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Development of tolerance against toxic *Microcystis aeruginosa* in three cladocerans and the ecological implications

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Three cladocerans pre-exposed to Microcystis developed different tolerance against toxic Microcystis, explaining zooplankton succession with blooms.

Abstract

This is the first experimental study to compare difference in the development of tolerance against toxic *Microcystis* among multi-species of cladocerans (*Daphnia*, *Moina* and *Ceriodaphnia*) pre-exposed to two *M. aeruginosa* PCC7820 strains (MC-containing and MC-free). Zooplankton were divided into S population (fed *Scenedesmus*), M-F population (fed *Scenedesmus* + MC-free *Microcystis*), and M-C population (fed *Scenedesmus* + MC-containing *Microcystis*). M-F and M-C populations were pre-exposed to *Microcystis* strains for 4 weeks, and their newborns were collected for experiments. A pre-exposure to MC-containing or MC-free *Microcystis* increased tolerance against toxic *Microcystis*. The marked increases in survival rate and median lethal time (LT_{50}, 100–194% increase) in the M-C population of *Ceriodaphnia* suggest that small-sized cladocerans may develop stronger tolerance against *Microcystis* than large-sized ones when both groups are exposed to toxic *Microcystis*. This may explain why dominant *Daphnia* is usually replaced by small-sized cladocerans when cyanobacteria bloomed in summer in eutrophic lakes.

Keywords: Cladoceran; *Microcystis aeruginosa* PCC7820; Microcystin; Tolerance development; Growth; Survival rate

1. Introduction

Eutrophication has resulted in a wide increase of cyanobacterial blooms (Shapiro, 1972; Paerl et al., 2001; Havens et al., 2003), often leading to changes in the structure of zooplankton communities (Nanazato and Yasuno, 1985; Trabeau et al., 2004). *Microcystis* is one of the most common and most studied bloom-forming cyanobacteria and a well-known producer of the hepatotoxic microcystins (Sivonen, 1999; Jungmann, 1992). Different cladocerans usually exhibited different responses to the toxicity of *Microcystis* (Lampert, 1981, 1982; Fulton and Paerl, 1987b; Hanazato and Yasuno, 1987; Bernardi and Giussani, 1990; Sartonov, 1995; Hietala et al., 1997; Versteeg et al., 1997; DeMott, 1999; Ferrão-Filho and Azevedo, 2003), and usually small-sized cladocerans exhibit higher tolerance to toxic *Microcystis* in field (Fulton and Paerl, 1987a; Jarvis et al., 1988; Ferrão-Filho and Azevedo, 2003). However, Fulton and Paerl (1987b) found similar tolerance to toxic *Microcystis* in different species of cladocerans, regardless of herbivore body size in laboratory experiments. It remains unknown why the field and laboratory experiments contradict each other. Hairston et al. (1999) suggest that a long-time exposure of *Daphnia* to cyanobacteria may enhance their resistance to the cyanobacteria. A detoxification mechanism for microcystin in aquatic organisms was also identified to explain the development of tolerance (Pflugmacher et al., 1998). Gustafsson et al. (2004) reported that *Daphnia magna* pre-exposed to toxic *Microcystis* have a development of tolerance to toxic *Microcystis*. Up to now, no studies have been conducted to compare differences

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in the development of such tolerance among muti-species of cladocerans pre-exposed to toxic *Microcystis*. The difference in the development of tolerance to cyanobacteria between large and small-sized cladocerans not only explain some of the contradictory results emerging from the field and laboratory experiments, but also help us know which genotype would be affected heavily by toxic *Microcystis* and virtually eliminated within a continued exposure to cyanobacteria.

Microcystin (MC) is always a main toxic factor of *M. aeruginosa*, while the MC-free strain of cyanobacteria is usually considered to be nutrient-deficient to zooplankton (DeMott and Müller-Navarra, 1997). However, the viewpoint of nutrient-deficient gives no explanation to some recent observations on the toxicity of MC-free strains (Rohrlack et al., 1999; Ferrão-Filho et al., 2000; Lürling, 2003). In field, MC-containing strains and MC-free strains usually coexist in the same lakes (Sivonen et al., 1990). MC-free cyanobacterial strain also has an important impact on zooplankton (Yasuno et al., 1998; Dittmann and Börner, 2005) and a comparison of the effects caused by pre-exposure to MC-containing and MC-free strains will help us to know the actual role of microcystins in the toxic test.

The main purpose of this study was to examine if there is a difference in the development of tolerance to toxic *Microcystis* among three species of cladocerans pre-exposed to toxic *Microcystis*. Both MC-containing and MC-free *Microcystis* strains were used, and each strain was added in different mixtures containing a palatable food species-the green alga *Scenedesmus obliquus*. The three cladoceran species were different in size.

2. Materials and methods

We used two strains of *Microcystis aeruginosa* PCC7820 in our study. One contained microcystin-LR, designated MC-containing strain, and the other was a mutant strain of *M. aeruginosa* PCC7820 without microcystins, designated MC-free strain. Both strains were cultured in the medium BG11. A food concentration of 10 mg/L (wet weight) mixed by *M. aeruginosa* and *Scenedesmus obliquus* was used in the experiment. Three cladoceran species were tested: *Daphnia carinata* (mean adult size 2.7 mm) was collected from Lake Chaohu, *Moina micrura* (mean adult size 1.0 mm) was collected from Lake Donghu and *Ceriodaphnia cornuta* (mean adult size 0.5 mm) was collected from Lake Chaohu. All animals and algae were cultured at 25 ± 0.5 °C and under a light/dark cycle of 14:10 h. The animals were cultured in the M7 medium (Samel et al., 1999) for 3 months prior to the experiment, and were fed *Scenedesmus obliquus* once every 2 days. The zooplankton populations were divided into three groups: S population (fed only *Scenedesmus*), M-F population (fed a mixture of *Scenedesmus* and MC-free *M. aeruginosa*), and M-C population (fed a mixture of *Scenedesmus* and MC-containing *M. aeruginosa*). During the pre-exposure, M-F and M-C populations were fed 10% *M. aeruginosa* for 1 week, then changed to 20% *M. aeruginosa* food for the next 2 weeks, and were finally transferred to 40% *M. aeruginosa* food in the 4th week. After the 4 weeks' exposure, parent cladocerans with eggs were collected and placed in glass jars with *Scenedesmus* as food. The newborns were collected and

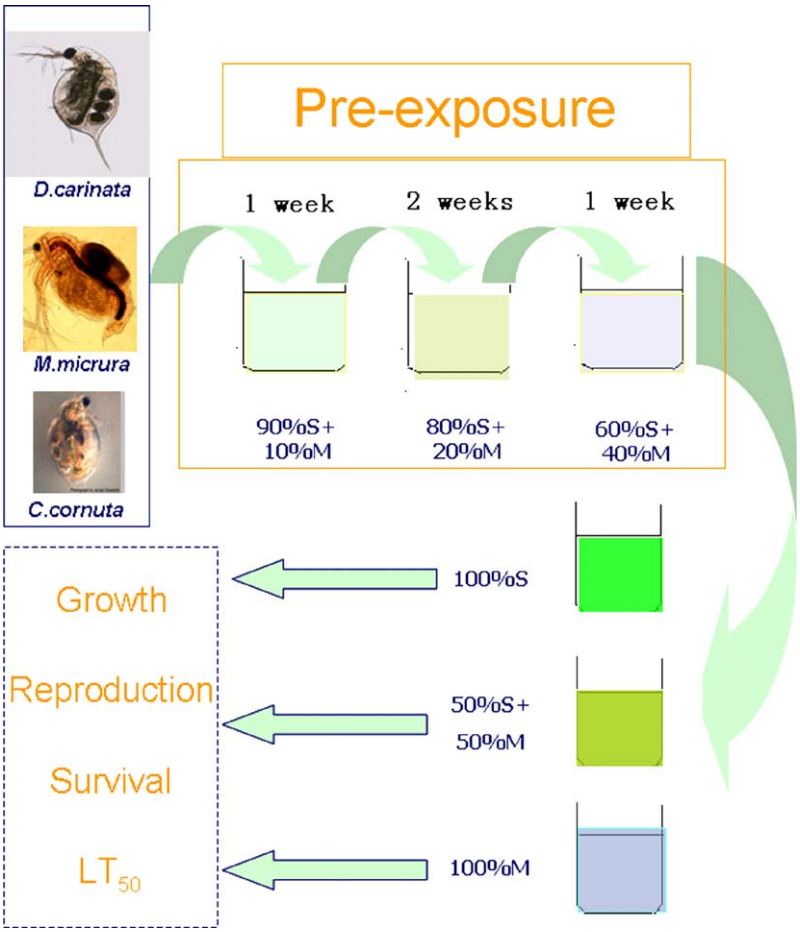

Fig. 1. The schematic diagram of the experiment.

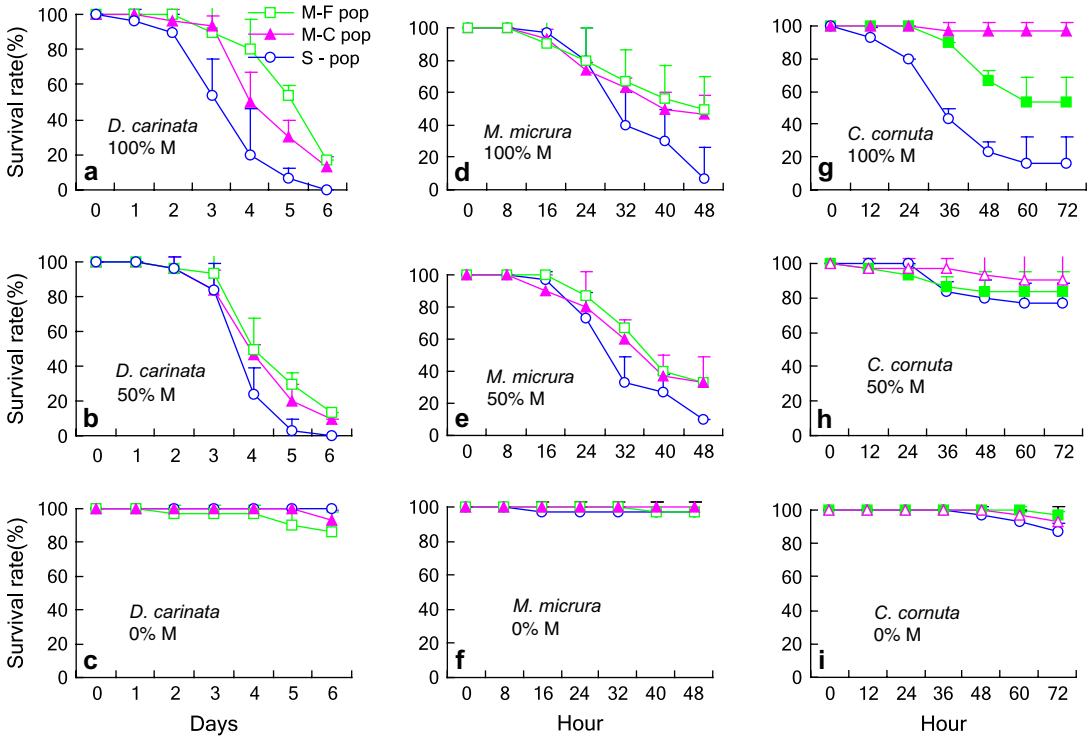

Fig. 2. The survival rates of the S, M-F and M-C populations of *Daphnia* (a–c), *Moina* (d–f), and *Ceriodaphnia* (g–i) in the 100%, 50%, and 0% *M. aeruginosa* treatments. Bars show the standard deviation of the mean.

placed in 200-ml vessels with three different concentrations (0%, 50% and 100%) of MC-containing *M. aeruginosa*. The survival animals and the number of eggs in the brood chamber were counted, and the length from top of the head to the end of the carapace was measured under a microscope. The schematic diagram of the experiment is shown in Fig. 1.

The microcystins in the MC-containing *M. aeruginosa* PCC7820 were extracted and analyzed using high performance liquid chromatography (HPLC) following the method of Zheng et al. (2004). The content of microcystin-LR per unit biovolume was 1.34×10^{-10} μg μm^{-3}, and no MC was detected in the MC-free strain. Variations in survival rate and body length of cladocerans were analyzed by ANOVA with repeated measures, including data of all time points.

3. Results

The survival rate of *Daphnia* and *Moina* reduced severely with 100% and 50% toxic *M. aeruginosa* as food. The M-C and M-F populations showed higher survival rate than the S population, but no significant difference was observed between the M-C and M-F populations (Fig. 2).

In the 100% and 50% *M. aeruginosa* treatments, few *Daphnia* and *Moina* survived, and no eggs were produced in the three populations throughout the experiment. On the contrary, all animals grew well with high survival rate in the 100% *Scenedesmus*, and body length and number of eggs was similar among the three populations.

Different responses were observed in the *C. cornuta* populations exposed to different proportions of toxic *M. aeruginosa*. In the 100% toxic *M. aeruginosa* treatment, the M-C, and M-F populations showed higher survival rate than the S population ($F = 30.14$, $p < 0.01$), while a significant difference was also detected between the M-F and M-C populations. In the 100% *Scenedesmus* treatment, body length was different between the M-C and M-F populations; compared with the S population, both M-C and M-F populations were shorter in body length ($F = 30.14$, $p < 0.01$); and body length was not significantly different between the M-C and M-F populations at the

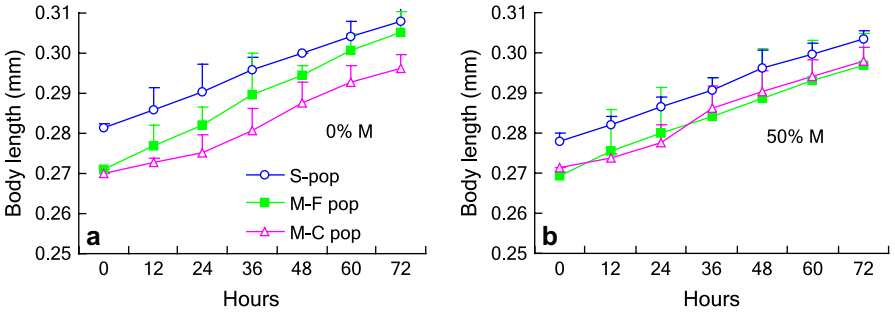

Fig. 3. The body length of *C. cornuta* in the 0% (a) and 50% (b) *Microcystis* treatments. Bars show standard deviation.

Table 1
Results of the repeated-measures ANOVA showing the effects of *Microcystis* addition (0, 50%) and populations (S, M-F and M-C pop) on the body length of *Ceriodaphnia*

Source	df	SS	*F*	*p*
Microcystis	1	1.3	1.3	0.260
Populations	2	18.4	9.1	<0.001
M × P	2	20.3	10.1	<0.001

Data of all time points were included.

beginning of the experiment (*t*-test, $p > 0.05$), but the M-F population had longer body length than the M-C population at the end of the experiment (*t*-test, $p < 0.01$). In the 50% *Scenedesmus* and *M. aeruginosa* treatments, there was no significant difference in survival rate among the three populations. The body length was also similar throughout the experiment between the M-C and M-F populations (Fig. 3). Repeated-measures ANOVA analysis shows that the body length of *Ceriodaphnia* was significantly affected by populations (S-, M-C- and M-F-pop) and the combination of populations and MC-containing *Microcystis* addition (Table 1).

In the 100% *M. aeruginosa* treatments, no eggs were produced in the three populations of *C. cornuta* throughout the experiment, whereas in the 50% *M. aeruginosa* treatments, *C. cornuta* began to produce eggs on the 6th day, but the mean sizes of their first broods were only 0.94 (0.33–1.33) per egg-bearing female for all the three populations.

LT_{50} (median lethal time) values of all cladoceran populations are shown in Table 2. Obviously, all cladoceran populations that were pre-exposed to *M. aeruginosa* had a higher LT_{50} than the S population: 49–69% increase for *Daphnia*, 35–52% increase for *Moina*, and as high as 100–194% for *Ceriodaphnia*.

4. Discussion

Exposure to MC-containing *M. aeruginosa* negatively influenced the survival, growth and reproduction of all zooplankton populations in our study. M-C and M-F populations of the three cladocerans showed higher survivorship than the S population in both 100% and 50% toxic *M. aeruginosa* treatments. The results indicate that pre-exposure to toxic *M. aeruginosa* could induce tolerance development of cladocerans against toxic *Microcystis*, which is consistent with the earlier study on *Daphnia magna* (Gustafsson and Hansson, 2004). The marked increases in survivorship and LT_{50} in the M-C population of *Ceriodaphnia* suggest that small-sized cladocerans may develop stronger tolerance against toxic *M. aeruginosa* than large-sized ones when both groups are exposed to toxic *Microcystis*. This may give us an explanation as to why no difference in sensitivity to toxic *M. aeruginosa* was detected between small and large body-sized cladocerans in laboratory experiments (Fulton and Paerl, 1987b), and why a senescence in *Daphnia* (Hülsmann and Weiler, 2000) and prosperity of small-sized cladocerans that may have a greater ability to utilize *Microcystis* as nutritional resource or a greater tolerance to the toxin succeed during *Microcystis* blooms in the field (Fulton and Paerl, 1987a). The greater development of tolerance to microcystins in small-sized cladocerans than in large-sized ones may be one of the important mechanisms to explain why the dominance of *Daphnia* is usually replaced by small-sized cladocerans following the occurrence of cyanobacterial blooms in summer in many eutrophic lakes (Hairston et al., 1999; Hülsmann and Weiler, 2000).

Table 2
Median lethal time (LT_{50}, h) of three cladoceran populations in 100% MC-containing *M. aeruginosa*

Species	S population	M-F population	M-C population
D. carinata	68 (53.9–86.1)	115 (98.2–135.6)	101 (84.4–119.7)
M. micrura	31 (25.9–36.5)	42 (29.1–60.0)	47 (30.9–72.4)
C. cornuta	34 (24.3–47.4)	68 (50.1–90.2)	>>100

Values in parentheses are 95% confidence limits.

In the present study, it remains unclear why differences in survival rates between the M-C and M-F populations were not significant for *Daphnia* and *Moina* but significant for *Ceriodaphnia*. In our study, a pre-exposure to *M. aeruginosa* induced slow growth and small individuals in *Ceriodaphnia,* which is consistent with the earlier studies in *Daphnia* (Lürling and Donk, 1997; Gustafsson and Hansson, 2004). The present study indicates that the M-F population of *Ceriodaphnia* showed an obvious recovery in body length in the 100% *Scenedesmus* treatment. Rohrlack et al. (2001) showed that MC-free strain produced a rich source of bioactive compounds instead of microcystins with weaker toxic effects on cladocerans. The present results indicate that perhaps similar compounds also enhanced the survival rate of cladocerans to toxic *M. aeruginosa,* while the toxic effects caused by these compounds on the growth of cladocerans disappeared readily when fed green algae.

The decline of the large-sized *Daphnia* population and their replacement by small-sized cladocerans in eutrophic lakes is usually attributed to predation by planktivorous fish (DeMott et al., 2001), while recent studies indicate that natural selection in eutrophic lakes also plays an important role in the change of the zooplankton community (Hairston et al., 1999). Eutrophication usually causes an increase in the abundance of nutritionally poor or even toxic cyanobacteria, and the cyanobacterial blooms often favor small-sized cladocerans and copepods at the expense of large-sized cladocerans (Fulton and Paerl, 1988; Gliwicz, 1990). Trabeau et al. (2004) report that the capsules of cyanobacteria have a greater impact on large-sized *Daphnia* than on small-sized cladocerans. Rohrlack et al. (2001) suggest that microcystins produced by *Microcystis* are important factors to change the cladoceran community in eutrophic lakes. It is interesting to speculate from our experiments that divergence (probably size-dependent) in tolerance development among cladocerans exposed to toxic cyanobacteria may result in significant changes of zooplankton community in eutrophic lakes. This conclusion is convincing for the following three reasons. First, *Microcystis* used in our experiments is a worldwide cyanobacterial species and a main producer of microcystin in eutrophic lakes, unlike some cyanobacterial

species with filament or capsule in special eutrophic lakes (DeMott et al., 2001; Trabeau et al., 2004). Second, as cyanobacterial species without microcystin usually comprise more than 50% of total cyanobacterial species of a cyanobacterial bloom (Sivonen et al., 1990), the MC-free cyanobacterial strain used in our experiments was also proved to cause an increase in the resistance of cladocerans. Third, a long-term exposure to cyanobacteria is a basic characteristic of natural cyanobacterial blooms (Carmichael et al., 1988; Sivonen et al., 1990), and genetic changes (on the scale of years) responding to natural selection in zooplankton have been proved to significantly affect the course of ecosystem change in a German lake (Hairston et al., 1999).

It should be noted, however, that the response shown in this study is an induced response in systems subject to period blooms of toxic cyanobacteria, and the strength and effectiveness of the detoxification mechanism might be affected by natural selection. Nevertheless, the present study indicates that it will be important to test for an induced detoxification mechanism when studying interactions between zooplankton and toxic cyanobacteria. Further experimental studies are needed to confirm if there is a strong and consistent size-dependent effect on the development of tolerance to toxic cyanobacteria by using a large number of zooplankton taxa with comparisons within genera.

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