Vol. 78: 103–113, 1991 | MARINE ECOLOGY PROGRESS SERIES
Mar. Ecol. Prog. Ser. | Published December 16

Growth and grazing rates of the herbivorous dinoflagellate *Gymnodinium* sp. from the open subarctic Pacific Ocean

Suzanne L. Strom*

School of Oceanography WB-10, University of Washington, Seattle, Washington 98195, USA

ABSTRACT: Growth, grazing and cell volume of the small heterotrophic dinoflagellate *Gymnodinium* sp. isolated from the open subarctic Pacific Ocean were measured as a function of food concentration using 2 phytoplankton food species. Growth and ingestion rates increased asymptotically with increasing phytoplankon food levels, as did grazer cell volume; rates at representative oceanic food levels were high but below maxima. Clearance rates decreased with increasing food levels when *Isochrysis galbana* was the food source; they increased with increasing food levels when *Synechococcus* sp. was the food source. There was apparently a grazing threshold for ingestion of *Synechococcus*: below an initial *Synechococcus* concentration of 20 µgC l^{-1} ingestion rates on this alga were very low, while above this initial concentration *Synechococcus* was grazed preferentially. Gross growth efficiency varied between 0.03 and 0.53 (mean 0.21) and was highest at low food concentrations. Results support the hypothesis that heterotrophic dinoflagellates may contribute to controlling population increases of small, rapidly-growing phytoplankton species even at low, oceanic phytoplankton concentrations.

INTRODUCTION

Heterotrophic dinoflagellates can be a significant component of the microzooplankton in marine waters. In the oceanic realm, Lessard (1984) and Shapiro et al. (1989) found heterotrophic dinoflagellates to be abundant in the North Atlantic, while these organisms can be numerous at least seasonally in the subarctic North Pacific (Taylor & Waters 1982, Strom & Welschmeyer 1991, B. Booth pers. comm.). Nearshore waters can also support sizable populations of heterotrophic dinoflagellates (e.g. Gifford 1988, Weisse & Scheffel-Möser 1990, Hansen 1991) which at times can be as large as populations of ciliated protozoans (Smetacek 1981, Garrison & Buck 1989, Lessard 1991).

Despite their potential importance, there have been few experimental laboratory studies of heterotrophic dinoflagellates. Distinguishing photosynthetic from heterotrophic species of common marine genera such as *Gymnodinium* and *Gyrodinium* is difficult or impossible using older preservation and microscopy techniques; experimental emphasis has been on more easily recognizable and collectable microzooplankton groups such as tintinnid ciliates. Swimming and feeding behaviors of heterotrophic dinoflagellates, however, differ substantially from those of ciliated protozoans, and the 2 groups of organisms may have very different responses to a given set of environmental conditions.

Many species of heterotrophic dinoflagellates are known to feed herbivorously (e.g. Lessard & Swift 1985, Jacobsen & Anderson 1986, Gaines & Elbrächter 1987, Buck et al. 1990). When abundant, such species may have a significant impact on phytoplankton populations. Lessard (1991) calculated that the dinoflagellate *Oblea rotunda* could have cleared up to 53 % of the daily primary production following a spring bloom in Chesapeake Bay, while Sherr et al. (1991) determined that > 5 µm flagellates (probably primarily dinoflagellates) cleared a larger proportion of the water in a salt marsh estuary than did the ciliate population in a

* Present address: Marine Science Institute, University of Texas at Austin, Port Aransas, Texas 78373-1267, USA

0171-8630/91/0078/0103/$ 03.00

third of their experiments. In Antarctic waters, herbivory by *Gymnodinium* sp. accounted for nearly all grazing losses to the natural phytoplankton assemblage in microcosm experiments (Bjørnsen & Kuparinen 1991). Given that heterotrophic dinoflagellates may be at least seasonally abundant and active as herbivores, their behavioral responses comprise an important part of the behavior of the entire microzooplankton community.

In nutrient-rich areas of the open ocean, the mechanisms that control phytoplankton biomass are not well understood (e.g. Banse 1990, Martin et al. 1990). For the open subarctic Pacific in particular, a long history of oceanographic research centered on Weather Station P (50° N, 145° W) has indicated that phytoplankton biomass remains low and nearly constant year-round, in spite of high nutrient concentrations and seasonal increases in primary production (Miller et al. 1988 and references therein). Recently, it has been hypothesized that microzooplankton grazing is responsible for maintaining phytoplankton standing stocks at observed low levels (Evans & Parslow 1985, Frost 1987, Miller et al. 1988). If true, individual microzooplankton species must have significant grazing rates at low natural food concentrations. Potential population growth rates must be nearly equivalent to those of phytoplankton to maintain low plant standing stocks in the face of increases in primary production. In this study, a herbivorous heterotrophic dinoflagellate was isolated from the open subarctic Pacific, and growth and grazing rates of this species were measured over a range of food concentrations. Grazer cell size and gross growth efficiency were also determined.

MATERIALS AND METHODS

Culture methods. The dinoflagellate was isolated from water collected at Station P in late September 1987. Water collected from the mixed layer using a Teflon-lined 30 l Go-Flo bottle was placed in acid-cleaned (10 % HCl), Milli-Q-rinsed 2 l polycarbonate bottles covered with one layer of neutral density screening and stored in an incubator cooled by flowing surface seawater during transit to shore (ca 3 d). Most bottles were enriched by additions of 2 or 3 species of cultured phytoplankton to encourage growth of herbivorous protozoans.

Laboratory isolation and culture maintenance procedures in general followed Gifford (1985). Protozoan culture medium was made by passing seawater (collected from the mixed layer at Station P) through a Gelman A/E filter followed by a 0.45 µm Nuclepore filter in a 147 mm Plexiglas filter holder A peristaltic pump fitted with silicone tubing was used for filtration. Trace metals and chelator were then added (Gifford 1985) and the medium was autoclaved and stored in 2 l polycarbonate bottles.

Cultures were maintained in 250 ml polycarbonate flasks that had been acid-cleaned (10 % HCl), Milli-Q rinsed, and autoclaved while containing about 150 ml Milli-Q water. The dinoflagellate was kept at 12 °C (= late summer mixed layer water temperature at Station P) on a 15 h light : 9 h dark cycle; irradiance was dim (≤ 5 µEin m^{-2} s^{-1}). The food supply was a mixture of 4 phytoplankton species (*Isochrysis galbana*, *Emiliania huxleyi*, *Micromonas pusilla*, *Synechococcus* sp. [strain DC-2]) on which the grazer grew best during isolation in multi-well plates. Protozoa were fed every 5 to 6 d with exponential-phase phytoplankon cultures and were transferred every 2 to 3 wk. Care was taken to maintain *Gymnodinium* sp. stocks on relatively low levels of food to prevent possible adaptation to unnaturally high phytoplankton concentrations.

Phytoplankton were grown in 250 ml borosilicate flasks on IMR medium (Eppley et al. 1967), with the exception of *Synechococcus* sp. and *Emiliania huxleyi*, which were grown on IMR medium diluted 1 : 4 with sterile filtered seawater. Cultures were maintained at 13 °C (*Synechococcus* at 18 °C) on a 15 h light : 9 h dark cycle and were transferred every 2 wk to maintain exponential-phase growth.

Experimental design. Prior to the experiment, a large stock (2.5 l) of dinoflagellates was grown on the food species *Isochrysis galbana* (cell diameter 4.5 µm) and *Synechococcus* sp. (strain DC-2) (cell dimensions 1.2 x 2.4 µm). Protozoan culture medium (140 ml) was added to each of 18 polycarbonate flasks (250 ml), precleaned as above. Aliquots of late exponential phase *I. galbana* and *Synechococcus* cultures were added to each flask for nominal total phytoplankton concentrations ranging from 10 to 300 µgC l^{-1} (3 flasks per food concentration). At the time of experiment set-up, *Synechococcus* contributed about one-third and *I. galbana* about two-thirds the total phytoplankton carbon. (This combination of food species yielded a higher dinoflagellate growth rate than any other paired combination of *I. galbana*, *Synechococcus*, *Emiliania huxleyi*, and *Micromonas pusilla* in preliminary experiments. The dinoflagellate would not grow when offered only a single food species.) Subsamples (10 ml) of the *Gymnodinium* stock culture were then added to each flask for an initial grazer concentration of ca 40 cells ml^{-1} An additional 4 control flasks containing phytoplankton only were set up as above: 2 each at total phytoplankton concentrations of 75 and 350 µgC l^{-1}.

The experiment was carried out under conditions of light and temperature specified for grazer culture maintenance. Flasks were incubated on slowly rotating turntables (1 rpm) to ensure an even light field for

all replicates and to minimize aggregation of phytoplankton foods. Dinoflagellates were acclimated to food concentrations for 42 h, then initial, 24 h, and 48 h samples were taken at approximately 13:00 h each day. Sampling procedure at each time point consisted of removing two 20 ml subsamples from each flask and adding them to 0.5 ml acid Lugol's (final preservative concentration 2 %) for grazer enumeration. An additional 5 ml subsample was taken from each flask for epifluorescence slide preparation and enumeration of phytoplankton.

Heterotrophic dinoflagellate abundance was determined by placing 1 to 3 ml from each Lugol-preserved subsample into an inverted microscope slide chamber and counting the entire slide contents with a Zeiss inverted microscope. The first 25 dinoflagellates encountered on one sample from each time point and food concentration were measured using an ocular micrometer.

Epifluorescence slides were prepared by placing 1 to 5 ml from each 5 ml subsample into a filtration tower and adding 3 drops 25 % glutaraldehyde. Samples then were filtered onto black 0.2 µm Nuclepore filters under gentle vacuum (< 200 mm Hg), rinsed with 0.45 µm filtered seawater, and mounted on glass slides using Cargill type B immersion oil. Filters were examined on a Zeiss epifluorescence microscope at either 500× (*Isochrysis galbana*) or 800× (*Synechococcus* sp.) with Zeiss filter set 47 77 09 (BP 450-490 excitation filter, FT 510 beam splitter, and LP 520 barrier filter) using xenon lamp illumination. Under this illumination system *I. galbana* fluoresces red and *Synechococcus* fluoresces yellow. Contents of either an ocular grid or the entire field of view (depending on cell density) were enumerated for random fields of view until a minimum of 50 cells (*I. galbana*) or 200 cells (*Synechococcus*) had been counted. In a few samples from the 48 h time point fewer cells were counted.

Phytoplankton growth and dinoflagellate clearance and ingestion rates were calculated according to the equations of Frost (1972) as modified by Heinbokel (1978) to account for growth of the dinoflagellate population during the experiment. Dinoflagellate growth was assumed to occur exponentially and was calculated for each incubation flask and time interval according to

$$\mu\,(\mathrm{d}^{-1}) = \frac{1}{t} \ln \frac{N_{t_2}}{N_{t_1}} \qquad (1)$$

where N_{t_1} and N_{t_2} = grazer abundances at the beginning and end of each time interval, respectively. Grazing and growth rates, *Gymnodinium* cell volumes, and gross growth efficiencies (GGE = grazer biomass produced/phytoplankton biomass ingested) were all plotted against average phytoplankton concentration (<C>; Frost 1972) for each time interval. Carbon content of the heterotrophic dinoflagellate and *Isochrysis galbana* were determined by measuring cells of each species with an ocular micrometer, calculating cell volumes using standard geometric formulae, and applying the equation of Strathmann (1967). Carbon content of *Synechococcus* was calculated using a value of 0.294 pgC cell^{-1} (Cuhel & Waterbury 1984). Reported *Gymnodinium* growth rates and GGEs are carbon specific, taking into account changes in average grazer cell volume over time.

RESULTS

Species description

The heterotrophic dinoflagellate was identified as belonging to the genus *Gymnodinium*, in accordance with Dodge & Lee (1985). Cells were athecate and spherical to slightly oblate, with the cingulum more-or-less medially located. Cell diameter (specimens preserved in 2 % acid Lugol's) was ca 12 µm, although cell size varied with feeding conditions (see below). The feeding mechanism of this species is not known; however, under epifluorescent illumination intact phytoplankton food cells were frequently observed inside the grazer, suggesting that it does not digest prey extracellularly by means of a pallium or peduncle. *Gymnodinium* sp. has proven very hardy in culture and can survive extended periods (at least 1 mo) of starvation.

Growth rate

Growth rates of both phytoplankton species were near zero under the low irradiance conditions of the incubations (avg. = −0.001 d^{-1} for *Isochrysis galbana*, −0.0003 d^{-1} for *Synechococcus* sp.). Parameters of the numerical response for Day 1 (Fig. 1A) were estimated using a Monod model:

$$\mu = \mu_{\max}\left[\frac{P}{K_p + P}\right] \qquad (2)$$

where $\mu_{\max}$ = maximum specific growth rate; P = phytoplankton concentration; K_p = half-saturation constant for growth (P at $\mu_{\max}/2$). The model predicted a $\mu_{\max}$ of 0.84 d^{-1} for *Gymnodinium*, slightly higher than the maximum observed growth rate of 0.75 d^{-1}, and a K_p of 19.8 µgC l^{-1}.

Growth rate versus food concentration data for Day 2 of the experiment did not follow the expected hyperbolic pattern (Fig. 1B). Growth rates at low food concentrations were consistently higher on Day 2 than on Day 1.

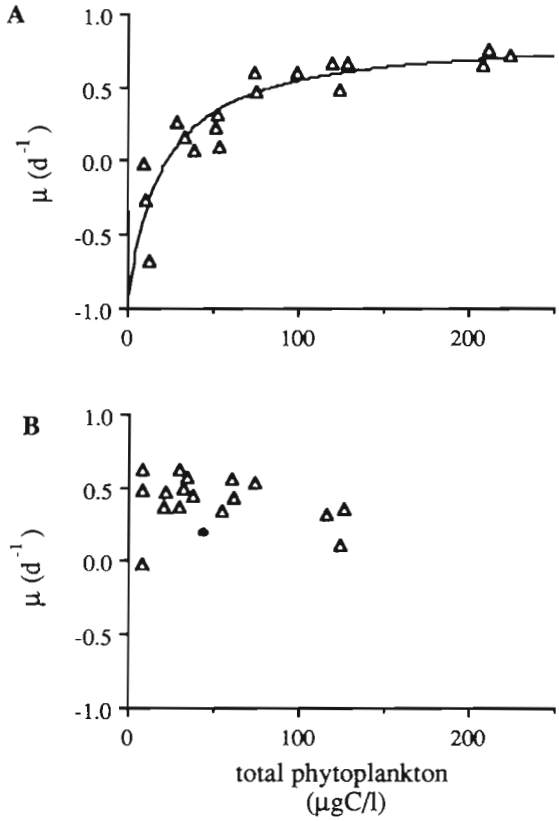

Fig. 1. *Gymnodinium* sp. Growth rate as a function of average food concentration. (A) Day 1 (data fit to Monod model); (B) Day 2

Cell volume

Gymnodinium sp. cell volume exhibited the same hyperbolic relationship to food concentration as did Day 1 growth rate (Fig. 2), ranging from a low of 600 μm^3 to a maximum of 1200 μm^3. Maximum cell volume as predicted by a Monod model fit to the data was 1111 μm^3.

Grazing rate

When fed a mixture of *Isochrysis galbana* and *Synechococcus* sp., *Gymnodinium* sp. exhibited total clearance rates ranging from 0.19 to 1.64 μl ind.$^{-1}$ h^{-1} and total ingestion rates ranging from 1.0 to 51.0 pgC ind. h^{-1}. Maximum volume-specific clearance (body volumes cleared body volume^{-1} h^{-1}) was 1.8×10^6 h^{-1}, while maximum carbon-specific ingestion (phytoplankton carbon ingested body carbon^{-1} h^{-1}) was 0.41 h^{-1}, the latter calculated using the volume : carbon conversion factor of Strathmann (1967) for determination of *Gymnodinium* carbon content. There is some

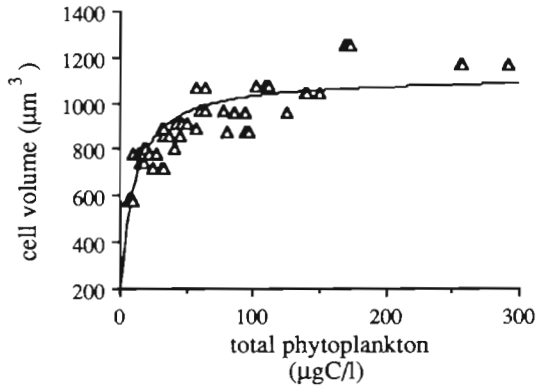

Fig. 2. *Gymnodinium* sp. Cell volume as a function of average food concentration (data fit to Monod model)

evidence that heterotrophic dinoflagellates may have higher carbon densities than photosynthetic flagellates. Carbon-specific ingestion calculated using a carbon density of 0.3 pg μm^{-3}, determined for the heterotrophic dinoflagellate *Protoperidinium parvum* (Jacobson 1988), gave a maximum of 0.19 h^{-1}.

Clearance and ingestion rates on *Isochrysis galbana* from Days 1 and 2 of the experiment are grouped

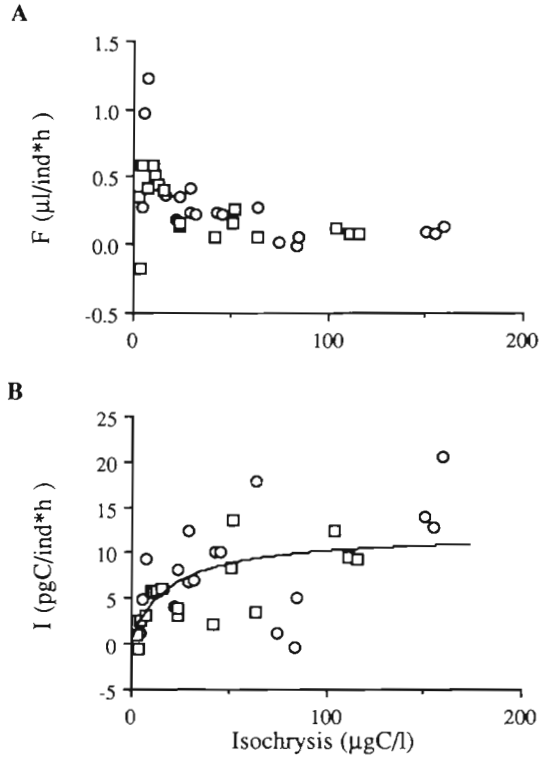

Fig. 3. *Gymnodinium* sp. Clearance (A) and ingestion (B) rates as a function of average food concentration for individuals grazing on *Isochrysis galbana*. (○) Day 1; (□) Day 2. (B) Data fit to Monod model

(there were no obvious differences between days). Clearance rates decreased sharply with food concentration up to ca 25 µgC l^{-1}, then decreased gradually with increasing food levels, reaching a minimum of ca 0.1 µl ind.$^{-1}$ h^{-1} (Fig. 3A). Ingestion of *I. galbana* (Fig. 3B) increased to a maximum of 12.1 pgC ind.$^{-1}$ h^{-1} as predicted by a Monod model fit to the data, with K_p = 19.2 µgC l^{-1}.

Grazing by *Gymnodinium* sp. on *Synechococcus* sp. differed on Days 1 and 2 of the experiment, so data are presented separately. Clearance rates on Day 1 varied at the lowest food concentrations but generally were near 0 below 20 µg phytoplankton C l^{-1}, then increased linearly to 0.5 µl ind.$^{-1}$ h^{-1} at the highest food concentrations (Fig. 4A). On Day 2, clearance rates from flasks with low initial *Synechococcus* concentrations were still near 0, while rates from flasks with high initial *Synechococcus* concentrations ranged from 0.6 to 0.8 µl ind.$^{-1}$ h^{-1} (Fig. 4B). This was in spite of the fact that average *Synechococcus* concentrations in these latter flasks were very low (< 15 µgC l^{-1}) on Day 2.

The same pattern was observed for *Gymnodinium* sp. ingestion of *Synechococcus* sp. (Fig. 5). Ingestion

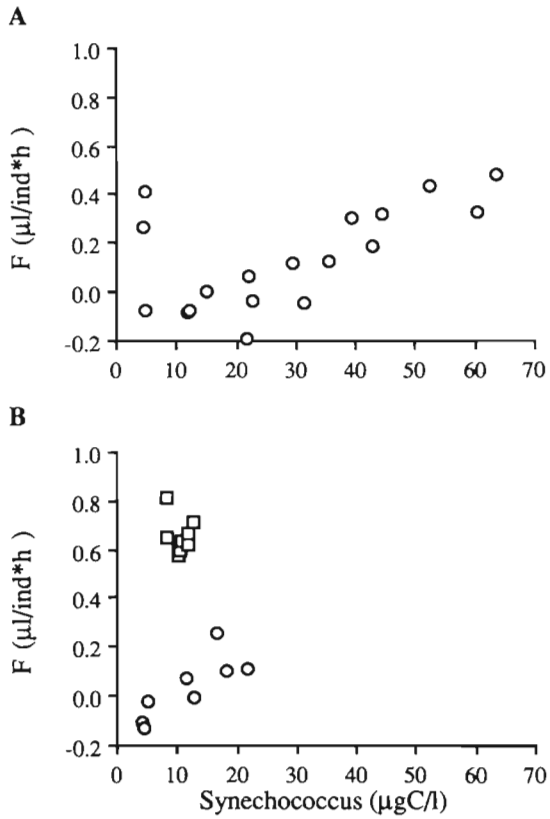

Fig. 4. *Gymnodinium* sp. Clearance rate as a function of average food concentration for individuals grazing on *Synechococcus* on Day 1 (A) and Day 2 (B). Day 2: (○) initial *Synechococcus* concentration < 20 µgC l^{-1}; (□) initial *Synechococcus* concentration > 20 µgC l^{-1}

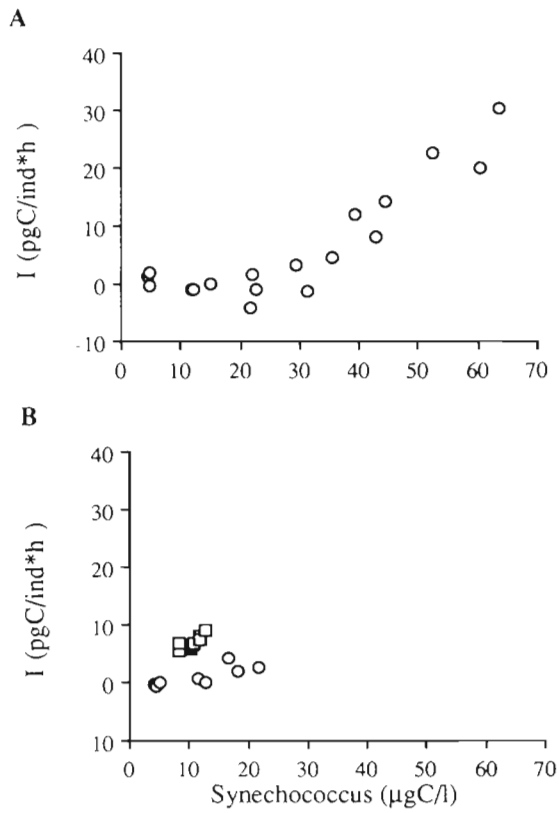

Fig. 5. *Gymnodinium* sp. Ingestion rate as a function of average food concentration for individuals grazing on *Synechococcus* sp. on Day 1 (A) and Day 2 (B). Day 2: (○) initial *Synechococcus* concentration < 20 µgC l^{-1}; (□) initial *Synechococcus* concentration > 20 µgC l^{-1}

rates were very low in any flask in which the initial *Synechococcus* concentration was below 20 µgC l^{-1}. Above this initial food level, ingestion rate increased linearly with food concentration on Day 1, reaching a maximum of 30.4 pgC ind.$^{-1}$ h^{-1}. Ingestion ranged from 5.4 to 9.2 pgC ind.$^{-1}$ h^{-1} on Day 2.

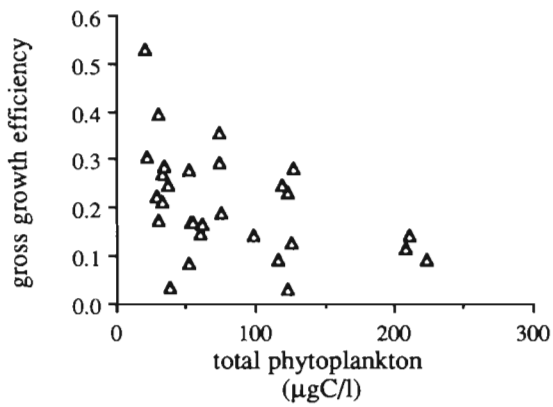

Fig. 6. *Gymnodinium* sp. Gross growth efficiency as a function of average food concentration

Gross growth efficiency

Gymnodinium sp. GGEs exhibited a great deal of variability (Fig. 6), ranging from 0.03 to 0.53 with a mean of 0.21 (SD = 0.11; n = 29). Maximum GGE appeared to be a function of food concentration, with the highest value of 0.53 observed at a total phytoplankton concentration of 20 $\mu gC\ l^{-1}$ and low values of 0.09 to 0.14 observed at total phytoplankton concentrations > 200 $\mu gC\ l^{-1}$.

DISCUSSION

Gymnodinium sp. growth rates reported here must be interpreted very cautiously. Rates were based on only 2 time points, initial and final, and could easily be biased by experimental error. Also, the 24 h time interval between samples may have been too long, such that critical portions of the exponential growth curve could have been missed. Given the paucity of data on heterotrophic dinoflagellates, however, it was decided that the data should be presented, at least for comparison with similarly-collected data for other protist species. *Gymnodinium* exhibited a maximum growth rate of 0.7 d^{-1}, reached at a concentration (critical concentration) of ca 80 $\mu gC\ l^{-1}$ on Day 1 of the experiment. Growth rates reported for other herbivorous dinoflagellates are similar, but critical concentrations are much higher (Table 1). Critical concentration differences may be due to differences in feeding mechanism, suitability of prey species, or isolation location (oceanic species may be adapted to lower food levels). When adjusted to 12 °C, reported maximum growth rates of microflagellates and ciliates are consistently higher than those of dinoflagellates (Table 1). It is not known whether this observation is generally true, perhaps owing to differences in feeding mechanism among the 3 groups of organisms, or whether the discrepancy is due to the small number of species thus far investigated.

The maximum observed *Gymnodinium* growth rate of 0.7 d^{-1} is approximately equal to growth rates determined for mixed layer phytoplankton at Station P. Average phytoplankton growth rates during spring and summer 1984, measured using 2 different techniques, ranged from 0.63 to 0.68 d^{-1} (Booth et al. 1988, Miller et al. 1988). During September 1987, when *Gymnodinium* was collected, pigment-specific phytoplankton growth rates as measured by the seawater dilution technique ranged from 0.07 to 0.59 d^{-1} (Strom & Welschmeyer 1991).

Dinoflagellate growth rates were high at relatively low phytoplankton concentrations, especially during Day 2 of the experiment. Again, these data must be interpreted cautiously, especially as decreases in phytoplankton concentration were larger during Day 2 than during Day 1 and it is less certain that the grazers were in a state of balanced growth. If real, the increased growth rate in low food treatments between Days 1 and 2 (Fig. 1) suggests that the grazer may have become gradually conditioned to low food concentrations over the course of the experiment. During Day 2, growth rates were maximal even at average phytoplankton concentrations of 10 $\mu gC\ l^{-1}$. Upper water-column algal carbon concentrations at Station P ranged from 1 to 24 $\mu g\ l^{-1}$ in May and August 1984 (Booth et al. 1988), and during 1987-88 ranged up to 50 $\mu g\ l^{-1}$ (Booth pers. comm.). Cells < 5 µm in size accounted for a large fraction of this biomass and thus may have been available to small protozoan grazers. In as much as *Gymnodinium* is representative of open subarctic microzooplankton species, sufficient concentrations of phytoplankton are present in the open subarctic Pacific to support high rates of protozoan growth. If phytoplankton biomass begins to increase, high growth rates of individual protozoan species should enable these grazer populations to keep pace with incipient blooms.

The cell volume of the heterotrophic dinoflagellate *Gymnodinium* was observed to vary by a factor of 2 over the range of phytoplankton concentrations employed in this experiment. While the relationship between grazer cell volume and food concentration (Fig. 2) resembled that between growth rate and food concentration (Fig. 1A), there was no direct relationship between grazer cell volume and growth rate. The same finding has been reported for the microflagellate *Pseudobodo* sp. grazing on the prasinophyte *Micromonas pusilla* (Parslow et al. 1986). Goldman & Dennet (1990) determined that the microflagellate *Paraphysomonas imperforata* could adjust its cell volume about 5-fold when fed a range of prey types, becoming very small when bacteria were the food source and larger when large phytoplankton species were offered. They theorized that feeding by direct interception is most efficient when the difference between predator and prey size is minimized. This hydrodynamic explanation, however, does not seem to apply in the present case: *Gymnodinium* cell volume apparently increased as *Synechococcus* (the smaller of the 2 food species) made up a progressively larger fraction of the diet (Fig 7A). Alternatively, since a positive relationship was observed between *Gymnodinium* cell volume and total phytoplankton ingestion (Fig. 7B), cell-volume changes in this species may simply reflect the amount of food contained within grazer food vacuoles at any given time. The average number of cells contained in a grazer should increase with food concentration until digestion, rather than encounter rate or rate of phagocytosis, limits ingestion.

Table 1. Growth rates and critical concentrations for various species of protozoans feeding herbivorously. Taxonomic affiliation: D = heterotrophic dinoflagellate; MF = microflagellate; T = tintinnid ciliate; A = aloricate ciliate. nd = not determined

Grazer	Taxonomic affiliation	Temp. (°C)	Food(s)	μ_{max} (d^{-1})	μ_{max} adjusted* (d^{-1})	Critical conc. ($\mu gC\ l^{-1}$)	Reference
Flagellates							
Gymnodinium sp.	D	12	*Isochrysis galbana* + *Synechococcus* sp	0.7	0.7	80	This study
Gymnodinium sp. (field population)	D	1	Naturally occurring phytoplankton	0.3	0.6	250	Bjørnsen & Kuparinen (1991)
Oxyrrhis marina	D	20	*Phaeodactylum tricornutum*	1.3	0.8	nd	Goldman et al. (1989)
			I. galbana	0.8	0.5		
			Dunaliella tertiolecta	0.8	0.5		
Protoperidinium hirobis	D	20	*Leptocylindricus danicus*	1.2	0.7	1250	Jacobson (1987)
Polykrikos kofoidii	D	nd	*Scrippsiella trochoidea*	0.7	–	450	Gaines (1988)
Oxyrrhis marina	D	nd	*D. tertiolecta*	0.7	–	175	
Paraphysomonas imperforata	MF	24	*D. tertiolecta*	2.4	1.1	nd	Goldman & Caron (1985)
			Chlorella stigmataphora	2.4	1.1		
			C. capsulata	2.3	1.0		
			I. galbana	2.4	1.1		
			Porphyridium sp	2.4	1.1		
			P. tricornutum	2.5–3.5	1.1–1.6		
Pseudobodo sp.	MF	18	*Micromonas pusilla*	2.0	1.4	nd	Parslow et al. (1986)
Ciliates							
Tintinnopsis cf. *acuminata*	T	18	*I. galbana* + *Monochrysis lutheri*	1.4	1.0	50	Heinbokel (1978)
Eutintinnus pectinis	T	18	*I. galbana* + *M. lutheri* + *D. tertiolecta*	1.4	1.0	80	
Helicostomella subulata	T	18	*I. galbana* + *D. tertiolecta*	0.8	0.6	60	
Favella sp.	T	15	*Heterocapsa triquetra*	1.1	0.9	95	Stoecker et al. (1983)
Tintinnopsis acuminata	T	15–25	*I. galbana*	1.3–2.0	1.1**	100	Verity (1985)
Tintinnopsis vasculum	T	5–15	*Dicrateria inornata*	0.5–1.1	0.9*	120	
Lohmaniella spiralis	A	15–23	Naturally occurring particulate matter	1.0	0.9	nd	Rassoulzadegan (1982)
Strombidium reticulatum	A	12	*Pyramimonas* sp.	0.9	0.9	450	Jonsson (1986)
Lohmaniella spiralis	A	12	*Pyramimonas* sp	1.0	1.0	450	
Balanion sp.	A	15	*H. triquetra*	2.2	1.9	nd	Stoecker et al. (1986)

*μ_{max} adjusted to 12 °C, assuming a $Q_{10} = 2$
**μ_{max} adjusted to 12 °C, using Q_{10} values determined for these organisms

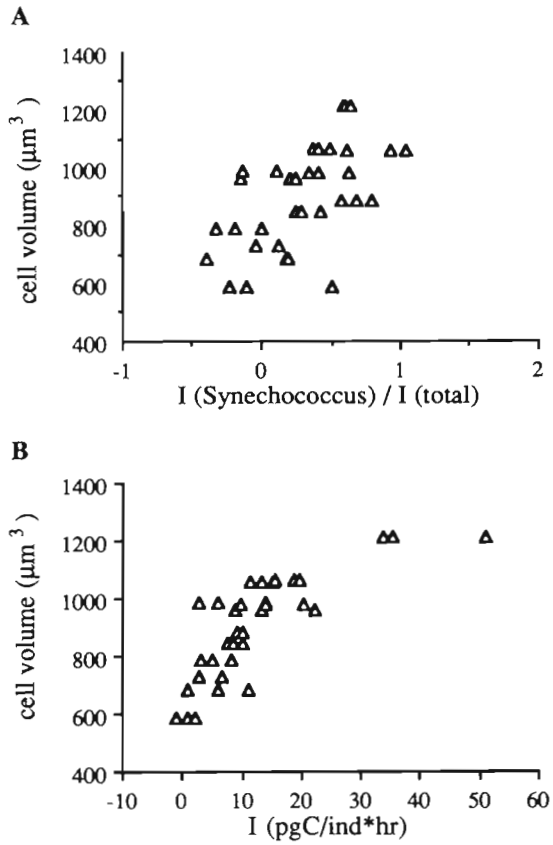

Fig. 7. Cell volume of *Gymnodinium* sp. versus *Synechococcus* sp. as a fraction of diet (A) and total phytoplankton ingestion (B)

Maximum volume-specific clearance rates for the heterotrophic dinoflagellate *Gymnodinium* sp. are higher than maximum rates observed for larger herbivorous protozoans, but appear reasonable given the small size of this grazer (Fig. 8) Clearance rates for the dinoflagellate *Oxyrrhis marina*, shown for comparison, appear anomalously low. Reported clearance rates for *O. marina* may not represent true maxima achievable by the organism at lower food concentrations. Alternatively, this species may employ a different feeding mechanism than *Gymnodinium*. *O. marina* appears to seek out and capture prey cells by extension of a thin filament (Goldman et al. 1989). Calculation of clearance rates may not be appropriate for this class of grazer, in which the capacity to ingest relatively large prey cells compensates for the inability to process large volumes of water. It is not known whether *Gymnodinium* feeds by utilizing a capture filament or, like chrysomonad microflagellates, by entrainment of food cells into a water current (e.g. Fenchel 1982).

Maximum carbon-specific ingestion for *Gymnodinium* is comparable to rates reported for other herbivorous protozoa (Fig. 8 references), given the smaller size of this grazer. The maximum carbon-specific ingestion rate of 0.19 h^{-1} (based on a relatively high *Gymnodinium* cell carbon content) is compatible with a growth rate of 0.7 d^{-1} and a GGE of 0.15, both typical values for this grazer feeding at high food concentrations (Figs. 1 & 6).

Clearance and ingestion of *Isochrysis galbana* by *Gymnodinium* exhibited a classic relationship to food concentration (Fig. 3), with clearance reaching a minimum and ingestion a maximum at 40 to 50 µg *I. galbana* C l^{-1}. Similar functional response curves have been obtained for a number of ciliate species (Heinbokel 1978, Fenchel 1980, Scott 1985, Verity 1985, Jonsson 1986). Maximum clearance rates on *I. galbana* and *Synechococcus* were similar (0.8 to 1.2 µl ind.$^{-1}$ h^{-1}), but maximum ingestion rates on *Synechococcus* were higher than rates on *I. galbana* (30 and 20 µgC ind.$^{-1}$ h^{-1}, respectively).

Clearance and ingestion rates as a function of food concentration for *Gymnodinium* sp. feeding on *Synechococcus* sp. exhibited an unusual pattern. Apparently there was no grazing on *Synechococcus* until initial concentrations of this species were > 20 µgC l^{-1} (Fig. 5). At initial concentrations above this level, ingestion gradually increased with food concentration; rates remained high even when *Synechococcus* concentrations were reduced well below the initial threshold level of 20 µgC l^{-1} on Day 2 of the

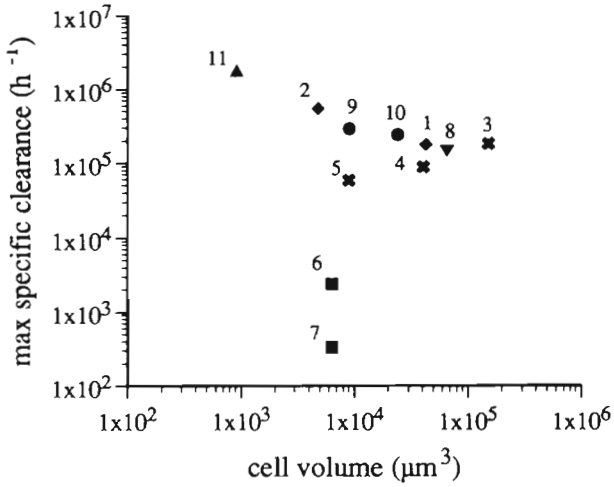

Fig. 8. Maximum volume-specific clearance rates of herbivorous Protozoa. 1, *Tintinnopsis vasculum*; 2, *T. acuminata* (◆: Verity 1985); 3, *Lohmaniella spiralis*; 4, *Strombidium reticulatum*; 5, *S. vestitum* (✖: Jonsson 1986); 6, *Oxyrrhis marina* fed *Isochrysis galbana*; 7, *O. marina* fed *Phaeodactylum tricornutum* (■: Goldman et al. 1989); 8, *Lohmaniella spiralis* (▼: Rassoulzadegan 1982); 9, *T.* cf. *acuminata*; 10, *Helicostomella subulata* (●: Heinbokel, 1978); 11, *Gymnodinium* sp. (▲: this study)

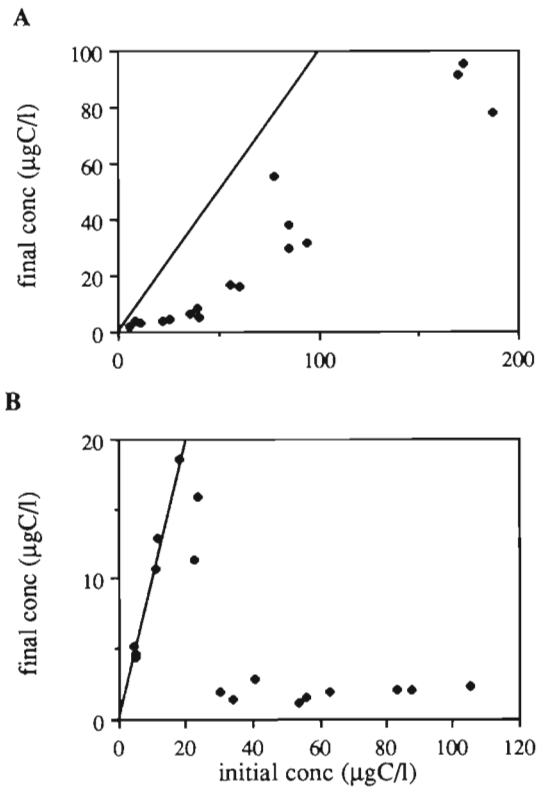

Fig. 9. Initial versus final concentration of *Isochrysis galbana* (A) and *Synechococcus* sp. (B). Solid lines represent equality (no change in concentration during experiment). *I. galbana* was grazed at all initial concentrations (all points fall below line), while *Synechococcus* was not grazed until initial concentrations exceeded 20 µgC l^{-1}

experiment (Fig. 9). An identical result was obtained for the flagellate *Paraphysomonas imperforata* feeding on *Dunaliella tertiolecta* both alone and in a food mixture (Goldman & Dennett 1990, their Fig. 6B), although the apparent threshold concentration for *P. imperforata* was very high (11 700 µgC l^{-1}).

The classic definition of a grazing threshold is the food concentration below which it is energetically unrewarding for a grazer to continue attempting to gather food (Frost 1975). The energy required to create feeding currents outweighs that gained from ingestion of the occasional phytoplankter, and the grazer goes into a reduced-activity or non-feeding mode. However, alternative types of grazing thresholds may be envisioned in which it is energetically unrewarding to either ingest or digest cells of certain species at low food concentrations. Perhaps certain structural or enzymatic modifications are necessary before a herbivore can feed on a given phytoplankton species effectively. Once these modifications are made, it would be energetically feasible for the herbivore to graze this phytoplankton species even at very low concentrations. Grazing thresholds of the latter type may not be apparent when grazers are offered only a single prey species.

Euphotic zone concentrations of *Synechococcus* spp. in the natural environment of the subarctic Pacific are consistently lower than the *Gymnodinium* threshold level of 20 µgC l^{-1}, ranging from < 1 to about 15 µgC l^{-1} (Booth et al. 1988, Booth pers. comm.). Once grazing is initiated, *Gymnodinium* can effectively consume *Synechococcus* at these low levels (Fig. 4B); perhaps transient peaks in prey abundance occur which suffice to trigger the grazing response. Alternatively, *Gymnodinium* may not be an important consumer of *Synechococcus* in this ocean region.

Prey selection by *Gymnodinium* was dependent on initial prey concentration (Fig. 10). In flasks with an initial phytoplankton concentration < 75 µgC l^{-1} (initial *Synechococcus* concentration < 25 µgC l^{-1}) *Isochrysis galbana* was always ingested in greater proportion than would be predicted by its relative abundance. In flasks with higher initial food concentrations, *Synechococcus* was generally the preferred prey species. In other words, once significant grazing on *Synechococcus* commenced, this species was grazed preferentially. These findings contrast with those of Heinbokel (1978), who observed no selective grazing by *Eutintinnus pectinnus* under conditions of low food concentration, preferential ingestion of *I. galbana* occurring only when average food concentration exceeded 60 µgC l^{-1}. The dynamics of prey selection by protozoan grazers may depend on whether there exists a threshold feeding response for any of the prey species available. Additionally, as suggested by Verity (1991), selective ingestion may be determined by the nutritional quality of the entire spectrum of potential prey species.

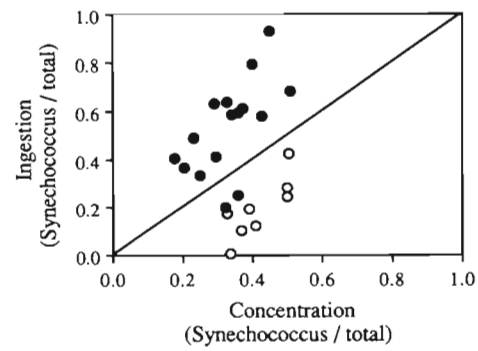

Fig. 10. *Gymnodinium* sp. Ingestion of *Synechococcus* as a fraction of total ingestion versus initial *Synechococcus* availability. Solid line represents no feeding preference (ingestion directly proportional to relative abundance of prey species). (○) Initial phytoplankton concentration < 75 µgC l^{-1} (initial *Synechococcus* concentration < 25 µgC l^{-1}); (●) initial phytoplankton concentration > 75 µgC l^{-1} (initial *Synechococcus* concentration > 25 µgC l^{-1})

GGEs determined for *Gymnodinium* are within the range of values reported for other heterotrophic protozoans, both herbivorous and bacterivorous (summarized by Caron & Goldman 1990). The tendency for protozoan GGEs to decrease at high food concentrations has been noted for a number of ciliate species (Heinbokel 1978, Stoecker & Evans 1985, Verity 1985, Jonsson 1986). Verity (1985) calculated that assimilation efficiency (growth + respiration/ingestion) did not decrease at high food levels during experiments with herbivorous tintinnids; however, Stoecker & Evans (1985) observed undigested phytoplankton cells in the fecal material of ciliates feeding at high food concentrations. This suggests that digestion may, in fact, be less efficient when food is very abundant. Lower assimilation efficiencies at high food levels have been reported for the herbivorous copepod *Calanus pacificus* (Landry et al. 1984), although long acclimation times (> 1 wk) apparently are necessary for the required changes in digestive enzyme activity to occur (Hassett 1986).

Increased GGEs at low food concentrations mean that transfer of material from phytoplankton to higher trophic levels is most efficient when food is scarce. By the same token, nutrient regeneration by grazers will be less efficient at low food concentrations. Changes in digestive efficiency, which may underlie GGE variation, indicate that the composition of fecal material produced by grazers can vary depending on their feeding regime.

CONCLUSIONS

The herbivorous dinoflagellate *Gymnodinium* sp., isolated from Station P in the open subarctic Pacific, reached maximum growth rates at relatively low food levels and grew rapidly even at low phytoplankton carbon concentrations. This was apparently due to the species' ability to feed effectively at these low concentrations: maximum volume-specific clearance and carbon-specific ingestion rates were high, and maxima were reached at low food levels. Increased GGE may also contribute to rapid growth rates at low phytoplankton concentrations.

Gymnodinium was able to feed on phytoplankton species similar in size and taxonomy to those found at Station P (Booth et al. 1982, Booth 1988). Depending upon the fraction of total phytoplankton biomass available to these grazers in the field, they may be capable of growth rates as high as those reported for Station P phytoplankton species. Threshold feeding behaviors and preferential grazing such as exhibited by *Gymnodinium* feeding on *Synechococcus* may provide a refuge from grazing for certain algal species, permitting them to reach a relatively high concentration before being grazed to low levels. Maximum volume-specific clearance rates for *Gymnodinium* were as high or higher than comparable rates for herbivorous ciliates, suggesting that heterotrophic dinoflagellates have the potential to be major consumers of phytoplankton in marine systems.

Acknowledgements. I thank the members of the SUPER program, in particular B. Frost, for encouragement and support. D. Gifford assisted with isolation of Station P protists; D. Montagnes and F. J. R. Taylor gave taxonomic advice. The manuscript was greatly improved by the comments of E. Lessard and an anonymous reviewer. This research was supported by N.S.F. grant OCE 86-13621 to B. W. Frost.

LITERATURE CITED

Banse, K. (1990). Does iron really limit phytoplankton production in the offshore subarctic Pacific? Limnol. Oceanogr. 35: 772–775

Bjørnsen, P. K., Kuparinen, J. (1991). Growth and herbivory by heterotrophic dinoflagellates in the Southern Ocean, studied by microcosm experiments. Mar. Biol. 109: 397–405

Booth, B. C. (1988). Size classes and major taxonomic groups of phytoplankton at two locations in the subarctic Pacific Ocean in May and August, 1984. Mar. Biol. 97: 275–286

Booth, B. C., Lewin, J., Lorenzen, C. J. (1988). Spring and summer growth rates of subarctic Pacific phytoplankton assemblages determined from carbon uptake and cell volumes estimated using epifluorescence microscopy. Mar. Biol. 98: 287–298

Booth, B. C., Lewin, J., Norris, R. E. (1982). Nanoplankton species predominant in the subarctic Pacific in May and June 1978. Deep-Sea Res. 29: 185–200

Buck, K. R., Bolt, P. A., Garrison, D. L. (1990). Phagotrophy and fecal pellet production by an athecate dinoflagellate in Antarctic sea ice. Mar. Ecol. Prog. Ser. 60: 75–84

Caron, D. A., Goldman, J. C. (1990). Protozoan nutrient regeneration. In: Capriulo, G. M. (ed.) Ecology of marine protozoa. Oxford Univ. Press, New York. p. 283–306

Cuhel, R. L., Waterbury, J. B. (1984). Biochemical composition and short term nutrient incorporation patterns in a unicellular marine cyanobacterium, *Synechococcus* (WH7803). Limnol. Oceanogr. 29: 370–374

Dodge, J D., Lee, J. J. (1985). Order 2. Dinoflagellida Butschli, 1885. In: Lee, J J., Hutner, S. H., Bovee, E. C. (eds.) An illustrated guide to the protozoa. Society of Protozoologists, Lawrence, Kansas, p. 22–41

Eppley, R. W., Holmes, R. W., Strickland, J. D. H. (1967). Sinking rates of marine phytoplankton measured with a fluorometer. J. exp. mar. Biol. Ecol. 1: 191–208

Evans, G. T., Parslow, J. S. (1985). A model of annual plankton cycles. Biol. Oceanogr. 3: 327–347

Fenchel, T (1980). Suspension feeding in ciliated protozoa: functional response and particle size selection. Microb. Ecol. 6: 1–11

Fenchel, T (1982). Ecology of heterotrophic microflagellates. I. Some important forms and their functional morphology. Mar. Ecol. Prog. Ser. 8: 211–223

Frost, B. W. (1972). Effects of size and concentration of food particles on the feeding behavior of the marine planktonic

copepod *Calanus pacificus*. Limnol. Oceanogr 17: 805–815
Frost, B. W. (1975). A threshold feeding behavior in *Calanus pacificus*. Limnol. Oceanogr. 20: 263–266
Frost, B. W. (1987). Grazing control of phytoplankton stock in the open subarctic Pacific Ocean: a model assessing the role of mesozooplankton, particularly the large calanoid copepods *Neocalanus* spp. Mar. Ecol. Prog. Ser. 39: 49–68
Gaines, G. (1988). Feeding and reproduction of heterotrophic dinoflagellates. In: Burkill, P. H., Reid, P. C. (eds.) NATO ASI: Protozoa and their role in marine processes. Workshop abstract. Plymouth Marine Laboratory, Plymouth, UK
Gaines, G., Elbrächter, M. (1987). Heterotrophic nutrition. In: Taylor, F. J. R. (ed.) The biology of dinoflagellates. Blackwell Scientific, Oxford, p. 224–268
Garrison, D. L., Buck, K. R. (1989). Protozooplankton in the Weddell Sea, Antarctica: abundance and distribution in the ice-edge zone. Polar Biol. 9: 341–351
Gifford, D. J. (1985). Laboratory culture of marine planktonic oligotrichs (Ciliophora, Oligotrichida). Mar. Ecol. Prog. Ser. 23: 257–267
Gifford, D. J. (1988). Impact of grazing by microzooplankton in the northwest arm of Halifax Harbor, Nova Scotia. Mar. Ecol. Prog. Ser. 47: 249–258
Goldman, J. C., Caron, D. A. (1985). Experimental studies on an omnivorous microflagellate: implications for grazing and nutrient regeneration in the marine microbial food chain. Deep-Sea Res. 32: 899–915
Goldman, J C., Dennett, M. R. (1990). Dynamics of prey selection by an omnivorous flagellate. Mar. Ecol. Prog. Ser. 59: 183–194
Goldman, J. C., Dennett, M. R., Gordin, H. (1989). Dynamics of herbivorous grazing by the heterotrophic dinoflagellate *Oxyrrhis marina*. J. Plankton Res. 11. 391–407
Hansen, P. J. (1991). Quantitative importance and trophic role of heterotrophic dinoflagellates in a coastal pelagial food web. Mar. Ecol. Prog. Ser 73: 253–261
Hassett, R. P. (1986). The effect of feeding history on the feeding behavior of *Calanus pacificus*. Ph.D. thesis, University of Washington
Heinbokel, J. F. (1978). Studies on the functional role of tintinnids in the Southern California Bight. I. Grazing and growth rates in laboratory cultures. Mar. Biol. 47: 177–189
Jacobson, D. M. (1987). The ecology and feeding behavior of thecate heterotrophic dinoflagellates. Ph.D. thesis, Massachusetts Institute of Technology and Woods Hole Oceanographic Institution
Jacobson, D. M. (1988). Growth and feeding rates of Protoperidinium. In: Burkill, P. H., Reid, P. C. (eds.) NATO ASI: Protozoa and their role in marine processes. Workshop abstract. Plymouth Marine Laboratory, Plymouth, UK
Jacobson, D. M., Anderson, D. M. (1986). Thecate heterotrophic dinoflagellates: feeding behavior and mechanisms. J. Phycol. 22: 249–258
Jonsson, P. R. (1986). Particle size selection, feeding rates and growth dynamics of marine planktonic oligotrichous ciliates (Cilophora: Oligotrichina). Mar. Ecol. Prog. Ser. 33: 265–277
Landry, M. R., Hassett, R. P., Fagerness, V., Downs, J., Lorenzen, C. J. (1984). Effect of food acclimation on assimilation efficiency of *Calanus pacificus*. Limnol. Oceanogr. 29: 361–364
Lessard, E. J. (1984). Oceanic heterotrophic dinoflagellates: distribution, abundance, and role as microzooplankton. Ph.D. thesis, University of Rhode Island
Lessard, E. J. (1991). The trophic role of heterotrophic dinoflagellates in diverse marine environments. Mar. microb. Fd Webs 5: 49–58
Lessard, E. J., Swift E. (1985). Species-specific grazing rates of heterotrophic dinoflagellates in oceanic waters, measured with a dual-label radioisotope technique. Mar Biol. 87: 289–296
Martin, J. J., Broenkow, W. W., Fitzwater, S. E., Gordon, R. M. (1990). Yes, it does: a reply to the comment by Banse. Limnol. Oceanogr. 35: 775–777
Miller, C. B., SUPER group (1988). Lower trophic level production dynamics in the oceanic subarcic Pacific Ocean. Bull. Ocean Res. Inst. Univ. Tokyo 26: 1–26
Parslow, J. S., Doucette, G. J., Taylor, F. J R., Harrison, P. J. (1986). Feeding by the zooflagellate *Pseudobodo* sp. on the picoplanktonic prasinomonad *Micromonas pusilla*. Mar. Ecol. Prog. Ser. 29: 237–246
Rassoulzadegan, F. (1982). Dependence of grazing rate, gross growth efficiency and food size range on temperature in a pelagic oligotrichous ciliate *Lohmaniella spiralis* Leeg., fed on naturally occurring particulate matter. Ann. Inst. océanogr., Paris 58 (S): 177–184
Scott, J. M. (1985). The feeding rates and efficiencies of a marine ciliate, *Strombidium* sp., grown under chemostat steady-state conditions. J. exp. mar Biol. Ecol. 90: 81–95
Shapiro, L. P., Haugen, E. M., Carpenter, E. J. (1989). Occurrence and abundance of green-fluorescing dinoflagellates in surface waters of the Northern Atlantic and Northwest Pacific Oceans. J. Phycol. 25: 189–191
Sherr, E. B., Sherr, B. F., McDaniel, J. (1991). Clearance rates of <6 µm fluorescently labeled algae (FLA) by estuarine protozoa: relative grazing impact of flagellates and ciliates. Mar Ecol. Prog. Ser. 69: 81–92
Smetacek, V. (1981). The annual cycle of protozooplankton in the Kiel Bight. Mar Biol. 63: 1–11
Stoecker, D. K., Cucci, T L., Hulbert, E. M., Yentsch, C. M. (1986). Selective feeding by *Balanion* sp. (Ciliata: Balanionidae) on the phytoplankton that best support its growth. J. exp. mar. Biol. Ecol. 95: 113–130
Stoecker, D. K., Davis, L. H., Provan, A. (1983). Growth of *Favella* sp. (Ciliata: Tintinnina) and other microzooplankters in cages incubated *in situ* and comparison to growth *in vitro* Mar. Biol. 75: 293–302
Stoecker, D. K., Evans, G. T (1985). Effects of protozoan herbivory and carnivory in a microplankton food web. Mar. Ecol. Prog. Ser. 25: 159–167
Strathmann, R. R. (1967). Estimating the organic carbon content of phytoplankton from cell volume or plasma volume. Limnol. Oceanogr. 12: 411–418
Strom, S. L., Welschmeyer, N. A. (1991). Pigment-specific rates of phytoplankton growth and microzooplankton grazing in the open subarctic Pacific Ocean. Limnol. Oceanogr. 36: 50–63.
Taylor, F. J. R., Waters, R. E. (1982). Spring phytoplankton in the subarctic North Pacific Ocean. Mar. Biol. 67: 323–335
Verity, P. G. (1985). Grazing, respiration, excretion and growth rates of tintinnids. Limnol. Oceanogr. 30: 1268–1282
Verity, P. G. (1991). Measurement and simulation of prey uptake by marine planktonic ciliates fed plastidic and aplastidic nanoplankton. Limnol. Oceanogr. 36: 729–750
Weisse, T., Scheffel-Möser, U. (1990). Growth and grazing loss rates in single-celled *Phaeocystis* sp. (Prymnesiophyceae). Mar. Biol. 106: 153–158

This article was presented by E. & B. Sherr, Corvallis, Oregon, USA

Manuscript first received: March 11, 1991
Revised version accepted: October 1, 1991