 doi:10.20944/preprints202105.0232.v1

Influence of Biotope and Biotic Factors on Cyanobacteria Abundance, Genotype and Toxin Production

Maria Iasmina MOZA[1,2,3] ☒, Carmen POSTOLACHE [1,4] ☒

[1]*Faculty of Biology, University of Bucharest, Department of Systems Ecology and Sustainable Development, Doctoral School in Ecology, 91-95 Splaiul Independenței St., district 5, 050095, Bucharest, Romania*

[2] *Foundation Conservation Carpathia, Wildlife Genetic Monitoring Laboratory, Calea Feldioarei nr. 27, 500450, Brasov, Romania*

[3] *Swiss Federal Institute for Environmental Science and Technology-Eawag, Überlandstrasse 133, P.O.Box 611, 8600, Dübendorf, Switzerland*

[4] *Research Institute of the University of Bucharest - ICUB, 36-46 Bd. M. Kogalniceanu St., district 5, 050107, Bucharest, Romania*

Correspondence 1: Maria Iasmina Moza, Faculty of Biology, University of Bucharest, Department of Systems Ecology and Sustainable Development, Doctoral School in Ecology, Splaiul Independenței no. 91-95, district 5, 050095, Bucharest, Romania and Foundation Conservation Carpathia, Wildlife Genetic Monitoring Laboratory, Calea Feldioarei 27, 500450, Brasov, Romania

Tel.: +40 743620132

E-mail: iasmina_moza@yahoo.com

Correspondence 2: Carmen Postolache, Faculty of Biology, University of Bucharest, Department of Systems Ecology and Sustainable Development, Doctoral School in Ecology, Splaiul Independenței no. 91-95, district 5, 050095, Bucharest, Romania

E-mail: carmen_postolache83@yahoo.com

 doi:10.20944/preprints202105.0232.v1

Abstract

Environmental genetics-related modern methods are shown as important indicators of various cyanotoxins syntheses, and their knowledge and use are critically analyzed. Microcystins and other cyanotoxins loads and syntheses are related to different drivers, like various chemical elements and compounds (especially nutrients, such as nitrogen and phosphorus, and their ratio), then to the light, conductivity, temperature, and other climatical and hydrological factors, to which spatial and geographical features (such as the surface of the water bodies) have to be added. The biotic relationships include different specific and supraspecific, uni- and bilateral links between the cyanobacteria, and subsequently their synthesized toxins, and protozoans (or protoctists), chromists, macrophytes, different systematical and ecological groups of zooplankton, and others. The importance of, but also the gaps in, the knowledge and the scarcity of studies involving ectocrines mediated interactions between different groups of algae and plants are highlighted. The paper ends with an interesting classification of lakes' trophicity, illustrated with conceptual diagrams, based on possible scenarios of cyanobacteria behavior.

Key words: cyanobacteria, toxic, biotic factors, abiotic factors, interactions, allelopathy

 doi:10.20944/preprints202105.0232.v1

1. Introduction

In the last decades, toxic or so-called harmful algal blooms (HABs) have been increasingly reported on a global scale in frequency, distribution and impact of their effects on public health, living resources, and local economies (1-3). Such blooms occur not only in marine and estuarine environment, where there are about 150 harmful or toxic microalgal species (4), but also in the freshwater ecosystems impacted by eutrophication, where cyanobacteria represent the dominant. Mass populations of toxic cyanobacteria are a global phenomenon and the recent recognition that incidences of blooms may increase significantly under future climate change serves to reinforce further the seriousness of the potential risks to human health (1). Due to this, toxic cyanobacteria have gained in recent years increasing amounts of attention by the scientists, authorities and general public worldwide (5).

The cyanotoxins are secondary metabolites produced by about 40 species of cyanobacteria (6), that include neurotoxic, hepatotoxic, genotoxic, inflammatory and cytotoxic agents (7). The genera known for their potential ability to produce toxic substances include *Anabaena*, *Aphanizomenon*, *Cylindrospermopsis*, *Lyngbya*, *Microcystis*, *Nodularia*, *Nostoc* and *Planktothrix* (8); however, even if the species able to produce toxins are present, they may release toxins only in special circumstances, under the pressure of several anthropogenic or environmental factors. With over 90 known congeners globally, hepatotoxic microcystins (*mcyE* gene is involved in their synthesis) are among the most potent and commonly encountered toxins (9) and they are mainly produced by species belonging to the *Microcystis*, *Anabaena* and *Planktothrix* genera, although many other taxa were shown to have toxic potential (10, 11). Genetic-based methods enhanced the understanding of the natural distribution of genes that are involved in cyanotoxins production, despite only indicating potential toxin producers (12). Quantitative real-time PCR (qPCR) has been increasingly applied to monitor potentially toxic cyanobacteria population shifts in diverse aquatic ecosystems worldwide (13, 14); because is sensitive and rapid.

Although the ecology of cyanobacteria is well described, the relationship between population dynamics and environmental factors that trigger the presence of genes involved in microcystins synthesis is poorly understood (15). For example, density of the hepatotoxic cells were direct correlated with the microcystins (MCs) concentration from water (16) and in concordance, with the gene (*mcyE* for example) copies number inside the cells. Some strains may produce higher MC concentration than others under the same laboratory conditions, while others can be more or less toxic depending on cultivating conditions (17). In addition, the succession of cyanobacteria species and biomass is influenced by seasonal changes of several factors including nutrients, grazing, light and temperature, which affect also the concentration of MC in the field (18). In environment, different strains produce different concentrations of MCs but not all the time target genes involved in MCs synthesis are well correlate with this concentrations as a recent study demonstrate (15). The effects of

 doi:10.20944/preprints202105.0232.v1

environmental factors on the abundance of MC-producing and non-toxic *Microcystis* genotypes have, however, been studied on a limited scale (15).

A brief overview on the influence of several physic-chemical parameters is provided in (18) and summarized below: a positive correlation between toxic genotypes (*mcyA*) and nitrate (NO_3-N) concentrations was noticed, while temperature and orthophosphate (PO_4-P) concentration seem not to influence *mcyA* abundance (19). In the same lake, a direct influence of the cyanophage assemblage in shifts of MC-producing and non-MC-producing subpopulations was suggested (20). NH_4 and NO_3 were also shown to increase toxic (*mcyA*), *Microcystis* strains abundance and MC concentrations in a hypereutrofic pond (21), without affecting the total *Microcystis* abundance. Total phosphorus was shown to positively correlate with both toxic (*mcyD*) and total *Microcystis* abundance in Lake Erie, USA, while *mcyD* genotypes correlated negatively with NO_3, total nitrogen, nitrogen to phosphorus ratio and pH (22). Phosphorus was also shown to correlate positively with the relative abundance of potentially hepatotoxic (*mcyE/ndaF*) cyanobacterial community of river Nile, Egypt, at a site showing strong phosphorus limitation (23).

In spite of the fact that both nitrogen and phosphorus influence the numbers/proportion of toxic genotypes, other factors such as low water depth, high pH, high temperature, lack of wind and water column stability, nutrient and light availability, favor cyanobacteria development (24) increasing the risk hazard.

Also, allelopathic relationships with macrophytes or other algal groups and grazing pressures of zooplankton or fish communities should be further considered as it has been noticed that competition and grazing stimulate the toxin production (25, 26). The cumulative impact of all these factors on toxin producing cyanobacteria requires more detailed investigations in order to elaborate effective management measures to prevent health hazards. Within this review we summarize the effect of biotic and abiotic factors on cyanobacteria abundance, genotype and toxicity.

2. Interaction with environmental factors

Is well know that organisms interact with their living and nonliving environment so, they are affected by *abiotic* (physical and chemical) and *biotic* (presence and activities of other organisms) factors. These adaptations are the result of evolution, the driving force of which is natural selection (27, 28). Cyanobacteria play an invaluable role in freshwater ecosystems because of their abilities to produce oxygen via oxygenic photosynthesis and convert atmospheric nitrogen to the biologically available form, ammonium (NH_4^+) (9, 29-31). Additionally their status as primary producers strengthens their connection to the aquatic environment, as they contribute to the foundation of the food web, especially stimulating bottom up food web shifts in shallow, eutrophic lakes (32).

2.1. Abiotic factors

2.1.1. Physical and chemical parameters

 doi:10.20944/preprints202105.0232.v1

Light conditions in shallow lakes may change on a time scale of days to weeks due to changes in cloudiness or wind-induced resuspension of sediments. It was demonstrated that light intensity is a critical factor influencing the production of cyanotoxins (33). Changes in light conditions may profoundly affect the microcystin composition and thereby the toxicity of cyanobacteria (34); the transcription of two genes responsible for microcystin production was already shown to be influenced by light quality (33). For example, the harmful cyanobacterium *Planktothrix agardhii*, a species that prefers mostly shallow, turbid lakes, produces a more toxic variant during periods of sunny weather, when recreational activities in lakes are most attractive (34). It has been documented that the excessive growth of cyanobacteria can reduce water transparency with light penetration to only few centimeters, and thus having important effects on both pelagic and benthic communities (35). The reduction of the euphotic zone together with the excessive increase in the ratio between the epilimnetic mixing layer and the euphotic depth is an unfavorable factor for other organisms (35).

Another physical parameter of the water, which has exhibited a high positive correlation with MCs concentration, but not with the number of *mcyE* gene copies, was the conductivity. Conductivity is a parameter related to the ability of electric conduction of water, and can indicate the ion concentration. *Microcystis* utilize various inorganic ions such as macronutrients and trace metal for growth (36). This confirms previous studies showing that alkaline pH (7.5 - 8.5), electrical conductivity from 241 to 367 µS/cm, and temperature ranging from 24.8 to 32ºC, are promoting microcystin development (37).

The pH of water may also influence toxin production. For *M. aeruginosa*, higher MC production occurred at pH values above and below their optimum growth threshold (33).

Temperature is a crucial parameter of the environment that influences the survival, metabolism, growth and reproduction of all cyanobacteria, as well as the interactions between them and other species (38) as well a fundamental relationship with cyanotoxin production (33). For example, some cyanotoxins are often found at temperatures that would be considered sub-optimum for cell growth, with maximum reported at 20 °C and production ceasing at temperatures exceeding 35 °C, namely cylindrospermopsin (33). Anatoxin-a production has also been shown to be highest at 20 °C, whereas maximum production of MC and nodularin has been reported to occur between 18 and 25 °C (33). Other studied showed that temperature, DO and *Microcystis* biomass positively correlated with MC accumulation as well (39). Temperature alone may only partly determine bloom formation and it is accepted that a combination of factors are responsible for a bloom to develop: increasing temperatures, decreasing nutrients and increased water column stability (40); temperature has the most pronounced effect on toxicity; the highest toxicity was found at 20°C, but reduced at temperatures in excess of 28°C.

 doi:10.20944/preprints202105.0232.v1

148 According to Paes and his colleagues (41), in all studied reservoirs that experienced toxic blooms water
149 transparency was reduced. *P. rubescens* abundance appears to be strongly influenced by water transparency, at least in pre-
150 alpine lakes (42).

151 A series of mild winters with earlier ice break-up can lead to an earlier stratification and a shift in the composition
152 of the phytoplankton from diatoms to cyanobacteria (43). After some scientists the bloom of cyanobacteria is associated
153 with thermal stratification (44). Other authors suggest that both growth and toxin production as *Anabaena* may be
154 controlled by salinity (45).

155 2.1.2. Nutrients

156 Freshwaters enrichment with nitrogen is a dynamic process, reflecting land use, hydrologic conditions, population
157 and economic growth (46, 47). Recent field studies have shown that bloom production by *Microcystis* sp. (a widespread
158 cyanobacterial genus frequently producing blooms) is often associated with high levels of nitrogen (48, 49). Other
159 experiments have demonstrated that toxic blooms are more likely to occur under elevated nitrogen concentrations (50).

160 Toxin production in non-nitrogen fixing cyanobacteria, such as *Microcystis* and *Planktothrix*, has been shown to
161 peak with high concentrations of nitrogen (33). According to a recent study (51), total MC concentrations could be
162 explained by a combination of abiotic factors like dissolved organic nitrogen (DIN), water temperature and ammonium
163 (NH_4^+). In this study, however, the best overall model to predict MC concentration combined both environmental variables
164 and species biomass (51). Total nitrogen, water temperature, ammonium and dissolved organic nitrogen influenced the
165 cyanobacterial community structure, which in turn resulted in differences in the dominant MC congener and the overall
166 toxicity. Ammonium (NH_4^+) did not emerge as a significant variable in the multivariate model although it is considered
167 important in structuring cyanobacterial communities based on empirical studies of some lakes (51).

168 The presence of N can contribute to both increased and decreased MC production, depending on the genera (33).
169 Nitrogen and phosphorus are the main elements for the matter and energy metabolism of the algae (52). Toxin production
170 in non-nitrogen fixing cyanobacteria, such as *Microcystis* and *Planktothrix*, has been shown to peak with high
171 concentrations of nitrogen (33). When attempting biomanipulation, the omission of nitrogen causes approximately ten fold
172 decrease in toxicity (40). High levels of nitrogen and phosphorus in freshwaters favour the growth of toxic strains
173 over nontoxic ones (53). Total nitrogen, pH, and the surface area of the lake predicted the occurrence probability of *mcyE*
174 genes, whereas total phosphorus alone accounted for MC concentrations (54).

175 Historically, phosphorus was seen as the primary limiting nutrient in freshwaters (55), and now it is generally
176 accepted that reduction in phosphorus input is an effective way to inhibit cyanobacterial dominance in freshwater
177 ecosystems. Unlike nitrogen, which can exist in gaseous form, phosphorus can only be found in dissolved ionic and

 doi:10.20944/preprints202105.0232.v1

178 particulate forms in natural waters. Cyanobacteria are able to dominate the phytoplankton communities at very low
179 phosphorus concentrations, because they have a very high natural affinity for this element (56, 57).

180 The findings related to phosphorous also demonstrate the importance of this nutrient to toxin production (33). Is
181 known that phosphorus was the limiting resource for phytoplankton biomass but the mixotroph *Dinobryon* biomass
182 increased with decreasing total phosphorus concentrations (58)**.** There are studies were the regression analysis showed that
183 the densities of phytoplankton and total phosphorus were positively and linearly correlated (R=0.715) (52). In the same
184 study the correlation between the concentration of TN and the density of phytoplankton was not remarkable (R=0.166). P
185 and N sources have been considered as the main factors to affect the growth of *Microcystis* cells and MC levels, and such
186 influencing process is so complicated that the different conclusions were obtained by different investigators from different
187 lakes (59).

188 N:P ratio in water is an important parameter in controlling cyanobacterial blooms, with several data suggesting that
189 total molar N:P ratios above 15 discourage the occurrence of these phenomena (60, 61). After a screening of more than
190 240 water bodies, scientist concluded that maximum concentrations of microcystins occurred in hypereutrophic lakes at
191 mass ratios of N:P below 23 and that many planktonic cyanobacteria have a benthic life stage where they engage in "luxury
192 uptake" of P from sediments, and consequently, episodes of cyanobacterial recruitment from sediments can dramatically
193 decrease the N:P ratio in the water column (62) **.** "TN/TP rule" hypothesis that cyanobacteria tended to dominate in the
194 lake when TN/TP <29, while decreased when TN/TP >29 but this rule is less applicable to highly eutrophic waters when
195 both N and P nutrients are very high. (59)**.** It was also suggested that, at least in one highly eutrophic lake TN/TP ratio is
196 not the factor bursting of blooms of *Microcystis* (59). *Microcystis* occurred only at higher TN, TP, temperature and pH
197 values (63) for example. The toxicity of *M. aeruginosa* is known to change depending on seasons (41, 64).

198 2.1.3. Variation in toxin concentration

199 Cyanotoxin production is dependent on a number of environmental conditions. Predominantly, these could include
200 nutrient concentration, light intensity, and temperature (33). Eutrophication increased the co-occurrence of potentially
201 MC-producing cyanobacterial genera, raising the risk of toxic-bloom formation (54).In general, studies considering the
202 effects of nutrients on toxin production find a positive correlation between the nutrient of interest and cellular toxin content.
203 Research focusing on nitrogen, cyanobacterial growth, and subsequent toxin production, report that increased nitrogen
204 corresponds to increased toxin production (65-67). Recent studied showed that physical and chemical parameters did not
205 significantly account for both intracellular and dissolved microcystins occurrence (68)

206 World Health Organization setting a guideline value of 1 µg/l for microcystin-LR in drinking water (69). Is notable
207 that typical environmental concentrations of MC are below 10 µg/L (33).

For some organisms that accumulate microcystins, total MC concentrations declined after October and began to increase in May , from the season point of view, that give us also a clue about the toxin production in the ecosystem (39). microcystins production did not correlate with the high level of nutrients (*t* test, $p > 0.1$). In fact, microcystins were never detected in the more eutrophic reservoirs (68). The cyanobacterial biomass in water, pH, and temperature explain the variability of MC concentration in the sediment of shallow lakes also (70). MC and abundances of total Microsytis and MC-producing Microsystis (MCM) were shown to be positively correlated with pH, DO and TP (59). A significant difference was found for conductivity, phosphates and the presence of microcystins Mc-LR and Mc-Tot in the oligo- and eutrophic reservoirs (68). It is speculated that N and P nutrients and the associated genes (e.g., *mcy*) may jointly drive MC concentration and toxigenicity (59)**.** Also species biomass was the best predictor of MC concentrations (51) and the highest growth rate is not correlated with highest toxicity (40) that reveal that cell density may not be the best predictor for the toxicity. *Microcystis* was often observed coexisting with *Anabaena*, *Planktothrix* and *Phormidium* (59) and therefore can bloom together.

2.2. Biotic factors

2.2.1. Zooplankton

When the density of phytoplankton was too high it was concluded that the multiplication of zooplankton was restrained or harmed by the worse eutrophication of the system (52). Zooplankton and cyanobacteria may interact directly via the feeding and excretion of zooplankton (71) and indirectly via trophic linkage pathways and behavioral responses to physical and chemical parameters (light, temperature, turbulence, pH, and dissolved oxygen). Results of laboratory studies (72) suggest that many colonial cyanobacteria are either not eaten or are a poor food for large zooplankton, particularly *Daphnia*. Therefore, at times when these colonial forms dominate the phytoplankton, *Daphnia* populations might be expected to show decreased growth and fecundity in response to food limitation or toxicity. Is very probably that any effect of cyanobacteria on zooplankton could be influenced by evolutionary mechanisms in natural systems with a long history of cyanobacterial blooms: zooplankton that co-occur with dense biomass of cyanobacteria have better chance to adapt than others that were not exposed to these conditions (41). It was already demonstrated that, if the proportion of any toxic cyanobacteria and any edible algae studied is in concordance with tested microorganism needs, the survival rate is growing (64, 73-76). This suggests that in nature, at least some of the total amount of *M. aeruginosa* is consumed, but only when the strain is not highly toxic. Therefore changes in the cyanobacterial abundances in nature are not only related to physical and chemical variables but are also likely due to grazing from zooplankton (64). There were species-specific differences in the filtration and feeding rates of zooplankton when offered mixed diets of green algae and toxic cyanobacteria. These probably explain the coexistence of different zooplankton species in *Microcystis*-dominant waterbodies. (76)

 doi:10.20944/preprints202105.0232.v1

238 2.2.2. Rotifers

239 It has been previously shown that some rotifers like *Brachionus calyciflorus* react negatively to the presence of
240 cyanobacteria (*Microcystis aeruginosa* and *Synechococcus elongatus*) because they lack essential compounds and not
241 because of their cell size (77). On the other hand, when nutrient-rich green algae serve as food, the difference in the cell
242 size matters and it is reflected directly in the lower growth rate of the rotifer population.

243 Among zooplankton, rotifers were shown to display a high range of tolerance to temperature. Thus, in some
244 subtropical shallow lakes, the rotifer density increased with the increase in temperature, reaching its maximum at
245 approximately 23°C but decreased slightly when the temperature exceeded 25°C (78). An experiment focused on the effect
246 of *Microcystis aeruginosa* on the survival and reproduction of the rotifer *Brachionus calyciflorus* using the life table
247 method at different temperatures showed that *M. aeruginosa* suppressed the survival and reproduction of *B. calyciflorus*,
248 particularly at a concentration of 10^6 cells/mL according to (79).

249 Some laboratory experiments have shown that temperature can play an important role in modifying the effect of
250 toxic cyanobacteria on freshwater, planktonic herbivores. Thus, in rotifers acclimated for many generations to low (12°-
251 14°C), intermediate (19°C), and high (25°-26°C) temperature, susceptibility to the cyanobacterium and its toxin increased
252 significantly with temperature. The results indicate that seasonal increases in water temperature, and climate warming, may
253 exacerbate the impact of toxic cyanobacteria on rotifers like *Brachionus calyciflorus* and *Asplanchna girodi*, and perhaps
254 other zooplankton taxa (80). So, is clear that a potentially modifying effect of temperature needs to be considered when
255 investigating the effect of the dynamics of zooplankton populations on toxic cyanobacteria also. For example temperature
256 is one of the important variables affecting the population growth and reproduction of rotifers including *B. calyciflorus*;
257 higher temperatures (30°C) stimulate their population growth (81). Usually when two or more rotifer species compete for
258 limited resources, one or more of them may be adversely affected by the presence of other (64) so the bad food quality is
259 not the only limited factors for rotifers to taken into account.

260 Even if the cyanobacteria strain is nontoxic or toxic, at least in the case of *B. calyciflorus* , this type of diet
261 suppresses the population growth; effects were less severe than that produced by toxic *M. aeruginosa* (73). Is was observed
262 that in eutrophic systems higher abundance of rotifers is often observed with higher abundance of cyanobacteria, which
263 indicates that not all cyanobacteria inhibit rotifer growth (41, 74). Additionally, rotifers are affected by three selected forms
264 (unicellular, filamentous, and colonial) of cyanobacteria (73). *Cylindrospermopsis raciborskii* is less harmful to the rotifer
265 *Brachionus calicyflorus* than the worldwide occurring cyanobacterium *Microcystis aeruginosa*; *Microcystis* did not support
266 growth of *Brachionus*, but even killed them within the 2 days experimental period (74). *Asplanchna girodi* was sensitive
267 to toxins of *Anabaena flos-aquae* and *L. majuscula* (82). More, the toxin-producing *Anabaena flos-aquae* has been shown

 doi:10.20944/preprints202105.0232.v1

to decrease lifespan, fecundity, and population growth rate of the rotifers *Brachionus calycijlorus* and *Synchaeta pectinata* (33). Usually, MCs are released into water after demise of cells and dissolved MCs during the collapse of heavy blooms can come in contact with a wide range of aquatic organisms including rotifers and have adverse effects on them by increasing mortality (81). Notably is that when different species coexist in the ecosystem react different to the toxins (64). For example the impact of *M. aeruginosa* in the diet had a more adverse effect on *B. calyciflorus* than on *B. havanaensis*, (64) but the same strain manage to kill the cladocerans *Ceriodaphnia dubia* and *Moina macrocopa* in previous study (75).

Important to be mentioned is that rotifer density and water temperature were negatively correlated (83). Depending of the ecosystem type (either temperate or tropical) rotifers react different: some species could survive if they are expose to *Cylindrospermopsis*, but can be profoundly affected if they are exposed to *Microcystis* for example (41). However, when analysed at the population level, rotifers and cladocerans showed a weak positive response to microcystin. For rotifers, negative relationships were observed for *Brachionus calyciflorus*, *Conochilus* sp., *Hexarthra* sp. with respect to the different orders, calanoid copepods showed a positive response to microcystin (41).

Rotifers consumed half of what a cladocerans consumed in term of food; that could be an explanation why rotifers resist longer in the nature if the ecosystem experience a toxic bloom (76). Also in the same study was reveal that cladocerans filtrate rate is growing if the food quality is lower, so again another motive why they survival rate is less: they manage to eat more *Microcystis* cells in comparison with rotifers in the same time interval.

Protists

Sometimes a higher density of zooplankton , mostly protists and rotifers showed the worse trophic state of the ecosystem (52). This suggests that whilst toxin production is likely to be linked with defense against protozoan grazing in some species (33). It has already been confirmed that toxins produced by *M. aeruginosa* did not change significantly the protozoan's mobility, morphology or viability in contrast, microcystins produced by *Gloeotrichia echinulara* was lethal for *Paramecium caudatum*, while extracts of *L. majuscula* acted very fast to provoke the lysis of the protozoan *Tetrahymena pyriformis* (82). Other study demonstrated that *Microcystis aeruginosa* was unable to produce MC in response to direct and indirect exposure to a protozoan grazer, this suggest that toxin production could be linked with defense against protozoan grazing only for some species (33).

A study conducted on the ciliate *Nassula sp*. isolated from a water body with no history of toxic blooms and fed with a toxic strain of *Planktothrix agardhii* for 8 months showed that this species can survive feeding exclusively on toxic cyanobacteria over an extended period of time, despite increasing MC concentrations (84). Conversely, a number of protozoan grazers are known to actively feed and grow on toxic cyanobacteria and in some cases *Microcystis aeruginosa* was unable to produce MC in response to direct and indirect exposure to a protozoan grazer (33). The toxins are probably

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 May 2021 doi:10.20944/preprints202105.0232.v1

accumulated in the ciliates and only fractions of MC remain in the water. Therefore, in water frequently can be found a small cyanotoxin concentration in comparison with the amount found in zooplankton tissues (85). For these reasons, this group of protist is seen as a potentially biotic agent to reduce toxins levels in freshwaters bodies that usually develop cyanobacterial blooms. However, the ability to synthesize microcystin does not seem to offer toxic *Microcystis* populations a significant defense against grazing by co-occurring zooplankton communities (86).

2.2.3. Other zooplankton

Unlike with rotifers, the presence of cladocerans can trigger the MC production in *Microcystis sp*. More exactly, in the presence of high zooplankton abundance (*Daphnia pulex* and *Brachionus calyciflorus*), at low cell density of *Microcystis* sp. the MC concentration was significantly higher as compared to controls (87). Therefore, cladocerans react different in comparison with other zooplankton groups, for example *Keratella cochlearis* was superior in competition with *Daphia pulex* under toxic *Microcystis* (88). One must consider that that natural mesozooplankton were better grazers of both toxic and non-toxic strains of *Microcystis* than their cultured counterparts (86).

A study designed to test the development of tolerance in several zooplankton species to MC in a range of temperatures showed that the ability to utilize *Microcystis* improved at 30°C in species like *Moina macrocopa*, *Daphnia carinata* and *Hexarthra mira*, but significantly decreased in case of rotifers (89). It was also shown that low concentrations of edible algae favors small-sized cladocerans, while high concentrations favor large-sized cladocerans, such as *Daphnia*, but the presence of cyanobacteria can affect the dominance status of large-bodied daphnid especially. Some cladocerans can coexist well with *Microcystis* sp. in nature but colony size affects cladoceran population and their interactions. (88). There are also strong intraspecific differences in the tolerance of different *Daphnia* clones to toxic/non-toxic cyanobacteria, and therefore the dynamics of the daphnid populations vary significantly in the presence of these microalgae in their diet (90).

Zooplankton groups may act as vector of the toxin uptake in the aquatic food web (91) and it seems that toxins are bioaccumulated in the ciliates and in the water remain mostly fractions of microcystins (referring to the their chemical structure) *(84)*. Therefore, in water frequently can be found a small cyanotoxin concentration in comparison with the amount found in zooplankton tissues (85).

2.2.4. Macrophytes and algae

Is well known that one of the more serious impacts of eutrophication on aquatic ecosystems is the disappearance of submerged macrophytes and the shift to a phytoplankton-dominated state (92). When aquatic plants cover the wetland by 60% of the water surface, the equilibrium of the ecosystem was maintain and the proportion of the cyanobacteria was

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 May 2021 doi:10.20944/preprints202105.0232.v1

maintain below 25%, almost the same with other algae groups namely Chlorophyta (36.8%) and Bacillariophyta (31.0%) (52).

Experiments provides evidence that resource competition can occur between benthic and water column primary producers (93) and studies have shown that macrophytes can successfully suppress the growth of algae through releasing allelochemicals in nature and in experimental systems (94). However, there is a lack of studies to integrate laboratory and field observations with respect to establishing allelopathic effects of macrophytes (33) There are good hints for allelopathic mode of action of cyanobacterial secondary metabolites within a lake phytoplankton community but not much is known on the mechanisms of interactions between cyanobacteria and algae, and how both sides contribute to phytoplankton dynamics during the year (92).

Some carophytes germinated less in the presence of MC in the sediment, and they also had lower chlorophyll concentrations. Different species have displayed variable sensitivity to the presence of MC in the water (95). MC-LR affects macrophyte *Ceratophyllum demersum* (lost of pigmentation, loss of leaves) (96). High concentrations of exudates and extracts of *M. aeruginosa* can allelopathically inhibit both seed germination and the early growth and photosynthesis of *Potamogeton malaianus* seedlings (97). A reduced growth in the presence of *M. aeruginosa* was observed for *Lemna minor* and for submerged plant *Ceratophyllum demersum* and also a significant decrease of chlorophyll a and b as well as total carotenoids (92). MC-LR has also been shown to exert inhibitory effects on aquatic plants, such as *Ceratophyllum demersum*, with the toxin inhibiting growth, morphology, and photosynthesis at environmentally relevant concentrations (5 μgL^{-1}) (33) or 90% inhibition of photosynthesis *E. canadensis* (92).

There is one study that shows that a golden algae have a great potential to biodegrading microcystin-LR (MC-LR) (98). They reported that the alga *Poterioochromonas sp* was able to degrade MC-LR in cells of *M. aeruginosa* while digesting the whole cells; degradation process was determined to be carried out inside the algae cell. As well, another study showed that Ochromonas sp., a mixotrophic chrysophyte, was able to feed on all four cyanobacterial strains tested, including the very toxic single-celled strain PCC 7806 (63).

Notably is that symbiosis between diatoms and cyanobacterial colonies may also occur in natural water ecosystems (99). Cyanobacterial toxin production can be regulated by complex growth phase dependent and environmental parameters and suppressed by the presence of extracellular products of a eukaryotic green alga like *Chlamydomonas reinhardtii* (100).

However, Microcystin-LR extracted from *Microcystis aeruginosa* had a negative effect on the growth of several green algae (33).

3. Conclusions

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 May 2021 doi:10.20944/preprints202105.0232.v1

In conclusion, since we cannot discuss about one or few direct factors that trigger the cyanobacteria mass development, target genes involved in cyanotoxin production and toxin production and release into environment, we formulate in this short review chapter five possible scenarios of cyanobacteria behavior in any freshwater ecosystem, especially shallow lakes (**Fig. 1-5**).

Scenarios 1 (**Fig. 1**). Interpretation\explanation: in this ideal case, in our lake ecosystem, there are also fish, zooplankton (especially daphnia), phytoplankton, bacterioplankton and aquatic plants, an ideal freshwater ecosystem. Cyanobacteria from here don't need to release toxins to any of other trophic compartment because: (i) there exists enough zooplankton that will eat microalgae, so those are not a stress factors for our cyanobacteria; (ii) there exists enough fish that will eat the zooplankton so that is not a stress factor for our cyano community; (iii) there are enough plants that will keep under control an algal bloom so that our zooplankton and fish could survive, but not that many that could disturb our cyanobacteria community. Conclusion: *this represents the ideal picture of a typical lake ecosystem and a scenario were cyanobacteria could live in "no stress"*. Lake type: oligo-mesotrophic

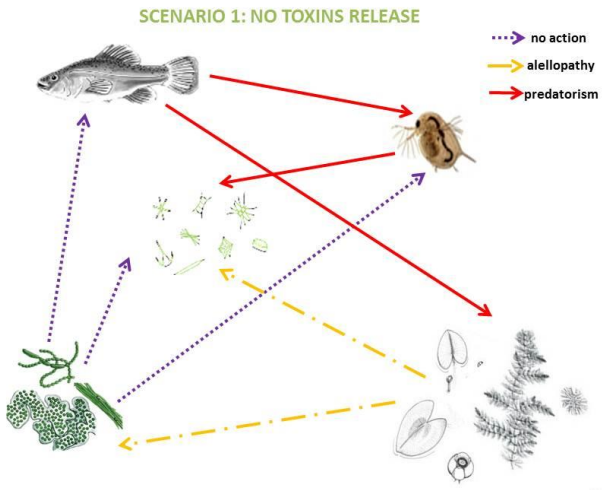

Figure 1. First scenario of cyanobacteria behavior into a freshwater ecosystem: no toxin release

Scenarios 2 (**Fig. 2**). Interpretation\explanation: in second case, in our lake ecosystem we have also fish, zooplankton, (especially daphnia), bacterioplankton and aquatic plants but less phytoplankton, because we have a cyanobacteria blooming. Cyanobacteria from here don't need to release toxins to any of other trophic compartment because:(i) exists enough zooplankton that will eat cyano (because those are the main food source), but will not survive enough to become a stress factor for our cyano population; (ii) there is enough fish that will eat plants and the zooplankton so that will be kept under control limit and not eat very much cyano; (iii) there are some plants that will keep under control an massive algal bloom so that our zooplankton and fish could survive, but not that many that could disturb our

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 May 2021 doi:10.20944/preprints202105.0232.v1

cyanobacteria community. Conclusion: *this represents the picture of a small cyanobacteria blooming but were the environmental changes are not that dramatic like in hipereutrofic lake for example, so our cyano have only "low stress"*. Lake type: meso-eutrophic.

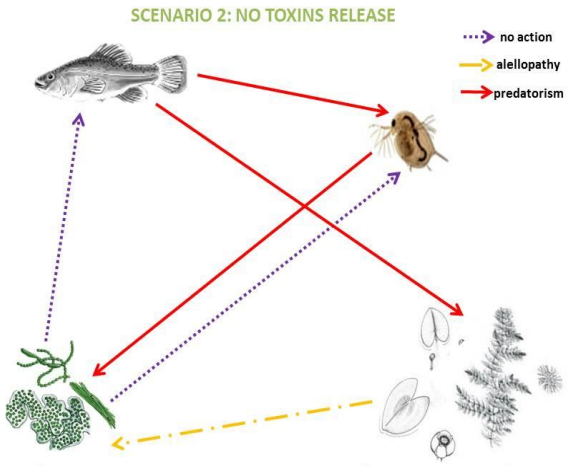

Figure 2. Second scenario of cyanobacteria behavior into a freshwater ecosystem: no toxin release

Scenarios 3 (**Fig. 3**). Interpretation\explanation: in this ideal case, in our lake ecosystem there are also fish, phytoplankton, bacterioplankton and aquatic plants, but no cladocerans for example. Cyanobacteria from here don't need to release toxins to any of other trophic compartment because: (i) there is enough fish that will eat other microalgae and zooplankton so that not represent as much an stress factor for our cyano community; (ii) there are enough plants that will keep under control an algal bloom by others microalgae so that our zooplankton and fish could survive, but not that many that could disturb our cyanobacteria community.Conclusion: *this represents the ideal picture of a typical lake ecosystem and a scenario were cyanobacteria could live in "medium stress"*. Lake type: meso-eutrophic.

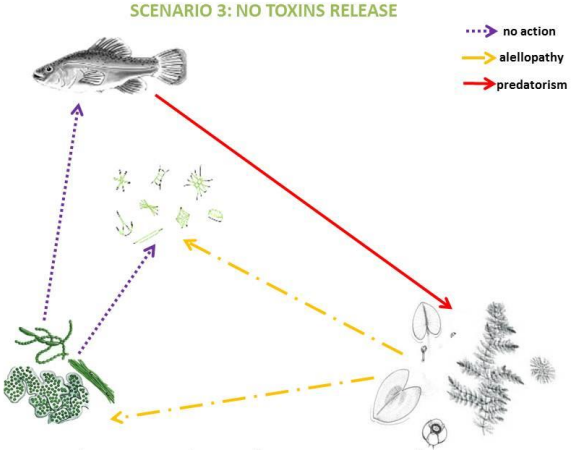

Figure 3. Third scenario of cyanobacteria behavior into a freshwater ecosystem: no toxin release

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 May 2021 doi:10.20944/preprints202105.0232.v1

Scenarios 4 (**Fig. 4**). Interpretation\explaination: in this ideal case, in our lake ecosystem are also fish, zooplankton, bacterioplankton and aquatic plants, but no other microalgae, because we have a cyano bloom. Cyanobacteria from here began to release toxins to any of other trophic compartment because: (i) exists enough zooplankton that will eat them, because don't have others microalgae, so those are a stress factors for our cyanobacteria; (ii) don't exist enough fish that will eat the zooplankton and keep them under control; (iii) there are some plants that are struggling to survive so they release alelopatthyc compounds against cyanobacteria, so this is another stress factor. Conclusion: *this represent the picture for instability of a typical lake ecosystem, if one important group is missing and a scenario were cyanobacteria live in "high stress" and became toxic.* Lake type: eu-hypetrophic.

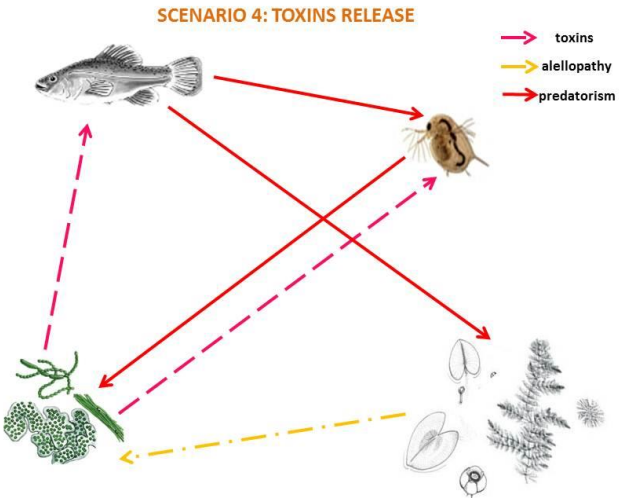

Figure 4. Fourth scenario of cyanobacteria behavior into a freshwater ecosystem: toxin release

Scenarios 5 (**Fig. 5**). Interpretation\explanation: in this ideal case, in our lake ecosystem there are also fish, zooplankton, phytoplankton, bacterioplankton and aquatic plants, but the diversity is low regarding species. We don't have a cyano bloom. Cyanobacteria from here began to release toxins to any of other trophic compartment because: (i) there exists too much zooplankton that will eat microalgae, but also cyanobacteria so those are a stress factors for our cyanobacteria; (ii) it doesn't exist enough fish that will eat the zooplankton so that is a stress factor for our cyano community; (iii) there are enough plants that will keep under control an algal bloom so that why the release allelopathyc substance and stress our cyanobacteria community. Conclusion: *this represents the ideal picture for instability of a typical lake ecosystem and a scenario were cyanobacteria live in "high stress" and became toxic*. Lake type: eu-hypetrophic.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 May 2021 doi:10.20944/preprints202105.0232.v1

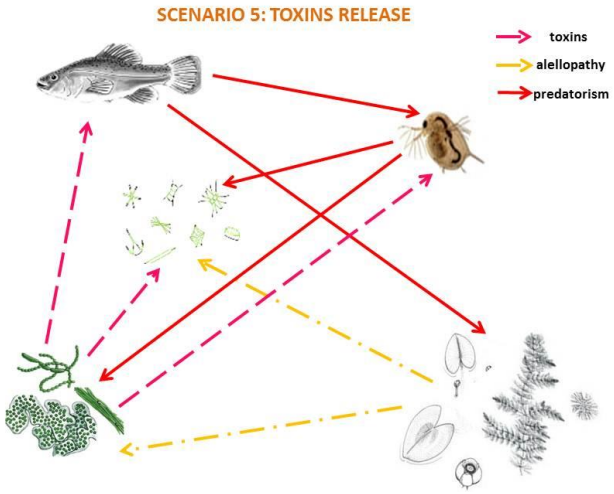

Figure 5. Five scenario of cyanobacteria behavior into a freshwater ecosystem: toxin release

Author Contributions: Conceptualization, M.M.I., C.P.; methodology, M.M.I., C.P.; data curation, M.M.I., C.P.; writing—original draft preparation, M.M.I.; writing—review and editing, M.M.I., C.P.; visualization, M.M.I., C.P.; supervision, C.P.; project administration, C.P.; funding acquisition, C.P.. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Swiss Enlargement Contribution (IZERZ0 – 142165, "CyanoArchive") in the framework of the Romanian-Swiss Research Programme.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: the authors express their deepest gratitude to dr. Carmen Chifiriuc and to dr. Ioan Sîrbu for uselfull observation and preliminary review. The authors also transmit their special thanks to CyanoArchive Project team and Sulina field team for all the support during the sampling trips, to dr. Piet Spaak, and dr. Cristina Sandu for making possible this project. The authors are grateful to the reviewers and Journal Editorial board.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. H. W. Paerl, J. Huisman, Blooms like it hot. *Science* **320**, 57-58 (2008).
2. F. M. V. Dolah, D. Roelke, R. M. Greene, Health and ecological impacts of harmful algal blooms: risk assessment needs. *Human and Ecological Risk Assessment: An International Journal* **7**, 1329-1345 (2001).
3. J. H. Landsberg, The effects of harmful algal blooms on aquatic organisms. *Reviews in Fisheries Science* **10**, 113-390 (2002).
4. J. H. Landsberg *et al.*, Saxitoxin puffer fish poisoning in the United States, with the first report of Pyrodinium bahamense as the putative toxin source. *Environmental Health Perspectives* **114**, 1502-1507 (2006).

 doi:10.20944/preprints202105.0232.v1

5. I. Stewart, A. A. Seawright, G. R. Shaw, Cyanobacterial poisoning in livestock, wild mammals and birds–an overview. *Cyanobacterial harmful algal blooms: state of the science and research needs*, 613-637 (2008).
6. J. A. Westrick, D. C. Szlag, B. J. Southwell, J. Sinclair, A review of cyanobacteria and cyanotoxins removal/inactivation in drinking water treatment. *Analytical and bioanalytical chemistry* **397**, 1705-1714 (2010).
7. A. N. Tyler *et al.*, Strategies for monitoring and managing mass populations of toxic cyanobacteria in recreational waters: a multi-interdisciplinary approach. *Environmental Health* **8**, 1-8 (2009).
8. W. W. Carmichael, Health effects of toxin-producing cyanobacteria:"The CyanoHABs". *Human and ecological risk assessment: An International Journal* **7**, 1393-1407 (2001).
9. G. A. Codd, L. F. Morrison, J. S. Metcalf, Cyanobacterial toxins: risk management for health protection. *Toxicology and applied pharmacology* **203**, 264-272 (2005).
10. F. F. Ngwa, C. A. Madramootoo, S. Jabaji, Comparison of cyanobacterial microcystin synthetase (mcy) E gene transcript levels, mcy E gene copies, and biomass as indicators of microcystin risk under laboratory and field conditions. *MicrobiologyOpen* **3**, 411-425 (2014).
11. K. Sivonen, Emerging high throughput analyses of cyanobacterial toxins and toxic cyanobacteria. *Cyanobacterial harmful algal blooms: state of the science and research needs*, 539-557 (2008).
12. K. Sivonen, T. Börner, in *The cyanobacteria*. (Caister Academic Press, 2008), pp. 159-197.
13. L. A. Pearson, B. A. Neilan, The molecular genetics of cyanobacterial toxicity as a basis for monitoring water quality and public health risk. *Current Opinion in Biotechnology* **19**, 281-288 (2008).
14. V. Ostermaier, R. Kurmayer, Application of real-time PCR to estimate toxin production by the cyanobacterium Planktothrix sp. *Applied and environmental microbiology* **76**, 3495-3502 (2010).
15. L. J. Beversdorf, T. R. Miller, K. D. McMahon, Long-term monitoring reveals carbon–nitrogen metabolism key to microcystin production in eutrophic lakes. *Frontiers in microbiology* **6**, 456 (2015).
16. K. Sivonen, G. Jones, Cyanobacterial toxins. *Toxic cyanobacteria in water: a guide to their public health consequences, monitoring and management* **1**, 43-112 (1999).
17. J. Vaitomaa *et al.*, Quantitative Real-Time PCR for Determination of Microcystin Synthetase E Copy Numbers for Microcystis and Anabaena in Lakes. *Applied and environmental microbiology* **69**, 7289-7297 (2003).
18. A. Martins, V. Vasconcelos, Use of qPCR for the study of hepatotoxic cyanobacteria population dynamics. *Arch Microbiol* **193**, 615-627 (2011).
19. M. Yoshida, T. Yoshida, Y. Takashima, N. Hosoda, S. Hiroishi, Dynamics of microcystin-producing and non-microcystin-producing Microcystis populations is correlated with nitrate concentration in a Japanese lake. *FEMS microbiology letters* **266**, 49-53 (2007).
20. M. Yoshida *et al.*, Ecological dynamics of the toxic bloom-forming cyanobacterium Microcystis aeruginosa and its cyanophages in freshwater. *Applied and environmental microbiology* **74**, 3269-3273 (2008).
21. J. H. Ha, T. Hidaka, H. Tsuno, Quantification of toxic Microcystis and evaluation of its dominance ratio in blooms using real-time PCR. *Environmental science & technology* **43**, 812-818 (2009).
22. J. M. Rinta-Kanto *et al.*, Lake Erie Microcystis: relationship between microcystin production, dynamics of genotypes and environmental parameters in a large lake. *Harmful algae* **8**, 665-673 (2009).
23. R. Amer, B. Díez, R. El-Shehawy, Diversity of hepatotoxic cyanobacteria in the Nile Delta, Egypt. *Journal of environmental monitoring* **11**, 126-133 (2009).
24. W. E. A. Kardinaal, P. M. Visser, Dynamics of cyanobacterial toxins. *Harmful Cyanobacteria. Aquatic ecology series*, 41-63 (2005).
25. E. M. Gross, C. Feldbaum, A. Graf, Epiphyte biomass and elemental composition on submersed macrophytes in shallow eutrophic lakes. *Hydrobiologia* **506**, 559-565 (2003).
26. C. Legrand, K. Rengefors, G. O. Fistarol, E. Graneli, Allelopathy in phytoplankton-biochemical, ecological and evolutionary aspects. *Phycologia* **42**, 406-419 (2003).
27. N. Botnariuc, A. Vădineanu, *Ecologie*. (Editura Didactică şi Pedagogică, 1982).
28. C. Darwin, The origin of species and The descent of man, New York (The Modern Library). (1859).
29. B. A. Whitton, M. Potts, in *Ecology of Cyanobacteria II*. (Springer, 2012), pp. 1-13.
30. J. Huisman, F. D. Hulot, in *Harmful cyanobacteria*. (Springer, 2005), pp. 143-176.
31. S. Visser, L. Stroosnijder, W. Chardon, Nutrient losses by wind and water, measurements and modelling. *Catena* **63**, 1-22 (2005).
32. K. Muylaert *et al.*, Relationship between bacterial community composition and bottom-up versus top-down variables in four eutrophic shallow lakes. *Applied and environmental microbiology* **68**, 4740-4750 (2002).
33. A. Holland, S. Kinnear, Interpreting the possible ecological role(s) of cyanotoxins: compounds for competitive advantage and/or physiological aide? *Marine drugs* **11**, 2239-2258 (2013).
34. L. Tonk *et al.*, The microcystin composition of the cyanobacterium Planktothrix agardhii changes toward a more toxic variant with increasing light intensity. *Applied and environmental microbiology* **71**, 5177-5181 (2005).
35. A. Sukenik, A. Quesada, N. Salmaso, Global expansion of toxic and non-toxic cyanobacteria: effect on ecosystem functioning. *Biodiversity and Conservation* **24**, 889-908 (2015).

36. M. Tao *et al.*, Use of a Generalized Additive Model to Investigate Key Abiotic Factors Affecting Microcystin Cellular Quotas in Heavy Bloom Areas of Lake Taihu. *PLoS One* **7**, 10 (2012).
37. L. R. d. Carvalho *et al.*, A toxic cyanobacterial bloom in an urban coastal lake, Rio Grande do Sul State, Southern Brazil. *Brazilian Journal of Microbiology* **39**, 761-769 (2008).
38. B. W. Ibelings *et al.*, Chytrid infections and diatom spring blooms: paradoxical effects of climate warming on fungal epidemics in lakes. *Freshwater Biology* **56**, 754-766 (2011).
39. Q. Xue, A. D. Steinman, X. Su, Y. Zhao, L. Xie, Temporal dynamics of microcystins in Limnodrilus hoffmeisteri, a dominant oligochaete of hypereutrophic Lake Taihu, China. *Environmental Pollution* **213**, 585-593 (2016).
40. P. Oberholster, A.-M. Botha, J. Grobbelaar, Microcystis aeruginosa: source of toxic microcystins in drinking water. *African Journal of Biotechnology* **3**, 10 (2004).
41. T. A. Paes, I. A. Costa, A. P. Silva, E. M. Eskinazi-Sant'Anna, Can microcystins affect zooplankton structure community in tropical eutrophic reservoirs? *Braz J Biol*, (2016).
42. B. Ernst, S. J. Hoeger, E. O'Brien, D. R. Dietrich, Abundance and toxicity of Planktothrix rubescens in the pre-alpine Lake Ammersee, Germany. *Harmful Algae* **8**, 14 (2009).
43. R. Adrian, N. Walz, T. Hintze, S. Hoeg, R. Rusche, Effects of ice duration on plankton succession during spring in a shallow polymictic lake. *Freshwater Biology* **41**, 621-634 (1999).
44. Ê. W. Dantas, A. N. Moura, M. d. C. Bittencourt-Oliveira, Cyanobacterial blooms in stratified and destratified eutrophic reservoirs in semi-arid region of Brazil. *Anais da Academia Brasileira de Ciências* **83**, 1327-1338 (2011).
45. J. Engström-Öst, S. Repka, M. Mikkonen, Interactions between plankton and cyanobacterium Anabaena with focus on salinity, growth and toxin production. *Harmful Algae* **10**, 530-535 (2011).
46. C. Martínez-Espinosa *et al.*, Denitrification in wetlands: A review towards a quantification at global scale. *Science of The Total Environment* **754**, 142398 (2021).
47. J. N. Galloway *et al.*, The nitrogen cascade. *Bioscience* **53**, 341-356 (2003).
48. H. Ma *et al.*, Growth inhibitory effect of Microcystis on Aphanizomenon flos-aquae isolated from cyanobacteria bloom in Lake Dianchi, China. *Harmful Algae* **42**, 43-51 (2015).
49. T. W. Davis *et al.*, Effects of nitrogenous compounds and phosphorus on the growth of toxic and non-toxic strains of Microcystis during cyanobacterial blooms. *Aquatic Microbial Ecology* **61**, 149-162 (2010).
50. C. Vézie, J. Rapala, J. Vaitomaa, J. Seitsonen, K. Sivonen, Effect of nitrogen and phosphorus on growth of toxic and nontoxic Microcystis strains and on intracellular microcystin concentrations. *Microbial ecology* **43**, 443-454 (2002).
51. M. E. Monchamp, F. R. Pick, B. E. Beisner, R. Maranger, Nitrogen forms influence microcystin concentration and composition via changes in cyanobacterial community structure. *PloS one* **9**, e85573 (2014).
52. L. Cao *et al.*, Biodiversity and water quality variations in constructed wetland of Yongding River system. *Acta Ecologica Sinica* **27**, 3670-3677 (2007).
53. C. Vezie, J. Rapala, J. Vaitomaa, J. Seitsonen, K. Sivonen, Effect of nitrogen and phosphorus on growth of toxic and nontoxic Microcystis strains and on intracellular microcystin concentrations. *Microb Ecol* **43**, 443-454 (2002).
54. A. Rantala *et al.*, Detection of Microcystin-Producing Cyanobacteria in Finnish Lakes with Genus-Specific Microcystin Synthetase Gene E (mcyE) PCR and Associations with Environmental Factors. *Applied and Environmental Microbiology* **72**, 10 (2006).
55. H. Paerl, in *Cyanobacterial harmful algal blooms: State of the science and research needs*. (Springer, 2008), pp. 217-237.
56. A. J. Posselt, M. A. Burford, G. Shaw, PULSES OF PHOSPHATE PROMOTE DOMINANCE OF THE TOXIC CYANOPHYTE CYLINDROSPERMOPSIS RACIBORSKII IN A SUBTROPICAL WATER RESERVOIR 1. *Journal of Phycology* **45**, 540-546 (2009).
57. A. M. Dolman *et al.*, Cyanobacteria and cyanotoxins: the influence of nitrogen versus phosphorus. *PloS one* **7**, e38757-e38757 (2012).
58. N. Kamjunke, T. Henrichs, U. Gaedke, Phosphorus gain by bacterivory promotes the mixotrophic flagellate Dinobryon spp. during re-oligotrophication. *Journal of Plankton Research* **29**, 39-46 (2006).
59. G. Yu *et al.*, Variation of Microcystis and microcystins coupling nitrogen and phosphorus nutrients in Lake Erhai, a drinking-water source in Southwest Plateau, China. *Environ Sci Pollut Res Int* **21**, 9887-9898 (2014).
60. H. W. Paerl *et al.*, Controlling cyanobacterial blooms in hypertrophic Lake Taihu, China: will nitrogen reductions cause replacement of non-N 2 fixing by N 2 fixing taxa? *PloS one* **9**, e113123 (2014).
61. V. H. Smith, D. W. Schindler, Eutrophication science: where do we go from here? *Trends in ecology & evolution* **24**, 201-207 (2009).
62. Diane M. Orihel *et al.*, High microcystin concentrations occur only at low nitrogen-to-phosphorus ratios in nutrient-rich Canadian lakes. *Canadian Journal of Fisheries and Aquatic Sciences* **69**, 1457-1462 (2012).

63. E. Van Donk *et al.*, The effect of a mixotrophic chrysophyte on toxic and colony-forming cyanobacteria. *Freshwater Biology* **54**, 1843-1855 (2009).
64. A. F. Alva-Martínez, R. Fernández, S. S. S. Sarma, S. Nandini, Effect of mixed toxic diets (Microcystis and Chlorella) on the rotifers Brachionus calyciflorus and Brachionus havanaensis cultured alone and together. *Limnologica - Ecology and Management of Inland Waters* **39**, 302-305 (2009).
65. C. Gobler, T. Davis, K. Coyne, G. Boyer, Interactive influences of nutrient loading, zooplankton grazing, and microcystin synthetase gene expression on cyanobacterial bloom dynamics in a eutrophic New York lake. *Harmful Algae* **6**, 119-133 (2007).
66. T. G. Downing, C. Sember, M. M. Gehringer, W. Leukes, Medium N: P ratios and specific growth rate comodulatemicrocystin and protein content in Microcystis aeruginosa PCC7806 and M. aeruginosa UV027. *Microbial ecology* **49**, 468-473 (2005).
67. C.-Y. Ahn, A.-S. Chung, H.-M. Oh, Rainfall, phycocyanin, and N: P ratios related to cyanobacterial blooms in a Korean large reservoir. *Hydrobiologia* **474**, 117-124 (2002).
68. A. D. Asencio, Determination of microcystins in reservoirs of different basins in a semiarid area. *Journal of Applied Phycology* **25**, 10 (2013).
69. X. Chen *et al.*, Fates of Microcystis aeruginosa cells and associated microcystins in sediment and the effect of coagulation process on them. *Toxins (Basel)* **6**, 152-167 (2014).
70. H. Song, L. X. Coggins, E. S. Reichwaldt, A. Ghadouani, The importance of lake sediments as a pathway for microcystin dynamics in shallow eutrophic lakes. *Toxins (Basel)* **7**, 900-918 (2015).
71. J. F. Haney, Field studies on zooplankton-cyanobacteria interactions. *New Zealand journal of marine and freshwater research* **21**, 467-475 (1987).
72. W. Lampert, Laboratory studies on zooplankton-cyanobacteria interactions. *New Zealand journal of marine and freshwater research* **21**, 483-490 (1987).
73. S. Zhao, Y. Wang, D. Li, Effects of toxic and non-toxicMicrocystis aeruginosain different mixtures withScenedesmus obliquuson growth ofBrachionus calyciflorus. *Journal of Freshwater Ecology* **29**, 377-386 (2014).
74. M. C. S. Soares, M. Lurling, V. L. M. Huszar, Responses of the rotifer Brachionus calyciflorus to two tropical toxic cyanobacteria (Cylindrospermopsis raciborskii and Microcystis aeruginosa) in pure and mixed diets with green algae. *Journal of Plankton Research* **32**, 999-1008 (2010).
75. A. F. Alva-Martínez, S. S. S. Sarma, S. Nandini, Population dynamics of Brachionus calyciflorus and Brachionus havanaensis (Rotifera) on mixed diets with Microcystis aeruginosa and green algae. *Hidrobiológica* **7**, 10 (2007).
76. A. Pérez-Morales, S. S. Sarma, S. Nandini, Feeding and filtration rates of zooplankton (rotifers and clodocerans) fed toxic cyanobacterium (Microcystis aeruginosa). *Journal of Environmental Biology* **35**, 8 (2014).
77. M. Lürling, W. Beekman, Influence of food-type on the population growth rate of the rotifer Brachionus calyciflorus in short-chronic assays *Acta Ecologica Sinica* **51**, 9 (2006).
78. G. Ji, X. Wang, L. Wang, Planktonic rotifers in a subtropical shallow lake: succession, relationship to environmental factors, and use as bioindicators. *ScientificWorldJournal* **2013**, 702942 (2013).
79. X. Zhang, H. Geng, Effect of Microcystis aeruginosa on the rotifer Brachionus calyciflorus at different temperatures. *Bull Environ Contam Toxicol* **88**, 20-24 (2012).
80. J. J. Gilbert, Effect of Temperature on the Response of Planktonic Rotifers to a Toxic Cyanobacterium. *Ecology* **77**, 8 (1996).
81. L. Huang, Y. Xi, X. Xu, X. Wen, Responses in population growth and reproduction of the freshwater rotiferBrachionus calyciflorusto microcystin-LR at different temperatures. *Annales de Limnologie - International Journal of Limnology* **48**, 383-390 (2012).
82. V. Vasconcelos, Cyanobacteria toxins: diversity and ecological effects. *Limnetica* **20**, 14 (2001).
83. I. Sellami *et al.*, Abundance and biomass of rotifers in relation to the environmental factors in geothermal waters in Southern Tunisia. *Journal of Thermal Biology* **34**, 267-275 (2009).
84. A. Combes, M. Dellinger, S. Cadel-six, S. Amand, K. Comte, Ciliate Nassula sp. grazing on a microcystin-producing cyanobacterium (Planktothrix agardhii): impact on cell growth and in the microcystin fractions. *Aquat Toxicol* **126**, 435-441 (2013).
85. A. S. Ferrão-Filho, B. Kozlowsky-Suzuki, S. M. Azevedo, Accumulation of microcystins by a tropical zooplankton community. *Aquatic Toxicology* **59**, 8 (2002).
86. T. W. Davis, C. J. Gobler, Grazing by mesozooplankton and microzooplankton on toxic and non-toxic strains of Microcystis in the Transquaking River, a tributary of Chesapeake Bay. *Journal of Plankton Research* **33**, 415-430 (2010).
87. A. Pérez-Morales, S. S. S. Sarma, S. Nandini, Microcystins production in Microcystis induced by Daphnia pulex (Cladocera) and Brachionus calyciflorus (Rotifera). *Hidrobiológica* **25**, 6 (2015).

 doi:10.20944/preprints202105.0232.v1

88. F. Chen, P. Xie, B. Qin, Different competitive outcomes among four species of cladocerans under different alga combinations of colonial Microcystis spp. and green alga Scenedesmus obliquus. *Hydrobiologia* **581**, 209-215 (2007).
89. S. Nandini, Responses of rotifers and cladocerans to Microcystis aeruginosa (Cyanophyceae): A demographic study. *Aquatic Ecology* **34**, 16 (2000).
90. B. Drugă, P. Turko, P. Spaak, F. Pomati, Cyanobacteria Affect Fitness and Genetic Structure of Experimental Daphnia Populations. *Environmental science & technology* **50**, 3416-3424 (2016).
91. J. L. Smith, J. F. Haney, Foodweb transfer, accumulation, and depuration of microcystins, a cyanobacterial toxin, in pumpkinseed sunfish (Lepomis gibbosus). *Toxicon* **48**, 580-589 (2006).
92. C. Wiegand, S. Pflugmacher, Ecotoxicological effects of selected cyanobacterial secondary metabolites: a short review. *Toxicol Appl Pharmacol* **203**, 201-218 (2005).
93. S. Cristofor *et al.*, Long-term changes of submerged macrophytes in the Lower Danube Wetland System. *Hydrobiologia* **506**, 625-634 (2003).
94. E. M. Gross, S. Hilt, P. Lombardo, G. Mulderij, Searching for allelopathic effects of submerged macrophytes on phytoplankton—state of the art and open questions. *Shallow Lakes in a Changing World*, 77-88 (2007).
95. C. Rojo, M. Segura, F. Cortés, M. A. Rodrigo, Allelopathic effects of microcystin-LR on the germination, growth and metabolism of five charophyte species and a submerged angiosperm. *Aquatic toxicology* **144**, 1-10 (2013).
96. S. Pflugmacher, Promotion of oxidative stress in the aquatic macrophyte Ceratophyllum demersum during biotransformation of the cyanobacterial toxin microcystin-LR. *Aquatic toxicology* **70**, 169-178 (2004).
97. G. Zheng, R. Xu, X. Chang, S. Hilt, C. Wu, Cyanobacteria can allelopathically inhibit submerged macrophytes: effects of Microcystis aeruginosa extracts and exudates on Potamogeton malaianus. *Aquatic botany* **109**, 1-7 (2013).
98. X. Zhang, H. Y. Hu, Y. Hong, J. Yang, Isolation of a Poterioochromonas capable of feeding on Microcystis aeruginosa and degrading microcystin-LR. *FEMS Microbiol Lett* **288**, 241-246 (2008).
99. P. Snoeijs, L. W. Murasi, Symbiosis between diatoms and cyanobacterial colonies *VIE MILIEU* **54**, 8 (2004).
100. K. D. Kearns, M. D. Hunter, Green algal extracellular products regulate antialgal toxin production in a cyanobacterium. *Environmental Microbiology* **2**, 7 (2000).