Implementation of surveillance plans for cyanobacteria in bathing waters: a case study from Lake Garda

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ABSTRACT

Since the beginning of the 1990s, Lake Garda has been affected by the development of surface blooms caused by the cyanobacterium *Dolichospermum lemmermannii*. Moreover, several other potentially toxigenic cyanobacteria were identified in the water samples collected during the monitoring activities, including the microcystins producer *Planktothrix rubescens* and a new species able to synthesize anatoxins (*Tychonema bourrellyi*). A plan has been activated by ARPAV (Regional Agency for Environmental Prevention of Veneto) to monitor algal communities and assess any risk of cyanobacterial proliferation that could affect the lake ecosystem and the bathing areas. In this work, we will briefly describe and critically comment on the main results obtained from the monitoring activities.

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Introduction

Lake Garda is the largest of the group of deep lakes located south of the Alps, between the regions of Veneto, Lombardia, and Trentino Alto Adige. According to its geomorphologic characteristics, the lake can be divided into two basins: the northwestern basin, with a maximum depth of 350 m, and the southeastern basin, with a maximum depth of 80 m (Figure 1A).

Due to its geographical position, Lake Garda should be classified among the warm monomictic basins. However, owing to its great depth, the lake is oligomictic, with a complete water circulation between the end of winter and the beginning of spring during cold and windy winters (Ambrosetti *et al*., 1992). The last complete circulation occurred in the winter of 2006 (Salmaso *et al*., 2020). The mixing depth of the lake is fundamental for replenishing nutrients from the hypolimnion to the trophogenic layers.

According to the Water Framework Directive 2000/60/CE (WFD; Directive 2000/60/EC, 2000), the ecological status of Lake Garda for the six-year period 2014-2019 was classified as 'Good' (ARPAV, 2023). During the analysed period, the mean annual epilimnetic Total Phosphorus (TP) concentrations measured at the deepest station (Brenzone; Figure 1A) were around 10 µg P L^{-1} (ARPAV Open Data: https://www.arpa.veneto.it/dati-ambientali/open-data), which allows to classify the lake as oligo-mesotrophic.

Since the beginning of the 1990s, Lake Garda has been affected by the development of surface blooms caused by the cyanobacterium *Dolichospermum lemmermannii* (Richter) P.Wacklin, L.Hoffmann & J.Komárek (Salmaso *et al*., 2015). Moreover, several other potentially toxigenic cyanobacteria were identified in the water samples collected during the monitoring activities, including a new species able to synthesize anatoxins, namely *Tychonema bourrellyi* (J.W.G.Lund) Anagnostidis & Komárek (Shams *et al*., 2015; Cerasino & Salmaso, 2020). Toxigenic cyanobacteria can pose significant challenges to the use of water resources for bathing, drinking water supply, and other uses (Chorus & Welker, 2021). In addition, algal blooms can have a major impact on ecosystem functioning (Amorim *et al*., 2021). For this reason and following the Directive 2006/7/EC (2006) on the management of bathing waters, in addition to routine microbiological analysis, a plan has been activated by ARPAV (Regional Agency for Environmental Prevention of Veneto) to monitor algal communities and assess any risk of cyanobacterial proliferation that could affect the bathing areas.

The main objective of this contribution was to briefly report the main results obtained from the monitoring activities carried out by ARPAV on the potential toxigenic species of Lake Garda.

Materials and Methods

The ARPAV algal surveillance plan for Lake Garda is based on the monthly monitoring of phytoplankton in the epilimnion (0-20 m) of the two deep pelagic stations located in the two main basins of the lake, namely Brenzone (northwestern basin) and Bardolino (southeastern basin) (Figures 1A, B). Sampling is carried out according to ISPRA (2014). The sampling frequency has been increased from 6 to 12 times/year for both the Brenzone and Bardolino stations to ensure adequate algal monitoring.

For the monitoring of the bathing areas, samples are taken between 5 and 10 cm from the surface at a monthly frequency during the summer months (June-September) at three stations located at Peschiera, Garda and Malcesine (Figures 2A, B). The basic approach followed the methods described in Funari *et al*. (2014).

In addition to these main activities, the possible presence of algal blooms is assessed during the bathing season in 65 bathing stations along the Verona coast through monthly visual checks. In the case of blooms, specific samples are taken for the analysis of cyanobacteria.

After collection, algal samples were fixed with Lugol's solution. Phytoplankton and cyanobacteria were identified and quantified using inverted microscopes (UNI EN, 2006; UNI EN, 2015; ISPRA, 2014). Taxonomic identifications were performed according to the manuals of the series Süßwasserflora von Mitteleuropa (Komárek J. and Anagnostidis K., 1999, 2008; Komárek J., 2013) and other

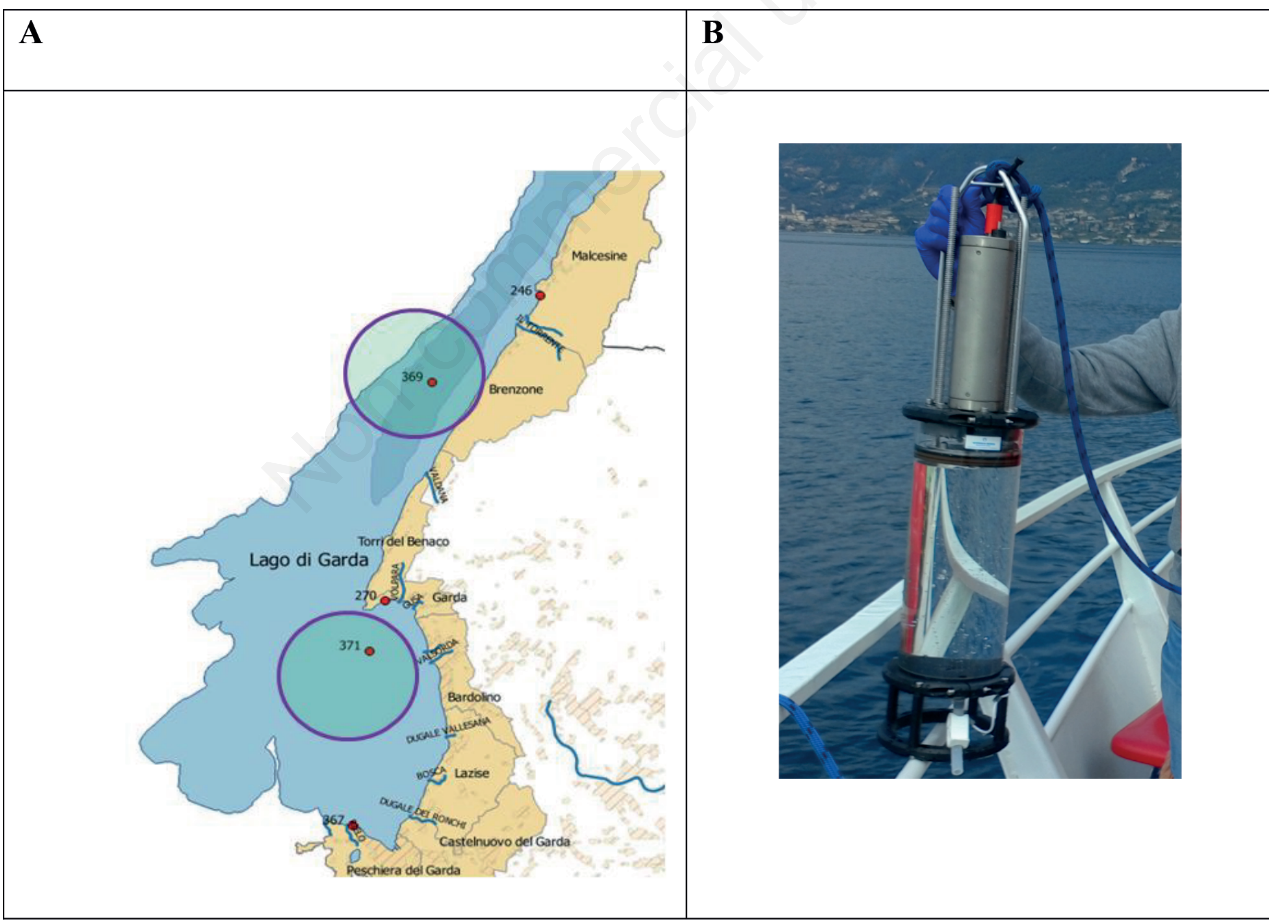

Figure 1. Monitoring activities in the deepest areas of Lake Garda. A) Map showing the two main pelagic monitoring stations located in the northwestern (Brenzone) and southeastern (Bardolino) basins. B) Integrating water bottle for the collection of samples in the euphotic zone.

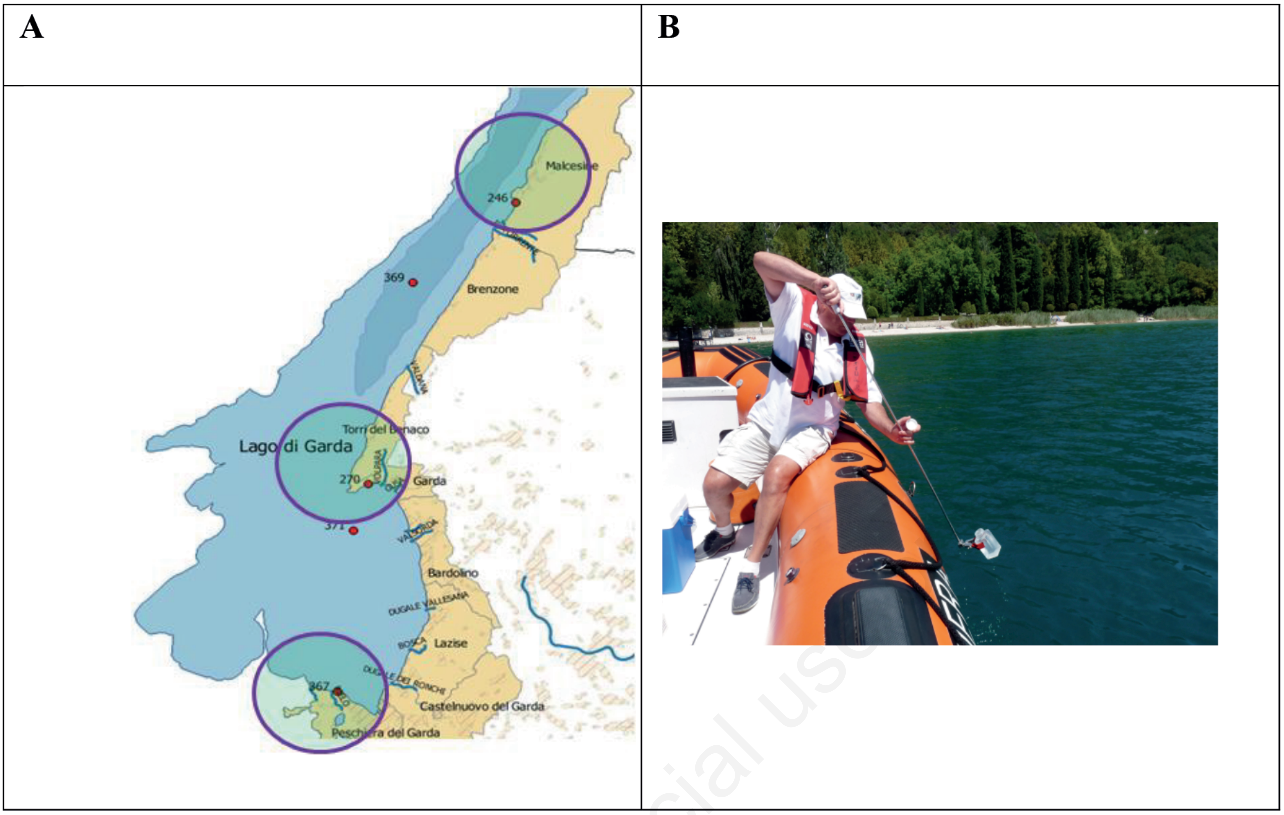

Figure 2. Monitoring activities in the bathing areas of the coastal zone. A) Map showing the main monitoring stations located along the eastern shores. B) Sampling of the surface bathing waters.

relevant scientific publications, with special attention to potentially toxic species as indicated by Funari *et al.* (2014).

Since 2010, the possible presence of algal toxins has been evaluated by the ARPAV laboratory of Verona using LC-MS/MS. The analyses are performed on not filtered water samples. The list of toxins analysed includes different congeners of microcystins (dmMC-LR, dmMC-RR, MC-LR, MC-RR, MC-YR, MC-LY, MC-LW, MC-LF), and nodularins (NOD) (LOQ 1 ng/ml, LOD 0.5 ng/ml).

Results

Algal monitoring in the deepest basin

The analyses carried out since 2015 in the Brenzone station showed a maximum annual biovolume of cyanobacteria ranging between around 200 mm^3 m^{-3} and 1700 mm^3 m^{-3}. The development of the biovolume of the potential toxigenic species almost coincided with that of the whole group of cyanobacteria (Figure 3). The

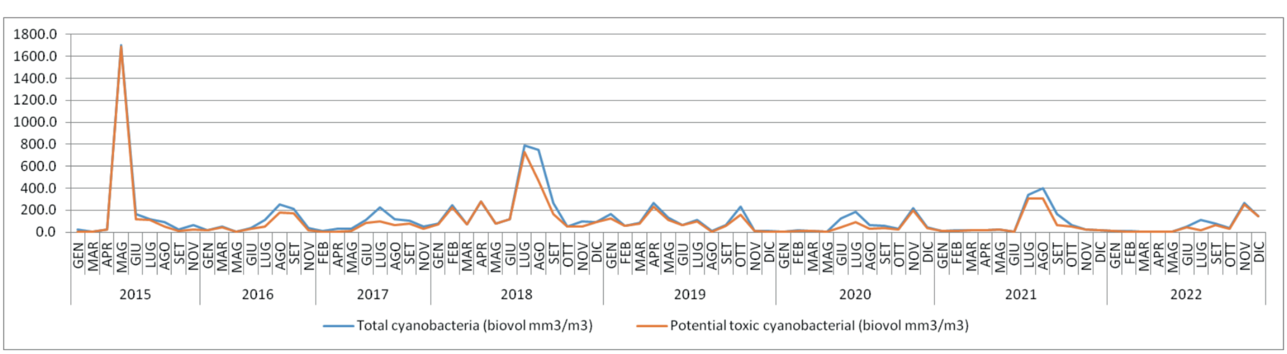

Figure 3. Temporal development of the biovolume (mm^3 m^{-3}) of total and potential toxic cyanobacteria from 2015 to 2022 in the pelagic station of Brenzone. Data refer to samples collected in the layer representative of the euphotic zone (0-20 m). The trends of total and potentially toxic cyanobacteria are almost identical.

Table 1. Potentially toxic cyanobacteria identified in Lake Garda.

Chroococcales	*Snowella lacustris* (Chodat) Komárek & Hindák
Chroococcales	*Microcystis aeruginosa* (Kützing) Kützing
Chroococcales	*Microcystis wesenbergii* (Komárek) Komárek ex Komárek
Chroococcales	*Coelosphaerium kuetzingianum* Nägeli
Chroococcales	*Woronichinia naegeliana* (Unger) Elenkin
Nostocales	*Dolichospermum lemmermannii* (Richter) P.Wacklin, L.Hoffmann & J.Komárek
Nostocales	*Aphanizomenon flos-aquae* Ralfs ex Bornet & Flahault
Oscillatoriales	*Pseudanabaena catenata* Lauterborn
Oscillatoriales	*Planktothrix rubescens* (De Candolle ex Gomont) Anagnostidis & Komárek
Oscillatoriales	*Tychonema bourrellyi* (J.W.G.Lund) Anagnostidis & Komárek

dominant potential toxigenic cyanobacteria were mainly represented by *Planktothrix rubescens* (De Candolle ex Gomont) Anagnostidis & Komárek and *Tychonema bourrellyi*. Their contribution (as the sum of both species) reached consistent biovolume values, particularly in May 2015 (1688 mm^3 m^{-3}), July 2018 (730 mm^3 m^{-3}), April 2019 (234 mm^3 m^{-3}), July 2021 (295 mm^3 m^{-3}) and November 2022 (170 mm^3 m^{-3}). During the period considered, a decrease in *P. rubescens* was followed by an increase in *T. bourrellyi*. *D. lemmermannii* was detected with biovolumes between 20 and 70 mm^3 m^{-3} until 2019 in the summer and autumn months and decreased in subsequent years. *Microcystis aeruginosa* (Kützing) Kützing was present with biovolumes above 30 mm^3 m^{-3} in October 2019 and September 2022. *Snowella lacustris* (Chodat) Komárek & Hindák was recorded with annual maximum biovolumes above 50 mm^3 m^{-3} since August 2018 (from 54-55 mm^3 m^{-3} in August 2018 and August 2021 to 122 mm^3 m^{-3} in November 2020).

East coast algal monitoring

The large number of samples collected and analyzed along the eastern shore of Lake Garda made it possible to monitor the presence of potentially toxic cyanobacteria in the principal areas frequented by bathers. At the same time, the activity allowed us to integrate the list of species obtained from the samples collected in the pelagic areas. The list of potentially toxic cyanobacterial species (Funari *et al.*, 2014; Cerasino & Salmaso, 2020) recorded so far in Lake Garda is reported in Table 1.

Among the species listed in Table 1, only *D. lemmermannii* was observed to form algal blooms (Salmaso *et al.*, 2024). Specifically, surface blooms were observed in years 2011, 2012, 2017 and 2020.

Cyanobacterial toxins

The analyses performed to date for the determination of microcystins and nodularin have always given negative results.

Discussion and Conclusions

The total biovolume of cyanobacteria recorded in the northwestern pelagic station showed a tendency to decrease annual maxima. Among the potentially toxigenic species identified in Lake Garda, experimental evidence based on isolation and cultivation indicated *P. rubescens* and *T. bourrellyi* as producers of microcystins and anatoxins, respectively (e.g., Salmaso *et al*., 2016; Cerasino *et al*., 2017). Conversely, several isolates of *D. lemmermannii* from Lake Garda and other deep perialpine lakes did not produce toxins (Capelli *et al*., 2017). The decrease of *P. rubescens*, followed by the new appearance of the filamentous cyanobacterium *T. bourrellyi* confirms the first observations made by Salmaso *et al*. (2015; 2018). At the same time, these changes were followed by a decrease in microcystins and an increase in anatoxin-a, determined by algal biomasses extracted from GFC filters. In any case, microcystin concentrations were always lower than 1 µg L^{-1}, while anatoxin-a concentrations were rarely higher than 1 µg L^{-1} (Cerasino & Salmaso, 2020).

The algal surveillance plan of ARPAV for Lake Garda in Veneto aims at monitoring bathing and algal blooms, to intervene promptly in case of incidents. This plan is facilitated by the fact that it is managed by a single Agency that provides competent facilities (sampling and laboratory analysis) and allows more timely surveillance and analysis of cyanobacteria in the event of blooms. It also allows for rapid communication with the public authorities that, in case of cyanobacteria blooms, must monitor these events, implement recovery plans, communicate risks to the public, and promote good collaboration between authorities.

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