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Feeding by the mixotrophic thecate dinoflagellate *Fragilidium* cf. *mexicanum* on red-tide and toxic dinoflagellates

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ABSTRACT: We investigated prey species, prey selectivity, growth rates, grazing rates, and the effect of light and nutrient environment on feeding for the thecate mixotrophic dinoflagellate *Fragilidium* cf. *mexicanum*. Among the red-tide and toxic dinoflagellate prey offered, *Lingulodinium polyedrum*, *Gymnodinium sanguineum*, *Prorocentrum micans*, *P. minimum*, and *Scrippsiella trochoidea* were ingested by *F.* cf. *mexicanum*, but *Amphidinium carterae* and *Cochlodinium polykrikoides* were not. The feeding frequency (FF), based on the percent of *F.* cf. *mexicanum* containing one or more target prey cells, was significantly affected by prey species. The maximum FFs of *F.* cf. *mexicanum* on *L. polyedrum* and *S. trochoidea* after 72 h incubation in a unialgal diet (50 and 89%, respectively) were much higher than those for *P. micans*, *P. minimum*, and *G. sanguineum* (6, 10, and 2%, respectively). FFs on *L. polyedrum* and *S. trochoidea* were significantly affected by prey concentration, but those on *P. micans* and *G. sanguineum* were not. *F.* cf. *mexicanum* strongly selected *L. polyedrum* over *S. trochoidea* in prey mixtures. With increasing mean prey concentration, growth and ingestion rates of *F.* cf. *mexicanum* feeding on *L. polyedrum* increased, with saturation at a mean prey concentration of approximately 500 cells ml^{-1}. The maximum specific growth rate (mixotrophic growth) of *F.* cf. *mexicanum* on *L. polyedrum* was 0.36 d^{-1}, under a 12 h light:12 h dark cycle of 20 µE m^{-2} s^{-1}, while its growth rate (phototrophic growth) under the same light conditions without added prey was −0.05 d^{-1}. The maximum ingestion rate of *F.* cf. *mexicanum* on *L. polyedrum*, 3.9 prey eaten $predator^{-1}$ d^{-1}, was comparable to those of the co-occurring heterotrophic dinoflagellates *Protoperidinium* cf. *divergens* and *P. crassipes* for the same prey. However, maximum clearance rate of *F.* cf. *mexicanum*, 6 µl $predator^{-1}$ h^{-1}, was much higher than those of *P.* cf. *divergens* and *P. crassipes*. The ingestion rate of *F.* cf. *mexicanum* on *L. polyedrum* was not significantly affected by light intensity or nutrient concentration when prey was plentiful.

KEY WORDS: Dinoflagellate · Growth · Grazing · Mixotrophy · Protist · Red tide

INTRODUCTION

Red tides, which consist of dense algal blooms visible at the sea surface, can upset the balance of food webs and cause large-scale mortalities in fish and shellfish (e.g. Norris & Chew 1975). Studies of red-tide formation and persistence suggest that grazing pressure may sometimes play an important role in red-tide dynamics (e.g. Watras et al. 1985). Grazing by microzooplankton such as rotifers, tintinnids, and heterotrophic or mixotrophic dinoflagellates is believed to contribute to the decline of blooms (Holmes et al. 1967, Eppley & Harrison 1975). A few studies have examined feeding by mixotrophic dinoflagellates on red-tide dinoflagellates (Jacobson & Anderson 1996, Skovgaard 1996a, b, Hansen & Nielsen 1997), while many studies have considered feeding by other microzooplanktonic grazers (Eppley & Harrison 1975, Stoecker et al. 1981, Watras et al. 1985, Jeong & Latz 1994). Recently, interest in

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phagotrophic feeding by phototrophic dinoflagellates and factors affecting their feeding has increased (Bockstahler & Coats 1993, Li et al. 1996, Granéli et al. 1997, Stoecker et al. 1997).

Species of the mixotrophic dinoflagellate genus *Fragilidium* have been found in coastal waters off many areas (Balech & Ferrando 1964, Eppley & Harrison 1975, Balech 1988, 1990, Skovgaard 1996a, Jeong et al. 1997). Several studies have reported that *Fragilidium* spp. fed intensively on some red-tide dinoflagellates, especially at the decline of blooms. For example, *F. heterolobum* was abundant and observed to feed intensively on *Lingulodinium polyedrum* (Stein) Dodge (previously *Gonyaulax polyedra*) during red tides (Balech & Ferrando 1964, Eppley & Harrison 1975). Similarly *F. mexicanum* was reported to feed heavily on *Alexandrium* sp. (Balech 1988). Phagotrophic feeding by *F. fissle* has not been observed yet, but this species might feed on red-tide dinoflagellates since it is sometimes abundant during blooms dominated by *Lingulodinium polyedrum* and *Alexandrium* sp. (Balech 1990). While these studies imply rapid feeding and growth of *Fragilidium* spp. during red tides of *L. polyedrum* and *Alexandrium* sp., few data are available to test this hypothesis.

Recently, Skovgaard (1996a) described the feeding process of *Fragilidium subglobosum*, a species which feeds exclusively on *Ceratium* spp., when offered diverse dinoflagellates and diatoms as prey. A subsequent report quantified growth and grazing rates of *F. subglobosum* when the feeding was on *Ceratium* spp. (Skovgaard 1996b, Hansen & Nielsen 1997). Other *Fragilidium* species may have different prey and varying degrees of selectivity. However, there are no reports on the feeding of *Fragilidium* species on other dinoflagellates that frequently form red tides. Therefore, to investigate the role of *Fragilidium* species in red-tide dynamics, it is important to quantify growth and grazing rates of *Fragilidium* species on red-tide dinoflagellates and to explore prey species and prey selectivity.

Feeding of some mixotrophic nanoflagellates and dinoflagellates is known to be affected by light intensity and/or nutrient concentrations (Keller et al. 1994, Skovgaard 1996b, Hansen & Nielsen 1997, Stoecker et al. 1997). However, feeding of a few mixotrophic flagellates appears unaffected by light and nutrient environments if prey are abundant (Sanders et al. 1990). Ingestion of *Ceratium* spp. by *Fragilidium subglobosum* was recently found to be affected by light intensity even when prey was plentiful (Skovgaard 1996b, Hansen & Nielsen 1997). However, no studies have considered the influence of nutrients on feeding of *Fragilidium* spp. Thus, it is worthwhile to explore the effects of light and nutrient on feeding by *Fragilidium* spp. on red-tide dinoflagellates.

The present study explores prey species, prey selectivity, grazing rate, growth rate, and the effects of light and nutrients on feeding for the mixotrophic thecate dinoflagellate *Fragilidium* cf. *mexicanum* when presented red-tide or toxic dinoflagellates as prey. Prey species included the most frequently encountered red-tide dinoflagellates of coastal waters off southern California where *F.* cf. *mexicanum* was isolated. *Amphidinium carterae* has not been reported to cause red tides, but it is toxic (Steidinger & Tangen 1996). Growth and ingestion rates of *F.* cf. *mexicanum* on *Lingulodinium polyedrum* under conditions that did not support phototrophic growth of the grazer were compared to those of *Protoperidinium* cf. *divergens* and *P. crassipes*, heterotrophic dinoflagellates that co-occur with *F.* cf. *mexicanum* (Jeong & Latz 1994). The results of the present study provide a basis for understanding the interactions between *F.* cf. *mexicanum* and red-tide or toxic dinoflagellates.

MATERIALS AND METHODS

Culture of phytoplankton prey. Seven dinoflagellate prey species (Table 1) were cultured in enriched f/2 seawater medium (Guillard & Ryther 1962) without silicate and maintained at 22°C with continuous illumination of 100 µE m^{-2} s^{-1} supplied by cool white fluores-

Table 1. Autotrophic (auto) and mixotrophic (mixo) species used in the present study listed in order of size. Mean equivalent spherical diameter (ESD) ± SD of the mean was measured by the PAMAS-SVSS particle counter. Volume (to the nearest hundred) was calculated according to the equation: volume = $\frac{4}{3}\pi(ESD/2)^3$. The number of cells measured was >2000

Species	ESD (µm)	Approximate volume (µm³)	Trophic mode
Prey			
Prorocentrum minimum	12.9 ± 3.6	1100	Mixo[b]
Amphidinium carterae	16.2 ± 2.5	2200	Mixo[c]
Cochlodinium polykrikoides	23.2 ± 3.1	6600	Auto
Scrippsiella trochoidea	25.1 ± 2.8	8300	Mixo[d]
Prorocentrum micans	26.0 ± 2.3	9200	Mixo[d]
Gymnodinium sanguineum	36.3 ± 5.6	25000	Mixo[e]
Lingulodinium polyedrum	37.9 ± 4.5	28500	Auto
Predator			
Fragilidium cf. *mexicanum*	54.5 ± 8.7[a]	84600	Mixo

[a]ESD of *Fragilidium* growing photosynthetically in f/2 medium under an illumination of ca 50 µE m^{-2} s^{-1}
[b]Li et al. (1996), [c]Cheng & Antia (1970), [d]Jacobson & Anderson (1996), [e]Bockstahler & Coats (1993)

cent lights. Cultures in exponential growth phase were used for all feeding experiments except the nutrient effect experiment, for which cultures in stationary growth phase were used (see 'Nutrient effects on feeding'). Carbon contents for dinoflagellates were estimated from cell volume according to Strathmann (1967).

Isolation and culture of *Fragilidium* cf. *mexicanum*. Plankton samples were taken at the end of the Scripps pier (La Jolla, CA, USA) during May, 1996, using a 35 cm diameter, 25 µm mesh plankton net and gently screened through 100 µm Nitex mesh before being placed in 150 ml plastic bottles for shipment to Korea. Detailed methods for culturing *Fragilidium* cf. *mexicanum* are described by Jeong et al. (1997).

Prey species and concentration effects. Seven experiments (1 to 7, sequentially) were designed to investigate the effects of prey species and prey concentration on feeding by *Fragilidium* cf. *mexicanum*. Feeding frequencies (FFs) of *F.* cf. *mexicanum* on prey after 2 h and 72 h incubation, rather than ingestion rates, were compared because ingestion rates for some prey species in preliminary experiments were too low to be measure reliably (e.g. the maximum ingestion rates of *F.* cf. *mexicanum* on *Gymnodinium sanguineum* and *Prorocentrum micans* were only 0.2 and 0.7 prey eaten predator^{-1} d^{-1}, respectively). FF is the proportion of *F.* cf. *mexicanum* cells that feed, as determined from the presence of ingested prey, and was calculated as the percentage of *F.* cf. *mexicanum* containing 1 or more target prey cells. FFs after 2 h show the relative ease of capture and/or ingestion of target prey by *F.* cf. *mexicanum* when unialgal diets were provided.

Lingulodinium polyedrum and *Scrippsiella trochoidea* cells were easily observed in the cytoplasm of *Fragilidium* cf. *mexicanum* for at least 2 h after being ingested. Thus, the number of prey observed in the cytoplasm of *F.* cf. *mexicanum* after 2 h of feeding represents the total number ingested only when *L. polyedrum* and *S. trochoidea* were used as prey. FFs after 72 h incubation show whether or not *F.* cf. *mexicanum* were able to feed on the target prey species, but cannot be used to calculate feeding rate. FFs as a function of prey concentration in a unialgal diet provide some insight on the effect of prey concentration on the grazer's feeding, while the average number of ingested target prey observed inside the grazer (APN) reveals whether or not a second and/or later target prey cell is attacked before the first is digested completely.

Eight hundred ml of a dense culture of *Fragilidium* cf. *mexicanum* maintained in f/2 media and growing photosynthetically on shelves illuminated with 50 µE m^{-2} s^{-1} of cool white fluorescent light on a 12 h light: 12 h dark cycle was transferred to a 1 l polycarbonate (PC) bottle containing approximately 200 ml freshly filtered seawater. Three 1 ml aliquots were then removed from the bottle and examined using a compound microscope to determine *F.* cf. *mexicanum* concentration. The diluted culture in the 1 l bottle was then used to set up an experiment.

In all experiments, the initial concentrations of *Fragilidium* cf. *mexicanum* and prey were established using an autopipette to deliver a predetermined volume of culture with known cell density to the experimental bottles. Six 32 ml PC bottles (mixtures of predator and prey) and duplicate 32 ml PC control bottles containing only *F.* cf. *mexicanum* were set up for each predator-prey treatment. Three ml of f/2 medium were added to all bottles, which were then filled to capacity with freshly filtered seawater. Initial concentrations of predators and prey were determined for each bottle by enumerating cells present in three 1 ml aliquots. The bottles were again filled to capacity with freshly filtered seawater, capped, and placed on a rotating wheel at 0.9 rpm at 22°C, where they received cool white fluorescent light on a 12 h light:12 h dark cycle. Bottles were illuminated from above, with light intensity (20 µE m^{-2} s^{-1}) calculated as the mean of measurements made using a light sensor (LI-COR LI-1000 DataLogger) placed inside a bottle hanging on the top (35 µE m^{-2} s^{-1}), bottom (8 µE m^{-2} s^{-1}), and 2 sides (19 µE m^{-2} s^{-1}) of the rotating wheel.

All bottles were taken from the rotating wheel at 2, 24, 48, and 72 h to observe interactions between *Fragilidium* cf. *mexicanum* and its prey. Observations were made with a dissecting microscope, which was used to look through the surfaces of the bottles without removing the caps. The contents of 1 set of triplicate experimental bottles were fixed at 2 h for each treatment, while those of the experimental bottles and the control bottles were preserved at 72 h. Samples were fixed with either glutaraldehyde (for *Gymnodinium sanguineum* prey) or Bouin's solution (for *Lingulodinium polyedrum*, *Scrippsiella trochoidea*, *Prorocentrum micans*, and *P. minimum* prey), depending on prey species and results of preliminary experiments to determine which fixative provided the best visibility of prey species inside the protoplasm of the grazer. Cells were counted within 2 d after preservation because *F.* cf. *mexicanum* cells gradually turned dark even when glutaraldehyde was used as the fixative. The number of prey cells observed inside *F.* cf. *mexicanum* (i.e. ingested prey) and in the medium and the numbers of *F.* cf. *mexicanum* with and without ingested prey were determined by observing and counting all or >300 cells in three to seven 1 ml Sedgwick-Rafter chambers using a compound microscope.

Pre-ingestion processes. In Expts 1 to 7, FFs were significantly affected by prey species. To investigate the cause for this difference in FFs, the pre-ingestion

process of *Fragilidium* cf. *mexicanum* was observed and video recorded for each prey species following the addition of dense predator and prey to 6-well plate chambers (3.5 cm in diameter and 18 ml in volume). Video recordings were taken at 10 to 100× using Olympus compound and dissecting microscopes equipped with a Watec video camera (Wat 202B).

Swimming speed. In the observation of pre-ingestion processes, we found that *Fragilidium* cf. *mexicanum* could not capture fast-swimming *Cochlodinium polykrikoides*. Thus, swimming speeds of prey and *F.* cf. *mexicanum* were measured at 22°C using a video analyzing system. For each species, aliquots from a dense culture were added to multiwell plates and allowed to acclimate for 30 min. Swimming was then observed and recorded at 40× as described above, with mean and maximum swimming velocity analyzed for fast-swimming cells that exhibited straight linear paths. Average swimming speed was calculated based on the linear displacement of cells in 1 s during single-frame playback. Swimming speeds of more than 10 cells were measured for each dinoflagellate species.

Growth and ingestion of *Fragilidium* cf. *mexicanum*. Expts 8 and 9 were designed to measure growth, ingestion, and clearance rates of *Fragilidium* cf. *mexicanum* feeding on *Lingulodinium polyedrum* as a function of the prey concentration (Table 2). About 1 or 2 d before these experiments were conducted, dense cultures of *F.* cf. *mexicanum* photosynthetically growing in f/2 medium under an illumination of 50 µE m^{-2} s^{-1} of cool white fluorescent light on a 12 h light:12 h dark cycle on shelves were transferred to 1 l PC bottles containing low concentrations of *L. polyedrum* (approximately 5 cells ml^{-1}). The bottles were filled to capacity with filtered seawater and placed on a rotating wheel at 0.9 rpm under a 12 h light:12 h dark cycle of illumination with 20 µE m^{-2} s^{-1} of cool white fluorescent light at 22°C to acclimate. Bottles were taken from the rotating wheel at intervals and examined under a dissecting microscope to determine the condition of *F.* cf. *mexicanum* and *L. polyedrum*, and then placed back on the rotating wheels. Once *L. polyedrum* cells were no longer detectable, three 1 ml aliquots from each bottle were counted using a compound microscope to determine cell concentrations of *F.* cf. *mexicanum*, and the cultures were then used to conduct experiments.

Initial concentrations of *Fragilidium* cf. *mexicanum* and prey were established using an autopipette, with triplicate 270 ml PC experiment bottles (mixtures of predator and prey) set up for each predator-prey concentration. In addition, triplicate control bottles for prey (*Lingulodinium polyedrum*) and duplicate control bottles for the predator (*F.* cf. *mexicanum*) were established at concentrations equal to those in predator-prey combinations. Thirty ml of f/2 medium were added to all bottles, which were then filled to capacity with freshly filtered seawater and capped.

Experimental and control bottles were incubated for 3 or 4 d using a rotating wheel and a mean light intensity of 20 µE m^{-2} s^{-1}, as previously described. We chose this light intensity to compare growth and ingestion rates of *Fragilidium* cf. *mexicanum* on *Lingulodinium polyedrum* with those of the heterotrophic dinoflagellates *Protoperidinium* cf. *divergens* and *P. crassipes* because it did not support photogrowth of *F.* cf. *mexicanum*, but did keep the prey *L. polyedrum* in a healthy condition. During preliminary experiments, *L. polyedrum* became unhealthy at light intensities ≤10 µE m^{-2} s^{-1}.

Ten ml aliquots were taken daily from each bottle and fixed with acidic Lugol's solution. Before taking subsamples, the condition and interaction of *Fragilidium* cf. *mexicanum* and *Lingulodinium polyedrum* were determined by carefully examining the contents of each bottle using a dissecting microscope. After sub-

Table 2. Experimental design. Density values indicate the actual initial concentrations (cells ml^{-1}) of prey and predator (*Fragilidium* cf. *mexicanum*)

Expt	Prey species	Prey density	*F.* cf. *mexicanum* density
1	*Lingulodinium polyedrum*	30, 120, 300, 1020, 2100	9, 20, 40, 70, 115
2	*Gymnodinium sanguineum*	20, 90, 280, 1100, 1960	12, 20, 44, 70, 90
3	*Prorocentrum micans*	27, 90, 250, 1160, 2160	15, 20, 30, 60, 90
4	*Scrippsiella trochoidea*	80, 490, 3350, 9130, 11850	18, 50, 100, 170, 270
5	*Cochlodinium polykrikoides*	10, 90, 620, 2600, 9100	13, 30, 50, 90, 120
6	*Amphidinium carterae*	1360, 4630, 15500, 59400, 110600	11, 25, 50, 80, 110
7	*Prorocentrum minimum*	1280, 4120, 12300, 49000, 90000	13, 25, 35, 88, 120
8	*L. polyedrum*	5, 10, 32, 63, 122	0.8, 1.6, 2.5, 4, 9
9	*L. polyedrum*	46, 90, 270, 740, 1400, 2230	7, 15, 24, 37, 60, 103
10	*L. polyedrum/S. trochoidea*	230/2830, 520/1880, 700/1470, 1180/720	56–67
11	*L. polyedrum*	1710–1770	74–80
12	*L. polyedrum*	1390–1480	57–64

samples were taken, bottles were again filled to capacity with freshly filtered seawater and placed back on the rotating wheel. The refilling diluted the cultures in the bottles, which was considered in calculating growth and ingestion rates. The concentrations of *F.* cf. *mexicanum* and *L. polyedrum* were determined for fixed samples as previously described.

The specific growth rate of *Fragilidium* cf. *mexicanum* (d^{-1}) was calculated by averaging the instantaneous growth rates (IGR), which were calculated as

$$IGR = \ln (F_{t1}/F_{t0})$$

where F_{t0} = the concentration of *F.* cf. *mexicanum* at Day t_0, and F_{t1} = the concentrations at Day t_1 (next day). The final day for this calculation was Day 3. Ingestion rates were calculated using the equations of Frost (1972) and Heinbokel (1978). Growth yield, defined as grazer biomass produced per prey biomass ingested, was calculated using Hansen & Nielsen's (1997) equations.

Prey selectivity in prey mixtures. Prey selectivity of *Fragilidium* cf. *mexicanum* was examined using mixtures of *Lingulodinium polyedrum* and *Scrippsiella trochoidea* as food. *F.* cf. *mexicanum* was observed to feed on both prey species when these unialgal diets were offered in Expts 1 and 4. *F.* cf. *mexicanum* cells growing photosynthetically in f/2 medium were added to 32 ml PC bottles containing different ratios of *L. polyedrum* and *S. trochoidea* (Expt 10 in Table 2). Five experimental bottles (containing both predator and prey) and 4 control bottles (containing only prey) were set up for each predator-prey treatment. Three ml of f/2 medium were added to each bottle. The contents of 1 experimental bottle and 1 control bottle were fixed with Lugol's solution to determine the actual initial concentrations of the predator and prey. The remaining bottles were incubated for 2 d using a rotating wheel and an illumination of 20 µE m^{-2} s^{-1}, as previously described. After incubation, the contents of each bottle were fixed with acidic Lugol's solution, with predator and/or prey concentrations and predator ingestion rates determined as previously described. The carbon ratio for ingestion rate of *F.* cf. *mexicanum* on *L. polyedrum* to that for total prey (*L. polyedrum* + *S. trochoidea*) was expressed as a function of prey availability (*L. polyedrum* carbon / total prey carbon).

Light effect on feeding. Expt 11 (Table 2) was conducted to investigate the effect of light intensity on the ingestion rates of *Fragilidium* cf. *mexicanum* when offered *Lingulodinium polyedrum* as prey. Dense cultures of *F.* cf. *mexicanum* growing mixotrophically on *L. polyedrum* in f/2 medium and under a 12 h light:12 h dark cycle of cool white fluorescent light at 50 µE m^{-2} s^{-1} were transferred to five 1 l PC bottles containing *L. polyedrum* in f/2 medium (ca 500 cells ml^{-1}). Bottles were wrapped with either 8, 4, 2, or 1 layer of screening to provide 20, 60, 100, and 200 µE m^{-2} s^{-1}, respectively. The bottles were placed on a vertically rotating plate, maintained at 22 ± 1°C, and evenly illuminated from one direction. Target light intensities, as determined inside the bottles, showed little variation during rotation of the plate.

Every 1 or 2 d the contents of each bottle were evenly divided into two 1 l PC bottles containing f/2 medium and *Lingulodinium polyedrum* at ca 500 cells ml^{-1} and placed back on the rotating plate. In this manner, *Fragilidium* cf. *mexicanum* cells were acclimated to target light intensities for 8 d. After acclimation, and once *L. polyedrum* cells were no longer detectable in the bottles, three 1 ml aliquots from each bottle were counted using a compound microscope to determine *F.* cf. *mexicanum* abundance. Aliquots from each bottle were then transferred to five 32 ml PC bottles containing *L. polyedrum*. Four 32 ml PC control bottles containing only *L. polyedrum* and another four 32 ml PC control bottles containing only *F.* cf. *mexicanum* were set up at each light intensity. Sixteen ml of f/2 medium was added to each PC bottle. The contents of 1 of the experimental bottles and 1 of each set of control bottles at each target light intensity were fixed with Lugol's solution to determine the initial concentrations of predator and/or prey.

Experimental and control bottles were incubated for 2 d using the vertically rotating plate and target illumination. After incubation, the contents from each bottle were fixed with Lugol's solution. The concentrations of *Fragilidium* cf. *mexicanum* and *Lingulodinium polyedrum* were determined by counting all grazers and >300 *L. polyedrum* cells in three 1 ml Sedgwick-Rafter chambers. Ingestion rates were calculated as previously described.

Nutrient effects on feeding. Expt 12 (Table 2) was conducted to investigate the effects of nutrient concentration on ingestion rate of *Fragilidium* cf. *mexicanum* when offered *Lingulodinium polyedrum* as prey. Dense cultures of *F.* cf. *mexicanum* growing mixotrophically on *L. polyedrum* were sieved through a 35 µm mesh. Cells retained on the sieve were transferred to 1 l PC bottles containing only oceanic seawater (nitrate + nitrite [hereafter N] and phosphate [P] concentrations <0.1 µM) and placed on an illuminated shelf. After 2 h, fluid from the upper third of the bottle was gently removed and distributed to five 1 l PC bottles containing 500 ml of either freshly filtered oceanic seawater (OC), f/2 medium (F), 500 ml f/2 medium without N (F – N), f/2 medium without P (F – P), or f/2 medium without both N and P (F – N – P). The bottles were then filled to capacity with freshly filtered oceanic seawater, capped, and placed on a vertically rotating wheel (0.9 rpm; 100 µE m^{-2} s^{-1}, 12 h light:12 h

dark cycle; 22 ± 1°C). Dense cultures of *L. polyedrum* were also concentrated on 35 µm mesh screening and transferred to two 2 l PC bottles containing oceanic water.

Every day thereafter, subsamples were taken from each bottle, gently filtered through GF/F filters, and stored frozen at −20°C until N and P concentrations were measured using a Nutrient AutoAnalyzing System (Bran and Luebbe Traacs 2000). After 5 d, N concentrations in bottles containing *Fragilidium* cf. *mexicanum* plus OC, F – N, or F – N – P were <1 µM, while P concentrations in bottles containing *F.* cf. *mexicanum* plus OC, F – P, or F – N – P were <0.3 µM. N and P concentrations in the bottles containing only *Lingulodinium polyedrum* cells also dropped to <1 and <0.3 µM, respectively, within 5 d.

These very low N and P cultures of *Lingulodinium polyedrum* (ca 500 cells ml^{-1}; hereafter *Lp*LNP) were used to feed the OC, F, F – N, and F – N – P cultures of *Fragilidium* cf. *mexicanum* during an additional 6 d acclimation period. Feeding was done at 1 to 2 d intervals by transferring the contents of each *F.* cf. *mexicanum* culture bottle to 2 new 1 l PC bottles containing target nutrients and *Lp*LNP. During this acclimation period, subsamples were taken daily for determination of N and P concentrations. Ranges (nd = not detectable) for N (and P) concentrations were 0.08–0.36 µM N (0.08–0.26 µM P) for the OC bottle, nd–0.09 µM N (0.06–0.22 µM P) in the F – N – P bottle, nd µM N (12–38 µM P) in the F – N bottle, 102–247 µM N (nd µM P) in the F – P bottle, and 137–243 µM N (12–32 µM P) in the F bottle.

After acclimation and once *Lingulodinium polyedrum* cells were no longer detectable, three 1 ml aliquots from each bottle were counted to determine cell concentrations of *Fragilidium* cf. *mexicanum*. Aliquots (2 to 3 ml) from each bottle containing acclimated *F.* cf. *mexicanum* cells were transferred to six 32 ml PC bottles containing *Lp*LNP (ca 6 ml) and 16 ml of matching target nutrients or oceanic water. One set of 4 control bottles containing only *Lp*LNP and another set of 4 control bottles containing only acclimated *F.* cf. *mexicanum* cells were set up at each nutrient condition. The bottles were filled to capacity with freshly filtered oceanic seawater and capped. For each nutrient condition, 10 ml aliquots were removed from 2 of the 6 experimental bottles and from 1 of each set of the 4 control bottles, filtered using GF/F filters, and stored frozen until N and P concentrations were determined. The remaining volume was fixed with Lugol's solution to determine the initial predator and prey concentrations.

The other experimental and control bottles were incubated for 2 d using a vertically rotating plate and an illumination of 100 µE m^{-2} s^{-1}. After incubation a 10 ml aliquot from each bottle was GF/F filtered and stored frozen for determination of N and P concentrations. The remaining volume from each bottle was fixed and the final concentrations of predator and/or prey were determined as previously described.

Statistical analysis. A 1-way ANOVA (Zar 1984) was used to test the effect of light intensity or nutrient concentration on ingestion rates of *Fragilidium* cf. *mexicanum* on *Lingulodinium polyedrum*.

RESULTS

Prey species and feeding frequencies

Fragilidium cf. *mexicanum* feeds on red-tide dinoflagellate prey by engulfment and can contain several prey cells simultaneously (Fig. 1). From the red-tide and toxic dinoflagellates offered as prey, *F.* cf. *mexicanum* ingested *Lingulodinium polyedrum* (Fig. 1A, B), *Gymnodinium sanguineum* (Fig. 1C), *Scrippsiella trochoidea* (Fig. 1D), *Prorocentrum micans* (Fig. 1E), and *Prorocentrum minimum* (Fig. 1F), but did not eat *Amphidinium carterae* or *Cochlodinium polykrikoides*. *F.* cf. *mexicanum* was observed to quickly attach to and easily engulf *L. polyedrum* and *S. trochoidea* cells as soon as they were offered, and usually engulfed a second prey cell soon thereafter. *F.* cf. *mexicanum* was able to ingest 3 *L. polyedrum* cells within 10 min. Feeding frequency (FF) values of *F.* cf. *mexicanum* on *L. polyedrum* and *S. trochoidea* >0 and an average number of ingested prey cells inside the grazer (APN) >1 after the 2 h incubation quantitatively confirm these observations. However, *F.* cf. *mexicanum* did not quickly attach to and sometimes had difficulty engulfing *G. sanguineum* and *P. micans*, even after capturing them. *F.* cf. *mexicanum* tried to ingest the longer lateral side of *G. sanguineum* and *P. micans* rather than the shorter apical side, and prey were able to escape after being captured. FF and APN values of 0 on these prey species after 2 h incubation confirm this observation. *F.* cf. *mexicanum* usually digested the prey cells offered after they had been completely engulfed.

FFs of *Fragilidium* cf. *mexicanum* on red-tide dinoflagellates were significantly affected by prey species (maximum FFs on 5 edible prey species; ANOVA, $p < 0.001$ after both 2 and 72 h) (Figs. 2 to 5).

For the *Lingulodinium polyedrum* diet, FF was significantly affected by prey concentration (ANOVA, $p < 0.01$ after 2 h and $p < 0.001$ after 72 h) (Fig 2A). FFs increased with increasing prey concentration below ca 300 and 800 cells ml^{-1} for the 2 h and 72 h incubations, respectively, but were saturated at higher concentrations. Maximum FFs were 20 and 44% for the 2 h and 72 h incubations, respectively. Maximum

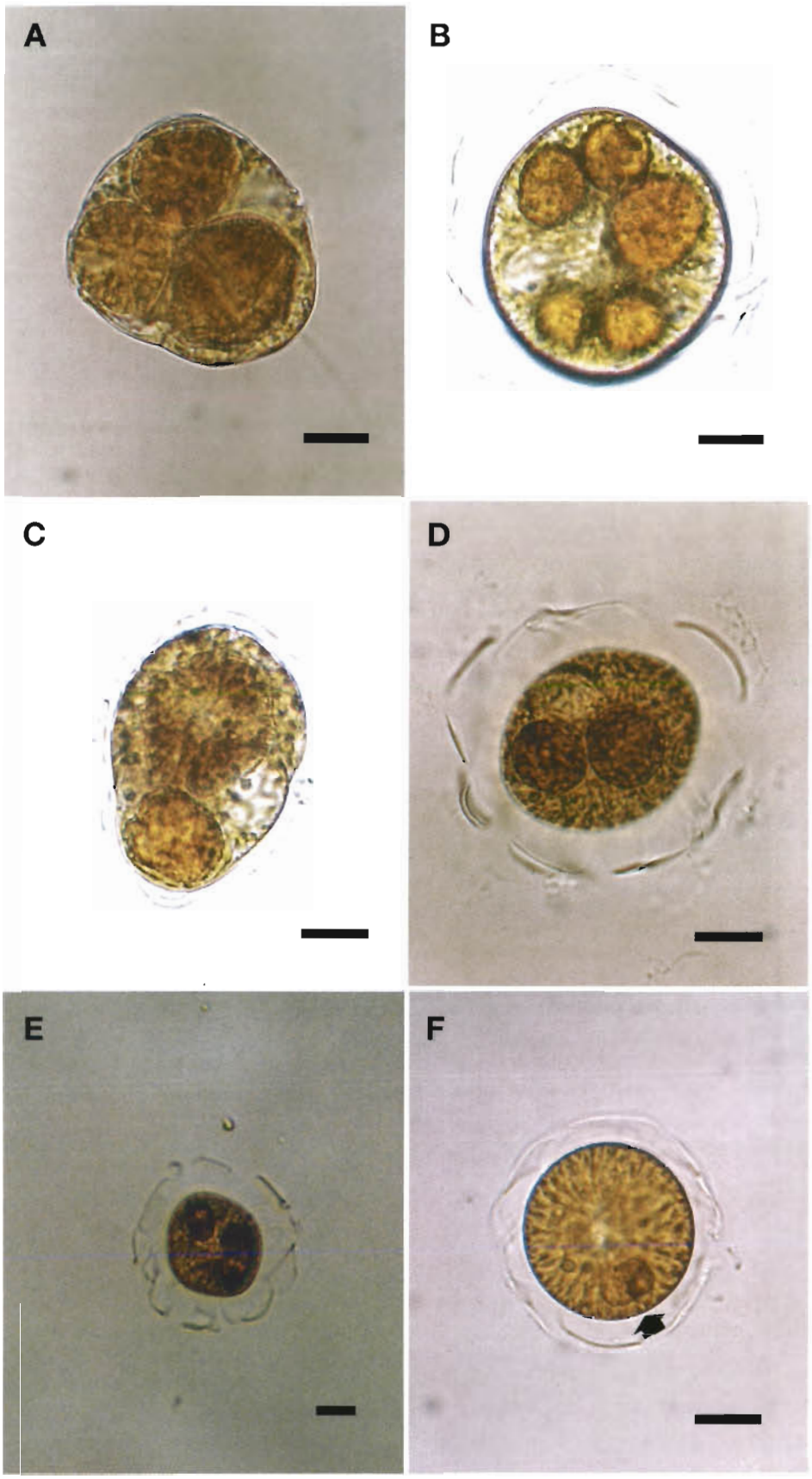

Fig. 1 *Fragilidium* cf. *mexicanum* with (A) 3 newly ingested *Lingulodinium polyedrum* cells, (B) 1 newly ingested and 4 semi-digested *L. polyedrum* cells, (C) 1 newly ingested *Gymnodinium sanguineum* (the larger prey cell) and 1 semi-digested *L. polyedrum* cell (the smaller cell), (D) 2 newly ingested *Scrippsiella trochoidea* cells, (E) 1 newly ingested and 1 semi-digested *Prorocentrum micans* cell, and (F) 1 *Prorocentrum minimum* cell (arrow). Scale bars = 20 µm

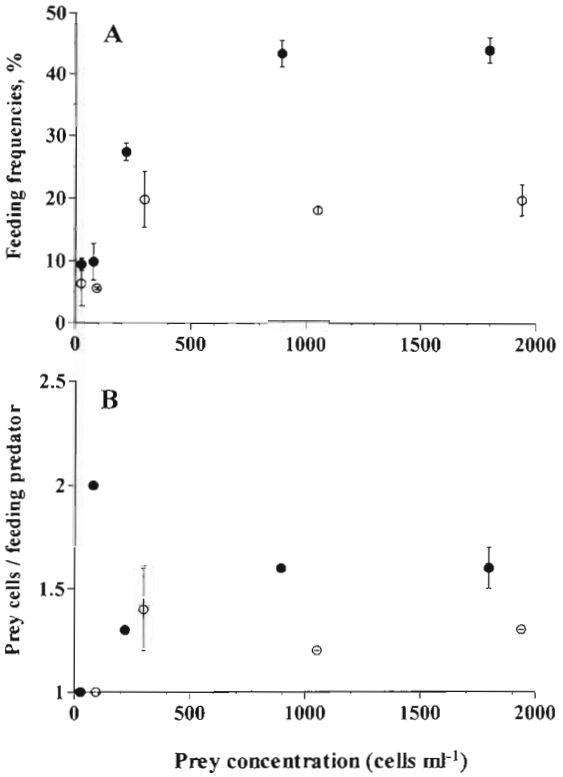

Fig. 2. *Fragilidium* cf. *mexicanum*. (A) Feeding frequency (%) on *Lingulodinium polyedrum* as a function of final prey concentration. Feeding frequency is based on the proportion of the *F.* cf. *mexicanum* cells observed to contain prey and is measured by calculating the percent ratio of *F.* cf. *mexicanum* containing 1 or more target prey cells to total *F.* cf. *mexicanum* after 2 h (○) and 72 h (●) incubations. (B) Average number of ingested prey in *F.* cf. *mexicanum* feeding on *L. polyedrum*, measured by calculating the average numbers of prey cells observed in the protoplasm of grazers that had fed after 2 h (○) and 72 h (●) incubations. Data points show treatment mean ± 1 SE

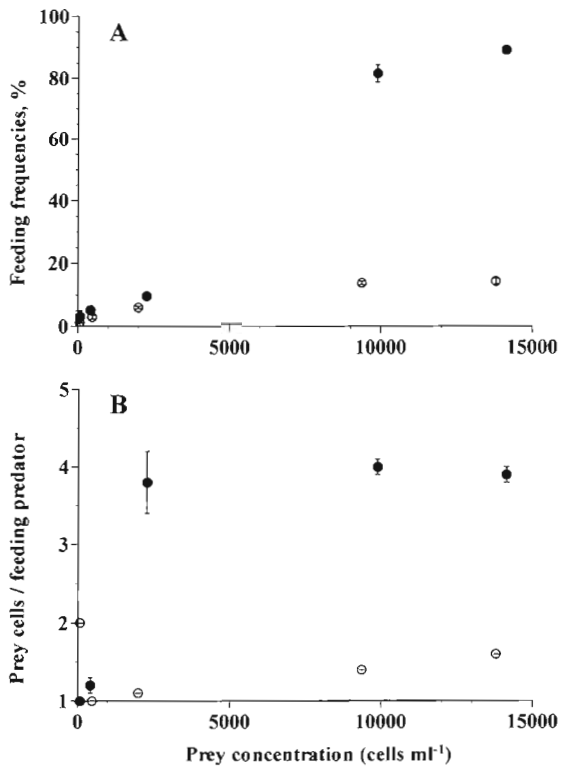

Fig. 3. *Fragilidium* cf. *mexicanum*. (A) Feeding frequency (%) on *Scrippsiella trochoidea* and (B) average number of ingested *S. trochoidea* after 2 h (○) and 72 h (●) incubations. Data points show treatment mean ± 1 SE

numbers of prey observed inside the protoplasm of *Fragilidium* cf. *mexicanum* (MPNs) after the 2 h and 72 h incubations were 3 and 6, respectively. The average number of prey cells observed in the grazer's protoplasm (APN) was between 1 and 2 prey cells predator^{-1} for *F.* cf. *mexicanum* feeding on *L. polyedrum* (Fig. 2B). APN was significantly affected by prey concentration (ANOVA, $p < 0.05$ after 2 h and $p < 0.001$ after 72 h) and generally increased with increasing prey concentration, with 1 exception (2 at 78 *L. polyedrum* ml^{-1}). After the 72 h incubation, densities of *F.* cf. *mexicanum* in experimental bottles were markedly higher than those in the control bottles.

For the *Scrippsiella trochoidea* diet, FF was significantly affected by prey concentration (ANOVA, $p < 0.001$ after both 2 and 72 h). It increased with increasing prey concentration < ca 9100 cells ml^{-1} for both 2 and 72 h incubations, with saturation at higher concentrations (Fig. 3A). MPNs after 2 h and 72 h incubations were 4 and 8, respectively. APN was ≤2 prey cells predator^{-1} at all prey concentrations after the 2 h incubation and at prey concentrations <400 cells ml^{-1} after the 72 h incubation, but was between 3 and 4 at the higher prey concentrations in the 72 h incubation (Fig. 3B). APN was significantly affected by prey concentration after 72 h (ANOVA, $p < 0.001$), but was not significant affected after 2 h ($p > 0.1$). After the 72 h incubation, there was no clear difference between the densities of *Fragilidium* cf. *mexicanum* in the control and experimental bottles.

For the *Gymnodinium sanguineum* diet, FFs were less than 2% (Fig. 4A), and were not significantly affected by prey concentration (ANOVA, $p > 0.1$), unlike the *Lingulodinium polyedrum* and *Scrippsiella trochoidea* diets. MPN after both the 2 and 72 h incubations was 3, while APN was 1 at all prey concentrations except 1920 cells ml^{-1} after the 72 h incubation, when it reached 2.2 prey cells grazer^{-1} (Fig. 4B).

For the *Prorocentrum micans* diet, FFs were zero after the 2 h incubation, but reached 5.6% in the 72 h incubation (Fig. 5A). FF was not significantly affected by prey concentration (ANOVA, $p > 0.1$), as with

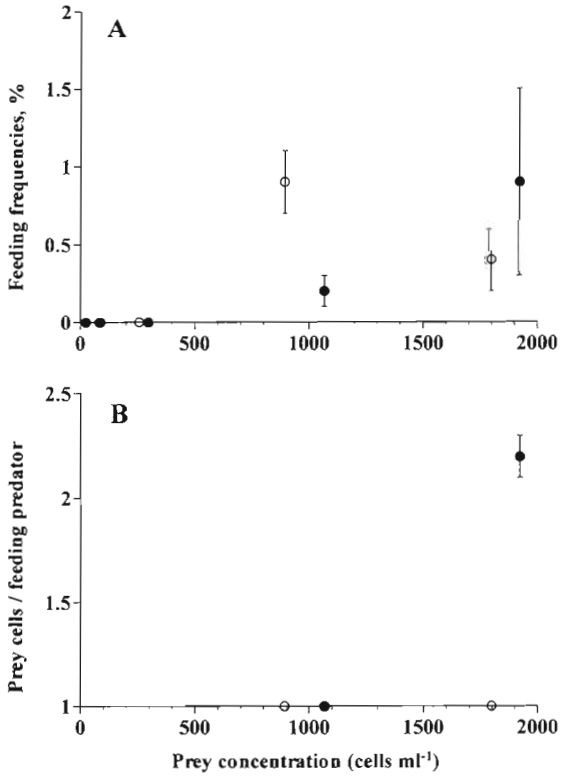

Fig. 4. *Fragilidium* cf. *mexicanum*. (A) Feeding frequency (%) on *Gymnodinium sanguineum* and (B) average number of ingested *G. sanguineum* after 2 h (○) and 72 h (●) incubations. Data points show treatment mean ± 1 SE

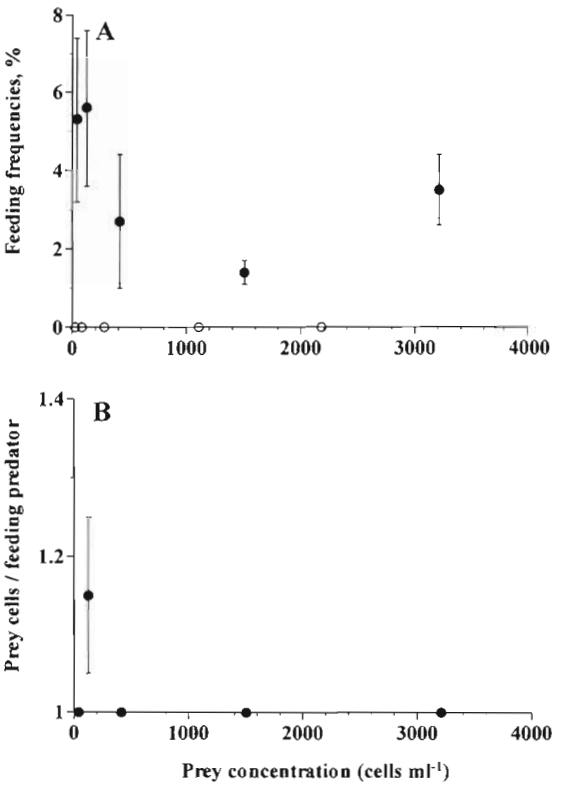

Fig. 5. *Fragilidium* cf. *mexicanum*. (A) Feeding frequency (%) on *Prorocentrum micans* and (B) average number of ingested *P. micans* after 2 h (○) and 72 h (●) incubations. Data points show treatment mean ± 1 SE

Gymnodinium sanguineum. MPN after the 72 h incubation was 2 and APN was almost 1 (Fig. 5B). APN was also not significantly affected by prey concentration after 72 h (ANOVA, $p > 0.1$).

For the *Prorocentrum minimum* diet, FFs were zero at the all prey concentrations except 70400 cells ml^{-1} in the 72 h incubation (9.6%). MPN after the 72 h incubation was 6 and APN was 1.6 prey cells $predator^{-1}$. Feeding by *Fragilidium* cf. *mexicanum* on *P. minimum* at high prey concentrations was ascertained through an additional experiment.

For the *Amphidinium carterae* and *Cochlodinium polykrikoides* diets, FFs were zero at the all prey concentrations offered in the present study.

Swimming speed

Average (± SE) and maximum swimming speeds of *Fragilidium* cf. *mexicanum*, 353 (± 17) and 448 μm s^{-1} respectively, were greater than those of all prey species except *Cochlodinium polykrikoides*, which had average and maximum speeds of 1063 (± 84) and 1449 μm s^{-1} respectively (Table 3).

Prey selectivity in prey mixtures

The preference of *Fragilidium* cf. *mexicanum* for a particular diet was determined from the ratio of ingestion rates for *Lingulodinium polyedrum* and *Scrippsiella trochoidea* as a function of the ratio of the mean prey concentrations (Fig. 6). All data points were above the 1:1 line (line of no preference), indicating that *F.* cf. *mexicanum* strongly preferred *L. polyedrum* to *S. trochoidea*.

Table 3. Swimming speeds (μm s^{-1}) of species used in the present study at 22°C

Species	Mean (±SE)	Maximum
Prorocentrum minimum	157 (±8)	194
Amphidinium carterae	111 (±3)	126
Cochlodinium polykrikoides	1063 (±84)	1449
Scrippsiella trochoidea	304 (±12)	348
Prorocentrum micans	268 (±21)	380
Gymnodinium sanguineum	193 (+10)	280
Lingulodinium polyedrum	282 (±15)	378
Fragilidium cf. *mexicanum*	353 (±17)	448

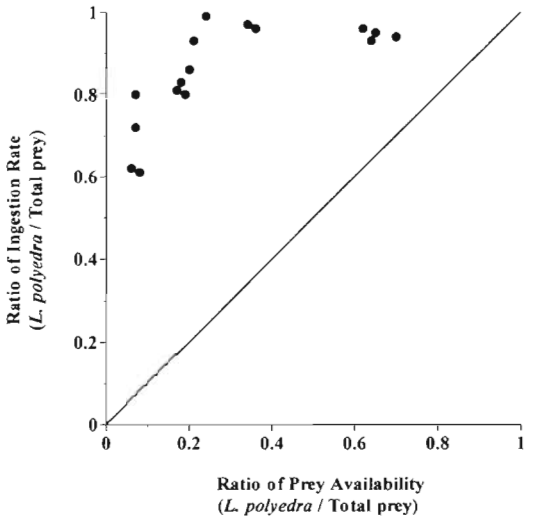

Fig. 6. *Fragilidium* cf. *mexicanum*. Prey selection in a mixed diet of *Lingulodinium polyedrum* and *Scrippsiella trochoidea*. Data points show results of single incubation bottles. The carbon ratio of the ingestion rate of *F.* cf. *mexicanum* on *L. polyedrum* to that on total prey (*L. polyedrum* + *S. trochoidea*) was expressed as a function of prey availability (*L. polyedrum* carbon/total prey carbon)

Growth and ingestion rates of *Fragilidium* cf. *mexicanum*

Specific growth rates of *Fragilidium* cf. *mexicanum* fed on *Lingulodinium polyedrum* increased rapidly with increasing mean prey concentration for prey concentrations <500 cells ml^{-1}, but were saturated at higher concentrations (Fig. 7). The maximum specific growth rate under a 12 h light:12 h dark cycle of illumination at 20 µE m^{-2} s^{-1}, obtained at a mean prey concentration of approximately 500 cells ml^{-1} in Expt 8, was 0.36 d^{-1}. Growth rate of *F.* cf. *mexicanum* without added prey (in 12 control bottles) was −0.05 ± 0.03 (mean ± SE).

The ingestion rate of *Fragilidium* cf. *mexicanum* feeding on *Lingulodinium polyedrum* increased with increasing mean prey concentration to a maximum of 3.9 prey $grazer^{-1}$ d^{-1}, with saturation at mean prey concentrations >500 cells ml^{-1} (Fig. 8A). Ingestion still occurred at mean prey concentrations <10 cells ml^{-1}. Clearance rates decreased with increasing mean prey concentration (Fig. 8B), with a maximum clearance rate of 6 µl $grazer^{-1}$ h^{-1} at a mean prey concentration of approximately 20 cells ml^{-1}.

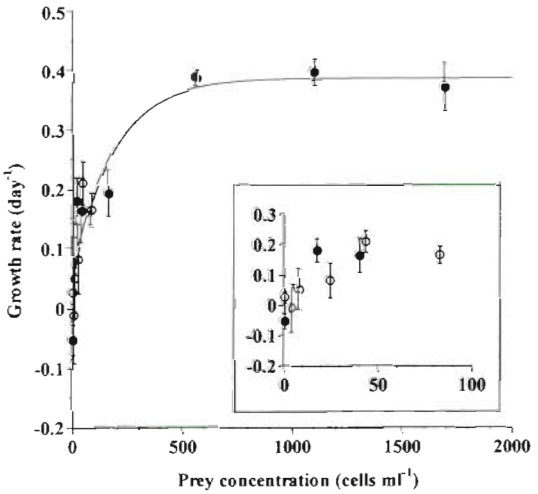

Fig. 7. *Fragilidium* cf. *mexicanum*. Specific growth rate on a diet of *Lingulodinium polyedrum* as a function of mean prey concentration (*c*). Data points show treatment means ± 1 SE. The equation of the regression line was obtained by pooling all treatments from Expts 8 (○) and 9 (●). The fitted curve is the Ivlev model. Growth rate (GR, d^{-1}) = 0.36 $(1 - e^{-0.0142c})$, R^2 = 0.762. Inset shows values at low prey concentrations

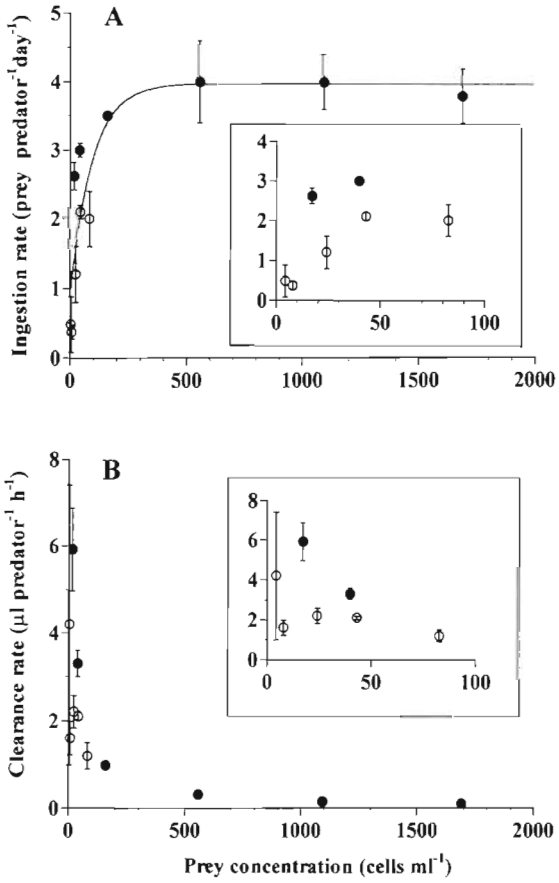

Fig. 8. *Fragilidium* cf. *mexicanum*. (A) Ingestion rate and (B) clearance rate on *Lingulodinium polyedrum* as a function of mean prey concentration (*c*). Data points show treatment means ± 1 SE. The regression line in (A) was obtained by pooling all treatments from the Expts 8 (○) and 9 (●). The fitted curve is the Ivlev model. Ingestion rate (IR, prey $grazer^{-1}$ d^{-1}) = 3.9$(1 - e^{-0.0202c})$, R^2 = 0.809. Insets show values at low prey concentrations

Growth yields were between 15 and 53%, and the value at the mean prey concentration for which the maximum growth rate was achieved was 39%.

Light and nutrient effects on ingestion rate

Ingestion rates of *Fragilidium* cf. *mexicanum* feeding on *Lingulodinium polyedrum* under food-satiated conditions at light intensities ranging from 20 to 200 µE m^{-2} s^{-1} were 3.0 to 3.8 prey $grazer^{-1}$ d^{-1}; however, they were not significantly different from one another (ANOVA, $p > 0.1$) (Fig. 9).

Ingestion rates of *Fragilidium* cf. *mexicanum* feeding on *Lingulodinium polyedrum* when food-satiated under different nutrient conditions (OC, F – N – P, F – N, F – P, and F) were between 3.4 and 4.2 prey $grazer^{-1}$ d^{-1}, but showed no significant difference among treatments (ANOVA, $p > 0.1$) (Fig. 10).

DISCUSSION

The present study shows that (1) *Fragilidium* cf. *mexicanum* can feed on diverse red-tide dinoflagellate species, (2) *F.* cf. *mexicanum* has a wide range of feeding frequencies (FFs) which were affected by prey species, (3) FFs on *Lingulodinium polyedrum* and *Scrippsiella trochoidea* were affected by prey concentration, but FFs on the other prey species offered under the same experimental conditions were not, (4) *F.* cf. *mexicanum* strongly selects *L. polyedrum* over *S. trochoidea* in prey mixtures, (5) the unialgal diet of *L. polyedrum* clearly supported the grazer's phagotrophic growth under conditions unfavorable to its phototrophic growth, and (6) the ingestion rate of *F.* cf. *mexicanum* on *L. polyedrum* was not significantly affected by light intensity or nutrient concentration.

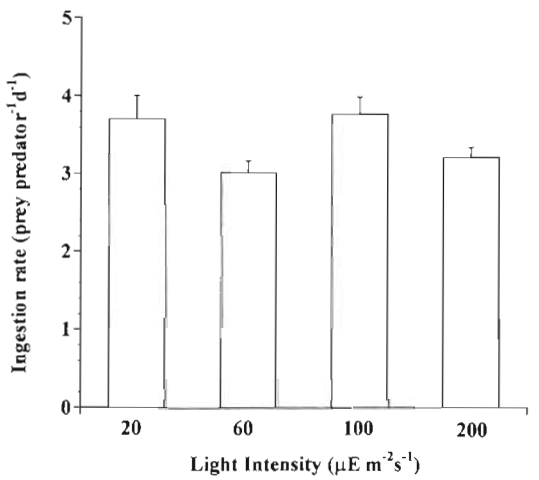

Fig. 9. *Fragilidium* cf. *mexicanum*. Ingestion rate on *Lingulodinium polyedrum* as a function of light intensity. Values are treatment means + 1 SE

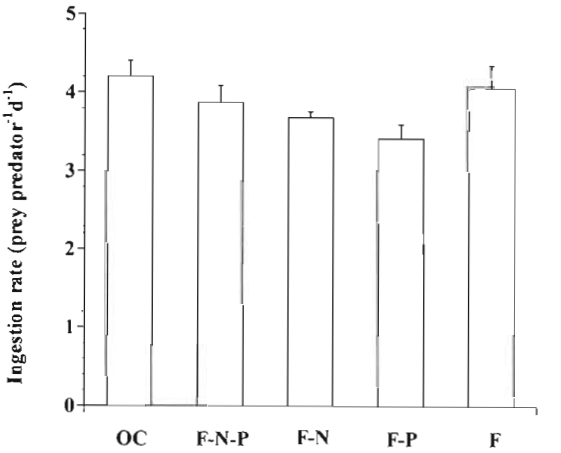

Fig. 10. *Fragilidium* cf. *mexicanum*. Ingestion rate on *Lingulodinium polyedrum* under different nutrient conditions. Values are treatment means + 1 SE. Media used were oceanic seawater (OC), f/2 medium without N and P (F – N – P), f/2 medium without N (F – N), f/2 medium without P (F – P), and f/2 medium (F) (see 'Materials and methods: nutrient effects on feeding'). During this experiment, initial and final concentrations of N (and P) were 0.18 and 0.12 µM N (0.17 and 0.04 µM P) in the OC bottles, 0.01 and 0.02 µM N (0.22 and 0.02 µM P) in the F – N – P bottles, nd (not detectable) µM N (37 and 18 µM P) in the F – N bottles, 205 and 159 µM N (nd µM P) in the F – P bottles, and 225 and 153 µM N (40 and 19 µM P) in the F bottles

Prey species and selection

Unlike *Fragilidium subglobosum*, which is known to feed exclusively on *Ceratium* spp. (Skovgaard 1996a), *F.* cf. *mexicanum* can feed on red-tide dinoflagellates belonging to diverse genera. *Lingulodinium polyedrum*, *Gymnodinium sanguineum*, *Prorocentrum micans*, *P. minimum*, and *Scrippsiella trochoidea*, species that most frequently form red tides in the coastal waters off southern Californian where the grazer was isolated, are all ingested.

The FF of *Fragilidium* cf. *mexicanum* feeding on different prey species and its selectivity in prey mixtures might be affected by several factors, including prey size, shape, taste, and swimming speed. When provided unialgal diets, *F.* cf. *mexicanum* had much higher feeding frequencies on *Lingulodinium polyedrum* and *Scrippsiella trochoidea* than on *Gymnodinium sanguineum*, *Prorocentrum micans*, and *P. minimum*. *L. polyedrum* and *S. trochoidea* may be nutritionally better prey for *F.* cf. *mexicanum*, or *F.* cf. *mexicanum* may have more difficulty capturing and/or

engulfing *G. sanguineum*, *Prorocentrum micans*, and *P. minimum*. The large ciliates *Favella* sp. (Jeong 1995) and *Strombidinopsis* sp. (ca 200 µm in cell length) (Jeong et al. 1999), the calanoid copepod *Calanus pacificus* (Huntley et al. 1983, 1987), and the larval northern anchovy *Engraulis mordax* (Scura & Jerde 1977) have higher maximum growth and/or development rates when feeding on *G. sanguineum* than on *L. polyedrum* or *S. trochoidea*. The C:N ratio of *G. sanguineum* (5.4) is lower than that of *L. polyedrum* (8.9) and *S. trochoidea* (9.1) (Fernández 1979). Therefore, it is unlikely that *L. polyedrum* and *S. trochoidea* are nutritionally better prey than *G. sanguineum*, *P. micans*, and *P. minimum*.

Lingulodinium polyedrum and *Scrippsiella trochoidea* have an almost spherical shape, while *Gymnodinium sanguineum*, *Prorocentrum micans*, and *P. minimum* are flat and long. While *Fragilidium* cf. *mexicanum* easily engulfed *L. polyedrum* and *S. trochoidea*, we found that it had difficulty ingesting *G. sanguineum* and *P. micans* because it tried to ingest the longer lateral side rather than the shorter apical side of these prey. Thus, *F.* cf. *mexicanum* failed to engulf *G. sanguineum* and *P. micans* even after capturing them. While *F. subglobosum* can ingest long *Ceratium tripos* cells from the apical or antapical side by gradually digesting part of the prey during engulfing (Skovgaard 1996a), *F.* cf. *mexicanum* appears unable to do this with *G. sanguineum* and *P. micans* cells. These prey might be strong enough to escape this type of gradual digestion or their large cell length and/or flat shape may prevent them from being engulfed by *F.* cf. *mexicanum*.

The maximum growth rates and/or feeding frequencies of the heterotrophic dinoflagellates *Protoperidinium* cf. *divergens* and *P. crassipes* on *Lingulodinium polyedrum* and *Scrippsiella trochoidea* are also higher than for *Gymnodinium sanguineum* and *Prorocentrum* spp. (Jeong & Latz 1994). Another heterotrophic dinoflagellate, *Noctiluca scintillans*, has much higher growth rates when feeding on *L. polyedrum* than on *G. sanguineum* (Jeong 1995). *Protoperidinium* spp., which feed using a pallium, and *Noctiluca* spp., which feed using a tentacle, also have more difficulty capturing and/or ingesting *G. sanguineum* cells than they do *L. polyedrum* and *S. trochoidea* cells. Since *Fragilidium* cf. *mexicanum* has a diet similar to that of co-occurring *P.* cf. *divergens*, *P. crassipes*, and *N. scintillans*, it may compete with these other grazers for *L. polyedrum* and *S. trochoidea* as prey.

Fragilidinium cf. *mexicanum* did not feed on *Amphidinium carterae* and *Cochlodinium polykrikoides*. *A. carterae* is known to be toxic (Steidinger & Tangen 1996), and it is also poor prey for the tintinnid ciliate *Favella ehrenbergii* (Stoecker et al. 1981) and the naked ciliate *Strombidinopsis* sp. (Jeong et al. 1999). Thus, the bad taste of *A. carterae* might be responsible for the zero FF found in this study. *F* cf. *mexicanum* might have difficulty in catching *C. polykrikoides* cells because it has a much slower swimming speed than this prey species. It is interesting that several studies have reported that *Fragilidium* spp. feed intensively on *Lingulodinium polyedrum* or *Alexandrium* spp. in natural water samples (Balech & Ferrando 1964, Eppley & Harrison 1975, Balech 1988), while there are no comparable reports that *Fragilidium* spp. feeds on the other prey species investigated in the present study.

Prey selectivity in prey mixture

The present study shows that *Fragilidium* cf. *mexicanum* has the ability to feed selectively when offered a prey mixture of red-tide dinoflagellates. *F.* cf. *mexicanum* strongly preferred *Lingulodinium polyedrum* to *Scrippsiella trochoidea*, although the maximum FF on *S. trochoidea* was similar to or higher than that on *L. polyedrum* when a unialgal diet was provided (Figs. 2 & 3). Prey selection by dinoflagellates in mixtures of prey species is common. Hansen & Nielsen (1997) concluded that *F. subglobosum* has a prey preference, showing a higher uptake rate on *Ceratium tripos* than on *C. furca* or *C. fusus* when unialgal diets were provided. *Protoperidinium* cf. *divergens* strongly selected *L. polyedrum* over *Gymnodinium sanguineum* (Jeong & Latz 1994) and round copepod eggs with a smooth surface over *L. polyedrum* (Jeong 1996). *Oxyrrhis marina* also has the ability to select prey among differently sized nanophytoplankton (Hansen et al. 1996). This differential feeding in a prey mixture may affect the population dynamics of the red-tide dinoflagellates when favorable conditions for their growth are provided in nature.

Growth and ingestion

A unialgal diet of *Lingulodinium polyedrum* can support population growth of *Fragilidium* cf. *mexicanum* under conditions under which its growth rate, without added prey, is negative or zero. This evidence suggests that *F.* cf. *mexicanum* can increase its population by feeding on *L. polyedrum* under conditions unfavorable for phototrophic growth. For the *Scrippsiella trochoidea* diet, the maximum FF was higher than that for *L. polyedrum* prey; however, there was no clear difference between the densities of *Fragilidium* cf. *mexicanum* in control and experimental bottles after 72 h incubation. This evidence suggests that the

unialgal diet of *S. trochoidea* does not support the phagotrophic growth of *F.* cf. *mexicanum* even though the grazer can actively feed on it, an observation that has also been made for the heterotrophic dinoflagellate *Protoperidinium* cf. *divergens* (Jeong & Latz 1994).

The pattern in the specific growth rate of *Fragilidium* cf. *mexicanum* feeding on *Lingulodinium polyedrum* was similar to those of *Protoperidinium* cf. *divergens* and *P. crassipes* feeding on the same prey (Jeong & Latz 1994). Maximum mixotrophic growth rate of *F.* cf. *mexicanum* on *L. polyedrum* under a 12 h light:12 h dark cycle of illumination at 20 µE m^{-2} s^{-1} was 0.36 d^{-1}, comparable to that achieved by *P. crassipes*, but lower than that achieved by *P.* cf. *divergens* (Table 4). Mean *L. polyedrum* concentration at which the maximum growth and ingestion rates of *F.* cf. *mexicanum* were achieved, 500 cells ml^{-1}, is also similar to that for *P. crassipes*, but is lower than that for *P.* cf. *divergens*, which achieves maximum growth and ingestion rates at 1100 to 1500 cells ml^{-1}. Therefore, phagotrophic growth of *F.* cf. *mexicanum* on *L. polyedrum* is comparable to that of *P. crassipes* on the same prey.

The maximum clearance rate of *Fragilidium* cf. *mexicanum* feeding on *Lingulodinium polyedrum*, 6 µl predator^{-1} h^{-1}, is much higher than that of *Protoperidinium* cf. *divergens* or *P. crassipes* (0.9 and 0.6 µl predator^{-1} h^{-1}). At low *L. polyedrum* concentrations, the engulfment process of *F.* cf. *mexicanum* may be more efficient in capturing and ingesting prey than the pallium feeding of *Protoperidinium* spp. An efficient feeding mechanism might enable *F.* cf. *mexicanum* to dominate *Protoperidinium* spp. under reciprocal predation in darkness (Jeong et al. 1997), even though the maximum ingestion rates of *F.* cf. *mexicanum* feeding on *L. polyedrum*, 3.9 prey predator^{-1} d^{-1}, were lower than that of *P.* cf. *divergens* (6.1 prey predator^{-1} d^{-1}) (Table 4).

The maximum clearance rate (6 µl predator^{-1} h^{-1}) of *Fragilidium* cf. *mexicanum* feeding on *Lingulodinium polyedrum* was slightly lower than that of *F. subglobosum* on *Ceratium tripos*, 7 µl predator^{-1} h^{-1} (Hansen & Nielsen 1997), when corrected to 22°C using a Q_{10} of 2.8 (Hansen et al. 1997).

Grazing of *Fragilidium subglobosum* on *Ceratium tripos* as a function of prey concentration is a Holling Type I curve (Hansen & Nielsen 1997) because *F. subglobosum* encysts after ingesting only 1 *C. tripos* cell. Grazing of *F.* cf. *mexicanum* on *Lingulodinium polyedrum* is close to a Holling Type 2 curve (Holling 1959). The ingestion rate of *F.* cf. *mexicanum* on *L. polyedrum* is likely to be affected mainly by encounter rate between predator and prey at prey concentrations <500 cells ml^{-1} and mainly by handling time at higher prey concentrations. *F.* cf. *mexicanum* can contain several *L. polyedrum* cells inside its protoplasm simultaneously.

Light and nutrient effects

When prey was plentiful, ingestion rates of *Fragilidium* cf. *mexicanum* feeding on *Lingulodinium polyedrum* under the light conditions provided in the present study (20, 60, 100, and 200 µE m^{-2} s^{-1}) were not significantly affected by light intensity (see Fig. 9). This is unlike the response of *F. subglobusum* feeding on *Ceratium lineatum* and *C. tripos*, for which ingestion rates were affected by light intensity from 0 to 150 µE m^{-2} s^{-1} and 10 to 45 µE m^{-2} s^{-1}, respectively (Skovgaard 1996b, Hansen & Nielsen 1997).

The mixotrophic dinoflagellate *Prorocentrum minimum* has been shown to have higher feeding frequency at lower nutrient concentrations and to stop feeding when high concentrations of nitrate and phosphate were added together (Stoecker et al. 1997). However, the ingestion rate of *Fragilidium* cf. *mexicanum* feeding on *Lingulodinium polyedrum* was not significantly affected by nutrient concentration when the prey was plentiful, and the grazer did not stop feeding after being incubated with the addition of high concentrations of nitrate plus phosphate (see Fig. 10). Therefore, phagotrophic feeding by *F.* cf. *mexicanum* on *L. polyedrum* may be primarily a strategy for obtaining carbon rather than inorganic nutrients.

Table 4. Comparison of growth and feeding by *Fragilidium* cf. *mexicanum*, *Protoperidinium* cf. *divergens* and *P. crassipes* on their optimal dinoflagellate prey, *Lingulodinium polyedrum*. μ_{max}: maximum growth rate. I_{max}: maximum ingestion rate. PCMG: prey concentration at which maximum growth rate was achieved. PCMI: prey concentration at which maximum ingestion rate was achieved. C_{max}: maximum clearance rate. μ_{max} of *F.* cf. *mexicanum* is its maximum mixotrophic growth rate under a 12 h light:12 h dark cycle of illumination at 20 µE m^{-2} s^{-1}, but it could be phagotrophic growth because there was no phototrophic growth without added prey (see text). Data on feeding by *Protoperidinium* spp. were obtained from Jeong & Latz (1994); μ_{max}, I_{max}, and C_{max} are corrected to 22°C using Q_{10} = 2.8 (Hansen et al. 1997)

	F. cf. *mexicanum*	*P.* cf. *divergens*	*P. crassipes*
Nutritional mode	Mixotrophic	Heterotrophic	Heterotrophic
Feeding mechanism	Engulfment	Pallium feeding	Pallium feeding
Volume (µm^3)	84600	119000	204000
μ_{max} (d^{-1})	0.36	0.66	0.42
I_{max} (prey predator^{-1} d^{-1})	3.9	6.1	2.9
PCMG and PCMI (cells ml^{-1})	500	1100	700
C_{max} (µl predator^{-1} h^{-1})	6	0.9	0.6

Under the light and nutrient conditions provided in the present study, *F.* cf. *mexicanum* fed on *L. polyedrum* much like a heterotrophic dinoflagellate, regardless of the light intensity and nutrient concentration.

Grazing impact

While there have been several reports of intensive feeding by *Fragilidium* spp. on red-tide dinoflagellates in natural samples (Balech & Ferrando 1964, Eppley & Harrison 1975), it is presently impossible to estimate grazing impact of *Fragilidium* spp. on red-tide populations due to the lack of quantitative data on the abundance of *Fragilidium* spp. Therefore, we can only infer the relative grazing impact. The ingestion rate of *F.* cf. *mexicanum* feeding on *Lingulodinium polyedrum* was between those of *Protoperidinium* cf. *divergens* and *P. crassipes*. Therefore, if *F* cf. *mexicanum* is as abundant as *Protoperidinium* spp., it may have a grazing impact on the populations of *L. polyedrum* similar to that of *Protoperidnium* spp. *Fragilidium* spp. might have a much lower grazing impact on populations of *Gymnodinium sanguineum*, *Prorocentrum micans*, or *P. minimum* than on *L. polyedrum* and *Scrippsiella trochoidea*, and may have no impact on the populations of *Amphidinium carterae* or *Cochlodinium polykrikoides*. To better understand the grazing impact by *Fragilidium* spp. on red-tide dinoflagellates, we will need to measure the abundance of *Fragilidium* spp. and red-tide dinoflagellates in the field.

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