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Mixotrophic feeding of *Fragilidium subglobosum* (Dinophyceae) on three species of *Ceratium*: effects of prey concentration, prey species and light intensity

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ABSTRACT: Growth and grazing responses of the mixotrophic dinoflagellate *Fragilidium subglobosum* were studied as a function of prey concentration, prey species, and light intensity in laboratory cultures. In monospecific (exclusively phototrophic) cultures the growth rate of *F. subglobosum* was 0.16 d^{-1} (doubling time 4.3 d) at a light intensity of 45 µmol photons m^{-2} s^{-1}. In cultures supplied with the phototrophic dinoflagellate *Ceratium tripos* at a similar light intensity, the growth rate of *F. subglobosum* reached a maximum level of 0.5 d^{-1} (doubling time 1.4 d) at a prey concentration of ca 10 *C. tripos* cells ml^{-1}. The functional response of *F. subglobosum* followed a Holling type I functional response. At prey concentrations which resulted in maximum growth rate, growth yield equalled ca 40%. However, at prey concentrations which led to lower growth rates, growth yield exceeded 100%, indicating that food uptake by *F. subglobosum* stimulated photosynthesis at low prey concentrations. When *C. tripos* cells were added in excess, growth and ingestion rate of *F. subglobosum* increased with light intensity within the studied range (9 to 45 µmol photons m^{-2} s^{-1}). Growth rates of *F. subglobosum* were higher with *C. tripos* as food than with *C. furca* and *C. fusus*.

KEY WORDS: Mixotrophy · Growth · Grazing · Holling type I functional response · Prey selection · Dinoflagellate · *Fragilidium subglobosum* · *Ceratium* spp. · Light

INTRODUCTION

Phototrophic dinoflagellates are an important group of phytoplankton, occasionally forming red tides in coastal seas. Ingestion of prey by photosynthetic (plastidic) dinoflagellates has been known for a long time, but until recently only found in a few species (Gaines & Elbrächter 1987, Schnepf & Elbrächter 1992). Recently, however, a large number of species collected from field samples have been found to contain food vacuoles, suggesting that mixotrophy is widespread in phototrophic forms (Bockstahler & Coats 1993a, b, Jacobson & Anderson 1996, Li et al. 1996). The term mixotrophy is here used for organisms which combine phototrophy and phagotrophy.

Three different feeding mechanisms have been described among dinoflagellates, all allowing the ingestion of relatively large prey (Jacobson & Anderson 1986, Gaines & Elbrächter 1987). The efficiency of prey capture depends on size, and experimental data suggest that dinoflagellates ingest prey corresponding to their own size most efficiently (Hansen 1992). Thus dinoflagellates are true raptorial feeders. A few studies have indicated that even large heterotrophic and mixotrophic dinoflagellates are able to feed substantially on bacteria-sized prey (Lessard & Swift 1985, Nygaard & Tobisen 1993, Neuer & Cowles 1995). However, these studies have all used methods (radioactively or fluorescently labelled bacteria) which po-

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tentially may have led to artefacts or misinterpretations (see Hansen in press for review).

The role of phagotrophy in mixotrophic flagellates varies considerably. Some species are primarily heterotrophic (e.g. *Poterioochromonas malhamensis*, *Ochromonas* spp.; Fenchel 1982, Anderson et al. 1989, Sanders et al. 1990). Others have an obligate requirement for light (e.g. *Dinobryon cylindricum*, *D. divergens*, *Ochromonas* sp., *Chrysochromulina brevifilum*), and growth is primarily due to photosynthesis (Caron et al. 1989, Veen 1991, Keller 1994). Acquisition of essential growth factors is yet another role for ingestion of prey in mixotrophic flagellates (e.g. *Uroglena americana*; Kimura & Ishida 1986, 1989). However our knowledge mainly derives from studies of chrysophytes and prymnesiophytes (Fenchel 1982, Anderson et al. 1989, Sanders et al. 1990, Jones et al. 1993). The role of phagotrophy in mixotrophic dinoflagellates has only been investigated in a single dinoflagellate, *Gymnodinium sanguineum* (Bockstahler & Coats 1993a). This dinoflagellate feeds selectively on small oligotrich ciliates. In field samples, the daily consumption of ciliate biomass by *G. sanguineum* was found to average 2.5% of body carbon and 4.0% of body nitrogen with maximal values of 11.6 and 18.5%, respectively. This suggests that *G. sanguineum* is primarily phototrophic. Nevertheless, when *G. sanguineum* occurs in high concentrations, its predation on ciliates may be substantial.

The present paper is the third in a series of papers dealing with the mixotrophic dinoflagellate *Fragilidium* (= *Helgolandinium*) *subglobosum*. In the first paper, Skovgaard (1996a) describes the feeding mechanism of *F. subglobosum*. Like Stosch (1969), Skovgaard was able to culture it photoautotrophically in monospecific cultures. In mixotrophic cultures, *F. subglobosum* feeds selectively on *Ceratium* species by direct engulfment, which is an unusual feeding mechanism for thecate dinoflagellates, but common in naked forms (Gaines & Elbrächter 1987, Schnepf & Elbrächter 1992). During the feeding process, the theca of *Ceratium* spp. is dissolved and the prey cell is transformed into a roundish food vacuole located in the center of *F. subglobosum*. When feeding on the small *C. lineatum*, *F. subglobosum* ingests up to 13 cells prior to encystment and subsequent cell division. However, when feeding on the large *C. tripos*, it only ingests a single cell before it divides. In both photoautotrophic and mixotrophic cultures, *F. subglobosum* forms division cysts as part of its life cycle.

In the second paper, Skovgaard (1996b) studied growth and ingestion responses of *Fragilidium subglobosum* as a function of light intensity in monospecific cultures and in cultures fed *Ceratium lineatum* in excess. In monospecific cultures, the maximum growth rate was obtained at a light intensity of ca 130 µmol photons m^{-2} s^{-1}. In mixotrophic cultures, the growth rate of *F. subglobosum* did not vary with light intensity within a wide range of light intensities, even though ingestion rates varied by a factor of 2 to 3. Maximum ingestion rates were obtained at intermediate light levels. At light levels below and above this level, lower ingestion rates were found.

The aim of the present paper is to study the functional and numerical response of *Fragilidium subglobosum* when fed *Ceratium tripos*. We also studied the growth and grazing response of *F. subglobosum* fed 3 *Ceratium* spp. separately at 3 light intensity levels.

MATERIALS AND METHODS

Culturing of organisms. The dinoflagellates *Ceratium furca*, *C. fusus*, and *C. tripos* were isolated from net (mesh size 20 µm) samples collected in the Kattegat (Denmark) in autumn 1992. The mixotrophic dinoflagellate *Fragilidium subglobosum* was isolated from the Kattegat (Skovgaard 1996a) and was provided by the culture collection of the Marine Biological Laboratory, Helsingør, Denmark. All organisms were grown as non-axenic cultures in B-medium (Hansen 1989) based on seawater (28‰) at 15 ± 1°C following a light:dark cycle of 14:10 h. Illumination was provided by cool white fluorescent lamps and the organisms were kept at a light intensity of ca 20 µmol photons m^{-2} s^{-1}. Light intensity was measured using a LI-COR LI-1000 radiation sensor equipped with a flat LI-COR underwater probe. The dimensions of *F. subglobosum* were determined by microscopic measurements of Lugol's fixed cells (n = 20 to 30), and volume was calculated assuming a sphere. Cell volumes of *Ceratium* species were estimated from the width of the sulcus using the equations of Thomsen (1992).

Experimental conditions. All experiments were carried out in 250 or 750 ml polystyrene bottles allowing light to penetrate from only 1 direction. The experimental bottles were mounted vertically on a rotating wheel (1 rpm) in order to keep the algae in suspension.

Numerical and functional responses of *Fragilidium subglobosum*. Experiments were carried out to study growth and grazing rates of *F. subglobosum* fed *Ceratium tripos* at prey concentrations ranging from 0 to 60 cells ml^{-1} at an illumination of 45 µmol photons m^{-2} s^{-1}. For each prey concentration, 3 bottles with *F. subglobosum* and 1 control bottle without *F. subglobosum* were incubated. The experiments were initiated by mixing cultures of *C. tripos* and *F. subglobosum* at a cell concentration ratio of 10:1, except at low prey concentrations (<10 *C. tripos* ml^{-1}), where a lower

concentration ratio of 5:1 was used. *F. subglobosum* was allowed to adapt to mixotrophic conditions for 3 d prior to subsampling once a day for up to 5 d. Subsamples were ca 10% of the experimental volume. Fresh B-medium was added to the experimental bottles, and the subsamples were fixed in 1% Lugol's (final concentration). Instantaneous growth rates of *F. subglobosum* were determined in steps of 24 h due to the daily dilution of the samples as: $\mu = [\ln(y_{t1}/y_{t0})]t^{-1}$, where y_{t0} = concentration of cells at Day t_0 (cells ml^{-1}), and y_{t1} = concentration of cells at Day t_1 (cells ml^{-1}), and t = the duration of each experiment (d). Ingestion rates of *F. subglobosum* were determined from the reduction in prey concentration over 24 h periods, using the control cultures to estimate prey growth rate. Ingestion rates, U, can be estimated using the following 2 equations:

$$dx/dt = \mu_x x - Uy \quad (1)$$

$$dy/dt = \mu_y y \quad (2)$$

where prey (x) is ingested by predator (y). It is assumed that the predator (y) grows exponentially with the rate constant of μ_y, and that the prey (x) grows with the rate constant of μ_x. The mortality due to predation is Uy, where U (dimension $x/y/t$) is the per capita ingestion rate, which is independent of x. As the solution to solve for μ is intractable, the ingestion rate (U) was iteratively calculated with time on a computer, allowing steps of 1 h (the programme is available upon request from P.J.H.). Clearance was calculated as: $C = U(x)/x$. In order to study the fate of the ingested biomass, estimates of growth yield (Y) were calculated as: $\{[\mu(m) - \mu(p)] \cdot V_y\}/(U \cdot V_x)$, where U is the ingestion rate (cells d^{-1}), $\mu(m)$ = growth rate of *F. subglobosum* in mixed cultures (d^{-1}), $\mu(p)$ = growth rate of *F. subglobosum* in purely phototrophic cultures (d^{-1}), and V_x and V_y refer to the cell volumes of *C. tripos* and *F. subglobosum* (μm^3), respectively.

Effects of light and prey selectivity on growth and ingestion rates. Experiments were carried out to study the effects of light and prey species on the growth and grazing response of *Fragilidium subglobosum*. Incubation bottles were shaded to reach light intensities of 9, 22.5 and 45 µmol photons m^{-2} s^{-1}. *F. subglobosum* was grown without prey prior to the experiment. *Ceratium furca*, *C. fusus* and *C. tripos* were used as prey at an initial biomass (cell concentration × cell volume) of 3.3 ppm (volume fraction); this corresponds to ca 30 *C. tripos*, which is well above saturating levels (see 'Results'). The prey:predator biomass relationship was 10:1. The duration of the experiments was 2 wk; otherwise the conditions were as stated above. In order to test the statistical significance of differences among treatments of ingestion and growth rates, a 1-way and 2-way ANOVA analysis, respectively, was carried out using a computer program (Sigmastat®, Jandel).

Initial prey uptake. This experiment was set up to test whether the initial prey uptake by *Fragilidium subglobosum* was determined by the physiology of *F. subglobosum* or, alternatively, was due to the food quality of the prey grown at different light intensities. Monospecific cultures of *F. subglobosum* were adapted to different light intensities. One set of *F. subglobosum* cultures was high-light adapted (45 µmol photons m^{-2} s^{-1}), while another set was low-light adapted (9 µmol photons m^{-2} s^{-1}). *Ceratium tripos* cultures which had been adapted to high light or low light intensity were fed to each set of the adapted *F. subglobosum* cells. Mixed cultures were subsampled after 4, 8, 12, 16, 20, 24, 30 and 47 h and fixed with glutaraldehyde (2% final concentration). The percentage of *F. subglobosum* containing a food vacuole was determined from more than 50 cells. Three replicates were set up for each combination.

RESULTS

Bioenergetics

The ingestion rate of *Fragilidium subglobosum* increased as a function of prey concentration and reached a maximum level of ca 0.6 *Ceratium tripos* d^{-1} (Fig. 1A). Maximum clearance was about 0.055 ml h^{-1} or 3.4×10^4 body volumes h^{-1} (Fig. 1B). In purely phototrophic cultures, the growth rate of *F. subglobosum* was 0.16 d^{-1} at a light intensity of 45 µmol m^{-2} s^{-1} (Fig. 2). In food-supplied cultures at a similar light intensity, the growth rate of *F. subglobosum* increased as a function of prey concentration and reached a maximum level of ca 0.5 d^{-1}, at a prey concentration of about 10 *C. tripos* cells ml^{-1} (Fig 2). The cell volume of *F. subglobosum* increased from ca 45000 μm^3 in monospecific cultures to about 90000 μm^3 in food satiated cultures (Fig. 3). At prey concentrations which resulted in maximum growth rate, growth yield equalled ca 40%. However, at prey concentrations which led to lower growth rates, growth yield increased to above 100% (Fig. 4).

Prey selection and light intensity

In monospecific cultures, the growth rate of *Fragilidium subglobosum* increased from about 0.04 to 0.11 d^{-1} as the light intensity increased from 9 to 45 µmol photons m^{-2} s^{-1} (Figs. 5 & 7). In food-supplied cultures, the increase in the growth rate of *F. subglobosum* was dependent on the prey species (Figs. 6

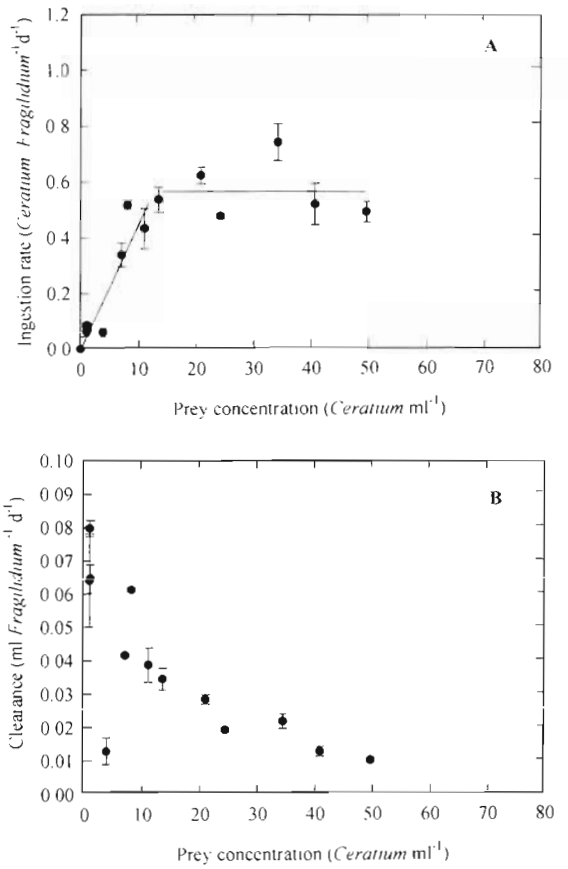

Fig. 1. *Fragilidium subglobosum*. (A) Ingestion rate as a function of cell concentration of *Ceratium tripos*. Data points represent treatment means ±1 SE (n = 3). Curves represent the rectilinear fit of data: $U = 0.0135 + 0.0413x$ for $x < 13.6$ cells ml^{-1}, $R^2 = 0.94$; $U = 0.5881 - 0.000906x$ for $x > 13.6$ cells ml^{-1}. (B) Clearance as a function of cell concentration of *C. tripos*. Data points represent treatment means ± 1 SE (n = 3)

& 7). The growth rate of *F. subglobosum*, when fed *Ceratium tripos*, increased from about 0.18 d^{-1} at the lowest light intensity to 0.4 d^{-1} at the highest light intensity. The increase in growth rate of *F. subglobosum* was significantly lower when fed the 2 smaller species (*C. furca* and *C. fusus*; $p < 0.01$). In fact, the growth rate of *F. subglobosum*, when fed the smallest species *C. fusus*, was only slightly different from growth rates obtained in monospecific cultures of *F. subglobosum* ($p < 0.05$). The change in growth rate of *F. subglobosum* as a function of light intensity was significantly larger in cultures fed *C. tripos* and *C. furca* compared to monospecific cultures ($p < 0.01$), indicating a positive interaction between photosynthesis and phagotrophy. When *F. subglobosum* was fed *C. tripos*, ingestion rates increased with light intensity, suggesting that light stimulates phagotrophy in *F. subglobosum* (Fig. 8).

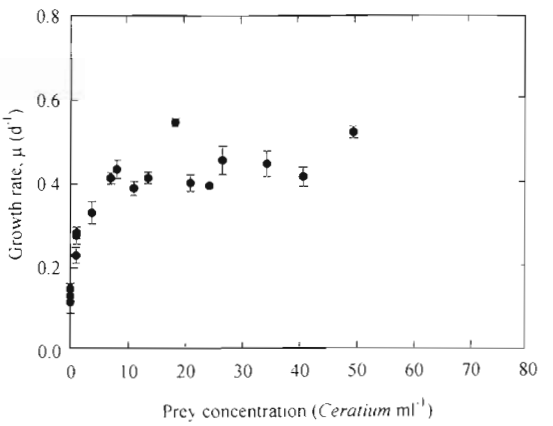

Fig. 2. *Fragilidium subglobosum*. Growth rate as a function of cell concentration of *Ceratium tripos*. Data points represent treatment means ± 1 SE (n = 3)

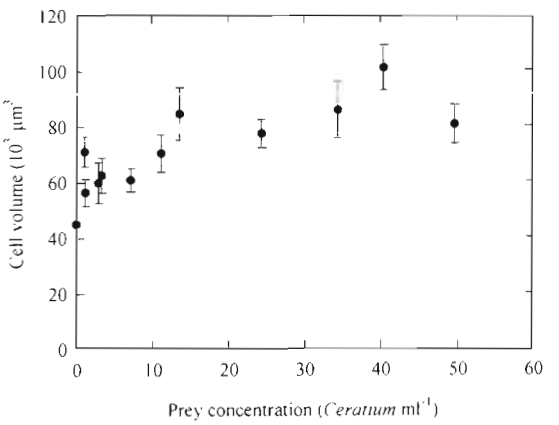

Fig. 3. *Fragilidium subglobosum*. Cell volume as a function of cell concentration of *Ceratium tripos*. Data points represent treatment means ± 1 SE (n = 3)

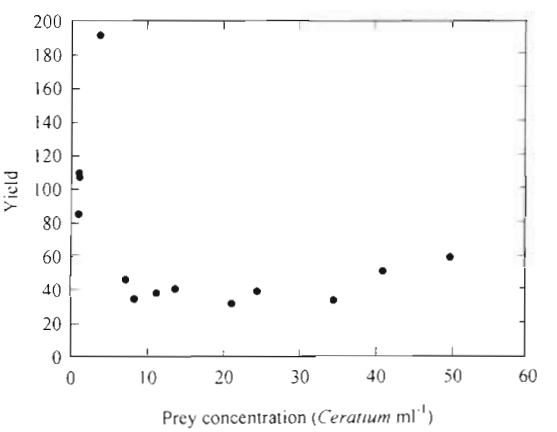

Fig. 4. *Fragilidium subglobosum*. Growth yield as a function of cell concentration of *Ceratium tripos*

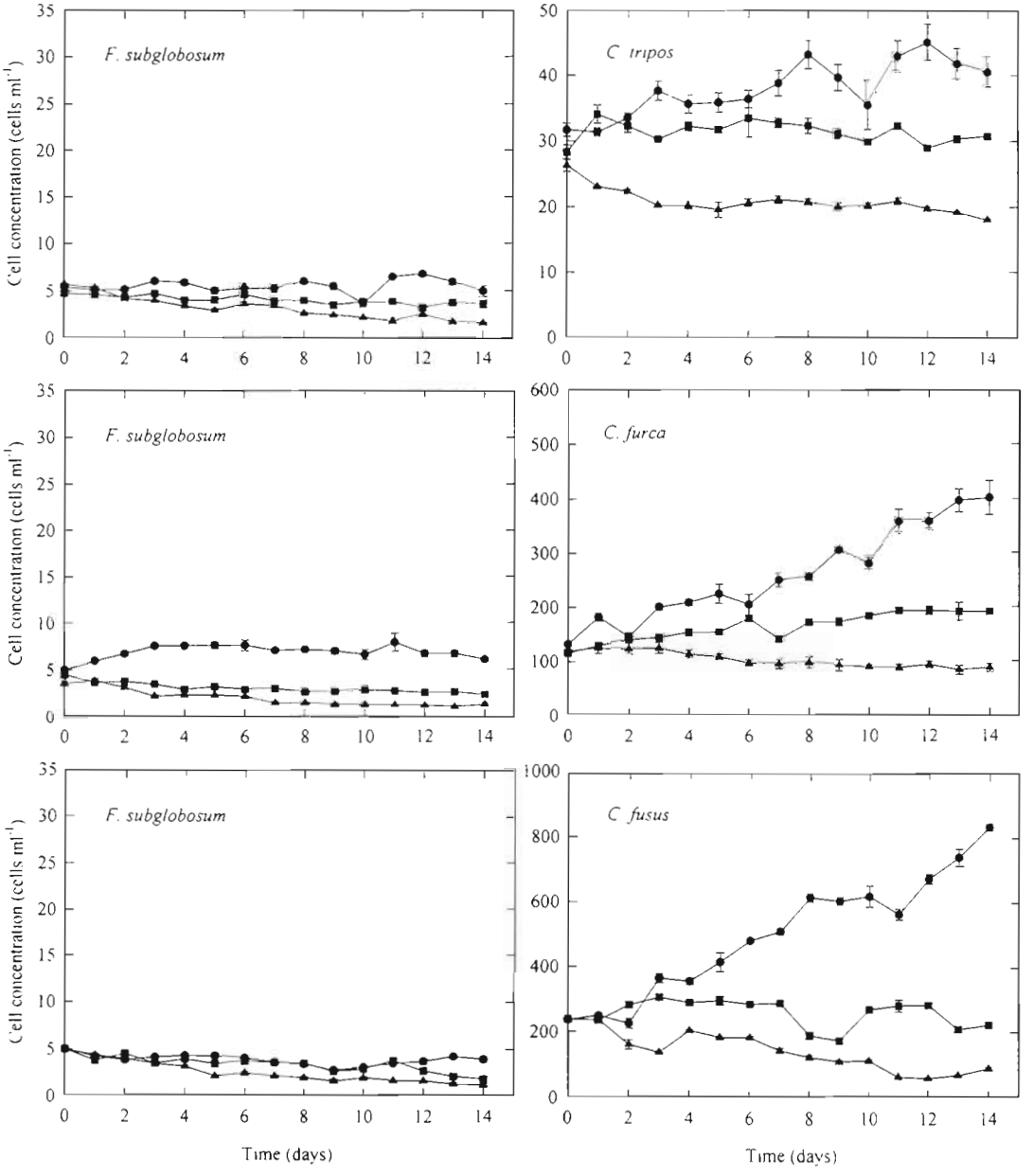

Fig. 5. Growth response of monospecific cultures of *Fragilidium subglobosum* and 3 *Ceratium* species: *C. tripos*, *C. furca* and *C. fusus*. Samples were taken daily, resulting in a dilution rate of the batch culture of ca 10% d^{-1}. Light intensities (in µmol photons m^{-2} s^{-1}): (●) 45; (■) 22.5; (▲) 9

Initial prey uptake

The initial uptake of *Ceratium tripos* cells by *Fragilidium subglobosum*, that had previously been grown photoautotrophically, depended on the light intensity to which *F. subglobosum* was exposed (Fig. 9). It made no difference whether *C. tripos* was preincubated at high or low light intensities. The number of *F. subglobosum* cells containing a food vacuole increased just around the time where light was turned on, indicating an initial diurnal rhythm in the prey uptake of *F. subglobosum*.

DISCUSSION

Holling type I functional response in *Fragilidium subglobosum*

Three types of functional responses have been used to described the relationship between the consumption rate by a predator and changes in prey concentration (Holling 1959). In studies dealing with suspension-feeding protozoa, data on ingestion rates have often been fitted to a hyperbolic function (e.g. Fenchel 1980,

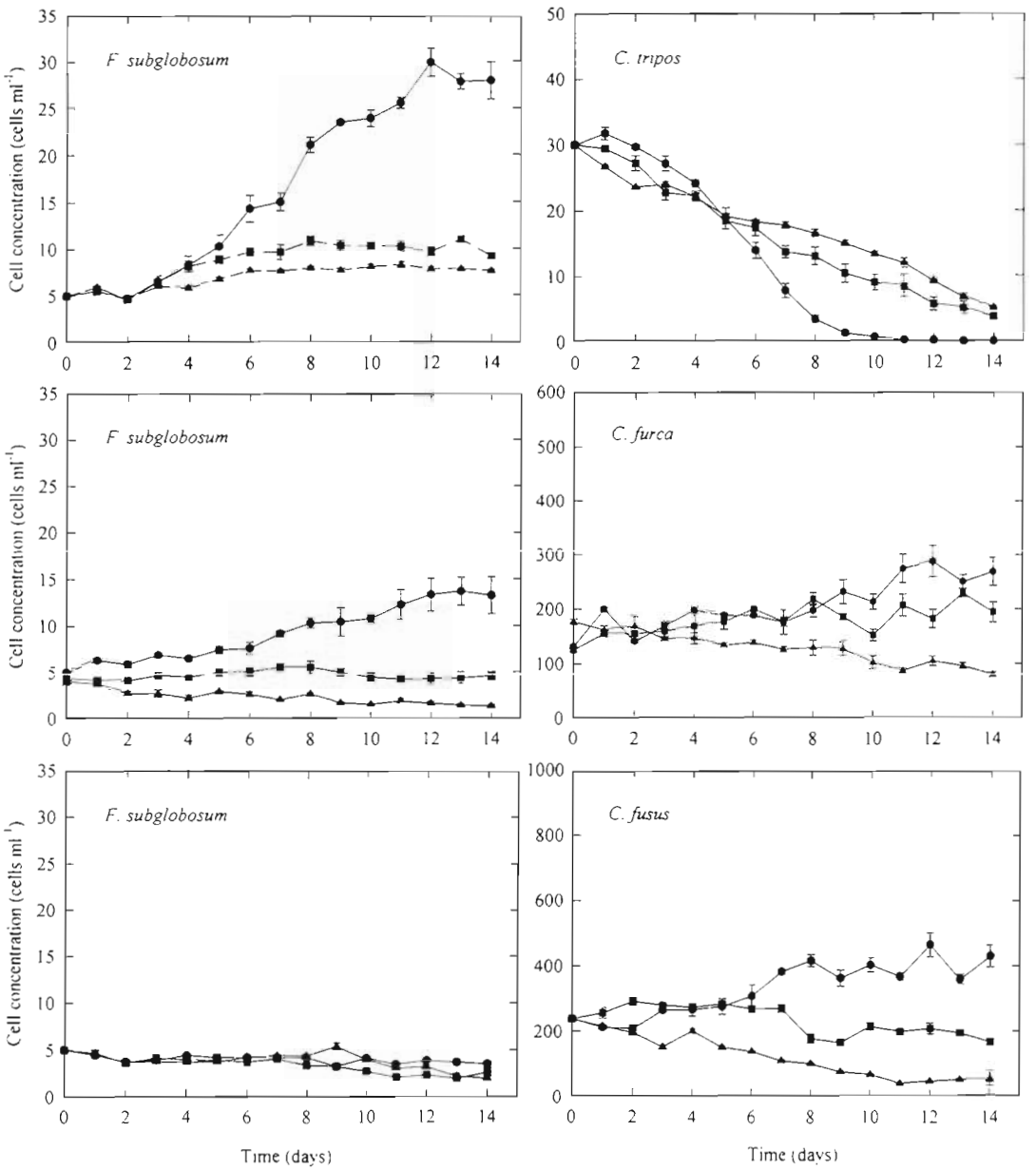

Fig. 6. Growth response of mixed cultures of *Fragilidium subglobosum* and 3 *Ceratium* species: *C. tripos*, *C. furca*, and *C. fusus*. Symbols as in Fig. 5

Jonsson 1986). Fenchel (1980) rationalized that this description is functionally analogous to the Holling type II functional response. The model assumes that it takes a finite time for the predator to process a prey. Thus, at low prey concentrations ingestion is limited by predator-prey encounter rate, while at high prey concentrations this 'handling time' will limit ingestion rate. Since *F. subglobosum* only ingests a single *C. tripos* prior to encystment and subsequent cell division, this feeding response is in fact a 'one step' process, based on encounter alone. Thus, the functional response of *F. subglobosum* is a rare example of the Holling type I functional response.

Prey recognition and selection

Growth and grazing responses of *Fragilidium subglobosum* are significantly higher when fed the larger species, *Ceratium tripos*, compared to the 2 smaller species, *C. fusus* and *C. furca* (Fig. 7). This observation cannot be explained solely by size selection, because Skovgaard (1996b) found growth rates of *F. subglobosum* fed *C. lineatum* (cell volume 8200 μm^3) which are similar to that obtained with *C. tripos* as food, and *C. lineatum* and *C. tripos* are the smallest and largest, respectively, of the 4 species which have been tested.

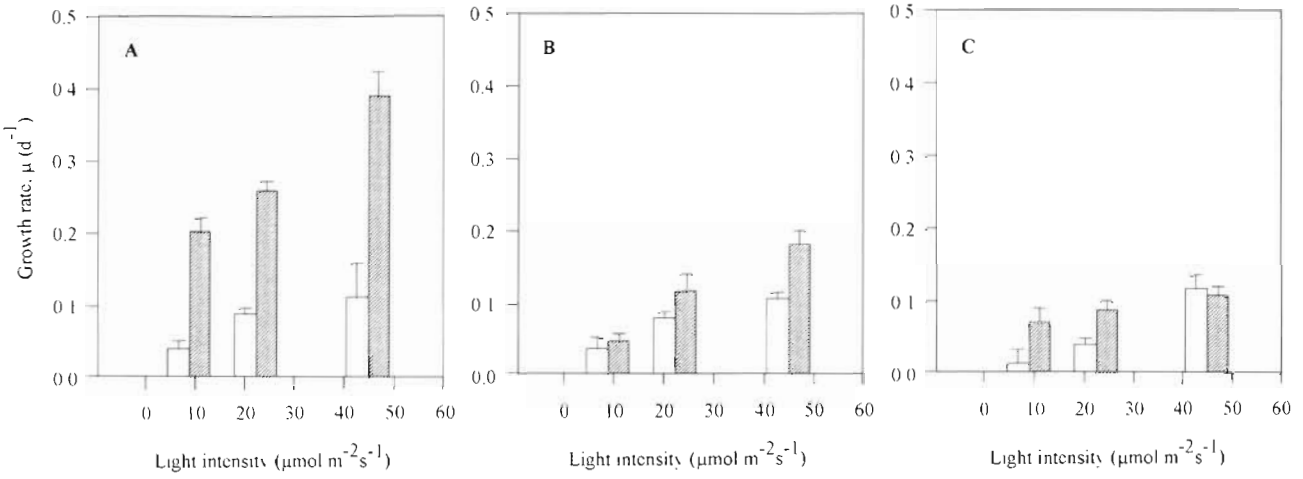

Fig. 7. Growth rates of *Fragilidium subglobosum* in monospecific culture (open bars) and in cultures supplied with 3 different species of *Ceratium* in excess (shaded bars). Bars represent treatment means ± 1 SD. (A) *C. tripos* (cell volume 110000 μm³), (B) *C. furca* (vol = 35000 μm³), (C) *C. fusus* (vol = 12500 μm³). Growth rate as a function of light intensity derived from data shown in Fig. 6. For calculation of growth rate, data from Days 3 to 14 were included, except for *C. tripos*, where only data from Days 3 to 6 or 8 were included

Since only 1 isolate of each species has been tested, it is uncertain whether this selectivity is species or even clone specific. The fact that dinoflagellates select prey on a basis other than size has been reported for a number of heterotrophic and mixotrophic species. Several species of dinoflagellates belonging to the genera *Dinophysis* and *Ceratium* have been shown to feed exclusively on ciliates (Hansen 1992, Bockstahler & Coats 1993b, Jacobson & Andersen 1994). In addition to this, some species of the heterotrophic genus *Protoperidinium* appear to feed on diatoms exclusively, while other species feed on dinoflagellate and/or prasinophyte prey (Jacobson & Anderson 1986). However, such a high degree of specificity in prey selection is not a general phenomenon in dinoflagellates; some species are indeed omnivorous (Jacobson & Anderson 1986, Hansen 1992). Prey selection which cannot be explained by size selection has also been documented in mixotrophic prymnesiophytes (e.g *Chrysochromulina*; Jones et al. 1993).

The mechanisms of prey selection other than size selection in phytoflagellates is unknown, but several authors have suggested that this may be due to chemosensory behaviour (e.g. Lewandowsky & Kaneta 1987). Alternatively, the identification of membrane-bound binding sites on the surface of prey and predator may lead to prey recognition. This mechanism of

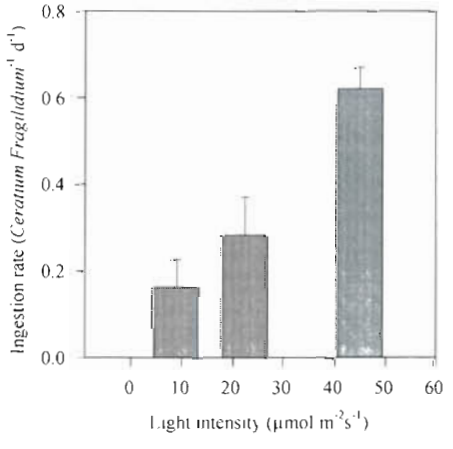

Fig. 8. *Fragilidium subglobosum* fed *Ceratium tripos*. Ingestion rates as a function of light intensity derived from data shown in Fig. 7

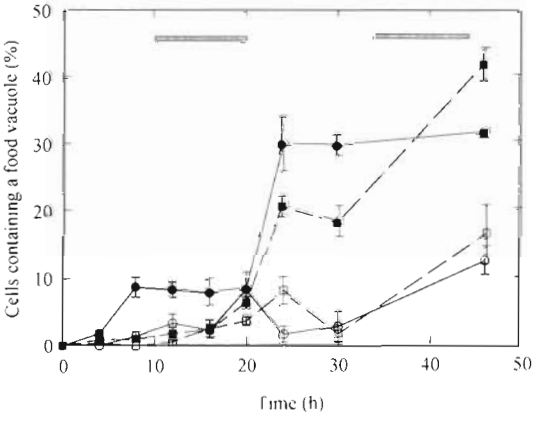

Fig. 9. Initial prey uptake by monospecific cultures of *Fragilidium subglobosum* fed *Ceratium tripos*. Percentage of *F. subglobosum* cells containing a food vacuole as a function of incubation time. (■ and ●) Cultures of *F. subglobosum* exposed to a 'high-light intensity' (9 μmol m⁻² s⁻¹) and fed *C. tripos* preadapted at a light intensity of 9 and 45 μmol⁻² s⁻¹, respectively. Shaded areas indicate dark periods (light:dark cycle 14:10 h)

recognition has been suggested to act in the relationship between symbiotic dinoflagellates and their host (e.g. Trench 1987).

Effects of light intensity and prey concentration on ingestion and growth rates in *Fragilidium subglobosum*

The present paper represents the first attempt to study the functional and numerical response of a mixotrophic dinoflagellate in laboratory culture. Cultures of *Fragilidium subglobosum* grown at 45 µmol photons m^{-2} s^{-1} increase their growth rate up to 3 times when fed *Ceratium tripos* in excess. The maximum specific ingestion rate compares to that of heterotrophic dinoflagellates of a similar size (Hansen et al. in press), indicating that the growth of *F. subglobosum* at this light intensity primarily depends on phagotrophy, when food is plentiful. This is supported by the fact that the growth yield of *F. subglobosum* grown at 45 µmol photons m^{-2} s^{-1} with excess food is not significantly different from the growth yield obtained from *F. subglobosum* grown in the dark and supplied with excess amounts of food (Skovgaard 1996b).

Growth yield increases from about 40% at prey concentrations which satiate prey ingestion to above 100% at lower prey concentrations (Fig. 4). Growth yields of this magnitude are of course not realistic, but may be an indication that food uptake by *Fragilidium subglobosum* stimulates photosynthesis at low prey concentrations. Thus, our data indicate that prey uptake by *F. subglobosum* may provide essential nutrients or growth factors apart from carbon for stimulation of photosynthesis.

Skovgaard (1996b) studied growth and ingestion responses of *Fragilidium subglobosum* as a function of light intensity in monospecific cultures and in cultures fed *Ceratium lineatum* in excess. In monospecific cultures of *F. subglobosum*, Skovgaard (1996b) found that growth rate increases as a function of light intensity, reaching a maximum level at ca 130 µmol photons m^{-2} s^{-1}. In mixotrophic cultures, growth rates of *F. subglobosum* did not vary with light intensity within the range of 0 to 365 µmol photons m^{-2} s^{-1}, even though ingestion rates varied by a factor of 2 to 3. Maximum ingestion rates were obtained at light levels of between 50 and 120 µmol photons m^{-2} s^{-1}. At light levels below and above this level, lower ingestion rates were found. In our study, however, both ingestion and growth rates of *F. subglobosum* fed *C. tripos* in excess increased by a factor of 2 to 3 as a function of light intensity between 9 and 45 µmol photons m^{-2} s^{-1}. This difference in growth response between the 2 studies may relate to differences in prey species. The maximum growth rate of *F. subglobosum* when fed *C. tripos* is similar to that obtained on *C. lineatum*. However, *C. tripos* is 10 times larger (in volume) than *C. lineatum* and this may potentially affect the ingestion rate.

Mixotrophs studied so far have either been primarily phototrophic or phagotrophic. For species that are mainly phototrophs, phagotrophy may be used to acquire major nutrients (nitrogen and phosphorus). Such a strategy for growth might be useful either in well-lit oligotrophic waters or at the pycnocline in stratified waters with nutrient limitation in the upper part (e.g. Bird & Kalff 1987, 1989, Arenovski et al. 1995, Havskum & Riemann 1996). Alternatively, the acquisition of essential growth factors, which the organism is unable to synthesize, is yet an other role for ingestion of prey in primarily phototrophic mixotrophs (Kimura & Ishida 1986, 1989).

For mixotrophs that are close to the phagotrophic end of the scale, maximum growth rates are only obtained phagotrophically. Consequently, photosynthesis by these mixotrophs has been interpreted as a survival strategy, when prey concentrations are low (Anderson et al. 1989, Sanders et al. 1990). Mixotrophy in *Fragilidium subglobosum* is not an adaptation to either low light or oligotrophic environments. Instead, *F. subglobosum* is a facultative mixotroph, capable of growing purely phototrophically in light without food and purely heterotrophically in the dark, achieving maximum growth rates which are not different from purely heterotrophic or phototrophic dinoflagellates (Skovgaard 1996b). When supplied with high prey concentrations and sufficient light intensity it is able to grow at a rate (μ = 0.5 d^{-1}, 15°C) which is faster than most dinoflagellates of this size.

Ecological significance

At present it is difficult to evaluate the role of *Fragilidum subglobosum* as a grazer of *Ceratium* species in nature, because data on the abundance of *F. subglobosum* are completely lacking. The lack of reports is probably due the fact that *F. subglobosum* is not easily identified. Identification of *F. subglobosum* is based on a close inspection of the plate pattern. Also, the division cysts of *F. subglobosum* may easily be overlooked in natural samples. However, *Ceratium* species are prominent members of the late summer phytoplankton community in temperate waters, occasionally forming dense blooms (Nordli 1957, Braarud et al. 1958, Mahoney 1979, Smetacek 1985). In Danish waters, concentrations of *C. tripos* and *C. lineatum*, which are the species preferred by *F. subglobosum*, often exceed 5 and 30 cells ml^{-1}, respectively, during late summer (Jensen 1994). Thus, judged from our experiments, *F*

subglobosum has the potential to be an important grazer on the 2 species in nature.

However, a number of factors which have not been dealt with may influence the actual grazing impact by *Fragilidium subglobosum*. Firstly, the studies conducted so far have only dealt with nutrient-replete cultures. It is a fact that *Ceratium* species often dominate the phytoplankton community when nutrient limitation may occur. As a consequence of this, *Ceratium* spp. often form a subsurface maximum at the pycnocline (e.g. Falkowski et al. 1980). Secondly, *Ceratium* spp. are themselves mixotrophs feeding specifically on ciliates (Bockstahler & Coats 1993b, Chang & Carpenter 1994). How this affects their growth rate is unknown. Thus, in order to evaluate the quantitative role of *F. subglobosum* as grazers on *Ceratium* spp. populations, field experiments need to be carried out.

Mixotrophy appears to be widespread among phototrophic dinoflagellates. This includes many of the bloom-forming species belonging to genera like *Gyrodinium*, *Gymnodinium*, *Dinophysis*, *Prorocentrum*, and *Ceratium* (see Hansen in press for review). We need to know more about the role of mixotrophy in the nutrition of the dinoflagellates. Also we need to study the role of mixotrophy at the community level. Mixotrophic dinoflagellates may gain a competitive advantage over strictly phototrophic forms by removing their competitors for nutrients and at the same time gain a higher growth rate. This raises the question of what role mixotrophy plays in bloom formation and persistence.

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