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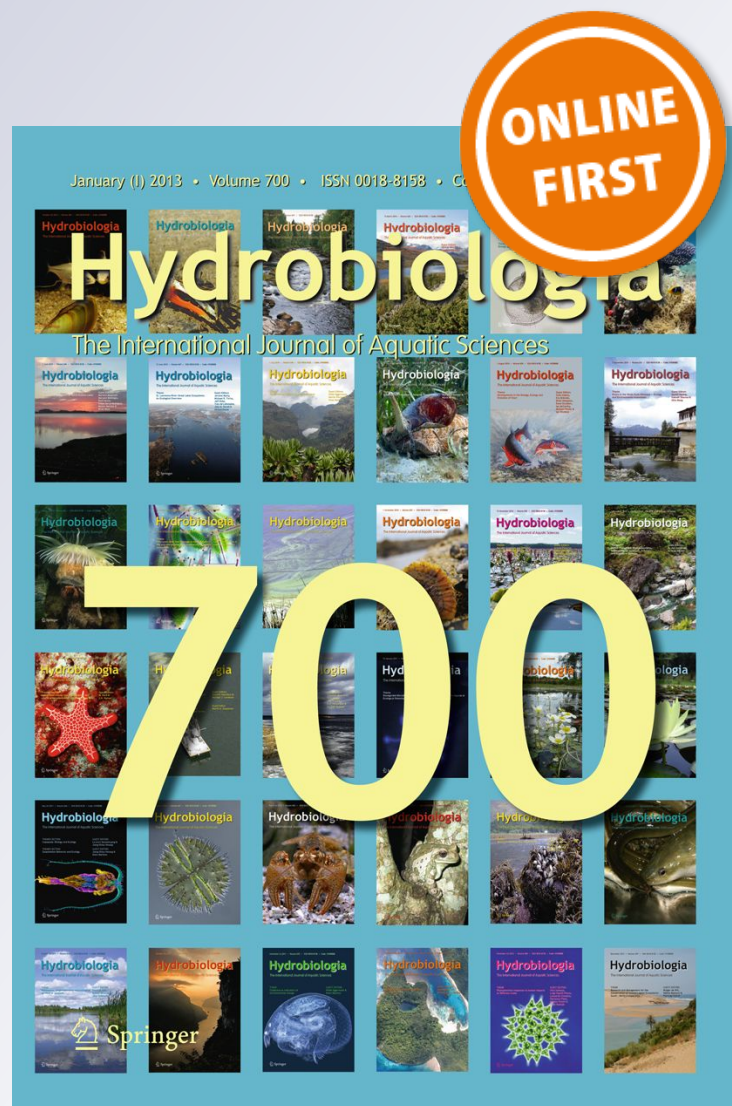
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Phytoplankton limitation in Patagonian and Pampean shallow lakes: effect of phosphorus and light

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Abstract In order to evaluate patterns of phytoplankton community structure and primary production limitation, we analyzed the phytoplankton communities (chlorophyll *a*, biovolume, species richness, and primary production) of a set of 20 shallow lakes encompassing a wide range of phosphorus and light levels. The degree of limitation or co-limitation by phosphorus and/or light was estimated using a combined index. We found a direct relationship between irradiance in the water column (I_{mean}) and the light saturation parameter (I_k) and, a negative relationship between I_{mean} and total phosphorus (TP)

concentration. Lakes at the lowest and highest ends of TP tended to display the simplest phytoplankton communities (i.e., only one or two algal classes accounted for the total phytoplankton biovolume). In contrast, lakes with intermediate TP exhibited a more complex phytoplankton composition. Low values of phytoplankton abundance proxies (chlorophyll *a* and biovolume), species richness, and primary production could be observed at any combination of phosphorus and light availability. However, the highest values of the previous variables were observed only when the relative availability of both resources was similar. The combined index developed provides a single metric for describing light versus nutrient limitation. The importance of its use is that can be applied to the analysis of clear lakes lacking vegetation and inorganic turbid lakes, besides organic turbid and clear lakes with vegetation.

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Introduction

The factors controlling phytoplankton primary production (PP) have long been studied. Understanding the factors and mechanisms involved in the PP limitation has not only great theoretical interest, but it is also an important issue from an applied perspective. Most studies have focused on the limitation of phytoplankton PP by one single resource (Hecky & Kilham, 1988; Beardall et al., 2001), although it may be simultaneously limited by more than one resource (i.e., co-limitation) (Tilman et al., 1982; Harpole et al., 2011; Yamaguchi et al., 2015). During the last decades, there has been a sustained interest in understanding the effect of PP limitation due to the combined effects of two or more limiting resources, both on the structure of the phytoplankton assemblages (Flynn, 2001; Brauer et al., 2012) and on overall lake functioning (Sterner et al., 1997; Dickman et al., 2006).

The evidence supporting the notion that phytoplankton biomass and production are directly related to nutrient load is overwhelming; however, there is also a great deal of variability resulting from the deviations from the predictions based on nutrient inputs (Phlips et al., 2000; Mao et al., 2008; Squires et al., 2009). Thus, nutrient inputs often, but not always, lead to increases in phytoplankton biomass. This apparent deficit in primary production may result from light limitation for algal growth. Therefore, in addition to quantifying the availability of nutrients, it is important to take into account the physical environment, particularly the underwater light climate and the factors controlling light availability (Bastidas Navarro et al., 2009; Bastidas Navarro & Modenutti, 2012; Gereá et al., 2016). As solar radiation is the source of energy for photosynthesis in phytoplankton, light availability can be a limiting resource to phytoplankton growth (Falkowski & Raven, 2007; Kirk, 2011). In many aquatic ecosystems with high transparency, the phytoplankton structure, standing crop, and dynamics are strongly correlated with the nutrient supply (Díaz et al., 1998; Queimaliños et al., 1998; Dzialowski et al., 2005; Abell et al., 2010; Bergström,

2010). In contrast, in turbid systems, light availability can be a major limiting factor for primary production (Irigoién & Castel, 1997; Schallenberg & Burns, 2004; Henry et al., 2006; Allende et al., 2009; Llamas et al., 2009; Torremorell et al., 2009).

In shallow lakes, the dominance of primary producers varies according to the prevailing limiting factor for phytoplankton primary production; nutrient limitation typically results in the dominance of aquatic macrophytes over phytoplankton, while increasing nutrient loads tend to favor phytoplankton, which in turn may outcompete macrophytes through increased water turbidity (Scheffer, 1998; Scheffer & van Nes, 2007; Genkai-Kato et al., 2012). Thus, as long as light availability is controlled by phytoplankton self-shading, nutrient levels may serve as a convenient descriptive metric for shallow lakes. However, if light availability becomes decoupled from phytoplankton self-shading, independent proxies for nutrient and light limitation are needed (Knowlton & Jones, 2000).

In order to evaluate a pattern related to the limitation of the phytoplankton community, we selected 20 shallow lakes within the Patagonia and Pampa regions of Argentina, encompassing a wide range of phosphorus and light levels. We expect that phytoplankton biomass proxies (chlorophyll *a* and biovolume), community descriptors (species richness), and primary production (PP) will reach maximum values in lakes that are not extremely limited by either phosphorus or light, i.e., within a range of conditions that would favor co-limitation by phosphorus and light. This prediction is based on the theory of synergistic interactions of limiting resources (Elser et al., 2007).

Materials and methods

Twenty shallow lakes were sampled during 2005 and 2006. Each shallow lake was visited once or up to three times, totaling 32 occasions. This set of 20 shallow lakes was selected so that they encompassed a wide gradient of optical and nutrient conditions. Abbreviations, sampling dates, geographic locations, and main morphometric features of the lakes are shown in Table 1.

Vertical profiles of downward irradiance (350–750 nm) were obtained with a spectroradiometer (USB2000, Ocean Optics, Dunedin, FL, U.S.A.)

Table 1 List of the studied shallow lakes, with their abbreviation, sampling dates, geographic location, and main morphometric features

Lake	Date	Geographic location	Z_{\max} (m)	Area (Ha)	Elevation (m)
Burro					
BU	2005/11/14	35°42'S; 57°55'W	2.2	1,020	6
Chascomús					
CH 1	2005/11/15	35°36'S; 58°02'W	1.9	3,010	7
CH 2	2006/06/07				
CH 3	2006/09/05				
Escondido					
ESC	2006/02/21	41°02'S; 71°33'W	8.0	10	770
Ezquerria					
EZQ	2006/02/21	41°03'S; 71°30'W	3.5	10	765
Fantasma					
FAN	2005/11/21	41°05'S; 71°27'W	0.5	1	780
Juncos					
JUN	2005/11/23	41°04'S; 71°03'W	1.5	7	907
Juventus					
JUV	2006/02/24	41°21'S; 71°31'W	11.0	4	1,010
Kakel Huincul					
KH1	2005/11/09	36°48'S; 57°47'W	1.5	2,000	8
KH2	2006/09/06				
Lacombe					
LC1	2005/11/11	35°49'S; 57°49'W	1.5	130	9
LC2	2006/09/07				
Limpia					
LI 1	2005/11/16	35°37'S; 57°48'W	1.9	560	8
LI 2	2006/06/07				
LI 3	2006/09/06				
Ñireco					
NIRE 1	2005/11/22	41°11'S; 71°19'W	1.0	1×10^{-2}	906
NIRE 2	2006/02/24				
Ñirihuau					
NIRI	2005/11/22	41°05'S; 71°11'W	1.0	1×10^{-2}	830
Patos					
PAT	2006/02/21	41°15'S; 71°17'W	0.7	1	1,461
Salada					
SA	2005/11/14	35°47'S; 57°52'W	2.0	450	8
San Jorge					
SJ 1	2005/11/10	35°40'S; 57°47'W	1.7	300	7
SJ 2	2006/06/07				
SJ 3	2006/09/05				
Trébol					
TRE	2006/02/21	41°03'S; 71°30'W	12.0	30	787
Triunfo					

Table 1 continued

Lake	Date	Geographic location	Z_{\max} (m)	Area (Ha)	Elevation (m)
TR1	2005/11/10	35°51'S; 57°52'W	1.2	150	9
TR2	2006/06/08				
TR3	2006/07/13				
TR4	2006/09/06				
Verde					
VER	2006/02/20	41°15'S; 71°17'W	6.0	0.23	1,545
Vitel					
VI	2005/11/08	35°32'S; 58°07'W	1.6	1,460	8
Yalka					
YA	2005/11/10	35°35'S; 57°54'W	1.4	1,060	11

attached to a fiber optic probe. For highly turbid lakes, irradiance profiles were performed at noon inside a black container (50 × 50 × 40 cm) filled with freshly collected lake water. This procedure was adopted to increase the accuracy of the measurements by eliminating the noise due to wave action observed in situ profiles. Light extinctions observed within a few centimeters of the surface and the waves do not allow to make these measurements every one to two centimeters. In clearer shallow lakes, the light profiles were measured in situ. Diffuse vertical attenuation coefficients for PAR, (K_d PAR) were calculated by regressing log-transformed irradiance measurements against depth.

Total phosphorus (TP) and total dissolved phosphorus (TDP) concentrations were measured in water samples collected at a point close to the surface (20–30 cm) in the central area of each shallow lake with a van Dorn bottle (10 l). The water samples were placed in polypropylene bottles and transported insulated and in darkness. Once in the laboratory, whole water samples were digested to determine TP by the molybdate-ascorbic method (APHA, 2005). For estimating TDP, the water was filtered through glass-fiber filters (Whatman GF/F, Whatman International Ltd., Maidstone, England) and determined by the molybdate-ascorbic method (APHA, 2005). Total alkalinity was determined by titration with 0.1 N H₂SO₄ and F2 of Gran's method (Gran, 1952). Dissolved inorganic carbon (DIC) was calculated following Rebsdorf (Rebsdorf, 1972).

Phytoplankton samples were collected directly in PVC bottles and fixed with acidified Lugol's iodine solution (1% final concentration). Additional samples

for qualitative analyses were collected using a 15 µm pore plankton net and fixed with 4% formaldehyde. Phytoplankton counting was carried out following the Utermöhl technique (Utermöhl, 1958) at 400× and the counting error was estimated according to Venrick (1978). The Shannon diversity index was calculated after Begon et al. (1987). The biovolume of each species was calculated according to the algal shape and the mean algal dimensions using the appropriate geometric formulae (Hillebrand et al., 1999; Sun & Liu, 2003). Phytoplankton composition was also analyzed in the context of the functional classification proposed by Reynolds et al. (2002) as updated by Padisák et al. (2009) and Kruk et al. (2010).

The chlorophyll *a* (Chl *a*) concentration was estimated from water samples (110–250 ml), filtered onto glass-fiber filters, following the acetone extraction method (Marker et al., 1980). The inorganic carbon fixation was obtained using the ¹⁴C technique (Steeman-Nielsen, 1952). Lake water samples were collected in PVC bottles and transported to the laboratory. Lake water aliquots were placed in 16 quartz tubes (45 ml) and each tube was inoculated with 1 µCi-labeled sodium bicarbonate. Incubations were performed inside a water bath during 3 h around noon. The quartz tubes were kept horizontally right under the water surface. In all cases, the incubation bath was filled with highly transparent tap water (optically similar to distilled water). The water temperature was adjusted during incubations so that it was the same as that measured in lakes. For each incubation series, eight different light intensities (transmittance ranging from 100 to <2%) were set up by covering the tubes with different layers of

neutral density filters. In addition, one tube was wrapped in aluminum foil and served as a dark control. All treatments were run with two replicates. At the end of the incubation, each replicate was filtered through Whatman GF/F filters. The filters obtained were subsequently placed in 4 ml scintillation vials in an HCl-saturated atmosphere and allowed to dry overnight. The activity was measured in a scintillation counter (Beckman LS 5000TD, Fullerton, CA, U.S.A.) after adding 2.5 ml of OptiPhase 'HiSafe'3 scintillation solution (Holm-Hansen & Helbling, 1995). The photosynthetic parameters for the PAR treatment were estimated from P versus I curves by fitting an exponential model (Platt et al., 1980):

$$P = P_s \times (1 - e(-\alpha \cdot I/P_s)) \times e(-\beta \cdot I/P_s),$$

where P is the photosynthetic rate at a given irradiance (I); P_s is the maximum light-saturated photosynthetic rate; α is the photosynthesis light efficiency at sub-saturating irradiances; β is the negative slope of the curve at high PAR irradiance (i.e., photoinhibition).

During phytoplankton PP determinations, PAR irradiance was measured with a GUV 511 radiometer (Biospherical Instruments, Inc., San Diego, CA, U.S.A.) located in San Carlos de Bariloche (41°09'S; 71°18'W) and with a portable radiometer ELDONET (Real Time Computers, Inc., Erlangen, Germany) in Chascomús city (35°37'S; 57°59'W). This information was used to derive the curves P versus I . The daily integrated phytoplankton PP was estimated using the exponential model. For comparative purposes, we used the mean irradiance measured on 2006/02/21. Mean irradiance was calculated after Ferrero et al. (2006):

$$I_{\text{mean}} = I_o \times \left(1 - e^{-K_d \times Z}\right) / K_d \times Z,$$

where I_o is the superficial daily mean irradiance; K_d is the vertical attenuation coefficients; Z is the lake depth.

In order to assess the degree of phytoplankton limitation by nutrients and light, we used the modeling approach originally developed for estuaries by Cloern (1999). Cloern's model of phytoplankton growth (Cloern et al., 1995) incorporates the rate of carbon assimilation (as a function of photosynthetic efficiency and light availability) and the ratio Chl:C (as a function of temperature, photoadaptation, and nutrient availability). As nitrogen (N) is typically the most

common limiting nutrient in estuaries whereas shallow lakes are more often limited by phosphorus (P), we modified the parameters of the model accordingly Hecky & Kilham (1988). The model uses the following inputs for each lake: PP, lake depth, PAR extinction coefficient (K_d), temperature, and TDP. The nutrient resource (P') is defined as the quotient of TDP divided by the half-saturation constant for TDP-uptake (K_p), i.e., $P' = \text{TDP}/K_p$. The constant K_p was calculated as the average of the K_p values reported for several freshwater phytoplankton species (Smith & Kalff, 1982). The light resource (I') is estimated as the daily mean irradiance (I_{mean}) divided by the light half-saturation constant, i.e., the daily irradiance at which growth rate is half the maximum ($I' = I_{\text{mean}}/K_i$). The constant K_i was calculated for each lake from the curves P versus I . Cloern and co-workers (Cloern, 1999; Cloern et al., 1995) took a step further by developing an index (R) that combines the estimates of nutrient (P') and light (I') availability into a single measure of nutrient and light limitation. R is a measure of the phytoplankton sensitivity to light and nutrient variability, R values greater than 10 correspond to phytoplankton assemblages severely limited by light, while values lower than 0.1 indicate severe nutrient limitation. R values close to 1 are indicative of co-limitation by both nutrient and light, and as rule of thumb, values between 0.1 and 10 indicate potential co-limitation. For more details of the calculations, see Supplementary Information.

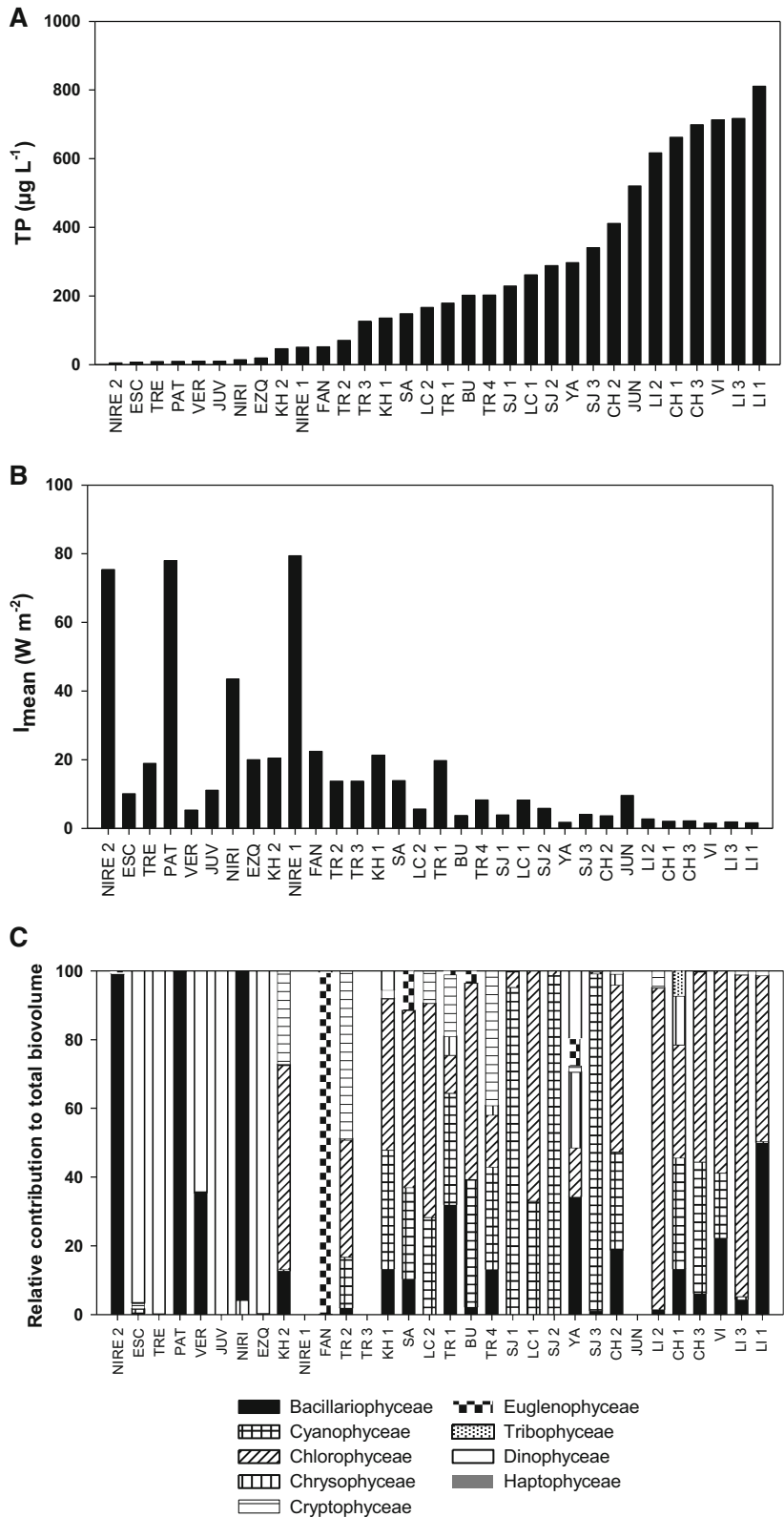
A canonical correspondence analysis (CCA) was performed based on the abundance of phytoplankton species and environmental factors. CCA was selected by performing previously detrended correspondence analysis (DCA), which indicated the unimodal distribution of the biological data. The significance of the axes was tested by Monte Carlo permutation test, and the significance of the environmental variables was analyzed by forward selection. The analysis was performed with the program CANOCO (terBraak & Šmilauer, 2002).

Results

As a first step, we ordered lake samples by increasing total phosphorus concentration. TP ranged widely from 4.7 to 915.2 $\mu\text{g l}^{-1}$ (Fig. 1A), while mean irradiance in the water column ranged from 1.5 to

Fig. 1 Nutrient levels, light conditions, and phytoplankton composition in the studied lakes.

A Distribution of total phosphorus; **B** Mean irradiance in the column water, and **C** relative composition of the different algal classes to total phytoplankton biovolume

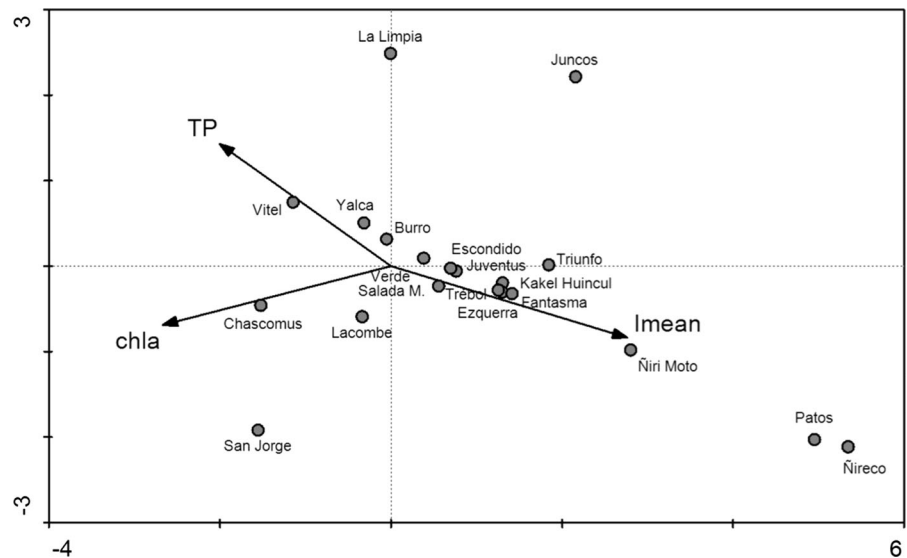


79.4 W m⁻² (Fig. 1B). These two resources show an inverse relationship ($R^2 = 0.55$, $P < 0.0001$) when both variables are log-transformed, but departures from this trend are evident when comparing Fig. 1A, B. In relation to biological parameters, the Shannon Diversity Index did not show a pattern along the TP gradient; however, lakes at the lowest and highest ends of TP displayed the simplest composition, with only one or two algal classes accounting for the total phytoplankton biovolume. In shallow lakes with low TP t, the Bacillariophyceae and Dinophyceae contributed mostly to the total biovolume, and in shallow lakes with a high TP the chlorophyceae contributed most to the total biovolume. In contrast, lake samples at intermediate TP displayed a more complex phytoplankton structure (Fig. 1).

The dominant phytoplankton functional groups differed among the studied lakes. According to the classification proposed by Kruk et al. (2010), our results showed that some of the lakes with lower light availability were mainly dominated by groups IV, and VII. Group IV includes organisms of medium size lacking specialized traits, mainly represented in the studied lakes by several species of Chlorococcales and desmids (particularly *Closterium aciculare* T. West very abundant in LI). Group VII is conformed by large mucilaginous colonial species, including both Chlorococcales and Chroococcales. Likewise, the turbid shallow lake SJ was almost exclusively represented by group III (large filaments with aerotopes), due to the absolute dominance of the cyanobacteria *Raphidiopsis mediterranea* Skuja. At the opposite extreme, some of the lakes with low nutrient availability exhibited a higher prevalence of group II, which includes small flagellated organisms with siliceous exoskeletal structures. This group was represented by several species of Chrysophyceae and Haptophyceae (e.g., *Dinobryon* spp., *Ochromonas* sp., *Chrysochromulina parva* Lackey). Group II was also important in terms of abundance in the lakes PAT and VER, but in terms of biovolume, the phytoplankton of these water bodies was better represented by group VII, which includes non-flagellated organisms with siliceous exoskeletons (basically diatoms). Lake NIRE showed a co-dominance of this group (with relatively high abundance of *Aulacoseira granulata* var. *granulata* (Ehrenberg) Simonsen and *Synedra acus* Kützing) and the dominance in biovolume of group IV (*Dictyosphaerium pulchellum* H.C.Wood). In lake EZQ, the group V

(unicellular flagellates of medium to large size) had the highest contribution to the algal biovolume, being represented by the species *Ceratium hirundinella* Schrank and *Gymnodinium paradoxum* Schilling, whereas group II was co-dominant. The functional classification proposed by Reynolds et al. (2002) and updated by Padisák et al. (2009), also reflected the differences among the lakes in the gradient of light and nutrients. Following this classification, most of the lakes more severely light-limited share some functional groups. The dominant groups were as follows: K, a codon that includes small-celled colonial, non-gasvacuolated Cyanobacteria, (represented in the studied lakes by several species of the genera *Aphanocapsa*, *Coelosphaerium*, and *Cyanodictium*); Sn (absolutely dominant in lake SJ), an association constituted by Cyanobacteria typical of mixed layers of light deficient environments, represented in lake SJ by *R. mediterranea*; F, codon frequent in meso-eutrophic lakes with high turbidity that includes colonial chlorophytes; P, an association comprising many diatoms and some desmids, frequently found in continuous or semi-continuous mixed eutrophic lakes, with a high contribution to the biovolume in Lakes LI and KH due to the abundance of *A. granulata* and *C. aciculare*. In addition, some of the relatively clearer enriched lakes showed a high contribution of associations X2 (mainly composed by the nanoflagellates *Plagioselmis* spp. and *Chlamydomonas* spp.), and Y (*Cryptomonas* spp.). In KH, the functional group Lm was also very important in terms of biovolume, and was represented by small colonies of *Microcystis* spp. On the other hand, most of the nutrient-limited lakes showed a marked dominance of the codon X3, typical in shallow oligotrophic environments, and was represented by nanoflagellated algae of the groups Chrysophyceae and Haptophyceae. Some of these lakes also had a relatively high contribution of group X2, more typical in meso-eutrophic lakes, and best represented by *Plagioselmis nanoplanctica* (Skuja) Novarina. In some lakes, other functional groups also contributed to the total algal biovolume. In particular, Lake VER was characterized by the dominance of the association C, frequent in mixed eutrophic small and medium-sized lakes, and includes species sensitive to the onset of stratification (e.g., *Asterionella formosa* Hassall). In Lake NIRE, the codon F was dominant in terms of biovolume due to the high abundance of *D. pulchellum* H.C.Wood. Finally, the codon MP including littoral

Fig. 2 CCA biplot of phytoplankton and environmental factors



diatoms drifted to the plankton (e.g., *Fragilaria construens* (Ehrenberg) Grunow and *Fragilaria* spp.) dominated in Lake PAT.

The ordination obtained of the CCA based on the abundance of phytoplankton species and environmental factors reflects the differences among the lakes in relation to light and P ; lakes with higher I_{mean} values placed towards the right part of the figure, whereas lakes with higher concentrations of TP falling towards the left side (Fig. 2). The Monte Carlo test confirmed that the environmental variables were significantly correlated with the first axis ($P = 0.004$), and the test of all axes was also significant ($P = 0.006$). The first two axes accounted for 75.1% of the variance (axis 1 44.7%; axis 2 30.4%). The forward selection procedure identified the following variables with significant contribution and low inflation factors: I_{mean} , Chl a and TP. The first axis was inversely correlated with I_{mean} (intra-set correlation coefficients: -0.81 , -0.61 , and 0.84 , respectively), whereas the second axis was positively correlated with TP (intra-set correlation coefficient: 0.44).

The photosynthetic parameters (P_s and α), PP, temperature, I_{mean} , and K_i of each shallow lake were used to calculate nutrient (P') and light availability (I'), and the combined index of nutrient versus Light limitation (R) (Table 2). None of the studied lakes was simultaneously rich in both resources. Low values of phytoplankton biomass proxies (Chl a , biovolume), community descriptors (species richness), and PP

could be observed at any combination of P and light availability. The highest values of the previous variables were observed only when the relative availability of both resources was comparable. In contrast, only low values could be observed in lakes where the availability of either P or light was disproportionately high compared to the availability of the other resource (Fig. 3). Essentially, similar results were obtained when using the index R , which combines the light and nutrient availability measurements in a single metric of P and light limitation (low values of R indicate nutrient limitation; high values, light limitation; and intermediate values are indicative of co-limitation by P and light). Low values of Chl a , biovolume, species richness, and primary production were measured all along the whole range of R , but the highest values were found within a narrow range of R ($0.1 > R > 10$) (Fig. 4). It is worth mentioning that the absolute values of R are only indicative, as they are heavily dependent on the choice of the model's parameters (see the discussion section). Within this set of lakes (i.e., $0.1 < R < 10$), the highest values of biovolume, Chl a , species richness and PP were observed in turbid organic lakes (Lakes CH, SA, SJ, and LC).

Primary production varied widely within the set of study lakes, from very low (NIRE: $0.03 \text{ gC m}^{-2} \text{ day}^{-1}$) to extremely high as in the most productive lakes (i.e., CH: $18.4 \text{ gC m}^{-2} \text{ day}^{-1}$). The photosynthetic parameters, P_s and α , derived from the

Table 2 Parameters used to calculate the R index

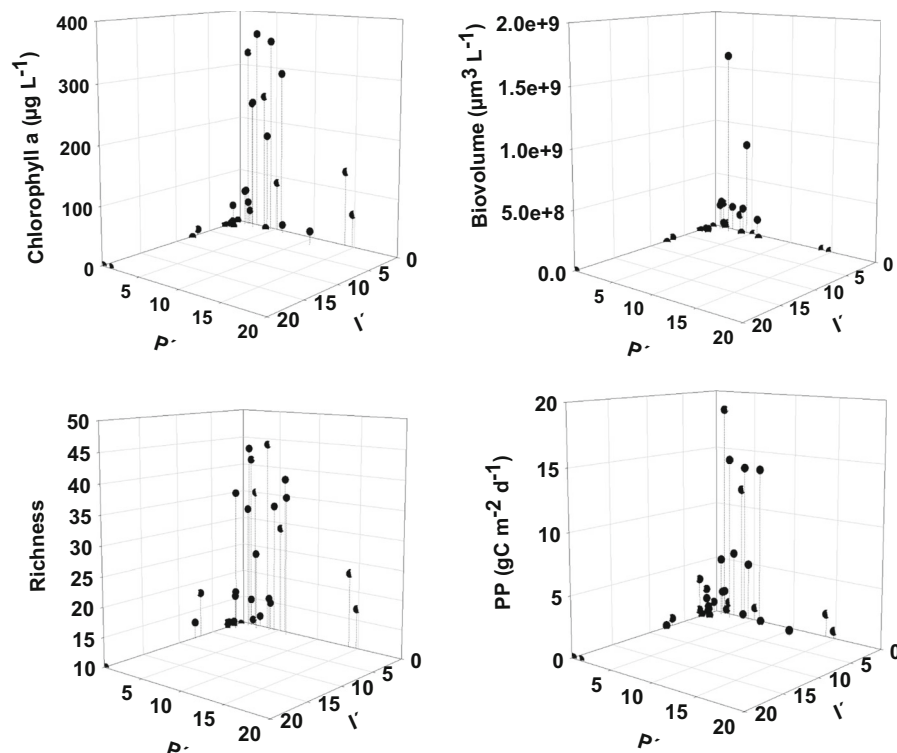
Lake	P_s (mgC mgChl a ⁻¹ h ⁻¹)	α (mgC m ² mgChl a ⁻¹ h ⁻¹ W ⁻¹)	PP (gC m ⁻² day ⁻¹)	Chl a (μ g l ⁻¹)	TDP (μ g l ⁻¹)	T (°C)	I_{mean} (W m ⁻²)	K_i (W m ⁻²)
BU	4.02	0.17	1.98	63.20	46.81	22.0	3.73	16.12
CH 1	13.84	0.52	11.50	244.83	160.24	21.5	2.05	18.30
CH 2	6.92	0.30	13.45	290.78	137.60	11.0	3.61	15.78
CH 3	11.42	0.61	18.40	323.65	57.50	9.0	2.17	12.94
ESC	3.24	0.64	0.69	1.29	6.80	20.5	10.09	3.49
EZQ	5.35	0.38	1.62	4.77	19.00	21.2	20.00	9.87
FAN	2.18	0.17	1.08	12.43	51.60	15.0	22.43	8.80
JUN	0.76	0.13	0.55	25.18	520.40	10.8	9.55	4.13
JUV	5.19	0.59	2.40	2.44	9.90	18.6	11.12	6.10
KH1	0.64	0.04	0.62	38.07	22.13	20.0	21.32	12.01
KH2	23.22	1.76	0.56	0.84	36.10	9.8	20.42	9.12
LC1	3.06	0.29	14.16	235.10	126.88	20.5	8.22	7.15
LC2	4.72	0.30	2.18	42.98	72.20	10.0	5.62	11.02
LI 1	6.75	0.20	1.95	131.50	646.66	23.0	1.62	22.71
LI 2	3.17	0.14	0.41	25.28	546.60	14.0	2.72	15.82
LI 3	4.68	0.13	0.63	58.16	688.70	8.0	1.87	24.74
NIRE 1	2.30	0.92	0.05	0.93	50.30	7.7	79.42	1.73
NIRE 2	2.69	0.60	0.03	0.47	4.70	21.0	75.41	3.09
NIRI	10.42	1.32	0.27	1.10	14.00	13.1	43.52	5.48
PAT	6.17	0.14	0.24	1.97	9.30	17.5	77.98	30.10
SA	3.80	0.16	5.09	64.45	62.16	21.0	13.91	17.00
SJ 1	8.64	0.56	13.48	173.20	99.00	18.0	3.90	10.76
SJ 2	1.34	0.07	4.98	347.67	74.20	14.0	5.82	13.31
SJ 3	2.68	0.10	5.69	359.05	118.20	8.0	4.03	17.84
TRE	7.93	0.84	3.48	1.41	9.00	22.7	18.92	6.58
TR1	1.42	0.04	0.30	16.20	157.00	20.0	19.73	22.35
TR2	2.20	0.77	0.69	10.95	62.20	18.0	13.74	1.97
TR3	4.84	0.56	0.21	1.90	126.00	11.5	13.74	5.94
TR4	16.98	1.07	0.50	2.53	194.30	10.0	8.24	11.04
VER	4.28	0.37	1.00	4.38	9.90	21.8	5.33	8.00
VI	5.19	0.08	1.08	228.87	80.18	22.5	1.51	43.87
YA	5.75	0.22	1.11	88.50	126.00	18.0	1.73	17.78

P_s , maximum light-saturated photosynthetic rate, α photosynthesis light efficiency at sub-saturating irradiances, PP primary production, $Chl a$ chlorophyll a concentration, TDP total dissolved phosphorus concentration, T temperature, I_{mean} mean irradiance, K_i light half-saturation constant

P versus I curves were significantly correlated ($r = 0.78$). In relation to the phytoplankton composition and the availability of light and phosphorus, we did not find correlation between the different phytoplankton classes and the diversity index with respect to R , I' , and P' (Supplementary Table). A significant

relationship was also observed between I_{mean} (mean irradiance in the water column) and the light saturation parameter (I_k) ($R^2 = 0.80$, $P < 0.0001$, Fig. 5), indicating that photosynthesis in the shallow lakes with greater availability of light in water saturates at higher irradiance. A significant negative relationship was

Fig. 3 3D scatter plots of chlorophyll a concentration, phytoplankton biovolume, species richness, and primary production versus I' (light availability) and P' (phosphorus availability)



observed between the I_{mean} and total phosphorus ($R^2 = 0.55$, $P < 0.0001$; Fig. 6). That is, the euphotic zone is lower in shallow lakes with higher P concentration.

Discussion

The wide range of nutrient and light conditions spanning in the studied lakes translated into very different scenarios for phytoplankton PP, from severe P limitation to strong light limitation. Thus, taken as a whole, the studied lakes encompass a great deal of the diversity of environmental conditions experienced by phytoplankton assemblages in temperate shallow lakes.

Regarding the phytoplankton structure, shallow lakes at either extreme (strongly limited by light or P) tended to exhibit phytoplankton communities dominated by one or two classes, while less severely limited environments displayed more varied communities (i.e., more equitative at the class level). On the other hand, the phytoplankton functional approach has demonstrated to be an adequate tool to analyze the

differences among lakes across this gradient of light and nutrients. Both functional classifications applied allowed discriminating lakes with severe light limitation from more transparent but nutrient-limited lakes. The functional groups approach stems from the phytosociological scheme of phytoplankton associations, coined many years ago (e.g., Margalef, 1978; Reynolds, 1984) to describe groups of species sharing certain common ecological attributes. The concept was refined and expanded, leading to different functional classifications (e.g., Reynolds et al., 2002; Salmaso & Padisák, 2007; Kruk et al., 2010). Although both functional approaches used in this study showed clear differences in composition among lakes, the Reynolds's classification (Reynolds et al., 2002) seems to be more sensitive to detect some particular groups present in lakes. For example, groups Y and X2 present in the vegetated shallow lakes from the Pampa plain that includes some species capable of mixotrophic nutrition (e.g., *Cryptomonas* spp. and *Plagioselmis* spp.). Nevertheless, in general terms, the morpho-functional approach tends to simplify the taxonomical approach and has been useful in regional characterizations of lakes, having potential utility in

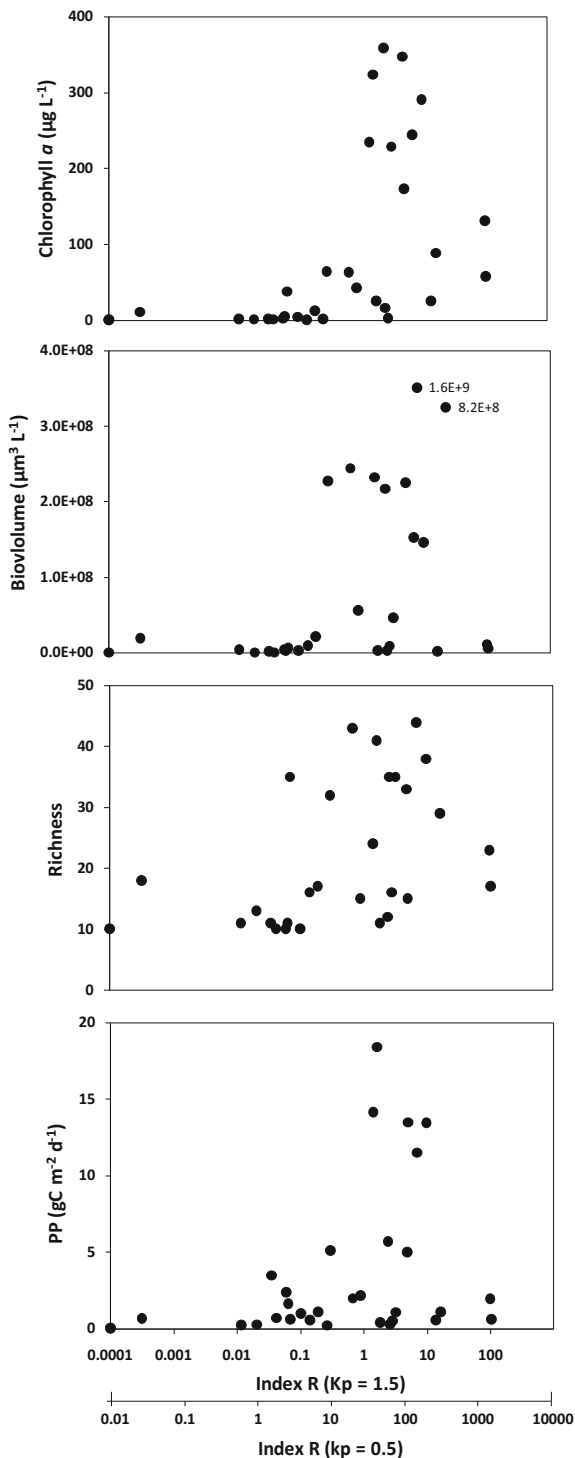


Fig. 4 Chlorophyll *a* concentration, phytoplankton biovolume, phytoplankton species richness, and phytoplankton primary production as functions of the *R* index. For each lake, two values of the *R* index were calculated using $K_p = 1.5 \mu\text{M}$ and $K_p = 0.5 \mu\text{M}$

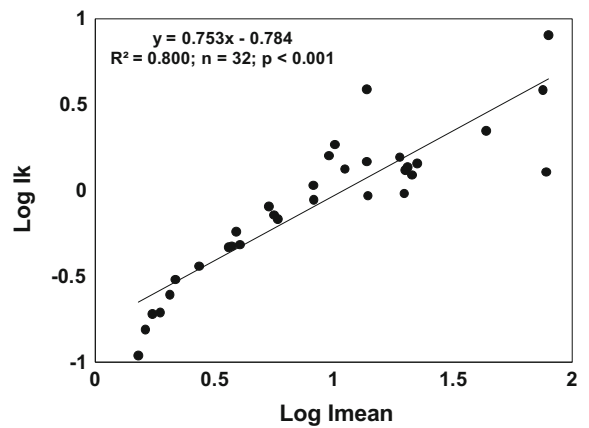


Fig. 5 Linear regression between the log-transformed irradiance in the water column (I_{mean}) and the log-transformed light saturation parameter (I_k) ($R^2 = 0.80$, $P < 0.0001$)

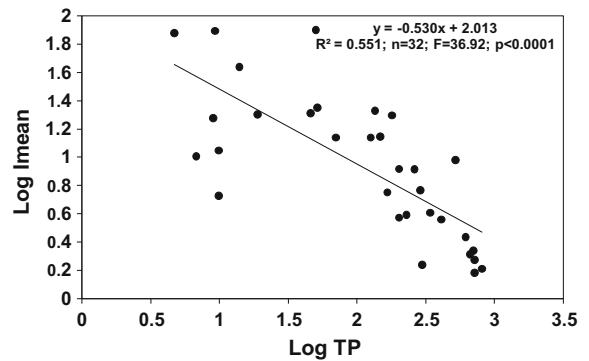


Fig. 6 Linear regression between log-transformed total phosphorus (TP) and log-transformed irradiance in the water column (I_{mean}) ($R^2 = 0.55$, $P < 0.0001$)

environmental biomonitoring (e.g., Tolotti et al., 2005; Izaguirre et al., 2012; Naselli-Flores & Barone, 2012). Differences in phytoplankton composition among the lakes were also evidenced from the results of the multivariate analysis CCA. Light conditions in the lakes and TP were the most important parameters explaining the variance in the abundance of the phytoplankton species.

There was a direct relationship between the photosynthetic parameters, which agrees well with the expectations based on previous studies (Côté & Platt, 1983; Behrenfeld et al., 2004). In addition, the significant direct relationship found between I_{mean} and I_k , suggested that phytoplankton acclimated to high light intensities displays the highest light saturation coefficient. Therefore, I_k could be taken as a fair

proxy of light acclimation, as also suggested by Sakshaug et al., 1997).

When plotted in the nutrient (P') versus light (I') availability diagram proposed by Cloern (1999), the distribution of lakes showed that (i) no single lake presented high availability of P and light simultaneously; (ii) only one lake, NIRE (sampled on two occasions) had extremely high I' values; (iii) a few “inorganic turbid” lakes, such as JUN and LI (sampled on three occasions) had extremely high P' values; and finally, (iv) most lakes displayed lower values of both I' and P' . This intermediate subset of lakes belongs to the universe of shallow lakes for which the shallow lakes theory was formulated (Kosten et al., 2012).

Following Cloern (1999), we computed the R -value for each lake. By combining measures of light (I') and nutrient (P') availability, R provides a single metrics for describing light versus nutrient limitation. Estimating I' and P' requires the estimation of the half-saturation constant of phytoplankton PP for light (K_i) and phosphorus (K_p), respectively. Here, we measured K_i from the individual P versus I response curve developed for each lake and sampling date. In contrast, the K_p values could not be measured for natural phytoplankton assemblages, and was therefore estimated using published values developed for algal cultures. The range of published K_p values is relatively narrow; nevertheless, it is wide enough to substantially affect the absolute value of R . Cloern (1999) concluded that $R < 0.1$ corresponds to nutrient limitation, $0.1 < R < 10$ to co-limitation by nutrient and light, and $R > 10$ to light limitation. For convenience, we set $K_p = 1.5 \mu\text{M}$ (Smith & Kalff, 1982) because doing so resulted in R values similar to those reported by Cloern and co-workers for estuaries. In the following discussion, $R < 0.1$ are referred to as low R values, $0.1 < R < 10$ as intermediated R values, and $R > 10$ as high R values, as a way to remind the reader (and ourselves) our uncertainty on the absolute value of R .

With the previous caveats in mind, all shallow lakes with low R ($R < 0.1$, $K_p = 1.5 \mu\text{M}$) were clear-vegetated. Intermediate values of R ($0.1 < R < 10$, $K_p = 1.5 \mu\text{M}$) corresponded to an assortment of lakes ranging from clear-vegetated to phytoplankton-turbid shallow lakes. Thus, this intermediate range of R appears to be the domain of P and light conditions in which lakes can adopt different stable states (clear-vegetated or phytoplankton-turbid). This range of

R also included the highest variability of phytoplankton community descriptors such as Chl a , biovolume, species richness, and PP. Low values of these descriptors were recorded regardless of the lake position within the R gradient, but high values were only observed at intermediate values of R . High values of R were measured exclusively in phytoplankton-turbid shallow lakes. However, the opposite does not necessarily apply, i.e., not all phytoplankton-turbid shallow lakes displayed high values of all community descriptors. Moreover, some phytoplankton-turbid shallow lakes displayed high values of certain descriptors and low values of others. For example, lake SJ presented an algal bloom of *R. mediterranea*, which resulted in high Chl a , biovolume, and production, but low species richness. In high values of R , phytoplankton community descriptors were low as a consequence of the low I_{mean} caused by inorganic turbidity.

Considering the math involved in the calculation of R , it is not surprising that phytoplankton-turbid lakes are associated with intermediate R values ($0.1 < R < 10$, given our choice of $K_p = 1.5 \mu\text{M}$). In these groups of lakes, phytoplankton growth is supposed to be limited by algae self-shading which implies low light availability (I'). On the other hand, most P is in the form of organic particulates (Lagomarsino et al., 2011), since high pH and high Ca concentrations in Pampean lakes favor the co-precipitation of dissolved P as apatite (Quirós et al., 2006). As a result, the availability of P , measured as P' is also low, which translates into intermediate values of R . According to Cloern (1999), those environments characterized by intermediate values of R ($0.1 < R < 10$) may be co-limited by nutrients and light. In our opinion, however, phytoplankton-turbid lakes are mostly (if not exclusively) limited by light, because the large amounts of P stored in the sediments could be mobilized to the water column if the phosphorus demand by phytoplankton increases (i.e., we suspect that P' actually underestimates the availability of P in phytoplankton-turbid lakes).

It is also not surprising that some clear-vegetated lakes also occur within the intermediate range of R ($0.1 < R < 10$), given that a substantial amount of light is attenuated by colored organic matter, resulting in low values of I' . Most likely all these lakes may be actually co-limited by P and light. In fact, preliminary experiments performed with phytoplankton from clear-vegetated lakes (Lagomarsino, pers. com.) failed

to stimulate phytoplankton growth by enrichment with P and/or light. It could be due to the limitation of phytoplankton PP by some other nutrient (e.g., nitrogen) in some clear-vegetated lakes (Sánchez et al., 2013), resulting in intermediate R values, that may be misleadingly interpreted as co-limitation by P and light.

From the above discussion, it appears that an intermediate range of R values is the natural domain within which different stable states are possible. On one hand, it is interesting to note that the coexistence of alternative states may be restricted to a narrow (two orders of magnitude) range of R values. The other side of the coin is that within this narrow range of R values, the actual value of R provides no clues for predicting the actual state of a lake.

In this study, inorganic turbid shallow lakes presented very high R values. This is reasonable because (i) inorganic turbidity results in very low I' and, (ii) fine grain particles have great affinity for P (Havens et al., 2007). However, in contrast to some turbid lakes a substantial proportion of P is present in the dissolved form, resulting in high P' . This group of lakes resembles the tidally energetic estuaries studied by Cloern and co-workers, but the available data are still insufficient to warrant a generalization.

To the best of our knowledge, this is the first study that attempted to use Cloern's model in freshwater shallow lakes, and although we attempted to cover a wide range of nutrient and light condition, the available data are still insufficient to generalize conclusions. Our exercise, however, revealed some potentialities and also some shortcomings. On the faulty side, we must emphasize that the absolute values of R are dependent on the choice of two important parameters, namely K_i and K_p . As mentioned before, K_i may be measured for each sample, while K_p was arbitrarily estimated here from bibliographic data. For the reasons discussed above, we set $K_p = 1.5 \mu\text{M}$. This figure is well within the range of values reported in the literature, but it is important to keep in mind that relatively small changes in this parameter can substantially affect the absolute value of R (Fig. 4). Therefore, our advice is to consider R as a relative metric, or better yet, to plot the data on the $I'-P'$ plane, rather than combining these two metrics into a single index. In addition, the inferences on potential co-limitation by phosphorus and light must be carefully evaluated along with the potential

limitation by some other resource (e.g., N), as discussed above.

Overall, our approach permitted the analysis of a wide set of lakes within a single conceptual framework. More specifically, the model allowed the treatment of clear lakes lacking vegetation and inorganic turbid lakes. Our study includes only one clear lake without vegetation, but this type of lakes is common in polar and alpine regions (Vincent & Laybourn-Parry, 2008). In principle, we see no reason for not including these lakes into a more general shallow lakes theory: increasing nutrient from extremely low levels would shift the dominance of primary producers from benthic algae to submersed macrophytes to phytoplankton or, it may be abbreviated from benthic algae to phytoplankton (as it is often the case in polar regions). The case of inorganic turbid lakes differs in that light limitation does not arise from increases in nutrient levels. In such cases, where light availability does not correlate with nutrient levels, the use of two independent axes appears as unavoidable.

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References

- Abell, J. M., D. Özkundakci & D. P. Hamilton, 2010. Nitrogen and phosphorus limitation of phytoplankton growth in New Zealand lakes: implications for eutrophication control. *Ecosystems* 13(7): 966–977.
- Allende, L., G. Tell, H. Zagarese, A. Torremorell, G. Pérez, J. Bustingorry, R. Escaray & I. Izaguirre, 2009. Phytoplankton and primary production in clear and turbid shallow lakes from the pampa plain (Argentina). *Hydrobiologia* 624: 45–60.
- APHA, 2005. Standard Methods for the Examination of Water and Wastewater. APHA, Washington, DC.
- Bastidas Navarro, M. & B. Modenutti, 2012. Precipitation patterns, dissolved organic matter and changes in the plankton assemblage in Lake Escondido (Patagonia, Argentina). *Hydrobiologia* 691: 189–202.
- Bastidas Navarro, M., B. Modenutti, C. Callieri, R. Bertoni & E. Balseiro, 2009. Balance between primary and bacterial production in North Patagonian shallow lakes. *Aquatic Ecology* 43: 867–878.
- Beardall, J., E. Young & S. Roberts, 2001. Approaches for determining phytoplankton nutrient limitation. *Aquatic Sciences – Research Across Boundaries* 63(1): 44–69.
- Behrenfeld, M. J., O. Prasil, M. Babin & F. Bruyant, 2004. REVIEW In search of a physiological basis for

- covariations in light-limited and light-saturated photosynthesis. *Journal of Phycology* 40(1): 4–25.
- Begon, M., J. L. Harper & C. R. Townsend, 1987. *Ecología. Individuos, Poblaciones, Comunidades*. Ediciones Omega, Barcelona: 886 pp.
- Bergström, A.-K., 2010. The use of TN:TP and DIN:TP ratios as indicators for phytoplankton nutrient limitation in oligotrophic lakes affected by N deposition. *Aquatic Sciences* 72(3): 277–281.
- Brauer, V. S., M. Stomp & J. Huisman, 2012. The nutrient-load hypothesis: patterns of resource limitation and community structure driven by competition for nutrients and light. *The American Naturalist* 179(6): 721–740.
- Cloern, J. E., 1999. The relative importance of light and nutrient limitation of phytoplankton growth: a simple index of coastal ecosystem sensitivity to nutrient enrichment. *Aquatic Ecology* 33: 3–16.
- Cloern, J. E., C. Grenz & L. Videgar-Lucas, 1995. An empirical model of the phytoplankton chlorophyll: carbon ratio – the conversion factor between productivity and growth rate. *Limnology & Oceanography* 40(7): 1313–1321.
- Côté, B. & T. Platt, 1983. Day-to-day variations in the spring-summer photosynthetic parameters of coastal marine phytoplankton. *Limnology & Oceanography* 28: 320–344.
- Díaz, M. M., F. L. Pedrozo & P. F. Temporetti, 1998. Phytoplankton of two Araucanian lakes of differing trophic status (Argentina). *Hydrobiologia* 369: 45–57.
- Dickman, E. M., M. J. Vanni & M. J. Horgan, 2006. Interactive effects of light and nutrients on phytoplankton stoichiometry. *Oecologia* 149(4): 676–689.
- Dzialowski, A. R., S.-H. Wang, N.-C. Lim, W. W. Spotts & D. G. Huggins, 2005. Nutrient limitation of phytoplankton growth in central plains reservoirs, USA. *Journal of Plankton Research* 27(6): 587–595.
- Elser, J. J., M. E. S. Bracken, E. E. Cleland, D. S. Gruner, W. S. Harpole, H. Hillebrand, J. T. Ngai, E. W. Seabloom, J. B. Shurin & J. E. Smith, 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters* 10(12): 1135–1142.
- Falkowski, P. G. & J. A. Raven, 2007. *Aquatic Photosynthesis*, 2nd ed. Princeton University Press, Princeton.
- Ferrero, E., M. Eöry, G. Ferreyra, I. Schloss, H. Zagarese, M. Vernet & F. Momo, 2006. Vertical mixing and ecological effects of ultraviolet radiation in planktonic communities. *Photochemistry & Photobiology* 82: 898–902.
- Flynn, K. J., 2001. A mechanistic model for describing dynamic multi-nutrient, light, temperature interactions in phytoplankton. *Journal of Plankton Research* 23(9): 977–997.
- Genkai-Kato, M., Y. Vadeboncoeur, L. Liboriussen & E. Jeppesen, 2012. Benthic-planktonic coupling, regime shifts, and whole-lake primary production in shallow lakes. *Ecology* 93(3): 619–631.
- Gerea, M., G. Pérez, F. Unrein, C. Soto Cárdenas, D. Morris & C. Queimaliños, 2016. CDOM and the underwater light climate in two shallow North Patagonian lakes: evaluating the effects on nano and microphytoplankton community structure. *Aquatic Sciences*. doi:10.1007/s00027-016-0493-0.
- Gran, G., 1952. Determination of the equivalence point in potentiometric titrations. *Analyst* 77(920): 661–671.
- Harpole, W. S., J. T. Ngai, E. E. Cleland, E. W. Seabloom, E. T. Borer, M. E. S. Bracken, J. J. Elser, D. S. Gruner, H. Hillebrand, J. B. Shurin & J. E. Smith, 2011. Nutrient co-limitation of primary producer communities. *Ecology Letters* 14(9): 852–862.
- Havens, K. E., K.-R. Jin, N. Iricanin & R. T. James, 2007. Phosphorus dynamics at multiple time scales in the pelagic zone of a large shallow lake in Florida, USA. *Hydrobiologia* 581: 25–42.
- Hecky, R. E. & P. Kilham, 1988. Nutrient limitation of phytoplankton in freshwater and marine environments: a review of recent evidence on the effects of enrichment. *Limnology & Oceanography* 33: 796–822.
- Henry, R., M. G. Nogueira, M. L. M. Pompeo & V. Moschini-Carlos, 2006. Annual and short-term variability in primary productivity by phytoplankton and correlated abiotic factors in the Jurumirim Reservoir (São Paulo, Brazil). *Brazilian Journal of Biology* 66(1B): 239–261.
- Hillebrand, H., C. D. Dürselen, D. Kirschtel, U. Pollinger & T. Zohary, 1999. Biovolume calculation for pelagic and benthic microalgae. *Journal of Phycology* 35: 403–424.
- Holm-Hansen, O. & E. W. Helbling, 1995. Técnicas para la medición de la productividad primaria en el fitoplancton. In Alveal, K., M. E. Ferrario, E. C. Oliveira & E. Sar (eds), *Manual de Métodos Ficológicos*. Concepción, Chile: 329–350.
- Irigoién, X. & J. Castel, 1997. Light limitation and distribution of chlorophyll pigments in a highly turbid estuary: the Gironde (SW France). *Estuarine, Coastal and Shelf Science* 44(4): 507–517.
- Izaguirre, I., L. Allende, R. Escaray, J. Bustingorry, G. Pérez & G. Tell, 2012. Comparison of morpho-functional phytoplankton classifications in human-impacted shallow lakes with different stable states. *Hydrobiologia* 698(1): 203–216.
- Kirk, J. T. O., 2011. *Light and Photosynthesis in Aquatic Ecosystems*, 3rd ed. Cambridge University Press, Cambridge.
- Knowlton, M. F. & J. R. Jones, 2000. Non-algal seston, light, nutrients and chlorophyll in Missouri reservoirs. *Lake and Reservoir Management* 16(4): 322–332.
- Kosten, S., M. Vernooij, E. Van Nes, M. D. L. A. González Sagrario, J. G. P. W. Clevers & M. Scheffer, 2012. Bimodal transparency as an Indicator for alternative state in South American lakes. *Freshwater Biology* 57: 1191–1201.
- Kruk, C., V. L. M. Huszar, E. T. H. M. Peeters, S. Bonilla, L. Costa, M. Lüring, C. S. Reynolds & M. Scheffer, 2010. A morphological classification capturing functional variation in phytoplankton. *Freshwater Biology* 55: 614–627.
- Lagomarsino, L., G. L. Pérez, R. Escaray, J. Bustingorry & H. E. Zagarese, 2011. Weather variables as drivers of seasonal phosphorus dynamics in a shallow hypertrophic lake (Laguna Chascomús, Argentina). *Fundamental and Applied Limnology/Archiv für Hydrobiologie* 178(3): 191–201.
- Llames, M. E., L. Lagomarsino, N. Diovisalvi, P. Fermani, A. Torremorell, G. Pérez, F. Unrein, J. Bustingorry, R. Escaray, M. Ferraro & H. Zagarese, 2009. The effects of light availability in shallow, turbid waters: a mesocosm study. *Journal of Plankton Research* 31: 1517–1529.
- Mao, J., Q. Chen & Y. Chen, 2008. Three-dimensional eutrophication model and application to Taihu Lake, China. *Journal of Environmental Sciences* 20(3): 278–284.

- Margalef, R., 1978. Life forms of phytoplankton as survival alternatives in an unstable environment. *Oceanologica Acta* 1: 493–509.
- Marker, A. F. H., A. Nusch, H. Rai & B. Riemann, 1980. The measurement of photosynthetic pigments in freshwater and standardization of methods: conclusions and recommendations. *Arch Hydrobiol Beih* 14: 91–106.
- Naselli-Flores, L. & R. Barone, 2012. Phytoplankton dynamics in permanent and temporary Mediterranean waters: is the game hard to play because of hydrological disturbance? *Hydrobiologia* 698(1): 147–159.
- Padisák, J., L. O. Crossetti & L. Naselli-Flores, 2009. Use and misuse in the application of the phytoplankton functional classification: a critical review with updates. *Hydrobiologia* 621: 1–19.
- Phlips, E. J., M. Cichra, F. J. Aldridge, J. Jembeck, J. Hendrickson & R. Brody, 2000. Light availability and variations in phytoplankton standing crops in a nutrient-rich blackwater river. *Limnology & Oceanography* 45(4): 916–929.
- Platt, T., C. L. Gallegos & W. G. Harrison, 1980. Photoinhibition of photosynthesis in natural assemblages of marine phytoplankton. *Journal of Marine Research* 38: 687–701.
- Queimaliños, C. P., B. E. Modenutti & E. G. Balseiro, 1998. Phytoplankton responses to experimental enhancement of grazing pressure and nutrient recycling in a small Andean lake. *Freshwater Biology* 40(1): 41–49.
- Quirós, R., M. B. Boveri, C. A. Petrachi, A. M. Renella, J. J. Rosso, A. Sosnovsky & H. T. von Bernard, 2006. Los efectos de la agriculturización de sus lagunas. In Tundizi, J. G., T. Matsumura-Tundisi & C. SidagisGalli (eds), *Eutrofização na América do Sul: Causas, conseqüências e tecnologias de gerenciamento e controle*. International Institute of Ecology, São Carlos: 1–16.
- Rebsdorf, A., 1972. The Carbon Dioxide System of Freshwater. A Set of Tables for Easy Computation of Total Carbon Dioxide and Other Components of the Carbon Dioxide System. Freshwater Biological Laboratory, Hillerod.
- Reynolds, C. S., 1984. Phytoplankton periodicity: the interactions of form, function and environmental variability. *Freshwater Biology* 14: 111–142.
- Reynolds, C. S., V. Huszar, C. Kruk, L. Naselli-Flores & S. Melo, 2002. Towards a functional classification of the freshwater phytoplankton. *Journal of Plankton Research* 24(5): 417–428.
- Sakshaug, E., A. Bricaud, Y. Dandonneau, P. G. Falkowski, D. A. Kiefer, L. Legendre, A. Morel, J. Parslow & M. Takahashi, 1997. Parameters of photosynthesis: definitions, theory and interpretation of results. *Journal of Plankton Research* 19(11): 1637–1670.
- Salmaso, N. & J. Padisák, 2007. Morpho-functional groups and phytoplankton development in two deep lakes (Lake Garda, Italy and Lake Stechlin, Germany). *Hydrobiologia* 578: 97–112.
- Sánchez, M. L., G. L. Pérez, I. Izaguirre & H. Pizarro, 2013. Influence of underwater light climate on periphyton and phytoplankton communities in shallow lakes from the Pampa plain (Argentina) with contrasting steady states. *Journal of Limnology* 72(1): e6.
- Schallenberg, M. & C. W. Burns, 2004. Effects of sediment resuspension on phytoplankton production: teasing apart the influences of light, nutrients and algal entrainment. *Freshwater Biology* 49(2): 143–159.
- Scheffer, M., 1998. *Ecology of Shallow Lakes*. Kluwer Academic Publishers, Dordrecht.
- Scheffer, M. & E. H. van Nes, 2007. Shallow lakes theory revisited: various alternative regimes driven by climate, nutrients, depth and lake size. *Hydrobiologia* 584: 455–466.
- Smith, R. E. H. & J. Kalff, 1982. Size-dependent phosphorus uptake kinetics and cell quota in phytoplankton. *Journal of Phycology* 18(2): 275–284.
- Squires, M. M., L. F. W. Lesack, R. Hecky, S. J. Guildford, P. Ramlal & S. N. Higgins, 2009. Primary production and carbon dioxide metabolic balance of a lake-rich Arctic river floodplain: partitioning of phytoplankton, epipelton, macrophyte, and epiphyton production among lakes on the Mackenzie Delta. *Ecosystems* 12: 853–872.
- Steeman-Nielsen, E., 1952. The use of radiocarbon (^{14}C) for measuring organic production in the sea. *Journal du Conseil International pour l'Exploration de la Mer* 18: 117–140.
- Sturner, R. W., J. J. Elser, E. J. Fee, S. J. Guildford & T. H. Chrzanowski, 1997. The light:nutrient ratio in lakes: the balance of energy and materials affects ecosystem structure and process. *The American Naturalist* 150(6): 663–684.
- Sun, J. & D. Liu, 2003. Geometric models for calculating cell biovolume and surface area for phytoplankton. *Journal of Plankton Research* 25: 1331–1346.
- ter Braak, C. J. F. & P. Šmilauer, 2002. *CANOCO reference manual and Cano Draw for Windows User's Guide: Software for Canonical Community Ordination (Version 4.5)*. Microcomputer power, New York.
- Tilman, D., S. S. Kilham & P. Kilham, 1982. Phytoplankton community ecology: the role of limiting nutrients. *Annual Review of Ecology and Systematics* 13: 349–372.
- Tolotti, M., E. Rott, H. Thies & R. Psenner, 2005. Functional species groups of phytoplankton in relation to lake restoration: a long term study of Piburger See, Austria. *Verhandlungen der Internationalen Vereinigung für theoretische und angewandte Limnologie* 29: 891–894.
- Torremorell, A., M. E. Llames, G. Pérez, R. Escaray, J. Bustingorry & H. Zagarese, 2009. Annual patterns of phytoplankton density and primary production in a large, shallow lake: the central role of light. *Freshwater Biology* 54: 437–449.
- Utermöhl, H., 1958. Zur Vervollkommnung der quantitativen Phytoplankton Methodik. *Mitteilungen Internationale Vereinigung Limnologie* 9: 1–38.
- Venrick, E. L. (1978) How many cells to count? . In Sournia, A. (ed), *Phytoplankton manual*. UNESCO, Paris: 167–180.
- Vincent, W. & J. Laybourn-Parry, 2008. *Polar Lakes and Rivers*. Oxford University Press, Oxford: 364 pp.
- Yamaguchi, H., N. Hirade, K. Higashizono, K. Tada, K. Kishimoto, K. Oyama & K. Ichimi, 2015. Light and nutrient limitation on phytoplankton production in the strait of an enclosed coastal sea (Bisan Strait, eastern Seto Inland Sea, Japan). *Journal of Sea Research* 103: 75–83.