



Physiological indicators of nutrient deficiency in phytoplankton in southern Chilean lakes

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Abstract

We assessed the nutrient status of phytoplankton in 28 lakes in southern Chile using two types of physiological indicators: specific alkaline phosphatase activity, and the elemental composition (carbon, nitrogen, and phosphorus) of seston. Alkaline phosphatase activity ranged from 0.001 to 0.11 $\mu\text{mol P } \mu\text{g chl}^{-1} \text{ h}^{-1}$, with P-deficiency indicated in about one-half the study lakes. C:N ranged from 3.9 to 24, C:P ranged from 86 to 919, and N:P ranged from 8.7 to 99. C:P and N:P ratios greater than the Redfield ratio were common, suggesting P deficiency in many of the lakes. C:N ratios were not generally indicative of N deficiency. Previous studies have suggested N may be the primary limiting nutrient in southern Chilean lakes, but our results indicate that P should not be discounted as a limiting nutrient.

Introduction

Southern Chile is undergoing a period of rapid human development; a growing salmon aquaculture industry, extensive logging, and other land-use changes are currently affecting many lakes in the region and have the potential to impact many more (Soto & Campos, 1995; Soto & Stockner, 1996). In addition, atmospheric deposition of oxidized nitrogen compounds to this part of the world, while currently very low, is predicted to increase in the next few decades (Galloway et al., 1994) and represents another potential impact on aquatic ecosystems in the region. The prospect of increased nutrient supply to lakes of this region underscores the importance of understanding their current nutrient status and its effect on aquatic productivity.

Phosphorus is generally thought to be the primary nutrient controlling the growth and standing crop of phytoplankton in many North Temperate lakes (e.g.,

Vollenweider, 1968; Dillon & Rigler, 1974; Schindler 1978; Smith, 1990), although there are exceptions (Goldman, 1988; Elser et al., 1990). A growing body of literature suggests that this pattern is not as widespread in the southern temperate and tropical zone lakes, with N, P, and micronutrient limitation observed in tropical lakes (Melack et al., 1982; Wurtsbaugh et al., 1985), and N limitation observed in many New Zealand lakes (White, 1983; White et al., 1985; Burns, 1991).

Previous investigations of nutrient limitation in Chilean lakes have been directed at inferring nutrient limitation from ratios of inorganic N and P concentrations in lake water (Soto et al., 1994; Soto & Stockner, 1996). Relationships between nutrient concentrations and chlorophyll *a* have also been examined, but interpretations of the available data differ. Soto et al. (1994) concluded that nitrogen may be limiting in many Chilean lakes, while Hedin & Campos (1991) found

that phosphorus–chlorophyll models developed for North Temperate Zone lakes appeared to apply fairly well to published data from several Chilean lakes and an Argentinian study. Baigún & Marinone (1995) used several published models to predict chlorophyll *a* in lakes in Argentine Patagonia and found that the models overestimated chl *a* in nearly all cases. In nutrient addition experiments, Steinhart et al. (1999) found evidence that both N and P are important in controlling biomass accumulation in a lake in Parque Nacional Alerce Andino, Chile. In this paper, we examine patterns of nutrient deficiency in southern Chilean lakes by using two physiological indicators of nutrient deficiency: alkaline phosphatase activity (APA) and seston C:N:P ratios. These assays provide information on the physiological status of phytoplankton, from which nutrient deficiency in the phytoplankton species present can be inferred.

Study sites

The 28 lakes included in this study span a latitudinal range from approximately 39° 05' S to 45° 50' S (Fig. 1) and range in size from about 0.05 to 870 km². Nearly all of the lakes in the region are of glacial origin (Soto & Stockner, 1996). Several of the lakes are in national parks or reserves, while others are in areas with considerable human influence (Table 1). Examples of human impacts on the lakes or their watersheds include salmon aquaculture, watershed deforestation and burning, agricultural activity, air pollution, and human settlement (Soto & Campos, 1995). In addition to human-caused disturbances, natural disturbances such as landslides, volcanism, and fire also occur (Alaback, 1991).

The climate is generally cool marine, with annual average precipitation ranging from ca. 1750 mm per year in the northern and southern areas (Muller, 1982; Organización de los Estados Americanos, 1969), to as high as 4000 mm per year in the area around Parque Nacional Alerce Andino and Lago Yelcho (W. Steffen, pers. comm.). Most precipitation occurs in the winter months, falling as snow at elevations greater than about 800 m. Some lakes in the region have active glaciers in their watersheds. These lakes were not included in the study, with the exception of Lago Yelcho, where glaciers occupy only a very small portion of the watershed.

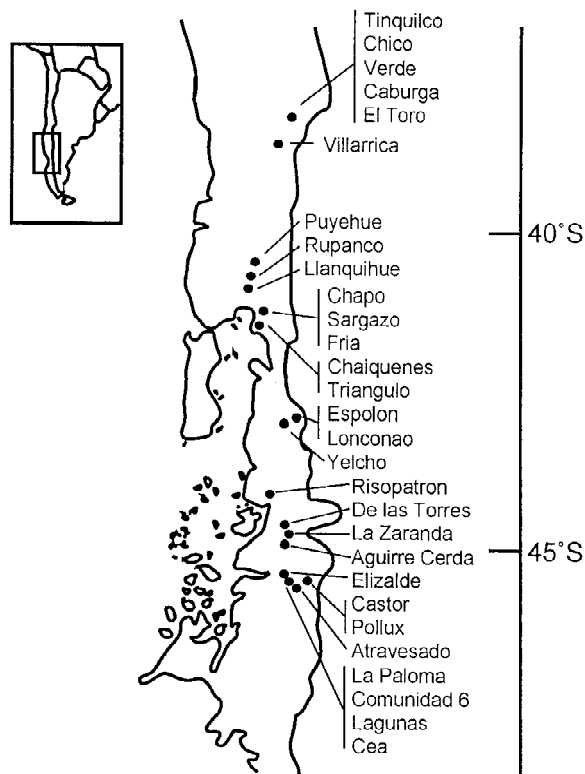


Figure 1. Map of study sites.

Methods

Sampling

Each lake was sampled once in February–April of 1995, with the exception of Lago Chaiquenes, which was sampled on three occasions. Lakes were sampled from an inflatable raft whenever possible, at least 100 m from the shore. When wind and waves prevented use of the raft, samples were taken from shore, and every effort was made to avoid areas with extensive littoral vegetation. Water for physiological assays was collected from just below the lake surface into clean polyethylene containers. Water samples for chemical analyses were collected into acid-washed polyethylene bottles and acidified with 6 N H₂SO₄ to a final pH of 2.

Analytical methods

Samples for chlorophyll *a* were collected by filtering lake water through 25-mm diameter Whatman GF/F filters, kept on ice until they could be frozen, and stored frozen for a maximum of 2 weeks before analysis. Samples were extracted for 20–24 h in

Table 1. Physiological indicators of nutrient deficiency in Chilean lakes: chlorophyll *a* ($\mu\text{g l}^{-1}$), specific alkaline phosphatase activity ($\mu\text{mol MUP } \mu\text{g chl } a^{-1} \text{ h}^{-1}$) and elemental ratios of seston. All ratios are molar. 'nd' indicates not determined. Neighboring National Parks and Reserves are also indicated. All lakes were sampled once, with the exception of Lago Chaquenes (*), which was sampled three times in February and March of 1995 (mean values reported)

Lake	Date Sampled (M/D/Y)	Adjoining park or reserve	Chl <i>a</i>	Specific APA	C:N	C:P	N:P
Aguirre Cerda	3/30/1995	—	1.2	0.030	4.2	149	42.8
Atravesado	4/1/1995	—	1.3	0.033	4.3	208	48.6
Caburga	2/26/1995	—	0.8	0.007	6.2	178	27.2
Castor	3/31/1995	—	0.5	0.020	23.5	431	4.6
Cea	4/1/1995	—	1.4	0.018	6.0	103	20.5
Chaquenes	1995*	Alerce Andino	0.8	0.048	6.5	366	53.5
Chapo	3/1/1995	Alerce Andino	0.4	0.041	7.3	159	29.4
Chico	2/25/1995	Huerquehue	1.2	0.007	5.0	164	36.0
Com. del las 6 Lagunas	4/1/1995	—	1.7	0.015	5.1	110	25.1
De las Torres	3/29/1995	Queulat	0.8	0.039	3.9	206	37.9
El Toro	2/25/1995	Huerquehue	0.7	0.007	11.5	121	17.7
Elizalde	3/31/1995	—	1.0	0.074	6.2	89	17.7
Espolón	3/28/1995	—	0.9	0.024	6.0	295	31.9
Fria	2/4/1995	Alerce Andino	1.3	0.034	9.3	919	49.6
La Paloma	4/1/1995	—	0.5	0.024	11.7	102	13.7
La Zaranda	3/30/1995	—	2.0	0.017	5.6	208	78.2
Llanquihue	3/3/1995	—	1.1	0.001	5.6	86	21.1
Lonconao	3/28/1995	—	0.5	0.081	11.0	358	31.8
Pollux	3/31/1995	—	1.7	0.018	7.8	130	16.5
Puyehue	3/2/1995	Puyehue	2.4	0.011	6.5	164	26.9
Risopatrón	3/29/1995	Queulat	1.2	0.022	7.7	216	30.5
Rupanco	3/3/1995	—	1.0	0.020	7.1	164	24.0
Sargazo	2/4/1995	Alerce Andino	0.5	0.111	5.2	389	81.0
Tinquilco	2/24/1995	Huerquehue	0.9	0.044	7.2	86	32.1
Triángulo	2/14/1995	Alerce Andino	0.8	0.029	10.8	302	31.6
Verde	2/25/1995	Huerquehue	0.9	0.013	nd	112	nd
Villarrica	2/26/1995	—	0.6	0.015	9.9	130	17.3
Yelcho	3/28/1995	—	0.9	0.010	5.6	95	20.8

basic methanol, and analyzed fluorometrically before and after acidification to correct for phaeopigments (Axler & Owen, 1994). Phaeopigment concentrations were low (nearly always $<0.1 \mu\text{g l}^{-1}$). Total phosphorus was determined by persulfate digestion and subsequent analysis for phosphate (Menzel & Corwin, 1965); total nitrogen was determined by basic persulfate digestion and analysis for nitrate (Solorzano & Sharp 1980).

Samples for determination of the carbon, nitrogen, and phosphorus content of seston were collected by filtering surface water through precombusted What-

man GF/F filters. The filters were dried overnight at 40°C and stored in a desiccator. Duplicate samples were analyzed for carbon and nitrogen on a Carlo-Erba CHN elemental analyzer. Phosphorus content was determined by acid persulfate digestion (Menzel & Corwin, 1965) and subsequent analysis of the solution for phosphate by the molybdenum-blue method of Strickland & Parsons (1968) on an Alpkem autoanalyzer. Molar elemental ratios of C:N:P were compared with the Redfield ratio of 106:16:1 to determine whether phytoplankton were nutrient deficient (Redfield, 1958; Hecky & Kilham, 1988).

The alkaline phosphatase activity (APA) assay is based on that described by Pettersson (1980). Six 4-ml subsamples of surface water were pipetted into borosilicate glass tubes. Half of the tubes were designated as controls and sterilized in a boiling water bath for at least 10 min. Four hundred μl of 165 μM MUP (4-methylumbelliferyl-phosphate, Sigma Chemical Co.) in sterile 0.1 M Tris buffer were added to each tube. Fluorescence was read at 10–15-min intervals for 1 h on a Turner 10AU fluorometer equipped with a near UV (310–390 nm) lamp, 300–400-nm excitation filter, >410- and 390–500-nm emission filters, and a red-sensitive photomultiplier tube. 4-Methylumbelliferone (MUF, 214 nM prepared in 0.1 M Tris buffer) was used to calibrate the fluorometer. The average change in fluorescence was used to calculate absolute APA, which then was normalized to algal biomass by dividing by the concentration of chlorophyll *a*.

Based on results from nutrient addition experiments in Lago Chaquenes (Steinhart, et al., 1999) and in Mirror Lake, New Hampshire (Steinhart, 1996), APA of less than 0.02 $\mu\text{mol P } \mu\text{g chl } a^{-1} \text{ h}^{-1}$ appears to indicate a lack of P deficiency. In those experiments, P was always required to cause an increase in algal biomass, indicating P limitation. The APA values in treatments that were not P-limited (P-fertilized treatments) were nearly always <0.015 $\mu\text{mol } \mu\text{g}^{-1} \text{ h}^{-1}$, while APA in the lakes and P-limited treatments (controls and N-fertilized treatments) was always at least 0.030 $\mu\text{mol } \mu\text{g}^{-1} \text{ h}^{-1}$. These values are higher than those suggested as indicator values by Healey & Hendzel (1979), but the pattern of low values in P-sufficient treatments and high values in P-deficient treatments, with a clear break in between, is consistent. They observed rapid increases in APA to near maximum rates with the induction of P-deficiency, and attributed a lack of intermediate values to the rapid response of phytoplankton to the onset of P-deficiency.

Human impacts within the lakes and their watersheds vary considerably from lake to lake. To compare relatively undisturbed lakes to ones which might be experiencing human impacts, lakes were classified in two groups: those located at least partially within a national park or reserve, and those that were entirely outside of parks or reserves (Table 1). All nutrient deficiency indicators were then compared for the two categories using a one-tailed *t*-test.

Results

Chlorophyll a and nutrient concentrations

Chlorophyll *a* and nutrient concentrations were almost universally low in the study lakes. Chlorophyll *a* ranged from 0.4 to 2.4 $\mu\text{g l}^{-1}$. Total phosphorus was below detection (detection limit of 10 $\mu\text{g P l}^{-1}$) in all except five lakes. Total nitrogen was below detection (detection limit of 70 $\mu\text{g N l}^{-1}$) in about half of the lakes. Because the majority of the nutrient measurements were below detection limits, they are of limited utility in exploring relationships between nutrients and chlorophyll and we do not report these data here.

Physiological indicators of nutrient status

Seston elemental ratios ranged from 3.9 to 24 for C:N, 86 to 919 for C:P, and 8.7 to 99 for N:P (Table 1). Ratios of C:P and N:P above the Redfield ratio are quite common, suggesting the occurrence of P deficiency in phytoplankton in many of the lakes surveyed (Fig. 2a,b). Ratios of C:N in nearly half the lakes sampled were greater than the Redfield ratio, but the departure from the Redfield ratio probably is not substantial enough to indicate N deficiency (Fig. 2c).

Specific alkaline phosphatase activity ranged from 0.001 $\mu\text{mol P } \mu\text{g chl}^{-1} \text{ h}^{-1}$ in Lago Llanquihue to 0.11 $\mu\text{mol P } \mu\text{g chl}^{-1} \text{ h}^{-1}$ in Lago Sargazo (Table 1). Our APA results indicate P deficiency in phytoplankton in about one-half the lakes surveyed. The correlation between APA and C:P was not significant (Fig. 3a, $p=0.10$) while that between APA and N:P was (Fig. 3b, $p < 0.01$). One lake, Lago Fría, had unusually high C:P and N:P ratios. When that lake is excluded from the analysis, both relationships are significant ($p=0.01$ for C:P versus APA; $p < 0.01$ for N:P versus APA). Non-algal material in the seston samples may explain some of the variance in the relationship between APA and elemental ratios.

Disturbance and variation in seston C:N:P and APA

Lakes wholly or partially located within National Parks or reserves tend to have higher APA, although the difference was not statistically significant ($p=0.18$). The C:P and N:P ratios also tend to be higher in lakes within parks although the difference was significant only for N:P ($p=0.09$ for C:P; $p=0.05$ for N:P). There was no significant difference in C:N ratios for the two categories (Table 2).

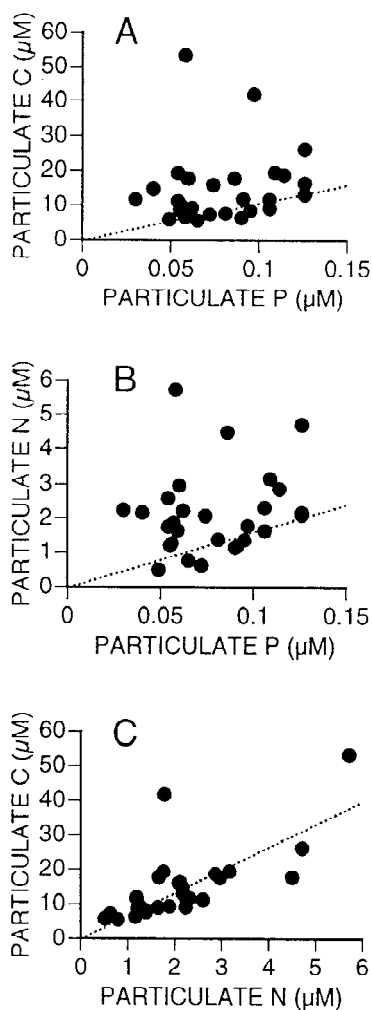


Figure 2. Seston composition for the study sites. Dashed line indicates the Redfield ratio. (A) Seston C and P; (B) Seston N and P; (C) Seston C and N.

Discussion

Our results indicate that phosphorus deficiency in the phytoplankton of southern Chilean lakes was common, though not universal, as evidenced by APA measurements and seston C:P and N:P ratios. Other studies have suggested that nitrogen is the most important limiting nutrient (Soto et al., 1994; Soto & Stockner, 1996), but our results suggest that the importance of phosphorus should not be discounted without further investigation. Nutrient enrichment experiments in Lago Chaquenes indicated that phosphorus and nitrogen co-limit biomass accumulation in that lake (Steinhart et al., 1999), and it is possible that N and P co-limit biomass accumulation in many other

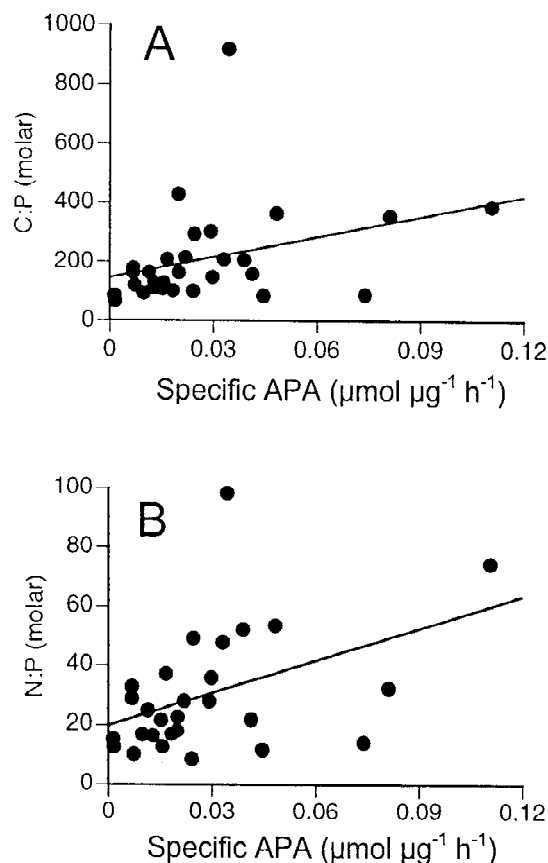


Figure 3. Relationship of seston composition to specific APA. Solid line indicates best fit to data. (A) Seston C:P and specific APA; (B) Seston N:P and specific APA.

Chilean lakes as well. It should be noted, however, that seasonal variation in nutrient limitation is common (Reynolds, 1984), and our results can only be

Table 2. Indicators of physiological nutrient status for 12 lakes entirely or partially included within national parks or reserves ('park lakes') and 16 lakes located outside of parks or reserves ('non-park lakes'). Differences between the two groups were tested using a one-tailed *t*-test

	Specific APA ($\mu\text{mol P}$ $\mu\text{g chl}^{-1}$ h^{-1})	C:P (mol)	N:P (mol)	C:N (mol)
Mean (park lakes)	0.034	267	39	7.4
Mean (non-park lakes)	0.025	177	28	7.8
<i>P</i> value	0.18	0.09	0.05	0.37

considered indicative of nutrient status in late austral summer to early fall.

Agreement between the methods of determining P status (seston ratios and APA) is reasonably good (Fig. 3). In addition to the possibility of a non-algal component influencing the seston analysis, there are other potential difficulties with the APA assay. Not all species of algae produce the enzyme (Healey & Hendzel, 1979), and some bacteria produce the enzyme (Currie et al., 1986). Differences between species of algae or between algae and bacteria in the ability to synthesize alkaline phosphatases might make it difficult to use the assay to make comparisons between different ecosystems. Among fairly similar systems, such as the oligotrophic southern Chilean lakes in this study, APA is still probably a good indicator of relative P availability.

There appear to be differences in physiological indicators of P-deficiency between lakes that are located at least partially within parks and preserves and those that are not, with a trend of higher N:P and C:P ratios and higher APA observed in lakes located within parks. The Auracanian region (north of about 41°S) is the most populous part of the study area, although several of those lakes are partially or entirely within Parque Nacional Huerquehue (Tinquilco, Chico, El Toro, and Verde; Fig. 1 and Table 1). The area around Lago Caburga is settled sparsely and almost completely forested. Llanquihue, Puyehue, Rupanco, and Villarrica all have towns around them, and all of these lakes but Villarrica have salmon farms. The central portion of the study area (approximately 41–44°S) is perhaps the least influenced by human activity, and with the exception of Lago Yelcho and Lago Chapo, none of the lakes in the central region have salmon culturing facilities in them. In general, the watersheds of the lakes in this region are completely forested or nearly so, although there is some modest agricultural activity near a few of the lakes. The southernmost part of the study area was extensively burned by colonists approximately 40–50 years ago (D. Soto, personal communication). Some reforestation projects are underway, but most of the area is completely deforested and is used for grazing livestock.

In addition to differences in human activity within the lakes and their watersheds, volcanic activity may affect nutrient supply to some lakes in the study area. In the Auracanian region, Volcán Villarrica erupted in 1984, while in the south, Volcán Hudson erupted in 1991. Weaire and Manly (1996) reported water quality data for lakes in the vicinity of Volcán Hudson 1.5

years following its eruption. Their results indicated an excess of NO_3 relative to PO_4 in most lakes, suggesting that P limitation of phytoplankton growth was likely even following a volcanic event that could have increased P input to the lakes.

Finally, it is possible that climate may interact with nutrient availability to influence the trophic status of lakes in this region. Soto et al. (1994), in a study of lakes in southern Chile, suggested that in areas where high winds are common, the depth of the mixed layer may exceed that of the photic zone and phytoplankton production may be light-limited some of the time. Baigún & Marinone (1995) also noted this possibility for lakes in Argentine Patagonia, which are mostly warm monomictic and have a shorter period of stratification than is typical of North Temperate zone lakes.

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