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Phytoplankton functional group classifications as a tool for biomonitoring shallow lakes: a case study

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Abstract – We assessed changes in phytoplankton community structure in relation to environmental variables in an urban eutrophic shallow lake (Lake Lugano, Argentina) throughout one year following two classification systems according to the morpho-functional groups (MFG) approach and morphologically based functional groups (MBFG). We aimed to compare the different approaches and find a simple tool to biomonitor urban freshwaters regarding their phytoplankton structure. Values of transparency, nutrients and chlorophyll *a* concentrations confirmed the eutrophic/hypertrophic conditions of the lake. The potentially toxic Cyanobacteria *Planktothrix agardhii* representing MFG 5a and MBFG III was generally dominant and reached bloom densities (>62,000 ind ml^{-1}). The multivariate analyses performed showed similar and overlapping results considering both approaches. Nutrients and transparency were the main environmental variables explaining the variance encountered. We conclude that MBFG classification was an adequate, easy-to-handle method for monitoring Lake Lugano. The functional approaches applied enabled the follow-up of potentially toxic Cyanobacteria in Lake Lugano. Further studies should include the estimation of cyanobacteria-derived toxin concentrations in water. We consider that the applicability of the MBFG approach deserves to be further explored as a promising tool for biomonitoring different types of urban water bodies.

Key words: urbanization / eutrophication / Cyanobacteria / bloom / morpho-functional approach

Résumé – Les groupes fonctionnels du phytoplancton en tant qu'outils de biosurveillance des lacs urbains : une étude de cas. Nous avons évalué les changements dans la structure de la communauté phytoplanctonique par rapport aux variables environnementales dans un lac peu profond eutrophique urbain (lac Lugano, Argentine) tout au long d'une année selon deux systèmes de classification ; l'approche morpho-functional groups (MFG) et morphologically based functional groups (MBFG). Nous avons cherché à comparer les différentes approches et à trouver un outil simple pour surveiller les eaux douces urbaines en ce qui concerne leur structure phytoplanctonique. Les valeurs de transparence, de nutriments et de concentrations de chlorophylle a ont confirmé les conditions eutrophiques/hypertrophiques du lac. Les cyanobactéries *Planktothrix agardhii* potentiellement toxiques représentant MFG 5a et MBFG III étaient généralement dominantes et ont atteint des densités d'efflorescence (>62,000 ind ml^{-1}). Les analyses multivariées effectuées ont donné des résultats similaires, se chevauchant pour les deux approches. Les nutriments et la transparence étaient les principales variables environnementales expliquant la variance rencontrée. Nous concluons que la classification MBFG était une méthode adéquate et facile à utiliser pour la surveillance du lac de Lugano. Les approches fonctionnelles appliquées ont permis le suivi des cyanobactéries potentiellement toxiques dans le lac de Lugano. D'autres études devraient inclure l'estimation des concentrations de toxines dérivées de cyanobactéries dans l'eau. Nous estimons que

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l'applicabilité de l'approche MBFG mérite d'être étudiée plus avant en tant qu'outil prometteur pour la biosurveillance de différents types de masses d'eau urbaines.

Mots-clés : urbanization / eutrophisation / Cyanobacteria / efflorescences algales / approximation morpho-fonctionnelle

1 Introduction

In the near future more than 50% of the humans will concentrate in cities as a result of population growth and migration from rural areas (Shochat *et al.*, 2006; WWAP, 2012). Urbanization is rapidly increasing and has become a matter of concern in conservation biology mainly due to the radical changes in habitat structure and the loss of endangered species by urban development. As deeply discussed by different authors, the term "urban" is difficult to define as it varies notably among countries. The definitions are based on human population density, legal municipal boundaries, degree of land use, and built areas (Pickett and Cadenasso, 2006; MacGregor-Fors, 2011; Francis and Chadwick, 2012). MacGregor-Fors (2011) recognizes the importance of homogenizing the term and re-defines the term "urban" considering that it refers to populated areas provided with basic services, where more than 1000 people/km^2 live or work, and an important proportion of the land (>50%) in a "city-scale" is covered by impervious surfaces. Besides the current debate, there is no doubt that the urban habitat conforms a unique setting, in which fundamental biological trends and processes can be decoupled by antrophogenic activities.

Water bodies form part of the urban landscape and constitute an element of interaction with their inhabitants; they have different uses and their water quality comprises an important issue for the urban development. They include naturally formed lakes with surrounding development, artificial quarry lakes, and recreational park-ponds, which can be subjected to an increasing negative environmental impact, and their water quality is often under great pressure (Olding *et al.*, 2000; Gledhill *et al.*, 2008; Henny and Meutia, 2014). Freshwater ecosystems provide the society with numerous ecosystem services but it is now recognized that human alterations of freshwaters have decreased their value (Taranu *et al.*, 2015). Cultural eutrophication of freshwater ecosystems is a problem worldwide, caused by increased nutrient loads resulting in water quality deterioration (Beckliogłu *et al.*, 2011; Araújo *et al.*, 2016). In this sense, urban areas constitute important sources of chemicals for freshwater ecosystems (Carpenter *et al.*, 2011). Eutrophication of urban ponds and shallow lakes is repeatedly reported in cities all around the world (Xiangcan, 2003; Fabre *et al.*, 2010; Waajen *et al.*, 2014; Yu *et al.*, 2016; Rodríguez-Flores, 2017) and can be nowadays considered a mainstream in aquatic ecology. Even though the available information for them is relatively scarce if compared with that for pristine water bodies, different aspects of the biotic components of urban aquatic systems have lately achieved an increased interest from ecologists. In particular, phytoplankton-focused studies have flourished (*e.g.*, Scasso *et al.*, 2001; Xiangcan, 2003; Hirose *et al.*, 2003; Meerhoff *et al.*, 2003; Fazio and O'farrell, 2005; Lv *et al.*, 2011; Segura *et al.*, 2013; Avigliano *et al.*, 2014; Waajen *et al.*, 2014; Jovanović *et al.*, 2017).

Pickett and Cadenasso (2006) stressed out that the flora and fauna of the metropolitan areas are mostly dominated by generalist species (*e.g.*, early successional trees and herbs, and species that can tolerate disturbance). They also highlight that a still open research question lies in the functional role of species in the ecological processes of urban ecosystems. Understanding the mechanisms to predict changes in plant and animal communities is a key challenge in ecology (Blaum *et al.*, 2011). In this sense, the need to transfer knowledge gained from single species to a more generalized approach has led to the development of categorization systems where species' similarities in life strategies and traits are classified into ecological groups (EGs) like functional groups/types or guilds. While approaches in plant ecology undergo a steady improvement and refinement of methodologies, and the progression in animal ecology is challenging (Blaum *et al.*, 2011), there is a strong case for attempting to categorize the phytoplankton species on the basis of their functional roles in aquatic ecosystem (Reynolds, 2006; Salmaso *et al.*, 2015). Phytoplankton is in general a highly diverse community and the occurrence of most species is hard to predict (Benincá *et al.*, 2008; Kruk *et al.*, 2011). Naselli-Flores and Barone (2011) suggested that a promising approach to solve this secret is the possibility to group phytoplankton species by means of their functional attributes. They also highlighted that trait-based classifications are increasingly used in phytoplankton ecology in order to explain and predict functional groups distributions along environmental gradients.

Recently, morphological and functional analyses of phytoplankton have become more and more popular. This fact mainly draws from the need to find easy-to-handle descriptors for management purposes (Naselli-Flores and Barone, 2011). When attempting to understand biodiversity response to environmental changes, the main advantage of applying functional groups classifications over single species approaches lies in the generalization of results (Blaum *et al.*, 2011). Salmaso *et al.* (2015) examined nine modes of classifying phytoplankton nontaxonomically. The classification methods assessed were based on either functional, morphometric, and morphological criteria (Margalef, 1978; Platt and Denman, 1978; Reynolds, 1988a,b; Kamenir *et al.*, 2004, 2006; Kruk *et al.*, 2010; Stanca *et al.*, 2013) or a combination of these with phenological, ecological, and/or taxonomic features when relevant (Reynolds, 1980; Reynolds *et al.*, 2002; Salmaso and Padisak, 2007). From these combined classifications, a largely influential and accepted approach among phytoplankton ecologists is that of Reynoldś (Reynolds *et al.*, 2002 updated by Padisák *et al.*, 2009). This classification is based on the functional traits of phytoplankton species, the ranges of environmental conditions over which they are found, as well as their co-occurrence. The success of this classification probably derives from the simplification of long taxonomic lists into about three dozens of functional groups (FG) or coda, sharing similar ecological characteristics

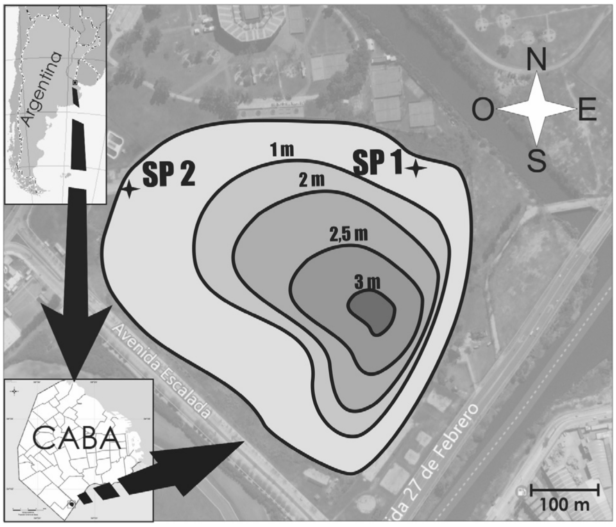

Fig. 1. Location of Lake Lugano in Parque Roca (Buenos Aires, Argentina), and the respective sampling points (SP1 and SP2).

(Naselli-Flores and Barone, 2011). Nonetheless, it requires specialist judgment in order to recognize algae up to the species level and classify them into the different coda. A relatively simpler method is the morpho-functional group (MFG) classification proposed by Salmaso and Padisak (2007) and updated recently (Tolotti *et al.*, 2012). This classification is based on morphological and structural features and includes taxonomy. It requires the ability to recognize the species from genus to order level and it is based on traits that strongly influence both ecological characteristics and functional processes. These authors based the MFGs on the criteria defined by Weithoff (2003) that are the presence of flagella, morphology (size, shape), ability to obtain alternative sources of nutrients and carbon, the presence of gelatinous envelopes, and taxonomy. As a result, phytoplankton species can be classified in 33 groups (after the subdivision proposed for diatoms by Tolotti *et al.*, 2012). An alternative classification based purely on morphological descriptors was proposed by Kruk *et al.* (2010). These authors defined seven species groups based on cluster analyses; this morphologically based functional group classification (MBFG) relies on the fact that the selected observable morphological traits (volume, maximum linear dimension, surface area, among others) are tightly linked to the autoecology of planktonic algae. In this case, the classification results in much greater simplification, leading to seven different groups.

With this paper, we attempt to monitor the changes in the phytoplankton community structure in an urban shallow lake throughout one year following two classification systems: morpho-functional groups (Salmaso and Padisak, 2007, updated by Tolotti *et al.*, 2012) and morphologically based functional groups (Kruk *et al.*, 2010). The aim is to compare the different approaches in order to find a relatively simple tool to biomonitor lentic aquatic ecosystems located in urban areas on the basis of their phytoplankton structure. As well, we aim to identify the dominant phytoplankton species and also detect the presence of potentially toxic and /or bloom-forming algae, as they represent a relevant aspect to determine the water quality of systems in close relation to the humans. Our study supports the application of the ecological group approach in the assessment of the phytoplankton response to environmental changes. It reinforces the idea of the use of a simple and sensitive enough method to monitor urban aquatic systems.

2 Materials and methods

2.1 Study site

Parque Roca constitutes the largest recreational park within the city of Buenos Aires (Argentina) with an area of 160 hectares (Sinistro *et al.*, 2004) (Fig. 1). It constitutes a green area surrounded by neighborhoods showing population densities greater than 10,371 inhabitants per km^2 and even surpassing 59,173 inhabitants per km^2, according to a recent population census (DGEC, 2016). Multiple activities (cultural, sportive, and social) take place in this park. It is an area with a high recreational value for the community. Lake Lugano is a man-made shallow water body (mean depth 1.5 m, maximum depth 3.0 m) located in this park. Its surface area is approximately 20 hectares, with a volume of 249.973 m^3. This water body was constructed in the 1950s. The water supply mainly consists on that derived from rains and superficial runoff waters. However, it has received untreated wastewater in an illegal manner during a long period (Rodríguez *et al.*, 2003), leading to the deterioration of its water quality. At the moment, the use of this water body for aquatic recreational activities is forbidden.

2.1.1 Environmental variables

Lake Lugano was monitored monthly during a 1-year period (28/5/2015 to 9/6/2016). Two sampling points (SP1 and SP2) were established in the littoral area based on previous monitoring studies held on this water body (Rodríguez *et al.*, 2003; Sinistro *et al.*, 2004). These sites were selected as they are highly accessible to the visitors and thus, represent areas of increased chances of contact between the public and the waters of the shallow lake. Environmental variables were measured *in situ* with portable instruments: pH, water temperature and conductivity (HANNA HI9811-5), dissolved oxygen (YSI ProODO), and turbidity (HACH 2100P). Transparency was estimated with a Secchi disc. Water samples were collected in acid-washed PVC bottles for the determination of the concentration of nutrients: ammonia (NH_4), nitrates (N-NO_3), dissolved reactive phosphorus (DRP), total nitrogen (TN), and total phosphorus (TP). Dissolved nutrients were analyzed after sample filtration through GF/F Whatman™ glass-fiber filters as follows: NH_4 with the phenate method, N-NO_3 with the cadmium reduction method, and DRP with the molybdovanadate method (APHA, 2005) using Hach® reagents. Total phosphorus (TP) and nitrogen (TN) were determined from unfiltered samples and posterior oxidation following Valderrama (1981). Daily air temperature and rainfall were provided by the Servicio Meteorológico Nacional (Argentina).

The phytoplanktonic chlorophyll *a* concentration was estimated following Marker *et al.* (1980). Water samples were obtained subsuperficially and filtered through GF/F Whatman™ glass-fiber filters. Samples for the study of the

Table 1. Ranges encountered for physical and chemical parameters in Lake Lugano throughout the studied period 2015–2016 (SD: standard deviation, min: minimum, max: maximum). Turbid: turbidity, NH_4: ammonia, N-NO_3: nitrates, TN: total nitrogen, DRP: dissolved reactive phosphorus, TP: total phosphorus, Cond.: conductivity, Water Temp.: water temperature, DO: dissolved oxygen, Secchi: Secchi transparency, Chlor *a*: phytoplanctonic chlorophyll *a* concentration, SP: sampling point.

	SP1			SP2		
	Mean	SD	Min–max	Mean	SD	Min–max
Turbid (NTU)	52.1	52.26	3–184	45.84	32.62	3–97
NH_4 (mg L^{-1})	0.40	0.72	0–2.08	0.73	0.77	0–2.24
N-NO_3 (mg L^{-1})	0.10	0.22	0–0.7	0.14	0.23	0–0.7
TN (mg L^{-1})	1.84	0.66	1.32–3.84	1.91	0.34	1.32–2.4
DRP (mg L^{-1})	0.83	0.34	0.33–1.56	0.78	0.24	0.35–1.2
TP (mg L^{-1})	1.26	0.44	0.60–2.16	1.35	0.38	0.84–1.92
pH	8.79	0.41	8.15–9.50	8.35	0.36	8–9
Cond (µS cm^{-1})	1459.75	262.97	1130–1860	1534.83	334.91	1060–2200
Water temp (°C)	18.07	6.29	10.20–28.30	18.44	5.79	10.60–27.60
DO (mg L^{-1})	12.16	4.44	4.98–20.65	7.77	3.27	0.21–11.2
Secchi (cm)	29.83	13.94	16–57	30.88	11.13	19–52
Chlor *a* (µg L^{-1})	98.56	70.91	1.53–235.31	108.70	74.45	0.51–210.25

phytoplankton structure and dynamics were collected as follows: for the qualitative analysis, water samples were obtained using a 15 µm phytoplankton net, fixed with 5% formalin and observed with a Zeiss™ optical microscope at ×1000 magnification. Quantitative samples were obtained subsuperficially and fixed with acetic Lugol's solution. Phytoplankton counts were performed using a Zeiss™ inverted microscope (Ütermöhl, 1958) and sedimentation chambers (2–5 ml sample). The counting error was estimated according to Venrick (1978). Individual algae (independently if it presented a colonial, a filamentous, cenobial, or unicellular habit) were considered as the unit. The phytoplankton organisms encountered throughout the study period were assigned to different ecological groups. We considered the MFG classification by Salmaso and Padisak (2007), considering potential mixotrophy, size (small <30–40 µm/large >30–40 µm), shape, presence of gelatinous envelopes, specific nutrient requirements and motility, and taxonomy when ecologically relevant. As for the MBFG, the classification was performed considering the presence/absence of flagella, siliceous structures, aerotopes, and/or mucilaginous envelopes, as well as morphometrical parameters (Kruk *et al.*, 2010).

2.1.2 Data analysis

Spearman's rank nonparametric correlations were performed between pairs of environmental variables. To characterize the structure of phytoplankton assemblages, we estimated the Shannon Diversity Index (H) and evenness (J) (based on Shannon index) for each sampling site and date, and functional group classifications. Moreover, we estimated the Bray–Curtis Similarity Index to evaluate the similitude between sites on each sampling date and both classification methods (Zar, 1999). The analyses were performed with Past 3.14 (Hammer *et al.*, 2001).

To assess for significant relationships between biological and environmental data, direct ordination analyses were used. First, a detrended correspondence analysis (DCA) was performed and, as data showed a linear response, a redundancy analysis (RDA) was applied. Environmental parameters that were highly correlated ($r > 0.7$) and with an inflation factor >10 were excluded from the analysis. MFG and MBFG were considered as the response variables and evaluated separately. Forward analysis of variables was used in all cases, considering the explained percentage of the variance and its significance. The Monte Carlo test was used for the statistical validation of the association between the ordination values of species, samples, and environmental variables. A total of 499 iterations were done for this test and considered to be significant at $p < 0.05$ level (ter Braak and Verdonschot, 1995). Multivariate analyses were performed using the software CANOCO 4.5.

3 Results

3.1 Environmental variables

Table 1 summarizes the ranges encountered for different physical and chemical variables throughout an annual cycle (late-autumn 2015/late-autumn 2016) for the studied urban water body. Lake Lugano presented a strong seasonal variability in water temperature ranging from 10.20 to 28.30 °C. The lowest temperatures were observed during late-autumns (2015 and 2016) and the highest during the summer, showing a clear seasonal pattern (Fig. 2a) and a high correlation with daily mean air temperature ($r = 0.910$, $p < 0.0001$). Conductivity values were relatively high and presented minimal differences between sampling sites, with the exception of the peak registered for SP2 (Fig. 2b) during April 2016. It was inversely correlated ($r = -0.525$, $p = 0.008$) with pluvial precipitation accumulated in the previous week (PP PW) of sampling date. As for pH, values remained alkaline during the whole studied period with a minimum value of pH = 8. The highest value registered (pH = 9.5) coincided with the warmer temperatures. SP1 presented higher values than SP2 during almost the whole studied period (Fig. 2c).

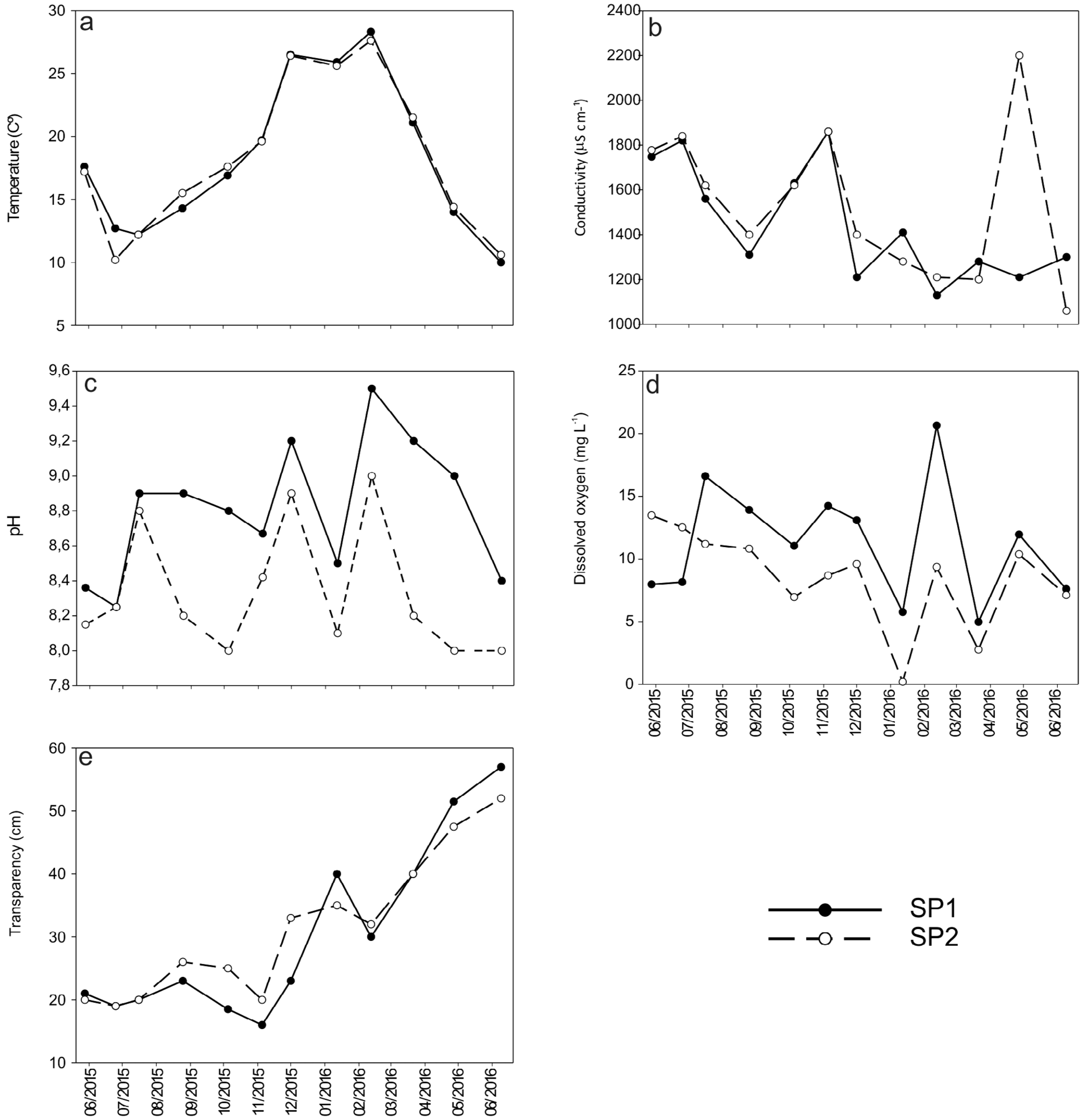

Fig. 2. Environmental variables measured in Lake Lugano during the annual cycle 2015–2016. (a) Water temperature. (b) Conductivity. (c) pH. (d) Dissolved oxygen concentration. (e) Transparency. SP: sampling point.

Dissolved oxygen (as well as oxygen percentage saturation) strongly fluctuated throughout the annual cycle, showing concentrations as low as 0.21 mg L^{-1} (representing 2.6% saturation) up to supersaturation values 20.65 mg L^{-1} (representing 265% saturation), SP1 generally presenting higher concentrations as compared with SP2 (Fig. 2d). Secchi depth was generally low (<40 cm), only exceeding that value during the cold months (Fig. 2e). This variable and conductivity were inversely correlated ($r = -0.629$, $p = 0.001$).

Nutrient concentrations presented marked variations (Fig. 3), mainly in the nitrogenous forms. As for the reduced nitrogen form (NH_4), figures were as dissimilar as two orders of magnitude (Fig. 3a). In general terms, the highest concentrations for this nutrient were estimated at the end of the studied period in the cold season (Austral autumn, March–June 2016). The oxidized nitrogen form (N-NO_3) followed a similar trend (Fig. 3b) with lowest values during the warmer season. NH_4 and N-NO_3 concentrations were positively correlated ($r = 0.832$, $p < 0.0001$). DRP presented a rather similar variation at both sampling points (Fig. 3c), and a positive correlation with turbidity ($r = 0.622$, $p = 0.003$). TP and TN concentrations oscillated between 1.80–6.48 mg L^{-1}

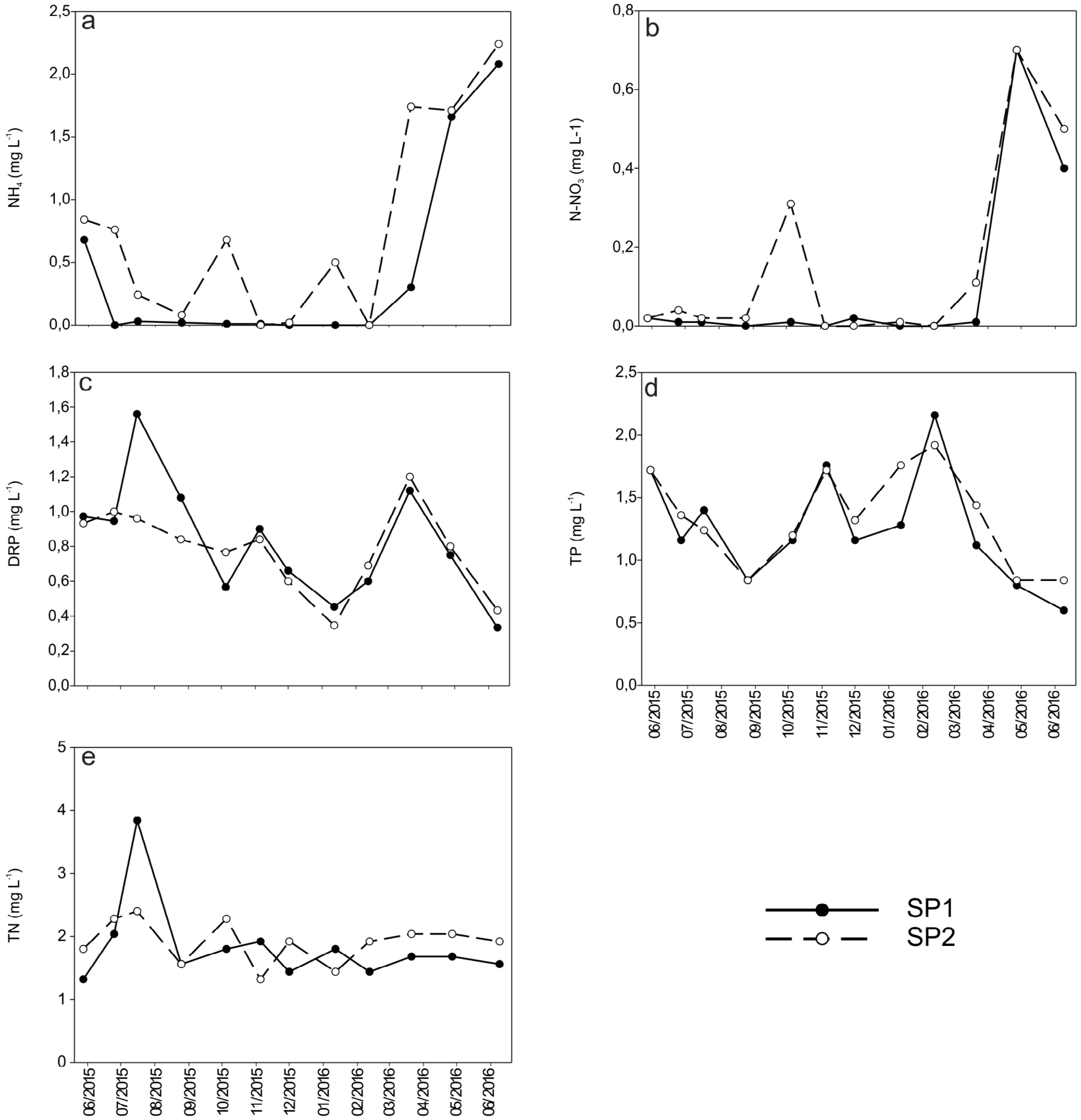

Fig. 3. Fluctuations in nutrient concentrations throughout the studied period in Lake Lugano. (a) NH_4: ammonia, (b) $N\text{-}NO_3$: nitrates, (c) DRP: dissolved reactive phosphorus, (d) TP: total phosphorus, (e) TN: total nitrogen. SP: sampling point.

and 1.32–3.84 mg L^{-1}, showing sharp fluctuations throughout the studied annual cycle (Fig. 3d and 3e). TP was positively correlated with water temperatures ($r = 0.592$, $p = 0.002$).

3.2 Phytoplanktonic community

Phytoplanktonic chlorophyll *a* concentrations differed strongly throughout the study, evidencing a variation of three orders of magnitude considering the maximum value 235.30 $\mu g\,L^{-1}$(July 2015) and its minimum 0.51 $\mu g\,L^{-1}$ (June 2016). The pattern of fluctuation was similar at SP1 and SP2, showing four and three clearly defined peaks (Fig. 4). Both, DRP concentration and turbidity were positively correlated with the chlorophyll *a* concentration ($r = 0.768$, $p < 0.0001$; $r = 0.768$, $p < 0.0001$, respectively).

Independent of the phytoplankton classification adopted (MFG or MBFG), the patterns of fluctuation of the community parameters, such as biodiversity or evenness, were very similar almost all throughout the study period and for both SP1 and SP2 (Fig. 5a and 5b). Moreover, Bray–Curtis similarity index revealed a very strong similitude assemblage (>0.8) composition between both sites almost at all sampling dates (Fig. 5c)

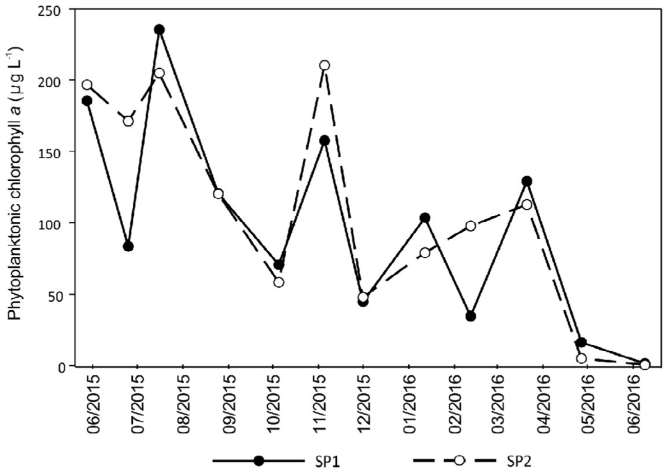

Fig. 4. Phytoplanktonic chlorophyll a fluctuations during the annual cycle 2015–2016 in Lake Lugano. SP: samping point.

whatever the classification approach was chosen. An exception was detected on spring 2015 and late-summer 2016 when this index was less than 50% (0.4). The Shannon Index never exceeded 1.6 and reached very low values (<0.2) during late-winter and spring 2015, mostly at SP1. The evenness associated to this index only presented high values (>0.8) occasionally and three clearly distinguishable peaks at autumn 2015 (May), summer (February 2016), and autumn again (June 2016). The autumn peaks coincided with moments of highest and minimum phytoplankton densities.

Phytoplankton density showed strong fluctuations at different moments throughout the study period oscillating between 92,639 and 707 ind ml^{-1} (Fig. 6a–6d), showing maximum and minimum values during the autumn seasons 2015 and 2016, respectively. After classifying the phytoplankton following the different approaches, it was observed that considering the MFG classification, the phytoplankton was generally dominated by MFG 5a (characterized by Cyanobacteria – filamentous Oscillatoriales) all throughout the annual cycle with the exception of late-autumn 2015, February 2016, and late autumn 2016 (Fig. 6a and 6b). However, it is worth to mention that even though this group was not dominant during autumn 2015, its densities were very high, reaching 26,660 ind ml^{-1} and 29,880 ind ml^{-1} at SP1 and SP2, respectively. At that moment, the MFGs co-dominating Lake Lugano's phytoplankton were MFG 5a and MFG 3a (characterized by autotrophic unicellular flagellates Phytomonadina), followed by MFG 7a (small centric diatoms). On the contrary, and for the same season in 2016, MFG 5a densities strongly dropped achieving minimum values at SP2 (22 ind ml^{-1}) and not being registered at SP1. February 2016 presented a phytoplankton community mainly represented by four MFG with very similar and relatively low densities each with an even structure (SP1 $J=0.78$; SP2 $J=0.71$), namely, MFG 5a, MFG 6a1 (characterized by large >30 μm – unicellular centric diatoms), MFG 11a (nonfilamentous chlorococcalean naked colonies), and MFG 11b (nonfilamentous chlorococcalean gelatinous colonies). The MFG dynamics throughout the studied period was very similar for both SP1 and SP2. Regarding the MBFG classification, a very similar pattern in group fluctuation was registered throughout the annual cycle, as compared with MFG (Fig. 6c and 6d). MBFG III (characterized by large filaments of Nostocales and Oscillatoriales with aerotopes) and MBFG V (represented by unicellular flagellates of medium to large size) were co-dominant during late-autumn 2015. After this date, a single group dominated the phytoplankton community at both study sites with the exception of February 2016 and autumn 2016. In this respect, MFBG III represented more than 80% of the total density during winter, spring 2015, and almost all throughout the summer. Again, for February 2016, an even phytoplankton structure was encountered (SP1 $J=1.0$; SP2 $J=0.87$). During this sampling date, MBFG III, MBFG IV (organisms of medium size lacking specialized structures), MBFG VI (nonflagellated organisms with siliceous exoskeletons), and MBFG VII (large mucilaginous colonies) represented the phytoplankton community at both sampling sites.

It is worth mentioning that the bloom-forming filamentous Cyanobacteria, *Planktothrix agardhii* (Gomont) Anagnostidis and Komárek 1988 (classified as MGF 5a and MBFG III) dominated Lake Lugano's phytoplankton almost all throughout the annual cycle and presented high densities for at least seven consecutive month-sampling dates. This species achieved extremely high values, peaking during early spring 2015 with values >62,000 ind ml^{-1}. It maintained high densities during late autumn 2015, spring, and summer and only decayed in numbers and relative importance during autumn 2016.

The RDA performed with the two phytoplankton classification approaches (MFG and MBFG) revealed strong similar results (Fig. 7a and 7b). The distributions of functional groups were influenced by temporal fluctuations of physical and chemical conditions. The cumulative percentage of variance of the species–environment relationship explained by the first axis was 95.1% ($p=0.002$) and 93.1% ($p=0.004$), for MFG and MBFG, respectively. It was directly associated to DRP ($r=0.5001$ and $r=0.5342$) and TN ($r=0.4879$ and $r=0.4887$), and inversely to NH_4 ($r=-0.6273$ and $r=-0.6607$) and Secchi transparency ($r=-0.833$ and $r=-0.8337$, MFG and MBFG, respectively, in all cases). The second axis explained 11.7 and 15.4% (MFG and MBFG, respectively) and was positively related to TP ($r=0.4538$ and $r=0.4888$, respectively). In both ordination analyses, samples were arranged according to a seasonal pattern. Samples of autumn 2015 were plotted in the upper side of the panel, related to high concentration of TP and characterized by the highest abundance of MFG 9b, MFG 3a, and MFG 7a (Fig. 7a) or MBFG IV, MBFG V, and MBFG VI (Fig. 7b). In turns, winter and spring 2015 samples were strongly influenced by the dominance of MFG 5a and MBGF III groups, situated in the right side of the panel when highest concentrations of TN and DRP, high turbidity conditions, and lowest water temperatures and NH_4 values were recorded. Contrarily, summer and autumn 2016 samples showing contrasting environmental conditions were represented in the left side of the graphics and were mainly related to a more diversified community.

4 Discussion

Major problems associated with eutrophication of urban shallow lakes include water quality deterioration with

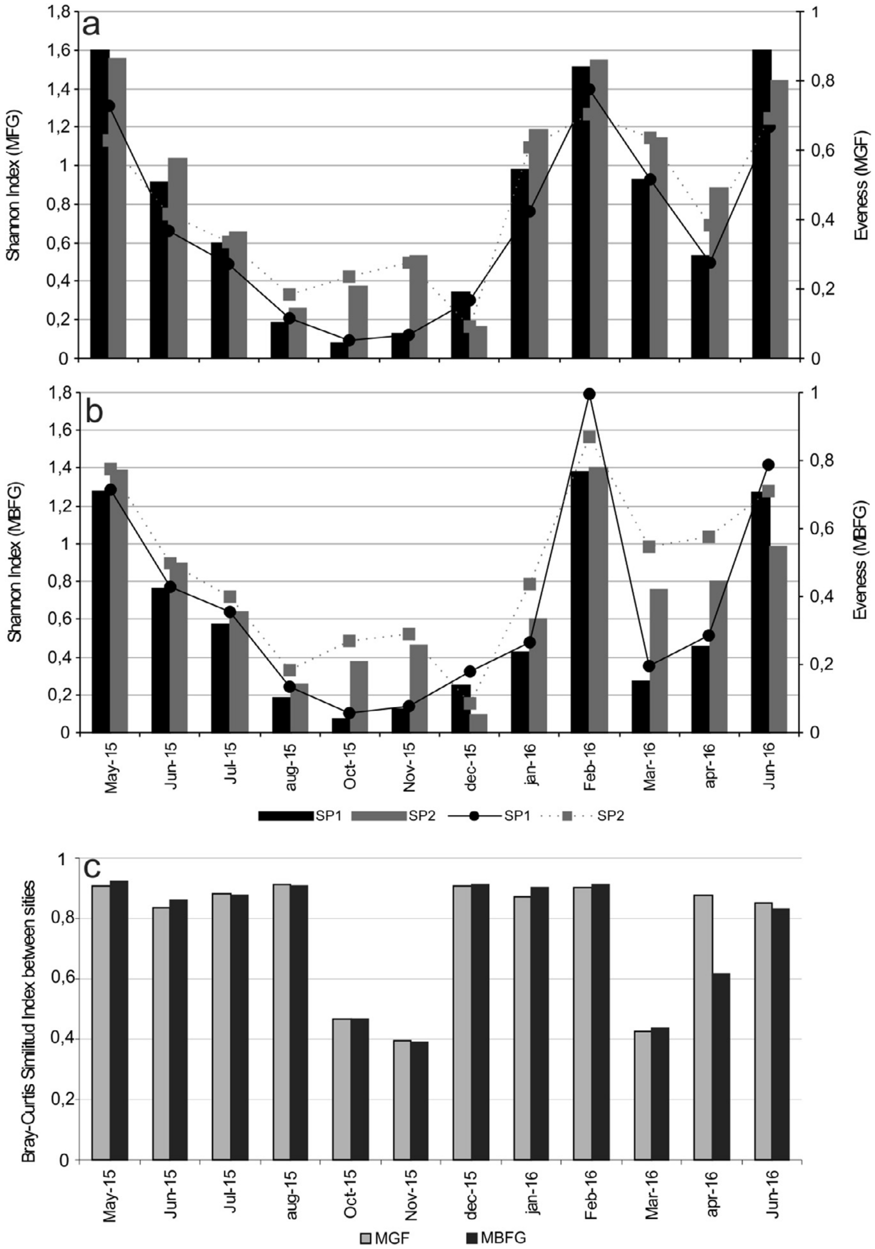

Fig. 5. Phytoplankton community attributes throughout the studied period in Lake Lugano, following the morpho-functional group (MFG) and the morphologically based functional group (MBFG) classifications. (a) Diversity and evenness for MFG. (b) Diversity and evenness for MBFG. (c) Similarity between sampling points considering both classification methods. SP: sampling point.

increased nutrient loads, such as phosphorus and nitrogen, leading to high densities of pelagic algae (increased phytoplankton chlorophyll *a* concentrations), which diminish water transparency. The increased turbidity due to phytoplankton, and the often predominance of Cyanobacteria, decrease the recreational value of urban superficial waters for the society. Lake Lugano constitutes one case under this scenario. It is the largest shallow lake in Buenos Aires City, as evidenced by this and past monitoring studies (Rodríguez *et al.*, 2003; Sinistro *et al.*, 2004). During the annual cycle 2015–2016, water remained turbid almost all throughout the year. In eutrophic ecosystems, Secchi depth transparency is below 1 m (Qiu *et al.*, 2001; Xiangcan, 2003; Chen *et al.*, 2009) or even lower than 0.5 m. In Lake Lugano, the values for this parameter fall within this range coinciding with previous observations (Rodriguez *et al.* op. cit.; Sinistro *et al.* op. cit.). Being already reported as an hypertrophic water body more than 10 years ago, Lake Lugano's DRP and TP concentrations showed more than a three-fold rise since then, evidencing an increased trend toward water deterioration. The extremely high TN and NH_4 concentrations also reveal the bad conditions of Lake Lugano and are similar or even higher than those encountered in highly impacted urban ponds and shallow lakes worldwide (Xiangcan, 2003; Segura *et al.*, 2013; Yu *et al.*, 2016; Rodríguez-Flores, 2017; Waajen, 2017). The increase in nutrient concentration can be the result of the input of runoff

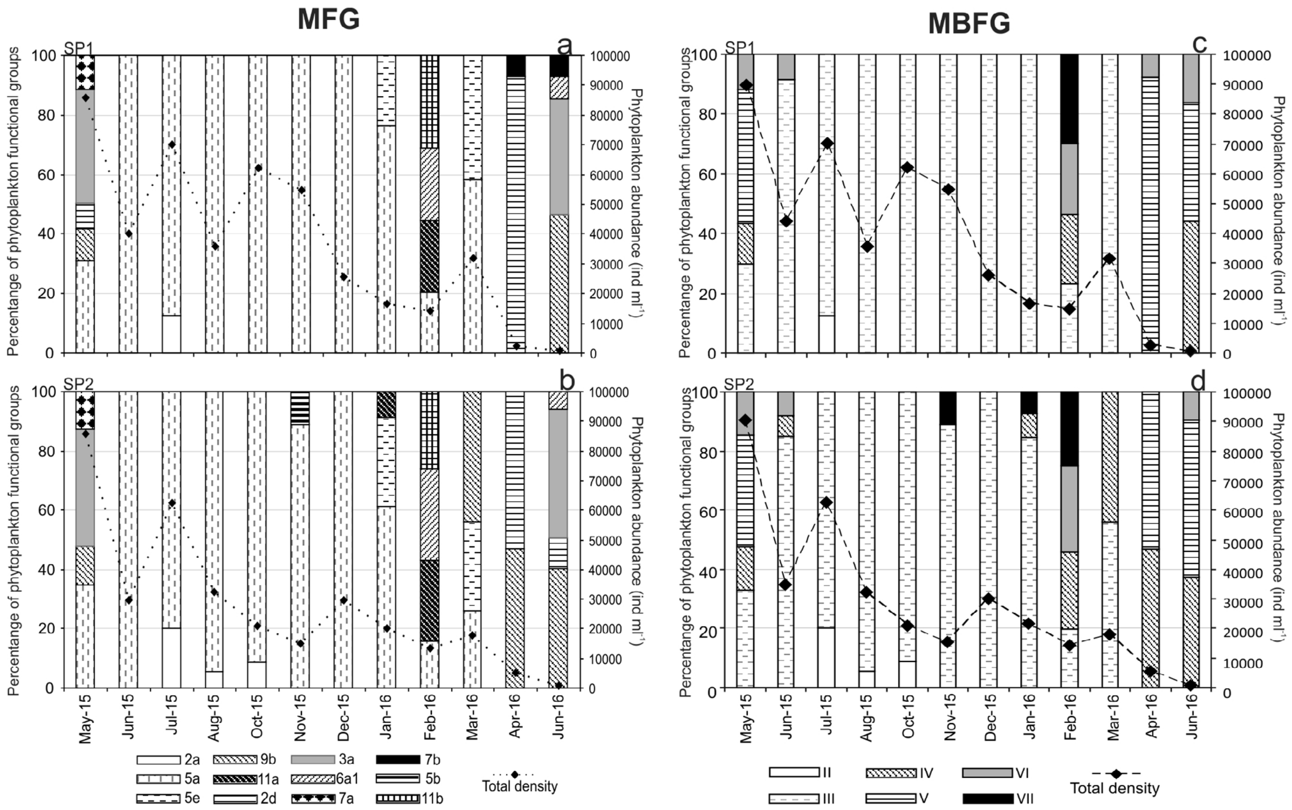

Fig. 6. Phytoplankton densities and proportions of the differing groups considering morpho-functional group (MFG) and the morphologically based functional group (MBFG) classifications in Lake Lugano for the studied period. SP: sampling point.

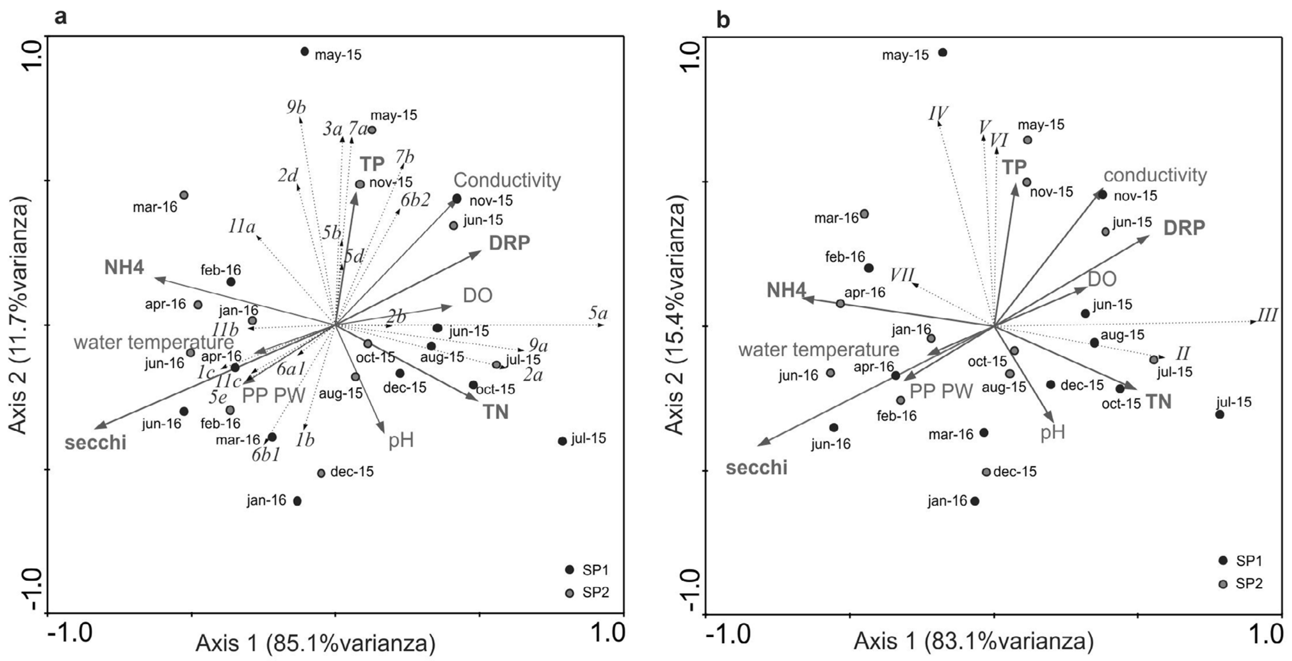

Fig. 7. Redundancy detrended analysis triplots based on the densities of (a) morpho-functional groups (MFG) proposed by Salmaso and Padisak (2007) and (b) morphologically based functional groups (MBFG) by Kruk et al. (2010).

waters as this shallow lake functions as an overflow system. Waters flowing on the nearby impervious surfaces enter the lake through a channel, and also illegal cloacal discharges have been reported (Rodríguez *et al.*, 2003). Urban population growth together with a limited investment in water supply and treatment infrastructure generate water quality problems (van der Bruggen *et al.*, 2010) such as what we have observed in Lake Lugano. Another aspect closely related to water deterioration in freshwater ecosystems is dissolved oxygen concentration. On some occasions, this parameter remained well below ($<5\,mg\,L^{-1}$) the guideline values for the maintenance of aquatic biota (Cuenca del Plata, 1987), confirming the poor water quality of this urban shallow lake. In hand with what was observed for transparency and nutrients, Lake Lugano's phytoplankton chlorophyll *a* concentrations were, in general, typical of eutrophic systems and exceeded $100\,\mu g\,L^{-1}$, evidencing its hypertrophic condition. The encountered values are within the ranges reported for other urban shallow lakes of differing latitudes (Fabre *et al.*, 2010; Segura *et al.*, 2013; Waajen *et al.*, 2014; Araújo *et al.*, 2016) and water bodies from Buenos Aires City (Avigliano *et al.*, 2014; Rodríguez-Flores, 2017). The sole exception to these eutrophic/hypertrophic values was recorded during the cold season 2016, associated with the collapse of the *P. agardhii* bloom evidenced in the decrease in MFG 5a/MBFG III densities.

The importance of assessing the ecosystems health (Meyer, 1997) includes not only ecological and functional aspects but also societal needs. Water systems deteriorate at high rates in megapolis and thus the development of procedures for diagnosing the ecological status and the monitoring urban freshwater ecosystems should be emphasized. The study of the aquatic biota represents an effective method to assess the integrity and health status of freshwater ecosystems, and it is now well known that biomonitoring is more advantageous than chemical monitoring (Lyche-Solheim *et al.*, 2013, and cites therein). Different biological components can be evaluated in this respect and lately, phytoplankton has been proposed for the assessment of the ecological status of freshwater ecosystems (Lepistö *et al.*, 2004; Padisák *et al.*, 2006; Lyche-Solheim *et al.*, 2013). In deteriorated shallow lakes, phytoplankton density increases, leading to diversity decline (Xiangcan, 2003). In Lake Lugano, biodiversity and evenness assessed following both functional groups approaches showed generally low values with an even community structure only occasionally. The lowest values characterized moments when MFG 5a/MBFG III were dominant; as already stated these groups include a bloom-forming Cyanobacterium (*P. agardhii*). Reduced diversity is characteristic of deteriorated systems and can be associated with the presence of Cyanobacteria. This aspect has been highlighted in particular for eutrophic/hypertrophic urban shallow lakes and ponds where *Planktothrix* spp. is a commonly reported case example (Scasso *et al.*, 2001; Crossetti *et al.*, 2008, Toporowska and Pawlik-Skowrońska, 2014).

The functional group approach was used in distinctive fields of ecological research. In particular for phytoplankton, the applicability of the morpho-functional group approach (Salmaso and Padisak, 2007) in the derivation of water quality indexes has been recently assessed (Salmaso *et al.*, 2015 and references therein). The criteria to define MFG are among the strongest traits for predicting the best competitors under different environmental conditions. In agreement with this, our results evidenced that the dynamics of MFG responded to temporal fluctuations of both physical and chemical conditions as evidenced by the high variance explained in the species–environment relationship by the multivariate analysis. During the study, Cyanobacteria in MFG 5a (filamentous algae Oscillatoriales), green-algae in MFG 9b (small $<30\,\mu m$ unicellular Chlorococcales), and in MFG 3a (autotrophic flagellates – Phytomonadina), and diatoms in MFG 7a (small $<30\,\mu m$ – centric diatoms) were the groups more abundantly represented in Lake Lugano's phytoplankton. The RDA revealed that MFG 5a was the functional group associated with high concentrations of TN and DRP. Represented by oscillatorian Cyanobacteria, the dominance of this group is generally related to the high availability of nutrients (Waajen, 2017). These water characteristics remained almost all throughout the study period. Eutrophic and hypertrophic lakes are susceptible to bloom formation and seasonal fluctuations in phytoplankton communities are commonly reduced (Xiangcan, 2003; Allende *et al.*, 2009; Toporowska and Pawlik-Skowrońska, 2014; Waajen, 2017). The almost persistent year-round bloom of *P. agardhii* (main representative of MFG 5a) increased the turbidity of the water column. This condition negatively affects the development of green-algae, which lack motility (Happey-Wood, 1988). In this respect, the small $<30\,\mu m$ unicellular Chlorococcales (MFG 9b) only became relatively abundant when MFG 5a was not dominant. Nonfilamentous chlorococcalean colonies in MFG 11a (naked colonies) and in MFG 11b (gelatinous colonies) only "appeared" (in spite of their relative low numbers) when the bloom collapsed and transparency increased. When transparency was low, only flagellated green-algae were able to codominate the phytoplankton community, represented by unicellular autotrophic Phytomonadina (MFG 3a). The presence of flagella is an advantageous trait under this condition, promoting their growth in combination with high nutrient concentrations. MFG 3a was also related to high turbidity values in eutrophic shallow lakes subjected to agricultural impact shallow lakes (Izaguirre *et al.*, 2012). Diatoms are susceptible to suffer sinking losses in stratified water columns due to their heavy siliceous walls, which distinguish them from other organisms (Padisák *et al.*, 2003). In Lake Lugano, small and centric diatoms in MFG 7a were only relatively abundant on autumn 2015, probably due to increased mixing conditions that allowed their maintenance in the water column. The lack of rooted macrophytes in shallow lakes makes them susceptible to wind action (Scheffer *et al.*, 1993) what guarantees the mixing of the water column and the concomitant sediment resuspension increasing the turbidity of the water column. This turbid environment provides adequate conditions for the development of algae adapted to cope with light constrains such as *Planktothrix* and can lead to blooms provided the nutrient requirements are fulfilled. As stated by Reynolds *et al.* (2002) in their trait-based functional classification of the freshwater phytoplankton, *Planktothrix agardhii* is a typical representative of the codon S1, characteristic of turbid mixed layers, presenting a high tolerance to light-deficient conditions. This scenario has been present in Lake Lugano and *P. agardhii* (MFG 5a; MBFG III) was the dominant species (and corresponding morpho-functional group) almost all throughout the annual cycle. In this respect, we can argue that under the

Alternative Steady States framework (Scheffer *et al.,* 1993), we can further recognize a phytoplankton turbid state dominated by low-light adapted Cyanobacteria, such as *P. agardhii*, when mixed and highly turbid conditions (provoked by increased sediment resuspension and not only due to increased algal densities) are met. The similarity encountered between the two sampling points both regarding the physical and chemical parameters and the structure and dynamics of the phytoplankton community provide further evidence of the characteristic mixed water of this shallow lake lacking associated vegetation during the 2015–2016 annual cycle. In this respect, we did not register the copious presence of palustrine, submerged and/or floating macrophytes throughout the study as reported previously for Lake Lugano (Rodríguez *et al.*, 2003). Note that those authors found that in the presence of macrophytes it was the cyanobacterium *Microsystis aeruginosa,* representative of codon M (Reynolds *et al.*, 2002), the typical bloom-former in Lake Lugano. Thus, in the previous study, more stagnant waters in eutrophic Lake Lugano might have provided the conditions required by this algae to show an exponential growth. In this respect, the dominance of morpho-functional group of Cyanobacteria under eutrophic conditions might depend on the stability of water column. Whereas *P. agardhii* (codon S1, MFG 5a/MBFG III) blooms are reported for mixed situations, *Microsystis* sp. (codon M, MFG 5c/MBFG III) requires more stagnant conditions (Reynolds *et al.*, 2002; Padisák *et al.*, 2009).

Interestingly, similar patterns in the seasonal development of the phytoplankton community as assessed considering the morphology-based functional group classification was encountered, and groups responded to the same set of physical and chemical variables as the morpho-functional groups. MBFG III (filaments of Nostocales and Oscillatoriales with aerotopes), MBFG V (unicellular flagellates of medium to large size), MBFG IV (organisms of medium size lacking specialized structures), MBFG VI (nonflagellated organisms with siliceous exoskeletons), and MBFG VII (large mucilaginous colonies) represented the phytoplankton community at different moments during the annual cycle. Again, the dominance and seasonal dynamic of the potentially toxic bloom-forming *P. agardhii* was detected by this classification method, representing the MBFG III. This group was also associated to high DRP and TN concentrations and low transparency. The close relationship between MBFG III and TN has been already reported for shallow lakes in highly human impacted areas (Izaguirre *et al.*, 2012) and its dominance was reported to be associated to low light conditions and high TN values (Kruk and Segura, 2012). The success of Oscillatoriales in this morpho-functional group is in agreement with studies eutrophic shallow lakes (Bonilla *et al.*, 2012; Waajen *et al.*, 2014) and confirms that they can succeed in turbid, eutrophic lakes as originally proposed for Oscillatoriales (Scheffer *et al.*, 1997). Studies on phytoplankton succession based on the MBFG approach revealed that the dominance of highly specialized groups, such as MBFG III, is expected to continue under stable conditions (Segura *et al.*, 2013). The other groups only evidenced limited representativeness occasionally, and responded to the same environmental variables as the morpho-functional group classification. They were associated to high TP concentrations and became relatively important following the same pattern as described for the MFG approach, mostly when MBFG III (represented as MFG 5a) was not dominant.

Several studies have evaluated the deficiencies and the benefits of the functional group approach comparing, among others, MFG and MBFG in different types of freshwater ecosystems (Litchman *et al.*, 2010; Izaguirre *et al.*, 2012; Abonyi *et al.*, 2014; Bortolini *et al.*, 2014; Salmaso *et al.*, 2015; Rodríguez-Flores, 2017). Some authors have stated that whereas the MFG approach can explain phytoplankton dynamics in relation to environmental variables (regional and temporal scales), the MBFG can better explain large-scale variations (Salmaso *et al.*, 2015 and cites therein). The strength in the assessment of phytoplankton dynamics by the MFG (based on 33 groups) resides in the fact that it can explain changes in the phytoplankton in response to major environmental drivers representing an adequate tool to monitor this community. However, expertise in taxonomy is required at least up to the genera/order level. In turn, the application of MBFG classification (based on seven groups) is beneficial due to the relative simplicity and independence of taxonomic affiliation (Izaguirre *et al.*, 2012), and diminishes the high dimensionality typical of phytoplankton community (Segura *et al.*, 2013). The obvious disadvantage of MBFG with respect to the MFG is that the simplification in a reduced number of groups does not allow for a more detailed description of the phytoplankton assemblage composition. In this sense, MBFG is not able to detect a number of aspects that might be relevant when studying the phytoplankton assemblage (*i.e.*, mixotrophic and autotrophic flagellates; chlorococcalean and chroococalean mucilagenous colonies). We coincide that, as stated by Kruk *et al.* (2010), the MBFG approach is coarse in comparison to other methods of phytoplankton classification (*i.e.*, morpho-functional group by Salmaso and Padisak, 2007). However, its relative simplicity turns it to be an easy-to-use tool for determining the phytoplankton assemblage in urban shallow lakes. In our study, these two phytoplankton classifications produced similar and overlapping results as evidenced by more than one parameter (diversity, evenness) and by the multivariate analyses. Even though there was greater functional group diversity when considering MFG (based in a greater number of possible groups), dominance, codominance, and dynamics patterns were very similar for both classifications. This provides evidence that the MBFG was sensitive enough to characterize the phytoplankton community of Lake Lugano.

Some considerations regarding the prevalence of *P. agardhii* represented in MFG 5a/MBFG III are worth mentioning. This group achieved very high densities in spite of the low water temperatures in the cold season 2015. Thus, in Lake Lugano, a decrease in water temperature might not necessarily result in Cyanobacterial density decay as it was also reported for other small urban aquatic ecosystems (Fabre *et al.*, 2010) at similar latitudes. Moreover, the absence of macrophytes in eutrophic systems favors the development of Cyanobacterial blooms (Allende *et al.*, 2009). These aspects constitute a matter of concern, as year-round blooms can persist in Lake Lugano with the evident impact in their recreational value. As well, a potential risk for aquatic biota and humans derives from the cyanobacterial toxic blooms (Marie *et al.*, 2012) and can result in fish kills. This issue has been already reported for Lake Lugano in 2003 when

Microcystis aeruginosa (Kützing) Kützing reached densities that exceeded 100,000 ind ml^{-1} (Rodríguez *et al.*, 2003). Common Cyanobacteria genera are known as toxin producers (*i.e.*, *Planktothrix* (MFG 5a/MBGF III), *Nostoc* (MFG 5e/MBGF III), *Dolychospernum* Syn. *Anabaena* (MFG 5e/MBGF III), and *Microcystis* (MFG 5c/MBGF VII) and are repeatedly reported in urban aquatic ecosystems (Scasso *et al.*, 2001; Rodríguez *et al.*, 2003; Lv *et al.*, 2011; Toporowska and Pawlik-Skowrońska, 2014; Waajen, 2017). Throughout our study, bloom-densities were registered for the filamentous Cyanobacteria *P. agardhii.* Even though we did not measure cyanobacterial-derived toxins, the potential toxicity of *Planktothrix* is recognized by different authors (Koramek and Komarková, 2004; Tonk *et al.*, 2005; Hulot *et al.*, 2012; Marie *et al.*, 2012). We did not register fish mortality during our year-round study but the repeated presence of *P. agardhii* should not be disregarded. Thus, the method employed for the study of the phytoplankton of these systems should include the possibility to identify and follow-up the dynamics of these potentially toxic algae. It is worth mentioning that the phytoplankton classification methods employed in our study were able to detect both the dominance and the dynamics of *P. agardhii* (MFG 5a/MBFG III).

5 Conclusion

The applicability of the MFG or the MBFG approach depends on the type of research being held and the aim of the study. Our results showed that the dynamics of the phytoplankton communities as assessed by MFG (Salmaso and Padisak, 2007) and MBFG (Kruk *et al.*, 2010) classifications similarly described their attributes and followed overlapping patterns of fluctuation responding to changes in environmental conditions in Lake Lugano. The two methods were sensitive enough to detect the presence of potentially toxic filamentous Cyanobacteria, as well as bloom episodes and their collapses. As the MFG classification requires more specialized skills in order to classify the organisms encountered than the MBFG, we consider that MBFG classification was an adequate, easy-to-handle method for monitoring Lake Lugano on an annual basis. Due to the similarity in the results obtained, the latter approach proved to be sensitive enough and could represent an objective way to study the phytoplankton dynamics by water quality managers. However, expert judgment is also required when alert signals are detected in the monitored ecosystem.

The application of the MBFG classification for biomonitoring purposes of urban shallow lakes and ponds should necessarily be done in combination with the identification of the dominant species and/or potentially toxic bloom-forming species. In particular, changes in MBFG III and MBFG VII should be closely followed as different highly toxic genera are classified in these groups. As well, further studies should include the estimation of cyanobacterial-derived toxin concentrations in water as a prerequisite to evaluate its quality in relation to the recreational value. We also consider that the applicability of the MBFG approach deserves to be further explored as a promising tool for the biomonitoring of different types of urban water bodies located in recreational parks, ecological reserves, and even in domestic ponds.

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