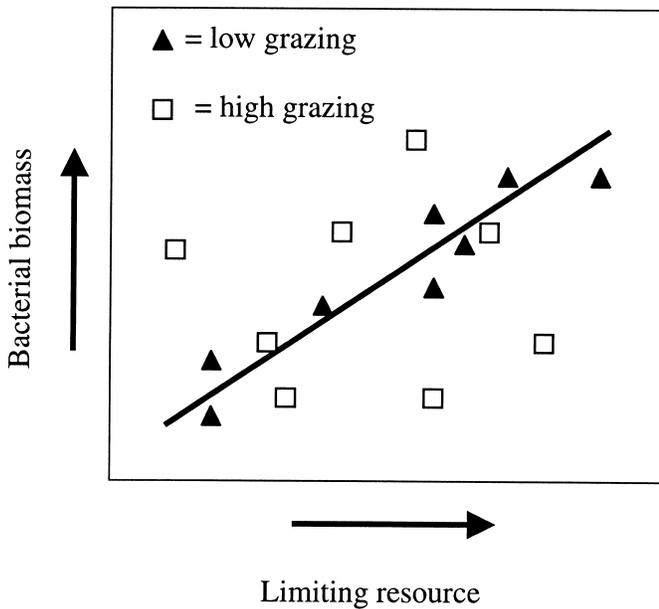


Fig. 1. Schematic of the model of Pace and Cole (1994) showing the theoretical response of bacteria to a limiting resource under conditions of low and high grazing pressure.

ner et al. 1986, 1989; Pernie et al. 1990), and bacterial production may exceed the supply of labile DOM used for growth when the lake is thermally stratified (Scavia et al. 1986; Scavia and Laird 1987). Thus heterotrophic bacteria may be supported by some other source of organic matter. Although it is understood that POM is extensively recycled, consumption of POM by bacteria may represent an important sink of POM that may affect the planktonic ecology of Lake Michigan.

Using stable isotope compositions as a tracer for POM, and ribosomal RNA (rRNA) concentrations as a molecular surrogate for active biomass, we studied the cycling of POM in Lake Michigan. Here we present data obtained from July and October 1997 that indicate a strong seasonal variation in the linkage between bacteria and POM. This combined analytical approach provided detailed information about the relationship between microbial population dynamics and water column processes.

**Field and laboratory methods**—Suspended POM and surface sediments were collected from the R/V *Neeskay* at a station 12 km offshore of Fox Point, Wisconsin (43°11'40"N, 87°40'11"W), at a water depth of roughly 100 m during mid-July and early October 1997. Samples were collected at mid-day.

Using a single 20-L Niskin bottle, water samples were retrieved from depth and immediately filtered. The suspended POM included detritus, bacteria, phytoplankton, and microzooplankton. Mesozooplankton were also captured at concentrations of a few individuals per 20 L. For particulate organic carbon concentration ([POC]) and rRNA analyses, separate 1-L aliquots of water were filtered through glass fiber filters (GF/F 0.7  $\mu\text{m}$ , Whatman) and membranes (0.2  $\mu\text{m}$ , Millipore), respectively. The remaining water from the

Niskin bottle was filtered on GF/F for stable isotope analysis, which requires a large quantity of POM. Only a small portion of suspended POM occurs in the size range between that which is retained by GF/F filters and membrane filters (Shafer and Armstrong 1994); therefore, we assumed that POM retained for [POC], stable isotopes, and rRNA analyses were nearly identical to one another. All filters and membranes were immediately frozen at  $-70^{\circ}\text{C}$  for transportation back to the laboratory.

Glass fiber filters were acidified in 1 N HCl overnight to remove carbonate and dried at  $50^{\circ}\text{C}$ . [POC] was determined using a Fisons NA 1500 elemental analyzer. Standard error for these analyses was within  $25 \mu\text{g L}^{-1}$ . C:N<sub>atomic</sub> ratios were also obtained via this analysis. Carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) stable isotope ratios were determined using an automated system composed of the aforementioned elemental analyzer coupled to a Micromass Optima isotope ratio-monitoring mass spectrometer. Samples were run in duplicate and standard materials were inserted into the automated sequence every sixth analysis. Errors are less than 0.3‰. DIC was precipitated from 250 mL of lake water with saturated NaOH and BaCl<sub>2</sub>, and  $\delta^{13}\text{C}$  was determined using an Iso-Carb system coupled to the Optima. All isotope values are reported versus Peedee belemnite (PDB) for  $\delta^{13}\text{C}$  and versus air for  $\delta^{15}\text{N}$ .

For rRNA analyses, the frozen membrane filters were crushed with sterile, baked spatulas and transferred to screw-cap Eppendorf tubes containing low-pH buffer, buffer-equilibrated phenol (pH 5.1), sodium dodecyl sulfate, and 0.5 g zirconium beads. RNA was isolated by bead-beating, phenol-chloroform extraction and ethanol precipitation as previously described (Stahl et al. 1988; MacGregor et al. 1997). RNA was transferred to nylon membranes in triplicate and 16S-like rRNA was probed with radiolabeled oligonucleotide probes targeting the domains Bacteria (S-D-Bact-0338-a-A-18), Archaea (S-D-Arch-0915-a-A-20), and Eucarya (S-D-Euca-1379-a-A-16), as well as with a universal probe (S\*-Univ-1390-a-A-18). Membranes were prehybridized at  $40^{\circ}\text{C}$  and washed at  $54^{\circ}\text{C}$ ,  $56^{\circ}\text{C}$ ,  $42^{\circ}\text{C}$ , and  $44^{\circ}\text{C}$ , respectively. Hybridization was quantified relative to known amounts of target RNA with a PhosphorImager (Model 400S; Molecular Dynamics Inc.).

Ideally, the sum of rRNA concentrations derived from the domain-level probes should equal hybridization to the universal probe. In July, summations of the domain-level probe totals were  $169 \pm 55\%$  of the universal probe. Past studies have shown that elevated domain summation usually reflects partial RNA degradation since the region of the small subunit rRNA targeted by the universal hybridization probe is very sensitive to enzymatic or chemical degradation (Raskin et al. 1997). We speculate the elevated summations observed in July reflect partial degradation of the universal target, for example, as might result from ingestion by grazers. In October, summations were  $107 \pm 18\%$ .

Following water column sampling, sediments were collected using a box corer that was immediately subcored on deck using 7.6-cm acrylic tubes and held at  $0-4^{\circ}\text{C}$  during transit to the laboratory. The subcores were sliced under anoxic conditions in a glove box and frozen. Sediment samples were analyzed as described above for POM, with only minor

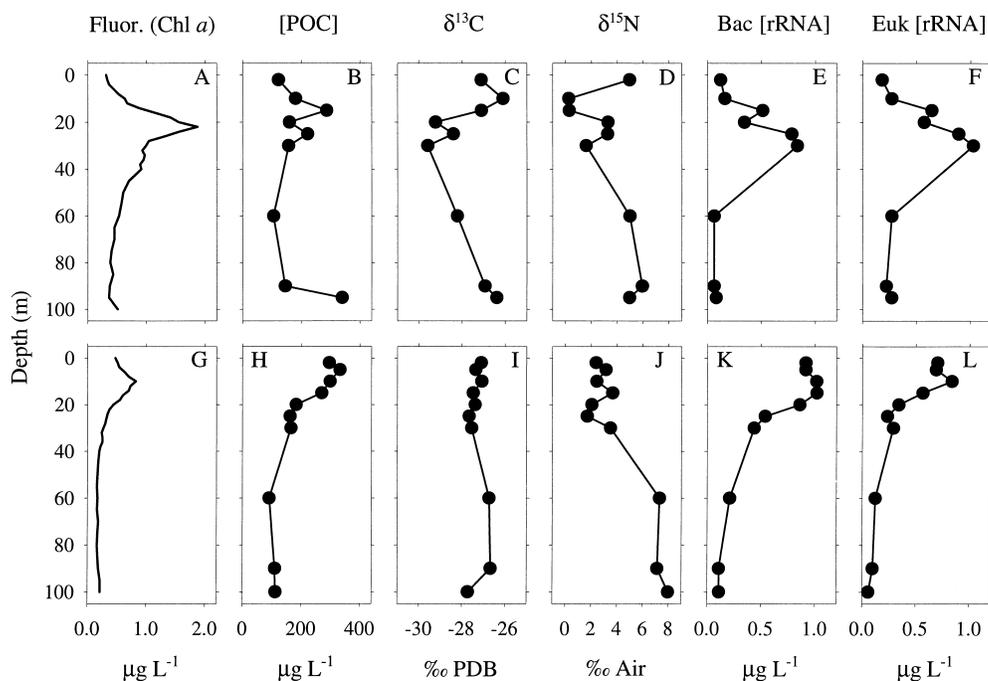


Fig. 2. Chl *a* fluorescence, POC concentrations, stable isotope compositions, and rRNA concentrations for Lake Michigan in July (panels A–F) and October (panels G–L). The scale and units for panels in the top row are the same as their complements in the bottom row.

modifications to the methods. Fundamental water column data was collected using a Seabird conductivity temperature depth (CTD) probe equipped with a chlorophyll *a* (Chl *a*) fluorescence detector. Casts were made immediately prior to POM sampling and coring.

*Distribution of Chl a fluorescence and [POC] during thermal stratification:* Depth profiles of [POC] and Chl *a* fluorescence revealed a distinct transition in the depth distribution of phytoplanktonic biomass between July and October. In July, a broad thermocline was in place between 8 and 16 m. This structure effectively partitioned the water column into a warm, nutrient-poor surface layer and a cold, nutrient-rich deep layer (Brooks and Edgington 1994). There were two distinct [POC] maximums in July (Fig. 2B): one in the thermocline at 15 m and one in the upper deep layer at 25 m. The latter maximum roughly coincided with the maximum Chl *a* fluorescence (Fig. 2A); this deep chlorophyll layer (DCL) is a common feature in Lake Michigan following the onset of thermal stratification (Fahnenstiel and Scavia 1987).

In October, the thermocline was deeper and sharper, extending between 18 and 20 m. Peak Chl *a* fluorescence was weaker than in July, and confined to the surface layer (Fig. 2G). The DCL is controlled by nutrient/light interaction and the decreased solar radiation in October may no longer support photosynthesis in the nutrient-rich deep layer (Scavia and Fahnenstiel 1987). The [POC] also peaked in the surface layer (Fig. 2H).

*Stable isotope ratios of suspended POM:* The stable carbon and nitrogen isotope ratios ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) of suspended

POM may be used as tracers of the dissolved source of these elements and the ecological setting in which they were transformed into organic matter (Fogel and Cifuentes 1993). Although myriad physical, chemical, and biological processes influence isotope ratios of suspended POM, these data may be applied to study the origin and fate of POM in marine and aquatic ecosystems (Ostrom et al. 1997; McCusker et al. 1999). Without isotopic analysis of all of the possible sources of carbon and nitrogen to an ecosystem, isotopic ratios of bulk POM cannot easily resolve specific sources of these elements to POM. However, they may be applied to identify seasonal and depth-dependent differences in POM synthesis.

In July, the POM was more enriched in  $^{13}\text{C}$  in the surface layer than in the DCL (Fig. 2C). This may be due in part to slight differences in the dissolved inorganic carbon (DIC) reservoir, which had a  $\delta^{13}\text{C}$  of  $-3.0 \pm 0.5\text{‰}$  and  $-4.0 \pm 0.4\text{‰}$  in the surface and deep layers, respectively. Additionally, the isotope effect during photosynthesis may be affected by dissolved  $\text{CO}_2$  availability and growth rate of phytoplankton (Fogel and Cifuentes 1993; references in McCusker et al. 1999). Although the precise reason for differences in the  $\delta^{13}\text{C}$  of POM with depth is difficult to resolve, these data indicate that POM was produced under different conditions in the surface layer versus the DCL.

The  $\delta^{15}\text{N}$  of POM in July also indicated biogeochemically distinct zones of POM cycling (Fig. 2D).  $\delta^{15}\text{N}$  values near the surface (2 m) and in the vicinity of the DCL (20, 25, and 30 m) vary in the range of 2–5‰ and may reflect the isotopic composition of  $\text{NO}_3^-$ , which was expected to be around 3.0‰ (McCusker et al. 1999). In contrast, in the thermocline at 10 and 15 m, the  $\delta^{15}\text{N}$  are significantly less

Table 1. Elemental and stable isotope\* composition of organic matter. Values for particulate organic matter (POM) are the average of July and October. Values for sediments are the average of four surface sediment samples from depths <2 cm below the sediment-water interface. Standard deviations are given in parentheses.

Sample	C:N <sub>atomic</sub>	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
Surface sediment	9.97 (0.28)	-27.36 (0.42)	5.54 (0.43)
>60 m POM	9.25 (1.70)	-27.93 (0.57)	6.50 (1.31)
Surface-layer POM	8.18 (1.34)	-27.12 (0.43)	2.32 (1.59)

\* Isotope ratios are reported versus PDB for  $\delta^{13}\text{C}$  and versus air for  $\delta^{15}\text{N}$ .

and approach 0‰, which may be indicative of the fixation of atmospheric N<sub>2</sub>. Although not the only mechanism to produce  $\delta^{15}\text{N}$  values in this range, this interpretation is further supported by the detection of heterocystous cyanobacteria and active transcription of cyanobacterial genes encoding N<sub>2</sub> fixation functions (nifH) at this location in Lake Michigan (MacGregor et al. 2001).

In contrast to July, stable isotope composition of POM in October was fairly homogeneous with depth across the thermocline (Fig. 2I,J). The relative lack of variation in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  indicates that POM was produced under uniform ecological conditions primarily in the surface layer and reflects the transition to recycling-based production. Perhaps surface POM, which is eventually mixed throughout the water column during winter overturn, persists in the deep layer for some time.

The composition of suspended POM in July and October at depths greater than 60 m is similar to the composition of sedimentary organic matter (OM, Table 1). The C:N<sub>atomic</sub> and  $\delta^{15}\text{N}$  of sedimentary OM are distinct from those of surface layer POM, whereas the  $\delta^{13}\text{C}$  are similar for all pools. These data are consistent with earlier observations of a bottom nepheloid layer in Lake Michigan that is maintained through resuspension of sediment (Hicks and Owen 1991).

*Biomass estimates using rRNA analysis:* We interpret the concentration of rRNA as a semiquantitative indicator of the biomass of active microorganisms within suspended POM. There is a one-to-one relationship between the quantity of ribosomes and the quantity of rRNA molecules in a cell. Ribosomes are central to protein synthesis (translation), and it is well known that microorganisms adjust ribosome content to meet the demands imposed by growth and maintenance (Bremer and Dennis 1987; Kerfhof and Kemp 1999). In slowly growing marine bacteria, Kemp et al. (1993) observed a strong linear correlation between RNA content and cell volume (and presumably cell mass). This relationship is consistent for a broad range of growth rates (0.01–0.25 h<sup>-1</sup>) that generally bracket growth rates reported for bacteria in Lake Michigan (Scavia and Laird 1987), including the slowly growing community associated with the nepheloid layer near the sediment-water interface (Hicks and Owen 1991). Additionally, Kemp et al. (1993) observed that near-dormant (0.01 h<sup>-1</sup>) marine bacteria do not retain excess ribosomes. RNA is generally related to biomass in zooplankton as well (Båmstedt and Skjoldal 1980), and in phytoplankton, RNA:DNA ratio is correlated with growth rate (Dortch et al.

1983). Because RNA is quickly degraded both enzymatically and chemically once outside the cell (reviewed in MacGregor 1999), rRNA recovered from POM is derived exclusively from active biomass.

The contribution of active plankton representing each of the three domains (Archaea, Bacteria, and Eucarya) was estimated using a set of three oligonucleotide probes, each designed to hybridize specifically to the 16S-like rRNA of each domain. For both sampling times and at all depths, archaeal rRNA was detectable but generally less than 1% of rRNA of bacteria (primarily heterotrophs) and eukaryotes (phytoplankton and zooplankton).

In July, profiles of bacterial and eukaryotic [rRNA] (Fig. 2E,F) roughly track Chl *a* fluorescence, reaching a maximum immediately below the DCL. These data suggest that the amount of active bacterial and eukaryotic biomass was spatially coupled to photosynthesis and the primary production of POM. Although there are small peaks in the [rRNA] profiles at the shallower [POC] maximum, they are not proportional to the concentration of [POC]. Therefore, the relative contribution of active biomass to suspended POM was different in the surface and deep layers during July.

The October profiles of bacterial and eukaryotic [rRNA] (Fig. 2K,L) also track Chl *a* fluorescence, peaking in the surface layer. Typically, growth of small phytoflagellates is supported entirely by regenerated phosphorus in the surface layer (Scavia and Fahnenstiel 1987), and in contrast to July, bacterial [rRNA] is higher than eukaryotic [rRNA]. This is consistent with previous observations of relatively enhanced production by bacteria, which are not nutrient limited in Lake Michigan during late thermal stratification (Moll and Brahe 1986). Also, it is evident that there was little active biomass in the deep layer, reflecting the seasonal destruction of the DCL and a transition to production at shallower depths.

The rRNA abundances also suggest that carbon from active bacteria made only a small contribution to bulk POC. For example, at 10 m in October, bacterial [rRNA] was  $\approx 1 \mu\text{g L}^{-1}$ , but [POC] was  $\approx 300 \mu\text{g L}^{-1}$ . Assuming that rRNA is 40% carbon and that 10% of bacterial carbon biomass is rRNA, roughly 1% of POC was from active bacteria. Alternatively, direct bacterial counts from this depth during the same cruise (compliments of R. Hicks, University of Minnesota) were  $2.1 \times 10^9 \text{ cells L}^{-1}$ , which when multiplied by an average carbon content of 24 fg cell<sup>-1</sup> for Lake Michigan (Scavia et al. 1986) indicates that total bacterial carbon biomass accounted for about 15% of POC. Despite the assumptions and disparate sources of data used for these two calculations, they independently suggest that active and total bacteria were only a minor component of POC.

Finally, there was little active biomass in the very deep layer despite abundant [POC] associated with a nepheloid layer. This result supports those of Hicks and Owen (1991), who did not find any increase in deep-layer heterotrophic bacterial activity associated with a bottom nepheloid layer in Lake Michigan.

*Analysis of factors controlling biomass:* Using the model of Pace and Cole (1994) (Fig. 1), relationships between bacterial rRNA, eukaryotic rRNA, and POM may be examined

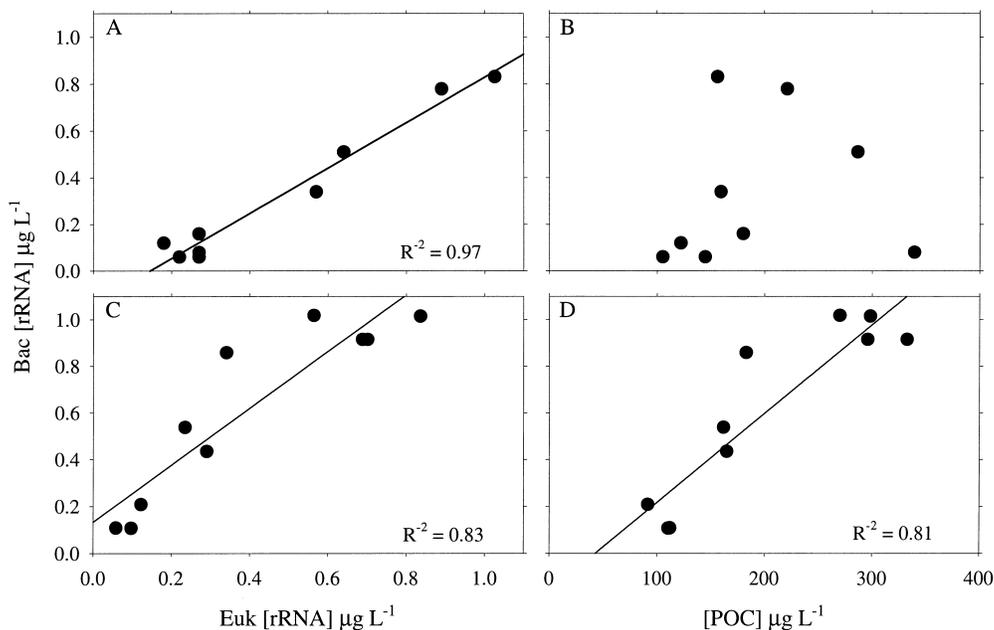


Fig. 3. Plots of relationships between bacterial and eukaryotic [rRNA] and between bacterial [rRNA] and [POC]. Panels A and B are for July; panels C and D are for October.

in the context of the relationship between active biomass and the bulk reservoir of suspended POM (Fig. 3). In Lake Michigan (Gardner et al. 1986; Scavia and Laird 1987; Pernie et al. 1990), as in many other environments (Cole et al. 1988), heterotrophic bacteria are primarily dependent on eukaryotes as sources of labile DOM released directly by phytoplankton (via excretion or autolysis) or released during grazing by zooplankton. Despite vertical variation in the chemical composition and origin of POM, the correlation between bacterial and eukaryotic [rRNA] throughout the water column in July (Fig. 3A) and October (Fig. 3C) is strong. This underscores the tight ecological relationship between these fundamental active components of the Lake Michigan planktonic ecosystem.

Using a similar approach, we may also examine the relationship between suspended POM and inferred active bacterial biomass. For July, bacterial [rRNA] and [POC] data do not covary in the surface layer (Fig. 3B); therefore, the fraction of POM composed of active bacterial biomass was not constant throughout the water column. One explanation for the lack of a constant relationship is a relaxed coupling between bacteria and the particulate pool of nutrients. According to the model of Pace and Cole (1994), this could result from more active grazing of bacteria. So in July, while the correlation between eukaryotes and bacteria was strong throughout the water column, the contribution of these active components of biomass to bulk POM varied, possibly because of some depth-dependent variation in grazing efficiency or preference. However in October, there was a strong correlation between [POC] and [rRNA] data (Fig. 3D). This suggests strong spatial coupling between inferred active biomass and concentration of suspended POM in the water column; the ecosystem became increasingly dependent on POM recycling for production as seasonal nutrient depletion in the surface layer progressed.

Whether the bacteria rely directly on POM as a source of carbon or their limiting resource covaries with POM is not known. Perhaps as phytoplanktonic production waned in the autumn, heterotrophic bacterial production became strongly limited by available DOM (Gardner et al. 1989), and the heterotrophic bacterial community was intimately associated with the demise of living eukaryotic particles. Alternatively, bacteria may rely on the direct hydrolysis of detrital POM, which may account for the deficit in bacterial carbon demand observed previously (Scavia and Laird 1987; Gardner et al. 1989; Pernie et al. 1990). Thus in October, there was little POC that was not coupled in some way with active bacterial biomass; this is evidenced by the  $x$ -intercept in Fig. 3D ( $43 \pm 37 \mu\text{g POC L}^{-1}$ ).

**Conclusions**—It has been asserted that the planktonic community in Lake Michigan is highly dependent on the recycling of POM by bacteria. By analyzing POM and rRNA at several depths in the summer and autumn, we have shown that the connection between heterotrophic bacteria and POM varies seasonally in this complex ecosystem. Although there appears to be a strong correlation between bacteria and eukaryotes throughout the year, the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of POM reflect the transition from primary production in the nutrient-rich deep layer to recycling-based production in the nutrient-poor surface layer as thermal stratification persists. It appears that the correlation between active heterotrophic bacteria and POM intensifies as this transition occurs, and it may be more correct to think of POM as recycling itself as opposed to being acted upon by independent microbial agents. Applying a combination of stable isotope and rRNA analyses has provided a new perspective on the relationship between planktonic organisms and POM not provided by more conventional measures.

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## References

- BÅMSTEDT, U., AND H. R. SKJOLDAL. 1980. RNA concentration of zooplankton: Relationship with size and growth. *Limnol. Oceanogr.* **25**: 304–316.
- BREMER, H., AND P. P. DENNIS. 1987. Modulation of chemical composition and other parameters of the cell by growth rate, p. 1527–1542. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger [eds.], *Escherichia coli* and *Salmonella Typhimurium* cellular and molecular biology. American Society for Microbiology.
- BROOKS, A. S., AND D. N. EDGINGTON. 1994. Biogeochemical control of phosphorous cycling and primary production in Lake Michigan. *Limnol. Oceanogr.* **39**: 961–968.
- COLE, J. J., S. FINDLAY, AND M. L. PACE. 1988. Bacterial production in fresh and saltwater ecosystems: A cross-system overview. *Mar. Ecol. Prog. Ser.* **43**: 1–10.
- DORTCH, Q., T. L. ROBERTS, J. R. CLAYTON, AND S. I. AHMED. 1983. RNA/DNA ratios and DNA concentrations as indicators of growth rate and biomass in planktonic marine organism. *Mar. Ecol. Prog. Ser.* **13**: 61–71.
- FAHNENSTIEL, G. L., AND D. SCAVIA. 1987. Dynamics of lake Michigan phytoplankton: The deep chlorophyll layer. *J. Great Lakes Res.* **13**: 285–295.
- FOGEL, M. L., AND L. A. CIFUENTES. 1993. Isotope fractionation during primary production, p. 73–98. *In* M. Engel and S. Macko [eds.], *Organic geochemistry*. Plenum.
- GARDNER, W. S., J. F. CHANDLER, AND G. A. LAIRD. 1989. Organic nitrogen mineralization and substrate limitation of bacteria in Lake Michigan. *Limnol. Oceanogr.* **34**: 478–485.
- GARDNER, W. S., J. F. CHANDLER, G. A. LAIRD, AND D. SCAVIA. 1986. Microbial response to amino acid additions in Lake Michigan: Grazer control and substrate limitation of bacterial populations. *J. Great Lakes Res.* **12**: 161–174.
- HICKS, R. E., AND C. J. OWEN. 1991. Bacterioplankton density and activity in benthic nepheloid layers of Lake Michigan and Lake Superior. *Can. J. Fish. Aquat. Sci.* **48**: 923–932.
- KEMP, P. F., S. LEE, AND J. LAROCHE. 1993. Estimating the growth rate of slowly growing marine bacteria from RNA content. *Appl. Environ. Microbiol.* **59**: 2594–2601.
- KERKHOF, L., AND P. KEMP. 1999. Small ribosomal RNA content in marine proteobacteria during non-steady-state growth. *FEMS Microbiol. Ecol.* **30**: 253–260.
- MACGREGOR, B. J. 1999. Molecular approaches to the study of aquatic microbial communities. *Curr. Opin. Biotechnol.* **10**: 220–224.
- , D. P. MOSER, K. H. NEALSON, AND D. A. STAHL. 1997. Crenarchaeota in Lake Michigan sediments. *Appl. Environ. Microbiol.* **63**: 1178–1181.
- , AND OTHERS. 2001. Microbiological, molecular biological, and stable isotope evidence for nitrogen fixation in the open waters of Lake Michigan. *Environ. Microbiol.* **3**: 209–219.
- MCCUSKER, E. M., P. H. OSTROM, N. E. OSTROM, J. D. JEREMIASON, AND J. E. BAKER. 1999. Seasonal variation in the biogeochemical cycling of seston in Grand Traverse Bay, Lake Michigan. *Org. Geochem.* **30**: 1543–1557.
- MOLL, R., AND M. BRAHCE. 1986. Seasonal and spatial distribution of bacteria, chlorophyll and nutrients in nearshore Lake Michigan. *J. Great Lakes Res.* **12**: 52–62.
- OSTROM, N. E., S. A. MACKO, D. DEIBEL, AND R. J. THOMPSON. 1997. Seasonal variation in the stable carbon and nitrogen isotope biogeochemistry of a coastal cold ocean. *Geochim. Cosmochim. Acta* **61**: 2929–2942.
- PACE, M. L., AND J. J. COLE. 1994. Comparative and experimental approaches to top-down and bottom-up regulation of bacteria. *Microb. Ecol.* **28**: 181–193.
- PERNIE, G. L., D. SCAVIA, M. L. PACE, AND H. J. CARRICK. 1990. Micrograzer impact and substrate limitation of bacterioplankton in Lake Michigan. *Can. J. Fish. Aquat. Sci.* **47**: 1836–1841.
- RASKIN, L., W. C. CAPMAN, R. SHARP, AND D. A. STAHL. 1997. Molecular ecology of gastrointestinal ecosystems, p. 243–298. *In* R. I. Mackie, B. A. White, and R. E. Isaacson [eds.], *Ecology and physiology of gastrointestinal microbes. V. 2: Gastrointestinal microbiology and host interactions*. Chapman and Hall.
- SCAVIA, D., AND G. L. FAHNENSTIEL. 1987. Dynamics of Lake Michigan phytoplankton: mechanisms controlling epilimnetic communities. *J. Great Lakes Res.* **13**: 103–120.
- , AND G. A. LAIRD. 1987. Bacterioplankton in lake Michigan: dynamics controls and significance to carbon flux. *Limnol. Oceanogr.* **32**: 1017–1033.
- , G. A. LAIRD, AND G. L. FAHNENSTIEL. 1986. Production of planktonic bacteria in Lake Michigan. *Limnol. Oceanogr.* **31**: 612–626.

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- SHAFFER, M. M., AND D. E. ARMSTRONG. 1994. Mass fluxes and recycling of phosphorus in Lake Michigan, p. 285–322. In L. A. Baker [ed.], *Environmental chemistry of lakes and reservoirs*. Advances in Chemistry Series 237. American Chemical Society.
- STAHL, D. A., B. FLESHER, H. R. MANSFIELD, AND L. MONTGOMERY. 1988. Use of phylogenetically based hybridization probes

for studies of ruminal microbial ecology. *Appl. Environ. Microbiol.* **54**: 1079–1084.

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## Phagotrophy and toxicity variation in the mixotrophic *Prymnesium patelliferum* (Haptophyceae)

**Abstract**—Phagotrophy was investigated in the photosynthetic and ichthyotoxic *Prymnesium patelliferum* (Haptophyceae) using fluorescent microspheres, fluorescently labeled bacteria, and live bacteria cells. Ingestion rates were estimated both from prey uptake and disappearance experiments in phosphorus (P)-limited and -replete algal cultures. *Prymnesium patelliferum* was feeding preferentially on bacteria (bact) compared to fluorescent microspheres (FM). Large fluorescent microspheres (1.6 and 3  $\mu\text{m}$ ) were ingested at very low rates ( $<0.1$  FM alga $^{-1}$  h $^{-1}$ ), and small microspheres (0.5  $\mu\text{m}$ ) were not ingested. Ingestion of bacteria (mean size 2  $\mu\text{m}$ ) was highest in P-limited *P. patelliferum* cultures (up to four bact alga $^{-1}$  h $^{-1}$ ) compared to P-replete cultures (0–1.2 bact alga $^{-1}$  h $^{-1}$ ). In addition, cellular P content of P-limited cells fed with bacteria became similar to those of P-replete cultures after 48 h, indicating a close relation between cellular P content and feeding behavior. Hemolytic activity of *P. patelliferum* was up to four times higher in P-limited cultures compared to P-replete cultures. During the transition from P-limiting to P-replete conditions, the addition of bacteria and/or the corresponding bacterial filtrate ( $<0.2$   $\mu\text{m}$ ) and/or  $\text{PO}_4^{3-}$  to P-limited cultures resulted in a decrease (50%) of the hemolytic activity after 24 h in relation to controls (no addition of bacteria, filtrate, or P). No  $\text{PO}_4^{3-}$  was detectable as a result of enriching cultures with bacterial cells or bacterial filtrates. These results indicate that *P. patelliferum* can use different sources of P (inorganic and dissolved, organic and particulate) and adapt its mode of nutrition in a short time. Furthermore, the decrease of hemolytic activity in the highly toxic P-limited cells also occurred rapidly following a recovery in cellular P status through mixotrophic feeding or uptake of inorganic phosphate, suggesting that toxicity in *P. patelliferum* cells can be minimized by nutrient manipulation.

Blooms of toxic haptophytes, mainly consisting of *Chrysochromulina polylepis*, *C. leadbeateri*, *Prymnesium parvum*, and *P. patelliferum*, have occurred in Scandinavia for the last 10 yr (Edwardsen and Paasche 1998). These blooms, mostly *C. polylepis*, have simultaneously killed hundreds of tons of farmed fish, resulting in large economic losses in this region. The toxic substances of the genera *Chrysochromulina* and *Prymnesium* have been largely studied in terms of their physiological effects (i.e., ichthyotoxic, cytotoxic, neurotoxic) and their responses to environmental conditions.

Toxicity can be greatly affected by the growth phase and stress conditions of the alga (i.e., light, salinity, nutrients; Edwardsen and Paasche 1998). Both nitrogen and phosphorus limitation have been shown to increase toxicity in *P. parvum* compared to nutrient repletion (Johansson and Granéli 1999).

Phagotrophy in photosynthetic haptophytes (i.e., mixotrophy) is well described in the genus *Chrysochromulina* (Jones et al. 1993; Nygaard and Tobiesen 1993) and to a lesser extent in the genus *Prymnesium* (Nygaard and Tobiesen 1993; Tillmann 1998). Most attention has been directed toward the physiology of these mixotrophs (i.e., the influence of biotic/abiotic factors on their heterotrophic capabilities). Nutrient limitation has been shown to have different effects on grazing activity in various mixotrophs (i.e., stimulation) (Caron et al. 1993; Legrand et al. 1998) or no effect (Skovgaard 1996). Phagotrophic nutrition may in turn affect cellular chemical composition and, thus, the synthesis of toxic substances. The percentage of naturally occurring *Dinophysis acuminata* cells containing food vacuoles has been shown to be related to the content of okadaic acid or dinophys toxin (DTX) per cell and seemed to be dependent on the nutrient conditions in the environment (see fig. 8 in Granéli and Carlsson 1998). However, the interaction between mixotrophic nutrition and toxicity in toxic phytoplankton has not been investigated.

In this study, we present data on bacterivory in *Prymnesium patelliferum*, showing that the ingestion of bacteria provided an immediate source of phosphorus, which enhanced the growth of phosphorus (P)-limited *P. patelliferum* cells and, in turn, decreased the hemolytic activity in previously P-limited cells.

**Culture and growth conditions**—A nonaxenic strain of *P. patelliferum* (Rhpat89 obtained from the Oslo Algal collection, Norway) was grown in triplicates in modified f/10 medium (Guillard 1995) corresponding to two different N:P ratios (160:1, 16:1), giving a limited P supply and no nutrient limitation with the following concentrations (N:P = 160:1:  $\text{NO}_3^- = 120$   $\mu\text{M}$ ,  $\text{PO}_4^{3-} = 0.75$   $\mu\text{M}$ ; N:P = 16:1:  $\text{NO}_3^- = 120$   $\mu\text{M}$ ,  $\text{PO}_4^{3-} = 7.5$   $\mu\text{M}$ ). The abundance of background bacteria (bact, mainly small cocci) ranged from 0.02 to  $0.05 \times 10^6$  bact ml $^{-1}$  in the cultures used in this study.