

Toxic Cyanobacterial Blooms in Reservoirs Under a Semiarid Mediterranean Climate: The Magnification of a Problem

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ABSTRACT: Sicilian reservoirs constitute the most important water resources available on the island. During summer 2001, the intense water utilization of Lake Arancio reservoir reduced the water level significantly, which coincided with the formation of intense blooms formed by the microcystin (MC)-producing cyanobacterium *Microcystis aeruginosa*. During summer 2003, Lake Arancio was continuously filled and the vertical stratification of the water column was maintained resulting in five to sixfold lower cell numbers of *M. aeruginosa*. For both years, a significant relationship between MC net production and *Microcystis* cell growth was observed, implying that *Microcystis* cell numbers can be used to infer MC concentrations in water. Unexpectedly, dense blooms of the MC-producing cyanobacterium *Planktothrix rubescens* occurred during winter 2005/2006 in the reservoirs Lake Pozzillo, Prizzi, Nicoletti, and Garcia but have not been reported earlier. In this season, MC concentrations higher than those recorded in summer were measured, implying that monitoring of Mediterranean drinking water reservoirs needs to be intensified during winter, a season usually considered to be less prone to the formation of cyanobacterial blooms.

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INTRODUCTION

Mediterranean reservoirs, especially those located in the semiarid part of the Mediterranean, represent a most important resource for human development. When compared with their temperate counterparts, semiarid environments

are characterized by longer periods of relatively high temperatures favoring algal growth. Human activities often negatively influence water quality and this may be enhanced due to the artificial nature of the reservoirs and the semiarid climate conditions. Because of its insularity, Sicily lacks both a permanent river network and aquifers large enough to ensure a minimum water supply to the reservoirs during the dry summer season (Naselli-Flores, 2003). Reservoirs fill up during the rainy season in autumn and winter and, in general, water residence times and nutrient loading increase during this time period (Kennedy et al., 2002). In consequence, a higher algal production and algal populations adapted to an increased water depth have been observed (Naselli-Flores and Barone, 2005).

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As a consequence of eutrophication processes algal blooms formed by cyanobacteria have increased in intensity and duration during the last years in Sicilian reservoirs (Naselli-Flores and Barone, 2007). Since 1979, the relative importance of *Microcystis* and other planktonic cyanobacteria gradually increased at the expense of green algae typically dominating those reservoirs (Naselli-Flores and Barone, 2003, 2005). From 2000 onwards *Microcystis aeruginosa* occurred with a frequency of 50–100% of total phytoplankton biovolume in 70% of the water stored in Sicilian reservoirs. Because of the Mediterranean climate characteristics, *Microcystis* starts growing at the end of February and grows until October/November. Surprisingly, during winter 2005/2006, intense blooms of the filamentous cyanobacterium, *Planktothrix rubescens* occurred in four reservoirs (Lakes Garcia, Nicoletti, Pozzillo, and Prizzi), which altogether contributed 30% of the total water volume stored in Sicily. Conversely to *P. agardhii*, which commonly develops in Sicilian eutrophic reservoirs at the end of summer, the occurrence of *P. rubescens* had never been recorded before in the island.

Planktonic cyanobacteria of the genera *Microcystis* and *Planktothrix* often produce small peptide molecules, most prominently microcystins (MC), that are toxic to a variety of organisms including humans (Chorus and Bartram, 1999). Besides the direct ingestion of MCs via drinking, water contaminated with MCs that is used for irrigation may inhibit germination and root growth potentially causing crop failures (Pflugmacher et al., 2006). In particular, inhibition of germination and root growth of alfalfa seedlings was observed at relatively low MC concentrations of $5.0 \mu\text{g L}^{-1}$. The total quantity of MCs produced by a cyanobacterial bloom varies in response to the proportion of MC-producing genotypes within a specific population (Kurmayer et al., 2002). Genotypes differing in morphological characteristics (so called morphospecies) in *Microcystis* sp. have been shown to vary in MC production (Via-Ordorika et al., 2004). Because of this potential hazard to human health cyanobacterial blooms need particular attention, and effective tools for surveillance need to be developed.

In this article, we present data on MC concentrations regularly gathered during summer 2001 and 2003 from the hypertrophic Lake Arancio, dominated by *Microcystis* sp. blooms, as well as data on MC concentrations collected

during winter 2005/2006 from other Sicilian reservoirs dominated by blooms of *Planktothrix rubescens*.

MATERIALS AND METHODS

Study Sites

Table I shows morphometric characteristics of the studied reservoirs. Detailed morphological and limnological descriptions of the reservoirs are given in Naselli-Flores (1999). Because of the strong decrease in water volume during summer 2001 the thermal stratification of Lake Arancio was atelomictic (*sensu* Barbosa and Padisák, 2002) with a pronounced warming of the surface water during the day and diurnal, even irregular, mixing events resulting in large fluctuations in the redox conditions of the water column (see Naselli-Flores, 2003). In 2003, changes in water level management resulted in a more stable water level and in the maintenance of thermal stratification during the summer period reducing the internal nutrient transport from the sediments to the water column (Naselli-Flores and Barone, 2005). Autumn 2005 was characterized by intense precipitation, especially in the central part of the island, and four reservoirs (Garcia, Nicoletti, Prizzi, and Pozzillo), which had maintained summer stratification, were subjected to floods which caused a sudden decrease in transparency and the abrupt breaking of their thermoclines.

Sampling

In Lake Arancio, water samples were collected from July to October weekly during the summer of 2001 and every 2 weeks during the summer of 2003. The Sicilian Regional Environmental Protection Agency (ARPA Sicilia) sampled the other four reservoirs during winter of 2005/2006. Water was collected 250 m from the dam at depths corresponding to 100, 50, and 1% of the subsurface irradiance, as measured with a LI-COR quantum sensor (LI-COR Biosciences, Lincoln, Nebraska). The samples from different depths were mixed and aliquots were used both for phytoplankton counting and biovolume estimation and for the analysis of microcystins (MC). Water samples were kept cool and dark during the transport to the laboratory and were filtered

TABLE I. Main characteristics of the studied reservoirs in Sicily

Reservoir	Volume (10^6 m^3)	Surface (km^2)	Maximum Depth (m)	Average Depth (m)	Use	Trophic State
Arancio	30	3.2	29	9	Irrigation	Eu-Hyper
Garcia	60	5.9	43	10	Drinking	Meso-Eu
Nicoletti	17	1.8	36	9	Irrigation	Meso
Pozzillo	154	7.7	50	18	Hydropower/irrigation	Meso-Eu
Prizzi	9	1.3	44	6	Drinking	Meso-Eu

through glass fiber GF/F filters (Whatman, Kent, Great Britain) within 2 h. The filters were folded and dried at 55–60 °C for 2 h. The dry filters were put in a 100 cc glass bottle containing silica gel and stored at –20 °C.

Microscopical methods of plankton enumeration and the analysis of population dynamics of *Microcystis* sp. in Lake Arancio are given in Naselli-Flores and Barone (2003, 2005). In particular, the different *Microcystis* morphospecies were identified after Komárek et al. (2002).

MCs were analyzed as described previously (Fastner et al., 1999; Kurmayer et al., 2003). Briefly, filters were cut into pieces and extracted in 70% methanol (w/v) for three times. The clear supernatant was injected into high performance liquid chromatography coupled to diode array detection (HPLC-DAD). MCs were identified by their characteristic absorption maximum at 240 nm and quantified using MC-LR as external standard. Given a filtered volume of 100 mL and assuming an average cellular MC content of 100 fg cell⁻¹ a reliable quantification of MC was possible above a concentration of 60,000 cells mL⁻¹. Consequently, for cell concentrations below this threshold no cellular MC contents were calculated.

From biovolume and MC concentrations growth rates (r day⁻¹) and MC (net) production rates (day⁻¹) were calculated using the formula $r = (\ln N_2 - \ln N_1)/\Delta t$, where $N_{1,2}$ were the cell concentrations/peptide concentrations at consecutive sampling days and Δt was the time interval in days.

RESULTS

Microcystin Production by *Microcystis aeruginosa*

During summer 2001, *Microcystis* spp. dominated the phytoplankton in Lake Arancio with a proportion of 93–100% of the total biovolume. Besides *Microcystis* spp., the assemblage included a few chlorophytes, such as *Botryococcus braunii*, *B. terribilis* and *Pediastrum* spp. The *Microcystis* morphospecies most frequently found included *M. aeruginosa* (91–100% with an average percentage of 97%). During most of the study period *M. panniformis* occurred with <2%, however, higher proportions were observed during the first 2 weeks of July and during the second week of October (min: 0; max: 6%; average: 1.5%). During the year 2003 *Microcystis* sp. reached a lower biovolume and occurred at subdominant proportions only (min: 1%; max: 37%; average: 10%). The seasonal maxima were observed during October of 2001 and September of 2003 (Fig. 1).

Total MC concentrations in the samples, expressed as MC-LR equivalents, ranged between 0 and 2753 $\mu\text{g L}^{-1}$. During both years *Microcystis* biovolume (BV) and the total MC concentration (expressed as MC-LR equivalents) were closely correlated: $\text{MC}[\mu\text{g L}^{-1}] = 2.24 \times \text{BV}[\text{mm}^3 \text{L}^{-1}] - 50.2$; $r^2 = 0.98$, $n = 16$, $P < 0.001$). The maxima

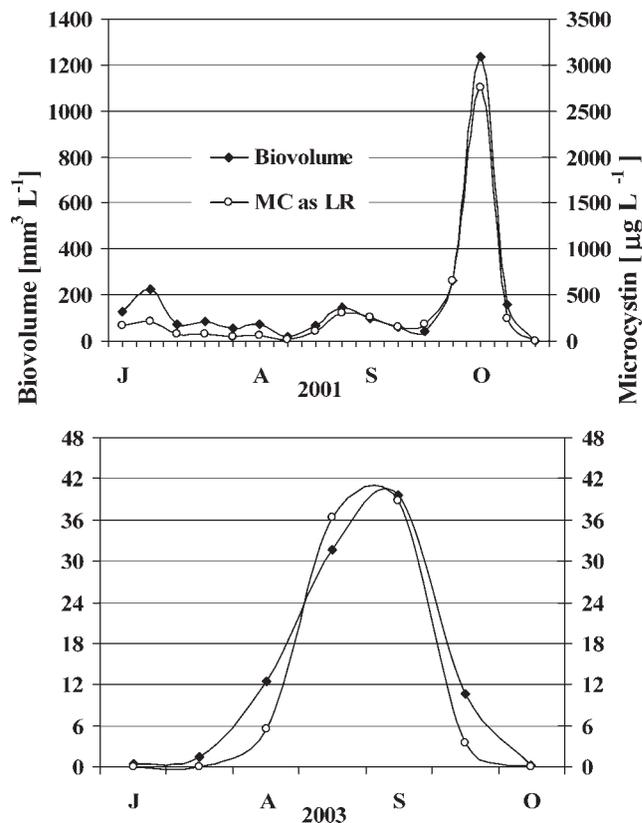


Fig. 1. Biovolume of *Microcystis aeruginosa* from July–October 2001 (upper graph) and 2003 (lower graph) in Lake Arancio and corresponding concentrations of microcystin (MC-LR and MC-YR estimated as MC-LR equivalents).

of MC concentrations, 2359 $\mu\text{g L}^{-1}$ and 40 $\mu\text{g L}^{-1}$ during 2001 and 2003, respectively, coincided with the maxima of *Microcystis* biovolume.

The dominant MC variants were MC-YR and -LR, while MC-RR occurred in lowest proportions only (<2%), and further variants were present in yet lower shares of the total content. During both years the proportion of MC-YR/MC-LR was almost constant (min: 60%; mean: 73% (± 1.6 SE); max: 79%). The MC content of the *Microcystis* biomass differed in the two years, with 0.9 to 3.5 μg (mean: 1.8 ± 0.2 SE) of MC-LR equivalents per mm^3 of biovolume during 2001 and 0.3 to 1.2 μg (mean: 0.72 ± 0.20 SE) during 2003 (Fig. 2). During 2001, the MC content was constant until the mid of August and then increased until the mid of September. This increase in MC content coincided with a period of low physical stability of the water column due to the yearly minimum content of water in the reservoir. This period was also characterized by an improvement in the underwater light availability and by a reoxygenation of the water column (Naselli-Flores, 2003; Naselli-Flores and Barone, 2003) favoring a strong increase in *Microcystis* growth. MC contents were significantly lower during the summer of 2003 ($P < 0.01$). Nevertheless, for both years

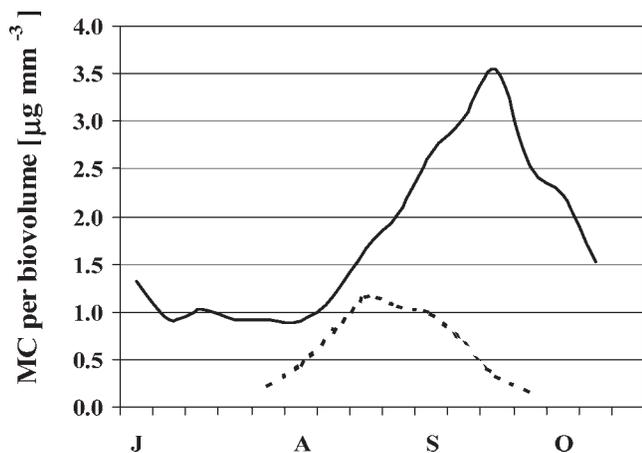


Fig. 2. Microcystin content in *M. aeruginosa* from July–October of 2001 (straight line) and 2003 (dashed line) in Lake Arancio.

the MC net production rates were related very closely, i.e., almost one to one, to the *Microcystis* growth rate calculated on the basis of biovolume (Fig. 3). Consequently, the seasonal variation in MC content was considered of minor importance relative to the dependence of MC net production on the increase or decrease of *Microcystis* biovolume.

Microcystin Production by *Planktothrix rubescens*

During winter 2005/2006 *P. rubescens* formed surface blooms in Lake Pozzillo, the largest Sicilian reservoir, reaching a population density peak $>50 \times 10^9$ cells L^{-1} (≈ 5600 $mm^3 L^{-1}$) at the end of December 2005. *P. rubescens* colored the water surfaces dull purple by a 60 cm thick layer floating on the surface. At the beginning of March the density of filaments decreased down to <20 filaments mL^{-1} (about 10×10^6 cells L^{-1} ; 1.1 $mm^3 L^{-1}$). *P. rubescens* was also observed in the other three reservoirs (Garcia, Nicoletti, and Prizzi) and showed lower cell numbers but a similar seasonal development in the population density growth. Growth of *Planktothrix* started in mid-November at the end of the stratification, and *Planktothrix* persisted until mid-February showing the lowest water temperatures (9–10 °C) of the year.

Analysis of MCs in a surface sample collected at highest cell density (December 2005) in Lake Pozzillo revealed the presence of demethylated variants of MC-RR (95%), MC-LR (3%), and small amounts of unknown MC variants (2%). The total concentration of MC (calculated as equivalents of MC-LR) was 34 $mg L^{-1}$. This concentration corresponded to a MC content of 6.0 $\mu g mm^{-3}$ of biovolume. After this peak, lower MC concentrations were detected, i.e., the MC concentration in a surface sample from Lake Prizzi at 15 February 2006 was 7 $\mu g L^{-1}$ (MC-RR determined as MC-LR equivalents).

DISCUSSION

In this study the MC content of the *Microcystis* population varied seasonally by a factor of four. In the majority of studies on the regulation of MC net production by various environmental conditions in the laboratory the different environmental factors (i.e., micro- and macronutrients, light, temperature, pH) were found to induce changes in MC content, usually by a factor of three to four (Sivonen and Jones, 1999).

In their unifying theory, Orr and Jones (1998) suggested MC net production to be coupled linearly to the cell division of the organism and concluded that—although MC is a secondary metabolite—it rather displays the attributes of essential intracellular nitrogenous compounds in cyanobacteria. According to this theory, the strongest influence of all possible environmental factors is indirect—through their effect on cell division and growth, whereas the direct effects of environmental conditions on MC biosynthesis are of minor importance.

The situation becomes more complex in the field since in contrast to the laboratory numerous different genotypes coexist and therefore may influence the MC concentration in the biovolume and water. The wax and wane of MC-producing versus non-MC-producing strains has been suggested as a most important factor regulating MC net production in water (Sivonen and Jones, 1999). In this study, a significant one to one relationship between MC net production and *Microcystis* growth was observed, implying that the number of coexisting MC-producing and non-MC-producing genotypes was seasonally stable, although it may have differed between the years 2001 and 2003. We cannot exclude the occurrence of shifts within the group of MC-

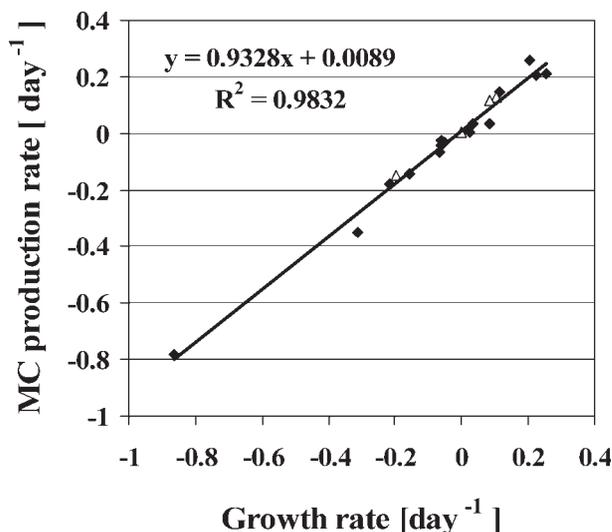


Fig. 3. Linear relationship between the growth rate of *Microcystis aeruginosa* calculated from biovolume estimates in the microscope and the net production rate of microcystin-LR equivalents during 2001 (◆) and 2003 (△).

producing genotypes. For example, Amé and Wunderlin (2005) identified iron and ammonium concentrations, and high temperature as factors that might influence the proportion of MC-producing genotypes. Indeed, during summer of 2001 Lake Arancio showed an atelomictic stratification type with diurnal and irregular mixing events resulting in large fluctuations in the redox conditions of the water column. However, nonetheless the proportion of MC variants, MC-LR, YR, and RR was relatively constant during both years implying that shifts among genotypes producing different MC variants did not occur. Notably, a number of field studies also reported significant linear relationships between MC net production and *Microcystis* biovolume (Kotak et al., 2000; Chorus et al., 2001; Kurmayer et al., 2003). Even the re-analysis of data of a study postulating that seasonal and spatial shifts in MC genotype composition determine MC net production in Lake Biwa (Ozawa et al., 2005) revealed a significant linear relationship between MC concentrations and *Microcystis* cell numbers ($y = 3.1526 + 0.0776x$, where y was the MC concentration in $\mu\text{g mL}^{-1}$ and x was the cell concentration in cells mL^{-1} , $R^2 = 0.59$, $n = 85$, cell contents of $>1000 \text{ fg cell}^{-1}$ were considered as outliers and have not been included). This evidence is of importance in order to use *Microcystis* cell numbers to predict MC concentrations in water. Thus, cell numbers or biovolume can be important surrogate parameters for authorities and water managers performing environmental monitoring and risk assessment. They become most meaningful through occasional MC analyses and determination of the ratio of MC per cell or per unit biovolume. Once this ratio is known to remain stable in a given water-body, conducting MC analyses once or 2–3 times per growing season would be sufficient to validate the prediction of MC concentrations in the water from cell density or biovolume determined more frequently, e.g., weekly or in 14-day intervals. This approach is particularly useful for water-bodies with unialgal cyanobacterial populations that persist for extended periods of time.

As shown by Naselli-Flores and Barone (2005), the water level reduction during summer in Sicilian reservoirs enhanced the growth of *Microcystis* sp. In contrary, a different water management, i.e., continuously refilling the reservoir and maintaining the vertical stratification reduced internal nutrient loading and by this the growth of *Microcystis* sp. On the other hand, these stratifying conditions may favor the occurrence of shade-adapted species, such as *Planktothrix rubescens*. *P. rubescens* typically occurs in deep, physically stratified and oligo- to mesotrophic waters in which they can form metalimnetic layers because light penetrates to this depth (Kurmayer et al., 2004). It has been shown both empirically and by modeling that during periods of low insolation such as in December the filaments receive low light doses only and react by floating up from deeper regions of the lake, thus forming visible water blooms (Walsby et al., 2005).

It is unclear why *P. rubescens* was observed only recently and not during the regular phytoplankton monitoring in the years before. During the last years the autumnal precipitation was increasing which has also been predicted by current climate models (Tin, 2006). High precipitation events occurred during early November 2005 (Sicilian Hydrological Service, unpublished data), causing a mixing of the water column but also decreasing the water transparency of the reservoirs. These conditions may have favored low light adapted phytoplankton species such as *Planktothrix* spp. In general *P. agardhii* has been shown to out compete green algae in shallow, eutrophic lakes (Scheffer et al., 1997). *P. rubescens* has a highly efficient light harvesting complex as well and frequently dominates deep reservoirs of the temperate climatic zone. *P. rubescens* has been suggested to outgrow *P. agardhii* under low light conditions and lower temperatures in the stratified lake Blelham Tarn ($<21^\circ\text{C}$) while *P. agardhii* may be more successful in shallow and warmer waters (Davis and Walsby, 2002).

The pinkish color of these blooms frequently alerts the population. Because of the concern of toxicity as well as public perception, local authorities had to stop the water use in this reservoir until the beginning of March when the density of filaments decreased below 20 per mL (about $10 \times 10^6 \text{ cells L}^{-1}$; 1.1 mg L^{-1}). The same measure was taken for the drinking water reservoirs Garcia and Prizzi. Red pigmented *P. rubescens* (*sensu* Suda et al., 2002) always produces MCs (Kurmayer et al., 2004). Similarly as for *Microcystis* sp. for *Planktothrix rubescens* significant correlations between the cell numbers and the MC concentrations have been reported (Briand et al., 2005). Consequently the counting of filaments/cell numbers can be useful to infer potential MC concentrations in water dominated by *P. rubescens*. However compared with *Microcystis* sp. in Lake Arancio the *Planktothrix* showed higher population densities leading to higher MC net production rates, and it contained two to sixfold more MC ($6.2 \mu\text{g mm}^{-3}$ biovolume). This study demonstrates that health risks caused by MC-producing cyanobacteria in reservoirs are present during winter as well as during summer, and if overlooked may have public health consequences. Consequently, water monitoring efforts need to be intensified during winter times, a season that is usually considered to be less prone to the formation of surface sums in the temperate region of the Northern hemisphere.

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