# Grazing by meso- and microzooplankton on phytoplankton in the upper reaches of the Schelde estuary (Belgium/The Netherlands)

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**Abstract**

In contrast with the marine reaches of estuaries, few studies have dealt with zooplankton grazing on phytoplankton in the upper estuarine reaches, where freshwater zooplankton species tend to dominate the zooplankton community. In spring and early summer 2003, grazing by micro- and mesozooplankton on phytoplankton was investigated at three sites in the upper Schelde estuary. Grazing by mesozooplankton was evaluated by monitoring growth of phytoplankton in 200 μm filtered water in the presence or absence of mesozooplankton. In different experiments, the grazing impact was tested of the calanoïd copepod *Eurytemora affinis*, the cyclopoid copepods *Acanthocyclops robustus* and *Cyclops vicinus* and the cladocera *Chydorus sphaericus*, *Moina affinis* and *Daphnia magna*/*pulex*. No significant grazing impact of mesozooplankton in any experiment was found despite the fact that mesozooplankton densities used in the experiments (20 or 40 ind. $l^{-1}$) were higher than densities in the field (0.1–6.9 ind. $l^{-1}$). Grazing by microzooplankton was evaluated by comparing growth of phytoplankton in 30 and 200 μm filtered water. Microzooplankton in the 30–200 μm size range included mainly rotifers of the genera *Brachionus*, *Trichocerca* and *Synchaeta*, which were present from 191 to 1777 ind. $l^{-1}$. Microzooplankton had a significant grazing impact in five out of six experiments. They had a community grazing rate of 0.41–1.83 $day^{-1}$ and grazed up to 84% of initial phytoplankton standing stock per day. Rotifer clearance rates estimated from microzooplankton community grazing rates and rotifer abundances varied from 8.3 to 41.7 μl ind.$^{-1}$ $h^{-1}$. CHEMTAX analysis of accessory pigment data revealed a similar phytoplankton community composition after incubation with and without microzooplankton, indicating non-selective feeding by rotifers on phytoplankton.

*Keywords:* Schelde estuary; grazing; rotifers; mesozooplankton; phytoplankton; HPLC; CHEMTAX

## 1. Introduction

In contrast to the downstream, marine reaches of estuaries, where the zooplankton community is usually dominated by marine calanoid copepods (e.g. Castel and Veiga,1990; Soetaert and Van Rijswijk, 1993; Tackx et al., 1995; Roman et al., 2001), freshwater zooplankton species tend to become more important in the upper reaches of estuaries, where the water is fresh or slightly brackish. In the upper reaches of estuaries, rotifers are often numerically the dominant zooplankton group.

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Rotifers have been found to dominate the zooplankton community in the upper reaches of the Hudson River estuary (Pace et al., 1992), the Hawkesbury-Nepean River estuary (Kobayashi et al., 1996), Chesapeake Bay (Park and Marshall, 2000) and the Schelde estuary (Muylaert et al., 2000a; Tackx et al., 2004). In this respect, the upper reaches of estuaries strongly resemble the lowland reaches of large rivers (e.g. Pourriot et al., 1982; Gosselain et al., 1994). Rotifers have a short generation time compared to crustacean zooplankton and are therefore well adapted to survive in ecosystems like rivers and estuaries, which often have a short retention time. Freshwater crustacean mesozooplankton like cladocera or cyclopoid copepods may occasionally become abundant in the upper reaches of estuaries, but rarely during prolonged periods.

Despite the fact that the upper reaches of estuaries tend to be very turbid they often show dense phytoplankton blooms. Phytoplankton biomass in upper estuarine reaches is often higher than in the marine reaches. It is not unusual for chlorophyll *a* concentrations in the upper reaches of estuaries to exceed 50 μg $l^{-1}$ (e.g. Schuchardt and Schirmer, 1991; Muylaert et al., 2005). While many studies have dealt with zooplankton grazing on phytoplankton in the marine zone of estuaries (e.g. Heinle et al., 1977; Tackx et al., 1995; Roman et al., 2001), much less is known about the fate of phytoplankton in the upper estuary. Park and Marshall (2000) suggested that rotifers may play an important trophic role in the upper reaches of estuaries. Grazing experiments carried out in the freshwater tidal Potomac River seem to confirm this hypothesis (Sellner et al., 1993). In the lowland reaches of rivers, of which the upper reaches of estuaries form a downstream continuation, rotifers were found to graze a considerable fraction of phytoplankton production or standing stock. In the River Meuse, rotifers grazed up to 113% of phytoplankton standing stock per day (Kobayashi et al., 1996; Gosselain et al., 1998). Using riverine ecosystem models, rotifers were predicted to exert a significant control on phytoplankton during summer (Seine river: Billen et al., 1994; Meuse river: Everbecq et al., 2001; Rhine river: Schol et al., 2002).

The Schelde estuary is one of the few European estuaries with an extensive freshwater tidal zone in its upper reaches. This freshwater tidal zone is characterized by the occurrence of dense phytoplankton blooms and a zooplankton community that is dominated by rotifers (Tackx et al., 2004). The first goal of this study was to determine whether zooplankton can exert a significant grazing pressure during phytoplankton blooms in the upper reaches of the Schelde estuary. The experiments were carried out during the spring and the summer blooms at three sites representative of riverine, freshwater tidal and oligohaline conditions. Grazing by mesozooplankton and microzooplankton (dominated by rotifers) were determined separately to evaluate the relative roles of rotifers and mesozooplankton in phytoplankton grazing. Using CHEMTAX analyses of HPLC derived pigment data phytoplankton groups selective grazing on the major was evaluated.

## 2. Materials and methods

### 2.1. Study site

The Schelde estuary (Fig. 1) is a macrotidal coastal plain estuary situated in Western Europe. In contrast to many other European estuaries, where locks have been constructed at the freshwater seawater interface, the Schelde estuary still possesses an extensive freshwater tidal zone in its upper reaches. In these upper reaches, dense phytoplankton blooms occur during spring and summer. The spring bloom tends to be mainly imported from the tributary river Schelde while the summer bloom reaches its maximum within the upper estuary. These phytoplankton blooms are dominated by diatoms but chlorophytes can be co-dominant in the tributary rivers and near the head of the estuary in summer (Muylaert et al., 2000b). The zooplankton community in the upper estuary is dominated by rotifers, which frequently attain abundances of about 1000 ind. $l^{-1}$ (Soetaert and Van Rijswijk, 1993; Muylaert et al., 2000a; Tackx et al., 2004). Mesozooplankton densities rarely exceed 20 ind. $l^{-1}$. The crustacean zooplankton in the freshwater tidal reaches and river is dominated by the cyclopoid copepod *Acanthocyclops robustus* with other cyclopoid copepods or cladocera like *Cyclops vicinus*, *Bosmina longirostris*, *Moina* spp. and *Daphnia* spp. often being codominant. Towards the brackish reaches of the estuary, the calanoid copepod *Eurytemora affinis* replaces the cyclopoid copepods and cladocera. This species has recently moved upstream into the freshwater tidal zone, possibly due to improved water quality (Appeltans et al., 2003).

For the experiments, water and zooplankton were collected in 2003 in spring (March) and early summer (June) at three sites situated at the upper reaches of the Schelde estuary (Fig. 1): the river Schelde just before it enters the estuary, the freshwater tidal reaches of the estuary in Dendermonde and the oligohaline reaches in Antwerpen. March and June were representative for the two phytoplankton blooms that occur annually in the upper Schelde estuary. Water and zooplankton for the experiments were sampled from the river bank using bucket hauls. A quantitative mesozooplankton sample (50 l) was collected by means of a 200 μm mesh size plankton net. Salinity and temperature were measured in situ using an YSI 650 MDS multimeter with an YSI 600 R sensor.

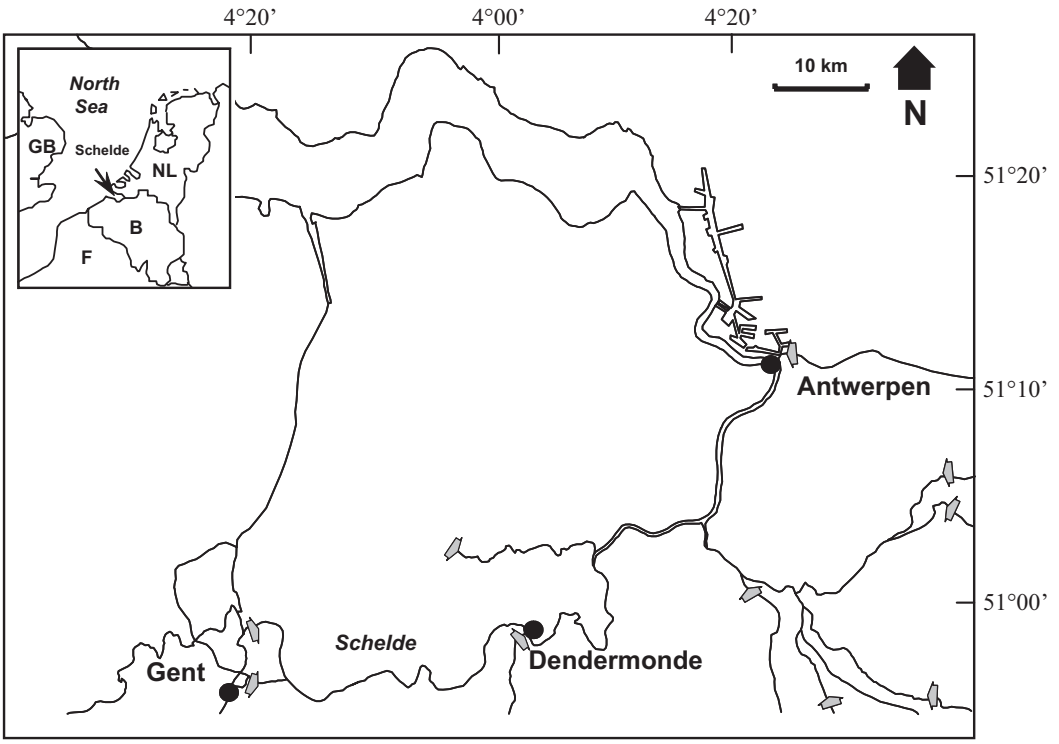


Fig. 1. Map of the Schelde estuary indicating the position of the sampling sites with black points. The grey arrows indicate the position of upper limit of tidal influence.

*2.2. Experimental setup*

Grazing rates of meso- and microzooplankton on phytoplankton were estimated by comparing phytoplankton growth rates in the presence and absence of grazers. Phytoplankton was separated from micro- and mesozooplankton by filtration over a 30 μm nylon mesh. Exploratory tests had demonstrated that this filter did not significantly retain phytoplankton. Mesozooplankton was separated from microzooplankton by filtration over a 200 μm nylon mesh. Microzooplankton grazing on phytoplankton was estimated by comparing phytoplankton development in the $<200$ μm and $<30$ μm filtrates. Microzooplankton therefore only included grazers in the 30–200 μm size range. Mesozooplankton grazing on phytoplankton was estimated by comparing phytoplankton development in $<200$ μm filtrates with and without added mesozooplankton. Mesozooplankton was collected at each sampling site by filtering 200–300 l of water through a 200–μm mesh size plankton net. A known number of individuals were picked out using a wide-bore pipette and a dissecting microscope to be added to the treatments. Two mesozooplankton treatments were set up for each experiment. If two species were co-dominant in the mesozooplankton community, the grazing impact of these two species was assessed separately. If only one species was dominant, this species was added to the treatments in different densities. The treatments were incubated in 1 l polycarbonate bottles during 1 day. Three replicates were prepared for each treatment. The bottles were incubated in a temperature and light controlled incubator. Temperature was set within approximately 1 °C of the field temperature (10 °C in March and 20 °C in June). Light intensity was set at 22 μmol $m^{-2}$ $s^{-1}$, which corresponds to the mean underwater irradiance at Dendermonde in spring. The mean underwater irradiance was estimated from a typical spring surface irradiance, the vertical light extinction coefficient and mean water column depth. The mean underwater irradiance experienced by phytoplankton will have been different at the other two sites and in summer. Therefore, growth rates measured in the experiments cannot be extrapolated to the field situation. In March, light was (accidentally) supplied continuously while a 12 h dark–12 h light cycle was supplied in June. Bottles were incubated on a rotating table (100 rpm) to keep the phytoplankton in suspension. All bottles were sampled for phytoplankton and microzooplankton at the start and at the end of the experiment.

Phytoplankton pigments were sampled by filtering 50–100 ml water over 25 mm GF/F filter. Filters were quickly dried between blotting paper and stored frozen at −80 °C until analysis. Microzooplankton was sampled by filtering 50–100 ml water over a 30 μm nylon mesh. Samples were fixed with formalin at a final

concentration of 4%. Samples for quantification of ciliates were fixed according to the Lugol-formalin-thiosulphate method (Sherr et al., 1989). In the mesozooplankton treatments, mesozooplankton individuals added to the bottles were collected on a 200 μm mesh at the end of the experiment for identification up to the species level. Mesozooplankton was fixed in a 4% formalin solution.

### 2.3. Analysis of samples

Phytoplankton biomass and community composition were investigated by means of HPLC pigment analysis. Pigments were extracted from the filters in 90% acetone by means of sonication (tip sonicator, 40 W for 30 s). Pigment extracts were filtered over a 0.2 μm nylon filter to remove particulates. Pigments were injected into a Gilson HPLC system equipped with an Alltima reverse-phase C18 column (25 cm×4.6 mm, 5 μm particle size). Pigments were analysed according to the method of Wright and Jeffrey (1997), which is an adaptation of the method of Wright et al. (1991) for marine phytoplankton. This method uses a gradient of three solvents: methanol 80%−ammonium acetate 20%, acetonitrile 90% and ethyl acetate. Three detectors were connected to the HPLC system: an Applied Biosystems 785A Programmable Absorbance Detector to measure absorbance at 785 nm, a Gilson model 121 fluorometer to measure fluorescence of chlorophylls and their derivates and a Gilson 170 diode array detector to measure absorbance spectra for individual pigment peaks. Pigments were identified by comparison of retention times and absorption spectra with pure pigment standards (supplied by DHI, Denmark).

Mesozooplankton added to the bottles, the quantitative mesozooplankton samples and microzooplankton present in the <200 μm filtrates were identified and enumerated using a dissecting microscope. Identification was based on Ruttner-Kolisko (1972), Pontin (1978) and Segers (1995). Ciliates were counted using an inverted microscope. Ciliates were identified up to class level Foissner et al. (1999). Samples for microzooplankton and ciliates were stained with Bengal Rose to aid in distinguishing between plankton and detritus.

### 2.4. Data analyses

One-way ANOVA was used to compare densities of potential grazers of phytoplankton and concentrations of total chlorophyll *a* at the end of the experiments between the treatments.

The CHEMTAX software was used to calculate the contribution of different algal groups to total chlorophyll *a* using concentrations of accessory pigments. This software package was developed specifically for the analysis of phytoplankton pigment data (Mackey et al., 1996). The CHEMTAX software makes use three matrices: (1) a matrix containing concentrations of all marker pigments in the samples; (2) an initial matrix containing marker pigment to chlorophyll *a* ratios for all algal groups; and (3) a ratio limit matrix defining limits on the theoretical marker pigment to chlorophyll *a* ratios. The CHEMTAX program optimizes the contribution of different algal groups to total chlorophyll *a* based on measured pigment concentrations (matrix 1), using the pigment ratio matrix (matrix 2) as a starting point and allowing pigment ratios to vary according to constraints defined in the limit matrix (matrix 3). The initial pigment ratio matrix (Table 1) was obtained from previous monitoring studies of phytoplankton pigments in the Schelde estuary in which biomass of major phytoplankton groups estimated by means of HPLC-CHEMTAX analyses was verified with data obtained by microscopical analyses (M. Lionard, unpublished data). Pigment data were processed separately using CHEMTAX for each experiment.

Community grazing rates and individual clearance were calculated according to Walz (1978) and Frost (1972). Grazing rates were calculated as $g = \ln(C_t/C_{zt})(1/t)$ where $C_t$ and $C_{zt}$ are the concentrations of the prey at the end of the incubation period, respectively in the absence and in the presence of the predator, and $t$ is the incubation time (in days). The percentage of initial phytoplankton biomass grazed per day was calculated as $100 - 100\,e^{-\ln(g)}$. The individual clearance rate was calculated as $F = g(V/P)$, where $P$ is the density of predators and $V$ is the bottle volume. For $P$ the average density of predators during the incubation period was used, which was calculated according to Marin et al. (1986): $P = (P_t - P_o)/\ln(P_t/P_o)$ where $P_t$ is

Table 1
Initial matrix with accessory pigments to chlorophyll *a* ratios in the major algal groups used in the CHEMTAX analyses

| | Peridinin | Fucoxanthin | Diatoxanthin diadinoxanthin | Alloxanthin | Lutein | Zeaxanthin | Echinenone | Chlorophyll *b* |
|---|---|---|---|---|---|---|---|---|
| Chlorophytes | 0 | 0 | 0 | 0 | 0.162 | 0.025 | 0 | 0.229 |
| Cryptophytes | 0 | 0 | 0 | 0.212 | 0 | 0 | 0 | 0 |
| Cyanophytes | 0 | 0 | 0 | 0 | 0 | 0.036 | 0.085 | 0 |
| Diatoms | 0 | 0.701 | 0.160 | 0 | 0 | 0 | 0 | 0 |
| Dinophytes | 0.760 | 0 | 0.302 | 0 | 0 | 0 | 0 | 0 |
| Euglenophytes | 0 | 0 | 0.333 | 0 | 0 | 0 | 0 | 0.372 |

the final predator density and $P_o$ is the initial predator density.

## 3. Results

As shown in Table 2 salinity was $<0.5$ in spring, as well as in summer, in Gent and Dendermonde. In Antwerpen, salinity was $<0.5$ in spring but was 1.75 in summer, which is indicative of oligohaline conditions. Temperature was 9.5 °C at all sites in spring and varied between 21.5 and 23.5 °C in summer. In spring, chlorophyll *a* concentration was highest in Gent and decreased in downstream direction towards Dendermonde and Antwerpen. In summer, chlorophyll *a* concentration was highest in Dendermonde and was lower in the river in Gent and in the oligohaline reaches in Antwerpen. The contribution of different algal groups to total chlorophyll *a* was assessed by means of CHEMTAX analysis of accessory pigment concentrations. In Gent and Dendermonde in spring and in Dendermonde in summer diatoms dominated the phytoplankton community with at least 78% of total chlorophyll *a*. In Gent in summer, chlorophytes were dominant (58% of total chlorophyll *a*) with diatoms being co-dominant. In Antwerpen, diatoms and chlorophytes were co-dominant in spring as well as in summer, with both groups contributing 60–80% to total chlorophyll *a*. In spring, euglenophytes contributed 11% to total chlorophyll *a* in Gent and 18% in Antwerpen. The contribution of other algal groups to total chlorophyll *a* was always $<10\%$.

Total mesozooplankton density was $<1$ ind. $l^{-1}$ in spring and summer in Gent and in spring in Dendermonde. Highest mesozooplankton densities were observed in Dendermonde in summer (about 7 ind. $l^{-1}$) and in Antwerpen in spring (about 9 ind. $l^{-1}$). Cyclopoid copepods (*Acanthocyclops robustus*, *Cyclops vicinus*) dominated the mesozooplankton community at all sites

Table 2
Environmental conditions and phytoplankton and zooplankton biomass or abundance and community composition at the sampling sites at the time of the experiments. n.d. indicates that no data were collected and – indicates zero abundance or biomass

| | Spring | | | Summer | | |
|---|---|---|---|---|---|---|
| | Gent | Dendermonde | Antwerpen | Gent | Dendermonde | Antwerpen |
| Abiotic factors | | | | | | |
| *T* (°C) | 9.47 | 9.45 | 9.54 | 23.7 | 23.1 | 21.51 |
| Salinity | 0.35 | 0.34 | 0.43 | 0.36 | 0.34 | 1.75 |
| ph | 9.58 | 7.76 | 7.64 | 7.37 | 7.43 | 7.53 |
| $O_2$ (%) | 82 | 65 | 35 | 49 | 41 | 22 |
| Phytoplankton | | | | | | |
| Chl *a* (μg $l^{-1}$) | 92.2 | 8.7 | 2.9 | 28.8 | 214.9 | 12.9 |
| Diatoms (%) | 78 | 79 | 27 | 35 | 89 | 43 |
| Chlorophytes (%) | 4 | 8 | 37 | 58 | 7 | 41 |
| Euglenophytes (%) | 11 | 3 | 18 | 1 | 0 | 5 |
| Dinoflagellates (%) | 6 | 4 | 0 | 1 | 0 | 0 |
| Cryptophytes (%) | 1 | 4 | 9 | 1 | 3 | 7 |
| Cyanophytes (%) | 1 | 1 | 9 | 4 | 0 | 5 |
| Microzooplankton | | | | | | |
| Rotifers (ind. $l^{-1}$) | 1157 | 191 | 860 | 1777 | 1433 | 796 |
| *Brachionus calyciflorus* (%) | 53 | 31 | 33 | 34 | 1 | 1 |
| *Brachionus angularis* (%) | 5 | 6 | 2 | 18 | 15 | 3 |
| *Polyarthra* sp. (%) | 11 | 7 | 7 | 0 | 9 | 0 |
| *Syncheata* sp. (%) | 5 | 11 | 35 | 1 | 3 | 18 |
| *Trichocerca* sp. (%) | 0 | 0 | 0 | 17 | 55 | 74 |
| Other rotifers (%) | 26 | 45 | 23 | 30 | 17 | 4 |
| *Copepod nauplii* (ind. $l^{-1}$) | – | 9.5 | 140 | – | 190 | 7 |
| Ciliates (ind. $ml^{-1}$) | n.d. | 80.8 | 46.5 | 75.4 | 55.3 | 31.7 |
| Mesozooplankton (ind. $l^{-1}$) | | | | | | |
| *Acanthocyclops robustus* | – | – | – | 0.2 | 6.9 | – |
| *Chydorus sphaericus* | 0.1 | – | 0.5 | – | – | – |
| *Cyclops vicinus* | 0.4 | 0.5 | 4.1 | – | – | – |
| *Daphnia pulex* | – | – | – | – | – | 0.2 |
| *Daphnia magna* | – | – | – | – | – | 0.2 |
| *Eurytemora affinis* | – | – | 4 | – | – | 1.5 |
| *Moina affinis* | – | – | – | 0.8 | – | – |

except in Antwerpen, where *Eurytemora affinis* was dominant in summer and was co-dominant with cyclopoid copepods in spring.

By comparing the $<30$ μm and $<200$ μm treatments we evaluated grazing by microzooplankton in the 30–200 μm size range on phytoplankton. Potential grazers in the 30–200 μm size range included ciliates, copepod nauplii and rotifers. Ciliate densities never differed significantly between the $<30$ μm and $<200$ μm size fractions indicating that ciliates were not significantly retained by the 30 μm mesh (Table 3). Densities of rotifers and copepod nauplii, on the contrary, were always significantly higher in $<200$ μm than in the $<30$ μm treatments. Densities of rotifers in the $<200$ μm size fraction at the start of the experiment varied from 191 to 1777 ind. $l^{-1}$. Rotifer densities were highest in Gent in spring as well as in summer. The rotifer community in Gent and Dendermonde was dominated by *Brachionus calyciflorus* in spring and by *Trichocerca* sp. in summer. In Antwerpen, the rotifer community was dominated by the genera *Synchaeta* and *Brachionus* in spring and by *Trichocerca* in summer. Densities of copepod nauplii were usually much lower than densities of rotifers. Densities of copepod nauplii exceeded rotifer densities by $>10\%$ only in spring in Antwerpen (16%) and summer in Dendermonde (13%). Abundances of rotifers and copepod nauplii often changed during the incubation period. Increases up to 340% and decreases down to 61% of initial densities were observed.

No significant difference in final chlorophyll *a* concentration was observed between the $<200$ μm treatments and the mesozooplankton treatments (Fig. 2) in any of the experiments, indicating no significant grazing by mesozooplankton on phytoplankton. Except for the experiment in Antwerpen in summer, chlorophyll *a* concentration at the end of the experiments was always significantly lower in the $<200$ μm treatments when compared to the $<30$ μm treatments, indicating significant grazing by microzooplankton on phytoplankton. Microzooplankton community grazing rates varied between 0.4 and 1.8 $day^{-1}$ (Table 4). The fraction of initial phytoplankton biomass grazed per day by microzooplankton varied between 33 and 84%. Given the fact that rotifers dominated the microzooplankton community it can be assumed that microzooplankton grazing was dominated by rotifers. Individual rotifer filtration rates were therefore estimated using average abundances of rotifers and total community grazing rates. Individual filtration rates varied between 8.3 and 41.7 μl ind.$^{-1}$ $day^{-1}$ (Table 4).

To evaluate whether selective grazing by microzooplankton on phytoplankton may influence the composition of the phytoplankton community, the phytoplankton community composition was compared at the end of the experiments in the presence and absence of microzooplankton (Fig. 3). No large differences in phytoplankton community were observed after incubation in the presence or absence of microzooplankton.

Table 3
Comparison of densities of potential phytoplankton grazers in the $<30$ μm and $<200$ μm filtrates. The two values separated by a hyphen represent densities measured at the start and at the end of the experiments, respectively. The *p*-level resulting from a one-way ANOVA test comparing densities of grazers in the 30 μm and 200 μm is given for each experiment. n.d. indicates that no data were collected and – indicates zero abundance

| | Spring | | | Summer | | |
|---|---|---|---|---|---|---|
| | Gent | Dendermonde | Antwerpen | Gent | Dendermonde | Antwerpen |
| Ciliates (cells $ml^{-1}$) | | | | | | |
| 30 μm | n.d. | 91–83 | 57–193 | 70–288 | 57–98 | 28–124 |
| 200 μm | n.d. | 81–81 | 46–137 | 75–241 | 55–74 | 32–111 |
| ANOVA *p*-level | – | 0.566 | 0.361 | 0.541 | 0.394 | 0.948 |
| Rotifers (ind. $l^{-1}$) | | | | | | |
| 30 μm | 117–n.d. | 10–47 | n.d.–n.d. | 107–410 | n.d.–n.d. | 360–140 |
| 200 μm | 1157–1693 | 191–843 | 860–550 | 1777–1720 | 1433–2893 | 797–610 |
| ANOVA *p*-level | $<0.001$ | 0.008 | – | $<0.001$ | – | $<0.001$ |
| Copepod nauplii (ind. $l^{-1}$) | | | | | | |
| 30 μm | 1.5–n.d. | 0–0 | n.d.–n.d. | 0–0 | n.d.–n.d. | 0–0 |
| 200 μm | 20–16.7 | 9.5-0 | 140–107 | 3.3–27 | 190–220 | 6.5–10 |
| ANOVA *p*-level | – | – | – | – | – | – |
| Mesozooplankton (ind. $l^{-1}$) | | | | | | |
| 30 μm | 0–0 | 0–0 | 0–0 | 0–0 | 0–0 | 0–0 |
| 200 μm | 0–0 | 10–21 | 0–0 | 0–0 | 0–0 | 0–0 |
| Zooplankton 1 | *Chydorus* (20) | *Eurytemora* (20) | *Eurytemora* (20) | *Moina* (20) | *Acanthocyclops* (20) | *Eurytemora* (20) |
| Zooplankton 2 | *Cyclops* (20) | *Cyclops* (20) | *Cyclops* (10) | *Acanthocylops* (40) | *Acanthocyclops* (40) | *Daphnia* (12) |

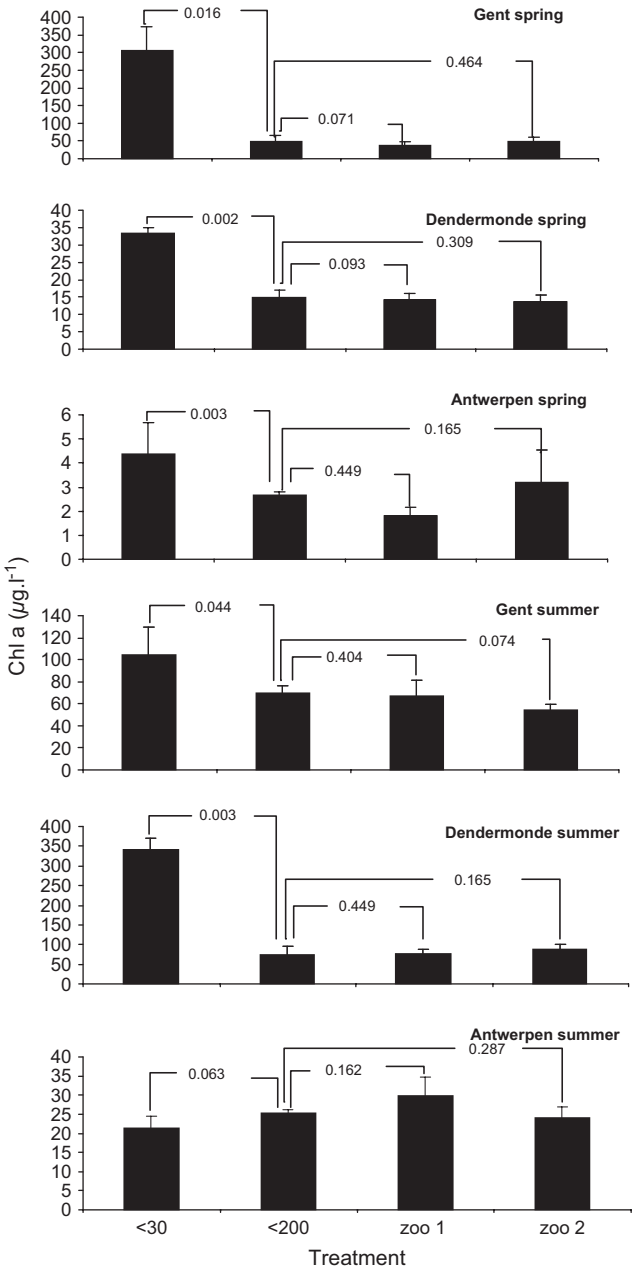


Fig. 2. Concentrations of chlorophyll *a* (µg $l^{-1}$) in the different treatments of the six experiments at the end of the incubation period. The mesozooplankton species and densities used in the "zoo1" and "zoo 2" are presented in Table 3. Error bars correspond to the standard deviation. The *p*-value of ANOVA analyses comparing the different treatments is shown above the bars.

## 4. Discussion

The goal of this study was to investigate whether micro- and/or mesozooplankton can exert a significant grazing pressure during phytoplankton blooms in the upper reaches of a macrotidal estuary, the Schelde estuary. The March experiments were typical for the phytoplankton spring bloom in the upper estuary, when phytoplankton is mainly imported from the river and the phytoplankton community is dominated by diatoms. The June experiments were typical of the summer bloom, when autochthonous phytoplankton populations develop in the freshwater tidal reaches and a succession in the phytoplankton community occurs from chlorophytes in the river to diatoms in the freshwater tidal and brackish zones (Muylaert et al., 2000a). Abundance and community composition of the microzooplankton community at the time of our experiments was typical for the upper Schelde estuary, being dominated by rotifers of the genera *Brachionus*, *Synchaeta* and *Trichocerca* (Tackx et al., 2004). Similar microzooplankton communities occur in other freshwater tidal estuaries (Rhode River estuary: Dolan and Gallegos, 1992; Elbe estuary: Meister, 1994; Chesapeake Bay estuary: Park and Marshall, 2000) or in lowland rivers (Loire river: Pourriot et al., 1982; Meuse river: Gosselain et al., 1994). Mesozooplankton densities in the field at the time of the experiments were relatively low compared to previous observations. The mesozooplankton densities used in the present experiments, however, were comparable to the maximal densities that have been observed in previous years. In at least two independent mesozooplankton experiments, the grazing impact of the two dominant mesozooplankton species that occur in the upper Schelde estuary was evaluated: *Eurytemora affinis*, which is dominant in the brackish zone of the estuary, and *Acanthocyclops vicinus*, which is the dominant species in the freshwater tidal reaches (Tackx et al., 2004). In addition, the impact of other species that are frequently encountered in the freshwater tidal and brackish reaches of the Schelde estuary but that are rarely dominant: was also tested the cyclopoid copepod *Cyclops vicinus* and the cladocera *Chydorus sphaericus*, *Daphnia pulex/magna* and *Moina affinis*.

No significant effect of mesozooplankton on phytoplankton was observed in any of the experiments. This was not due to the low mesozooplankton densities at the time of the experiments as mesozooplankton in the experimental bottles was at a density comparable to the maximal densities that occur in the estuary. This suggests that mesozooplankton did not exert a significant grazing pressure on phytoplankton during the spring and summer blooms and that its impact on phytoplankton blooms is probably insignificant even when higher densities occur. Because mesozooplankton densities in that bottles were higher than those in the field, that results should be interpreted with some caution as increasing mesozooplankton densities in experimental bottles may perturb the zooplankton feeding behaviour (Roman and Rublee, 1980). The lack of a significant grazing impact of mesozooplankton on phytoplankton does not necessarily imply that mesozooplankton does not feed on phytoplankton in the upper Schelde estuary. *Eurytemora affinis*, one of the species used in this study has been shown to exert

Table 4
Mean rotifer abundance, contribution of rotifers to combined abundance of rotifers and copepod nauplii, microzooplankton community grazing rate, fraction of initial phytoplankton biomass grazed and estimated individual filtration rates for rotifers (estimated from microzooplankton community grazing rate and rotifer abundance). Data are presented only for the experiments where microzooplankton exerted a significant grazing pressure on phytoplankton

| | Spring | | | Summer | |
|---|---|---|---|---|---|
| | Gent | Dendermonde | Antwerpen | Gent | Dendermonde |
| Mean rotifer abundance (ind. $l^{-1}$) | 1408 | 440 | 693 | 1748 | 2079 |
| % of rotifers in microzooplankton | 99 | 100 | 84 | 98 | 93 |
| Microzooplankton community grazing rate ($day^{-1}$) | 1.83 | 0.81 | 0.50 | 0.41 | 1.53 |
| % initial phytoplankton biomass grazed | 84 | 55 | 39 | 33 | 78 |
| Estimated rotifer filtration rate (µl ind.$^{-1}$ $h^{-1}$) | 41.7 | 76.3 | 29.8 | 8.3 | 30.7 |

a clearance rate up to 1 ml ind.$^{-1}$ $h^{-1}$ on diatoms in the brackish reaches of the Schelde estuary (Tackx et al., 2003). In the upper Schelde estuary and river, chlorophyll *a* concentrations are often high (up to 215 µg $l^{-1}$ in this study) relative to the densities of mesozooplankton. While mesozooplankton may feed on phytoplankton in the upper Schelde estuary, their impact on phytoplankton standing stocks may have been too low to be measurable by means of the method used in this study.

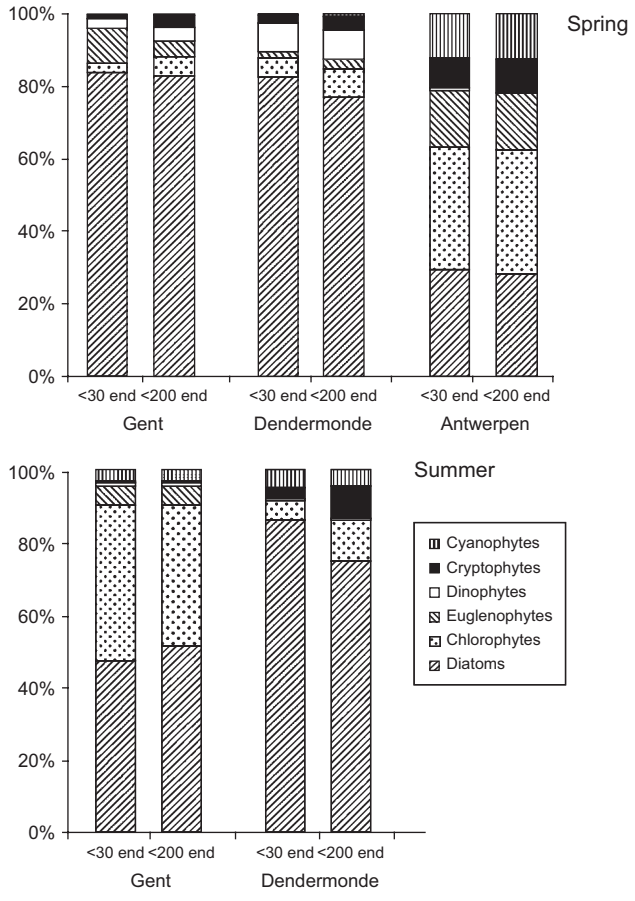


Fig. 3. Comparison of phytoplankton community composition at the end of the experiment in the <30 and <200 µm treatments.

In contrast to mesozooplankton, a significant effect of microzooplankton on phytoplankton was observed in all experiments except in Antwerpen in June. In the experiments, the microzooplankton included organisms between 30 and 200 µm in size. This size range encompasses rotifers, copepod nauplii and ciliates. As ciliates were not retained by the 30 µm mesh, microzooplankton grazing on phytoplankton in the present experiments did not include grazing by ciliates. Both copepod nauplii and rotifers were strongly retained by the 30 µm mesh. Grazing by microzooplankton in the experiments should mainly be attributed to copepod nauplii and/or rotifers. On all occasions, abundance of copepod nauplii was much lower (maximal 16%) than rotifer abundance and in two experiments copepod nauplii were even absent. Grazing by microzooplankton in the present experiments should probably mainly be attributed to rotifers. To evaluate whether rotifers were capable of exerting the observed grazing pressure on phytoplankton, individual rotifer filtration rates were estimated from total rotifer densities and microzooplankton community grazing rates and compared these filtration rates with literature data. These estimated individual filtration rates should be interpreted with caution as they do not take into account additional grazing by copepod nauplii. Moreover, individual filtration rates are difficult to assess when rotifer abundances change during the incubation, which was the case in several experiments. Apart from the high filtration rate of 76 µl ind.$^{-1}$ $h^{-1}$ measured in Dendermonde in spring, the estimated filtration rates were in the range of published rates measured for the same species as those occurring in the upper Schelde estuary using radioactively labelled prey method in previous laboratory or field experiments (Starkweather and Gilbert, 1977: 45–50 µl ind.$^{-1}$ $h^{-1}$ for *Brachionus calyciflorus* in a laboratory study; Rothhaupt, 1990a: 30 µl ind.$^{-1}$ $h^{-1}$ for *Brachionus calyciflorus* in a laboratory study; Sellner et al., 1993: 5.4 µl ind.$^{-1}$ $h^{-1}$ for *Brachionus angularis* in a field study; Gosselain et al., 1994: 14 µl ind.$^{-1}$ $h^{-1}$ for *Brachionus calyciflorus* in a laboratory study, and from 2.14 to 19.71 µl ind.$^{-1}$ $h^{-1}$ for *Brachionus calyciflorus* in a field study in the Meuse

river; Havens, 1991: 30 µl ind.$^{-1}$ h$^{-1}$ for *Brachionus calyciflorus* and 30 µl ind.$^{-1}$ h$^{-1}$ for *Synchaeta stylata* in a field study). The high value estimated for Dendermonde in spring may have been due to an overestimation of clearance rates related to the large increase in rotifer abundance during the incubation period.

The importance of rotifers as grazers of phytoplankton in the upper reaches of the Schelde estuary is in contrast with the marine reaches of the estuary, where mesozooplankton is often assumed to dominate grazing on phytoplankton (e.g. Soetaert et al., 1994). Dilution experiments, however, have indicated that microzooplankton may also be important grazers of phytoplankton in the marine reaches of estuaries (McManus and Ederington-Cantrell, 1992; Ruiz et al., 1998). In these studies, however, ciliates were identified as the dominant microzooplankton grazers while, in the present study, microzooplankton grazing was mainly ascribed to rotifers and grazing by ciliates was not taken into account. A similar microzooplankton grazing pressure on phytoplankton has been observed in lowland rivers where phytoplankton biomass and rotifer abundances were in the same order of magnitude as in the upper reaches of the Schelde estuary (e.g. Billen et al., 1994; Descy and Gosselain, 1994; Kobayashi et al., 1996).

In this study, the grazing pressure of microzooplankton on phytoplankton varied from non-significant (in Antwerpen in June) to a maximum of 1.83 day$^{-1}$ (in Gent in March). That the grazing pressure of rotifers on phytoplankton in Antwerpen in June was non-significant can probably be ascribed to the absence of efficient phytoplankton grazers, as the rotifer community at that time was dominated by 74% by the small rotifer *Trichocerca*. In the experiments where microzooplankton had a significant impact on phytoplankton biomass, the grazing rate varied considerably (from 0.41 to 1.83 day$^{-1}$). No clear relationship could be observed between microzooplankton grazing rate and rotifer abundance, community composition or temperature. The minimal grazing rate occurred at relatively high rotifer abundance (in Gent in summer). *Brachionus* spp. dominated the rotifer community both when the highest and the lowest grazing rates were measured. Both high and low grazing rates were measured during the spring as well as the summer bloom, despite a difference in water temperature of more than 10 °C. The lack of a clear pattern in microzooplankton grazing rates may be due to the importance of alternative food sources of rotifers in the Schelde estuary. Rotifers have a broad diet and are capable of feeding on bacteria, heterotrophic flagellates or ciliates (Dolan and Gallegos, 1991; Arndt, 1993; Gilbert and Jack, 1993; Ooms-Wilms, 1997). These alternative food sources may be important in the Schelde estuary, which is a net heterotrophic ecosystem (Soetaert et al., 1994). Certainly, more studies are needed to investigate what regulates seasonal and spatial variations in grazing pressure of rotifers on phytoplankton.

Despite the high biomass of phytoplankton during the spring and summer blooms, microzooplankton nevertheless grazed a large fraction of phytoplankton standing stock per day (up to 84%). The impact of microzooplankton on the development of phytoplankton blooms in the upper Schelde estuary might be significant, especially because phytoplankton growth rates are low due to severe light-limitation (Muylaert et al., 2005). The potential impact of microzooplankton feeding on phytoplankton seasonal succession in upper reaches of the Schelde estuary remains unclear. In the Schelde estuary, dense rotifer populations occur from spring onward (Muylaert et al., 2000b). Despite the presence of these rotifer populations and the strong grazing impact they may have on phytoplankton, dense phytoplankton blooms develop in the upper Schelde estuary in summer. These blooms are only terminated when discharge increases in autumn (Muylaert et al., 2000b), suggesting that discharge is more important in regulating phytoplankton blooms than rotifer grazing. The reason why rotifers are incapable of controlling phytoplankton biomass or causing a clear water phase in the upper Schelde estuary may be twofold. First, rotifers require high food levels for growth (Hansen et al., 1997; Walz, 1978) and can therefore never reduce phytoplankton to very low levels without becoming food-limited. Second, rotifers in the upper Schelde estuary may never reach sufficiently high population densities to control phytoplankton biomass because rotifer populations are kept low by the short retention time of the water (<10–12 days for the freshwater tidal reaches, Muylaert et al., 2000b).

Selective feeding by rotifers has frequently been reported in the literature (e.g. Pourriot, 1977; Starkweather, 1980; Bogdan and Gilbert, 1984). Selective grazing by the microzooplankton community on different phytoplankton groups was evaluated by comparing the composition of the phytoplankton community after incubation in the presence (<200 µm treatments) and absence (<30 µm treatments) of microzooplankton grazing. No difference in phytoplankton community composition was observed between the <30 µm and <200 µm treatments, indicating no strong selective grazing by the microzooplankton. Algal groups were consumed in approximately the same proportion as in which they occurred in situ. This was surprising because diatoms were always an important component of the phytoplankton community and selection against diatoms was expected as diatoms are difficult to ingest by rotifers due to their silica frustules. The lack of significant selective feeding in these experiments may be related to the importance of *Brachionus* spp. in the rotifer community. Compared to other rotifers,

members of the family Brachionidae are considered to be rather non-selective filter feeders (Bogdan and Gilbert, 1984). Moreover, several studies have shown that *Brachionus* spp. are capable of ingesting diatoms or other large phytoplankton species that are similar in size to the *Stephanodiscus* and *Cyclotella* species that occur in the upper Schelde estuary (Hansen et al., 1997; Hotos, 2003).

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