Blooms of the blue-green alga *Cylindrospermopsis raciborskii* in Emilia Romagna region (Italy): case reports managed by the Regional Agency for Environmental Protection and Energy of Emilia Romagna (ARPAE)

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ABSTRACT

Cylindrospermopsis raciborskii is a toxic cyanobacterium that can form blooms potentially harmful to humans and animals. The proliferation of this species is difficult to predict, and its blooms are hard to detect as they cause just a moderate change in water colour. Global warming might increase the incidence of its proliferation worldwide. This trend was observed also by ARPAE that detected a growth in the presence of this alga in Emilia Romagna lakes and reservoirs in the last three years. This note describes the monitoring activities conducted by ARPAE during these algal blooms and the risk management actions implemented.

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Introduction

Cyanobacterial blooms have become an increasingly relevant issue in Italy in recent years, representing a major environmental problem that can have significant ecological and human health impacts (Lucentini and Ottaviani, 2011). Among toxic bloom-forming cyanobacteria, the freshwater species *Cylindrospermopsis raciborskii* (Wołoszyńska) Seenayya & Subba Raju) is a growing environmental and human concern as it is spreading worldwide thanks to its adaptation mechanism and its capacity to outcompete other phytoplankton species (Burford *et al*., 2016). This cyanobacterium is currently regarded as a synonym of *Raphidiopsis raciborskii* (Wołoszyńska) (Aguilera *et al*., 2018). Moreover, this species can produce dangerous toxins like cylindrospermopsin and saxitoxins that cause a range of health problems in humans and animals, including gastroenteritis due to lesions in the intestinal wall, hepatitis due to damage to liver cells, renal dysfunction due to damage to kidney cells, and haemorrhages due to damage to blood vessels (Lucentini and Ottaviani, 2011). Additionally, cylindrospermopsin is considered a potential carcinogen (Humpage *et al*., 2000).

The mechanism of toxins release is still a controversial issue as some studies affirmed that the production is triggered at the cellular or molecular level by environmental stimuli, while other authors stated that production of cylindrospermopsin is a constitutive process (Davis *et al*. 2014; Rzymski and Poniedziałek, 2014).

Blooms of *C. raciborskii* have been increasingly reported also in Italian lakes and reservoirs, raising concerns about their potential impacts on water quality and public health. *C. raciborskii* blooms have been documented more frequently in central and northern regions and in particular in inland waters and confined water bodies that are more susceptible to eutrophication and human exploitation (Bogialli *et al*., 2005; Messineo *et al*., 2010). The increase in these blooms might be correlated to environmental changes as well as to an increase in citizens' sensibility to the correlation between environmental quality and human health. However, also as the Regional Agency for Environmental Protection of Emilia Romagna (ARPAE), we observed an appearance of *C.*

raciborskii blooms not previously reported in our region and an increase of these phenomena in the last three years.

In this note, we describe some cases of *C. raciborskii* blooms reported by citizens as green colourations of surface water and outline the monitoring actions and risk management activities implemented by ARPAE during these blooms.

Results and Discussion

As reported by other studies (Fabbro and Duivenvoorden, 1996; Bogialli *et al*., 2005; Messineo *et al*., 2010), also in our cases, blooms of *C. raciborskii* were mainly observed in artificial lakes, reservoirs, and inland water as they are characterized by high nutrient concentrations, lower flow, decreased turbulence, and low turbidity. Moreover, all these blooms were reported between the summer months and the end of the summer and were detected due to a change in water colouration that appeared with a strong green colour, but without the formation of scum.

Two bloom events of this blue-green alga were observed during the late summer of 2022. One bloom occurred on the 5th of September 2022 in an artificial lake within a golf club (Lake Foschi Golf Club) in the province of Forlì. The lake covers an area of 93,700 m^2 and is characterized by a maximum depth of 1-2 m. During the bloom, the water of the lake displayed a strong green colour, high turbidity, and the absence of unpleasant odours. The chemical-physical parameters detected at the water surface revealed a water temperature of 21.9°C, high dissolved oxygen concentration (13.67 mg L^{-1}), and high pH value (8.59). The second bloom was observed on the 14th of September 2022 in the Gallego Canal in the province of Bologna and was characterized by the absence of superficial scum and water with a strong green colour (Figure 1a).

Since this was the first time we detected a bloom of this species, its correct identification was made by expert taxonomists who confirmed the presence of *C. raciborskii* by the shape of the filament, the terminal heterocyst, and the presence of akinete (Figure 1b).

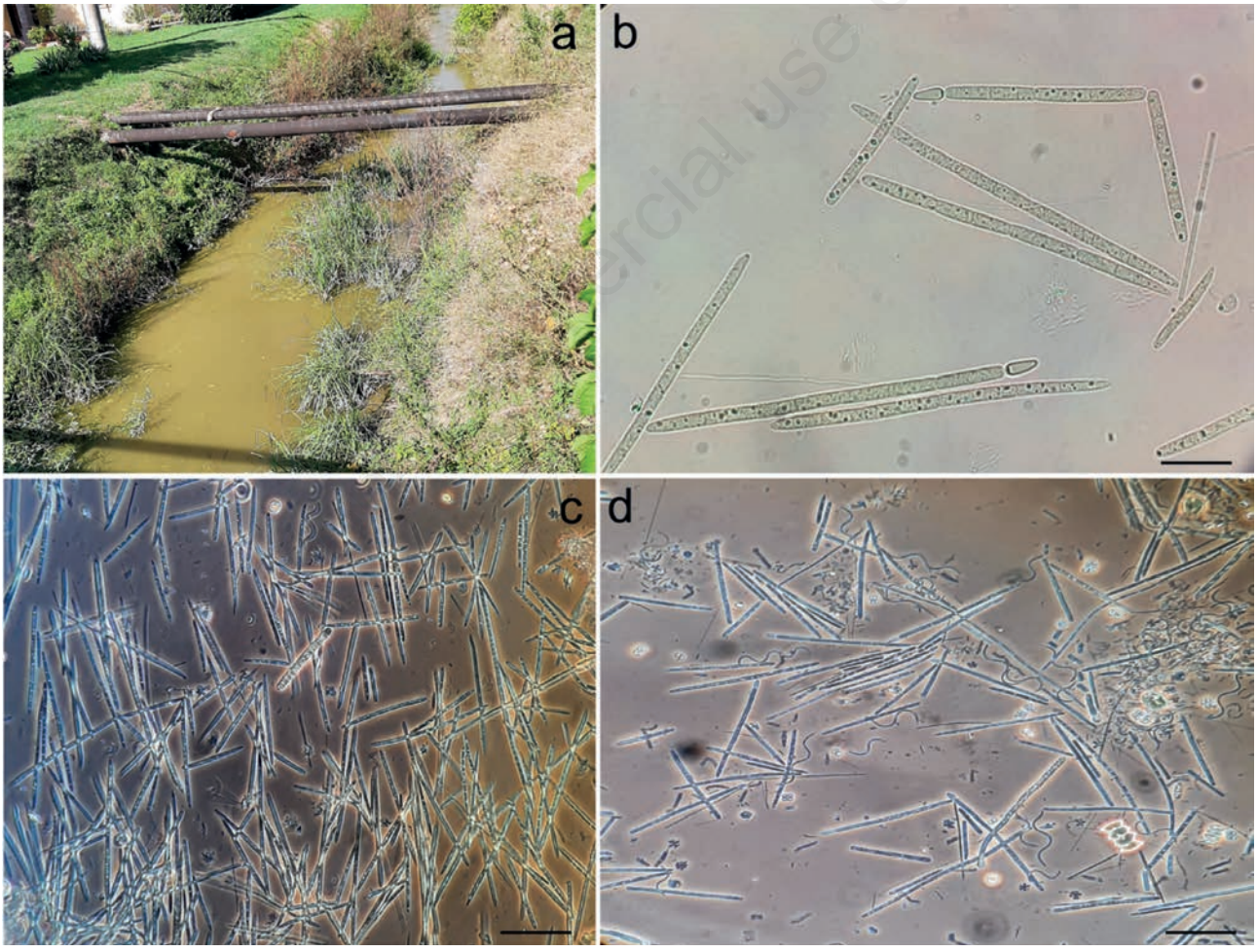

Figure 1. Blooms of *Cylindrospermopsis raciborskii* in Emilia Romagna lakes. a) Green water discoloration of surface water in the Gallego canal (BO) caused by *C. raciborskii* bloom. b) Typical form of *C. raciborskii* detected in the Gallego canal (Scale bar 20 µm). c) A dominant presence of *C. raciborskii* during a bloom in the Paul Harris artificial lake located in a public park in the province of Forlì (Scale bar 50 µm). d) Bloom of *C. raciborskii* detected in the artificial lake Paul Harris in the province of Forlì with the presence of other cyanobacteria, diatoms, and chlorophytes (Scale bar 50 µm).

In both cases, the quantification of the algae under an optical microscope revealed a cell density of approximately 5,000 cells/mL, which exceeds the alert level established by the World Health Organization and reported in the ISTISAN 11/35 report (Lucentini and Ottaviani, 2011). Despite the limitations of our laboratory, which include only microscopic identification and a lack of instruments for selective screening analysis for cyanobacterial toxin quantification (ELISA essay or LC/MS methods), we were able to conduct *in vivo* bioassays with the crustacean *Daphnia magna* to assess the toxic effect of the algae in the analysed samples. Despite the lack of toxicity detected in biological assays (EC_{50} value =0%), a fish kill was observed during the bloom in the Lake Foschi Golf Club. Therefore, due to the lack of more specific analyses for toxin quantification, it was not possible to attribute this mortality event to the presence of cyanotoxins or to the typical chemical-physical characteristics of the bloom event, such as high pH and oxygen deficiency. Risk management actions, in this case, were based on the banning of water recreational use as well as water irrigation use and on a monitoring program of 1-2 weeks consisting of algal cell counting.

Another significant *C. raciborskii* blooming event occurred in August 2023 and persisted until September 2023. This event affected an artificial lake situated inside the Paul Harris public park in the Forlì province that has a surface dimension of 460 m and a maximum depth of 3 m. In line with previous observations, the bloom was characterized by a green water discoloration and by the absence of scum or other solids on the surface. Chemical-physical parameters detected at the surface revealed a water temperature of 24.7°C, a high pH value, the high value of dissolved organic carbon, and a low value of dissolved oxygen, respectively 9.5, 110, and 2.75 mg L^{-1}.

During the first bloom event that occurred in early August, an abundant and dominant presence of *C. raciborskii* was observed, with a cell count of 500,000 cells mL^{-1} and a total absence of other phytoplankton species (Figure 1c). Subsequent monitoring, carried out throughout August and September, revealed periods of reduced bloom with a minimum concentration of this alga (11,000 cells mL^{-1}) towards the end of August, followed by a new increase in September, with a maximum density of 330,000 cells mL^{-1}. In this case, light microscopy analysis revealed the presence of other cyanobacteria belonging to the genera *Pseudanabaena* and *Merismopedia*, diatoms of the genus *Nitzschia,* and chlorophytes (Figure 1d). Risk management actions mirrored those implemented during previous bloom events and were based on the closure of lake access, thereby restricting recreational and irrigation water use.

Conclusions

The major concern about cyanobacterial bloom, including *C. raciborskii*, is the phenomenon of massive contamination of cyanobacteria in reservoirs for the production of drinking water and, accordingly, the establishment of correct activities to assess and manage the risk associated with the presence of harmful toxins in water intended for human consumption (Lucentini and Ottaviani, 2011).

C. raciborskii's ability to thrive in diverse environmental conditions poses a significant challenge in predicting its presence and proliferation. Unlike other algal blooms that produce visible scums, blooms of this species manifest as subtle changes in water colour, making their detection particularly difficult. This characteristic further complicates efforts to monitor and manage these blooms effectively (McGregor and Fabbro, 2000; Briand *et al*., 2002; Burford *et al*., 2016).

The experience gained by ARPAE from the bloom events described above demonstrates that the management of emergencies arising from uncontrolled cyanobacterial growth with potential toxin production in water bodies presents a multifaceted challenge that requires a prompt and informed decision-making. However, the process is often complicated by a convergence of pressing factors that can hinder effective risk management. Firstly, the elements for risk assessment such as algal identification and toxins quantifications might be insufficient due to operator inexperience or the lack of adequate instrumentation. Moreover, the stakeholder groups associated with the utilization of the water body can be diverse and demand conflicting actions aligned with their specific interests.

In this complex scenario, as an environmental protection agency, we resolve to develop monitoring plans that incorporate both collaboration with research institutions where screening methods for cyanotoxin determination are already developed and certified, as well as collaboration with other services inside our Agency to establish timely and effective risk assessment and management plans.

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