

# Water residence time and the dynamics of toxic cyanobacteria

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## SUMMARY

1. Climate change affects aquatic ecosystems differently depending on local conditions. In the Mediterranean region, predicted drier seasons could especially affect lake water residence time and in consequence cyanobacteria and cyanotoxin dynamics.
2. We carried out a 3-year study of a shallow, Mediterranean lake (Lake Albufera, Spain), to study the effects of water residence time and other drivers on the dynamics of harmful cyanobacteria and microcystin concentrations (MCYST).
3. Longer water residence time in dry years and dry seasons increased total cyanobacteria biomass, *Microcystis aeruginosa* populations and MCYST concentrations in the lake water and seston. Droughts increased water retention time by about 45%, and *M. aeruginosa* populations and MCYST were 1–2 orders of magnitude higher.
4. All samples analysed contained MCYST, and among them 70% had values above the recommended guidelines. Flows lower than  $10 \text{ m}^3 \text{ s}^{-1}$  raised toxicological risk from low to moderate-high according to international standards. Mean MCYST concentrations bound in the cells were one order of magnitude higher than in the water ( $11 \pm 2.9$  and  $1.2 \pm 0.3 \mu\text{g L}^{-1}$ , respectively).
5. The microcystin content per unit biovolume of *M. aeruginosa* was generally higher at the start of population growth (April–May) than at the population maximum (July–October). This was related to increase in water residence time, total phosphorus concentration and mean colony size within the edible range ( $<50 \mu\text{m}$ ). The maximum MCYST content corresponded with average populations of  $10^3 \text{ colonies L}^{-1}$  and  $2 \text{ mm}^3 \text{ L}^{-1}$ , which could additionally be used to evaluate toxicological risks in the lake.
6. *Microcystis aeruginosa* colonies were larger with increasing water residence time and more closely related to the lake hydrology and water column stagnation than to MCYST colony content.
7. Feasible measures for restoration and conservation of shallow Mediterranean lakes in a future climate scenario are discussed.

**Keywords:** mediterranean, microcystin, *Microcystis*, restoration, shallow lake

## Introduction

Ecosystem responses vary from place to place, and it is still hard to predict how different ecosystems will be modified by climate change, which influences a variety of ecological processes, such as ice-cover, wind regimes, solar radiation and precipitation (Harvey, 2000). Shallow lakes, owing to their morphology and large interfaces with both the atmosphere and sediment, can react more

rapidly to changes in nutrients and temperature than deeper lakes (Moss, 1998; Mooij *et al.*, 2007). Changes in water column stability, light and nutrient loading may have implications for phytoplankton dynamics and hence for food webs in shallow lakes (Moss, 1998). In some shallow northern European lakes, predictions about the effects of climate change have highlighted an increase in lake water nutrients and turbidity, with a loss of aquatic biodiversity, affecting especially aquatic plants and birds,

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together with a greater presence in the plankton of cyanobacteria (Jeppesen, Søndergaard & Jensen, 2003; Mooij *et al.*, 2007; Wagner & Adrian, 2009).

Similar studies on shallow lakes in other climatic zones are scarce and deserve more attention. The Mediterranean region will be one of the most sensitive areas regarding extreme climate changes, because of marked decrease and fluctuation in seasonal precipitation (Harvey, 2000; Sánchez *et al.*, 2004). For the Mediterranean zone, temperature is expected to increase twice as rapidly as in northern Europe ( $0.1\text{--}0.4\text{ }^{\circ}\text{C decade}^{-1}$ ) (IUCN (the World Conservation Union), 2002; Sánchez *et al.*, 2004), while mean annual precipitation will decrease south of  $45^{\circ}\text{N}$  (Harvey, 2000). Consequently, shortage of rainfall is expected to accentuate water-level fluctuations and residence times in freshwater bodies of the Mediterranean zone. These changes can severely affect other variables, such as salinity, nutrient concentrations, underwater light and structure of food webs in shallow Mediterranean lakes (Beklioglu *et al.*, 2007). For example, nitrate-nitrogen availability is anticipated to decrease as a result of reduced leaching, probably a prolonged terrestrial growing season and increased terrestrial nitrogen assimilation, and, on the other hand, in-lake assimilation of nitrogen by increased biological activity (Beklioglu *et al.*, 2007), and these predictions are complicated by unknown future use of fertilisers in agriculture. Furthermore, this is an area where water is limited and the possible negative effects of climatic change on water quality can restrict even more the water supply for human uses and conservation of natural lakes.

Some authors suggest that global warming will lead, in temperate lakes, to a seasonal shift towards greater presence of cyanobacterial blooms (Paerl & Huisman, 2009). This is partly attributed to their preference for higher temperatures compared with other phytoplankton groups (Reynolds, 1997; Butterwick, Heaney & Talling, 2005; de Senerpont Domis, Mooij & Huisman, 2007). However, in warmer lakes this group of algae often already predominates (Cook, Vardaka & Lanaras, 2004; Romo *et al.*, 2004; Kosten *et al.*, 2012) for a variety of reasons, such as direct and indirect effects of temperature, high nutrient levels, turbidity and low zooplankton grazing (Romo *et al.*, 2004; Gyllström *et al.*, 2005; Kosten *et al.*, 2012). Internal nutrient recycling from the sediment is increased by temperature (Søndergaard, Jensen & Jeppesen, 2003), and nutrients can be concentrated in the water column by evaporation in shallow Mediterranean lakes (Özen *et al.*, 2010). High irradiance and temperature combined with low rainfall and consequent longer water residence times can promote cyanobacterial growth dur-

ing the whole year in the Mediterranean zone (Beklioglu *et al.*, 2007).

Cyanobacteria can benefit not only from their higher optimum growth temperatures, but also from the increase in vertical water stability imposed by temperature (Paerl & Huisman, 2009). Different cyanobacteria can respond variously to water conditions, and large, buoyant colonial cyanobacteria, like *Microcystis*, can take advantage of stable water columns (Paerl, 1988; Visser *et al.*, 2005; Elliott, 2010). *Microcystis* has a low growth rate, and its population losses are reduced by gas vesicles counteracting sedimentation and by colony formation that additionally may reduce grazing (Reynolds, 1997). Buoyancy confers an advantage to this colonial species to accumulate and form blooms on the water surface or epilimnia under stiller conditions (Visser *et al.*, 2005). Recent field evidence has also linked warmer summers with the proliferation of buoyant cyanobacteria in some temperate stratified lakes (Jöhnk *et al.*, 2008; Wagner & Adrian, 2009). Since *Microcystis* tends to accumulate in the water surface, flushing has been used as a restoration measure to prevent toxic cyanobacteria blooms, and toxicological and environmental problems in some shallow lakes (Jagtman, Van Der Molen & Vermij, 1992; Verspagen *et al.*, 2006). All of this suggests more outbreaks of inedible and toxic algae in freshwater water bodies with climate warming.

Cyanobacteria and cyanotoxins can cause major water quality problems (Chorus, 2001; Huisman, Matthijs & Visser, 2005) through neurotoxins, hepatotoxins, cytotoxins and skin irritants, which can threaten humans, other vertebrates and invertebrates (Chorus & Bartram, 1999). Microcystins are hepatotoxins, which are mainly produced by species of *Microcystis*, *Anabaena*, *Anabaenopsis*, *Planktothrix*, *Oscillatoria*, *Hapalosiphon* and *Nostoc* (Chorus & Bartram, 1999; Codd *et al.*, 2005). Assessments of cyanotoxin occurrence and distribution from natural lakes are fewer than those reported from reservoirs used for drinking water, despite the unquestionable importance of natural lakes for recreation, wildlife and conservation (Chorus & Bartram, 1999; WHO, 2003). Surveys of cyanobacteria and cyanotoxins, as well as the factors affecting their presence, are poorly documented for shallow Mediterranean lakes (Cook *et al.*, 2004; Kardinaal & Visser, 2005), even though *Microcystis* appears in almost all the algal blooms reported (Cook *et al.*, 2004). Recent studies in some shallow Greek lakes have revealed a widespread occurrence of harmful cyanobacteria and MCYST (Cook *et al.*, 2004; Gkelis *et al.*, 2005; Vardaka *et al.*, 2005; Kagalou *et al.*, 2008; Papadimitriou *et al.*, 2010).

Some studies on how climate changes affects water stability and phytoplankton succession in stratified lakes have been reported recently (Jöhnk *et al.*, 2008; Wagner & Adrian, 2009; Elliott, 2010). However, how lake water residence time may drive dynamics of toxic cyanobacteria and cyanotoxins in polymictic lakes remains almost unreported. This is especially the case for shallow Mediterranean and other warm lakes, where future droughts may severely increase water residence times and cyanobacteria, as well as affect the uses, management and conservation of such lakes.

This work examines the effect of water residence time on cyanobacteria and microcystin dynamics for 3 years in a shallow Mediterranean lake (Lake Albufera, East Spain) and is one of the longest monitoring of microcystin concentrations (MCYST) in a shallow lake, extending the study period during the whole year. We tested whether longer water residence time during drier years and seasonal stagnation increased the presence of toxic cyanobacteria, and of MCYST in the water and seston. Other factors relevant to their ecology and dynamics, such as lake nutrient concentrations and colony size of *Microcystis*, were also studied. Critical water renewal rates and other restoration measures to avoid or mitigate cyanobacterial blooms and lowering health and environmental risks are discussed in the framework of a future climate scenario.

## Methods

### Study area

Lake Albufera is the largest Spanish coastal lake, with a surface area of 23.2 km<sup>2</sup>. It is shallow (mean depth of 1.2 m), polymictic and oligohaline (salinity 1–2‰) and is located in the Natural Park of the Albufera (210 km<sup>2</sup>) on the Mediterranean Spanish coast (39°20'N, 0°21'W). The Park is a wetland protected by the Ramsar Convention and the European Habitat list NATURA 2000. Since the 18th century, rice has been cultivated intensively in the areas surrounding the lake. The lake water is regulated by sluice gates situated on its three outlet channels that flow into the Mediterranean Sea. Daily outflow has been automatically recorded since late 2005. The hydrological cycle of the lake is related to seasonal rainfall and rice cultivation. The lake has a longer water residence time from April–May to September, but new inflow increases in October and especially in January–February with seasonal rain and emptying of the paddy fields (Romo *et al.*, 2005). The annual mean air temperature for 1980–2008 was 18.3 ± 0.1 °C (mean ± SE) and annual mean

rainfall, 614 ± 40 mm (mean ± SE). During the period studied, 2005 and 2006 were drier (349 and 432 mm) than 2007 and 2008 (687 and 825 mm, respectively). Accordingly, the annual lake water turnover was 5.5, 7.7 and 9.5 year<sup>-1</sup> for 2006, 2007 and 2008, respectively, and calculations were made following the method suggested by Soria & Vicente (2002).

The Albufera Lake has one of the longest phytoplankton data sets for shallow Mediterranean lakes (1980–2008). The lake has been eutrophic since the 1970s and cyanobacteria predominate throughout the year, except for sporadic periods (few days or weeks) of clear water after partial reduction in external nutrient loading (Villena & Romo, 2003; Romo *et al.*, 2005). Since 2002, potentially toxic cyanobacteria have generally increased (*Microcystis aeruginosa* (Kützinger) Kützinger and *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya et Subba Raju (Romo *et al.*, 2008). Zooplankton is mainly dominated by rotifers and copepods (Romo *et al.*, 2005). Fisheries are still an economic activity in the lake, and about 200 tons of mugilids (mainly *Liza* spp. and *Mugil cephalus* Linnaeus) are removed annually for human consumption (Blanco *et al.*, 2003; Blanco & Romo, 2006). More detailed information about the lake is given elsewhere (Romo *et al.*, 2005).

### Sampling and analyses

A total of 68 water samples were collected between November 2005 and December 2008 at weekly, bimonthly or monthly intervals. Samples were taken from the upper 50 cm of the water column at three representative points in the lake dependent on water inputs (Romo & Miracle, 1993). Concentrations of nitrate, ammonium, orthophosphate, total phosphorus and total nitrogen in the lake water were analysed according to standard methods (APHA, 1992). Chlorophyll *a* was extracted with 95% acetone after filtration with GF/F filters and measured spectrophotometrically (APHA, 1992).

Phytoplankton community composition was determined according to Romo & Miracle (1993) and algal biovolume according to Hillebrand *et al.* (1999). Biovolume of *M. aeruginosa* colonies was calculated as the sum of cell volumes per colony to avoid overestimation when biovolume is estimated considering colony volume. Colony mean length was measured in at least 25 individuals per sample.

Dissolved and cell-bound microcystins were analysed by filtering onto GF/F fibreglass filters and frozen for subsequent total microcystin analysis. For analysis of dissolved microcystins, the filtered lake water was analysed directly by enzyme-linked immunosorbent assay

(ELISA). Cell-bound microcystins were extracted from the filters with methanol (90%) followed by vacuum centrifugation to remove the organic solvent. The extract was suspended in distilled water, and the supernatant after centrifugation was subjected to ELISA assay in duplicate or triplicate (Fastner, Flieger & Neumann, 1998). For each ELISA test, the commercial EnviroGard microcystin kit was used, and the control and six calibrates (range 0.1–1.6 ppb) were assayed and dilutions of the samples were used when necessary. The ELISA is an indirect-competitive method using the b-amino acid 6E-ADDA as the epitope for antibody recognition for the quantitative analysis of all the microcystin analogues and nodularins. It was assumed that all the MCYST analogues determined by ELISA have equivalent toxicity to microcystin-LR, and results are expressed as micrograms of cellular MCYST equivalents per litre (Kotak *et al.*, 1995).

### Statistical analyses

Data were log-transformed if necessary for statistical normality. ANOVA and Tukey's test were used to examine significant differences of the variables between years and sampling points. Spearman correlation coefficient was used to determine significant relationships between pairs of variables. Data were explored using the statistical package SPSS 17.0 for Windows.

### Results

There were no significant differences in the concentration of algal biomass and MCYST in the lake water or cell-

bound among the three sampling points during the study period ( $P > 0.05$ ), thus the average values for all sampling points are reported.

Total cyanobacteria biovolume and abundance during the study period averaged 70 and 78% of total phytoplankton biovolume and abundance, respectively (maxima of 97 and 98%, respectively). Chroococcal cyanobacteria, *M. aeruginosa* and MCYST values were higher during drier years with longer water residence times (Table 1). There were no significant differences in nutrient levels among the study years (Table 1). There were significant negative correlations between water renewal and total algal biovolume and abundance ( $r = -0.49$  and  $r = -0.46$ ,  $P < 0.01$ ), as well as with chlorophyll *a* ( $r = -0.28$ ,  $P < 0.05$ ). *Microcystis aeruginosa* populations were also negatively correlated with rate of water flushing ( $r = -0.40$  and  $-0.38$ ,  $P < 0.001$  for biovolume and abundance, respectively) (Fig. 1), and their biovolume varied by two orders of magnitude among hydrological years (Table 1). Populations of *M. aeruginosa* represented up to 14% of total biovolume during the study period and developed mainly during April–October at the time of longer water residence time in the lake (Fig. 2). Diameter of *M. aeruginosa* colonies ranged between 18 and 106  $\mu\text{m}$  during the study period (Table 1). Mean colony size changed significantly between years (Table 1) and was negatively correlated with water renewal ( $r = -0.40$ ,  $P < 0.001$ ) (Fig. 1).

All samples analysed contained MCYST in the lake water and seston, and 70% of them had values above  $1 \mu\text{g L}^{-1}$ . In the water samples, mean MCYST concentration was  $1.2 \pm 0.3 \mu\text{g L}^{-1}$  (mean  $\pm$  SE) and ranged

**Table 1** Mean, minimum and maximum values of the variables studied (Nov 2005–Dec 2008)

	Mean	Min	Max	2006	2007	2008	<i>P</i>
Water flushing ( $\text{m}^3 \text{s}^{-1}$ )	4.30	0.02	23.5	3.3	4.0	4.8	*
Nitrate ( $\text{mg N L}^{-1}$ )	1.36	0.09	5.08	1.65	1.41	1.31	ns
Ammonium ( $\text{mg N L}^{-1}$ )	0.28	0.01	1.94	0.52	0.35	0.24	ns
DIN ( $\text{mg N L}^{-1}$ )	1.65	0.12	5.48	2.26	1.77	1.54	ns
Total nitrogen ( $\text{mg N L}^{-1}$ )	4.68	2.56	6.16	nd	4.57	4.66	ns
Total phosphorus ( $\text{mg P L}^{-1}$ )	0.23	0.09	0.64	0.25	0.19	0.30	ns
Orthophosphate ( $\text{mg P L}^{-1}$ )	0.015	0.001	0.051	nd	0.013	0.015	ns
Chlorophyll <i>a</i> ( $\mu\text{g L}^{-1}$ )	156	20	515	127	162	202	ns
Cyanobacteria total abundance (ind $\text{mL}^{-1}$ )	378 147	3729	1 009 772	516 522	356 607	347 234	ns
Chroococcal cyanobacteria abundance (ind $\text{mL}^{-1}$ )	78 015	792	492 925	165 458	69 949	52 445	*
Cyanobacteria total biovolume ( $\text{mm}^3 \text{L}^{-1}$ )	83	0.25	387	187	79	56	**
<i>Microcystis aeruginosa</i> abundance (ind $\text{mL}^{-1}$ )	1429	9	7058	3502	1500	376	***
<i>Microcystis aeruginosa</i> biovolume ( $\text{mm}^3 \text{L}^{-1}$ )	3.08	0.01	30.49	10.48	2.82	0.24	***
<i>Microcystis aeruginosa</i> colony diameter ( $\mu\text{m}$ )	41	18	106	57	41	29	**
Water microcystins ( $\mu\text{g L}^{-1}$ )	1.2	0.1	15.9	6.0	1.6	0.4	*
Seston microcystins ( $\mu\text{g L}^{-1}$ )	10.9	0.1	120.5	7.0	22.9	1.7	*

The last column summarises results from the ANOVA to test for differences between years.

Probabilities are: \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , ns = not significant differences, nd = not determined.

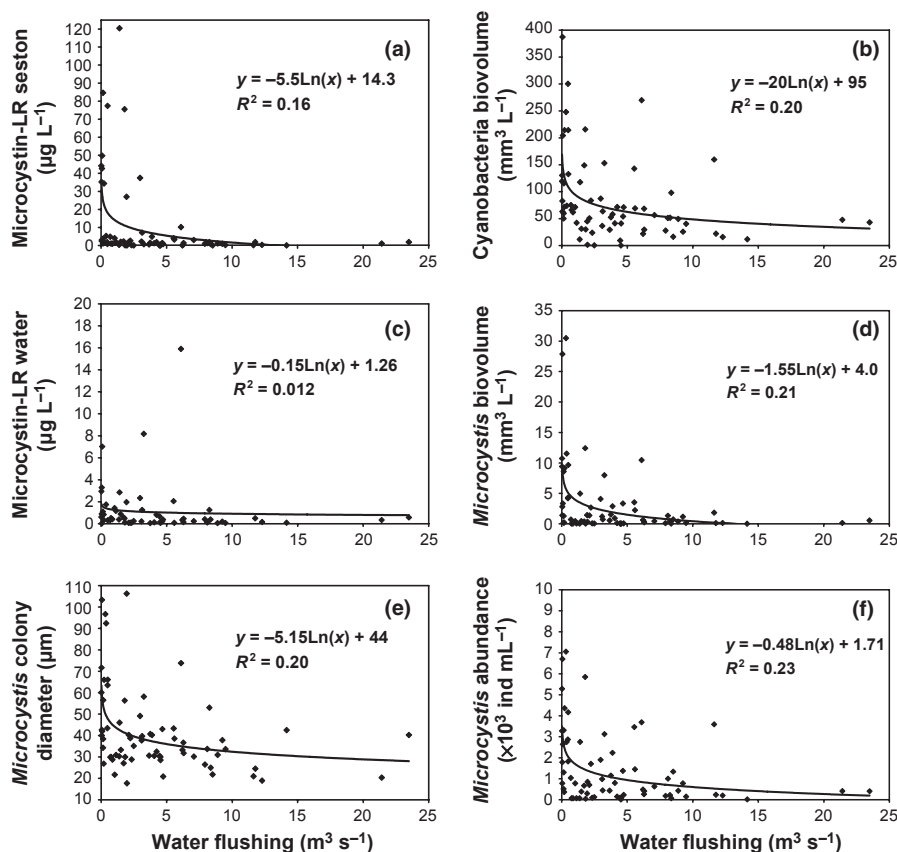


Fig. 1 Relationships of seston and water MCYST, total cyanobacteria biovolume, *Microcystis aeruginosa* colony diameter and *M. aeruginosa* biovolume and abundance with water flushing of the lake during the study period (Nov 2005–Dec 2008). *M. aeruginosa* is abbreviated as *Microcystis*.

between 0.1 and 16 µg L⁻¹ for the study period (Table 1). The mean percentage of water MCYST in relation to cell-bound was 11%, with mean seston MCYST values of  $11 \pm 2.9$  µg L⁻¹ (mean ± SE) and changing widely during the study (Table 1). MCYST concentrations in water and in the seston were positively correlated ( $r = 0.45$ ,  $P < 0.01$ ), and in general MCYST appeared in the lake water after the seston peak.

Chlorophyll *a* was not related to seston and water MCYST concentrations ( $P > 0.05$ ). However, abundance and biovolume of chroococcal cyanobacteria were significantly correlated with MCYST (water:  $r = 0.32$  and  $0.43$ ; seston:  $r = 0.76$  and  $0.70$ ,  $P < 0.01$ , respectively), and correlation coefficients were higher than with total cyanobacteria (water:  $r = 0.38$  and  $0.40$ ; seston:  $r = 0.59$  and  $0.67$ ,  $P < 0.01$ , respectively). This is explained by the strong relationship of MCYST with abundance and biovolume of *M. aeruginosa* populations ( $r = 0.65$  and  $r = 0.70$ ,  $P < 0.01$ , respectively). Increasing populations of this species above  $10^3$  colonies L⁻¹ and with seston MCYST above 10 µg L⁻¹ were observed with water discharges lower than

10 m³ s⁻¹ (Fig. 1a,f). Microcystin content per unit biovolume of total cyanobacteria diminished with flushing ( $r = -0.37$ ,  $P < 0.01$ ).

There was a significant negative correlation between the MCYST content per unit biovolume of *M. aeruginosa* and abundance and biovolume of this species ( $r = -0.38$  and  $r = -0.37$ ,  $P < 0.01$ ), which shows that MCYST content tended to be higher at the onset of its population development in April–May when mean colony size was less than 50 µm (Fig. 2). At this time, populations of this species were on average  $10^3$  colonies L⁻¹ and  $2$  mm⁻³ L⁻¹ (Fig. 2). Although in the lake mean MCYST content per biovolume of *M. aeruginosa* declined thereafter during summer, populations of this species remained high until water renewal increased by October (Fig. 2). There was not a significant relationship between MCYST content of *M. aeruginosa* and its colony size ( $r = -0.22$ ,  $P > 0.05$ ). Total phosphorus was correlated with MCYST content per unit biovolume of *M. aeruginosa* ( $r = 0.39$ ,  $P = 0.001$ ), but no relationship was found with this species abundance, biovolume or colony diameter. Other cyanobacteria

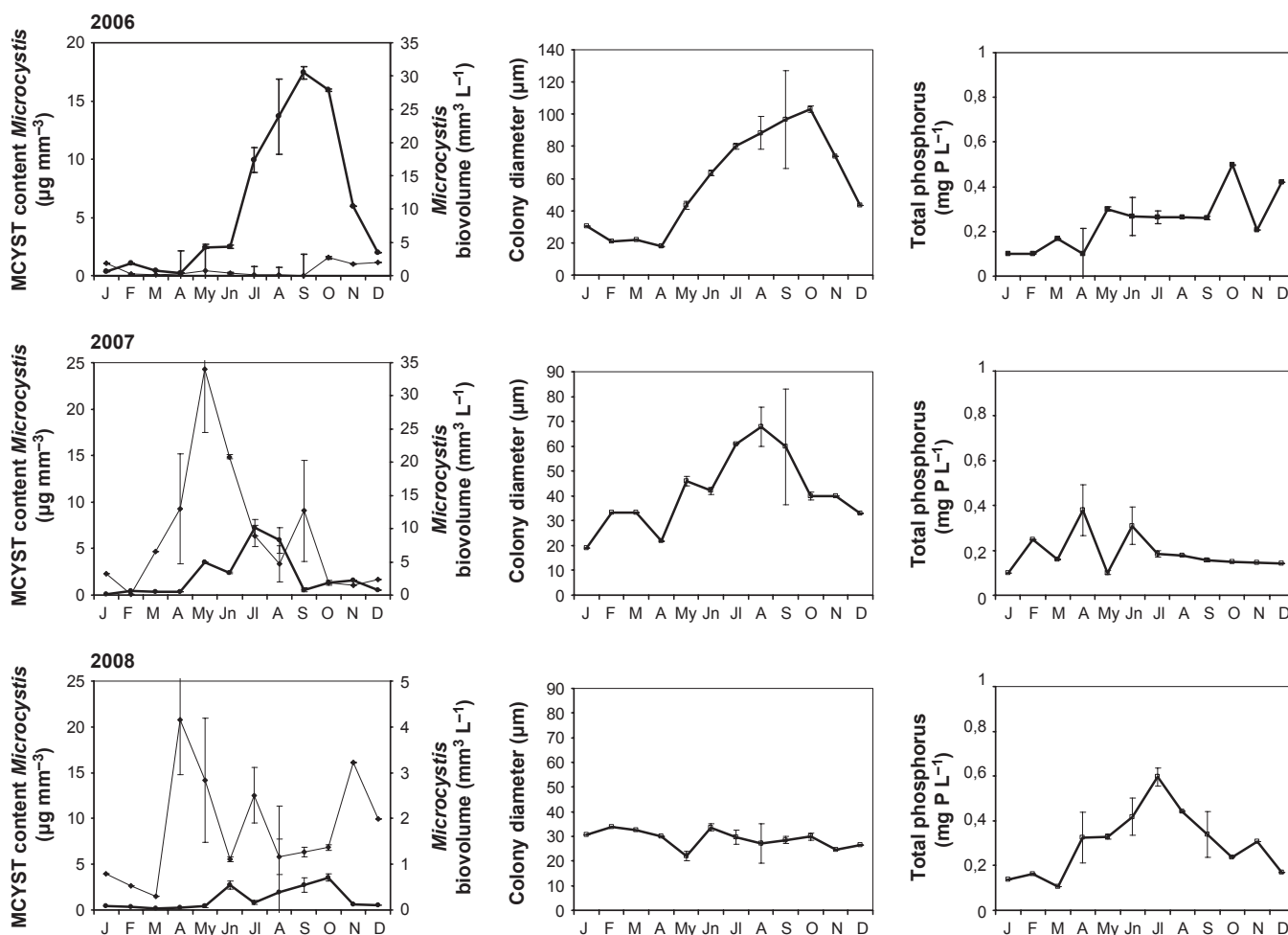


Fig. 2 Seasonal changes of MCYST content per *Microcystis aeruginosa* biovolume related to population of this species, its colony diameter and water total phosphorus concentrations for 2006, 2007 and 2008. Left y-axis variable is plotted as thin line and right y-axis variable as thick line. Data are monthly means for each year and vertical bars SE.

potentially producing MCYST (*Anabaena*, *Anabaenopsis* and *Planktothrix*) made up <5% of the lake total phytoplankton biovolume during the study period.

## Discussion

Some of the predictions about water residence time and increase in cyanobacteria and MCYST were confirmed. Longer water residence times during dry years and spring-summer stagnation enhanced total cyanobacteria biomass, *M. aeruginosa* populations and MCYST concentrations in the lake water and seston. Droughts increased water retention time by about 45%, and *M. aeruginosa* populations and MCYST were 1–2 orders of magnitude higher. There was a relationship between populations of *M. aeruginosa* and MCYST, and complementary research identified toxic genotypes of this species in the lake (Ouahid & Fernández, 2009). The long-term data series on

the lake phytoplankton also showed that other buoyant chroococcal cyanobacteria favoured by water stability have increased significantly since 2002 (*Aphanocapsa incerta* (Lemmermann) Cronberg et Komárek and *Aphanothece clathrata* W. et G. S. West, Romo et al., 2008). Size of *M. aeruginosa* colonies was larger with increasing water residence time and was more closely related to the lake hydrology and water column stagnation than to MCYST colony content.

The presence of MCYST in the lake water and seston in all the samples demonstrates the potential risks of toxicity all year around and for the whole lake. Presence of MCYST in the Albufera lake water and seston have changed from almost undetectable levels in 1999 (Bradt & Villena, 2002), when the lake was dominated by filamentous cyanobacteria (Villena & Romo, 2003) to concentrations that exceeded the recommended international standards during the present study (WHO, 2003). MCYST

accumulates in the top levels of the food web (fish) and constitutes a threat for fisheries and wildlife inhabiting the lake and related habitats (Romo *et al.*, 2011). Similar patterns of increasing presence of toxic cyanobacteria and accumulation in commercial fish species or target taxa (e.g. fish, mussels, crayfish, gastropods) has recently also been reported in some shallow Greek Mediterranean lakes (Gkelis, Lanaras & Sivonen, 2006; Kagalou *et al.*, 2008; Papadimitriou *et al.*, 2010). Significant geographic differences may underlie the dominance of microcystin-producing cyanobacteria. Toxic filamentous cyanobacteria appear more often in northern European lakes, while *Microcystis* more commonly dominates cyanobacterial blooms in the Mediterranean zone (Cook *et al.*, 2004). Longer water retention times for shallow Mediterranean lakes could account for these differences. Our results agree with Cook *et al.* (2004) in the view that the presence of toxic cyanobacteria and MCYST in shallow Mediterranean lakes might be more widespread than hitherto believed. Furthermore, Lake Albufera, like other coastal lakes located in a transitional zone which links epicontinental and marine waters, may extend problems to other related ecosystems (e.g. rice fields, beaches and marine water), and this should be considered when assessing monitoring and management programmes. The combination in the Albufera Lake of high cyanobacteria biomass and presence of MCYST in the lake water and in the food web could cause adverse and unexpected health and environmental effects.

Welker *et al.* (2003) highlighted in Lake Müggelsee a negative relationship between the MCYST content per unit biovolume of *Microcystis* and its biomass. This seems a common pattern in lakes, which suggest that toxigenic colonies are relatively more abundant or more toxigenic at the onset of the *Microcystis* growth (Kardinaal & Visser, 2005). Similarly, in Lake Albufera, the MCYST content of *M. aeruginosa* generally peaked at the onset (April–May) of its maximum populations when water residence and phosphorus increased (Fig. 2), but earlier in the season than that reported in northern European lakes (June–July, Kardinaal & Visser, 2005). This maximum MCYST content corresponded with average populations of  $10^3$  colonies  $L^{-1}$  and  $2\text{ mm}^{-3}\text{ L}^{-1}$ , which could additionally represent thresholds for toxicological risks in the lake. Although, in Lake Albufera, MCYST content tended to be lower thereafter during summer, populations were kept high until they were flushed out by October and partly rested in the plankton and on the sediment (microscopic observations). The sediment can act as an important source of colony recruitment into the water column in shallow waters (Hansson, 1996; Verspagen *et al.*, 2005).

The factors driving the seasonal pattern of MCYST content per unit biovolume of *Microcystis* and its biomass have not been yet fully elucidated. Competitive low-light adaptation of non-toxic strains compared with toxic strains could determine this response (Kardinaal *et al.*, 2007), providing self-regulating toxin production at times when it might be favourable to maintain a chemical defence against potential grazers (Kardinaal & Visser, 2005). In Lake Albufera, colony size of *M. aeruginosa* during April–May of high MCYST content was within an edible range ( $<50\text{ }\mu\text{m}$ ), which could support the hypothesis of toxin content regulation per colony against zooplankton grazing (Kardinaal & Visser, 2005). Experimentally, increases in both temperature and phosphate concentrations have yielded the highest growth rates of toxic *Microcystis*, suggesting that climate warming and eutrophication can additively promote the growth of more toxic *Microcystis* blooms (Davis *et al.*, 2009). Synthesis and types of microcystins that *Microcystis* can produce seem dependent also on available carbon and nitrogen that stimulated toxic strains in laboratory experiments (Wiedner *et al.*, 2003; Kardinaal *et al.*, 2007; Van de Waal *et al.*, 2009).

The present study also suggests that flushing could be used to shift the lake towards a clear state and open a window for macrophyte recolonisation, as observed in some shallow Dutch lakes of similar size (Jagtman *et al.*, 1992; Meijer & Hosper, 1997). If lake residence time can be managed and water balance in the watershed allows lake flushing, this can be used as a restoration measure to minimise toxic cyanobacteria blooms and toxicological and environmental problems (Jagtman *et al.*, 1992; Verspagen *et al.*, 2006). For a large Dutch lake, Verspagen *et al.* (2006) estimated that water discharges of  $75\text{ m}^3\text{ s}^{-1}$  throughout the year or a slightly lower rate during summer ( $65\text{ m}^3\text{ s}^{-1}$ ), in combination with a higher flushing in winter ( $125\text{ m}^3\text{ s}^{-1}$ ), could suppress *Microcystis* blooms. This applies if sediment acts more like a sink than a source of *Microcystis* populations, which might be unrealistic for shallow waters (Verspagen *et al.*, 2004).

In Lake Albufera, discharge rates above  $10\text{ m}^3\text{ s}^{-1}$  lowered *M. aeruginosa* populations ( $<10^3$  colonies  $L^{-1}$ ) and seston MCYST to low risk levels ( $1\text{--}10\text{ }\mu\text{g L}^{-1}$ ), as according to recommended international guidelines to minimise toxicological risks (Chorus & Bartram, 1999; WHO, 2003). During drier years, water retention time increased by about 45%, *M. aeruginosa* populations were two orders of magnitude higher, and MCYST reached a moderate-high risk level (Chorus & Bartram, 1999). These differences between years suggest that annual water inputs to the lake should be at least about  $200\text{ Hm}^3$  to

reduce water residence and undesirable *M. aeruginosa* proliferation (target year 2008, Table 1). A critical period is between April and October when water residence in the lake increased considerably ( $<5 \text{ m}^3 \text{ s}^{-1}$ ). These estimated thresholds of water input could be attainable only with a new policy of water uses and management in the zone, which should be strengthened during drier years. However, since water resources in most lake watersheds of the Mediterranean zone are restricted and climate change predictions are for less rain in the future, flushing seems infeasible as a long-term management method. As an additional restoration measure, some authors suggest a rise of salinity in estuarine and coastal lakes to prevent or reduce *Microcystis* populations (Verspagen *et al.*, 2006). However, *Microcystis* can tolerate relatively high salinities (10–20‰, Tonk *et al.*, 2007), and increase in salinity by water evaporation or by flushing with sea water is unlikely to remove this species once it has been established; even worse, salt stress could cause cell leakage and massive toxin excretion into the water increasing toxicological risks, or additionally allowing some toxic marine algae to invade the phytoplankton (Tonk *et al.*, 2007; Paerl & Huisman, 2009).

Further oligotrophication of Lake Albufera and reversal to a macrophyte-dominated state are the foreseen strategies. Nutrients are a driver in shaping phytoplankton carrying capacity and cyanobacteria contribution in shallow lakes, and thereby a key factor for water quality (Moss *et al.*, 2004). Reduction in nutrient inputs seems particularly relevant to counteract climate warming in temperate shallow lakes (Jeppesen *et al.*, 2003; Mooij *et al.*, 2005) and can become even more important in warm shallow lakes (Kosten *et al.*, 2012). In shallow Mediterranean lakes, cyanobacteria can dominate phytoplankton under a wide range of nutrient levels (Romo *et al.*, 2004), but replacement by smaller and edible chroococcal cyanobacteria under water stagnation can take place at low nutrient concentrations ( $\text{TP} < 0.03\text{--}0.05 \text{ mg L}^{-1}$ , Romo & Villena, 2005). For this response, zooplankton grazing is also relevant in structuring phytoplankton size (Romo *et al.*, 2004; Gyllström *et al.*, 2005). Therefore, together with strategies for nutrient control, management of fish communities should be also considered (Jeppesen *et al.*, 2003), despite the possibility that increase in temperature can increase omnivory and shorten aquatic food webs (Arim, Bozinovic & Marquet, 2007). Total phosphorus levels in Lake Albufera need to be lowered at least four times ( $\text{TP} < 0.03\text{--}0.05 \text{ mg L}^{-1}$ , compared with Table 1) and TN halved ( $<2 \text{ mg L}^{-1}$ ), and this should be complemented with selective regulation of fish communities (Romo *et al.*, 2005).

However, it is likely that other toxic cyanobacteria (e.g. *Cylindrospermopsis*) will profit from future climate scenarios if nutrients are reduced in the lake (e.g.  $\text{TN}:\text{TP} < 20$ , Reynolds, 1997). Some studies suggest that with increasing temperatures, N-fixing cyanobacteria, well adapted to water stability (e.g. *Anabaena*), but with lower nutrient requirements, will replace *Microcystis* in nutrient-rich lakes or in the final stages of the phytoplankton epilimnetic succession (Cook *et al.*, 2004; Paerl & Huisman, 2009; Wagner & Adrian, 2009). However, this will be unlikely to avoid toxicological problems, because several genera of N-fixing cyanobacteria produce toxins (Codd *et al.*, 2005). As reported from other lakes (Paerl & Huisman, 2009), *Cylindrospermopsis raciborskii* has increased its presence in Lake Albufera in recent years, with adaptation to summer temperatures, a wide range of nitrogen levels and prolonged water retention (Romo *et al.*, 2008). Restoration of eutrophic warm shallow lakes could thereby be at a crossroad of uncertain solutions in future climate scenarios.

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