J. Phycol. **36,** 33–45 (2000)

MIXOTROPHY IN *GYRODINIUM GALATHEANUM* (DINOPHYCEAE): GRAZING RESPONSES TO LIGHT INTENSITY AND INORGANIC NUTRIENTS[1]

Aishao Li,[2,3] *Diane K. Stoecker*

Horn Point Laboratory, University of Maryland Center for Environmental Science, Cambridge, Maryland 21613

and

D. Wayne Coats

Smithsonian Environmental Research Center, Box 28, Edgewater, MD 21037

This paper presents results of field and laboratory studies on mixotrophy in the estuarine dinoflagellate *Gyrodinium galatheanum* (Braarud) Taylor. We tested the hypotheses that this primarily photosynthetic organism becomes phagotrophic when faced with suboptimal light and/or nutrient environments. In Chesapeake Bay, incidence of feeding of this species on cryptophytes is positively correlated with prey density and concentrations of nitrate and nitrite, but negatively correlated with depth, salinity, and phosphate concentration. Feeding in natural assemblages and cultures increased hyperbolically with light intensity. The stoichiometric proportions of dissolved inorganic P and N (DIP:DIN) at the stations where *G. galatheanum* was present were far below the optimal growth P:N (1:10). Incidence of feeding was negatively related to the ratio of DIP to DIN, suggesting that P limitation may have induced feeding. Addition of nitrate, or addition of both nitrate and phosphate, inhibited feeding in a natural population, indicating that N limitation may also induce feeding. Ingestion of the cryptophyte, *Storeatula major*, by cultured *G. galatheanum* was higher in media low in nitrate or phosphate or both, but moderate rates of feeding occurred in nutrient-replete cultures. When cells were grown in media with varying concentrations of nitrate and phosphate, N deficiency resulted in greater cellular N and Chl *a* losses than did P deficiency, but P deficiency stimulated feeding more than N deficiency. Both N and P deficiency, or P:N ratios that deviated greatly from 1:10, result in an increase of cellular carbon content and an increase in propensity to feed. Our results suggest that feeding in *G. galatheanum* is partly a strategy for supplementing major nutrients (N and P) that are needed for photosynthetic carbon assimilation. Feeding in *G. galatheanum* may also be a strategy for supplementing C metabolism or acquiring trace organic growth factors, since feeding occurs, although at a reduced rate, in nutrient-replete cultures.

Key index words: **feeding responses; light and nutrient limitation; Redfield ratio; mixotrophic dinoflagellate; *Gyrodinium galatheanum***

Phagotrophic activity has been documented in many photosynthetic algal taxa, and is particularly common in phototrophic chrysophytes, prymnesiophytes, and dinoflagellates (Boraas et al. 1988, Sanders and Porter 1988). Mixotrophy (i.e. the capability for simultaneous heterotrophy and autotrophy) could be advantageous to species living under varying environmental conditions. Particularly when cells are experiencing light or nutrient limitation, phagotrophy could serve as a mechanism of acquiring organic carbon or major inorganic nutrients (nitrogen or phosphorus), respectively (Caron et al. 1993, Sanders et al. 1990). Mixotrophy may also be important in acquiring specific growth factors (Kimura and Ishida 1989, Raven 1997).

Previous investigations of mixotrophy in nondinoflagellate photosynthetic flagellates have revealed that, besides prey density, several environmental variables including light intensity and inorganic nutrient concentrations can regulate phagotrophy (Caron et al. 1990, 1993, Sanders et al. 1990, Sibbald and Albright 1991, Jones et al. 1993). The feeding responses to these factors appear to be highly variable from species to species (Raven 1997). Different feeding responses to light intensity and inorganic nutrients have also been observed in mixotrophic dinoflagellates. Feeding by *Fragilidium subglobosum* is stimulated by light under dim light conditions, but is inhibited at high light intensities (Skovgaard 1996, Hansen and Nielsen 1997). Feeding in *Prorocentrum minimum*, however, is stimulated by light but inhibited by addition of inorganic nutrients (Stoecker et al. 1997).

Although the importance of light and inorganic nutrients as factors that regulate feeding in mixotrophic flagellates has been recognized, detailed examinations of how these factors influence physiological state of the cells, and therefore feeding capability, are scarce (Skovgaard 1996). The present study treats the ecology and physiology of the mixotrophic dinoflagellate *Gyrodinium galatheanum* (Braarud) Taylor (=*Gymnodinium galatheanum*), a bloom-forming and sometimes toxic species commonly found in estu-

[1] Received 9 August 1998. Accepted 23 September 1999.

[2] Present address: Biology Department, Mail Stop 32, Redfield 330, Woods Hole Oceanographic Institution, Woods Hole, MA 02543-1049.

[3] Author for reprint requests; e-mail ali@whoi.edu.

arine and coastal waters (Nielsen and Stromgren 1991; Nielsen 1993, 1996). In Chesapeake Bay, grazing of phycoerythrin-containing cryptophytes and several other nanoplanktonic organisms by *Gyrodinium galatheanum* (reported as *G. estuariale*) is very common (Li et al. 1996). The co-occurrence of cryptophytes and the dinoflagellate in the Bay, along with the ready detection of ingestion due to the prey's orange fluorescence, provides an ideal system to examine the factors that influence feeding in this mixotrophic dinoflagellate. This study was undertaken by a combination of field observations and laboratory experiments to test the following hypotheses: (1) phagotrophy by *G. galatheanum* is a response to light limitation; (2) phagotrophy is a response to N and P limitation.

MATERIALS AND METHODS

Field sampling. During monthly cruises in Chesapeake Bay between April and September 1995 and 1996, we took samples to examine the factors that may control feeding in *G. galatheanum.* Samples were taken at 2- to 3-m intervals from the surface to near bottom at stations along the main stem of the Bay (Stoecker et al. 1997). Samples were also taken from transect stations in the mesohaline area of the Bay where relatively high abundances ($\geq$50 cells·mL^{-1}) of *G. galatheanum* were found. Vertical CTD (conductivity, temperature, depth) Niskin bottle casts were made at each station during daylight.

For microscopic analysis, whole-water samples (20 mL for each) were immediately preserved with glutaraldehyde at a final concentration of 1% and stored at 4° C until 5- to 10-mL subsamples were filtered onto 2-μm-pore black membrane filters (Poretic Corp.). Filters were mounted on glass slides with immersion oil (Resolve) and capped with a coverslip. Slides were stored frozen at −20° C and subsequently examined at room temperature with epifluorescence microscopy (Zeiss filter set 487709; BP450-490 exciter filter, FT 510 dichromatic beam splitter, and LP520 barrier filter). *Gyrodinium galatheanum* and orange-fluorescent cryptophytes were enumerated at 400×. The presence of food vacuoles with ingested orange-fluorescent cryptophytes was recorded for the first 100 *G. galatheanum* encountered on each slide.

For inorganic nutrient analyses (ammonia, nitrate, nitrite, and dissolved phosphate), samples were gently filtered (<110 mm Hg) through GF/F filters and stored frozen (−20° C) in acid-washed scintillation vials until analysis (Technicon Autoanalyzer II, Bran & Luebbe detector, TAOS software).

Shipboard experiments. Several sets of shipboard experiments examined the influence of light and inorganic nutrients on phagotrophic activity by *G. galatheanum.* The first experiment examined the effects of light level and light history. On 14 September 1995, a surface sample was collected at 38°58′ N, 76°23′ W. Twenty 250-mL polycarbonate bottles were filled with aliquots of the water at 14:35 h when incident light intensity was ~900 μmol photons·m^{-2}·s^{-1}. Of these, ten bottles were used for a 4-h light-exposure experiment while the others were used for a 24-h light-exposure experiment. For the 4-h experiment, duplicates were wrapped with aluminum foil (dark treatment), with 3 (~16% I_o), 2 (~33% I_o), or 1 (~67% I_o) layers of neutral density screening or without added screening (100% I_o). Five milliliters of *Storeatula major* (strain g, called *Cryptomonas* sp. in Li et al. 1996) culture was added to each replicate to achieve densities of ~5×10^3 cells·mL^{-1}. The reason for using this prey density is that ingestion of the cryptophyte by this dinoflagellate was saturated at ~5×10^3 cells·mL^{-1} (Li et al. 1996). Bottles were gently mixed and a 20-mL aliquot from each bottle was taken and preserved with 1% (final conc.) glutaraldehyde immediately after addition of prey and at 1-h intervals over 4 h. Incubation was conducted in an on-deck incubator with running seawater to maintain water at ambient surface water temperature. The second set of bottles was treated in a similar way as the first set, except that the prey *S. major* (strain g) was added to each replicate after samples had been acclimated for 24 h under the different light treatments as described above. Procedures for enumeration of *G. galatheanum* and cryptophytes and for determination of ingeted cryptophytes were similar to those used for field sampling, except that 400 cells were scored for the presence of food vacuoles. Changes in the number of ingested cryptophyte prey with time were used to estimate ingestion rates of *G. galatheanum.*

The second field experiment was carried out to determine the effect of inorganic nutrients on feeding by *G. galatheanum.* A surface water sample containing *G. galatheanum* was collected at the same station and time as described above. The treatments were set up at 14:50 h, when duplicate 250-mL bottles were filled with aliquots of the water. The controls were unamended, 883 μM nitrate was added to the +N replicates, 36 μM phosphate was added to the +P replicates, and both nutrients were added to the +NP replicates. Control and treatments were acclimated for 24 h at near *in situ* temperature (19–21° C) and irradiance (0 to 1200 μmol photons·m^{-2}·s^{-1}). After 24 h of incubation, cultured *Storeatula major* was added to each bottle to achieve ~5×10^3 cell·mL^{-1}. Procedures for taking subsamples, fixation, and enumeration of cell density and determination of ingested cryptophytes by *G. galatheanum* were as in the first experiment.

Laboratory experiments. Based on the results of field observations and shipboard experiments, several laboratory experiments were performed for the purposes of: 1) defining the effects of irradiance on feeding, 2) quantifying the effects of concentrations of nitrate (NO_3^-) and phosphate (PO_4^{3-}) on feeding, and 3) investigating the relationship between intracellular C, N, and P and the feeding capacity of *G. galatheanum.*

Gyrodinium galatheanum (strain GE), isolated from the mesohaline portion of Chesapeake Bay (USA), was used for all laboratory experiments. *Storeatula major* (strain g) was obtained from Dr. T. Kana at Horn Point Laboratory (HPL, Cambridge, MD, USA). Stock cultures of these two species were maintained at 20° C, in 10 psu f/2–Si medium (Guillard 1975) on a light regime of 12:12 h light: dark (L:D) at a photon irradiance rate of 150 μmol·m^{-2}·s^{-1}.

To examine effects of irradiance on feeding, triplicate bottles with 250 mL f/2–Si medium were inoculated with *G. galatheanum* and allowed to acclimated for 3.7 days on a 12:12 L:D cycle at the following irradiances: darkness, 15, 30, 60, 100, 200, 300, 550, and 800 μmol photons·m^{-2}·s^{-1}. The medium was made with GF/F filtered Sargasso Sea water (37 psu), diluted with deionized water to achieve a salinity of 10 psu. The incubation temperature was 21 °C. At the end of the acclimation period, cultured *Storeatula major* was added to each replicate to achieve ~5×10^3 cell·mL^{-1} (saturated prey density). Aliquots (10 mL) from each replicate were then taken at intervals of 1 h over a 4-h period and fixed with 1% glutaraldehyde for determination of feeding rates.

The effect of concentrations of nitrate and phosphate on the feeding rate of *G. galatheanum* was examined in a factorial experiment with four levels of NO_3^- and four levels of PO_4^{3-}. Three replicates were used. Nutrient-free medium used for this experiment was prepared as described above (Sargasso seawater diluted to salinity of 10).

No trace metals and vitamins were added, and concentrations of $NO_3^- + NO_2^-$ as well as PO_4^{3-} were undetectable (<0.03 μM and <0.01 μM, respectively). A stock culture of *G. galatheanum* was concentrated above a 5-μm-pore membrane filter (Poretics Corp.), and washed four times with the nutrient-free medium. The washed cells were then inoculated in 48 acid-rinsed 250-mL polycarbonate bottles containing the nutrient-free medium. The inoculated cells were allowed to absorb the residual nutrients for 24 h. The following treatments were then set up and incubated over a 3-day period under 200 μmol photons·m^{-2}·s^{-1} at 21° C: nutrient-free medium, media with daily additions of different concentrations of PO_4^{3-} (1, 4, and 36 μM respectively), media with daily additions of NO_3^- (16 μM) and

TABLE 1. Summary of abundance ranges of *Gyrodinium galatheanum* (*G. gal*) and cryptophytes, as well as ranges of mean ingested cryptophytes per *G. galatheanum* during cruises in 1995 and 1996 in the Chesapeake Bay

Cruise	No. of stations	Sample size	Abundance range		Range of ingested cryptophytes per *G. galatheanum*
			G. gal (cells·mL^{-1})	Cryptophytes (cells·mL^{-1})	
7–10 May 1995	9	34	4–4040	143–1076	0–0.33
17–21 June 1995	19	70	3–3141	143–6302	0–0.47
7–11 July 1995	21	75	3–1364	227–7974	0–0.18
21–25 Aug. 1995	10	27	3–342	115–2822	0–0.16
14–17 Sept. 1995	14	53	3–935	84–1224	0–0.12
14–16 April 1996	8	28	2–26	5–7010	0–0.08
9–12 May 1996	16	91	2–244	89–2504	0–0.18
1–5 June 1996	11	50	1–425	20–1205	0–0.28
6–10 July 1996	17	66	3–593	0–5890	0–0.45
14–18 Aug. 1996	8	29	3–230	67–3315	0–0.10
7–11 Sept. 1996	11	48	3–341	126–2262	0–0.04

PO_4^{3-} (0, 1, 4, and 36 μM respectively), media with daily additions of NO_3^- (64 μM) and PO_4^{3-} (0, 1, 4, and 36 μM respectively); and media with daily additions of NO_3^- (883 μM) and PO_4^{3-} (0, 1, 4, and 36 μM respectively). After a 3-day acclimation, aliquots were taken from each bottle for cell counts (10 mL), Chl *a* (10 mL), and POC and PN (10 mL) analyses. *Storeatula major* was rinsed with nutrient-free medium and then added to the replicate bottles to achieve a concentration of ~5 × 10^3 cells·mL^{-1}. Twenty-milliliter aliquots were taken from each bottle at intervals of 1 h over a 4-h period and preserved with 1% glutaraldehyde for determination of feeding rates as described earlier.

Another laboratory experiment was designed to examine the relationship between physiological state and the feeding capability of *G. galatheanum*. The experiment was carried out in a manner similar to the previous one, except that the phosphate concentration was kept constant at 4 μM, while the nitrate concentration was varied between 0 and 200 μM. Trace metals and vitamins were added for all treatments. After a 3-day acclimation, aliquots were taken from each bottle for cell counts and determination of cell volume (20 mL), Chl *a* (20 mL), POC and PN (20 mL), and particulate phosphorus (20 mL) analyses. *Storeatula major* that had been rinsed with nutrient-free medium was then added to each replicate to achieve concentrations of ~5 × 10^3 cells·mL^{-1} (saturated prey density). Twenty-milliliter aliquots from each bottle were taken at hourly intervals for 4 h and preserved with 1% glutaraldehyde for determination of feeding rates, as described earlier.

TABLE 2. Spearman rank order correlation analysis of mean number of ingested cryptophytes per *Gyrodinium galatheanum*, with biological and physical-chemical parameters in the Chesapeake Bay. Samples were collected from the Chesapeake Bay during cruises in 1995 and 1996.

Parameter	Coefficient	*P*	Number of samples
Cryptophytes (cells·mL^{-1})	0.377	<0.0001	571
G. galatheanum (cells·mL^{-1})	0.51	<0.0001	571
Depth (m)	−0.329	<0.0001	560
Temperature (°C)	0.0198	0.641	560
Salinity (psu)	−0.386	<0.0001	560
NH_4^+ (μM)	0.0469	0.474	236
NO_2^- + NO_3^- (μM)	0.411	<0.0001	236
PO_4^{3-} (μM)	−0.396	<0.0001	236

Cell numbers and volumes were determined using a Coulter Multisizer II electronic particle counter. Chlorophyll *a* concentrations were determined fluorometrically. Samples were filtered onto Whatman GF/C filters and extracted in the dark in 90% acetone for 24 h at 4° C; the extracted samples were read on a Turner Model II fluorometer and corrected for phaeopigments according to Strickland and Parsons (1972). C, N, and P contents of the cryptophyte and *G. galatheanum* were measured. Particulate C and N were analyzed with a Leeman Lab 440 HA elemental analyzer in samples retained on precombusted Whatman GF/C filters. Particulate P was analyzed as soluble reactive P following persulfate oxidation of samples filtered onto Whatman GF/C filters (Anderson 1976).

Statistical analyses. Spearman rank order correlation analysis for field data was done with SigmaStat Version 2.0 (Jandel Scientific software). Comparison of ingestion rate in the control with those in the treatments in the shipboard nutrient experiment was done using a Bonferroni-adjusted Kolmogorov–Smirnov two-sample test (Sokal and Rohlf 1995). For the laboratory nutrient experiments, two-way ANOVAs with replication were run using STATISTICA Version 5.0A (StatSoft, 1984–1995). Feeding data for this experiment were rank-transformed.

RESULTS

Field observation on mixotrophy. Gyrodinium galatheanum and orange-fluorescent cryptophytes were found in 571 samples collected from Chesapeake Bay during cruises in 1995 and 1996 (Table 1). Among these samples, abundance of *G. galatheanum* and the cryptophyte ranged from 1 cells·mL^{-1} to 4040 cells·mL^{-1} and from 0 to 7974 cells·mL^{-1}, respectively (Table 1). Mean ingested cryptophytes ranged from 0 to 0.47 per *G. galatheanum* cell (Table 1). Mean ingested prey was positively associated with cryptophyte abundance (Table 2). Incidence of feeding was negatively correlated to depth and salinity (Table 2). Of the 571 samples, only 236 were analyzed for inorganic nutrient concentration. Within this subset, mean ingested prey by *G. galatheanum* was positively correlated with concentration of NO_2^- and NO_3^-, but negatively correlated with concentration of PO_4^{3-} (Table 2).

The relationship between incidence of feeding in *G. galatheanum* and the ratio of dissolved inorganic phosphorus (PO_4^{3-}) to dissolved inorganic nitrogen (NH_4^+, NO_2^-, and NO_3^-) is presented in Fig. 1. Con-

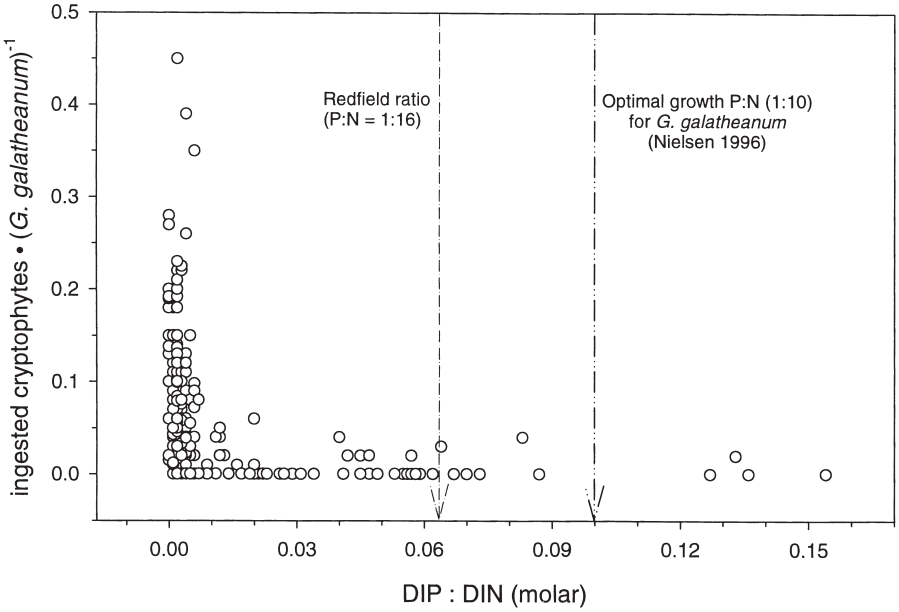

FIG. 1. Incidence of ingested cryptophytes in Chesapeake Bay *Gyrodinium galatheanum* populations in relationship to ratio of dissolved inorganic phosphorus to nitrogen (DIP:DIN). Number of ingested cryptophytes was negatively correlated with DIP:DIN (Spearman rank order correlation analysis: $r = -0.58$, $P < 0.001$ and $n = 236$). Samples were collected during cruises in 1995 and 1996.

centrations of PO_4^{3-} in some samples were below the detectable level (<0.01 μM). Higher feeding occurred where the ratio of dissolved inorganic phosphorus (DIP) to dissolved inorganic nitrogen (DIN) was lower than the Redfield ratio (P:N = 1:16). The negative correlation of ingestion of cryptophytes by *G. galatheanum* with ratio of DIP:DIN (Spearman rank order correlation: $r = -0.576$, $P < 0.0001$, $n = 236$) indicates that phosphorus limitation may have induced feeding (Fig. 1).

Shipboard experimental results. Feeding trials were done with *G. galatheanum* in a natural assemblage to determine whether different light levels and history of light exposure influence the feeding capabilities of this mixotrophic dinoflagellate (Fig. 2). Similar feeding responses to light were found in both short- and long-term experiments. Increased irradiance resulted in increased feeding rate up to 33% of incident light, and then the rates became saturated. At each level of irradiance, the feeding rate was significantly higher in treatments incubated on deck for 24 h than in treatments incubated for only 4 h before the feeding trial (*t*-test, $P < 0.01$).

In the on-deck nutrient addition experiment with *G. galatheanum* in a natural assemblage, the dinoflagellates were exposed to the nutrient treatments for 24 h prior to an afternoon feeding trial (Fig. 3). Addition of nitrate and a combination of both nitrate and phosphate significantly reduced feeding. However, addition of phosphate alone appeared to reduce

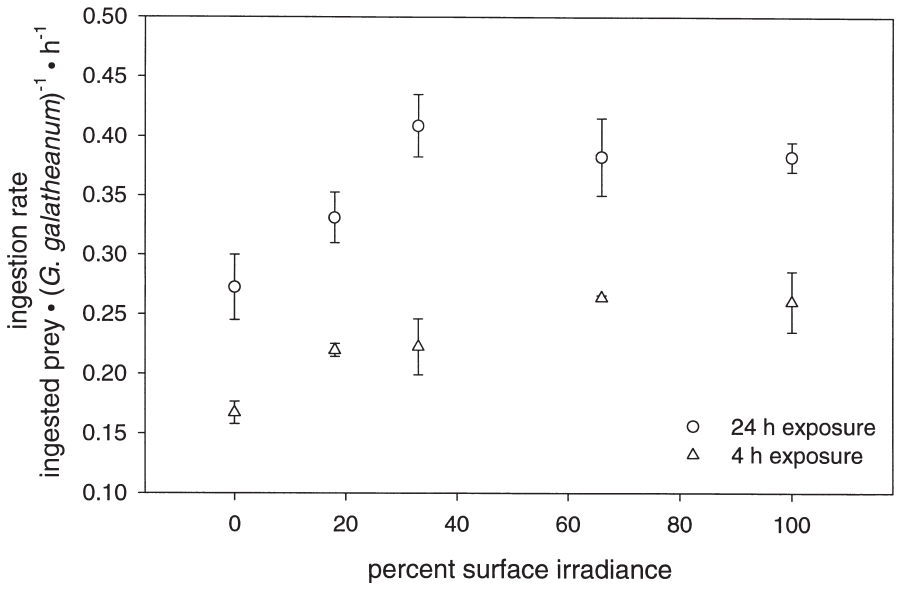

FIG. 2. Effects of light level on ingestion rate of *Gyrodinium galatheanum* in natural assemblages from Chesapeake Bay. Cultured *Storeatula major* was added as prey. Feeding trials were done with *G. galatheanum* in natural assemblages exposed to light treatments for 4 h (triangles) or 24 h (circles) prior to addition of prey. Error bars indicate standard error of mean ($n = 2$). The samples were collected from surface water at a mid-Bay station (38°58′ N, 76°23 W) on 14 September 1995.

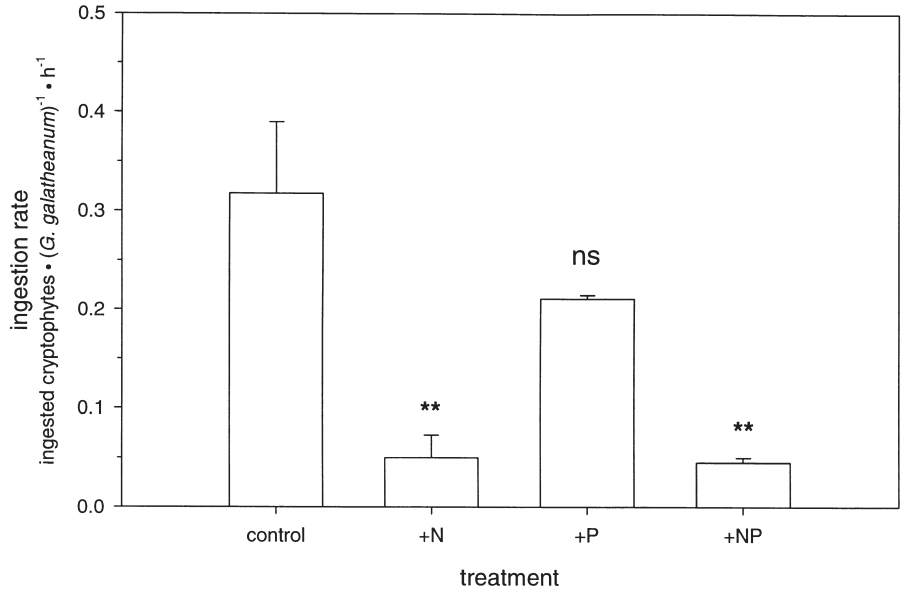

FIG. 3. Effects of addition of inorganic nutrients on ingestion rate of *Gyrodinium galatheanum* in natural assemblages from Chesapeake Bay. Cultured *Storeatula major* was added as prey. Treatments were designed as control (unamended), +N (addition of 883 μM nitrate), +P (addition of 36 μM phosphate), and +NP (addition of 883 μM nitrate and 36 μM phosphate). ** Indicates that the ingestion rates in the treatment and control are significantly different ($P < 0.05$); ns indicates that the ingestion rates in the treatment and control are not significantly different ($P > 0.05$). Error bars indicate standard error of mean ($n = 2$). The samples were collected from surface water at a mid-Bay station (38°58′ N, 76°23 W) on 14 September 1995.

feeding slightly, if at all (Fig. 3). Pairwise comparisons between the +N and the +P as well as the +NP and the +P treatments were significant ($P < 0.05$), but the difference between the +N and the +NP treatments was not significant ($P > 0.05$).

Laboratory experiments. Feeding responses were measured in *G. galatheanum* cultures that had been acclimated for 3.7 days at different light intensities (Fig. 4). Ingestion rate hyperbolically increased with increasing light intensity. At irradiances higher than 60 μmol photons·m^{-2}·s^{-1}, feeding rates no longer increased with increasing irradiance. No photoinhibition of feeding occurred, even at light intensities of up to 800 μmol photons·m^{-2}·s^{-1}.

To test the hypotheses that feeding is stimulated by inorganic nutrient limitation or depends on P:N ratios, *G. galatheanum* cultures were grown in media containing varying P and N contents. The physiological parameters (cellular Chl *a* and cellular contents of C, N) (Figs. 5A–5C) and the feeding responses to the various inorganic nutrients were examined (Fig. 6). High cellular C content occurred when cells were grown in media depleted of N or P and in media with P:N that deviated significantly from the Redfield ratio (P:N = 1:16) (Fig. 5A). Cellular contents of N and Chl *a* were affected more significantly by concentration of NO_3^- than by concentration of PO_4^{3-}. Cellular contents of N and Chl *a* increased with increased concentration of NO_3^- in media at four levels of PO_4^{3-} (Figs. 5B and 5C). The pattern of feeding responses to varying concentrations of NO_3^- and PO_4^{3-} was generally similar to that of cellular C content to varying

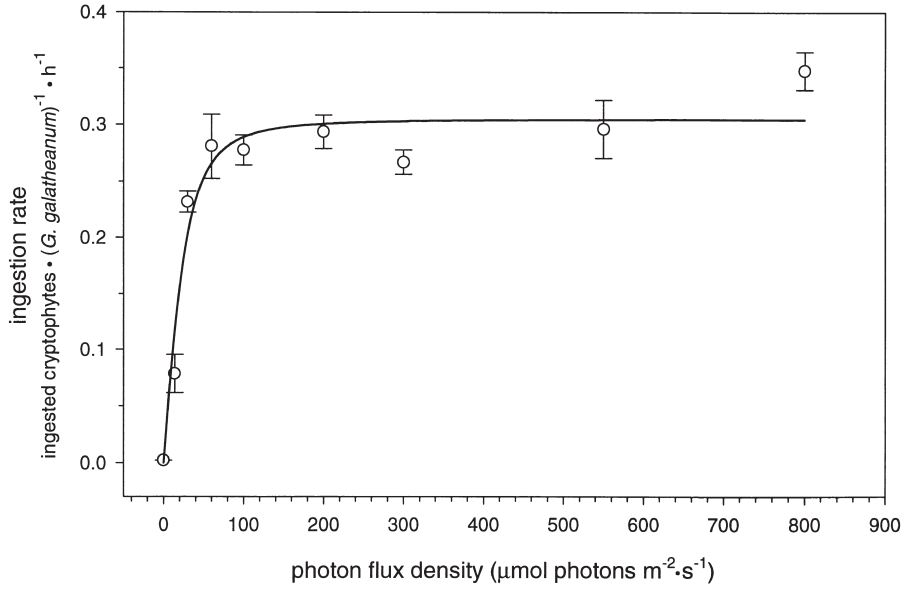

FIG. 4. *Gyrodinium galatheanum.* Ingestion rates as a function of light intensity. Prior to addition of cultured *Storeatula major* as prey, *G. galatheanum* cells in cultures were acclimated for 3.7 days under different light treatment conditions. Error bars indicate standard error of mean ($n = 3$). Curve fitted by hyperbolic tangent function.

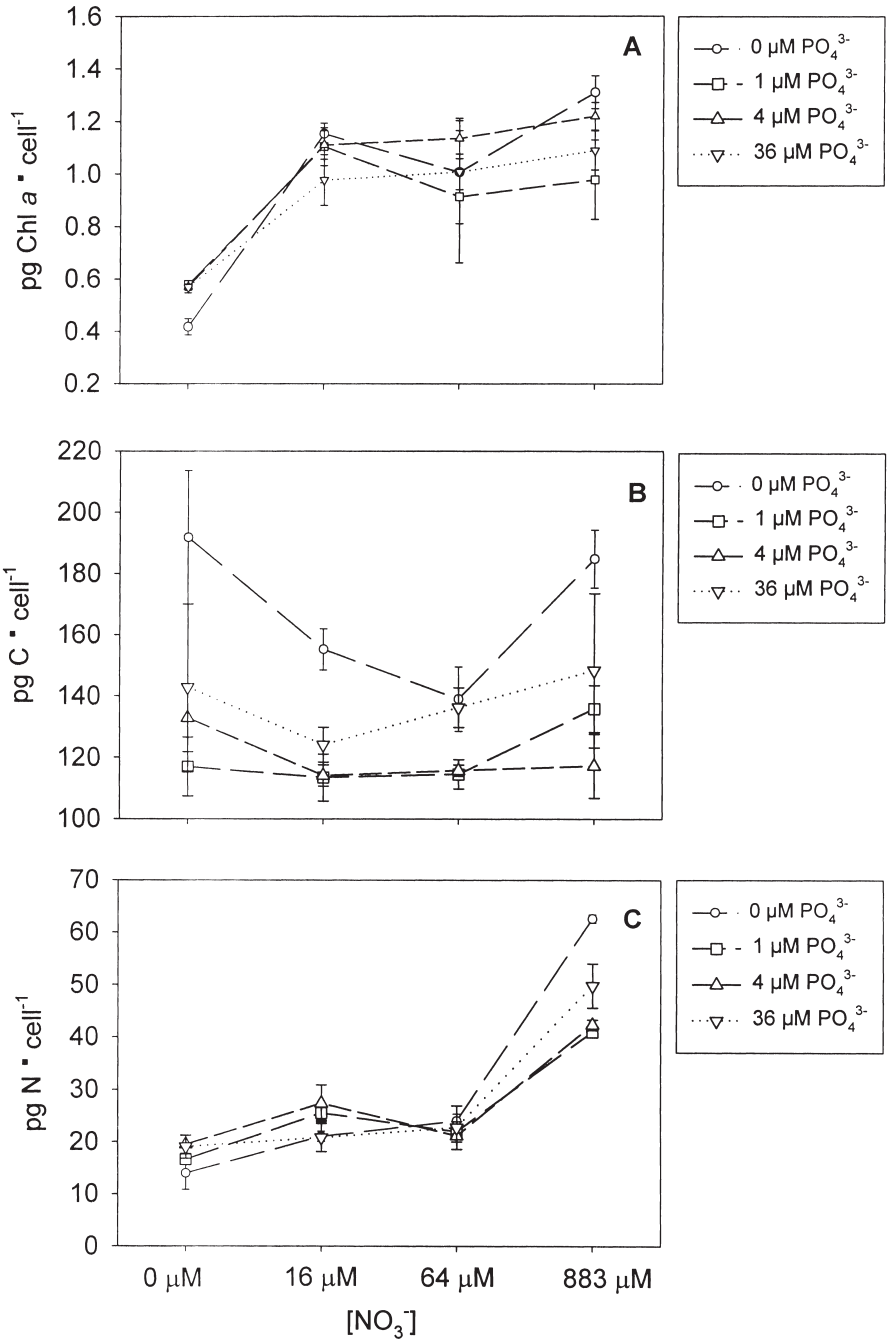

FIG. 5. *Gyrodinium galatheanum*. Effects of added nitrate and phosphate concentrations on cellular Chl *a* (A), cellular carbon (B), and cellular nitrogen (C). *G. galatheanum* cells were acclimated for 3 days under the different nutrient addition treatments. Error bar = 1 SE.

concentrations of NO_3^- and PO_4^{3-} (Figs. 5 and 6). The highest feeding rate was found when cells were grown in media depleted of N and P, and somewhat lower rates were observed when cells were grown in media depleted in one nutrient (Fig. 6). Lowest feeding rates occurred where P:N ratios in media were close to the Redfield ratio (P:N = 1:16). A two-way ANOVA for this nutrient experiment indicated that concentrations of nitrate and phosphate both significantly influenced feeding and that their interaction was significant (Table 3).

The relationship of several parameters (cellular contents of carbon and Chl *a*, cellular C:N, and cell volume) with feeding rates was also analyzed for this experiment (Table 4). Feeding rates were positively associated with cellular C ($P < 0.01$) and cellular C:N ratios ($P < 0.01$), but negatively associated with cellular Chl *a* ($P < 0.01$). The correlation between cell volume and feeding rates was not significant ($P > 0.05$). Ingestion rates linearly declined with increased Chl *a*:C ratio (w/w) (Fig. 7A) and linearly increased with increased cellular C content (Fig. 7B).

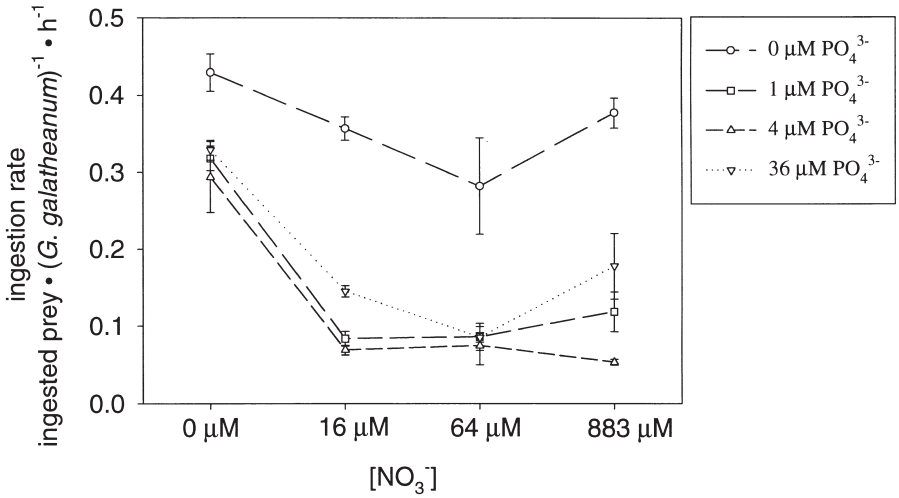

FIG. 6. *Gyrodinium galatheanum.* Effects of added nitrate and phosphate concentrations on feeding capability of *G. galatheanum* in a laboratory experiment. Prior to addition of cultured *Storeatula major* prey, *G. galatheanum* cells were acclimated for 3 days under the different nutrient addition treatments as in Fig. 5. Error bar = 1 SE.

The relationship between physiological state and the feeding capability of *G. galatheanum* was further examined in a second experiment. *Gyrodinium galatheanum* was grown in media in which P:N was varied by changing only the nitrate concentration (Figs. 8–10). This experiment differed from the first nutrient experiment in that phosphate concentrations were held at 4 µM, nitrate concentration was varied, and vitamins were added to the media. Cellular carbon content appeared to be a good physiological indicator for cells grown under nutrient stress or under nutrient-unbalanced conditions. The cells grown at nitrate concentrations < 40 µM (N:P $<$ 10:1 in media) and at nitrate concentrations > 80 µM (N:P $>$ 20:1) had higher carbon contents than the cells grown in media with N:P near 10:1 (Fig. 8A). Cells grown at N:P $<$ 10:1 had lower cellular contents of N, P, and Chl *a* than cells living in the media with N:P $>$ 10:1 (Figs, 8B–8D). Nitrogen limitation also led to increased cell volume (Fig. 8E), which indicates that the accumulation of cell carbon under low N:P ratios in media was due to increased cell volume. Higher cellular C:N and C:P ratios but lower Chl *a*:C ratios occurred when the cells were N limited (Figs. 9A–9C). Correspondingly, higher feeding rates occurred when cells were N limited (Fig. 9D).

Slight increases in cellular C content and in ingestion rate were also apparent when nitrate concentrations in the media were elevated, particularly when N:P ratios were markedly higher than 10:1 (Figs. 8A and 9D). A regression analysis further revealed that there was a positive linear correlation between feeding rates and cellular carbon contents (Fig. 10).

When the results of the two laboratory nutrient manipulation experiments are compared, some differences between the physiological state of the cells under nutrient-replete conditions are obvious. In the first experiment, in which vitamins were not added to the media, the maximum Chl *a* per cell was lower than in the second experiment, in which vitamins were added (Figs. 5 and 8). The minimum C content per cell was higher in the first experiment than the second (Figs. 5B and 8A). Maximum feeding rates were higher in the first experiment (without added vitamins) and feeding rate appeared to be more strongly correlated with Chl *a*:C and cellular C content in the second experiment (with added vitamins) than in the first (Figs. 7 and 10).

DISCUSSION

The results of on-deck and laboratory light experiments do not support the hypothesis that the photosynthetic dinoflagellate *G. galatheanum* becomes phagotrophic when light is limited. Although addition of prey might carry over trace amounts of inorganic nutrients into the samples in the on-deck light experiment and nutrient uptake during the incubation peri-

TABLE 3. Analysis of two-way ANOVA with replicate for laboratory nutrient experiment. Dependent variable: rank (ingested prey·(*Gyrodinium galatheanum*)$^{-1}$·h^{-1}). Data are shown in Fig. 6.

Source of variance	df	MS	*F*	*P*
NO_3^-	3	920.1	30.99	<0.0001
PO_4^{3-}	3	1592.3	53.63	<0.0001
$NO_3^- \times PO_4^{3-}$	9	80.2	2.70	0.0184

TABLE 4. Correlation of feeding rate of *Gyrodinium galatheanum* with physiological parameters (Pearson product moment correlation) in laboratory experiment. Data are shown in Figs. 5 and 6.

Parameter	Coefficient	*P*
Cellular C (pg·cell^{-1})	0.6017	<0.01
Cellular ChI *a* (pg·cell^{-1})	−0.3898	<0.01
Cellular C:N (molar)	0.5505	<0.01
Cell volume (µm3·cell^{-1})	−0.0923	0.5329 ns[a]

[a] ns, nonsignificant.

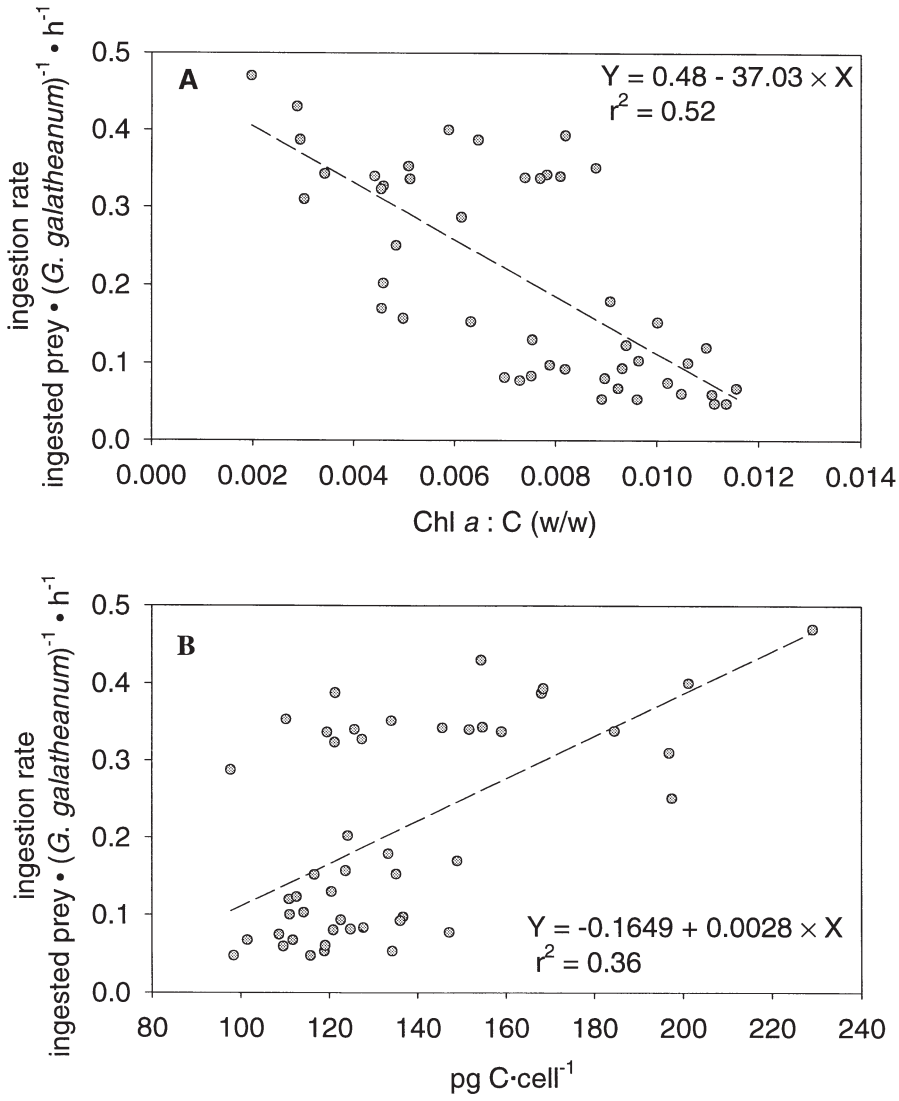

FIG. 7. *Gyrodinium galatheanum*. Correlation of feeding capability of *G. galatheanum* with cellular Chl *a*:carbon ratio (A) and with cellular carbon (B). Prior to addition of cultured *Storeatula major* as prey, *G. galatheanum* cells that were acclimated under different treatment conditions as in Fig. 5 were taken for Chl *a* and carbon measurements. The dashed line represents a linear regression fit of data.

ods might have changed the original nutrient regime, consistent results were observed in both the on-deck and laboratory light experiments. For example, higher ingestion rates were found for the cells with short-term (4 h) and long-term (24 h) exposure to light than for cells kept in the dark (Fig. 2). In the laboratory experiment, no feeding was detected in *G. galatheanum* acclimated for 3.7 days to total darkness. Additionally, the ingestion rates increased hyperbolically with increasing light intensity, even in cells that had been acclimated to nutrient-replete conditions (Fig. 4). These results indicate that feeding in *G. galatheanum* is light dependent, rather than a response to light limitation.

A light-dependent feeding pattern appears to be common in mixotrophic dinoflagellates. Similar feeding response to light has been found in the mixotrophic dinoflagellates *Prorocentrum minimum* (Stoecker et al. 1997), *Gymnodinium sanguineum* (A. L., unpublished data), and *Ceratium furca* (personal communication with G. W. Smalley). The effects of light on feeding in the mixotrophic dinoflagellate *Fragilidium subglobosum*, however, are somewhat complicated. Skovgaard (1996) reported that ingestion rates in this dinoflagellate could change from relatively constant low feeding rates in low light (within light levels ≤50 μmol photons·m^{-2}·s^{-1}), to increased ingestion rate with increased light intensities up to 100 μmol photons·m^{-2}·s^{-1}, and then decreased feeding rates with increasing light at irradiances >100 μmol photons·m^{-2}·s^{-1}. This species is also capable of growing almost equally well phototrophically or phagotrophically in the dark (Skovgaard 1996). In contrast to *Fragilidium subglobosum*, *G. galatheanum* can grow phototrophically in monocultures, but cannot survive in the dark even in the presence of food (*Storeatula major*) (Li et al. 1999). The differences between these two species suggest that they exploit different nutritional strategies: the former is a facultative phagotroph or phototroph, and the energy source (i.e. organic car-

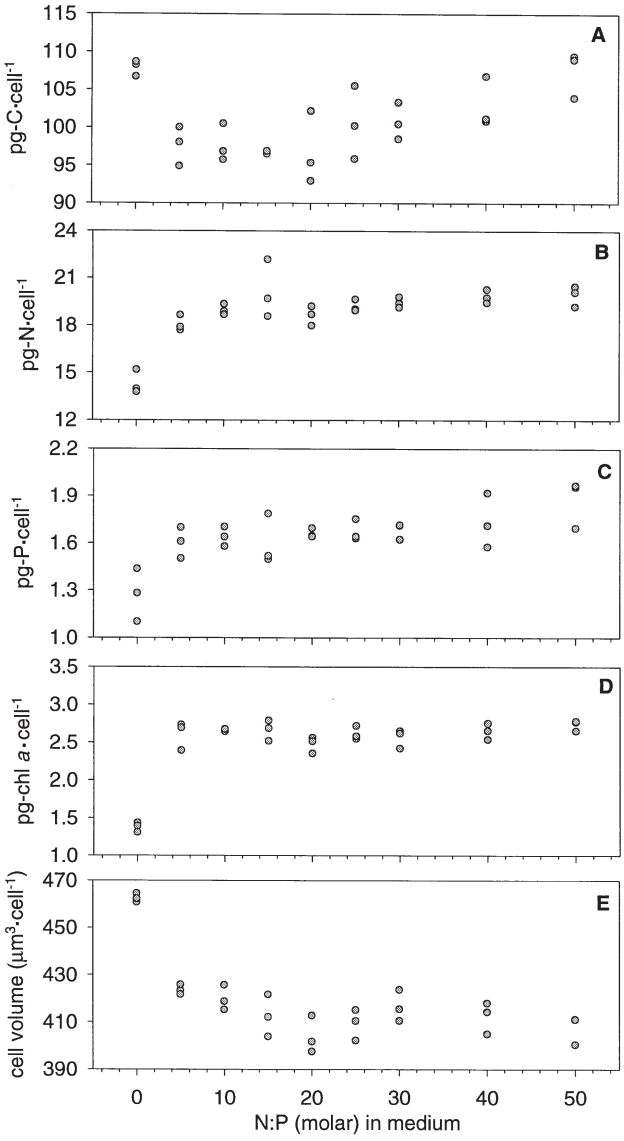

FIG. 8. *Gyrodinium galatheanum.* Cellular contents of carbon (A), nitrogen (B), phosphorus (C), Chl *a* (D), and cell volume (E) as a function of N:P ratios in media. *G. galatheanum* cells were acclimated for 3 days in media with varying N:P (i.e. initial $[PO_4^{3-}]$ = 4 μM and initial $[NO_3^-]$ = 0–200 μM).

bon) from the ingested prey alone can support its growth; the latter is an obligate phototroph, and light is its essential energy source.

Several possibilities could explain the light-dependent feeding response in *G. galatheanum.* One explanation is that the synthesis of feeding components (e.g. feeding organelles and the enzymes involved in ingestion and digestion or the energy, such as ATP, necessary to drive the feeding process) may be dependent on photosynthesis. This theory may explain the feeding response to light in a mixotrophic chrysophyte *Dinobryon cylindricum* (Caron et al. 1993) and the dinoflagellate *Fragilidium subglobosum* (Skovgaard 1996), but it has not been experimentally tested. Another explanation is that ingestion of prey may be induced by requirements for trace factors and/or inorganic nutrients for growth driven by photosynthetic carbon assimilation. In the on-deck experiment, the feeding rates of *G. galatheanum* in natural assemblages were higher in the long-term incubations than in the short-term incubation under a range of irradiance (Fig. 2). Prolonged incubation probably led to nutrient stress in the cells, which stimulated feeding. In the laboratory light experiment, the dinoflagellates were phagotrophic in the light treatments even when they were grown in inorganic nutrient-replete medium (f/2–Si) (Fig. 4). This indicates that feeding might also supplement carbon and/or supply trace growth factors as well as supply major nutrients (i.e. N and P). The light saturation curve of feeding in *G. galatheanum* is similar to the light saturation kinetics of inorganic nutrient uptake and $^{14}CO_2$ incorporation existing in many photosynthetic algae (MacIsaac and Dugdale 1972, Paasche et al. 1984, Lomas et al. 1996). These similarities suggest that feeding in this species depends on simultaneous photosynthetic carbon assimilation and/or generation of ATP.

Our results are consistent with the hypothesis that nutrient limitation stimulates feeding in *G. galatheanum.* In the on-deck experiment with natural assemblages, addition of nitrate and both nitrate and phosphate inhibited feeding, but the magnitude of feeding response to different nutrients varied (Fig. 3). In the laboratory nutrient experiments, the cells grown in N-depleted or P-deplete or both-depleted media had greater capability to eat (Figs. 6 and 9D).

Besides absolute concentrations of inorganic P and N, inorganic P:N ratios in the water appear to be an important factor in regulating feeding in *G. galatheanum.* In the laboratory nutrient experiment, higher feeding occurred when cells were acclimated to media in which P:N ratios deviated significantly from the Redfield ratio (1:16) (Fig. 6). In the experiment in which P:N in the media was varied by only changing nitrate concentrations, feeding rates increased when P:N ratios deviated from a ratio of P:N = 1:10 (Fig. 9D). This appears to be the optimal P:N ratio for *G. galatheanum* growth, since signs of P or N limitation (increase of C contents) occurred when P:N ratio decrease or increase widely from 1:10 (Fig. 8A). A previous study by Nielsen (1996) on the growth and chemical composition of *G. galatheanum* suggested that growth was limited by P or N on either side of the optimum P:N ratio of 1:10.

Although our cultures used in laboratory experiments were not axenic and thus the potential contribution of bacteria to C, N, and P composition cannot be ruled out, the use of actively growing cultures and GF/C filters (>1.2 μm pore size) for retaining our targeted cells for particulate C, N, and P analyses minimizes bacterial interference. Furthermore, the cellular contents of C, N, P, and Chl *a* in *G. galatheanum* found in this present study are within the ranges of

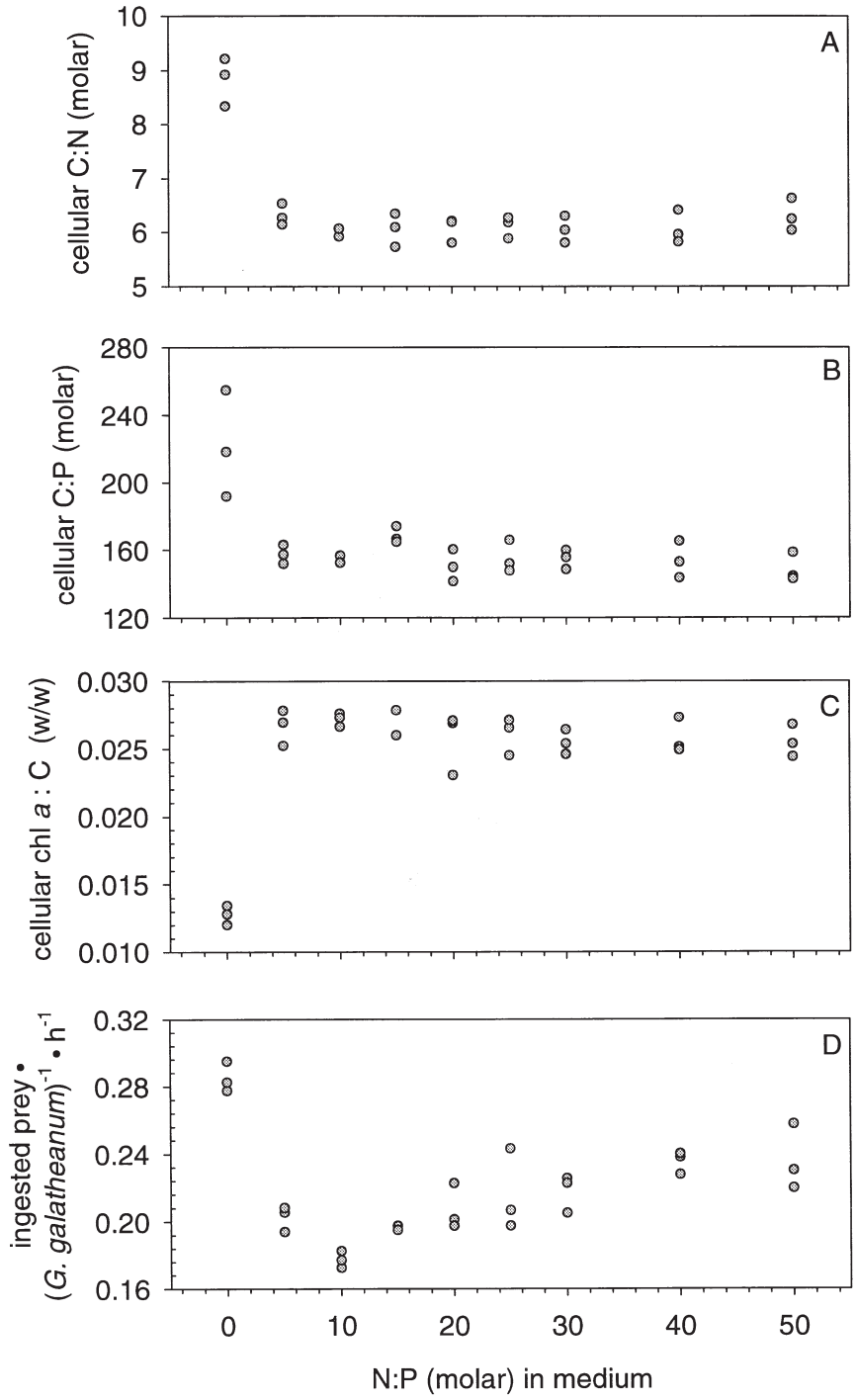

FIG. 9. *Gyrodinium galatheanum*. Ratios of cellular carbon:nitrogen (A), carbon:phosphorus (B), Chl *a*:carbon (C), and corresponding ingestion rates (D) as a function of N:P ratios in media. *G. galatheanum* cells were grown for 3 days in media with varying N:P (i.e. initial $[PO_4^{3-}]$ = 4 μM and initial $[NO_3^-]$ = 0–200 μM) before addition of *Storeatula major* as prey.

the values reported by Nielsen (1996). These cellular parameters ranged from 93 to 229 pg·cell^{-1} for C, 9 to 64 pg·cell^{-1} for N, 14 to 22 pg·cell^{-1} for P, and 1 to 14 pg·cell^{-1} for Chl *a* (Figs. 5A–5C and 8A–8D). Nitrogen deficiency resulted in greater cellular N and Chl *a* losses than did P deficiency (Fig. 5). However, the C:P ratios we found are higher than previously reported for this species (Nielsen, 1996). Similar responses of these physiological parameters to nutrient status appear have been found in many dinoflagellates and other groups of phytoplankton (Latasa and Berdalet 1994, Perry 1976, Sakshaug et al. 1983, 1984). Cellular C content appears to be a good indicator of nutrient-unbalanced growth conditions for *G. galatheanum*, because both N and P deficiency, or ambient P:N ratios that deviate significantly from 1:10, result in increased cellular carbon content (Figs. 5B and 8A). The cells of *G. galatheanum* grown under nutrient-depleted or nu-

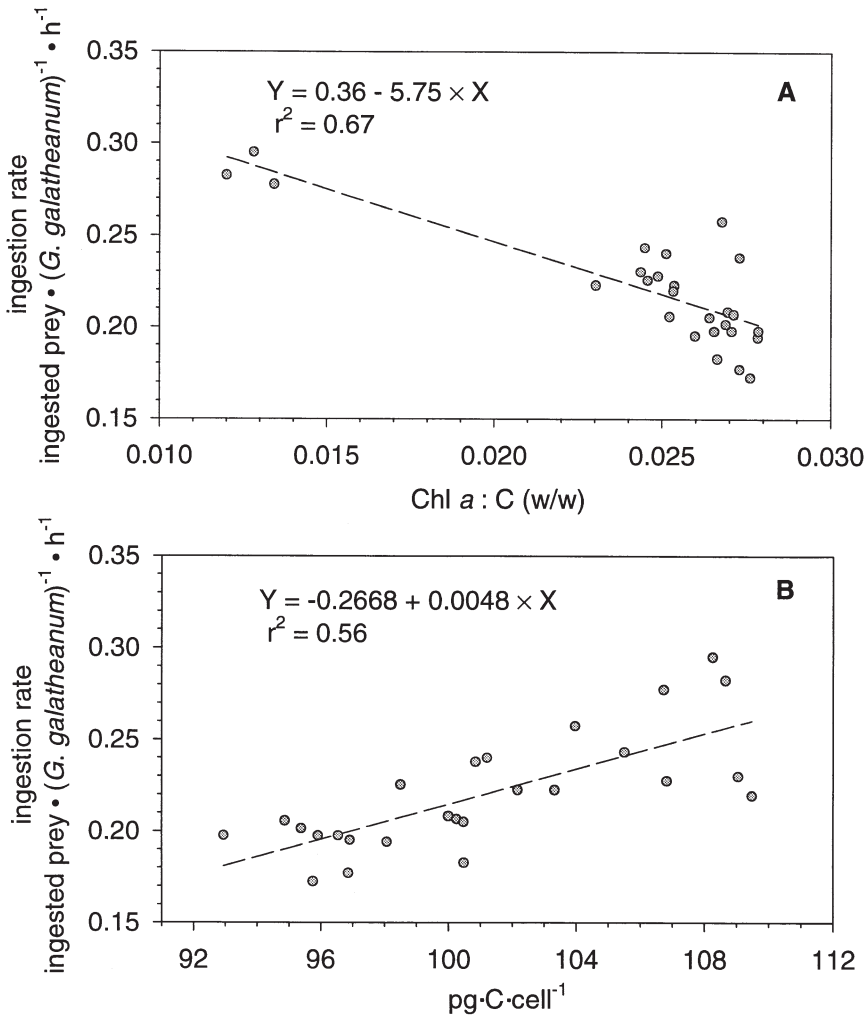

FIG. 10. *Gyrodinium galatheanum*. Ingestion rates as a function of cellular Chl *a*:C (A) and as a function of carbon contents (B). Prior to addition of cultured *Storeatula major* as prey, *G. galatheanum* cells that were acclimated under different nutrient treatment conditions as in Fig. 9 were taken for cellular carbon measurements. The dashed line represents a linear regression fit of data.

trient-unbalanced conditions have greater capability to eat than did the cells that had been acclimated under nutrient-replete and nutrient-balanced growth conditions (Figs. 6, 7, 9D, and 10).

In Chesapeake Bay, phytoplankton growth in general appears to be limited by dissolved inorganic P during spring and by inorganic N in summer (Fisher et al. 1992). Spatial variations in the limiting nutrients are also evident, characterized by P limitation in the North Bay and N limitation in the South Bay (Fisher et al. 1992, Glibert et al. 1995). Populations of *G. galatheanum* in Chesapeake Bay generally follow 7–13 salinity isopleths and thus they are usually located from the mid-Bay to the northern part of the Bay, depending on the season (A. L., unpublished data). Our field data show that the ratios of dissolved inorganic P and N were far below the Redfield ratio (P:N = 1:16) in most of the samples in which *G. galatheanum* was abundant (Fig. 1). Given that the optimal P:N ratio is 1:10 for *G. galatheanum*, it is most likely that the populations of this species in the Bay experience potential P limitation rather than N limitation and that feeding is stimulated primarily by P limitation (Fig. 1). However, our laboratory results show that cells grown in nutrient-replete media of an P:N ratio of 1:10 still feed, but at a lower rate than nutrient-stressed or nutrient-unbalanced cells. These results suggest that feeding is advantageous to *G. galatheanum* for more reasons than the supply of P or N.

The discrepancies between the two laboratory nutrient manipulation experiments suggest that feeding may also supply vitamins. In the first experiment, in which vitamins were not added to the experimental media, the ingestion rates of the dinoflagellate at roughly similar inorganic nutrient concentrations were higher than in the second experiment. Furthermore, in the second experiment, in which vitamins were added, the feeding response was more tightly correlated with cellular C content and Chl *a*:C ratios than in the first experiment. These observations suggest that *G. galatheanum* may be stimulated to feed by both lack of vitamins and inorganic nutrient depletion or unbalance. Alternatively, in these two laboratory experiments the absolute inorganic nutrient concentrations probably changed during the 3-day acclimation period because nutrients were added daily;

however, the nutrient ratios probably remained relatively constant. Differences in the absolute nutrient concentrations between the two experiments might have contributed to differences in the responses in feeding and other aspects of the cell physiology in these two experiments.

Our data set as a whole, including field and laboratory observations, supports the conclusion that multiple factors can regulate feeding in *G. galatheanum*. Prey availability apparently controls feeding on cryptophytes in Chesapeake Bay (Table 2). The negative correlation with salinity might be related to stratification and mixed-layer depth conditions that actually affect feeding by altering the irradiance and chemical environments of the *in situ* population. Thus, although in the laboratory feeding is readily induced by imposing N or P limitation, feeding was not detected in all natural populations in N- or P-depleted waters (Fig. 1). For example, one might expect that high rates of ingestion should also show up on the far right hand side of Fig. 1. They did not, but that may be a function of the low number of data points on that end of the graph. Alternatively, factors such as inadequate irradiance or absence of prey that could be detected by epifluorescence microscopy might have been responsible for lack of detectable feeding in these populations. In addition, the Chesapeake Bay populations may be feeding, but not on cryptophytes, and hence feeding would not have been detected with our technique. Our laboratory results indicate that cells always feed to some extent in the light, although either N or P limitation can increase feeding (Figs. 4, 6, and 9D). The only laboratory condition that completely inhibited feeding was darkness (Fig. 4). These results suggest that feeding regulation in the light occurs in response to unbalanced metabolism that can be imposed by limitation of potentially any element necessary for photosynthesis and growth.

Mixotrophy appears to be very common among bloom-forming photosynthetic dinoflagellates (Sanders and Porter 1988, Bockstahler and Coats 1993, Jacobson and Anderson 1996, Li et al. 1996). Given that they can have relatively high feeding rates (Bockstahler and Coats 1993, Hansen and Nielsen 1997, Skovgaard 1996, and data presented herein), mixotrophic dinoflagellates may play significant roles in aquatic food webs as grazers and predators. Combinations of inorganic nutrient uptake, phagotrophy and photosynthesis may provide nutritional flexibility for these species in variable environments. Feeding on other protists, including competitors for resources, may contribute to their bloom formation and persistence. Not enough is known about the extent to which ingested prey contribute to the nutritional needs of mixotrophic dinoflagellates. Little is known about how feeding influences their photophysiology. Further studies on these matters are needed to better understand the roles of mixotrophy in dinoflagellate bloom formation and persistence and the role of mixotrophic dinoflagellates in the trophodynamics of aquatic ecosystems.

We thank the Captain and crew of the RV *Cape Henlopen* for ship operation and assistance during field sampling. Dr. T.R. Fisher kindly provided us the facilities for particulate P analyses. We thank D.E. Gustafson for his excellent technical help in the laboratory work. We thank Dr. K.A. Steidinger for identification of strain GE and Dr. P. Kugrens for identification of strain g. Discussion with J.E. Adolf and suggestion from two anonymous reviewers contributed to the improvement of this paper. Support was derived from NSF grant OCE931772 awarded jointly to D.W.C. and D.K.S and a Horn Point Laboratory Graduate Assistantship awarded to A.L. Contribution No. 3243 of the University of Maryland Center for Environmental Science.

Anderson, J. M. 1976. An ignition method for the determination of total phosphorus in lake sediments. *Water Res.* 10:329–31.

Bockstahler, K. R. & Coats, D. W. 1993. Spatial and temporal aspects of mixotrophy in Chesapeake Bay dinoflagellates. *J. Eukaryot. Microbiol.* 40:49–60.

Boraas, M. E., Estep, K. W., Johnson, P. W. & Sieburth, J. M. 1988. Phagotrophic phototrophs: the ecological significance of mixotrophy. *J. Protozool.* 35:249–52.

Caron, D. A., Porter, K. G. & Sanders, R. W. 1990. Carbon, nitrogen and phosphorus budgets for the mixotrophic phytoflagellate *Poterioochromonas malhamensis* (Chrysophyceae) during bacterial ingestion. *Limnol. Oceanogr.* 35:433–43.

Caron, D. A., Sanders, R. W., Lim, E. L., Marrase, C., Amaral, L. A., Whitney, S., Aoki, R. B. & Porter, K. G. 1993. Light-dependent phagotrophy in the freshwater mixotrophic chrysophyte *Dinobryon cylindricum*. *Microb. Ecol.* 25:93–111.

Fisher, T. R., Peele, E. R., Ammerman, J. W. & Harding, L. W., Jr. 1992. Nutrient limitation of phytoplankton in Chesapeake Bay. *Mar. Ecol. Prog. Ser.* 82:51–63.

Glibert, P. M., Conley, D. J., Fisher, T. R., Harding, L. W., Jr. & Malone, T. C. 1995. Dynamics of the 1990 winter/spring bloom in Chesapeake Bay. *Mar. Ecol. Prog. Ser.* 122:27–43.

Guillard, R. R. L. 1975. Culture of phytoplankton for feeding marine invertebrates. *In* Smith, W. L. & Chanley, M. H. [Eds.] *Culture of Marine Invertebrate Animals.* Plenum Publishing Corp., New York, pp. 29–60.

Hansen, P. J. & Nielsen, T. G. 1997. Mixotrophic feeding of *Fragilidium subglobosum* (Dinophyceae) on three species of *Ceratium*: effects of prey concentration, prey species and light intensity. *Mar. Ecol. Prog. Ser.* 147:1877–96.

Jacobson, D. M. & Anderson, D. M. 1996. Widespread phagocytosis of ciliates and other protists by marine mixotrophic and heterotrophic thecate dinoflagellates. *J. Phycol.* 32:279–85.

Jones, H. L. J., Leadbeater, B. S. C. & Green, J. C. 1993. Mixotrophy in marine species of *Chrysochromulina* (Prymnesiophyceae): ingestion and digestion of a small green flagellate. *J. Mar. Biol. Assoc. U.K.* 73:283–96.

Kimura, B. & Ishida, Y. 1989. Phospholipid as a growth factor of *Uroglena americana*, a red tide Chrysophyceae in Lake Biwa. *Nippon Suisan Gakkaishi* 55:799–804.

Latasa, M. & Berdalet, E. 1994. Effect of nitrogen or phosphorus starvation on pigment composition of cultured *Heterocapsa* sp. *J. Plankton Res.* 16:83–94.

Li, A., Stoecker, D. K., Coats, D. W. & Adam, E. J. 1996. Ingestion of fluorescently-labeled and phycoerythrin-containing prey by photosynthetic dinoflagellates. *Aquat. Microb. Ecol.* 10:139–47.

Li, A., Stoecker, D. K. & Adolf, J. E. 1999. Feeding, pigmentation, photosynthesis and growth of the mixotrophic dinoflagellate *Gyrodinium galatheanum*. *Aquat. Microb. Ecol.* 19:163–76.

Lomas, M. W., Glibert, P. M., Berg, G. M., & Burford, M. 1996. Characterization of nitrogen uptake by natural populations of *Aureococcus anophagefferens* (Chrysophyceae) as a function of incubation duration, substrate concentration, light, and temperature. *J. Phycol.* 32:907–16.

MacIsaac, J. J. & Dugdale, R. C. 1972. Interactions of light and inorganic nitrogen in controlling nitrogen uptake in the sea. *Deep-Sea Res.* 19:209–32.

Nielsen, M. V. 1993. Toxic effect of the marine dinoflagellate *Gymn-*

odinium galatheanum on juvenile cod *Gadus morhua*. *Mar. Ecol. Prog. Ser.* 95:273–7.

Nielsen, M. V. 1996. Growth and chemical composition of the toxic dinoflagellate *Gymnodinium galatheanum* in relation to irradiance, temperature and salinity. *Mar. Ecol. Prog. Ser.* 136:205–11.

Nielsen, M. V. & Stromgren, T. 1991. Shell growth response of mussels (*Mytilus edulis*) exposed to toxic microalgae. *Mar. Biol.* 108:263–7.

Paasche, E., Bryceson, I. & Tangen, K. 1984. Interspecific variation in dark nitrogen uptake by dinoflagellates. *J. Phycol.* 20:394–401.

Perry, M. J. 1976. Phosphate utilization by an oceanic diatom in phosphorus-limited chemostat culture and in the oligotrophic waters of the central North Pacific. *Limnol. Oceanogr.* 21:88–106.

Raven, J. A. 1997. Phagotrophy in phototrophs. *Limnol. Oceanogr.* 42:198–205.

Sakshaug, E., Andresen, K., Myklestad, S. & Olsen, Y. 1983. Nutrient status of phytoplankton communities in Norwegian waters (marine, brackish, and fresh) as revealed by their chemical composition. *J. Plankton Res.* 5:175–96.

Sakshaug, E., Granèli, E., Elbrächter, M. & Kayser, H. 1984. Chemical composition and alkaline phosphatase activity of nutrient-saturated and P-deficient cells of four marine dinoflagellates. *J. Exp. Mar. Biol. Ecol.* 77:241–54.

Sanders, R. W. & Porter, K. G. 1988. Phagotrophic phytoflagellates. *Adv. Microb. Ecol.* 10:167–92.

Sanders, R. W., Porter, K. G. & Caron, D. A. 1990. Relationship between phototrophy and phagotrophy in the mixotrophic chrysophyte *Poterioochromonas malhamensis*. *Microb. Ecol.* 19: 97–109.

Sibbald, M. J. & Albright, L. J. 1991. The influence of light and nutrients on phagotrophy by the mixotrophic nanoflagellate, *Ochromonas* sp. *Mar. Microb. Food Webs* 5:39–47.

Skovgaard, A. 1996. Mixotrophy in *Fragilidium subglobosum* (Dinophyceae): growth and grazing responses as functions of light intensity. *Mar. Ecol. Prog. Ser.* 143:247–53.

Sokal, R. R. & Rohlf, F. J. 1995. *Biometry*. 3rd ed. W.H. Freeman & Co., New York, 887 pp.

Stoecker, D. K., Li, A., Coats, D. W. Gustafson, D., & Nannen, M.K. 1997. Mixotrophy in the dinoflagellate *Prorocentrum minimum*. *Mar. Ecol. Prog. Ser.* 152:1–12.

Strickland, J. D. H. & Parsons, T. R. 1972. *A Practical Handbook of Seawater Analysis*. Bull. 167. Fisheries Research Board of Canada, Ottawa, 310 pp.