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Phytoplankton phosphorus deficiency and alkaline phosphatase activity in the McMurdo Dry Valley lakes, Antarctica

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Abstract

We assessed the nutrient (N, P) deficiency of phytoplankton from the perennially ice-covered lakes in Taylor Valley, Antarctica, with ^{14}C-based bioassays. Phytoplankton in the near-surface waters from three of the four lakes displayed elevated photosynthetic carbon assimilation in the presence of added P relative to controls. Carbon assimilation in samples from deep chlorophyll layers was also enhanced by P addition in two of the lakes. No effect of N addition without simultaneous P addition was noted in any lake. Lake Bonney near-surface phytoplankton consistently showed a strong response to P and N + P additions, suggesting P deficiency. The near-surface phytoplankton of Lake Hoare responded to N + P but not N or P addition alone, suggesting a more general nutrient deficiency. Further investigation of phytoplankton phosphorus deficiency was carried out in the east lobe of Lake Bonney and in Lake Hoare. Water column dissolved and sestonic elemental ratios and planktonic alkaline phosphatase activity measurements corroborated the results of the bioassays. Despite the proximity of the lakes and their similarity in ice cover and watershed, a high degree of phytoplankton phosphorus deficiency was evident in Lake Bonney but not in Lake Hoare. We hypothesize that the difference in the degree of phytoplankton P deficiency between these lakes may not be due to differences in external P fluxes, but rather to differences in the internal cycling of phosphorus stemming from large differences in water column chemistry.

The McMurdo Dry Valleys region of Antarctica is a unique environment in which the biological communities have had to adapt to some of the most extreme conditions available on Earth. The largest ice-free region on the continent, it is among the coldest and driest deserts in the world, having a mean annual temperature of $\leq -20°C$ and ≤ 10 cm yr^{-1} precipitation (Heywood 1984). Within this desert are a number of lakes, many of which maintain a body of liquid water beneath a permanent ice cover. This ice cover protects the underlying water column from experiencing the extreme low temperatures and large temperature variations that are common in the environment above the lake ice (Spigel and Priscu 1998). These lakes offer habitats for specialized aquatic communities that have adapted to low light levels, lack of water column mixing, and intensely stratified chemical environments ranging from extreme oxygen supersaturation to anoxia (Simmons et al. 1993; Priscu et al. 1999).

Life in the dry valley lakes is primarily microbial, with a conspicuous absence of metazoans (Takacs and Priscu 1998). The plankton of the dry valley lakes is composed primarily of microalgae, bacteria, and protozoans (James et al. 1998; Priscu et al. 1999). Among the phytoplankton, flagellated cryptophytes dominate shallow populations, and chrysophytes and chlorophytes make up deep chlorophyll maxima; cyanobacteria may also be present (Lizotte and Priscu 1998). Because of the thick ice cover, light levels in the lake water columns are generally less than 3% of incident irradiation and are seldom sufficient for saturation of phytoplankton photosynthesis (i.e., primary production is primarily light-limited; Neale and Priscu 1998). The layer in which net productivity is nonzero, or the trophogenic zone, deepens with increasing summer light. In the east lobe of Lake Bonney, for example, the trophogenic zone extends to a maximum depth of about 20 m (Priscu et al. 1999). Because of the consistently low light levels and high water column stability, antarctic lake phytoplankton are extremely shade-adapted (Neale and Priscu 1998). They also have an abundance of light-harvesting carotenoid pigments that are efficient at absorbing the blue-green wavelengths of light that penetrate the ice (Lizotte and Priscu 1998). As within any aquatic community, the phytoplankton in antarctic lakes must have an adequate supply of nutrient elements for balanced growth, whether introduced exogenously or through the biochemical recycling of organic matter. Because of losses, such as the escape of lift-off cyanobacterial mats and associated sediments through the ice cover (Parker et al. 1982), recycling can never be 100% efficient, so new supplies of nutrients must be brought into the system from its surroundings. An inadequate supply of nitrogen, phosphorus, or other required nutrient during the summer months may limit the photosynthetic capacity of the phytoplankton and, hence, limit their ability to utilize the available light energy (Lizotte and Pris-

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cu 1994; Neale and Priscu 1998). Nitrogen deficiency has been implicated in some antarctic lakes (Vincent and Vincent 1982; Priscu et al. 1989); however, the phytoplankton of others appear to suffer from potential phosphorus deficiency (Parker and Simmons 1985; Priscu 1995).

Because of the protective ice cover of the lakes and the strong salinity gradients within some of them, convective mixing and turbulence are virtually absent (Spigel and Priscu 1996). Because of this extreme water column stability, vertical fluxes of dissolved nutrients from the deep pools are essentially controlled by molecular diffusion, providing only a slow refueling of nutrient supplies (Priscu 1995; Spigel and Priscu 1998). New nutrients from outside the lakes may enter with seasonal meltwater streams for only a few weeks per year, and much of this limited freshwater input appears to spread across the lake in a thin layer just below the ice cover (Spigel and Priscu 1998). Material transport to the lakes is also achieved through aeolian transport of terrestrially derived sediment onto the ice cover. Although the ice cover prevents the lakes from trapping as much windblown sediment as would ice-free lakes, some sediment does get trapped and can slowly migrate downward through the ice (Priscu et al. 1998). Such migration is facilitated by cracks and liquid water channels in the ice; thus, this form of material transport to the water column is also enhanced by the elevated temperatures during the summer season (Fritsen et al. 1998). Through absorption of solar radiation, the sand and gravel deposited on the ice surface also may form their own localized meltwater pockets, speeding its gravitational settling through the ice cover (Psenner et al. 1999). However, nutrients introduced in this fashion can be used by microbial communities in meltwater pockets within the ice cover itself (Paerl and Priscu 1998; Priscu et al. 1998); these communities may act as a ''biofilter,'' removing readily leachable nutrients from deposited material into less available particulate organics before the gravel-associated material can pass through the ice and into the lakes.

In this study, we set out to evaluate the potential phosphorus deficiency of phytoplankton in the major lakes of Taylor Valley, Antarctica. In the assessment of phytoplankton nutrient status, individual methods can provide misleading results if considered alone; the best approach is to conduct several independent yet complementary assays simultaneously (Vincent 1981). Investigators have used a number of indicators for nutrient deficiency in studies of lake ecosystems, including the response of phytoplankton in nutrient addition bioassays, the elemental compositions of the dissolved nutrient supplies and of the seston, and the activity of alkaline phosphatases (Healy and Hendzel 1980; Morris and Lewis 1988; Hecky et al. 1993). The McMurdo Dry Valley lakes represent extreme aquatic environments in which to examine the relative roles of nitrogen and phosphorus in nutrient deficiency and have a number of unique aspects relevant to the study of nutrient limitation. For example, the limited stream flow and lack of terrestrial vegetation in the watershed provide an opportunity to study sestonic elemental ratios in the near absence of potentially confounding terrestrial detritus (Hecky et al. 1993). Also, because the lakes are in a hydrologic balance between limited seasonal stream inflow and ablation at the ice surface with no outflows (Chinn 1993), they have extremely low rates of flushing. These lakes therefore represent end members in which the results of short-term biological assays of nutrient deficiency should converge on the predictions based on elemental ratios of seston (Hecky et al. 1993). In addition, the virtual absence of external inputs of nutrients during the early part of the season (prior to the initiation of stream flow) allows for examination of nutrient deficiency in a relatively simple, practically closed system. We therefore proceeded to (1) conduct an initial nutrient assessment using ^{14}C-based N and P deficiency bioassays on these lakes and then (2) generate complementary data on phosphorus deficiency (dissolved inorganic and organic N : P ratios, sestonic C : N : P ratios, alkaline phosphatase activity assays) from two lakes displaying different degrees of P deficiency in the ^{14}C bioassays.

Materials and methods

All field work was conducted in Taylor Valley, McMurdo Dry Valleys, Antarctica. Four lakes, each permanently covered with an ice cover approximately 4 m thick, were examined: Lake Bonney (east and west lobes), Lake Fryxell, and Lake Hoare. The two lobes of Lake Bonney are connected by a shallow constriction only and are considered as distinct basins. The Taylor Valley lakes represent a central focus of the ongoing McMurdo Dry Valleys Long-Term Ecological Research (LTER) project, which uses these systems as sentinals of global environmental change (see http://huey.colorado.edu/LTER/). The exact geographic location and physical setting of each of the lakes have been well described elsewhere (Priscu 1998).

^{14}C-based nutrient bioassays were performed during late spring and summer (November–December) 1994 on samples from immediately beneath the ice and from the deep chlorophyll layers within each lake. Associated profiles of chlorophyll *a* (Chl *a*), bacterial numbers and nutrients were collected at this time. Additional field work focusing on phosphorus was conducted during late winter and early spring (September–October) 1995 in the east lobe of Lake Bonney and Lake Hoare only. Particulate carbon, nitrogen, phosphorus, and Chl *a* were measured, and a variety of alkaline phosphatase assays were carried out. Dissolved inorganic and organic pools of nitrogen and phosphorus were also assessed. Dissolved organic carbon was not measured at this time, but results from November 1996 are presented here for comparison to other nutrient data. Lake water samples were collected with 2- or 5-liter Niskin samplers on a cable lowered by winch through a hole melted in the ice cover. All depths are reported with respect to the water level in the sampling hole (piezometric depth), which was about 30–50 cm below the surface of the ice. Temperature and conductivity were measured with a SEABIRD Model 25 CTD sonde; salinity was computed as outlined by Spigel and Priscu (1996) and is reported in practical salinity units (PSU). Photosynthetically active radiation was measured within the water column and at the ice surface during November–December 1996 using Licor spherical and flat plate sensors, respectively. Experiments and sample preparation

were carried out in the field laboratories at Lake Bonney and Lake Hoare. Analyses were conducted at McMurdo Station (Crary Laboratory) or at the University of Hawaii (UH).

^{14}C bioassays—Water samples (20 liters) were collected from selected depths in each lake and transported by helicopter within 30 min of collection to the Lake Bonney field laboratory. Samples were kept dark and near the temperature of collection during transport. From each sample, a 5.6-liter subsample was drawn and amended with ^{14}C-bicarbonate to a final activity of 4.1 kBq ml^{-1}. This subsample was then used to prepare eight 700-ml aliquots in 1,000-ml acid-washed high-density polyethylene (HDPE) bottles. Nutrient supplements were added to duplicate bottles to yield the following enrichments: N = 20 μM NH_4^+, P = 2 μM PO_4^{3-}, N + P = 20 μM NH_4^+ + 2 μM PO_4^{3-}, control = no nutrient additions. The bottles were incubated in an environmental chamber at the temperature of collection and at saturating irradiance (70 μmol photons m^{-2} s^{-1}; Lizotte and Priscu 1992). Subsamples (60 ml) were withdrawn at 24-h intervals over 4–6 d and filtered through Whatman GF/F filters. The filters were placed in 20-ml glass scintillation vials and acidified (0.5 ml of 3 N HCl) to remove inorganic carbon. The filters were dried at 50°C and counted using standard liquid scintillation spectrometry. Nutrient addition response factors (cf. Elser et al. 1990) were calculated as the ratios of the slopes of the least-squares fits of the natural log of the radioactivity (disintegrations per minute) on the filter against time of nutrient-enriched samples versus control samples. The statistical significance of these results was determined by comparing the treatment slopes to the control slope with a one-way analysis of variance followed by a post hoc Dunnett's test (Zar 1984).

Dissolved nutrients—Samples for dissolved inorganic nitrogen (DIN) analyses were vacuum-filtered (<0.5 atm) through combusted Whatman GF/F filters into acid-washed HDPE bottles and immediately frozen at −20°C. These samples were returned to Crary Laboratory, where they were thawed at room temperature and analyzed for NH_4^+, NO_2^-, and NO_3^- using an autoanalyzer (Lachat Instruments). No special modifications to standard autoanalytical methods (Strickland and Parsons 1972) were required, except that samples with salinities exceeding 35 PSU were diluted with deionized water to final salinities at or below this value before analysis. Samples for dissolved organic carbon (DOC) were similarly filtered into combusted acid-washed amber glass bottles, preserved with phosphoric acid, and returned unfrozen to Crary Laboratory, where they were analyzed using an OI Model 700 carbon analyzer (Oceanography International Corp.).

Samples for soluble reactive phosphorus (SRP), total dissolved phosphorus (TDP), and total dissolved nitrogen (TDN) were filtered through acid-washed, combusted GF/F filters and were frozen in HDPE bottles. These samples were returned to UH and kept frozen until the day of analysis. SRP was determined with a manual colorimetric method (Strickland and Parsons 1972); highly saline samples from Lake Bonney were diluted with deionized water to about 50 PSU prior to analysis. TDP was measured as SRP after UV oxidation (1,200 W, 2.5 h) of the sample (Armstrong et al. 1966). Before the oxidation step, hypersaline samples were diluted with deionized water, and fresh samples were amended with an NaCl solution such that all samples fell within the range of 10–50 PSU. Oxidation was enhanced by adding 75 μl of 30% H_2O_2 to 20 ml of sample prior to UV exposure. Dissolved organic phosphorus (DOP) was calculated by subtracting SRP from TDP. TDN was measured using high-temperature combustion with chemiluminescent detection (Walsh 1989). Hypersaline TDN samples were diluted with deionized water to 50 PSU before analysis. Dissolved organic nitrogen (DON) was calculated by subtracting DIN from TDN.

Particulate bioelements, Chl a*, and bacteria*—Particulate organic carbon (POC) and particulate nitrogen (PN) samples were collected on combusted GF/F filters and stored frozen for later acidification and analysis at Crary Laboratory with a Carlo-Erba model 1500 CHN analyzer. Samples for particulate phosphorus (PP) were collected on acid-washed, combusted GF/F filters and returned frozen to UH. PP was later analyzed as SRP after high-temperature ashing with subsequent acid extraction (Karl et al. 1991). Particulate Chl *a* samples were collected on GF/F filters and frozen for transport to Crary Laboratory. The filters were then extracted in 90% acetone and analyzed by fluorometry (Holm-Hansen et al. 1965). For bacterial enumeration, formalin-preserved water samples (10 ml) were stained with acridine orange, filtered onto black 0.2-μm polycarbonate filters, and counted by epifluorescence microscopy (Takacs and Priscu 1998). Non-autofluorescing rods and cocci were counted on each filter at ×1,000 magnification.

Alkaline phosphatase activity—The enzymatic activity of alkaline phosphatase (APase) in lake water samples was estimated using the compound 4-methylumbilliferyl phosphate (MUP) as a substrate analog during timed, temperature-controlled incubations, with subsequent analysis of the hydrolysis product 4-methylumbilliferone (MUF) by fluorometry (Pettersson and Jansson 1978). Incubations were carried out in acid-washed 15-ml polystyrene centrifuge tubes. In each tube, 0.5 ml of buffered (Tris, pH 8.3) MUP was added to a 4.5-ml water sample and incubated in the dark. The concentration of the MUP addition was varied to adjust the final MUP to the desired concentration (from 0 to 100 μM, depending on the particular experiment). Denatured controls were prepared by boiling the samples for 5 min and cooling back to incubation temperature prior to the addition of MUP. Selected samples were enriched with 5 μM KH_2PO_4 prior to MUP addition. At time zero, and at selected time points thereafter, the enzymatic reaction was stopped by freezing of the sample in liquid nitrogen (Chróst and Velimirov 1991). After rapid freezing, the samples were maintained between −20 and −40°C until returned to Crary Laboratory for analysis. A 20°C water bath was used to rapidly thaw the samples, which were then immediately analyzed with a Perkin-Elmer LS-50B luminescence spectrometer. An APase substrate–response curve was generated using an iterative nonlinear regression technique (Marquardt-Levenberg algo-

Table 1. Results and background data from Nov–Dec 94 nutrient bioassay experiments. SRP concentrations below the detection limit of 0.02 μM are treated as 0.02 μM for ratio calculations. DIN is nitrate + nitrite + ammonium, each with a 0.03-μM detection limit used for ratio calculations. Nutrient bioassay response factors represent the ratios of treatment to control responses, as outlined in Materials and methods.

Lake	Depth (m)	Chl *a* (μg L^{-1})	Bacteria (cells L^{-1} $\times$ 10^9)	Nutrient concentration (μM)			Response factor		
				DIN	SRP	DIN : SRP	+N	+P	+(N + P)
Bonney (west lobe)	5	2.8	7.7	8.8	0.02	440	1.05	1.38**	1.32**
	13	5.6	2.2	32.0	0.02	1600	0.98	1.22*	1.20*
Bonney (east lobe)	5	1.1	5.3	9.2	0.02	460	0.95	1.42**	1.30**
	13	0.7	2.5	26.3	0.02	1315	1.06	0.99	1.19
	18	0.4	3.8	110.5	0.03	3683	1.01	1.16	1.15
Hoare	5	9.5	2.8	1.0	0.02	50	1.08	1.05	1.47**
	14	1.8	1.1	3.0	0.02	150	0.89	0.79	0.80
Fryxell	5	6.8	0.99	0.09	0.11	0.8	0.73	1.78**	2.02**
	9	8.0	15.25	3.32	0.18	18.4	1.04	1.38*	1.31*

Statistical results of bioassays (two-tailed Dunnett's test) were different from control at * $\alpha = 0.05$ and ** $\alpha = 0.01$.

rithm). Because of logistic constraints, only one time-course APase incubation from Lake Hoare was performed.

Results

^{14}C bioassays of dry valley lakes—Phosphorus stimulated carbon assimilation significantly in all samples except those from Lake Hoare and from the deep chlorophyll layers in the east lobe of Lake Bonney (Table 1). The addition of nitrogen alone never stimulated carbon assimilation relative to controls in samples at the $\alpha = 0.05$ level. Simultaneous addition of nitrogen and phosphorus had nearly the same effect as the addition of phosphorus alone in all but the 5-m sample from Lake Hoare. The results from this near-surface sample from Lake Hoare indicated a synergistic stimulatory effect of N + P addition but no effect of N or P when added singly. Nutrient concentrations during the spring–summer 1994 season varied considerably among lakes, with elevated inorganic nitrogen levels generally occurring in deeper water, whereas SRP levels were relatively low at all depths (Table 1). Bulk nutrient concentrations were not related to phytoplankton biomass (Chl *a*) among the lakes. DIN : SRP ratios in all samples except the near-surface water of Lake Fryxell exceeded, often considerably, the 16 : 1 Redfield ratio that is typically required for balanced algal growth (Redfield et al. 1963), implying a deficiency of P relative to N for phytoplankton growth.

Physical limnology and microbial biomass—Lake Hoare and the east lobe of Lake Bonney differed markedly in temperature and salinity (Fig. 1). Salinity in the east lobe of Lake Bonney was nearly zero immediately under the ice, then rose with depth through a steep chemocline to a hypersaline 140 PSU in the waters below 25 m. Temperature in this lake displayed a maximum of about 6°C at 15 m, with values around 0°C immediately under the ice and −2°C near the bottom. Lake Hoare was nearly fresh throughout and had only a slight salinity gradient down to 14 m (Fig. 1). Temperature in Lake Hoare fell in a narrow range (0.2–0.8°C) with a maximum at about 8 m. Light penetration through the ice was low in both lakes, but higher in the east lobe of Lake Bonney (~3%) than in Lake Hoare (~0.3%). The water column concentrations of Chl *a* were substantially higher and the bacterial abundances somewhat lower in Lake Hoare than in the east lobe of Lake Bonney (Fig. 1).

Nutrient pools and elemental ratios—Vertical profiles of DOC, TDN, and DIN during the winter–spring 1995 season revealed high concentrations below the chemocline and low concentrations through most of the trophogenic zone in the east lobe of Lake Bonney (Fig. 2). In Lake Hoare, these pools were of similar magnitude in the trophogenic zone but did not change with depth except for a small increase at the lake bottom. POC and PN were similar within the trophogenic zones of the two lakes but stayed mostly constant with depth in Lake Hoare, while rising through large maxima just below the chemocline (about 22 m) in the east lobe of Lake Bonney (Fig. 2). The SRP profile obtained from the east lobe of Lake Bonney revealed a depleted layer to 18 m, a sharp gradient (coincident with the gradient in salinity) to a maximum at 24 m, and a more gradual decline to the bottom (Fig. 2). This profile is consistent with data presented earlier (Priscu et al. 1999), but the trophogenic zone SRP concentrations we measured (0.02–0.04 μM) are on the low end of those measured previously. This already extremely low level should be considered an upper limit to the actual free orthophosphate concentration for three reasons: (1) the SRP assay may measure some acid-labile organics as well as orthophosphate (Strickland and Parsons 1972), (2) potential arsenate interference was not examined separately (Karl and Tien 1997), and (3) metallophosphate complexes and other ''bound'' forms of phosphate that may not be truly available to the plankton in situ may be released by the acid molybdate assay (Cembella et al. 1984). The DOP profile resembled that of SRP, except that DOP in the trophogenic zone was not completely depleted, having a rather constant concentration averaging 0.23 μM in the upper 18 m. The trophogenic zone PP was also extremely low (0.03–0.04 μM) but rose below the chemocline to a maximum of 0.38 μM at 24 m. Total phosphorus (TP = SRP + DOP + PP) was composed

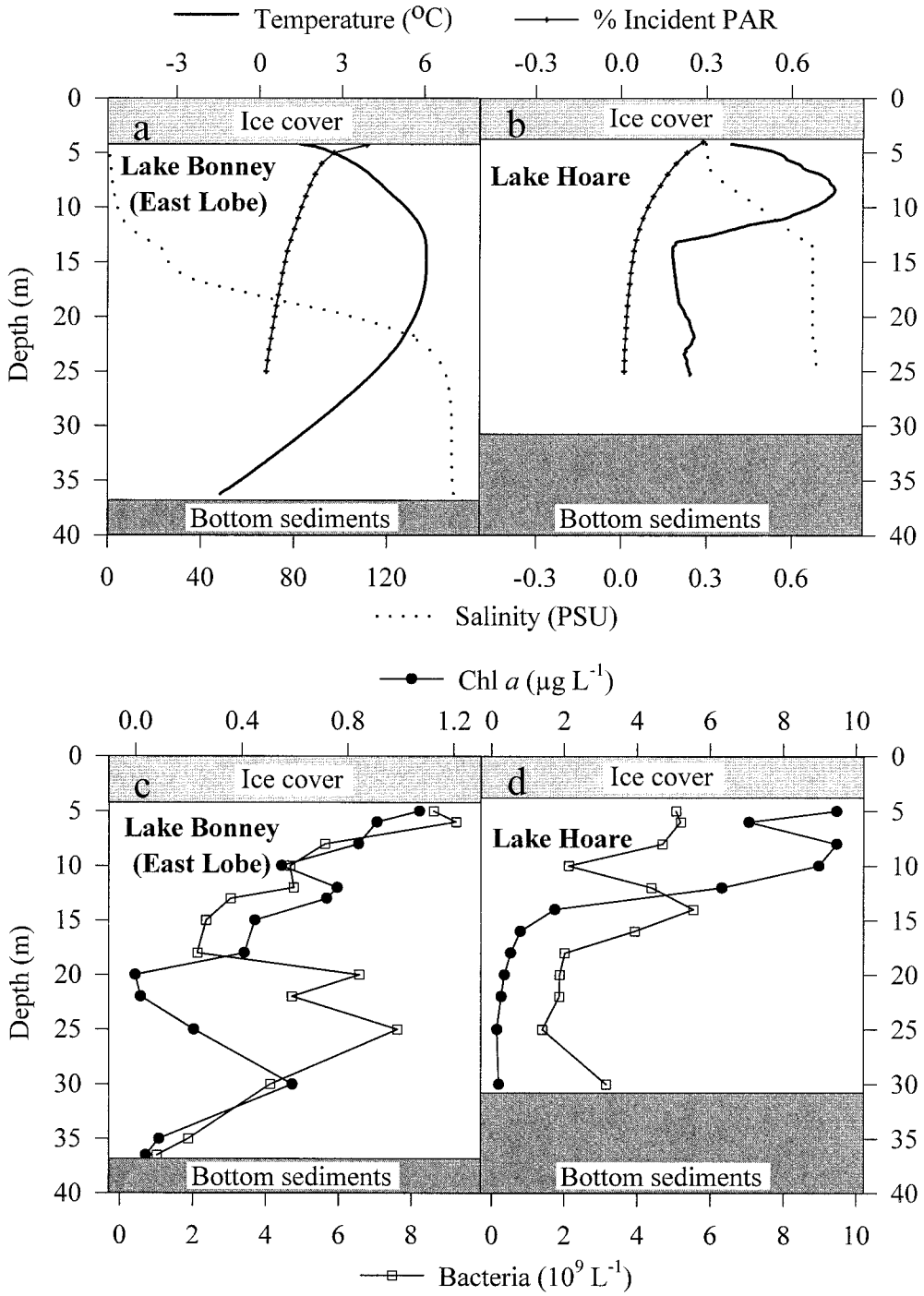

Fig. 1. Temperature (°C), photosynthetically active radiation (PAR, % incident PAR at the ice surface; 400–700 nm), salinity (PSU), Chl *a* (μg L^{-1}), and bacterial numbers (10^9 cells L^{-1}) in the water columns of the east lobe of (a, c) Lake Bonney and (b, d) Lake Hoare.

primarily of DOP in the trophogenic zone, but included significant proportions of SRP and PP within and below the chemocline (Fig. 2). The concentration of SRP in Lake Hoare was very low (0.01–0.20 μM) throughout the water column, except for a sharp increase to 2.53 μM just above the lake bottom (Fig. 2). This enrichment was associated with anoxic bottom water, not with a salinity gradient as was seen in Lake Bonney. DOP showed no such near-bottom enrichment, remaining nearly constant with depth at an average concentration of 0.21 μM. PP was low (0.24 μM) throughout the water column, with the highest values just below the ice cover at 5–6 m. PP in the anoxic bottom water (30 m depth) was only 0.10 μM. The TP enrichment observed in the bottom water was therefore almost entirely

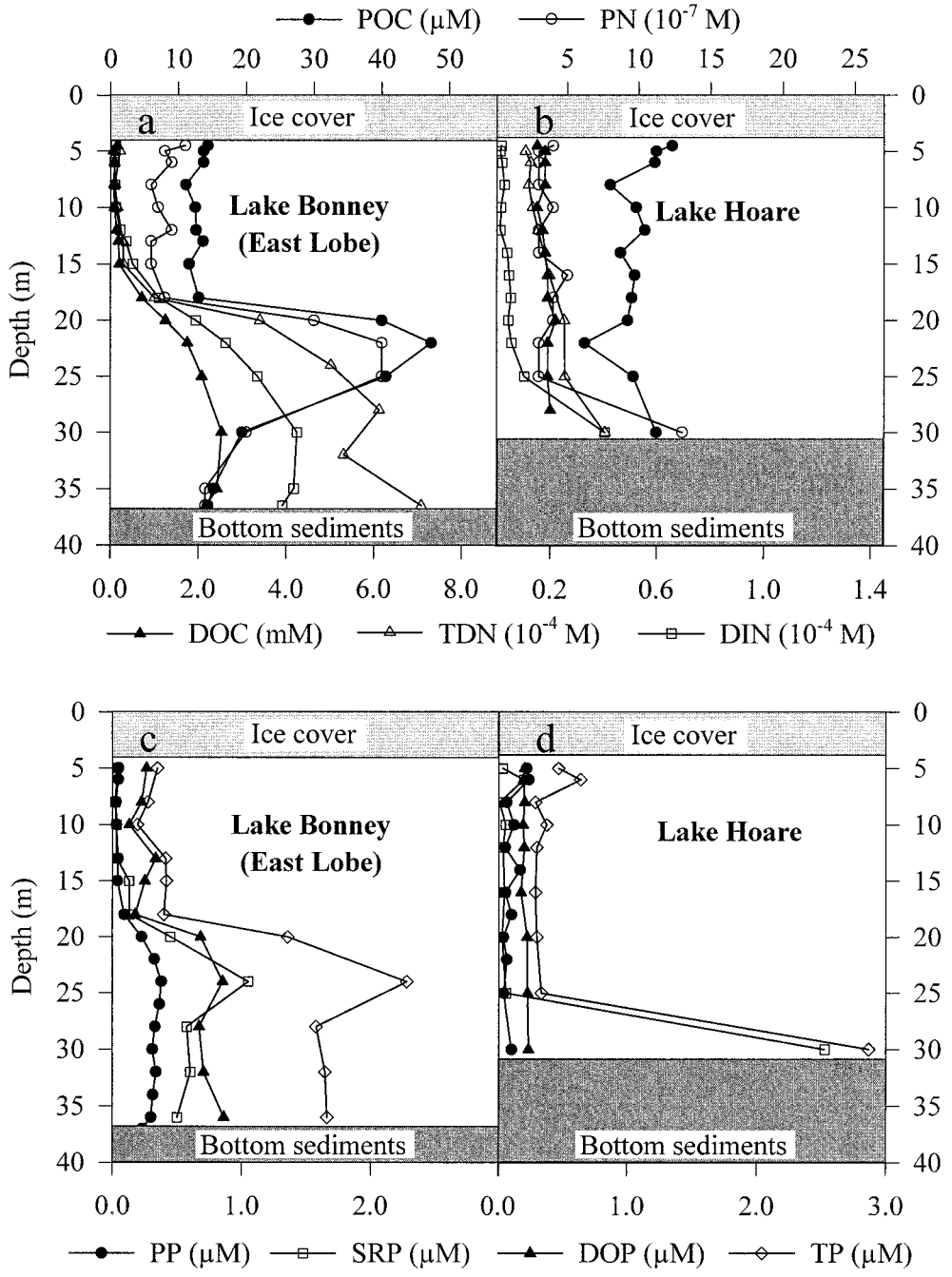

Fig. 2. Carbon, nitrogen, and phosphorus pools of the water columns of (a, c) the east lobe of Lake Bonney and (b, d) Lake Hoare: particulate organic carbon (POC), particulate phosphorus (PP), soluble reactive phosphorus (SRP), total phosphorus (TP), and dissolved organic phosphorus (DOP) concentrations (μM); dissolved inorganic nitrogen (DIN) and total dissolved nitrogen (TDN) (10^{-4} M units); particulate nitrogen (PN, 10^{-7} M units); dissolved organic carbon (DOC, mM).

composed of dissolved acid-labile phosphorus compounds (i.e., SRP).

Molar carbon-to-phosphorus and nitrogen-to-phosphorus ratios showed a tremendous degree of variability with depth between operationally defined nutrient pools and between lakes (Fig. 3). The POC : PP and POC : PN ratios in the trophogenic zone of Lake Bonney were at or above their respective 106 : 1 and 106 : 16 Redfield ratios, suggesting general planktonic nutrient deficiency. In this same layer, PN : PP was at or slightly above the 16 : 1 Redfield ratio, possibly indicating a sestonic deficiency of P relative to N. At the same time, POC : PP and PN : PP ratios below the trophogenic zone were lower than Redfield, indicating an excess of P over N and C in this deep particulate matter. DIN : SRP, DIN : TDP and TDN : TDP ratios throughout the water column of the east lobe of Lake Bonney were high (36–1,042),

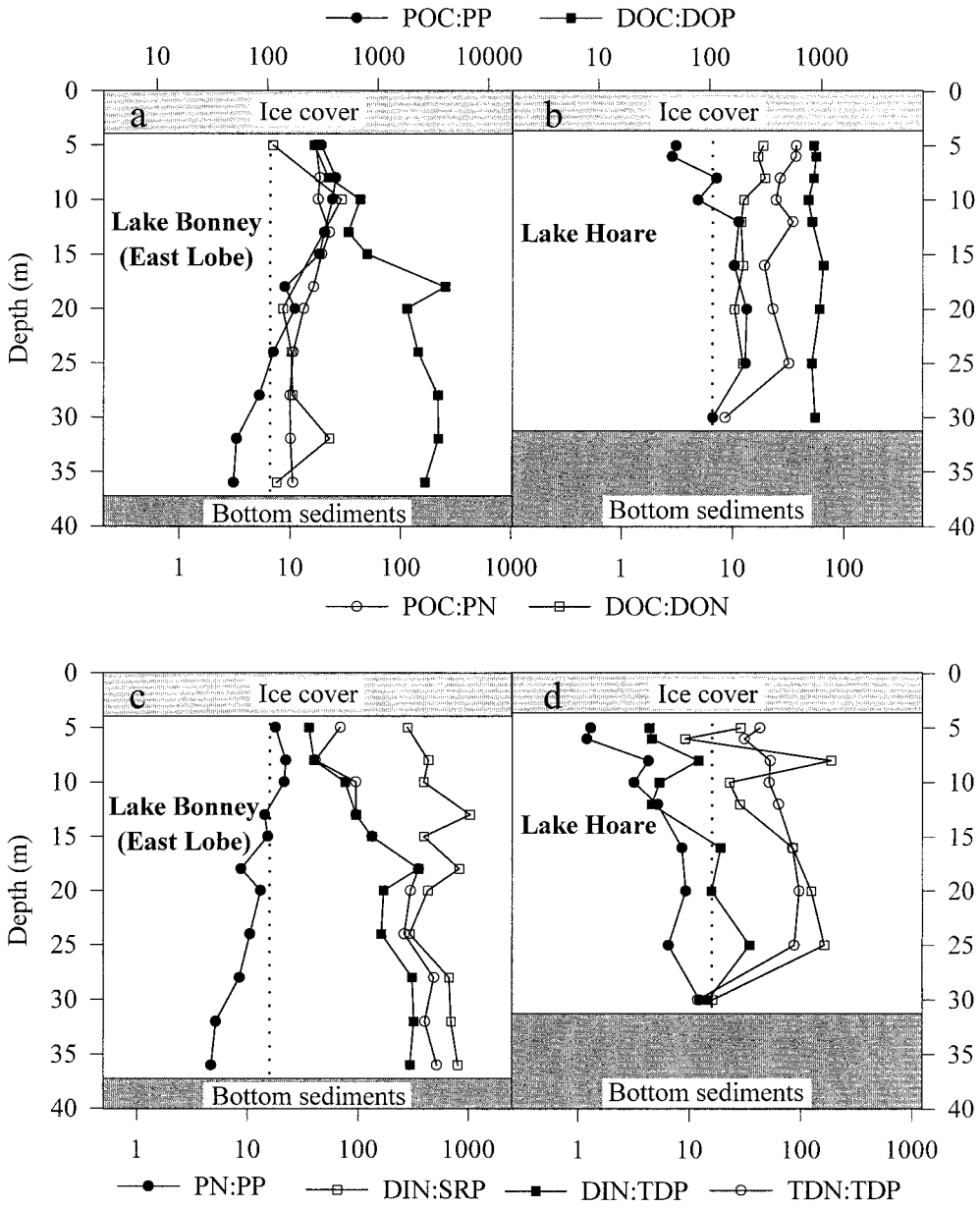

Fig. 3. Elemental ratios in the particulate and dissolved pools of the water columns of (a, c) the east lobe of Lake Bonney and (b, d) Lake Hoare. All ratios (mol : mol) are presented on logarithmic scales. Upper panels present C : P (top axis) and C : N (bottom axis) ratios. Lower panels present N : P ratios. Particulate and dissolved pools are as given in Fig. 2. The vertical dotted line in each panel indicates the Redfield C : N : P stoichiometry of 106 : 16 : 1.

indicating an extreme deficiency of phosphorus relative to nitrogen in the dissolved nutrient pools throughout the lake. Dissolved inorganic and total nutrient pools in Lake Hoare were also depleted in P relative to N, but to a lesser degree, except for the anoxic bottom water, which approached the Redfield 16 : 1 N : P ratio in both dissolved organic and inorganic pools (Fig. 3). However, the DIN : TDP ratio was less than 16 : 1 in the trophogenic zone of Lake Hoare. The particulate pool in Lake Hoare had an N : P ratio well below 16 : 1 and a C : P ratio near 106 : 1, suggesting sestonic N deficiency (relative to P), except in the anoxic bottom water, where the particulate C : N : P approached the Redfield 106 : 16 : 1 ratio.

APase activity in Lake Hoare and Lake Bonney—The hydrolysis of the fluorescent MUP substrate analog by alkaline phosphatases in 5-m water samples from the east lobe of Lake Bonney followed a linear time course from time zero to 10–20 h incubation time (Fig. 4), with hydrolysis rate decreasing thereafter. Based on this observation, all subsequent APase activity measurements were determined from incubations of $<$20 h. The addition of 5 μM phosphate to

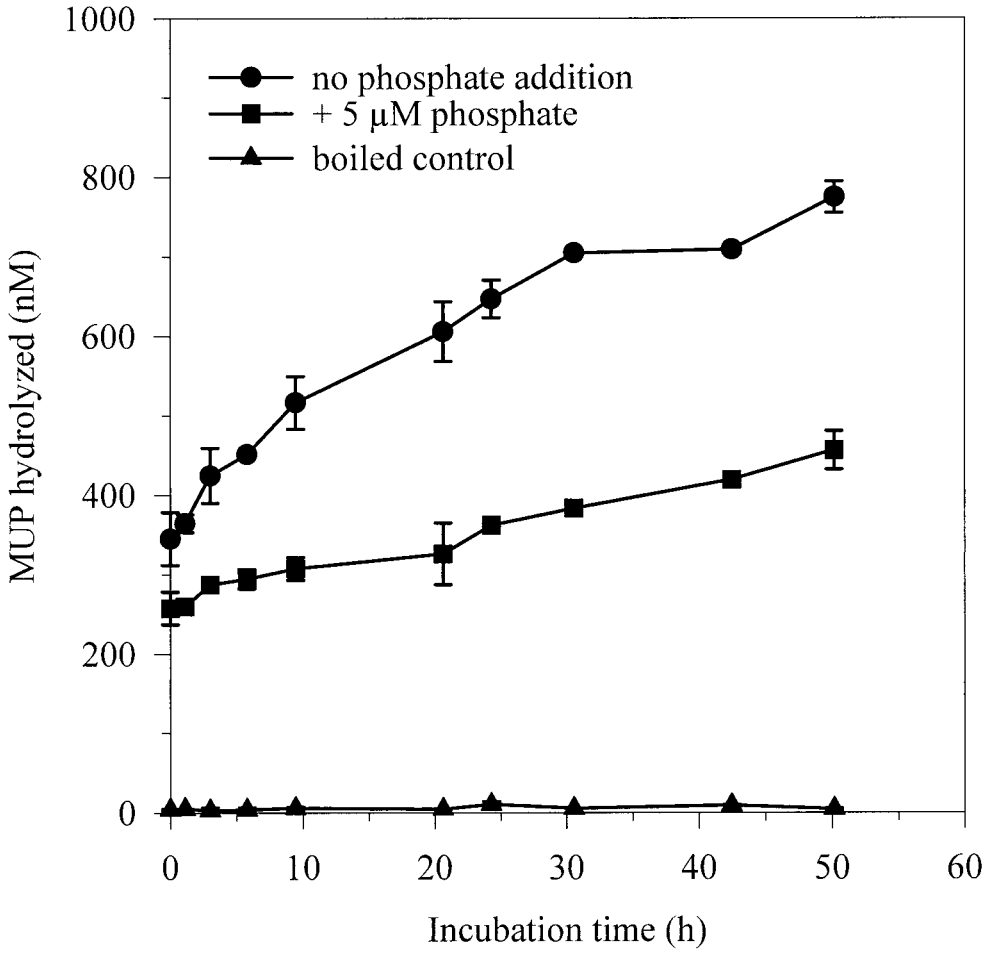

Fig. 4. Time course of hydrolysis of MUP substrate during incubation for the measurement of alkaline phosphatase activity at 5 m in the east lobe of Lake Bonney. Incubations were performed after addition of 1 μM MUP, with and without the previous addition of 5 μM phosphate. Control samples were boiled and cooled before addition of MUP. Incubation temperature was 4°C. Error bars are ±1 SD of triplicate incubations.

APase incubations resulted in significantly reduced rates of MUP hydrolysis. Boiled control samples showed virtually no APase activity out to 50 h incubation time. A large fraction of the total MUP hydrolyzed was hydrolyzed during the first few seconds after substrate addition, as evidenced by the high time-zero measurements of MUF produced, but linear MUF production continued thereafter (Fig. 4). Subsequent APase activity rates have been determined after time-zero correction.

The substrate–response curve for alkaline phosphatase activity at 5 m in the east lobe of Lake Bonney was well described by standard Michaelis–Menten enzyme kinetics.

$$V = (V_{max}S)/(K_m + S)$$

V is the reaction velocity at a given substrate concentration S, V_{max} is the maximum potential reaction velocity at the temperature of incubation, and K_m is the substrate concentration at which V is half of V_{max} (Fig. 5). The results shown are for incubation temperatures of 5°C, but the temperature optimum for APase activity was about 35°C (Fig. 6). Most of the rate measurements in this study were made at 4–5°C with 1 μM MUP substrate to more closely simulate in situ conditions; however, it should be noted that because of the suboptimal temperatures and subsaturating substrate concentrations used, maximum potential APase activity (cf. Healy and Hendzel 1979) may actually have been considerably higher. A single time-course experiment from 5 m in Lake Hoare made at saturating substrate concentration (100 μM MUP) yielded an order of magnitude less APase activity than was measured in Lake Bonney (Fig. 5). To more directly compare the results from the two lakes, normalizations of the APase activities to Chl *a* and particulate phosphorus were performed. It should be noted that the Chl *a* values used for normalization were collected from the same samples used for APase analyses (September–October 1995); these Chl *a* values were considerably lower than the ones presented in Fig. 1 (November 1994) because they were collected early in the season while biomass was accumulating. Kinetic parameters from Lake Bonney (determined by nonlinear regression for both the raw data and the normalized data), when compared to the single substrate-saturated rate measurement from Lake Hoare, revealed that the large dif-

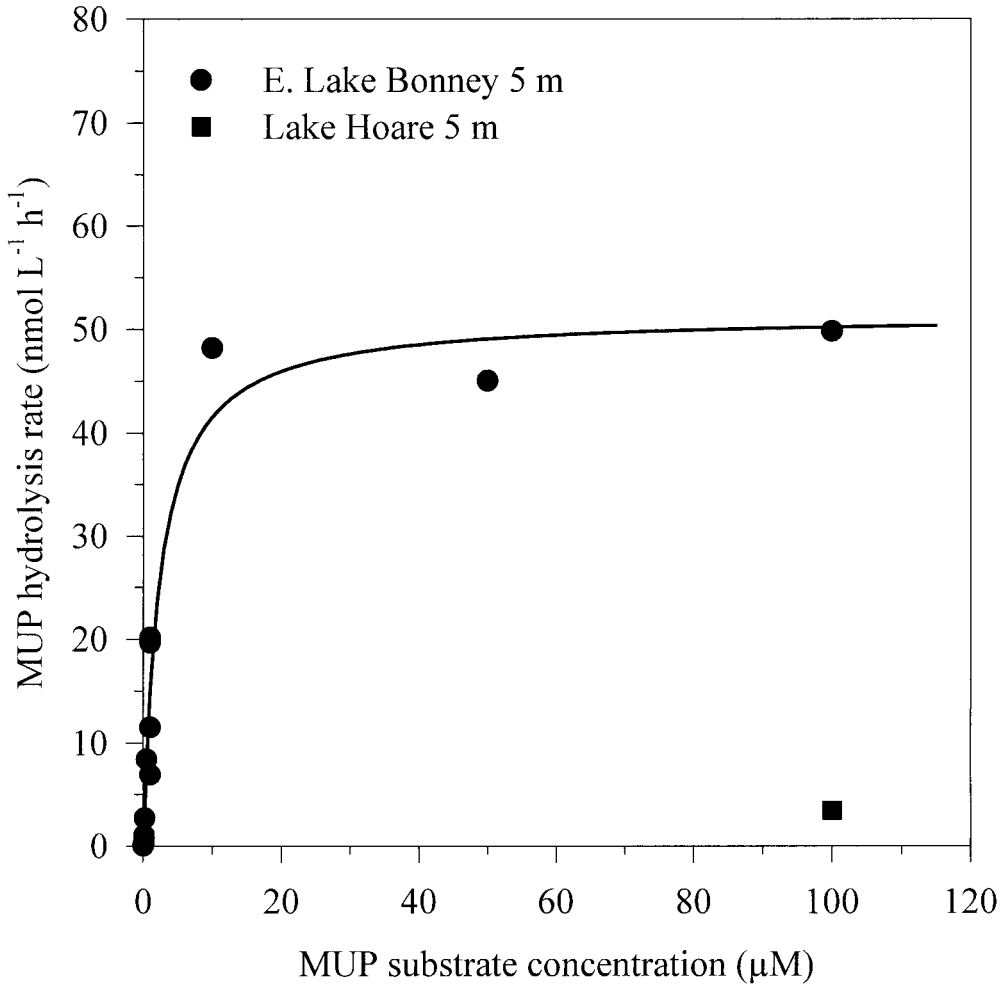

Fig. 5. Substrate response of APase activity at 5 m in the east lobe of Lake Bonney. APase activity is estimated as the hydrolysis rate of added MUP substrate during incubation at 5°C. Solid curve is fit to the data by iterative nonlinear regression. The MUP hydrolysis rate from a single time-course incubation of 5-m water from Lake Hoare at 5°C is included for comparison.

ference in APase activity observed between the lakes was not an artifact produced by a difference in biomass (Table 2).

The depth distribution of APase activity in the trophogenic zone of the east lobe of Lake Bonney showed the highest rates near the ice cover at 5 m decreasing to near-zero rates at 18 m (Fig. 7). The addition of 5 μM phosphate to these samples suppressed APase activity at all depths, but the degree of suppression was greatest in the 5-m sample. Size fractionation of a 5-m sample from the east lobe of Lake Bonney revealed very little APase activity in the free dissolved (<0.2 μm) pool (Fig. 8). Most of the activity occurred in the 0.2–2.0-μm size class. APase activity in all fractions was suppressed by the addition of 5 μM phosphate.

Discussion

Phytoplankton P deficiency as revealed by ^{14}C bioassays—The results of the ^{14}C bioassays imply that photosynthetic carbon assimilation by phytoplankton during the 1994 season was potentially phosphorus limited in the trophogenic layers of all four lakes examined, except perhaps in Lake Hoare, where phytoplankton responded to N + P addition but not to N or P added individually (Table 1). In addition, deep chlorophyll populations in Lake Fryxell and the west lobe of Lake Bonney exhibited signs of phosphorus deficiency (Table 1). In none of the lakes was significant nitrogen deficiency exhibited at any depth, except for the synergistic response to N + P addition seen at 5 m in Lake Hoare. With the exception of the 5-m sample from Lake Fryxell, all samples used for bioassays had DIN : SRP ratios above the balanced 16 : 1 Redfield ratio. The P-deficient response from 5 m in Lake Fryxell, in spite of apparently low DIN : SRP, may be explained by the difficulty in precisely quantifying SRP and DIN concentrations at levels near and below their respective detection limits.

Elemental ratios and implications for phytoplankton nutrient deficiencies—The trophogenic zones of both the east lobe of Lake Bonney and Lake Hoare were characterized by

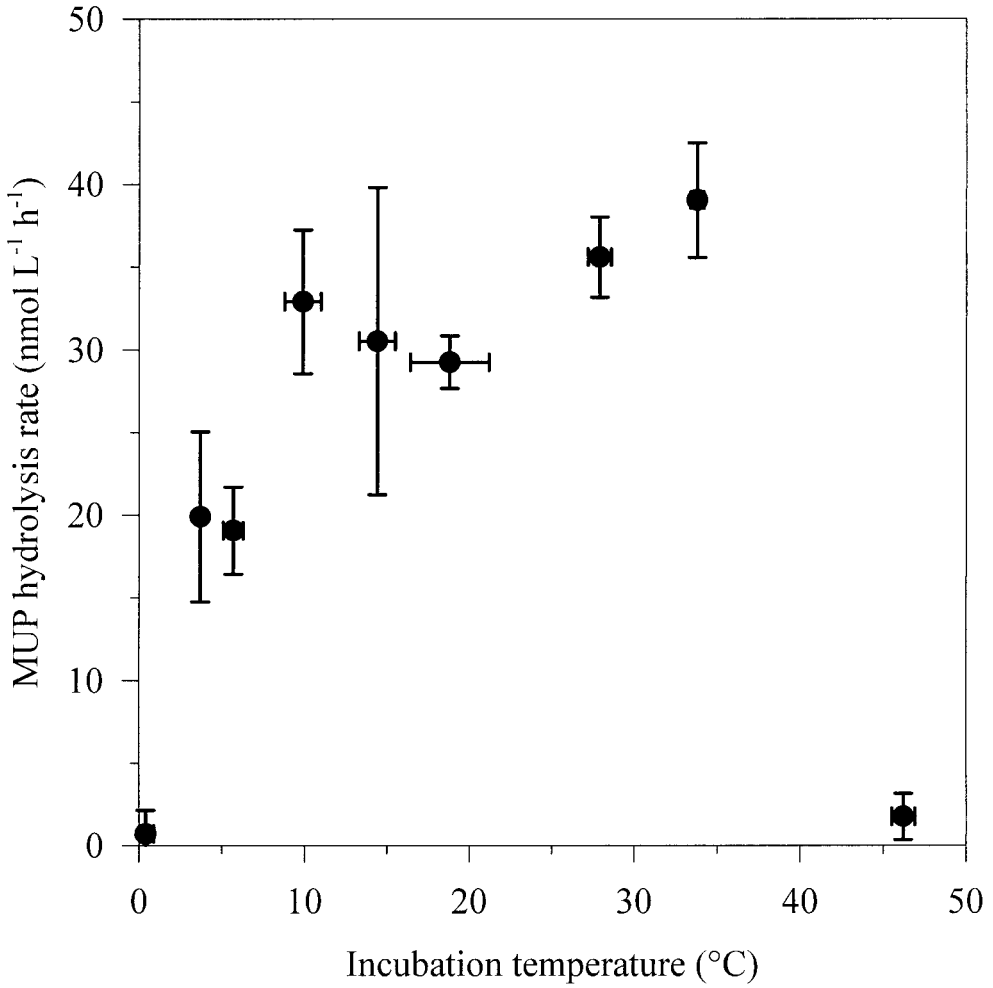

Fig. 6. Temperature response of alkaline phosphatase activity at 5 m in the east lobe of Lake Bonney, as estimated by the rate of hydrolysis of a 1-μM MUP addition in parallel incubations. Vertical error bars are $\pm$1 SD of triplicate incubations at a given temperature; horizontal error bars are $\pm$1 SD of three temperature measurements (beginning, middle, and end of incubation).

very low SRP concentrations (Fig. 2). High DIN : SRP ratios in Lake Bonney and low DIN : SRP ratios in Lake Hoare have been cited as one line of evidence for P deficiency in the former and N deficiency in the latter (Priscu 1995). The present results (Fig. 3) are consistent with the previous data set from the east lobe of Lake Bonney, but the DIN : SRP ratios for Lake Hoare measured here (9.2–163 in the upper 14 m) are considerably higher than earlier results (0.3–0.5; Priscu 1995). This is apparently a result of higher SRP values in the prior study, possibly because of analytical difficulties in the accurate measurement of phosphate at very low levels. A recent investigation, using a steady-state radio-

Table 2. Alkaline phosphatase kinetic parameters from two dry valley lakes.

Lake	Depth (m)	V_{max}	Units of V_{max}	K (μM substrate)
Bonney (east lobe)	5	51.4 $\pm$ 2.8	nmol L^{-1} h^{-1}	2.4 $\pm$ 0.5
		122.6 $\pm$ 6.4	nmol (μg Chl *a*)$^{-1}$ h^{-1}	3.8 $\pm$ 0.8
		36.8 $\pm$ 2.0	nmol (μg PP)$^{-1}$ h^{-1}	2.4 $\pm$ 0.5
Hoare†	5	3.03	nmol L^{-1} h^{-1}	ND‡
		8.23	nmol (μg Chl *a*)$^{-1}$ h^{-1}	ND
		0.43	nmol (μg PP)$^{-1}$ h^{-1}	ND

* Parameters (mean $\pm$ standard error) determined by nonlinear regression of 15 rate measurements.
† Single rate measurement at saturating substrate concentration (100 μM).
‡ ND, not determined.

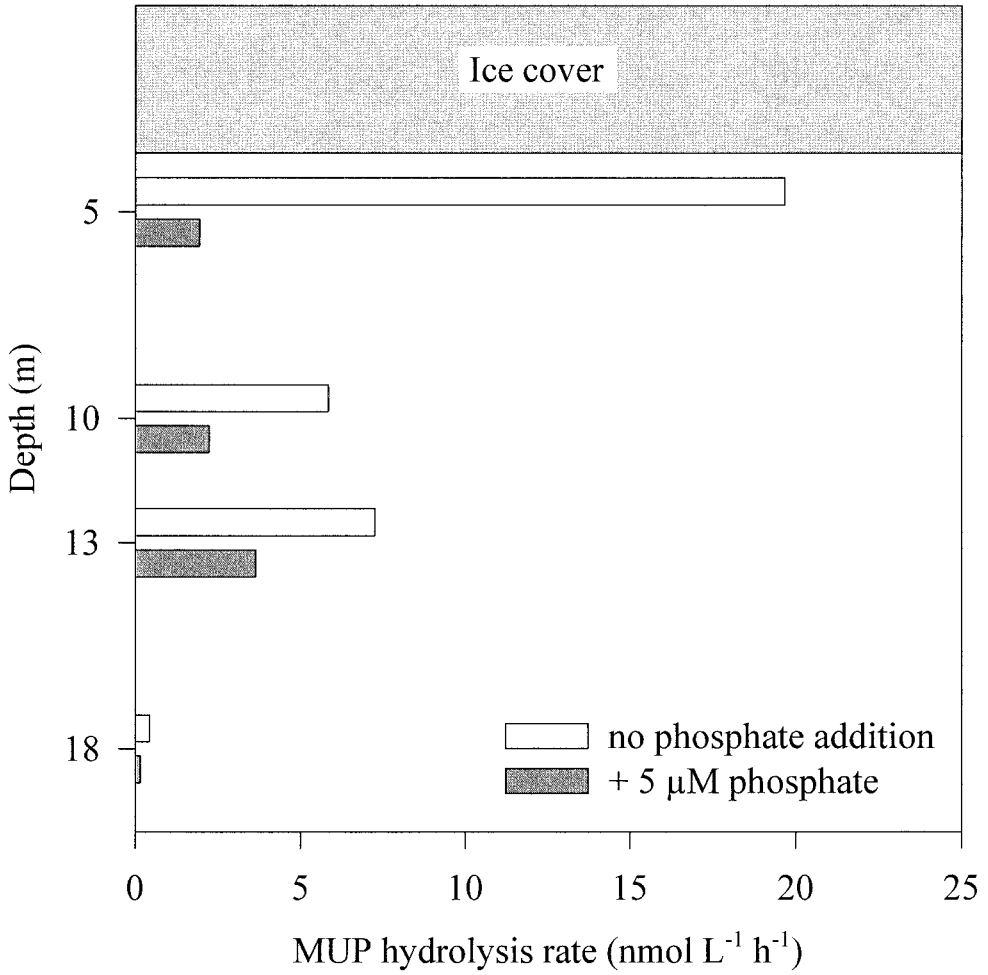

Fig. 7. Depth profile of alkaline phosphatase activity in trophogenic zone of the east lobe of Lake Bonney. Both unamended samples and those treated with 5 μM phosphate are shown at 5, 10, 13, and 18 m. APase activity is estimated as the rate of hydrolysis of a 1-μM MUP substrate addition during incubation at 5°C.

bioassay technique to measure phosphate in a diverse set of North American lakes, found that the standard SRP analysis overestimated true phosphate concentrations by two to three orders of magnitude (Hudson et al. 2000). Notwithstanding this potential artifact, the measured DIN : SRP ratios in the trophogenic zone of Lake Bonney were about an order of magnitude higher than those in Lake Hoare, implying lower availability of P relative to N for phytoplankton growth in the former lake.

When considering nutrient availability, one should not focus solely on the inorganic nutrients, as many organic forms are potentially available through direct assimilation, and more importantly, after exoenzymatic hydrolysis (Cembella et al. 1984). The TDN : TDP ratio in aquatic environments may therefore be more indicative of the nutrient status of the ecosystem than the DIN : SRP ratio (Smith et al. 1986). Furthermore, based on their concentrations in glacial meltwater, organic nutrients are suspected to be important to the maintenance of antarctic lake ecosystems (Downes et al. 1986). Our results revealed that TDN : TDP in the lakes studied was similar to DIN : SRP, except in the trophogenic zone of Lake Bonney (Fig. 3). From 5 to 20 m, the TDN : TDP ratio in the east lobe of Lake Bonney was substantially lower than the DIN : SRP ratio, implying that the dissolved organic nutrients were depleted in nitrogen relative to phosphorus. However, the lack of a vertical gradient in DOP (Fig. 2) and other evidence based on APase activity (see below) suggest that this organic material, although hydrolyzable by UV-oxidation, is resistant to enzymatic hydrolysis and thus is probably not readily available for phytoplankton growth. Therefore, if labile DOP inputs are important as a phosphorus source, they are likely used rapidly after entering the lake and do not accumulate from one season to the next. Another potential index for discriminating between N and P limitation is the DIN : TDP ratio, which may be preferable to the DIN : SRP and TDN : TDP ratios because of the relatively high bioavailability of DOP relative to DON in aquatic ecosystems (Morris and Lewis 1988). In the east lobe of Lake Bonney, DIN : TDP ratios are substantially higher than Redfield, and differ little from the TDN : TDP ratios (Fig. 3). In Lake Hoare, however, DIN : TDP in the trophogenic zone is low, indicating N deficiency, in contrast to the P-deficient

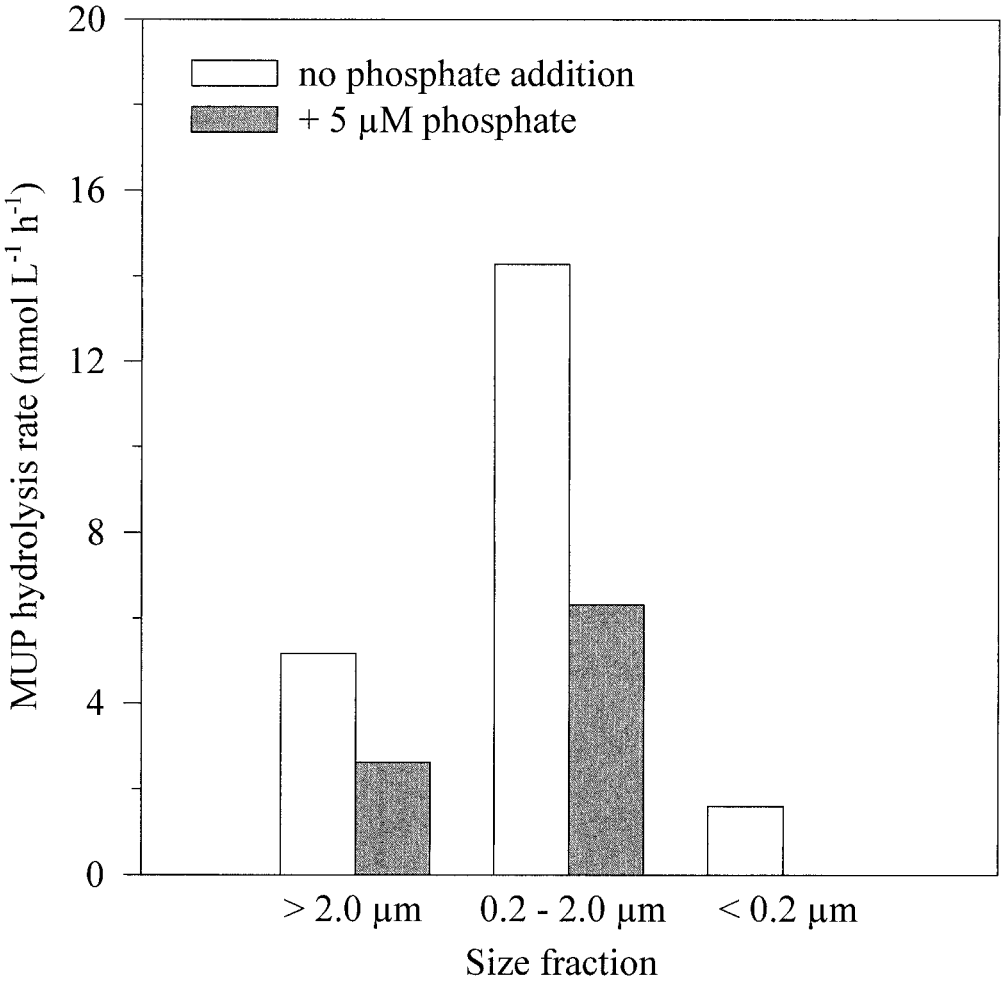

Fig. 8. Size fractionation of alkaline phosphatase activity at 5 m in the east lobe of Lake Bonney. Samples were size-fractionated by differential filtration prior to any additions. APase activity is estimated as the rate of hydrolysis of a 1-μM MUP substrate addition during incubation at 4°C. Both unamended samples and those treated with 5 μM phosphate are shown. There was no measurable activity in the <0.2-μm fraction with 5 μM phosphate added.

TDN : TDP results. Taken together, the various elemental ratios of the dissolved pools strongly support P deficiency relative to N in the east lobe of Lake Bonney but are somewhat ambiguous regarding N versus P deficiency in Lake Hoare.

Particulate phosphorus was low in the trophogenic zones of both lakes studied (Fig. 2), though a slight increase toward the under-ice surface was noted in Lake Hoare. The deep water in the east lobe of Lake Bonney contained higher levels of PP, but the lack of carbon assimilation (Priscu et al. 1999) and the virtual absence of bacterial thymidine incorporation (Takacs and Priscu 1998) in this layer suggests that a majority of this particulate material is nonliving. This deep PP pool may therefore represent organic detritus, mineral phases, or both. Particulate elemental ratios were quite different between the two lakes (Fig. 3). In the trophogenic zones, where much of the particulate matter is composed of phytoplankton (Priscu 1995), we saw slightly high PN : PP ratios (compared to Redfield) in the east lobe of Lake Bonney and low PN : PP ratios in Lake Hoare. This suggests that phytoplankton in Lake Bonney are deficient in phosphorus relative to nitrogen and that the opposite holds true for Lake Hoare phytoplankton. Lake Hoare particulate matter was N-deficient throughout the water column, except in the anoxic bottom water, where all nutrient pools appeared to be near a balanced Redfield 106 : 16 : 1 C : N : P ratio. The particulate matter below the chemocline in Lake Bonney was enriched in C and P relative to N, suggesting that it was composed primarily of mineral or organic detrital matter, because the primary potential source of living organic matter (phytoplankton) is depleted in P relative to N. We suspect that at least some of this material represents either fine aeolian mineral particles (which are P-rich; J. E. Dore unpubl. data) that sank through the less dense surface waters and became suspended in the denser hypersaline bottom waters, or mineral precipitates that formed in situ (carbonates, phosphates). A combination of these scenarios is also possible.

Alkaline phosphatase activity and phytoplankton P deficiency—The time course of APase activity revealed some important features (Fig. 4). First, it can be seen that the ob-

served APase activity as measured by the hydrolysis of MUP to MUF was probably not an artifact of variable background fluorescence or nonenzymatic hydrolysis, as evidenced by the near-zero fluorescence of boiled controls. Second, there was a distinct suppression of APase activity produced by the addition of phosphate, to be expected under P-deficient environmental conditions (Cembella et al. 1984). Finally, an intriguing result of this experiment was the high time-zero hydrolysis evident in the live treatments. The reaction in all samples was stopped by immediate freezing in liquid nitrogen after addition of the MUP substrate, with an elapsed time of no more than 2 min; nevertheless, this large time-zero fluorescence was evident in all experiments. Because there is presumably a suite of enzymes responsible for the observed activity, an enzyme with particularly rapid kinetics may have caused the time-zero fluorescence. Alternatively, it is possible that some background fluorescence from dissolved compounds in the water samples may have contributed to the time-zero fluorescence but that these compounds were denatured by boiling in the control samples. However, the time-zero fluorescence of 0.2-μm filtered samples was less than half that of parallel unfiltered samples (data not shown), suggesting that the time-zero fluorescence was, at least in part, due to particulate enzyme activity, not dissolved fluorescent compounds. A third potential explanation for the high time-zero response involves enzymatic ''burst'' kinetics (see for example Copeland 2000).

Consider the following multistep enzymatic reaction,

$$E + S \underset{k_{-1}}{\overset{k_{+1}}{\Leftrightarrow}} ES \overset{k_2}{\Rightarrow} P + EQ \overset{k_3}{\Rightarrow} E + Q,$$

where E is the enzyme, S the substrate, P and Q are products, and the k_n are the rate constants for the reactions indicated. If the final step is rate-limiting, then in the steady-state, the enzyme will exist almost entirely as EQ, and the overall release of both P and Q will be governed by the rate constant k_3. Consider the case when in the initial condition S is very small but there is a substantial amount of enzyme. Then the enzyme exists primarily as E, and upon addition of a pulse of S, P and EQ are produced rapidly, but the production of Q and release of E cannot keep up. In this transient phase, P is rapidly produced without production of Q; as steady-state is reached, the production rates of P and Q become equal.

In the case of these alkaline phosphatase experiments, P represents the hydrolysis product MUF, and Q represents the phosphate released. S is the MUP substrate added (see reaction scheme for APase outlined in Jansson et al. 1988). Initially, available phosphate monoesters are nearly absent from the system, but a substantial amount of uncomplexed alkaline phosphatase is on hand. When the MUP is added, MUF is produced in a burst (governed by k_{+1} and k_2), but the phosphate removed from the MUP remains complexed to the APase, releasing free APase and phosphate at a slower rate (governed by k_3). What we presumably see in the time-zero samples is the burst of MUF, but the actual rate of release of phosphate is seen in the steady state kinetics following time zero.

The burst kinetics phenomenon, if confirmed, has important analytical and ecological implications. Analytically, it implies that time-zero corrections must be applied following time zero, or gross overestimates of potential steady-state rates of phosphate liberation may result. Ecologically, it implies that in situ substrate concentrations are vanishingly low, yet high levels of enzyme are maintained. The maintenance of high levels of APase may represent an adaptation to an environment in which substrate inputs are rapid and stochastic. Such inputs could be expected from release of dissolved organic matter from gravel-associated ice cap communities (Priscu et al. 1998) as these gravel packets fall into the water column during the formation of summer meltwater channels in the ice cover or from the delivery of the organic-rich initial melt of nearby glacial faces (Downes et al. 1986).

The substrate response of APase activity in the east lobe of Lake Bonney was described by a standard Michaelis–Menten function (Fig. 5). The apparent half-saturation constant K_m of 2.4–3.8 μM (Table 2) was on the low end of the reported range for APase in aquatic environments (about 1–100 μM; Jansson et al. 1988). The low K_m implies a high affinity of Lake Bonney APase for available phosphate monoesters. The maximum reaction velocity of 51.4 nmol L^{-1} h^{-1} indicates the potential for turnover of the SRP pool in less than 1 h following a sufficiently large input of phosphomonoester substrate. The single data point from Lake Hoare has been included on this plot, demonstrating the much reduced APase activity there compared with the east lobe of Lake Bonney. This comparison reveals more severe phosphorus deficiency in the east lobe of Lake Bonney than in Lake Hoare, consistent with the unambiguous stimulation of photosynthesis by P addition observed among near-surface phytoplankton in Lake Bonney but not in Lake Hoare (Table 1; see also Priscu 1995).

Maximum APase activity has been evaluated experimentally as a function of the degree of P deficiency in a variety of algal cultures; slight P deficiency is indicated by maximum potential hydrolysis rates of >2 nmol (μg PP)$^{-1}$ h^{-1} or >3 nmol (μg Chl *a*)$^{-1}$ h^{-1}, whereas severe P deficiency is indicated by maximum potential hydrolysis rates of >10 nmol (μg PP)$^{-1}$ h^{-1} or >5 nmol (μg Chl *a*)$^{-1}$ h^{-1} (Healy and Hendzel 1979). Maximum APase activities from the east lobe of Lake Bonney, whether normalized to PP (36.8 nmol [μg PP]$^{-1}$ h^{-1}) or to Chl *a* (122.6 nmol [μg Chl *a*]$^{-1}$ h^{-1}), were clearly indicative of extreme P deficiency (Table 2). The degree of P deficiency implied by the maximum APase rate in Lake Hoare was somewhat ambiguous; when normalized to PP (0.43 nmol [μg PP]$^{-1}$ h^{-1}), no P deficiency was suggested, but when normalized to Chl *a* (8.23 nmol [μg Chl *a*]$^{-1}$ h^{-1}), severe P deficiency was implied (Table 2). This apparent contradiction may be due to a substantial fraction of the APase activity (in both lakes) being associated with bacterial and free dissolved pools, rather than the algal pool alone. Microscopic analysis of phytoplankton in the dry valley lakes shows that most species are larger than 2.0 μm (J.C. Priscu unpubl. data). When APase activity in Lake Bonney was measured on whole water from 5 m deep and on <2.0- and <0.2-μm filtrates to evaluate the relative proportions of algal, bacterial, and dissolved APase, the results showed that the 0.2–2.0-μm (bacterial) fraction held

most (68%) of the total APase activity, and the <0.2-μm (dissolved) fraction also held some (8%) of the activity (Fig. 8). Normalization to PP rather than to Chl *a* may therefore be more appropriate here if APase activity is considered as an overall community P deficiency indicator.

The temperature response of APase activity in the east lobe of Lake Bonney showed a maximum potential rate at about 35°C (Fig. 6), typical of alkaline phosphatases in other aquatic environments (Healy and Hendzel 1979). Although APases with temperature optima as low as 5°C have previously been described from antarctic microbial sources (Banerjee et al. 2000), no special adaptation to the relatively cold water temperature of the east lobe of Lake Bonney (Fig. 1) was evident in our study. The relatively rapid rise in activity between 0 and 10°C suggests that in situ rates may be strongly influenced by even minor changes in temperature. Although potential rates of APase activity were higher at 5 m than at 13 m (Fig. 7), it is still conceivable that true in situ APase activities reached on substrate input may actually be higher in the lower trophogenic zone than immediately below the ice because of the approximately 3°C higher temperature at 13 m. Also, it is clear that even should adequate substrate at times become available, APase activity as a whole will be suboptimal owing to the low overall temperature of the lake environment.

Sources, sinks, and internal cycling of P in antarctic lakes—The four antarctic lakes studied here all lie within the same valley and have similar ice covers and watersheds (Fountain et al. 1999; Lyons et al. 2000). Nevertheless, their phytoplankton communities exhibit different degrees of phosphorus deficiency. Because the lakes have no significant outflows (Chinn 1993), these differences must be related to the supply of exogenous phosphorus to the lakes and the internal cycling of phosphorus within the lakes. New phosphorus may enter the lakes via intermittent stream flow during the summer or possibly via release from the permanent ice cover. Such exogenous nutrient inputs are likely to be important on an annual basis. However, the field work in this study was conducted when air temperatures rarely exceeded −20°C, and flowing water was absent and had been so for several months. Primary photosynthetic production in these effectively closed systems must therefore have been supported during this period by endogenous phosphorus supplies. Because of the strong stability of the lake water columns, this phosphorus is presumably supplied via upward diffusion across the lakes' chemoclines (Priscu 1995). Another possible yet undemonstrated mechanism for bringing phosphorus from the chemocline into the trophogenic layer is via the vertical phototactic migrations of microorganisms. Many of the phytoplankton species in these lakes are flagellated and may move vertically in order to achieve optimal light-harvesting potential and possibly nutrient supply (Priscu and Neale 1995; Lizotte and Priscu 1998). This process was not examined in our study, yet remains an important consideration for future research.

If the primary source of phosphorus supporting phytoplankton growth prior to the onset of stream flow is upward diffusion across the chemocline, then we expect that the degree to which phosphorus (vs. nitrogen) limits primary productivity during this period will be determined by the N : P ratio of the upward diffusive flux of inorganic and bioavailable organic nutrients. Priscu (1995) showed that the response of phytoplankton carbon assimilation to N and P enrichments in these lakes was consistent with the DIN : SRP flux ratios from nutrient gradients immediately below the phytoplankton populations sampled. In the present study, we find a more consistent response of carbon fixation to phosphorus amendment in Lake Bonney than in Lake Hoare, as well as much higher APase activity in the east lobe of Lake Bonney than in Lake Hoare. These results are in accord with our measured diffusive flux ratios across the chemoclines of DIN : SRP (12.3 in Lake Hoare vs. 284 in Lake Bonney), DIN : TDP (12.3 in Lake Hoare vs. 166 in Lake Bonney) and TDN : TDP (6.1 in Lake Hoare vs. 302 in Lake Bonney).

It is possible, therefore, that because of the extremely high DIN : SRP and TDN : TDP ratios of the Lake Bonney deepwater nutrient source, the lake ecosystem is driven toward phosphorus deficiency by factors producing these deepwater nutrients. However, regeneration of organic matter with moderate N : P sinking out of the trophogenic zone cannot explain the observed N : P values of the dissolved nutrients in the deep water. The dense deepwater brine of Lake Bonney is thought to have originated from several evaporative periods over the last 10,000 yr and represents a resource legacy of past events (Chinn 1993; Lyons et al. 2000). Atmospheric dinitrogen fixation appears today to be an insignificant process in Lake Bonney (Priscu 1995), but it is possible that past periods of higher dinitrogen fixing activity could have contributed to the high N : P ratios in the deep water. A more likely scenario is that enhanced precipitation of phosphate minerals during evaporative periods selectively removed phosphorus from the water column. Conversely, Lake Hoare is a younger lake and has not experienced such evaporative periods; thus, it remains relatively fresh from top to bottom (Fig. 1; Fountain et al. 1999; Lyons et al. 2000). Dissolved inorganic and organic nutrients in the anoxic bottom water of Lake Hoare are balanced in phosphorus relative to nitrogen. However, this bottom water is of limited areal extent; hence, the significance of its overall contribution to phytoplankton nutrient supply in the lake is unclear.

We have presented several lines of evidence that the Lake Bonney ecosystem is extremely P-deficient. However, the different approaches taken to assessing nutrient status have yielded somewhat ambiguous results in Lake Hoare that suggest N and P codeficiency. In the east lobe of Lake Bonney, the stage appears to have been set for phosphorus deficiency through the legacy left by past geological and geochemical modifications of the lake. The relative roles of stream-borne and diffusive phosphorus supplies, the mechanisms by which microbial populations of this lake have adapted to their P-deficient environment, and the relative roles of phytoplankton and bacteria in phosphorus cycling are important areas for future research.

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