Marine Biology (1994) 121: 373–380

E. J. Buskey · C. J. Coulter · S. L. Brown

Feeding, growth and bioluminescence of the heterotrophic dinoflagellate *Protoperidinium huberi*

Received: 13 June 1994 / Accepted: 21 July 1994

Abstract Feeding, growth and bioluminescence of the thecate heterotrophic dinoflagellate *Protoperidinium huberi* were measured as a function of food concentration for laboratory cultures grown on the diatom *Ditylum brightwellii*. Ingestion of food increased with food concentration. Maximum ingestion rates were measured at food concentrations of ~600 µg C l^{-1} and were ~0.7 µg C individual^{-1} h^{-1} (1.8 *D. brightwelli* cells individual^{-1} h^{-1}). Clearance rates decreased asymptotically with increasing food concentration. Maximum clearance rates at low food concentration were ca. 23 µl ind^{-1} h^{-1}, which corresponds to a volume-specific clearance rate of 5.9×10^5 h^{-1}. Cell size of *P. huberi* was highly variable, with a mean diameter of 42 µm, but no clear relationship between cell size and food concentration was evident. Specific growth rates increased with food concentration until maximum growth rates of ~0.7 d^{-1} were reached at a food concentration of 400 µg C l^{-1} (~1000 cells ml^{-1}). Food concentrations as low as 10 µg C l^{-1} of *D. brightwellii* (~25 cells ml^{-1}) were able to support growth of *P. huberi*. The bioluminescence of *P. huberi* varied with its nutritional condition and growth rate. Cells held without food lost their bioluminescence capacity in a matter of days. *P. huberi* raised at different food concentrations showed increased bioluminescence capacity, up to food concentrations that supported maximum growth rates. The bioluminescence of *P. huberi* varied over a diel cycle, and these rhythmic changes persisted during 48 h of continuous darkness, indicating that the rhythm was under endogenous control.

Introduction

Heterotrophic dinoflagellates are common protozoans in many marine ecosystems, but their trophic role is poorly understood compared to other groups of zooplankton. Photosynthetic dinoflagellates have been intensively studied because of the toxic and nuisance blooms they often form in coastal regions and because of their role in marine primary productivity. The quantitative importance of heterotrophic dinoflagellates, however, has not been fully appreciated until recently, because their heterotrophic nature is often not recognized without careful preservation of samples and examination with epifluorescent microscopy for chlorophyll autofluorescence. Gaines and Elbrachter (1987) estimated that >50% of the species of dinoflagellates are heterotrophic or mixotrophic, and at least 50% of open-ocean dinoflagellate standing stocks can consist of heterotrophs (Lessard 1984). These organisms can be major contributors to microzooplankton community biomass (Smetacek 1981; Hansen 1991; Lessard 1991; Verity et al., 1993); this community, in turn, can be responsible for the bulk of phytoplankton grazing in both coastal and oceanic waters (Gifford 1988 and references therein; Miller et al. 1991; Burkill et al. 1993).

A number of different feeding modes are found among the heterotrophic dinoflagellates (Gaines and Elbrachter 1987; Elbrachter 1991). *Protoperidinium huberi*, the subject of this study, is a pallium feeder. Pallium feeding (Gaines and Taylor 1984; Jacobson and Anderson 1986) is employed by thecate species that cannot engulf their food; prey cells are captured individually using a thin filament, and are then surrounded by a membrane (the pallium) and digested completely outside the theca. These grazers appear to prefer diatoms and, in some cases, dinoflagellates as prey (Jacobson and Anderson 1986; Jeong and Latz 1994), sometimes preying on species as large or larger than themselves (Strom and Buskey 1993).

Recent work has indicated that diatoms may be particularly fast-growing members of some oceanic communities (Furnas 1990; Strom and Welshmeyer 1991) and large diatoms may be key species in terms of new production (Goldman 1993). Pallium-feeding heterotrophic dinoflagellates may have a significant grazing impact on populations of large diatoms; this in turn has important implications for the coupling of primary producer and grazer

Communicated by N. H. Marcus, Tallahassee

E. J. Buskey (✉) · C. J. Coulter · S. L. Brown
Marine Science Institute, The University of Texas at Austin, P.O. Box 1267, Port Aransas, Texas 78373,USA

populations, and for the fate (sinking vs suspended) of this fraction of the primary production.

Numerous species of heterotrophic dinoflagellates are also capable of bioluminescence (Buskey et al. 1992), and in several oceanic regions heterotrophic dinoflagellates may be responsible for the majority of epipelagic bioluminescence (Lapota et al. 1989; Buskey et al. 1994; Swift et al. 1994). The bioluminescence capacity of these dinoflagellates appears to depend on their nutritional state; their bioluminescence decreases rapidly in the absence of food (Buskey et al. 1992). A decrease in bioluminescence capacity could increase the susceptibility of these organisms to predation. Bioluminescence in dinoflagellates is thought to act as a defense mechanism which deters predation by nocturnal grazers. Copepods ingest dimly bioluminescent dinoflagellates at a greater rate than highly bioluminescent dinoflagellates (Esaias and Curl 1972; White 1979) and exhibit photophobic responses to natural and simulated dinoflagellate bioluminescence (Buskey et al. 1983; Buskey and Swift 1983). The present study examines the relationships between food concentration, feeding, growth and bioluminescence for the thecate heterotrophic dinoflagellate *Protoperidinium huberi*.

Materials and methods

Plankton samples for isolating *Protoperidinium huberi* were collected with a 20 μm-mesh, 20 cm-diam net in the Aransas Ship Channel in Port Aransas, Texas (27°50′N; 97°03′W) during December of 1992 (ambient water temperature ~20°C). The net was allowed to stream with the tide for a few minutes during a flood tide. The sample was then gently screened through a 100 μm-mesh sieve to remove mesozooplankton and debris. Aliquots of the microzooplankton samples were placed in 1-liter polycarbonate centrifuge bottles, diluted with ciliate media (Gifford 1985), and enriched with several species of cultured phytoplankton including *Ditylum brightwellii*, *Thalassiosira* sp. and *Prorocentrum micans*. These enrichments were then placed on a bottle roller rotating at ~2 rpm to keep food in suspension in a temperature-controlled incubator (20°C), with a 12 h light:12 h dark cycle under low-light conditions (~2 μ*M* photons m^{-2} s^{-1}). Samples of the enrichments were examined under a stereomicroscope every few days for the growth of *Protoperidinium* species. Various species of *Protoperidinium* were isolated from the enrichments and brought into culture. The species used in this study was identified as *P. huberi* based on the description of Balech (1988).

Phytoplankton species used as food in these studies were cultured in *f*/2 medium (Guillard and Ryther 1962). Phytoplankton were grown in polycarbonate flasks at 20°C on a 12 h light:12 h dark cycle at ~120 μ*M* photons m^{-2} s^{-1} (photosynthetically available radiation measured with Biospherical Instruments QSL-100 quantum scalar irradiance meter). Only actively growing phytoplankton cultures were used. Phytoplankton cells for elemental analysis were filtered onto precombusted GF/F filters, dried at 50 °C, and combusted in a Carlo-Erba EA1108 elemental analyzer. The volume of *Protoperidinium huberi* cells was determined from measurements made at 200× magnification and using appropriate geometric formulas; the carbon content of *P. huberi* was then estimated using a carbon:cell volume ratio of 0.14 pg C μm^{-3} (measured for *Oblea rotunda* by Lessard, cited in Strom and Buskey 1993).

Protoperidinium huberi cultures were maintained in polycarbonate containers on a bottle roller as described above. Cultures were fed a mixture of *Thalassiosira* sp., *Nitzschia thermalis*, *Biddulphia* sp., *Ditylum brightwellii* and *Corethron hystrix*, in equal proportions at 2 to 3 d intervals, and were diluted with fresh medium at 1 wk intervals.

Preliminary studies were conducted to survey the species of phytoplankton consumed by *Protoperidinium huberi*. A single species of cultured phytoplankton was placed in each well of a tissue-culture plate and several (~50) *P. huberi* were added individually to each well. The wells were examined under a stereomicroscope over a period of a few hours to determine if *P. huberi* was feeding on that species of phytoplankton. Observations of feeding behavior were easy, for these pallium feeders tow their food particles around for up to an hour.

Specific growth-rate constants were measured by adding ~2 to 3 *Protoperidinium huberi* per ml to 150 ml of ciliate media with *Ditylum brightwellii* at a range of food concentrations. For experiments done at low food concentrations, *P. huberi* were taken from cultures which had not been fed for several days, so that their initial division rates would not reflect their prior culture conditions. Controls containing *P. huberi* without food were also prepared, to ensure that there was no division based on energy reserves. Triplicate 10 ml samples were removed daily, placed in 20 ml scintillation vials and held in the dark at 20°C for 1 h. These samples were assayed for their bioluminescence during the preservation process by adding 1 ml of formaldehyde to the sample after it was placed within the integrating sphere of the photon-counting system. Following the bioluminescence measurement, the sample was stained with three drops of a 10 $g l^{-1}$ Calcofluor solution. Calcofluor binds to the cellulose plates of thecate dinoflagellates, causing them to fluoresce a bright blue-white under UV illumination (Fritz and Triemer 1985). Samples were stored in the dark at 4°C until enumeration. In one experiment, samples were taken at 12 h intervals to more accurately examine the relationship between division rate and bioluminescence over a 24 h period. *P. huberi* abundances from these samples were determined by filtering the samples onto 0.4 μm pore-size polycarbonate filters. These filters were then mounted on slides and counted under epifluorescent illumination. The diameters of 30 cells were also measured to examine for size variations under different food regimes. Specific growth rates (μd^{-1}) were calculated as the linear portion of ln (*P. huberi* ml^{-1}) regressed against time (a specific growth rate of 0.693 d^{-1} corresponds to a division rate of one division per day). Growth-rate experiments were carried out for a period of 4 d, with samples collected after 24, 48, 72 and 96 h. During the last day of the experiment, sample volumes were reduced from the original 150 to 60 ml. This reduction in volume had no effect on growth rates or bioluminescence.

Grazing-rate determinations followed a similar protocol, except that higher concentrations of *Protoperidinium huberi* were used at higher food concentrations (up to 10 *P. huberi* ml^{-1} at 1000 μg C l^{-1}), and control containers containing phytoplankton without grazers were also prepared for each food concentration. Grazers were allowed to acclimate to their new food conditions for 24 h before initial sampling. A second sample for determination of grazing rates was taken 24 h after the initial sample. Grazer and food-cell counts were determined microscopically using the blue fluorescence of the Calcofluor-stained dinoflagellates and the red autofluorescence of their photosynthetic food. Ingestion, clearance and growth rates were determined using the equations of Heinbokel (1978). Gross growth efficiencies (grazer biomass produced/phytoplankton biomass consumed) were determined using measured phytoplankton cell carbon values for *Ditylum brightwellii* and cell volume:cell carbon relationships for *P. huberi*.

The time required for *Protoperidinium huberi* to feed on *Ditylum brightwellii* cells was determined by direct observation. A few *P. huberi* were placed in a petri dish with a suspension of *D. brightwellii*. When a cell was observed to attach to a diatom it was gently transferred to another dish and the time when the capture occurred was noted. The feeding process was observed until the cell had dropped the remains of the diatom.

Bioluminescence was quantified using a low-light level detection system consisting of an integrating sphere collector (Labsphere, Polane-coated) and a photon-counting photometer (Hammamatsu C1230 photon-counting system and a R 464 photomultiplier tube). The photometer system was calibrated using cultures of biolumines-

cent bacteria (*Photobacterium* sp.) and a Quantalum 2000 luminescence photometer with a highly stable silicon photodiode sensor. Chemical stimulation using formalin was used in this study to avoid damaging *Protoperidinium huberi* cells through mechanical stimulation, since all cells must be counted to obtain growth rates. Bioluminescence in dinoflagellates can be chemically stimulated with the addition of a small amount of acid (e.g. Sweeney 1969), or by the addition of a variety of other chemicals (Hamman and Seliger 1972). Formalin was found to stimulate the same amount of bioluminescence in *P. huberi* as addition of acid or mechanical stimulation.

Experiments were performed to determine if there was a diel rhythm of feeding, growth or bioluminescence in *Protoperidinium huberi*. In these experiments, *P. huberi* grown at a *Ditylum brightwellii* concentration of 350 µg C l^{-1} were sampled at 4 h intervals over a 48 h period. In the first experiment, they were grown under a 12 h light:12 h dark cycle to determine if there were diel patterns of feeding, growth or bioluminescence; in the second experiment they were grown in continuous darkness to determine if the diel rhythms were circadian.

Results

Protoperidinium huberi was observed to feed on 15 of the 18 diatom species offered in preliminary feeding experiments [*Nitzschia frustulum*, *Skeletonema* sp., *Leptocylindrus danicus*, *Amphora coffeaeformis*, *Amphiprora paludosa* var. *duplex*, *Thalassiosira* sp., *Nitzschia thermalis*, *Cylindrotheca fusiformis*, *Biddulphia* sp., *Navicula salinarum*, *Coscinodiscus radiatus*, *Nitzschia curvilineata*, *Ditylum brightwellii*, *Corethron hystrix* and *Coscinodiscus* sp. (CCMP 312)]. *P. huberi* did not feed on the diatoms *Minutocellis* sp., *Navicula incerta* or *Coscinodiscus* sp. (CCMP1585); it fed on only 2 of the 5 dinoflagellate species offered as food, *Heterocapsa niei*, and *Prorocentrum micans*; it did not feed on a variety of other small flagellates offered as food (Table 1). In preliminary experiments, a number of phytoplankton species were found to support growth of *Protoperidinium huberi*; *D. brightwellii* appeared to support the highest growth rates and was chosen as the food for more detailed studies.

Clearance rates of *Protoperidinium huberi* feeding on *Ditylum brightwellii* ranged from 0.6 to 23.0 µl h^{-1}, with highest clearance rates measured at the lowest food concentrations (Fig. 1A). Ingestion rates ranged from 63 to 741 pg C individual^{-1} h^{-1} (0.2 to 1.8 cells individual^{-1} h^{-1}), with maximum ingestion rates occurring at a food concentration of ~600 µg C l^{-1} (1500 cells ml^{-1}; Fig. 1B). The average time required for *P. huberi* to consume one *D. brightwellii* cell was 24.1±6.4 min (mean ±1 SD based on 24 measurements). This suggests that at maximum ingestion rates, *P. huberi* spent ~70% of its time with a food particle attached. The amount of carbon per cell of *D. brightwellii* ranged from 372 to 427 pg C $cell^{-1}$, with a mean value of 395 pg C $cell^{-1}$ (n=8). The carbon content of *P. huberi* was estimated based on the calculated volume of the cell and the volume:biomass conversion for *Oblea rotunda*, another thecate heterotrophic dinoflagellate. Determinations of gross growth efficiency of *P. huberi* were highly variable in our study, ranging from 0.17 to 0.59, with a mean value of 0.37±0.11 (±1 SD). Gross growth efficiencies tended to be higher for cells grown at low food concentrations (mean values ranging from ~0.3 to 0.6 at < 300 µg C l^{-1}), and somewhat lower (mean values ranging from ~0.2–0.4 at >300 µg C l^{-1}) for cells grown at higher food concentrations (Fig. 2). *P. huberi* may have spent less time feeding on food particles at higher food concentrations and may have less completely digested their prey.

Specific growth rates of *Protoperidinium huberi* measured as a function of food concentration (*Ditylum brightwellii*) ranged from 0.04 to 0.72 d^{-1} (Fig. 3). Maximum growth rates were reached at food concentrations of ~400 µg C l^{-1} (1000 cells ml^{-1}). Positive growth was ob-

Table 1 Taxa and sizes of phytoplankton species offered as food to *Protoperidinium huberi* (*Ph*) in preliminary feeding experiments. Ratio of cell volumes of phytoplankton food to volume of *P. huberi* is presented. Experiments were scored as Y if *P. huberi* were observed with captured food cells, and as N if no captured cells were observed

Species	Size (µm)	Ratio (prey: P_m vol)	Feeding
Prymnesiophyceae			
Emiliania huxleyi	4 × 4	0.001	N
Isochrysis galbana	5 × 5	0.002	N
Cryptophyceae			
Pyrenomonas salina	6 × 10	0.007	N
Cryptomonas sp.	7 × 11	0.010	N
Chlorophyceae			
Dunaliella tertiolecta	6 × 7	0.004	N
Dinophyceae			
Heterocapsa pygmaea	9 × 14	0.020	N
Heterocapsa niei	12 × 18	0.045	Y
Gonyaulax polyedra	32 × 33	0.463	N
Prorocentrum micans	30 × 39	0.554	Y
Gyrodinium dorsum	35 × 47	0.930	Y
Bacillariophyceae			
Minutocellis	3 × 4	0.001	N
Nitzschia frustulum	5 × 6	0.003	Y
Skeletonema sp.	5 × ≤ 145[a]	0.073	Y
Leptocylindrus danicus	5 × 18[b]	0.009	Y
Amphora coffeaeformis	8 × 16	0.021	Y
Amphiprora paludosa	9 × 16	0.026	Y
Navicula incerta	6 × 20	0.014	N
Thalassiosira sp.	12 × 16	0.047	Y
Nitzschia thermalis	13 × 24	0.082	Y
Cylindrotheca fusiformis	6 × 35	0.025	Y
Biddulphia sp.	17 × 27	0.158	Y
Navicula salinarum	5 × 49	0.025	Y
Coscinodiscus radiatus	24 × 32	0.373	Y
Nitzschia curvilineata	15 × 48	0.218	Y
Ditylum brightwellii	14 × 62	0.246	Y
Corethron hystrix	15 × 68	0.310	Y
Coscinodiscus sp. (CCMP312)	61 × 68	5.12	Y
Coscinodiscus sp. (CCMP1585)	67 × 83	7.54	N

[a] Forms chains of variable length
[b] Forms chains of variable length; this measure is for a single cell

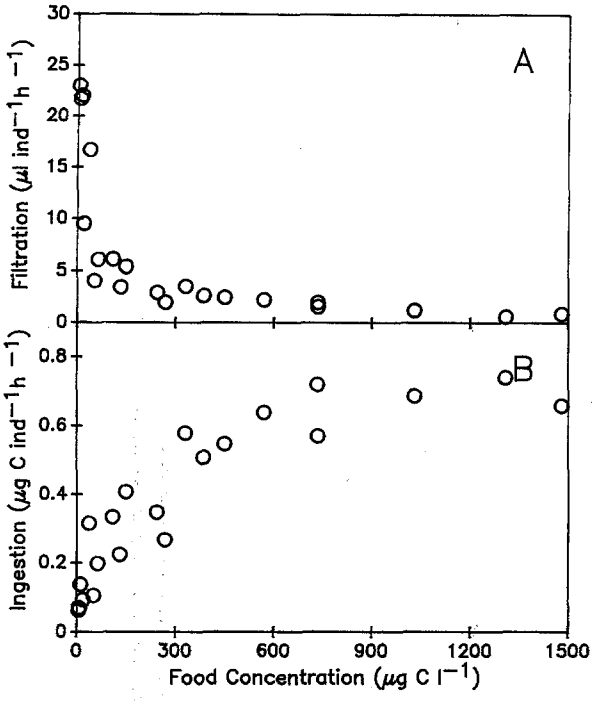

Fig. 1 *Protoperidinium huberi* fed *Ditylum brightwelli*. Clearance (**A**) and ingestion (**B**) as a function of mean food concentration over 24 h experiment

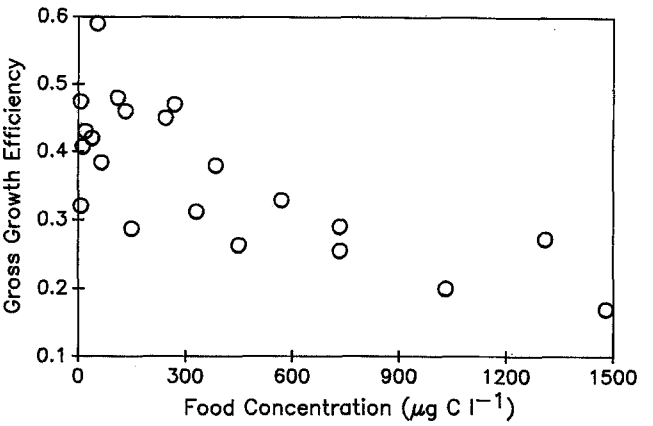

Fig. 2 *Protoperidinium huberi*. Gross growth efficiency as a function of mean concentration of *Ditylum brightwellii* over 24 h period during which growth and grazing rates were calculated

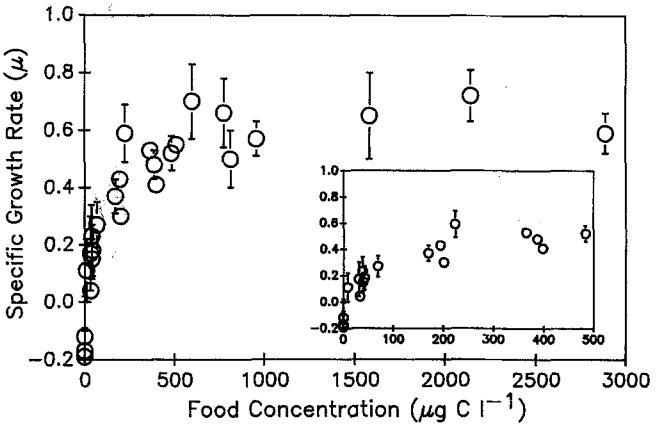

Fig. 3 *Protoperidinium huberi*. Specific growth rate (d^{-1}) as a function of mean food concentration (*Ditylum brightwellii*) over the 4 d of experiment. Inset shows expanded view of same data at low food concentrations

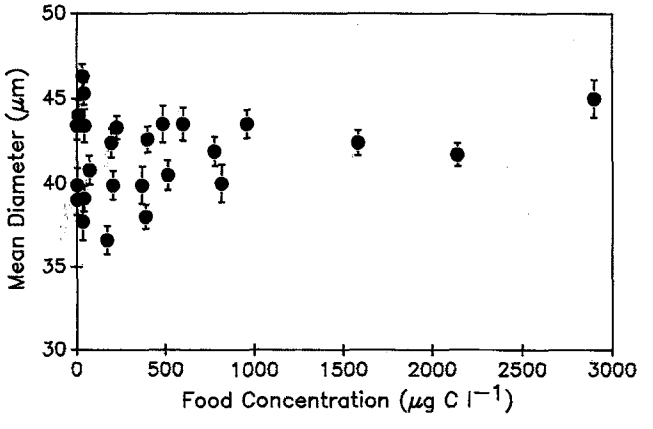

Fig. 4 *Protoperidinium huberi*. Mean cell diameters from all growth experiments as a function of mean food concentration

tained with *D. brightwellii* concentrations as low as 10 µg C l^{-1} (25 cells ml^{-1}). Cultures of *P. huberi* held without food always decreased in numbers over the 4 d experimental period. Cannibalism may have be partly responsible for the decrease in numbers; cells held individually showed little mortality over similar time intervals. However, cannibalism was only directly observed once in cultures where even a small amount of food was present. Cell size of *P. huberi* was highly variable, ranging from 30 to 67 µm in diameter. The mean diameter of cells measured in all experiments was 41.7±2.5 µm (±1 SD). There was no clear relationship between food concentration and cell diameter (Fig. 4).

Bioluminescence per cell decreased rapidly for cells held individually without food (Fig. 5A). For cells held in groups in larger volumes, the decrease in bioluminescence was somewhat less rapid (Fig. 5B). Cannibalism may have occurred in cultures held without food, providing the surviving *Protoperdinium huberi* with some nutrition. In growth experiments where samples were taken at 12 h intervals over a 4 d period, the relationship between bioluminescence and growth rate was examined for *P. huberi* grown at different food concentrations. During the first 24 h period, there was little difference between the bioluminescence of cells grown at different food concentrations, and only small differences in growth rate. By Day 3, the differences in bioluminescence and growth rate were greatest, and bioluminescence per cell was highly correlated to specific growth rate (Fig. 6). At higher food concentrations, cells grew faster and bioluminescence capacity per

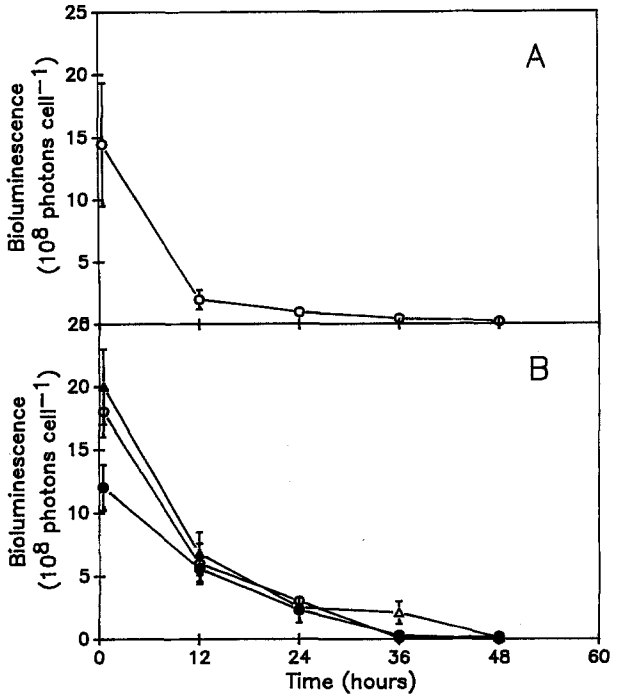

Fig. 5 *Protoperidinium huberi.* Changes in bioluminescence over time for cells held without food individually (**A**) or held in groups without food (**B**). Experiments in **B** served as controls in growth experiments run at low food concentrations

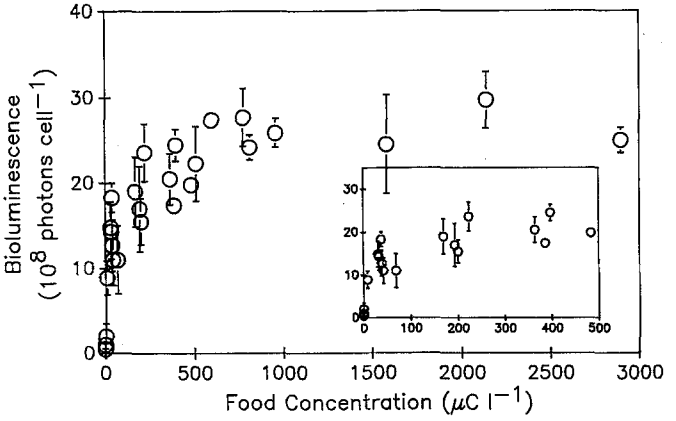

Fig. 7 *Protoperidinium huberi.* Bioluminescence per cell at end of 4 d growth experiments as a function of mean food concentration. Inset shows expanded view of same data at low food concentrations

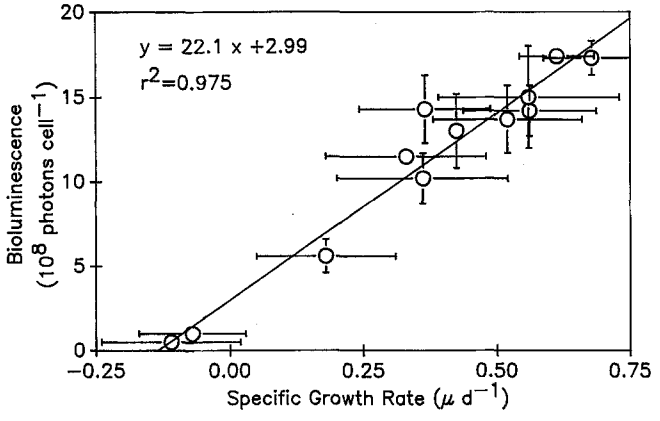

Fig. 6 *Protoperidinium huberi.* Relationship between bioluminescence per cell and specific growth rates for dinoflagellates grown at different concentrations of *Ditylum brightwellii*. Growth rates were determined over 24 h period, based on samples taken at 12 h intervals

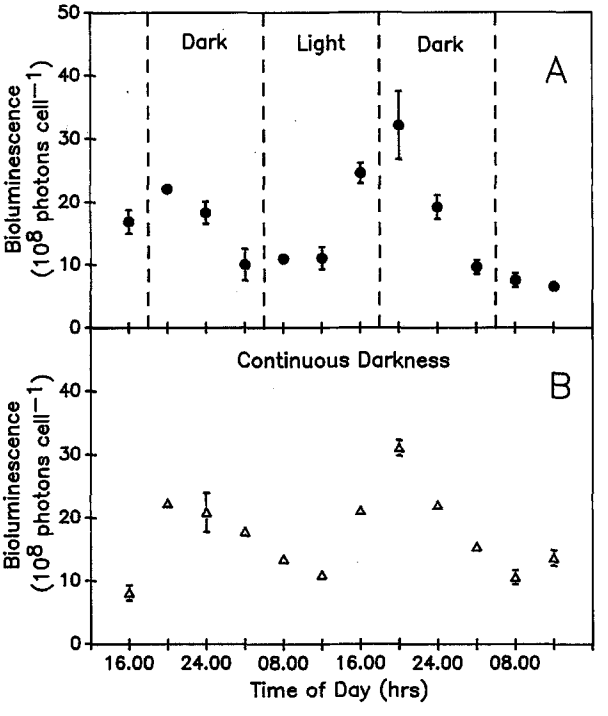

Fig. 8 *Protoperidinium huberi.* Diel pattern of bioluminescence of dinoflagellates held in a 12 h light:12 h dark cycle (**A**), and of dinoflagellates held in continuous darkness (**B**)

cell was higher in the more rapidly growing cells. For all experiments, the bioluminescence per cell at the end of the experiment generally increased with food concentration (Fig. 7) with a maximum bioluminescence per cell found at similar food concentrations to those that support maximum growth rates (400 µg C l^{-1}).

The experiments that were designed to examine if there was a diel pattern of feeding, growth or bioluminescence, revealed a clear pattern of diel changes in bioluminescence capacity per cell (Fig. 8A). Rhythmic changes in bioluminescence intensity were also found to persist over a 48 h experiment when cells were moved from a 12 h light:12 h dark cycle to continuous darkness, suggesting that the rhythm was under endogenous control (Fig. 8B). No diel variations or endogenous rhythms in feeding or growth were detected.

Discussion

Protoperidinium huberi preyed on most species of diatoms tested, and on a smaller proportion of the dinoflagellates species tested (Table 1). This is in agreement with a previous study by Jacobson and Anderson (1986), who studied feeding of a number of species of *Protoperidinium*. *Oblea rotunda*, another species of thecate heterotrophic dinoflagellate, is more of a feeding generalist, feeding on prymnesiophytes, cryptophytes, chlorophytes and prasinophytes, as well as a variety of dinoflagellates and diatoms (Strom and Buskey 1993). *O. rotunda* (~23 μm diam) is considerably smaller that *P. huberi* (~42 μm diam), however, and both species feed on cells ranging from < 1% to >100% of their volume (see Table 1; and Strom and Buskey 1993). Food selection by *P. huberi* may have been somewhat limited by food size in this study. *P. huberi* did not feed on any of the small prymnesiophytes, cryptophytes or chlorophytes tested, nor did it feed on the smallest diatom (*Minutocellis*) tested, all of which had cell volumes < 1% of that of *P. huberi*. *P. huberi* was able to feed on one species of diatom with a volume larger than itself, *Coscinodiscus* sp. (CCMP312), but did not feed on the largest species of *Coscinodiscus* sp. tested (CCMP1585). Only three species within the size range of preferred food items were not fed on by *P. huberi*, the dinoflagellates *Heterocapsa pygmaea* and *Gonyaulax polyedra*, and the diatom *Navicula incerta*. It is unclear why *P. huberi* would not feed on these phytoplankton species. In these feeding tests, *P. huberi* were not starved or conditioned to different foods for a long period of time; under other conditions its feeding preferences might broaden.

The maximum specific-growth rate of *Protoperidinium huberi* grazing on the diatom *Ditylum brightwellii* was 0.72 d^{-1}, slightly more than one division per day. This is similar to the maximum growth rate for *Oblea rotunda* feeding on *D. brightwellii*, nearly a doubling per day (Strom and Buskey 1993), but less than the maximum growth rates of *Oxyrrhis marina* (1.3 d^{-1}, Goldman et al. 1989) and of *Gyrodinium* sp. (1.15 d^{-1}, Hansen 1992). The growth rates of heterotrophic dinoflagellates are generally lower than those reported for similarly sized ciliates (Banse 1982; Hansen 1992). Jacobson and Anderson (1993) measured considerably higher growth rates for *P. hirobis* grazing on *Leptocylindrus danicus*, with a maximum growth rate of 1.2 d^{-1}, which corresponds to 1.7 divisions d^{-1}. The maximum growth rate of *P. huberi* was also lower than the maximum growth rate of the food used in this experiment. Growth rates of *D.brightwellii* of 1.5 (Baars 1981) and 2.1 (Paasche 1968) doublings d^{-1} have been reported. Under conditions favorable to their growth, *D. brightwellii* can easily outgrow a population of *P. huberi*.

Maximum clearance rates for *Protoperidinium huberi* were 23 μl h^{-1}. This corresponds to a maximum volume-specific clearance rate of 5.93×10^5 h^{-1}, which is similar to values reported for *P. hirobis* (Jacobson and Anderson 1993) and for other heterotrophic dinoflagellates and ciliates (Strom 1991). Clearance rates were within the range of values reported for species of *Protoperidinium* by Lessard and Swift (1985) of 1 to 28 μl individual^{-1} h^{-1}. *P. huberi* has a mean swimming speed of 0.33 mm s^{-1} (Buskey unpublished data), which corresponds to 8 body lengths per second (2.8×10^4 body lengths h^{-1}). This is similar to the swimming speeds of other heterotrophic dinoflagellates (Buskey et al. 1993). This swimming speed (2.8×10^4 body lengths h^{-1}) makes it difficult to explain the maximum clearance rate (5.93×10^5 h^{-1}), if it is assumed that *P. huberi* locates its food only by direct interception of food particles.

In order to search the larger volume of water as suggested by the maximum clearance rates, either prey must be unevenly distributed and *Protoperidinium huberi* must be capable of remaining in high-density food patches once they are encountered, or *P. huberi* must be capable of remote detection of prey. *Oblea rotunda* has been shown to have a behavioral response to phytoplankton exudates, supporting the possibility of remote chemosensory detection of prey (Strom and Buskey 1993). *Protoperidinium* spp. cells have been observed swimming around a prey cell for several seconds before attaching to the cell and beginning pallium feeding (Jacobson and Anderson 1986; and observations during present study); some form of sensory perception must be used to remain in the vicinity of the cell until feeding begins. Chemoreception seems to be the most likely explanation.

Gross growth efficiencies (GGE) for *Protoperidinium huberi* feeding on *Ditylum brightwellii* averaged 0.37, which is lower than that reported for *Oblea rotunda* feeding on *D. brightwellii* (0.60, Strom and Buskey 1993), but within the range of values reported for heterotrophic protozoans (Caron and Goldman 1990). The cell carbon:cell-volume conversion factor used in determining GGE of *P. huberi* was measured for the thecate heterotrophic dinoflagellate *O. rotunda*. Since we did not determine the carbon:volume ratio directly for *P. huberi*, our calculated GGEs must be interpreted with caution.

Heterotrophic dinoflagellates of the genus *Protoperidinium* are often common members of the oceanic microzooplankton community, and their abundances can exceed 200 individuals l^{-1} in oceanic regions (Buskey 1994; Swift et al. 1994) and 60 000 individuals l^{-1} in coastal bays (Jacobson 1987). Given their high grazing rates, their ability to consume a wide range of particle sizes and their potential growth rates of about a doubling per day, *Protoperidinium* spp. have the potential to be important grazers in the marine environment and, in some cases, may have more grazing impact on diatom populations than do copepods (Jacobson 1987). These small grazers and other protists are themselves subject to being preyed on by planktonic predators such as copepods (Sherr et al. 1986; Stoecker and Capuzzo 1990). Bioluminescent dinoflagellates such as *P. huberi* may have a competitive advantage over other protozoan grazers in environments where predation by mesozooplankton is important in controlling microzooplankton populations, since bioluminescence is thought to function as a defense against nocturnal predators (Esaias and Curl 1972; Buskey et al. 1983).

In this study, we have shown that *Protoperidinium huberi* grown under growth-limiting food conditions have lower levels of bioluminescence than those grown at higher food concentrations, and that cells that do not feed lose the majority of their bioluminescence capacity in a matter of days. Previous studies with autotrophic bioluminescent dinoflagellates have demonstrated that nocturnal grazers such as copepods feed more on dinoflagellates with reduced bioluminescence capacity than on brightly bioluminescent cells (Esaias and Curl 1972; White 1979). In the study of Esaias and Curl (1972), bioluminescence of *Gonyaulax acatenella* was reduced by two orders of magnitude through photoinhibition. Complete starvation for 48 h only reduced bioluminescence of *P. huberi* by one order of magnitude (Fig. 5A), and differences in bioluminescence were only a factor of 2 to 3 for cells grown at different food concentrations (Fig. 7). It is uncertain if changes in bioluminescence of the magnitude observed in the present study would have affected predation on *P. huberi* by nocturnal grazers, but changes in light intensity of this magnitude were insufficient to cause a change in behavioral response of the copepod *Acartia hudsonica* to simulated dinoflagellate bioluminescence (Buskey and Swift 1983).

It is not clear whether the normal range of variations in food availability observed in nature will cause changes in intensity of bioluminescence similar to those observed in our laboratory studies. Swift et al. (1994) reported not to have found any relationship between bioluminescence per cell and chlorophyll concentrations in the north Atlantic over a range of chlorophyll from 4.5 to 75 ng l^{-1}. As these authors point out, this could be because the *Protoperidinium* spp. were not exposed to low food concentrations for sufficient time to affect their bioluminescence, or that food concentrations were still above the saturation values for maximum bioluminescence, and that chlorophyll is a poor measure of food availability for *Protoperidinium* spp. The change in bioluminescence with food availability shown for *P. huberi* in the present study is analogous to the photoenhancement of bioluminescence of autotrophic dinoflagellates, where bioluminescence intensity is dependent on the amount of light the cells received during the previous day (Sweeney et al. 1959; Swift and Meunier 1976).

Protoperidinium huberi exhibited a diel variation in the amount of bioluminescence per cell; more bioluminescence was produced in response to chemical stimulation during the dark period than during the light period (Fig. 8). This pattern was also found for *P. huberi* exposed to constant darkness, indicating the ability of the cells to keep track of time over an interval of ~24 h without an external light stimulus; such rhythms are known as circadian, because the period is about a day. Circadian rhythms in the bioluminescence of autotrophic dinoflagellates have been known for a long time (Haxo and Sweeney 1955; Sweeney and Hastings 1957; Hastings and Sweeney 1958). Circadian rhythms of bioluminescence intensity should be of adaptive value, since maximum bioluminescence intensity can be produced during the dark period when it would be of most value as a deterrent against grazers.

Spatial and temporal distribution patterns of bioluminescence in the sea are sometimes estimated from direct measurements of bioluminescence of identified species and estimates of their abundance based on plankton samples (e.g. Swift et al. 1985; Batchelder and Swift 1989; Buskey 1992). This bioluminescence budget approach assumes that the amount of bioluminescence that can potentially be produced per organism is constant over a range of environmental conditions. The present study provides further evidence that the bioluminescence of *Protoperidinium* spp. may vary with food availability, although these relationships have not yet been demonstrated to exist in nature. If the relationship between growth rate and bioluminescence proves to be robust, measurements of bioluminescence per cell of known *Protoperidinium* species may provide insight into their in situ growth rates and recent feeding history. Cells in a population that exhibit less bioluminescence per dinoflagellate could indicate a recent history of poor nutritional conditions with resulting lower growth rates. Conversely, observations of greater bioluminescence per dinoflagellate could indicate a recent history of better nutritional conditions and more rapid growth rates for cells in that population. More study is needed of growth rates and bioluminescence of *Protoperidinium* species in natural food assemblages.

Acknowledgements This study was supported by ONR Grant NOOO14-91-J-1879 and NSF Grant OCE 93-14036 to EJB. Technical assistance was provided by J. O. Peterson; these studies benefited greatly from discussions with S. L. Strom. This is University of Texas Marine Science Institute Contribution #894.

References

Baars JWM (1981) Autecological investigations on marine diatoms 2. Generation times of 50 species. Hydrobiol Bull 15:137–151

Balech E (1988) Los dinoflagelados del Atlantic Sudoccidental. Publnes esp Inst españ Oceanogr, Madrid: 1:1–310

Banse K (1982) Cell volumes, maximal growth rates of unicellular algae and ciliates, and the role of ciliates in the marine pelagial. Limnol Oceanogr 27:1059–1071

Batchelder HP, Swift E (1989) Estimated near-surface mesoplanktonic bioluminescence in the western North Atlantic during July 1986. Limnol Oceanogr 34:113–128

Burkill PH, Edwards ES, John AWG, Sleigh MA (1993) Microzooplankton and their herbivorous activity in the northeastern Atlantic Ocean. Deep-Sea Res 40:479–493

Buskey EJ (1992) Epipelagic planktonic bioluminescence in the marginal ice zone of the Greenland Sea. Mar Biol 113:689-698

Buskey EJ (1994) Bioluminescence and growth rates of heterotrophic dinoflagellates on varying algal diets: implications for studies of bioluminescence in the Arabian Sea. In: Thompson MF, Tirmizi NM (eds) Arabian Sea living marine resources and the environment. Vanguard Press, Karachi (in press)

Buskey EJ, Coulter CJ, Strom SL (1993) Locomotory patterns of microzooplankton: potential effects on food selectivity of larval fish. Bull mar Sci 53:29–43

Buskey EJ, Mills L, Swift E (1983) The effects of dinoflagellate bioluminescence on the swimming behavior of a marine copepod. Limnol Oceanogr 28:575–579

Buskey EJ, Strom SL, Coulter CJ (1992) Bioluminescence of heterotrophic dinoflagellates from Texas coastal waters. J exp mar Biol Ecol 159:37–49

Buskey EJ, Swift E (1983) Behavioral responses of the coastal copepod *Acartia hudsonica* to simulated dinoflagellate bioluminescence. J exp mar Biol Ecol 72:43–58

Caron DA, Goldman JC (1990) Protozoan nutrient regeneration, In: Capriulo GM (ed) Ecology of marine Protozoa. University Press, New York, Oxford, pp 283–306

Elbrachter M (1991) Food uptake mechanisms in phagotrophic dinoflagellates and classification. In: Patterson DJ, Larsen J (eds) The biology of free-living heterotrophic dinoflagellates. Clarendon, New York, pp 303–312

Esaias WE, Curl HC Jr (1972) Effect of dinoflagellate bioluminescence on copepod ingestion rates. Limnol Oceanogr 17:901–906

Fritz L, Triemer RE (1985) A rapid, simple technique utilizing Calcofluor White M2R for the visualization of dinoflagellate thecal plates. J Phycol 21:662–664

Furnas MJ (1990) *In situ* growth rates of marine phytoplankton: approaches to measurement, community and species growth rates. J Plankton Res 12:1117–1151

Gaines G, Elbrachter M (1987) Heterotrophic nutrition. In: Taylor FJR (ed) The biology of dinoflagellates. Blackwell Scientific Publications, Palo Alto, pp 224–268 (Bot Monogr 21)

Gaines G, Taylor FJR (1984) Extracellular digestion in marine dinoflagellates. J Plankton Res 6:1057–1061

Gifford DJ (1985) Laboratory culture of marine planktonic oligotrichs (Ciliophora, Oligotricha). Mar Ecol Prog Ser 23:257–267

Gifford DJ (1988) Impact of grazing by microzooplankton in the Northwest Arm of Halifax Harbour, Nova Scotia. Mar Ecol Prog Ser 47:249–258

Goldman JC (1993) Potential role of large oceanic diatoms in new primary production. Deep-Sea Res 40:159–168

Goldman JC, Dennett MR, Gordin H (1989) Dynamics of herbivorous grazing by the heterotrophic dinoflagellate *Oxyrrhis marina*. J Plankton Res 11:391–407

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

Hamman JP, Seliger HH (1972) The mechanical triggering of bioluminescence in marine dinoflagellates: chemical basis. J cell Physiol 80:397–408

Hansen PJ (1991) Quantitative importance and trophic role of heterotrophic dinoflagellates in a coastal pelagic food web. Mar Ecol Prog Ser 55:217–227

Hansen PJ (1992) Prey size selection, feeding rates and growth dynamics of heterotrophic dinoflagellates with special emphasis on *Gyrodinium spirale*. Mar Biol 114:327–334

Hastings JW, Sweeney BM (1958) A persistent diurnal rhythm of luminescence in *Gonyaulax polyedra*. Biol Bull mar biol Lab, Woods Hole 115:440-458

Haxo FT, Sweeney BM (1955) Bioluminescence in *Gonyaulax polyedra* In: Johnson FH (ed) The luminescence of biological systems. American Association for the Advancement of Science, Washington, DC, pp 415–420

Heinbokel JF (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 DM (1987) The ecology and feeding of thecate heterotrophic dinoflagellates. Ph.D. thesis. Massachusetts Institute of Technology and the Woods Hole Oceanographic Institution, Boston

Jacobson DM, Anderson DM (1986) Thecate heterotrophic dinoflagellates: feeding behavior and mechanisms. J Phycol 22:249–258

Jacobson DM, Anderson DM (1993) Growth and grazing rates of *Protoperidinium hirobis* Abe, a thecate heterotrophic dinoflagellate. J Plankton Res 15:723–736

Jeong HJ, Latz MI (1994) Growth and grazing rates of the heterotrophic dinoflagellates *Protoperidinium* spp on red tide dinoflagellates. Mar Ecol Prog Ser 106:173–185

Lapota D, Geiger ML, Stiffey AV, Rosenburg DE, Young DK (1989) Correlations of planktonic bioluminescence with other oceanographic parameters from a Norwegian fjord. Mar Ecol Prog Ser 55:217–227

Lessard EJ (1984) Oceanic heterotrophic dinoflagellates: distribution, abundance and role as microzooplankton. Ph.D. thesis. University of Rhode Island, Kingstown

Lessard EJ (1991) The trophic role of heterotrophic dinoflagellates in diverse marine environments. Marine Microb Fd Webs 5:49–58

Lessard EJ, 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

Miller CB, Frost BW, Booth B, Wheeler PA, Landry MR, Welschmeyer N (1991) Ecological processes in the subarctic Pacific: iron limitation cannot be the whole story. Oceanography, Wash 4:73–78

Paasche E (1968) Marine plankton algae grown with light-dark cycles 2. *Ditylum brightwellii* and *Nitzschia turgidula*. Physiologia Pl 21:66–77

Sherr EB, Sherr BF, Paffenhöfer G-A (1986) Phagotrophic protozoa as food for metazoans: a "missing" trophic link in marine pelagic food webs? Mar microb Fd Webs 1:61–80

Smetacek V (1981) The annual cycle of protozooplankton in the Kiel Bight. Mar Biol 63:1–11

Stoecker DK, Capuzzo JM (1990) Predation on protozoa: its importance to zooplankton. J Plankton Res 12:891–908

Strom SL (1991) Growth and grazing rates of the herbivorous dinoflagellate *Gymnodinium* sp from the open subarctic Pacific Ocean. Mar Ecol Prog Ser 78:103–113

Strom SL, Buskey EJ (1993) Feeding, growth and behavior of the thecate heterotrophic dinoflagellate *Oblea rotunda*. Limnol Oceanogr 38:965–977

Strom SL, Welschmeyer NA (1991) Pigment-specific rates of phytoplankton growth and microzooplankton grazing in the open subarctic Pacific Ocean. Limnol Oceanogr 36:50–63

Sweeney BM (1969) Transducing mechanisms between circadian clock and overt rhythms in *Gonyaulax*. Can J Bot 47:299–308

Sweeney BM, Hastings JW (1957) Characteristics of the diurnal rhythm of luminescence in *Gonyaulax polyedra*. J cell comp Physiol 49: 115–128

Sweeney BM, Haxo FT, Hastings JW (1959) Action spectra for two effects of light on luminescence in *Gonyaulax polyedra*. J gen Physiol 43:285–299

Swift E, Lessard EJ, Biggley WH (1985) Organisms associated with stimulated epipelagic bioluminescence in the Sargasso Sea and the Gulf Stream. J Plankton Res 7:831–848

Swift E, Meunier VA (1976) Effects of light intensity on division rate, stimulable bioluminescence, and cell size of the oceanic dinoflagellates *Dissodinium lunula*, *Pyrocystis fusiformis* and *P. noctiluca*. J Phycol 12:14–20

Swift E, Sullivan JM, Batchelder HP, Van Keuren J, Vaillancourt RD, Bidigare RR (1994) Bioluminescent organisms and bioluminescence measurements in the Northern Atlantic Ocean. J geophys Res (Sect Oceans) (in press)

Verity PG, Stoecker DK, Sieracki ME, Burkill PH, Edwards ES, Tronzo CR (1993) Abundance, biomass and distribution of heterotrophic dinoflagellates during the North Atlantic spring bloom. Deep-Sea Res 40:227–244

White HH (1979) Effects of dinoflagellate bioluminescence on the ingestion rates of herbivorous zooplankton. J exp mar Biol Ecol 36:217–224