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Looking for general trends in trophic interactions among estuarine micro- and mesozooplankton

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Trophic interactions among micro- and mesozooplankton were investigated in the Schelde estuary. Field populations of plankton were separated by selective filtrations (30 and 200 μm). Predation was measured by comparing ciliate and rotifer abundance in treatments with and without potential predators (cladocerans, cyclopids, the calanoid Eurytemora affinis and rotifers). To deal with variability in the data, a cross-calculation method using all replicates separately is proposed. In order to look for general trends in predation behaviour, the predation rates were ranked and analysed in relation to ranked characteristics of the potential prey: numerical abundance, growth rate, individual and population biomass. Cyclopids fed selectively on the ciliates having the highest population biomass and growth rates among the ciliate population. Cyclopids selected the least abundant rotifers. No trends were detected for cladocerans and E. affinis. As predators, rotifers selected the least abundant ciliates in spring. Although no general selectivity patterns for the predators studied can be drawn yet, the potential of the method employed is illustrated and discussed. Its main advantage is the possibility to extend the obtained data set with data from new experiments as well as with extant data on selectivity of the predators.

INTRODUCTION

The connection of the microbial loop [bacteria—heterotrophic nanoflagellates (HNF)—ciliates] to the classical food chain (mesozooplankton—fish) has been subject to numerous studies in both marine and freshwater systems (Adrian and Schneider-Olt, 1999; Carrias *et al.*, 2001; Samuelsson and Anderson, 2003). These studies often point to complex and variable links between micro- and mesozooplankton. This complexity and variability is often related to the selective feeding of mesozooplankton predators on microzooplankton preys which influences the structure of the plankton community (Karabin, 1978; Fussmann, 1996; Plassmann *et al.*, 1997; Yoshida *et al.*, 2000). A prey species can be selected by a given predator solely because of its specific characteristics such as size, palatability or defense mechanisms (Williamson, 1983, 1987; Stemberger and Gilbert, 1987; Roche, 1990). It can also be selected because of what makes its characteristics specific within the context of the total potential prey community in the feeding medium. A large-sized species, for example, can be selected because it is easily detectable and offers an important energy intake (Ramakrishna Rao and Kumar, 2002). A large prey species might also be selected because it is the biggest species within the available prey range under the given circumstances, and might not be selected by the same predator in the presence of other, bigger species. In other instances, small prey might be preferred (Kerfoot, 1977; Brandl and

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Fernando, 1978; Confer and Applegate, 1979; Ramakrishna Rao and Kumar, 2002). A species might be selected because the combination of its body size and the population's abundance makes it the main contributor to the total population in the feeding medium (Brandl and Fernando, 1978; Karabin, 1978; Ramakrishna Rao and Kumar, 2002). Prey may also be selected when they have a considerable growth rate, most likely a condition consistent with a good nutrient status, and which coincidentally also makes them a well-replenished food source.

Most data on selective feeding interactions are obtained from experiments considering one specific field situation or, at best, a series of experiments within one specific system. Such data do not easily allow investigations of the selective feeding behaviour of mesozooplankton predators as a function of quantifiable characteristics of the prey community. Therefore, it has hitherto been difficult to generalize about the mechanisms structuring the complex interactions between the microbial food web and the classical food chain. In order to detect general patterns in prey selection under natural conditions, we need to be able to combine data from various field experiments that cover a broad range of situations, both in terms of the above-mentioned prey characteristics and of environmental conditions such as, for instance, temperature. Due to the fact that predation pressure varies with prey abundance, prey characteristics and physical conditions (Mullin, 1963; Frost, 1972), the measured predation pressures might be considered on a relative rather than absolute scale. The potential prey present in various experiments will inevitably cover various species and even various types of organisms. Therefore, we also need to consider the prey characteristics mentioned above in a relative scale which is independent of the specific composition of the prey community in each experiment.

Estuaries are systems which have characteristically high concentrations of particulate matter. This makes it difficult to obtain measurable differences in particle concentrations between mean control and experimental bottles in an incubation counting type experiment and renders microscopic analysis extremely laborious and limited in its resolution (Tackx *et al.*, 2003); see introduction in Joaquim-Justo *et al.* (2004) for a recent summary. Due to the presence of strong spatial environmental gradients and a high temporal variability related to fluctuating freshwater inputs, estuaries are moreover very dynamic ecosystems characterized by a high spatio-temporal variability of the planktonic community composition. It is hence very difficult to obtain consistent series of experiments on one prey or predator taxon or even on one prey or predator functional group under estuarine field conditions. Estuaries therefore represent rather extreme instances in relation to the problems explained above. Faced with this complexity, relatively few predation experiments have been performed using natural estuarine populations (Havens, 1991; Griffin and Rippingale, 2001; Froneman, 2002; Sipura *et al.*, 2003; Tackx *et al.*, 2003).

In an attempt to elucidate general trends in the feeding interactions between micro- and mesozooplankton under natural conditions in the complex situation offered within the Schelde estuary (Belgium and the Netherlands), we report data from incubation experiments with copepods, cladocerans and rotifers as predators and with rotifers and ciliates as prey in a 'functional group' approach. In order to optimize the information obtained from zooplankton feeding experiments under estuarine field conditions, we propose a method to cover the variability in prey abundances measured between replicate control—or experimental bottles (see Methods). To be able to combine data from various experiments involving different communities of potential prey and physicochemical conditions, a ranking method of relative intensity of predation pressure on the one hand and ranking of prey characteristics (numerical abundance, individual size, population biomass, growth rate) on the other hand is employed.

METHOD

Experimental setup

Predation of mesozooplankton (copepods, cladocerans) on microzooplankton (rotifers, copepod nauplii and ciliates) and by rotifers on ciliates was studied by comparing growth rates of potential prey items in the presence and absence of predators. Experiments were carried out in spring (March) and early summer (June) 2003, at three sites in the Schelde estuary (Belgium): Antwerpen (brackish), Dendermonde (freshwater tidal) and Gent (riverine). Water for the experiments was collected from a pontoon using bucket hauls. Figure 1 presents the experimental setup. Predation of rotifers on ciliates was evaluated by comparing the abundance of ciliates in 30-μm filtrates (containing ciliates but no rotifers) with their abundance in 200-μm filtrates (containing both ciliates and rotifers). Predation of mesozooplankton on rotifers and ciliates was evaluated by comparing the abundance of rotifers and ciliates in a 200-μm filtrate (containing rotifers and ciliates but no mesozooplankton) with their abundance in a 200-μm filtrate to which a known number of individuals from a mesozooplankton species were added. Two mesozooplankton

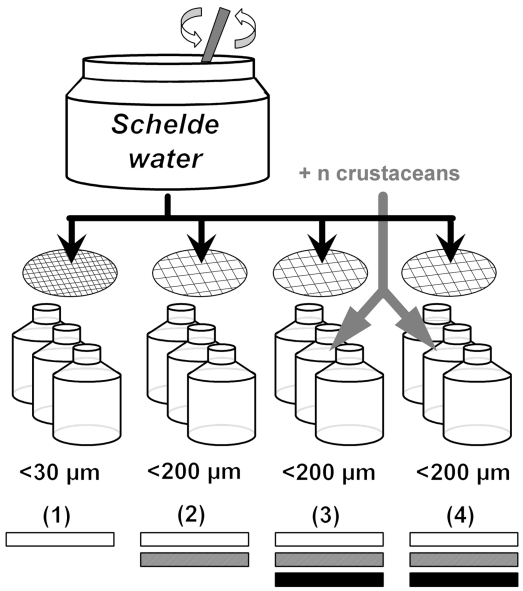

Fig. 1. Experimental setup, showing the filtration fractions (<30 and <200 µm). Four treatments were performed, each in triplicate, containing the assemblage of phytoplankton, flagellates and ciliates (white bars), rotifers (striped bars) and copepods or cladocerans added (black bars) or not.

treatments were prepared for each experiment. Filtrates were prepared by reverse filtration through nylon meshes. Preliminary tests were carried out to determine which mesh sizes were most efficient for separating ciliates, microzooplankton and mesozooplankton. Mesozooplankton to be added to the experimental bottles were collected by filtering a large volume of water through a 200-µm plankton net. Mesozooplankton individuals were picked out in the laboratory under a stereomicroscope using a wide-bore pipette. If two species were co-dominant, the impact of these two species was assessed separately. If only one species was dominant, this species was added to the treatments in different numbers. A summary of the species used and the number of specimen added in each experiment is given in Table I. Each treatment was prepared in triplicate in 1-L polycarbonate bottles. The bottles were incubated in a temperature- and light-controlled incubator. Temperature was set within ~1°C of the field temperature at the time of sampling (10°C in March and 20°C in June). Light intensity was set at 22 µmol m^{-2} s^{-1}. In March, light was (accidentally) supplied continuously while a 12-h dark–12-h light cycle was supplied in June. Bottles were incubated during 3 days on a rotating table (100 rpm) and manually shaken three times a day in order to avoid sedimentation effects. The bottles were sampled at the beginning (t_0) and at the end of the experiment (t_{end}). For enumeration of ciliates, a 40-mL sample was fixed according to the Lugol, formalin, thiosulphate method (Sherr *et al.*, 1989). Rotifers were sampled by filtering 50–100 mL water over a 30-µm nylon mesh, which was stored in 4% formalin.

Data acquisition

Salinity and temperature were measured *in situ* using a YSI 650 MDS multimeter with a YSI 600 R sensor. Ciliates were identified, measured and enumerated using an inverted microscope. To distinguish ciliates from suspended matter, Bengal rose was added to the samples and only a small subsample was concentrated in the sedimentation chambers. Ciliates were identified to the class level using Foissner *et al.* (Foissner *et al.*, 1999) and a minimum of 100 individuals counted per sample. Up to 30 individuals of each ciliate class were measured (body length and wide) using the inverted microscope micrometer, in order to estimate their biomass. Rotifers were washed off the mesh on which they were collected and a sub-sample was enumerated in a counting wheel using a dissection microscope. Identifications were based on Ruttner-Kolisko (Ruttner-Kolisko, 1972), Pontin (Pontin, 1978) and Segers (Segers, 1995) and were carried out up to the species level if possible. The individual biomass (IB) of ciliates and rotifers was estimated using bibliographic

Table I: Mesozooplankton predator species used in the spring and summer experiments

	Spring			Summer		
	Gent	Dendermonde	Antwerpen	Gent	Dendermonde	Antwerpen
Chydorus sphaericus	20	—	—	—	—	—
Moina affinis	—	—	—	20	—	—
Daphnia longispina, Daphnia magna	—	—	—	—	—	12
Eurytemora affinis	—	20	20	—	—	20
Acanthocyclops trajani	—	—	—	40	20 and 40	—
Cyclops vicinus	20	20	10	—	—	—

The number of specimen introduced in the 1 L bottles of the two mesozooplankton treatments is indicated for every station. With the exception of the Dendermonde experiment in summer, in which one species (*A. trajani*) was used at two different densities, two species were employed separately in all experiments.

data (Dumont *et al.*, 1975) and the measurements mentioned above. In this last instance, the individual volume was calculated following an appropriate geometrical form (Bottrel *et al.*, 1976) and the biomass was estimated as 10^6 $\mu m^3 = 1$ μg of wet weight (Lohmann, 1908). The individual biomasses of ciliate and rotifer species observed in this study are presented in Table II. The IB of each prey taxon (PB) was calculated by multiplying its numeric abundance (Ab) with its IB. Mesozooplankton used in the experiments was identified to the species level using a dissection microscope.

Data analyses

The experiments performed covered a variety of predator–prey combinations and physico-chemical conditions, typical for this type of field studies.

For each predator, the predation rate g on each potential prey species considered was calculated as

$$g = \ln(C_t/Cz_t) \cdot \frac{1}{t}$$

with C_t and Cz_t being the abundance of the prey in, respectively, the absence and presence of predators at the end of the experiment and t being the incubation time in days.

Table II: Individual biomass, in micrograms fresh weight, of the different taxa used for the determination of the IB-ranks

Taxon	Individual biomass (μg FW)
Rotifers	
Brachionus angularis	0.470
Brachionus calyciflorus	0.400
Brachionus leydigi	0.150
Brachionus rubens	0.150
Other *Brachionus*	0.400
Bdelloids	0.110
Filinia brachiata	0.450
Filinia longiseta	0.450
Keratella cochlearis	0.110
Keratella quadrata	0.340
Notholca sp.	0.460
Polyarthra sp.	0.740
Synchaeta sp.	0.260
Trichocerca sp.	0.110
Other rotifers	0.300
Ciliates	
Colpodea	30.000
Gymnostomatea	0.106
Heterotrichida	0.037
Hypotrichia	0.007
Oligotrichida	0.010
Peritrichia	0.077
Prostomatida	0.005
Scuticociliata	0.002
Tintinnids	0.068

Growth rate k was calculated as

$$k = \ln(C_t/C_0) \cdot \frac{1}{t}$$

with C_t and C_0 being the abundance of the prey in the absence of predators at the end and the start of the experiment, respectively, and t being the incubation time in days.

Ab was estimated as

$$\mathrm{Ab} = \frac{(C_t - C_0)}{\ln(C_t/C_0)}$$

with C_0 and C_t being the abundance of the taxa at the start and the end of the experiment, respectively, in the control bottles.

In the type of experiments carried out for this study, the significance of a predator–prey interaction is generally tested by comparing the average prey density in the presence and absence of a predator and g is calculated from the mean prey abundance in the presence and absence of the predator at the end of the experiment. The probability of finding significantly different prey abundances in the presence and absence of the predator decreases with variability between the replicates. Hence, in this approach, predation interactions often go undetected due to, for instance, one outlier in a control bottle or high variability among replicates because only a low number of individuals were observed during the counts.

In order to take into account the variability of the prey abundances, growth rates and predation activity among the experimental bottles, Ab, k and g were calculated for all control or experimental bottles or combinations thereof. For example, k was calculated for each of the three control bottles whereas g was calculated for each possible control bottle–experimental bottle combination. A detailed scheme illustrating the procedure followed for these cross-calculations is given in Fig. 2.

In order to be able to combine the results obtained from all of these experiments, we propose the use of considering ranks of predation pressure (measured as g). This should allow general conclusions with respect to the feeding behaviour of a given planktonic predator or functional group to be drawn. In this study, we analyse these ranked predation pressures in relation to a set of easily quantifiable, ranked prey characteristics: (i) their mean numerical abundance (Ab), (ii) their growth rate (k), (iii) their individual biomass (IB) and (iv) their population biomass (PB). Only the most abundant prey taxa, together representing 90% of the prey numeric population were considered in the analysis.

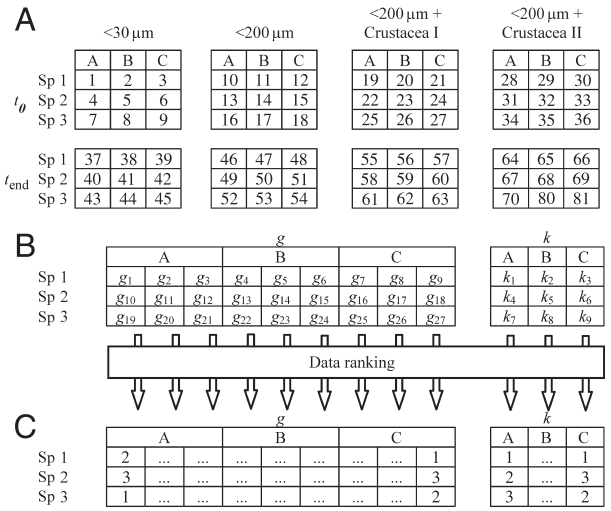

Fig. 2. (**A**) Each cell contains the abundance of a species in one of the three incubation bottles (A–C) at t_0 or t_{end} for the four treatments. The cells are numbered from 1 to 81. (**B**) Calculation of the predation rates g (for one of the two treatments with mesozooplankton) and the growth rates k using all possible cross-calculations. g_i is the grazing rate calculated using the abundances of the prey species, at t_{end}, in the treatments with and without predator (see the formula in Materials and Methods). For example, using pairs of cells 46–55, 46–56 and 46–57, respectively, for g_1, g_2 and g_3; 47–55, 47–56 and 47–57 for g_4, g_5 and g_6. k_i is the growth rate calculated using the abundance of a species at t_0 and t_{end} in each control bottle (see the formula in Materials and Methods). For example, using cells 1 and 37 for k_1, or 17 and 53 for k_8. (**C**) All columns were then ranked according to the g or k values, resulting in the scheme in Fig. 2. For example, the ranks 2-3-1 shown in the first row correspond to $g_{19} > g_1 > g_{10}$ in (**B**), and the last row 1-3-2 correspond to $k_3 > k_9 > k_6$. See Materials and Methods for further explanation.

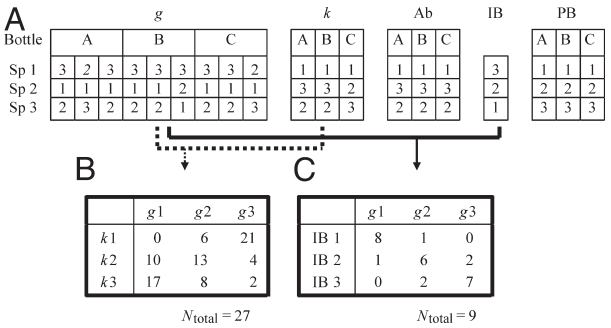

Fig. 3. Example illustrating the calculation of predation rate rank frequencies on prey as a function of their characteristic's ranks (growth rate k, abundance Ab, individual biomass IB and population biomass PB). A simple fictitious table with the rank-values of three prey species (sp 1–3) is presented in section (**A**). (**B**) Indicates the frequencies obtained as a function of growth rate (k) and (**C**) the frequencies obtained as a function of prey numerical abundance (Ab). See text for further explanation.

In each experiment, characteristics of each prey taxon were observed in each bottle and were ranked between the taxa according to g or k (Fig. 2). Only the positive values were considered for the ranking. Nine g-ranks were estimated for each prey, with cross-calculations, by comparing its abundances between the two triplicates sets of bottles—with and without predators; three k-ranks, three Ab-ranks and three PB-ranks were estimated with the three bottles without predator; the IB-rank was estimated using measurements and the literature. In order to illustrate the method, we present a simplified fictitious table with only three prey species and their associated ranks (Fig. 3). With crossed combinations, the frequencies, for each experiment, were based on 9 possibilities for the IB (9 g-ranks × 1 IB-rank) or 27 (9 g-ranks × 3 k, Ab or PB ranks) for k, Ab and PB. Using these ranked data, the frequency of the g-ranks as a function of the prey ranked characteristics was inventoried considering all experiments in which the same predator, in terms of functional groups (for example, cyclopids), was calculated. Spring and summer experiments were considered separately, because of a substantial difference in predator species occurrence. In Fig. 3, an example of the method is given for the calculation of the g-rank frequencies as a function of the k-rank frequencies.

When g was calculated from differences in prey abundance between control bottle 1 and experimental bottle 1 (column 1), species 1 was ranked third with respect to its g (it was the least predated species among the three potential preys). Hence $g_{1,1}(\text{sp1}) = 3$, noted in bold in Fig. 3A—*g*. It was ranked second with respect to its g when g was calculated from differences in its relative abundance in control bottle 1 and experimental bottle 2, hence $g_{1,2}(\text{sp1}) = 2$, noted in italics in Fig. 3A—*g*. This species had the highest k (it was the fastest grower) in control bottle 1, hence $k_1(\text{sp1}) = 1$, in bold in Fig. 3A—*k*. We count a score of 3 for $g3$ with $k1$ meaning in three instances the third highest g value was measured on the fastest growing prey. The second g-rank estimated for sp1, with a value of 2 (in italics), also corresponds with $k1$ in the three bottles, giving a score of 3 for $g2$ with $k1$. In this way, scores were calculated for the nine g-ranks of each species. The sum of the scores of the g-ranks with the k-ranks is given in Fig. 3B. The same reasoning was applied with all prey characteristic ranks. Figure 3C shows the results obtained for IB-ranks. N_{total} is the number of possible combinations.

A Spearman rank test at $P < 0.05$ was used in order to test this distribution for each g-rank and for the different cumulated g-ranks (i.e. $g1 + g2$, $g1$ to $g3$, $g1$ to $g4$, etc.). If these frequencies increased or decreased as a function of the prey characteristics ranks, the predation was considered to be selective; otherwise the predation

was unselective with regard to the prey characteristic considered.

In our fictitious example (Fig. 3), the distribution of the occurrences shows that prey which have the lowest growth rate are the most grazed, with a score of 17 *g*1 for *k*3, 13 *g*2 for *k*2 and 21 *g*3 for *k*1. Figures 4–7, showing our results, are a visual representation of this kind of table where the number of occurrences was transformed in frequencies, becoming contingency tables.

RESULTS

Mean numeric abundances of the prey

Ciliate abundance at the sampling sites was comparable between spring and summer, with about 75 ind. mL^{-1} in Antwerpen and Dendermonde and about 300 ind. mL^{-1} in Gent (Fig. 8). Scuticocilates dominated the ciliate community in Gent in spring and summer and in Dendermonde and Antwerpen in summer. Oligotrichids

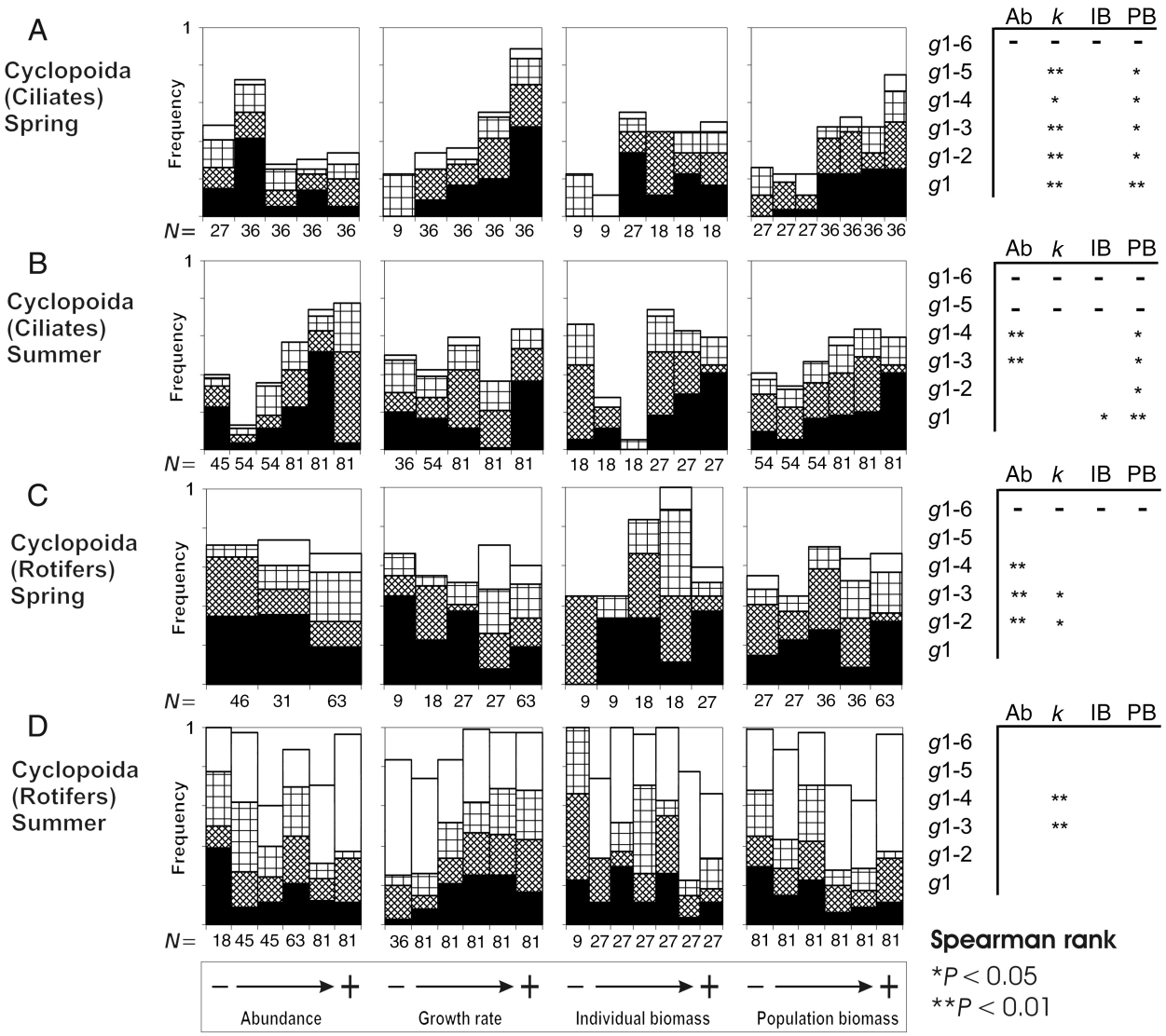

Fig. 4. Cumulated frequencies of the predation rate (*g*) ranks of Cyclopoida on ciliates [(**A**) spring and (**B**) summer] and on rotifers [(**C**) spring and (**D**) summer] in relation to the abundance (Ab) rank, the growth rate (*k*) rank, the individual biomass (IB) rank and the population biomass (PB) rank of the prey. The black, cross-hatched and squared bars are the first, second and third *g*-ranks. The white bars are the fourth and more *g*-ranks. The white area above the bars represents the frequency of the negative or zero *g*. *N* is the number of occurrences used for the calculation of the frequencies. Significant positive correlations between *g* frequencies and an increase/decrease in the prey characteristics frequencies are shown on the right. Spearman rank correlation was applied to each of the cumulative series of the positive *g* ranks, from the first *g*-rank (*g*1) to the sixth *g*-rank (*g*1–6); the minus sign indicates the absence of cumulative series and significances are shown as asterisks.

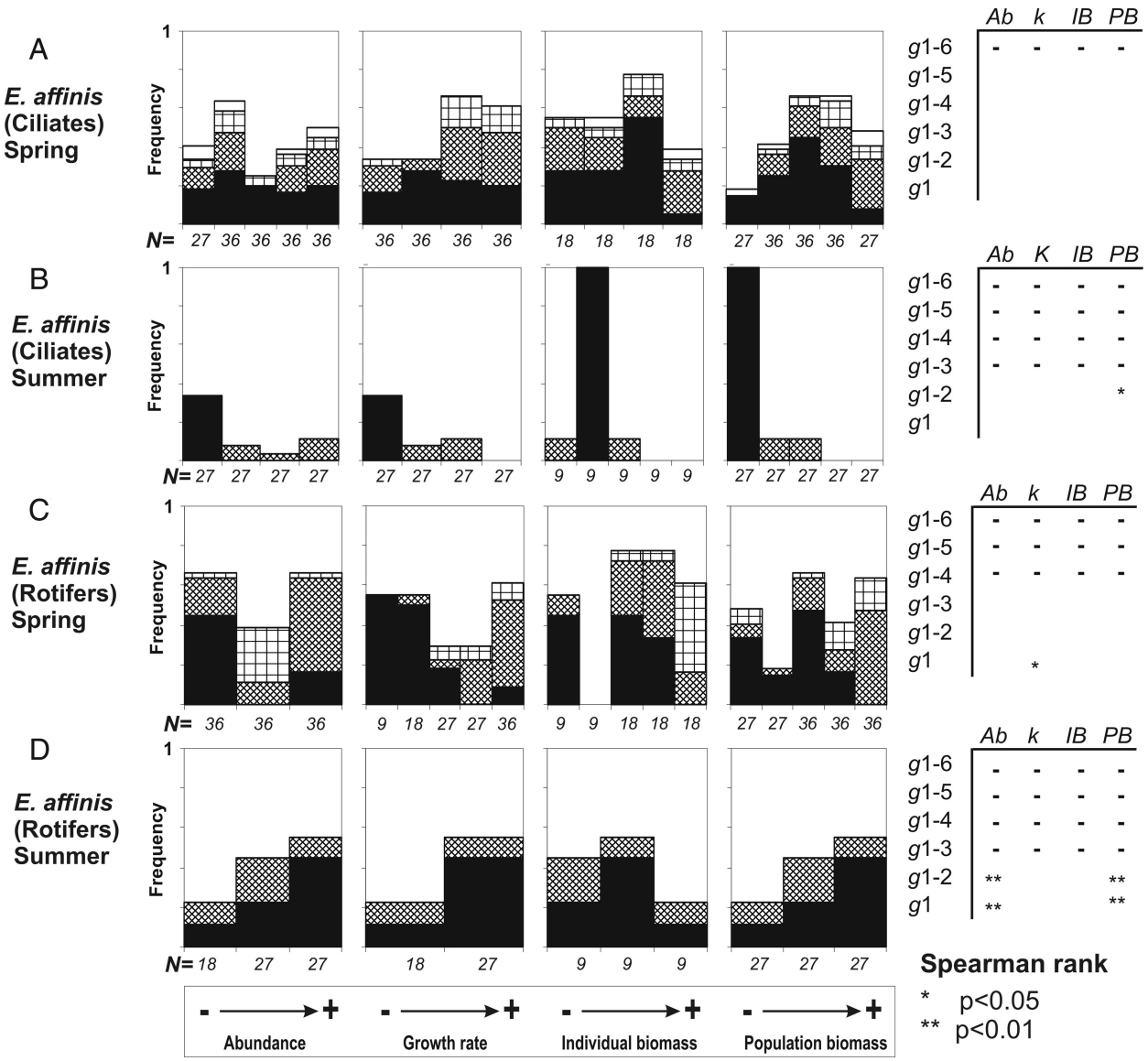

Fig. 5. Cumulated frequencies of the predation rate (*g*) ranks of *E. affinis* on ciliates [(**A**) spring and (**B**) summer] and on rotifers [(**C**) spring and (**D**) summer], with the abundance (Ab) rank, the growth rate (*k*) rank, the individual biomass (IB) rank and the population biomass (PB) rank of the prey. See Fig. 4 for further details on legend.

dominated the ciliate community in Antwerpen and Dendermonde in spring. Ciliate classes other than scuticociliates and oligotrichids were relatively rare and never exceeded 40% of total ciliate abundance. In spring, rotifer abundance decreased from Gent to Antwerpen (Fig. 8), mainly due to a decrease in the abundance of the dominant species *Brachionus calyciflorus*. This tendency was also observed at t_0 of the summer experiment, but the small *Trichocerca* sp., dominant everywhere at t_0, considerably increased during incubation in the Dendermonde experiment. So, the highest mean abundances were reached in summer for this station (Fig. 8).

Selectivity patterns

In the spring experiments with cyclopoid copepods as predators, a selective feeding of cyclopoid copepods on ciliate taxa that attained high biomass and achieved high growth rates was detected (Fig. 4A). In the summer experiments with cyclopoid copepods, the *g*-ranks of ciliates were also positively correlated with the PB-ranks but not with the *k*-ranks (Fig. 4B). With respect to predation by cyclopoid copepods on rotifers, a significant negative correlation was only found between *g*-ranks and Ab in the spring experiment (Fig. 4C). This

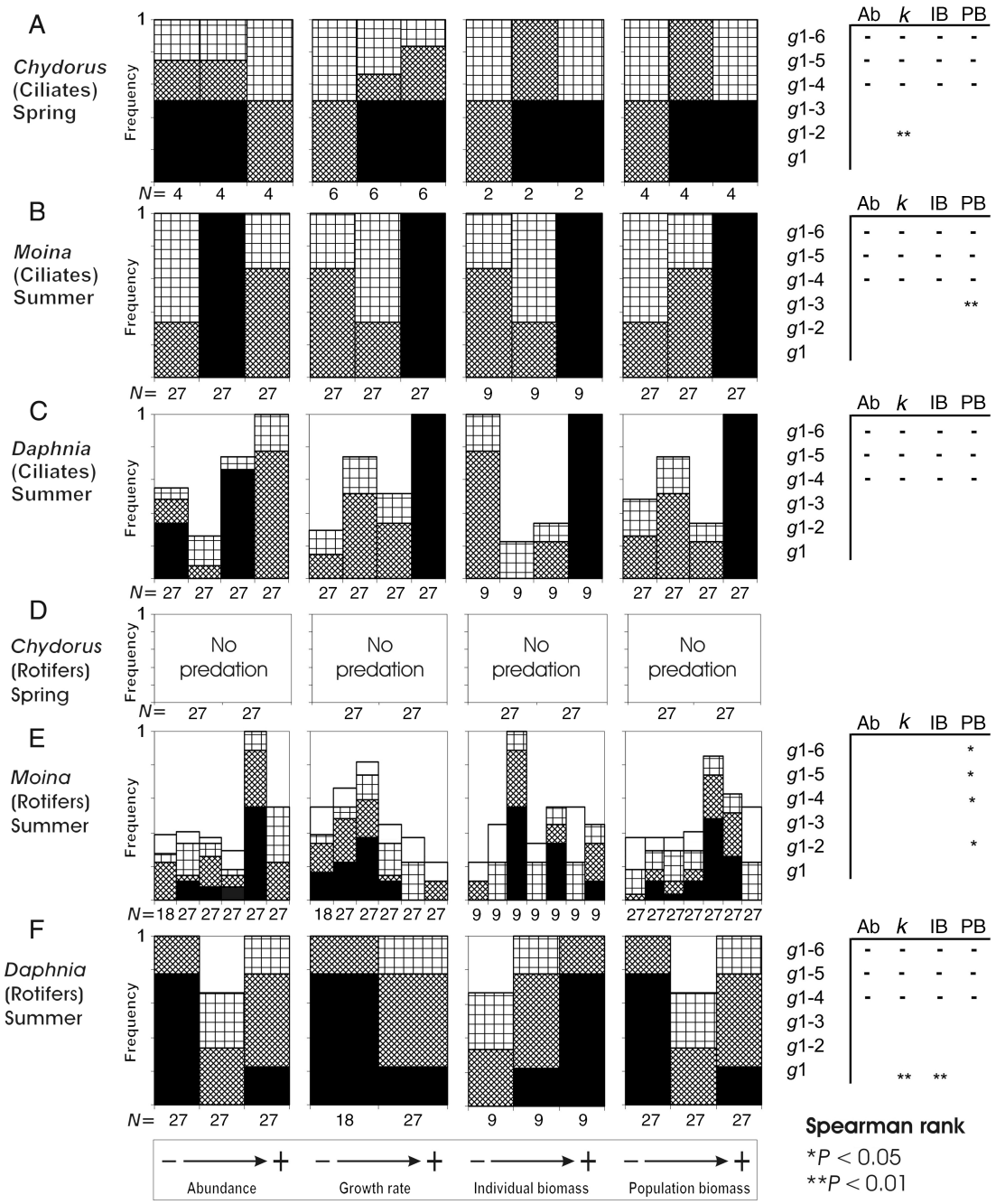

Fig. 6. Cumulated frequencies of the predation rate (*g*) ranks of Cladocera on ciliates (**A**–**C**) and on rotifers (**D**–**F**) with the abundance (Ab) rank, the growth rate (*k*) rank, the individual biomass (IB) rank and the population biomass (PB) rank of the prey. Cladocerans considered were *Chydorus* (**A** and **D**) in spring and *Moina* (**B** and **E**) and *Daphnia* (**C** and **F**) in summer. See Fig. 4 for further details on legend.

correlation was negative, indicating selective feeding on rotifers with a low relative abundance.

The only significant correlations observed in the experiments with the calanoid copepod *Eurytemora affinis*, were between *g*-ranks and rotifer Ab and PB-ranks in the summer experiments, (Fig. 5D). No significant correlations between *g*-ranks and any other ranks were detected for cladocerans (Fig. 6). For rotifers feeding on ciliates, a significant negative correlation was observed between *g*-ranks and Ab in the spring experiments (Fig. 7), indicating selective feeding on the less abundant ciliates.

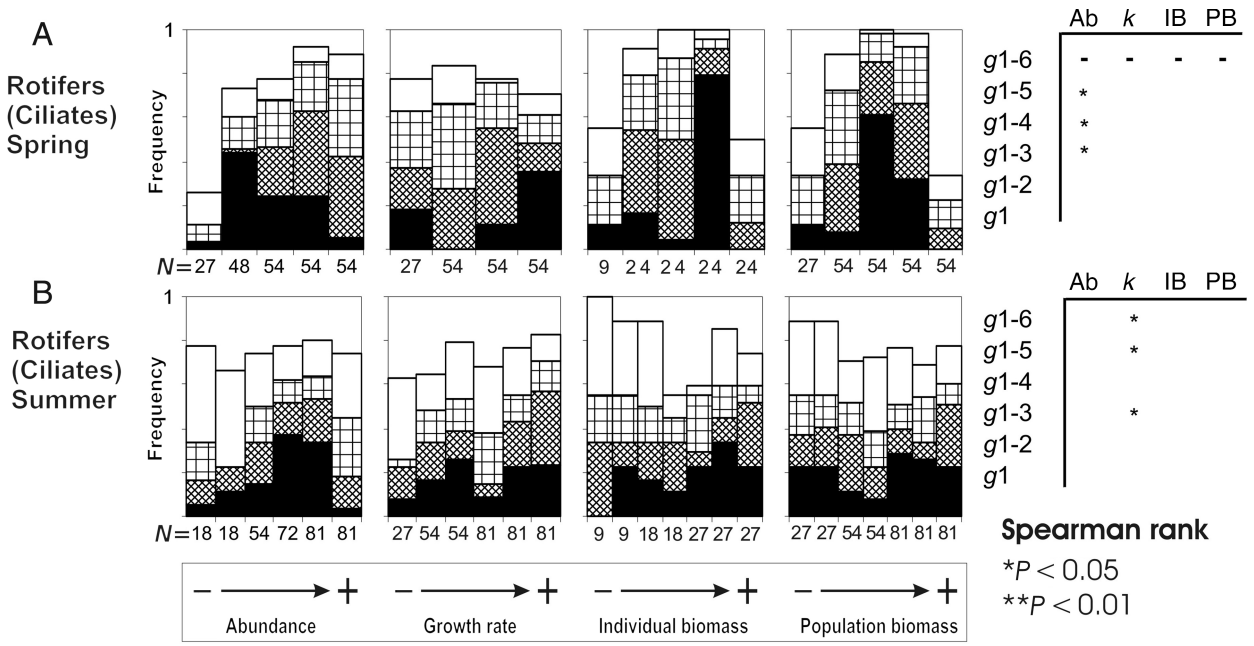

Fig. 7. Cumulated frequencies of the predation rate (*g*) ranks of rotifers on ciliates [(**A**) spring and (**B**) summer] with the abundance (Ab) rank, the growth rate (*k*) rank, the individual biomass (IB) rank and the population biomass (PB) rank of the prey. See Fig. 4 for further details on legend.

DISCUSSION

In order to approach field conditions as much as possible, this study was performed with the natural plankton assemblages present in the Schelde estuary at the time/site of the experiments. This resulted in a wide variety of predator–prey combinations and physico-chemical conditions, typical for this type of field studies. These types of data do not readily allow general conclusions to be drawn from a limited number of experiments with a given set of conditions (predator, prey, environmental circumstances).

With the cross-calculations performed in this study, the information from an experiment with a non-significant difference in mean prey abundance between the experimental and the control bottle does not need to be discarded. Although this procedure leads to an inflation of the number of instances per experiment, it does not bias the results: variability within control bottles can generate positive or negative *g* values alike. However, in order to guarantee the objectivity of the approach, it is also important to consider the number of instances in which no positive predation was calculated, as was done in the Figs. 4–7.

The ranking of predator intensity measured as *g* as applied here accounts for the standardization of predation intensity over various potential feeding situations with regard to both potential prey composition and environmental conditions. If a predator selects a prey because it is the largest among the ones present in the feeding medium, but does not differentiate between which species it is, the different prey species selected in various experiments will all score a high-ranked *g* value. As to environmental conditions, temperature, for example, these are likely (expected) to influence total feeding activity of the predators, but not the selectivity pattern, so not the ranks of *g*.

In this first approach, prey characteristics considered for ranking were numerical abundance, individual size, population biomass and growth rate. The importance of the prey characteristics features in zooplankton selection patterns has amply been shown in the literature (Poulet, 1973; Gamble, 1978; Bollens and Penry, 2003; Lapesa *et al.*, 2004; Atienza *et al.*, 2006). These features also have the advantage of being quite easy to quantify, either by using microscopic analysis, which can be assisted by an image analysis apparatus, or by an automatic counting device such as the Coulter counter (Tackx *et al.*, 1989; Billones *et al.*, 1999).

To synthesize the selection patterns observed in the different experiments with the same predator or functional group, we finally considered correlations between *g*-rank frequencies and ranked prey characteristics frequencies as observed in the totality of the experiments with a given predator. In this regard, we had some difficulty in deciding which *g*-rank(s) to consider. The highest observed value in a given experiment, *g*1, may be very close to *g*2, the second highest

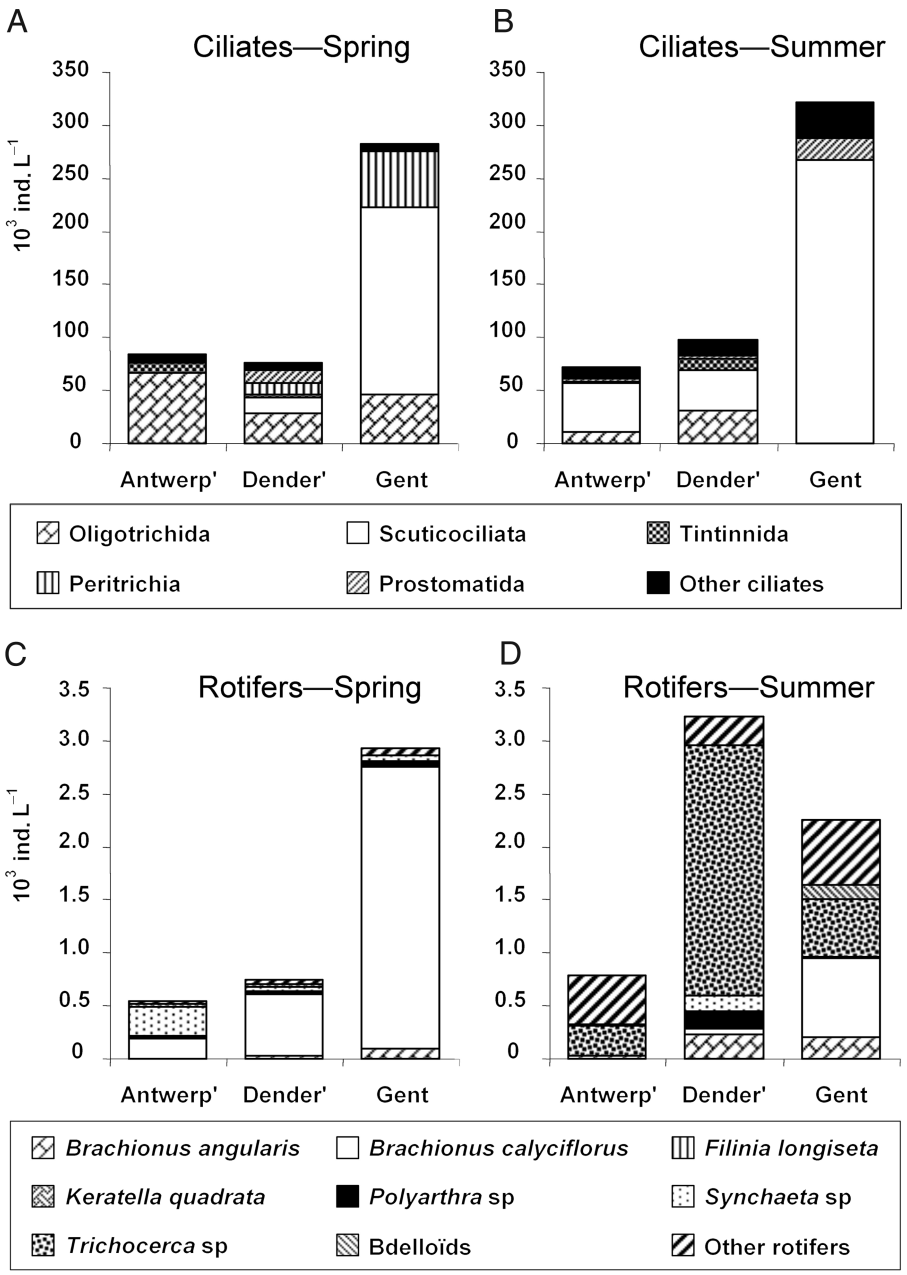

Fig. 8. Mean numerical abundance of prey measured in the three control bottles at t_0 for Ciliates [(**A**) spring and (**B**) summer] and rotifers [(**C**) spring and (**D**) summer] at the three stations (Antwerpen, Dendermonde and Gent).

value, and in fact not functionally different. The cumulative value of *g*1 and *g*2 (*g*1-2) may therefore better represent the 'highest' predation activity, than *g*1 only. In order for the detection of the selectivity patterns to be conservative, we have opted—in an arbitrary way—to consider only those instances as significant, in which a consistent significance of at least three consecutive cumulative values, starting from *g*1 or *g*2 onwards, showed a significant correlation with the prey characteristic considered. For example, in Fig. 4A, *g*-rank series are significantly related to *k* and to PB from *g*1 to *g*1-3 onwards, and in these instances even till *g*1-5. We consider however that the correlation between *g*1-4 and following cumulative *g* values are generated by the addition of randomly distributed frequency values to the *g*1-3 series. In Fig. 4B, we observe a significant correlation from *g*1-3 onwards with prey abundance. Following our selection criteria

this instance is not considered significant, as the correlation is not from $g1$ or $g2$ onwards.

In our dataset, on average, 39% of the instances did not result in positive predation. This can be due to variability in the data, but also to the fact that the potential prey considered is simply not eaten by the predator. The coefficient of variation between prey abundance data in the three replicate control bottles at t_{end} varied between 3% and 31% for ciliates and 3% and 34% for rotifers. No significant difference in variability was observed between the spring and summer experiments, and variability in the experimental bottles was also in the same range for both ciliates and rotifers in both seasons. The taxon abundance was—logically—the most important factor influencing its variability, stressing the importance of counting a sufficiently high number of individuals for each species to be included in the analysis. On the other hand, differences in feeding activity of a given predator under identical incubation conditions can also occur as a result of his history prior to the incubation (stress during handling, for example). As long as the feeding selectivity pattern is not influenced, ranking will allow to combining the data in the analysis, whereas the absolute predation pressure rates may differ substantially.

In our present dataset, instances with a very clear trend such as for example 100% positive gs for cladocerans (e.g. *Chydorus sphaericus*) on ciliates (Fig. 6A) and 0% on rotifers (Fig. 6D), are based on only one experiment with this predator. Clearly more equilibrated sets on each predator type are necessary to be able to analyse the information obtained from 'negative' results in depth.

As the number of experiments that were carried out for this study was limited and as each experiment was characterized by often different combinations of prey and predator populations, we cannot yet draw any general conclusions on selective feeding behaviour of the predators investigated. They do, however, allow the feasibility of the proposed ranking and cross-calculation method to be demonstrated.

The selection of the ciliate biomass population peak by cyclopoids in the spring experiments is in agreement with the findings of several previous studies. In grazing experiments using Coulter-counter or microscopical analysis of the potential prey volume distribution in the feeding medium selective predation on the biomass peak has been reported for several species of suspension feeders (Poulet, 1973; Allan *et al.*, 1977; Richman *et al.*, 1977; Gamble, 1978; De Mott, 1988; Tackx *et al.*, 1989; Tackx *et al.*, 2003). Our results indicate that this 'peak tracking' is also exerted by cyclopoids when feeding on ciliates. The population peak selected consisted of the fastest growing ciliates (Scuticocilates, Prostomatida or Colpodea depending on the station and the season). Such a situation (biomass bulk of rapid renewable prey) could represent an easy-detectable and sustainable prey resource for the predator. In contrast, selection of the least abundant prey occurred for cyclopoids feeding on rotifers and for rotifers feeding on ciliates, both in the spring experiments. In this instance, the low prey abundance could be a consequence of the high predation pressure rather than a cause, though nonetheless it suggests that predators may continue to graze a selected prey item long after its abundance has declined to low relative if not absolute levels (Flynn *et al.*, 1996). In the experiments using *E. affinis* or cladocerans as predators, the low number of experiments and/or the low diversity of prey selected probably lead to a lack of a significant trend.

Further experiments will indeed have to reveal if the trends detected for other taxa are confirmed and if any other ones arise.

The advantage of the proposed method over selectivity indices (Ivlev, 1961; Vanderploeg and Scavia, 1979; Chesson, 1983) is that it enables us to complement the analysis with data from past or future experiments, performed under different conditions or even in different systems but involving the same predator species or predator functional group. However, when combining data from experiments using various incubation volumes, clearance rate 'F' should be used instead of 'g', as predation pressure 'g' measured for a same feeding activity of the predator will vary among experiments with the incubation volume used. Caution will also be needed when combining datasets of experiments using rather widely different experimental conditions. Predator density as well as incubation time, for instance, can influence the selectivity pattern measured, because of stimulated production of small particles (notably algae) in the experimental bottles as compared to the controls (Roman and Rublee, 1980; Tackx and Polk, 1986). Such effects influence the ranking of predation pressure values depending on the experimental setup used. Further exploration of this method should permit to evaluate and possibly adjust the choice of criteria for considering correlations between g and prey characteristics as valid.

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