

# Nutrient limitation in Lago Chaiquenes (Parque Nacional Alerce Andino, Chile): evidence from nutrient enrichment experiments and physiological assays

Limitación por nutrientes en el Lago Chaiquenes (Parque Nacional Alerce Andino): evidencia de experimentos de enriquecimiento y ensayos fisiológicos

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## ABSTRACT

Local human activity, in the form of aquaculture and watershed development, and the long-range transport of pollutants can potentially increase inputs of both nitrogen and phosphorus to lakes in southern Chile. We used enrichment experiments in enclosures and physiological assays of nutrient deficiency to examine whether N or P or both control the growth of phytoplankton in Lago Chaiquenes. Addition of both N and P was required to cause an increase in algal biomass in the enclosure experiments. Alkaline phosphatase activity and seston C:P and N:P ratios also indicated P deficiency. Nitrogen deficiency was not indicated by C:N or N:P ratios. Both N and P appear to be important factors limiting the growth of phytoplankton in this lake.

**Key words:** nutrient limitation, phytoplankton, nitrogen, phosphorus.

## RESUMEN

Actividades humanas locales, tales como acuicultura, desarrollo antrópico de las cuencas y el transporte de polución área pueden aumentar los ingresos de fósforo y nitrógeno a los lagos del Sur de Chile. Se llevaron a cabo experimentos de enriquecimiento nutritivo para determinar el efecto del N y de P sobre el crecimiento del fitoplancton en el lago Chaiquenes. En estos experimentos se observaron aumentos de la biomasa de algas en limnocorrales experimentales en respuesta a la adición tanto de N como P. La actividad de fosfatasa alcalina y las razones C:P y N:P del seston indicaron deficiencia de P habiendo menos evidencia para la limitación por N de acuerdo a las razones C:N y N:P. Sin embargo ambos elementos en conjunto parecen limitar el crecimiento del fitoplancton en este lago.

**Palabras clave:** limitación por nutrientes, fitoplancton, nitrógeno, fósforo.

## INTRODUCTION

Nutrient limitation of the growth and abundance of phytoplankton has long been an important focus of limnological studies. Phosphorus is generally thought to be the nutrient which limits potential net primary production in North Temperate Zone lakes. The most convincing evidence comes from whole-lake nutrient enrichment experiments such as those conducted at the Experimental Lakes Area in Ontario (Schindler & Fee

1974, Schindler 1977) and the success with which P loading or P concentrations have been used to predict phytoplankton standing crop (e.g. Vollenweider 1968, Dillon & Rigler 1974, Oglesby 1977, Schindler 1978). There is some evidence to suggest that P limitation may not be universal in North Temperate lakes. For example, Goldman and colleagues argue that historically, Lake Tahoe was nitrogen limited and only became P limited in the last twenty years (Goldman 1988, Goldman et al. 1993).

In a survey of published enrichment studies, Elser et al. (1990) found that N caused an increase in biomass or production as frequently as P, and even more positive responses were encountered when N and P were added in combination. Reviewing whole-lake experiments, the investigators pointed out that the majority of whole-lake nutrient enrichment experiments involved the addition of both N and P, and that the individual roles of N and P in controlling productivity may not have been distinguished adequately. Thus, N may play a more important role in controlling lake productivity than is sometimes acknowledged.

Less is known about nutrient limitation in tropical and South Temperate Zone lakes. Both P limitation (Melack et al. 1982) and

N limitation (Wurtsbaugh et al. 1985) have been observed in tropical lakes. Many New Zealand lakes are thought to be primarily N limited, particularly on the central volcanic plateau of the North Island (Burns 1991, White 1983). The evidence for N limitation in New Zealand lakes comes from a combination of experimental work and a general failure of P loading models derived for North Temperate lakes to predict accurately phytoplankton standing crop (White 1983, White et al. 1985).

Southern Chile is relatively sparsely populated and contains many pristine lakes, but the area is undergoing rapid development that may adversely affect water quality. Potential threats to water quality include shoreline development, deforestation,

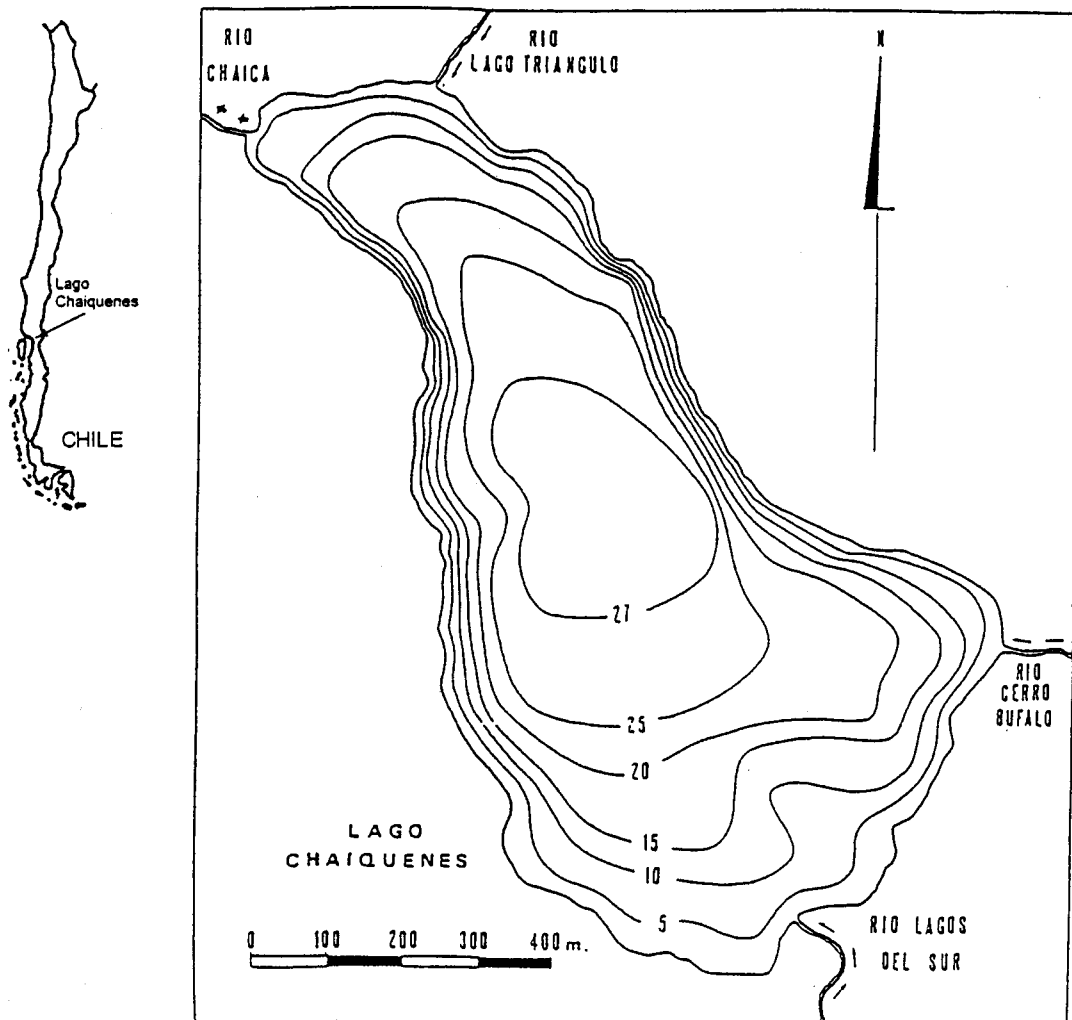


Fig. 1. Bathymetric map of Lago Chaiquenes with depth contours (m).

Mapa Batimétrico del Lago Chaiquenes. Contornos de profundidad (metros).

a rapidly growing salmon culture industry (Soto & Campos 1995, Soto & Stockner 1996), and predicted increases in atmospheric deposition of nitrogen compounds (Galloway et al. 1994). This makes an understanding of nutrient limitation of phytoplankton in this region imperative, but much previous work is based only on inferences drawn from nutrient concentrations, and the resulting conclusions are varied. Experimental evidence and nutrient concentrations suggest that N may play a key role in regulating phytoplankton standing crop in lakes in the Araucanian region (Soto & Campos 1995) and near Torres del Paine National Park (Soto et al. 1994), as well as in Argentinean Patagonia (Pedrozo et al. 1993). Phosphorus was generally a better predictor of algal biomass than N in a survey of Argentinean lakes (Quiros 1988). When biomass and total P data for several southern Chilean lakes were compared with similar data for North Temperate Zone lakes, the relationship between total P and biomass in Chilean lakes did not appear to be fundamentally different (Campos 1984, Hedin & Campos 1991), and P has been suggested as a factor which may limit the growth of phytoplankton in lakes in the Araucanian region (Campos 1984).

In this paper we describe nutrient enrichment experiments performed during the summer of 1995 in Lago Chaiquenes, Parque Nacional Alerce Andino, to test directly whether N or P limits the accumulation of algal biomass on short time scales, and compare the results of those experiments with physiological indicators of nutrient deficiency.

We employ two types of physiological indicators of nutrient deficiency: alkaline phosphatase activity (APA) and seston elemental ratios. Alkaline phosphatases are repressible enzymes produced by many species of phytoplankton and bacteria to utilize organically bound P, the synthesis of which is inhibited when phosphate is available (Perry 1972, Pettersson 1980). Investigators have shown APA to be related to the growth of algae in culture (Healey & Hendzel 1979), to decrease with phosphate enrichment of natural phytoplankton assemblages (Elser & Kimmel

1986), and to increase as N:P in nutrient supply to lakes increases (Healey & Hendzel 1980). These findings demonstrate the utility of APA as a measure of P deficiency in lake microorganisms. Similarly, algal C:N:P ratios have been employed in algal cultures (Healey & Hendzel 1979) and in natural phytoplankton assemblages (Healey & Hendzel 1980) as indicators of nutrient status. Where algal C:N:P has been compared directly to results from nutrient enrichment experiments, elemental ratios have accurately indicated phytoplankton nutrient status (Melack et al. 1982, Paasche & Erga 1988, Dodds & Priscu 1990).

## METHODS

### *Site description*

Lago Chaiquenes (41°35'S, 72°32'W, elev. 256 m; Fig. 1) is one of about 15 lakes within Parque Nacional Alerce Andino, located approximately 40 km southeast of Puerto Montt. The park is 392 km<sup>2</sup> in area and was established as a preserve for several stands of alerce (*Fitzroya cupressoides*) trees. The park receives approximately 4,000 mm of precipitation per year (W. Steffen, personal communication). The lake's watershed is completely forested, and is typical of southern Chilean temperate rainforests. The most abundant tree species is the southern beech, *Nothofagus dombeyi*. Other common taxa include *Drymis winteri*, *Eucryphia cordifolia*, *Saxegothea*, and *Podocarpus*. There are three permanent streams that flow into the lake, as well as several intermittent streams. The Río Chaica is the lake's outlet. A landslide occurred along the southern shore of the lake in 1987 (J. Cisternas, personal communication), but there is no other evidence of recent disturbance in or near the lake or its watershed. A hiking trail skirts the northern shore of the lake for about 200 m; there are no trails near two of the three inflowing streams. Camping is prohibited near the lake. It is highly unlikely that humans have had a substantial impact on the lake or watershed since the park was established in 1984.

### Chemical Analyses

In January of 1995, water samples from two of the inflowing streams, the lake outlet, and the lake surface were collected into acid-washed polyethylene bottles. Samples were kept on ice, returned to the laboratory at the University of Southern Chile in Puerto Montt, and immediately frozen until analysis at the Institute of Ecosystem Studies in Millbrook, NY. The chemical analyses were performed within 3 weeks of sample collection. Ammonium was determined by the phenolphthorite method (Solorzano 1969); nitrate was analyzed by ion chromatography, and soluble reactive phosphorus (SRP) was analyzed by the molybdenum blue method (Strickland & Parsons 1968). Minimum detection limits were 0.02 mg L<sup>-1</sup> for ammonium, 0.03 mg L<sup>-1</sup> for nitrate, and 0.002 mg L<sup>-1</sup> for SRP. We should note that there is some disagreement regarding the use of plastic bottles for the storage of samples which are to be analyzed for SRP. In some published methods the use of plastic bottles is considered inadvisable (e.g. American Public Health Association 1995), while in others plastic bottles are considered suitable for this purpose (Wagner 1998). Our own tests comparing HDPE and glass showed no significant loss of SRP from samples stored in HDPE bottles (Steinhart & Hedin, unpublished data). Samples for determination of the C, N, and P content of seston were collected by filtering 400-500 mL of surface water through precombusted Whatman GF/F filters. The filters were dried and stored in a desiccator. Duplicate samples were analyzed for C and N on a Carlo-Erba CHN elemental analyzer. Duplicate samples were analyzed

for C and P content by acid persulfate digestion and subsequent analyses of the headspace gas for CO<sub>2</sub> by gas chromatography and of the solution for phosphate.

Samples (400-500 mL) for chlorophyll analysis were filtered through Whatman GF/F filters (manufacturer's specified pore size of 0.7 µm) and frozen. Chlorophyll a was measured on a Turner 10AU fluorometer following extraction in methanol. Fluorescence was measured before and after acidification to correct for the presence of phaeopigments (Axler and Owen 1994).

### Alkaline Phosphatase Activity

The alkaline phosphatase (APA) assay is based on that described by Pettersson (1980). Surface water was collected into acid-washed polyethylene bottles and six 4-mL subsamples were pipetted into borosilicate glass tubes. Three tubes were sterilized by placing them in a boiling water bath for ten minutes and served as killed controls. When the controls were cool, 400 µL of 165-µM MUP (4-methylumbelliferyl-phosphate) in sterile 0.1-M tris buffer was added to each tube. Fluorescence was read at 10-15 minute intervals for up to one hour on a Turner 10AU fluorometer equipped with a near UV (310-390-nm) lamp, 300-400-nm excitation filter, >410-nm and 390-500-nm emission filters, and a red-sensitive photomultiplier tube. Aliquots of 4-methylumbelliferone (MUF, 214-nM prepared in 0.1-M Tris buffer) were used to calibrate the fluorometer. The increase in fluorescence over time was always linear, and fluorescence in the controls seldom increased appreciably over the course of

Table 1

Water chemistry for L. Chaiquenes and streams (samples collected January 1995)

Composición química del agua del Lago Chaiquenes y arroyos afluentes (muestras colectadas en enero de 1995)

Location	NH4 (mg L <sup>-1</sup> )	NO3 (mg L <sup>-1</sup> )	PO4 (mg L <sup>-1</sup> )
L. Chaiquenes	<0.02	<0.03	<0.002
Rio L. Triangulo	<0.02	0.07	<0.002
Rio Cerro Bufalo	<0.02	0.03	<0.002
Rio Chaica	<0.02	0.03	<0.002

the incubations. The minimum detection limit for absolute APA was  $7 \text{ nmol L}^{-1} \text{ h}^{-1}$ . Alkaline phosphatase activity was normalized to algal biomass by dividing by the concentration of chlorophyll a.

### Enrichment Experiments

Three nutrient addition experiments were performed in Lago Chaiquenes over the

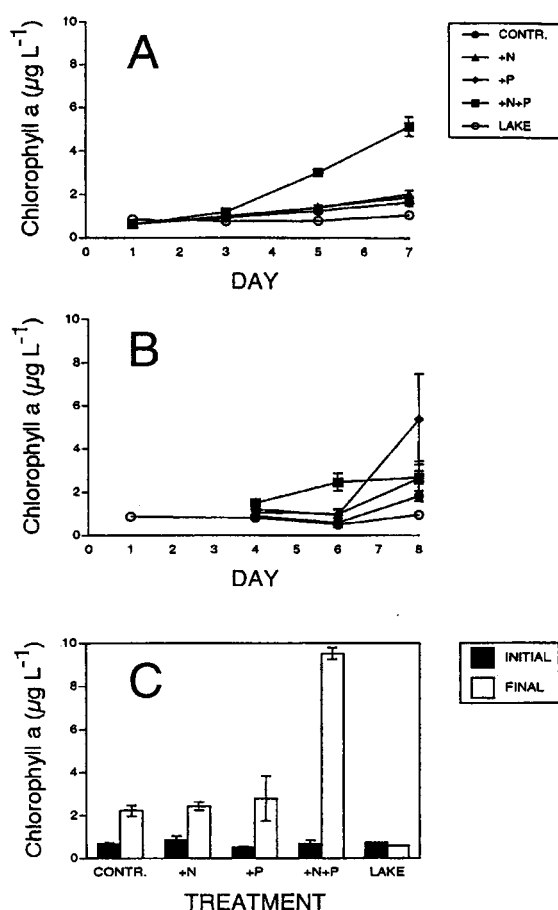


Fig. 2. Change in chlorophyll a in enclosures in nutrient enrichment experiments in L. Chaiquenes (treatment means  $\pm$  standard error). Lake values shown for comparison. Chl a was measured only on the first and final days of Experiment III. Contr. = control. (A) Experiment I. (B) Experiment II. (C) Experiment III.

Cambios en la concentración de clorofila a en los experimentos de enriquecimiento de nutrientes en L. Chaiquenes. (Promedio por tratamiento  $\pm$  error estándar). Para comparar, también se muestran los valores medidos en el lago. La clorofila se midió solo el primer y último día del experimento III. Contr. = control. (A) Experimento I. (B) Experimento II. (C) Experimento III.

course of the summer (January - April 1995). We chose to repeat the experiment because nutrient availability may vary with changes in supply or recycling, and we wished our results to be more broadly applicable than those obtained in a single experiment. Treatments consisted of controls, N addition, P addition, and combined N+P addition, with four replicates of each treatment. The enclosures were filled with 80 L of unfiltered surface water and nutrients added to approximate final concentrations of  $30 \mu\text{g P L}^{-1}$  and  $210 \mu\text{g N L}^{-1}$  (N was added as  $\text{NH}_4\text{Cl}$ , P as  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ ). Nutrients were added approximately every other day in experiments I and II, and only on the first day in experiment III. The experiments lasted 7-12 days; experiments were not run longer because chlorophyll concentration in the control enclosures began to increase relative to the lake within 3-7 days. In the first two experiments, chlorophyll was measured approximately every other day; in the final experiment, chlorophyll was measured only on the first and last days. Results for each experiment were analyzed by repeated measures ANOVA with contrasts to identify significant responses relative to the control treatment. APA was measured on the fourth day of the second experiment and on the final day of the third experiment, and seston C:N:P was determined on the final day of the third experiment. Seston and APA results were analyzed by one-way ANOVA.

## RESULTS

### Stream and Lake Chemistry

Lago Chaiquenes and its inflowing streams are very dilute (conductivity  $<20 \mu\text{S cm}^{-1}$ ) and nutrient-poor. Ammonium, nitrate, and SRP concentrations were usually below the limit of detection (Table 1).

### Enrichment Experiments

In general, both N and P were required to cause a significant increase in biomass in the enrichment experiments in Lago

Chaiquenes (Fig. 2), suggesting consistent colimitation of algal biomass over the course of the study period. In the first experiment, a significant response relative to the control treatment was observed only for the combined N and P treatment ( $P < 0.001$ , Fig. 2A). In the second experiment (Fig. 2B), chlorophyll a in the +P treatment was significantly higher than in the control ( $P = 0.022$ ), and marginally higher in the combined N and P treatment ( $P = 0.069$ ). There was substantial within treatment variability in the second experiment, with noticeable chlorophyll a increases in one +N enclosure and two of the +P enclosures. However, the most consis-

tent response was observed in the combined N and P treatment, with higher chlorophyll a concentrations in those enclosures on the fourth and sixth days, but not the eighth. In the third experiment (Fig. 2C), chlorophyll a was only measured on the initial and final days. Chlorophyll a increased in all treatments but only in the combined N and P treatment was it significantly higher than the control ( $P < 0.001$ ). Chlorophyll concentrations in the control enclosures generally increased relative to the lake over the course of all three experiments. Containment of phytoplankton near the lake surface likely altered light and temperature regimes in the experimental enclosures as compared to the epilimnion of the lake, and may have been the cause of the observed increase in algal biomass.

Results from physiological assays done on the enclosures are consistent with P deficiency, but these results do not exclude the possibility of colimitation by N and P. In the second enclosure experiment (Fig. 3A), APA in the treatments which received P alone or in combination with N was significantly lower than in the controls ( $P < 0.001$ ). APA in the lake also appeared to be slightly lower than in the controls, but was still considerably higher than the treatments which received P additions. The results from the third enclosure experiment are less clear (Fig. 3B); APA in the combined N and P treatment is significantly lower than in the controls ( $P = 0.009$ ), but P alone did not have the same effect ( $P = 0.23$ ). APA in the lake was about twice that in the control enclosures. Addition of phosphorus caused seston C:P and N:P ratios to decline significantly (Fig. 4A and 4C,  $P < 0.001$ ). Although the addition of nitrogen was required to cause an increase in biomass, N addition had little effect on C:N ratios (Fig. 4B).

Alkaline phosphatase activity values for Lago Chaiquenes were among the highest observed in any Chilean lakes where it has been measured (Steinhart 1996), and indicated severe P deficiency (Table 2). Seston C:P and N:P ratios ranged from 295-398 and 44-69, respectively, over the course of the summer, which also indicated P deficiency. Ratios of C:N ranged from 5.1-7.7

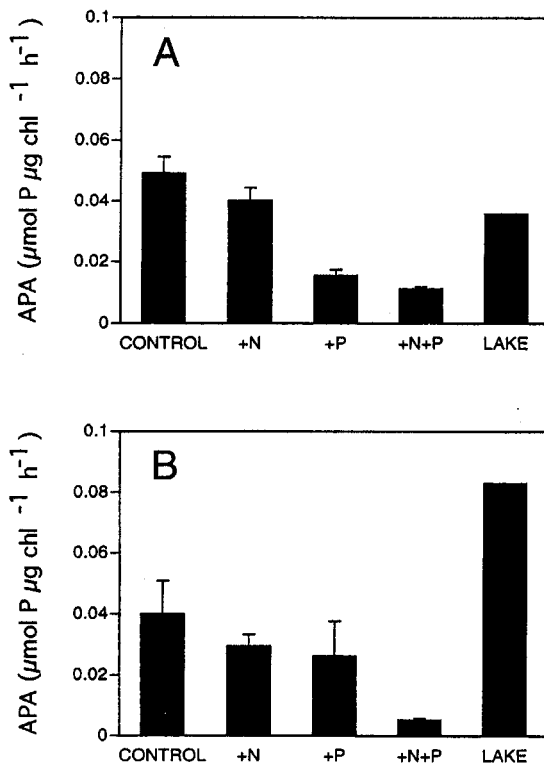


Fig 3. APA results in Lago Chaiquenes nutrient enrichment experiments (treatment means + standard error). Lake value shown for comparison. (A) APA measured on the fourth day of experiment II and (B) on the last day of experiment III.

Resultado del análisis APA en los experimentos de enriquecimiento de nutrientes en el Lago Chaiquenes (promedio por tratamiento +/- error estandard). También se indican los valores en el lago para la comparación. (A) valores APA medidos en el cuarto día del experimento II y (B) en el último día del experimento III.

and were not indicative of N deficiency (Table 2).

#### DISCUSSION

The nutrient enrichment experiments clearly demonstrate that both N and P were required to cause an increase in algal biomass in Lago Chaquenes, at least on the time

scale of the experiments (Fig. 2). Results from the APA assays and seston C:N:P data also suggest P deficiency, but seston data did not indicate N deficiency. The results from the experiments and the physiological assays were consistent over the course of the study period, suggesting a persistent limitation by both N and P in this lake during the summer. Lago Chaquenes is similar to other lakes in the region in that phytoplankton exhibit symptoms of P deficiency, although it appears to be more severe than in other lakes (Steinhart 1996). In other studies, it has been suggested that N may be the most important factor limiting primary production in many Chilean lakes (Soto et al. 1994, Soto & Campos 1995, Soto & Stockner 1996). While different methods of assessing nutrient limitation were used in those studies, our finding of colimitation of algal biomass by both N and P is not inconsistent with previous results indicating N limitation, although the results we report here suggest that phosphorus should not be discounted as a factor limiting the growth of phytoplankton in southern Chilean lakes.

While there was little change in the physiological nutrient status of lake phytoplankton or in results from nutrient enrichment experiments during the three-month period of study, it would be interesting to extend this type of study over the course of an entire year, as nutrient supply to the lake almost certainly varies seasonally with different hydrologic inputs of nutrients. It has been shown that high stream flow events are extremely important in regulating fluxes both of dissolved and particulate substances, especially the latter (e.g. Bukaveckas et al. 1993, Bormann et al. 1969). Our study was done during summer, the driest part of the year (Muller 1982), when hydrologic inputs were likely to be low relative to other times of the year.

Algal picoplankton (0.2-2 $\mu$ m) can be an important constituent of phytoplankton communities both in terms of biomass and primary production (Stockner 1988). Our results probably do not reflect their nutrient status or response to fertilization, and we cannot predict what consequences this would have for our conclusions.

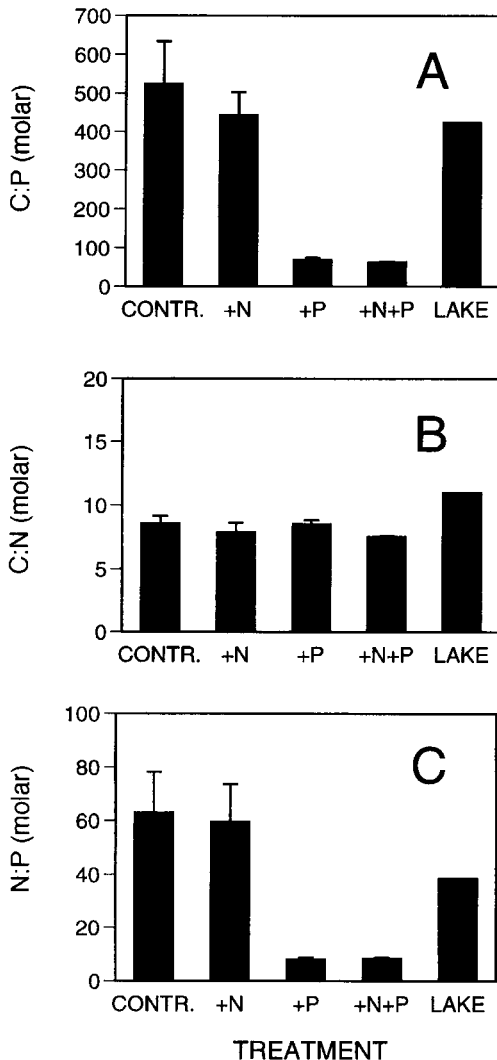


Fig. 4. Seston elemental ratios (molar) on the final day of nutrient enrichment experiment III (treatment means + standard error). Lake value shown for comparison. Contr. = Control. (A) seston C:P, (B) seston C:N, (C) seston N:P.

Razones elementales (molares) en el seston medidas el último día del experimento III (Promedios por tratamiento + error estandar). También se indican los valores medidos en el lago para la comparación. Contr.+ Control. (A) seston C:P, (B) seston C:N, (C) seston N:P.

No effort was made in this study to examine how in-lake processes affecting nutrient cycling may affect nutrient limitation. For example, various investigators have argued that when N does limit primary production, it should not do so for long as nitrogen-fixing cyanobacteria may contribute enough N to the ecosystem to eventually reverse N limitation (Schindler 1977, Howarth et al. 1988). This result has been observed in experimentally fertilized lakes (Flett et al. 1980) and enclosures (Levine & Schindler 1992). It is possible that P fertilization could induce N limitation and consequently stimulate N fixation, but our experiments were probably too short to allow such a response to occur. Though we lack direct evidence of the importance of nitrogen fixation in this lake, it is unlikely to be an important process in this lake for two reasons. First, nitrogen-fixing cyanobacteria are uncommon in other lakes in the region (Campos 1984, Soto & Campos 1995). Second, N fixation has been shown to be positively correlated with total P concentration (Smith 1990), and it is probable that planktonic nitrogen fixation is inconsequential in such a phosphorus-poor lake as Lago Chaquenes.

Zooplankton community composition may affect nutrient recycling and availability (e.g. Elser et al. 1988, Lehman 1984). Copepod dominance may exacerbate N-limitation as copepod fecal pellets tend to be rich in nitrogen and sink rapidly, exporting N from the photic zone (Knauer et al. 1979). Zooplankton communities in the

lakes of southern Chile tend to be dominated by calanoid copepods (Campos 1984, Soto & Campos 1995, Soto & Zuñiga 1991), so this may be an important process in Lago Chaquenes. Investigators have argued that zooplankton may excrete soluble nutrients that are relatively enriched in P (e.g. Lehman 1984) or in N, in the case of cladocerans (Elser et al. 1988, Sterner et al. 1994). Either case could tend to exacerbate N or P limitation when zooplankton are increasing in biomass. Rotifers can also affect nutrient cycling in lakes by cycling P more rapidly than copepods or cladocerans (Makarewicz & Likens 1979), although their importance in Lago Chaquenes is unknown.

The results of this study demonstrate that both N and P are important in regulating the growth of phytoplankton in Lago Chaquenes. The possible effects of sustained increases in nutrient inputs, allowing for changes in community composition, remain unknown and are an important concern as the region undergoes further human development.

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Table 2

Chlorophyll a, specific alkaline phosphatase activity ( $\mu\text{mol } \mu\text{g chl}^{-1}\text{h}^{-1}$ ) and seston C:N:P ratios in Lago Chaquenes

Clorofila a, actividad específica de fosfatasa alcalina ( $\mu\text{mol } \mu\text{g chl}^{-1}\text{h}^{-1}$ ) y razones de C:N:P en el seston del Lago Chaquenes

Date	Chl a ( $\mu\text{g L}^{-1}$ )	Specific APA (molar)	C:N (molar)	C:P (molar)	N:P
5 February 1995	0.4	0.084	5.1	317	61.8
20 February 1995	1.0	0.031	5.7	398	69.2
4 March 1995	0.9	0.030	6.5	295	45.2
21 March 1995	0.9	—	7.7	367	47.6
6 April 1995	1.3	—	7.2	316	43.7

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