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Grazing on colonial and filamentous, toxic and non-toxic cyanobacteria by the zebra mussel *Dreissena polymorpha*

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Colony forming and toxic cyanobacteria form a problem in surface waters of shallow lakes, both for recreation and wildlife. Zebra mussels, Dreissena polymorpha, have been employed to help to restore shallow lakes in the Netherlands, dominated by cyanobacteria, to their former clear state. Zebra mussels have been present in these lakes since they were created in the 19th century by the excavation of peat and are usually not considered to be an invasive species. Most grazing experiments using Dreissena have been performed with uni-cellular phytoplankton laboratory strains and information on grazing of larger phytoplankton taxa hardly exists. To gain more insight in to whether D. polymorpha is indeed able to decrease cyanobacteria in the phytoplankton, we therefore performed grazing experiments with zebra mussels and two species of cyanobacteria, that greatly differ in shape: colony forming strains of Microcystis aeruginosa and the filamentous species Planktothrix agardhii. For both species a toxic and a non-toxic strain was selected. We found that zebra mussels cleared toxic Planktothrix at a higher rate than non-toxic Planktothrix, toxic or non-toxic Microcystis. Clearance rates between the other strains were not significantly different. Both phytoplankton species, regardless of toxicity, size and shape, were found in equal amounts (based on chlorophyll concentrations) in the excreted products of the mussels (pseudofaeces). The results show that zebra mussels are capable of removing colonial and filamentous cyanobacteria from the water, regardless of whether the cyanobacteria are toxic or not. This implies that the mussels may be used as a biofilter for the removal of harmful cyanobacterial blooms in shallow (Dutch) lakes where the mussels are already present and not a nuisance. Providing more suitable substrate for zebra mussel attachment may lead to appropriate mussel densities capable of filtering large quantities of cyanobacteria.

INTRODUCTION

Cyanobacteria are a nuisance in freshwater ecosystems due to bloom formation and toxin production (Chorus and Bartram, 1999). Mass mortality of aquatic organisms in the field is often ascribed to cyanobacteria (e.g. Krienitz *et al.*, 2003), although usually direct evidence is lacking (Ibelings *et al.*, in press). Human illness and even death have been also linked to toxicity by cyanobacteria (Carmichael *et al.*, 2001). The first and most crucial step in decreasing the negative effects of cyanobacterial blooms is by decreasing the nutrient load to lakes (Gulati and Van Donk, 2002). As an additional measure, blooms may be decreased by promoting growth of filter-feeders in the food web, a strategy known as biomanipulation (Reeders and Bij de Vaate, 1990; Meijer, 2000). Traditionally attempts have focussed on stimulating a higher daphnid biomass (Gulati and Van Donk, 2002). Densities of daphnids however, usually decline during summer, due to fish predation (Sommer *et al.*, 1986) when toxic cyanobacterial blooms reach their peak density (Gliwicz, 1990). Large colonies or filaments of cyanobacteria may also be difficult to handle for zooplankton, because their size and shape interfere with the filtering system of zooplankton (Lampert, 1987a). Results of

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biomanipulation using zooplankton have been mixed (Meijer *et al.*, in preparation), and it would be sensible to look for alternative means. Here we report on using zebra mussels, *Dreissena polymorpha* (Pallas) as biofilters in eutrophic lakes.

Dreissena is often considered to be a nuisance species because it attaches to hard substrata, such as the intake pipes of water treatment plants (Nalepa and Schloesser, 1993; MacIsaac, 1996; Enserink, 1999). The clogging by zebra mussels decreases the flow in pipes (Nalepa and Schloesser, 1993) and can, therefore, cause severe economic problems (Ahlstedt, 1994). Ecosystems are also threatened by the invasion of zebra mussels. The mussels completely change phytoplankton and zooplankton composition in the pelagic zone due to their high filtration rate (Strayer *et al.*, 1999). North American endemic bivalve species are currently under threat of extinction because zebra mussels attach on their shells and compete with them for food (Ricciardi *et al.*, 1998). On the other hand, zebra mussels may improve ecosystem quality by greatly decreasing phytoplankton biomass, resulting in a higher transparency which may be beneficial for, e.g. biodiversity and resilience of shallow lakes (Strayer *et al.*, 1999). For instance, in Lake IJsselmeer (the Netherlands), a reverse gradient exists between zebra mussel densities (which are mainly found in the south) and the cyanobacterial biomass (which is mainly found in the north) (Ibelings *et al.*, 2003). It has been suggested that zebra mussels could be used as a potential tool in the biomanipulation of shallow lakes, suffering from harmful cyanobacterial blooms (Noordhuis *et al.*, 1992).

Past grazing studies have provided insight into the effectiveness of different types of filter-feeders in removing cyanobacteria and other phytoplankton from the water (Lampert, 1987b). Most grazing studies focused on the clearance of single-celled phytoplankton by feeding laboratory-reared cultures to the grazers (e.g. Baker *et al.*, 1998; Dionisio Pires and Van Donk, 2002). In nature however, bloom forming cyanobacteria occur mainly as colonies or filaments (Mur *et al.*, 1999). Several grazing studies with filamentous cyanobacteria exist, especially using zooplankton as grazers (e.g. Gulati *et al.*, 2001). Grazing studies on colonial cyanobacteria like *Microcystis*, grown under controlled conditions in the laboratory, however are nearly absent, with the exception of Vanderploeg *et al.* (Vanderploeg *et al.*, 2001) for zebra mussels and Panosso *et al.* (Panosso *et al.*, 2003) and Rohrlack *et al.* (Rohrlack *et al.*, 1999) for zooplankton. One reason for this lack of information is that *Microcystis* in culture looses its mucilage matrix resulting in a strain that no longer grows as colonies but instead consists of single cells. Importantly, if zebra mussels are to be used in biomanipulation, data on the specific clearance of cyanobacteria of different size and shape, including filaments and colonies are required.

Toxicity and/or poor food quality have been shown to affect grazing in different organisms (e.g. DeMott, 1999 for zooplankton; Madon *et al.*, 1998 for zebra mussels). Microcystin has been shown to depress grazing rates in zooplankton (DeMott, 1999) but this has not been found for zebra mussels. Previously we showed, using laboratory strains of single-celled phytoplankton, that zebra mussels are capable of filtering toxic cyanobacteria from the water (Dionisio Pires and Van Donk, 2002; Dionisio Pires *et al.*, 2004a,b). In this study, we approach more natural circumstances by performing grazing experiments with zebra mussels feeding on colonial strains of *Microcystis aeruginosa* and the filamentous cyanobacterium *Planktothrix agardhii*. Both cyanobacterial species occur frequently and in high abundance in shallow lakes in the Netherlands. In some Dutch lakes, *Planktothrix* (*Oscillatoria*) has been recorded to reach a density of 200×10^3 filaments mL^{-1} (Gulati and Van Donk, 2002). In Lake IJsselmeer, *M. aeruginosa* is the main producer of toxic blooms (Ibelings *et al.*, in press). Although the *Microcystis* strains, used in this study, lack a mucilage matrix, they are different from other laboratory-reared *Microcystis* strains because their cells stick together, resulting in colonial particles comparable in size and shape to their naturally occurring counterparts. We still performed grazing experiments with laboratory-reared cultures of *Microcystis* and *Planktothrix* rather than lake seston because in this way we were able to disentangle the effects of size from those of toxicity on the grazing behaviour of the mussels. In our experiments, a toxic (microcystin-LR) and a non-toxic strain of both cyanobacterial species was used. In the study, we test three hypotheses about grazing of *Dreissena* upon filamentous or colonial cyanobacteria. Our null hypotheses are mainly based upon literature about the interactions between cyanobacteria and filter-feeding zooplankton. The first hypothesis states that grazing on the toxic strains will be lower than that on the non-toxic strains. The second hypothesis states that mussels will graze more efficiently on smaller size fractions because smaller colonies will more easily pass the filtering apparatus of the mussels. Finally, we expect that filaments (as in *Planktothrix*) are less easily filtered from the water by the mussels than by spherical-shaped cells (as in *Microcystis*) because filaments may cause clogging more than do (small) colonies at the inhalent siphon. In order to be used as an efficient biofilter, *Dreissena* should be indiscriminant in its grazing, cyanobacteria must be taken up irrespective of size, shape or toxicity.

METHODS

Collection of zebra mussels

Zebra mussels from Lake IJsselmeer were collected with a box corer (780 cm^2) in winter 2003 and transported to the laboratory within 2 hours. About 250–300 individuals were placed in 10 L lake water (3°C). They were gradually (8–10 days) acclimated to the ambient temperature in the laboratory (17°C) and daily fed (>2 mg C L^{-1}) with the green alga *Scenedesmus obliquus* (CCAP 276/3A). An earlier study showed that the mussels feed well on this single-celled alga (Dionisio Pires *et al.*, 2004a). The water was completely refreshed three times per week. The mussels were immediately used in the experiments after temperature acclimation was completed.

Culturing of cyanobacteria

All strains were grown in batch culture at room temperature (18–20°C) in 2L Erlenmeyers containing 2L sterile O_2 medium (Van Liere and Mur, 1978). The flasks were aerated and stirred at 70 r.p.m. Incident irradiance was about 11 μmol photons m^{-2} s^{-1} and was provided continuously. The strains were harvested in the exponential growth phase prior to the experiments.

Microcystis aeruginosa strains V40 and V131 were originally isolated from Lake Volkerak (the Netherlands) and kindly provided by Edwin Kardinaal from the Institute for Biodiversity and Ecosystem Dynamics (University of Amsterdam, the Netherlands). The V40 strain contains the toxin microcystin-LR, whereas V131 lacks microcystin. Although both strains are classified as colony forming, they lack a significant mucilage matrix. The spherical-shaped cells form colonies by sticking together. The colony 'sizes' (number of cells per colony, n = 150) of the V131 and V40 strain are 134 (range: 41–265) and 202 (range: 51–722) μm, respectively. The diameter of the cells from the V131 and V40 strain is 2.18 μm and 2.9 μm, respectively.

For *P. agardhii*, strains NIVA-CYA116 and NIVA-CYA126 were used. These strains were kindly provided by Dr. Randi Skulberg from the Norwegian Institute for Water Research (NIVA, Oslo, Norway). The CYA126 strain contains microcystin-LR, whilst CYA116 is not toxic. The average length of the CYA116 strain (n = 150) is 187 μm (range: 29–530 μm) and of the CYA126 strain (n = 150) is 1166 μm (range: 400–2163 μm). Both strains had a diameter of 1.45 μm.

Microcystin determination

For the determination of the concentration of the toxin microcystin-LR, 10 mL of all cyanobacterial cultures were filtered over a pre-weighed GF/C filter (after flushing with 100 mL milliQ water) and freeze dried. Filters were then weighed to determine microcystin-LR on a weight-specific basis. This was done in triplicate. Microcystin was extracted from the filters using 10 mL 75% MeOH in an ultrasonic bath; cells were spun down at 1530 *g* (Hettich centrifuge) for 10–12 min. The supernatant was placed in a new tube and the residue was extracted for a second time with 7.5 mL 75% MeOH in an ultrasonic bath, followed by a second centrifuge step. The liquid from the second extraction was pooled with the liquid from the first extraction and dried under nitrogen flow (45°C). The dried material was dissolved in 50% MeOH, sonified for 5 min and centrifuged. Samples were measured on a reverse-phase high-performance liquid chromatography (HPLC) equipped with a C-18 column [LichoCart 250–4; Lichrosphere 100 RP-18 (5 μm) (Merck, Darmstadt, Germany)], using a gradient of 30–70% (vol/vol) of aqueous acetonitrile (together with 0.05% vol/vol trifluoroacetic acid) at a flow rate of 1 mL min^{-1}, as recommended by Lawton *et al.* (Lawton *et al.*, 1994). The detection limit of the HPLC for microcystin was 50 μg L^{-1} (2.5 ng solved in 50 μL MeOH). Purified microcystin-LR (from Sigma Chemicals) was used as a standard for the calculation of microcystin concentrations of the samples.

Grazing

Grazing with zebra mussels was performed with five mussels, of 13–17 mm shell length, in a grazing vessel containing 500 mL 0.45 μm filtered lake water and food at a concentration of 2 mg C L^{-1}. This was done in triplicate. The temperature was set at 17°C and incident irradiance was 8 μmol photons m^{-2} s^{-1}. Mussels were acclimated to the experimental setup by placing them in filtered lake water with the appropriate food treatments, as to be provided in the grazing experiments. This acclimation was done twice: food was supplied 24 and 2 hour prior to the actual grazing experiment. As a control, three vessels with lake water and no mussels were used to correct for changes in cyanobacterial biomass other than those related to grazing. The grazing experiments were performed with the mussels feeding on the cyanobacteria as single food, since other studies by the same authors already investigated the role of mixtures of cyanobacteria and green algae (Dionisio Pires and Van Donk, 2002; Dionisio Pires *et al.*, 2004a). In the grazing experiments on *Microcystis*, we filtered the two strains over 60 μm and performed the experiments with two size fractions: smaller and larger than 60 μm. All treatments were run simultaneously.

To be able to quantify grazing, we took 1.5 mL samples from the vessels at 0 and 60 min and analysed the

cyanobacterial biomass by means of pulse amplitude modulated fluorometry (Phytoplankton-Analyzer, Phyto-PAM), as has been done in other zebra mussel grazing experiments on laboratory-reared phytoplankton (Dionisio Pires and Van Donk, 2002).

After the grazing experiment, the mussels were rinsed under running deionized water and placed in 500 mL 0.45 μm filtered lake water for 30 min to allow for excretion of cyanobacteria by (pseudo) faeces. This water was thoroughly shaken to dissolve particles into the water. Samples (1.5 mL) were taken from the water at the end of this period and analysed for chlorophyll content. These examinations will provide indications about whether cyanobacteria are really ingested and/or assimilated by the mussels after they have been filtered out of the water. If all cyanobacteria are rejected by the mussels, they may potentially resume growth, which would make the zebra mussel an unsuitable tool for the control of cyanobacterial blooms.

Data analysis

Grazing was measured by calculating clearance rates of the mussels on the different cyanobacterial species/strains. It is calculated as the volume of water (mL) from which the mussel has removed all of the food particles per unit time (Bunt *et al.*, 1993). Clearance rate (CR mL mg DW^{-1} $hour^{-1}$) was calculated as follows (Coughlan, 1969):

$$CR = \frac{V}{nt} \times \left(\ln \frac{C_0}{C_t} - \ln \frac{C_0'}{C_t'} \right),$$

in which V is the volume of the food suspension (500 mL), n the dry weight of the five mussels (mg), t the duration of the experiment (in hour), C_0 the cyanobacterial concentration (μg chlorophyll L^{-1}) in vessels with mussels at $t = 0$, C_t the concentration at time t, C_0' the concentration of cyanobacteria in the control vessels at $t = 0$ and C_t' at time t.

In the experiments, a proportion of the filtered cells may return to the water in the form of pseudofaeces and hence their chlorophyll may be reanalyzed. The clearance rates reported here for the mussel experiments are therefore net clearance rates.

Differences in clearance rates and the excretion of phytoplankton between the different *Microcystis*(including also the different size fractions) and *Planktothrix* strains were tested using a type III general linear model at the $P = 0.05$ level. Tukey was used as a post-hoc test for all pair-wise multiple comparisons. Prior to analysis, data were checked for normality and homogeneity of variance. All statistics were performed in SPSS 11.5 (SPSS Inc. 2002).

RESULTS

Microcystin

The *Microcystis* strain V40 contained microcystin-LR at a concentration of 76.6 μg g DW^{-1}, and (as expected) no microcystin was found in V131. The *Planktothrix* strain NIVA-CYA126 contained microcystin-LR at a concentration of 2581.7 μg g DW^{-1}. The NIVA-CYA116 did not contain any microcystin.

Clearance rate

In the control vessels, mean chlorophyll concentrations of all strains were constant during the grazing period. These data are shown in Table I. Because the chlorophyll concentrations in the control treatments did not change during the grazing period, the decreased concentrations of chlorophyll in the mussel treatments can be attributed to grazing.

Significant differences in clearance rates of zebra mussels were found between the different strains (Fig. 1, $F_{5,11} = 9.299$, $P < 0.05$). The Tukey test revealed that this was caused by a significant higher clearance rate on the *Planktothrix* strain CYA126 than on all other strains (Fig. 1, $P < 0.05$), despite the long filament length and high toxicity of this strain. Within the *Microcystis* treatments, no significant differences in clearance rates of zebra mussels on toxic and non-toxic *Microcystis* were found (Fig. 1, $F_{1,7} = 0.139$, $P > 0.05$). Zebra mussels also cleared the two different size fractions within a *Microcystis* strain at equal rates (Fig. 1, $F_{1,7} = 0.106$, $P > 0.05$). The observed power of the general linear model on the clearance rate data was 0.992. This suggests that the number of replicates was adequate.

Table I: Mean (±SE) chlorophyll concentrations (μg L^{-1}) of Microcystis (V40 and V131) and Planktothrix (CYA116 and CYA126) at the begin and end (1 hour later) in the control treatments of the grazing experiment

Strain	Begin	End
V40 <60 μm	2.92 (±0.01)	2.72 (±0.09)
V40 >60 μm	3.35 (±0.14)	2.71 (±0.02)
V131 <60 μm	9.81 (±0.08)	8.90 (±0.10)
V131 >60 μm	11.05 (±0.19)	10.41 (±0.15)
CYA116	5.86 (±0.11)	5.95 (±0.03)
CYA126	13.34 (±0.26)	14.02 (±0.33)

The *Microcystis* strains are further divided in size fractions >60 and <60 μm. *Microcystis* strain V40 and *Planktothrix* strain CYA126 contain microcystin-LR, whilst *Microcystis* strain V131 and *Planktothrix* strain CYA116 do not.

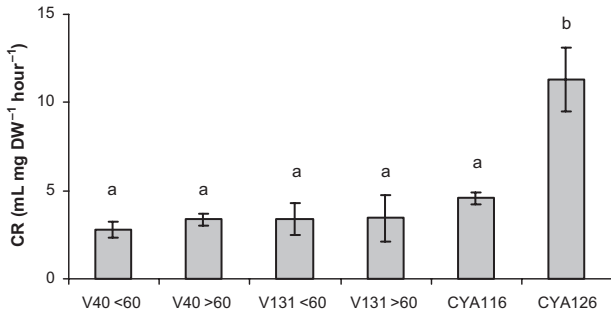

Fig. 1. Mean (±SE) clearance rate (mL mg DW^{-1} hour^{-1}) of zebra mussels on two species of cyanobacteria: colony forming *Microcystis aeruginosa* (V40 and V131) and filamentous *Planktothrix agardhii* (CYA116 and CYA126). The *Microcystis* strains are further divided in size fractions >60 and <60 μm. *Microcystis* strain V40 and *Planktothrix* strain CYA126 contain the toxin microcystin-LR, while *Microcystis* strain V131 and *Planktothrix* strain CYA116 do not. CYA126 forms the largest filaments of the two *Planktothrix* strains. Similar symbols (a, b) indicate homogeneous groups that are not statistically different at 95% level. CR, clearance rate; DW, dry weight.

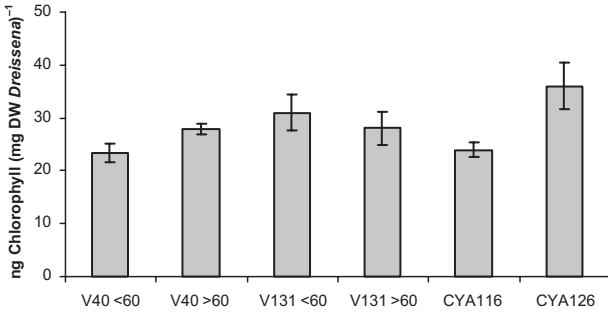

Fig. 2. Mean (±SE) excretion, as measured by chlorophyll content, of colony forming *Microcystis aeruginosa* (V40 and V131) and filamentous *Planktothrix agardhii* (CYA116 and CYA126) by zebra mussels. The *Microcystis* strains are further divided in size fractions >60 and <60 μm. *Microcystis* strain V40 and *Planktothrix* strain CYA126 contain microcystin-LR, whilst *Microcystis* strain V131 and *Planktothrix* strain CYA116 do not. CYA126 forms the largest filaments of the two *Planktothrix* strains. DW, dry weight.

Pseudofaeces

In the excreted products of the zebra mussels [(pseudo) faeces], no significant differences in chlorophyll concentrations between and within *Microcystis* and *Planktothrix* strains were found (Fig. 2, $F_{5,11} = 2.796$, $P > 0.05$). It made no difference whether the strains were toxic or differed in size and shape. The observed power of the general linear model on the chlorophyll concentrations in the excreted products was 0.609, which is not optimal but sufficient (Sokal and Rohlf, 1995).

DISCUSSION

This study is the first to describe grazing by zebra mussels on strains of *Microcystis* that maintain their capacity for colony formation when grown in the laboratory and that furthermore differ in toxicity. It is also the first study to report on grazing by *Dreissena* on toxic and non-toxic strains of *Planktothrix*, and the first to combine mussel grazing on both filamentous and colonial cyanobacteria in one study. Our hypothesis, that zebra mussels would graze less efficiently on toxic strains than on non-toxic ones is rejected since no significant differences were found. Also, size or shape of the food particles, be it filamentous or colonial did not affect filtration capacity of the mussels, i.e. the second and third hypothesis are rejected as well. Overall, the mussels showed a higher clearance rate on *Planktothrix* CYA126 than on the three other strains. This result is surprising at first sight considering the long filament length and the high toxicity of CYA126. It is known that zebra mussels are capable of collecting large particles (Ten Winkel and Davids, 1982) and taking up toxic cyanobacteria (Dionisio Pires *et al.*, 2004b), but this still does not explain why clearance on CYA126 exceeded that on the other strains. Differences in morphology that were not observed by us or a range of undetermined cellular compounds may have reduced clearance rates on the other strains. For instance, Jungmann (Jungmann, 1992) found that additional compounds, isolated from a microcystin-containing cyanobacterial species, were more toxic to daphnids than the actual microcystins.

In our experiments, we provided the mussels with a single type of food. We therefore did not study selective feeding of the mussels in the presence of alternative food (e.g. green algae or diatoms). One could argue that the mussels filtered the *Microcystis* and *Planktothrix* from the water simply because no choice was offered to them. However, in another study (Dionisio Pires *et al.*, 2004a; Dionisio Pires *et al.*, in press), we exposed mussels to natural seston and some of this seston included toxic *Microcystis* colonies (Dionisio Pires *et al.*, in press). In that study, the zebra mussels filtered the cyanobacteria from the water at equal rates to other phytoplankton and detritus. In some instances, the clearance rate on cyanobacteria was even higher than on other seston components (Dionisio Pires *et al.*, 2004a). Therefore, although we did not offer a variable food assemblage to the mussels in this study, there is no reason to assume that they would not filter these cyanobacteria if mixed with other phytoplankton.

Grazing on colony-forming *Microcystis*

Zebra mussels cleared both *Microcystis* strains at equal rates, regardless of toxin content and size. Vanderploeg *et al.* (Vanderploeg *et al.*, 2001) showed that zebra mussels filtered a <53 μm fraction of the non-toxic *Microcystis* strain CCAP 1450/11 at a higher rate than the >53 μm fraction, suggesting that size matters for the removal of particles

from the water by *Dreissena*. In the same study of Vanderploeg *et al.* (Vanderploeg *et al.*, 2001), zebra mussels showed filtration rates on the toxic unicellular *Microcystis* strain PCC 7820 that were as high as the rates on the non-toxic strain, suggesting that toxin content of cyanobacteria does not inhibit filtration, as it does for zooplankton (Lampert, 1981; DeMott *et al.*, 1991; Ferrão-Filho *et al.*, 2000). Also, Dionisio Pires and Van Donk (Dionisio Pires and Van Donk, 2002) found that the clearance rate of zebra mussels on single-celled, toxic *Microcystis* was as high as the clearance rate on single-celled, non-toxic *Microcystis*. Toxicity of *Microcystis* does therefore not seem to hamper zebra mussel filtration. In addition, Dionisio Pires (unpublished data) performed grazing experiments with zebra mussels, feeding on natural seston. Hereafter, the mussels were exposed for 7 days either to toxic *Microcystis* or to the heterokont alga *Nannochloropsis* (good food). After this long-term exposure, the zebra mussels were allowed again to feed on natural seston. The results showed that long-term exposure to toxic *Microcystis* did not affect the feeding behaviour of the mussels, since the clearance rates of the mussels on the different seston components, after these 7 days, were equal between the *Microcystis* and *Nannochloropsis* treatments. This implies that toxicity of *Microcystis* does not affect zebra mussel feeding behaviour over a longer term.

No differences in the excretion of toxic and non-toxic *Microcystis* strains by the mussels were found. The amount of cyanobacteria (i.e. chlorophyll concentrations) found in the pseudofaeces were low which indicates that *Microcystis* was actually ingested after filtration from the water. Vanderploeg *et al.* (Vanderploeg *et al.*, 2001) reported that zebra mussels rejected *Microcystis* colonies as pseudofaeces, which is contradictory to what was found in our study. An explanation may be that the cyanobacteria in the study of Vanderploeg *et al.* (Vanderploeg *et al.*, 2001) maintained their mucilage and perhaps therefore became less palatable. On the other hand, using natural seston containing colonies of *Microcystis*, Dionisio Pires *et al.* (Dionisio Pires *et al.*, in press) showed that the zebra mussels preferentially ingested the (toxic) cyanobacteria from the field.

Grazing on filamentous Planktothrix

Zebra mussels cleared the toxic *Planktothrix* strain at a higher rate than the non-toxic form and both strains were found in equal amounts in the pseudofaeces. Our results are in contradiction with the findings of McNaught and DeSorcie (McNaught and DeSorcie, 1993), although they used a different cyanobacterial species with different characteristics. These authors did not find differences in clearance rate of zebra mussels feeding on toxic and non-toxic *Anabaena flos-aquae* and the mussels produced more pseudofaeces when fed the toxic strain, suggesting more rejection of toxic than non-toxic strains after filtration. From their study however, it is unclear what type of toxins is produced by the *Anabaena* strain. *Anabaena* is capable of producing different types of toxin, including the hepatotoxic microcystin and neurotoxins (Sivonen and Jones, 1999). The differences between our strains and the *Anabaena* strain of McNaught and DeSorcie (McNaught and DeSorcie, 1993) make comparison of the grazing results difficult. As far as we know, our study and that of McNaught and DeSorcie (McNaught and DeSorcie, 1993) are the only studies on the grazing of mussels on filamentous cyanobacteria. *Anabaena flos-aquae* may even be considered spiral forming rather than filamentous, which makes our study the first on grazing of mussels on (toxic and non-toxic) filamentous cyanobacteria.

Surprisingly, we found no difference in excretion of the two *Planktothrix* strains. The toxic CYA126 had on average about 10 × the length of the CYA116. Our study contradicts the work of Ten Winkel and Davids (Ten Winkel and Davids, 1982), who showed that the electivity index (a measure for particle selection) of zebra mussels decreased with increasing algal length and was negative (i.e. rejection of particles before ingestion) for phytoplankton $\geq$ 60 μm. On the other hand, Horgan and Mills (Horgan and Mills, 1997) found that zebra mussels, feeding on seston from Oneida Lake, New York (USA), cleared particles between 0 and 150 μm with equal efficiency. The Oneida Lake seston included cyanobacteria like *Aphanizomenon* and *Anabaena*, which apparently did not interfere with grazing. Also Roditi *et al.* (Roditi *et al.*, 1996) found that zebra mussels removed all particle size classes of the Hudson River seston with equal efficiency.

Grazing studies on filamentous cyanobacteria are usually performed with zooplankton, especially *Daphnia* spp., because zooplankton species have been the classical filter-feeders for use in lake restoration (Gulati and Van Donk, 2002). These studies are however, not completely clear about whether daphnids can or cannot feed on filamentous cyanobacteria. In general, it is assumed that filaments clog at the feeding groove of the daphnids, making ingestion impossible (Hawkins and Lampert, 1989; Gliwicz and Lampert, 1990; Gliwicz, 1990). However, it has also been shown that daphnids can retard bloom formation of species as *Planktothrix* (Sarnelle, 1993). Gulati *et al.* (Gulati *et al.*, 2001) showed that *Daphnia galeata* had a higher clearance rate on the shorter filaments of *Oscillatoria limnetica*. Pattinson *et al.* (Pattinson *et al.*, 2003) found filamentous cyanobacteria like *Oscillatoria* spp. and *Lyngbya* spp. in the guts of three different species of daphnids. These mixed reports on the efficacy with which filter-feeding zooplankton is able to take up cyanobacteria were the direct impetus for our study on grazing by *Dreissena*.

Biomanipulation in the Netherlands (a comparison with daphnids) and impacts of mussels in the United States

In Lake IJsselmeer, the Netherlands, a reverse gradient exists between the occurrence of cyanobacteria and zebra mussels (Ibelings *et al.*, 2003). In Lake Veluwe, the Netherlands, the return of zebra mussels coinciding with a return of charophytes and a decrease in fish (bream) are believed to have been instrumental in a regime shift: after more than two decades of turbid water—during which period *Dreissena* was absent—Lake Veluwe returned to its former clear state (Lammens *et al.*, 2002). In the United States however, zebra mussel invasion is believed to have strengthened the dominance of cyanobacteria, especially of *Microcystis* (e.g. Makarewicz *et al.*, 1999). This effect was observed in lakes with a total dissolved phosphorous (TP) <25 μg P L^{-1} while no effect was observed when TP was >25 μg P L^{-1} (Raikow *et al.*, 2004). In Lake IJsselmeer, the TP concentrations are between 50 and 100 μg P L^{-1}. We performed our grazing experiments under nutrient rich conditions since shallow lakes in the Netherlands are still nutrient rich. Our results (mussels filtering cyanobacteria) are therefore in agreement with the findings of Raikow *et al.* (Raikow *et al.*, 2004) and also lend support to the idea that the observed gradient in cyanobacteria in Lake IJsselmeer is a result of mussel grazing. In Lake Veluwe, on the other hand, TP concentrations dropped to <10 μg P L^{-1} (from >500 μg L^{-1}) in the 1990s; return of zebra mussels did not promote growth of the (formerly) dominant cyanobacterial species *P. agardhii*. Perhaps in support of Raikow's findings, small *Microcystis* blooms have appeared in Lake Veluwe for the first time in its existence in the 1990s, i.e. after the return of the zebra mussels to the lake (Meijer *et al.*, in preparation).

Traditional biomanipulation measures aim at improving conditions in lakes for daphnids in order for these filter-feeders to exert a high grazing pressure on algae (Gulati and Van Donk, 2002). Although daphnids are held responsible for clear-water phases in spring, their density usually declines in summer, probably as a combination of fish predation (Sommer *et al.*, 1986) and bloom formation of cyanobacteria (Gliwicz, 1990). Size and toxicity of the cyanobacteria are two characteristics that explain the negative impact of these blooms on the daphnids (Gliwicz, 1990). In the summer of 2002, using seston from Lake IJsselmeer, we demonstrated that *Daphnia galeata* cleared moderately toxic cyanobacteria from Lake IJsselmeer as efficient as other seston groups, but in the summer of 2003, when the cyanobacteria were 3-fold more toxic, the feeding of this cladoceran on the smaller size fractions of cyanobacteria was reduced (Dionisio Pires *et al.*, in press). In the same study, *Dreissena* showed prefential selection of the cyanobacteria, regardless of the microcystin content. These results suggest that zebra mussels may be used as an alternative means for biomanipulation in cases where zooplankton grazing is suppressed. The results presented in this study support the role that *Dreissena* could play as a key grazer in shallow lakes in the recovery from eutrophication (Lammens *et al.*, 2002).

We conclude from this study that zebra mussels have no problems in filtering and ingesting multicellular cyanobacteria. This implies that for the removal of cyanobacteria in shallow lakes in the Netherlands, the mussels may be considered as a biofilter, perhaps more so than daphnids. We do not intend however, to promote the spreading of zebra mussels in lakes around the world and are well aware that *Dreissena* may cause severe economic and ecological problems (Nalepa and Schloesser, 1993). Rather, with respect to the use of *Dreissena* in biomanipulation, we only refer to shallow lakes in the Netherlands where the zebra mussels are usually not considered to be a nuisance by lake management (quite the opposite). For instance, in Lake IJsselmeer (a wetland of international importance under the Ramsar Convention) the mussels are the major food source for overwintering diving ducks (Van Eerden, 1998). If water managers would desire to use these bivalves as a biofilter, establishment of a higher biomass of zebra mussels may be achieved by providing hard substrate, like stones, for the settlement of mussel larvae (Reeders and Bij de Vaate, 1990). Noordhuis *et al.* (Noordhuis *et al.*, 1992) calculated that a density of about 500 mussels m^{-2} was required for light to penetrate to the bottom of small and medium-sized shallow waterbodies. Compared to the levels found in the United States and Canada (800 000 m^{-2}, Kovalak *et al.*, 1993), this seems to be a non-nuisance density, which may result in improved water clarity.

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