

Feeding of the heterotrophic freshwater dinoflagellate *Peridiniopsis berolinense* on cryptophytes: analysis by flow cytometry and electronic particle counting

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ABSTRACT: We measured feeding rates and food selectivity of the heterotrophic freshwater dinoflagellate *Peridiniopsis berolinense* by analytic flow cytometry (AFC), electronic particle counting and sizing (EPCS), and conventional microscopy. Several differently sized strains of the common cryptophytes *Rhodomonas minuta* and *Cryptomonas* sp. were offered in short-term (1 to 4 h) laboratory experiments as food in concentrations ranging from 3.0×10^4 to 1.0×10^5 cells ml^{-1} . *P. berolinense* is a raptorial species that attacks its prey by peduncle-feeding. Since *P. berolinense* sucks out its prey but does not take up whole cells, grazing rates cannot be quantified by established techniques such as fluorescently labeled algae. We measured dinoflagellate ingestion rates as loss rates of algal prey cells, by the increase of autofluorescence within food vacuoles of *P. berolinense*, and by the direct formation of food vacuoles. Biovolumes of prey and predator were measured by EPCS. The grazing response was variable and depended on the nutritional status of *P. berolinense*. Heavily starved cells were small, started feeding immediately and were less selective than moderately starved specimens. We show how nutritional status can be assessed by AFC and EPCS prior to the beginning of an experiment.

KEY WORDS: Heterotrophic dinoflagellates · Feeding rates · Flow cytometry · *Peridiniopsis berolinense*

INTRODUCTION

Protozoa, by their feeding and excretion, play a pivotal role in aquatic food webs. Interactions between planktonic protozoa and their bacterial and algal prey have been studied intensively over the last 2 decades (reviewed by Capriulo 1990, Laybourn-Parry 1992). The majority of those investigations, however, assessed feeding rates in broad categories such as 'heterotrophic bacteria' and 'microzooplankton'. Reports that studied feeding interactions at the species level concentrated on ciliates and small heterotrophic nanoflagellates. The feeding ecology of the larger heterotrophic dinoflagellates has recently been investigated in some marine species (Strom 1991, Hansen 1992,

Jacobson & Anderson 1993, Strom & Buskey 1993, Neuer & Cowles 1995, Hansen et al. 1996). A summary of the trophic role of heterotrophic dinoflagellates considered marine dinoflagellates exclusively (Lessard 1991). This probably reflects the fact that almost 90% of the total of 2000 extant dinoflagellate species live in the ocean (Laybourn-Parry 1992), and that most freshwater species are photoautotrophic. Recent evidence suggests, however, that heterotrophic dinoflagellates are more common and contribute more to total protozoan biomass in lakes than has been hitherto assumed (Arndt & Mathes 1991, Mathes & Arndt 1994).

In this paper we analyse the feeding behaviour of an obligate heterotrophic freshwater dinoflagellate, *Peridiniopsis berolinense*. Like at least 9 other species in 6 dinoflagellate genera (Hansen 1991) it sucks out the cellular contents of its algal prey by means of a dinoflagellate-specific cell organelle, the peduncle

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(Kollmann 1977, Spero 1982, Wedemeyer & Wilcox 1984, Gaines & Elbrächter 1987, Elbrächter 1991). This feeding mode is known as myzocytosis (Schnepp & Deichgräber 1984). Ingestion rates of myzocytotic species cannot be measured by the direct uptake of prey surrogates such as fluorescently labeled microspheres or fluorescently labeled bacteria and algae (summarized by Sherr & Sherr 1993). Ingestion can, however, indirectly be demonstrated and quantified by the formation of food vacuoles and corresponding increase in autofluorescence within the predator cells. We used analytical flow cytometry (AFC; e.g. Burkill 1987) to quantify the latter and conventional microscopy to measure the former. Flow cytometry is a powerful laser based technology that characterizes and quantifies cells according to their size and cellular constituents. Although originally developed for biomedical research, AFC is being used increasingly in aquatic ecology, namely to characterize and quantify autotrophic cells (see Olson et al. 1993, Tarran et al. 1993 for review). Yet in spite of its tremendous potential, AFC has only sporadically been used to study the feeding ecology of either marine (Cucci et al. 1989, Monger & Landry 1992, Burkill et al. 1993) or freshwater (Kenter et al. 1996) planktonic protozoa.

We investigated the size-selective feeding of *Peridiniopsis berolinense* on 2 common cryptophyte algae by combining flow cytometry, conventional microscopy, and electronic particle counting. The latter not only provides a useful control of the counting precision of the flow cytometer, but gives additional information on cell size of the algae and dinoflagellates studied. In this first paper we present a protocol that may be used in future studies to consider the nutritional status of heterotrophic protists at the beginning of an experiment. The nutritional condition of protozoan predators which in most cases is unknown has been shown to affect both total ingestion rates and food selectivity (Choi 1994, Jürgens & DeMott 1995). The major goal of our study was to demonstrate how new technologies and conventional microscopy can be combined to obtain new qualitative and quantitative information on the feeding ecology of aquatic protozoa.

MATERIALS AND METHODS

Study organisms. *Peridiniopsis berolinense* (Lemmermann) Bourrelly is a 26 to 33 μm long, 22 to 30 μm wide thecate dinoflagellate common in freshwater ponds and lakes (Huber-Pestalozzi 1968, Popovsky & Pfiester 1990, Kirchhoff 1992). A previous survey revealed that *P. berolinense* occurs in the plankton of

several north German lakes and ponds in abundances up to 600 cells ml^{-1} (B. Meyer, MPIL, Plön, unpubl. data). The culture material used in this study was isolated from cells which had germinated from a sediment sample of Lake Plußsee, a small (14 ha), deep (29 m), naturally eutrophic lake located close to the Max Planck Institute for Limnology in northern Germany (Overbeck & Chróst 1994). *P. berolinense* has been kept in batch culture in our laboratory on a diet of 2 unidentified *Cryptomonas* species for several years now. The *P. berolinense* used in this study had been grown on the large dinoflagellate *Ceratium furcoides* that were reared in WC medium (Guillard & Lorenzen 1972) under continuous light at 55 to 60 $\mu\text{E m}^{-2} \text{s}^{-1}$ and 20°C. The length of *C. furcoides* ranges from 149 to 191 μm , its width from 45 to 56 μm .

Size-selective feeding of *Peridiniopsis berolinense* was measured on 3 cryptophyte species. The largest species, an unidentified *Cryptomonas* sp., is 10.2 to 27.6 μm long, 7.3 to 16.0 μm wide with a mean bio-volume ranging from 650 to 1200 μm^3 . This species had been isolated from Lake Constance by A. Giani (Limnological Institute Konstanz, Germany). A distinctly smaller cryptophyte species, *Cryptomonas* sp. strain no. 26.80 (formerly named *Rhodomonas* sp. strain no. 26.80), had been obtained from the Culture Collection of Algae in Göttingen (Germany). With an average cell volume of 280 μm^3 this species is slightly larger than the third one, *Rhodomonas minuta* (mean volume 195 μm^3), which had been isolated from Lake Michigan in 1981 (Stemberger & Gilbert 1985) and has been kept in our laboratory for several years. All cryptophytes were kept in batch cultures in WC medium under continuous light at 20°C.

Experimental design. *Peridiniopsis berolinense* were harvested from batch cultures in their late stationary phase when food was already depleted. We separated *P. berolinense* from *Ceratium furcoides* by means of a 41 μm mesh gauze 24 h before the beginning of the experiments. The *P. berolinense* were then kept without food in Erlenmeyer flasks of 100 ml volume that were also used for the experiments. Prior to the experiments we measured the cell concentrations of the cryptophyte cultures with an electronic particle counter (outlined below). We prepared the experimental algal concentrations by combining subsamples of 2 algal species and diluting the mixture with WC medium in Erlenmeyer flasks. In each experiment, the larger *Cryptomonas* sp. was offered together with one of the smaller *Rhodomonas minuta* or *Cryptomonas* sp. strain no. 26.80 at similar cell numbers. Experiments were conducted in continuous light at 55 to 60 $\mu\text{E m}^{-2} \text{s}^{-1}$ and 20°C.

To start the experiments, *Peridiniopsis berolinense* was added to the algal containers. Experimental

bottles without *P. berolinense* that we prepared in all but the first experiment served as controls. Algal and dinoflagellate cell concentrations and dimensions were measured in samples taken at the beginning and at intervals ranging from every 5 min to 1 h over a period of 1 to 4 h. We mixed the experimental containers gently before taking samples. One aliquot of about 3 ml was analysed by AFC, a second one (1 ml) by electronic particle counting. Both subsamples were analysed alive immediately after sampling. A third subsample (1 to 4 ml) was fixed with Lugol's iodine solution and counted by conventional inverted microscopy.

Flow cytometry. We used a PAS-III (Partec) flow cytometer equipped with a tunable (457 to 515 nm) argon laser, a high-pressure HBO-100 mercury arc lamp, and 6 photomultipliers. In the present investigation we excited the cells at a wavelength of 488 nm by the laser and measured in list mode. Parameters considered were right-angle light scatter (side scatter, SSC), forward angle light scatter (FALS), red (auto-) fluorescence (560 nm and 610 nm dichroic beam splitters, 610 nm longpass cutoff filter) and green fluorescence (500 nm dichroic beam splitter, 520 nm interference filter). Green fluorescence was used to calibrate the instrument with yellow-green (YG) latex beads (Polysciences) of 2.13 μm diameter. Algae and protozoa did not show measurable fluorescence at the high voltage we used in the green channel. The beads could therefore be removed from the analysis of red fluorescing and scattering particles by gating for non to low green fluorescence after data acquisition. We used the former 2 parameters to identify the algal and dinoflagellate populations. Red fluorescence is a measure of chlorophyll *a* autofluorescence, SSC is related to cell size. Sample volume measured by AFC was 0.5 ml. The beginning and end of each measurement were recorded automatically. Experimental results were analysed using the integrated Partec software package.

Electronical particle counting and sizing (EPCS). Cell concentrations and dimensions were determined using a CASY 1-Model TTC (Schärfe System) electronic particle counter. This device measures particle concentrations, equivalent spherical diameter (ESD) and volume according to the resistance-measuring principle combined with pulse area analysis. Results are presented in 1024 channels. The orifice of the measuring capillary was 150 μm . Between 0.2 and 1.0 ml of the sample volume was diluted to 10 ml with isotonic solution (CASYton, Schärfe System), and 0.2 or 0.4 ml of the total volume was then measured in 4 replicates. The typical analysis time was 12 to 15 s per measurement. To analyse 1 sample in 4 replicates therefore took about 1 min. Values reported are

arithmetic means. The integrated software CASYstat (Schärfe System) allows calculation of mean size distributions of parallel measurements and standard deviations for all measurement parameters. We thus produced mean size distributions at each sampling time. The effect of size-selective grazing can be visualized by the changing size distribution over the course of the experiment. To quantify the grazing effect, we subtracted mean size distributions of samples taken later during the experiment from those taken at the beginning (difference curves). Finally, we overlaid the difference size distribution thus calculated onto the initial size distribution measured at the beginning of the experiment.

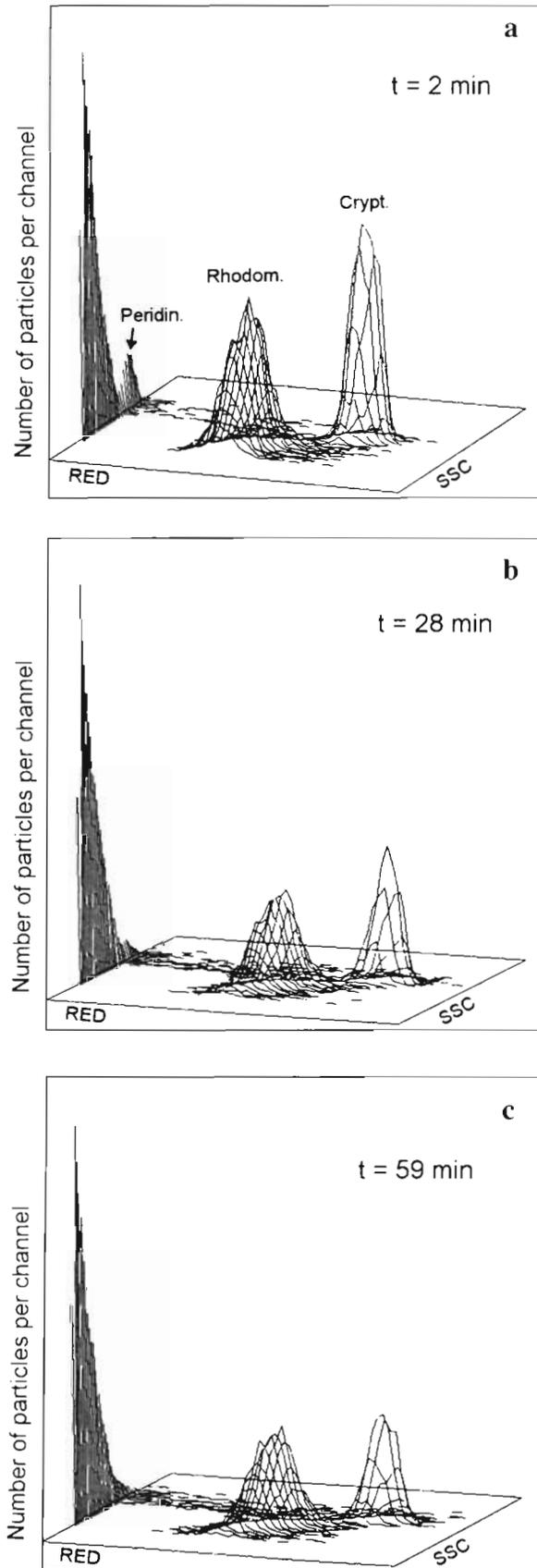
Calculation of ingestion and clearance rates. In the first experiment changes in algal abundance during the experiment and ingestion rates reported were based on AFC measurements performed in short intervals (1 to 5 min) during the initial 30 min. In subsequent experiments we calculated ingestion rates from changes of algal concentrations measured by both AFC and EPCS (second experiment) or EPCS alone (third experiment). Cell size (ESD) and biovolumes of the algae and dinoflagellates were also measured by EPCS in unpreserved samples. We checked the EPCS measurements of cellular abundance in each case by inverted microscopy.

Per capita ingestion rates I (cells *Peridiniopsis*⁻¹ h⁻¹) were calculated from the negative slope of least squares linear regression equations of prey population cell numbers versus time divided by the average abundance of *P. berolinense* during each experiment. Except for the first experiment, cell numbers in experimental bottles were corrected for changes in the controls if the latter were significant. To convert ingestion rates to carbon uptake, we multiplied the calculated ingestion rates by the mean initial biovolume of each algal species measured by EPCS and a carbon to volume conversion factor of 0.11 pg C m⁻³ (Rocha & Duncan 1985). Although *P. berolinense* is not a filter feeder, we calculated an equivalent clearance rate (nl *Peridiniopsis*⁻¹ h⁻¹) by dividing cell ingestion rates by the average prey concentration during the feeding interval to compare our results with literature data (see discussion in Goldman et al. 1989).

RESULTS

Species-specific feeding of *Peridiniopsis berolinense*

In the first experiment, we offered 2 cryptophyte species in high concentrations, (0.8 to 1.0) $\times 10^5$ cells ml⁻¹ each, as food to *Peridiniopsis berolinense*. Flow



cytometric analysis revealed distinctly higher loss rates of *Cryptomonas* sp. than of the smaller *Rhodomonas minuta* (Fig. 1). The 2 algal populations and the dinoflagellates were clearly separated from each other in the red fluorescence versus side scatter histogram. The second, larger peak at the SSC axis without any red fluorescence was due to bacteria and detritus particles. At the beginning of the experiment (Fig. 1a), *P. berolinense* showed almost no red fluorescence. With progressing time, *P. berolinense* was increasingly distributed along the chlorophyll axis (Fig. 1b, c). After 28 min the larger *Cryptomonas* sp. was distinctly more reduced relative to *R. minuta*. During the next half hour the peak height of the 2 algae, i.e. their cell numbers, changed only little (Fig. 1c).

The majority of the *Peridiniopsis berolinense* population was located along the y-axis at the beginning of the experiment, i.e. they did not show any red fluorescence and were thus heavily starved. With increasing time and uptake of chlorophyll-bound autofluorescence originating from the algae, more and more *P. berolinense* showed measurable red fluorescence, they 'migrated' along the x-axis. In accordance with measured changes in cryptophyte abundance, this process mainly occurred during the initial half hour. It therefore appears that *P. berolinense* fed heavily on the larger *Cryptomonas* sp. during the first half hour before loss rates levelled off (Fig. 2). Cell numbers of the smaller *Rhodomonas minuta* assessed by AFC decreased moderately and linearly over the whole experimental hour while the abundance measured by EPCS did not change significantly during the second 30 min of the experiment. It seems therefore likely that the low *R. minuta* concentration obtained by AFC after 62 min was an outlier. Ingestion rates can be calculated from the negative slope of the linear regression equation lines given in Fig. 2 and the average *P. berolinense* concentration of 3467 cells ml⁻¹. For the larger *Cryptomonas* sp., the grazing rate was high, 0.29 cells *Peridiniopsis*⁻¹ min⁻¹, during the first 30 min of the experiment.

In another experiment with 3-fold lower total algal concentrations compared to the first experiment, *Cryptomonas* sp. was ingested at low rates while changes in cell concentrations of the smaller *Cryptomonas* strain no. 26.80 were not significantly different from zero (Fig. 3). Feeding on *Cryptomonas* sp.

Fig. 1. Selective feeding of *Peridiniopsis berolinense* on *Cryptomonas* sp. and *Rhodomonas minuta* measured in live samples by flow cytometry during the first experiment. Peak height (z-axis) corresponds to cell concentrations, the x-axis indicates red autofluorescence (chlorophyll *a*), the y-axis right angle side scatter (SSC)

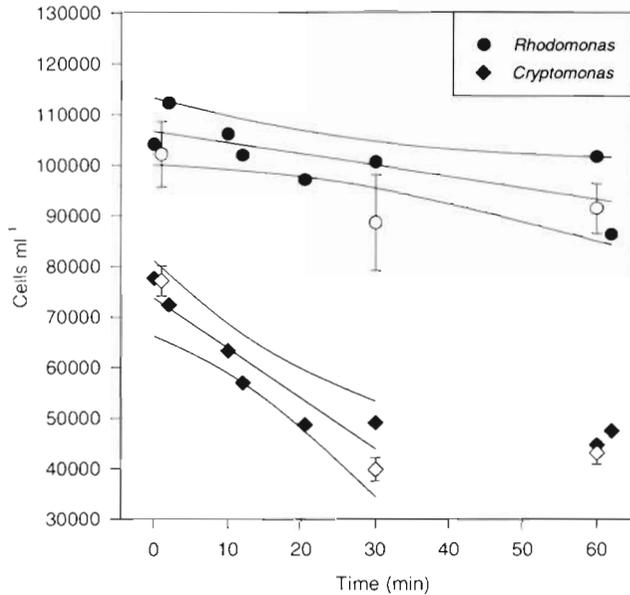


Fig. 2. Change of cell numbers of *Cryptomonas* sp. and *Rhodomonas minuta* measured in live samples by analytical flow cytometry (AFC) and electronic particle counting (EPCS) during the first experiment. Solid symbols and least-squares regression equation lines correspond to AFC. Open symbols with error bars (SD) denote EPCS measurements, each performed in triplicate

continued over the first 2 h of the experiment. The regression coefficients of the least-squares linear regression equations of the cellular decrease of *Cryptomonas* sp. versus experimental time calculated from measurements by the electronic particle counter and the flow cytometer were not significantly different (Student's *t*-test). The average *Peridiniopsis berolinense* concentration was 2797 cells ml⁻¹ during this experiment. The instantaneous ingestion rates of *Cryptomonas* sp. were thus 10-fold lower, 0.03 cells *Peridiniopsis*⁻¹ min⁻¹, than in the previous experiment. A third experiment with intermediate food and high dinoflagellate abundances yielded ingestion

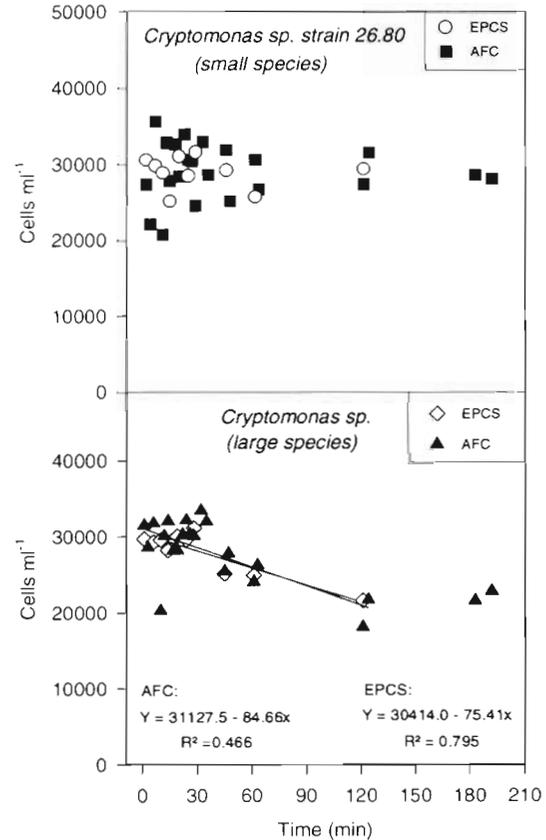


Fig. 3. Cell concentrations of the smaller *Cryptomonas* strain no. 26.80 and the larger *Cryptomonas* sp. measured by AFC and EPCS during the second experiment

rates close to the second experiment (Table 1). Similar to the second experiment, feeding on *Cryptomonas* sp. continued during the entire experimental period (3 h) whilst grazing on *Rhodomonas minuta* was insignificant (time course not shown). The food concentrations, abundances of *P. berolinense*, and the per capita ingestion rates calculated for all 3 experiments are summarized in Tables 1 & 2.

Table 1. Initial cell concentrations of prey and predator, and ingestion rates *I* (in cells *Peridiniopsis*⁻¹ h⁻¹) of *P. berolinense* during the experiments. Algal cell concentrations and grazing rates were calculated from significant linear regression equations of algal abundance versus time measured by electronic particle counting and/or flow cytometry (AFC). ns: not significantly different from changes in controls

Expt no.	<i>Rhodomonas</i> (cells ml ⁻¹)	<i>Cryptomonas</i> (cells ml ⁻¹)	Total prey conc. (cells ml ⁻¹)	<i>Peridiniopsis</i> (cells ml ⁻¹)	<i>I</i> _{Rho}	<i>I</i> _{Cry}	<i>I</i> _{tot}
1	102050	77095	179145	3467	3.87 ^b	8.61 ^b	12.48 ^b
2	31161 ^a	30414	61575	2797	ns	0.68/0.66 ^b	0.68
3	70360	52927	123287	9306	ns	0.88	0.88

^a*Cryptomonas* sp. strain no. 26.80 (formerly *Rhodomonas* sp. strain no. 26.80)
^bCalculated from AFC

Assessment of the nutritional status, increase of autofluorescence and formation of food vacuoles of *Peridiniopsis*

To quantify the increase of autofluorescence in the food vacuoles of *Peridiniopsis berolinense* during the experiments (cf. Fig. 1), we divided the *P. berolinense* gate as defined in the SSC versus red fluorescence histogram into 240 channels along the x-axis (Fig. 4). The location of the *P. berolinense* population at the x-axis is indicative of their nutritional status. Only 21 min after the beginning of the first experiment, *P. berolinense* individuals were spread over a wide range of channel numbers of the histogram whereas more than 90% of the population were measured in channel numbers 0 to 20 during the initial measurement. Only about 2% of all individuals showed red autofluorescence beyond channel number 40 at the beginning (Fig. 5a). This is in agreement with our microscopic observation that food was already depleted when we separated *P. berolinense* from their *Ceratium furcoides* prey 24 h prior to the experiment. The share of individuals with relatively high red fluorescence (>channel 40) increased

linearly during the first half hour of the experiment before levelling off. In contrast to the first experiment, 13.3% of the population was located in channel numbers >80 in the second experiment, i.e. they were still relatively well fed. About 2% were recorded even beyond channel number 140 in the initial sample. This share increased up to about 25% during the course of the experiment (Fig. 5b). The time course of the increase in autofluorescence agreed well with the finding that ingestion of *Cryptomonas* sp. virtually ceased after the first half hour in the first experiment (cf. Fig. 2) but continued over 2 h in the second experiment (cf. Fig. 3). We did not measure the increase of autofluorescence in *P. berolinense* in the third experiment for technical reasons.

Cell size was another indicator of the different nutritional status of *Peridiniopsis berolinense* at the beginning of the experiments (Table 2). The average cell size of *P. berolinense* was distinctly smaller in the first (2695 μm^3) than in the second (4944 μm^3) and third (5692 μm^3) experiment. The small cells used in the initial experiment were close to the minimum cell size typical of heavily starved cells (Kirchhoff & Weisse unpubl.).

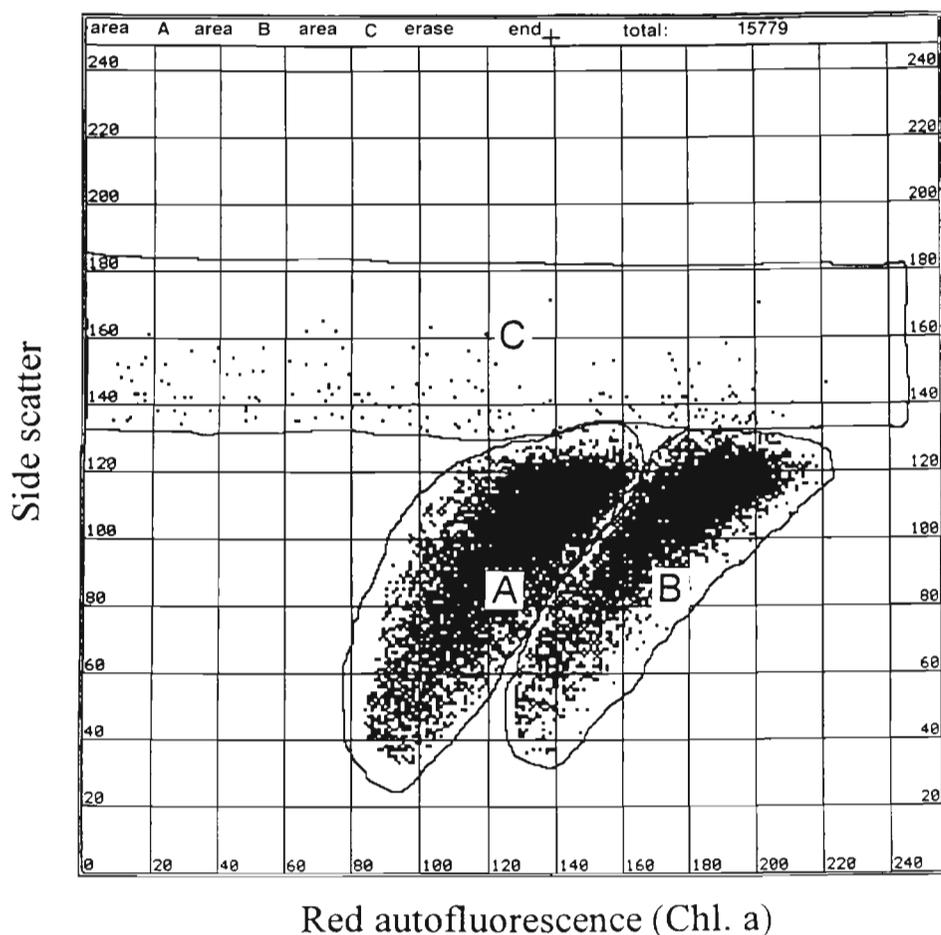


Fig. 4. Gates of the algae (A) *Rhodomonas minuta* and (B) *Cryptomonas* sp., and (C) the dinoflagellate *Peridiniopsis berolinense* in the side scatter versus chlorophyll a histogram measured by AFC in the first experiment 21 min after the beginning. Each data point denotes 1 cell. A part of the *P. berolinense* population located at the y-axis (channel no. 0 of the x-axis), i.e. without any red fluorescence, is not visible in the histogram. Because red fluorescence of *P. berolinense* originates from algal pigments, the dinoflagellates are increasingly shifted along the x-axis to the right the more algae they have ingested

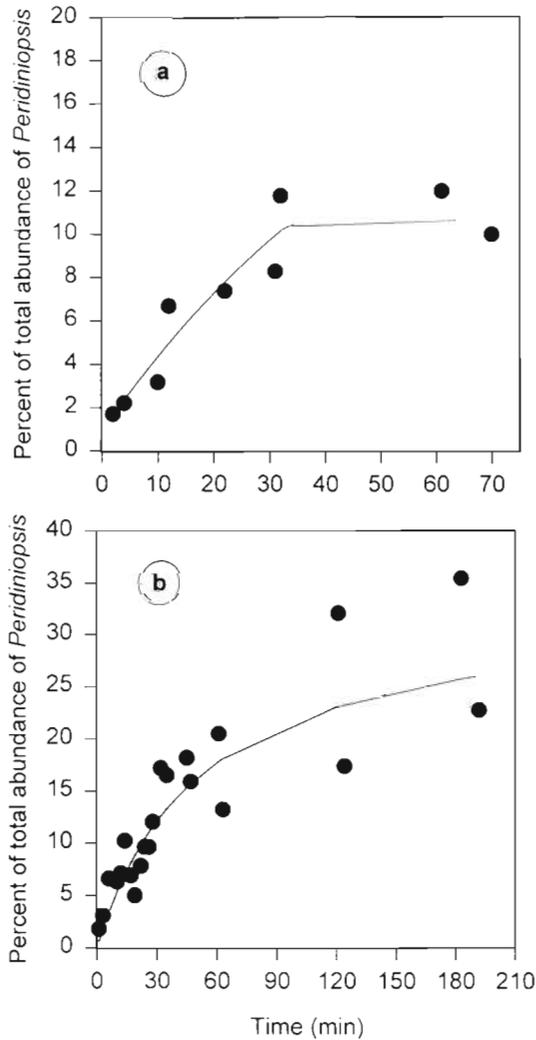


Fig. 5. Relative increase of red autofluorescence of *Peridiniopsis berolinense* assessed by AFC when feeding on the cryptophytes in the (a) first and (b) second experiments. Symbols indicate the percentage of the *P. berolinense* population beyond a given channel number, i.e. with higher autofluorescence, in the SSC versus red fluorescence diagram (cf. Fig. 4). In the first experiment, the percentage of cells > channel 40 is shown; in the second experiment, the percentage of cells > channel 140 is depicted

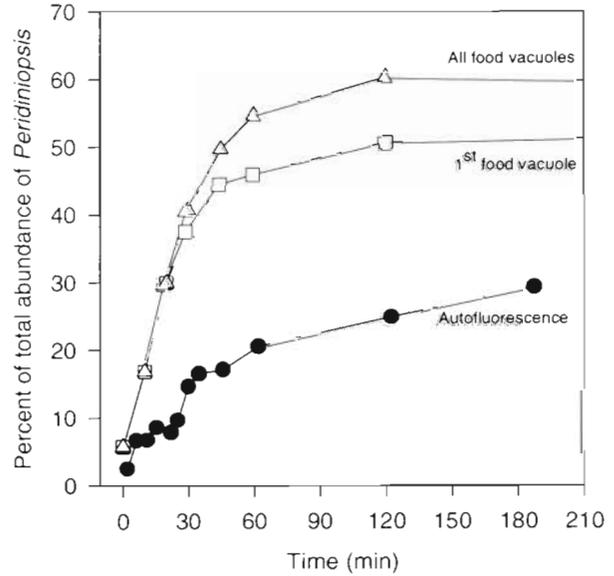


Fig. 6. Increase of red autofluorescence of *Peridiniopsis berolinense* assessed by AFC (mean values of 2 consecutive measurements; ●) compared to the formation of food vacuoles (open symbols) in the second experiment. (□) Percentage of the dinoflagellate population with 1 food vacuole; (Δ) combined percentage of *P. berolinense* with 1, 2 or 3 food vacuoles

When *Peridiniopsis berolinense* feeds, ingestion is followed by the formation of food vacuoles. The increase of autofluorescence shown in Fig. 5 originates from an accumulation of chlorophyll inside the food vacuoles. We compared the formation of food vacuoles as observed from microscopic observations with the increase of autofluorescence measured by AFC during the second experiment (Fig. 6). Note that the percentage of autofluorescence is a relative measure depending on the channel number chosen and does not necessarily have to correspond numerically to the percentage of food vacuoles formed. The increase of autofluorescence mainly indicated the formation of the first food vacuole that occurred in almost every second specimen during the first hour of the experiment. The

Table 2. Algal and dinoflagellate cell volumes, and carbon ingestion (I) and clearance rates of *Peridiniopsis berolinense*. ns: not significantly different from changes in control

Expt no.	<i>Rhodomonas</i> vol. (μm^3)	<i>Cryptomonas</i> vol. (μm^3)	<i>Peridiniopsis</i> vol. (μm^3)	I_{Rho} (pg C <i>Peridiniopsis</i> ⁻¹ h ⁻¹)	I_{Cry} (pg C <i>Peridiniopsis</i> ⁻¹ h ⁻¹)	I_{tot} (pg C <i>Peridiniopsis</i> ⁻¹ h ⁻¹)	Clearance rate (nl <i>Peridiniopsis</i> ⁻¹ h ⁻¹)
1	192	633	2695	81.7	599.5	681.2	69.7
2	281 ^a	989	4944	ns	72.9	72.9	22.0
3	200	1020	5692	ns	98.7	98.7	16.6

^a*Cryptomonas* sp. strain no. 26.80 (formerly *Rhodomonas* sp.)

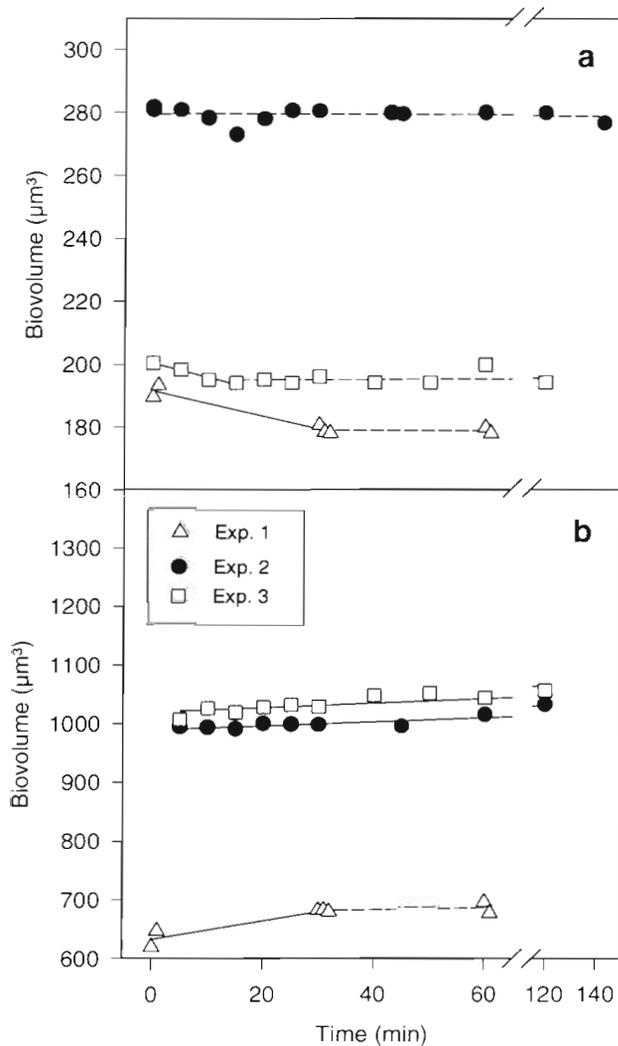


Fig. 7. Change of mean cell volumes of the algal populations during the experiments: (a) *Rhodomonas minuta*/*Cryptomonas* sp. strain no. 26.80, and (b) *Cryptomonas* sp. Significant changes ($p < 0.05$) are indicated by solid lines, dashed lines indicate insignificant changes

prolonged increase of autofluorescence up to at least the second hour was probably caused by the formation of a second and, in a few cases, even a third food vacuole by some *P. berolinense* specimen during the experiment.

Size-selective feeding by *Peridiniopsis berolinense*

We monitored changes in algal cell volumes during the experiments. In the first experiment, the average cell size of the *Rhodomonas minuta* population decreased significantly ($p < 0.001$; Fig. 7a) while the mean cell size of *Cryptomonas* sp. increased ($p < 0.05$; Fig. 7b) during the initial 30 min. Thereafter, cell size of both cryptophyte species remained constant. In the

second and third experiment, we found a highly significant ($p < 0.01$ and $p < 0.001$, respectively) increase of the average biovolume of the large *Cryptomonas* sp. over the first hour that continued for another hour (Fig. 7b). The size of the smaller of the 2 *Cryptomonas* species offered in the second experiment, *Cryptomonas* strain no. 26.80, did not change. In the third experiment, cell size of the small *R. minuta* decreased significantly ($p < 0.05$) during the first 15 min and remained constant thereafter. The relative increase in the average size of the large *Cryptomonas* sp. was high in the first ($0.25\% \text{ min}^{-1}$) and moderate in the second ($0.035\% \text{ min}^{-1}$) and third ($0.037\% \text{ min}^{-1}$) experiment. It should be noted, however, that the average cell volume of *Cryptomonas* sp. was distinctly larger at the beginning of the later experiments compared to the first one.

To visualize the grazing effect, we subtracted the mean size distribution measured in the first experiment after 30 min from the initial size distribution. The resulting difference curve indicates the range where feeding was effective and the number of algae that had been grazed in each of the 1024 channels. Apparently, *Peridiniopsis berolinense* mainly fed in the size range between 8 and 15 μm ESD (Fig. 8). When compared to the initial size distribution it becomes obvious that *P. berolinense* fed in direct response to the *Cryptomonas* sp. peak while among *Rhodomonas minuta* the dinoflagellates selected for the largest cells. At the size of the *R. minuta* peak (6.7 μm ESD), no algae had been ingested. We obtained similar results in the other experiments in which both the peak of the *Cryptomonas* sp. distribution and the maximum of the ingestion were located in the range between 11.6 to 14.3 μm .

DISCUSSION

The present study is the first that investigated feeding rates of a freshwater dinoflagellate using analytical flow cytometry (AFC) and electronic particle counting and sizing (EPCS) in short-term (1 to 3 h) experiments. A similar approach has been used recently to measure grazing rates of the naked heterotrophic dinoflagellate *Oxyrrhis marina* in long-term (2 d) experiments (Hansen et al. 1996). Flow cytometry alone had been applied earlier by Tarran (1992) to measure selective grazing by *O. marina*. *O. marina* is a widespread marine raptorial species and comparable in size to *Peridiniopsis berolinense* (Goldman et al. 1989, Hansen et al. 1996). Hansen et al. (1996) used AFC and EPCS to characterize different algal populations and to measure their specific loss rates due to dinoflagellate feeding. Unlike the present study, these authors did

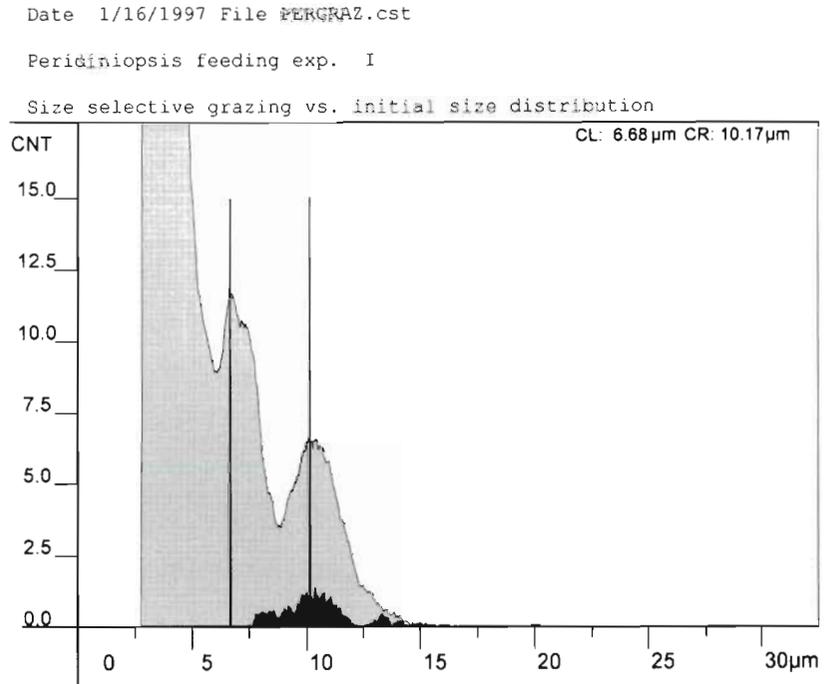


Fig. 8. Size-selective grazing of *Peridiniopsis berolinense* (dark shaded area) versus the initial algal size distribution (light shaded area) measured by EPCS in the first experiment. The y-axis denotes count rate, i.e. number of particles per each of the 1024 channels of the equivalent spherical diameter (ESD, x-axis); the latter is a measure of cell size. The dark area thus indicates the size range where feeding was effective and the number of algae in each channel that have been grazed during the experiment. Cursors mark the abundance peak of *Rhodomonas minuta* (left) and *Cryptomonas* sp. (right), respectively. The corresponding ESD are given in the top right corner (CL: cursor left, *R. minuta*; CR: cursor right, *Cryptomonas* sp.)

not attempt to measure the uptake of algal material directly by AFC.

In comparison to conventional microscopy, AFC and EPCS offer several significant advantages that render them very attractive for short-term feeding studies with aquatic protozoa. First, statistical reliability of the results obtained is better because compared to time-consuming microscopy a much larger number of cells can be analysed in a much shorter time. Ingestion rates presented in this paper were derived from regression analyses on results from subsamples taken in short intervals. This is superior to the conventional end point method where results are based on the difference between initial and final experimental results only, and the shape of the feeding response curve versus time remains unknown. Secondly, AFC and EPCS do not require any manipulation of the cells such as fixing, staining, or filtering. Fixation has been shown to potentially severely underestimate feeding rates of protozoa due to egestion of ingested material upon fixation (Sieracki et al. 1987). Shrinking as a result of fixation (e.g. Müller & Geller 1993, Montagnes et al. 1994) is another factor with a potential bias on the results from conventional grazing experiments. Filtration may also yield biased results because of cell breakage and leaky filters (Stockner et al. 1990). In addition to these technical advantages, AFC provides an objective measure of ingestion based upon the increase of autofluorescence in the food vacuoles of the protozoa during the experiment. This is of special

significance in feeding studies with myzocytotic dinoflagellates where ingestion rates cannot be measured by the increasing number of particles inside food vacuoles. The latter approach was successfully employed to study grazing of the heterotrophic marine dinoflagellate *Gyrodinium spirale*, which feeds by direct engulfment of its prey (Hansen 1992).

Shortcomings of the current AFC and EPCS method are that they require some *a priori* knowledge of the organisms used, work best at relatively high prey and predator abundances, and need costly equipment. To overcome the first problem, we combined AFC/EPCS with conventional microscopy. If it is only for the sake of unequivocal identification, the latter can be replaced by novel automatic techniques. The potential of flow cytometry to identify and count dinoflagellates in mixed algal populations using direct immunofluorescence has already been demonstrated (Vrieling et al. 1996 and references therein). Relatively high cell numbers are necessary to obtain statistically reliable results in short-term grazing experiments if feeding rates are low. Both the algae and the dinoflagellate concentrations used in our experiments were somewhat higher than in their natural environments. In Lake Plußsee, from which *Peridiniopsis berolinense* had been isolated, maximum concentrations of *Rhodomonas* spp. exceed 20×10^3 cells ml⁻¹ (B. Meyer, MPIL, Plön, unpubl. data), and the total number of autotrophic nanoflagellates in the lake reaches up to 63×10^3 cells ml⁻¹ (Meier & Reck 1994). The experimental concen-

tration of *P. berolinense* was about 5- to 15-fold higher than their maximum numbers of 600 cells ml⁻¹ found in north German lakes (B. Meyer, MPIL, Plön, unpubl. data).

We were able to link the increase of autofluorescence measured by AFC to the microscopically measured number of food vacuoles formed by *Peridiniopsis berolinense* during the feeding and digestion process. These 2 techniques measure somewhat different aspects of the feeding process. The microscopic observations provided information about the time when the first, second, and third food vacuoles were formed consecutively, and the fraction of the *P. berolinense* population that actively fed on the cryptophytes during the experiment. The autofluorescence of *P. berolinense* measured by AFC is an integrative signal that increases as long as ingestion exceeds digestion of plant material. Two hours after the beginning of the second experiment, when about 50% of the population had 1 food vacuole formed, a steady state between ingestion and digestion was seemingly reached. Because cytometric measurements of autofluorescence scattered widely after 2 and 3 h it is difficult to judge whether the autofluorescence of the *P. berolinense* population further increased during this period. The scattering resulted from the fact that at the end of this experiment many individuals in the SSC versus red fluorescence histogram were located close to the chosen channel boundary at channel number 140. The red fluorescence originating from the algal pigments inside the food vacuoles of *P. berolinense* is a continuum while the channel number selected for the whole experimental period was arbitrarily set to illustrate the results. *Cryptomonas* sp. loss rates measured by the particle counter that also levelled off 2 h after the beginning of the experiment revealed that feeding had virtually stopped. We therefore conclude that because feeding was close to zero, but the number of food vacuoles and the autofluorescence did not start to decline until after 2 to 3 h, lifetime of the food vacuoles is at least 3 h. This conclusion is in agreement with Hansen's (1992) observation that prey particles in the food vacuoles of *Gyrodinium spirale* became digested beyond recognition only after 3 h.

Microscopic observations of the feeding process as well as the time course of the formation of food vacuoles led us to conclude that 1 food vacuole was formed as a result of 1 feeding act. When *Peridiniopsis berolinense* feeds upon the cryptophytes which are smaller than the dinoflagellates this is not surprising. We do not know yet whether this also holds when *P. berolinense* collectively feeds upon the much larger *Ceratium*, i.e. when several *P. berolinense* attack 1 large prey item. Because in the present study formation of 1 food vacuole can be equated with ingestion of

1 cryptophyte cell (1 food vacuole *Peridiniopsis*⁻¹ = 1 cell ingested *Peridiniopsis*⁻¹), we can derive an independent estimate of ingestion rates: since 1 h after the beginning of the experiment 42% of the *P. berolinense* population showed 1 food vacuole, 8.5% a second and another 0.7% a third one, total ingestion I_{tot} can be calculated according to

$$I_{\text{tot}} \text{ (cells } Peridiniopsis^{-1} \text{ h}^{-1}) = (0.42 \times 1 \text{ cell } Peridiniopsis^{-1} \text{ h}^{-1}) + (0.08 \times 2 \text{ cells } Peridiniopsis^{-1} \text{ h}^{-1}) + (0.007 \times 3 \text{ cells } Peridiniopsis^{-1} \text{ h}^{-1})$$

The result of 0.60 cells *Peridiniopsis*⁻¹ h⁻¹ is in close agreement with ingestion rates calculated from loss rates of prey cells, 0.68 cells *Peridiniopsis*⁻¹ h⁻¹ determined by EPCS and 0.66 cells *Peridiniopsis*⁻¹ h⁻¹ calculated from AFC (cf. Table 1). The latter values were calculated after correcting for changes occurring in the control bottle. Cell numbers of *Cryptomonas* sp. in the control significantly decreased during the second experiment (data not shown). Without taking this decline into consideration, the grazing rate would have been distinctly higher, 1.61 cells *Peridiniopsis*⁻¹ h⁻¹.

Ingestion (0.68 and 0.88 *Cryptomonas Peridiniopsis*⁻¹ h⁻¹) and clearance rates (16.6 and 22.0 nl *Peridiniopsis*⁻¹ h⁻¹, cf. Table 2) of *P. berolinense* that we calculated for moderately starved animals from the second and third experiment are in the range of results obtained from a number of marine dinoflagellate species of similar size (Goldman et al. 1989, Hansen 1992, Strom & Buskey 1993, Hansen et al. 1996 and references therein). The feeding rate measured for heavily starved individuals in the first experiment of the present study was, however, much higher. Since we did not use control bottles in the first experiment and therefore could not correct for a potential decline of algal abundance due to effects other than grazing, we may have overestimated the ingestion rates of *P. berolinense* in the initial experiments. In spite of this caveat we conclude that the duration and relative grazing impact on the 2 algae was correctly stated because we found a remarkable coincidence in the respective time courses of ingestion rates and changes in algal cell size in the first as well as in the other experiments. The high grazing rates we calculated for starved *P. berolinense* may, however, be misleading for 2 other reasons. Firstly, we assumed in these calculations that entire prey cells were ingested by *P. berolinense* which is not the case. Remains of the pellicles of the target cells were clearly visible under the microscope. The extent to which the cell content of a prey cell is taken up by *P. berolinense* is yet unknown. It remains to be investigated whether the prey hunting and feeding processes depend on the nutritional status of the dinoflagellates. At present we cannot rule out that heavily starved *P. berolinense* attack more prey cells in

the same time but take up relatively less of their cell content than satiated individuals. With respect to the ecological significance, the term 'per capita cell destruction rate' appears to be more adequate than cell ingestion rate to describe the grazing impact of peduncle-feeding heterotrophic dinoflagellates. Secondly, the contrasting experiments that we presented in this paper suggest that the time course of the protozoan feeding response is variable depending on the nutritional status. Small, heavily starved cells like the ones we used in the first experiment started feeding almost immediately at the beginning of the experiment. We therefore obtained very high *initial* feeding rates of 0.29 *Cryptomonas* cells *Peridiniopsis*⁻¹ min⁻¹ averaged over the first 30 min of the experiment. The 'migration' of the *P. berolinense* population along the red fluorescence axis measured by AFC (cf. Figs. 1 & 4) suggests that almost every cell had taken up some algal material within the first half hour. This was clearly different in the second experiment with the larger, less severely starved specimens, where only 40% of the population showed food vacuoles after 30 min (cf. Fig. 6).

Concerning size-selective feeding, our results are similar to those obtained for *Oxyrrhis marina* (Hansen et al. 1996). Of 7 algal species tested, *O. marina* preferentially consumed the 2 largest species, the prasinophyte *Tetraselmis suecica* (cell volume 492 µm³) and an unidentified marine *Rhodomonas* species (cell volume 250 µm³). In a preliminary experiment, we had checked by offering fluorescently labeled bacteria that *Peridiniopsis berolinense* does not feed on bacteria.

We use the location of heterotrophic dinoflagellates and ciliates along the red autofluorescence axis to monitor the nutritional status of our cultures routinely. If the same population is measured by AFC repeatedly under various food conditions, a 'gate', i.e. the range in the SSC versus red autofluorescence or similar histograms (cf. Fig. 4), can be easily defined over which the population can vary. This gate can then be subdivided into a number of appropriate channels, and the nutritional status can be related to a channel number. This technique can also be applied to natural populations if protozoa and their algal food can be separated from each other based upon their size or additional parameters such as their relative DNA content (Kenter et al. 1996). Additional information on the nutritional status of heterotrophic protists can be obtained by EPCS if the range of the biovolume is known. The powerful combination of AFC and EPCS that we have outlined in this study should allow for consideration of the physiological status of prey and predator prior to an experiment. Consequently, feeding experiments with starved or well-fed specimens can be precisely timed in future studies with aquatic protozoa.

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