Vol. 26: 247–257, 2002 | AQUATIC MICROBIAL ECOLOGY
Aquat Microb Ecol | Published January 18

Dinoflagellate grazing on the raphidophyte *Fibrocapsa japonica*

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ABSTRACT: Laboratory experiments were performed to determine the growth and grazing capabilities of 2 heterotrophic dinoflagellates with different feeding modes (pallium feeder: *Oblea rotunda*; engulfment feeder: *Oxyrrhis marina*) when fed the raphidophyte *Fibrocapsa japonica*. Both dinoflagellates readily ingested prey and exhibited positive growth when feeding on monocultures of *F. japonica*. Maximum growth rates at food saturation were 0.54 and 0.72 d^{-1} for *O. rotunda* and *O. marina*, respectively. Both dinoflagellates are thus able to grow faster than their prey, for which a maximum growth rate of 0.45 d^{-1} has been previously reported. In the case of *O. rotunda*, it was found that a rather high food concentration of 300 *F. japonica* cells ml^{-1} (corresponding to 142 µg C l^{-1}) was needed to sustain half-saturated growth. This is consistent with the quantification of behavioural aspects of the feeding process. In about 55% of cases, a failure to attach the tow filament after prey encounter was recorded, and in about 83% of cases, *F. japonica* was able to escape from the attached tow filament, indicating that motility of *F. japonica* is a quite effective defence mechanism against pallium-feeding dinoflagellates. In addition, qualitative observations suggest that trichocysts of *F. japonica* may act mechanically as a grazer deterrent.

KEY WORDS: *Fibrocapsa japonica* · Heterotrophic dinoflagellates · *Oblea rotunda* · *Oxyrrhis marina* · Protozoan grazing · Trichocysts

INTRODUCTION

Heterotrophic protozoa are an important part of the marine plankton (Smetacek 1981, Lessard 1991) contributing significantly to the transfer of matter and energy through the food web. Their short generation times and ability to react quickly to short-term variation in food conditions enables them to fulfil the function of a heterotrophic buffer, which might balance the flow of matter in case of a sudden overload with primary material, as is the case at the beginning of the coastal spring bloom (Tiselius & Kuylenstierna 1996). For an algal bloom to develop, the growth rate of the bloom-forming species must exceed the sum of all loss processes. Among these loss processes, grazing is generally believed to be one of the more important factors. The importance of grazing for the dynamics of algal blooms becomes most apparent by its failure; if zooplankton are able to control initial stages of bloom development, there are no blooms and the importance of grazing goes unnoticed (Turner et al. 1998). If a certain algal species is difficult to graze, e.g. due to size or specific defence mechanisms, a reduced grazing pressure will certainly favour bloom development. Predation resistance has recently been speculated to be a major driving force shaping plankton evolution (Geider et al. 2001, Smetacek 2001). However, behavioural aspects of the feeding interactions among protists, which, in turn, may help to reveal species-specific algal defence mechanisms, have received little attention.

Fibrocapsa japonica Toriumi and Takano is an ichthyotoxic, neurotoxin-producing raphidophyte flagellate (Khan et al. 1996b). The species, first described as *Botryococcus* sp., has been reported to cause blooms

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and heavy mortalities of caged fish in Japan (Toriumi & Takano 1973). Since the early 1990s, *F. japonica* has been reported in European waters. After the first observation in 1991 on the French coast (Billard 1992), there have been reports of *F. japonica* occurrences from the Dutch part of the North Sea (Vrieling et al. 1995) as well as from the German North Sea coast (Rademaker et al. 1998). Since then *F. japonica* has been regularly detected in the summer plankton of the coastal North Sea, although neither dense blooms nor toxic effects of this species in the North Sea area have been described up to now. The potential impact of grazing on *F. japonica* population dynamics is poorly known. Compared to the number of papers studying the effects of toxic dinoflagellates on zooplankton grazers (e.g. Teegarden 1999 and references therein), there are only a few studies dealing with zooplankton grazing on raphidophycean species, which mainly focus on species of the genera *Chattonella* (e.g. Uye 1986, Nakamura et al. 1992) or *Heterosigma* (e.g. Sykes & Huntley 1987). So far only Uye & Takamatsu (1990), in their study of the feeding interaction between copepods and red-tide flagellates from Japanese coastal waters, have investigated the effects of *F. japonica* as a food organism.

The aim of the present investigation was to study the ability of heterotrophic dinoflagellates to grow using *Fibrocapsa japonica* as a food source. The study was performed using *Oblea rotunda* (Lebour) Balech ex Sournia as a representative of the pallium-feeding thecate dinoflagellates and *Oxyrrhis marina* Dujardin, generally considered benthic, as a representative of athecate, prey-engulfing dinoflagellates. For pallium-feeding dinoflagellates, most aspects of the feeding interaction can be easily observed and quantified with a microscope. For example, it was recently shown that feeding selectivity of *Protoperidinium pellucidum* may, in part, be explained by the lower capture success with motile prey (Buskey 1997). The present study therefore included observations and quantification of behavioural components of *O. rotunda* feeding on *F. japonica*.

MATERIAL AND METHODS

Fibrocapsa japonica was isolated by capillary isolation from the German Wadden Sea in 1995. Cells were grown in artificial seawater (Harrison et al. 1980) at a salinity of 29‰ and enriched with F/2 (Guillard & Ryther 1962) and $NaSeO_3$ (final concentration 1.0×10^{-2} µM). Cultures were maintained at 20°C at an irradiance of 50 to 150 µmol quanta m^{-2} s^{-1} and a photoperiod of 12 h light:12 h dark.

The heterotrophic dinoflagellate *Oblea rotunda* was isolated by capillary isolation from a brackish pond near Büsum (Germany) in 1993. Stock cultures held in multiwell plates or 100 ml flasks were fed *Dunaliella* sp. Several weeks prior to the experiments, subcultures fed *Fibrocapsa japonica* were established. Cultures were transferred about once a week to fresh medium containing late exponential *F. japonica* cells. Similarly, *Oxyrrhis marina* (Göttingen culture collection, Strain B21.89) growing with *Dunaliella* sp. as a food source was transferred to *F. japonica* medium 2 mo before the experiments started.

Cell sizes of at least 50 Lugol's solution-fixed grazers and prey cells were measured in a Zeiss inverted microscope equipped with a calibrated ocular micrometer (Table 1).

All growth and feeding experiments were conducted at a constant temperature (20°C) and low irradiance (ca 3 µmol quanta m^{-2} s^{-1}) to ensure only a very low growth of prey during the experiment. Experimental bottles (100 ml DURAN flasks) were gently shaken by hand twice a day to minimise settling.

***Oxyrrhis marina*—batch culture growth and grazing.** The grazing and growth response of *Oxyrrhis marina* fed *Fibrocapsa japonica* (in triplicate) was monitored for 5 d after inoculating *O. marina* (initial concentration 240 ml^{-1}) in 100 ml DURAN flasks containing 2400 *F. japonica* ml^{-1}. The *O. marina* culture that was used had been pre-cultured with *F. japonica* but was starved for 2 d prior to the onset of the experiment. The ingestion rate of *O. marina* feeding on *F. japonica* was estimated by counting the number of prey cells inside the food vacuoles (Fig. 1D,E), ingested during a known period of time. During the first 4 h, subsamples for counting were taken each $^1/_2$ h. Thereafter, sampling intervals were increasingly prolonged, resulting in final sampling rates of 2 d^{-1}. Ingestion rate was calculated from the linear part of a plot of particles $cell^{-1}$ against time.

***Oblea rotunda*—batch culture growth and grazing.** In a first experiment, batch culture growth of *Oblea rotunda* (initial concentration 80 ml^{-1}) was monitored

Table 1. Cell volume and calculated cellular carbon of species used in the present study. Volume is the median volume of at least 50 individuals calculated from size measurement assuming a sphere (*Oblea rotunda*) or a prolate spheroid (*Fibrocapsa japonica*, *Oxyrrhis marina*) as geometric shape. In the case of *O. marina*, only cells without ingested food were measured, as feeding cells are considerably enlarged (Fig. 1D,E). Cellular carbon was calculated using a carbon:cell volume ratio of 0.14 pg C $µm^{-3}$, as measured for *O. rotunda* by Lessard (1991)

Species	Volume ($µm^3$)	Cellular carbon (pg C $cell^{-1}$)
Fibrocapsa japonica	4445	622
Oblea rotunda	6125	858
Oxyrrhis marina	2800	392

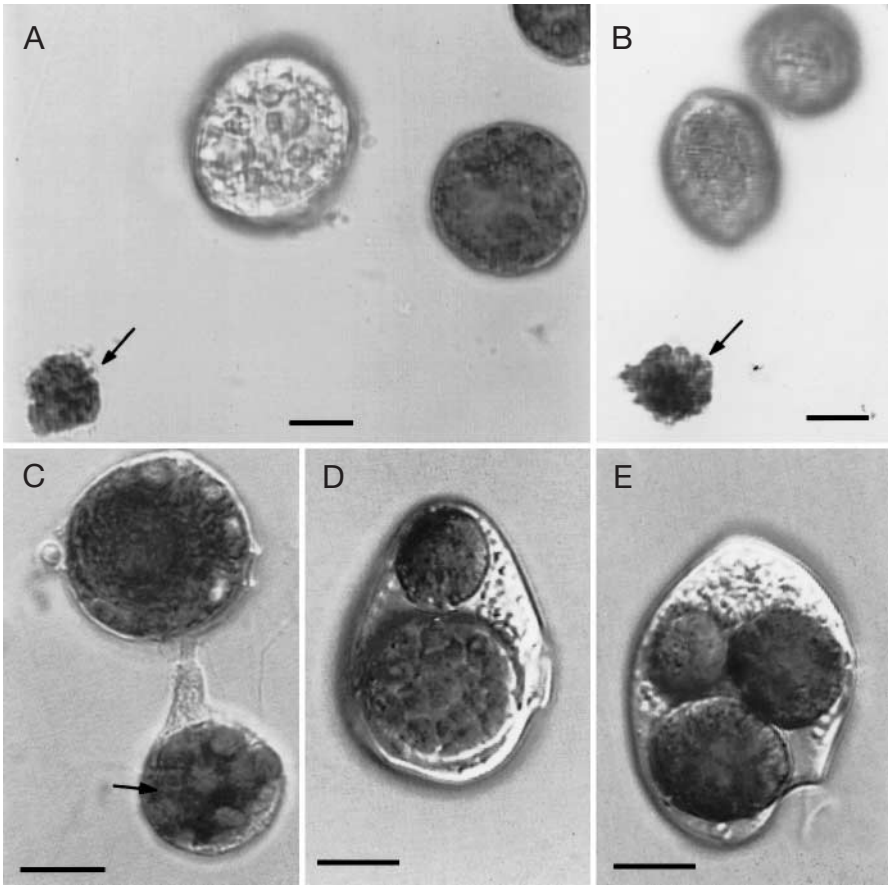

Fig. 1. Video prints from the grazing experiments with (A to C) *Oblea rotunda* and (D,E) *Oxyrrhis marina*. (A,B) grazing residues (arrows) are easily distinguished from *Fibrocapsa japonica* or *O. rotunda* cells. (C) Lugol's solution-fixed sample showing *O. rotunda* with *F. japonica* (arrow) partly digested in the pallium. (D,E) *O. marina* with 2 or 3 ingested *F. japonica* cells. Scale bars = 10 µm

in the presence of *Fibrocapsa japonica* (initial concentration 3600 ml^{-1}) in triplicate for 10 d. Triplicate flasks containing phytoplankton only served as a control. For cell counts of grazers, food cells and grazing residues ('faecal pellets', see below), 1 to 2 subsamples were taken per day. From the regression coefficient (slope) of numbers of *O. rotunda* and *F. japonica* during the period of constant exponential growth of *O. rotunda* (μ d^{-1}) and zero growth of *F. japonica* (as revealed from control bottles), ingestion rate, *I* (prey ingested grazer^{-1} d^{-1}), was calculated as follows:

$$I = -\text{slope} \times \mu \ (\text{d}^{-1})$$

***Oblea rotunda*—growth and grazing at different food concentrations.** A second set of experiments was conducted to determine the growth and grazing rates of *Oblea rotunda* as a function of food concentration. A series of equivalent dinoflagellate inoculate (30 ml^{-1}) was exposed to 6 different food densities ranging from 220 to 3000 cells ml^{-1}. Different food concentrations were established by appropriate dilution of an exponential batch culture of *Fibrocapsa japonica*. Three replicates were set up for each food concentration. Triplicate flasks containing phytoplankton only (initial concentration: 1300 cells ml^{-1}) served as control. Grazers were allowed to acclimate to their new food conditions for 24 h before initial sampling. The final sample for determination of growth and grazing rates was taken 48 h after the initial sampling. Final samples were fixed with Lugol's solution, and grazer and food cell counts were determined microscopically. Growth rate, ingestion and clearance were calculated using the equations of Frost (1972) and Heinbokel (1978). In addition, ingestion and clearance were estimated by a different method. This method is based on the fact that residues of digested *F. japonica* cells can be recognised by their irregular shape and by their colour (Fig. 1A,B) and thus could be quantified. These red-brown residues were never seen in non-grazed *F. japonica* culture and deteriorating cells were present even if lysed. This method of counting the appearance of faecal particles as a direct consequence of grazing avoids the assumptions and errors inherent in the measurement of the disappearance of food, i.e. the assumption of identical growth characteristics of phytoplankton with and without grazers and the error inherent in calculating the difference between 2 large numbers (Jacobson & Anderson 1993). Calculation of ingestion based on

enumeration of faecal particles, however, may be biased when faecal material degrades during the incubation period. Data gathered during the long-term batch culture experiment were used to assess the rate of disintegration and this value was than used to correct counts of faecal pellets for degradation in the short-term experiments with different food concentrations. Ingestion was calculated according to Jacobsen & Anderson (1993). Michaelis-Menten kinetics relating specific growth rate or ingestion rate to food concentration were fitted to the data by non-linear regression. Gross growth efficiency (GGE) was calculated as grazer biomass produced per phytoplankton biomass consumed.

***Oblea rotunda*—behavioural aspects of the feeding process.** The duration of the feeding process was determined by direct observations under a stereomicroscope. *Oblea rotunda* cells observed just to have attached to a *Fibrocapsa japonica* cell were gently transferred into a small water droplet (ca 200 µl) and observed every 5 min until *O. rotunda* had dropped the remains of the food particle. The feeding process of *O. rotunda* from encounter to digestion was observed in detail in Utermöhl chambers using an inverted microscope (Zeiss Axiovert 35, low magnification, dark field illumination). A set of observations was made to determine the proportion of cells that were successfully captured. If *O. rotunda* circling the food cell, in its stereotypical feeding behaviour, failed to attach the tow filament, this interaction was scored as a 'lost contact'. If *O. rotunda* attached to an algal cell, began towing it around and then lost it before the pallium could envelope the prey, this event was scored as an 'escape'. In total, 471 and 609 feeding events were observed to count lost contact and escape, respectively. In addition, qualitative observations of the feeding process were made using a trichocyst-bearing strain of *F. japonica*. Observations were documented with a Panasonic video system. From videotapes, single frames were printed by a Sony video-printer.

RESULTS

Oxyrrhis marina—batch culture growth and grazing

After inoculating starved *Oxyrrhis marina* cells into a *Fibrocapsa japonica* culture, *O. marina* cell numbers remained constant or even slowly decreased during the fist 12 h (Fig. 2A). After that lag period, *O. marina* cell numbers increased exponentially with a growth rate of 0.72 d^{-1} (doubling time 23.2 h). At the end of the experiment, maximum concentrations of about 1800 dinoflagellates ml^{-1} were reached. Whereas *F. japonica* numbers in ungrazed control bottles increased slightly with time, concentrations in the experimental bottles strongly declined and reached values near zero at the end of the experiment (Fig. 2B). Enumeration of food particles per grazer, which were clearly visible inside the *O. marina* cells (Fig. 1), showed a linear increase during the first hours of the experiment, resulting in a grazing rate of 1.9 *F. japonica* per *O. marina* d^{-1} (Fig. 3). Further on, the curve of particles per grazer flattened, reaching maximum values of about 1.2 *F. japonica* per *O. marina* , and then decreased continuously until the end of the experiment.

Oblea rotunda—batch culture growth and grazing

Oblea rotunda grew well with *Fibrocapsa japonica* as food. Fig. 4A shows exponential growth for about 4 d, resulting in a growth rate of 0.39 d^{-1}, which corresponds to a doubling time of 43 h. This is comparable to a doubling time of 37 h when *Dunaliella* sp. is offered as food (results not shown). After 4 d, cell numbers reached maximum values of about 400 ml^{-1} and subse-

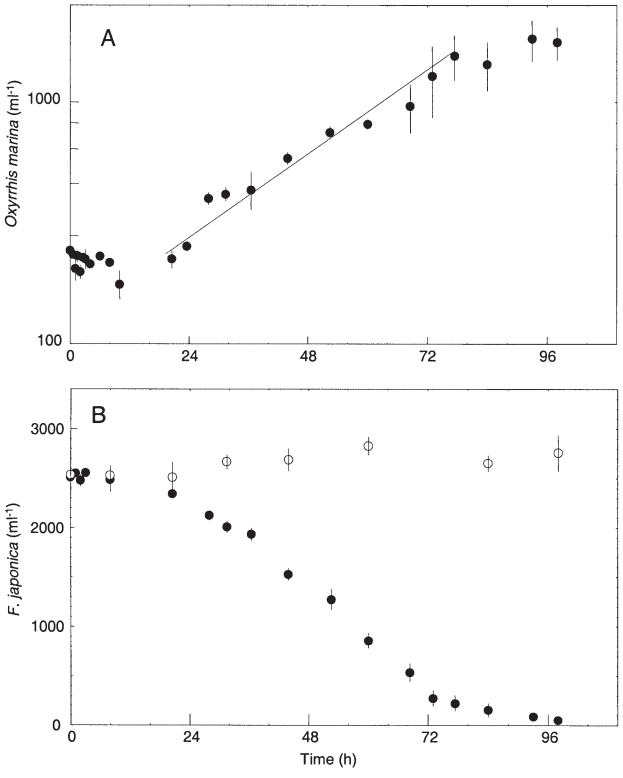

Fig. 2. Batch culture growth of *Oxyrrhis marina* fed *Fibrocapsa japonica*. (A) Growth curve of *O. marina* (log cell concentration versus time); the regression line indicates the curve section used to calculate growth rate and (B) *F. japonica* cell concentrations in experimental (●) and control (○) bottles. Data points represent mean values (n = 3) with error bars (±1 SD)

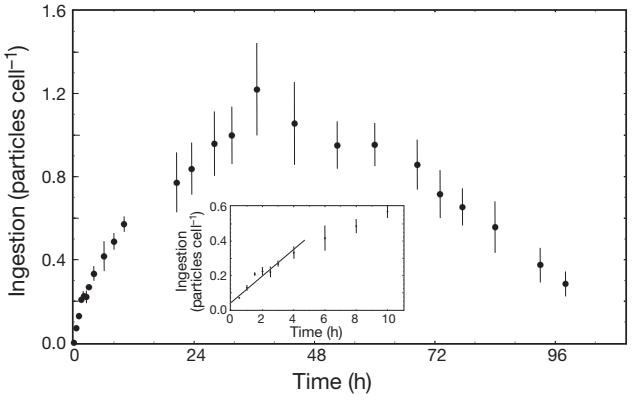

Fig. 3. *Oxyrrhis marina*. Number of particles inside food vacuoles, clearly resembling *Fibrocapsa japonica*, as a function of incubation time over the complete time course of the experiment; (insert) only values from the beginning of the experiment. A linear regression of the first 4 h was taken to calculate the ingestion rate. Data points represent mean values (n = 3) with error bars (±1 SD)

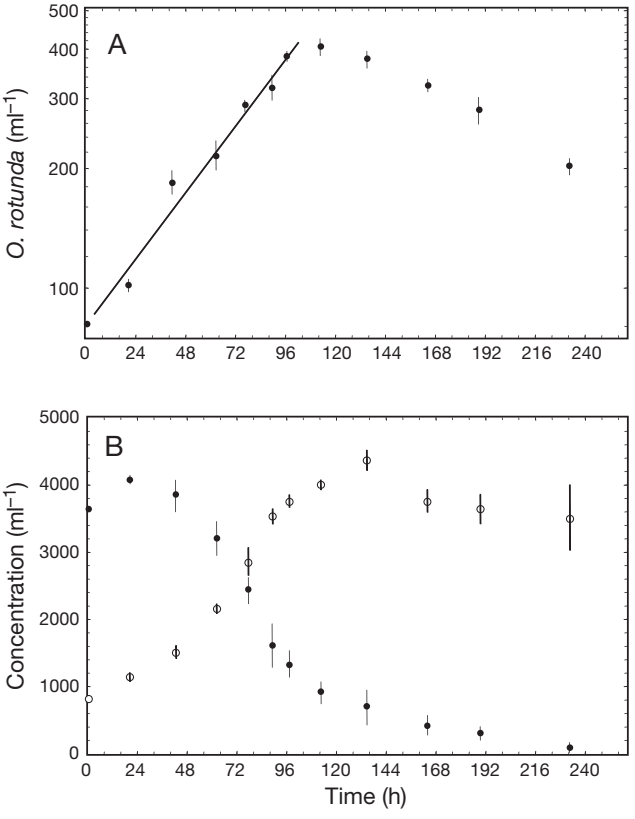

Fig. 4. Batch culture growth of *Oblea rotunda* fed *Fibrocapsa japonica*. (A) Growth curve of *O. rotunda*; the regression line indicates the curve section used to calculate the growth rate; (B) *F. japonica* (●) and faecal pellet (○) concentrations versus time. Data points represent mean values (n = 3) with error bars (±1 SD)

quently declined slowly. In control flasks, *F. japonica* cell numbers increased around 35% over the first 2 d and thereafter remained stable (results not shown). In experimental flasks, food concentrations continuously declined after 2 d, reaching near zero values at the end of the experiment (Fig. 4B). Correspondingly, concentrations of faecal pellets strongly increased. At the end of the experiment, there was a slight decrease of faecal pellets (Fig. 4B). A log-linear regression analysis of the sum of *F. japonica* and faecal pellets versus time shows, after an initial increase due to cell division of *F. japonica*, a slow linear decline, which results in a degradation rate of 0.060 d^{-1} (Fig. 5). This value was used to correct counts of faecal pellets for degradation in the short-term experiments with different food concentrations. Regression analysis of *O. rotunda* and food (Fig. 6) was used to calculate ingestion rates (see 'Material and methods'). Using the decline of food cells, an ingestion rate of 4.7 *F. japonica* per *O. rotunda* d^{-1} was calculated.

Oblea rotunda—growth and grazing at different food concentrations

The specific growth rate of *Oblea rotunda* increased with increasing food concentration until it became saturated at about 1500 *Fibrocapsa japonica* cells ml^{-1} (Fig. 7) with a maximum growth rate of 0.54 d^{-1} (doubling time = 31 h). The lowest food concentration tested in this experiment (220 cells ml^{-1}) sustained positive growth. Fitting the data to Michaelis-Menten kinetics, the best fit was achieved assuming a thresh-

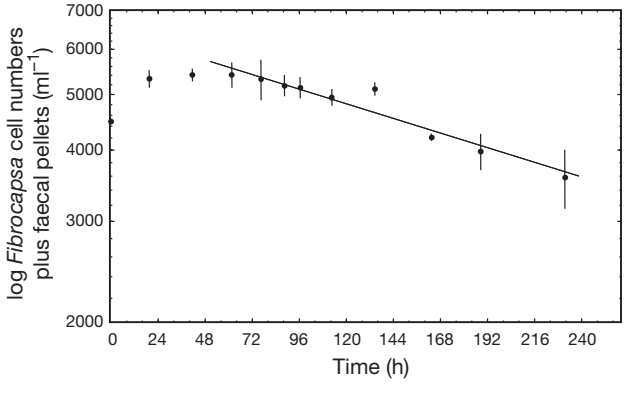

Fig. 5. *Fibrocapsa japonica* plus faecal pallet concentrations versus time, as revealed from the long-term batch growth experiments with *Oblea rotunda*. A log-linear regression of the decline yielded a disintegration coefficient of faecal material of 0.060 d^{-1}. Data points represent mean values (n = 3) with error bars (±1 SD)

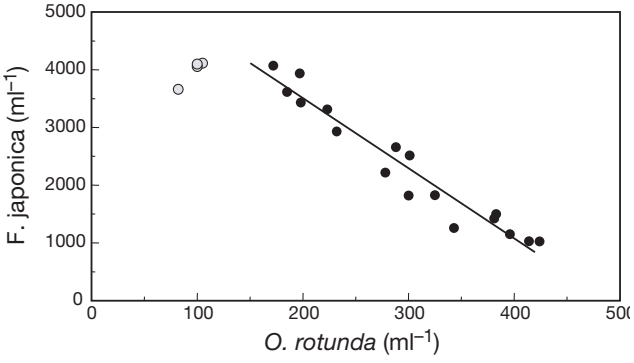

Fig. 6. Batch culture growth of *Oblea rotunda* fed *Fibrocapsa japonica*. The regression line of *O. rotunda* versus *F. japonica* cell concentrations was used to calculate the ingestion rate. The slope is –12.1 (r^2 = 0.94; n = 17). The initial values (shaded data points) when cell division led to an increase in cell numbers were not taken into account. For the final calculation of the ingestion rate see text

old of 150 *F. japonica* cells ml^{-1}, below which growth is assumed to be negative.

Ingestion, clearance and GGE in this experiment (Fig. 8) were estimated by 2 different methods: (1) based on prey cell counts according to Frost (1972), and (2) based on faecal pellet counts, corrected for disintegration (see above). Both methods led to the same pattern of parameters as a function of food concentration, even though ingestion and clearance calculated according to Frost (1972) were slightly higher than rates calculated by enumeration of digested residues. Ingestion rates increased to a maximum of about 5 prey cells dinoflagellate^{-1} d^{-1} and became saturated at a prey concentration of about 1500 *Fibrocapsa japonica* ml^{-1} (Fig 8A). Clearance rates determined by either method increased with decreasing prey concentration (Fig. 8B) up to a maximum value of about 0.35 µl dinoflagellate^{-1} h^{-1} at 250 *F. japonica* ml^{-1}. GGE was 7 to 13% and 10 to 17% for methods 1 and 2, respectively (Fig. 8C). Although GGE was relatively constant over the range of food concentrations tested, lower values were found at the lowest and highest food concentrations.

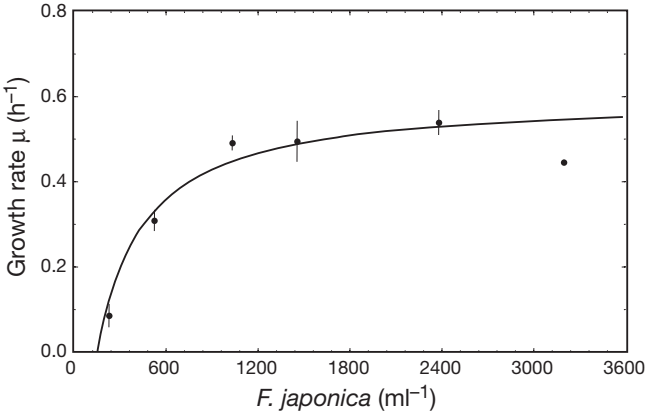

Fig. 7. *Oblea rotunda*. Growth rate (μ) as a function of initial prey (*Fibrocapsa japonica*) concentration. Data points represent mean values (n = 3) with error bars (±1 SD). The curve is numerically fitted to Michaelis-Menten kinetics. μ_{max} = 0.6 d^{-1}; K_s = 300 *F. japonica* ml^{-1}; growth threshold = 150 *F. japonica* ml^{-1}

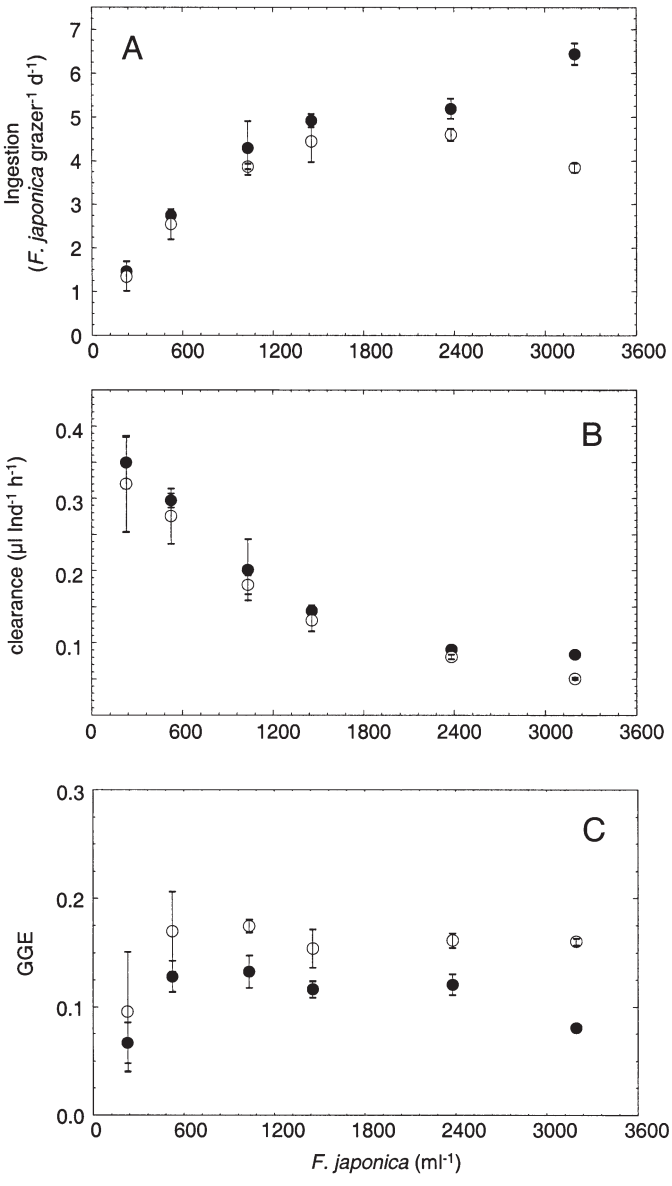

Fig. 8. *Oblea rotunda*. (A) Ingestion, (B) clearance and (C) gross growth efficiency as a function of initial *Fibrocapsa japonica* concentration, when calculated from changes in *F. japonica* (●) or faecal pellet (○) concentrations. Data points represent mean values (n = 3) with error bars (±1 SD)

Oblea rotunda: behavioural aspects of the feeding process

Pallium-feeding dinoflagellates capture and consume their prey as individual targets, and many aspects of the feeding process can be easily observed and quantified by direct microscopical observation. When *Oblea rotunda* encounters a prey, it swims around in tight circles for a short period. After a successful attachment with a tow filament, the pseudopodial pallium begins to emerge (Fig. 9). After closure of the pallium around the food cell, digestion starts. The duration of the whole feeding process from attachment to the release of faecal material was quite variable, ranging from 68 to 118 min with a mean time of 105 min (based on 17 measurements). The ultimate feeding success, however, may be hampered in the following ways, which were quantified by direct observation: (1) *O. rotunda* circling the cell in its stereotypical feeding behaviour may fail to attach the tow filament (scored as 'lost contact'). (2) Prey cells still beating their flagella may be lost after the tow filament was attached (scored as 'escape'). A percentage quantification of these steps (Fig. 10) shows that prey capture is a tedious task. In 55% of cases, *O. rotunda* lost contact after prey encounter and characteristic pre-feeding motion (number of observations: 471). After the attachment of the tow filament, only 17% of cases led to a successful digestion, whereas in 83% of cases the algal cell was detached and managed to escape (number of observations: 609). In total, only about 8% of encounters exhibited by the typical 'pre-feeding' swimming behaviour led to successful digestion.

In their original description, Toriumi & Takano (1973) specified trichocyst rods concentrated in the posterior part of the cells as typical features of *Fibrocapsa japonica*. However, the *F. japonica* strain used in the feeding experiments was absolutely free of trichocysts (Fig. 11). The factors inducing or needed for the formation of trichocysts are under investigation. An important factor is probably the use of artificial seawater as culture medium, as preliminary investigations have shown that in another strain, cultured in natural seawater originating from the German Wadden Sea, a number of large trichocyst are present. Anyway, preliminary observations of the feeding process using trichocyst-bearing *F. japonica* cells revealed that trichocyst extrusion may substantially complicate the feeding process. Upon contact of the pallium with the *F. japonica* cell, trichocysts can be extruded and prevent further development of the pallium, eventually allowing *F. japonica* to escape. Trichocysts thus may act mechanically as a grazer deterrent. The peculiarity of this 'defence' seems to depend on the strength of trichocyst action, as it was also observed that the pallium may, in some cases, envelop extruded trichocysts completely so that digestion can take place subsequently. In addition, it was observed that after a first 'shot' of trichocysts, a second discharge of trichocysts may occur, completely both the *F. japonica* cell and the pallium (Fig. 12).

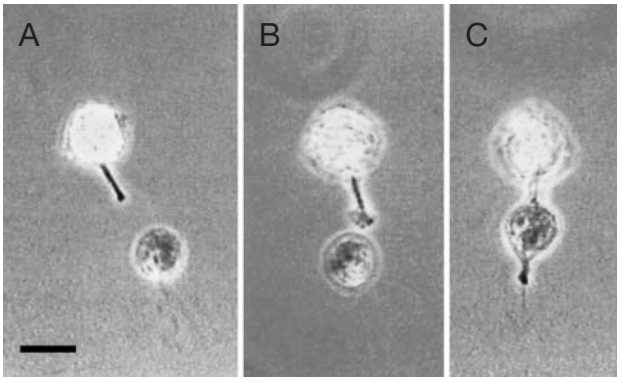

Fig. 9. Video sequence of *Oblea rotunda* catching *Fibrocapsa japonica*. (A) The pseudopodial pallium emerges around a thin tow filament (not visible); (B) reaching the food cell, the pallium begins to spread out; and (C) finally completely covers the prey cell, including the long trailing flagellum. Scale bar = 20 µm

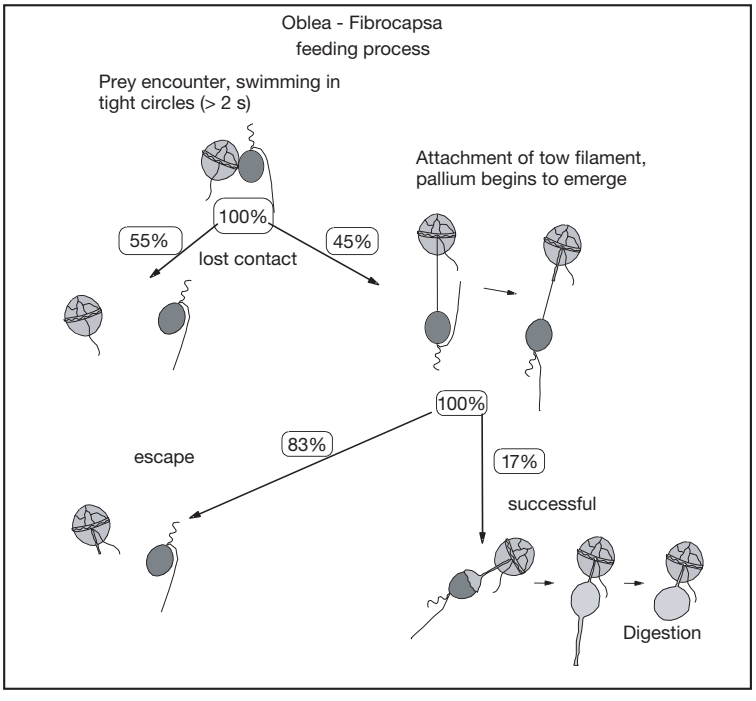

Fig. 10. *Oblea rotunda.* Observational quantification of different steps in the feeding process. Total numbers of observations were 471 and 609 for quantifying 'lost contact' or 'escape', respectively

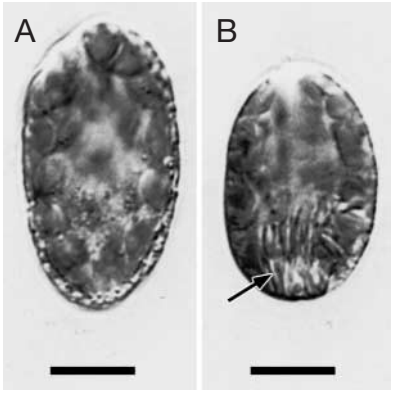

Fig. 11. Comparison of *Fibrocapsa japonica* cells (A) without and (B) with trichocysts (arrow). Scale bars = 10 µm

DISCUSSION

We found that both thecate and athecate heterotrophic dinoflagellates in this study readily ingest prey and exhibit positive growth when feeding on monocultures of *Fibrocapsa japonica*, which has been reported to produce a potent neurotoxin (Khan et al. 1996b). Little is known about the toxic action of *F. japonica*. The toxin has been classified as a neurotoxin similar to brevetoxin, but with a higher toxicity (Khan et al. 1996b, Nannen 1998). Moreover, Oda et al. (1997) demonstrated that raphidophycean flagellates (including *F. japonica*) may produce substantial quantities of toxic oxygen radicals. However, no direct toxic effects of feeding and growth were apparent in the present study. Long-term negative effects of *F. japonica* can also be ruled out, as cultures of grazers with *F. japonica* as the sole food have been successfully maintained for several months since the experiments were carried out. There is only one other study available in which zooplankton feeding interactions with *F. japonica* as food were analysed. Uye & Takamatsu (1990) report that *F. japonica*, among other red-tide flagellates, was almost entirely rejected by the planktonic copepod *Acartia amorii*. For the other copepod species tested, *Pseudodiaptomus marinus*, negative effects on faecal pellet production were observed. However, in this as well as in the present study, toxicity or cellular toxin concentrations of *F. japonica* were not estimated, making it impossible to relate the observed effects to toxin concentration.

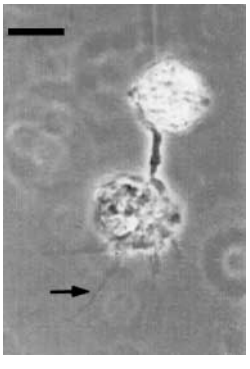

Fig. 12. Trichocysts (arrow) discharged from a captured *Fibrocapsa japonica* cell led to a completely broken algal cell and tore up the pallium of *Oblea rotunda*. Scale bar = 20 µm

It has been repeatedly established that cultured strains of toxic algae are typically less toxic than those collected from natural populations (White 1986, Cembella et al. 1988, Edvardsen 1993) and may vary considerably with respect to cellular toxin content (Anderson 1990, Chang et al. 1997, Edvardsen & Paasche 1998, Parkhill & Cembella 1999). There is evidence that strains of *Fibrocapsa japonica* grown in culture produce toxins. In their study on ichthyotoxicity and neurotoxin production of *F. japonica*, Khan et al. (1996b) used an *F. japonica* culture isolated from the Dutch part of the North Sea in 1993 showing that—in principle—the European population produces toxins. In preliminary experiments, toxin was also detected in the same isolate as that used in the present experiments (de Boer et al. 2000). However, the toxicity of *F. japonica* may be quite variable, depending on cell density, growth conditions or culture age (Khan et al. 1996b, de Boer et al. 2000). Fish tests performed by Khan et al. (1996b) showed no ichthyotoxicity until cell density reached 4.1×10^3 cells ml^{-1}, which is around the highest cell densities used in the present study. Furthermore, ichthyotoxicity varied with culture age, being highest in late-logarithmic growth and decreasing to low levels as the cultures entered the stationary phase (Khan et al. 1996b). It may thus be possible that the use of *F. japonica* cultures with relatively low cell numbers (compared to the study of Khan et al. 1996b) may have prevented toxic effects from becoming effective. Unfortunately, Uye & Takamatsu (1990), who showed adverse effects of *F. japonica* on the copepod *Acartia omorii*, did not provide the exact cell concentration used in their experiment, but the lowest prey concentration they report was 2000 ml^{-1}. This is at the upper end of the concentration range used in our experiments. Cell numbers during toxic blooms of *F. japonica* are not available. The highest cell concentration observed in the field available so far is from the German Wadden Sea (327 ml^{-1}; Rademaker et al. 1998). The threshold of 4.1×10^3 cells ml^{-1}, upon which ichthyotoxic effects are reported (Khan et al. 1996b), roughly corresponds to a carbon content of 2.6 mg C l^{-1}, which would require an amount of 32 µmol l^{-1} nitrogen (applying the Redfield ratio). The formation of such a high biomass is supposed to be restricted to a very few coastal sites where large amounts of nutrients

are continuously discharged. However, extremely high cell densities of *F. japonica* may locally be formed as a result of its pronounced vertical migratory behaviour. During the light period, cells concentrate at the surface, forming a yellowish-green thin layer (pers. obs., Khan et al. 1996a).

The maximum specific growth rate of *Oblea rotunda* feeding on *Fibrocapsa japonica*, as estimated from growth at different food concentrations, was 0.54 d^{-1}. This is well within the range of food-saturated growth rates of 0.45 to 0.66 d^{-1} found for *O. rotunda* grazing on 6 different algal species (Strom & Buskey 1993). With a growth rate of 0.72 d^{-1} when fed *F. japonica*, *Oxyrrhis marina* can grow even faster, although the prey:predator ratio of about 10:1 used in these experiments was rather low. Thus, both dinoflagellates are able to grow faster than their prey, for which a maximum growth rate of 0.45 d^{-1} (20°C, optimal nutrient and irradiance conditions) has been reported (Khan et al. 1996a). However, in the case of *O. rotunda*, relatively high food concentrations are required to sustain maximum growth. A Michaelis-Menten fit of growth rate versus food concentration revealed that positive growth required a concentration of >150 prey cell ml^{-1} and that half-maximum growth was achieved at about 300 prey cell ml^{-1}. Applying a volume-to-carbon conversion, this corresponds to 70 µg C l^{-1} and 142 µg C l^{-1} for the growth threshold and the half-saturation constant, respectively. Growth thresholds of only 10 to 24 µg C l^{-1} have been reported for thecate heterotrophic dinoflagellates feeding on diatoms (Strom & Buskey 1993, Buskey et al. 1994, Naustvoll 1998), which is approximately equivalent to the lower food limit of many ciliates (Jonsson 1986). For *Diplopsalis lenticula* growing on a diatom (*Ditylum brightwellii*), growth is half saturated at a food concentration of only 29 µg C l^{-1} (Naustvoll 1998). Ingestion, clearance and GGE as a function of food concentration were estimated by 2 different methods. The close agreement between both methods clearly shows the applicability of the more direct method of counting faecal particles in short-term grazing experiments of *O. rotunda* and *F. japonica*. In similar comparisons of these 2 methods, Naustvoll (1998) and Jeong & Latz (1994) found that rates estimated according to Frost (1972) gave rates consistently higher than those calculated with the increase of faecal pallets. In contrast to the present study, no correction for faecal pellet disintegration was applied in these studies. However, it cannot be ruled out that the disintegration rate of 0.06 d^{-1} for *F. japonica* faecal particles, as estimated in this study, may be variable depending, for example, on temperature, concentration of bacteria or turbulent motion of the experimental flasks.

The findings of rather high food concentration needed to sustain maximum growth are consistent with the observational quantification of the feeding process. In 55% of cases, a failure to attach the tow filament after prey encounter was recorded and in 83% of cases, *Fibrocapsa japonica* was able to escape from the attached tow filament, indicating that motility of *F. japonica* is a quite effective defence mechanism against pallium-feeding dinoflagellates. This is in line with results presented by Buskey (1997), who observed a lower capture success of *Protoperidinium pellucidum* feeding on motile prey than on non-motile diatoms. Comparing *Ditylum brightwelli* with *Dunaliella tertiolecta* as food for *Oblea rotunda*, Strom & Buskey (1993) noted that the non-motile diatom consistently supported higher rates of growth and grazing than the motile flagellate. In addition to prey size (Hansen 1992) and chemosensory signals (Hauser et al. 1975, Spero 1985, Buskey 1997), feeding selectivity among pallium-feeding heterotrophic dinoflagellates may thus partly be ascribed to prey motility as an active defence mechanism. The quantification of motility as an effective defence mechanism presented here, however, may be variable and may mainly depend on the swimming activity of the *F. japonica* cells, which in turn depend on both environmental factors and physiological conditions (Khan et al. 1998).

In addition to motility as a defence mechanism, preliminary observations using a trichocyst-bearing culture indicated that trichocysts of *Fibrocapsa japonica* may act mechanically as a grazer deterrent. A comparable defensive function of trichocysts is known for the freshwater ciliates. Recent results strongly suggest that trichocyst in *Paramecium* spp. function as defence organelles against many predatory ciliates (Harumoto 1994, Miyake & Harumoto 1996). For *F. japonica*, trichocyst effects on the algae/grazer interactions still have to be quantified in detail.

To conclude, although no toxic effects of *Fibrocapsa japonica* on grazing and growth of *Oblea rotunda* were apparent, prey capture seemed to be the most crucial link in the feeding process from encounter to digestion. As a consequence, pallium-feeding dinoflagellates may not be able to control *F. japonica* bloom development in its early stages but may potentially contribute to the decline of dense populations.

Acknowledgements. Special thanks are due to Cornelia Reineke for technical assistance. I greatly appreciate constructive criticism and suggestions from Victor Smetacek, John Dolan and 3 unknown reviewers. This work was supported by the European Commission (Research Directorate General Environment Programme Marine Ecosystems) through the BIOHAB project 'Biological control of harmful algal blooms in European coastal waters: role of eutrophication' (contract EVK3-CT99-00015). The BIOHAB project is part of the EC EUROHAB cluster.

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Editorial responsibility: John Dolan, Villefranche-sur-Mer, France

Submitted: May 5, 2001; Accepted: October 8, 2001
Proofs received from author(s): December 7, 2001