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APPLIED ISSUES

Selective grazing by adults and larvae of the zebra mussel (*Dreissena polymorpha*): application of flow cytometry to natural seston

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SUMMARY

1. Selective grazing of adults and larvae of the zebra mussel (*Dreissena polymorpha*) on phytoplankton and detritus from both laboratory cultures and natural seston was quantified using flow cytometry.
2. Mean clearance rate of adult zebra mussels was higher on a mixture of the green alga *Scenedesmus* and the cyanobacterium *Microcystis* than when *Scenedesmus* was offered as single food, suggesting selective feeding by the mussels.
3. Feeding on lake seston both adults and larvae showed a higher clearance rate on phytoplankton than on detritus particles, suggesting that zebra mussels select for phytoplankton. Furthermore, it was noted that adults preferred seston particles in the 0–1 and 30–100 μm size ranges.
4. In our study, zebra mussels did not discriminate against cyanobacteria, and our results indicate that they may even ingest them preferentially.

Keywords: biomanipulation, clearance rate, EurOPA, phytoplankton, toxic *Microcystis aeruginosa*

Introduction

Water quality management in the Netherlands is often concerned with controlling cyanobacterial blooms (Hosper, 1997) that occur mainly in eutrophic lake systems (Mur, Skulberg & Utkilen, 1999). A number of studies have shown that cyanobacterial blooms can have an enormous impact on aquatic ecosystem health and wildlife (e.g. Sivonen & Jones, 1999) and even human health (e.g. Chorus & Bartram, 1999). Removal by grazers is one way of reducing the negative effects of cyanobacterial blooms (Reeders & Bij de Vaate, 1990; Reeders, Bij de Vaate & Noordhuis, 1993). Grazing studies have provided insight into the effectiveness of filter feeders in the removal of cyanobacteria and other phytoplankton particles (Lampert, 1987).

Selection by grazers for good or poor quality food is an important topic in grazing studies on phytoplankton (Richman & Dodson, 1983; DeMott, 1999). Differences in food quality are usually attributed to differences in polyunsaturated fatty acids (PUFAs) (DeMott & Müller-Navarra, 1997; Von Elert & Stampfl, 2000), phosphorus limitation (Gulati & DeMott, 1997) and toxicity (Engström, Viherluoto & Viitasalo, 2001). PUFAs are generally found in higher concentrations in green algae compared with cyanobacteria (Ahlgren, Gustafsson & Boberg, 1992; Vanderploeg, Liebig & Gluck, 1996) and certain cyanobacteria taxa may be toxic (Chorus & Bartram, 1999). Hence, mixtures of a green alga (considered as high quality food) and a cyanobacterium (considered as poor quality food) have been used in many studies on selective grazing (e.g. DeMott, 1999). However, the possibility of

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distinguishing the food quality of the two phytoplankton groups under field conditions remains limited (e.g. Richman & Dodson, 1983).

In the last decade, several techniques have been used in grazing experiments to distinguish the food quality of different phytoplankton groups. All techniques have, however, their inherent limitations (Cucci *et al*., 1989). For example, light microscopy (e.g. Zhang, Watanabe & Inouye, 1996) is time-consuming and quantification of detritus is difficult using this technique. Coulter Counter measurements, on the other hand, are rapid, but cannot distinguish between phytoplankton groups and detritus within the same size range (Cucci *et al*., 1989). Some studies have used radioactive labelling, and this method is considered as a precise and accurate method for the determination of zooplankton feeding rates on laboratory cultures (e.g. Lampert, 1981). Recently, the PhytoPAM has been shown to be a useful tool for grazing experiments with mixtures of different phytoplankton species (Dionisio Pires & Van Donk, 2002). This method allows for the quantitative differentiation between cyanobacteria, green algae and diatoms using chlorophyll fluorescence of specific pigments (Dionisio Pires & Van Donk, 2002; Dionisio Pires, Kusserow & Van Donk, 2003). However, one disadvantage of the PhytoPAM technique is that samples cannot be preserved, but need to be analysed immediately. Furthermore, the PhytoPAM is probably not useful for measuring grazing rates on natural seston because it does not detect non-fluorescent (i.e. non-living detritus) particles. Using flow cytometry it is possible to measure selection on different types of natural particles (Hofstraat *et al*., 1994).

Flow cytometry has been developed especially for biomedical applications, such as immunofluorescence and analyses of DNA and RNA (Ormerod, 1994). On a smaller scale, it has found its application in aquatic ecology, e.g. in the study of cell physiology, phycology and cell counting [see Collier (2000) for a review]. The majority of the studies using flow cytometry are from marine systems (Li, 1997, 2002; Partensky, Hess & Vaulot, 1999; Legendre, Courties & Troussellier, 2001). In The Netherlands a flow cytometer, The Optical Plankton Analyzer (OPA), was developed for the analysis of different phytoplankton groups and for natural seston (Dubelaar *et al*., 1989; Peeters *et al*., 1989; Balfoort *et al*., 1992; Hofstraat *et al*., 1994). For the assessment of algal grazing by filter feeders, flow cytometry could be useful because the technique combines high discriminative power with quick and quantitative analysis of large numbers of particles (Collier, 2000).

In this study, we assessed selective grazing of both adult and veliger larvae zebra mussels [*Dreissena polymorpha* (Pallas)] on phytoplankton and detritus from both laboratory cultures and natural seston. Zebra mussels are among the most important filter-feeding grazers in Dutch lakes, and they have been considered as a useful tool in the restoration of eutrophic systems by biomanipulation (Reeders & Bij de Vaate, 1990; Reeders *et al*., 1993). Earlier studies have, however, shown that grazing rates depend on food quality. For example, Shumway & Cucci (1987) found that the soft shell clam *Mya arenaria* (Linnaeus) and the ribbed mussel *Geukensia demissa* (Dillwyn) had reduced clearance rates when exposed to toxic dinoflagellates. As poor food quality has been shown to have negative effects on clearance (Stoeckmann, 2003), we hypothesise that mussels will have lower clearance rates when toxic cyanobacteria are present in the food. Ward *et al.* (1997) showed that pseudofaeces of the oysters *Crassostrea gigas* (Thunberg) and *C. virginica* (Gmelin) were enriched with detritus, indicating selection for live food. Our second hypothesis is therefore that, when offered natural seston, clearance rates of the mussels will be higher for phytoplankton than for detritus. For distinguishing between different particles we used an Optical Plankton Analyzer, the EurOPA (Jonker *et al*., 1995). This flow cytometer cannot discriminate between diatoms and green algae, therefore, only distinctions between cyanobacteria and other phytoplankton are reported here.

Methods

Collection and maintenance of D. polymorpha

Zebra mussels were provided by the State Institute for Inland Water Management and Waste Water Treatment (RIZA, Lelystad, The Netherlands) on 6 February 2001. The mussels were collected with a trawl from Lake IJsselmeer and kept at 4 °C until the next day when they were transported to the Centre for Limnology. Upon arrival, the mussels (approximately 250 individuals) were immediately transferred to 550 L flow-through aquaria containing 400 L of copper-free water. A bacterial filter, consisting of aqua

gravel (Velda B.V., Enschede, The Netherlands) and lava stone (Eheim GmbH & Co. KG, Diezisau, Germany) ensured constant cleaning of aquaria water. The animals were maintained on a diet of the green alga *Scenedesmus obliquus* [(Turpin) Kützing] fed daily at approximately 2 mg C L^{-1}, and twice weekly the mussels were fed 'Algae Diet C-6', a concentrated culture of *Thalassiosira pseudonana* (Hasle and Heimdal), *Skeletonema* sp., *Chaetoceros calcitrans* (Paulsen) and *Isochrysis galbana* (Parke) (Coast Seafoods Co., South Bend, WA, U.S.A.). A 16 : 8 h light : dark cycle was used, and water temperature was held at 12 °C.

Phytoplankton cultures

Grazing studies were done using the laboratory-reared green alga *S. obliquus* and the cyanobacterium *Microcystis aeruginosa* (Kützing). The *Scenedesmus* strain (CCAP 276/3A; cell diameter size: 4.7 μm) was provided by the Culture Collection of Algae and Protozoa. *M. aeruginosa* [NIVA-CYA 140 (toxic, microcystin-LR: 5091 μg L^{-1}_{medium}); cell diameter size: 3.8 μm] was provided by the Norwegian Institute for Water Research. Both phytoplankton species were cultured as single cells in WC medium (Guillard & Lorenzen, 1972, as modified by Lürling (1999)) in chemostat cultures [1 L, 20 ± 1 °C, 7 μmol s^{-1} m^{-2}, dilution rates: 0.2 day^{-1} (*Microcystis*) and 1 day^{-1} (*Scenedesmus*)].

Grazing on cultured phytoplankton

Adult zebra mussels, measuring 18–22 mm shell length, were used in the grazing experiments. One week before the start of the experiments, 50 mussels were transferred to a microcosm with 3 L synthetic freshwater medium (Ralph Tollrian medium; Tollrian, 1993) and fed daily with *S. obliquus*. The 22 of the 50 animals that were used for the grazing experiments were acclimated to the experimental setup for 24 h. The animals were placed individually in 250 mL vessels (Schott Duran®, Mainz, Germany) filled with 200 mL medium of food (*Scenedesmus* or a mixture of *Scenedesmus* and *Microcystis*) and placed on a shaker at 50 rotations per minute to keep the food in suspension. Visual observations showed that the mussels quickly attached themselves and were seemingly undisturbed by the shaking. Before being transferred to the grazing vessels, the mussels were gently cleaned with a brush under running de-ionised water. Eleven mussels were placed in medium with food for the grazing measurements and the remaining eleven mussels were placed in medium without food to correct for expelled food particles from the acclimation period. Another 11 vessels with food, but without mussels, were used as a control to check for growth of phytoplankton cells during the experimental period. A low light intensity was used (<5 μmol s^{-1} m^{-2}). The grazing experiment lasted 8 h, during which time 5 mL water samples were taken at $t = 0$, 0.5, 1, 2, 4 and 8 h and fixed with a Paraformaldehyde (PF) and Glutaraldehyde (GA) solution (final concentration: 0.01% PF and 0.1% GA). At the end of the experiments, the mussels were freeze-dried and weighed (without shell and byssus threads) to calculate specific clearance rate (mL mg DW Dreissena^{-1} h^{-1}).

Grazing experiments on cultured phytoplankton were performed with (i) *Scenedesmus* at a cell concentration of 10^8 cells L^{-1} and (ii) a 50 : 50 mixture of *Scenedesmus* and *Microcystis* on volume basis, but at the same concentration as single food (Table 1). The food concentrations in both treatments were above the incipient limiting concentration of approximately 2 mg C L^{-1} (Walz, 1978): 2.7 mg C L^{-1} for *Scenedesmus* as single food and 2.1 mg C L^{-1} for the mixture.

Measuring of samples by flow cytometry

In flow cytometers, individual particles pass through one or more laser beams, and at the moment of passage a particle scatters the laser light and cell pigments are excited, resulting in fluorescence. The

Table 1 Cell concentrations and biovolume of phytoplankton species used in the grazing experiments with laboratory cultures

Food source	Number of cells *Scenedesmus* (×10^8 L^{-1})	Biovolume *Scenedesmus* (mm^3 L^{-1})	Number of cells *Microcystis* (×10^8 L^{-1})	Biovolume *Microcystis* (mm^3 L^{-1})
Scen	1	14.34		
Mixture	0.59	7.17	2.53	7.17

Scen, *Scenedesmus* as a single food; Mixture, mixture of *Scenedesmus* and *Microcystis*.

scattering and fluorescence signals are measured and quantified by photomultipliers and particles can be optically analysed based on these signals. A schematic drawing of the principle can be found in Dubelaar & Jonker (2000).

Samples were analysed using the EurOPA flow cytometer (TNO, Delft, The Netherlands) equipped with 633 nm (red) and 532 nm NdYAG (uGreen laser 50 mW; Uniphase, Witney, U.K.) lasers to distinguish between green algae and cyanobacteria (Fig. 1). Cyanobacteria are characterised by the presence of phycocyanins, which fluoresce upon excitation with the 633 nm laser. The EurOPA flow cytometer was designed especially for the analysis of phytoplankton in freshwaters by using a 1 mm diameter cuvette and by designing the data processing in such a way that both small (1 μm in diameter) as well as large (approximately 500 μm in diameter) particles could be processed (Jonker *et al*., 1995). In addition, the size of a particle could be calculated from the particle pumping rate and the time that a particle was inside a laser beam (known as 'Time-of-Flight'). Calibration of the EurOPA was carried out with 2 μm Fluoresbrite fluorescent monodisperse carboxylated beads (Poly-Sciences, Inc., Warrington, PA, U.S.A.).

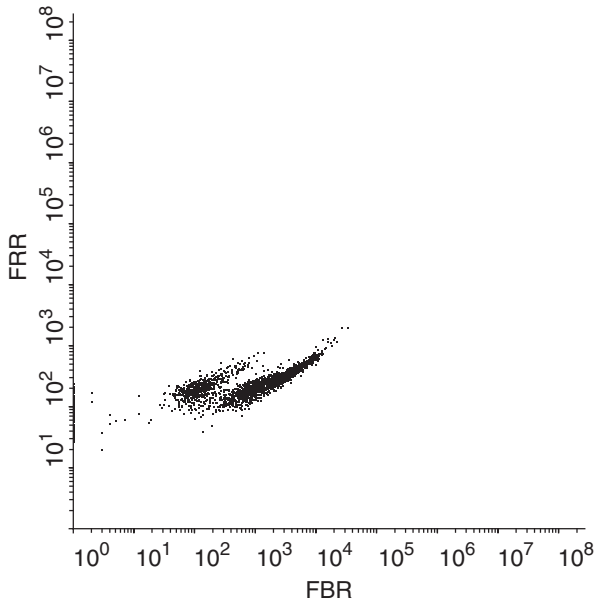

Fig. 1 Distinction of phytoplankton species (a mixture of the cyanobacterium *Microcystis* and the green alga *Scenedesmus*) on the EurOPA flow cytometer by plotting red fluorescence of the green laser (FBR) against red fluorescence of the red laser (FRR). The left cluster represents *Microcystis* cells and the right cluster represents *Scenedesmus* cells.

Grazing by adult zebra mussels on natural seston

Grazing rates of adult mussels on natural seston were measured in the same way as the experiments on cultured phytoplankton. For the grazing experiments, water from Lake Zwemlust (located in the Province of Utrecht, The Netherlands) was collected in the spring and transported to the laboratory within 30 min and filtered through a 100 μm mesh net to remove most of the zooplankton. During the 24 h acclimation period, 30 mussels measuring 18–22 mm shell length were taken from the stock culture, individually placed in vessels containing 200 mL lake water and placed on a shaker at 50 rotations per minute. Ten mussels were individually transferred to vessels containing 200 mL of lake water, and another 10 animals were transferred to vessels containing 200 mL of 0.2 μm filtered water for correcting clearance rates for expelled particles originating from the acclimation period. Control vessels ($n = 10$) with 200 mL lake water, but without zebra mussels, were used to correct for changes in seston biomass during the experimental period. Five millilitre water samples were taken from each of the vessels at $t = 0$ and $t = 1$ h and fixed with a PF and GA solution (final concentration: 0.01% PF and 0.1% GA) to measure clearance rate. The mussels were then removed and treated as described above. Phytoplankton species composition was also determined by light microscopy at 400× magnification.

Grazing by zebra mussel larvae on natural seston

Laboratory reared, 3-day-old larvae (size approximately 100 μm) were transferred to water from Lake Zwemlust and from the River Vecht, near Lake Zwemlust. In both cases, the water was filtered through a 3 μm Nitex^{TM} screen (Nitex Ltd, Sofia, Bulgaria) because zebra mussel larvae do not feed on particles >4 μm (Sprung, 1989). Larvae ($n = 100$) were placed in 25 mL Erlenmeyer flasks (five replicates) containing 10 mL water, and on a shaker at 20 rotations per minute. A 16 : 8 h L : D cycle and low light intensity (<5 μmol s^{-1} m^{-2}) was used. As a control, seston without larvae was incubated (five replicates) as described above. From the experiments with both lake and river seston, five 10 mL aliquots were sampled to determine the concentration of suspended particles at $t = 0$, and fixed with a PF and GA solution (final concentration: 0.01% PF and 0.1% GA). After the

grazing period of 24 h, the contents of all the flasks were filtered through a 70 μm Nitex™ screen and the larvae retained. The water was fixed with PF and GA as described above. Samples were analysed using the EurOPA flow cytometer equipped with a 633 nm (red) laser and a 488 nm Argon ion laser (200 mW; Coherent, model Annova 90, Palo Alto, CA, U.S.A.). At this point, we decided to use a 488 nm instead of the 532 nm laser to try to separate green algae from diatoms. However, this could only be achieved at the cost of the distinction of green algae and cyanobacteria. Because in the Netherlands cyanobacteria have higher impacts on freshwater ecosystems than diatoms, as a result of bloom formation and toxin production by the former, we decided to maintain the distinction cyanobacteria and other phytoplankton.

Data analyses

Clearance rate is a measure of grazing activity by zebra mussels and is calculated as the volume of water (mL) from which the mussel (mg DW) has removed all of the food particles of given size per unit time (Bunt, MacIsaac & Sprules, 1993). Specific clearance rate (mL mg DW Dreissena^{-1} h^{-1}) was calculated according to Coughlan (1969) as:

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

where V is the volume of the food suspension (200 mL), n is the weight of an adult mussel (mg DW) or the number of larvae ($n = 100$), t is the duration of the experiment (in hour), C_0 is the particle concentration (number mL^{-1}) at $t = 0$, C_t is the particle concentration at time t in vessels with animals, C_0' is the concentration of particles in the control vessels at $t = 0$ and C_t' is the concentration of particles at time t. In the grazing experiments on cultured phytoplankton, the last term $(\ln(C_0'/C_t'))$ was omitted from the equation if the slope of regression between cell concentrations (y) and time (x) in the controls did not differ significantly from zero. Because a proportion of the filtered cells may return to the water in the form of pseudofaeces and hence potentially be recounted, the clearance rates reported here for the experiments involving adult zebra mussels are not referred to as collection rates but as net clearance rates. Clearance rates of the different phytoplankton species (grazing on cultured phytoplankton) or different seston groups (grazing by adults on natural seston) were tested by one-way ANOVA and in the grazing experiments with larvae a two-way ANOVA was used to test clearance rates between seston groups (cyanobacteria, other phytoplankton and detritus) and between systems (Zwemlust and Vecht). Tukey ($P = 0.05$) and Tukey–Kramer ($P = 0.01$) contrast tests were used to distinguish between the differences found by ANOVA ($P = 0.05$) (Sokal & Rohlf, 1995; Fowler, Cohen & Jarvis, 1999).

Results

Grazing on laboratory cultures

The mussels in the vessels containing medium without food did not significantly enrich the water with particles resulting from the acclimation period (ANOVA, $P > 0.05$). Hence, for the calculation of clearance rates no correction was applied for expelled food particles resulting from the acclimation period. In the mixture of *Scenedesmus* and *Microcystis*, no difference in clearance rate was found (one-way ANOVA, $P > 0.05$). Clearance rates after 8 h ranged from 4.04 to 7.54 mL mg DW Dreissena^{-1} h^{-1} and were higher on a mixture of *Scenedesmus* and *Microcystis* than on *Scenedesmus* alone (Fig. 2; one-way ANOVA, $F_{2,23}$, 6.89, $P < 0.01$).

Grazing on natural seston by adults

The seston of Lake Zwemlust was dominated by diatoms [>82 mm^3 L^{-1}, mainly *Asterionella* (Hassall)]. Green algae (mainly *Chlorococcales*) were approxi-

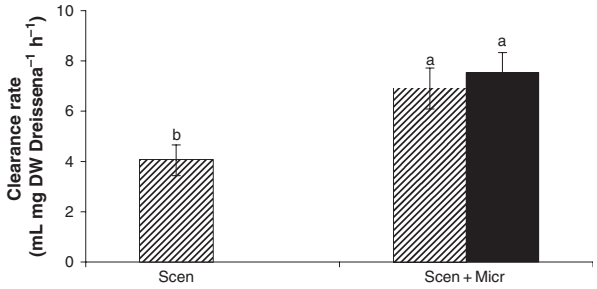

Fig. 2 Mean (±SE) clearance rate after 8 h (mL mg DW Dreissena^{-1} h^{-1}) of adult zebra mussels, *Dreissena polymorpha*, feeding on *Scenedesmus* as single food (Scen; dashed bars) and on a mixture of *Scenedesmus* and *Microcystis* (Scen and Micr; dashed and black bars, respectively). Similar letters represent groups that are not significantly different ($P > 0.05$, Tukey's test).

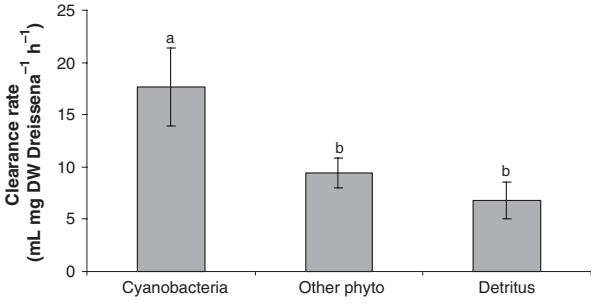

Fig. 3 Mean (±SE) clearance rate (mL mg mussel DW^{-1} h^{-1}) of adult zebra mussels on Cyanobacteria, other phytoplankton (Other Phyto) and detritus particles of Lake Zwemlust. Similar lettering represent groups that are not significantly different ($P > 0.05$, Tukey's test).

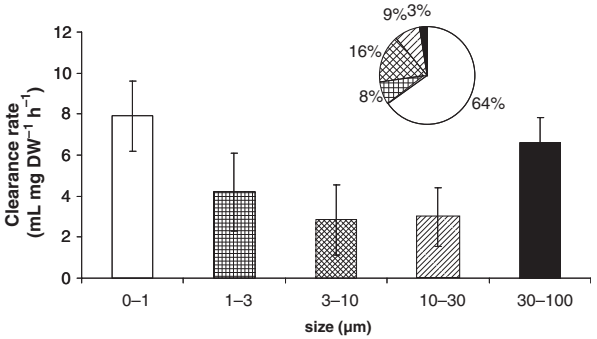

Fig. 4 Mean (±SE) clearance rate (mL mg mussel DW^{-1} h^{-1}) of adult zebra mussels on different seston size-classes of Lake Zwemlust. The relative amount (%) of each size-class in the seston is given in the pie chart.

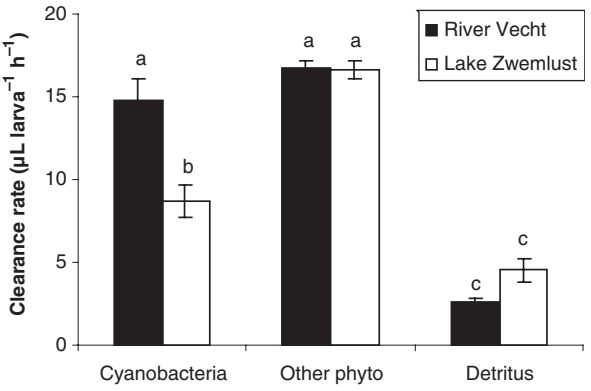

Fig. 5 Mean (±SE) clearance rate (µL larva^{-1} h^{-1}) of zebra mussel larvae on cyanobacteria, other phytoplankton (Other Phyto) and detritus particles in seston from the River Vecht (black bars) and Lake Zwemlust (white bars). Similar lettering represent groups that are not significantly different ($P > 0.05$, Tukey's test).

mately 1% of the diatoms, cyanobacteria (50% filament forming species and 50% single cells) approximately 0.5%.

As in the experiments on grazing on laboratory reared phytoplankton, the mussels in the vessels containing water without food did not significantly enrich the water with particles resulting from the acclimation period (ANOVA, $P > 0.05$). Consequently, in the calculation of clearance rates no correction was applied for expelled food particles originating from the acclimation period. Clearance rates of adult zebra mussels between seston groups ranged from 6.75 to 17.67 mL mg DW Dreissena^{-1} h^{-1} (Fig. 3). Clearance rates of adult zebra mussels varied between seston groups (Fig. 3; one-way ANOVA, $F_{2,30}$, 4.09, $P < 0.05$). Mean clearance rate was significantly (ANOVA, $P < 0.05$) higher for cyanobacteria than for other phytoplankton and detritus (Fig. 3). Clearance rates on other phytoplankton and detritus were not significantly different (ANOVA, $P > 0.05$).

When the seston was divided into different size-classes, clearance rates ranged from 2.84 to 7.90 mL mg DW Dreissena^{-1} h^{-1} and differed between size-classes (Fig. 4; $F_{4,76} = 8.11$, $P < 0.01$). This was probably because of higher clearance rates on size-classes 0–1 and 30–100 µm as compared with the other size-classes (Fig. 4).

Grazing on natural seston by larvae

Clearance rates of larvae ranged from 2.58 to 16.66 µL larva^{-1} h^{-1} (Fig. 5). Clearance rates of larvae on the different seston components differed significantly (two-way ANOVA, $F_{2,18}$, 41.33, $P < 0.01$). Clearance rates on cyanobacteria and other phytoplankton were significantly higher (ANOVA, $P < 0.05$) than on detritus seston from both Lake Zwemlust and the River Vecht (Fig. 5). Clearance rates on cyanobacteria from Lake Zwemlust were significantly lower (ANOVA, $P > 0.05$) than the clearance rates on cyanobacteria from the River Vecht and on other phytoplankton (Fig. 5). Between the two locations no other significant differences in clearance rates were found (two-way ANOVA, $F_{1,18}$, 1.42, $P > 0.05$).

Discussion

Adult zebra mussels were shown to graze effectively on small cyanobacteria. Surprisingly, this finding contradicts our expectations, namely that mussels

would exhibit lower clearance rates on food of poor quality (toxic cyanobacteria) (Stoeckmann, 2003). The grazing experiments on natural seston, using adult mussels, showed higher clearance rates on cyanobacteria than on detritus, whereas clearance rates on other phytoplankton did not differ from clearance rates on detritus. Our second hypothesis, that mussels will exhibit higher grazing pressure on phytoplankton than on detritus was supported, but only by the comparison between cyanobacteria and detritus. Larvae were shown to be more effective than adult mussels in selecting phytoplankton from detritus thereby supporting this hypothesis.

Grazing on laboratory cultures

The higher clearance rates of zebra mussels on a mixture of *Scenedesmus* and *Microcystis* than on *Scenedesmus* alone can be the result of two different mechanisms. First, pumping rate may be affected by the motivation of the mussels to feed, which may be influenced by the sensory quality of the food composition as a whole. Secondly, selective rejection of food particles by mussels may occur once the particles are filtered (Vanderploeg *et al*., 2001). Endoscopic examinations of particle transportation on zebra mussel gills has shown that single cells of *Microcystis* were transported to the mouth, whereas *Scenedesmus quadricauda* (Turpin) were expelled as pseudofaeces (Baker, Levinton & Ward, 2000). The higher clearance rates in the mixture of *Scenedesmus* and *Microcystis* may be explained by lower pseudofaeces production than when fed *Scenedesmus* as single food. It is also possible that the sensory impact of both species is higher than that by just *Scenedesmus* alone, resulting in mussels being motivated to pump at a faster rate. Dionisio Pires & Van Donk (2002) found higher clearance rates on the green alga *Chlamydomonas* in a mixture with *Microcystis* than when *Chlamydomonas* was provided as single food. However, these authors showed that if *Microcystis* was toxic, clearance rates on *Chlamydomonas* did not differ in the presence or absence of *Microcystis*. The *Microcystis* strain used in this study consists of single cells that are within the size range of particles normally ingested by *Dreissena* (Baker *et al*., 2000; Vanderploeg *et al*., 2001), hence the mechanism of this observation is still not clear. In nature, however, *Microcystis* occurs mainly as colonies (Mur *et al*., 1999) and hence colony size could play an important role in food selection. For instance, Vanderploeg *et al*. (2001) showed that phytoplankton >53 μm, containing mainly toxic *Microcystis*, was filtered less efficiently than phytoplankton <53 μm. Further, using natural seston, Bastviken, Caraco & Cole (1998) found the highest clearance rates of zebra mussels when fed unicellular *Microcystis* and the lowest clearance rates were found when fed colonial *Microcystis*.

Grazing on natural seston by adults

The high clearance rates of adult zebra mussels on cyanobacteria compared with other phytoplankton and detritus indicates selective feeding. As mentioned above, selection of particles occurs at the gills where particles are rejected as pseudofaeces (see e.g. Baker *et al*., 2000). Baker *et al*. (1998) showed, with artificially prepared seston, that zebra mussels preferred *Microcystis* single cells to other phytoplankton and detrital particles. Ward *et al*. (1997, 1998) showed that both the marine mussel *Mytilus trossulus* (Gould) and the oyster *C. gigas* were capable of sorting *Rhodomonas* (Pascher & Ruttner) cells (small cryptophyte) from similarly-sized cord grass [*Spartina* (Loisel)] detritus.

Zebra mussels had the highest clearance rates on seston in the size ranges 0–1 and 30–100 μm. Most phytoplankton cells were <10 μm and some larger ones, like *Asterionella*, were >30 μm. Between 10 and 30 μm the seston consisted mainly of detritus. This finding suggests that zebra mussels are capable of distinguishing phytoplankton from non-phytoplankton. Horgan & Mills (1997) found no differences in clearance rates between size-classes <100 μm. However, these authors used small mussels (9–11 mm) and measured clearance rates after 2 h only, hence it is plausible that the food became depleted in their experiments. Microscopic techniques showed that zebra mussels are able to ingest bacteria from ≤1 to 4 μm (Silverman *et al*., 1995; Silverman, Lynn & Dietz, 1996a; Silverman *et al*., 1996b). In addition, Frischer *et al*. (2000) clearly showed that clearance rates on bacteria increased as bacterial size increased. Zebra mussels had a retention efficiency of 6 and 29% for plastic microspheres of 0.5 and 1.1 μm, respectively, but for microspheres ≥1.5 μm the efficiency was 100% (Lei, Payne & Wang, 1996). Compared with natural seston, however, filtration of these microspheres decreased when they passed through the gut, suggesting constipation by these non-nutritious particles.

Flow cytometry can distinguish between different particle types of the same size, which is an important advantage over electronic particle counters. In this study, however, we were not able to test the effects of grazing on particles of the same size because concentrations of some particles were too low to calculate reliable clearance rates.

Grazing on natural seston by larvae

Our study revealed that zebra mussel larvae were able to discriminate phytoplankton from detritus. This result probably reflects differentiation of particles by the larvae based on food quality rather than on size, as the water used in the experiments did not contain particles >3 μm. Dionisio Pires *et al.* (2003) recently showed that zebra mussel larvae had different feeding behaviours when fed laboratory cultures of toxic and non-toxic *Microcystis*; namely they showed higher clearance rates on non-toxic *Microcystis* than on toxic *Microcystis*.

In contrast to adult zebra mussels, the larvae showed a preference for phytoplankton other than cyanobacteria in Lake Zwemlust. However, this difference between adults and larvae may be an artefact of our study design. Larvae were offered seston ≤3 μm and adults were exposed to seston ≤100 μm, hence diet compositions differed. Mean clearance rate of larvae on cyanobacteria was higher on seston from the River Vecht than from Lake Zwemlust. This could be due to differences in seston composition, food concentration or water quality.

On a daily basis, the clearance rates of the larvae ranged from 62 to 402 and from 108 to 400 μL larva^{-1} day^{-1} on seston from the River Vecht and Lake Zwemlust, respectively. MacIsaac *et al.* (1992) found clearance rates for *Dreissena* larvae ranging from 245 to 418 μL larva^{-1} day^{-1}. The difference in the lower ranges in clearance rates between this study and the values found by MacIsaac *et al.* (1992) could be due to the smaller veligers used in our study. Further, the fact that MacIsaac *et al.* (1992) used beads, which as recognised by the authors can lead to an overestimation of clearance rates, may also explain the differences in clearance rates.

The application of flow cytometry in grazing studies is increasing (Dubelaar & Jonker, 2000), especially in marine waters (Cucci *et al.*, 1985, 1989; Shumway *et al.*, 1985; Bougrier, Hawkins & Héral, 1997; Reckermann & Veldhuis, 1997; Ward *et al.*, 1997, 1998; Christaki *et al.*, 1999; Kuipers & Witte, 1999; Bratvold, Srienc & Raub, 2000). For example, Shumway *et al.* (1985) demonstrated that flow cytometry was useful in measuring clearance rates and monitoring cell types in faeces and pseudofaeces. In fresh waters, only a few studies have used flow cytometry in grazing measurements. Kenter, Zimmermann & Müller (1996); Baker *et al.* (1998) and Weisse & Kirchhoff (1997) studied grazing in the laboratory using one or two prey items or artificial seston. Pile *et al.* (1997) measured *in situ* grazing, but these authors only looked at picoplankton and neglected major phytoplankton and detritus. Hence, to our knowledge this is the first grazing study on freshwater natural seston using flow cytometry.

The advantages of flow cytometry in grazing studies over other techniques become clear in experiments involving natural seston. We have shown that flow cytometry allows us to measure grazing on different components (based on pigment composition and size) of natural seston. Sorting of algal particles from non-algal particles is an important advantage of flow cytometry over ordinary phytoplankton counting methods. With improvement in discriminating between diatoms and green algae and the development of *in situ* flow cytometers (Dubelaar *et al.*, 1999; Dubelaar & Gerritzen, 2000), flow cytometry has the potential to become the preferred tool for measuring grazing on heterogeneous assemblages of particles.

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