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## SHALLOW LAKES

# Functional response of *Anodonta anatina* feeding on a green alga and four strains of cyanobacteria, differing in shape, size and toxicity

Babette M. Bontes · Antonie M. Verschoor ·  
L. Miguel Dionisio Pires · Ellen van Donk ·  
Bas W. Ibelings

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**Abstract** We studied the functional response of the freshwater unionid bivalve *Anodonta anatina*, feeding on five phytoplankton strains differing in food quality: the small green alga *Scenedesmus obliquus*, a toxic and a non-toxic strain of the filamentous cyanobacterium *Planktothrix agardhii* and a toxic and a non-toxic strain of the coccoid cyanobacterium *Microcystis aeruginosa*. On *S. obliquus*, *A. anatina* had a type II functional response with a maximum mass-specific ingestion rate ( $IR_{max}$ ) of  $5.24 \text{ mg C g DW}^{-1} \text{ h}^{-1}$  and a maximum mass-specific clearance rate ( $CR_{max}$ )

of  $492 (\pm 38) \text{ ml g DW}^{-1} \text{ h}^{-1}$ , the highest values for all the phytoplankton strains that were investigated. On toxic and non-toxic *P. agardhii* filaments, *A. anatina* also had a type II functional response, but  $IR_{max}$  and  $CR_{max}$  were considerably lower ( $IR_{max}$  1.90 and  $1.56 \text{ mg C g DW}^{-1} \text{ h}^{-1}$ ;  $CR_{max}$   $387 (\pm 97)$  and  $429 (\pm 71) \text{ ml g DW}^{-1} \text{ h}^{-1}$ , respectively) than on *S. obliquus*. Toxicity of *P. agardhii* had no effect on the filtration rate of the mussels. On the non-toxic *M. aeruginosa* (small coccoid cells), we also observed a type II functional response, although a type I functional response fitted almost as good to these data. For the colonial and toxic *M. aeruginosa*, a type I functional response fitted best to the data: IR increased linearly with food concentration and CR remained constant.  $CR_{max}$  and  $IR_{max}$  values for the (colonial) toxic *M. aeruginosa* ( $383 (\pm 40) \text{ ml g DW}^{-1} \text{ h}^{-1}$ ;  $3.7 \text{ mg C g DW}^{-1} \text{ h}^{-1}$ ) demonstrated that *A. anatina* filtered and ingested this cyanobacterium as good as the other cyanobacterial strains. However, on the non-toxic *M. aeruginosa* we observed the lowest  $CR_{max}$  of all phytoplankters ( $246 (\pm 23) \text{ ml g DW}^{-1} \text{ h}^{-1}$ , whereas  $IR_{max}$  was similar to that on toxic *M. aeruginosa*. The high maximum ingestion rates on *S. obliquus* and *M. aeruginosa* indicate a short handling time of these phytoplankton species. The high clearance rates on *S. obliquus*, toxic *M. aeruginosa* and *P. agardhii* reflect a high effort of the mussels to filter these particles out of the water

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Shallow lakes in a changing world

B. M. Bontes · A. M. Verschoor ·  
L. M. Dionisio Pires · E. van Donk ·  
B. W. Ibelings  
Department of Food Web Studies, Centre for  
Limnology, Netherlands Institute of Ecology  
(NIOO-KNAW), P.O. Box 1299, 3600 BG Maarssen,  
The Netherlands

*Present Address:*

B. M. Bontes (✉)  
Royal Netherlands Institute of Sea Research (NIOZ),  
P.O. Box 59, 1790 AB Den Burg, The Netherlands  
e-mail: bontes@nioz.nl

L. M. Dionisio Pires  
Great Lakes Environmental Research Laboratory,  
NOAA, 2205 Commonwealth Blvd, Ann Arbor, MI  
48105-2945, USA

column at low concentrations. The low clearance rates on non-toxic *M. aeruginosa* may be explained by the small size and coccoid form of this cyanobacterium, which may have impaired *A. anatina* to efficiently capture the cells. Although *A. anatina* had relatively high maximum clearance rates on non-toxic and toxic *P. agardhii*, this cyanobacterium does not seem to be a good food source, because of the observed high rates of pseudofaeces production and hence low ingestion rates.

**Keywords** Bivalves · Cyanobacteria · Functional response · Grazing · Pseudofaeces · *Unionidae*

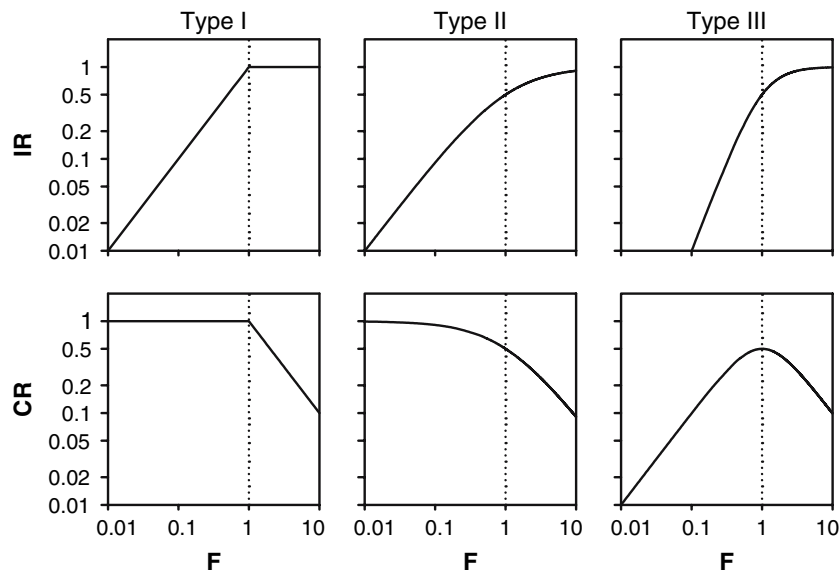
### Introduction

Shallow lakes in The Netherlands are inhabited by large freshwater bivalves of the family of Unionidae, such as *Anodonta anatina* L. (pond mussel). We were interested to see if *A. anatina* may contribute—like the zebra mussel *Dreissena polymorpha* Pallas (Reeders & Bij de Vaate, 1990)—to the improvement of water quality by reducing algal biomass and increasing water transparency. Enhanced grazing on phytoplankton may help shift shallow lake ecosystems with high and intermediate nutrient loading from a turbid, phytoplankton dominated, to a clear, water state dominated by macrophytes (Scheffer, 1998). One reason to predict a substantial impact of Unionid bivalve grazing on ecosystem processes is that, in marine and estuarine systems, similarly sized bivalves have been shown to have large ecosystem impacts on the benthic-pelagic coupling of energy cycles (Prins et al., 1998). A second reason to focus on Unionid species for the control of the effects of eutrophication is the potential risk of zebra mussel spreading in ecosystems. Although zebra mussels—with their high filtration rates on phytoplankton (Fahnenstiel et al., 1995)—have been promoted as a tool in biomanipulation of lakes in the Netherlands (Reeders & Bij de Vaate, 1990), they have also been shown to promote blooms of (toxic) cyanobacteria in North America (Vanderploeg et al., 2001). Moreover, we believe that for Dutch shallow lakes, with soft substrates, Unionids are

better adapted to these habitats than zebra mussels that require hard substrate for settlement.

We studied the feeding of *A. anatina* on cultures of the green alga *S. obliquus* Turp. Kütz., toxic and non-toxic strains of the filamentous cyanobacterium *P. agardhii* Gomont and colonial *M. aeruginosa* Kütz., each at five different concentrations to determine the functional response. The functional response is the relation between feeding rate and food density and as such may provide indications of underlying mechanisms, such as an increase in handling time related to the size of food particles. When knowing the functional response of *A. anatina* for a certain type of food, we know more about which mechanism may be limiting to the food uptake and hence we can predict how the grazer may respond to this food type in the field. In bivalves particles are first filtered from the water column. In the mantle cavity (where gills and palps are situated), ingestible particles are directed straight to the mouth, whereas unwanted particles are sorted on the gills and palps, embedded in mucus and then expelled as pseudofaeces (PF) (Dionisio Pires, 2005).

Because the functional response of *Anodonta* has never been analysed before, and different types of functional responses have been described in bivalve literature, we compared three basic types of functional responses (Holling, 1959; Hassell, 1978). The simplest type, type I (rectilinear) functional response, shows a linear increase of the ingestion rate (IR) with the food concentration, up to a concentration where the maximum amount of food that can be ingested by an organism is reached. This concentration is called the incipient limiting level (ILL: Rigler, 1961). Above ILL, the IR remains constant and maximal irrespective of food concentration. This implies that above ILL, further increases in cell concentration result in higher PF production rates (Sprung & Rose, 1988). The clearance rate (CR), a measure for the efficiency with which food is filtered from the water column, decreases accordingly (Fig. 1). Often, such a discontinuous transition at ILL is not observed, unless the food is filtered from the environment with negligible handling of the food. For many organisms, handling time of food is a limiting factor for the



**Fig. 1** Graphic representation of the three basic types of functional responses for ingestion rates (IR) and clearance rates (CR), respectively, as a function of food concentration (F) (solid lines). The dotted vertical lines indicate for Type I functional response the incipient limiting level (ILL), whereas they indicate the half-saturation concentration (K) for Type II and Type III functional responses. For comparability, all functional responses are scaled to a

maximum IR or CR of 1, and to and ILL or K of 1, on axes with arbitrary units. Therefore, for type I, IR is maximum at ILL, and (for types II and III) IR is at half its maximum value (0.5) at K. Note that—for comparability with experimental data—graphs are plotted on logarithmic scales, which makes it difficult to immediately recognize the sigmoid shape of the Type III functional response

amount of food that can be ingested. In a type II (hyperbolic, curvilinear) functional response, this is visible in CR, which decreases progressively towards zero with increasing food concentrations. Consequently, IR decelerates at higher food concentrations and goes asymptotically towards the maximum ingestion rate. With a type III functional response, IR accelerates more than linearly at low food densities, which for example may be the case with reward-dependent feeding behaviour. The initial CR will be 0, and maximal at intermediate food densities (Fig. 1). Because IR will still saturate (and thus decelerate) at higher food concentrations, as in a type II functional response, this will result in a sigmoidal (S-shaped) functional response (Fig. 1). With a type III functional response, CR will be maximal at the half-saturation food concentration K (Fig. 1).

To investigate the functional response of *A. anatina*, we chose the different food items based on differences in food quality (green algae vs. cyanobacteria), shape (filamentous, colonial or

single cells), and toxicity (toxic or non-toxic strains). Our null hypothesis (1) is that *A. anatina* has a negligible handling time of the food, meaning that it has a type I functional response. Therefore, (2) production of PF starts only above ILL and increases linearly with food concentration. We expect that *A. anatina* is able to discriminate between green algae and (often less nutritious) cyanobacteria so that: (3) CR on *S. obliquus* is higher than on all the cyanobacteria. Furthermore because retention efficiency is species specific and depends on particle size (Ward & Shumway, 2004); we expect that (4) small species like *M. aeruginosa* are more easily retained than large filaments of *P. agardhii*, which may congest the siphon. Finally (5) with respect to toxicity (i.e. microcystin-LR), CR and IR on microcystin producing cyanobacterial strains will be lower than on non-toxic strains. Our study adds new data on the functional response of the rarely studied freshwater bivalve *A. anatina* feeding on different food sources. These data are relevant for studies of behaviour and populations, as well as

for the possible application of these bivalves in biomanipulation of turbid shallow lakes.

## Materials and methods

### Grazing experiments

*Anodonta anatina* mussels were collected in spring 2005 in the Babbelaar (Lake Lauwersmeer, The Netherlands). Mussels were kept in aquaria filled with filtered lake water (0.45 µm, Lake Maarsseveen) on a layer (10 cm) of river sand (Aqua-colisa, Gemert-Bakel). Mussels were kept at 17–20°C, under a light: dark regime of 16:8 h and fed every day with *S. obliquus* CCAP276/3A. Water in the aquaria was replaced once a week. Five phytoplankton strains were used in the experiments: four cyanobacteria (*P. agardhii* strains CYA116 and CYA126 and *M. aeruginosa* strains V131 and V40) from exponentially growing batch cultures, and one green alga (*S. obliquus* CCAP276/3a), which was cultured in a chemostat. For size characteristics, origin and toxicity of the strains we refer to Table 1. Before the experiments, the *M. aeruginosa* strains were filtered over a 70 µm filter, to remove extremely large colonies.

Grazing experiments were performed in the laboratory, with *A. anatina* feeding on the five phytoplankton strains as single food sources. For each strain, the mussels were exposed to a range of five food concentrations (0.5; 1; 2; 5; 10 mg C L<sup>-1</sup>), with 5 replicates per strain per concentration. Food concentrations were based on regressions between ash-free dry weight of the strains (4 h at 550°C) and optical density at 750 nm (Helios-δ, Unicam, UK). Per grazing vessel, one mussel, with an average shell length between 8 and 10 cm and a shell width between 5 and 6 cm, was cleaned with a brush under running tap water to remove phytoplankton adhered to the shell and incubated in 2 l of the appropriate food concentration (suspended in 0.45 µm filtered lake water). The grazing vessels were 2 l batch systems and contained no sediment, as a pilot study showed that sediment absence did not affect the grazing behaviour of *A. anatina*.

Acclimation with the appropriate food concentrations started 24 h prior to the actual grazing

experiment (19–20°C; 10–15 µmol photons m<sup>-2</sup> s<sup>-1</sup>). Two hours before the grazing experiment food was added again to ensure that the mussels were well fed before the actual start of the experiment (Dionisio Pires et al., 2005). The 2 l were enough to prevent complete food depletion during the second acclimation and the grazing experiment period. Before the actual grazing period, the mussels were rinsed carefully under running tap water. We included duplicate vessels for each of the food concentrations without mussels as controls to correct for changes in the phytoplankton biomass other than those related to grazing. During the experiment the vessels were constantly mixed by aeration to keep food in suspension, without disturbing the mussels. Samples (15 ml) were taken from all vessels (both mussels and controls) at the start ( $T_{0 \text{ min}}$ ), before the introduction of the mussels, and at the end ( $T_{60 \text{ min}}$ ) of the experiment. After the grazing period the mussels were rinsed under running tap water and transferred to a vessel with 0.45 µm filtered lake water and samples were taken from these vessels after 30 min ( $T_{90 \text{ min}}$ ) to assess PF production. Samples were stored in the dark, until being measured at the end of the experiment. Measurements (chlorophyll concentrations (µg l<sup>-1</sup>)) were done using a PhytoPAM (Walz, Germany). Carbon concentrations of the phytoplankton were measured on a UniQuant Universal Carbon and Nitrogen Analyzer (Calanus, Finland). After the grazing period, the mussels were stored at -20°C. At a later stage, the shells were removed, the soft tissue was freeze-dried, and weighed.

### Determination of clearance rates, PF production rates and ingestion rates

Mass-specific clearance rates (CR, ml mg DW<sup>-1</sup> h<sup>-1</sup>) were determined from chlorophyll fluorescence measurements and calculated as follows (Coughlan, 1969):

$$CR = \frac{V}{nt} * \left\{ \ln \frac{A_0}{A_t} - \ln \frac{A'_0}{A'_t} \right\},$$

in which  $V$  is the volume in the grazing vessel (2,000 ml),  $n$  the dry weight of a single *A. anatina*

**Table 1** Characteristics of the phytoplankton strains used in the experiments

Strain code	Shape	Origin	Size and volume	Mean ( $\pm$ SE)	Range	Toxin
<i>Scenedesmus obliquus</i> CCAP276/3A	Fusiform, mainly single cells	Collection of algae, culture and protozoa Windermere, UK	Length ( $\mu\text{m}$ ) Width ( $\mu\text{m}$ ) Biovol ( $\mu\text{m}^3$ )	9.8 (1.6) 4.3 (2.2) 162.45 (20.2)	9.1–18.1 3.0–17.1 42.1–1,816.2	N.T.
<i>Planktothrix agardhii</i> CYA116	Multicellular filaments	Norwegian Institute of Water Research, Oslo, Norway	Length ( $\mu\text{m}$ ) Diameter ( $\mu\text{m}$ ) Biovol ( $\mu\text{m}^3$ )	426.0 (18.9) 3.7 (0.1) 4,683.2 (207.4)	38.3–889.5 3.1–4.5 286.8–14,138.9	N.T.
CYA126	Multicellular filaments		Length ( $\mu\text{m}$ ) Diameter ( $\mu\text{m}$ ) Biovol ( $\mu\text{m}^3$ )	830.8 (80.1) 3.8 (0.2) 9,438.0 (909.5)	100.3–2,345.2 3.1–4.5 754.8–37,295.6	Microcystin-LR 2,581.7 $\mu\text{g g DW}^{-1}$ (Dionisio Pires, 2005)
<i>Microcystis aeruginosa</i> V131	Coccioid single- and double cells	Culture collection University of Amsterdam; isolated from Lake Volkerak, The Netherlands.	Diameter ( $\mu\text{m}$ ) Biovol ( $\mu\text{m}^3$ )	6.5 (0.3) 164.4 (19.0)	4.1–10.2 33.5–523.6	N.T.
V40	Coccioid single cells & multicellular colonies		Diameter ( $\mu\text{m}$ ) Biovol ( $\mu\text{m}^3$ )	15.1 (10.0) 463.3 (19.8)	4.5–70.2 129.8–2,021.9	Microcystin-LR 76.6 $\mu\text{g g DW}^{-1}$ (Dionisio Pires, 2005)

The toxin column indicates the main type of toxin that has been found, and it's cellular concentration. N.T. = no toxins described for this strain in the literature

mussel (mg),  $t$  the duration of the experiment (in h),  $A_0$  the algae concentration ( $\mu\text{g}$  chlorophyll  $\text{l}^{-1}$ ) in the vessels with mussels at  $t = 0$ ,  $A_t$  the algal concentration in the vessels with mussels at time  $t$ ,  $A_0'$  and  $A_t'$  the concentration of algae in the control vessels at  $t = 0$  at time  $t$ , respectively. Note that each replicate contained one animal. The average algal carbon concentrations ( $\bar{A}$  mg C  $\text{l}^{-1}$ ) were determined by multiplying the geometric mean of initial and final algal concentrations with the slope ( $s$ ) of previously made regressions for each phytoplankton species between carbon and chlorophyll concentrations:

$$\bar{A} = s \cdot \sqrt{A_t \cdot A_0}$$

Slopes of the regressions between chlorophyll and carbon were all forced through 0, and all had  $r^2$  values higher than 0.99. The average algal carbon concentrations and clearance rates were used to calculate gross mass-specific ingestion rates (Lürling & Verschoor, 2003) ( $\text{IR}_g$ , mg C mg DW $^{-1}$  h $^{-1}$ ):

$$\text{IR}_g = \bar{A} \cdot \text{CR}$$

To calculate net ingestion rates, we first calculated mass-specific PF production rates (PPR, mg C mg DW $^{-1}$  h $^{-1}$ ) from the algal concentrations in the PF production treatments ( $A_P$ ):

$$\text{PPR} = \frac{s \cdot V \cdot A_P}{\Delta t \cdot n}$$

so that net mass-specific ingestion rates ( $\text{IR}$ , mg C mg DW $^{-1}$  h $^{-1}$ ) could be calculated by subtracting PPR from gross ingestion rates:

$$\text{IR} = \text{IR}_g - \text{PPR}$$

For ease of comparison among different treatments, individuals, and food concentrations, we also calculated PF production as a fraction of gross ingestion ( $F_P$ ):

$$F_P = \frac{\text{PPR}}{\text{IR}_g}$$

Because for one strain (*S. obliquus* CCAP276/3A) PF data were available for only three concentrations (0.5, 1, and 5 mg C  $\text{l}^{-1}$ ), we pooled the data for only these concentrations within each

phytoplankton strain. Due to non-normality and heteroscedasticity of these data, even after arcsin transformation, we applied nonparametric tests for comparisons among these strains. Overall differences of these fractions ( $F_P$ ) between the different strains were compared using the Kruskal–Wallis test, followed by pairwise (two-tailed) Mann–Whitney  $U$ -tests for comparisons between strains.

For *S. obliquus* CCAP276/3A, the original PF data were considered to be too limited in terms of number of data points and range of food concentrations to make reliable model fits of the functional response models. Therefore, the average of these  $F_P$  values was used to estimate the net ingestion rates from gross ingestion rates.

#### Fitting and intercomparison of functional response models

Functional response models were fitted by iterative nonlinear regression of functional response models on mass-specific ingestion and clearance rates. Because variance increased with food concentration, we minimized the squared residuals between  $\log_{10}$ -transformed observations and model predictions. The functional response model that best fitted to a particular data set (i.e.: leaving the smallest squared residuals) was used for comparisons between phytoplankton strains.

The type I (rectilinear) functional response fitting functions used were:

$$\begin{aligned} \text{if } \bar{A} < \text{ILL then } \text{IR} &= \text{IR}_{\max} \frac{\bar{A}}{\text{ILL}}, \\ \text{and if } \bar{A} \geq \text{ILL then } \text{IR} &= \text{IR}_{\max}, \end{aligned}$$

with ILL being the incipient limiting level: the food concentration where the ingestion rate becomes constant maximum, and  $\text{IR}_{\max}$  being the maximum ingestion rate. Clearance rates were fitted similarly, and for reasons of comparability, maximum clearance rates ( $\text{CR}_{\max}$ ) were calculated as derived parameters:

$$\begin{aligned} \text{if } \bar{A} < \text{ILL then } \text{CR} &= \frac{\text{IR}_{\max}}{\text{ILL}} = \text{CR}_{\max}, \\ \text{and if } \bar{A} \geq \text{ILL then } \text{CR} &= \frac{\text{IR}_{\max}}{\bar{A}} \end{aligned}$$

Type II (curvilinear) fitting functions used were

$$\text{IR} = \text{IR}_{\max} \frac{\bar{A}}{\bar{A} + K}, \quad \text{CR} = \frac{\text{IR}_{\max}}{\bar{A} + K}$$

$$\text{so } \text{CR}_{\max} = \frac{\text{IR}_{\max}}{K},$$

with  $K$  being the half-saturation food concentration at which half of  $\text{IR}_{\max}$  is reached, which is a measure for the initial slope and curviness of the functional response. With low  $K$ , the functional response starts steeply and saturates rapidly with increasing concentration, whereas at high  $K$ , the functional response rises and saturates slowly, and at very high  $K$  the functional response is practically linear.

Type III (sigmoid) fitting functions used were

$$\text{IR} = \text{IR}_{\max} \frac{\bar{A}^2}{\bar{A}^2 + K^2}, \quad \text{CR} = \text{IR}_{\max} \frac{\bar{A}}{\bar{A}^2 + K^2},$$

$$\text{so } \text{CR}_{\max} = \text{IR}_{\max} \frac{\text{IR}_{\max}}{2K},$$

with  $K$  being the half-saturation food concentration, as well as the inflection point of the sigmoid curve, and the food concentration at which  $\text{CR}_{\max}$  is reached.

For the comparisons of  $\text{CR}_{\max}$  and  $\text{IR}_{\max}$  between phytoplankton strains, we performed pairwise comparisons between each possible strain pair. Our null hypothesis ( $H_0$ ) for comparisons was that the separate functional response models were not significantly better predictors than when the model was fitted to the pooled observations for both strains. This hypothesis was compared against the alternative hypothesis ( $H_1$ ) that the separate models were better predictors, using the significance of the maximum likelihood ratio statistic  $G$  (also known as  $G^2$ , Bishop et al., 1975),

$$G = 2N \times \ln \left( \frac{\text{MS}_{\text{among}}}{\text{MS}_{\text{within}}} \right)$$

with  $N$  being the total number of observations (i.e. samples),  $\text{MS}_{\text{among}}$  the total variance of the residuals between the  $\log_{10}$ -transformed predicted and observed values of the alternative model ( $H_1$ ), and  $\text{MS}_{\text{within}}$  the variance of the residuals of the

null model ( $H_0$ ).  $G$  has a  $\chi^2$  distribution with degrees of freedom numbers corresponding to the difference between the numbers of parameters of the two models being compared.

## Results

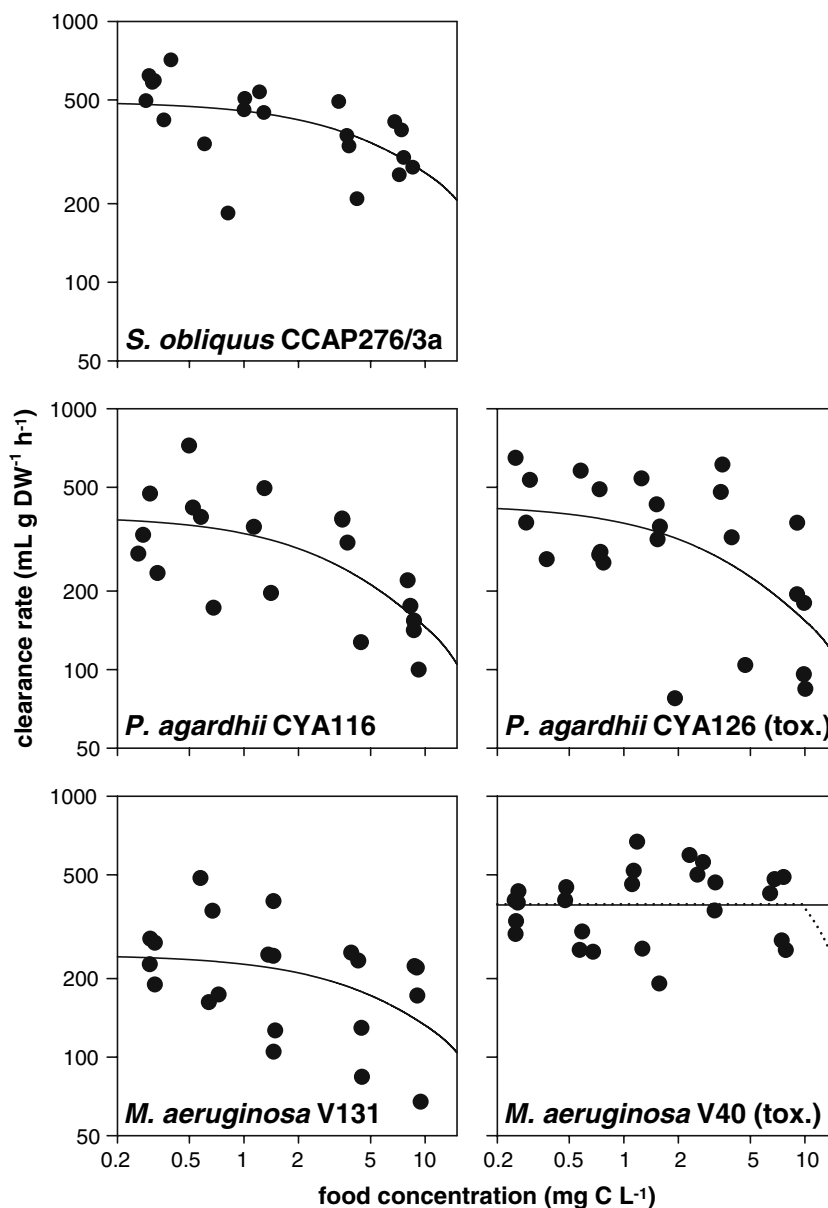
### Clearance rates

CR decreased with increasing food concentrations, except for toxic *M. aeruginosa* (V40) where the CR was essentially constant (Fig. 2). The residuals, indicating the goodness of fit of the different functional response models (Table 2, lower indicates better fit), showed that clearance rates on *S. obliquus* (CCAP276/3A), both *P. agardhii* strains (non-toxic CYA116 and toxic CYA126) and non-toxic *M. aeruginosa* (V131) yielded a type II response. For toxic *M. aeruginosa* both type I and II fitted equally well (similar residuals) (Table 2).  $\text{CR}_{\max}$  decreased in the order *S. obliquus* > toxic *P. agardhii* > non-toxic *P. agardhii* > toxic *M. aeruginosa* > non-toxic *M. aeruginosa* (Fig. 2, Table 3).  $K$  is extremely large ( $4.7 \times 10^{12}$ ) for toxic *M. aeruginosa* (Table 3), meaning no decrease in CR at higher concentrations, and indicating that a linear (type I) functional response applies here. However, for reasons of comparability between strains, we used the type II functional response model with this extreme value for  $K$ , which yields a practically linear function.  $K$  is high for non-toxic *M. aeruginosa* and *S. obliquus*, and low for non-toxic and toxic *P. agardhii* (Fig. 2, Table 3). The functional responses based on clearance rates were all significantly different from each other, except for between toxic and non-toxic *P. agardhii* (Table 4).

### Ingestion rates

On *S. obliquus* and both *P. agardhii* strains (non-toxic CYA116 and toxic CYA126), *A. anatina* had a type II response (Fig. 3 and Table 2), but for both *M. aeruginosa* strains (non-toxic V131 and toxic V40) a type I response was a better fit (smaller residuals for I than II; Table 2). For the type II functional response,  $K$  values as well as

**Fig. 2** Mass-specific clearance rates (CR) of *Anodonta anatina* grazing on different concentrations of five different phytoplankton strains. Food concentrations are geometric means of initial and final concentrations. Dots represent measured clearance rates, solid lines represent Type II functional response model regressions, dotted lines represent Type I functional response model regressions



$IR_{\max}$  decreased in the order toxic *M. aeruginosa* > non-toxic *M. aeruginosa* > *S. obliquus* > non-toxic *P. agardhii* > toxic *P. agardhii* (Table 3), meaning that handling times were shortest for both *M. aeruginosa* strains (non-toxic V131 and toxic V40). For both *M. aeruginosa* strains,  $K$  and  $IR_{\max}$  were smaller with type I than with type II functional responses, yet they were still larger than for non-toxic and toxic *P. agardhii*. Strains that differed significantly in their functional response based on CR also differed

significantly in their functional responses based on IR (Table 4), and if a type I functional response would be fitted on both *M. aeruginosa* strain data, these responses would still be significantly different (Table 4).

#### Pseudofaeces production

For all phytoplankton strains, PF production increased more than linearly with increasing food concentrations, and we did not observe absence



**Table 2** Residual sum of squares for the fits of the three different functional response models to data of clearance rates (CR) and ingestion rates (IR) of *Anodonta anatina* on five different phytoplankton strains

Strains	N	Type I (rectilinear)	Type II (curvilinear)	Type III (sigmoidal)
<b>CR</b>				
<i>S. obliquus</i> CCAP276/3A	21	0.4179	<b>0.3514</b>	1.264
<i>P. agardhii</i> CYA 116	20	0.9712	<b>0.5331</b>	1.902
<i>P. agardhii</i> CYA 126 (toxic)	23	1.7411	<b>1.1939</b>	1.753
<i>M. aeruginosa</i> V131	21	0.9196	<b>0.7656</b>	1.326
<i>M. aeruginosa</i> V40 (toxic) V40	25	<b>0.4538</b>	<b>0.4538</b>	1.113
<b>IR</b>				
<i>S. obliquus</i> CCAP276/3a <sup>a</sup>	12	2.0248	<b>0.3365</b>	0.5960
<i>S. obliquus</i> CCAP276/3A <sup>b</sup>	21	0.4179	<b>0.3514</b>	1.2644
<i>P. agardhii</i> CYA116	20	1.2203	<b>0.7187</b>	0.7685
<i>P. agardhii</i> CYA 126 (toxic)	23	3.2579	<b>2.5232</b>	2.8503
<i>M. aeruginosa</i> V131	21	1.5167	<b>1.502</b>	2.2005
<i>M. aeruginosa</i> V40 (toxic) V40	25	<b>0.4803</b>	0.4804	1.1123

Also given are the number of data points used per regression (N). Bold numbers indicate best fits

<sup>a</sup> Original data CCAP276/3A

<sup>b</sup> Estimated data CCAP276/3A (see Materials and Methods)

**Table 3** Parameter estimates of nonlinear regression of type II (curvilinear) and type I (rectilinear) functional response models of clearance and ingestion rates of *Anodonta anatina* on five different phytoplankton strains

	N	Clearance rates		Ingestion rates	
		CR <sub>max</sub>	K	IR <sub>max</sub>	K
Type II					
<i>S. obliquus</i> CCAP276/3A	21	492.70	11.44	5.24	11.44
<i>P. agardhii</i> CYA116	20	387.42	6.04	1.90	5.59
<i>P. agardhii</i> CYA126 (toxic)	23	429.27	5.60	1.56	4.51
<i>M. aeruginosa</i> V131	21	246.58	11.60	7.48	48.24
<i>M. aeruginosa</i> V40 (toxic)	25	382.92	$4.77 \times 10^{12}$	$4.23 \times 10^3$	$1.14 \times 10^4$
Type I					
<i>M. aeruginosa</i> V131	21	199.24	17.89	3.55	24.32
<i>M. aeruginosa</i> V40 (toxic)	25	382.92	9.74	3.73	10.01

Given are N, the number of data points used per regression; CR<sub>max</sub>, the estimated maximum mass-specific clearance rates (ml g DW<sup>-1</sup> h<sup>-1</sup>); K, (in case of type II) the half-saturation food concentration (mg C l<sup>-1</sup>), or (in case of type I) the incipient limiting food concentration (ILL, also mg C l<sup>-1</sup>); and IR<sub>max</sub>, the estimated maximum ingestion rates (mg C g DW<sup>-1</sup> h<sup>-1</sup>)

of PF production at low food levels (i.e. below the incipient limiting level). The Kruskal–Wallis test revealed significant differences in PPR by *A. anatina*, when grazing on the five different strains ( $p < 0.0001$ ,  $H = 30.41$ , d.f. = 4,  $N = 64$ ). Pairwise comparisons (Mann–Whitney *U*-test) showed that *A. anatina* had significantly lower PPR for toxic *M. aeruginosa*, compared to the other cyanobacterial strains ( $p < 0.05$ ), but PPR was not significantly different from *S. obliquus* ( $p > 0.05$ , Fig. 4). Significantly higher PPR were found for non-toxic *M. aeruginosa* than for *S. obliquus* ( $p < 0.05$ ).

## Discussion

The type I (rectilinear) functional response is often thought to be applicable for ‘true’ filter feeders such as bivalves, which is illustrated by the frequent use of the term ‘incipient limiting level’ in the literature. Unfortunately, such terminology is often not supported by a full functional response analysis. In our experiments, we found that *A. anatina* had a type II (curvilinear, hyperbolic) functional response on 4 out of 5 investigated phytoplankton strains, indicating that the handling time of the food plays an

**Table 4** Pairwise comparisons of the best-fitting functional response models of clearance and ingestion rates of *A. anatina* on five different phytoplankton strains

Strain code	CYA116 <i>P. agardhii</i>		CYA126 <i>P. agardhii</i> (toxic)		V131 <i>M. aeruginosa</i>	V40 <i>M. aeruginosa</i> (toxic)		
Clearance rates (type II)								
<i>S. obliquus</i> CCAP276/3A	24.399	<b>[&lt;0.001]</b>	11.699	<b>[0.003]</b>	51.372	<b>[&lt;0.001]</b>	9.648	<b>[0.008]</b>
<i>P. agardhii</i> CYA116			0.685	[0.710]	12.172	<b>[0.002]</b>	32.424	<b>[&lt;0.001]</b>
<i>P. agardhii</i> CYA126 (toxic)					14.318	<b>[&lt;0.001]</b>	19.414	<b>[&lt;0.001]</b>
<i>M. aeruginosa</i> V131							52.588	<b>[&lt;0.001]</b>
Ingestion rates (type II)								
<i>S. obliquus</i> CCAP276/3A <sup>a</sup>	8.913	<b>[0.012]</b>	4.683	[0.096]	32.333	<b>[&lt;0.001]</b>	5.429	[0.066]
<i>S. obliquus</i> CCAP276/3A <sup>b</sup>	28.575	<b>[&lt;0.001]</b>	16.501	<b>[&lt;0.001]</b>	55.830	<b>[&lt;0.001]</b>	7.946	<b>[0.019]</b>
<i>P. agardhii</i> CYA116			0.244	[0.885]	18.538	<b>[&lt;0.001]</b>	38.294	<b>[&lt;0.001]</b>
<i>P. agardhii</i> CYA126 (toxic)					11.792	<b>[0.003]</b>	23.298	<b>[&lt;0.001]</b>
<i>M. aeruginosa</i> V131							60.600	<b>[&lt;0.001]</b>
Ingestion rates (type I)								
<i>M. aeruginosa</i> V131							56.863	<b>[&lt;0.001]</b>

Shown are the values of the maximum likelihood ratio statistic ( $G$ ) and corresponding  $p$ -values [in square brackets]. Model fits were considered to be significantly different when  $p < 0.05$ , shown in bold, indicating that the separate models were better predictors than when fitting a single model through the pooled observations of these pairs

<sup>a</sup> Original data CCAP276/3A

<sup>b</sup> Estimated data CCAP276/3A (see Materials and methods section)

important role in the ingestion process. Therefore, we reject hypothesis 1 (clearance and ingestion rates are according to a type I functional response) for all strains except for the toxic *M. aeruginosa* (V40).

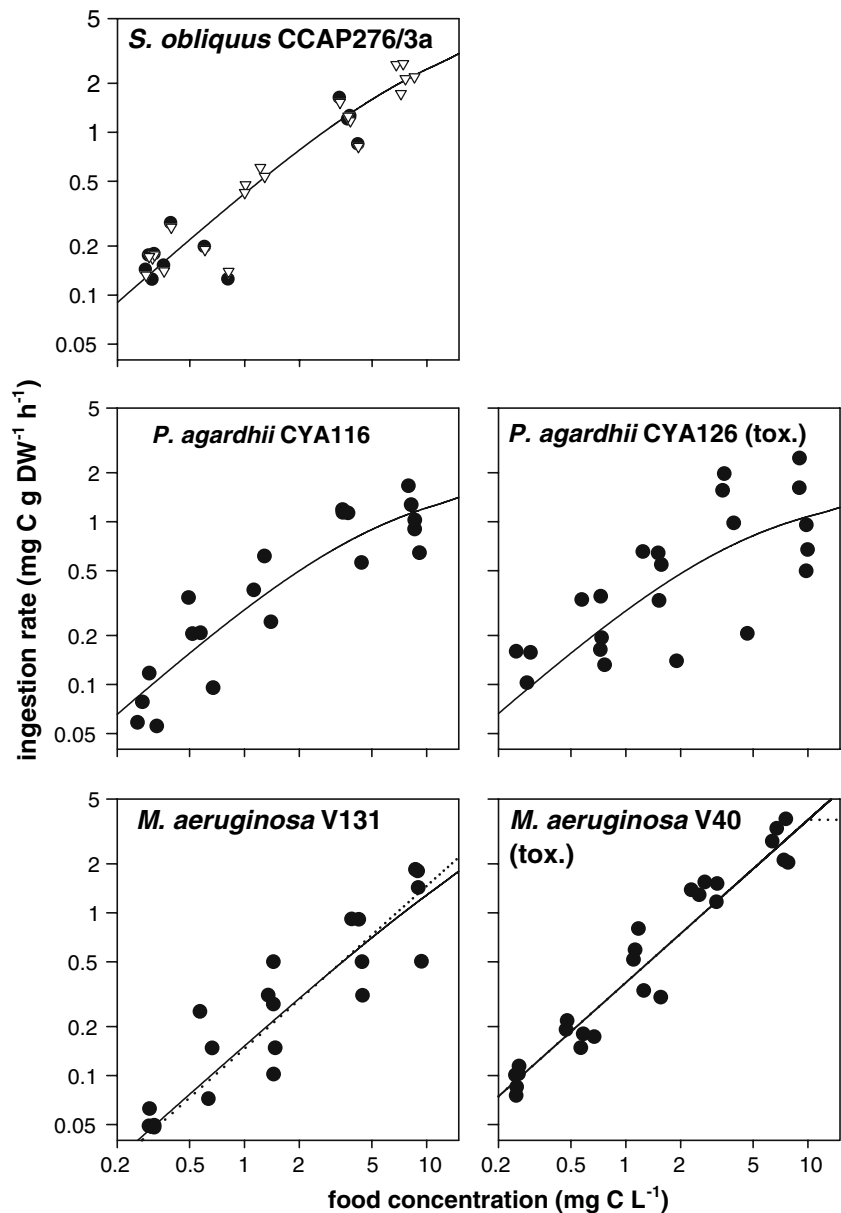
The striking exception to the observed functional responses is the CR on the toxic *M. aeruginosa*, both on the basis of the functional response type (type I) and our parameter estimates. These suggest that toxic *M. aeruginosa* should be very small but in fact this strain consists of single cells and intermediately sized colonies (Table 1). Although these colonies are easily retained on the gills, maybe even better than the smaller sized single cells, ingestion of such particles would still involve some handling time. Visual observations of the colonies of toxic *M. aeruginosa* showed that these are not solid and packed in a thick mucus layer, as is often observed in large *M. aeruginosa* colonies but loose aggregates that disintegrate easily. It is not difficult to imagine that such colonies could disintegrate due to shear forces in the feeding process. Thus, the actual size of the particles once ingested could be much smaller, as small as single cells, leading to very short handling times. The combination of easy capture on the gills (of intermediately sized

aggregates) and easy ingestion (of small cells) could thus explain why we found a type I functional response of *A. anatina* for this strain.

We did not observe absence of PF production at low food levels (i.e. below the incipient limiting level), so that hypothesis 2 is rejected as well. Since PF production increased more than linearly with food concentration, this again indicates that a type II functional response is more appropriate for the feeding behaviour of *A. anatina*. The differences in fractions of PF production relative to gross ingestion (Fig. 4) suggest that *A. anatina* is able to discriminate between food sources by varying the degree of excretion (Sprung & Rose, 1988). The high fractions of PF production for non-toxic *M. aeruginosa* (V131) (30% of gross ingestion) and both *P. agardhii* strains (non-toxic CYA116 and toxic CYA126) (15–20% of gross ingestion) indicate that these strains were the least preferred. Both PF and feeding data show that *S. obliquus* (CCAP276/3A) is the preferred food type, which is in concordance with our hypothesis 3.

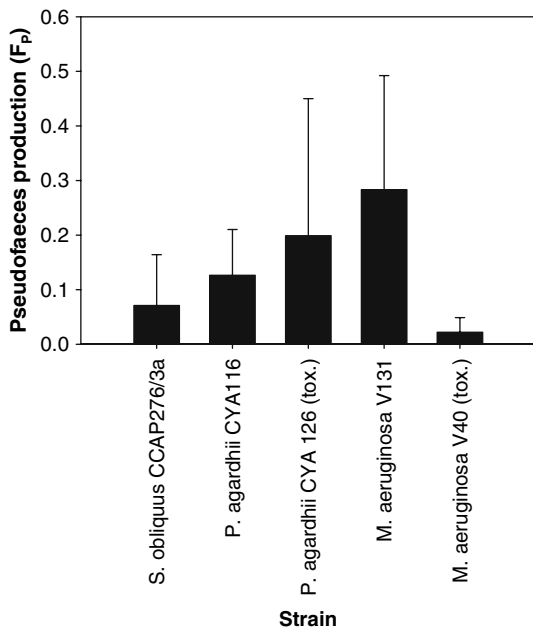
Hypothesis 4, coccal/colonial *M. aeruginosa* is preferred over filamentous *P. agardhii*, is less easy to accept or reject. Maximum clearance rates were higher on both non-toxic and toxic

**Fig. 3** Mass-specific ingestion rates (IR) of *Anodonta anatina* grazing on different concentrations of five different phytoplankton strains. Food concentrations are geometric means of initial and final concentrations. Dots represent measured ingestion rates, white triangles are estimated ingestion rates (see Materials and methods), solid lines represent Type II functional response model regressions, dotted lines represent Type I functional response model regressions



filamentous *P. agardhii* than on non-toxic *M. aeruginosa*, and equal to toxic *M. aeruginosa*, showing that *P. agardhii* is filtered at least equally efficient from the water column at low food densities. On the other hand, maximum ingestion rates were higher for both non-toxic and toxic *M. aeruginosa*, which indicates that *M. aeruginosa* is preferred over *Planktothrix* at high food densities. Probably the higher maximum clearance rates on both *P. agardhii* strains are

artefacts caused by the larger size of these algae, which makes it more likely that they are filtered by chance. The high PF production on non-toxic and toxic *P. agardhii* species suggests that they are not very suitable food sources. At higher concentrations, long handling times of the filaments rapidly becomes a serious limiting factor in the ingestion process, which is visible in the curviness of the functional response (Figs. 2, 3, low  $K$  values in Table 3). With *M. aeruginosa*,



**Fig. 4** Fraction of PF production relative to gross ingestion ( $F_p$ ) of *Anodonta anatina* grazing on five different phytoplankton strains. Bars represent averages, error bars represent standard deviations

however, the functional responses did not reach satiation (Figs. 2, 3).

Thus, at very high food concentrations, the feeding of *A. anatina* was not limited anymore by the amount of food in the environment, but solely by the handling time of the food and the estimated maximum ingestion rates were the inverse of the handling time of the food. For the filamentous *P. agardhii* we expected long handling times (e.g. due to clogging of the siphon), and indeed this species yielded the lowest maximum ingestion rates of all (Table 3). In our study, biovolume (Table 1) was inversely related to the maximum ingestion rate (Table 3), which shows that food particle size can be used as a predictor of feeding rates.

Contrary to our hypothesis 5, toxicity did seem not seem to affect the grazing behaviour of *A. anatina*. For *M. aeruginosa*, the toxic strain is preferred over the non-toxic strain. Moreover, the higher maximum ingestion rate of the toxic *M. aeruginosa*, as compared to *S. obliquus*, suggests that toxin production did not interfere with filtration and ingestion. Previous findings describe

that mussels are relatively insensitive to microcystin producing cyanobacteria (e.g. Dionisio Pires et al., 2004). We are unable to distinguish the effects of size (toxic *P. agardhii* twice as long as non-toxic *P. agardhii* (Table 1)) and toxicity of the toxic *P. agardhii* on the lower ingestion rates by *A. anatina* on this strain as compared to the non-toxic *P. agardhii* strain. However, because we did not find a significant effect of microcystin on the feeding behaviour of *A. anatina* in the *Microcystis* treatments, it seems more plausible that size rather than toxin caused the lower ingestion rate of *A. anatina* on the toxic *Planktothrix* strain.

For the discussion on the applicability of *A. anatina* as a biofilter, we compare the filtration rates of *A. anatina* (mean dry weight: 3.5 g) with that of *Corbicula leana* in the field (Hwang et al., 2004) and *D. polymorpha* in the laboratory (Dionisio Pires, 2005). Hwang et al. (2004) showed in the hypertrophic Lake Ilgam, ( $3 \times 10^5$ – $8 \times 10^5$  cells  $\text{ml}^{-1}$  of *M. aeruginosa* and  $0.6$ – $2.8$  mg C  $\text{l}^{-1}$  of *P. agardhii*) that *C. leana* filtered  $1.6$ – $7.8$  l mussel $^{-1}$  day $^{-1}$ . Furthermore, field experiments with *D. polymorpha* by Reenders & Bij de Vaate (1990) in a Dutch lake revealed a filtration rate of  $1.2$  l mussel $^{-1}$  day $^{-1}$  (with a shell length of 18 mm). Comparing grazing capacities per unit biomass when offering the same phytoplankton species (concentration  $2$  mg C  $\text{l}^{-1}$ ), we found that *A. anatina* cleared  $0.38$ ;  $0.22$ ;  $0.34$ ;  $0.22$  and  $0.42$  ml  $\text{mg}^{-1}$  h $^{-1}$ , and *D. polymorpha* (mean individual dry weight of 9 mg; Dionisio Pires, 2005)  $4.0$ ;  $4.6$ ;  $11.3$ ;  $3.4$  and  $3.1$  ml  $\text{mg}^{-1}$  h $^{-1}$  of *S. obliquus* (CCAP276/3a), non-toxic- and toxic *P. agardhii* (CYA116 and CYA126) and non-toxic- and toxic *M. aeruginosa* ( $<60$   $\mu\text{m}$ ) (V131 and V40), respectively. This means that for the phytoplankton strains used in this study, the grazing capacity, on a weight-specific basis, of *D. polymorpha* is  $\sim 7$ – $33$  times higher than that of *A. anatina*. Despite the low clearance per unit of biomass, the grazing capacities per individual of *A. anatina* per day ( $\text{CR}_{\text{max}}$  of  $42.6$ ;  $33.5$ ;  $37.1$ ;  $21.3$  and  $33.1$   $\text{l}^{-1}$  day $^{-1}$  of *S. obliquus*, non-toxic *P. agardhii*; toxic *P. agardhii*, non-toxic *M. aeruginosa* and toxic *M. aeruginosa*, respectively) were comparably high with those of marine

bivalves (*M. edulis* 35.3 l mussel<sup>-1</sup> day<sup>-1</sup> and *Cerastoderma edule* (Linnaeus, 1758) 31.2 l mussel<sup>-1</sup> day<sup>-1</sup>; Ward & Shumway, 2004). On a weight-specific basis, the grazing capacity of *Anodonta* is low compared to mussel species as *D. polymorpha*. However, per individual, *Anodonta*'s grazing capacity is equal or higher than other species.

The current abundance of Unionid bivalves in the field (0–5 mussels or 17,500 mg DW m<sup>-2</sup>, as found in a field study in four shallow lakes in The Netherlands during 2005, unpublished data) however, is too low for *A. anatina* to have a strong effect on algal biomass. In contrast, in high density areas of Lake IJsselmeer and Lake Markermeer, *D. polymorpha* has been described to remove ±70% of the algal population (Lamens, 1999). When we take into account the number of 1,000 individuals per m<sup>-2</sup> of *Dreissena* observed in Lake IJsselmeer (Bij de Vaate, 1991), an average individual DW for *D. polymorpha* and *A. anatina* of 9 and 3,600 mg respectively, and the grazing rates for *Anodonta* found in this study and for *Dreissena* found in Dionisio Pires (2005), we would need 83 *Anodonta* mussels per m<sup>-2</sup> to clear the same volume of water from e.g. the toxic *P. agardhii* (CYA126) (at a food density of 2 mg C l<sup>-1</sup>) as would 1,000 *Dreissena* mussels. However, densities of Unionid mussels in the field appears to be low (unpublished data). On the other hand, the observed high densities of *D. polymorpha* (individuals per m<sup>-2</sup>) 500–1,000 and 400–1,000 individuals per m<sup>-2</sup> in Lake IJsselmeer and Lake Markermeer, respectively (Bij de Vaate, 1991); 30,000 individuals per m<sup>-2</sup> in Lake Zürich; 20,000 individuals per m<sup>-2</sup> in Lake Garda and 21,000 individuals per m<sup>-2</sup> in Lake Constance (Burla & Lubini-Ferlin, 1976; Franchini, 1978 and Suter, 1982 in Bij de Vaate, 1991) make it more likely that these smaller mussels have a larger effect on the primary productivity and a more realistic achievement for biomanipulation. If *A. anatina* is to be used as a biofilter in shallow Dutch lakes, its densities must be promoted. Current densities may be low because after years of eutrophication the stability of the sediments has changed, providing unsuitable habitat for these mussels.

## Summary and conclusion

From this study we conclude that *A. anatina* mussels can filter and ingest colonial as well as larger filamentous cyanobacteria. This observation indicates that *A. anatina* may be useful as a biofilter even when cyanobacteria blooms are toxic, provided that densities of these mussels in the field are increased through suitable restoration measures (to be developed).

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