

Evidence for constant and highly specific active food selection by benthic ciliates in mixed diatoms assemblages

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Abstract

Observational and experimental studies have shown that phagotrophic ciliates are highly selective predators. However, little is as yet known about the actual mechanisms involved in prey selection. We used direct behavioral observations to study the mechanism of prey selection in benthic algalivorous ciliates feeding on mixed assemblages of diatom species. Four ciliate species, viz. three *Strombidium* species and a *Pseudochilodonopsis* species, and three diatom species from intertidal sediments in the Schelde estuary were used for the experiments. In each experiment, a single ciliate species was offered a mixture of two diatom species. The feeding preferences of the ciliates were estimated as well as relative encounter rates, attack probabilities, and capture successes for the prey species. The feeding preferences of the ciliates were distinctly predator-specific and highly discerning with respect to the nature of the prey species. They appeared to result mainly from active selection at the time of the encounters and between the encounter and attack of the diatoms. Our observations suggest that selective encounters with the diatoms were caused by noncontact detection of individual prey items, at least for the *Strombidium* species. Additional experiments confirmed that these ciliates were able to distinguish between diatom species on the basis of soluble chemical cues. Grazing was also constant, i.e., prey switching was not observed. Moreover, neither total prey density nor feeding history influenced the preferences. We hypothesize that these highly specialized trophic interactions may be an important driving force in shaping structure and diversity of benthic diatom communities in intertidal sediments.

Many protozoans in subtidal and intertidal benthic ecosystems feed on diatoms (Fenchel 1968, 1969; Finlay et al. 1993). Diatoms are important primary producers and an important food source in estuaries (Admiraal 1984; Underwood and Kromkamp 1999). Few investigations have attempted to quantify the influence of benthic protozoan grazing on diatom communities. These studies generally reported a rather small direct impact of protozoan algalivory on microphyto-benthic standing stock and production (Fenchel 1975; McCormick 1991; Epstein et al. 1992; Balczon and Pratt 1996; Hamels et al. 1998). On the other hand, benthic algalivorous ciliates, just like protozoa in general (Verity 1991), are selective predators (e.g., McCormick 1991; Balczon and Pratt 1995). According to Epstein et al. (1992), only 4 of the 42 extant diatom species on a sandy intertidal flat were extensively grazed by ciliates. The selective predation on diatom species suggests that benthic algalivorous ciliates might influence more the species composition and succession of diatoms than their numbers. However, the actual mechanisms involved in this selection are far from clear.

Selective feeding is defined as the imbalance between the

proportion of prey types in a predator's diet and the proportion of the same prey types in the environment (Chesson 1983). This can involve passive mechanisms, which are governed by the relative availability and predation susceptibility of the prey species, as well as active predator behavior. Mechanical constraints related to size or growth form of the diatoms and size of the ciliates and design of their feeding apparatus can determine the susceptibility of diatoms to ciliate predation (Fenchel 1968; Patterson et al. 1989; McCormick 1991). Finlay et al. (1993) suggested that benthic algalivorous ciliates select diatom prey on a merely mechanistic basis. However, size and other morphological features alone did not adequately explain the observed feeding patterns of algalivorous ciliates in other studies (McCormick 1991; Balczon and Pratt 1995). Prey may also differ in their behavior (e.g., motility) and therefore differ in their susceptibility to predation (e.g., Müller and Schlegel 1999; Broglio et al. 2001). However, discrimination between prey of similar size, shape, or behavior was found in several protozoan species (e.g., Nygaard et al. 1988; Landry et al. 1991) and suggests that active selection may also determine protozoan feeding preferences (Verity 1991). For example, Balczon and Pratt (1995) found that algalivorous ciliates selected against stained diatoms when stained and unstained diatoms of the same species were offered simultaneously to the ciliates. Active selection by protozoa is generally attributed to differences in the nutritional value of the particles, the palatability of the particles, or the production of toxic or inhibitory compounds by certain taxa, and is thought to be mediated by chemical cues (Verity 1988, 1991; Balczon and Pratt 1995; Müller and Schlegel 1999). However, it is as yet largely unknown how chemical cues might affect the feeding behavior of protozoa. Chemosensory behavior is probably involved in the localization of prey patches, but it remains

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Table 1. Mean dimensions of the diatom species (± 1 SD). The approximate relative biovolume is given in brackets. Growth of the ciliate species (length ± 1 SD in parentheses) with each of the diatom species as a sole prey species is rated qualitatively. Condition of the ciliate cultures approx. 1 week after the inoculation at 10°C of a few ciliate cells with fresh diatoms. –, no ciliates found or only a few unhealthy ciliates (i.e., prey is unsuitable); +, ciliate number had increased but not enough to use the ciliates for experiments; ++, a large number of ciliates; +++, with this prey species, the largest number of ciliates was obtained.

	<i>Navicula arenaria</i> var. <i>rostellata</i>	<i>Navicula</i> <i>phyllepta</i>	<i>Stauropora</i> <i>salina</i>
Dimensions of the diatoms			
Length (μm)	55.1 \pm 2.7	13.1 \pm 0.6	27.8 \pm 1.4
Valve width (μm)	10.5 \pm 1.1	4.98 \pm 0.19	8.5 \pm 0.6
Pleural height (μm)	10.1 \pm 1.4	3.3 \pm 0.4	9.8 \pm 0.5
Biovolume (μm^3)	3,740 (27)	137 (1)	1,810 (13)
Growth of the ciliates			
<i>Strombidium cinctum</i> (48.9 \pm 5.4 μm)	+++	++	–
<i>Strombidium sauerbreyae</i> (67.7 \pm 4.8 μm)	+	++	+++
<i>Strombidium</i> sp. (33.8 \pm 2.4 μm)	–	+++	+
<i>Pseudochilonopsis</i> sp. (49.1 \pm 7.7 μm)	+	+++	–

unclear to what extent it may be involved in the selection of individual prey in mixed prey assemblages (Stoecker et al. 1981; Taniguchi and Takeda 1988).

The available data on selective feeding of benthic algivorous ciliates are derived from the comparison of food vacuole contents with the composition of the available prey assemblages (McCormick 1991; Epstein et al. 1992; Finlay et al. 1993; Balczon and Pratt 1995). However, this approach gives only limited insight in the actual mechanisms involved in individual cell selection and the relative importance of passive and active selection. In the present study, direct behavioral observations were used to study the mechanisms of prey selection in benthic algivorous ciliates feeding on mixed assemblages of diatom species from estuarine intertidal sediments. The feeding preferences of four ciliate species were established, as well as relative encounter rates, attack probabilities, and capture successes in various two-species prey mixtures. The influence of prey ratio, prey abundance, and feeding history was also determined.

Materials and methods

Organisms for the experiments—The ciliate and diatom species used in our experiments originated from two intertidal locations in the polyhaline reaches of the Schelde estuary (southwest Netherlands): the Molenplaat intertidal flat and the Paulina salt marsh. The Molenplaat was the study site for the ECOFLAT (Eco-metabolism of an estuarine tidal flat) project. A site description of this tidal flat is given in Herman et al. (2001). The Paulina salt marsh is situated downstream of the Molenplaat (Moens et al. 2002).

Three diatom species were used in our experiments (Table 1): *Stauropora salina* (W. Smith) Mereschkowsky, *Navicula phyllepta* Kützing, and *Navicula arenaria* Donkin var. *rostellata* Lange-Bertalot. For convenience, the latter will be referred to as *N. arenaria* in the text. The species were isolated from Molenplaat sediments and grown semicontinuously in unialgal, nonaxenic batch cultures with f/2 medium (Guillard, Sigma-Aldrich) prepared with filtered (Whatman GF/C) and autoclaved Schelde water. Diatom species were

identified and monospecificity of the cultures was verified after oxidation of the diatom valves (Sabbe 1993). Permanent slides of these oxidized diatoms have been deposited in the permanent slide collection of the laboratory. For each diatom species, the dimensions of 50 cells were measured with an ocular micrometer; biovolume was calculated on the basis of formulas in Hillebrand et al. (1999) (Table 1). Diatoms were grown in sterilized 1-liter glass Erlenmeyer flasks. To provide a solid substratum for the benthic diatoms, the bottom of each flask was covered with a layer of sand grains (median grain size 168 μm) from the Molenplaat; the sand had been washed in distilled water and oven sterilized at 170°C for 4 h. The flasks were incubated in an incubator with a 12:12 light:dark cycle at 10°C or 16°C. They were shaken daily to prevent diatoms from clumping together and to remove diatoms from the glass walls and sand grains. New diatom cultures were started by subsampling within a few days to weekly in order to provide exponentially growing diatoms for both the enrichment and the monospecific ciliate cultures (see below) and for the experiments. For the ciliate cultures, diatom suspensions were poured directly from the culture flasks. For the experiments, diatoms were harvested from the cultures by concentration on a 3- μm Nuclepore polycarbonate filter. The cells were then resuspended from the filters in fresh f/2 medium and densities were determined microscopically on formaldehyde-preserved (2% final concentration) subsamples in a Bürker or Sedgewick Rafter counting chamber. If necessary, densities were adjusted to the density desired for the experiment (see below) by dilution with f/2 medium or by means of further concentration.

Four ciliate species were used in the experiments (Table 1): *Strombidium cinctum* and *Strombidium sauerbreyae*, an unidentified *Strombidium* sp. and *Pseudochilonopsis* sp. Species of the genus *Strombidium* (order Oligotrichida) are ovoid, have a circle of apical membranelles surrounding the oral area, and swim in a helical pattern. *Strombidium* species are very common in marine sediments, especially in sand, and most feed on diatoms (Fenchel 1968). *Pseudochilonopsis* species, like other cyrtophorids, are flattened dorso-

Table 2. Summary of the experiments and the experimental conditions. Density and biomass ratios are for prey 1 : prey 2. For experiments 1, 2, and 3, prey 2 corresponds to the unsuitable diatom species. For the full names of the organisms, see Table 1.

Experiment	Ciliate species	Prey species		Factor tested	Prey ratio(s) used	
		1	2		Density	Biomass
1	<i>S. cinctum</i>	<i>N. arenaria</i>	<i>S. salina</i>	Feeding preference	1:2	1:1
					1:25	1:12.5
					1:50	1:25
2	<i>Strombidium</i> sp.	<i>N. phyllepta</i>	<i>N. arenaria</i>	Feeding preference	27:1	1:1
					1:1	1:27
					1:10	1:270
3	<i>Pseudochilodonopsis</i> sp.	<i>N. phyllepta</i>	<i>S. salina</i>	Feeding preference	13:1	1:1
					1:1	1:13
					1:10	1:130
4	<i>S. cinctum</i>	<i>N. arenaria</i>	<i>N. phyllepta</i>	Influence of prey ratio	1:100	1:3.7
					1:27	1:1
					1:10	2.7:1
					1:1	27:1
5	<i>S. cinctum</i>	<i>N. arenaria</i>	<i>N. phyllepta</i>	Influence of feeding history	10:1	270:1
					1:27	1:1
6	<i>S. sauerbreyae</i>	<i>S. salina</i>	<i>N. arenaria</i>	Influence of total diatom biomass	2:1	1:1

ventrally with an oral area located on the ventral side. They possess cilia on their ventral side by which they usually creep along sediment particles. The great majority of the ciliates are algivores (Fenchel 1968). Ciliate species were isolated from batch enrichment cultures according to the protocols outlined by Caron (1993). The ciliates were grown monospecifically in Petri dishes containing diatom suspensions from cultures. For each ciliate species, the size of 25 living cells was measured using an ocular micrometer (Table 1). These measurements are approximate, as the cell volume of ciliates is subject to fluctuations related to, among other things, the nutritional status of the cells (Fenchel and Jonsson 1988). However, relative differences between the species were distinct and probably exceeded size fluctuations within each species. The ciliate cultures were stored under the same conditions as the diatom cultures and were renewed weekly by transferring a few cells to Petri dishes with fresh diatom suspensions. Attempts to sustain ciliate growth on suspensions of single diatom species revealed that the four ciliate species differed strongly in their ability to grow on a certain diatom species (Table 1). Diatom species were considered to be an unsuitable prey species for a given ciliate species if all attempts to sustain a culture of the ciliate species on this diatom species failed. Prey-predator combinations for the experiments were chosen on the basis of these culture experiences. Preceding an experiment, ciliates were (unless stated otherwise) precultured for several generations on a mixture of the two diatom species used for the subsequent experiment (see below). These cultures were started a few days before the experiments to yield ciliates in a healthy, exponential growth phase.

General experimental conditions—Foraging behavior of the ciliates was observed in Petri dishes with a thin agar layer (Difco agar noble 1.5%, added to f/2 medium) on the bottom surface ($\sim 21 \text{ cm}^2$). In each experiment, a mixture of

two diatom species, at a certain density and biomass ratio, was presented to one ciliate species (Table 2). Diatom suspensions from both prey species were combined so that, for all experiments (unless stated otherwise), 1 ml of the diatom mixture contained a total diatom biovolume of $\sim 2.9 \times 10^8 \mu\text{m}^3 \text{ ml}^{-1}$. One ml of the two-species mixture was dispensed on the agar plates. Because diatoms settled, this resulted in a diatom biovolume of $\sim 1.4 \times 10^7 \mu\text{m}^3 \text{ cm}^{-2}$, corresponding to $0.4\text{--}8 \times 10^4 \text{ cells cm}^{-2}$. This compares well with diatom densities at the sediment surface of intertidal sediments ($\sim 1 \times 10^4 \text{ cells cm}^{-2}$; Paterson 1986). The arbitrary but fixed biomass allows standardization among experiments with differently sized diatom species and minimizes overlap among cells. The ciliates were starved for 8–12 h at 10°C before each experiment to standardize the conditions and to assure prey uptake during observations; they were acclimated to room temperature for 1 h before the experiments.

During preliminary observations, the ciliate species were found to adopt typical raptorial feeding, capturing each particle individually. This feeding mode predominates if the prey:predator size ratio exceeds about 0.1 (Fenchel 1987). The following predation sequence was discerned and defined on the basis of these preliminary observations: *Strombidium* spp. frequently interrupted their helical swimming pattern to stop in the vicinity of a diatom cell (=encounter, or E). Subsequently, the ciliate either swam away without any attempt to ingest the diatom (only E), or started to engulf the diatom (=attack, or A; at least a part of the diatom enters the ciliate cell). Complete ingestion without loss or rejection of the diatom was defined as a capture (or C). *Pseudochilodonopsis* spp. usually creep or walk along sediment particles. These ciliates walked on the agar surface in a very characteristic pattern resembling circles and ignored many of the diatom cells they walked over. An encounter (E) was defined as an interruption of the normal creeping pattern, lingering some seconds while the diatom was apparently examined. Attack

(A) and capture (C) were defined as for the *Strombidium* spp.

To start the experiment, a few 10s of ciliates (in ~1 ml) were carefully added to a Petri dish with settled diatoms by pipetting. The swimming or creeping ciliates were observed using a dissecting microscope with transillumination. Once an encounter was noticed, this interaction was followed until the ciliate swam away, carefully noting the diatom species involved and whether the encounter was followed by an attack and capture or not. This procedure was continued for 1 h. For each of the 20 treatments (see below and in Table 2), interactions between predator and prey were observed for mostly four, sometimes two, consecutive periods (replicates) of 1 h. For each replicate, fresh agar slants and prey and predator cells were used. Different treatments within one experiment were performed on the same or consecutive days.

Experimental design—A first series of experiments tested whether a diatom species that was unsuitable for ciliate growth in cultures and another diatom species that supported ciliate growth in cultures were differentially encountered, attacked, and captured by the algivorous ciliate species when offered as a mixture. The inability of the ciliates to grow on a certain diatom species does not necessarily mean that diatoms of this species cannot be ingested or attacked and used as an additional food source. For the three experiments in this series, the ciliate species *S. cinctum* (experiment 1), *Strombidium* sp. (experiment 2), and *Pseudochilodonopsis* sp. (experiment 3), respectively, were used as the grazer species (Table 2). In each experiment, three different prey ratios were used, viz. from an equal biomass ratio (1:1) for both prey species, to a large predominance of the unsuitable prey species (Table 2).

In a second series of three experiments (experiments 4, 5, and 6), we examined the feeding behavior of ciliates offered a mixture of two diatom species that, on the basis of earlier culture experiences, proved both to be suitable prey for the ciliate species (Table 2). In experiment 4, *S. cinctum* was offered a mixture of *N. arenaria* and *N. phyllepta* (Table 2). Five different prey ratios (see Table 2) were used to test for possible switching behavior. In case of no switching, the preference remains constant as the ratio of available prey changes. Experiment 5 used the same ciliate and diatom species as experiment 4 and tested the influence of feeding history on selective feeding. Both prey species were offered in biomass-equivalent mixtures (1:1), but ciliates were pre-cultured for several generations with a mixture of both prey species (as for the other experiments) or either *N. phyllepta* or *N. arenaria* as a sole prey species. In experiment 6, the influence of the total amount of diatom biomass on selective feeding behavior was tested with *S. sauerbreyae* offered a mixture of *S. salina* and *N. arenaria* at an equal biomass ratio (Table 2). Total biovolumes were $1.4 \times 10^7 \mu\text{m}^3 \text{cm}^{-2}$ (as for the other experiments), 1.8×10^6 or $1.8 \times 10^8 \mu\text{m}^3 \text{cm}^{-2}$.

Data analysis—Prey preference was analyzed using Chesson's α selectivity index (Chesson 1983),

$$\alpha = \frac{r_i/p_i}{\sum_i r_i/p_i}$$

where p_i is the proportion of diatom species i in the offered mixture of two species and r_i is the proportion of the captured diatoms that belongs to species i . This index varies between 0 and 1 and is unaffected by the relative abundance of food types, thus allowing meaningful comparisons between treatments with different prey ratios (Lechowicz 1982). The null hypothesis for no preference (i.e., $\alpha_1 = \alpha_2 = 0.5$) was tested by calculating a t -statistic (Chesson 1983).

The observed (capture) preferences are the product of the relative encounter rates, attack probabilities (the probability of an attack after an encounter; A/E) and capture successes (the probability of a capture after an attack; C/A) (Sih 1993). In order to evaluate the relative contribution of these factors, preference was evaluated after each step in the feeding process. To this end, two derivatives of Chesson's selectivity index α were calculated for the preferentially captured diatom species: preference at the time of encounters (α_E) and at the time of the attacks (α_A). They were calculated using the formula for α , but with r_i replaced by the proportion of the encountered and attacked diatoms that belongs to species i , respectively.

The relative number of encounters with both prey species was compared with the number of encounters expected based on the relative abundances of the prey species, using a heterogeneity G -test (Sokal and Rohlf 1995). Significance of differences in attack probabilities and capture successes between the prey species was tested using t -tests. Between treatment differences in Chesson's index (α), the relative number of encounters, A/E, and C/A were tested using one-way analysis of variance (ANOVA). The Student–Newman–Keuls (SNK) multiple comparisons test was used for post hoc pairwise comparisons. These statistical analyses were performed with STATISTICA 5.1 for Windows (StatSoft). Chesson's indices, attack probabilities, and capture successes were arcsine square-root transformed to meet the normality and variance equality criteria.

T-maze experiments—The behavioral observations strongly suggested that the *Strombidium* spp. were able to discriminate between diatom species on the basis of soluble chemical cues (see below). An additional experiment was set up to test this hypothesis. This experiment was performed with the two ciliate and diatom species combinations used for experiments 1 and 2 of the behavioral observations (see Table 2). Three-way glass stopcocks (T-mazes) with two-way plugs (straight bore) were used as described by Van Houten et al. (1982). The ciliates were introduced into the plug via the central arm and got equal access to the two test arms when the plug was turned 90°. Five experiments were run, with six replicates each. In the first and second experiments, diatom suspensions were introduced into the test arms (different species in each arm). *N. arenaria* and *S. salina* were used for the first experiment, *N. phyllepta* and *N. arenaria* for the second experiment. The diatom suspensions were subsamples directly taken from the diatom cultures. The cultures of the two prey species for each experiment had ap-

Table 3. Total number of encounters, attacks, and captures observed in experiments 1, 2, and 3 (for the three replicates together) and relative numbers of encounters, attacks, and captures with the suitable prey species.

Experiment	Suitable prey offered (% of total number)	Encounters		Attacks		Captures	
		Total number	% with suitable prey	Total number	% with suitable prey	Total number	% with suitable prey
1	33.3	257	96.0	48	100	22	100
	3.8	65	47.7	14	100	8	100
	2	62	42.2	12	100	9	100
2	96.4	731	99.5	90	100	90	100
	50	278	98.2	25	100	25	100
	9.1	103	85.1	7	100	7	100
3	92.9	709	96.2	79	100	65	100
	50	631	53.8	55	100	49	100
	9.1	633	33.2	44	100	39	100

proximately the same diatom biomass per unit volume. Diatom abundances in the cultures were determined by counts on fixed subsamples as described above. In the third and fourth experiments, diatom cells were removed from the diatom suspensions through filtration (3- μm Nuclepore polycarbonate filters), and the experiments were repeated with these diatom-free fluids instead of the diatom suspensions. For the fifth experiment, bacterial cells were also removed from the suspensions through filtration on a 0.2- μm Nuclepore polycarbonate filter. This experiment was only performed with *N. phyllepta* and *N. arenaria* and the ciliate species *Strombidium* sp. (as in the T-maze experiments 2 and 4).

The ciliates were pipetted directly from the cultures. At least 200 ciliates were used in each T-maze experiment; if necessary, ciliate cultures were concentrated by reverse filtration using a 5- μm Nitex mesh. Experiments were performed in the dark to eliminate any phototactic responses and at room temperature ($\sim 21^\circ\text{C}$). One hour after the start of the experiment, the stopcocks were closed. The test arms, which were sealed with Parafilm, were emptied into separate Petri dishes. The ciliates in these Petri dishes were fixed with an acid lugol solution and enumerated immediately using a dissecting microscope with transillumination. After each trial, the T-mazes were disassembled, washed with 1 N HCl, and rinsed in hot running tap water, then with distilled water.

For each experiment, the significance of differences in ciliate numbers between the test arms with different diatom species was tested using *t*-tests for dependent samples. Paired differences were normally distributed (Shapiro Wilk's *W*-test). Statistical analyses were performed with STATISTICA 5.1 for Windows (Statsoft). Controls, in which the same diatom species (*N. phyllepta*) was introduced into the test arms, did not reveal any significant difference in ciliate migration into the two test arms. Numbers of the ciliate *Strombidium* sp. did not differ significantly between the test arms when *N. phyllepta* suspensions were used (*t*-test for dependent samples, $P = 0.15$) nor when diatom-free fluid of these suspensions was used (*t*-test for dependent samples, $P = 0.67$).

Results

Mixtures of suitable and unsuitable prey—The observations in the first three experiments yielded comparable results. The unsuitable diatom species were not captured at all (Table 3), even when they outnumbered the other prey species by a factor of 25, 270, or 130 in terms of biomass, respectively (see Table 2). Consequently, the value of Chesson's selectivity index α for the suitable prey species always equalled one. The fact that unsuitable prey were not captured did not result from failure of attacks. Although encounters with both prey species were observed in each experiment (see Table 3), encounters with the unsuitable prey species never resulted in an attack (i.e., A/E = 0). Attack probability after an encounter with a diatom of the suitable prey species, on the other hand, averaged 0.38 ± 0.16 in experiment 1, 0.1 ± 0.02 in experiment 2, and 0.17 ± 0.04 in experiment 3 (Table 3).

The relative number of encounters increased with the relative abundance for both prey species in the three experiments (Fig. 1). However, in each case, the suitable prey species was encountered more than expected on the basis of its relative abundance (Fig. 1, Table 3). This difference between the observed and expected encounter proportions was highly significant (*G*-test, all $P < 0.005$), except for two of the three treatments in the experiment with *Pseudochilonopsis* sp. (experiment 3). The differences were most pronounced when the suitable prey were rare: for the treatment in each experiment with the lowest numbers of suitable prey, the observed relative encounter rates for the suitable prey were a factor 21, 9.4, or 3.6 higher, respectively, than expected on the basis of their relative abundances (Table 3).

Mixtures of two suitable prey—In contrast with the first series of experiments, both diatom species were captured by the ciliates in experiments 4, 5, and 6 (but not necessarily in each treatment). Nevertheless, in each of these experiments, a clear preference for either of the diatom species was found.

In the fourth and fifth experiments, *S. cinctum* showed a strong preference for the larger species *N. arenaria*, inde-

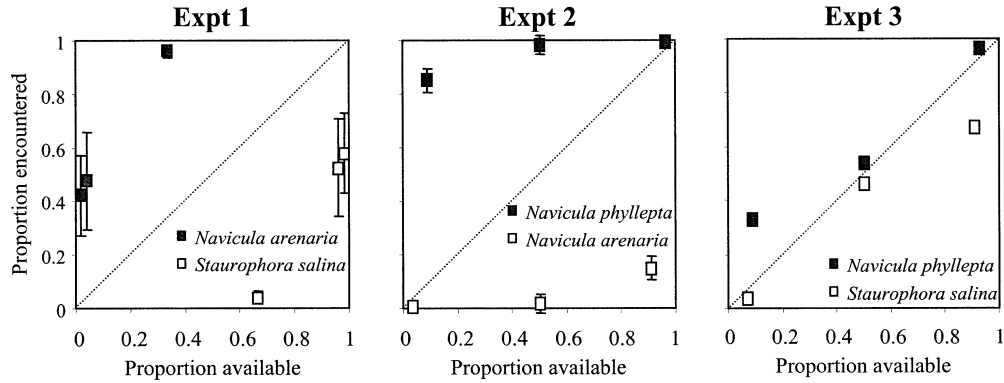


Fig. 1. Proportion of each prey species encountered by the ciliates (mean \pm 1 standard deviation [SD], $n = 4$ for experiments 1 and 2, $n = 2$ for experiment 3) in relation to its relative abundance (as a proportion). The dotted line represents random encounters. Black boxes represent the suitable prey species; unfilled boxes represent the unsuitable prey species. SD for experiment 3 are smaller than the symbols.

pendent of prey ratio or feeding history (t -test of Chesson's index vs. 0.5, degrees of freedom = 3, all $P < 0.01$; Fig. 2). Neither feeding history nor prey ratio significantly affected the value of the selectivity index (ANOVA, $P > 0.05$), except for a slightly significant difference between the values at prey ratios 1:27 and 10:1 (SNK, $P < 0.05$; Fig. 2).

The relative number of encounters with either of the prey species increased with its relative abundance (Fig. 3) and was not significantly affected by differences in feeding history (ANOVA, $P > 0.5$). *N. arenaria*, the preferred prey species, was encountered significantly more than expected on the basis of its relative abundance for all prey ratios and for different feeding histories (G -test, all $P < 0.005$; Fig. 3). At equal abundances for both prey species, for instance, only on average $1.2 \pm 1.5\%$ of the observed encounters were with *N. phyllepta* (Fig. 3).

Although encounters of *S. cinctum* with *N. arenaria* resulted more often in an attack than encounters with *N. phyllepta*, the difference was not always significant (Fig. 4). Attack probability averaged 0.27 ± 0.09 with *N. arenaria* and 0.19 ± 0.03 with *N. phyllepta* (averaged for experiments 4 and 5) and was not significantly affected by food history (ANOVA, $P > 0.1$). The attack probability for *N. arenaria*

increased when its relative abundance decreased (Fig. 4). Although encountered and attacked less frequently than *N. arenaria*, *N. phyllepta* was practically always captured whenever attacked: capture success ranged from 0.85 to 1 for this prey species (Fig. 4). Capture success for *N. arenaria*, which averaged 0.35 ± 0.13 , was highest when ciliates were precultured with *N. arenaria*, but was not significantly affected by prey ratios or culture conditions (ANOVA, $P > 0.1$).

In the last set of experiments, *S. sauerbreyae* preferentially captured *S. salina* (Fig. 2). Due to high variability between the replicates, the preference for *S. salina* was not significant for the treatment with the lowest total prey biomass (t -test of Chesson's index 0.71 ± 0.21 vs. 0.5, degrees of freedom = 3, $P > 0.1$), but the preference was significant for the two other treatments ($P < 0.05$). Nevertheless, Chesson's α did not significantly differ among the treatments (ANOVA, $P > 0.05$).

Analogous to the previous experiments, the preferred prey species in this experiment was encountered significantly more by its grazer than expected on the basis of its relative abundance (G -test, all $P < 0.005$; not shown). Whereas the relative abundance of *S. salina* was fixed at 66.7% of total abundance in this experiment, the relative number of en-

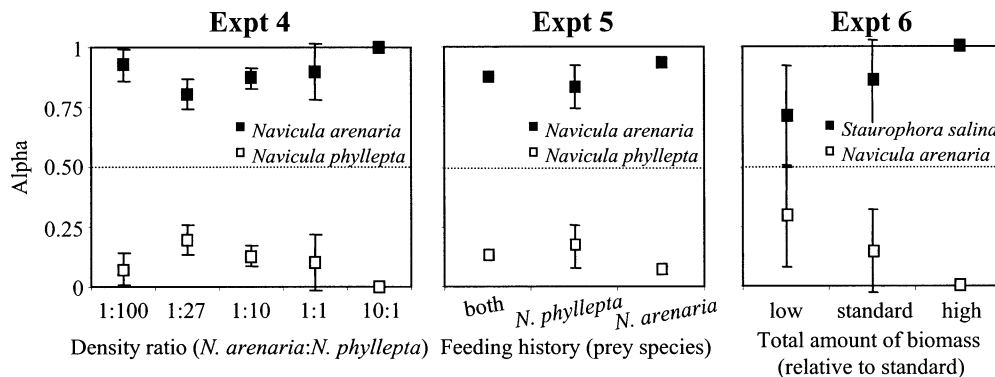


Fig. 2. Chesson's coefficient of selectivity for the different treatments in experiments 4, 5, and 6 (mean \pm 1 SD, $n = 4$). The dotted line represents neutral selection.

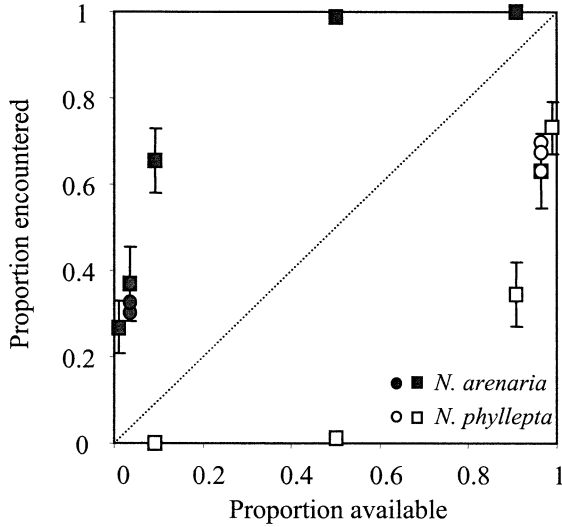


Fig. 3. Proportion of either of the prey species encountered by the ciliates (mean \pm 1 SD, $n = 4$) in relation to its relative abundance (as a proportion) in experiments 4 (squares) and 5 (circles). The dotted line represents random encounters. Black boxes represent the suitable prey species; unfilled boxes represent the unsuitable prey species.

counters with *S. salina* averaged $79.6 \pm 0.5\%$ and was not significantly affected by the total amount of prey biomass (ANOVA, $P > 0.5$).

Although encounters of *S. sauerbreyae* with the preferred prey species *S. salina* resulted more often in an attack than encounters with *N. arenaria*, differences between the prey

species were insignificant (Fig. 4). Attack probabilities averaged 0.23 ± 0.07 and 0.16 ± 0.03 , respectively, and were not significantly affected by differences in total prey biomass (ANOVA, $P > 0.05$). The number of attacks with *N. arenaria* was very low. Capture successes for this prey species could only be calculated for the treatment with high prey biomass, for which it equalled 0 (Fig. 4). When *S. salina* cells were attacked, the probability of a successful capture averaged 0.89 ± 0.06 and did not significantly differ for differences in total prey biomass (ANOVA, $P > 0.1$; Fig. 4).

Relative importance of encounter, attack, and capture (α_E , α_A , and α_C or α).—The preferentially captured prey species was the preferred species at the encounter stage ($\alpha_E > 0.5$) in each of the six experiments (Fig. 5). In experiments 1, 2, 4, and 5, this preference at the encounter stage (α_E) increased only slightly at the attack stage (α_A), whereas the preferences remained unchanged or decreased slightly after the attack (α_C) (Fig. 5). Accordingly, preference was mainly established at the encounter stage in these experiments. In contrast, the attack stage was the most crucial stage in the experiment with *Pseudochilodonopsis* sp. (experiment 3), as all encountered specimens of the preferred prey were attacked (i.e., $\alpha_A = 1$), with α_E being relatively low (Fig. 5). In experiment 6, preference was clearly established at time of the encounters, but, in contrast with the other experiments, this preference increased at the attack as well as the capture stage of the feeding process (Fig. 5). In summary, the contribution of higher attack probabilities, and especially of

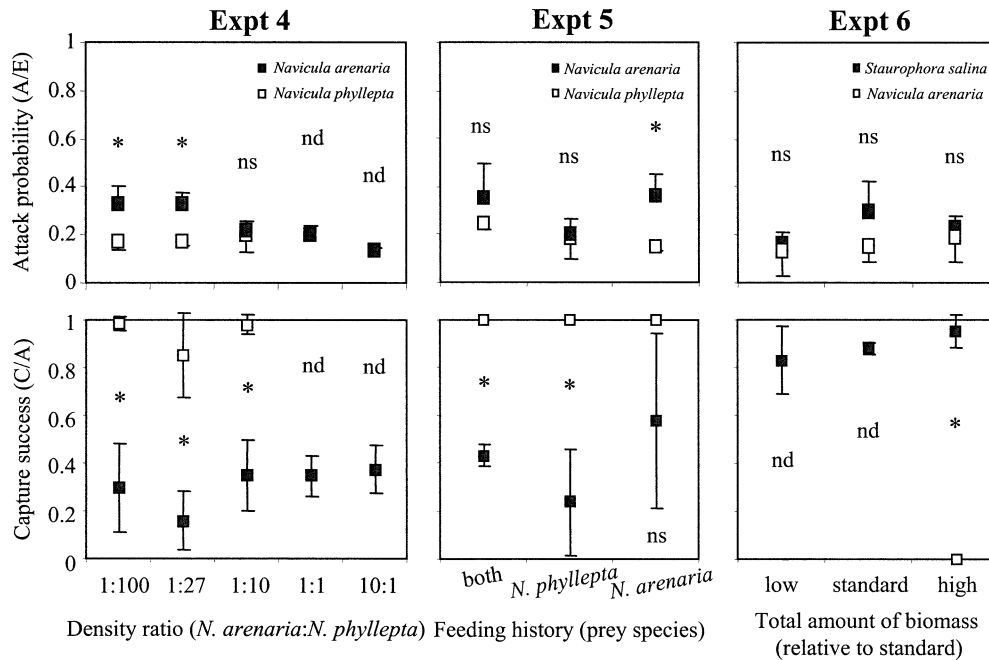


Fig. 4. Mean attack probability (± 1 SD, $n = 4$) and capture success (± 1 SD, $n = 4$) for the different treatments in experiments 4, 5, and 6. * Value differs significantly between both prey species (t -tests, $P < 0.05$); ns, difference between both prey species is not significant; nd, the difference between both prey species could not be determined because, for one of the prey species, attack probability or capture success could not be calculated.

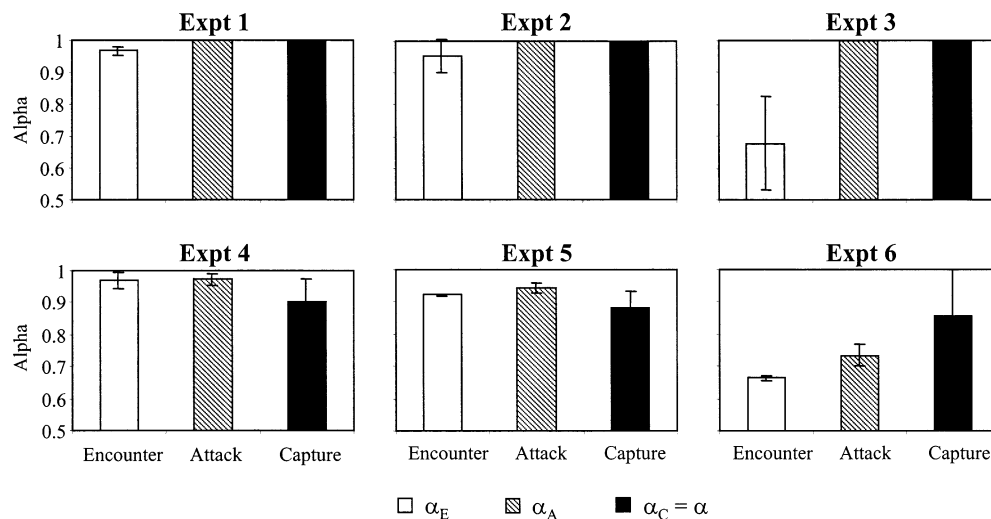


Fig. 5. Preferences evaluated after each step of the feeding process, using derivatives of Chesson's coefficient of selectivity (α = preference after capture) viz. α_E (i.e., preference after encounter) and α_A (i.e., preference after attack). Values are means of the average values for each treatment in the experiment \pm 1 SD.

higher capture successes to prey preferences (α) was small relative to the importance of preferential encounters.

T-maze experiments—*S. cinctum* as well as *Strombidium* sp. numbers were significantly higher in the test arms with a suspension of suitable diatoms (*N. arenaria* and *N. phyllepta*, respectively) than in the test arms with unsuitable diatoms (Fig. 6A). Ciliate numbers were also significantly higher in the test arms with diatom-free fluid derived from these suspensions of suitable diatoms (Fig. 6B). When the suspensions were filtered through a 0.2- μ m filter, *Strombidium* sp. numbers were still significantly higher in the test arms with *N. phyllepta* compared with *N. arenaria* (*t*-test for dependent samples, $P < 0.05$; not shown).

Discussion

Highly selective grazing by estuarine algivorous ciliates—Our observational experiments show that diatom pre-

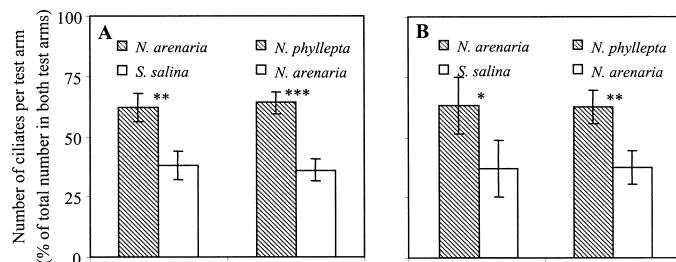


Fig. 6. Mean number (% of total, \pm 1 SD) of ciliates in the test arms with (A) diatom suspensions and (B) diatom-free fluid derived from these suspensions. Levels of significance (*t*-tests for dependent samples) for differences in ciliate abundance between the test arms with different diatom species are * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. The ciliate *S. cinctum* was used in the experiments with *N. arenaria* and *S. salina*; *Strombidium* sp. was used with *N. phyllepta* and *N. arenaria*.

datation by ciliates from intertidal estuarine sediments can be highly selective. The four benthic algivorous ciliate species fed selectively among the diatom species that were presented to them in pairs, even when these prey species belonged to the same genus. Likewise, the feeding preferences of the ciliates differed strongly, even for ciliates belonging to the same genus. For example, with a mixture of the diatom species *N. phyllepta* and *N. arenaria*, *Strombidium* sp. was never observed to ingest *N. arenaria* cells, whereas *S. cinctum* showed a marked preference for *N. arenaria* over *N. phyllepta*. Our experiments also show that the preferences of the ciliates were not affected by total diatom biomass or feeding history and that switching (i.e., the tendency of a predator to change from one prey to another according to the abundance of the prey; Lincoln et al. 1998) was absent. This supports the suggestion by Strom and Loukos (1998) that it is possibly uncommon in protozoa to provide a refuge for preferred prey species at low prey concentrations. A possible explanation for this phenomenon could lie in the specific feast-and-famine life history strategy reported for protozoa (Fenchel 1987). Because food resources in aquatic environments are frequently patchy or temporally variable, many protozoan species are well adapted to cope with starvation. Some protozoan taxa in aquatic environments form cysts in order to survive adverse environmental conditions such as depletion of food resources (Fenchel 1987). However, a great variety of other survival mechanisms are also found in protozoa (e.g., decrease in metabolic activity, the formation of highly motile swarmer cells; Fenchel 1987; Finlay 1990).

Selective ingestion of diatom species by benthic algivorous ciliates had previously been reported (McCormick 1991; Epstein et al. 1992; Balczon and Pratt 1995). These studies assessed selectivity on the basis of a comparison of the relative abundances of diatom species in the food vacuoles of the ciliates with the prey ratios in situ or in experimental mixtures. The present study, however, based on direct behavioral observations, allowed us to dissect the actual

mechanisms involved in prey selection during the feeding process and more specifically to evaluate the relative importance of active versus passive selection mechanisms in benthic ciliates.

Importance of active versus passive selection mechanisms in estuarine algivorous ciliates—Finlay et al. (1993) suggested that benthic algivorous ciliates select diatoms merely on a mechanistic basis. McCormick (1991) quoted size differences as well as growth form and microspatial distribution of the diatom species as possible explanations for differences in the consumption of diatom species by ciliates. Growth form and prey microlocation were the same for all diatom species in our experiments. In addition, relative prey abundance and prey size did not play a primary role in determining prey selection. Our experiments show that passive selection, governed by the relative availability and predation susceptibility of the prey items, only played a secondary role in prey selection by the algivorous ciliates. Moreover, the feeding preferences of the ciliates appeared to result mainly from active selection at the encounter stage and, to a lesser degree, also the attack stage of the feeding process.

Evaluation of the preferences after encounter, attack, and capture of the diatoms revealed that the feeding preferences were mainly established at the time of the encounters. The preferred prey species were encountered more than expected on the basis of their relative abundances in all experiments, and this appeared to be the major determinant of the observed feeding preferences for the *Strombidium* species. For *Pseudochilonopsis* sp., however, preferential encounters with the preferred prey species were only significant when these diatoms were scarce relative to diatoms of the other prey species. For the four ciliate species in our experiments, neither the relative abundance nor the size of the diatom species explains the preferential encounters with the preferred diatom species. Supposing size differences between the diatom species influenced encounter rates, the larger of the two diatom species would be expected to have a higher relative encounter rate than expected on the basis of its relative abundance. However, in three out of the five prey-predator combinations in our experiments (experiments 2, 3, and 6), the smallest of both prey species was preferred and encountered more than expected on the basis of its relative abundance. In another experiment (experiment 1), the larger *N. arenaria* was preferred above the smaller *S. salina*. However, the small size difference between these prey species (approximately a factor of two) cannot explain why the larger *N. arenaria* was encountered on average 12 times, and up to 21 times, more than expected on the basis of its relative abundance. Finally, we can also safely assume that possible differences in the gliding velocities between the diatom species hardly influenced the encounter rates between the ciliates and the diatom species. Diatom movement in general is very slow compared with ciliate speed (by a factor 10^2 – 10^3 ; Fenchel 1987; Hay et al. 1993). In conclusion, the observed encounter patterns can only be explained by assuming that the ciliates recognized the diatoms before an encounter and subsequently actively favored encounters with preferred diatoms and/or actively avoided encounters with the unpreferred prey species.

The fact that none of the encounters with an unsuitable prey species resulted in an attack demonstrates that diatoms were also actively selected after encounter and before an attack. Likewise, in the experiments with two suitable prey species, the preferences established at the time of encounters were invariably consolidated by higher attack probabilities for the preferred diatom species (Fig. 5). However, compared with the preferential encounters, a higher attack probability for the preferred diatom species only contributed substantially to the magnitude of the feeding preferences in the experiment with *Pseudochilonopsis* sp. (experiment 3; Fig. 5).

The probability of a successful capture after an attack appeared to be related to the size of the diatoms, suggesting that mechanical constraints did influence capture successes. The smallest diatom species, *N. phyllepta*, was nearly always captured whenever attacked whether it was the preferred species or not and independent of the predator species. For the largest diatom species, *N. arenaria*, which was mainly captured by *S. cinctum* in the experiments, capture successes varied between 0.16 and 0.74 (0.42 on average). The observations revealed that *S. cinctum*, trying to capture *N. arenaria* cells, frequently could not engulf these cells entirely, presumably because of their length, and were therefore rejected. On several occasions, these diatoms, which were always attacked at the cell apex, appeared to prevent ingestion by moving in the opposite direction. However, despite the fact that mechanical constraints, and possibly even escape behavior of the diatoms, appeared to affect capture successes, capture success by itself hardly contributed to the overall feeding preferences. In the experiments with mixtures of suitable and unsuitable prey species (experiments 1, 2, and 3), differences in the capture successes for the prey species could not influence the feeding preferences at all because unsuitable prey were never attacked. In only one of the remaining experiments (experiment 6), the smallest prey species was preferred. Here, the higher capture success for this prey species did contribute to the overall feeding preference (Fig. 5). In two other experiments (experiments 4 and 5), a lower capture success for the preferred prey species lowered the magnitude of the feeding preferences, without ultimately changing the preferences established at the encounters and attacks (Fig. 5).

Evidence for active, noncontact selection of closely related diatom species in mixed assemblages—It has been shown before that ciliates can discriminate between prey on the basis of other factors than size or shape (Verity 1991). In the case of the pelagic filter-feeding ciliate *Favella* sp., high-speed video observations of the feeding behavior revealed that, in mixtures of microspheres and algal cells from similar size and shape, capture by the oral membranelles and subsequent capture in the peristomal area of the ciliates was similar for all particles (Stoecker et al. 1995). Selection was only accomplished after contact of the particles with the cell surface of the oral cavity and was executed by active rejection of undesired particles (Taniguchi and Takeda 1988). Balczon and Pratt (1995) showed that benthic algivorous ciliates were able to distinguish between stained and unstained diatoms of the same species, when these cells were offered

simultaneously to the ciliates. However, in the absence of direct observations, it could not yet be established whether prey recognition by benthic algivorous ciliates was contact or noncontact. For the three *Strombidium* species in our experiments, the selection mechanism appeared to be based mainly on discrimination between the diatom species before the encounters (see above). In order to test the hypothesis that the ciliates in our experiments detected and recognized individual prey items in mixed assemblages while swimming and *before* any physical contact with the diatoms, i.e., that they were recognized through prey-specific, soluble chemical cues, we performed additional T-maze experiments. In these experiments only *Strombidium* species were used, as the *Pseudochilonopsis* individuals move by creeping over the substrate. As they most likely had contact with the diatoms before an encounter (defined here as an interruption of the normal creeping pattern), it is impossible to determine whether their motility pattern was influenced by solute or contact recognition. The T-maze experiments with diatom-free fluids (3- μm filtration) derived from diatom cultures unambiguously showed that the *Strombidium* ciliates were able to actively distinguish between different, closely related diatom species without any physical contact between prey and predator. In order to rule out contact between predators and possible prey-associated satellite microorganisms, which have been shown to be species-specific in marine diatoms (Schäfer et al. 2002), an additional filtration (0.2- μm filtration) was carried out. Even after removing possible associated bacteria, the ciliate *Strombidium* sp. was still able to distinguish between the diatom species. However, at present, it remains impossible to assess whether the actual recognition was based on diatom cues or chemical cues exuded by satellite microbes. Ricci et al. (1996) showed that a predatory ciliate was able to distinguish between closely related ciliate prey without any physical contact between prey and predator (using T-mazes), but to our knowledge, this ability was not shown before for algivorous ciliates and closely related algal prey.

The observed chemosensory behavior might be comparable with the behavioral responses involved in several other ecological processes in ciliate communities, such as orientation in oxygen gradients (Fenchel and Bernard 1996), location of prey patches (Fenchel and Jonsson 1988; Fenchel and Blackburn 1999; Morelli et al. 1999), and supposedly also in the congregation of ciliates from complementary mating types (Stock et al. 1999). In all these processes, the ciliates respond to chemical cues by changing their locomotory behavior, ultimately leading to a considerable increase or reduction of motility. The present study highlights the extreme fine tuning of the chemosensory mechanisms involved in prey recognition, which allows at least some ciliates to detect individual diatom cells in mixed assemblages through noncontact recognition, and not only the accumulation in patches of high prey abundances (as in Fenchel and Jonsson 1988; Morelli et al. 1999).

The present study suggests that trophic interactions between algivorous ciliates and diatoms are far more complex than generally thought. Our experiments unambiguously show that the ciliates were highly selective and distinguished between similar (with respect to size and shape) and phy-

logenetically closely related diatom species in mixed assemblages. The feeding preferences were distinctly predator-specific, and prey ratio, total prey density, nor feeding history influenced the feeding preferences. For the *Strombidium* species, our results also show that the feeding preferences resulted mainly from active selection based on soluble chemical cues.

The pronounced specificity of diatom predation by the ciliates in the present study and the recognition by the ciliates of chemical cues excreted by the diatoms should be kept in mind for the design of grazing experiments. For instance, grazing-rate estimates obtained by the addition of only a single diatom species as prey (see, e.g., in Epstein 1997a,b) should be treated with caution. Likewise, experimental consumption rates for dead versus living (i.e., excreting) diatoms by ciliates might differ. Apart from possible toxicity of the dyes, heat killing during staining in Balczon and Pratt (1995) might have accounted for the preferential ingestion by ciliates of unstained diatoms, offered together with stained diatoms.

Although the available data are scarce, it is generally assumed that protozoa do not have an important quantitative impact on diatom standing stocks in benthic marine and estuarine ecosystems (Epstein et al. 1992; Hamels et al. 1998). Some studies, on the other hand, have shown temporary and largely monospecific bursts of algivorous ciliates, especially in densely packed diatom films (e.g., Webb 1956; Hamels et al. 1998). A bloom of the epibenthic algivorous ciliate *Chlamydomonad triquetrus* has been estimated to consume more than 35% of the benthic primary production in silty sediments of the Molenplaat intertidal flat (Hamels et al. 1998). Given the fact that this grazing can be highly species specific, even in mixed assemblages, as shown in the present study, it is not unlikely that algivorous ciliates are a major driving force in shaping structure and diversity of benthic diatom communities' intertidal sediments. Whether highly selective ciliate grazing can actually drive diatom populations to extinction on local scales remains an open question, given the high habitat microheterogeneity and more complex trophic interactions in natural communities compared with experimental conditions.

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