Vol. 41: 145–152, 2005 | AQUATIC MICROBIAL ECOLOGY
Aquat Microb Ecol | Published November 25

Growth rates and starvation survival of three species of the pallium-feeding, thecate dinoflagellate genus *Protoperidinium*

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ABSTRACT: We measured growth rates and starvation survival capacity of 3 thecate heterotrophic dinoflagellate species (*Protoperidinium conicum*, *P. depressum*, *P. excentricum*; Peridiniacea: Dinophyceae), isolated from surface waters in Puget Sound, Washington, USA. Feeding on the diatom *Ditylum brightwellii*, the 3 species achieved maximum specific growth rates of 1.13, 0.21 and 0.33 d^{-1} respectively. Maximum growth rates were observed at prey concentrations between 50 and 280 µg C l^{-1}. Prey concentrations <20 µg C l^{-1} supported only negative or low growth rates. Predators survived in the presence of 11 phylogenetically diverse phytoplankton species for several days, but only the diatom *D. brightwellii* supported measurable predator growth. Grazing rates of up to 6 µg C l^{-1} (22 *D. brightwellii*) *Protoperidinium*$^{-1}$ d^{-1} were calculated from limited data. All species were able to starve for extended periods; *P. depressum* survived up to 71 d at diatom prey concentrations <1 µg C l^{-1}. This extended starvation survival provides *Protoperidinium* species with a distinct advantage when prey availability is heterogeneous in time or space. Our results suggest that resistance to starvation could affect *Protoperidinium*'s energy allocation and could help explain previously observed dominance of *Protoperidinium* species in wintertime plankton communities despite low phytoplankton-prey concentrations. The viability of *Protoperidinium* species in the absence of prey has important implications for their function as both predators of phytoplankton and prey for zooplankton.

KEY WORDS: *Protoperidinium* · Dinoflagellate · Starvation · Growth rate · Heterotrophic protists · Microzooplankton · Food web

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INTRODUCTION

Research over the past 2 decades has shown that heterotrophic protists (i.e. microzooplankton) are significant grazers of bacterial and phytoplankton biomass, contribute to the cycling of organic matter and nutrients, and serve as important trophic links in marine microbial food webs (e.g. Smetacek 1981, Sherr & Sherr 1994, Landry et al. 2000). The significance of heterotrophic protists can largely be attributed to the fact that unicellular organisms can grow rapidly, on the order of 1 division d^{-1}. Heterotrophic protists act both as predators of phytoplankton and as prey for zooplankton. The population dynamics of these organisms therefore can have far-reaching effects on the structure and function of planktonic communities and thus the distribution and flux of organic matter and energy in marine microbial food webs.

Quantitative estimates of protistan growth and ingestion rates are essential for assessing population dynamics. The numerical and functional responses of a variety of heterotrophic protists have been empirically determined in laboratory cultures (e.g. Hansen et al. 1997). Many of these measurements have been made

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with relatively abundant prey. In the ocean, however, phytoplankton concentrations fluctuate frequently, in both time and space. Low phytoplankton concentrations may persist for several months of the year, even in areas with regular phytoplankton blooms such as coastal areas of polar and temperate waters. Recent analyses have shown that phytoplankton abundance is spatially heterogeneous, with prey concentrated in patches and thin layers (Cowles et al. 1998, Franks & Jaffe 2001, McManus et al. 2003). Finally, even if phytoplankton availability were constant and high, the species composition might not include suitable prey for the predators. Many heterotrophic protists show a high degree of selectivity with regard to their prey (e.g. Buskey 1997, Jakobsen & Hansen 1997, Naustvoll 2000). Thus, the availability of suitable prey at high density is uncertain at best and possibly rare for any given predator species. Consequently, protistan predators may frequently experience low prey or even starvation conditions. However, few studies have thus far quantified growth or mortality rates of protistan predators at very low prey concentrations.

To better understand the quantitative significance of heterotrophic protists during times of low prey abundance, we investigated growth and starvation survival in the cosmopolitan and exclusively heterotrophic dinoflagellate genus *Protoperidinium* (Peridiniacea: Dinophyceae). *Protoperidinium* species feed by deploying a pseudopodium, termed the pallium, around the prey cell, dissolving and absorbing the cell content of the prey before retracting the pallium (Jacobson & Anderson 1986). *Oblea*, *Zygabikodinium* and *Diplopsalis* are the only other dinoflagellate genera known to use this particular feeding mechanism (Jacobson & Anderson 1986, Strom & Buskey 1993, Naustvoll 1998). The pallium allows predators to ingest prey much larger than itself, reversing the typical predator:prey size ratio. *Protoperidinium* species are therefore competing for prey with larger, multicellular organisms such as copepods, rather than with other unicellular predators such as ciliates. When large phytoplankton cells are 'repackaged' in this manner, their biomass is made available to predators with smaller prey size spectra. For example, copepods have been shown to graze upon protists, including *Protoperidinium* species (Kiørboe et al. 1996, Nejstgaard et al. 1997, Levinsen et al. 2000b). Thus, *Protoperidinium* species gain some of their significance by simultaneously preying upon dominant primary producers and transferring that biomass to larger organisms. As a result, the genus *Protoperidinium* has been relatively well studied in recent years and several growth rates have been published (see Table 2). However, these data primarily reflect *Protoperidinium* spp. growth rates at high prey concentrations. In this study, we quantified the numerical responses of 3 species of the genus *Protoperidinium* with an emphasis on low prey concentrations. Further, we estimated how long *Protoperidinium* spp. could survive in the absence of prey. Our goal was to examine the genus's population dynamics and functional role at low prey concentrations.

MATERIALS AND METHODS

Isolation and cultivation. The heterotrophic dinoflagellates *Protoperidinium conicum* (Gran) Balech, *P. depressum* (Bailey) Balech and *P. excentricum* (Paulsen) Balech were isolated from fresh field samples collected from surface waters in Puget Sound, Washington, USA. Taxonomic identification was based on morphology and thecal plate arrangements according to Dodge (1982). Single cells of *Protoperidinium* spp. were micropipetted into 20 ml culture flasks containing filtered seawater and 1 of 11 potential prey species (Table 1). These cultures were monitored for up to 10 d for predator survival and growth. Clonal, but not axenic, *Protoperidinium* cultures were maintained feeding solely on the diatom *Ditylum brightwellii* (West) Grunow (Strain CCMP 358). *D. brightwelli* was chosen as the prey species, because it was the only test prey species that supported growth. Prey algae and each *Protoperidinium* species were cultured at 12°C on a 16:8 h light:dark cycle in *f*/2 medium (Guillard 1975) at a salinity of 30 PSU. Batches of the diatom prey were grown in 1000 ml polycarbonate bottles at 100 µmol photons m^{-2} s^{-1}. Cultures of all *Protoperidinium* species were kept at 30 µmol photons m^{-2} s^{-1} on a plankton wheel rotating at 1 rpm.

Growth and ingestion experiments. For each *Protoperidinium* species, 6 polycarbonate bottles (275 ml) were prepared at each of 6 final prey concentrations

Table 1. *Protoperidinium* spp. Survival (d) in the presence of phylogenetically diverse phytoplankton. Incubation was terminated after 10 d. Growth was observed only in the presence of the diatom *Ditylum brightwellii*

Species	Class	Survival
Micromonas pusilla	Prasinophyceae	5
Pyraminomonas sp.	Prasinophyceae	5
Dunaliella tertiolecta	Chlorophyceae	10
Amphidinium carterae	Dinophyceae	7
Gymnodinium simplex	Dinophyceae	9
Prorocentrum micans	Dinophyceae	10
Rhodomonas lens	Cryptophyceae	9
Rhodomonas salina	Cryptophyceae	10
Isochrysis galbana	Prymnesiophyceae	8
Pavlova lutheri	Prymnesiophyceae	10
Ditylum brightwellii	Coscinodiscophyceae	10

ranging from 0 to 2500 cells ml^{-1}, equivalent to 0 to 600 µg C l^{-1} in filtered seawater. At each prey concentration, 3 control bottles were set aside to monitor changes in diatom abundance in the absence of predation. The remaining 3 bottles were amended with dinoflagellate stock culture. Initial dinoflagellate concentrations were 0.1 to 1 cell ml^{-1}. These concentrations were chosen because Jeong & Latz (1994) measured higher growth rates with initial *Protoperidinium* species concentrations of 1 compared to 7 cells ml^{-1}. The starvation treatment without added prey contained maximally 0.8 µg C l^{-1} due to carryover of diatoms from the predator inocula. Experimental bottles were maintained at 12°C on a plankton wheel rotating at 1 rpm at low light (30 µmol photons m^{-2} s^{-1}) to minimize diatom growth. Initially, 10 ml aliquots were taken daily and replaced with 10 ml filtered seawater. After 2 to 3 wk, sampling frequency was decreased to twice a week. Samples were fixed immediately with glutaraldehyde (1% final concentration), stained with DAPI and gently (5 mm Hg) filtered onto a 2 µm black polycarbonate filter (Poretics). Samples from the starvation experiments were inspected live under a dissecting microscope before fixation to ensure cells were alive and actively swimming.

Microscopic analysis. Cell concentrations were determined using a standard epifluorescence microscope (Zeiss) at 200× magnification. For low-concentration samples (<50 cells) whole samples were counted, and for high-concentration samples a minimum of 50 cells was counted.

Dinoflagellate and diatom cell sizes were determined from approximately 30 preserved cells with an inverted microscope (Zeiss) equipped with a digitizer pad and Microbiota software (Roff & Hopcroft 1986). The average cellular carbon content of the diatom prey *Ditylum brightwellii* was 230 pg C $cell^{-1}$ based on an average cell volume of 5500 $µm^3$ (Menden-Deuer & Lessard 2000). No correction for fixation-induced cell size changes was applied (Menden-Deuer et al. 2001).

Growth and ingestion rate calculations. Growth rates were calculated as the slope of the least-squares regression of abundance data during the longest period of increasing cell density, i.e. the linear portion of the semi-natural log plot of cell concentration vs. time in each flask. Mean and standard deviations were calculated independently for each of the triplicate flasks, at each prey concentration. A minimum of 3 and maximum of 15 time points were used as the basis for the growth rate concentrations. The 4% dilution due to replacing the sample volume was taken into account in the growth rate calculations. Prey concentration was calculated from the geometric mean prey concentration in each flask during the predator growth interval. Only initial diatom abundance data were available for some *Protoperidinium excentricum* incubations, and prey concentration was based on initial inoculum concentration. Ingestion rates were calculated using the method described by Frost (1972) and Heinbokel (1978).

RESULTS

Growth rates

Specific growth rates for all 3 *Protoperidinium* species increased with increasing prey concentration up to a maximum prey concentration of 280 µg C l^{-1} (Fig. 1). A maximum observed specific growth rate of 1.13 was measured at approximately 60 µg C l^{-1} for *P. conicum*, and 0.21 and 0.33 d^{-1} at approximately 280 and 250 µg C l^{-1} for *P. depressum* and *P. excentricum*, respectively. Maximum growth rates obtained from fitting the Michaelis-Menten equation to the data resulted in very similar growth rates for *P. depressum* (0.24, $r^2 = 0.81$) and *P. excentricum* (0.29, $r^2 = 0.84$); the data for *P. conicum* did not allow a reasonable fit.

Growth rates measured for *Protoperidinium conicum* at prey concentrations of approximately 600 µg C l^{-1} were notably lower than at lower prey concentrations (Fig. 1a). The reason for this decrease is not clear. However, in our cultures we regularly observed that extremely high prey concentrations (>1200 µg C l^{-1}) prevent growth and can kill growing *Protoperidinium* cultures. Specific growth rates for all species changed rapidly with only minimal changes in prey concentration, and our results would have benefited from greater resolution of prey concentration treatments between 50 and 200 µg C l^{-1}. Positive growth rates observed at nearly 0 µg C l^{-1} were probably due to residual growth.

The results of our growth rate calculations were critically dependent upon the time interval considered. To quantify the significance of frequency of samplings on growth rate estimates, we calculated growth rates for hypothetical, fixed time intervals based on the initial and final concentrations only. Depending on the length of the time interval between initial and final samples, calculated growth rates for *Protoperidinium depressum* could vary by >100%, irrespective of initial prey concentration (Fig. 2). The variability in growth rate estimates decreased with increasing duration of the experiment for all initial prey concentrations.

Ingestion rates

In control bottles, there was little or no net growth of diatoms, due to low light levels. Diatom-prey concen-

tration in the treatments with predators decreased measurably relative to the controls only in incubations where predator abundance had increased substantially (>10 *Protoperidinium depressum* ml^{-1}; Fig. 3). Thus, we could only calculate ingestion rates for a few incubations for *P. conicum* and *P. depressum*. Despite their size difference, both predators had similar ingestion rates. *P. conicum* ingested 22 ± 1 and *P. depressum* ingested 15 ± 3 *D. brightwellii* predator^{-1} d^{-1} at prey concentrations of 400 and 600 µg C l^{-1}, respectively.

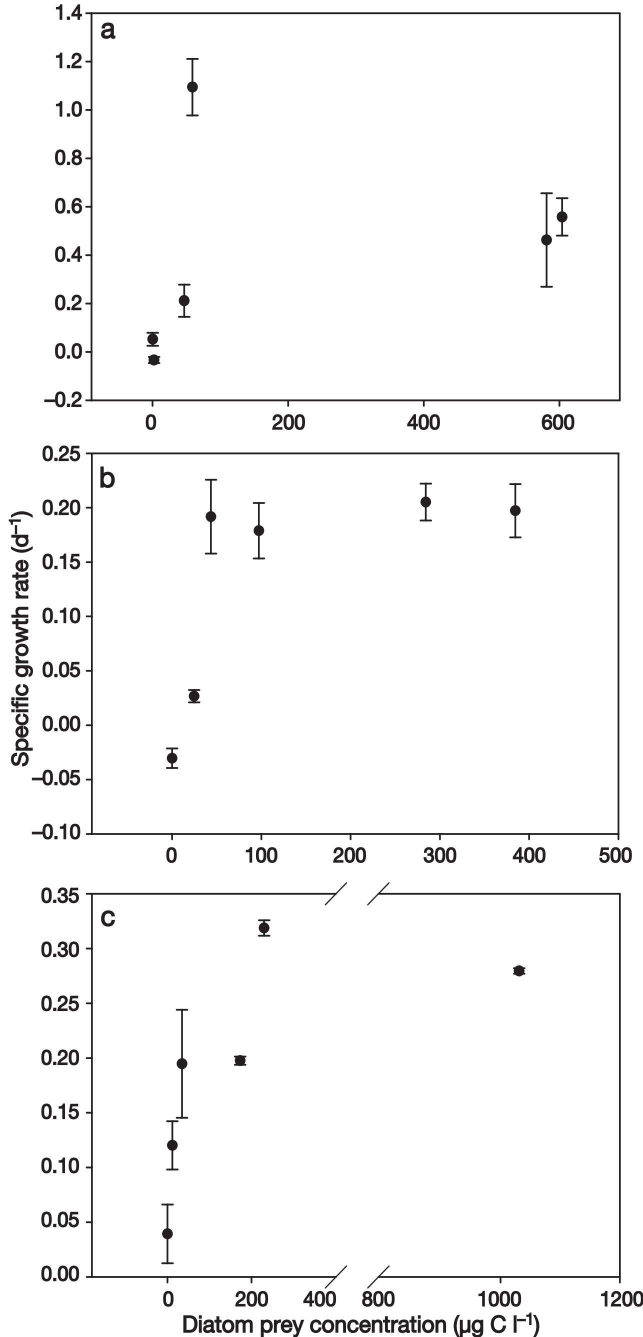

Fig. 1. (a) *Protoperidinium conicum*, (b) *P. depressum*, (c) *P. excentricum*. Specific growth rates as a function of mean diatom prey concentration. Growth rate estimates based on longest continuous exponential phase. Data are means ± 1 SD (n = 3)

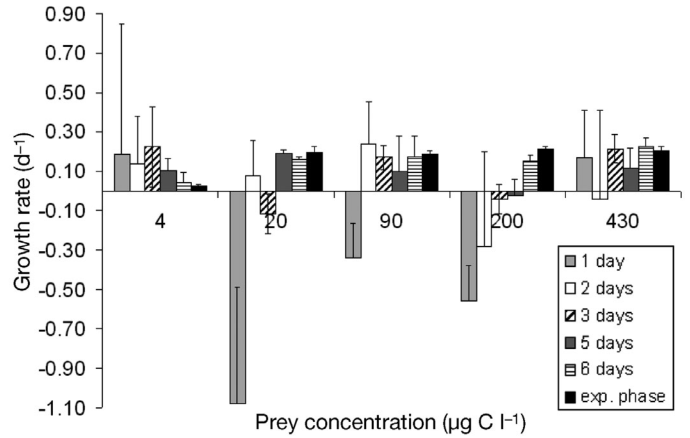

Fig. 2. *Protoperidinium depressum.* Growth rate estimates calculated from initial and final samples only. Categories represented by initial prey concentration. Actual growth rate determined by fitting regression to daily samples is shown for comparison (exp. phase). Data are means (+1 SD)

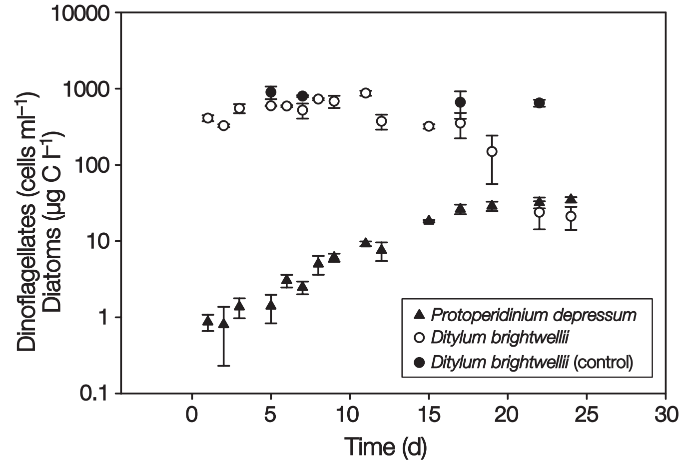

Fig. 3. *Protoperidinium depressum* and *Ditylum brightwellii*. Changes in concentrations vs. time

Starvation survival rates

Protoperidinium spp. were able to survive for a minimum of 5 d in cultures with 11 phylogenetically diverse phytoplankton species (Table 1). However, growth was observed only with the diatom *Ditylum brightwellii*. All species of *Protoperidinium* were able to survive prolonged periods of starvation in cultures with prey concentrations of <1 µg C l^{-1} (Fig. 4). At the time the cultures were terminated, the cells were not dead; thus, they may have survived even longer.

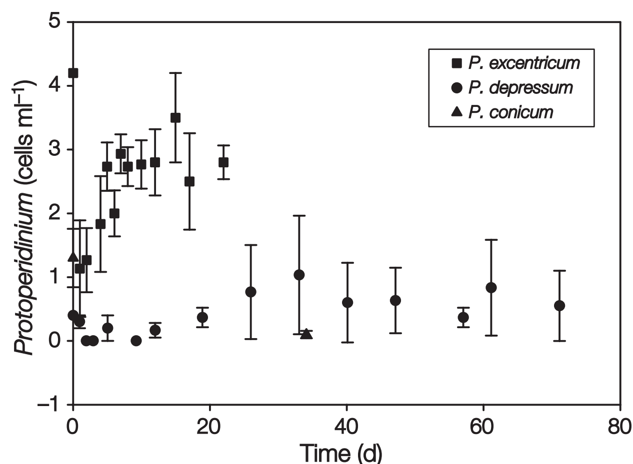

Fig. 4. *Protoperidinium* spp. Cell concentration vs. time in incubations with extremely low diatom-prey concentrations of <1 µg C l^{-1}. Note that there are only initial and end-point measurements for *P. conicum*

Observation of live *Protoperidinium* cells, taken from starving cultures after 1 mo and longer, showed that cells qualitatively appeared much more transparent and swam much slower than well-fed cells. All 3 *Protoperidinium* species were able to resume growth after starvation upon exposure to *D. brightwellii* prey. Unfortunately, we did not conduct a quantitative analysis to estimate how long, if at all, the lag phase was before predators resumed growth.

DISCUSSION

Maximum specific growth rates for the 3 *Protoperidinium* species investigated here ranged from 0.21 to 1.13 d^{-1}. This range corresponds to a population doubling time of 80 to 14 h for closely related species. The difference of 1 order of magnitude in cell volume amongst species cannot explain the difference in measured growth rates, as the largest and smallest species had similar growth rates. The experiments were conducted under the same culture conditions for all 3 species. Neither the temperature nor the prey species offered were optimized for any of the species, and the difference in growth rate may simply be a reflection of this lack of optimization.

Hansen et al. (1997) established a predictive relationship between dinoflagellate size and growth rate. The growth rates for *Protoperidinium depressum* and *P. excentricum* measured here correspond well with those predicted by Hansen et al.'s (1997) relationship. Populations of these 2 species would take more than 2 d to double in biomass. Olseng et al. (2002) attributed rapid increases in *Protoperidinium* abundance to advection because growth rates of >1 division d^{-1} were considered highly unlikely. However, the measured maximum growth rate for *P. conicum* was more than twice the rate predicted from Hansen et al.'s (1997) regression, and was greater than most reported growth rates for the genus *Protoperidinium* (Table 2). The importance of experimental conditions such as temperature and prey species to measured growth rates needs to be investigated in greater detail to determine whether *P. conicum* intrinsically grows faster than other dinoflagellate species or whether the particular experimental conditions favored higher growth rates for *P. conicum*.

Sampling frequency and duration of the experiment were key factors affecting growth rate estimates. With the exception of the highest prey concentrations, growth was not continuous during the incubation; thus, initial and final sampling alone would not have been adequate. Our analysis of growth rate calculations showed that estimates based only on initial and final samples would have resulted in significant differences in estimates of the specific growth rate, because initial decreases in *Protoperidinium* cell abundance are not measured and duration of lag and onset of stationary phases are unknown.

While rapid growth was only observed in *Protoperidinium conicum*, all species sustained continuous periods of growth for up to 2 wk at prey concentrations >280 µg C l^{-1}. The variability in measured growth rates shows that some *Protoperidinium* species can achieve high growth rates over short time periods, and all can sustain lower growth rates for periods of several days. Therefore, depending on prey concentration, these thecate heterotrophs can undergo rapid or gradual changes in population size and subsequently have

Table 2. *Protoperidinium* species. Maximum specific growth rates (d^{-1})

Species	Cell volume (µm³)	Growth rate	Source
P. bipes	1430	1.37	Jeong et al. (2004)
P. cf. *divergens*	119 000	0.49	Jeong & Latz (1994)
P. conicum	50 000	1.13	This study
P. crassipes	204 000	0.31	Jeong & Latz (1994)
P. depressum	278 000	0.21	This study
P. excentricum	24 000	0.33	This study
P. hirobis	6 400	1.23	Jacobson & Anderson (1993)
P. huberi	39 000	0.72	Buskey et al. (1994)
P. pallidum	53 000	0.28	Naustvoll (2000)
P. pellucidum	24 600	0.7	Buskey (1997)
P. pellucidum	29 300	0.33	Hansen (1992)
P. spiniferum	47 700	0.3	Jacobson & Anderson (1986)
P. steinii	9000	0.18	Naustvoll (2000)

immediate or sustained effects on organism abundance and biogeochemical fluxes in marine microbial food webs.

It is noteworthy that at prey concentrations of <10 µg C l^{-1} some positive growth rates were measured for all 3 species. This growth may partially be explained by a post-feeding, residual cell division as observed by Jakobsen & Hansen (1997). Growth at prey concentrations <10 µg C l^{-1} has also been measured in some other dinoflagellate and ciliate species (Jakobsen & Hansen 1997).

In our experiments, a significant reduction in diatom biomass in treatments with predators relative to the diatom controls was only observed when *Protoperidinium* spp. concentrations were relatively high (>10 *Protoperidinium* cells ml^{-1}; Fig. 3). Published coastal abundances of *Protoperidinium* spp. are usually <5 cells ml^{-1} (Lessard 1991, Kjæret et al. 2000, Olseng et al. 2002). Our own laboratory cultures have infrequently reached maximum concentrations of about 140 cells ml^{-1} for *P. conicum* and *P. excentricum*. *Protoperidinium* spp. are frequently observed in plankton samples and, because of their conspicuous morphology, are easily identified and routinely reported in plankton surveys. It is unlikely that *Protoperidinium* abundances are greatly underestimated. Nonetheless, high *Protoperidinium* spp. concentrations of >100 cells ml^{-1} have rarely been reported for field samples (Jeong et al. 2004). If our observations apply to natural plankton communities, *Protoperidinium* spp. would significantly reduce phytoplankton population size only if its abundance was higher than typically observed concentrations.

All 3 *Protoperidinium* species investigated herein showed a remarkable ability to survive in the absence of prey for extended periods (over 2 mo for 1 species). No cyst or swarmer formation was observed during this period. Growing and recently fed *Protoperidinium* spp. appeared optically dense and contain lipid vesicles when viewed under a microscope. *P. conicum* and *P. depressum* cultures occasionally show a bright pink coloring. However, these pigments were not observed before or during the experiments. Over the course of the starvation experiment, we observed increasing transparency in starved cells and disappearance of visible organelles. We suspect that increases in transparency were due to *Protoperidinium* cells metabolizing their cell content. These observations suggest that, in addition to a reduced metabolism, *Protoperidinium* species utilize storage reserves to survive famine conditions.

Several studies have previously reported starvation survival in some heterotrophic protist species (Jackson & Berger 1984, Fenchel 1990, Jeong & Latz 1994). To our knowledge, the longest survival previously observed was for an athecate dinoflagellate that could starve for 30 d (Strom 1991). In contrast, many other protistan predators, particularly ciliates, die rapidly, within hours to a few days of starvation (Jackson & Berger 1984, Fenchel 1990, Jakobsen & Hansen 1997). The ability to survive extended periods without food should be considered when comparing growth rates amongst different groups of heterotrophic protists. Whilst ciliates may allocate all acquired energy to cell division, we propose that *Protoperidinium* species convert part of their energy to storage products not immediately measured as growth. Therefore, to assess and compare predator energetics, both growth rate and starvation survival ability must be considered to estimate realized growth rate and growth efficiency.

Given that phytoplankton are rare or absent over significant spatial (e.g. thin layers, McManus et al. 2003) and temporal scales (e.g. winter-time abundance, Levinsen et al. 2000a), it appears likely that phytoplankton predators have evolved survival strategies to cope with low prey abundances. One of these strategies may be to prey upon a diverse cell size spectrum, which is consistent with the observation that dinoflagellates have the largest predator:prey size ratios known for planktonic predators (Hansen et al. 1994). Another possible strategy is to prey upon a wide prey-species spectrum. Jeong (1994) described *Protoperidinium* cf. *divergens* preying on copepod eggs and nauplii. An extensive prey-species spectrum would certainly expand the times of year during which suitable prey were available. *Protoperidinium* cysts can be observed in live samples. However, no cysts were observed in our experiments and we are not aware that cyst formation occurs in response to starvation. Nonetheless, several different strategies in addition to a reduced metabolism and resistance to starvation may be employed as survival mechanisms and thus deserve further attention.

Viability in the absence of prey provides *Protoperidinium* species and possibly other dinoflagellates with a competitive advantage. Heterotrophic protists that do not possess the ability to survive without prey for even a short time will undergo drastic fluctuations in abundance, including local extinction. In contrast, protists that do survive starvation can seek out patchy prey and may gain numeric dominance not only through growth but also through reduced mortality rates. Levinsen et al. (2000a) reported that *Protoperidinium* spp. constituted up to 60% of the winter heterotrophic dinoflagellate biomass in a Greenland bay. The authors could not trace the source of the population, but hypothesized that reduced metabolic rates allowed protists to survive periods with low phytoplankton concentrations. Our results show that *Protoperidinium* species can indeed survive prolonged

periods of adverse conditions, and thereby gain numeric dominance in the plankton community.

Thus far, we do not know by what mechanisms *Protoperidinium* species survive for such long periods. Knowledge of this mechanism, together with estimates of growth rates of starved cultures, after they have been inoculated with prey, will provide a more complete assessment of the role these predators play in marine microbial food webs, particularly as prey to zooplankton during times of low phytoplankton abundance.

Acknowledgements. We thank R. A. Horner for her kind support of this research. H. H. Jakobsen and 3 anonymous reviewers made valuable comments and suggestions, improving this manuscript. This research was funded by NSF grant OCE-9403426 to E.J.L. Support for S.M.-D. was provided by the German Academic Exchange Service (DAAD) and D.G. received support from NOAA Washington SeaGrant Program (NA040AR170032).

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Editorial responsibility: David A. Caron, Los Angeles, California, USA

Submitted: November 9, 2004; Accepted: September 6, 2005
Proofs received from author(s): November 18, 2005