Freshwater Biology (1994) **31**, 183–190

Influence of phytoplankton on the response of bacterioplankton growth to nutrient enrichment

LIZHU WANG* AND JOHN C. PRISCU†
Department of Biology, Montana State University, Bozeman, MT 59717, U.S.A.

* *Present address: DNR Area Fisheries Office, 7372 State Highway 25 SW, Montrose, MN 55363, U.S.A.*
† *Author to whom correspondence should be sent*

SUMMARY

1. Laboratory experiments were conducted to test the effect of nutrient enrichment on bacterioplankton growth in the presence and absence of phytoplankton.
2. In one series of experiments, bacterioplankton growth in terms of specific activity [^{3}H-thymidine incorporation (cell number)$^{-1}$] was greater in whole lake water samples than in samples from which phytoplankton had been removed by filtration (1.0 μm), regardless of the nutrient enrichments (control, NH_4^+ plus PO_4^{3-}, and mannitol). Organic C enhanced bacterioplankton growth in both whole and filtered lake water.
3. In another series of experiments (with the same nutrient enrichments as in the first experiment except that glucose replaced mannitol), bacterioplankton growth in whole lake water enriched with PO_4^{3-} plus NH_4^+ and incubated in the light was greater than in two treatments designed to inhibit photosynthetic activity (+DCMU and dark). Bacterioplankton response to nutrient addition was greatest in the PO_4^{3-} plus NH_4^+ enrichment under all three conditions (light +DCMU, and dark).
4. These results indicate that bacterioplankton growth could be directly limited by inorganic P and N when these elements are in short supply. Enhancement of bacterioplankton growth by phytoplankton occurs only under PO_4^{3-} and NH_4^+ replete environments.

Introduction

Heterotrophic bacterioplankton are not only responsible for the degradation of organic matter and recycling of nutrient, but also are important producers of particulate organic matter (Azam *et al.*, 1983). Thus, they may provide a route for the assimilation of dissolved organic matter into classical food chain. Because of the ecological importance of bacterioplankton in aquatic ecosystems, knowledge of the factors regulating their activity will lead to a more thorough understanding of ecosystem processes.

It is commonly believed that phytoplankton growth is limited by inorganic P and N (Dodds, Johnson & Priscu, 1989; Elser, Marzolf & Goldman, 1990) but that bacterioplankton growth is limited by organic C (Azam *et al.*, 1983; Riemann & Søndergaard, 1986; Bjørnsen *et al.*, 1989). Phytoplankton are thought to be the main source of the organic C used by bacterioplankton (Currie, 1990). Inorganic P and N concentrations may therefore determine phytoplankton abundance, which in turn, can regulate bacterioplankton growth (Currie, 1990). Such a relationship is supported by the positive correlations observed between bacterioplankton production and phytoplankton biomass (Fuhrman, Ammerman & Azam, 1980; Cole, Findlay & Pace, 1988; White *et al.*, 1991). Recent studies have shown that bacterioplankton growth rates can be stimulated directly by inorganic P enrichment in both oligotrophic (Coveney & Wetzel, 1992) and mesotrophic lake water (Toolan, Wehr & Findlay, 1991). These reports are consistent with the fact that bacterioplankton inorganic P and N uptake systems have higher affinities for P and N than those of phytoplankton (Currie & Kalff, 1984; Currie, Bentzen & Kalf, 1986; Vadstein & Olsen, 1989). Col-

lectively, these latter findings indicate that bacterioplankton growth in nature is not controlled solely by the availability of organic C substrates.

To address the questions of whether inorganic P and N or organic C limit bacterioplankton growth, and whether this limitation is influenced by phytoplankton photosynthetic activity, we conducted two types of experiments using samples from a eutrophic lake. In the first, bacterioplankton growth response to nutrient enrichment in whole lake water samples was compared with samples from which phytoplankton were removed. In the second, bacterioplankton growth response to nutrient enrichments in lake water with a photosynthetically viable phytoplankton assemblage was compared with treatments where photosynthesis was inhibited. In general, our results indicate that bacterioplankton growth can be limited directly by inorganic N and P when these elements are in short supply.

Materials and Methods

Initial conditions

Organisms were collected from Hebgen Lake, a 50 km^2 eutrophic reservoir located on the upper Madison River, Montana, U.S.A. on 22 September 1989 (phytoplankton exclusion experiment) and 19 June 1990 (inhibition of photosynthesis experiment). In both experiments, 900 ml lake water was placed in duplicate autoclaved 1 l flasks on a G10 Gyrotory Shaker at 60 rpm. An irradiance of 120 $\mu E\, m^{-2}\, s^{-1}$ was provided by 40 W 'cool-white' fluorescent lamps and water temperature was maintained at approximately 25°C.

Phytoplankton exclusion experiment

Two treatments, one with and one without phytoplankton, were used in the experiment. In treatments without phytoplankton, water was filtered through 1.0 µm membrane filters under weak suction (<8 cmHg) to remove phytoplankton, then examined under an epifluorescence microscope to confirm the absence of chlorophyll autofluorescence. In treatments with phytoplankton, water was filtered through 280 µm Nitex mesh (no vacuum) to remove large grazers and detritus. In both treatments (with and without phytoplankton), three initial nutrient enrichments were established: control (ambient concentrations), 140 µg NH_4Cl-N l^{-1} + 93 µg KH_2PO_4-P l^{-1}, and 91 mg mannitol l^{-1}. Concentrations of N, P, and C addition were predetermined from results of *in situ* microcosm experiments (Wang, Miller & Priscu, 1992) to ensure that no nutrient depletion occurred during the incubation.

Chlorophyll *a* subsamples were collected from each flask at the start of the incubation and at the end of the experiment on Day 5. Chlorophyll *a* concentrations were measured by initial extraction in 95% ethanol heated to boiling followed by overnight extraction at 4°C (Sartory & Grobbelaar, 1984). Fluorescence of the extract was measured with a Turner model 112 fluorometer and compared with a chlorophyll *a* standard curve made using pure *Anacystis* chlorophyll *a* (Sigma Chemical Co.). Ammonium was determined by the phenol hypochlorite method (Solorzano, 1969) and soluble reactive phosphorus (SRP) by the mixed molybdate method modified for arsenate interference (Downes, 1978). Dissolved organic carbon (DOC) was determined with a Dorhmann Carbon Analyzer standardized with glucose.

Three subsamples for bacterial ^{3}H-thymidine incorporation and cell number were taken from each replicate 4 h after starting the incubation, and on Days 1, 3, and 5. Bacterial thymidine incorporation was determined by adding high activity (55 Ci $mmol^{-1}$) methyl-^{3}H-thymidine (ICN Radiochemical Inc.) to 10 ml water samples (final concentration 10 nM) in 20 ml glass scintillation vials. Pilot experiments showed that 10 nM thymidine saturated bacterial uptake mechanisms and autoradiography confirmed that thymidine was utilized exclusively by bacteria. ^{3}H-thymidine was evaporated to dryness and rehydrated with deionized water before use to eliminate products of self radiolysis and to remove ethanol. The inoculated sample was incubated at 25°C in the dark for 30 min. Activity was terminated by adding 10 ml ice-cold 10% trichloroacetic acid (TCA) to each vial. Following overnight extraction at 4°C, samples were filtered onto 0.2 µm membrane filters (Poretics Corporation). After rinsing five times (2 ml each rinse) with ice-cold 5% TCA, the filter was transferred to a 20 ml scintillation vial and 7 ml Cytoscint scintillation cocktail (ICN Radiochemical, Irvine, CA) was added. Radioactivity of each sample was determined by standard liquid scintillation spectrometry using a Beckman LS-100C counter. Counting efficiency was

determined by the external standard ratio method using ^{3}H-toluene as a reference and acetone as the quenching agent. Samples for bacterial counts were fixed with formaldehyde (3% final concentration) and enumerated using the acridine orange direct count technique (Hobbie, Daley & Jasper, 1977).

Inhibition of photosynthesis experiment

Three treatments (light, light + photosynthetic inhibitor, dark) were tested with water gravity filtered through 280 μm Nitex mesh to remove large zooplankton and detritus. The photosynthetic inhibitor, 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), was added to a final concentration of 5×10^{-6} M in one treatment, an amount we found to be sufficient to inhibit oxygenic photosynthesis completely. Flasks were covered with two layers of aluminium foil for the dark treatment. All three treatments were further subjected to three levels of nutrient addition: control (ambient concentration); 140 μg NH_4Cl-N l^{-1} + 93 μg KH_2PO_4-P l^{-1}; and 90 mg glucose l^{-1}. Chlorophyll *a* concentrations were measured 4 h after the start of incubation and on Day 5; bacterial thymidine incorporation and cell number were measured daily over the entire 5 days of the experiment. Sample size and analytical methods were the same as described for the phytoplankton exclusion experiment.

Test of significance

To determine the effect of inorganic P, inorganic N and organic C on bacterioplankton specific activity (thymidine incorporation per cell number), each nutrient addition was compared with controls by analysis of variance (three-way ANOVA; Snedecor & Cochran, 1980). The comparisons were done on two replicate samples (three subsamples for each replicate) from each treatment for all time course data. A *t*-test was used to compare the influence of phytoplankton on bacterioplankton cell number, bacterioplankton thymidine incorporation and bacterioplankton specific activity after 72 h incubation in whole lake water with samples from which phytoplankton were removed or photosynthesis was inhibited. The nutrient influences on phytoplankton chlorophyll *a* at the end of the experiments (120 h) for different nutrient additions were also compared with controls using a *t*-test. Because grazing by protozoans can influence cell number and thymidine incorporation, specific activity should yield the most realistic estimate of bacterial response to experimental manipulation. Hence, we confined most of our comparisons to changes in bacterial specific activity.

Results

Background conditions

During the phytoplankton exclusion experiment, the dominant phytoplankton species in the whole lake water treatment were the cyanobacterium *Anabaena spiroides* var. *crassa* Lemm (69% of total biovolume) and the chrysophyte *Ochromonas* sp. (13% of total biovolume). Before starting the incubation, the experimental water had the following conditions: chlorophyll $a = 3.5\,\mu g\,l^{-1}$, SRP $= 10\,\mu g\,l^{-1}$, NH_4^+-N $= 11\,\mu g\,l^{-1}$, and DOC $= 6.6\,mg\,l^{-1}$. During the inhibition of photosynthesis experiment, the dominant phytoplankton in the experimental water were the chlorophytes *Schroederia* sp. (48% of total biovolume) and *Closteridum* sp. (43% of total biovolume). At the beginning of the latter experiment, the lake water had the following conditions: chlorophyll $a = 178\,\mu g\,l^{-1}$, SRP $= 8\,\mu g\,l^{-1}$, NH_4^+-N $= 9\,\mu g\,l^{-1}$, and DOC $= 7.5\,mg\,l^{-1}$.

Nutrient effects on bacterioplankton

Organic C enrichments significantly increased bacterioplankton specific activity in both whole water and filtered lake water treatments in the phytoplankton exclusion experiment ($P < 0.01$). At the end of the incubation, bacterial specific activities in organic C treatments were two to ten times higher compared with controls (Fig. 1a & b, Table 1). Inorganic P and N significantly stimulated bacterioplankton specific activity in the whole lake water treatment only ($P < 0.01$). In the photosynthesis inhibition experiment, bacterioplankton specific activity was stimulated significantly only by inorganic P and N enrichment ($P < 0.01$) (Fig. 2, Table 1).

Phytoplankton effect on bacterioplankton

Bacterioplankton thymidine incorporation, cell number and specific activity in all nutrient enrichments with phytoplankton present were significantly

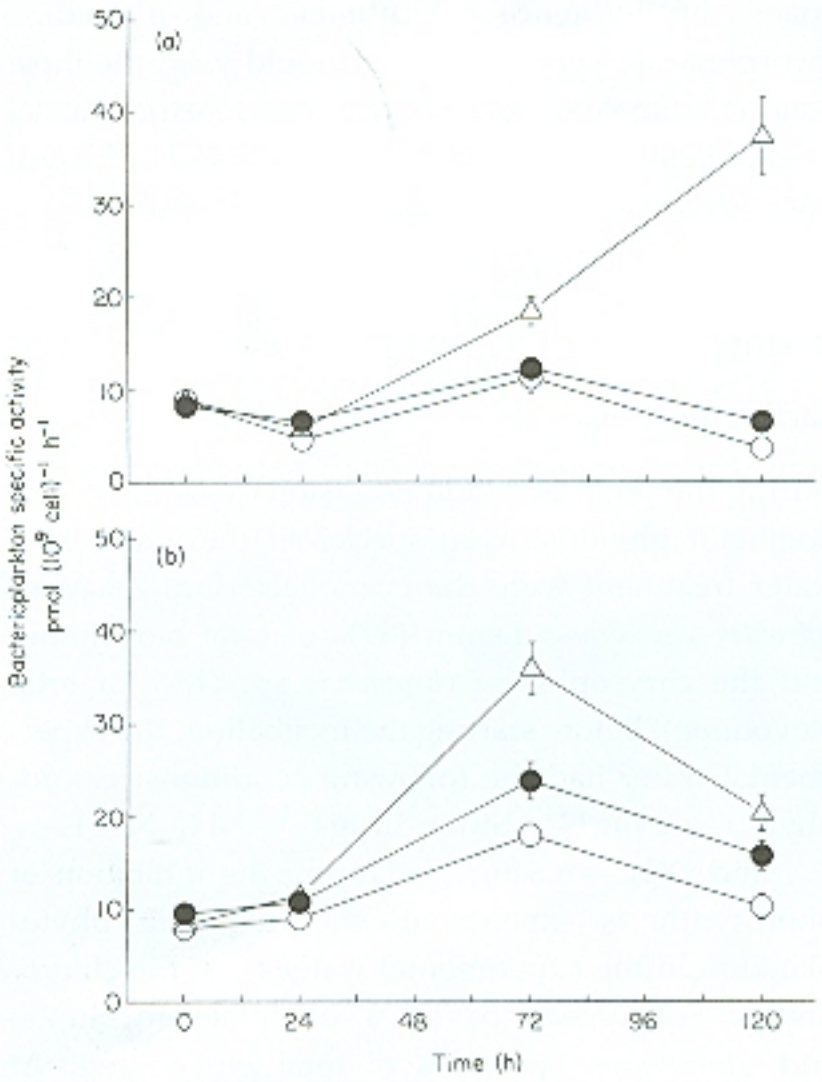

Fig. 1 Bacterioplankton specific activity in filtered lake water (a) and whole lake water (b) treatments in the phytoplankton exclusion experiment. Error bars are ±1 SE ($n = 2$) and are shown only if larger than the symbol. ○, Control; ●, N + P; △, C.

higher ($P < 0.05$) than in the same enrichments with phytoplankton absent during the phytoplankton exclusion experiment (Fig. 3). In the photosynthesis inhibition experiment, active photosynthesis yielded significantly ($P < 0.05$) higher bacterial thymidine incorporation and bacterial specific activity in the inorganic P plus N enrichments only (Fig. 4, Table 1). Photosynthesis inhibition did not significantly influence bacterial activity in control and organic C enrichments.

Nutrient effect on phytoplankton

Phytoplankton chlorophyll *a* increased significantly in the inorganic P plus N ($P < 0.01$) and organic C ($P < 0.05$) enrichments compared with controls in the whole lake water treatment of the phytoplankton exclusion experiment (Fig. 5a). Phytoplankton chlorophyll *a* was also stimulated significantly ($P < 0.01$) by inorganic P and N enrichment in the light treatment during the 5 day photosynthetic inhibition experiment (Fig. 5b).

Discussion

Stimulation of bacterial activity by organic C addition occurred during the 1989 phytoplankton exclusion experiment only, implying that ambient organic C supply limited bacterial growth at this time. Conversely, lack of stimulation by organic C during the 1990 experiment indicated a system with sufficient organic carbon. These results may be related to the ambient phytoplankton biomass (i.e. trophic state) at the time of the experiment, which was almost 50-fold greater in 1990. The large particulate organic C pool (mostly phytoplankton) in 1990 presumably increased the supply of dissolved organic C and

Table 1 Time course means of bacterial thymidine incorporation (^{3}H-THY, nmole l^{-1} h^{-1}), bacterial cell number (CELL, 10^6 ml^{-1}), and specific activity [SP-ACT, pmol $(10^9$ cell$)^{-1}$ h^{-1}] in the phytoplankton exclusion experiment and the photosynthesis inhibition experiment. All measured points were used in the multifactor analysis of variance to test the significant difference between control and the other treatments

	Phytoplankton exclusion experiment						Photosynthesis inhibition experiment								
	Algae present			Algae absent			Light			Light + DCMU			Dark		
Nutrient additions	^{3}H-THY	CELL	SP-ACT	^{3}H-THY	CELL	SP-ACT	^{3}H-THY	CELL	SP-ACT	^{3}H-THY	CELL	SP-ACT	^{3}H-THY	CELL	SP-ACT
Control	0.04	3.23	11.3	0.02	2.54	7.12	0.08	2.07	36.3	0.08	2.16	37.3	0.08	2.08	38.2
P + N	0.06	3.78*	15.0	0.03*	3.18*	8.55*	0.43	4.29	99.1	0.18	2.88	63.4	0.19	2.89	66.0
C	0.08	3.82	19.0	0.06	3.51	17.6	0.09*	2.04*	39.7*	0.08*	2.17*	38.3*	0.08*	2.20*	36.0*

* Not significantly different from control, $P > 0.05$.
All the other nutrient enrichments are significantly different from control at $P < 0.05$.

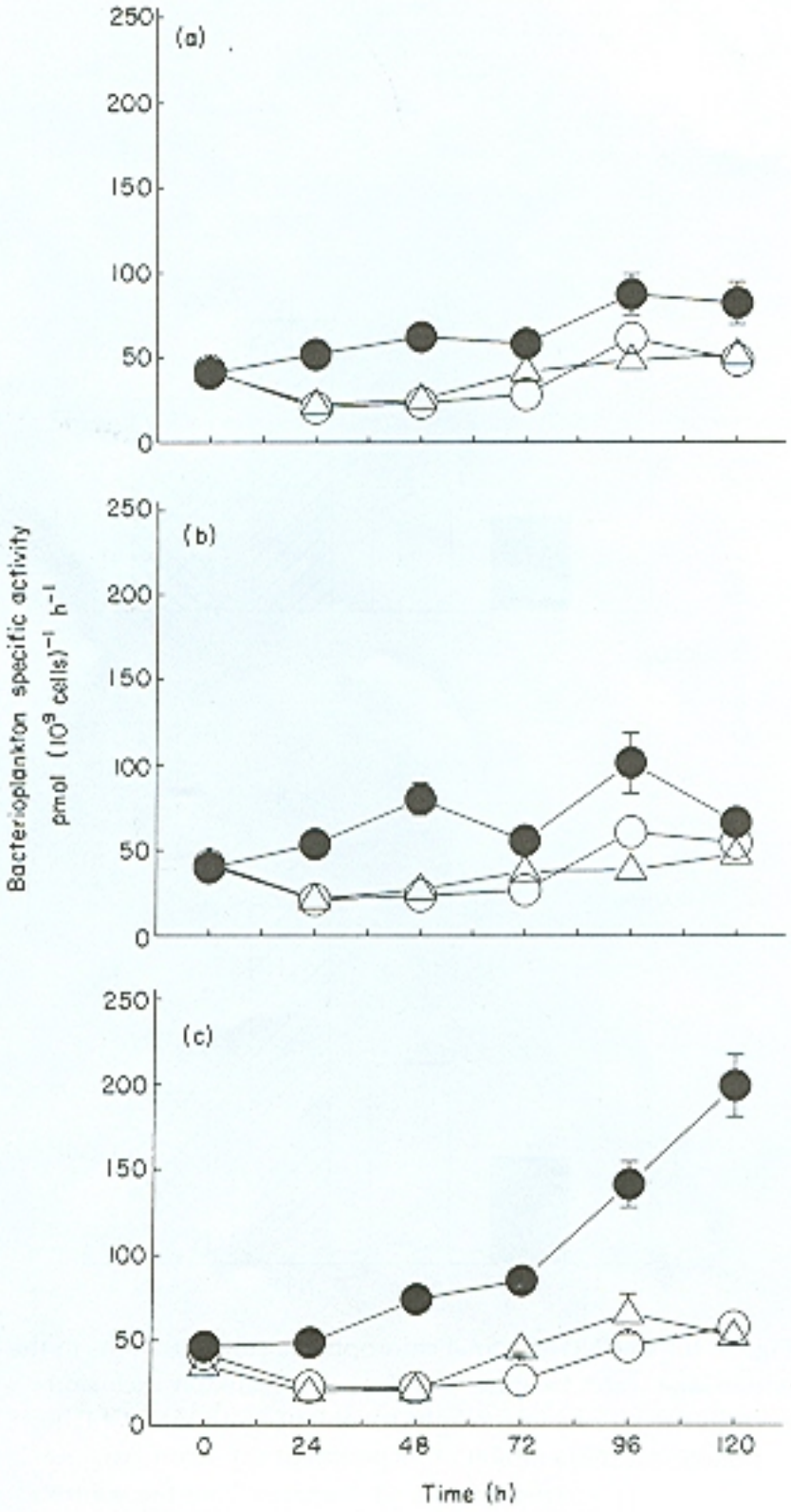

Fig. 2 Bacterioplankton specific activity in the DCMU (a), dark (b), and light (c) treatments in the inhibition of photosynthesis experiment. Error bars are ±1 SE ($n = 2$) and are shown only if larger than the symbol. ○, Control; ●, N + P; △, C.

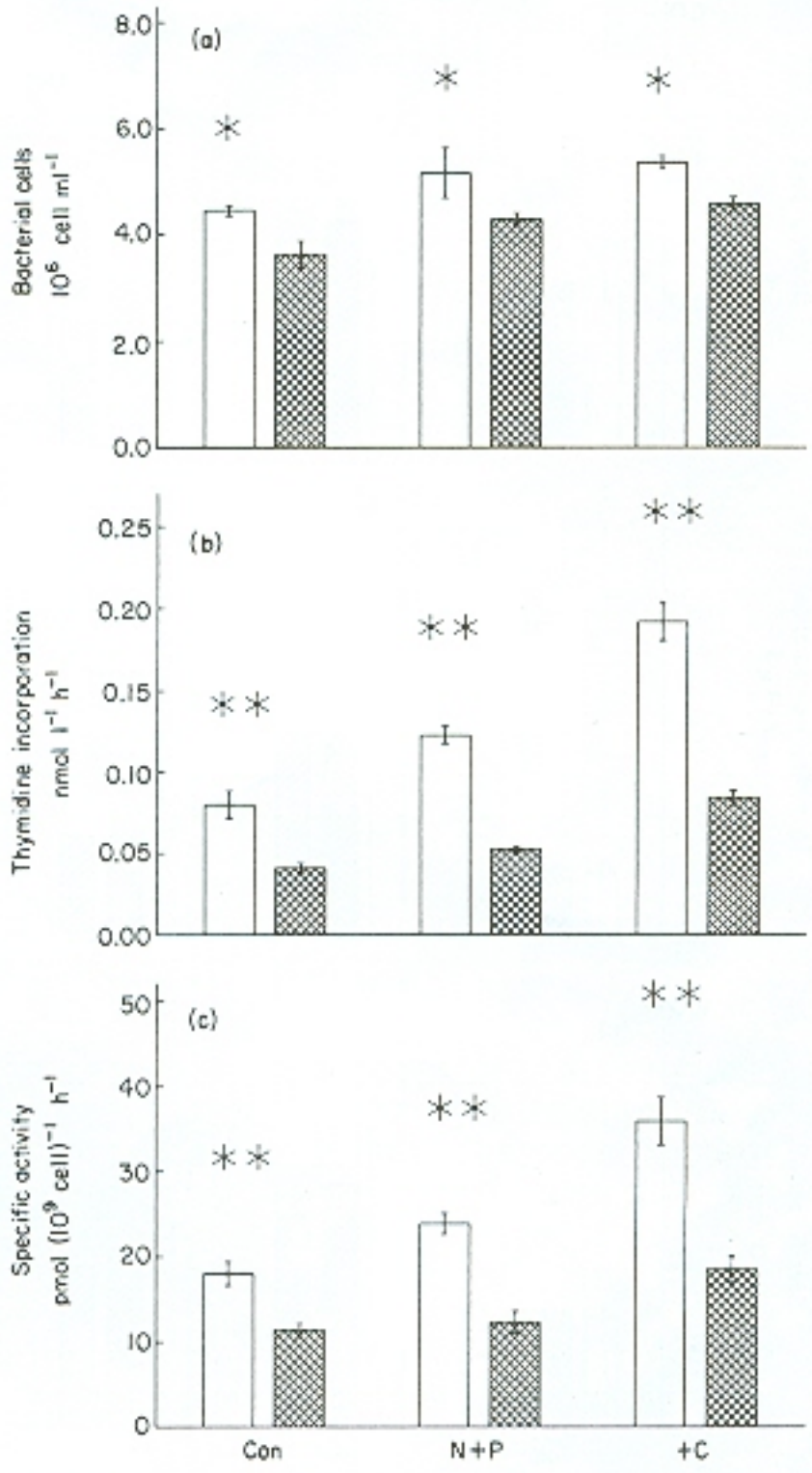

Fig. 3 Bacterioplankton cell number (a), thymidine incorporation (b), and specific activity (c) after 72 h of incubation in the phytoplankton exclusion experiment. Open bars indicate whole lake water (phytoplankton present); cross-hatched bars represent filtered lake water (phytoplankton absent). Error bars show ±1 SE (n = 2). Asterisks denote statistical differences between whole lake water and filtered lake water for each treatment; * $P < 0.05$, ** $P < 0.01$.

supported relative high ambient bacterioplankton specific activity (5-fold higher in 1990 than in 1989). Although we contend that the phytoplankton DOC supply rate was greater in 1990, the DOC pool size was similar to that in 1989. Similar DOC pool sizes can occur, despite different supply rates, if DOC is in a dynamic equilibrium between uptake and supply. Such an equilibrium concept is supported by the data of Herbst & Overbeck (1978), who found that algal products never accumulated in growth medium because bacterial utilization of DOC balanced algal release. Bell & Sakshaug (1980) also reported that a 4-fold increase in bacterial activity was enough to prevent a large accumulation of dissolved extracellular products during an algal bloom in Trondheimsfjord, Norway.

Inorganic P and N enrichments stimulated bacterioplankton specific activity in four of our five

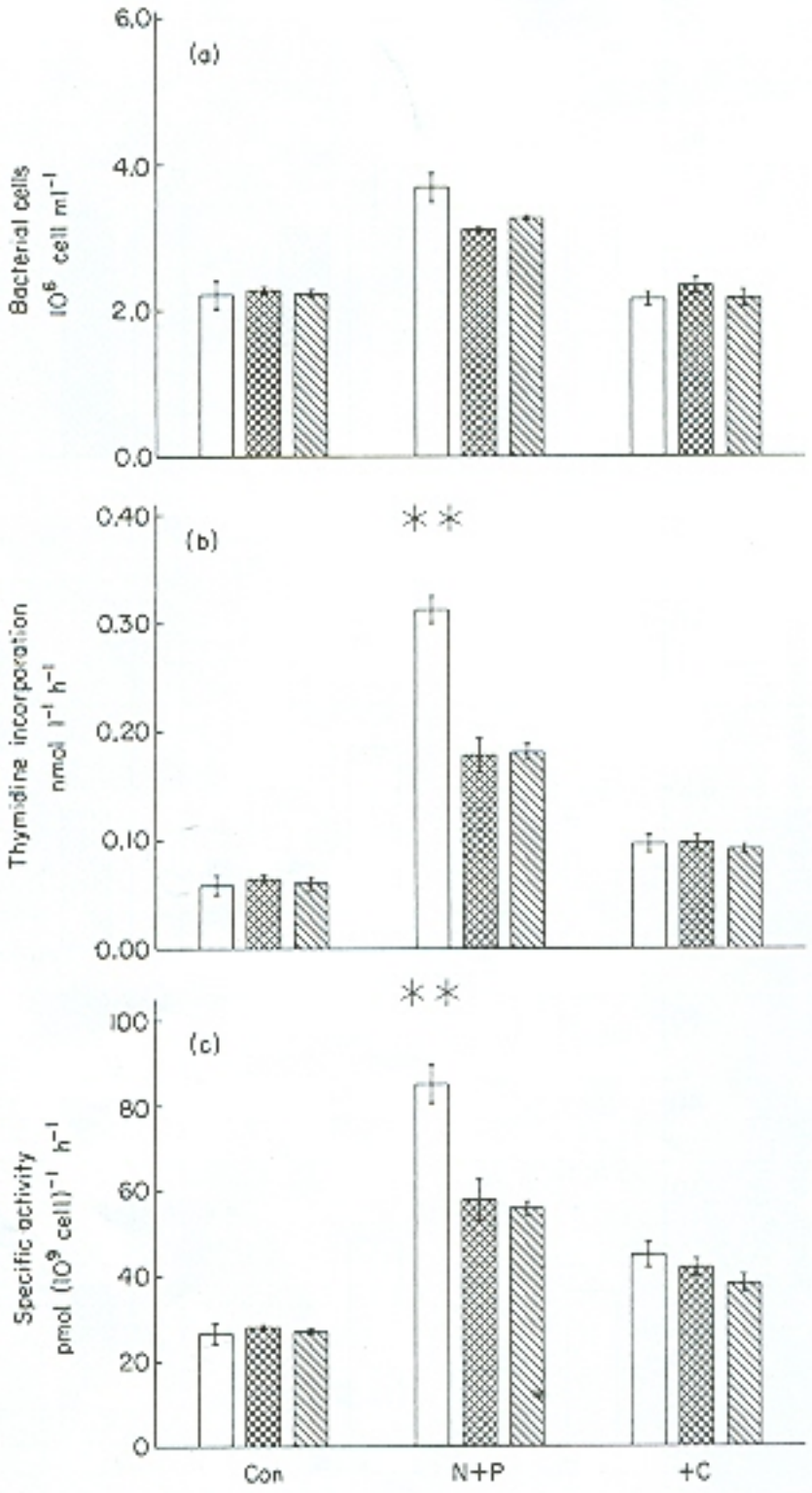

Fig. 4 Bacterioplankton cell number (a), thymidine incorporation (b), and specific activity (c) after 72 h of incubation in the photosynthesis inhibition experiment. Open bars = light incubation (photosynthesis present); cross-hatched bars = DCMU (photosynthesis inhibited); hatched bars = dark (photosynthesis inhibited). Error bars show ±1 SE ($n = 2$). Asterisks denote statistical differences between DCMU and dark samples, and the control for each of the treatments; ** $P < 0.01$.

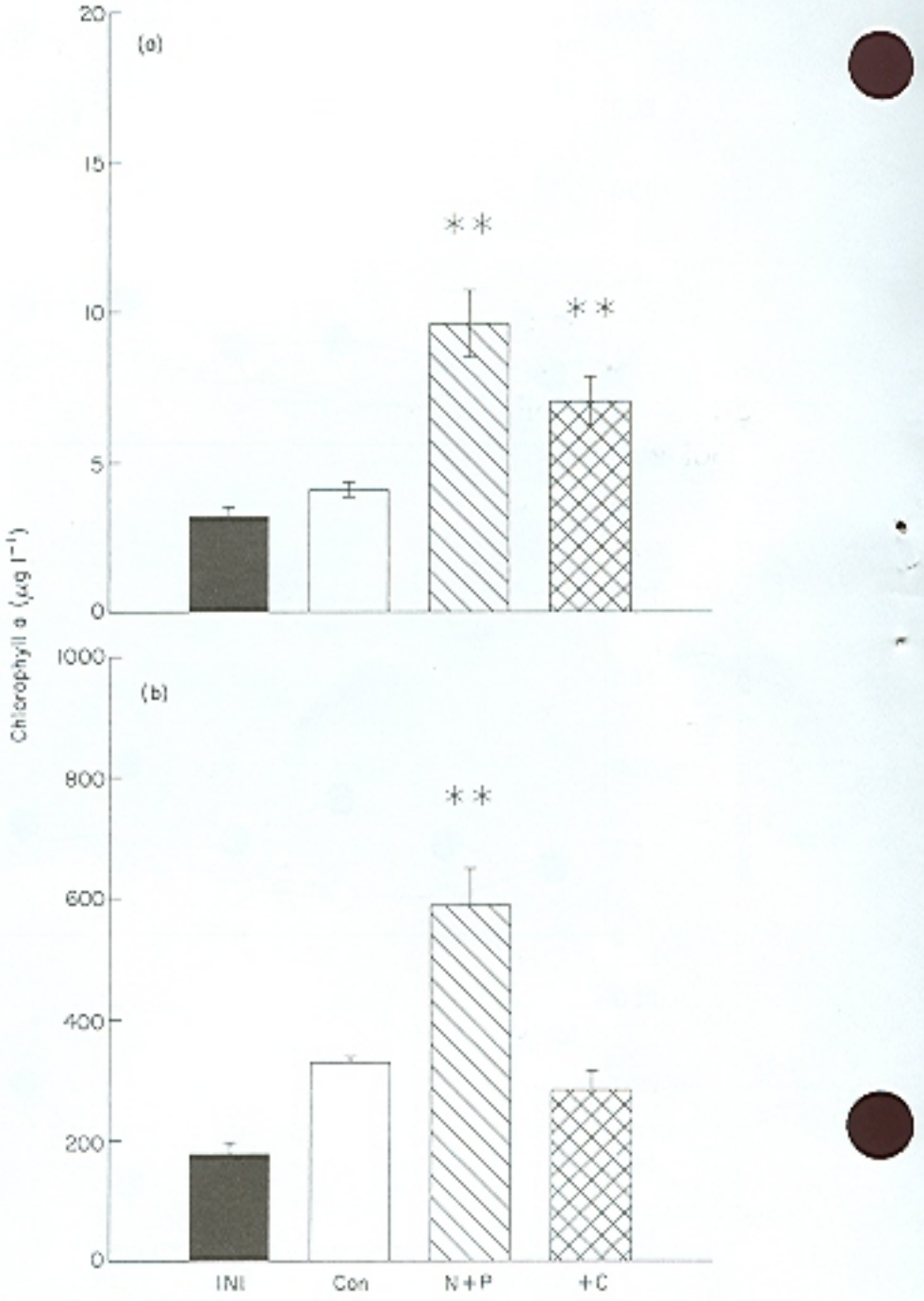

Fig. 5. Initial (INI) and final chlorophyll *a* concentrations in the whole lake water treatment of the phytoplankton exclusion experiment (a) and in the light treatment (without DCMU) of the photosynthesis inhibition experiment (b). Error bars are ±1 SE ($n = 2$). Asterisks denote differences from the control (Con); * $P < 0.05$, ** $P < 0.01$. Con = final chlorophyll *a* in the control, N + P = final chlorophyll *a* in the N + P-enriched treatment, +C = final chlorophyll *a* in the carbon-enriched treatment. Chlorophyll *a* did not change significantly in DCMU and dark incubations (data not shown).

treatments regardless of the presence or absence of phytoplankton activity (Figs 1b, 2a, b & c), indicating that inorganic P and N can directly limit bacterioplankton growth in our study lake. Such results are supported by recent reports of inorganic P stimulation of bacterioplankton growth in an oligotrophic lake (Coveney & Wetzel, 1992) and in a mesoeutrophic lake (Toolan *et al.*, 1991). Our results, in concert with those of others, do not support the common view that the growth of bacterioplankton in nutrient-deficient lakes is always limited by the availability of reduced carbon substrates (Currie & Kalff, 1984).

Phytoplankton stimulated bacterioplankton specific activity during our 1989 experiment, where there was an apparent deficiency of organic C, regardless of nutrient enrichments (Fig. 3). The higher bacterio-

plankton activity in the whole water compared to the filtered water was apparently not the result of filtering injury of bacteria, because high bacterial activity was observed in the organic C enrichment after filtration (Fig. 1a). Such a result implies that materials released from phytoplankton might have compensated for the shortages of organic C for bacterioplankton. Our contention is supported by the results from other studies showing enhanced bacterial activity in the presence of algae (Peterson *et al.*, 1985; Murray, Cooksey & Priscu, 1986). However, the stimulation of bacterioplankton by phytoplankton during our 1990 experiment occurred only in the inorganic P and N enrichment (Fig. 4). This latter result indicates that phytoplankton products control bacterial growth only when excess P and N are available, and that the commensalistic relationship between phytoplankton and bacterioplankton, with respect to DOC exchange, occurs only when there is sufficient inorganic P and N. This scenario is supported by our results showing that inorganic P and N enrichment stimulated bacterioplankton activity in the 1989 experiment (an apparently C-deficient environment) when phytoplankton were present (Fig. 1b).

We have used specific activity as a primary index of bacterioplankton growth. The use of this index has several advantages in interpreting our results. Firstly, specific activity (a physiological parameter) would respond rapidly and directly to a limiting nutrient enrichment. Secondly, specific activity should be independent of small changes in initial cell density (which could result from filtration), allowing direct comparison between whole water and filtered water treatments. Finally, direct grazing effect on bacterioplankton cell density is eliminated.

In summary, our experiments demonstrated that bacterioplankton growth can be stimulated by direct additions of inorganic P and N in the absence of phytoplankton. Indirect stimulation of bacterioplankton from inorganic P and N via phytoplankton products occurred only when organic C was apparently in short supply. Collectively, our results from two seasons indicate that nutrient limitation of phytoplankton and bacterioplankton varies temporally in Hebgen Lake. Moreover, the commensalistic relationship between phytoplankton and bacterioplankton is not always present. The intensity of this latter relationship apparently depends on rates of DOC supply.

Acknowledgments

We thank T. Miller and T. Sharp for their assistance in the field. K. Lohman, M. Lizotte and an anonymous reviewer provided comments that improved the manuscript. This study was conducted under a grant from the Soap and Detergent Association to J.C.P. Additional support was provided by National Science Foundation Grants DPP-9117907 and DPP-88-20591 to J.C.P.

References

Azam F., Fenchel T., Field J.G., Gray J.S., Meyer-Reil L.A. & Thingstad F. (1983) The ecological role of water-column microbes in the sea. *Marine Ecology Progress Series*, **10**, 257–263.

Bell W.H. & Sakshaug E. (1980) Bacterial utilization of algal extracellular products. 2. A kinetic study of natural populations. *Limnology and Oceanography*, **25**, 1021–1033.

Bjørnsen P.K., Riemann B., Pock-Steen J., Nielsen T.G. & Horsted S.J. (1989) Regulation of bacterioplankton production and cell volume in a eutrophic estuary. *Applied Environmental Microbiology*, **55**, 1512–1518.

Cole J.J., Findlay S. & Pace M.L. (1988) Bacterial production in fresh and saltwater ecosystems: a cross-system over-view. *Marine Ecology Progress Series*, **43**, 1–10.

Coveney M.F. & Wetzel R.G. (1992) Effects of nutrients on specific growth rate of bacterioplankton in oligotrophic lake water cultures. *Applied Environmental Micobiology*, **58**, 150–156.

Currie D.J. (1990) Large-scale variability and interactions among phytoplankton, bacterioplankton, and phosphorus. *Limnology and Oceanography*, **35**, 1437–1455.

Currie D.J. & Kalff J. (1984) A comparison of the abilities of freshwater algae and bacteria to acquire and retain phosphorus. *Limnology and Oceanography*, **29**, 298–310.

Currie D.J., Bentzen E. & Kalff J. (1986) Does algal–bacterial phosphorus partitioning vary among lakes? A comparative study of orthophosphate uptake and alkaline phosphatase activity in freshwater. *Canadian Journal of Fisheries and Aquatic Science*, **43**, 311–318.

Dodds W.K., Johnson K.J. & Priscu J.C. (1989) Simultaneous nitrogen and phosphorus deficiency in natural phytoplankton assemblages: theory, empirical evidence, and implications for lake management. *Lake and Reservoir Management*, **5**, 21–26.

Downes M.T. (1978) An automated determination of low reactive phosphorus concentrations in natural waters in the presence of arsenic, silicon acid and mercuric

chloride. *Water Research*, **12**, 743–745.

Elser J.J., Marzolf E.R. & Goldman C.R. (1990) Phosphorous and nitrogen limitation of phytoplankton growth in freshwaters of North America: a review and critique of experimental enrichment. *Canadian Journal of Fisheries and Aquatic Science*, **47**, 1468–1477.

Fuhrman J.A., Ammerman J.W. & Azam F. (1980) Bacterioplankton in the coastal eutrophic zone: distribution, activity and possible relationships with phytoplankton. *Marine Biology*, **60**, 201–207.

Herbst V. & Overbeck Y. (1978) Metabolic coupling between alga *Oscillatoria redekei* and accompanying bacteria. *Naturwissenchaften*, **65**, 598–608.

Hobbie J.E., Daley J. & Jasper S. (1977) Use of nuclepore filters for counting bacteria by fluorescence microscopy. *Applied Environmental Microbiology*, **33**, 1225–1228.

Murray R.E., Cooksey K.E. & Priscu J.C. (1986) Stimulation of bacterial DNA synthesis by algal exudates in attached algal-bacterial consortia. *Applied Environmental Microbiology*, **52**, 1177–1182.

Peterson B.J., Hobbie J.E., Hershey A.E., Lock M.A., Ford T.E., Ventullo R.M. & Volk G.S. (1985) Transformation of a tundra river from heterotrophy to autotrophy by the addition of phosphorus. *Science*, **229**, 1383–1386.

Riemann B. & Søndergaard M. (1986) Regulation of bacterial secondary production in two eutrophic lakes and in experimental enclosures. *Journal of Plankton Research*, **8**, 519–536.

Sartory D.P. & Grobbelaar J.U. (1984) Extraction of chlorophyll *a* from freshwater phytoplankton for spectrophotometric analysis. *Hydrobiologia*, **114**, 177–187.

Snedecor G.W. & Cochran W.G. (1980) *Statistical Methods*, 7th edn, pp. 298–330. Iowa State University Press, Ames, Iowa.

Solorzano L. (1969) Determination of ammonia in natural water by the phenol–hypochlorite method. *Limnology and Oceanography*, **14**, 799–801.

Toolan T., Wehr J.D. & Findlay S. (1991) Inorganic phosphorus stimulation of bacterioplankton production in a meso-eutrophic lake. *Applied Environmental Microbiology*, **57**, 2074–2078.

Vadstein O. & Olsen Y. (1989) Chemical composition and phosphate uptake kinetics of limnetic bacterial communities cultured in chemostats under phosphorus limitation. *Limnology and Oceanography*, **34**, 939–946.

Wang L., Miller T.D. & Priscu J.C. (1992) Bacterioplankton nutrient deficiency in a eutrophic lake. *Archiv für Hydrobiologie*, **125**, 423–439.

White P.A., Kalff J., Rasmussen J.B. & Gasol J.M. (1991) The effect of temperature and algal biomass on bacterial production and specific growth rate in freshwater and marine habitats. *Microbial Ecology*, **21**, 99–118.

(*Manuscript accepted 11 October 1993*)