

Use of the ‘food vacuole content’ method to estimate grazing by the mixotrophic dinoflagellate *Gyrodinium galatheanum* on cryptophytes

AISHAO LI^{1,3}, DIANE K. STOECKER¹ AND D. WAYNE COATS²

¹HORN POINT LABORATORY, UNIVERSITY OF MARYLAND CENTER FOR ENVIRONMENTAL SCIENCE, CAMBRIDGE, MD 21613 AND ²SMITHSONIAN ENVIRONMENTAL RESEARCH CENTER, BOX 28, EDGEWATER, MD 21037, USA

³PRESENT ADDRESS: BIOLOGY DEPARTMENT, WOODS HOLE OCEANOGRAPHIC INSTITUTION, WOODS HOLE, MA 02543-1049, USA

We measured in situ grazing rates of the mixotrophic dinoflagellate Gyrodinium galatheanum (Braarud) Taylor 1995 on populations of phycoerythrin-containing cryptophytes in Chesapeake Bay. Rates were estimated from instantaneous food vacuole contents, in situ temperatures, cryptophyte abundances and experimentally determined digestion rates. Laboratory digestion experiments showed that specific digestion rate constants increased sigmoidally with temperature, but were unrelated to the initial food vacuole content when it was <0.46 cryptophytes dinoflagellate⁻¹. These results allowed us to establish an empirical model to estimate in situ ingestion of cryptophyte prey by G. galatheanum. The estimated rates ranged from 0 to 0.26 cryptophytes dinoflagellate⁻¹ day⁻¹, corresponding to daily ingestion of 0–12.29 µg carbon, 0–2.48 µg nitrogen and 0–0.34 µg phosphorus dinoflagellate⁻¹. Estimated daily consumption of cryptophyte biomass by G. galatheanum was equivalent to 0–12% of body carbon, 0–13% of body nitrogen and 0–21% of body phosphorus. Estimated in situ clearance rates for cryptophytes ranged from 0 to 0.27 µl dinoflagellate⁻¹ day⁻¹, representing daily removal of 0–4% of the cryptophyte standing stock. Although G. galatheanum may increase its growth rate through phagotrophy, it appears to have little grazing impact on cryptophyte prey populations.

INTRODUCTION

Mixotrophy by organisms that combine phototrophy and phagotrophy is common among dinoflagellates (Sanders and Porter, 1988; Bockstahler and Coats, 1993a; Jacobson and Anderson, 1996; Li *et al.*, 1996; Uchida *et al.*, 1997; Stoecker, 1999). Many mixotrophic dinoflagellates form blooms, and some of them are potentially toxic species (Nielsen and Stromgren, 1991; Bockstahler and Coats, 1993a; Nielsen, 1993; Stoecker *et al.*, 1997; Li *et al.*, 2000b). Although the nutritional benefits of phagotrophy to mixotrophs, as well as the significance of mixotrophs as consumers, have been elucidated in other groups of phytoflagellates (Bird and Kalf, 1987; Boraas *et al.*, 1988; Caron *et al.*, 1990; Rothhaupt, 1996a,b; Thingstad *et al.*, 1996), until recently only a few studies have focused on these aspects in mixotrophic dinoflagellates (Bockstahler and Coats, 1993a,b; Skovgaard, 1996; Jeong *et al.*, 1999;

Li *et al.*, 1999, 2000a,b; Smalley *et al.*, 1999). Bockstahler and Coats (Bockstahler and Coats, 1993b) employed a ‘food vacuole content’ method to estimate the grazing impact by the mixotrophic dinoflagellate *Gymnodinium sanguineum* on ciliate prey populations. They reported *in situ* ingestion rates from 0 to 0.06 prey dinoflagellate⁻¹ h⁻¹, which represented an average of 2.5% of body carbon (C) and 4.0% of body nitrogen (N) from daily ingestion of ciliate prey. The daily removal of ciliate biomass by *G. sanguineum* was estimated as 6–67% of the <20 µm oligotrich standing stock.

The food vacuole content method, an increasingly popular approach for the estimation of protist grazing rates (Fenchel, 1975; Kopylov and Tumentseva, 1987; Bernard and Rassoulzadegan, 1990; Dolan and Coats, 1991; Bockstahler and Coats, 1993b), is based on the use of data on instantaneous food vacuole content (prey protist⁻¹) and experimentally determined digestion rates.

This method is modified from the ‘gut fluorescence/gut content’ approach, which has been used to infer macrozooplankton grazing rates (e.g. Dagg and Grill, 1980; Kiørboe *et al.*, 1985; Dam and Peterson, 1988; Purcell and Nemazie, 1992; Irigoien, 1998). Rather than use of gut passage time, the food vacuole content method employs the disappearance rate of food vacuole content (or digestion rate). It is proposed that the instantaneous food vacuole content of a protist (F) is a function of its ingestion rate (I) and disappearance rate (D) of food vacuole content (or digestion rate). Mathematically, changes in food vacuole content over time can be expressed as:

$$dF/dt = I - D \quad (1)$$

Assuming that the disappearance rate (D) of food vacuole content is proportional to food vacuole content (i.e. $D = U \times F$, where U is the digestion rate constant), then under the assumption of steady state, $dF/dt = 0$ and

$$I = U \times F \quad (2)$$

The ingestion rate I (number of ingested prey protist⁻¹ h⁻¹) is given by the product of the food vacuole content F (prey protist⁻¹) and the digestion rate constant U (h⁻¹). The reciprocal of U gives the food vacuole turnover time.

Application of the food vacuole content method to estimate the *in situ* ingestion rate requires obtaining an accurate assessment of the average number of ingested prey per predator (F) and experimental determination of digestion rate constants (U) (Landry, 1994). To obtain accurate estimates of ingested prey per predator, appropriate techniques for field sampling and preservation, and for identification of prey in food vacuoles are obligatory (Sieracki *et al.*, 1987; Bockstahler and Coats, 1993b; Li *et al.*, 1996). Determination of digestion rate constants for protists can be difficult since it involves separating delicate, small-size prey and predator. Furthermore, the digestion rate constant (U) is often influenced by environmental and biological factors, such as temperature (Sherr *et al.*, 1988; Dolan and Coats, 1991), food type or quality (Bernard and Rassoulzadegan, 1990), and amount or number of ingested prey (Capriulo, 1990; Capriulo and Degnan, 1991). Among these, temperature appears to be the most important factor that influences digestion of specific prey by a protist, since digestion is enzyme controlled (Capriulo, 1990). Therefore, when the food vacuole content method is applied to estimate *in situ* ingestion rates for specific prey, the digestion rate constant U is often corrected for temperature using an assigned Q_{10} value (Bockstahler and Coats, 1993b), or by using an experimentally determined functional relationship between temperature and U (Dolan and Coats, 1991).

Application of the food vacuole content method must obey the steady-state assumption, i.e. ingestion and

digestion rates must be in equilibrium. This assumption may be true within short time frames (i.e. hours). However, rates of ingestion and digestion may vary over longer periods of time (i.e. a day), since diel oscillation of these rates could exist. Application of this method also requires that U is independent of F .

In this study, we dealt with *Gyrodinium galatheanum* (Braarud) Taylor 1995, a mixotrophic dinoflagellate that forms blooms in Chesapeake Bay (Li *et al.*, 2000b) and that feeds on cryptophytes and other small protists (Li *et al.*, 1996). Mean food vacuole contents as high as 0.47 cryptophyte prey dinoflagellate⁻¹ have been observed in *G. galatheanum* from Chesapeake Bay in summer (Li *et al.*, 2000b). These observations stimulated us to examine whether the food vacuole content method (Bockstahler and Coats, 1993b) could be applied to estimate *in situ* feeding rates of *G. galatheanum*. We experimentally determined digestion rate constants using cultured *G. galatheanum* and cryptophyte prey, and examined the influence of temperature on U and the dependency of U on initial food vacuole contents. Furthermore, we estimated *in situ* grazing by *G. galatheanum* on cryptophytes using data on instantaneous food vacuole contents (cryptophytes dinoflagellate⁻¹), *in situ* temperature and experimentally determined digestion rates. The grazing impacts on cryptophyte prey populations by *G. galatheanum* and the potential nutritional contribution of ingested cryptophyte prey to the dinoflagellate were also considered.

METHOD

Cultures of organisms

The mixotrophic dinoflagellate *G. galatheanum* (Braarud) Taylor 1995 (strain GE, 10–15 µm), isolated from the mesohaline portion of Chesapeake Bay, was used for laboratory experiments. The phycoerythrin-containing cryptophyte *Stoeatula major* (strain g, 4–9 µm) was provided by Dr T. Kana at Horn Point Laboratory, University of Maryland, Cambridge, MD. Stock cultures of these two species were maintained at 20°C in f/2-Si medium (salinity = 10) (Guillard, 1975), with a light regime of 12:12 h light:dark (L:D) and a photon irradiance rate of 150 µmol photons m⁻² s⁻¹. The medium was made with filtered (GF/F) Sargasso Sea water (salinity = 37), diluted with deionized distilled water to achieve a salinity of 10. Light intensity was measured using a LI-COR LI-185B radiation sensor equipped with a flat LI-COR underwater probe.

Digestion experiments

The rate at which *G. galatheanum* digested cryptophyte prey was determined in time course, temperature-controlled

experiments using cultured *G. galatheanum* and *S. major*. Twelve laboratory experiments were conducted to determine the specific digestion rates (k) of *S. major* by *G. galatheanum* over a temperature range of 10–29.7°C (Table I). For all experiments, cultures of *G. galatheanum* in exponential growth were acclimated for 3–5 days at the incubation temperatures at which experiments were to be run. Different temperatures were obtained by using a temperature-controlled water bath equipped with a water cooling system. A light intensity of 200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, with a 12:12 h L:D cycle, was provided. Digestion experiments were conducted during the light periods.

In order to obtain feeding cells of *G. galatheanum*, cultures of the cryptophyte *S. major* were used as prey. Since the size of *S. major* cells ranges from 4 to 9 μm , these cultures were first filtered by gravity through 8 μm membrane filters to remove large cells (>8 μm). This process was to ensure that after the feeding incubations, residual prey cells could be efficiently removed by filtration through the same pore size membrane filters. Depending on the experiments, 50–100 ml of *S. major* culture with cell size <8 μm were added to the incubation bottle with 300 ml of *G. galatheanum*. The density of added cryptophytes varied, ranging from 3×10^3 to 1×10^5 cells ml^{-1} . Varying densities of added prey, combined with different incubation times and temperatures, were used to produce different initial food vacuole contents in the different experiments.

After addition of prey, *G. galatheanum* were allowed to feed for 2–4 h at the assigned temperature. *Gyrodinium*

galatheanum were then washed free of the residual cryptophyte prey using repeated reverse filtration through 8 μm membrane filters. In this process, 300 ml of incubated sample were gently reduced to ~30 ml and then returned to 300 ml using GF/F-filtered f/2-Si medium. The sample was carried through six additional washes, each representing a 1:10 dilution, and then brought to 900 ml using GF/F-filtered f/2-Si medium. During this wash process, the temperature was controlled to match that to which the dinoflagellate had been acclimated. Three milliliters of subsample were then taken from the manipulated sample to check for residual prey in the medium. By this filtration process, the density of the residual prey in medium was diluted $>3 \times 10^7$ times and >98–99% of the residual prey in the medium was removed. The entire filtration process required 2–3 h to be completed. *Gyrodinium galatheanum* appeared to be unharmed by the cleaning procedures as there was no detectable change in their swimming patterns compared to unmanipulated cultures. The ‘cleaned’ samples were then each divided into three aliquots and placed into triplicate, 500 ml polycarbonate bottles for determination of digestion time.

The samples in triplicate bottles were incubated under the assigned temperature and light intensity conditions as described above. A 10 ml aliquot was taken hourly from each bottle for 6 h, except for the experiments that were conducted at 21.5°C, in which a 10 ml aliquot was taken hourly for 5 h. Aliquots were fixed with cooled 1% glutaraldehyde and stored at 4°C. Fixed samples were

Table I: *Gyrodinium galatheanum*. Specific digestion rate constant (k) of cryptophyte by *G. galatheanum*

Temperature (°C)	Initial food vacuole contents (ingested cryptophytes G^{-1})	k (h^{-1})	t-test for k	r^2	No. rep. exp ⁻¹
10	0.44 (0.006)	0.004 (0.001)	$P < 0.05^*$	0.25	3
15	0.11 (0.001)	0.004 (0.001)	$P < 0.01^{**}$	0.56	3
20.1	0.87 (0.019)	0.044 (0.002)	$P < 0.01^{**}$	0.75	3
21	0.28 (0.008)	0.017 (0.002)	$P < 0.01^{**}$	0.64	3
21.5 ^a	0.28 (0.004)	0.016 (0.004)	$P < 0.01^{**}$	0.68	2
21.5 ^a	0.67 (0.015)	0.045 (0.004)	$P < 0.01^{**}$	0.76	3
21.5 ^a	0.42 (0.004)	0.032 (0.001)	$P < 0.01^{**}$	0.85	3
23	0.27 (0.005)	0.023 (0.001)	$P < 0.01^{**}$	0.83	3
23.2	0.51 (0.026)	0.035 (0.005)	$P < 0.01^{**}$	0.71	3
25	0.25 (0.016)	0.024 (0.003)	$P < 0.01^{**}$	0.88	3
25	1.73 (0.013)	0.081 (0.005)	$P < 0.01^{**}$	0.95	3
29.7	2.01 (0.042)	0.088 (0.009)	$P < 0.01^{**}$	0.84	3

Estimates determined from time course experiments at different temperatures using cultured *G. galatheanum* and *S. major*. Numbers in parentheses represent SE. G, *G. galatheanum*; *significant; **highly significant.

^aExperiments with a duration of 5 h, other experiments were run for 6 h.

thereafter 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 by epifluorescence microscopy (Zeiss filter set 487709, BP450–490 exciter filter, FT 510 dichromatic beam splitter and LP520 barrier filter) at room temperature ($\sim 20^{\circ}\text{C}$) (Li *et al.*, 1996).

The presence of ingested cryptophytes was recorded for the first 400 *G. galatheanum* encountered per slide, giving a total of 1200 cells per treatment examined. These data were used to calculate the mean number of recognizable cryptophytes in food vacuoles per *G. galatheanum* cell for each sampling time in each replicate. Data on the disappearance of ingested cryptophytes with time were used to calculate specific digestion rate constants (k) by linear regression analysis for each experiment. Influences of temperature and initial food vacuole content on digestion rate constants U were analyzed by plotting temperature against specific digestion rate constants (k) and plotting initial food vacuole content against k . The functional relationships between temperature and digestion rate constant U , and between initial food vacuole content and U , were fitted with linear, exponential and sigmoidal models to find the best fit. Combination effects of initial food vacuole contents and temperature on digestion rates were also estimated by multiple non-linear regression analysis.

Estimates of daily *in situ* ingestion rates and the impact of grazing by *G. galatheanum* on cryptophyte populations

We estimated *in situ* ingestion rates for *G. galatheanum* using equation (2) as described in the Introduction. The daily *in situ* ingestion rate [$\text{prey } (G. \text{galatheanum})^{-1} \text{ day}^{-1}$] is given by the product of the instantaneous food vacuole content [F : $\text{prey } (G. \text{galatheanum})^{-1}$] for the field *G. galatheanum* populations and the ingestion rate constant, $U(\text{h}^{-1}) \times 24$. Data on instantaneous food vacuole contents were derived from examination of field samples collected at main-stem stations on Chesapeake Bay during May through September 1995 and during April through September 1996 (Figure 1). The digestion rate constant (U) was determined by laboratory digestion experiments and corrected for ambient temperature. This approach assumes steady-state conditions (i.e. ingestion and digestion are in equilibrium).

Estimates of daily *in situ* clearance for discrete samples were calculated using the following equation:

$$CR = 1000 \times I/V \quad (3)$$

where CR ($\mu\text{l } G. \text{galatheanum } \text{day}^{-1}$) is the *in situ* clearance on a daily basis, I is the estimated daily ingestion rate

[cryptophytes ($G. \text{galatheanum})^{-1} \text{ day}^{-1}$] and V is the corresponding *in situ* prey density (cells ml^{-1}). This approach assumes that *G. galatheanum* fed on all phycoerythrin-containing cryptophytes and that there was no diel variation in *G. galatheanum* feeding.

Estimated ingested C, N and phosphorus (P) on a per cell basis by *G. galatheanum* are given by the product of the daily ingestion rate (I) and the average cellular content of C, N or P of cultured *S. major*. We assumed that the cellular content of cryptophyte prey consumed *in situ* is similar to that of cultured *S. major*. We used an average cellular C, N and P content of *G. galatheanum* and *S. major* derived from data given in Li *et al.* for phototrophically grown cells (Li *et al.*, 2000). Estimates of the potential daily contributions of ingested C, N and P to *G. galatheanum* are given by the following equation:

$$\% \text{ ingested body C, N or P} = \frac{100 \times (\text{cellular C, N or P content of prey}) \times I}{\text{cellular C, N or P content of } G. \text{galatheanum}} \quad (4)$$

The daily removal of cryptophyte standing stock by *G. galatheanum*, expressed as a proportion of prey abundance, was calculated as:

$$\% \text{ prey population removed day}^{-1} = \frac{100 \times I \times (G. \text{galatheanum } \text{ml}^{-1})}{\text{prey } \text{ml}^{-1}} \quad (5)$$

The cell abundances of *G. galatheanum* used here are from Li *et al.* (Li *et al.*, 2000b).

To estimate the effect of grazing by *G. galatheanum* on prey populations and the potential contribution of ingested prey to *G. galatheanum* nutrition in the mixed layer of Chesapeake Bay, depth-integrated values for abundances of *G. galatheanum* and cryptophytes, as well as the estimated values described above, were generated for the surface mixed layer of main-stem stations in the bay using data collected in 1995 and 1996 (Li *et al.*, 2000b). These values were calculated as depth-weighted averages of data for samples taken between the surface and the greater of two depths represented by either the pycnocline or the depth above which samples contained detectable *G. galatheanum*.

Statistical analyses

The relationships between the specific digestion rate constant k and temperature, and between k and initial food vacuole contents, were analyzed using Pearson's product moment correlation (SigmaStat Version 2.0, Jandel Scientific software). The same method was also applied to analyze the relationships between prey abundance and estimated *in situ* ingestion rates and *in situ* clearance rates. Linear and non-linear regressions were run using SigmaStat Version 2.0 (Jandel Scientific software).

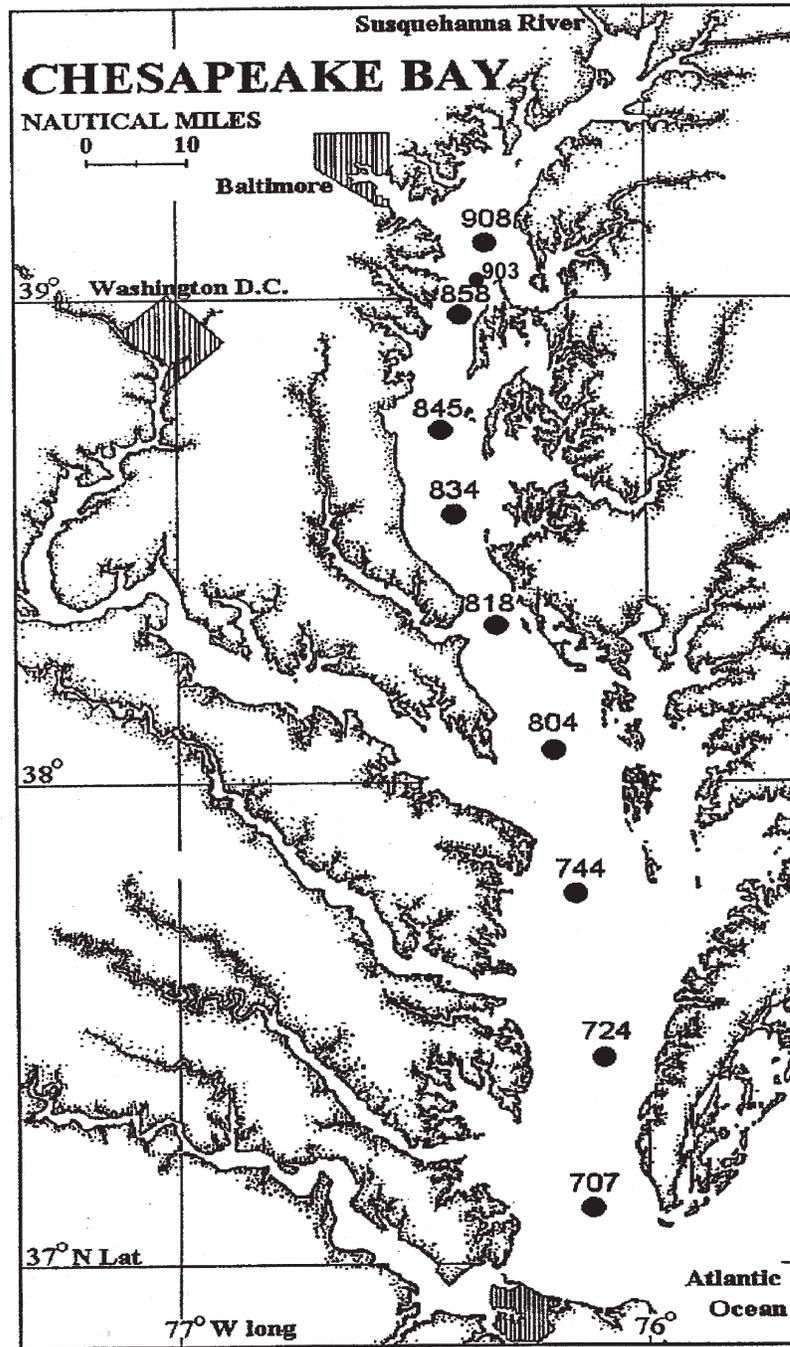


Fig. 1. Vicinity map showing main-stem stations (●) in Chesapeake Bay occupied in 1995 and 1996 cruises. Station designations from the south to the north are 707 (37°07'N; 76°07'W); 724 (37°24'N; 76°05'W); 744 (37°44'N; 76°11'W); 804 (38°04'N; 76°13'W); 818 (38°18'N; 76°17'W); 834 (38°34'N; 76°26'W); 845 (38°45'N; 76°26'W); 858 (38°08'N; 76°23'W); 903 (39°03'N; 76°21'W); 908 (39°08'N; 76°20'W).

RESULTS

Digestion experiments

Results from laboratory digestion experiments are summarized in Table I. Data for five of 12 digestion experiments are

presented graphically in Figure 2. For all experiments, mean ingested cryptophytes per *G. galatheanum* declined linearly over time within the experimental periods (5 h for three experiments conducted at 21.5°C and 6 h for nine experiments conducted within a temperature range of

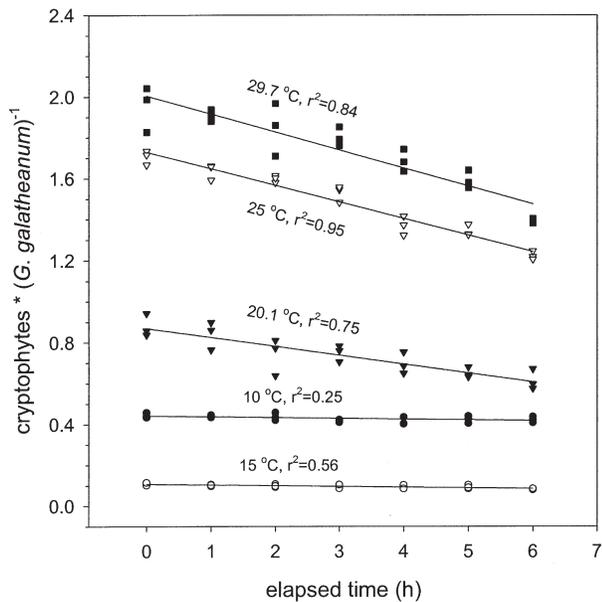


Fig. 2. Temporal decline in the number of ingested cryptophyte prey in *G. galatheanum*. Data are from five representative digestion experiments conducted at 10, 15, 20.1, 25 and 29.7°C. The results of the linear regressions are shown in Table I.

10–29.7°C). Linear regression models consistently fitted the data and gave specific digestion rate constants k (slopes of the linear regressions) at different temperatures and for *G. galatheanum* with different initial food vacuole contents (Table I; Figure 2). Values of these specific digestion rate constants ranged from a minimum of 0.004 h⁻¹ at 15°C for *G. galatheanum* with an average initial food vacuole level of 0.11 prey dinoflagellate⁻¹ to a maximum of 0.088 h⁻¹ at

29.7°C for *G. galatheanum* with an average initial food vacuole content of 2.01 prey dinoflagellate⁻¹ (Table I).

Digestion rate constants, irrespective of initial food vacuole contents, were strongly dependent on temperature (Figure 3). The relationship between digestion rate constant and temperature can be described by a simple exponential model given in Figure 3. We use the exponential model to fit our data here because effects of temperature on the metabolism of many protists often follow exponential function within their optimal temperature range (Caron *et al.*, 1990).

The digestion rate constant was also dependent on initial food vacuole content, particularly when the initial food vacuole contents were >0.5 cryptophytes (*G. galatheanum*)⁻¹ (Table III). The digestion rate constant, irrespective of temperature, increased with initial food vacuole content and can be described by a linear model given in Figure 4. A backward stepwise regression for the variables, including temperature (T), initial food vacuole content (iniFV), T^2 (iniFV)² and $T \times$ (iniFV), indicated that the specific digestion rate constant (k) could be predicted from a combination of temperature (T) ($P < 0.001$), initial food vacuole content (iniFV) ($P < 0.01$) and T^2 ($P < 0.01$):

$$k = -0.068 + 0.0067 \times T - 0.00014 \times T^2 + 0.041 \times \text{iniFV} \quad (6)$$

This model is highly significant ($r^2 = 0.96$ and $P < 0.01$).

Since instantaneous food vacuole contents in field populations of *G. galatheanum* were <0.47 cryptophytes (*G. galatheanum*)⁻¹, the relationships among digestion rate constant, temperature, and initial food vacuole constant were re-examined using laboratory data only from those

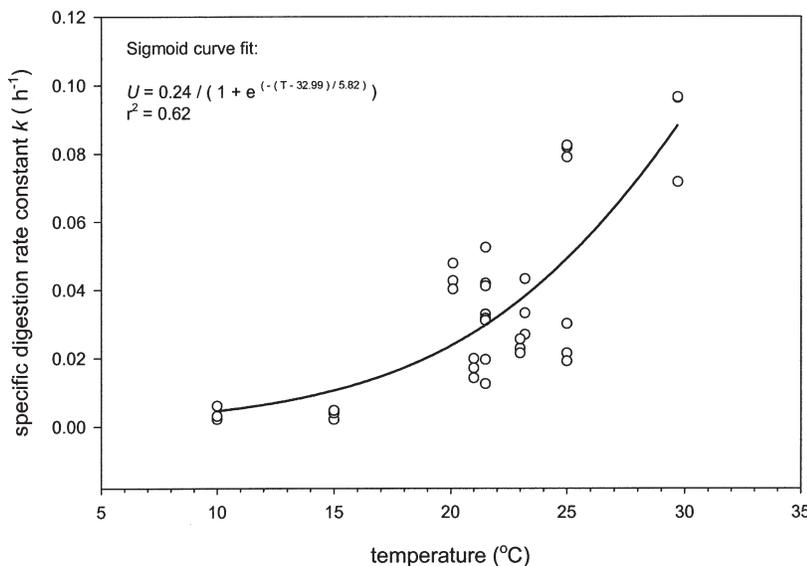


Fig. 3. Functional relationship between the specific digestion rate constant (k) and temperature (T) for *G. galatheanum*. The line represents the sigmoidal curve fit to the data by the equation shown at the top of the plot.

Table II: Average cellular C, N and P contents for cultured *G. galatheanum* and *S. major*

	pg C cell ⁻¹	pg N cell ⁻¹	pg P cell ⁻¹	Growth conditions
<i>G. galatheanum</i>	101.83 (1.17)	18.73 (0.35)	1.64 (0.04)	Grown in media with varying nitrate and phosphate concentrations at 20°C under a light intensity of 200 μmol photons m ⁻² s ⁻¹ and with a D:L cycle of 12:12 h. Sample size = 27. Data from Li et al. (2000a)
<i>S. major</i>	47.01 (1.11)	9.41 (0.32)	1.29 (0.09)	Grown in f/2-Si medium at 20°C under a light intensity of 150 μmol photons m ⁻² s ⁻¹ and with a D:L cycle of 12:12 h. Sample size = 6. Data from Li et al. (2000a)

Numbers in parentheses represent SE.

Table III: Summary of statistical models fitted to the data on digestion rate constant versus temperature

Model	F	r ²
(1) $U = -0.051 + 0.004 \times T$	38.6**	0.54
(2) $U = 0.002 \times e^{(0.134 \times T)}$	52.31**	0.61
(3) $U = 0.243 / (1 + e^{-(T - 32.99) / 5.82})$	26.35**	0.62

Sample size = 35; U = digestion rate constant (h⁻¹); T = temperature (°C); F = statistic for regression. **P < 0.01.

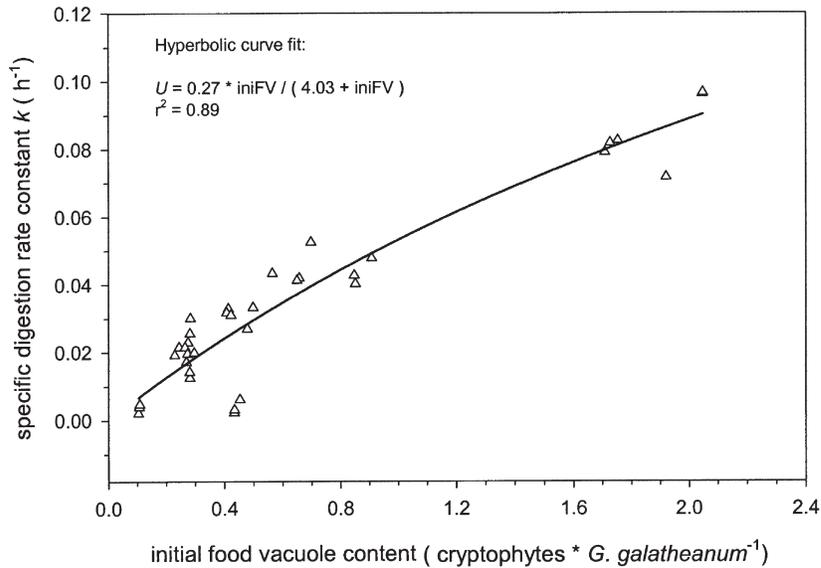


Fig. 4. Functional relationship between the specific digestion rate constant (*k*) and the initial food vacuole content (iniFV) for *G. galatheanum*. The line presents a hyperbolic fit to the data using the equation shown at the top of the plot.

Table IV: Pearson product moment correlation analysis. Relationship of specific digestion rate constant (k) to temperature (T) and initial food vacuole contents

Specific digestion rate constant k , calculated for:	Temperature (°C)			Initial food vacuole content		
	r	P	n	r	P	n
Full range of initial food vacuole contents	0.73	<0.01**	35	0.94	<0.01**	35
Only initial food vacuole contents of <0.47 cryptophytes dinoflagellate ⁻¹	0.81	<0.01**	20	0.29	0.21 ns	20

n , sample size; **highly significant; ns, not significant.

Table V: Summary of statistical models fitted to the data on digestion rate constant versus temperature

Model type	Equation	F	r^2	Q_{10} (15–30°C)
Linear	(1) $U = -0.016 + 0.002 \times T$	34.03**	0.65	2.35
Simple exponential	(2) $U = 0.002 \times e^{(0.11 \times T)}$	24.44**	0.58	3.05
Sigmoidal	(3) $U = 0.025 / (1 + e^{-(T - 17.95) / 1.73})$	27.12**	0.76	3.49

Only those data with initial food vacuole contents of ≤ 0.47 cryptophytes (*G. galatheanum*)⁻¹ were included for the following regressions. F -test indicates that the sigmoidal model fits the data better than the linear model ($F = 4.26$, $P < 0.05$) and the simple exponential model ($F = 6.59$, $P < 0.05$). In this table, U represents digestion rate constant (h^{-1}), T represents temperature (°C), F represents statistic for regression and ** indicates that the regression is significant ($P < 0.01$). Sample size = 20.

experiments in which initial food vacuole contents were < 0.47 cryptophytes dinoflagellate⁻¹. Pearson product moment correlation analysis showed that digestion rate constant was significantly correlated with temperature, but not with initial food vacuole contents for this subset of experiments (Table IV). This result indicated that the food vacuole content method was suitable only when the initial food vacuole content was < 0.47 cryptophytes (*G. galatheanum*)⁻¹, because this method requires that the digestion rate U is independent of initial food vacuole content. Further analysis for this subset of data indicated that the following sigmoidal model could best describe the relationship between digestion rate constant (U) and temperature (T) compared with the linear and exponential models (Table V):

$$U = 0.025 / (1 + e^{-(T - 17.95) / 1.73}) \quad (7)$$

This sigmoidal model is highly significant ($P < 0.01$) and 76% of variance can be explained by temperature (Table V). This empirical equation, combined with equation (2) as described in the Introduction, was applied to estimate

in situ ingestion rates using data on instantaneous food vacuole contents and *in situ* temperatures.

Estimates of *in situ* ingestion rate and clearance rate of cryptophytes by *G. galatheanum*

For samples collected in 1995 and 1996, *G. galatheanum* was estimated to ingest an average of 0.02 cryptophytes day⁻¹ with maximal rates reaching 0.26 prey day⁻¹ (Table VI). When laboratory-measured cellular C, N and P contents for cultured *S. major* (Table II) were used to infer the cellular C, N and P contents for field cryptophyte populations, average ingestion rates of 0.91 pg C dinoflagellate⁻¹ day⁻¹ with a maximal value of 12.39 pg C dinoflagellate⁻¹ day⁻¹, an average of 0.18 pg N dinoflagellate⁻¹ day⁻¹ with a maximal value of 2.48 pg N dinoflagellate⁻¹ day⁻¹, and an average of 0.03 pg P dinoflagellate⁻¹ day⁻¹ with a maximal rate of 0.34 pg P dinoflagellate⁻¹ day⁻¹ were calculated. Correspondingly, *G. galatheanum* was estimated to clear an average of 0.02 μ l day⁻¹ with maximal clearance rates up to 0.27 μ l dinoflagellate day⁻¹ (Table VI).

Table VI: *Gyrodinium galatheanum*. Abundance and estimated grazing rates on phycoerythrin-containing cryptophytes

	Abundance (cells ml ⁻¹)	Ingestion				Clearance (μl G ⁻¹ day ⁻¹)
		Prey G ⁻¹ day ⁻¹	pg C G ⁻¹ day ⁻¹	pg N G ⁻¹ day ⁻¹	pg P G ⁻¹ day ⁻¹	
Range	>1–4040	0–0.26	0–12.39	0–2.48	0–0.34	0–0.27
Mean	142	0.02	0.91	0.18	0.03	0.02
SE	22	0.002	0.11	0.02	0.003	0.002
<i>n</i>	302	302	302	302	302	302

G, *Gyrodinium galatheanum*. Discrete samples for 1995 (May–September) and 1996 (April–September) cruises.

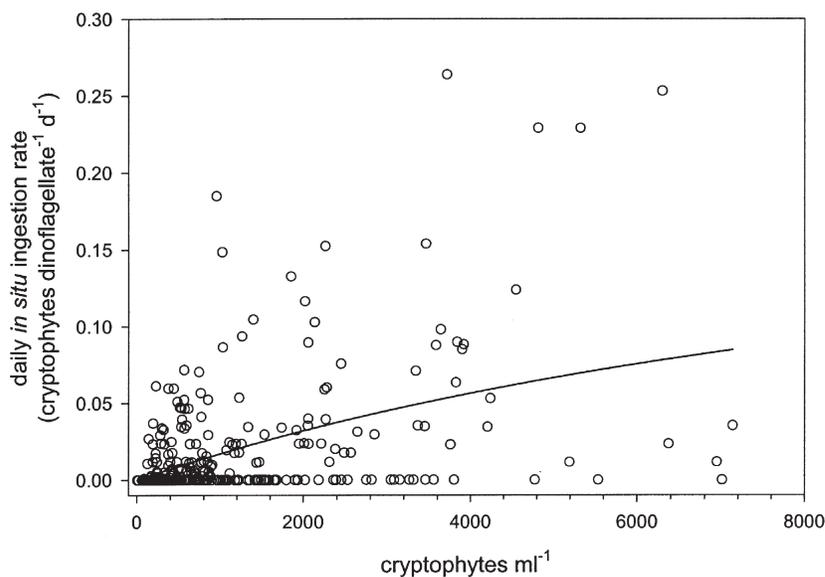


Fig. 5. *Gyrodinium galatheanum*. Estimated daily *in situ* ingestion rate (I) as a function of prey abundance (F). The line represents a hyperbolic (Michaelis–Menten) curve fit to the ingestion data by the equation $I = 0.23 \times F / (12\,580 + F)$ ($r^2 = 0.19$, $P < 0.01$).

In situ ingestion rates of *G. galatheanum* on cryptophytes were strongly influenced by prey abundance (Figure 5). The functional relationship between ingestion rates and prey abundance could be described by a hyperbolic (Michaelis–Menten) equation, as shown in Figure 5 ($P < 0.01$).

Seasonal and spatial variations of estimated ingestion and clearance rates, as well as abundances of *G. galatheanum* and cryptophytes, for 1995 and 1996 cruises are presented for depth-integrated data in Figures 6 and 7. Depth-integrated data indicate that *G. galatheanum* occurred at high abundances (>400 cells ml⁻¹) in the upper bay during late spring and early summer in 1995 (Figure 6A). Cryptophyte abundances showed substantial

fluctuation spatially, although relatively high abundances could be found through the entire bay during the cruises in 1995 (Figure 6B). In contrast, relatively high ingestion and clearance rates were limited to the upper bay during May through September (Figures 6C and 7A).

Depth-integrated data also indicate that relatively high abundances of *G. galatheanum* (>60 cells ml⁻¹) occurred in the mid-bay during summer and in the upper bay during early fall in 1996 (Figure 6D). Depth-integrated abundances of cryptophytes showed different seasonal trends from *G. galatheanum* abundance. In the upper bay, high abundance of cryptophytes (>1500 cells ml⁻¹) was associated with low abundance of *G. galatheanum* (<30 cells ml⁻¹) during spring (Figure 6D and E), whereas relatively low

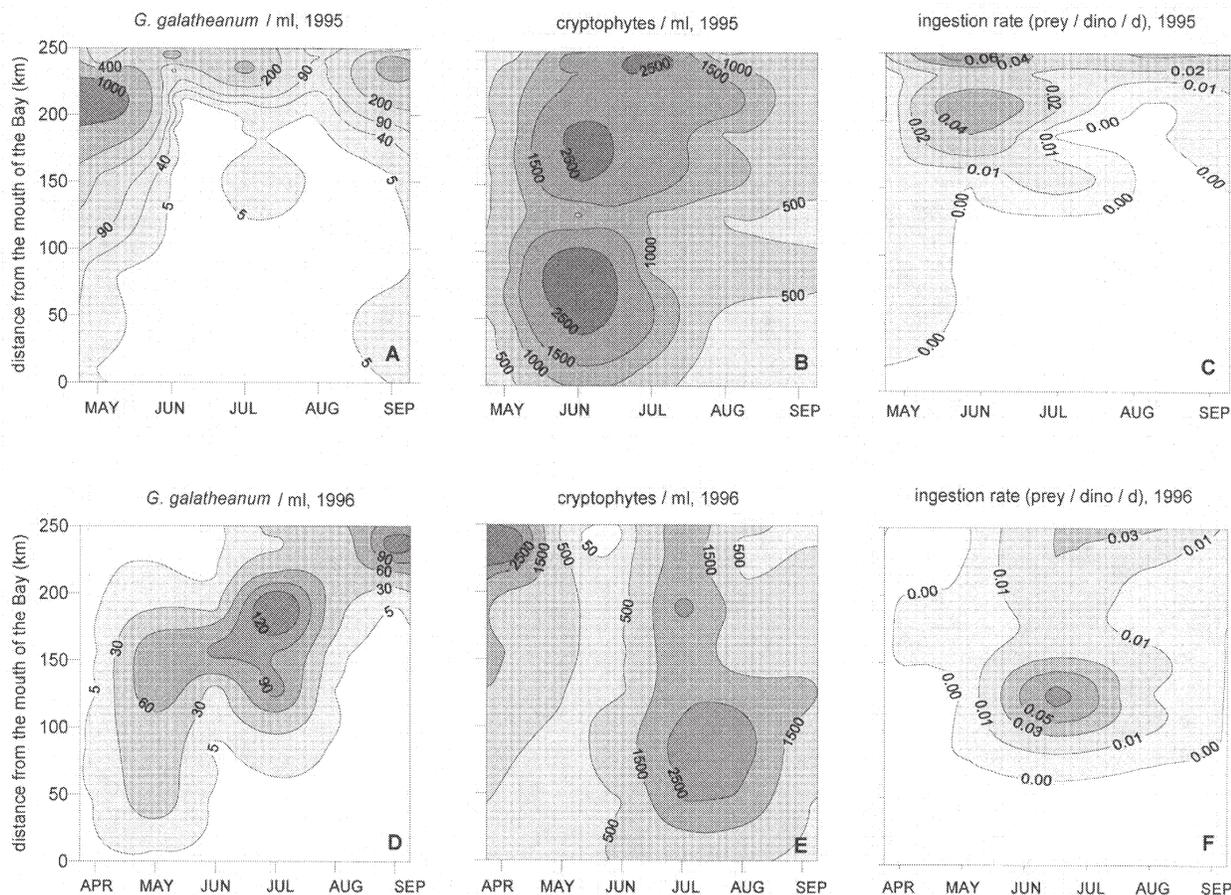


Fig. 6. Depth-integrated abundances of *G. galatheanum* and cryptophytes, and *in situ* ingestion rates of cryptophytes by *G. galatheanum* in Chesapeake Bay during May–September 1995 are presented in (A), (B) and (C), respectively. Depth-integrated values for these variables during April–September 1996 are shown in (D), (E) and (F), respectively.

abundance of cryptophytes (<500 cells ml⁻¹) was associated with relatively high abundance of *G. galatheanum* (>60 cells ml⁻¹) during early fall (Figure 6D and E). Depth-integrated data indicate that relatively high ingestion and clearance rates occurred during summer 1996, but showed substantial fluctuation spatially (Figures 6F and 7C). Interestingly, these rates were lowest during spring in the upper bay even though this was when and where cryptophyte abundances were highest (Figures 6F and 7C).

Grazing impact on cryptophyte prey populations

Estimates of the impact of feeding by *G. galatheanum* on cryptophyte populations in Chesapeake Bay are given in Table VII (discrete samples), while depth-integrated data on seasonal and spatial variations of grazing impact are presented in Figure 7B and D. Averaged discrete data show that *G. galatheanum* could remove only a small proportion (0.23%) of cryptophyte standing stock daily in Chesapeake Bay, although maximal removal values for

discrete samples could reach 4.1% of cryptophyte standing stock daily (Table VII).

Depth-integrated data indicated a striking up-bay increase in percentage removal of cryptophyte prey in 1995, with relatively high values (>1%) occurring during late spring and early summer in the upper bay (Figure 7B). Compared with the data for 1995, lower daily removal of cryptophyte populations by *G. galatheanum* (<0.5%) and more noticeable spatial and temporal fluctuations of grazing impact were observed in 1996 (Figure 7D).

Potential contribution of feeding on cryptophytes to *G. galatheanum* nutrition

Estimates of the potential contribution of ingested cryptophytes to *G. galatheanum* nutrition were expressed as the percentage ratio of daily ingested C, N and P to cellular C, N and P content of *G. galatheanum*. The estimated values from discrete samples show that the maximum consumption rates were equivalent to 12.2, 13.2% and 20.8% of *G. galatheanum* body C, N and P, respectively (Table VII).

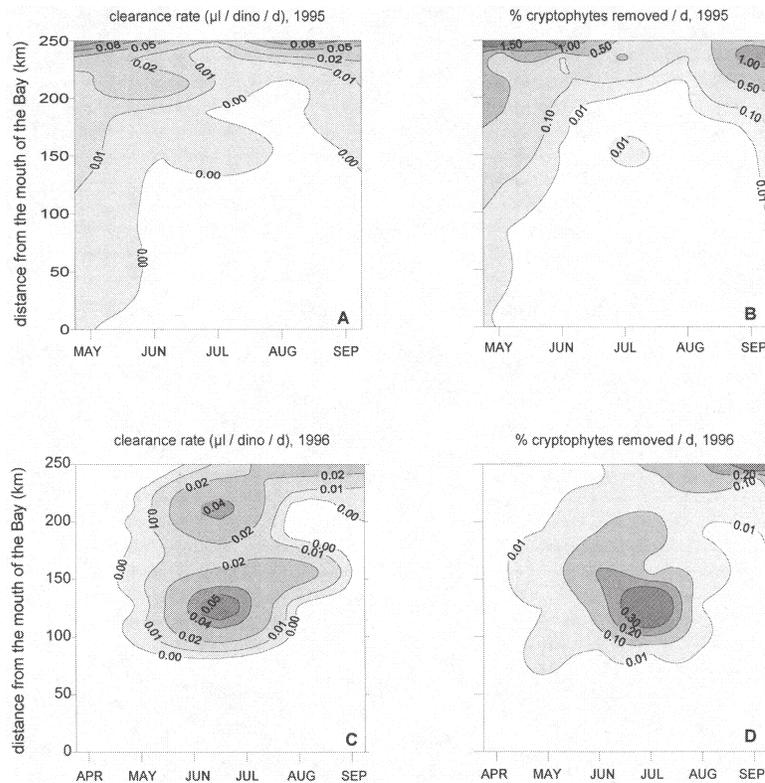


Fig. 7. Depth-integrated *in situ* clearance rates of *G. galatheanum* and daily removal of cryptophyte biomass by *G. galatheanum* populations in Chesapeake Bay during May–September 1995 are represented in (A) and (B), respectively. Depth-integrated values for these variables during April–September in 1996 are shown in (C) and (D), respectively.

Table VII: Estimated biomass-specific ingestion of cryptophytes by G. galatheanum and impact of grazing by G. galatheanum on cryptophyte populations. Discrete samples for 1995 (May–September) and 1996 (April–September) cruises

	Estimated C, N and P specific ingestion			Cryptophyte abundance (cells ml ⁻¹)	% cryptophyte standing stock removed day ⁻¹
	% body C day ⁻¹	% body N day ⁻¹	% body P day ⁻¹		
Range	0–12.2	0–13.2	0–20.8	0–7136	0–4.1
Mean	0.9	1.0	1.5	1219	0.23
SE	0.11	0.11	0.18	76	0.03
n	302	302	302	302	302

The relatively high potential contribution of ingestion to cellular P is due to the low C:P (47.01:1.29) ratio in cryptophytes (Table II).

Depth-integrated data on the potential contribution of predation on cryptophytes to *G. galatheanum* nutrition are presented in Figure 8. The potential contributions of C, N and P to *G. galatheanum* appear to be more pronounced

in the upper bay than in the mid and lower bay during cruises in 1995 (Figure 8A–C). Calculated values for 1996 indicate that ingestion of cryptophytes contributed more C, N and P to *G. galatheanum* during summer than other seasons (Figure 8D–F). Spatial fluctuations of these values were striking for the 1996 cruises, with the potential contribution of C, N and P from ingestion being relatively

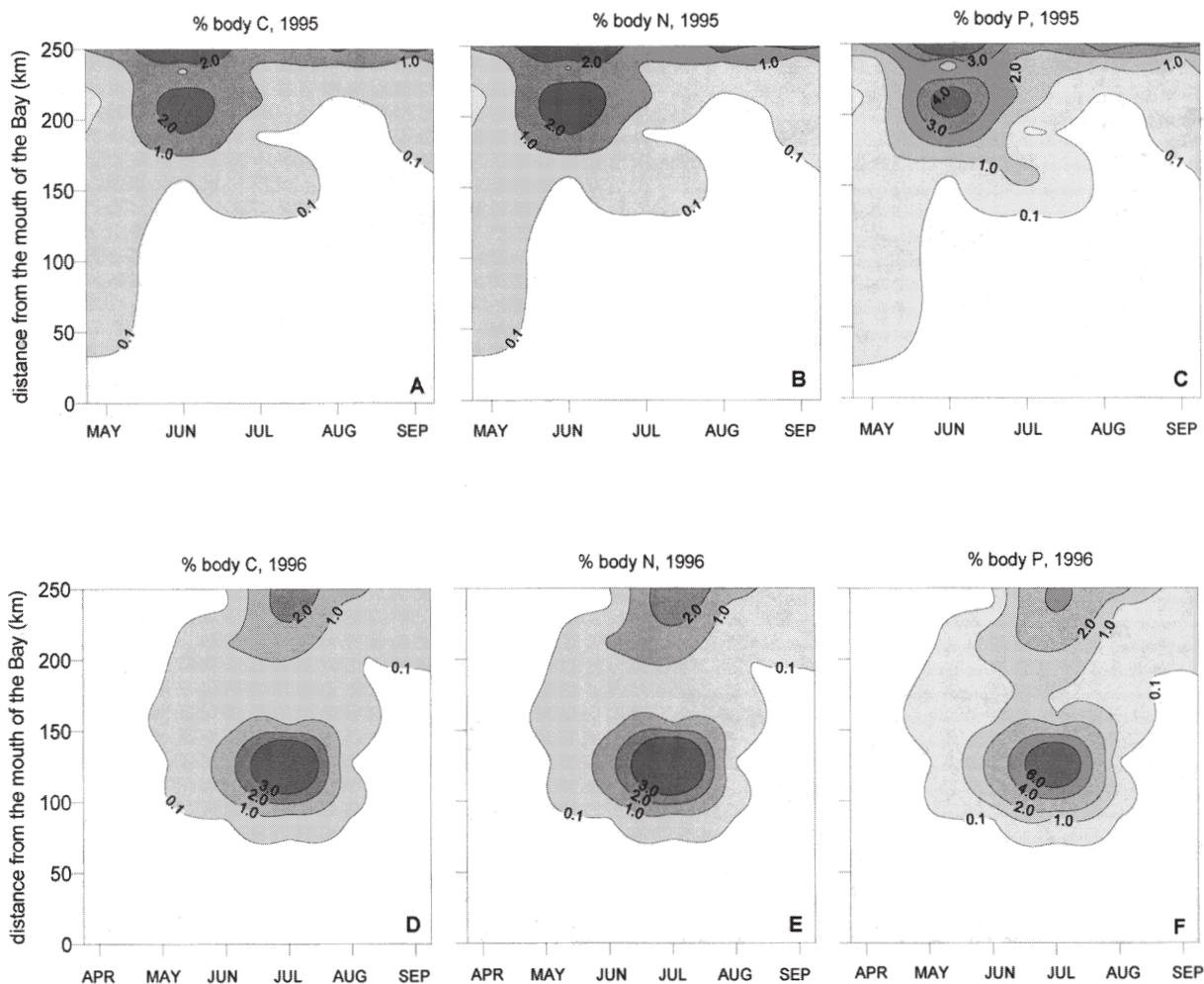


Fig. 8. Depth-integrated, biomass-specific, daily consumption of cryptophyte C, N and P by *G. galatheanum* in Chesapeake Bay during May–September 1995 (**A**, **B** and **C**, respectively). Depth-integrated values for these variables during April–September 1996 are shown in (**D**), (**E**) and (**F**), respectively.

high (>2, >2 and 6% for C, N and P, respectively) at several stations in the mid and upper bay (Figure 8D–F).

DISCUSSION

Experimental digestion data presented in this study showed that ingested prey (cryptophytes) in *G. galatheanum* were digested linearly with time (Figure 2; Table I). Other studies, which have focused on heterotrophic flagellates and ciliates, have found that digestion of food vacuole content is linear with time (Fenchel, 1975; Sherr *et al.*, 1988; Capriulo and Degnan, 1991). In contrast, Bockstahler and Coats (1993b) reported that the digestion of ciliate prey by the mixotrophic dinoflagellate *G. sanguineum* followed an exponential decay model. Exponential decay patterns of prey digestion are also found in

several heterotrophic ciliates (Berger and Pollock, 1981; Fok and Shockley, 1985; Dolan and Coats, 1991). It was proposed that these types of patterns were due to food items being randomly mixed during digestion (Dolan and Coats, 1991, 1997). This theory was based on the fact that in some protozoa the food vacuoles that contained digested prey fuse with the food vacuole that contained newly ingested prey (Dolan and Coats, 1991; Kaneshiro *et al.*, 1992). In *G. galatheanum*, however, we found that the digestion of food items followed linear patterns, suggesting that food items were digested in a manner of ‘first in, first out’. Theoretically, this sequential digestion should be more efficient than randomly mixed digestion in terms of utilization of energy and material (Penry and Jumars, 1986, 1987).

Digestion rates of cryptophytes by *G. galatheanum*

ranged from 0.004 to 0.088 prey (*G. galatheanum*)⁻¹ h⁻¹ under experimental conditions (Table I). The corresponding half-life of food vacuoles ranged from 5.7 h at 29.7°C to 139 h at 10°C. The long half-life of food vacuoles in *G. galatheanum* at 10°C may reflect the possibility that at low temperatures *G. galatheanum* can retain chloroplasts from its prey. Retention of functional chloroplasts derived from algal prey, or 'cleptoplastidy', is common in dinoflagellates (Schnepf and Elbrächter, 1992).

The rates at which the cryptophytes were digested by *G. galatheanum* depended on temperature (Figure 3). Dependency of digestion rate on temperature is expected, because digestive processes, including synthesis and recycling of food vacuole membranes, are enzyme controlled (Capriulo and Degnan, 1991). Calculation of Q_{10} from a modified sigmoidal model of digestion rate constant versus temperature gave a value of 3.49 over a temperature range of 15–30°C (Table V). This value is comparable to those for heterotrophic protists. A range of 1.5–10 of Q_{10} factors for food vacuole processing has been reported for ciliates (Dolan and Coats, 1991). For example, Fenchel reported Q_{10} values of 3 and 10 over a temperature range of 10–25°C for the algivorous *Stylonychia mytilus* and bacterivorous *Tetrahymena pyriformis*, respectively (Fenchel, 1975).

Our experimental data indicate that an increase in the number of food vacuoles per *G. galatheanum* cell was associated with a decrease in vacuole processing time (i.e. an increase in disappearance rates of food vacuoles) (Table IV; Figure 4). There are at least two possible explanations for this result. First, it is possible that more enzymes are added and that the digestion process is faster in cells with more food vacuoles. Secondly, the food vacuole contents may be digested at the same rate regardless of food vacuole number, but the encounter rate between food vacuoles and the site of defecation may increase, which in turn increases the possibility of a particular food vacuole being defecated. In the second case, the cells would not assimilate as much C, N and P per prey item as at low food vacuole numbers as the prey would only be partially digested when defecated. The consequence of either of these two possibilities would be that more membrane would be available to form new food vacuoles.

When the number of food vacuoles was low, the vacuole processing time was unrelated to the number of food vacuoles per dinoflagellate (Table IV). Under this condition, the probability of defecation does not have a major influence on food vacuole processing time. As a result, temperature, a major factor that controls digestive enzyme activity, becomes the dominant element in determining food vacuole processing time.

The estimates of *in situ* rates of ingestion and clearance, based on instantaneous food vacuole contents and

experimentally determined digestion rate constants that were corrected by *in situ* temperature, gave a maximal ingestion rate of 0.26 prey dinoflagellate⁻¹ day⁻¹ and a maximal clearance rate of 0.27 µl dinoflagellate⁻¹ day⁻¹. Estimated *in situ* grazing rates were positively correlated with prey abundance ($r = 0.43$, $P < 0.01$), while there was no significant relationship between clearance rates and prey abundance ($r = -0.08$, $P = 0.17$). These results may reflect the fact that the *in situ* rates of ingestion and clearance were not only influenced by prey abundance, but also controlled by physical (e.g. temperature and light intensity), chemical (e.g. inorganic nutrients) and biological (e.g. prey selection) factors (Li *et al.*, 2000a). For example, we found that high light intensity and low ambient concentrations of inorganic nutrient (nitrate or phosphate) could stimulate ingestion of cryptophytes by *G. galatheanum* (Li *et al.*, 2000a).

Another mixotrophic dinoflagellate in Chesapeake Bay, *G. sanguineum*, feeds on <20 µm oligotrichine ciliates and had *in situ* clearance rates of 0–5.8 µl dinoflagellate⁻¹ h⁻¹, or 0–139.2 µl dinoflagellate⁻¹ day⁻¹ (Bockstahler and Coats, 1993b). Considerably lower *in situ* clearance rates, 0–0.27 µl dinoflagellate⁻¹ day⁻¹ for *G. galatheanum*, are reported here. These low values may reflect the fact that *G. galatheanum* selectively feeds on some species over others, even within the same category of prey [i.e. phycoerythrin-containing cryptophytes (Li *et al.*, 1996)]. In addition, the relatively low clearance of *G. galatheanum* compared to *G. sanguineum* is probably due to its smaller size, since it is very common that clearance and ingestion rates decrease with decreasing predator volume (Hansen *et al.*, 1997). We found that the mean cell volume of cultured *G. galatheanum* was 418 µm³ cell⁻¹ (Li *et al.*, 2000a), while *G. sanguineum* in nutrient-replete cultures is 11-fold larger [4735 µm³ cell⁻¹ (Doucette and Harrison, 1991)] than *G. galatheanum*.

At maximal rates, *G. galatheanum* was capable of ingesting 12.4 pg C, 2.5 pg N and 0.3 pg P daily (Table VI). These calculations were based on data on cellular C, N and P contents derived from a cultured cryptophyte (*S. major*), assuming that the chemical compositions of cultured cryptophytes represent the chemical compositions of field cryptophytes. C and N contents in our cultured cryptophyte (Table II) were similar to those reported for other cryptophytes of similar size (Verity *et al.*, 1992; Montagnes *et al.*, 1994). Although *S. major* was originally isolated from Chesapeake Bay and is a dominant phycoerythrin-containing cryptophyte species in the bay (Marshall, 1994), cellular contents of cultured cryptophytes may vary with culture conditions and differ from field cryptophytes to some extent. Extrapolating these data to field cryptophytes may have resulted in some biases. Also, *G. galatheanum* ingests non-cryptophyte prey as

well as cryptophyte prey (Li *et al.*, 1996), so total daily ingestion of C, N and P may have been higher. Our estimate, based on cryptophyte prey, is therefore a minimum estimate of total ingestion.

Daily ingestion of cryptophyte biomass by *G. galatheanum* averaged 0.9% of its body C with a maximal value of 12.2%, 1.0% of its body N with a maximal value of 13.2%, and 1.5% of its body P with a maximal value of 20.8%. These mean values for *G. galatheanum* appear to be relatively low compared with those for *G. sanguineum* (Bockstahler and Coats, 1993b), but these mean values represented averages of data over 2 years. Depth-integrated data indicate that the potential contribution of ingested C, N and P to *G. galatheanum* biomass varied temporally and spatially, with maximal values occurring in the mid to upper bay during summer of 1995 and 1996 (Figure 8).

Under optimally phototrophic growth conditions, the doubling time for *G. galatheanum* is ~2 days (Li *et al.*, 1999). At maximal growth and ingestion rates, and assuming a gross growth efficiency (GGE) of 40% based on C (Fenchel, 1987; Strom, 1991), *G. galatheanum* could meet 10% of its C, 11% of its N and 17% of its P requirements for reproduction through phagotrophy. However, under nutrient-limiting conditions, protistan grazers can excrete very little N or P, and GGE for these elements may be considerably higher than for C (Caron and Goldman, 1990). Thus, *G. galatheanum* may be gaining >11 and 17%, respectively, of its N and P from grazing. These budgetary considerations support our previous hypothesis that feeding may supplement nutrient requirements for *G. galatheanum* bloom formation and persistence during summer in the mid and upper bay where inorganic nutrients were potentially limited in supply (Li *et al.*, 1999, 2000b). However, because mixotrophic growth rates of this dinoflagellate are higher than phototrophic growth rates even in nutrient-replete media (Li *et al.*, 1999), phagotrophy may contribute to the growth of this dinoflagellate in the bay even when inorganic nutrients are not limiting.

In Chesapeake Bay, the distribution of inorganic nutrient inputs varies spatially and temporally (Fisher *et al.*, 1992; Malone *et al.*, 1996). This variability in resources may favor mixotrophs over strict autotrophs or heterotrophs (Stoecker *et al.*, 1997). Theoretically, since high nutrient inputs can lead to high standing stocks of phytoplankton, increased phytoplankton biomass may also lead to severe competition for limiting inorganic nutrients when the rate of input decreases. For example, high abundances of *G. galatheanum* often co-occurred with high abundances of its prey, cryptophytes, during summer in the upper bay where the phosphate concentration was low (Li *et al.*, 2000b). In addition, this dinoflagellate may not be able to compete with its prey in term of utilization of inorganic nutrients since cryptophytes are smaller in size

and smaller size cells usually have an advantage in competition for inorganic nutrients (Malone, 1980). Under these highly competitive conditions, 'eating your competitor' (Thingstad *et al.*, 1996) appears to be an adaptive strategy for this mixotrophic dinoflagellate.

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