

Seasonal dynamics of nutrient limitation in a tropical high-altitude lake (Lake Titicaca, Peru-Bolivia): Application of physiological bioassays¹

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Abstract

Five types of physiological assay were applied to samples from three sites in Lake Titicaca, Peru-Bolivia (16°S), to assess the magnitude and seasonality of N or P limitation of algal growth. Phosphorus appeared to be in abundant supply relative to growth requirements throughout 1982. Alkaline phosphatase levels were low (<3 nmol substrate converted $\cdot \mu\text{g}^{-1}$ Chl *a* $\cdot \text{liter}^{-1}$), $^{32}\text{PO}_4^{3-}$ turnover times were always long (> 10³ min in the main body of the lake), and little or no phosphorus was accumulated by the seston during short term enrichments. Ammonium enhancement assays suggested a persistent cellular shortage of nitrogen throughout all seasons in the plankton of a large shallow bay, and in the main basin when the water was stratified. High ammonium uptake capacities, as measured with the NH_4^+ analogue [¹⁴C]methylammonium, and selective accumulation of nitrogen from N plus P enrichments provided supportive evidence of cellular N deficiency. Winter mixing brought large quantities of NO_3^- and SRP into the surface euphotic zone of the main basin, and throughout this period neither N nor P appeared to be limiting. Polymixis in the shallow bay resulted in large variations in nitrogen supply and deficiency, and these effects were modulated in part by changes in depth of the euphotic zone. Nitrogen was always in low abundance relative to phosphorus in Lake Titicaca, but the extent of nutrient control on algal growth varied considerably with site and time of year.

The controlling nutrients for freshwater algal production have been little explored outside the northern regions of the Northern Hemisphere. Many north-temperate lakes are characterized by growth-limiting levels of phosphorus (Schindler 1978). In apparent contrast, some tropical waters (e.g. Lake Lanao: Lewis 1974; Lake Tanganyika: Coulter 1977; Lake Victoria: Talling 1966; Nile River: Prowse and Talling 1958) seem poor in dissolved inorganic nitrogen (DIN) relative to soluble reactive phosphorus (SRP); this has led to speculation that nitrogen may be generally limiting at low latitudes (e.g. Talling and Talling 1965; Viner 1975). Although these DIN and SRP data are highly suggestive, nutrient limitation in the tropics has only rarely been tested by direct bioassay. Many such assays have

suffered from prolonged incubation in small containers under artificially extreme enrichments, and perhaps as a consequence they have sometimes proved inconclusive, if not contradictory.

A more sophisticated approach was adopted by Melack et al. (1982) who used large volume, open-ended polyethylene tubes to measure enrichment responses by the plankton of an equatorial soda lake. They reported, for the one time of year tested, compelling evidence of phosphorus limitation. Analysis of seston from a second lake suggested less availability of phosphorus than nitrogen there also, while in a third lake low seston N:P ratios and abundant SRP were indicative of N limitation. These findings led Melack et al. to question the generality of any statement identifying a single nutrient of importance in the tropics.

In Lake Titicaca, a high altitude lake in the tropical Andes, we have applied a variety of methods to estimate nutrient demand and deficiency of the phytoplankton. We report here observations from five types of physiological bioassay. From these, together with nutrient and chlorophyll measurements, we examine the extent and sea-

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sonality of N and P limitation to algal growth in the surface waters at several sites across the lake.

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Methods and materials

Lake Titicaca is a large (8,100 km²), deep (max depth, 275 m), alpine (3,803 m ASL) lake which lies on the Peru-Bolivian border at 16°S latitude. Two sites were routinely sampled: station 4 in the main body of the lake (Lago Grande, 6,315 km²; mean depth 147 m) and station 23 in a shallower bay on the western shore (Bahia de Puno, 525 km²; mean depth in the open water region, 14 m; max depth, 37 m). A third site (station 50) located in the eutrophic inner bay near Puno (Inner Harbor, 14 km²; mean depth <5 m) was also sampled at several times throughout the year. Chlorophyll *a* measurements were made along a transect between these sites, and toward the shoreline in Lago Grande (Fig. 1). Lago Grande is stratified during most of the year but mixes more or less thoroughly late in each dry season, August–September. The shallower bays are polymictic. Surface water temperatures range from 11°C during the dry season (May–September) to 16°C during the rainy season (October–April). Additional limnological data for Titicaca are presented elsewhere (Richerson et al. 1977; Widmer et al. 1975; Kittel and Richerson 1978; Lazaro 1981).

Samples for bioassay and chemical analysis were collected with an opaque Van Dorn sampler, from 5 m in Bahia de Puno and Lago Grande and from 0.5 m in the eutrophic Inner Harbor. Water was immediately filtered through acid-washed, glass-fiber filters (Whatman, GF/C) and frozen for up to 4 weeks for chemical analysis; there was no evidence of major shifts in nutrient chemistry during storage. The short term physiological assays were performed either im-

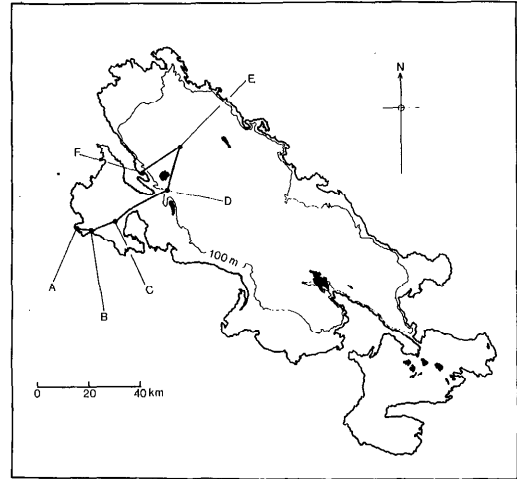


Fig. 1. Location of sampling sites and transect line on Lake Titicaca. A—Station 50, Inner Harbor; B—harbor mouth; C—station 23, Bahia de Puno; D—Rocas Misteriosas; E—station 4, Lago Grande; F—Ccotos.

mediately (ammonium enhancement) or within 2–5 h of collection (³²P turnover, seston accumulation, methylammonium transport, alkaline phosphatase).

Ammonium was measured with the indophenol blue method (Solórzano 1969); the detection limit (below which there was no significant difference between sample and blank) was 0.20 µg-atom NH₄-N·liter⁻¹. SRP and silica were measured according to Strickland and Parsons (1968); detection limits were 0.03 and 0.05 µg-atoms·liter⁻¹. Nitrate was determined by hydrazine reduction and subsequent diazotization (Kamphake et al. 1967). Samples for this analysis had to be diluted 1:5 to 1:10 with deionized, distilled water because of marked, but unidentified, interference of the reduction step in full-strength lake water; detection limits for NO₃-N were consequently ca. 0.25 µg-atom·liter⁻¹.

Plankton for Chl *a* analysis was filtered through 158-µm netting to remove zooplankton or 10-µm netting to remove all but ultraplankton. The filtrates were passed through glass-fiber filters (Whatman GF/C) which were then extracted in 100% methanol for 24 h. Extracts were cleared by centrifugation and analyzed fluorometrically

before and after acidification (Holm-Hansen and Riemann 1978). The Turner 111 fluorometer was calibrated against spinach chlorophyll *a* and *b* standards assayed spectrophotometrically. The ultraplanktonic fraction may have been slightly underestimated if very small algal cells ($<1 \mu\text{m}$) passed through the GF/C filters (Yentsch 1983). However, the total biomass contribution of such cells appeared negligible in our routine counts from the surface mixed layer.

Five short-incubation physiological bioassays were applied throughout the year. Samples for ammonium enhancement, a measure of nitrogen deficiency (Yentsch et al. 1977), were dispensed into six 60-ml Pyrex bottles. H^{14}CO_3 was injected into each (final concn of ca. $40 \mu\text{Ci}\cdot\text{liter}^{-1}$) and then NH_4Cl added to three bottles (final concn $7 \mu\text{g}\cdot\text{atoms N}\cdot\text{liter}^{-1}$). The bottles were wrapped in aluminum foil and placed inside a light-tight box which was incubated at 10 m (a depth below the region of daily surface heating) for 4 h. The labeled plankton was then filtered onto $0.45\text{-}\mu\text{m}$ Millipore membranes which were air-dried and counted by liquid scintillation. Results were expressed as the percent enhancement of dark CO_2 fixation by ammonium enrichment.

Ammonium transport capacity was measured with a radioisotopically labeled structural analogue of ammonium, ^{14}C methylammonium (Vincent 1979; Wheeler and McCarthy 1982). Lake water was dispensed into 130-ml Pyrex bottles which were injected with 0.2, 0.4, 0.6, 0.8, 1.0, or $1.4 \mu\text{Ci}$ of $^{14}\text{CH}_3\text{NH}_3^+$ (ICN Pharmaceuticals, $4.17 \mu\text{Ci}\cdot\mu\text{mol}^{-1}$) and incubated at $180 \mu\text{Einst}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at $12^\circ\text{--}14^\circ\text{C}$ (within 2°C of lake water temperatures) for 20 min. Subsamples were then filtered through $0.45\text{-}\mu\text{m}$ Millipore membranes, which were subsequently rinsed with filtered lake water, air-dried, and counted by liquid scintillation. Samples filtered within 30 s of ^{14}C methylammonium addition provided an adsorption control and these values were subtracted from uptake at each substrate concentration. V_{max} and apparent K_s values were estimated from nonlinear regression fits (Snedcor and Cochran 1974) to a Michaelis-Menten model (Li 1983).

As a measure of phosphate demand and availability, lake water samples were injected with $^{32}\text{PO}_4^{3-}$ and its accumulation by the seston measured after 2, 4, 8, 16, 30, 60, and 120 min. Uptake curves were plotted as the percentage of label remaining in solution at each interval. Turnover times (theoretical time required for complete removal of $^{32}\text{PO}_4^{3-}$ from solution) were calculated from natural log regressions to the uptake data over the first 60 min. Since uptake rates were generally slow these data were reasonably approximated by the log-linear model (r^2 generally >0.95).

Alkaline phosphatase activity was measured as a further guide to phosphorus deficiency. The enzyme was assayed with *o*-methyl fluorescein phosphate (MFP) under optimal standard conditions ($10 \mu\text{M}$ MFP, 35°C) (Healey and Hendzel 1979).

The luxury uptake capacity of *Titicaca* phytoplankton was used as a fifth measure of nutrient demand. Lake water samples were incubated with additions of ammonium ($11 \mu\text{g}\cdot\text{atoms N}\cdot\text{liter}^{-1}$) and phosphate ($0.7 \mu\text{g}\cdot\text{atom P}\cdot\text{liter}^{-1}$) for 2 h under $180 \mu\text{Einst}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at $12^\circ\text{--}14^\circ\text{C}$. The nitrogen and phosphorus content of the filtered seston was measured before and after enrichment. Each filter was digested for 4 h at 360°C with 0.5 ml of concentrated sulfuric acid containing 10.4 g Se, 24 g $\text{CuSO}_4\cdot 5\text{H}_2\text{O}$, and 680 g K_2SO_4 per liter. The digest was then diluted to 10 ml and centrifuged. Dissolved ammonium was analyzed by the method of Searle (1975) and phosphate by the method of Downes (1978). The methodology and theoretical background for this and the other assays is described in detail elsewhere (Vincent 1981*a,b*).

Temperature was measured in the field with a Whitney TC-5C thermistor. The attenuation of underwater irradiance was determined with a Lambda quantum irradiance probe with a $4\text{-}\pi$ sensor.

Results

Chlorophyll a nutrients and mixing—Chlorophyll *a* concentrations were always highest in the eutrophic Inner Harbor. Throughout 1982 this shallow, almost isolated harbor contained $10\text{--}30 \mu\text{g Chl } a\cdot\text{liter}^{-1}$, an order of magnitude higher than

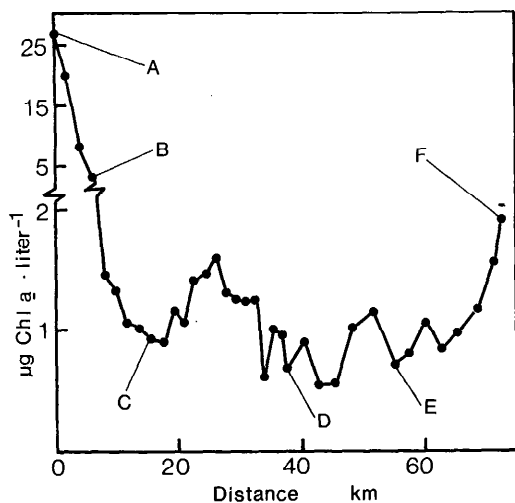


Fig. 2. Chlorophyll *a* concentrations in the surface waters of Lake Titicaca, 27 October 1982. Cruise transect and sites as on Fig. 1.

elsewhere in the lake. For four dates distributed over the year, $\text{NH}_4\text{-N}$ was $\leq 1 \mu\text{g-atom}\cdot\text{liter}^{-1}$, $\text{NO}_3\text{-N}$ was undetectable, and SRP concentrations ranged from 0.15 to 0.60 $\mu\text{g-atom}\cdot\text{liter}^{-1}$.

Within the channel that connects the Inner Harbor with Bahia de Puno there was an abrupt decrease in Chl *a* levels (Fig. 2). Chlorophyll biomass in Bahia de Puno was of a magnitude comparable to that in Lago Grande (Fig. 2), but on any one date concentrations could differ twofold–fourfold between the two regions of the lake. Slightly higher Chl *a* concentrations were sometimes recorded at the mouth of Bahia de Puno, and toward the shoreline and in small sheltered bays of the main lake basin (e.g. Ccotos, F, Fig. 2).

Levels of Chl *a* were relatively high in Bahia de Puno throughout 1982 until late July (mean concn at 5 m \pm 2 SE, $3.63 \pm 0.59 \mu\text{g}\cdot\text{liter}^{-1}$) when there was an abrupt decline to much lower levels which persisted until the end of the sampling program in December (for August–December, $1.25 \pm 0.20 \mu\text{g Chl } a\cdot\text{liter}^{-1}$). This decline in surface euphotic chlorophyll was paralleled by a decrease in the percentage of ultraplankton, which fell from 67% in July to 38% by mid-September (Fig. 3A). After that the

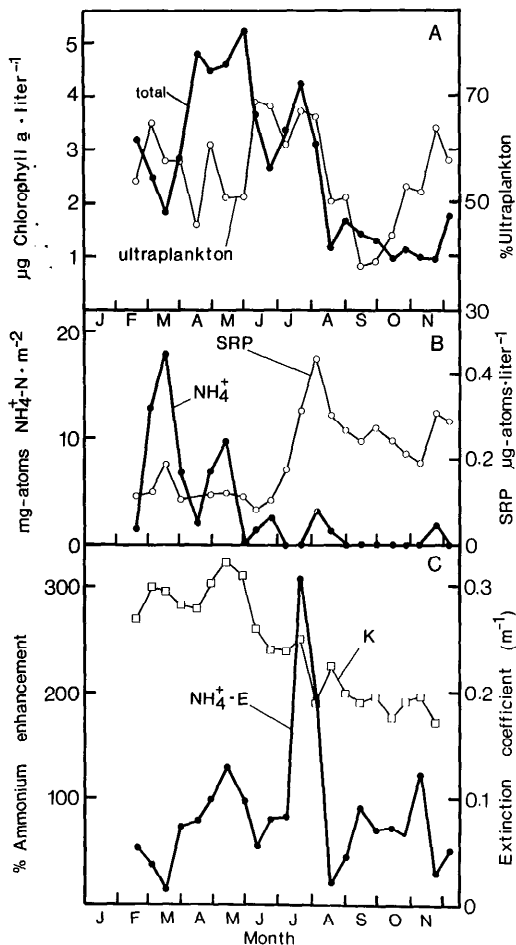


Fig. 3. Seasonal characteristics of Bahia de Puno (station 23). A. Chlorophyll *a* levels at 5 m (●) and percentage chlorophyll passing through a 10- μm filter (% ultraplankton, ○). B. Integral $\text{NH}_4\text{-N}$ levels in the region 15–24 m (●) and average SRP levels in the upper euphotic zone (0–15 m, ○). C. Extinction coefficient for the water column measured by log-linear regressions to underwater irradiance profiles (□) and ammonium enhancement ($\text{NH}_4^+\text{-E}$) values for 5-m water samples (●).

$<10\text{-}\mu\text{m}$ chlorophyll fraction steadily increased, while total Chl *a* concentration changed little.

The shift from high to low Chl *a* in July–August also corresponded with changes in dissolved nutrient concentrations. Over the first half of the year SRP values in the surface waters of Bahia de Puno were ca. 0.1 $\mu\text{g-atom}\cdot\text{liter}^{-1}$, but in July this increased

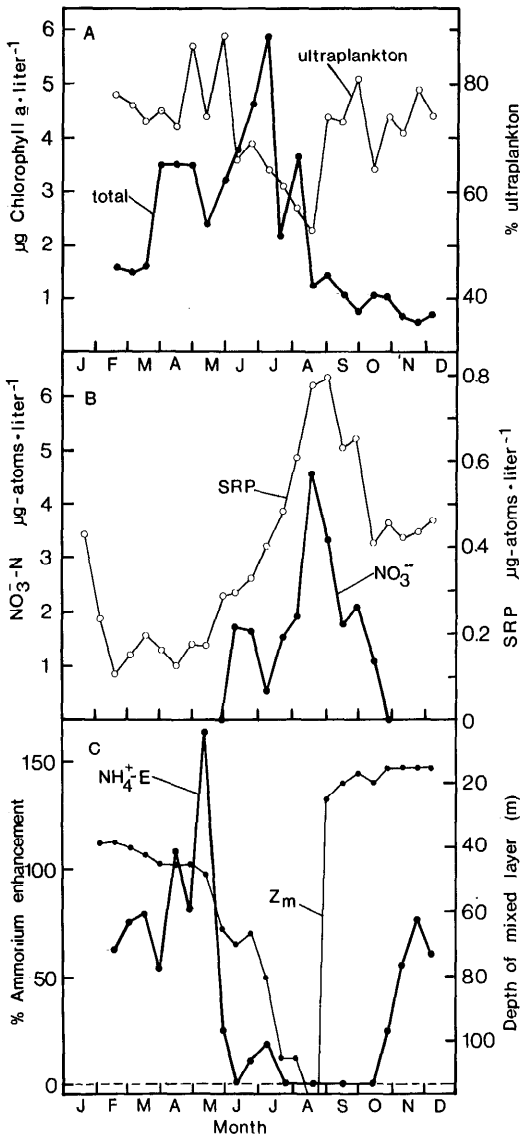


Fig. 4. Seasonal characteristics of Lago Grande (station 4). A. Chlorophyll *a* levels at 5 m (●) and percentage chlorophyll passing through a 10- μm filter (○) ultraplankton, (○). B. Average $\text{NO}_3^- \text{-N}$ (●) and SRP (○) concentrations in the surface region, 0–25 m. C. Depth of mixing (Z_m , ○) and ammonium enhancement values for 5-m water samples ($\text{NH}_4^+ \text{-E}$, ●).

to 0.2–0.4 $\mu\text{g-atoms} \cdot \text{liter}^{-1}$ and persisted through the rest of the year (Fig. 3B). However in both periods, dissolved inorganic nitrogen (both nitrate and ammonium) in the upper region of the water column (0–15

m) was generally at or below our limits of detection. Over 1982, silicate values ranged from 2–10 $\mu\text{g-atoms} \cdot \text{liter}^{-1}$.

Bahia de Puno did not show the well defined seasonality in stratification and mixing that is characteristic of Lago Grande. Water was stratified (below the diel thermocline) in mid-March, mid-April, June, late July–August, and for a more prolonged period from September through December.

In the first half of the year these several-week stratifications resulted in a buildup of NH_4^+ in the bottom waters (0–25 m) which was subsequently mixed into the surface euphotic zone during each polymictic cycle (Fig. 3B). This hypolimnetic accumulation of ammonium was much less obvious in the second half of the year, when the surface waters at station 23 were more transparent (Fig. 3C) and the euphotic zone extended to the lake floor (but to about 20 m earlier in 1982; Richerson et al. unpubl. data). Irradiance levels at 25 m changed from about 0.05% of surface levels during February–May to about 0.9% during September–December (Fig. 3C).

Chlorophyll *a* levels in Lago Grande (station 4) and Bahia de Puno (station 23) during 1982 were similar (mean concn \pm 2 SE at 5 m was 2.24 ± 0.62 in Lago Grande and 2.59 ± 0.60 in Bahia de Puno). The percentage of ultraplankton was generally higher in Lago Grande (mean for 1982 was $72.0 \pm 3.8\%$ compared to $55.6 \pm 3.8\%$ in Bahia de Puno). The mid- to late stratification period in Lago Grande during early 1982 showed relatively high Chl *a* (Fig. 4A) with a dominant ultraplankton component (70–90% < 10 μm). Chl *a* concentrations rose further during autumnal and winter mixing, but were diluted during two episodes of deep mixing: 8–22 July when the mixed layer was deepened to about 110 m, and 5–19 August when mixing extended to the bottom. During this period there was a steady decline in the percentage of ultraplankton, from about 88% in May to 53% by mid-August (Fig. 4A). Surface euphotic Chl *a* remained low in Lago Grande with the onset of stratification in early September, but there was an immediate return to ultraplankton dominance (Fig. 4A). Chlorophyll biomass at 5 m declined slowly but steadily over the last

few months of the year. A deep chlorophyll maximum developed at greater depths (25–35 m) then (W. F. Vincent unpubl. data), but the nutritional state of this community was not tested.

Throughout 1982, $\text{NH}_4^+\text{-N}$ levels in the surface waters of Lago Grande were close to or below our limit of detection. Surface $\text{NO}_3^-\text{-N}$ was $<0.25 \mu\text{g-atom}\cdot\text{liter}^{-1}$ until winter circulation in May–August, when values abruptly increased, to a maximum of about $4.5 \mu\text{g-atoms}\cdot\text{liter}^{-1}$ by the final date of mixing (Fig. 4B). SRP also increased over this period, from about $0.15 \mu\text{g-atom}\cdot\text{liter}^{-1}$ during late stratification to about $0.8 \mu\text{g-atom}\cdot\text{liter}^{-1}$ at the end of mixing. During early stratification there was a steady loss of both DIN and SRP—between 19 August and 28 October these elements decreased in the surface euphotic zone in the atomic ratio $14.6 \text{NO}_3^-\text{-N}$ to 1.0 SRP. By the latter date $\text{NO}_3^-\text{-N}$ was again undetectable, while SRP concentrations remained at about $0.4 \mu\text{g-atom}\cdot\text{liter}^{-1}$ through November–December.

Ammonium enhancement—This assay of nitrogen deficiency was applied to samples from Lago Grande and Bahia de Puno at 2-week intervals from February–December 1982. Positive dark fixation responses to ammonium addition were recorded in Bahia de Puno on all dates (Fig. 3C). A steady rise in enhancement from 17 March to 12 May corresponded with a threefold rise in Chl *a* concentrations over the same period (Fig. 3A). Much greater enhancement was recorded in late July–August, when Chl *a* levels were still high ($3\text{--}4 \mu\text{g}\cdot\text{liter}^{-1}$) and when SRP rose to the highest concentration observed.

The ammonium enhancement values dropped abruptly to a minimum of 21% on 18 August (Fig. 3C), corresponding with the sharp decrease in surface Chl *a* (Fig. 3A). Over the next 4 weeks enhancement returned to values more typical of earlier in the year (60–80%) while Chl *a* slowly declined. During the 6-week period in October–November that Bahia de Puno was stratified, enhancement rose to 123% of controls, and then fell below 50% when stratification began to break down in late November–December.

The seasonal pattern of nitrogen deficiency as measured by NH_4^+ enhancement was very different in the main body of the lake (Fig. 4C). Lago Grande samples responded strongly to ammonium enrichment from the first measurements in February until mid-May. Strongest enhancements (78%) were from 16 April to 13 May, the time of highest Chl *a* over the final weeks of stratification. With the onset of winter mixing, ammonium enhancement abruptly declined and by 10 June there was no significant response to NH_4^+ addition. A very small enrichment effect ($<20\%$ enhancement) was recorded in late June–early July, but there was a consistent lack of enhancement over the next 3.5 months. Stratification began again in late August–early September, but it was not until late October, when surface DIN had been completely exhausted, that significant responses to NH_4^+ were again recorded. From 20 October to 7 December enhancement increased while Chl *a* levels in the surface waters of Lago Grande declined to their annual minimum.

Ammonium uptake capacity—The [^{14}C]methylammonium uptake data were

Table 1. V_{max} and apparent K_s values for [^{14}C]methylammonium uptake by plankton samples from Lake Titicaca. Each value was determined by a non-linear regression fit of uptake data for 5–6 substrate concentrations to a Michaelis-Menten curve; r^2 values express the goodness-of-fit to this model; $P < 0.05$ for all regressions.

	V_{max}^*	V_{max}^\dagger	K_s^\ddagger	r^2
Lago Grande				
18 Mar	23.6	14.7	0.8	0.905
29 May	4.7	1.3	0.6	0.788
19 Aug	7.3	5.8	6.3	0.929
25 Nov	3.5	5.9	0.6	0.817
Bahia de Puno				
18 Mar	9.3	5.2	0.9	0.996
29 May	16.6	8.7	0.9	0.831
19 Aug	17.3	14.4	2.5	0.965
25 Nov	9.4	9.8	1.3	0.928
Inner Harbor				
18 Mar	136.8	5.6	0.4	0.873
29 May	35.1	1.9	0.2	0.836
19 Aug	36.5	1.7	2.7	0.949
25 Nov	56.9	10.9	0.6	0.847

* $\text{ng-atoms N}\cdot\text{liter}^{-1}\cdot\text{h}^{-1}$.

† $\text{ng-atoms N}\cdot\mu\text{g}^{-1}\text{ Chl } a\cdot\text{h}^{-1}$.

‡ $\mu\text{mol}\cdot\text{liter}^{-1}$.

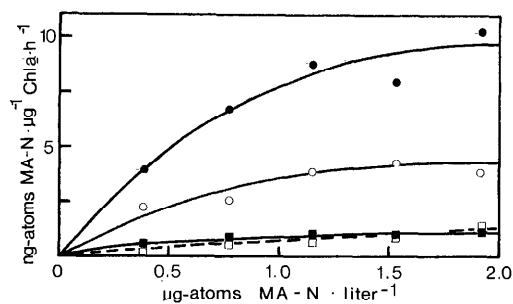


Fig. 5. Uptake of [^{14}C]methylammonium (MA) by Lago Grande plankton at four times of year: ●—18 March; ■—29 May; □—19 August; ○—25 November.

closely fitted by the Michaelis-Menten model (Table 1). Maximum uptake velocities (V_{\max}) for this ammonium analogue varied considerably with site and time (Fig. 5, Table 1). $V_{\max} \cdot \text{liter}^{-1}$ from the Inner Harbor was consistently 5–10 times higher than elsewhere in the lake (Table 1). Bahia de Puno plankton had a much higher V_{\max} than Lago Grande for all but the late stratification period (18 March); transport capacity ($V_{\max} \cdot \text{liter}^{-1}$) in Lago Grande on 18 March was over twice that in Bahia de Puno, and about four times higher than the rates subsequently recorded at station 4 (Lago Grande) during winter.

These site-to-site and temporal differences are further emphasized when V_{\max} values are normalized against Chl *a*. The V_{\max} per unit chlorophyll value of about 7 $\text{ng-atoms} \cdot \text{h}^{-1}$ has been found to correlate with other indications of pronounced nitrogen deficiency in various temperate lakes (Vincent 1981a). This level of uptake capacity was approached or exceeded at all three stations on Lake Titicaca at various times of the year (Table 1). Highest $V_{\max} \cdot \mu\text{g}^{-1}$ Chl *a* was recorded in Lago Grande on 18 March, but with the onset of mixing in mid-May uptake capacity per unit chlorophyll declined by an order of magnitude. Toward the end of winter mixing, uptake rates were low at all substrate concentrations tested (Fig. 5), but the apparent K_s value was very large by comparison with other times of year, and the calculated V_{\max} value was consequently much higher than in the early winter period (Table 1). With

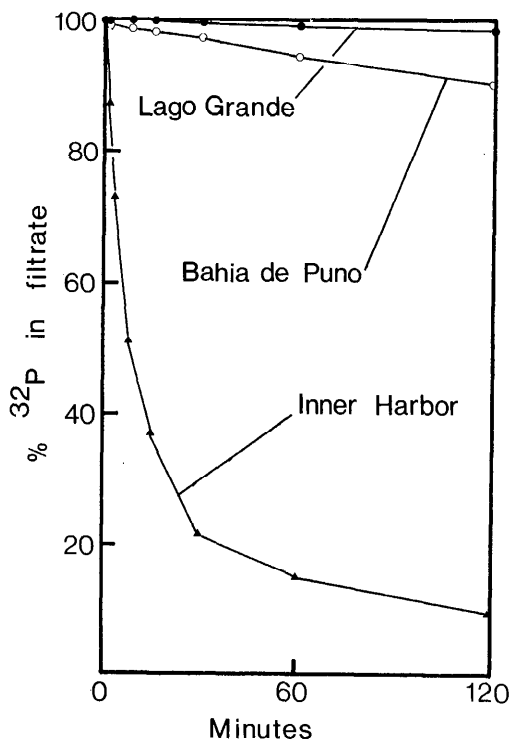


Fig. 6. $^{32}\text{PO}_4^{3-}$ loss from solution for near-surface water samples collected 29 May 1982.

the onset of stratification apparent K_s dropped, but V_{\max} remained high, suggesting conditions of increased N deficiency. However on 25 November uptake capacity was still well below that recorded in March.

Bahia de Puno samples exhibited less variation in $V_{\max} \cdot \mu\text{g}^{-1}$ Chl *a* than those from Lago Grande, with all capacities in the range 5–15 $\text{ng-atoms} \cdot \mu\text{g}^{-1}$ Chl *a* · liter $^{-1}$. Lower values were recorded for two dates in the Inner Harbor (station 50), but, on 25 November during a period of usually low Chl *a* per liter, a methylammonium uptake capacity above 7 $\text{ng-atoms} \cdot \text{N} \cdot \mu\text{g}^{-1}$ Chl *a* · liter $^{-1}$ was recorded for this part of the lake.

Orthophosphate turnover—The rate of net transfer of $^{32}\text{PO}_4^{3-}$ from solution to seston was slow in relation to biomass at all three stations throughout the year. Fastest turnover times (inverse of uptake rate constant) were in the Inner Harbor (Fig. 6) and varied from 13 min in the middle of the rainy sea-

Table 2. $^{32}\text{PO}_4^{3-}$ turnover times (T/O, min) and soluble reactive phosphorus concentrations (SRP, $\mu\text{g}\cdot\text{atom}\cdot\text{liter}^{-1}$) for samples from Lake Titicaca, 1982.

	Lago Grande		Bahia de Puno		Inner Harbor	
	T/O	SRP	T/O	SRP	T/O	SRP
20 Feb	5.9×10^3	0.13	6.0×10^1	0.12	1.3×10^1	*
18 Mar	2.8×10^3	0.19	9.3×10^2	0.14	1.1×10^2	0.36
20 May	8.9×10^3	0.29	1.3×10^3	0.11	3.2×10^1	0.27
17 Jul	8.7×10^4	0.44	1.2×10^4	0.31	3.4×10^3	*
25 Sep	$>10^5$	0.63	$>10^5$	0.28	2.1×10^3	0.55
7 Dec	$>10^5$	0.47	8.2×10^4	0.29	3.0×10^3	*

* No data.

son (20 February) to $>3,000$ min from 17 July onward (Table 2).

Turnover times were always shorter in Bahia de Puno than Lago Grande samples (Table 2). In water from both sites, ^{32}P uptake was fastest in the middle of the rainy season and slowed progressively through the dry season (April–November). In general, slowest orthophosphate turnovers corresponded with highest SRP concentrations (Table 2). In the final months of the year $^{32}\text{PO}_4^{3-}$ uptake was too slow to be measured in Lago Grande. Negligible or barely measurable uptake over a 2-h incubation was also recorded for Bahia de Puno at this time.

Alkaline phosphatase—Activities of this enzyme were measured on two dates during stratification of Lago Grande when SRP values were at or near their seasonal minimum and phosphorus limitation therefore was most likely. On both dates alkaline phosphatase activity was extremely low, both per liter of lake water and per unit of Chl *a* (Table 3). Highest levels were recorded in Bahia de Puno and Inner Harbor. However, when normalized against Chl *a*, these values all fall below 3 nmol of substrate converted per μg Chl *a* $\cdot\text{h}^{-1}$ (Table 3), which Healey and Hendzel (1979) recognized as a lowermost limit for slight phosphorus deficiency.

Luxury nutrient uptake—Seston levels of nitrogen and phosphorus were measured in samples from Lago Grande (station 4) on four dates, with and without 2 h of enrichment of $\text{NH}_4^+\text{-N}$ and $\text{PO}_4^{3-}\text{-P}$. N:P ratios of natural seston did not differ significantly from the Redfield ratio (Redfield et al. 1963) (overall mean for controls with no enrich-

ment was 14.96 g-atoms N : g-atoms P) but the response to addition of N+P varied greatly with time of year (Table 4).

During the midstratification period the seston accumulated nitrogen rapidly and phosphorus at much slower rates. These results clearly indicate luxury uptake and storage of nitrogen: such rates of accumulation could support theoretical growth rates of $6.22\cdot\text{d}^{-1}$ (V_N) or $1.76\cdot\text{d}^{-1}$ (V_P). Previously reported production and biomass estimates from Lake Titicaca suggest actual phytoplankton growth rates (estimated biomass-C turnover time) of $0.3\cdot\text{d}^{-1}$ (Richerson et al. 1977).

In the late stratification period N was similarly accumulated at faster rates than P ($V_N = 5.88$, $V_P = 1.60$) and the seston N:P quotient was slightly, but not significantly, higher in the enrichment than in the control ($t = 0.98$, $P = >0.1$). In the early stratification period there was a slight but not sig-

Table 3. Alkaline phosphatase activity in Lake Titicaca. Each value is the mean (± 2 SE) for triplicates expressed as nmoles of methylfluorescein produced from methylfluorescein phosphate.

	Alkaline phosphatase (nmol $\cdot\text{liter}^{-1}\cdot\text{h}^{-1}$)	Activity (nmol $\cdot\mu\text{g}^{-1}$ Chl <i>a</i> $\cdot\text{h}^{-1}$)
17 April		
Lago Grande	0.88 ± 0.28	0.29 ± 0.10
Bahia de Puno	4.65 ± 1.68	1.21 ± 0.54
Inner Harbor	13.31 ± 1.25	0.74 ± 0.07
29 October		
Lago Grande	0.36 ± 0.29	0.30 ± 0.25
Bahia de Puno	1.91 ± 0.74	1.87 ± 0.73
Inner Harbor	34.89 ± 2.67	2.11 ± 0.66

Table 4. Seston nitrogen and phosphorus levels in Lago Grande samples after a 2-h incubation with and without (control) NH_4^+ and PO_4^{3-} enrichment. Each value is the mean of duplicates in $\mu\text{g}\text{-atoms N or P}\cdot\text{liter}^{-1}$. *F*-test values are from orthogonal comparisons from ANOVA of the seston N or P data pooled for all four experiments.

	Control	Enriched	% Change	<i>F</i> -test
Midstratification (18 Feb 82)				
Seston N	3.33	5.59	68	45.9(<i>P</i> > 0.005)
Seston P	0.19	0.22	16	4.3(<i>P</i> > 0.05)
Seston N:P	17.53	25.41	—	—
Late stratification (11 Apr 82)				
Seston N	2.58	4.21	63	23.9(<i>P</i> < 0.005)
Seston P	0.21	0.24	12	3.4(<i>P</i> > 70.1)
Seston N:P	12.29	17.55	—	—
Late mixing (5 Aug 82)				
Seston N	3.68	3.25	-12	1.2(<i>P</i> > 0.25)
Seston P	0.24	0.24	0	0.8(<i>P</i> > 0.25)
Seston N:P	15.33	13.54	—	—
Early stratification (15 Oct 82)				
Seston N	1.91	2.37	24	2.0(<i>P</i> > 0.1)
Seston P	0.13	0.12	-7	1.2(<i>P</i> > 0.25)
Seston N:P	14.69	19.75	—	—

nificant accumulation of nitrogen ($V_N = 2.59$).

Discussion

Throughout 1982 dissolved phosphorus appeared to be in abundant supply for algal production in both Lago Grande and Bahia de Puno. SRP concentrations rarely fell below $0.10 \mu\text{g}\text{-atom}\cdot\text{liter}^{-1}$ in Bahia de Puno or 0.15 in Lago Grande. ^{32}P turnover times were always slow and decreased to negligible during the second half of the year when surface SRP rose quite high at both stations. Even in the eutrophic Inner Harbor that receives domestic waste from Puno, SRP values were always high ($>0.3 \mu\text{g}\text{-atom}\cdot\text{liter}^{-1}$) and ^{32}P turnover slow relative to biomass. Alkaline phosphatase activity was low relative to the threshold of deficiency suggested by Healey and Hendzel (1979), and negligible relative to activities reported in P-limited phytoplankton communities (Vincent 1981*b*). The seston N:P ratio in Lago Grande samples approximated the Redfield ratio and little or no phosphorus was accumulated during N and P enrichment. These observations argue against phosphorus as an important control on algal growth in Lake Titicaca.

A variety of evidence suggests the greater

importance of nitrogen than phosphorus in this lake. Ammonium-N was either low or undetectable in the surface waters at all sites. Nitrate concentrations were also generally low, except in Lago Grande during and immediately after winter circulation. Over the first month of mixing, DIN to SRP ratios fell from 5.9 (9 June) to an extreme low of 1.2 (8 July) as nitrogen was selectively accumulated by the phytoplankton (Fig. 4B). After restratification NO_3^- -N was brought down to concentrations below detection while SRP remained abundant. In Bahia de Puno, hypolimnetic NH_4^+ -N was rapidly lost during each mixing event, while surface SRP concentrations remained high (Fig. 3B).

Ammonium enhancement assays confirm a persistent cellular deficiency of nitrogen in the plankton of Bahia de Puno for all seasons and in that of Lago Grande at two times of year. Assays of Lago Grande phytoplankton during the first of these two sustained periods of positive ammonium enhancement demonstrated a much greater demand for N than for P. Similarly, just before the second period of enhancement (16 October), nitrogen, not phosphorus, was selectively accumulated from a P+N enrichment. For certain times of year at all three stations ammonium uptake capaci-

ties, as measured by [^{14}C]methylammonium, were high and indicative of nitrogen shortage over much of the lake.

These physiological observations from Lake Titicaca suggest a greater importance of nitrogen relative to phosphorus than is often assumed for nutrient-limited algal growth. They are consistent with results from a series of 4–6-day enrichment bioassays conducted during 1981–1982 which generally demonstrated a growth response to NH_4^+ , but not to PO_4^{3-} , SiO_2 , or micronutrients (W. Wurtsbaugh unpubl. data). However, these short term assay findings also show strong seasonality in the degree of nutrient limitation. Nitrogen deficiency was apparently greatest in Lago Grande during late stratification, when DIN was undetectable and ultraplanktonic abundance maximal. At this time of year, SRP was at an annual minimum (but $>0.1 \mu\text{g-atom}\cdot\text{liter}^{-1}$), ^{32}P turnover rates were fastest, and P was accumulated into the seston (albeit slowly) from N+P enrichments. Although uptake demand for phosphorus was highest during these final months of stratification, it was very low relative to the demand for nitrogen, as indicated by strong ammonium enhancement responses, highest V_{max} values for methylammonium uptake, and rapid, selective accumulation of N from an enrichment with N+P.

Winter mixing brought quantities of NO_3^- -N and SRP into the surface euphotic zone and throughout this period there was no evidence of cellular shortages of either N or P. NH_4^+ transport capacity dropped by an order of magnitude, NH_4^+ enhancement fell to zero, ^{32}P turnover times increased to $>10^5$ min, and neither N nor P was accumulated from an enrichment. Factors other than macronutrient supply must therefore limit algal production in Lago Grande during deep mixing.

Nutrient levels in the surface euphotic zone dropped slowly during early stratification, yet there was no concomitant increase in Chl *a*. Ammonium enhancement remained low or zero, and ^{32}P turnover continued to be slow. These observations imply that loss processes, rather than nutrient-limited production, were exerting a dominant control on community biomass at that

time of year. Unusually dense concentrations of the large-bodied *Daphnia pulex* found during this period (A. Pawley unpubl. data) suggest that zooplankton grazing might have been particularly important.

In early November surface NO_3^- dropped below detection and cellular N deficiency began to rise. This final period was characterized by an increased seston demand for N, raised methylammonium transport capacities, and strong ammonium enhancement. SRP concentrations remained high and ^{32}P turnover extremely slow. These data indicate a resumption of N-limiting conditions.

In Bahia de Puno there was less, but not negligible, seasonal variation in the extent of nitrogen deficiency. In the first half of the year there were two polymictic cycles. Water transparency was low and during both stratification events NH_4^+ accumulated in the absence of algal uptake below the euphotic zone (>20 m). In the first stratification period Chl *a* biomass was low and NH_4^+ enhancement at an annual minimum. When this stratified water column began to mix there was a sudden growth of phytoplankton, but NH_4^+ was rapidly exhausted and the plankton became increasingly nitrogen deficient. Ammonium enhancement rose to a maximum when the water column restratified, which thereby reduced the supply of deep-water and sediment NH_4^+ . The cellular N shortage was apparently alleviated during subsequent mixing in late May.

Extreme enhancement responses were recorded 8–20 July when there was a large increase in SRP, but not of NH_4^+ or NO_3^- , in the surface waters of Bahia de Puno. Although the origin of this SRP remains unclear, the dates correspond with deep water mixing in Lago Grande, which might suggest entry of P-rich water from Lago Grande into Bahia de Puno.

In mid- to late July both the DIN/SRP imbalance and cellular N shortage were extreme. Restratification then began and the availability of N was further reduced. High biomass levels could no longer be sustained in the surface euphotic zone and populations crashed to near the annual minimum by 20 August. This abrupt drop in surface Chl *a* cannot be explained simply by sedi-

mentation losses in the less turbulent stratified regime because the fraction of larger-celled phytoplankton did not diminish over this period, but increased significantly.

After mid-August, SRP was present in high concentrations but Chl *a* biomass remained low (ca. $1 \mu\text{g}\cdot\text{liter}^{-1}$). Cellular N deficiency recovered after the population collapse and then persisted at higher levels from 15 September onward. Over this period the water was stratified and relatively transparent (extinction coefficient $\leq 0.2\cdot\text{m}^{-1}$; Fig. 3C). A phytoplankton community developed in the hypolimnion under this improved light climate (Richerson et al. unpubl. data) and perhaps stripped out sediment-derived nutrients before they could be transferred to the upper euphotic waters of the bay. An increase in both hypolimnetic and surface nutrients between 9 and 25 November corresponded with a sharp decrease in cellular N shortage, as measured by NH_4^+ enhancement. Over the following 2 weeks these improved cellular nitrogen conditions were translated into growth and a pronounced increase in Chl *a* was recorded on 6 December.

Bahia de Puno therefore demonstrates a complex intraannual variation in nitrogen supply and cellular deficiency. Stratification and mixing events operate in different ways through the year, and nutrient supply to the near-surface phytoplankton community may be modulated in part by changes in light regime and the vertical extension of the euphotic zone toward the sediments.

These observations from Lake Titicaca support the utility of short-incubation physiological bioassays. The results are consistent with two other approaches toward understanding nutrient limitation in Lake Titicaca—in situ growth-enrichment assays, and longer term (20 day) semicontinuous culture enrichments (W. Wurtsbaugh and H. Carney unpubl. data). However, the last two types of growth assay necessarily suffer the effects of prolonged incubation in small-volume containers. In physiological assays of the type used here the incubation period is too short to force an artificial species succession or to induce large-scale shifts in the nutritional state of the plankton. Such assays, however, are subject to transient

physiological effects which may not be translated into growth (Vincent 1981a). These effects may obscure changes in cellular deficiency if, for example, there is a strong diel uncoupling between nutrient uptake and growth or if spatial differences in nutrient demand and deficiency are large. The confounding influence of these transient physiological responses was reduced to a minimum in the present study by using a wide array of assays at regular intervals and at three distinct locations in the lake.

The persistent shortage of nitrogen relative to phosphorus in Lake Titicaca finds parallels in several tropical studies. Lake George phytoplankton demonstrated a strong uptake demand for nitrogen, and enrichments with N alone resulted in elevated respiratory rates (Viner 1973). Growth of phytoplankton from lagoons of various salinities on the Ivory Coast responded strongly to enrichments with N while SRP and seston N:P ratios indicated an abundance of available phosphorus (DuFour and Slepoukha 1981). Peters and MacIntyre (1976) reported fast ^{32}P turnover times for three East African waters, but in two other lakes ^{32}P turnover was slow and SRP present in high concentration, suggesting a deficiency other than phosphorus. In a series of growth bioassays on a tropical reservoir in Brazil, nitrogen was identified as the primary limiting nutrient while phosphorus did not seem to play an important role (Henry and Tundisi 1982). Growth responses to nitrogen enrichment have also been observed in bioassays from Lake Jacaretinga in the Amazon (Zaret et al. 1981) and Lake Valencia, Venezuela (Lewis 1983).

Bioassays with *Selenastrum* suggested N limitation in Lake McIlwaine, a manmade lake in Zimbabwe (Robarts and Southall 1977). However, other data from the lake did not support this conclusion and the relative importance of N:P and light remains unclear (Viner et al. 1981). The same *Selenastrum* assay indicated N limitation in Little Connemara and Upper Umgusa impoundments, but in four other artificial waters the test alga responded to phosphorus enrichments (Robarts and Southall 1977).

In addition to these direct bioassay mea-

surements, many limnologists have argued from chemical data the greater importance of N than P for algal growth in many tropical waters. Talling (1966) reported that NO_3^- -N levels in the euphotic zone of Lake Victoria were undetectable ($<7 \mu\text{g-atoms}\cdot\text{liter}^{-1}$) through most of the year, and he thereby considered nitrogen most likely to limit algal production. Viner (1975) reviewed chemical observations from many tropical African waters and concluded that nitrogen is the most important limiting nutrient. In Lake Tanganyika, DIN was conspicuously low relative to SRP and therefore appeared to exert critical control on the annual production cycle (Coulter 1977). DIN:SRP ratios are also low in Lake Waiau, Hawaii (Massey 1981), and Lake Lanao, Phillipines (Lewis 1974), leading to speculation that nitrogen is limiting in both systems.

Melack et al. (1982) reported convincing bioassay evidence of P limitation in one African soda lake. They thereby questioned that tropical waters differ nutritionally from their temperate counterparts and suggested that, as at any other latitude, N, P, Si, or micronutrients are just as likely to be limiting for algal growth. However, the high frequency among tropical waters of low DIN:SRP conditions and of bioassay responses to N but not to P is striking. N-limited algal populations have also been recently reported as typical of certain midlatitude regions (Canfield 1983; White et al. 1982). Nitrogen limitation of freshwater algal growth may be geographically more widespread than observations from glaciated basins in the north temperate zone (Schindler 1978) would suggest.

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