Vol. 262: 137–151, 2003 | MARINE ECOLOGY PROGRESS SERIES Mar Ecol Prog Ser | Published November 7

Feeding in the mixotrophic dinoflagellate *Ceratium furca* is influenced by intracellular nutrient concentrations

Gabriela W. Smalley[1,2,3], D. Wayne Coats[1,*], Diane K. Stoecker[2]

[1]Smithsonian Environmental Research Center, PO Box 28, Edgewater, Maryland 21037, USA

[2]Horn Point Laboratory, University of Maryland Center for Environmental Science, PO Box 775, Cambridge, Maryland 21613, USA

[3]*Present address:* Georgia Institute of Technology, 10 Ocean Science Circle, Savannah, Georgia 31411, USA

ABSTRACT: In field populations of the mixotrophic dinoflagellate *Ceratium furca*, feeding on microzooplankton is commonly observed and is influenced by inorganic nutrient concentrations and ratios. Using batch and 'semi-continuous' laboratory cultures, we examined in more detail the nutrient conditions that trigger feeding in *C. furca*. Cultures were maintained at a range of nitrogen (N) and phosphorus (P) concentrations for 31 to 36 d. Several parameters were determined at regular intervals, including inorganic nutrient concentrations of the medium, *C. furca* abundance and size, cellular chlorophyll *a* (chl *a*), C, N, and P contents and densities, photosynthetic rates, and ingestion of ciliate prey. We detected significant differences between treatments in all cellular parameters measured. Most notably, feeding only occurred in cultures that had been growing under N- or P-deplete conditions for 11 to 16 d, whereas nutrient-replete cells did not ingest prey. Feeding increased markedly as cellular C:N:P ratios deviated farther from those found under optimum growth conditions. Specifically, feeding in P-deplete cultures increased at C:P ratios >130 and at N:P ratios >19, whereas N-deplete cultures required a C:N ratio of ~10 and an N:P ratio <7 to commence feeding. Growth and photosynthetic rates were reduced compared to those of nutrient-replete cells regardless of the limiting nutrient. In N-limited cultures, cell size and chl *a* density decreased compared to nutrient-replete cells, whereas the size of P-limited cells significantly increased. This change in average cell size was caused by the development of a bimodal size distribution under N- and P-reduced conditions, raising the possibility of sexual reproduction in *C. furca*. Changes in cellular parameters were reversible upon nutrient addition, and feeding decreased or ceased within hours to days. The findings presented here indicate that cellular nutrient quotas and ratios are more critical than absolute inorganic nutrient concentrations in regulating feeding behavior of *C. furca*.

KEY WORDS: Dinoflagellate mixotrophy · Phagotrophy · Nutrient limitation · Photosynthesis · *Ceratium furca*

Resale or republication not permitted without written consent of the publisher

INTRODUCTION

Many phytoflagellates, once thought to be strictly photosynthetic, are capable of phagotrophy. This form of mixotrophy seems particularly common among chrysophytes, prymnesiophytes, and dinoflagellates (Porter 1988, Sanders & Porter 1988). In many cases, feeding has been hypothesized to be a response to nutrient limitation. For example, feeding in mixotrophic nanoflagellates in the Sargasso Sea was more pronounced in nutrient-depleted surface water. No feeding occurred in the nutrient-rich deep chlorophyll *a* (chl *a*) maximum, where phagotrophic carbon acquisition would have been advantageous due to low light conditions (Arenovski et al. 1995). Likewise, in the coastal waters of Norway, Nygaard & Tobiesen (1993)

*Corresponding author. Email: coatsw@si.edu

© Inter-Research 2003 · www.int-res.com

found that increased feeding on bacteria by certain mixotrophic chrysophytes coincided with low inorganic phosphorus (P) concentrations and suggested that bactivory served as a survival strategy during nutrient limitation. In many primarily photosynthetic dinoflagellates, feeding also seems to be related to inorganic nutrient concentrations. In Chesapeake Bay, ingestion of ciliates by *Ceratium furca* and of cryptophytes by *Gyrodinium galatheanum* increased with decreasing inorganic P concentrations (Li et al. 2000a,b, Smalley & Coats 2002), whereas *Prorocentrum minimum* contained a greater number of food vacuoles at higher NH_4 concentrations (Stoecker et al. 1997).

Nutrient addition experiments with natural populations support a relationship between feeding and nutrient concentrations. Consistent with the suggested role of phagotrophy as a means to supplement the nutrient budget, Arenovski et al. (1995) reported a decrease in phagotrophic activity or abundance of mixotrophic nanoflagellates in response to nutrient addition. Nutrient addition assays using field populations of the 3 dinoflagellates *Gyrodinium galatheanum*, *Prorocentrum minimum*, and *Ceratium furca* also showed a decrease in feeding when nitrogen (N) and/or P were added (Stoecker et al. 1997, Li et al. 2000b, Smalley 2002). However, differences in the response pattern among and within species were apparent. Whereas the ingestion rate of *G. galatheanum* dropped upon the addition of N and N+P (Li et al. 2000b), feeding in *P. minimum* actually increased when N or P were added separately, but decreased when these nutrients were added together (Stoecker et al. 1997). In feeding trials involving *C. furca* collected from 3 different sampling sites, ingestion rates consistently dropped when N and P were added together. However, the response to separate N or P addition varied, with ingestion rates decreasing, increasing, or remaining unaltered depending on feeding trial (Smalley 2002).

For a few mixotrophic species, the influence of nutrient concentrations in the growth medium on ingestion has been investigated in the laboratory. Legrand et al. (1998) found that the mixotrophic dinoflagellate *Heterocapsa triquetra* failed to ingest prey under nutrient-replete growth conditions, but did so in nutrient-deplete medium regardless of light environment. Likewise, feeding by *Gyrodinium galatheanum* was stimulated by low N and/or P concentrations in the growth medium and increased as N:P ratios of the medium deviated farther from the Redfield ratio (Li et al. 2000a,b). Feeding by field populations of *Ceratium furca* and *G. galatheanum* showed a similar relationship with inorganic nutrient ratios and was positively correlated with inorganic N:P ratios (Li et al. 2000b, Smalley & Coats 2002). These observations suggest that nutrient ratios, rather than absolute nutrient concentrations, may play a role in regulating phagotrophy in these mixotrophs (Raven 1997, Stoecker et al. 1997, Li et al. 2000a,b, Smalley & Coats 2002).

Whereas feeding in field populations of *Ceratium furca* was clearly influenced by inorganic nutrient concentrations and ratios (Smalley 2002, Smalley & Coats 2002), the feeding response observed during nutrient addition assays exhibited no consistent relationship with these parameters (Smalley 2002). This illustrates the difficulty of inferring nutrient limitation of a cell or population based on inorganic nutrient concentrations alone. The biological availability of inorganic nutrients (especially PO_4), the ability to utilize dissolved organic nutrients, and the internal storage capabilities of organisms can all influence the interpretation of inorganic nutrient data (Cembella et al. 1984, Fisher et al. 1992). We thus examined the relationship between nutrient concentrations and feeding in *C. furca* in a controlled laboratory setting. Using batch and 'semi-continuous' cultures, we were able to manipulate the nutrient environment and the nutritional history of *C. furca*, allowing us to gain a better understanding of the relationships between inorganic nutrient concentrations, cellular nutrient quotas, and feeding.

MATERIALS AND METHODS

Culture of organisms. Cultures of *Ceratium furca* originally isolated from Chesapeake Bay were maintained at 20°C in 15‰ modified f/2-Si medium (Guillard & Ryther 1962) and exposed to an irradiance level of 160 µmol m^{-2} h^{-1} on a 14:10 h light:dark (L:D) cycle. Ciliates of the genus *Strobilidium* were isolated from the Rhode River, a Maryland tributary of Chesapeake Bay, and grown at 4°C in 15‰ water enriched to f/2-levels with vitamins and trace metals. Ciliates were maintained at an irradiance of 15 µmol m^{-2} h^{-1} on a 14:10 h L:D cycle and fed a mixture of the flagellates *Isochrysis galbana* and *Storeatula major*. The genus *Strobilidium* was chosen as prey because analysis of *C. furca* food vacuole content and potential prey availability in the field indicated that *C. furca* preyed mainly on small (10 to 40 µm) choreotrich ciliates of this genus (Smalley et al. 1999).

Batch culture experiment. To determine the relative effect of inorganic N and P concentrations on feeding in *Ceratium furca*, we conducted a time-course experiment using batch cultures. At the beginning of the experiment, *C. furca* stocks were concentrated ~7-fold by gentle reverse filtration through 20 µm Nitex mesh. Cells were then evenly distributed into ten 2 l polycarbonate bottles and diluted to approximately 2500 cells ml^{-1} using 5 different target media to yield duplicate 2 l

cultures for the following treatments: (1) nutrient-replete (f/2-Si; 883 µM NO_3, 36 µM PO_4), (2) 50% reduced N levels (f/2-½N), (3) no added N (f/2-N), (4) 50% reduced P levels (f/2-½P), and (5) no added P (f/2-P). Treatments were incubated under the same light and temperature conditions as stock cultures. After a recovery period of 1½ h, aliquots were removed to determine inorganic nutrient concentrations of the medium, *C. furca* abundance and size, cellular chl *a*, C, N, and P contents and densities, photosynthetic rates at growth irradiance, and ingestion of ciliate prey. This process was repeated weekly for inorganic nutrient analysis and 2 to 3 times a week for the other parameters for a total of 36 d.

'Semi-continuous' culture experiment. A 'semi-continuous' culture experiment was used to explore relationships among relatively stable external nutrient concentrations, cellular nutrient quotas, and feeding in *Ceratium furca*. For this experiment, 1.2 l of *C. furca* stock culture at 5000 cells ml^{-1} was distributed to ten 2 l polycarbonate bottles. Duplicate bottles were assigned to each of 5 different nutrient regimes. While f/2 concentrations of N were added to all treatments, P concentrations ranged from very low to high (0, 3, 6, 13, or 15 µM PO_4). Treatment bottles were incubated for 31 d under the same light and temperature conditions as stock cultures. Daily, 25% (300 ml) of the culture medium and a sufficient number of *C. furca* to retain cell density at ~5000 ml^{-1} were removed and replaced with 300 ml of fresh target medium. To accomplish this, *C. furca* densities were first determined for each bottle and a volume of culture fluid containing *C. furca* equivalent in number to those produced during the preceding 24 h was removed. The remainder of the 300 ml of medium to be replaced was then withdrawn by siphon using silicone tubing capped with 20 µm Nitex mesh to prevent removal of additional *C. furca*. Whereas 300 ml was removed daily from each bottle, the proportional removal of culture medium with cells to cell-free medium varied slightly from day to day and depended on net growth of *C. furca*. Samples for analysis of dissolved inorganic nutrient concentrations in the spent medium, cell size, and cellular C, N, and P contents, and densities of *C. furca* were taken every 3 d. Photosynthesis, chl *a* content and density, and ingestion of ciliates by *C. furca* were assessed every 6 d. Cultures were assumed to have reached steady state when cellular parameters (i.e. cell size, feeding, photosynthetic rates, chl *a*, C, N, and P contents) deviated by no more than 10% from the 'steady-state' mean of those parameters.

Nutrient addition experiment. *Ceratium furca* cultures were maintained in P-deplete f/2 medium for 2 mo under the same light and temperature conditions described above. At the start of the experiment, 400 ml of culture was added to each of eight 500 ml glass flasks. Duplicate flasks were then spiked with 0, 1.5, 9, or 20 µM PO_4 to yield 4 different nutrient regimes. Subsamples were immediately withdrawn for analysis of inorganic nutrient concentrations, *C. furca* abundance and size, cellular chl *a*, C, N, and P contents, photosynthetic rates, and ingestion rates. This process was repeated after 3, 6, 9, 27, and 51 h, except that feeding assays were not conducted at the 6 and 9 h time points. In addition, cell size was determined for all treatments at the beginning of the experiment.

Analytical procedures. *Strobilidium* sp. and *Ceratium furca* densities were determined microscopically after Lugol's fixation (2% final concentration). Successive microscope transects (100× to 125×) of triplicate Sedgwick-Rafter chambers were examined until 100 *C. furca* cells had been encountered for each chamber. The entire chamber was scanned for determination of *Strobilidium* sp. abundance. Cell length of 100 *C. furca* specimens was measured using an ocular micrometer, and cells were grouped into 2 size categories, <60 µm and ≥60 µm. To determine cell volume and equivalent spherical diameter (ESD), 2 ml of *C. furca* culture preserved in 2% gluteraldehyde were analyzed using a Coulter Multisizer II electronic particle counter (Coulter Corporation) interfaced with AccuComp particle characterization software (Coulter AccuComp version 2.01, 1998). Growth rates were calculated assuming exponential growth/decay of *C. furca* over the experimental period.

The ability of *Ceratium furca* to ingest ciliate prey was determined using the labeling technique described in Smalley et al. (1999). These authors used fluorescent latex microspheres (Fluoresbrite plain YG 1.0 micron; Polysciences) to label natural prey and followed ingestion of labeled prey by *C. furca* in the field. The preferred prey of *C. furca*, small choreotrich ciliates, were labeled quickly (>95% labeled in 15 min) and retained the microspheres for the duration of the experiment (>99% labeled after 6 h), whereas *C. furca* was unable to ingest microspheres alone (Smalley et al. 1999). For the feeding assays employed here, we added fluorescent microspheres at 5×10^6 ml^{-1} to between 10 and 20 ml of *Strobilidium* sp. culture in 20 ml scintillation vials. The vials were placed in a temperature-controlled incubator until the culture medium had equilibrated at 20°C (~15 min), which also allowed sufficient time for a majority of *Strobilidium* sp. to ingest microspheres. *C. furca* was then added at a target prey:predator ratio of 1:1.5. The vials were incubated for 6 h under the same light and temperature conditions as experimental and stock cultures, and subsequently fixed with modified Bouin's solution (Coats & Heinbokel 1982). Ingestion rate was determined by placing each sample in a 5 ml Zeiss settling

chamber. After allowing sufficient time for settling, the chamber was scanned at 200× to 400× on an inverted microscope equipped with epifluorescence optics (Leitz Diavert; 450 to 490 nm excitation, 520 nm barrier filter). The number of labeled food vacuoles per *C. furca* was recorded for the first 100 specimens encountered. Hourly ingestion rate was obtained by dividing the mean number of food vacuoles per *C. furca* by incubation time. Feeding assays were conducted at the same time of day to avoid differences in ingestion rate due to diel feeding patterns.

Photosynthetic rates of *Ceratium furca* were determined at treatment temperature and irradiance using the ^{14}C method. Rates were consistently measured at 12:00 h to avoid differences due to diel fluctuations in photosynthesis. One ml aliquots of the cultures were spiked with $NaH^{14}CO_3$ (ICN Chemicals) to a final activity of 0.25 µCi ml^{-1} and incubated for 1 h at 20°C and 160 µmol m^{-2} h^{-1}. Parallel dark vials were treated likewise, but wrapped in aluminum foil during incubation. Incubations were terminated by adding 0.25 ml of 10% HCl to each vial to remove unincorporated ^{14}C. After placing the vials on an orbital shaker (150 rpm) overnight, 5 ml Ecolume scintillation cocktail was added to each vial, and activity was determined on a liquid scintillation counter (Packard TriCarb model 1600TR). To determine total activity added, 20 µl of ^{14}C stock solution was added in triplicate to vials containing 200 µl phenethylamine and 5 ml scintillation cocktail. Dissolved inorganic carbon was measured with a Capni-Con 5 Total Carbon Dioxide Analyzer. Photosynthetic rates were calculated using the equation given by Parsons et al. (1984) and normalized to cell number and chl *a* concentrations to yield cell-specific (pg C $cell^{-1}$ h^{-1}) and chl *a*-specific (mg C mg chl a^{-1} h^{-1}) rates. Chl *a* content of *Ceratium furca* was determined fluorometrically on 20 ml aliquots concentrated on GF/C filters and extracted in 90% acetone for 24 h in the dark at 4°C. Chl *a* density was obtained by normalizing chl *a* $cell^{-1}$ to cell volume ($µm^3$).

Cellular C, N, and P contents of *C. furca* were measured using 50 ml aliquots that were washed 3 times (GF/F filtered, autoclaved 15 psu seawater) by gentle gravity filtration through 12 µm Nuclepore filters to remove bacteria and cell debris. For analysis of cell abundance, 5 ml of the washed cell suspension were fixed in Lugol's solution. Twenty ml each were collected on precombusted Whatman GF/F filters for CHN and particulate P analyses. Cellular C and N were analyzed with an Exeter Analytical CE-440 Elemental Analyzer, and particulate P was determined as soluble reactive P following high temperature combustion and hydrochloric acid digestion (Anderson 1976). Cellular C, N, and P contents were divided by cell volume to yield C, N, and P densities.

Samples (20 ml) for the analysis of inorganic nutrient concentrations (nitrate and nitrite, ammonia, dissolved inorganic phosphate) of the culture medium were filtered through GF/F filters and stored frozen (–20°C) in acid-washed scintillation vials. They were analyzed using a Technicon Autoanalyzer II equipped with a Bran and Luebbe detector (Technicon Engineering).

Statistical analyses. All data were examined for homogeneity of variance and normality. Data that failed to meet these criteria were transformed as indicated in text or figure legends. If transformation did not correct the problem, non-parametric tests were used. The cellular parameters measured for *Ceratium furca* were analyzed for significant differences between treatments (1-way ANOVA) and/or over time (linear regression or 2-way ANOVA with time as second factor) using SAS/MIXED procedures (SAS System for Windows, Release 8.01; SAS Institute). The Tukey test was used for pairwise mean comparison (SAS/MIXED). Correlations between feeding and cellular nutrient ratios were determined using a Spearman rank order correlation (batch and 'semi-continuous' culture experiments) or a Pearson product moment correlation (nutrient addition experiment; SigmaStat version 2.03, SPSS). Means were reported ± SE of the mean unless otherwise indicated. Means and SE of transformed data were back-transformed for graphical representation to facilitate comparison with non-transformed data. When non-parametric tests were used to analyze data, we reported the median and the 25th and 75th percentiles.

RESULTS

Batch culture nutrient experiment

Initially, an increase of cell numbers was observed in all 5 treatments (Fig. 1A). After several days, however, cells in the f/2-N and f/2-P treatments stopped growing and eventually began to decline in numbers. Growth rates calculated over the entire length of the experiment exhibited significant treatment-specific differences ($p < 0.0001$). Growth was highest in the nutrient-replete treatment (0.044 ± 0.0005 d^{-1}), followed by the f/2-½N and f/2-½P conditions (0.034 ± 0.0020 d^{-1} and 0.027 ± 0.0001 d^{-1}, respectively). Cells in the f/2-N treatment exhibited a growth rate of 0.009 ± 0.0007 d^{-1}, whereas the growth rate of f/2-P cultures was negative (–0.014 ± 0.0030 d^{-1}).

Cell size also exhibited treatment-specific changes over time ($p < 0.0001$; Fig. 1B). By Day 11, average cell size in the f/2-N treatment was significantly smaller than in the nutrient-replete treatment ($p < 0.0001$), whereas average size of cells in the f/2-P treatment

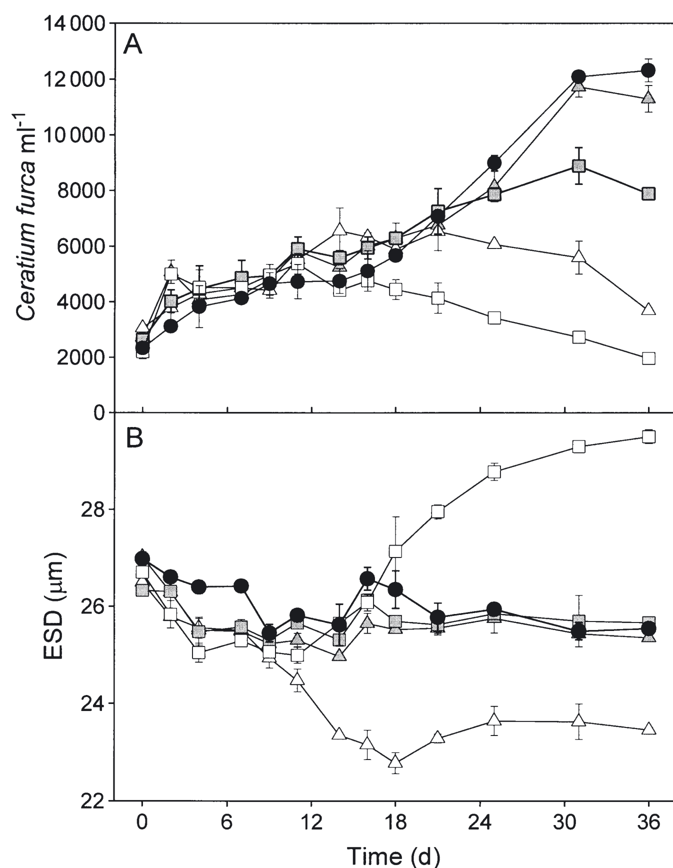

Fig. 1. *Ceratium furca*. (A) Cell density and (B) equivalent spherical diameter (ESD) of the dinoflagellate grown in batch cultures. Cells were grown at 5 different inorganic nutrient levels: f/2-Si (●), f/2-½N (▲), f/2-N (△), f/2-½P (■), f/2-P (□) for 36 d. Data are presented as mean ± SE

was significantly larger by Day 18 ($p = 0.008$). This change in average cell size reflected the development of a bimodal size distribution in some treatments. Within the same treatment, the size of some cells increased slightly or remained unchanged, while the size of others decreased dramatically. This resulted in 2 size categories with average equivalent spherical diameters (mean ± SD) of 22.0 ± 1.05 µm and 28.3 ± 1.12 µm (or approximately <60 µm and ≥60 µm in length, as *Ceratium furca* is not spherical). The cells also differed in pigmentation and morphology, with the smaller cells exhibiting lower chl *a* content and shorter apical and antapical horns. By the end of the experiment, the percentage of small cells differed significantly between treatments ($p = 0.002$; arcsin square-root transformation). The N-limited cultures contained the highest percentage of small cells (39 ± 0.0%), followed by the nutrient-replete and f/2-½N and f/2-½P conditions (21 ± 1.5%, 19 ± 1.0%, and 22 ± 2.5%, respectively). P-deficiency resulted in the lowest percentage of small cells (9 ± 2.8%).

The ability of *Ceratium furca* to feed when provided with prey was also strongly influenced by nutrient concentrations in the growth medium. Initially, little or no feeding was observed in subsamples of *C. furca* from all 5 treatments incubated with prey. However, ingestion rates increased after 11 d in cells from the f/2-P treatment (Fig. 2A) and to a lesser extent after 16 d in cells grown under f/2-N conditions (Fig. 2B). In these 2 treatments, ingestion rates continued to increase until Day 36, whereas no feeding was detected in cells from the remaining treatments. The increase in feeding lagged 5 to 10 d behind a sharp drop in inorganic P or N concentrations to trace levels in the f/2-P and f/2-N treatments, respectively (Fig. 2A,B). Throughout the experiment, food vacuoles were only observed in cells ≥60 µm in length, whereas small cells failed to ingest prey ciliates. When we considered feeding of larger cells only, the differences and patterns of feeding rates among the various treatments persisted, despite the treatment-specific differences in the percentage of small cells.

Cellular chl *a* density and cell-specific photosynthetic rate also exhibited treatment-specific changes over time (chl *a*: $p < 0.0001$, log-transformed; photosynthesis: $p < 0.0001$, log-transformed; Fig. 3). While chl *a* density decreased in all treatments after ~9 d, this decrease was not significantly different in the f/2-Si, f/2-½N, f/2-½P, and f/2-P treatments ($p = 0.06$). However, chl *a* density of *Ceratium furca* grown in f/2-N dropped sharply after Day 9 and was significantly lower than that of cells grown in f/2-Si by Day 11 ($p = 0.049$; Fig. 3A). Cell-specific photosynthesis was similar in the f/2-Si, f/2-½N, and f/2-½P treatments throughout the experiment ($p = 0.07$), but was significantly lower in the f/2-N and f/2-P cultures compared to f/2-Si cultures by Days 11 and 18, respectively (f/2-N: $p = 0.0044$; f/2-P: $p = 0.0068$; Fig. 3B). Photosynthetic rate of the P-deplete cells recovered somewhat on the last day of the experiment and was no longer significantly different from that of nutrient-replete cells ($p = 0.789$; Fig. 3B).

Cellular C and P densities were also affected by the various nutrient treatments and exhibited significant treatment-specific changes over time (C: $p < 0.0001$; P: $p < 0.0001$). Both N- and P-deficient cultures contained significantly more C per µm^3 at the end of the experiment than other cultures (Fig. 4A). Cellular P density also increased significantly in N-deficient cells, but decreased in f/2-P cultures (Fig. 4C). On the other hand, cellular N density remained similar in all treatments over time ($p = 0.148$), and no significant differences between treatments were detected after 36 d (Fig. 4B). Ratios of C:N, C:P, and N:P also changed depending on the nutrient treatment. By the end of the experiment, P-limited cells had significantly higher

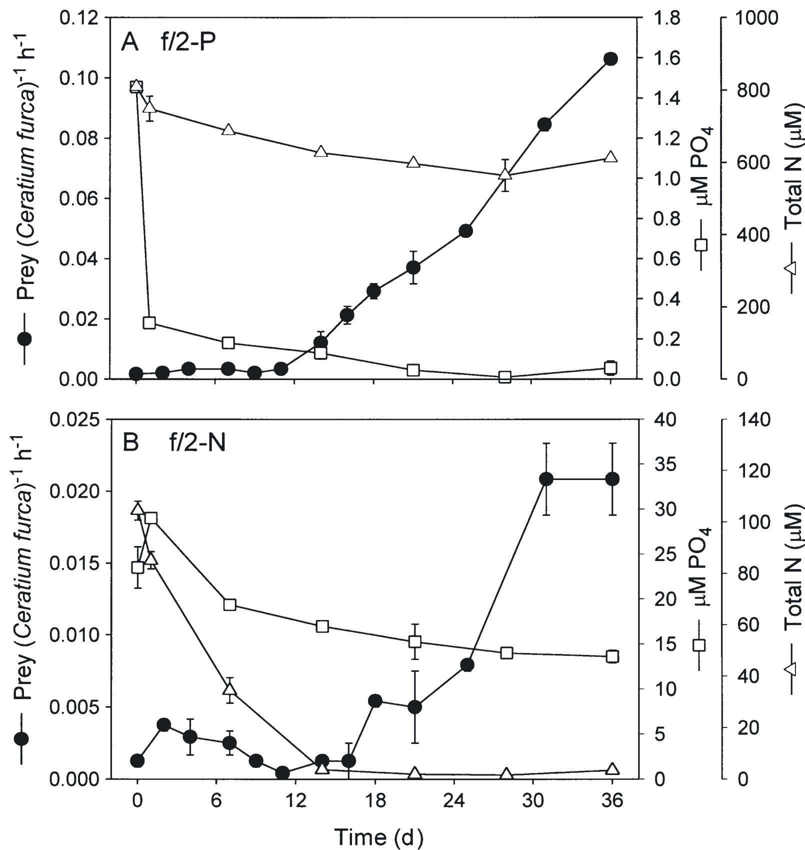

Fig. 2. *Ceratium furca*. Ingestion rates of cultures compared to inorganic phosphorus (PO_4) and nitrogen (NO_2+NO_3 and NH_4) concentrations over time. (A) f/2-P, (B) f/2-N. Data are presented as mean ± SE

C:N, C:P, and N:P ratios than nutrient-replete cells (Fig. 4D,E,F). The C:N ratio of N-deficient cells was also higher, whereas N:P was significantly lower. No change was detected in the C:P ratio of N-deficient cultures. Ratios of C:N, C:P, and N:P were positively correlated with ingestion rates of cells from P-reduced treatments (Table 1). Similar trends were observed in the N-reduced treatments, where ingestion rate was positively correlated with C:N, but negatively with N:P; Table 1).

'Semi-continuous' culture experiment

By replacing 25% of the spent medium with fresh medium on a daily basis, we were able to provide a relatively stable inorganic nutrient environment for *Ceratium furca* cultures. Inorganic N (NH_4+ NO_2+NO_3) was present at high concentrations in all treatments and averaged 746 ± 3.6 µM N. Daily fluctuations were similar between treatments and averaged 2.4 ± 0.28% across all treatments (Fig. 5A). Inorganic P concentrations varied across the different treatments, but after an initial decrease remained stable within treatment (Fig. 5A). We were able to maintain *C. furca* at the target level of 5000 cells ml^{-1} in most treatments (Fig. 5B). However, P concentrations in the treatment receiving no P addition were insufficient to sustain cell growth after 7 d, and cell number decreased by 5.8 ± 4.2% d^{-1} thereafter. On the other hand, cultures exposed to the various P-addition treatments grew by an average of 12.2 ± 0.48% d^{-1}. While the average daily increase in cell numbers was similar across these treatments, it varied drastically from day to day within treatments (3 µM

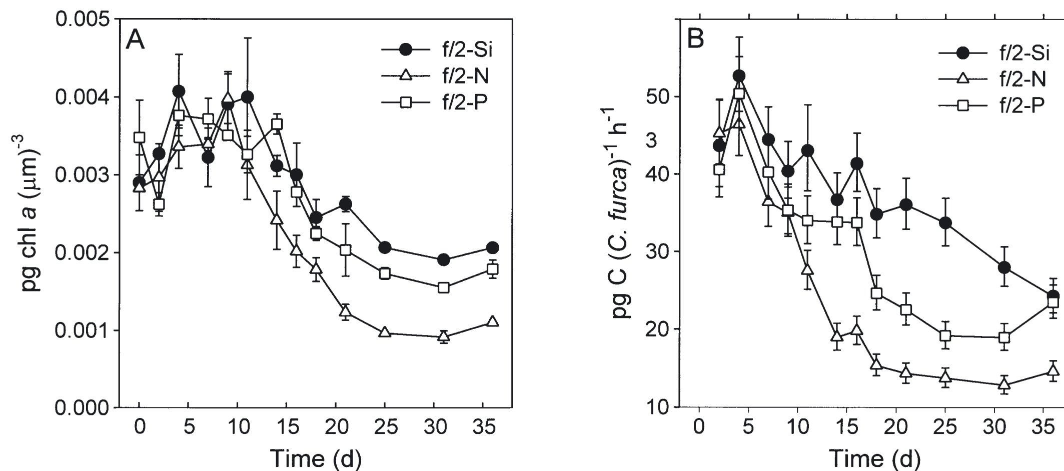

Fig. 3. *Ceratium furca*. (A) Cellular chl *a* density and (B) cell-specific photosynthetic rate of cultures grown under nutrient replete and deplete conditions over time. Data are presented as mean ± SE

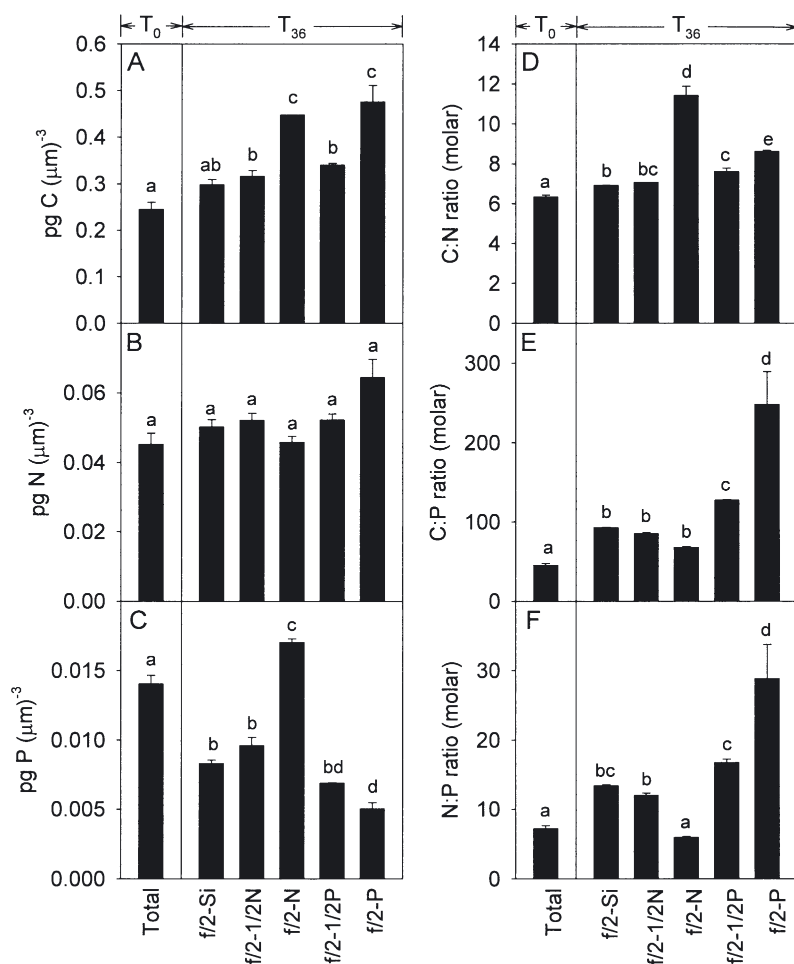

Fig. 4. *Ceratium furca*. Cellular C, N, and P densities and ratios of the dinoflagellate grown in batch cultures at different inorganic nutrient concentrations. T_0: start of experiment; T_{36}: end of experiment. To obtain total value, T_0-data for all treatments were averaged. The following data were transformed for statistical analysis, but back-transformed for graphic representation: C:N (log), C:P (reciprocal), N:P (reciprocal). Data are presented as mean ± SE. Bars with the same letter(s) are not significantly different from one another

Table 1. *Ceratium furca*. Correlation analysis of feeding and cellular nutrient ratios of the dinoflagellates grown in batch and 'semi-continuous' cultures. Coeff. = correlation coefficient

	Batch culture (n = 78) N-reduced	Batch culture (n = 78) P-reduced	Semi-continuous culture (n = 30)
C:N ratio			
Coeff.	0.439	0.497	0.657
p-value	<0.0001	<0.0001	<0.0001
C:P ratio			
Coeff.	0.151	0.480	0.875
p-value	0.186	<0.0001	<0.0001
N:P ratio			
Coeff.	−0.452	0.558	0.900
p-value	<0.0001	<0.0001	<0.0001

PO_4: 0.0 to 30.1%; 6 µM PO_4: −2.8 to 32.0%; 13 µM PO_4: −5.7 to 31.5%; 16 µM PO_4: −5.2 to 25.0%).

After 12 to 19 d, 85 to 90% of the values obtained for the various cellular parameters differed by no more than 10% from mean values (data not shown), suggesting that the *Ceratium furca* cultures had attained steady state. Steady-state means for these parameters were thus calculated using data from Days 19 to 30. We found significant differences between treatments in all cellular characteristics measured (Fig. 6). Notably, feeding was only observed in cells receiving 0 and 3 µM PO_4 (Fig. 6A). *C. furca* from the 0 µM PO_4 treatment were also significantly larger and had a higher C density (Fig. 6B,G), whereas chl *a* and N densities were not significantly different from those of other treatments (Fig. 6D,H). On the other hand, both cell- and chl *a*-specific photosynthetic rates and P density were severely reduced in P-deficient cells (Fig. 6E,F,I). Cell size exhibited a bimodal distribution (mean ± SD of 23.7 ± 1.11 and 33.8 ± 1.12 µm over all treatments). Whereas severe P-deficiency resulted in a very low percentage of cells <60 µm in length, moderate P-limitation actually led to an increase in the percentage of small cells compared to nutrient-replete cultures (Fig. 6C). Food vacuoles were only observed in cells ≥60 µm in length.

As in the batch culture experiment, feeding was significantly correlated with cellular C:N, C:P, and N:P ratios (Table 1). Furthermore, combining data from both laboratory experiments indicated threshold C:N:P ratios beyond which feeding was induced (Fig. 7). Specifically, feeding in P-deplete cultures did not markedly increase until a C:P ratio of ~130 and an N:P ratio of 19 were reached (Fig. 7B,C), whereas N-deplete cultures required a C:N ratio of ~10 and an N:P ratio below 7 to commence feeding (Fig. 7A,C).

Nutrient addition experiment

Total nitrogen (NH_4+NO_2+NO_3) was present at high concentrations in all treatments and averaged 633 ± 7.7 µM N during the 3 d experiment. Conversely, PO_4 concentrations decreased significantly over time in each treatment except for the control, where PO_4 con-

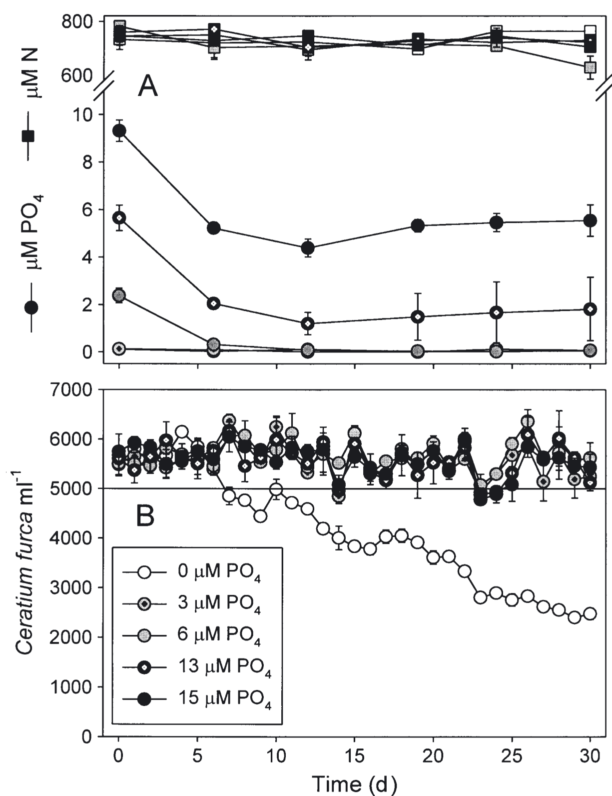

Fig. 5. *Ceratium furca*. (A) Inorganic phosphorus (PO_4) and nitrogen (NH_4+NO_2+NO_3) concentrations over time in the culture medium. (B) Cell density of the dinoflagellate grown in 'semi-continuous' cultures. From the culture medium, 25% was removed daily with and/or without *C. furca* cells and replaced by fresh medium containing 0, 3, 6, 13, or 15 μM PO_4 to maintain cell density at a target level of 5000 cells ml^{-1} (solid line). Figure legend applies to both graphs. Data are presented as mean ± SE

centrations were already at detection level (0 μM PO_4: p = 0.164; 1.5 μM PO_4: p = 0.006; 9 μM PO_4: p < 0.001; 20 μM PO_4: p < 0.001; Fig. 8). Over the course of the experiment, cell growth of *Ceratium furca* did not differ significantly between nutrient treatments (p = 0.168) and averaged –0.014 ± 0.0132 d^{-1} for the 0 μM P-addition, –0.005 ± 0.0102 d^{-1} for the 1.5 μM P-addition, –0.028 ± 0.0127 d^{-1} for the 9 μM P-addition, and –0.005 ± 0.0132 d^{-1} for the 20 μM P-addition. We did not detect significant changes in cell density for treatments except the 9 μM P-addition, where cell density decreased slightly, but significantly (p = 0.0403). Average cell size at the beginning of the experiment was 33.2 ± 1.13 μm (ESD, mean ± SD). The percentage of small cells was very low in all treatments (average 1.8 ± 0.24%) and did not change over time (p = 0.718).

Feeding in the control showed no significant change over the course of the experiment (p = 0.227) and averaged 0.05 ± 0.004 prey *Ceratium furca*$^{-1}$ h^{-1}. However, 4 h after nutrient addition, cells in the other treatments already exhibited a significantly reduced feeding rate, averaging 0.02 ± 0.001 prey *C. furca*$^{-1}$ h^{-1} (p = 0.016), a decrease of ~55% compared to the control (Fig. 9). Feeding in the 1.5 μM PO_4 addition treatment remained at ~45% of control values for the remainder of the experiment. On the other hand, feeding in the 9 and 20 μM PO_4 addition treatments further decreased, reaching an average of 0.002 ± 0.0007 prey *C. furca*$^{-1}$ h^{-1} or 3.5% of control rates the following day.

We observed significant changes over time in several physiological parameters measured, including cellular chl *a* content (p < 0.0001), cell- and chl *a*-specific photosynthetic rates (p < 0.0001 and p < 0.0001, respectively), and cellular P content (p < 0.0001), but not in C and N contents of *Ceratium furca* (p = 0.097 and p = 0.06, respectively; Table 2). Chl *a* per cell increased significantly in the treatments that had received 1.5 and 9 μM PO_4 (p = 0.0013 and p < 0.0001, respectively), but remained stable in both the control and the 20 μM PO_4 addition treatment (p = 0.926 and p = 0.963, respectively; Fig. 10A, Table 2). Photosynthetic rates in the control did not change significantly over time (p = 0.113), but increased rapidly in all other treat-

Table 2. *Ceratium furca*. Daily rates of change in cellular parameters measured in P-deplete cells after P-addition. Different letters indicate significant differences between treatment means (α ≤ 0.05). Log-transf.: data log-transformed for statistical analysis; reciprocal: data transformed for statistical analysis by calculating reciprocal values

Parameter	P-addition treatment				Remarks
	0 μM PO_4	1.5 μM PO_4	9 μM PO_4	20 μM PO_4	
Chl *a*					
pg chl *a* (*C. furca*)$^{-1}$ d^{-1}	–0.03 ± 0.343[a]	2.8 ± 0.63[b]	2.1 ± 0.30[b]	–0.01 ± 0.285[a]	log-transf.
Photosynthetic rates					
cell-specific (pg C $cell^{-1}$ h^{-1})	1.4 ± 0.81[a]	14.8 ± 1.04[b]	13.4 ± 1.49[b]	9.2 ± 0.79[b]	log-transf.
chl *a*-specific (mg C [mg chl *a*]$^{-1}$ h^{-1})	0.34 ± 0.126[a]	1.12 ± 0.153[b]	1.13 ± 0.073[b]	0.87 ± 0.054[b]	
Cellular nutrients					
pg C (*C. furca*)$^{-1}$ d^{-1}	–296 ± 231.3[a]	–2 ± 117.9[a]	–691 ± 287.4[a]	–155 ± 342.8[a]	
pg N (*C. furca*)$^{-1}$ d^{-1}	4 ± 34.8[a]	102 ± 17.1[a]	–26 ± 39.1[a]	95 ± 66.3[a]	
pg P (*C. furca*)$^{-1}$ d^{-1}	–3.0 ± 3.75[a]	53.9 ± 17.16[b]	95.6 ± 12.06[c]	141 ± 15.4[d]	Reciprocal

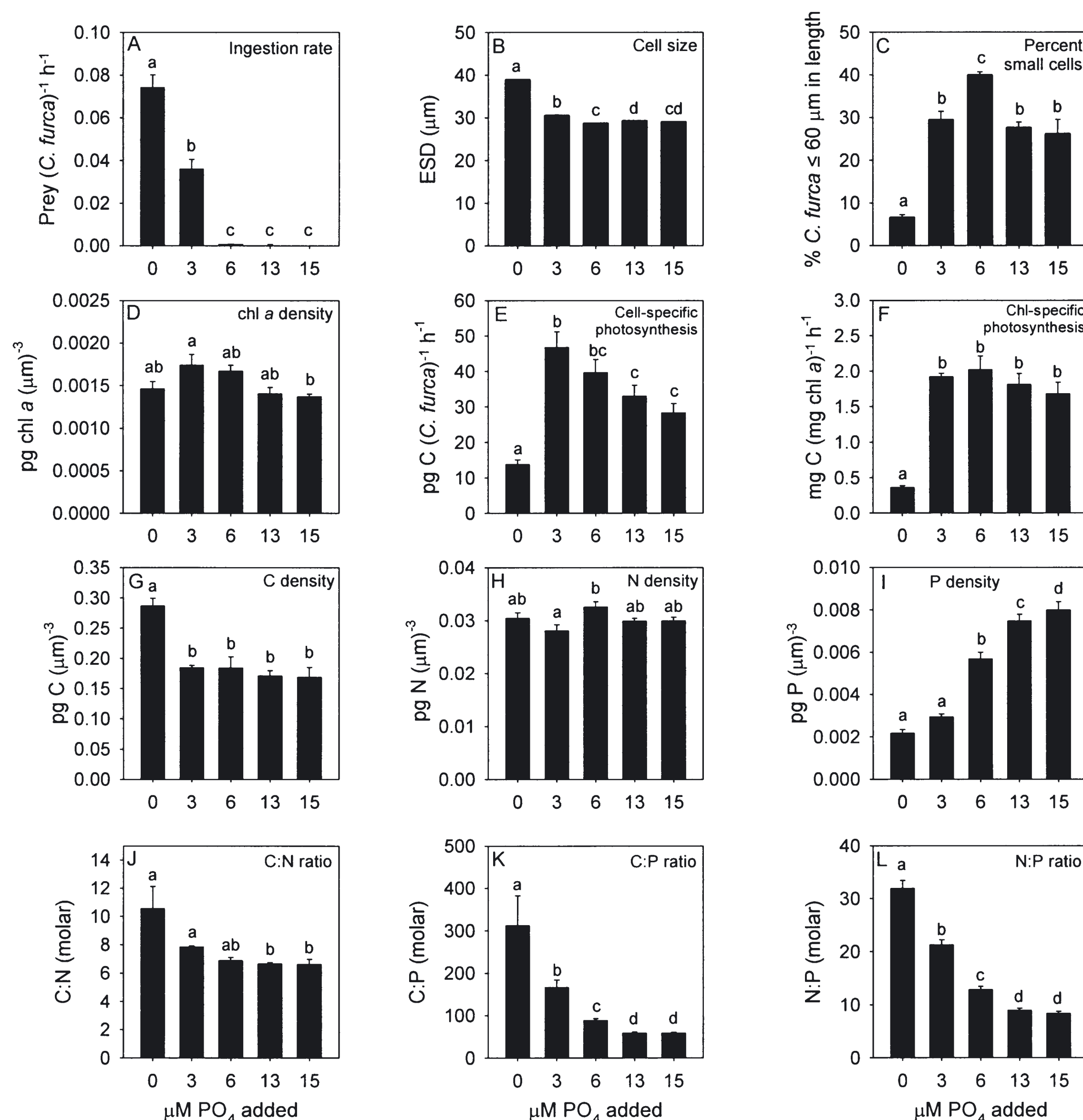

Fig. 6. *Ceratium furca*. Physical and biochemical parameters for cells in 'semi-continuous' cultures. Average values were determined for each parameter once steady state had been reached (Days 19 to 30). The following data were transformed for statistical analysis, but back-transformed for graphic representation: cell-specific photosynthesis (log), C density (rank), C:N (rank), C:P (reciprocal), N:P (log). Data are presented as mean ± SE except for cellular C density and C:N ratio (G, J), where the median, 25th, and 75th percentiles are reported. Bars with the same letter(s) are not significantly different from one another

ments after an initial depression, especially in cells that had received 1.5 µM PO_4 at the beginning of the experiment ($p < 0.0001$; Fig. 10B,C, Table 2). Cellular P content of *C. furca* in the control remained stable ($p = 0.447$). Cellular P content in treatments receiving PO_4 increased at similar rates on Days 1 and 2 in all P-addition treatments, but began to show treatment-specific differences on Day 3 (Fig. 10D, Table 2). Rates of cellular P increase (Table 2) were not significantly different from those of inorganic PO_4 disappearance (Fig. 8) at the 0.05 probability level, indicating that most of the decrease in PO_4 was accounted for by the concurrent increase in cellular P. This rapid increase in cellular P led to significant decreases in C:P and N:P ratios in all P-addition treatments (C:P: $p < 0.0001$; N:P: $p < 0.0001$; all data were log-transformed), while no treatment-specific changes in the C:N ratio were detected over time ($p = 0.343$). Cellular nutrient ratios were again correlated with feeding, although the relationships were different from those obtained for the 2 previous experiments, and no obvious threshold ratios could be determined (Fig. 11).

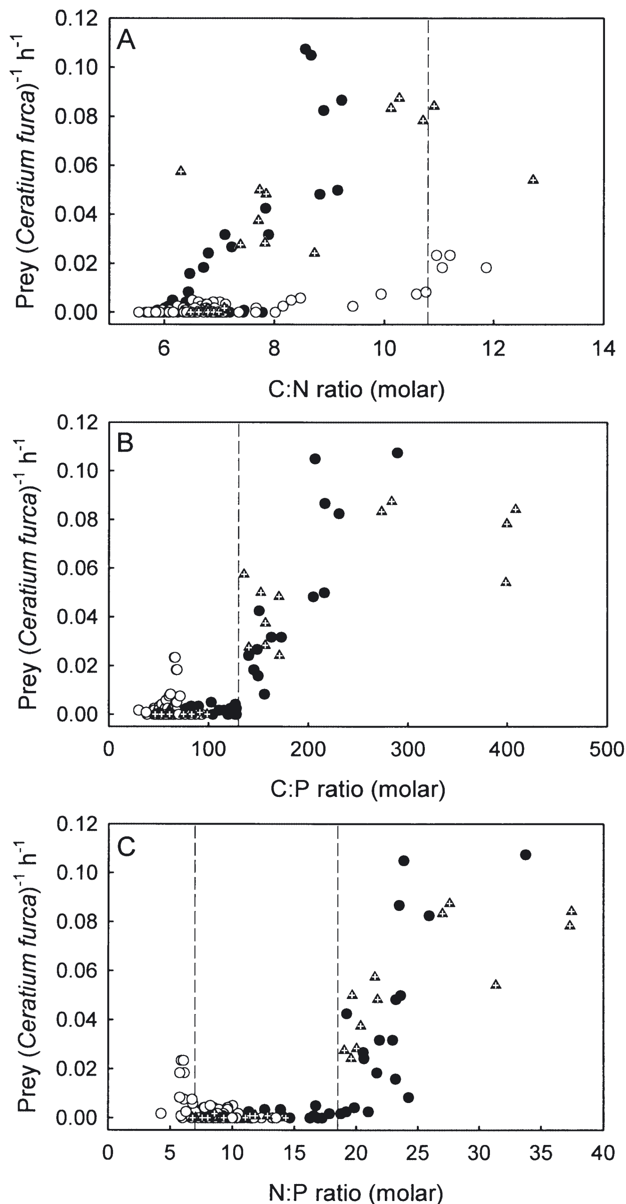

Fig. 7. *Ceratium furca*. Correlation between ingestion rate and (A) cellular C:N, (B) C:P, and (C) N:P ratios. *C. furca* was grown in N-limited (○) and P-limited (●) batch cultures and P-limited 'semi-continuous' cultures (▲, white cross hair). Dashed lines represent apparent threshold C:N:P ratios that had to be crossed before feeding was induced

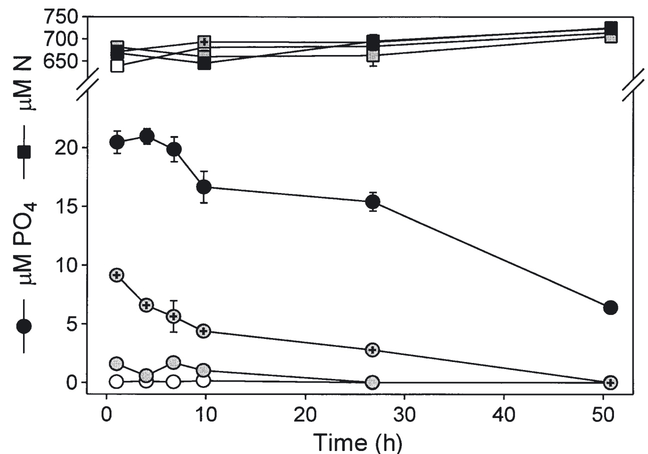

Fig. 8. *Ceratium furca*. Inorganic phosphorus (PO_4) and nitrogen (NH_4+NO_2+NO_3) concentrations in the culture medium over time. At T_0, inorganic PO_4 was added to P-limited batch cultures at 0 (white symbols), 1.5 (gray symbols), 9 (gray symbols, cross hair), and 20 µM PO_4 (black symbols). Data are presented as mean ± SE

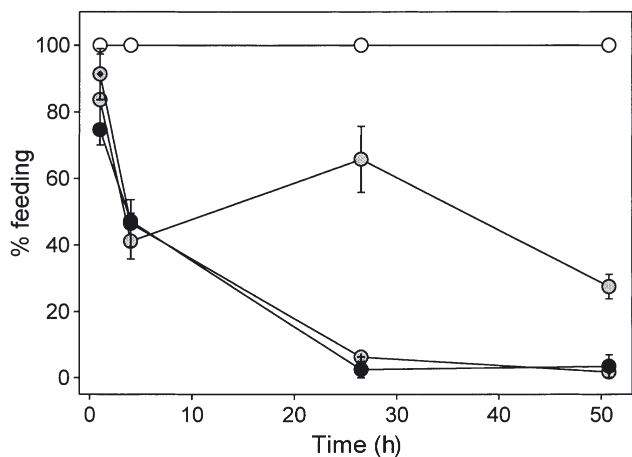

Fig. 9. *Ceratium furca*. Feeding after P-addition expressed as a percentage of the ingestion rate measured in the control. At T_0, inorganic PO_4 was added to P-limited batch cultures at 0 (white symbols), 1.5 (gray symbols), 9 (gray symbols, cross hair), and 20 µM PO_4 (black symbols). Data are presented as mean ± SE

DISCUSSION

Feeding in *Ceratium furca* was strongly influenced by nutrient concentrations. Feeding did not occur in treatments where nutrients were plentiful, but increased in the nutrient-depleted treatments. However, while inorganic nutrients dropped rapidly to trace levels, an increase in feeding lagged several days behind. This suggests that the effect of inorganic nutrients on feeding was neither immediate nor direct, but rather was mediated through internal cellular nutrient concentrations and ratios. Data on cellular nutrient content from both batch and 'semi-continuous' culture experiments support this hypothesis. As cellular C:N:P ratios deviated farther from those found under optimum growth conditions (f/2-Si), feeding increased markedly. However, there was a wide range of ratios around the optimum where little or no feeding

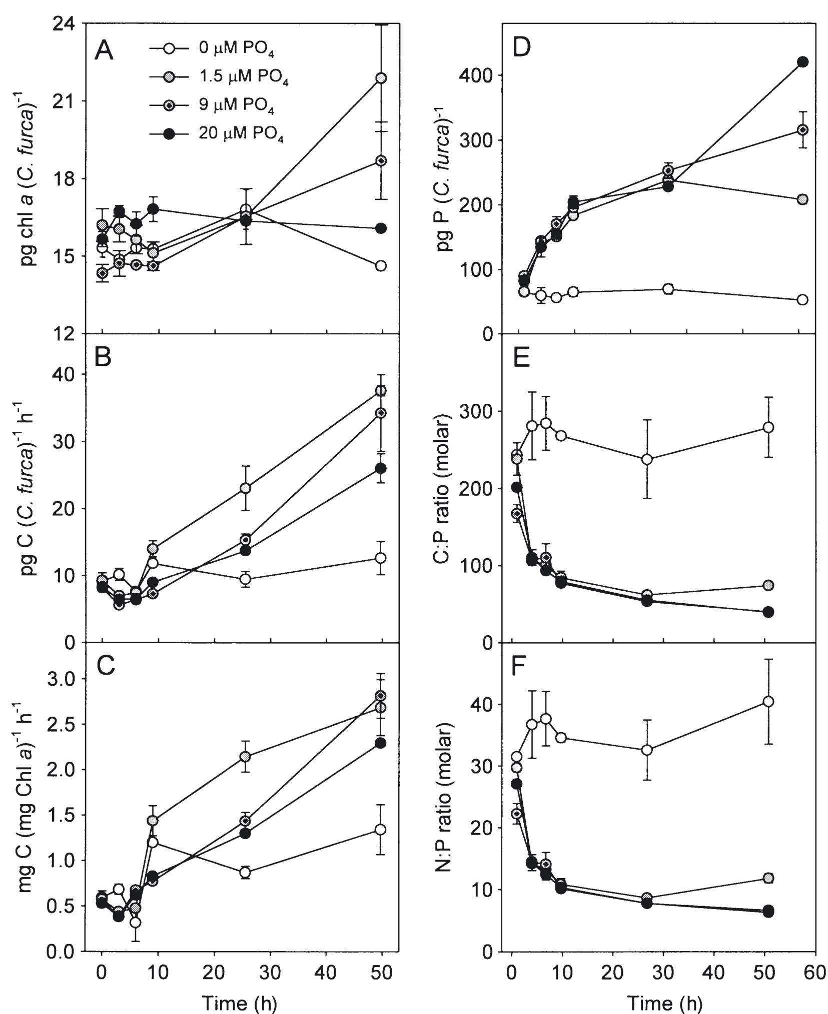

Fig. 10. *Ceratium furca*. (A) Cellular chlorophyll *a* (chl *a*) content, (B) cell-specific and (C) chl *a*-specific photosynthesis, (D) cellular P content, and (E) cellular C:P and (F) N:P ratios upon P-addition. At T_0, inorganic PO_4 was added to P-limited batch cultures at 0, 1.5, 9, and 20 µM PO_4. Figure legend applies to all graphs. Data are presented as mean ± SE

occurred, suggesting that threshold ratios had to be crossed before feeding could be induced.

Interestingly, no such threshold ratios were evident in the nutrient addition experiment, although feeding was again correlated with C:N:P ratios. While little or no feeding was observed in batch and 'semi-continuous' culture experiments at C:P and N:P ratios below 130 and 19, respectively, ingestion rates measured in the 1.5 µM PO_4 treatment of the nutrient addition experiment were still relatively high at C:P and N:P ratios of 60 and 9, respectively. This indicates that simply taking up the limiting nutrient (PO_4 in this case) was not enough to reduce feeding and suggests that the nutrient also had to be metabolized to some degree. When inorganic P is transported across the algal plasma membrane, it becomes part of a dynamic intracellular phosphate pool (Cembella et al. 1984). From this pool, it is incorporated into phosphorylated metabolites or transported to polyphosphate storage vesicles or vacuoles (Elgavish et al. 1980, Cembella et al. 1984). When P-deficient cells are resupplied with inorganic P, they often take up excess P at increased maximum rates and incorporate it into the polyphosphate fraction (Healey 1973, Perry 1976, Brown & Harris 1978, Elgavish et al. 1980). Thus, the relatively high cellular P content in feeding *Ceratium furca* cells from the 1.5 µM PO_4 treatment may have resided largely in the unincorporated intracellular phosphate pool or the polyphosphate fraction used for storage, which may have little direct effect on feeding regulation. Therefore, while cellular C:N:P ratios are good indicators of feeding response when the nutrient environment is stable or changing slowly, they may not be as effective in predicting feeding when nutrient availability increases rapidly, and thus leads to excess luxury P-uptake and an uncoupling of P-uptake and growth.

While induction of feeding in laboratory cultures was only observed after several days of nutrient deprivation, the inhibitory effect of nutrient addition on ingestion rates in field (Smalley 2002) and laboratory experiments could be detected within hours. This discrepancy in response time was likely due to the involvement of different physiological processes. Since laboratory cultures had been growing phototrophically for many years, they probably had to synthesize a variety of structures and enzymes before prey could be ingested and processed. In contrast, cells in the nutrient addition experiments were already feeding. Thus, a decrease in ingestion rate might not require de novo synthesis and could be accomplished by relatively quick downregulation of certain enzymes.

In addition to modulating cellular C, N, and P contents and ratios, *Ceratium furca* underwent other physiological changes in response to nutrient limitation. Cell-specific photosynthetic rate was reduced significantly in both N- and P-deplete *C. furca*. In the N-depleted cultures, this decrease in cell-specific photosynthesis was accompanied by a striking reduc-

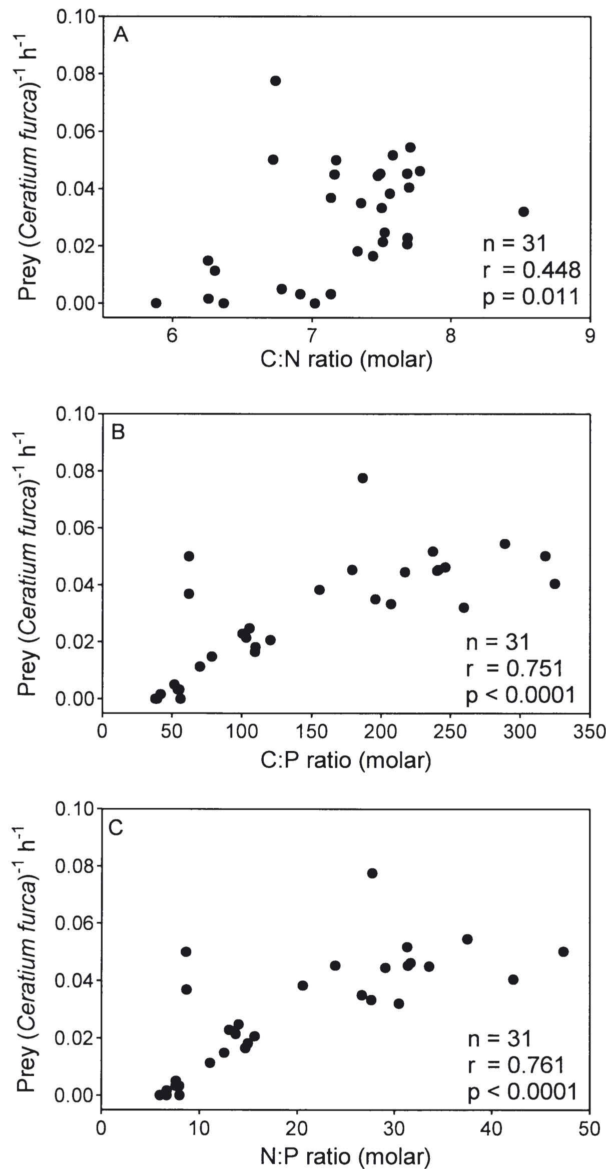

Fig. 11. *Ceratium furca*. Correlation between ingestion rate and (A) cellular C:N, (B) C:P, and (C) N:P ratios of P-limited cultures that had received 0, 1.5, 9, and 20 µM PO_4 at the start of the experiment

tion of cellular chl *a*, while chl *a*-specific photosynthetic rates remained similar to or slightly higher than that observed in nutrient-replete cultures. In the P-limited cultures, on the other hand, cellular chl *a* density did not decrease compared to that of nutrient-replete cells, whereas chl *a*-specific photosynthetic rate was significantly lower. These findings are consistent with patterns found in various other phytoplankton species. Cells commonly react to nutrient limitation by reducing cell-specific photosynthesis (Lehman 1976, Everest et al. 1986, Osborne & Geider 1986, Herzig & Falkowski 1989, Levy & Gantt 1990), and several mechanisms have been proposed to explain this phenomenon. As both N and P are needed for protein synthesis, the over-all protein content of the cell is reduced when either nutrient is in limiting supply (Healey 1973, Everest et al. 1986, Olson et al. 1986). This decrease affects several enzymes and protein complexes needed for both dark and light reactions of photosynthesis, including reaction center proteins, chl *a*-binding proteins, pigment-protein complexes, and RUBISCO (Kolber et al. 1988, Falkowski et al. 1989, Falkowski & Raven 1997). In addition, N-limited cells exhibit a reduction of chl *a* and other pigments of the light-harvesting system, thus effectively decreasing the amount of photons that can be harvested (Everest et al. 1986, Osborne & Geider 1886, Kolber et al. 1988, Roy 1988, Herzig & Falkowski 1989, Levy & Gantt 1990, Sciandra et al. 2000). Finally, cellular ATP levels are reduced during P-limitation, leading to an overall impairment of the energy metabolism (Cembella et al. 1984).

It has been suggested that during times of nutrient-limitation, mobilization of proteins and pigment-protein complexes associated with photosynthetic C-uptake could provide the nutrient requirements for basal metabolic demands, such as respiration (Perry et al. 1981, Prézelin 1982, Roy 1988, Falkowski et al. 1989). In our experiment, dilution through cell growth was not enough to account for the drop of chl *a* in N-deficient cultures after Day 9, and it is thus conceivable that this N-rich pigment was deliberately digested to obtain more N. Under P-limitation, on the other hand, cellular chl *a* density remained stable, while chl *a*-specific photosynthetic rate decreased, indicating that RUBISCO and/or other proteins associated with the dark reaction may have been metabolized. Besides cell maintenance, nutrient-limited *Ceratium furca* may have used the recycled N or P for synthesis of enzymes associated with inorganic nutrient uptake. For example, in *Chlorella stigmatophora* increasing N-limitation was accompanied by increases in the activity of nitrate reductase, NADPH-glutamate dehydrogenase, and glutamine synthetase (Everest et al. 1986). In addition, as *C. furca*'s ability to phagocytize prey increased with increasing nutrient-limitation, the dinoflagellate probably increasingly redirected N and P to the synthesis of morphological structures and enzymes needed for ingestion and digestion.

The decreased photosynthetic rates observed under nutrient-limitation were reversible upon nutrient addition, and increased rapidly over the course of the experiment. However, during the first few hours after P-addition, rates were actually slightly depressed. This phenomenon has been reported by several investigators and is thought to be due to energy competition

between processes of C-fixation and nutrient uptake (Healey 1973, 1979, Falkowski & Stone 1975, Lean & Pick 1981, Levy & Gantt 1990). If a cell is starved for N or P, there is little need for increased C-fixation, and energy is preferentially used for nutrient uptake. Only once a critical level of cellular N or P is reached or most of the available P is taken up does C-fixation resume (Lean & Pick 1981). This may also explain why both photosynthetic rates and chl *a* content of *Ceratium furca* increased more rapidly in the lower P-addition treatments. In these treatments, the available P was taken up within 1 or 2 d, after which energy was again available for pigment synthesis and C-fixation. On the other hand, PO_4 concentrations in the medium of those cultures that had received the highest P-addition remained high throughout the experiment, and cells continued to take up P, as indicated by the substantial increases in cellular P even on the last day. In these cultures, most energy may have been allocated to processes associated with P-uptake at the expense of C-fixation.

Changes in nutrient concentrations also caused modifications of growth rate of *Ceratium furca*. Growth rates of batch cultures were significantly reduced in the nutrient-limited treatments compared to the nutrient-replete control. However, these differences in growth did not become apparent until ~11 d into the experiment, suggesting that *C. furca* was able to draw on nutrients mobilized from its internal storage pools to maintain growth for almost 2 wk. The cessation of growth at that time, presumably due to the depletion of cellular nutrient pools, correlated well with the increase in feeding observed around the same time. Interestingly, growth rates of the 'semi-continuous' cultures were remarkably similar, despite the wide range of daily P-additions (excluding the treatment where no P was added). This similarity in growth contrasted with the pronounced differences found in both inorganic nutrient concentrations and cellular nutrient quotas. Thus, the low daily P-additions appeared sufficient for *C. furca* to maintain cell growth, given the lower P requirements for reproduction due to reduced cellular P contents in these treatments. On the other hand, the high daily variation in growth observed for all these treatments (–5.7 to 32.0%) may have masked any difference that could have resulted from the various P concentrations. In the nutrient addition experiment, no growth was observed over the 3 d period. Such a delay in growth upon nutrient addition has been observed in other studies as well (Healey 1979, Levy & Gantt 1990) and may be explained by the energy allocation hypothesis outlined above (i.e. most energy may have been allocated to processes associated with P-uptake rather than to cell growth).

The nutritional status of *Ceratium furca* also influenced cell size. While N-limitation led to a decrease in average cell size, severe P-deficiency resulted in increased average cell size. Such observations have been reported before for a variety of algal groups, including dinoflagellates (Lehman 1976, Prézelin 1982, Olson et al. 1986). P-limitation can disrupt the cell cycle by restricting nucleic acid synthesis and interfering with normal mitotic events (Cembella et al. 1984). The inability of cells to divide thus leads to increased cell size (Lehman 1976, Cembella et al. 1984). In our experiments, *C. furca* was apparently unable to divide after 3 to 7 d in P-depleted medium, and cell volume subsequently increased. The interpretation of cell size, however, was complicated by the development of a bimodal size distribution in some of the treatments. This raises the possibility of the presence of sexual life history stages in *C. furca*. Sexual reproduction has been well documented in numerous dinoflagellate species (Pfiester & Anderson 1987), including several of the genus *Ceratium* (von Stosch 1964, 1972). In addition, Weiler & Eppley (1979) reported the formation of 'gametes' in *C. furca*, although zygote formation was not observed. Typically, sexual reproduction in dinoflagellate cultures is induced by nutrient starvation, more specifically, N-depletion (Pfiester & Anderson 1987). However, other environmental factors, such as temperature and light intensity, have also been reported to cause gamete formation (von Stosch 1964, Weiler & Eppley 1979). The small cells observed in our experiments resembled gametes described for other *Ceratium* species. As in other dinoflagellates, nutrient limitation (both N and P to some degree) triggered the formation of these cells. However, we did not observe zygote formation and are thus unable to confirm that these cells were indeed involved in sexual reproduction.

The formation of 'gametes' further complicated the interpretation of our feeding data, as these cells failed to ingest the prey ciliates. The *Strobilidium* sp. used in our experiments may simply have been too large for small *Ceratium furca* cells to ingest. On the other hand, if these small cells represent a temporary life-history stage involved in sexual reproduction, they may lack certain structural and/or biochemical features that make phagocytosis possible in vegetative stages. Alternatively, cells containing food vacuoles may be zygotes, while vegetative stages may be incapable of ingesting prey. Phagocytosis could provide zygotes with additional energy for division and/or the formation of a resting stage. Sexuality may thus be necessary for feeding to occur. However, the treatments where feeding was most pronounced also contained the lowest percentage of small cells, making the above scenario less probable. At present, we do not

have enough information on the life cycle of *C. furca* to resolve this question.

In the present study, we have shown that feeding in *Ceratium furca* is clearly influenced by inorganic nutrient concentrations, indicating that *C. furca* employed phagotrophy as a means for obtaining limiting macronutrients. Nutrient-limitation seemed to stimulate feeding via cellular nutrient quotas and ratios, as ingestion rates increased markedly when cellular C:N:P ratios deviated farther from those found under optimum growth conditions. In addition, phagotrophy was induced only after threshold C:N:P ratios were crossed. While nutrient availability and uptake rates obviously have a profound effect on cellular C:N:P ratios, C-fixation rates can also influence this ratio, as changes in photosynthesis will lead to changes in stored C reserves. At non-inhibitory light levels, increasing irradiance should lead to increased C-fixation, and thus to a greater biosynthetic need for N and P in order to utilize the additional C. *C. furca* could meet this increased need by increasing feeding. This relationship between feeding and irradiance was indeed observed for some field populations of *C. furca* (Smalley 2002), thus supporting the outlined hypothesis. Our hypothesis can easily be modified to include other limiting growth factors such as essential vitamins and trace metals, which were implicated in feeding regulation of several other primarily photosynthetic mixotrophs (Stoecker 1998).

Acknowledgements. We thank D. H. Smalley, M. Goodison and M. Johansson for technical assistance with laboratory experiments. This research was supported by National Science Foundation grant OCE 931772 awarded to D.W.C. and D.K.S. and OCE 9819670 to D.K.S. G.W.S. was supported by a Smithsonian Environmental Research Center Internship and Graduate Fellowship and a Horn Point Graduate Research Assistantship.

LITERATURE CITED

Arenovski AL, Lim EL, Caron DA (1995) Mixotrophic nanoplankton in oligotrophic surface waters of the Sargasso Sea may employ phagotrophy to obtain major nutrients. J Plankton Res 17:801–820

Anderson JM (1976) An ignition method for the determination of total phosphorus in lake sediments. Water Res 10: 329–331

Brown EJ, Harris RF (1978) Kinetics of algal transient phosphate uptake and the cell quota concept. Limnol Oceanogr 23:35–40

Cembella AD, Antia NJ, Harrison PJ (1984) The utilization of inorganic and organic phosphorus compounds as nutrients by eukaryotic microalgae: a multidisciplinary perspective. Part 2. CRC Crit Rev Microbiol 11:13–81

Coats DW, Heinbokel JF (1982) A study of reproduction and other life cycle phenomena in planktonic protists using an acridine orange fluorescence technique. Mar Biol 67: 71–79

Elgavish A, Elgavish GA, Halmann M, Berman T (1980) Phosphorus utilization and storage in batch cultures of the dinoflagellate *Peridinium cinctum* f. Westii. J Phycol 16: 626–633

Everest SA, Hipkin CR, Syrett PJ (1986) Enzyme activities in some marine phytoplankters and the effect of nitrogen limitation on nitrogen and carbon metabolism in *Chlorella stigmatophora*. Mar Biol 90:165–172

Falkowski PG, Raven JA (1997) Aquatic photosynthesis. Blackwell Scientific Publications, Oxford

Falkowski PG, Stone DP (1975) Nitrate uptake in marine phytoplankton: energy sources and interaction with carbon fixation. Mar Biol 32:77–84

Falkowski PG, Sukenik A, Herzig R (1989) Nitrogen limitation in *Isochrysis galbana* (Haptophyceae). II. Relative abundance of chloroplast proteins. J Phycol 25:471–478

Fisher TR, Peele ER, Ammermann JW, Harding LW (1992) Nutrient limitation of phytoplankton in Chesapeake Bay. Mar Ecol Prog Ser 82:51–63

Guillard RRL, Ryther JH (1962) Studies on marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* (Cleve) Gran. Can J Microbiol 8:229–239

Healey FP (1973) Inorganic nutrient uptake and deficiency in algae. CRC Crit Rev Microbiol 3:69–113

Healey FP (1979) Short-term responses of nutrient-deficient algae to nutrient addition. J Phycol 15:289–299

Herzig R, Falkowski PG (1989) Nitrogen limitation in *Isochrysis galbana* (Haptophyceae). I. Photosynthetic energy conversion and growth efficiencies. J Phycol 25:462–471

Kolber Z, Zehr J, Falkowski P (1988) Effects of growth irradiance and nitrogen limitation on photosynthetic energy conversion in Photosystem II. Plant Physiol 88:923–929

Lean DRS, Pick FR (1981) Photosynthetic response of lake plankton to nutrient enrichment: a test for nutrient limitation. Limnol Oceanogr 26:1001–1019

Legrand C, Granéli E, Carlsson P (1998) Induced phagotrophy in the photosynthetic dinoflagellate *Heterocapsa triquetra*. Aquat Microb Ecol 15:65–75

Lehman JT (1976) Photosynthetic capacity and luxury uptake of carbon during phosphate limitation in *Pediastrum duplex* (Chlorophyceae). J Phycol 12:190–193

Levy I, Gantt E (1990) Development of photosynthetic activity in *Porphyridium purpureum* (Rhodophyta) following nitrogen starvation. J Phycol 26:62–68

Li A, Stoecker DK, Coats DW (2000a) Spatial and temporal aspects of *Gyrodinium galatheanum* in Chesapeake Bay: distribution and mixotrophy. J Plankton Res 22:2105–2124

Li A, Stoecker DK, Coats DW (2000b) Mixotrophy in *Gyrodinium galatheanum* (Dinophyceae): responses to light intensity and inorganic nutrients. J Phycol 36:33–45

Nygaard K, Tobiesen A (1993) Bacterivory in algae: a survival strategy during nutrient limitation. Limnol Oceanogr 38: 273–279

Olson RJ, Vaulot D, Chisholm SW (1986) Effects of environmental stress on the cell cycle of two marine phytoplankton species. Plant Physiol 80:918–925

Osborne BA, Geider RJ (1986) Effect of nitrate-nitrogen limitation on photosynthesis of the diatom *Phaeodactylum tricornutum* Bohlin (Bacillariophyceae). Plant Cell Environ 9:617–625

Parsons TR, Maita Y, Lalli CM (1984) A manual of chemical and biological methods for seawater analysis. Pergamon Press, Oxford

Perry MJ (1976) Phosphate utilization by an oceanic diatom in phosphorus-limited chemostat culture and in the oligotrophic waters of the central North Pacific. Limnol Oceanogr 21:88–107

Perry MJ, Talbot MC, Alberte RS (1981) Photoadaptation in marine phytoplankton: response of the photosynthetic unit. Mar Biol 62:91–101

Pfiester LA, Anderson DM (1987) Dinoflagellate reproduction. In: Taylor FJR (ed) The biology of dinoflagellates. Blackwell Scientific Publications, Oxford, p 611–648

Prézelin BB (1982) Effects of light intensity on aging of the dinoflagellate *Gonyaulax polyedra*. Mar Biol 69: 129–135

Porter KG (1988) Phagotrophic phytoflagellates in microbial food webs. Hydrobiologia 159:89–97

Raven JA (1997) Phagotrophy in phototrophs. Limnol Oceanogr 42:198–205

Roy S (1988) Effects of changes in physiological condition on HPLC-defined chloropigment composition of *Phaeodactylum tricornutum* (Bohlin) in batch and turbidostat cultures. J Exp Mar Biol Ecol 118:137–149

Sanders RW, Porter KG (1988) Phagotrophic phytoflagellates. Adv Microb Ecol 10:167–192

Sciandra A, Lazzara L, Claustre H, Babin M (2000) Responses of growth rate, pigment composition and optical properties of *Cryptomonas* sp. to light and nitrogen stresses. Mar Ecol Prog Ser 201:107–120

Smalley GW (2002) Mixotrophy in the red-tide dinoflagellate *Ceratium furca*, with emphasis on feeding biology and ecology. PhD thesis, University of Maryland, College Park

Smalley GW, Coats DW (2002) Ecology of the red-tide dinoflagellate *Ceratium furca*: distribution, mixotrophy, and grazing impact on ciliate populations of Chesapeake Bay. J Eukaryot Microbiol 49:64–74

Smalley GW, Coats DW, Adam EJ (1999) A new method using fluorescent microspheres to determine grazing on ciliates by the mixotrophic dinoflagellate *Ceratium furca.* Aquat Microb Ecol 17:167–179

Stoecker DK (1998) Conceptual models of mixotrophy in planktonic protists and some ecological and evolutionary implications. Eur J Protistol 34:281–290

Stoecker DK, Li A, Coats DW, Gustafson DE, Nannen MK (1997) Mixotrophy in the dinoflagellate *Prorocentrum minimum*. Mar Ecol Prog Ser 152:1–12

von Stosch HA (1964) Zum Problem der sexuellen Fortpflanzung in der Peridineengattung *Ceratium*. Helgol Wiss Meeresunters 10:140–152

von Stosch HA (1972) La signification cytologique de la ‘cyclose nucléaire’ dans le cycle de vie des dinoflagallés. Mem Soc Bot Fr:201–212

Weiler CS, Eppley RW (1979) Temporal pattern of division in the dinoflagellate genus *Ceratium* and its application to the determination of growth rate. J Exp Mar Biol Ecol 39:1–24

Editorial responsibility: John Dolan (Contributing Editor), Villefranche-sur-mer, France

Submitted: April 8, 2003; Accepted: July 16, 2003
Proofs received from author(s): October 24, 2003