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ORIGINAL ARTICLE

High chlorophyll *a* concentration in a low nutrient context: discussions in a subtropical lake dominated by Cyanobacteria

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ABSTRACT

Temporal variability in some water quality parameters can play an important role in determining the presence and abundance of primary producers, and consequently in the trophic state and other characteristics and uses of lake ecosystems. In this sense, the present study aimed at understanding temporal dynamics of some trophic relevant water quality parameters in different time scales and their correlation and influence in phytoplankton biomass (chlorophyll a*) in a shallow subtropical coastal lake. Peri Lake is located in Florianópolis island in Southern Brazil and samples were taken monthly between March 2007 and February 2013. The lake showed low dissolved nutrients concentration, especially phosphorus (P) (median dissolved P: 2.0 µg L^{-1}) and high chlorophyll* a *(median: 20.8 µg L^{-1}) concentration. Total nitrogen (TN) concentration varied broadly, with a median of 672.8 µg L^{-1}, and total P (TP) concentration was low (median: 13.5 µg L^{-1}). A seasonal pattern of variation concerning dissolved and total P and chlorophyll* a *concentration was observed, associated mainly with temperature and wind speeds, but no clear pattern was observed for nitrogen (N) fractions. Significant differences were observed in different years for some parameters, with higher chlorophyll* a *and lower N concentration in the last three years sampled. The lake was considered potentially P limited during the majority of the study period and a positive correlation was found between chlorophyll* a *and total and dissolved P concentration. Phytoplankton biomass (as chlorophyll* a*) was apparently controlled by water temperature and P availability (TN:TP ratio and dissolved P). Water transparency (as Secchi depth) was strongly and negatively influenced by chlorophyll* a *concentration.* Cylindrospermopsis raciborskii *abilities to compete for P and light seem to be important factors determining its success and dominance in this low P coastal ecosystem. The fluctuating P supply, probably associated to sediment resuspension by wind in this shallow waterbody, is an advantageous factor for cyanobacteria and has an important role in chlorophyll* a *dynamics. Thus, high chlorophyll* a *concentration in this subtropical lake seems to be related to the P-limited condition, shallowness and low water column transparency, which are probably favouring the dominance of* C. raciborskii*, especially in higher summer temperatures, and leading to high chlorophyll* a *concentration even in a low dissolved nutrient environment.*

Key words: Cylindrospermopsis*; subtropical lake; phosphorus limitation; chlorophyll* a*; water quality; Southern Brazil.*

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INTRODUCTION

The most important chemical elements affecting trophic state are nitrogen (N) and phosphorus (P), what makes very important the evaluation of the availability and quality of long-term records of these changes in trends and rates (Heathwaite *et al.*, 1996). Total N (TN) and total P (TP) pools in freshwaters can include a complex set of chemical fractions that may vary in their availability for biological uptake. In spite of that, the TN:TP ratio can be a good proxy for the relative availability of these two important elements to the biota over ecologically meaningful time and space scales (Sterner, 2008).

TN:TP ratios have been commonly used to infer phytoplankton nutrient limitation in experimental and field studies (Chislock *et al.*, 2014; Figueredo *et al.*, 2014). The exact ratio at which either nutrient becomes limiting may vary among lakes as well as within lakes, depending on the phytoplankton community that is present, and therefore, a range of N:P ratios have been proposed to classify the nutrient limitation status of lakes (Abell *et al.*, 2010). On the other hand, reviews and studies indicate that the molar Redfield ratio of 16:1 can be used for inferring potential nutrient limitation (Klausmeier *et al.*, 2004; Abell *et al.*, 2010; Loladze and Elser, 2011). Klausmeier *et al.* (2004) suggested that field surveys should focus less on average values and more on the variation in particulate and dissolved nutrient ratios, by using higher spatial and temporal resolution and presenting the range of values observed rather than just averages. That is what we attempted in the present study.

Concerning the discussion of which nutrient is predominantly limiting in freshwater ecosystems, 'The Phosphorus Limitation Paradigm' states that P generally controls biological primary production in oligotrophic lakes over multi-annual time scales, but co-limitation by multiple factors would be the rule in most lakes over shorter time scales (Sterner, 2008). In a review concerning global nutrient cycles, Arrigo (2005) highlighted that co-limitation of primary producers by multiple resources can occur in some parts of the world's ocean, and that this phenomenon is most commonly observed in oligotrophic systems.

Cyanobacteria can be an important component of the primary producers in freshwater ecosystems. *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya and Subba-Raju is a highly adaptive bloom forming cyanobacteria capable of producing toxins and commonly observed over a wide range of ecological conditions, that has been expanding its global distribution from the tropics toward temperate climates (Padisák, 1997; Hamilton *et al.*, 2005; Stuken *et al.*, 2006; Vidal and Kruk, 2008). The migration of *C. raciborskii* toward more southern latitudes in South America is relatively recent, and demonstrates the wide tolerance, plasticity and success of the species in subtropical climates (Piccini *et al.*, 2011).

In this context, Peri Lake is a freshwater subtropical system with low P concentration and dominance of *C. raciborskii*. Understanding the characteristics that allow high chlorophyll *a* concentration and cyanobacteria dominance in this low nutrient fragile ecosystem is important before more intensive *C. raciborskii* blooms occur and, more importantly, before the occurrence of toxin release, which would affect the entire biota and ecological equilibrium in the lake, as well as the water supply to thousands of people. Moreover, understanding the processes underlying nutrient cycles in freshwater ecosystems are particularly important in the face of increasing anthropogenic nutrient release and climate change (Arrigo, 2005).

In this sense, the present study aimed at understanding temporal dynamics of some trophic relevant water quality parameters in different time scales and their correlation and influence in phytoplankton biomass (chlorophyll *a*) in a subtropical coastal lake ecosystem (Peri Lake) over a 6-years period. Our hypotheses were: i) water quality parameters will show a seasonal pattern of variation because of the subtropical location of the lake, but no significant variation among years, since the watershed is inside a protected/preserved area; ii) phytoplankton biomass (as chlorophyll *a*) will be constantly limited by P, reflecting in high N:P ratios; iii) chlorophyll *a* will be controlled by P availability and water temperature.

METHODS

Study area

Peri Lake is located in Santa Catarina State, Southern Brazil (27°44'S and 48°31'W). It has a surface area of 5.07 km² surrounded by mountains covered by Atlantic Rain Forest and sandy Restinga (forest formation typical of sandy coastal plains and dunes), and almost the entire drainage basin is within a conservation area (Municipal Park) with relatively low human influence since 1981. Peri Lake is considered a coastal lagoon due to the geographic location and geological origin, but presents some features that are quite different from other coastal lagoons worldwide, such as a maximum depth of 11.0 m, an average depth of approximately 4.2 m, and no sea water influence (freshwater). It is a polymictic water body, the water column is well oxygenated, and presents a relative spatial homogeneity (vertically and horizontally) concerning water quality features (Hennemann and Petrucio, 2011). Since 2000, the lake supplies potable water for approximately 100,000 people. The climate in the area is characteristically subtropical (Köppen-Geiger *cfa* – Kottek *et al.*, 2006), with rainfall and winds well distributed along the year, but relatively more frequent and stronger in spring and summer months (September-February).

Studies developed intermittently in the last 18 years have shown that phytoplankton in Peri Lake is dominated most of the year by the potentially toxic cyanobacterium *Cylindrospermopsis raciborskii*, and that its density and dominance are increasing (Laudares-Silva, 1999; Tonetta *et al.*, 2013). In monthly samplings in 2009-2010, Tonetta *et al.* (2013) found *C. raciborskii* densities between 11,074 and 231,886 ind. mL^{-1} (mean: 86,734 ind. mL^{-1}), with dominance ranging between 33% and 96% (mean: 73%).

Sampling and analysis

The present study consisted of monthly sampling of physical, chemical and biological parameters in four depths in one central site (≈9.0 m depth): surface (≈0.1 m), Secchi depth (≈1.0 m), photic zone limit (≈3.0 m) and aphotic zone (≈6.0 m). Samples were taken during 72 months (March 2007 to February 2013), using a 3 L van Dorn bottle. A map of Peri Lake watershed and the location of the central site sampled in the present study can be seen in Hennemann *et al.* (2015). Transparency (with a Secchi disk), pH, dissolved oxygen (DO) and water temperature (WTemp) were measured *in situ* with specific probes (YSI and WTW). Climate data (air temperature, wind speed and rainfall) was provided by ICEA (*Instituto de Controle do Espaço Aéreo*) from the Defence Ministry, Brazilian Federal government, taken from the Florianópolis airport station (5.5 km from Peri Lake). Dissolved inorganic nitrogen (DIN) was determined as the sum of nitrite (N-NO_2^- - Golterman *et al.*, 1978), nitrate (N-NO_3^- - Mackereth *et al.*, 1978) and ammonium (N-NH_4^+ - Koroleff, 1976); inorganic phosphorus was measured as soluble reactive phosphorus (SRP - Strickland and Parsons, 1960); total phosphorus and nitrogen (TP and TN - Valderrama, 1981) were also determined. Detection limit was around 1.0 µg L^{-1} for all methods. Nutrients were measured in laboratory from filtered and unfiltered frozen water samples kept in polyethylene bottles at -20°C. Chlorophyll *a* (Chl-*a*) concentration was obtained by filtering 500 mL water samples through glass fibre filters Millipore AP40 followed by extraction with 90% acetone according to the method and equations described by Lorenzen (1967).

In order to work with six complete datasets of 12 months to compare among years variation, we considered December 2007, January 2008 and February 2008 as summer 2007; December 2008, January 2009 and February 2009 as summer 2008, and so on. Differences among seasons and years were tested by Kruskal-Wallis analysis of variation, followed by multiple comparisons. Spearman's correlation was also applied between all variables, including climate data of the seven days previous to sampling dates. General Linear Model (GLM) was applied with Chl-*a* concentration as dependent variable, and physical, chemical and climate parameters as continuous predictors. Statistical analyses and graphs were made in the software Statistica 7® (StatSoft) and Microsoft Excel 2007®.

RESULTS

Dynamics of trophic relevant parameters

Monthly rainfall varied between 0 mm and 478 mm, with a mean of 112 mm/month in the 2007-2012 period, and accumulated year precipitation ranged between less than 1000 mm in 2008 and 2012, and around 2000 mm in 2010 and 2011. Air temperature varied according to the subtropical climate between 0.0°C and 38.6°C during the study period, and mean annual temperature gradually increased from 2008 (19.8°C) to 2012 (21.4°C) (Fig. 1).

The four sampled depths did not show clear significant differences concerning the parameters analyzed (water column was well mixed), so the four depths were grouped in monthly means for the statistical analysis. Water temperature (Fig. 2a) varied seasonally (summer > fall > spring > winter), with all seasons significantly differing from each other (P<0.01). Dissolved oxygen (DO) was significantly higher in winter (Fig. 2a), and WTemp and DO varied inversely to each other, as expected because of lower O_2 solubility in water with increasing WTemp. Water column was always well oxygenized. Water pH (Fig. 2b) remained close to neutrality and did not varied among years, but it was significantly higher in spring when compared to fall and winter (P <0.01). Secchi depth (Fig. 2b) remained around 1.0 m most of the time and showed a decrease in 2011 and 2012 (≈0.8m) (P <0.01). Total P (Fig. 3a) varied from 4.0 to 39.1 μg L^{-1} (median: 13.5 μg L^{-1}) and showed higher concentration in summer months when compared to fall (P<0.01). In spite of the ten times difference between minimum and maximum values, TP remained around the median in most months sampled in 2007-2012. Total P was positively correlated with Chl-*a* and negatively with rainfall (Tab. 1). Median TN (Fig. 3a) was 672.8 μg L^{-1}, ranging from 90.6 and 1752.6 μg L^{-1}. No clear seasonal pattern of variation was observed for TN, but it varied significantly among years

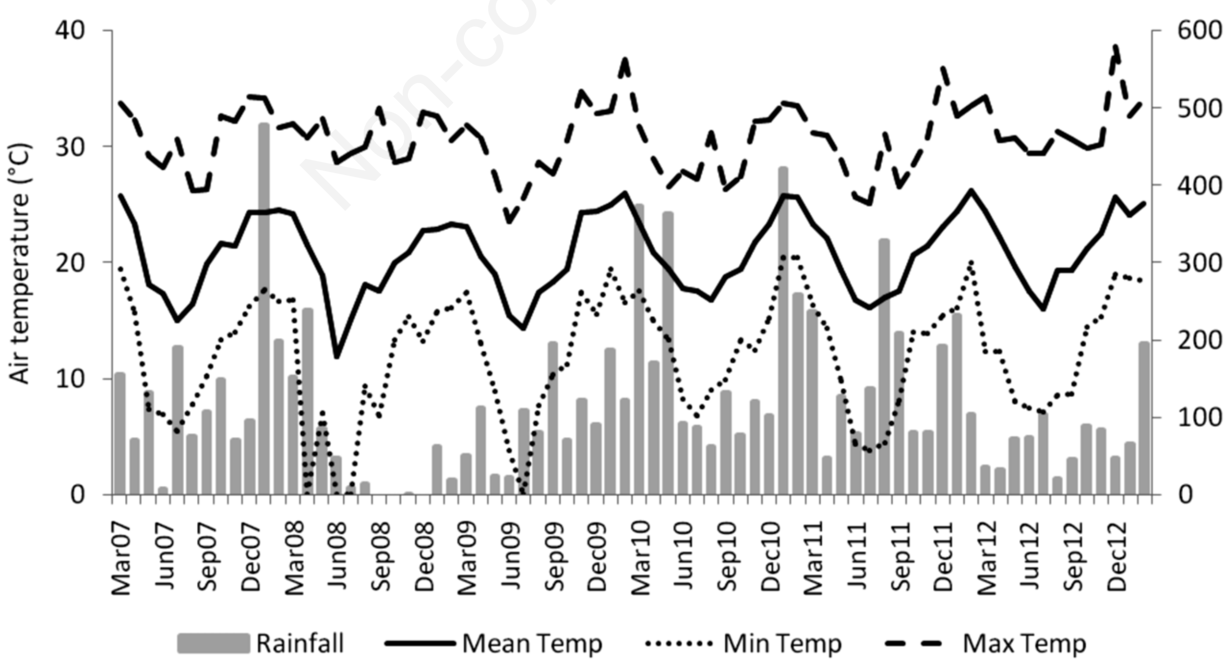

Fig. 1. Monthly climatic variables (rainfall and minimum, maximum and mean air temperature) in Peri Lake along the study period (March 2007-February 2013). Data provided by ICEA - Ministry of Defence - Brazilian Government.

(P <0.01), with lower concentration in 2010-2011 than in 2007-2009 and 2012.

Dissolved nutrients concentration were very low in general, and showed no clear seasonal pattern of variation, except for SRP, which showed a tendency of higher concentration in summer months. SRP (Fig. 3b) varied between undetectable and 10.8 μg L^{-1} (median: 2.0 μg L^{-1}), with considerable variation among months and years. A positive correlation was observed between SRP and Chl-*a* concentration, wind speeds and Wtemp, but a negative correlation was found with N-NH_4^+ (Tab. 1). Concerning dissolved N fractions, the broad monthly variation in the N-NH_4^+ (Fig. 3b) concentration (from undetectable to 96.5 μg L^{-1}; median: 11.1 μg L^{-1}), resulted in non-significant variation among years and seasons, except for lower values in 2012 (P<0.01). Nitrate (Fig. 3c) varied from undetectable to 26.7 μg L^{-1} (median: 6.4 μg L^{-1}) and showed lower concentration in 2008 and 2012 in comparison to 2009 and 2010 (P <0.05). Nitrate varied considerably among months, but spring concentration was significantly lower than other seasons (P<0.01). Nitrate was negatively correlated with wind (Tab. 1). Nitrite (Fig. 3c) had very low concentration (median: 0.4 μg L^{-1}, range: undetectable to 1.3 μg L^{-1}) and can be considered negligible in the N cycle in Peri Lake for the biota.

In spite of the low dissolved nutrients and TP amounts, Chl-*a* concentration (Fig. 4) was high, varying from 4.3 μg L^{-1} to almost 60.0 μg L^{-1} (median: 20.8 μg L^{-1}), and

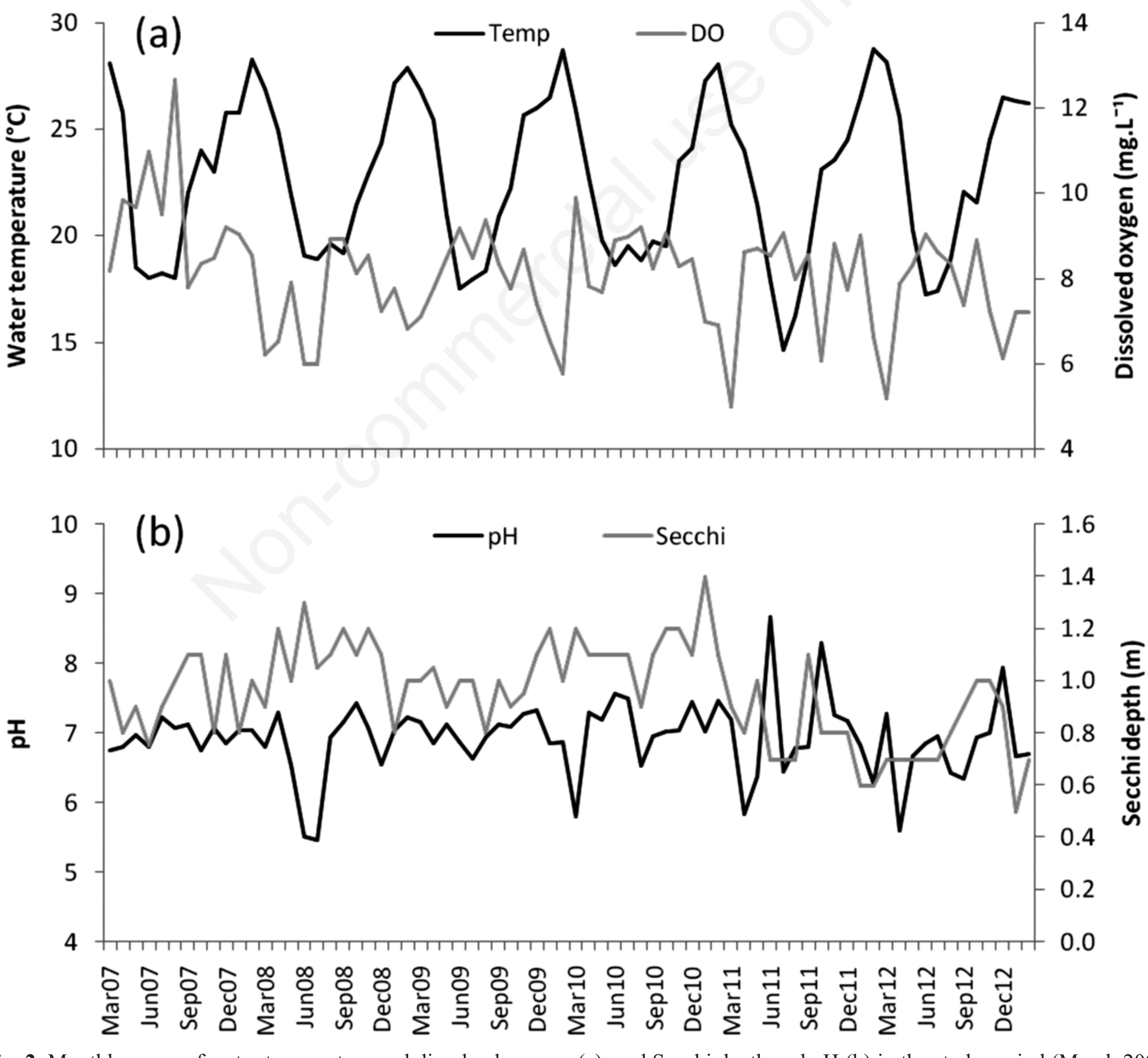

Fig. 2. Monthly mean of water temperature and dissolved oxygen (a), and Secchi depth and pH (b) in the study period (March 2007-February 2013).

showed the expected pattern of higher concentration in summer when compared to the other seasons ($P<0.01$). The last years sampled (2011 and 2012) had higher concentration ($P<0.01$) than previous years, and 2007 was significantly lower than all sampled years. Additionally, Chl-*a* was negatively correlated to N-NH_4^+, Secchi depth and DIN:SRP.

Nutrient limitation and chlorophyll-a relationships

TN:TP molar ratio in Peri Lake (Fig. 4) varied from 9 to 451 (median: 105). Higher values were observed in 2007, 2009 and 2012 ($P<0.05$), decreasing in 2008 and especially in 2010 and 2011. A tendency of lower TN:TP ratios could be observed in summer months. On the other hand, when the ratio for dissolved nutrients DIN:SRP is considered (Fig. 4), the median ratio decreases considerably to 20, ranging from 1 to 482. The year 2012 showed significantly lower DIN:SRP than the previous years ($P<0.01$).

Results from the GLM were significant ($F=7.237$; $R^2=0.60$; adjusted $R^2=0.51$; $P=0.000$) with transparency (Secchi depth), Wtemp and TN:TP ratio as the stronger most significant predictors of Chl-*a* variation (Tab. 2).

DISCUSSION

The results found in the present study show that water quality parameters present considerable temporal variation, some of them showing clear seasonal patterns and others showing yearly variation. The importance of Wtemp and P in directly controlling Chl-*a* concentration in Peri Lake was demonstrated. High Chl-*a* concentration causes low transparency in the water column. N:P ratios showed considerable temporal fluctuations, but TN:TP were higher than 50 the majority of the sampling period, indicating a prevailing condition of P limitation of primary producers.

Dynamics of trophic relevant parameters

Water temperature, DO and pH were influenced by seasonal air temperature variation, with Wtemp closely following air temperature, lower DO concentration in higher temperatures, and higher pH in the warmer most active growing season (spring), probably associated to higher CO_2 production. Lower DO was observed in periods of high Chl-*a*, what can also be a consequence of higher consumption by respiration processes. Previous studies in Peri Lake showed that the seston is predominantly composed by organic particles and living cells (Laudares-Silva, 1999), which explains the negative correlation found between Secchi depth and Chl-*a*, and the lower transparency values observed in the last years sampled, when higher Chl-*a* concentration was also detected.

Concerning total nutrients, high and low TP concentrations observed in some occasions could be related to winds and rainfall previously to sampling dates (Tonetta *et al.*, 2013). The tendency of higher TP concentration in summer is probably also related to higher wind speed and frequency usually observed in this season, as well as to the higher phytoplankton biomass and P storage inside the cells; this explanation is reinforced by the positive correlation between TP and Chl-*a*. Negative correlation between TP and rainfall can be a consequence of the dilution effect of rainfall water over the already low TP concentration, since the preserved watershed would not significantly contribute to P-inputs to the lake. Concentration of

Tab. 1. Spearman's correlation between chlorophyll-a, nutrients and climate data in Peri Lake during the study period (March 2007-February 2013). Marked correlations (underlined) are significant at $P<0.01$.

	Chl	TP	TN	SRP	NO_2^-	NO_3^-	NH_4^+	TN:TP	DIN:SRP	Temp	pH	DO	Secchi	Wind	Rain
Chl	1.00														
TP	0.26	1.00													
TN	0.02	0.04	1.00												
SRP	0.24	0.10	0.05	1.00											
NO_2^-	-0.64	-0.11	0.29	-0.05	1.00										
NO_3^-	0.07	0.16	0.04	0.15	-0.03	1.00									
NH_4^+	-0.32	0.09	0.07	-0.15	0.29	0.17	1.00								
TN:TP	-0.14	-0.51	0.80	0.00	0.26	-0.07	0.03	1.00							
DIN:SRP	-0.25	0.03	-0.06	-0.73	0.14	0.22	0.70	-0.07	1.00						
W temp	0.32	0.02	0.07	0.18	-0.05	0.04	0.07	0.05	0.02	1.00					
pH	-0.11	-0.11	-0.21	-0.24	0.06	0.06	0.20	-0.09	0.30	0.09	1.00				
DO	-0.27	-0.19	-0.03	-0.13	0.08	-0.04	0.03	0.08	0.05	-0.48	-0.07	1.00			
Secchi	-0.43	-0.07	-0.36	-0.01	0.40	0.05	0.19	-0.31	0.16	0.05	0.18	-0.16	1.00		
Wind	0.08	0.03	0.07	0.34	0.11	-0.16	-0.08	0.07	-0.25	-0.04	0.03	-0.02	0.04	1.00	
Rain	0.13	-0.24	-0.13	-0.04	-0.26	0.07	-0.18	0.02	-0.05	0.09	0.11	0.03	-0.09	-0.21	1.00

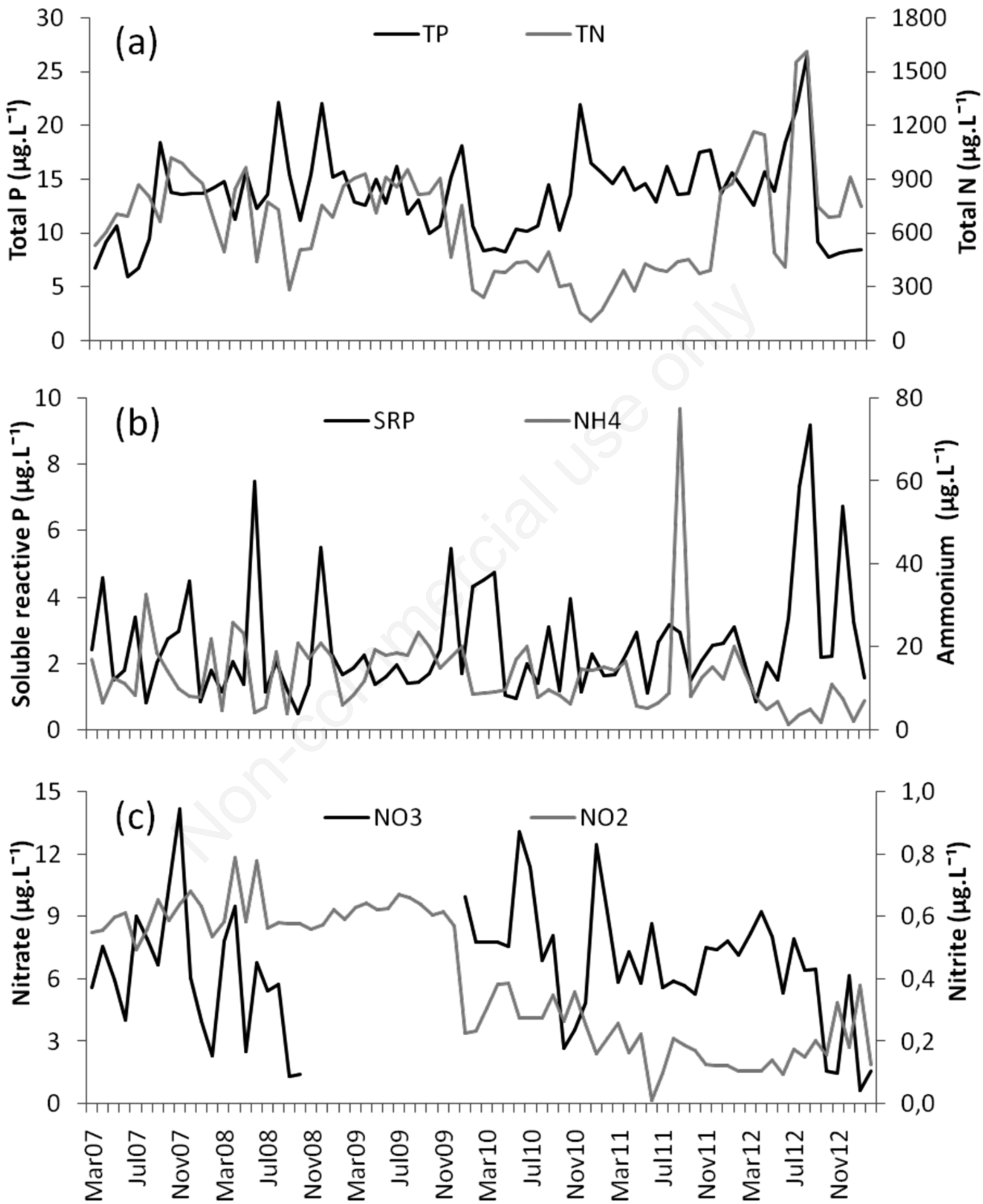

Fig. 3. Monthly mean of TP and TN (a), SRP and N-NH_4^+ (b), N-NO_3^- and N-NO_2^- (c) concentration (µg L^{-1}) for the period of March 2007-February 2013 in Peri Lake.

TP similar to the observed in Peri Lake is usually found in relatively protected tropical and subtropical lakes, considered oligotrophic and showing low Chl-*a* concentration, such as Cabiúnas Lagoon (Marotta *et al.*, 2010), Lake Annie (Torres *et al.*, 2012) and some reservoirs in Australia (Burford *et al.*, 2007). This becomes clearer when we observe datasets with several lakes around the world, such as the one provided by Solomon *et al.* (2013). Most subtropical shallow lakes show much higher TP concentration (James *et al.*, 2009; Fabre *et al.*, 2010; Andrade *et al.*, 2012).

In relation to TN concentration, the years of 2010 and 2011 showed periods of high rainfall, what could have had a diluting effect in TN concentration, leading to lower concentration of this nutrient in the water. A low rainfall period followed in 2012, which lowered lake water level, could also have influenced in the higher TN concentration observed in the lake this year. The lack of seasonal pattern for TN is probably associated to high variance among months and decoupling with temperature and Chl-*a*, differently from TP. Total N concentration similar to Peri Lake is usually found in more eutrophic lakes, with higher TP concentration, but with similar Chl-*a* amounts (Solomon *et al.*, 2013).

Low dissolved nutrients concentration and lack of a clear seasonal pattern of variation in general (except for SRP) could be associated to the relatively well distributed rainfall along the year. However, rainfall can vary broadly among months and even years, which could be also a factor influencing in temporal variation in dissolved nutrients concentration in the water column. The low dissolved nutrients concentration observed in the lake may be related to high recycling rates, the well oxygenated water column and high assimilation by the phytoplankton and bacterial communities, which result especially in low nitrate and SRP concentrations (Hennemann and Petrucio, 2011). Lack of significant point and non-point sources of pollution associated with the preserved watershed also contribute with this low dissolved nutrients condition. The tendency of higher SRP observed in summer months can be related to stronger and more constant winds in this season, what is corroborated by the positive correlation ($P<0.01$) found between SRP and wind. A positive correlation was also observed between SRP and Chl-*a* concentration in Peri Lake along the present long term study, what is expected in water bodies limited by P. The positive correlation between total and dissolved P fractions and Chl-*a* means that when there is a higher availability of dissolved P (increasing SRP in the water column), part of it is rapidly assimilated by phytoplankton, increasing both Chl-*a* and TP. Variation in N-NH_4^+ could not be directly related to any other parameter, except in the last seasons

Tab. 2. General Linear Model (GLM) results, with Chl-*a* as dependent variable. Multiple R^2=0.596; adjusted R^2=0.513; P=0.00000.

	beta	F	P
Intercept		4.946	0.030
TN:TP ratio	−0.570	4.868	0.031
Water temperature	0.279	6.785	0.012
Secchi depth	−0.617	37.211	0.000

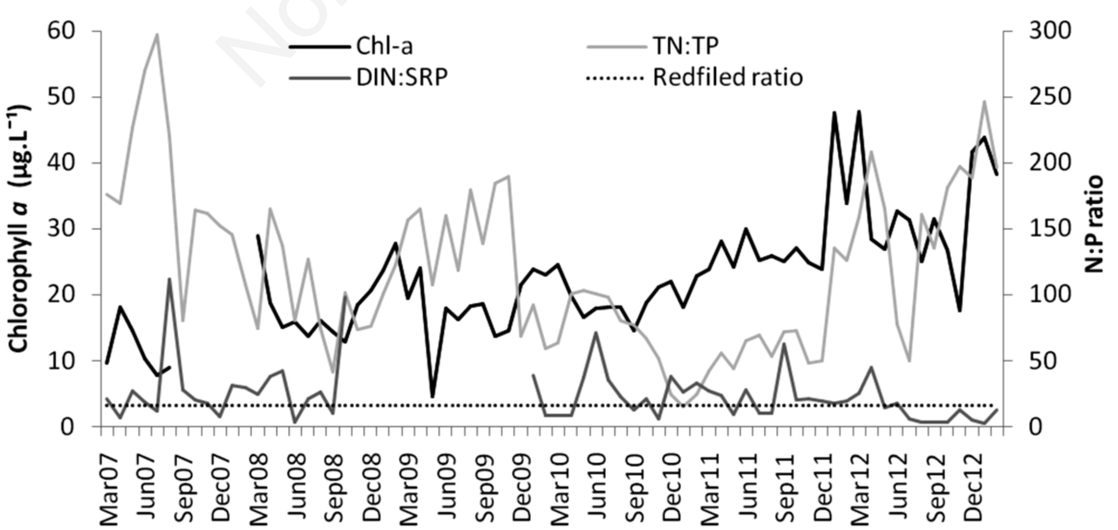

Fig. 4. Monthly mean of Chl-*a* concentration (µg L^{-1}) and TN:TP and DIN:SRP ratios for the period of March 2007-February 2013 in Peri Lake. The dashed line is the Redfield mass ratio (7.2:1).

sampled, in which $N\text{-}NH_4^+$ low concentration was followed by high Chl-*a* and though may be associated to consumption of $N\text{-}NH_4^+$ by primary producers. This hypothesis is reinforced by the negative correlation found between Chl-*a* and $N\text{-}NH_4^+$. Concerning $N\text{-}NO_3^-$ variation, spring is typically a high activity growing season for phytoplankton, after the cold temperatures and lower light availability in winter, and could have led to higher $N\text{-}NO_3^-$ consumption and its depletion in the water column.

Chlorophyll *a* demonstrated the expected seasonal pattern, positively correlated with water temperature and showed a gradual increase from 2007 to 2012. Since this pattern was not observed for nutrients and no important alteration was observed in the lake watershed in the last two decades, especially due to the fact that almost the entire lake watershed is preserved and within a conservation area (Municipal Park) with limited human influence, the increasing Chl-*a* concentration, especially in 2012, could be related to higher temperatures (mean annual temperature gradually increased from 2008 to 2012, from 19.8 to 21.4°C) and/or lower rainfall (a significant drop in the lake water level could be observed in its margins throughout 2012). Chl-*a* was also negatively correlated with DIN:SRP, which means that when there is a higher proportion of SRP, phytoplankton primary production is stimulated. Chlorophyll-*a* concentration higher than 20 $\mu g\ L^{-1}$ is usually observed in eutrophic and highly human influenced ecosystems (Huszar *et al.*, 2000; Torres *et al.*, 2012). Water bodies with Chl-*a* concentration similar to Peri Lake usually have much higher P concentration. In a study in five subtropical shallow lakes in Uruguay (characteristics similar to Peri Lake), TP concentration were considerably higher than Chl-*a* (Pacheco *et al.,* 2010) in comparison with the concentration observed in Peri Lake. Fragoso *et al.* (2011) also found Chl-*a* values similar to our study lake, but in much higher dissolved P concentration in a subtropical shallow lake in southern Brazil. Large subtropical shallow lakes in USA and China also show considerably higher TP concentration than Peri Lake, but similar Chl-*a* content (James *et al.*, 2009).

These high levels of Chl-*a* in low-P Peri Lake are difficult to explain, but can be associated to the dominance of *C. raciborskii* in the phytoplankton community, which is a superior competitor for nutrients and light, as will be further discussed in the next section, and to a low predation pressure, since the zooplankton community presents only 16 taxa and is dominated by rotifers, which was also attributed to the presence and dominance of filamentous Cyanobacteria (N.D. Gerzson, *unpublished data*). Results from Hennemann *et al.* (2015) show increasing nutrient accumulation in the sediments of Peri Lake in more recent times, which can mean that the system is becoming more eutrophicated, but these nutrients are being buried in the unsatured sediments, especially P, because of the well oxygenated water column. This explanation also contributes in the understanding of high N:P ratios in the lake, which are discussed below.

Nutrient limitation and chlorophyll a relationships

TN:TP molar ratio in Peri Lake showed a condition of potential P limitation during almost the entire period of study, considering the Redfield molar ratio of 16:1 (Redfield, 1958). This is in agreement with studies that say that freshwater oligotrophic ecosystems not subjected to pollution sources are usually P-limited (Downing and McCauley, 1992; Sterner, 2008).

In the present study, periods of lower TN:TP ratios, especially in summer, can be associated to P inputs from sediments resuspension, which are more intense in spring and summer, as previously discussed, and are the most likely explanation for the lower TN:TP ratios in warmer more windy periods. The dissolved nutrients ratio DIN:SRP showed a condition of potential light P limitation and probably co-limitation by N and P during most of the studied period. Significantly lower DIN:SRP in 2012 was a consequence of both an increase in SRP and a decrease in $N\text{-}NH_4^+$, and was accompanied by high Chl-*a*. Higher availability of SRP probably promoted phytoplankton growth and depletion of $N\text{-}NH_4^+$. Dissolved nutrients concentration (of both N and P) are so low in Peri Lake that both nutrients can be considered limiting most of the time indeed, although the positive correlation between Chl-*a* and P indicates that P-limitation is more intense and more important in the long term.

Variations in nutrient stoichiometry can be associated not only with different species composition in the phytoplankton community, but also with different cellular components, which have their own unique stoichiometric properties (Arrigo, 2005). In the case of Peri Lake, the great time variability in N:P ratios may also reflect periods of resource acquisition (high N:P) and periods of exponential growth with cellular assembly (low N:P). The high TN:TP ratios observed in the majority of the sampling period can mean that primary producers contain high proportion of resource-acquisition machinery inside their cells and that the community has achieved a state of competitive equilibrium (Klausmeyer *et al.*, 2004).

Another possible explanation for the high TN:TP ratios found in Peri Lake comes from Elser *et al.* (2009), that suggested that enhanced N inputs from the atmosphere during the past several decades of human industrialization and population expansion appear to have produced regional phytoplankton P limitation, and are favouring those relatively few species that are best able to compete for the limiting P. Indeed, Hennemann *et al.* (2015) showed that paleolimnological records indicate increased N deposition in Peri Lake in recent decades, which could be reflecting in the dominance and high densities of *C. raciborskii* observed in the system.

The success of *C. raciborskii* has being attributed to several intrinsic competitive factors, including a high P storage capacity and a high-affinity cellular P uptake system (Istvánovics *et al.*, 2000). Additionally, it has been demonstrated that the species has the ability to grow under conditions of P-limitation that are already limiting to other Cyanobacteria (Jensen *et al.*, 1994; Padisák, 1997). Posselt *et al.* (2009) showed that dominance of this cyanobacterium can be favoured in lakes with fluctuating P supply, and Amaral *et al.* (2014) recently demonstrated growth optimization of phosphate-deficient *C. raciborskii* to short-term nutrient fluctuations in P supply, which was attributed to its physiological flexibility. *Cylindrospermopsis raciborskii* tolerance to low light levels (Briand *et al.*, 2004), the capacity to fix atmospheric N_2 (Moisander *et al.*, 2012), phenotypic plasticity concerning pigments, size and growth rates (Bonilla *et al.*, 2012), together with the competitive advantages concerning P mentioned above, probably have contributed to the success of this species, maintaining high Chl-*a* concentration in the low nutrient context (specially P) of Peri Lake. According to Amaral *et al.* (2014), the adaptive behavior of this species may help to explain its invasive success in a wide range of aquatic ecosystems where P is frequently the limiting resource. Presence and dominance of *C. raciborskii* could even be contributing to the ongoing state of P limitation observed in Peri Lake. A more detailed discussion on *C. raciborskii* dominance in low light and low-P conditions in Peri Lake can be found in Tonetta *et al.* (2015). Similarly to Peri Lake, subtropical Lakes Javier and Leandro in Uruguay are coastal shallow lakes limited by light and P, showing co-dominance by *C. raciborskii* and other colonial Cyanobacteria (Fabre *et al.*, 2010). In a review in temperate German lakes, Dolman *et al.* (2012) also found that *C. raciborskii* reached higher bio-volumes in lakes with high N relative to P concentrations. In tropical lake of Lagoa Santa (Brazil), a persistent bloom of *C. raciborskii* also occurs under low P availability (Figueredo and Giani, 2009).

Concerning GLM results, the negative influence of TN:TP ratio on Chl-*a* concentration corroborates the previous discussion, since lower N:P ratios indicate a higher P availability and lead to higher phytoplankton biomass, especially in a P limited environment such as Peri Lake. The negative relationship with Secchi depth (transparency) is probably a consequence of the fact that high Chl-*a* concentration in the lake causes low water transparency, and not the opposite. Additionally, lower transparency was observed in periods of lower rainfall (e.g. 2012), which resulted in lower water levels in the lake and higher concentration of phytoplankton biomass. Lower light availability could also be favouring dominance of *C. raciborskii* as previously mentioned. Temperature showed a positive influence in Chl-*a* concentration; in fact, cyanobacteria usually grow better at higher temperatures (Huszar *et al.*, 2000; Paerl and Huisman, 2008), such as the ones observed in Peri Lake during summer. Although Lürling *et al.* (2012) showed that chlorophytes growth rates are similar or even higher than cyanobacteria in temperature experiments, their results also showed that *C. raciborskii* had higher growth rates in water temperature of 27.5°C, which is the mean water temperature observed in the hottest month in Peri Lake.

As already pointed out, P may generally control biological production in oligotrophic lakes over multi-annual time scales but co-limitation of multiple nutrients is probably the rule in most lakes over shorter time scales (Sterner, 2008). This seems to be the case in the oligotrophic Peri Lake ecosystem, since TN:TP over a longer time scale indicated P-limitation, while an analysis in shorter time periods and considering the dissolved nutrient fraction showed that co-limitation can occur relatively frequently. Time fluctuations observed in DIN:SRP ratio in Peri Lake are advantageous to *C. raciborskii*, since the species shows several adaptations to fluctuations in P supply, including tolerance to low nutrients concentration and capacity of rapid assimilation of available nutrients, as previously discussed.

It is quite clear in the literature that several aspects can influence nutrient cycling, dynamics and availability in aquatic ecosystems, including sediment characteristics, bacterial community, land-use patterns in the watershed, food web structure, oxygen concentration, physical characteristics of the lake, among others (Heathwaite *et al.*, 1996; Elser *et al.*, 2007). According to Heathwaite *et al.* (1996), even in the absence of a land-use change, nutrient transport and transformation can be affected by a combination of internal and external factors. The author showed an example according to which increasing inputs of atmospheric N from increased emissions from fossil fuel combustion combined with the decreasing nutrient requirement of forests in a more steady state could result in N leaching, even though major land-use change had not occurred. This could be the case in Peri Lake and together with the N_2-fixing capacity of Cyanobacteria could be contributing to the P-limited condition observed in the present study.

CONCLUSIONS

Peri Lake showed a seasonal pattern of variation concerning dissolved and total P and Chl-*a* concentration, associated mainly with temperature and wind speeds, but no clear pattern was observed for N fractions, partially confirming our first hypothesis. On the other hand, significant variations were observed among the years sampled, with the last three years (2010-2012) showing significant differences in comparison to the 2007-2009 period, especially in relation to Chl-*a* and N concentration. Climate differences among years, especially rainfall and air temperature, seem to be important factors influencing in this yearly variation.

The lake showed a condition of potential P limitation during most of the study period (second hypothesis) and a positive correlation between Chl-*a* and P. GLM showed that Chl-*a* seems to be controlled mainly by temperature and P availability, confirming our third hypothesis. Sediment resuspension by wind is probably an important P source, influencing in Chl-*a* patterns. *C. raciborskii* abilities to compete for the limiting P seem to be an important factor determining its success and dominance in the lake.

These conclusions have very important management consequences for coastal ecosystems such as Peri Lake, especially in the context of future global climatic changes associated with temperature elevation and alteration in rainfall and wind patterns, as well as increase in cultural eutrophication. Additional nutrient and temperature variation experiments with the phytoplankton community and research concerning sediment influence in the water quality dynamics of this shallow lake are important future studies to be conducted in order to better understand the dynamics of trophic relevant parameters and Cyanobacteria dominance and behaviour in low-P subtropical water bodies.

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REFERENCES

Abell JM, Özkundakci D, Hamilton DP, 2010. Nitrogen and phosphorous limitation of phytoplankton growth in New Zealand lakes: implications for eutrophication control. Ecosystems 13:966-977.

Amaral V, Bonilla S, Aubriot L, 2014. Growth optimization of the invasive cyanobacterium *Cylindrospermopsis raciborskii* in response to phosphate fluctuations. Eur. J. Phycol. 49:134-141.

Andrade CFF, Niencheski LFH, Attisano KK, Milani MR, 2012. [Fluxos de nutrientes associados às descargas de água subterrânea para a Lagoa Mangueira (Rio Grande do Sul, Brasil)].[Article in Portuguese]. Quim. Nova 35:5-10.

Arrigo KR, 2005. Marine microorganisms and global nutrient cycles. Nature 437:349-355.

Bonilla S, Aubriot L, Soares MCS, González-Piana M, Fabre A, Huszar VLM, Lürling M, Antoniades D, Padisák J, Kruk C, 2012. What drives the distribution of the bloom-forming cyanobacteria *Planktothrix agardhii* and *Cylindrospermopsis raciborskii*? FEMS Microb. Ecol. 79:594-607.

Briand J-F, Leboulanger C, Humbert J-F, Bernard C, Dufour P, 2004. *Cylindrospermopsis raciborskii* (cyanobacteria) invasion at mid-latitudes: selection, wide physiological tolerance, or global warming? J. Phycol. 40:231-238.

Burford MA, Johnson SA, Cook AJ, Packer TV, Taylor BM, Townsley ER, 2007. Correlations between watershed and reservoir characteristics, and algal blooms in subtropical reservoirs. Water Res. 41:4105-4114.

Chislock MF, Sharp KL, Wilson AE, 2014. *Cylindrospermopsis raciborskii* dominates under very low and high nitrogen-to-phosphorous ratios. Water Res. 49:207-214.

Dolman AM, Rucker J, Pick FR, Fastner J, Rohrlack T, Mischke U, Wiedner C, 2012. Cyanobacteria and cyanotoxins: the influence of nitrogen versus phosphorous. PLoS One 7:e38757.

Downing JA, McCauley E, 1992. The nitrogen:phosphorous relationship in lakes. Limnol. Oceanogr. 37:936-945.

Elser JJ, Andersen T, Baron JS, Bergström AK, Jansson M, Kyle M, Nydick KR, Steger L, Hessen DO, 2009. Shifts in lake N:P stoichiometry and nutrient limitation driven by atmospheric nitrogen deposition. Science 326:35-837.

Elser JJ, Bracken MES, Cleland EE, Gruner DS, Harpole WS, Hillebrand H, Ngai JT, Seabloom EW, Shurin JB, Smith JR, 2007. Global analysis of nitrogen and phosphorous limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecol. Lett. 10:1135-1142.

Fabre A, Carballo C, Hernández E, Píriz P, Bergamino L, Mello L, González S, Pérez G, León JG, Aubriot L, Bonilla S, Kruk C, 2010. [El nitrógeno y la relación zona eufótica/zona de mezcla explican la presencia de cianobacterias en pequenõs lagos subtropicales, artificiales de Uruguay].[Article in Spanish]. Panam. J. Aquat. Sci. 5:112-125.

Figueredo CC, Giani A, 2009. Phytoplankton community in the tropical lake of Lagoa Santa (Brazil): conditions favoring a persistent bloom of *Cylindrospermopsis raciborskii*. Limnologica 39:264-272.

Figueredo CC, Ruckert G, Cupertino A, Pontes MA, Fernandes LA, Ribeiro SG, Maran NRC, 2014. Lack of nitrogen as a causing agent of *Cylindrospermopsis raciborskii* intermittent blooms in a small tropical reservoir. FEMS Microbiol. Ecol. 87:557-567.

Fragoso Jr CR, Marques DMLM, Ferreira TF, Janse JH, van Nes EH, 2011. Potential effects of climate change and eutrophication on a large subtropical shallow lake. Environ. Model. Softw. 26:1337-1348.

Golterman HL, Clymo RS, Ohnstad MAM, 1978. Methods for physical and chemical analysis of freshwater. Blackwell Scientific Publ., Oxford: 213 pp.

Hamilton PB, Ley LM, Dean S, Pick FR, 2005. The occurrence of the cyanobacterium *Cylindrospermopsis raciborskii* in Constance Lake: an exotic cyanoprokaryote new to Canada. Phycologia 44:17-25.

Heathwaite AL, Johnes PJ, Peters NE, 1996. Trends in nutrients. Hydrol. Proces. 10:263-293.

Hennemann MC, Petrucio MM, 2011. Spatial and temporal dynamic of trophic relevant parameters in a subtropical coastal

lagoon in Brazil. Environ. Monit. Assess. 181:347-361.
Hennemann MC, Simonassi JC, Petrucio MM, 2015. Paleolimnological record as an indication of incipient eutrophication in an oligotrophic subtropical coastal lake in Southern Brazil. Environ. Monit. Assess. 187:513.
Huszar VLM, Silva LHS, Marinho M, Domingos P, Sant'Anna CL, 2000. Cyanoprokaryote assemblages in eight productive tropical Brazilian waters. Hydrobiologia 424:67-77.
Istvánovics V, Shafik HM, Presing M, Juhos S, 2000. Growth and phosphate uptake kinetics of the cyanobacterium, *Cylindrospermopsis raciborskii* (Cyanophyceae) in throughflow cultures. Freshwater Biol. 43:257-75.
James RT, Havens K, Zhu G, Qin B, 2009. Comparative analysis of nutrients, chlorophyll and transparency in two large shallow lakes (Lake Taihu, P.R. China and Lake Okeechobee, USA). Hydrobiologia 627:211-231.
Jensen P, Jeppesen E, Olrik K, Kristensen P, 1994. Impact of nutrients and physical factors on the shift from cyanobacterial to chlorophyte dominance in shallow Danish lakes. Can. J. Fish. Aquat. Sci. 51:1692-1699.
Klausmeier CA, Litchman E, Daufresne T, Levin S, 2004. Optimal nitrogen-to-phosphorous stoichiometry of phytoplankton. Nature 429:171-174.
Koroleff F, 1976. Determination of nutrients, p. 117-181. In: K. Grasshoff (ed.) Methods of sea water analysis. Verlag Chemie, Weinhein.
Kottek M, Grieser JC, Beck BR, Rubel F, 2006. World map of the Köppen-Geiger climate classification updated. Meteorol. Z. 15:259-263.
Laudares-Silva R, 1999. [Aspectos limnológicos, variabilidade espacial e temporal na estrutura da comunidade fitoplanctônica da Lagoa do Peri, Santa Catarina, Brasil].[PhD Thesis in Portuguese]. Universidade Federal de São Carlos.
Loladze I, Elser JJ, 2011. The origins of the Redfield nitrogen-to-phosphorous ratio are in a homoeostatic protein-to-rRNA ratio. Ecol. Lett. 14:244-250.
Lorenzen CJ, 1967. Determination of chlorophyll and phaeopigments: spectrometric equations. Limnol. Oceanogr. 12: 343-346.
Lürling M, Eshetu F, Faassen EJ, Kosten S, Huszar VLM, 2013. Comparison of cyanobacterial and green algal growth rates at different temperatures. Freshwater Biol. 58:552-559.
Mackereth FJH, Heron J, Talling JF, 1978. Water analysis: some revised methods for limnologists. Freshwater Biological Association, Scientific Publication 36.
Marotta H, Duarte CM, Meirelles-Pereira F, Bento L, Esteves FA, Enrich-Prast A, 2010. Long-term CO_2 variability in two shallow tropical lakes experiencing episodic eutrophication and acidification events. Ecosystems 13:382-392.
Moisander PH, Cheshire LA, Braddy J, Calandrino ES, Hoffman M, Piehler MF, Paerl HW, 2012. Facultative diazotrophy increases *Cylindrospermopsis raciborskii* competitiveness under fluctuating nitrogen availability. FEMS Microbiol Ecol 79:800-811.
Pacheco JP, Iglesias C, Meerhoff M, Fosalba C, Goyenola G, Mello FT, García S, Gelós M, García-Rodríguez F, 2010. Phytoplankton community structure in five subtropical shallow lakes with different trophic status (Uruguay): a morphology-based approach. Hydrobiologia 646:187-197.
Padisák J, 1997. *Cylindrospermopsis raciborskii* (Woloszyska) Seenayya et Subba Raju, an expanding, highly adaptive cyanobacterium: worldwide distribution and review of its ecology. Arch. Hydrobiol. Supp. 107:563-593.
Paerl HW, Huisman J, 2008. Blooms like it hot. Science 320: 57-58.
Piccini C, Aubriot L, Fabre A, Amaral V, González-Piana M, Gianni A, Figueredo CC, Vidal L, Kruk C, Bonilla S, 2011. Genetic and eco-physiological differences of South American *Cylindrospermopsis raciborskii* isolates support the hypothesis of multiple ecotypes. Harmful Algae 10:644-653.
Posselt AJ, Burford MA, Shawn G, 2009. Pulses of phosphate promote dominance of the toxic cyanophyte *Cylindrospermopsis raciborskii* in a subtropical water reservoir. J. Phycol. 45:540-546.
Redfield RC, 1958. The biological control of chemical factors in the environment. Am. Sci. 46:205-222.
Solomon CT, Bruesewitz DA, Richardson DC, Rose KC, Van de Bogert MC, Hanson PC, Kratz TK, Larget B, Adrian R, Leroux Babin B, Chiu C-Y, Hamilton DP, Gaiser EE, Hendricks S, Istvànovics V, Laas A, O'Donnell DM, Pace ML, Ryder E, Staehr PA, Torgersen T, Vanni MJ, Weathers KC, Zhu G, 2013. Ecosystem respiration: Drivers of daily variability and background respiration in lakes around the globe. Limnol. Oceanogr. 58: 849-866.
Sterner RW, 2008. On the phosphorous limitation paradigm for lakes. Intern. Rev. Hydrobiol. 93:433-445.
Strickland JDH, Parsons TR, 1960. A manual of seawater analysis. J. Fish. Res. Board Can. 167:1-311.
Stuken A, Rucker J, Endrulat T, Preussel K, Hemm M, Nixdorf B, Karsten U, Wiedner C, 2006. Distribution of three alien cyanobacterial species (Nostocales) in northeast Germany: *Cylindrospermopsis raciborskii*, *Anabaena bergii* and *Aphanizomenon aphanizomenoides*. Phycologia 45:696-703.
Tonetta D, Hennemann MC, Brentano DM, Petrucio MM, 2015. Considerations regarding the dominance of *Cylindrospermopsis raciborskii* under low light availability in a low phosphorus lake. Acta Bot. Bras. 29:448-451.
Tonetta D, Petrucio MM, Laudares-Silva R, 2013. Temporal variation in phytoplankton community in a freshwater coastal lake of southern Brazil. Acta Limnol. Brasil. 25:99-110.
Torres IC, Inglett PW, Brenner M, Kenney WF, Reddyet KR, 2012. Stable isotope (d13C and d15N) values of sediment organic matter in subtropical lakes of different trophic status. J. Paleolimnol. 47:693-706.
Valderrama JC, 1981. The simultaneous analysis of total nitrogen and phosphorous in natural waters. Mar. Chem. 10: 1109-1122.
Vidal L, Kruk C, 2008. *Cylindrospermopsis raciborskii* (Cyanobacteria) extends its distribution to latitude 34°53'S: taxonomical and ecological features in Uruguayan eutrophic lakes. Panam. J. Aquat. Sci. 3:142-151.