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INCREASED CONSUMER FITNESS FOLLOWING TRANSFER OF TOXIN TOLERANCE TO OFFSPRING VIA MATERNAL EFFECTS

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Abstract. Adaptations and counteradaptations are common in coevolving predator–prey systems, but little is known of the role of maternal transfer of adaptive traits in mediating species interactions. Here, we focused on tolerance against cyanobacterial toxins and asked whether this tolerance was an induced defense developed during *Daphnia*'s lifetime, whether it was a trait that is constantly expressed, and whether such tolerance to the toxin can be transferred to the next generation through maternal effects. These questions were addressed by feeding a single clone of *Daphnia magna* a diet with and without algal toxin and recording changes in fitness (as intrinsic rate of population increase). Analysis of F1, F2, and F3 generations revealed that the increased tolerance to toxic *Microcystis* was an inducible defense developed during an individual's lifetime, and that this trait could be transferred from mother to offspring. This maternal effect was expressed in several fitness parameters, including shorter time to maturity and first reproduction, and higher numbers of offspring compared to inexperienced individuals. In some circumstances, such maternal effects may increase population production by up to 40% and may help to stabilize material and energy transfer to higher trophic levels.

Key words: *adaptation; algal toxin;* Daphnia magna; *cyanobacteria; inducible enzyme defense; maternal effects; microcystin;* Microcystis aeruginosa; *predator–prey coevolution; toxin tolerance.*

REPORTS

INTRODUCTION

Dense blooms of cyanobacteria in lakes and coastal waters have become increasingly frequent and widespread during the last century (Hallegraeff 1993). In addition to diminishing the quality of water resources for human use, cyanobacteria also may have considerable impact on zooplankton and, through trophic interactions, the whole ecosystem (Reynolds 1994). Cyanobacteria have several characteristics that make them insufficient as a food source for zooplankton. For example, grazing-resistant forms, such as filaments and colonies, may mechanically interfere with feeding (Porter and Orcutt 1980), while their low content of fatty acids can suppress growth rates of zooplankton (Müller-Navarra et al. 2000). Moreover, their ability to produce toxins (Carmichael 1994) can constrain zooplankton in different ways, from reduced filtering rates to severe intoxication and rapid death (Christoffersen 1996).

Cyanobacteria are able to produce a wide range of toxic secondary metabolites as well as other harmful compounds (Sivonen and Jones 1999). The most common and widely studied toxin is microcystin, which is a hepatotoxin that inhibits the phosphatase 1 and 2A activities involved in diverse biological processes (MacKintosh et al. 1990). Many aquatic organisms including fish, macrophytes, and zooplankton are negatively affected by microcystins (Christoffersen 1996). For example, by comparing clones of the common lentic herbivore *Daphnia* established from diapausing eggs from sediment layers deposited during the different phases of eutrophication in Lake Constance, Hairston et al. (1999) showed an increase of resistance in *Daphnia galeata* to *Microcystis aeruginosa* during the eutrophication process.

Exposure to microcystin during cyanobacterial blooms may select against individual *Daphnia* that lack tolerance to toxic *Microcystis* (Gustafsson and Hansson 2004). The remaining part of the population will therefore have higher tolerance to microcystin, possibly because of the presence of one or more detoxifying enzymes (Pflugmacher et al. 1998). Any gene responsible for the synthesis of such a potential detoxifying enzyme either could be constantly expressed, meaning that the enzyme is synthesized regardless of toxin presence, or could be induced in response to a specific activator (Beattie et al. 2003). Such an inducible enzyme system could allow for a female to switch on a toxin-defense mechanism, which is then passed on to the offspring via cytoplasmic factors such as enzymes (Mousseau and Fox 1998). If these offspring then face the same environmental conditions as their mothers, they will be better prepared than progeny from mothers lacking cytoplasmic factors or genes coding for the putative detoxifying enzyme. This phenomenon of passing on information about the environment from females to the offspring is known as maternal effects (Mousseau and Fox 1998), and has been demonstrated for diverse taxa such as insects (Mousseau and Dingle 1991), lizards

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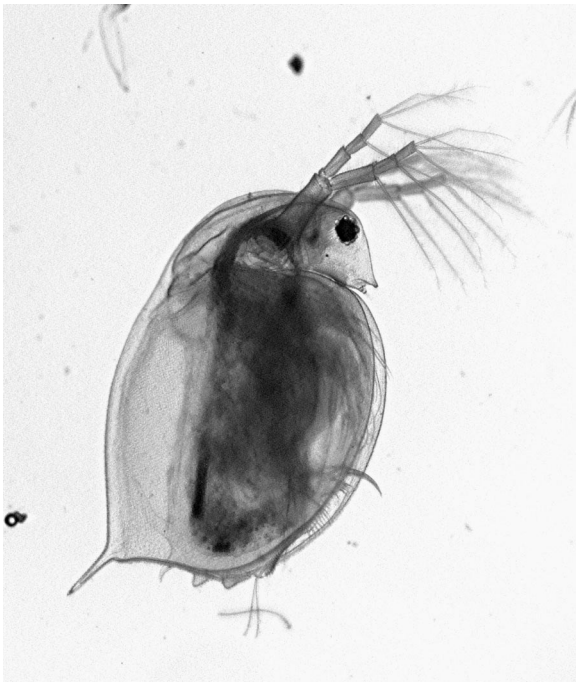

PLATE 1. *Daphnia magna*. Photo credit: Gertrud Cronberg.

(Uller 2004), and crustaceans. *Daphnia* is a genus in which maternal effects are well known, including predator-induced morphological defenses (Tollrian 1995, Agrawal et al. 1999) and the alternation between asexual and sexual reproduction (LaMontage and McCauley 2001).

The purpose of our experiment was to determine whether increased microcystin tolerance in *Daphnia magna* is an induced defense developed during an individual *Daphnia*'s lifetime, or whether it is a trait that is constantly expressed. Furthermore, we tested whether the tolerance to the toxin can be transferred to the next generation through maternal effects. This study was conducted using a clone of *Daphnia magna* developed from a single female and followed over three generations (see Plate. 1). We hypothesized that, in the case of an inducible defense, an improved tolerance to microcystin would be observed within an individual's lifetime. Furthermore, we hypothesized that we would observe an improved fitness of the offspring due to maternal effects; that is, offspring of a mother exposed to toxins would have higher fitness than offspring of mothers with no experience of the toxins. On the other hand, if the clone lacked a detoxifying mechanism, exposure to microcystin would reduce the fecundity of the exposed individuals during their lifetime. Likewise, if there was no maternal effect, the following generations would have equal or lower fitness than their predecessors.

MATERIAL AND METHODS

Daphnia magna were collected from a pond in Lund, southern Sweden, which had exhibited no toxic cyanobacterial blooms during the last five years. We chose *Daphnia* from this population to assure that any potential detoxifying mechanism was not activated. Animals were kept in a walk-in incubator at 20°C with a light:dark cycle of 12:12 in 50-L aquaria filled with a suspension of tap water and the green alga *Scenedesmus obliquus* as the food source. Prior to the start of the experiment, the daphnids were grown for five months, and at least seven generations, in the laboratory under cyanobacteria-free conditions to ensure that any possible prior exposure to microcystin should not affect the experiments. The clone used in the experiment was established from a single parthenogenetic female. Two strains of *Microcystis aeruginosa* were used in the experiment: one that produced microcystin (NIVA-CYA 228/1) and one that lacked the toxin (NIVA-CYA 143). Both algae were provided by the Norwegian Institute for Water Research (NIVA) culture collection. The green alga *Scenedesmus obliquus* (NIVA) was used as a nontoxic food source. Both *M. aeruginosa* strains grow as single or paired cells and were chosen to make sure that any mechanical interference could not independently influence *Daphnia* fitness. The algae were cultured in Z8 medium (Ahlgren 1977) at 20°C with a light:dark cycle of 12:12 and a light intensity of 15–20 μmol quanta·m^{-2}·s^{-1}.

Life history tables were constructed for F1, F2, and F3 generations of *Daphnia magna*, to study the effect of microcystin on *Daphnia* fitness. The control treatment consisted of 5% nontoxic *Microcystis aeruginosa* NIVA-CYA 143 and 95% *Scenedesmus obliquus*, whereas the experimental treatment consisted of 5% toxin-producing *M. aeruginosa* NIVA-CYA 228/1 and 95% *S. obliquus*, hereafter referred to as control (C) and microcystin (M) treatments, respectively. A non-microcystin-producing strain of *M. aerugionsa* was chosen for the C treatment to account for possible differences in food quality among treatments associated with the presence of *M. aeruginosa*. For both C and M treatments, the algal concentrations corresponded to 1 mg C/L, which was equivalent to 60 000 cells/mL of *Scenedesmus obliquus* and 140 000 cells/mL of either *M. aeruginosa* strain. Animals were provided twice this concentration every second day, resulting in a mean food concentration of 1 mg C·L^{-1}·d^{-1}. Algal cells were enumerated in a 110-μL Palmer-Maloney sedimentation chamber (Wildlife Supply, Buffalo, New York, USA) using an inverted Nikon microscope at 400× magnification.

For C and M treatments, each of 10 offspring (<24 h old) from one parthenogenetic female were transferred to individual 60-mL beakers and were placed in a walk-in incubator at 20°C with a light:dark cycle of 16:8. Every second day, the animals were checked for

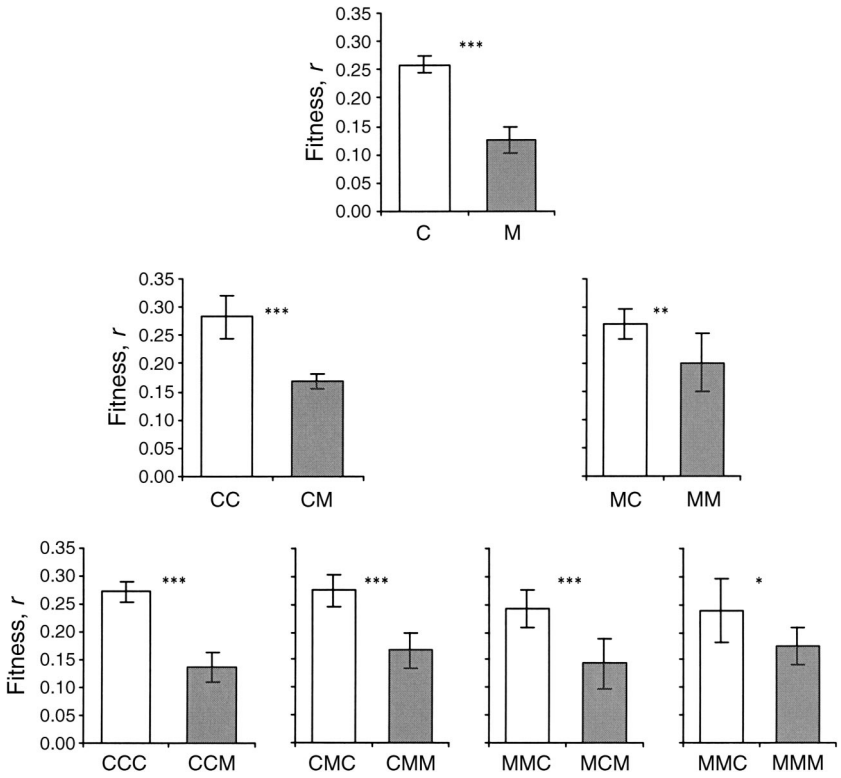

FIG. 1. Fitness values (means ± SD), estimated as intrinsic rate of increase (*r*), for *Daphnia magna* in control (C; open bars) and microcystin (M; gray bars) treatments over three generations F1, F2, and F3 (upper to lower panels). Asterisks indicate significant differences by ANOVA: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

presence of eggs and were transferred to fresh medium with their respective food type and density. At each sampling date, *Daphnia* were scored for their age when first and second clutch of eggs appeared in the egg chamber, age when the first and second clutches were delivered, and number, length, and sex of the offspring. Maturation in *Daphnia* was defined as the age at which the first clutch of eggs was visible in the brood chamber. Remaining offspring in the clutches were preserved in Lugol's solution, the sex was determined, and the length was measured from the eye to the end of the carapace. The biomass was calculated according to Bottrell et al. (1976).

The F2 generation was obtained from the second brood from F1 animals. The second brood was used because the offspring in the first brood of *Daphnia* are generally smaller (Lampert and Trubetskova 1996) and have a higher mortality than the following broods (S. Gustafsson, *unpublished data*). Newly hatched offspring (12–36 h old) from control treatments were transferred to control and microcystin treatments in the F2 generation (labeled CC and CM, respectively). Similarly, offspring from the microcystin treatment were transferred to C and M treatments in the F2 generation (MC and MM, respectively), resulting in four treatments (Fig. 1). The same procedure was repeated for the F3 generation when the F2 *Daphnia* delivered their second brood. By this arrangement we could follow the clonal offspring for three generations and study the outcome from different combinations of control and microcystin treatments. The phenotypic plasticity results were based on results from the F1 generation. Unfortunately, when the animals in the third generation reached maturity, an epibiontic ciliate, *Vorticella*, infected the experiment and some replicates of the F3 generation had to be excluded from the analysis; therefore, only the first two F3 broods were used in calculations and interpretation of results.

In this study we used the intrinsic rate of population increase, *r*, as a measurement of fitness. The Euler equation (Stearns 1992) was used to calculate *r*:

$$1 = \sum e^{-rx} l_x m_x.$$

Here x is age (in days), l_x is the probability of surviving to age x, and m_x is fecundity at age x. Mortality due to treatment was 0 in the experiment; thus l_x was set to 1 for all animals. We calculated *r* for the first two broods. In *Daphnia*, the production of eggs starts two instars before they are deposited in the brood chamber (Zaffagnini 1987), and is affected by both food quality and abundance (Ebert 1992), and possibly also by microcystin.

Microcystin concentrations were quantified by taking a 1-mL sample from the C and M algal suspensions

TABLE 1. Fitness dimensions (mean ± SD) of *Daphnia magna* in the control (C) and microcystin (M) treatments, with Mann-Whitney *U* test (*Z* statistics).

Fitness dimensions	Unit	Microcystin	Control	*Z*	*P*
No. offspring, clutch 1	no.	4.7 ± 2.4	10 ± 4.1	−7.1	<0.001
No. offspring, clutch 2	no.	8.1 ± 3.9	17 ± 5.0	−8.0	<0.001
Ind. biomass of offspring, clutch 1	μg	4.6 ± 2.2	4.5 ± 1.8	−0.3	0.775
Ind. biomass of offspring, clutch 2	μg	5.2 ± 2.0	6.3 ± 2.6	−3.4	0.001
Age at maturity	d	9.6 ± 1.9	6.8 ± 1.0	−8.2	<0.001
Age when clutch 1 was born	d	12 ± 2.6	9.5 ± 1.4	−7.9	<0.001
Age when clutch 2 was born	d	16 ± 2.6	12 ± 1.7	−7.9	<0.001

Notes: Life history parameter values are calculated for M and C treatments from the three generations, F1, F2, and F3. ''Ind.'' is individual *Daphnia magna*.

that were given to the experimental animals every second day. These samples were freeze–thawed three times and sonicated to release the cell-bound microcystins. The suspensions were centrifuged and the supernatant was analyzed for concentration of microcystins with the Enzyme-Linked-Immuno-Sorbent Assay (ELISA; Chu et al. 1989) using a Hyperion Microreader 3 (Hyperion, Miami, Florida, USA). Commercial analysis kits (EnvironGard, Strategic Diagnostics, Newark, Delaware, USA) were used to quantify microcystin levels. They consist of polyclonal antibodies that bind either microcystins or microcystin–enzyme conjugates.

The microcystin concentration in the M treatments fluctuated with time during the experiment, ranging from 0.73 to 1.37 μg/L. For each M treatment, we calculated the average concentration of microcystin experienced by the animals over the period from two instars before maturity until the second clutch of eggs was deposited in the brood chamber. For all M treatments, the microcystin value was 1.02 ± 0.17 μg/L (mean ± SD), except for the CM treatment, which was 0.74 μg/L. This difference was entirely attributed to natural fluctuations in the toxicity of algae, which happened to coincide with the timing for the second egg clutch deposition in the CM treatment. Consequently, animals in the CM treatment were possibly less affected by microcystin than were animals in the other M treatments. Therefore, to be able to perform an adequate comparison between life history parameters among treatments, we adjusted reproductive parameters for the CM treatment. Specifically, we multiplied the number of offspring in the first and second clutch by a factor of 0.74/1.02 = 0.725, and we corrected the time to reach maturity and the interclutch time by dividing by the same factor. This adjustment assumes a linear response of *Daphnia* to toxin concentration, such as has been demonstrated elsewhere (DeMott 1999).

All statistical analyses were performed with SPSS (Version 10 for Macintosh; SPSS 2000). A Mann-Whitney *U* test was used to compare the life history parameters for the C vs. M treatments. This approach was used because some life history parameters (e.g., days to reach maturity, age when fist and second clutch were delivered, and interclutch time) were not normally distributed, according to Levene's test of normality. In addition, the intrinsic rates of population increase were compared using an ANOVA or an independent *t* test after testing for normality and equal variance. Intergenerational effects of toxins were compared using ANOVA and Tukey's test.

RESULTS

Treatments with toxin-producing *Microcystis aeruginosa* affected life history parameters of *Daphnia magna* negatively (Table 1). Specifically, individuals in M treatments produced fewer offspring in both clutch 1 and clutch 2 than did those in control trials. As well, the biomass of individual offspring in clutch 2 was lower than in controls when the females had been exposed to microcystin, but did not differ in clutch 1. Moreover, daphnids in the M treatment also matured later and delivered their first and second brood later compared to *Daphnia* in the C treatment (Table 1). In addition to delayed maturation, some females in the exposed group (M) aborted their eggs, which resulted in large differences between the treatments when comparing age for delivery of the first clutch.

Daphnia in all M treatments had significantly lower fitness than those in C treatments ($F_{1, 124} = 244.68$, $P < 0.001$), with *r* values ranging from 0.12 to 0.20 for M treatments and from 0.25 to 0.28 for the C treatments (Fig. 1). Comparison of M and C treatments for offspring that shared mothers throughout the experiment revealed that the M treatment *Daphnia* had lower fitness in all cases (Fig. 1). Further, the number of offspring increased with time over the first three clutches for both C and M treatments, and the females in the C treatment produced significantly more offspring (Fig. 2). However, the number of offspring per clutch leveled off after the third clutch for both C and M treatments, and differences between the two treatments were, with the exception of clutch 5, no longer significant after clutch 4 (Fig. 2).

To evaluate maternal effects, we compared daughters in M treatments that had mothers without experience of microcystin (''inexperienced'' mothers M, CM, CCM, and MCM) with daughters from ''experienced'' mothers (MM, CMM, and MMM). In all cases, individuals in treatments with ''experienced'' mothers had higher fitness than those with mothers lacking expe-

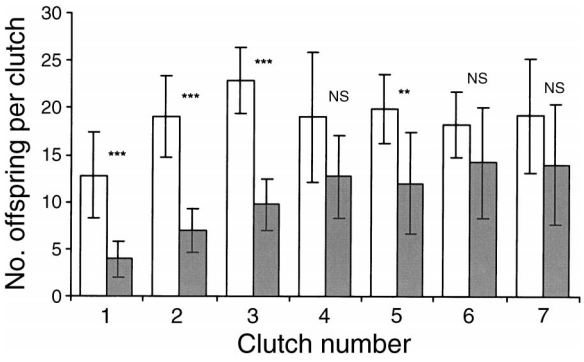

FIG. 2. Number of offspring (means ± SD) in control (C) and microcystin (M) treatments (open and gray bars, respectively) for the F1 generation in clutches 1–7. Asterisks indicate significant differences by Student's *t* test: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS, not significant.

rience with microcystin ($t_{60} = -3.95$, $P < 0.001$) (Fig. 3). Similarly, the time required to reach maturity and delivery of the first clutch also differed between the two groups, with maturation being delayed 1.5 d for daughters with inexperienced mothers ($z = -3.35$, $P = 0.001$), and delivery of the first clutch being delayed 2.5 d ($z = -4.22$, $P < 0.001$; Fig. 3). In addition, this inexperienced group had significantly ($t_{60} = -2.18$, $P = 0.033$) fewer offspring in the second clutch: 7.2 ± 3.1 offspring compared to 9.4 ± 4.5 in females with ''experienced'' mothers (values are means ± SD). In contrast, no grandmaternal effects were found when we compared the fitness values for treatment CCM and MCM ($t_{14} = 0.264$, $P = 0.796$). In these two treatments, F2 and F3 had experienced the same environment, whereas that of their grandmothers differed.

Juveniles generally spent <24 h (but occasionally up to 36 h) in the same environment as their mother. To test whether this exposure may have affected the fitness of offspring, we compared the individuals exposed to microcystin in their mother's environment to those born into nontoxic treatments. Thus, individuals transferred from their mother's M treatment to C treatment (MC, MMC, CMC; Fig. 1) were compared with offspring born in the C treatment and subsequently transferred to another C treatment (C, CC, CCC and MCC; Fig. 1). If exposure to the mother's environment during these first hours had affected the juveniles, we would have expected differences in fitness between these groups. However, this was not the case: neither fitness nor life history parameters differed between the two groups ($t_{62} = 0.369$, $P = 0.713$). This result demonstrates that there were no environmental effects on newly hatched *Daphnia* due to exposure to microcystin in their mother's environment, and further supports the suggestion that toxin tolerance was passed on through maternal effects. Fitness of the control line (C, CC, and CCC) was compared with that of the microcystin line (M, MM, and MMM) to evaluate the effect of microcystin exposure over three generations. Although fitness of the control line did not change from the first to the third generation ($F_{2,27} = 1.98$, $P = 0.158$), fitness of the microcystin line increased from the first to the second generation (Tukey test, $P = 0.001$), but did not increase further in the third generation (Tukey test, $P = 0.278$) (Fig. 4).

DISCUSSION

In this paper, we provide evidence that *Daphnia* has an inducible defense mechanism against toxic cyanobacteria and that it can pass on its tolerance against cyanotoxins to its offspring. Overall, exposure to toxin-producing *Microcystis aeruginosa* had a negative effect on fitness of *Daphnia magna*, a pattern that is in accord with previous studies (e.g., de Bernardi and Guisanni 1990). However, because our control treatments consisted of nontoxic *Microcystis* within an otherwise nutritious food source, we infer that observed fitness reductions were attributable to the toxic effects of microcystin-containing algal strains. For example, females exposed to microcystin matured later and produced fewer offspring than did females in control treatments. Furthermore, the animals exposed to toxins enhanced their ability to deal with toxic cyanobacteria

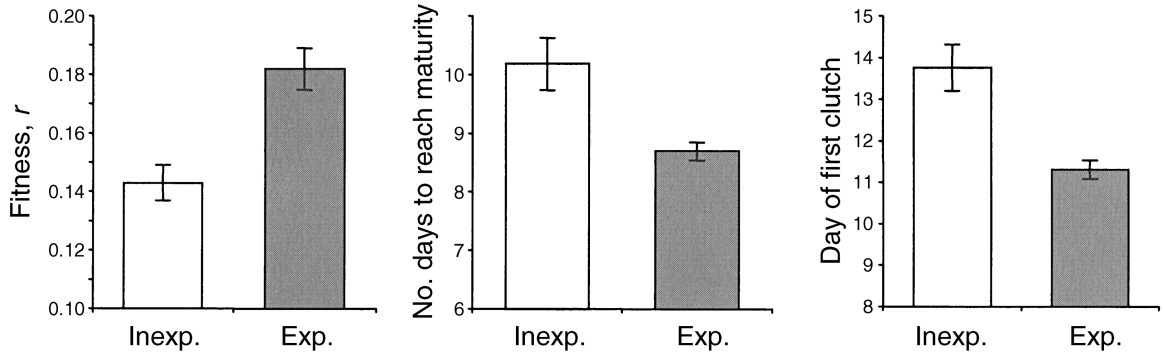

FIG. 3. Differences in fitness (*r*), days to reach maturity, and day of delivery of the first clutch between the groups with ''inexperienced'' (inexp.) mothers (M, CM, CCM, and MCM) and ''experienced'' (exp.) mothers (MM, CMM, and MMM), where M is the microcystin treatment and C is the control. Values are mean ± SE.

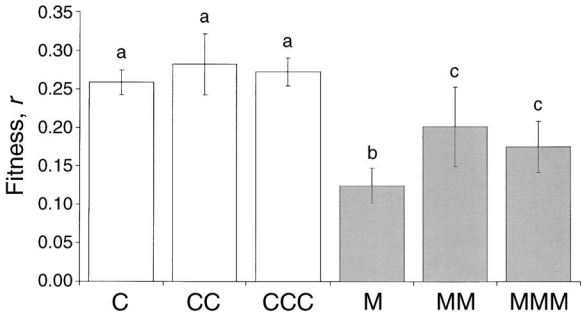

FIG. 4. Fitness (*r*) for three generations in the control line (C, CC, and CCC) and the microcsytin line (M, MM, and MMM). Different lowercase letters indicate significantly different means (Tukey's test; $P = 0.001$ for the difference between bars designated b and c).

during their lifetime, as demonstrated by increases in clutch size through time. This pattern may reflect a lag between exposure to microcystin and induction of the detoxification mechanism. Such phenotypic plasticity could be an important adaptation for clonal animals to withstand rapid variations in toxin concentrations within the environment. Further, because *Daphnia* play such a central role in lake food webs, the availability of such an inducible defense to toxins should help to maintain energy and material transfer to higher trophic levels.

Our results also show that if the previous generation had been exposed to toxic *Microcystis*, subsequent offspring had higher fitness. Such a pattern is consistent with the presence of maternal effects, in which experienced mothers transferred information about their environment to their offspring to improve their survival. In contrast, offspring of inexperienced mothers matured later, delivered their first clutch approximately two days later than the other group, and had fewer offspring. Such a delayed maturation could arise either because of reductions in individual growth rates, and therefore time to reach the size threshold for reproduction (Ebert 1992), or because individuals exposed to toxins are often smaller than those from nontoxic environments (Nandini and Rao 1998, Gustafsson and Hansson 2004). Regardless of the precise mechanism, delays in maturity may leave individuals vulnerable to predation by other invertebrates (e.g., *Chaoborus*, *Leptodora*) that partly regulate overall secondary production. The fitness of the offspring from experienced mothers increased between the first two generations, but did not improve further in the third generation. A plausible explanation for this pattern may be that after one generation in the toxic environment, the offspring in the second generation are born with the gene for synthesis of detoxifying enzymes turned on, and thus can improve their performance in the toxic environment. The third generation, however, may also be born with the defensive enzyme system enabled; therefore, no further improvement in fitness could occur between the F2 and F3 generations. In addition, this study shows that the environment experienced by the first generation did not affect the performance of the third generation, suggesting that there were no grandmaternal effects.

Our study clearly shows that the tolerance to microcystin is an inducible defense that can be transferred from mother to offspring. Such maternal effects have been reported from both animals and plants in terrestrial, as well as aquatic, ecosystems (Mosseau and Fox 1998, Agrawal et al. 1999). Although such induced tolerance may be less important for individuals with short lifetimes (Vijverberg and Ritcher 1982, Lampert 1991), through the maternal effect induced tolerance may have a prolonged and important impact on overall population survival. For example, because *Daphnia* mortality rates can be as much as 10–40% per day (Vijverberg and Ritcher 1982, Lampert 1991), daphnids from experienced mothers could produce 45% more offspring after two clutches than do individuals from inexperienced mothers. Even though the number of offspring is a vital part of a fitness value, the age of maturation also has a considerable impact when the daily mortality rate is high. Further, because experimental microcystin concentrations (1 μg/L) were well within natural summer concentrations observed for south Swedish lakes (S. Gustafsson, *unpublished data*), it seems likely that maternal effects may be a common and important mechanism for sustaining herbivore production and availability for higher trophic levels, such as fish.

In conclusion, we find that the tolerance to toxic *Microcystis* in *Daphnia* is an inducible defensive mechanism developed during an individual's lifetime. In addition, this trait can be transferred from mother to offspring: a maternal effect (Mousseau and Dingle 1991, Mousseau and Fox 1998, Agrawal et al. 1999). Finally, this ability of a *Daphnia* mother to pass on her experience of the environment to her offspring is of considerable importance for her fitness, both through an increased number of progeny and a faster development to maturity.

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