

Daphnia growth on microcystin-producing and microcystin-free *Microcystis aeruginosa* in different mixtures with the green alga *Scenedesmus obliquus*

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Abstract

The hypothesis that negative effects of *Microcystis* on *Daphnia* growth and reproduction can be explained from the presence of microcystin in the *Microcystis* cells was tested by comparing the effects on *Daphnia* life history characteristics of a microcystin-free mutant strain and microcystin-containing strain of the cyanobacterium *Microcystis aeruginosa* PCC7806. To avoid nutritional deficiency, *Microcystis* was offered to *Daphnia* alone and in various mixtures with the high-quality green alga *Scenedesmus obliquus*. In contrast to expectation, growth of *Daphnia* on microcystin-free cells was not much better than growth on microcystin-containing cells. Because nutritional insufficiency, morphology, and feeding inhibition could not explain the observed effects, the results show that *Microcystis* must contain substances other than microcystins that are poisonous to *Daphnia*.

It is generally accepted that cyanobacteria can cause major disruptions of the aquatic ecosystem (Christoffersen 1996). In particular, the effects on the grazer *Daphnia*, which is a key species in freshwater food chains (Lampert 1987), have been documented extensively (e.g., Lampert 1987; De-Benardi and Giussani 1990; DeMott 1999; Müller-Navarra et al. 2000).

Cyanobacteria have strong negative effects on *Daphnia* and are undoubtedly an inadequate food source (Lampert 1987). There is, however, much less consensus on the causal factors; hence, several causes for the negative effects of cyanobacteria on *Daphnia* have been proposed. First, many cyanobacteria possess toxins that might cause death in *Daphnia* (DeMott et al. 1991; DeMott and Dwahale 1995). Second, a “bad taste factor” or feeding deterrents might reduce the food intake of *Daphnia* (Nizan et al. 1986; Haney et al. 1995). Third, cyanobacteria might lack essential fatty acids or lipids, and the poor nutritional value could have severe effects on *Daphnia* growth and reproduction (Müller-Navarra 1995; DeMott and Müller-Navarra 1997; Von Elert and Wolffrom 2001). Finally, morphological features, such as size or mucus, might hamper ingestion or digestion of cyanobacteria by *Daphnia* (Fulton and Paerl 1987; De-Benardi and Giussani 1990). All these factors could contribute to reduced growth, lower reproduction, and, eventually, a *Daphnia* population decline thereby reducing the efficiency of the energy transfer from primary producers up the trophic levels. As a result, an accumulation of cyanobacteria occurs and notorious blooms are formed that are indicative of water quality deterioration.

Microcystis aeruginosa is one of the most common cya-

nobacteria in freshwater bodies all around the world. Many *M. aeruginosa* strains produce bioactive intracellular chemicals, so-called microcystins (MCs), of which dozens of variants have been detected (Codd et al. 1997). Numerous studies on the detrimental effects of cyanobacteria on *Daphnia* have been performed with *Microcystis* strains in which MCs are suspected of being the major cause of reduced growth and increased mortality in *Daphnia* (e.g., DeMott et al. 1991; Reinikainen et al. 1994; Rohrlack et al. 1999a). However, a major problem in research on the detrimental effects of *Microcystis* on *Daphnia* is that the potential causes (i.e., toxicity, poor nutritional value, bad taste, and morphology) might all be strain-specific properties. Therefore, single-strain experiments and experiments that compare strains varying in MC content could be of limited use because other strain-specific properties could vary as well. One of these properties, the feeding inhibitory effect, has already been shown to be independent of microcystins (Rohrlack et al. 1999a, 2001).

In a recent and inspiring series of studies, Rohrlack and coworkers have focused on the feeding and survival of *Daphnia* on the MC-producing strain of *M. aeruginosa* PCC7806 and its MC-free mutant as the sole food (Rohrlack et al. 1999a,b, 2001). This mutant is genetically engineered and cannot synthesize any variant of microcystin because of an insertion mutation of a microcystin synthetase gene (Dittmann et al. 1997). Hence, the availability of this mutant provides an opportunity to test the hypothesis that negative effects of *Microcystis* on *Daphnia* growth and reproduction can be explained by the presence of MCs in the cells, because the wild-type and mutant strains only differ in their ability to produce MCs. To overcome nutritional insufficiency in an essential compound, such as unsaturated fatty acids (cf. DeMott and Müller-Navarra 1997; Ferrao et al. 2000; Von Elert and Wolffrom 2001), both the wild-type and mutant strain of *M. aeruginosa* PCC7806 were added in different mixtures with a good food (*Scenedesmus*). It was hypothesized that growth and life history characteristics of *Daphnia* will only be adversely affected when MC-containing *Microcystis* is present in the food, but not when MC-

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Acknowledgments

The research of M.L. was made possible by a fellowship from the Royal Netherlands Academy of Arts and Sciences. I cordially thank M. Dionisio-Pires (Centre for Limnology, The Netherlands) for providing both the wild-type and mutant strains of *M. aeruginosa* PCC7806. Bill DeMott and an anonymous reviewer are thanked for valuable comments on the manuscript.

free cells are fed together with *Scenedesmus*. In addition to the life history experiments, short-term grazing experiments were performed to assess the effect of different food mixtures on *Daphnia* feeding.

Materials and methods

Organisms—Both the wild-type strain and the mutant strain of *M. aeruginosa* Kützing PCC7806 (originally isolated in 1972 from Braakman Reservoir, The Netherlands) were obtained from the collection at the Centre for Limnology (Nieuwersluis, The Netherlands). The mutant is not able to synthesize any microcystins because of an insertional inactivation of the microcystin synthetase gene. This insertion completely knocks out microcystin synthesis but has no effect on the production of other oligopeptides, such as cyanopeptolines (Dittmann et al. 1997). Both strains were grown in cellulose plug-stoppered 250-ml erlenmeyer flasks that contained 125 ml of sterile, slightly modified WC (Woods Hole CHU) medium (Lürling and Beekman 1999). The flasks were incubated on a rotating shaking device (60 rpm) in $30 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ supplied in a 16:8 light:dark (LD) rhythm at 18°C. Culture densities and size distributions were determined in the range of 2–25 μm equivalent spherical diameter (100- μm capillary) with a Coulter Multisizer II electronic particle counter. After 3 weeks of acclimatization to our laboratory growth conditions, new batches of *Microcystis* were started daily with identical initial *Microcystis* concentrations of $1.5 \times 10^7 \mu\text{m}^3 \text{ ml}^{-1}$. These batches were incubated as described above for 1 week, after which the cyanobacteria were used in life history experiments. The wild-type cultures had reached average (± 1 SD) densities of $1.68 (\pm 0.43) \times 10^8 \mu\text{m}^3 \text{ ml}^{-1}$, whereas the mutant cultures were $1.70 (\pm 0.59) \times 10^8 \mu\text{m}^3 \text{ ml}^{-1}$. Under the conditions employed, both strains exhibited excellent growth with identical (*t*-test, $P = 0.868$) growth rates of $0.34 (\pm 0.04) \text{ d}^{-1}$ and $0.34 (\pm 0.06) \text{ d}^{-1}$ for the wild-type and mutant strains, respectively.

The green alga *Scenedesmus obliquus* (Turpin) Kützing (formerly known as *Scenedesmus acutus* Meyen) originated from the Max Planck Institute for Limnology (Plön, Germany). The green algae were maintained in 1.0-liter chemostat systems in continuous light of $120 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ at 20°C on WC medium and with a dilution rate of 1.1 d^{-1} .

The cladoceran *Daphnia magna* Straus was isolated from Lake Zwemlust (The Netherlands) and cultured in the laboratory at 20°C in 1-liter jars containing 800 ml RT medium (Tollrian 1993). *Daphnia* stock cultures were fed three times a week with *S. obliquus* ($\sim 4 \text{ mg C L}^{-1}$); culture medium was refreshed every 3 weeks, and the number of animals was reduced to approximately 10 *Daphnia* per jar.

Daphnia growth experiment—To examine the influence of microcystins on life history parameters, *D. magna* were grown on *Microcystis*, on mixtures of wild-type (MC+) or mutant (MC-) *M. aeruginosa* PCC7806 with *S. obliquus*, and on green algal food only. Before the life history experiment, juvenile *Daphnia* born on the same day were collected from the stock cultures and placed individually in sep-

arate 125-ml test tubes containing 100 ml of *Scenedesmus* food suspension with a concentration of $10^7 \mu\text{m}^3 \text{ ml}^{-1}$ (equivalent to $\sim 5 \text{ mg C L}^{-1}$). These *Daphnia* were transferred daily to new tubes with fresh food, and newborns from the third broods were used as experimental animals. The newborns were joined in a 500-ml beaker with RT medium. For each treatment, five neonates were randomly selected and transferred into 125-ml test tubes containing 100 ml of a food suspension (in RT medium).

The different food treatments comprised either 100% *S. obliquus* (So), 75% So and 25% *M. aeruginosa* (Ma), 50% So and 50% Ma, 25% So and 75% Ma, or 100% Ma. The different treatments received equal food concentrations of $10^7 \mu\text{m}^3 \text{ ml}^{-1}$ (equivalent to $\sim 5 \text{ mg C L}^{-1}$). In an additional series, *D. magna* was grown on different concentrations of *S. obliquus* only comparable to the amount of green algae in the mixtures (i.e., 100%, 75%, 50%, and 25% So and no food). Cyanobacterial and green algal densities were determined in the range of 2–25 μm equivalent spherical diameter (100- μm capillary) with a Coulter Multisizer II electronic particle counter. The tubes, each with one experimental animal, were incubated in a climate-controlled room at 20°C in 16:8 LD low light of $\sim 4 \mu\text{mol m}^{-2} \text{ s}^{-1}$. The animals were transferred daily to clean tubes with fresh food, inspected for molting, and measured from just above the eye to the base of the tail spine with a dissecting microscope. Time to reach maturity, mortality, and number of newborns were recorded. Neonates were removed from the tubes. Growth and reproduction were followed until animals had reached the third adult instar. The intrinsic rate of population increase was determined using the Euler equation. In the case of no reproduction (*b*), the intrinsic rate of population increase (*r*) was determined from mortality rates (*d*) because $r = b - d$.

Mortality rates were estimated from Type II survivorship curves after $\ln(n + 1)$ transformation by linear regression, assuming that death of *Daphnia* was entirely determined by their interaction with the environment (Hutchinson 1978).

Results and discussion

The presence of *Microcystis* in the food had a tremendous effect on growth, development, survival, and body size of *D. magna*. Animals fed a mixture of *M. aeruginosa* and *S. obliquus* or *M. aeruginosa* only not only remained much smaller, but also exhibited bad survivorship (Fig. 1). By contrast, in control populations fed *S. obliquus* only, all animals reached the second adult instar (instar 7) and had the largest body sizes (Fig. 1). A repeated measures analysis of variance (SPSS version 10.1) on the body length of *Daphnia* yielded a significant instar effect ($F_{6,48} = 966$; $P < 0.001$), a significant food type effect ($F_{2,8} = 39.5$; $P < 0.001$), and a significant strain (MC+/MC-) effect ($F_{2,8} = 140.4$; $P < 0.001$). However, no post hoc test was performed because of poor survivorship, especially in the MC+ treatments (Fig. 1). When the repeated measure analysis of variance was restricted to the first three instars, the results indicated that the size of juvenile *Daphnia* was significantly affected by the food type (Table 1). Tukey's post hoc test revealed that the

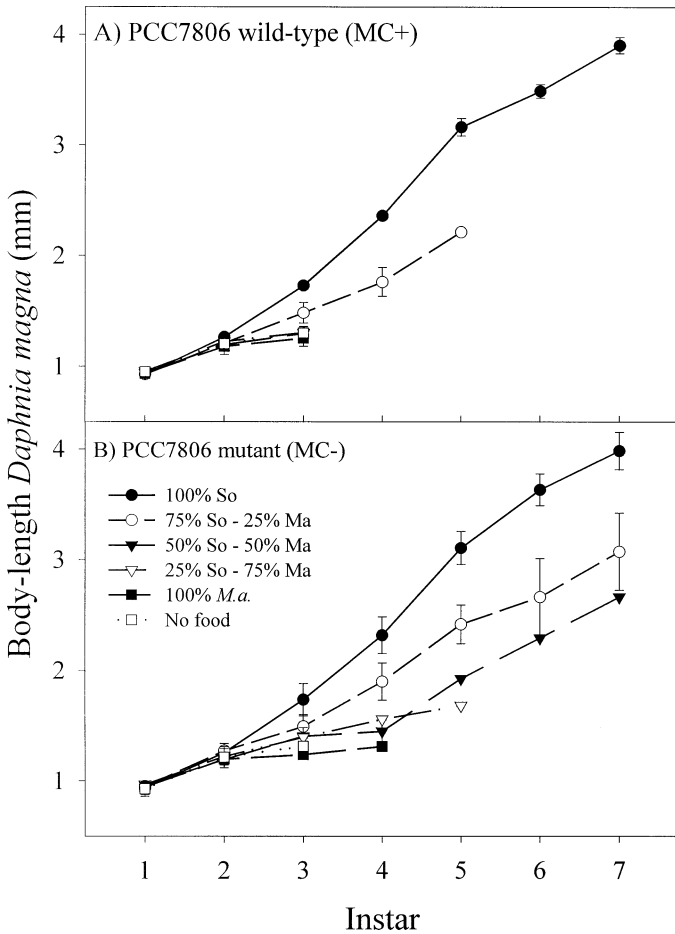


Fig. 1. Mean body length (mm) of *Daphnia magna* in successive instars cultured on either *Scenedesmus obliquus* (100% So), on 3:1 mixtures (75% So/25% Ma), 1:1 mixtures (50% So/50% Ma) and 1:3 mixtures (25% So/75% Ma) of *S. obliquus* with *Microcystis aeruginosa* PCC7806 on *Microcystis* only (100% Ma) and without food (No food). (A) The microcystin-containing wild-type *M. aeruginosa* PCC7806 was supplied. (B) The microcystin-free mutant strain was added. Error bars indicate 1 SD.

Table 2. Time needed to kill 50% of the *Daphnia* (LT₅₀, day ± one SE) fed with different food. The food supplied to *Daphnia* was *Scenedesmus* only (100% *S. obliquus* [So]; 3:1, 1:1, and 1:3 mixtures of *Scenedesmus* with a wild-type microcystin-containing *Microcystis* (MC+) and a MC-free mutant (MC-); *Microcystis* only (100% *M. aeruginosa* [Ma]); or no food at all. Also included are controls in which *Daphnia* was fed only *Scenedesmus* corresponding to the amounts in the mixtures (*Scenedesmus*).

Food type	LT ₅₀		
	MC+	MC-	<i>Scenedesmus</i>
100% So	>10	>10	>10
75% So/25% Ma	8.12(0.36)	8.60(0.32)	>10
50% So/50% Ma	5.89(0.12)	8.59(0.33)	>10
25% So/75% Ma	7.62(0.60)	7.85(0.46)	>10
100% Ma	6.79(0.21)	7.36(0.66)	—
No food	8.17(0.32)		5.33(0.22)

100% *Scenedesmus* treatments formed one homogeneous group, whereas the *Microcystis* treatments and no food treatments formed another homogeneous group. However, no differences in *Daphnia* growth on either the MC+ or the MC- strain over the first three instars were found (Table 1). Where growth and survival were very poor in the presence of the MC+ strain, they were better when food was offered as mixtures of MC- with *S. obliquus* (Fig. 1; Table 2). The effects of the various food types on *Daphnia* survival were analyzed by Kaplan-Meier Survival Analysis in the statistical tool pack SPSS version 10.1. The mean time needed to kill 50% of the *Daphnia* was shorter when MC+ was a food component than when MC- was part of the food (Table 2). Hence, MC-containing cells killed *Daphnia* faster than MC-free cells, but differences between the MC+ and MC- strains were less pronounced in this study than those found in other studies (Rohrlack et al. 1999a, 2001). All *D. magna* fed with different amounts of *S. obliquus* only (i.e., 100, 75, 50, and 25% So) survived until the end of the experiment (Fig. 1; Table 2).

In addition to strong effects on somatic growth, the pres-

Table 1. Summary of repeated measures analysis of variance* of the effect of food type (100% *Scenedesmus*; 100% *Microcystis*; 3:1, 1:1, and 1:3 mixtures of both food species; and no food) and strain (microcystin-free mutant and microcystin-containing wild-type *Microcystis aeruginosa* PCC7806) on the body length of juvenile *Daphnia magna* over the first three consecutive instars.

Source	df	MS	F	P
Body length (between subjects)				
Strain	1	2.662	0.56	0.459
Food type	5	63.142	13.4	<0.001
Strain × food type	4	1.757	0.37	0.827
Error	32	4.729		
Body length (within subjects)				
Instar	2	1243.922	1125	<0.001
Instar × Strain	2	3.347	3.03	0.055
Instar × Food type	10	49.288	44.592	<0.001
Instar × Strain × Food type	8	2.290	2.07	0.052
Error	64	1.105		

* df, degrees of freedom; MS, men squares.

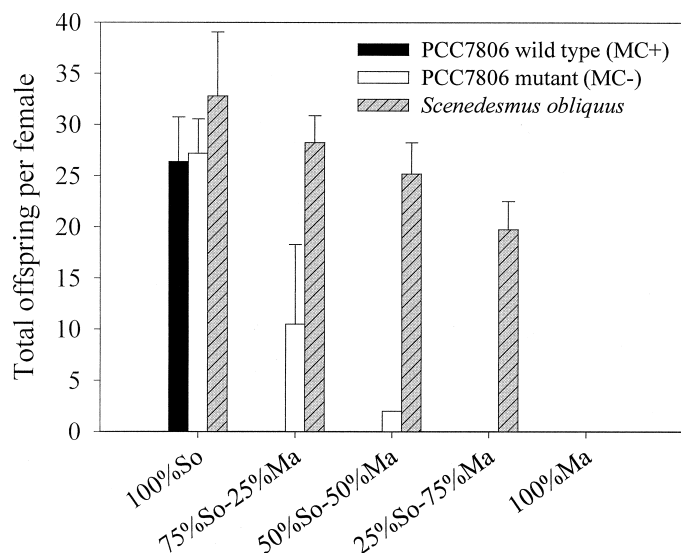


Fig. 2. Total offspring per *Daphnia* fed with *Scenedesmus obliquus* only (100% So), with 3:1 mixtures (75% So/25% Ma), 1:1 mixtures (50% So/50% Ma), and 1:3 mixtures (25% So/75% Ma) of *Scenedesmus* with microcystin-containing (MC+) and microcystin-free (MC-) *Microcystis aeruginosa* PCC7806 and with *Microcystis* only (100% Ma). Error bars indicate 1 SD.

ence of *Microcystis* in food strongly influenced reproduction (Fig. 2). No animals reached maturity without food on MC- only, when 75% of the food was MC-, or when MC+ was a food component. Consequently, in all these treatments, reproduction was zero (Fig. 2). In the treatment where 50% of the food was composed of MC-, one female reached maturity and released two neonates, whereas two animals reached maturity and reproduced in the presence of 25% MC- (Fig. 2). In all *Scenedesmus* treatments, *Daphnia* exhibited good reproduction.

Poor somatic growth, poor survivorship, and lack of reproduction resulted in negative *Daphnia* population growth when MC+ was a component of the food (Fig. 3). Offering the MC-free mutant in mixtures hardly improved this pattern, and only in the 75% So/25% Ma treatment was a positive population growth rate found (Fig. 3). In contrast, *Daphnia* population growth was positive for all pure *Scenedesmus* food quantities.

Because the lack of MCs in the mutant had only a marginal effect on *Daphnia* growth, the results do not support the hypothesis that the negative effects of *Microcystis* on *Daphnia* growth and reproduction can be explained just from the presence of MCs in the cells. For instance, whereas the amount of food was sufficient to support excellent growth in the 50% *S. obliquus* treatment, the addition of a similar amount of MC-free PCC7806 to 50% *Scenedesmus* suppressed *Daphnia* growth to such an extent that the population growth rate became negative (Fig. 3). The major cause of negative effects on *Daphnia* population growth was death of the animals. One of the most important prerequisites for a positive population growth is that animals survive long enough for reproduction. However, despite observations that MC-containing PCC7806 killed *Daphnia* faster than MC-

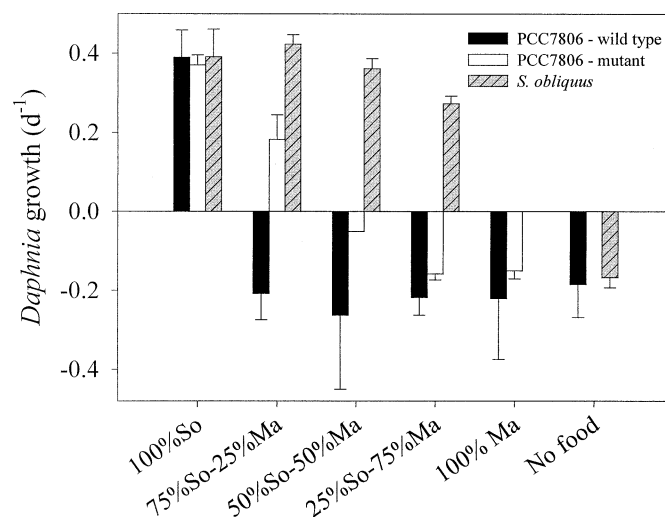


Fig. 3. Intrinsic rate of population increase of *Daphnia magna* fed with *Scenedesmus obliquus* (100% So), with 3:1 mixtures (75% So/25% Ma), 1:1 mixtures (50% So/50% Ma), and 1:3 mixtures (25% So/75% Ma) of *Scenedesmus* with microcystin-containing (MC+) and microcystin-free (MC-) *Microcystis aeruginosa* PCC7806, with *Microcystis* only (100% Ma), and in treatments without food (No food). Error bars represent 1 SD.

free PCC7806 (Rohrlack et al. 1999a, 2001; this study), >50%, if not all, of the animals died off within one generation in these studies. This means that the majority of the animals died before they reproduced, which exerts devastating effects on *Daphnia* population development (see Fig. 3) and indicates the limitation of mortality or acute lethality as a toxicological endpoint in assessment of ecological risks of *Microcystis*.

The detrimental effect of the MC-free mutant on *Daphnia* growth also could not be explained from a deficiency in an omega-3 fatty acid (Müller-Navarra 1995; DeMott and Müller-Navarra 1997) or in another lipid (Von Elert and Wolf from 2001). The nutritional insufficiency would have been overcome in the mixtures of MC-free *Microcystis* with *Scenedesmus* and should have resulted in *Daphnia* growth, as observed in the treatments where *Daphnia* was feeding on *Scenedesmus* only (DeMott and Müller-Navarra 1997; Ferrao et al. 2000). This is corroborated by the results from experiments with the wild-type strain that also run counter to the nutritional inadequacy hypothesis (DeMott 1999). Another factor that could be excluded was colonial morphology hampering ingestion (Fulton and Paerl 1987). Under the conditions employed in this study, both PCC7806 strains were completely uni- and bicellular. Also under different culturing conditions, both the wild type (DeMott 1999) and the mutant grew as single cells without any sign of a mucilage envelope (Rohrlack et al. 2001).

Hence, in the current study, a factor other than MCs, a fatty acid or morphology apparently is depressing *Daphnia* growth. Jungmann (1992, 1995) demonstrated that the wild-type PCC7806 contains compounds other than microcystins that are toxic to *Daphnia*, because an MC-free fraction from the wild-type strain PCC7806 remained toxic to *Daphnia*.

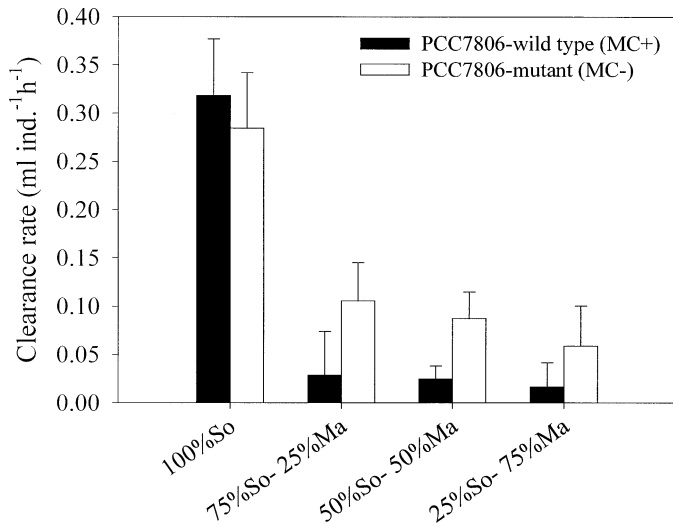


Fig. 4. Clearance rate of *Daphnia magna* feeding for 2 h on *Scenedesmus obliquus* (100% So) and on 3:1 mixtures (75% So/25% Ma), 1:1 mixtures (50% So/50% Ma), and 1:3 mixtures (25% So/75% Ma) of *Scenedesmus* with microcystin-containing (MC+) and microcystin-free (MC-) *Microcystis aeruginosa* PCC7806. Error bars indicate 1 SD ($n = 4$).

Additional compounds that could be poisonous to *Daphnia* (Jakobi et al. 1996) are cyanopeptolines (Martin et al. 1993). These compounds could partly explain the discrepancy between studies that favor the MC toxicity hypothesis (DeMott et al. 1991; Rohrlack et al. 1999a) and other studies that do not (Jungmann 1992, 1995).

Recently, Rohrlack et al. (2001) provided an alternative explanation for the poor performance of *Daphnia* on the MC-free PCC7806. Visual observations, the death of *Daphnia* at almost the same rate as starved animals, and low feeding rates on PCC7806 led them to suggest that daphnids died of starvation. To test this feeding inhibition hypothesis, I performed a short-term grazing experiment.

Daphnia grazing experiment—The grazing experiment was run in 24-well culture plates. Each well was filled with 2.5 ml of food suspensions (100% So, 75% So and 25% Ma, 50% So and 50% Ma, or 25% So and 75% Ma for both wild-type and mutant PCC7806). The food suspensions were made up in six replicates at equal concentrations of $10^7 \mu\text{m}^3 \text{ml}^{-1}$. Non-egg-bearing *D. magna* from the same cohort were transferred individually into four wells per food treatment. The *D. magna* used in the experiment were of similar size and had a mean (± 1 SD) body length of 1.63 (± 0.13) mm ($n = 51$). Two wells per food treatment without *D. magna* served as controls. The well plates were incubated for 2 h at 20°C in the dark. The clearance rates were calculated from the decrease in chlorophyll *a* (Chl *a*) derived from dark-adapted fluorescence using a PHYTO-PAM (Walz) according to the equation $\text{CR} = [\ln(\text{Chl}_0) - \ln(\text{Chl}_t)] \times t^{-1} \times 2.5$, in which CR is the clearance rate ($\text{ml ind.}^{-1} \text{h}^{-1}$), Chl_0 is the Chl *a* content ($\mu\text{g L}^{-1}$) at the start of the experiment, Chl_t = the Chl *a* content ($\mu\text{g L}^{-1}$) at the end of the experiment after incubation period t (h), and 2.5 is the amount of me-

Table 3. Two-way analysis of variance* on clearance rates of *Daphnia* fed with *Scenedesmus* only or 3:1, 1:1, and 1:3 mixtures of *Scenedesmus* with a wild-type microcystin-containing *Microcystis* (MC+) and a MC-free mutant (MC-).

Source	df	MS	F	P
Strain	1	0.01085	6.35	0.019
Food type	3	0.124	72.6	<0.001
Strain \times Food type	3	0.004843	2.83	0.060
Error	24	0.001709		

* df, degrees of freedom; MS, mean squares.

dium per individual *D. magna* (ml ind.^{-1}). Clearance rates were compared using two-way analysis of variance with strain (MC+/MC-) and food composition (100% So, 75% So/25% Ma, 50% So/50% Ma, 25% So/75% Ma) as the two fixed factors, followed by Tukey's test.

Daphnia clearance rates on mixtures with the MC- strain were higher than on mixtures with the MC+ strain (Fig. 4). The two-way analysis of variance on *Daphnia* clearance rates indicated a significant difference between food types in the two *Microcystis* strains (Table 3). Moreover, the analysis of variance indicated a significant effect of the proportion of *Microcystis* in the food, but no interaction effect (Table 3). Tukey's post hoc comparison revealed two homogeneous groups: (1) the 100% So treatments and (2) the 75% So/25% Ma, 50% So/50% Ma, and 25% So/75% Ma treatments. Clearance rates on the *Scenedesmus*-only food were significantly higher than on the other food types (Fig. 4). Feeding inhibition in the presence of MC-free PCC7806 compared to feeding solely on *Scenedesmus* varied between 63% in the 75% So/25% Ma treatment and 79% in the 25% So/75% Ma treatment. The reduction is less than the initial 90–95% feeding inhibition found for *D. magna* grazing on 50% and 80% mixtures of MC-containing PCC7806 and *Scenedesmus*, but in that study, a strong recovery after 24 h was observed (DeMott 1999). Extrapolation to the life history experiment means that *Daphnia* could still harvest more *Scenedesmus* in the 75% So/25% Ma treatment than in the 25% *Scenedesmus*-only treatment. Taking into account the complete feeding recovery after 24 h in 50% So/50% Ma treatments (DeMott 1999), *Daphnia* could be expected to perform at least as well on the 50% So/50% Ma (MC-) mixtures as on the 50% *Scenedesmus*-only treatment. However, growth, reproduction, and survival were much lower in the presence of MC-free PCC7806 than in treatments with only *Scenedesmus* as food. These results suggest that, although an important influence of feeding inhibition cannot be excluded in determining the detrimental effect of *Microcystis* on *Daphnia*, compounds that exist in *Microcystis* that have not yet been structurally resolved could be involved as well (Jungmann 1992, 1995). Therefore, the results of the current study are not fully in line with the conclusions by Rohrlack et al. (2001) that detrimental effects of *Microcystis* on *Daphnia* can be estimated from a parameter as simple as the MC ingestion rate.

Poor somatic growth, survivorship, and reproduction of *Daphnia* were found when the animals were cultured on mixtures of a good green algal food with *Microcystis*. Dif-

ferences between animals grown on an MC-free mutant and on the MC-containing wild-type *Microcystis* were much smaller than expected on the basis of the MC toxicity hypothesis. Undoubtedly, MCs are toxic to *Daphnia* and one of the causes of death (Rohrlack et al. 1999a) because they are potent inhibitors of phosphatases (DeMott and Dwahale 1995). The detrimental effects of *Microcystis* on *Daphnia* also depend on feeding rate inhibition, which appears independent of MCs (Rohrlack et al. 1999a, 2001; this study), because it will determine not only the dose of toxins, but the amount of ingested food. Sensitivity to feeding inhibition, however, can vary remarkably between *Daphnia* species (DeMott et al. 1991; DeMott 1999), and the feeding deterrence can differ among various *Microcystis* strains (Nizan et al. 1986; Rohrlack et al. 1999b). Inasmuch as a different MC-free *Microcystis* strain (NIVA-CYA 43) was much less harmful to *Daphnia* than the MC-free mutant used in this study, despite the feeding inhibition it caused (Lürling and van der Grinten 2003), a potentially important role of other non-MC poisonous compounds cannot be excluded. This means the proposed MC ingestion rate (Rohrlack et al. 2001) might not be the sole predictor for the harmful effects of *Microcystis* on *Daphnia*. In the risk assessment of *Microcystis* blooms, mostly field samples are analyzed for the presence of MCs using enzyme-linked immunosorbent assay and reversed-phase high-performance liquid chromatography (e.g., Wirsing et al. 1999). However, any growth-inhibiting and lethal effects on *Daphnia* could contribute to deterioration of water quality. In view of the results of this study, these deteriorating effects might not necessarily relate to the presence and concentration of MCs because *Microcystis* could contain toxins other than MCs that strongly affect *Daphnia* growth and survival.

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Received: 18 December 2002

Accepted: 26 May 2003

Amended: 23 June 2003