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Phytoplankton-based water quality metrics: feasibility of their use in a Neotropical shallow lake

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Abstract. Urban lakes constitute important recreational areas, but often they are eutrophicated. In this study we discuss the utility of 12 ecological quality metrics to test whether they: (1) can be applied to Neotropical lakes; (2) are sensitive to environmental variations throughout the year; and (3) are affected by heterogeneous spatial distribution of phytoplankton. Phytoplankton and environmental variables (including nutrients) were sampled monthly in an urban lake (four littoral and one limnetic station) throughout 1 year ($n = 60$ samples). Twelve ecological quality metrics were tested using total phosphorus as a proxy of eutrophication through general lineal models. The best adjusted metrics were then transformed to an ecological quality ratio (EQR) to allow comparisons. The Phytoplankton Assemblage Index (Q-index) and the Cyanobacteria Bloom Index (CBI) were the most accurate. Differences in water quality estimation occurred across the year, with an overestimation of water quality in the absence of cyanobacteria blooms. There were no differences due to effects of the spatial distribution of phytoplankton. The Q-index was related to temperature and soluble reactive phosphorus, whereas the CBI was related to conductivity. We conclude that the Q-index is the most accurate metric for monitoring purposes, responding well to variations in phosphorus.

Additional keywords: eutrophication, hypertrophic, phosphorus.

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Introduction

Urban lakes are often man-made ecosystems that increase the quality of life in urban centres, providing areas suitable for recreational and educational activities, and even improving air quality (Martínez-Arroyo and Jáuregui 2000). These lakes have a small direct catchment and much of the water feeding them is drained from metropolitan areas through storm water channels and pipelines (Naselli-Flores 2008). Because urban lake basins are part of a city, the environmental problems of metropolitan areas have a negative effect on them (Naselli-Flores 2008), making eutrophication of these standing waters the most common issue (Birch and McCaskie 1999; Verma *et al.* 2011). Consequently, the excess of nutrients and organic matter stored in lakes is unable to be completely processed, frequently resulting in dense cyanobacteria blooms (Smith and Schindler 2009; Schindler 2012).

In the Neotropical region, long-term monitoring of aquatic systems is uncommon and there is a lack regulatory frameworks such as the Water Framework Directive (European Commission 2000) in Europe or the *Federal Water Pollution Control Act* (2008, L110–L288) in the US, which establish regular monitoring of the health of water bodies. Moreover, surface waters in the Neotropical region are under increasing ecological stress due to anthropogenic activities beyond eutrophication, such as deforestation, soil erosion and contamination (United Nations Environment Program 2002; Food and Agriculture Organization 2003; Srebotnjak *et al.* 2012). All of this contributes to an extensive ecological degradation of environments by decreasing their ability to provide goods and services (Tejerina-Garro *et al.* 2005). Under this scenario, the need to develop practical and effective ecological tools to monitor water resource quality is imperative (Hughes and Oberdorff 1999), with many issues currently needing to be resolved in the Neotropical region. For example, in inshore regions of shallow lakes, chemical interactions with macrophytes may generate differences in phytoplankton assemblages between these areas and the deeper open-water zone (for a review, see Gross *et al.* 2007). In addition, when cyanobacteria blooms occur, these may accumulate in certain areas, mostly depending on weather conditions (Chorus *et al.* 2000; Bonilla 2009; Wu *et al.* 2015). Consequently, this may affect water quality estimation depending on sampling design. Moreover, phytoplankton assemblage structure integrates biological responses to previous environmental conditions (Madgwick *et al.* 2006; Thackeray *et al.* 2013). This may have an effect on phytoplankton water quality metrics, because unexplained temporal variability in metrics scores may likely arise due to the temporal dimension inherent in phytoplankton–environment interactions. Indeed, although several water quality metrics have been developed and tested in recent years (for a review, see Phillips *et al.* 2011), there is still a lack of information concerning the ability of such metrics to predict water quality in Neotropical shallow lakes. The aim of the present study was to test and discuss the utility of different ecological quality metrics that use phytoplankton as indicator organisms. We aimed to answer the following questions: (1) are these metrics useful indicators to describe the trophic state of shallow Neotropical lakes; (2) are these metrics sensitive to temporal changes in physical and chemical variables; and (3) is water quality estimation using these metrics affected by heterogeneous distribution of phytoplankton among sampling sites?

Materials and methods

Study area

Quillá Lake is a shallow urban lake (31°39′S, 60°42′W) in Argentina that has a surface of 12 ha, a flat-bed topography, a mean depth of 2.7 m and a maximum depth of 4 m in its central area. Quillá Lake has profuse littoral emergent macrophytes dominated by *Panicum elephantipes* Nees ex Trin., *Echinochloa polystachya* (Kunth), *Typha latifolia* Linnaeus and *Schoenoplectus californicus* (C.A.Mey.) Soják (Fig. 1). Quillá Lake was built in 1943 and constitutes an important urban recreational area. However, for more than 40 years frequent cyanobacteria bloom events have been recorded in this water body and hence recreational and sports activities (i.e. fishing and swimming) are banned due to frequent unpleasant odours and the potential risk to human health (Frau *et al.* 2018).

Fig. 1. Study area being indicated the five sampling points selected.

Table 1. The 12 metrics evaluated in the present study (6 compositional and 6 relative abundance metrics)

Index abbreviations are: Cyanobacteria Bloom Index (CBI); Functional Traits Index (FTI); Morpho-Functional Groups Index (MFGI); Phytoplankton Trophic Index (PTI); Phytoplankton Assemblage Index (Q-index); Size Phytoplankton Index (SPI). Variables in this table are: *S*, the number of species in a sample or a population; *N*, the number of individuals in a population or community; *ni*, the number of individuals in a species *i* of a sample from a population; $pi = ni/N$, the fraction of a sample of individuals belonging to species *i*; $aj = ni/N$, the fraction of a sample of individuals belonging to species *i*; *sj*, optimum of *aj* found in the sample; S_{max}, the maximum number of species in a sample; *R*, $\log_{10}$ of number of the total number of taxa; *Bvi*, biovolume of the species *i*; *TSi*, trophic score of the species *i*; *IVi*, indicator value of the species *i*

Index	Formula	References
Margalef	$M = \frac{S-1}{\ln N}$	Margalef (1958)
Shannon's H′	$H' = \sum pi \times \ln pi$	Shannon and Weaver (1949)
Richness	$r = \sum n\,species$	Whittaker (1972)
Simpson	$S = 1 - \sum \frac{ni \times (ni-1)}{N \times (N-1)}$	Simpson (1949)
Pielou	$J' = \frac{H'}{\ln H_{max}}$	Pielou (1975)
Equitability	$E = \frac{H'}{\log_{10} R}$	Harper (1999)
Q-index	$Q = \sum pi \times F$	Padisák *et al.* (2006)
PTI	$PTI = \sum \frac{aj \times sj}{aj}$	Phillips *et al.* (2011)
MFGI	$MFGI = \sum \frac{Bvi \times TSi \times IVi}{Bvi \times IVi}$	Phillips *et al.* (2011)
SPI	$SPI = \sum \frac{Bvi \times TSi \times IVi}{Bvi \times IVi}$	Phillips *et al.* (2011)
FTI	$FTI = \sum \left(\frac{MFGI+SPI}{2}\right)$	Phillips *et al.* (2011)
CBI	$\log_{10}(cyan + 1)$	Mischke *et al.* (2011)

Samplings and water quality analyses

From June 2014 to May 2015, 14 environmental variables were measured or estimated monthly at five sampling sites (four littoral and one limnetic). The sampling sites were chosen to encompass the spatial heterogeneity produced by the wind, which varies with seasons, and by differences between the littoral (macrophytes present and a shorter water column) and limnetic (no macrophytes and a deeper water column) areas ($n = 60$ samples for each variable; Fig. 1). The physical and chemical variables considered were temperature (°C), dissolved oxygen (DO) concentration (mg L^{-1}) and saturation (DO% saturation), pH and conductivity (mS cm^{-1}); these were determined using HANNA multiparameter probes. (Hanna Instruments, Woonsocket, RI, USA) Depth (Z_d; m) was measured with an ultrasonic probe. The photic zone (Z_{eu}) was estimated according to Koenings and Edmundson (1991) for turbid environments. Water volume (m^3) entering the lake (urban water run-off + direct precipitation) was estimated using the criteria suggested by UNESCO (2006). Water samples for nutrient determination (i.e. nitrite–nitrate, ammonium and soluble reactive phosphorus (SRP) concentrations, µg L^{-1}) were analysed in the laboratory according to the American Public Health Association (2005). Throughout the text, the term 'dissolved inorganic nitrogen' (DIN) is used in reference to nitrite–nitrate + ammonium. Variations in total phosphorus (TP) concentrations have been found to be powerful predictors of phytoplankton, even better than total nitrogen concentration (Brown *et al.* 2000; Phillips *et al.* 2008; Søndergaard *et al.* 2011). For this reason, TP concentrations (µg L^{-1}) were used to validate the different ecological quality metrics evaluated in the present study. TP was estimated by digestion with nitric and sulfuric acids followed by determination of SRP (American Public Health Association 2005).

Phytoplankton samples ($n = 60$) were collected using 100-mL bottles, fixed immediately with 1% acidified Lugol's solution and obtained from the same sites and at the same sampling frequency as samples for environmental variables. Taxonomic classification was done according to Lee (2008) following the keys and specific bibliography for each algal group, such as Krammer and Lange-Bertalot (1991), Zalocar de Domitrovic and Maidana (1997), Tell and Conforti (1986), Komárek and

Table 2. Mean (±s.d.) of each environmental variable measured during the study period (modified from Frau *et al.* 2018)
Z_d, depth (m); Z_{eu}, photic zone; DO, dissolved oxygen; TN, total nitrogen; DIN, dissolved inorganic nitrogen; TP, total phosphorus; SRP, soluble reactive phosphorus

	Winter	Spring	Summer	Autumn
Temperature (°C)	15.0 ± 0.7	19.4 ± 1.8	27.3 ± 0.7	24.0 ± 3.1
pH	7.4 ± 0.4	7.6 ± 0.7	7.7 ± 0.5	7.7 ± 0.6
Conductivity (mS cm^{-1})	2.4 ± 0.7	3.4 ± 0.3	3.3 ± 0.4	5.9 ± 4.5
$Z_d : Z_{eu}$	1.3 ± 0.8	1.1 ± 1.1	1.1 ± 0.9	1.4 ± 1.2
DO (mg L^{-1})	14.2 ± 3.8	12.1 ± 2.3	8.8 ± 2.9	9.8 ± 1.1
DO% sat.	100 ± 19	91 ± 32	79 ± 9	84 ± 47
Water volume (m^3)	5687 ± 2724	8405 ± 4098	20 350 ± 12 511	23 383 ± 26 590
TN (μg L^{-1})	2475.3 ± 1870.7	1326.4 ± 837.4	959.3 ± 216.1	894.7 ± 155.8
DIN (μg L^{-1})	2083.7 ± 1603.0	152.2 ± 104.5	120.4 ± 102.7	195.8 ± 218.3
TP (μg L^{-1})	212.1 ± 25.1	269.2 ± 104.3	357.1 ± 125.6	400.6 ± 157.3
SRP (μg L^{-1})	109.9 ± 51.8	66.0 ± 52.3	97.4 ± 64.0	89.9 ± 31.7

Fott (1983) and Komárek and Anagnostidis (1999, 2005), among others and recent revisions. Quantitative phytoplankton analyses were conducted following the method of Utermöhl (1958). Counting error was estimated according to Venrick (1978) accepting a maximum error of 20% while species were counted at 400× magnification. Phytoplankton biovolume (mm^3 L^{-1}) was estimated following Hillebrand *et al.* (1999) by measuring 20 individuals of each species. Mean values of total phytoplankton biovolume on different sampling dates and its variation coefficient (CV), expressed as a percentage, were calculated.

Twelve metrics of phytoplankton abundance and composition were used to analyse the ecological status of the lake. Six of these metrics use biovolume information for each species, whereas the other six are diversity metrics that use compositional data or the relative biovolume of the species (Table 1). All these metrics have been used and validated in different intercalibration exercises in the context of the Water Framework Directive in European countries (e.g. Spatharis and Tsirtsis 2010; Thackeray *et al.* 2013; Borics *et al.* 2014).

To compare response sensitivity of the different metrics (described in Table 1), generalised linear models (GLM) with Gaussian fitting were used. All metrics were regressed against TP ($\log_{10}$-transformed data). The models obtained were compared using the Akaike information criterion (AIC) and selecting only those models with statistical significance ($P < 0.05$).

To assess the effects of environmental variation throughout the year on those metrics that showed a good statistical correlation with TP, multiple regression models were used, with metric values as response variables and physicochemical variables (nitrite–nitrate, SRP, ammonium, temperature, pH, DO concentration, $Z_d : Z_{eu}$, conductivity and water volume entering the lake) as predictors. The models were run including all possible subsets of these variables (including the null model, with all the environmental variables) and were then ranked using the AIC criterion to find the optimal combination that encompass the minimum number of statistically significant predictor variables. Randomised block design (RBD) analysis of variance (ANOVA) was used (after verifying the requirements of the parametric tests) to explore differences among the five sampling sites (four littoral, one limnetic) isolating the temporal effect (season of the year = blocks). Finally, the metrics that fitted the best in the GLM were converted to a normalised ecological quality ratio (EQR), ranging from 0 (the worst trophic status) to 1 (the best trophic status), to allow for comparisons. EQR scaling (bad, poor, moderate, good and high) was performed following the criteria recommended by the Water Framework Directive (European Commission 2000) using the TP concentration across sampling periods using the following equation:

$$EQR = (TP_{\text{obs}} - TP_{\text{max}}) \div (TP_{\text{min}} - TP_{\text{max}})$$

where TP_{obs} is the TP concentration measured at the sampling station, and TP_{max} and TP_{min} are the maximum and minimum TP concentrations recorded during the whole sampling period respectively. The different categories were obtained by estimating the cero, the first, second, third and fourth quartile, and then the different ecological categories (bad, poor, moderate, good and high) were assigned. For every metric, the same equation was used. Mean (±s.d.) index values that had the best fit were calculated for each sampling date.

Results

Environmental variation

Water temperature, water volume and TP concentration were higher in summer and autumn than in winter and spring. The opposite was found for total nitrogen (TN) concentration during the whole year, which was twofold higher than TP. DIN and SRP fractions were very high in winter, but dropped during spring, summer and autumn. DO was always >8 mg L^{-1}, but the DO concentration decreased during autumn and summer. A similar pattern was observed for DO% saturation. Conductivity was always high, ranging from 2.4 to 5.9 mS cm^{-1}; pH remained rather neutral throughout the study period and the $Z_d : Z_{eu}$ ratio was >1 across seasons (Table 2).

Water quality metrics

The 12 phytoplankton metrics evaluated varied widely in their relationship with TP concentration, highlighting different strengths of the metrics to indicate the primary pressure of nutrient enrichment (Fig. 2). The metric–phosphorus

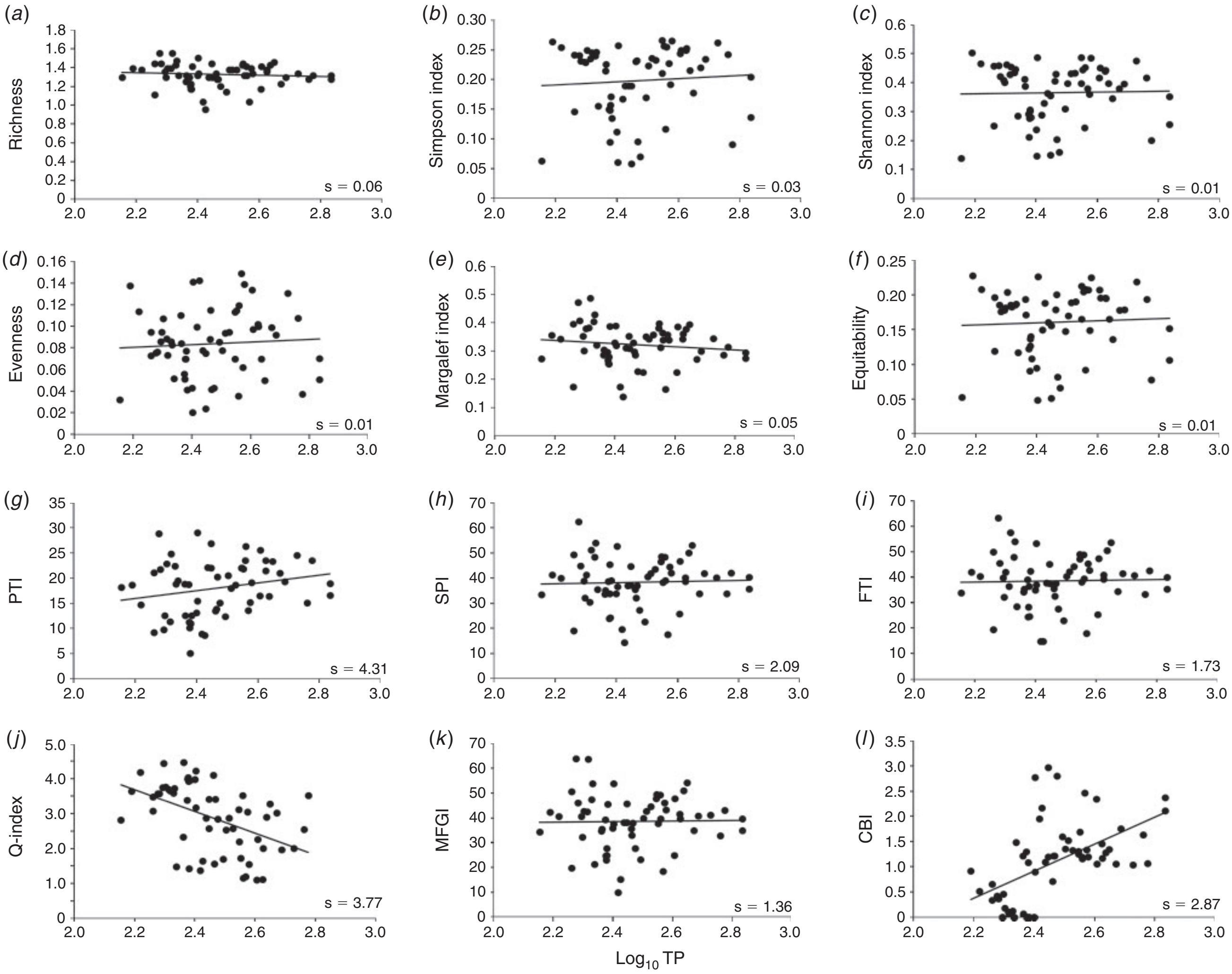

Fig. 2. Scatterplots of values of the 12 phytoplankton metrics at the sampling points *v.* $\log_{10}$ total phosphorus (TP) concentrations. The absolute value of the slope (s) for each straight-line equation is given. Q-index, Phytoplankton Assemblage Index; PTI, Phytoplankton Trophic Index; MFGI, Morpho-Functional Groups Index; SPI, Size Phytoplankton Index; FTI, Functional Traits Index; CBI, Cyanobacteria Bloom Index.

relationships were strongest for the Phytoplankton Trophic Index (PTI) (Fig. 2*g*), the Phytoplankton Assemblage Index (Q-index; Fig. 2*j*) and the cyanobacteria bloom index (CBI; Fig. 2*l*), as indicated by higher absolute values of the slopes of the straight-line equations for these relationships. The other nine metrics tested showed low absolute slope values, indicating a small association with TP concentration across sampling sites and seasons. The GLM analyses confirmed these patterns, indicating statistically significant models for the Q-index and CBI, but not for the PTI (Table 3).

Effects of environmental variations on metrics

The GLM performed with the set of environmental variables and the two best fitted metrics (Q-index and CBI) showed that both models were associated with environmental variations. The Q-index was negatively correlated with temperature and SRP, whereas the CBI was positively associated with conductivity (Table 4).

Metric responses to phytoplankton distribution patterns

In all, 115 species of phytoplankton were recorded during the study. Total phytoplankton biovolume varied among sampling dates, ranging between 14.5 $mm^3\ L^{-1}$ in July and 684.6 $mm^3\ L^{-1}$ in September. Variations in biovolume were also recorded within sampling dates (i.e. among sampling sites in the same sampling month), with the highest variations (CV $>50\%$) recorded in September, December, February and May (Fig. 3).

In response to these variations in biovolume, the Q-index showed variable results throughout the year, with higher variation (s.d.) among sampling sites in November, February and May (Fig. 4*a*). A similar pattern was observed for the CBI, but these variations where more evident (high s.d.) during September, October, December, February and May (Fig. 4*c*). The RBD ANOVA showed a lack of significance when the Q-index ($F = 0.12$ $P = 0.97$) and the CBI ($F = 1.01$ $P = 0.41$) were compared among sampling sites, but in both cases the effect

Table 3. Relationships between metrics and total phosphorus (TP) concentration as determined by linear regression models
The Akaike information criterion (AIC) and AIC variation between the most optimal model and the corresponding model (ΔAIC) are shown. For each model, *F*- and *P*-values are given. Statistically significant models ($P < 0.05$) are in bold. Index abbreviations are: Cyanobacteria Bloom Index (CBI); Functional Traits Index (FTI); Morpho-Functional Groups Index (MFGI); Phytoplankton Trophic Index (PTI); Phytoplankton Assemblage Index (Q-index); Size Phytoplankton Index (SPI)

Index	Regression formula ($y = a + bx$)	AIC	ΔAIC	*F*-value	*P*-value
CBI	**CBI = −4.11 + 2.13($\log_{10}$(TP))**	**138.7**	**−23.4**	**12.4**	**0.008**
Equitability	Equitability = 0.34 – 0.04($\log_{10}$(TP))	−49.6	164.9	0.1	0.7
Evenness	Evenness = 0.12 – 0.03($\log_{10}$(TP))	−115	230.8	0.2	0.6
FTI	FTI = 34.14 + 1.73($\log_{10}$(TP))	452.3	−336.9	0.04	0.08
Margalef	Margalef = 7.84 – 2.10($\log_{10}$(TP))	43.8	71.5	1.4	0.2
MFGI	MFGI = 35.16 + 1.26($\log_{10}$(TP))	462.9	−347.5	0.02	0.9
PTI	PTI = −0.95 + 0.70($\log_{10}$(TP))	376.8	−261.4	3.1	0.08
Q-index	**Q index = 12.13 – 3.77($\log_{10}$(TP))**	**159.9**	**−44.5**	**5.2**	**<0.001**
Richness	Richness = 32.34 – 4.01($\log_{10}$(TP))	381	−265.6	1.02	0.3
Shannon	Shannon = 1.36 – 0.04($\log_{10}$(TP))	91.2	24.2	0.01	1.0
Simpson	Simpson = 0.37 – 0.08($\log_{10}$(TP))	−13.5	128.9	0.2	0.6
SPI	STI = 32.38 + 2.40($\log_{10}$(TP))	445.6	−330.2	0.09	0.7

Table 4. Relationships between metrics and environmental drivers in the optimal linear mixed-effects models
The number of estimated model parameters (*k*), the variables used as predictors in the model and the difference in Akaike information criterion (AIC) between the optimal model and the corresponding null model (ΔAIC_{null}) are shown. For each predictor, the sign of the corresponding relationship is given as positive (+) or negative (−). Q-index, Phytoplankton Assemblage Index; CBI, Cyanobacteria Bloom Index; SRP, Soluble Reactive Phosphorus

Metric	*k*	Predictor	ΔAIC_{null}	*F*-value	*P*-value
Q-index	5	Temperature (−); SRP (−)	7.42	8.74	<0.0001
CBI	3	Conductivity (+)	6.79	3.38	0.04

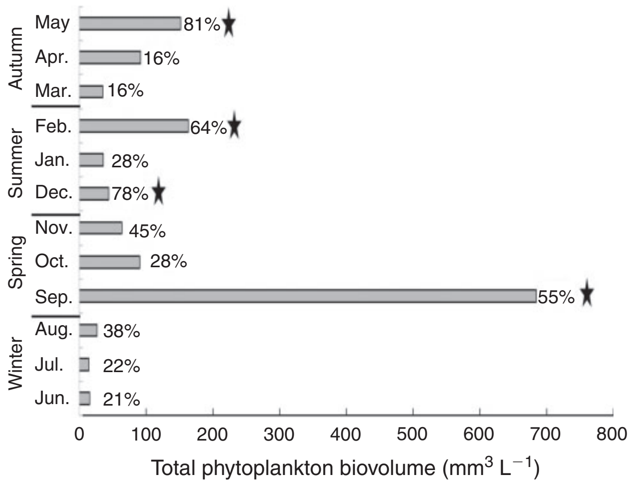

Fig. 3. Mean total phytoplankton biovolume across sampling months. The percentage coefficient of variation (CV) is given above the columns for each sampling date. Stars indicate the months in which cyanobacteria scum-forming blooms occurred according to Frau *et al.* (2018).

of seasons (blocks) was significant ($P < 0.001$). Finally, when the two metrics were standardised by applying the EQR for this lake (Table 5), the following differences were observed in the water quality estimation (Fig. 4*c*). The CBI showed high water quality in almost 67% of months, with poor water quality in <20% of months and good water quality in 5% of months, whereas for the Q-index, 77% of samples showed poor water quality, 20% showed moderate water quality and 5% showed good water quality (Fig. 4*d*).

Discussion

In the present study we tested 12 metrics of water quality based on phytoplankton composition and biovolume that had been developed and tested previously in temperate areas. The aim was to assess the usefulness of these water quality metrics to describe the trophic state of a shallow Neotropical hypertrophic lake (mean TP range 200–400 µg L^{-1}; see Table 2), and their ability to appropriately reflect changes in water quality (spatial, temporal and environmental variations). Comparison of metric scores showed a heterogeneous ability to characterise the eutrophication of the Neotropical lake evaluated here. In the present study, 2 of the 12 metrics tested, namely the Q-index (Padisák *et al.* 2006) and the CBI (Mischke *et al.* 2011), showed a strong association with TP concentration, suggesting that these metrics reflected well the eutrophication gradient throughout the year. These results also suggest that the relationship holds despite possible environmental variations, and are consistent with the idea that phosphorus boosts phytoplankton biomass (Reynolds 2006). The latter relationship has been proven empirically by others (e.g. Phillips *et al.* 2008; Søndergaard *et al.* 2011), particularly for cyanobacteria blooms (Elliott *et al.* 2006; Thackeray *et al.* 2013). However, we did not find an association between the other 10 metrics tested and TP. This was particularly true for the composition (PTI; Functional Traits Index, FTI; Morpho-Functional Group Index, MFGI, Size Phytoplankton Index, SPI) and diversity (richness, Shannon,

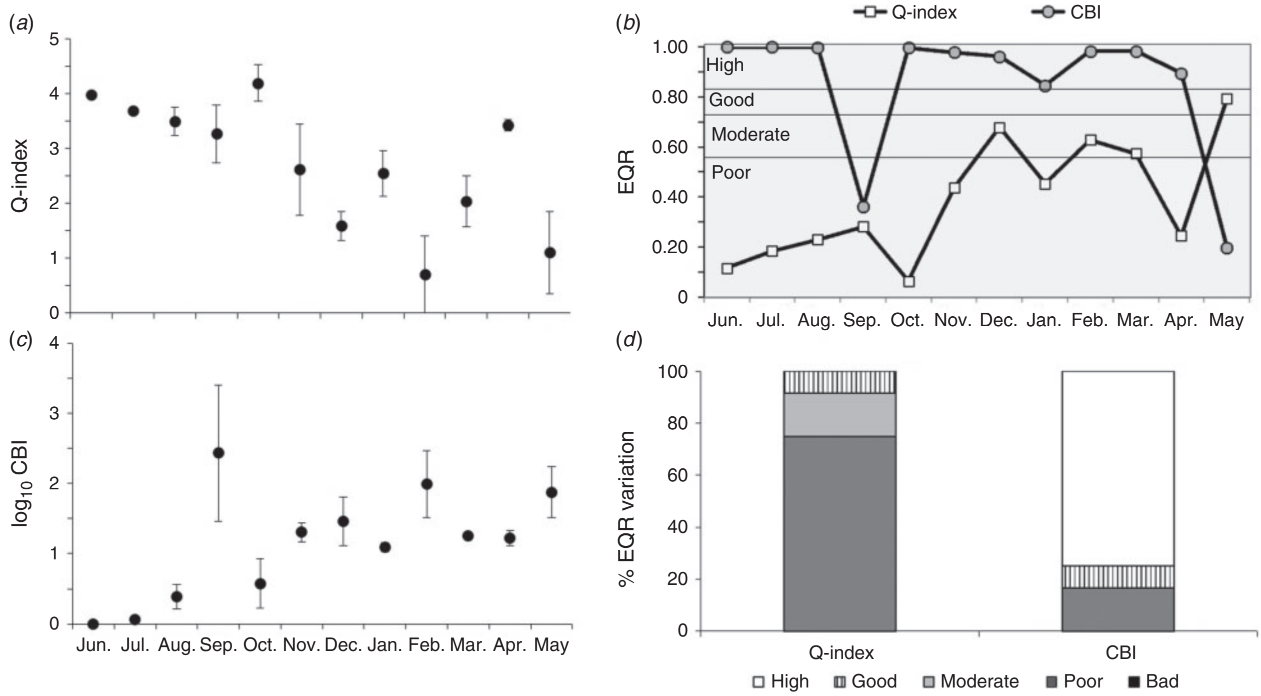

Fig. 4. Mean (±s.d.) values for each sampling month for the (*a*) Phytoplankton Assemblage Index (Q-index) and (*c*) Cyanobacteria Bloom Index (CBI). (*b*) Mean ecological quality ratios (EQR) calculated for each sampling month and metric and (*d*) total mean percentage variation in EQR for the whole study.

Table 5. Boundaries of the ecological quality ratio (EQR) values identified for total phosphorus concentration (TP) in Quillá Lake

EQR	TP
0	Bad
0.1–0.58	Poor
0.59–0.74	Moderate
0.75–0.86	Good
0.87–1	High

Simpson, Margalef, equitability and evenness) metrics. These results obtained here contrast with those reported by several studies following the European Water Framework Directive, in which all the metrics proved to be good indicators of water quality (see Spatharis and Tsirtsis 2010; Phillips *et al.* 2011; Borics *et al.* 2014).

Although the cyanobacteria fraction could be analysed more easily and in a more timely manner than the whole phytoplankton fraction, which could encompass several phyla and up to hundreds of species in one single monitoring period, the results of the present study suggest that the CBI could be very sensitive to changes in phytoplankton biovolume across the year in Quillá Lake. Indeed, when cyanobacteria blooms were unnoticed (particularly during the winter and spring seasons; see Fig. 3), the CBI suggested an excellent water quality state (EQR value high). Conversely, the Q-index seems to have given more reliable results because it uses all the phytoplankton species to assess water quality. However, the disadvantages of the Q-index are that it requires expert knowledge of Reynolds functional groups, considerable training in phytoplankton taxonomy to reach at least the genus level, and environmental information not always accessible. These drawbacks may be overcome by using statistical model classifications (Kruk *et al.* 2017), which decrease the need for expert knowledge and environmental information.

The results of the present study suggest that variations in metrics associated with environmental gradients should be considered in water body assessment and monitoring programs because statistically significant relationships were found for temperature, conductivity and SRP. The Q-index was negatively correlated with temperature and SRP. This suggests that with an increase in temperature, the Q-index (which ranges from 1 to 5, with 5 being an excellent score) may show lower scores. Indeed, Reynolds (2006) found a positive relationship between phytoplankton biovolume and temperature. This relationship is especially important for the development of cyanobacteria blooms (Paerl *et al.* 2016). The negative relationship between the Q-index and SRP suggests a high sensitivity of this metric to detect changes in phosphorus concentrations. This may be an advantage in hypertrophic lakes, like the one studied here, where phosphorus concentrations are always high. The CBI showed a positive association with conductivity, which, in the lake assessed here increased during summer. The latter scenario favoured the development of cyanobacteria species adapted to high conductivity, such as *Anabaenopsis arnoldii* Aptekar and *Raphidiopsis curvata* Fritsch et Rich (Frau *et al.* 2018). Compared with similar studies performed in deeper, temperate lakes (Thackeray *et al.* 2013), we did not find any significant correlations between either the Q-index or the CBI and lake depth. Depth is usually highly correlated with variables such as nutrients resuspension, light availability and thermal stratification in deeper lakes (Phillips *et al.* 2008; Thackeray *et al.* 2013). In shallow lakes, like the one studied herein, frequent mixing of the water column may secure nutrient availability and light, and phytoplankton may be more affected by competition for resources and environmental variations (e.g. temperature) than by depth, a conclusion that was also reached by Thackeray *et al.* (2013).

The effects of heterogeneous spatial phytoplankton distribution seemed unrelated to estimates of the Q-index and CBI. This was particularly true for the CBI, despite the presence of scum-forming blooms of *Microcystis aeruginosa* (Kützing) Kützing and *A. arnoldii*, heterogeneously distributed across sampling sites in the lake during blooms events. The CBI metric may decrease the effect of spatial heterogeneity due to the input of log-transformed data. Conversely, the Q-index computes the whole phytoplankton assemblage, and this could buffer the effect of a heterogeneous distribution of the few species of cyanobacteria that caused blooms. This may also indicate that other phytoplankton taxa, which also reflect poor water quality, may be well represented in the whole phytoplankton assemblage. Other sources of variation not confirmed for the lake studied here were differences in the littoral and limnetic areas (shorter water column and the presence of macrophytes *v.* deeper water and the absence of vegetation; Clarke *et al.* 2002; Thackeray *et al.* 2013). A lower water column in the littoral area may favour resuspension of algae and nutrients and secure a better light climate. In contrast, littoral vegetation could compete with phytoplankton for light and nutrients, even through allelopathic effects. However, the absence of significant differences among sampling locations in the present study suggests that the effect of vegetation would be only evident at high vegetation percentage coverage, an effect that has been examined previously (e.g. Sinistro *et al.* 2006; Frau *et al.* 2015).

Conclusions

The results obtained for Quillá Lake suggest that 2 of 12 candidate phytoplankton assemblage metrics could potentially reflect well variations in lake water quality in hypertrophic shallow lakes of the Neotropical region. This is particularly true for the Q-index, which appears to respond well to variations in TP concentration despite continuous high concentrations. The Q-index was robust beyond the spatial heterogeneous distributions of phytoplankton and was responsive to environmental variations, such as temperature. The results obtained here highlight the need to develop monitoring programs able to capture environmental variations throughout the year, because changes in environmental conditions may have an effect on the way we estimate water quality using phytoplankton.

Conflict of interest

The authors declare that they have no conflicts of interest.

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