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Growth of *Favella* sp. (Ciliata: Tintinnina) and other microzooplankters in cages incubated *in situ* and comparison to growth *in vitro* *

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Abstract

Microplankton cages with porous polycarbonate membrane sides were used to investigate the population growth of *Favella* sp., a large tintinnid which preys on dinoflagellates, *Balanion* sp., a non-loricate ciliate which also preys on dinoflagellates, and two other tintinnids, *Eutintinnus pectinis* and *Tintinnopsis kofoidi,* at close to *in-situ* conditions in a small estuary during a spring dinoflagellate bloom. The effects of temperature and food concentration on the growth of *Favella* sp. and *Balanion* sp. were also investigated in culture. Growth rates in the field were variable from day to day. The highest net growth constant (base e) observed for *Favella* sp. in the cages was 0.032 (generation time 21.7 h). This was lower than growth constants which can be achieved in culture. Food availability, parasitism by the dinoflagellate *Duboscquella* sp., and perhaps life cycle events all contributed to the lower net growth rate of *Favella* sp. in the field. The highest net growth constant observed in the cages for *Balanion* sp. was 0.068 (generation time = 10.7 h), which is also lower than growth constants achieved in culture. The growth of *Balanion* sp. populations in the cages was limited by the availability of small-sized dinoflagellates and by predation. The highest net growth constants observed for *E. pectinis* and *T. kofoidi* were 0.030 and 0.068, respectively; we know little about the factors controlling the growth of these tintinnids.

Introduction

Determination of growth rates is a major problem in plankton ecology. Although planktonic protozoa can, at times, contribute significantly to secondary production (Heinbokel and Beers, 1979) few data are available on their in-situ growth rates (Coats and Heinbokel, 1982). We investigated the population dynamics of planktonic ciliates in microplankton cages (enclosures) with porous polycarbonate membrane sides. The membranes allow water exchange with the environment, thus reducing containment effects (Roman and Rublee, 1980), while retaining all but the smallest of the microplankton. Using the cages, net growth can be determined at close to *in-situ* conditions. In addition, species interactions can be observed and sometimes manipulated by excluding a certain size fraction of the larger plankton from the cages.

Our field experiments focused on the population dynamics of ciliates associated with estuarine dinoflagellate blooms. These ciliates include *Favella* sp., a large tintinnid (oral lorica diameter 65–75 μm), and *Balanion* (sp. nov., Coats and Small, in preparation) a non-loricate ciliate about $32 \times 34\ \mu$m in size. Laboratory studies have shown that *Favella* sp. requires dinoflagellates (Gold, 1970; Stoecker *et al.,* 1981) and preys selectively on this taxon in algal mixtures (Stoecker *et al.,* 1981). Two other tintinnids, *Eutintinnus pectinis* (Kofoid) (oral lorica diameter 17–23 μm) and *Tintinnopsis kofoidi* Hada (oral lorica diameter 40 μm), were abundant in some of the caging experiments and are included in the data analyses.

To aid in interpreting results of the field experiments, we determined the effects of temperature and dinoflagellate abundance on the growth rates of laboratory cultures of *Favella* sp. and *Balanion* sp. The dinoflagellate *Heterocapsa triquetra* (Ehrenberg) Stein ($16 \times 22\ \mu$m) was used as food in these experiments. It is one of the most common dinoflagellates in the estuaries we have studied and it was relatively abundant during 4 out of the 6 caging experiments. *H. triquetra* is often present in the digestive vacuoles of field collected *Favella* sp. and *Balanion* sp.

Materials and methods

Microplankton cages

The microplankton cages are made of polished Plexiglas with sides of 12-μm pore size polycarbonate membrane

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(Nuclepore Corp.) and contain a volume of approximately 417 ml (Fig. 1). This design was modified from phytoplankton cages described by Owens *et al.* (1977). The cages have no metal parts in order to avoid toxicity to the enclosed microplankton. The cages were soaked in seawater for several days to leach potential toxins from the materials. After each experiment, the cages with membranes attached were soaked for several hours and then rinsed in distilled water before re-use. Individual membranes were re-used up to three times.

The half-time for water exchange between the chamber and the environment was measured in a cage that had been suspended *in situ* for 24 h at a water temperature of 13 °C. At the end of this period, 6 mM Rhodamine B was added and 1-ml samples were taken periodically for 1 h. The dilution of the dye was determined from a standard curve based on fluorometer readings. The half-time for exchange was 120 min.

The ability of the cages to retain moderate-size dinoflagellates and naked ciliates was tested using the dinoflagellate *Heterocapsa triquetra* (Ehrenberg) Stein (Strain HT984) and *Balanion* sp. (Strain CILY II). Diluted cultures of *H. triquetra* and *Balanion* sp. were added to each cage while the cage was partially submerged in filtered (0.45 μm) seawater. The cage was then lifted and water allowed to seep through the membranes. The water seeping through the membranes was fixed with acid Lugol's solution. The inverted microscope technique (Utermohl, 1931; Hasle, 1978) was used to count both ciliates and dinoflagellates. The percentage of the cells of each type which leaked through the cage was determined. The cages quantitatively retained *H. triquetra* (no leakage) and *Balanion* sp. (0.01% leakage).

In-situ experiments

We did microplankton caging experiments on calm days during the spring of 1982 when *Favella* sp. was abundant in Perch Pond, Falmouth, Massachusetts, USA. All sampling and incubations were done at a station marked by a buoy in 4 m of water. The depth of suitable *Favella* sp. concentrations was located by an initial sampling at mid day. One-liter samples were collected using a Mityvac hand vacuum pump (Neward Enterprises) and Teflon tubing from 0, 1, 2, 3, and 4 m depths. Temperatures were measured immediately, and aliquots were saved for salinity determination. 250-ml sub-samples were concentrated on a 41-μm Nitex mesh and examined under a dissecting microscope for *Favella* sp.

When *Favella* sp. was present at concentrations greater than about 200 individuals l^{-1}, we did a caging experiment. Six liters were collected with the pump sampler from the depth judged to have the highest *Favella* sp. concentration. Subsamples (417 ml) were either filtered through a 41-μm Nitex mesh to remove *Favella* sp. and the larger microzooplankton or not filtered. At time zero, duplicate filtered and unfiltered subsamples were preserved

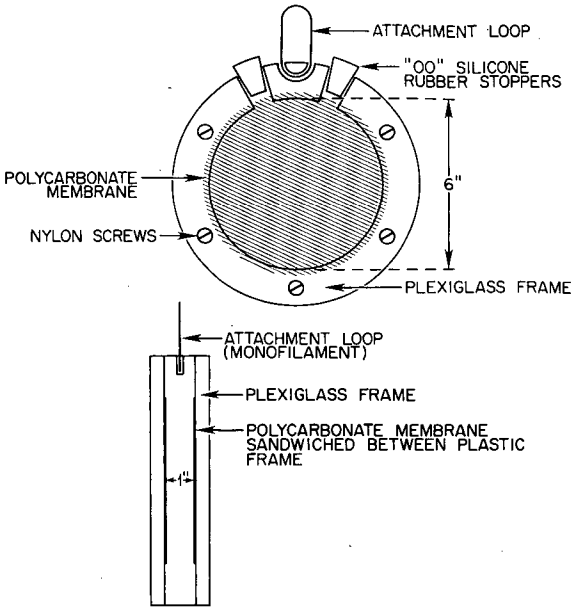

Fig. 1. Microzooplankton cage

with 2% formaldehyde for later counts of ciliates and food. Two or three cages were filled with filtered aliquots of the sample and two or three cages with unfiltered aliquots. Because the 41-μm meshes sometimes clogged, thereby retaining dinoflagellates, we eventually filtered through a 64-μm mesh. The cages were suspended on 60 pound test monofilament line from poles supported by buoys. The depth at which the cages were hung was approximately the same as the depth from which the sample was collected. The time between collection of the sample and suspension of the cages was on the order of 30 min. After 24-h incubation, the cages were retrieved and their contents immediately fixed in 2–4% formaldehyde.

The volume of the cage samples was measured to correct for the decrease in sample volume as the cages were lifted. Any large zooplankton present in the samples were enumerated. Microplankton in 25- or 100-ml aliquots of the time 0 and 24 hour caged samples were then counted. The abundant dinoflagellates were enumerated by species. The less common dinoflagellates were not identified to species, but were enumerated.

Laboratory experiments

In the laboratory we investigated the influences of temperature and food concentration on the growth rates of *Favella* sp. (Strain P081) and *Balanion* sp. (Strain CILY II). The dinoflagellate *Heterocapsa triquetra* (clone A984) was used as food. Both of the ciliates and the dinoflagellate were originally isolated from Perch Pond, Falmouth, Massachusetts, USA.

The alga was grown in enriched seawater medium f/2 (Guillard, 1975), with silicic acid omitted, on a 14hL:10hD cycle under 291 μ Ein m^{-2} s^{-1} of cool white (Sylvania Co.) flourescent light at 20 °C. *Favella* sp. was cultured as previously described (Stoecker *et al.*, 1981) except that

aged, diluted Sargasso seawater (5 parts seawater to 1 part distilled water) was used to make the medium SWT. *Heterocapsa triquetra* was the sole algal food, and cultures were maintained at 15°–17 °C. Culture methods for *Balanion* sp. were similar to those for *Favella* sp.

One experiment with *Favella* sp. and two experiments with *Balanion* sp. were done to determine the effect of temperature on growth rate (Table 5). To determine the effect of food density on growth rate, two experiments were done using *Favella* sp. and one experiment was done using *Balanion* sp. (Tables 6 and 7, respectively).

The ciliates were grown in 100–150 ml of medium (SWT) in 250-ml polycarbonate flasks for all but one experiment. This exception was a food density experiment using *Favella* sp. (Table 6; experiment no. 2) in which the ciliates were grown in 600 ml of medium in 1-l flasks. Initial inocula were 1–2 *Favella* sp. or *Balanion* sp. ml^{-1}. In the experiments determining temperature effects (Table 5), the flasks were incubated at 7°, 10°, 15°, or 20 °C in dim light on a 14 h L:10 h D cycle. In the experiments testing food density effects (Table 6 and 7), the flasks were incubated at 15 °C in the dark to minimize dinoflagellate growth.

To allow the cultures to acclimate to the experimental conditions, the first sampling was done 24 h after inoculation. Subsequent samples were taken every 24 h in the *Favella* sp. experiments and every 8–16 h in the *Balanion* sp. experiments. Each flask was sampled 3–4 times. For *Balanion* sp., the volume sampled was 10 ml; for *Favella* sp., the volume sampled was 25 ml.

Ciliate growth was exponential between the first and second samplings in all experiments, and changes in ciliate number for this interval were used for the calculation of growth rates. Algal numbers changed during the incubations. The range of food densities for the samples used in the calculation of ciliate growth rates are given for each treatment (Tables 5–7).

Calculation of growth constants

The net growth constant, K_e, for field and laboratory experiments was calculated as:

$$K_e = \frac{\ln N_1 - \ln N_0}{t_1 - t_0},$$

where the subscripts "0" and "1" denote values at the beginning and end of the incubation, respectively, of the concentration of cells (N) and elapsed time in hours (t). K_e is equivalent to 'μ" (Stanier *et al.*, 1970) or "r" (Wilson and Bossert, 1971).

Generation time, in hours, was calculated as:

$$G = \frac{\ln 2}{K_e}.$$

Calculation of dinoflagellate cell volumes

In order to compare the results of the food density experiments with earlier work done on optimal food densities for *Favella* sp. (Stoecker *et al.*, 1981) and with the results of the *in-situ* incubations, approximate cell volumes were determined for a number of dinoflagellates. For cultures of *Heterocapsa triquetra* (Strain A984), *H. pygmaea* Loeblich III (Strain Gymno), *Gonyaulax tamarensis* Lebour (Strain GT429), *G. polyedra* F. Stein (Strain GP60e), and *Scrippsiella trochoidea* (Stein) Loeblich III (Strain Peri), average cell volumes were determined from Coulter counter (Model TAII) data as described by Harbison and McAlister (1980). Because we did not have a culture of *G. spinifera* (Clap. et Lachm.) Diesing, the approximate cell volume was calculated from microscopic measurements of cells in plankton samples. An ellipsoid shape was assumed for this species.

The approximate cell volumes are: *Heterocapsa pygmaea*, 467 μm^3; *H. triquetra*, 1 595 μm^3; *Gonyaulax tamarensis* 17 157 μm^3; *G. polyedra*, 20 580 μm^3; *G. spinifera* 14 074 μm^3; *Scrippsiella trochoidea* 8 579 μm^3.

Results

In-situ microplankton caging experiments

Seven caging experiments were done in Perch Pond between May 18 and June 3, 1982. Water temperature at the times and depths of the incubations ranged from 15° to 21 °C and salinities from 26.70 to 28.56‰ S (Table 1). Some samples were lost when membranes tore. The success rate exceeded 80%, except when the membranes were used more than twice.

Initial *Favella* sp. densities at the depth where the tintinnid was most numerous, and where the cages were deployed, ranged from 386 to 7 409 individuals l^{-1} (Table 2). In five of the experiments there was a net increase in *Favella* sp., in two experiments *Favella* sp. declined (Table 2). We did not find organisms judged capable of preying on *Favella* sp. in the cages, but some *Favella* sp. were parasitized by the dinoflagellate *Duboscquella* sp. Chatton (Fig. 2). The *Favella* sp. population of May 27, 82 was heavily parasitized by *Duboscquella* sp., which may partially explain the decline of *Favella* sp. in this experiment (Table 2). During some caging experiments (Table 2), conjugating pairs (Fig. 3) or cysts of *Favella* sp. (Fig. 4) were present.

Table 1. Caging experiments: physical-chemical parameters, Perch Pond, Spring, 1982

Date	Depth (m)	Temperature (°C)	Salinity (‰)
May-18	2	17.5	No data
May-19	2	18.5	26.70
May-24	1	15.3	26.90
May-25	1.5	16.0	27.44
May-27	2	19.0	28.22
May-31	1	18.5	28.57
June-3	3	21.0	27.76

Table 2. *Favella* sp. populations. Caging experiments, Perch Pond, Spring, 1982

Date[a]	*Favella* sp. (l^{-1}) + SD[b] (N)[c]		Percent of population						Net[d] K_e
			Conjugating		Parasitized		Cysts		
	t=0	t=24	t=0	t=24	t=0	t=24	t=0	t=24	
May-18	1 867 ± 180 (2)	4 048 ± 521 (2)	0	0	0	0	0	0	0.032
May-19	7 409 ± 479 (2)	9 038 (1)	0	3	0	1	0	0	0.008
May-24	4 507 ± 21 (2)	3 383 ± 813 (3)	0	0	2	7	2	5	−0.012
May-25	831 ± 198 (2)	1 238 ± 94 (3)	0	0	6	9	1	20	0.017
May-27	386 ± 31 (2)	57 ± 18 (2)	0	0	31	27	3	9	−0.080
May-31	727 ± 182 (2)	1 485 ± 139 (2)	0	0	4	5	0	0	0.030
June-3	1 270 ± 150 (2)	2 112 ± 5 (2)	0	1	6	2	0	2	0.021

[a] Cages set
[b] Standard deviation
[c] N = number of replicates
[d] Growth constant

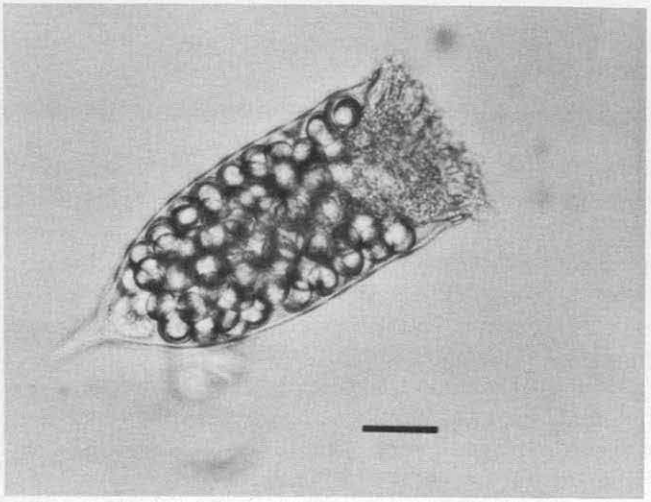

Fig. 2. *Favella* sp. infected by the dinoflagellate parasite *Duboscquella* sp. Scale bar = 30 μm

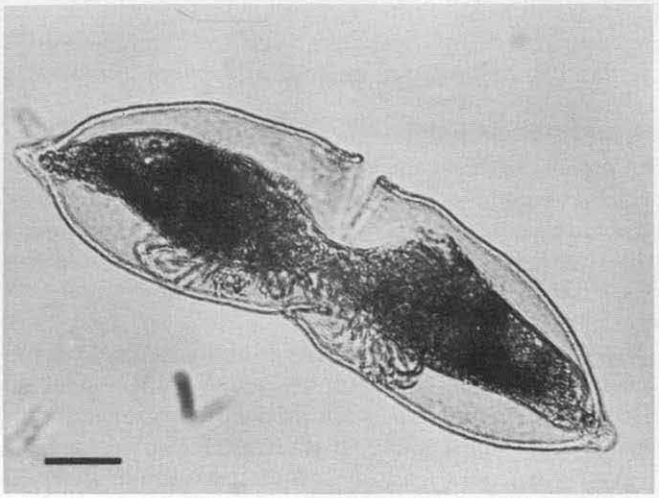

Fig. 3. Conjugating *Favella* sp. Scale bar = 30 μm

In the cages with *Favella* sp. (unfiltered samples), initial dinoflagellate densities ranged from 39 to 212 ml^{-1} (Table 3). On May 18 and May 19, 82, *Gonyaulax spinifera* was the dominant dinoflagellate, on the other dates *Heterocapsa triquetra* and *Scrippsiella trochoidea* were dominant. All three of these dinoflagellate species could be seen in digestive vacuoles of *Favella* sp. Total dinoflagellate cell volume, excluding very large dinoflagellates, such as *Dinophysis* sp., which are not eaten by *Favella* sp., was estimated from densities and average cell volumes. On the first caging date, the total dinoflagellate cell volume was initially relatively high, approximately 2.0×10^6 μm^3 ml^{-1}, but was lower on subsequent dates (Table 3). Dinoflagellate numbers decreased in both unfiltered and filtered treatments during all *in-situ* incubations, but the decreases were greater in the unfiltered than in the filtered treatments. In those experiments where the 41-μm mesh clogged, thereby reducing the initial dinoflagellate densities below ambient concentrations, the cage samples from

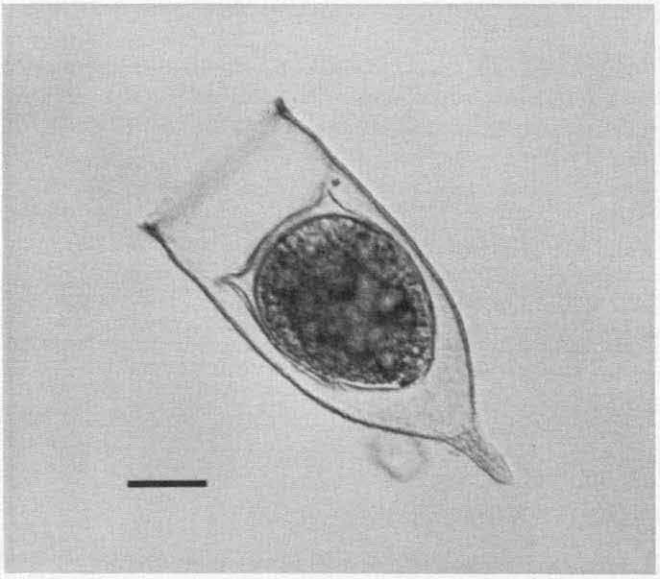

Fig. 4. Cyst of *Favella* sp. Scale bar = 30 μm

Table 3. Dinoflagellate populations. Caging experiments, Perch Pond, Spring, 1982

Date[b]	Treatment[c]	N[d]	Density (ml^{-1} + SD)[a]								Estimated total cell volume[f] (μm^3 ml^{-1})	
			Heterocapsa triquetra[e]		*Scrippsiella trochoidea*[e]		*Gonyaulax spinifera*[e]		Total dinoflagellates[f]			
			t=0	t=24	t=0	t=24	t=0	t=24	t=0	t=24	t=0	t=24
May-18	UF	2	–	–	–	–	100± 5	22±2	212± 10	74±21	2.0×10^6	1.3×10^6
	41	1	–	–	–	–	89±12	55	180± 1	152		
May-19	UF	1	–	–	–	–	63± 4	1	102±112	18	0.6×10^6	0.4×10^6
May-24	UF	3	47±10	4± 1	14± 4	3±1			73± 2	10± 3	0.3×10^6	0.05×10^6
May-25	UF	3	25± 3	10± 2	6± 1	4±2			39± 1	20± 4	0.2×10^6	0.09×10^6
May-27	UF	2	19± 3	7± 1	16± 4	5±1			39± 1	17± 1	0.2×10^6	0.09×10^6
	41	3	18± 1	5± 2	12± 2	2±1			34± 2	10± 4		
May-31	UF	2	21± 5	4± 1	26± 2	8±1			74± 19	36± 5	0.4×10^6	0.1×10^6
	41	3	26± 1	17± 4	38±13	12±7			92± 25	57±12		
June-3	UF	2	104±15	11± 2	36±10	7±3			161± 23	30± 0	0.5×10^6	0.08×10^6
	64	3	95±21	24±10	38± 4	16±3			148± 20	61±11		

[a] SD = Standard deviation
[b] Cages deployed
[c] U.F. = unfiltered
41 = 41-μm mesh filtered
64 = 64-μm mesh filtered
[d] N = no. replicates, t = 24 h (for t = 0, N = 2)
[e] Densities for species shown only when initial densities > 10 ml^{-1}
[f] Includes dinoflagellates no greater than 20 000 μm^3 in volume

Table 4. Populations of abundant ciliates (except *Favella* sp.). Caging experiments. Perch Pond, Spring, 1982

Date[b]	Treatment[c]	N	Species	Density (no. l^{-1} ± SD)[a]		Net K_e
				t=0	t=24	
May-18	UF	2	*Eutintinnus pectinis*	11 015±1 379	14 983±2 244	0.0128
	41	1	*Eutintinnus pectinis*	6 678± 764	11 240	0.0217
May-19	UF	1	*Eutintinnus pectinis*	9 285±1 153	9 677	0.0017
May-24	UF	3	*Eutintinnus pectinis*	3 517± 762 (2% parasitized)	2 619±1 676 (3% parasitized)	−0.0123
May-25	UF	3	*Eutintinnus pectinis*	4 585± 749 (3% parasitized)	9 448± 630 (2% parasitized)	0.0301
May-27	UF	2	*Eutintinnus pectinis*	4 550± 55 (11% parasitized)	5 371±1 813 (2% parasitized)	0.0069
	41	3	*Eutintinnus pectinis*	1 603± 558 (6% parasitized)	2 779± 33 (1% parasitized)	0.0229
	UF	2	*Balanion* sp.	308± 155	219± 33	−0.0142
	41	3	*Balanion* sp.	426± 32	120± 49	−0.0528
May-31	UF	2	*Eutintinnus pectinis*	10 409±1 996	9 063± 346	−0.0058
	41	3	*Eutintinnus pectinis*	5 075± 951	10 228±1 924	0.0292
	UF	2	*Balanion* sp.	523± 288	418± 122	−0.0093
	41	3	*Balanion* sp.	326± 173	262± 68	−0.0091
	UF	2	*Tintinnopsis kofoidi*	7 303± 477	4 666± 327	−0.0187
	41	3	*Tintinnopsis kofoidi*	122± 57	657± 251	0.0682
June-3	UF	2	*Balanion* sp.	282± 108	1 281± 101	0.0631
	64	3	*Balanion* sp.	286± 5	1 472± 484	0.0683

[a] SD = Standard deviation
[b] Cages set
[c] UF = unfiltered; 41 = 41-μm mesh filtered; 64 = 64 μm mesh filtered

the filtered treatments were discarded. Other smaller algae besides dinoflagellates were present but were not enumerated.

In addition to *Favella* sp., other microzooplankton initially present were rotifers, including *Synchaeta* spp., polychaete larvae, copepod larvae, smaller tintinnids, and aloricate ciliates. Except for the large *Synchaeta* spp., these survived in the cages. The ciliates greater than 30-μm diameter were enumerated when they were abundant (Table 4).

Eutintinnus pectinis (Kofoid) was abundant on the first six dates with initial densities as high as 11 000 l^{-1} (Table 4). Growth was usually higher for this species in the filtered than in the unfiltered treatments where numbers sometimes declined during incubation (Table 4). *E. pectinis* was parasitized by *Duboscquella* sp. in the May 24, May 25, and May 27, 82 experiments.

The tintinnid *Tintinnopsis kofoidi* Hada was abundant only on May 31, 82. It increased in the filtered treatments and decreased in the unfiltered treatments (Table 4).

The aloricate ciliate *Balanion* sp. was present, but not initially abundant, on May 27, May 31, and June 3, 82. On the first two dates, it decreased in both the filtered and unfiltered treatments but on June 3, 82 it increased in both treatments (Table 4).

Laboratory experiments

The growth rate of *Favella* sp. and *Balanion* sp. was determined in culture at temperatures from 7° to 20 °C (Table 5). *Favella* sp. grew faster at 20 °C, with a net K_e of 0.058 (generation time of 12 h), than at 15° or 7 °C. At 15°–20 °C *Balanion* sp. had a higher net K_e, around 0.1 (generation time of 5–6 h) than did *Favella* sp. The growth rate of *Balanion* sp. appeared to be less sensitive than that of *Favella* sp. to decreases in temperature.

The growth rates of *Favella* sp. and *Balanion* sp. at varying densities of *Heterocapsa triquetra* were determined at 15 °C (Table 6). In experiment no. 1 with *Favella* sp., growth was fastest at 1 000–1 270 *H. triquetra* cells ml^{-1} with inhibition occurring at food densities above 3 000 cells ml^{-1}. In the second experiment, *Favella* sp. grew fastest at *H. triquetra* densities between 406 and 1 000 cells ml^{-1}. The net growth constants were comparable to those obtained in the 15 °C temperature treatment.

Balanion sp. grew fastest at the highest *Heterocapsa triquetra* density tested, 2 000 cells ml^{-1} (Table 7). In the temperature experiment at 15 °C even higher food densi-

Table 5. *Favella* sp. and *Balanion* sp. Effect of temperature on the growth (in culture)

Temperature (°C)	$K_e \pm$ (Mean + SE)[a]		
	Favella[b]	*Balanion* sp.	
		Exp. 1[c]	Exp. 2[d]
7	0.016 ± 0.001	0.064 ± 0.008	
10	–	0.058 ± 0.004	
15	0.041 ± 0.003	0.099 ± 0.008	0.102 ± 0.011
20	0.058 ± 0.007	0.120 ± 0.001	0.101 ± 0.012

[a] Mean (± standard deviation) of individual growth constants determined for 3 replicate flasks
[b] Growth constants determined for a 24-h interval; food densities during this interval ranged from 1 704 to 2 019 *Heterocapsa triquetra* cells ml^{-1}
[c] Growth constants determined for a 9-h interval; food densities during this interval ranged from 6 795 to 10 245 *H. triquetra* cells ml^{-1}
[d] Growth constants determined for a 9-h interval; food densities during this interval ranged from 6 526 to 8 713 *H. triquetra* cells ml^{-1}

Table 6. *Favella* sp. Effect of algal food concentration on the growth (in culture) at 15 °C

Range of food concentrations[a] (*Heterocapsa triquetra* cells ml^{-1})	K_e (Mean ± SD)[b]
Experiment no. 1	
48– 100	0.022 ± 0.007
116– 250	0.025 ± 0.012
420– 500	0.029 ± 0.005
750– 924	0.033 ± 0.005
1 000–1 270	0.042 ± 0.001
2 000–3 754	0.023 ± 0.006
3 000–5 670	0.023 ± 0.001
Experiment no. 2	
44– 50	0.028
77– 100	0.033
191– 250	0.030
406– 500	0.047
855–1 000	0.047
1 720–2 000	0.044
2 700–3 000	0.034

[a] Range during interval for which growth constants were calculated
[b] Experiment no. 1: Mean (± standard deviation) of individual growth constants determined in duplicate flasks for a 24-h interval
Experiment no. 2. 1 flask per treatment; duplicate subsamples. Growth constants determined for a 24-h interval using average of the duplicate counts

Table 7. *Balanion* sp. Effect of algal food concentration on the growth (in culture) at 15 °C

Range of food concentrations (*H. triquetra* cells ml^{-1})[a]	K_e (Mean ± SD)[b]
25– 75	0.054 ± 0.011
73– 209	0.066 ± 0.003
138– 429	0.074 ± 0.013
359– 679	0.084 ± 0.012
520– 882	0.085 ± 0.008
1 172–1 927	0.091 ± 0.003
1 921–2 151	0.100 ± 0.008

[a] Range during interval for which growth constants calculated
[b] Mean (± standard deviation) of individual growth constants determined for duplicate flasks for a 24-h interval

ties, 6 526 to 10 245 cells ml^{-1} (Table 5), had been used without decreasing the net K_e. Growth was not inhibited by high food concentrations.

Discussion

Microplankton populations in Perch Pond changed during the 17-d experimental period in late May and early June although dinoflagellates predominated throughout this time. The net growth rate of *Favella* sp. (Table 2) and of other abundant ciliates (Table 4) varied among the seven experiments. On some dates rapid growth occurred, whereas on other dates populations of a given species declined.

In comparing the growth of *Favella* sp. and *Balanion* sp. in the *in-situ* incubations to their growth in culture it is important to appreciate that the K_e's are not strictly equivalent. It was not feasible to make counts except at the beginning and end of the *in-situ* incubations and thus we did not establish that growth was in fact exponential, which is an assumption implicit in using logistic equations. However, comparison of field and laboratory growth rates is a useful, although imperfect, tool for evaluating the role, in controlling the dynamcis of ciliate populations, of factors which may influence cell division rates, such as temperature, salinity, food availability and life cycle events and the role of factors which cause mortality, such as predation among the microplankton, parasitism, and disturbance. In the following discussion we assume that the differences between field and laboratory results, which cannot be explained by physical-chemical parameters or by food availability, are due to mortality factors present in the field but not in the laboratory experiments (predation, parasitism, and disturbance) or to life cycle events.

Temperature was probably not an important factor in the fluctuations in ciliate net growth. Water temperature varied from 15.3° to 21 °C during the 17-d study period. However, *Favella* sp., which had been isolated from this bloom, grew well in culture at 15° and 20 °C with net growth constants (base e) of 0.041 and 0.058 respectively (Table 5). In the field, within the same temperature range, net growth constants ranged from −0.080 to 0.032 (Table 2). Salinity was probably not responsible for the lower net growth rates observed in the field because in the laboratory, *Favella* sp. grows well at salinities from 27 to 32‰ s (Trowbridge and Stoecker, unpublished data).

We suspect food availability was the major factor controlling the growth rate of *Favella* in the *in-situ* incubations. This tintinnid is a selective predator on dinoflagellates, which it requires as food (Stoecker *et al.*, 1981). In laboratory experiments, the fastest growth rates for *Favella* sp. were achieved when *Heterocapsa triquetra* densities were about 1 000 cells ml^{-1} (Table 6). Because cell size is an important factor, these results cannot be directly related to the field situation where a mixture of dinoflagellate species was present (Table 3). For *Favella* sp. optimum dinoflagellate density is negatively correlated ($r = 0.96984$; $P < 0.01$) with cell volume (Fig. 5; data from Stoecker *et al.*, 1981). From these data the optimum food volume for *Favella* sp. is estimated to be from 2×10^6 to 5×10^6 μm^3 ml^{-1}. The optimum volume, with *H. triquetra* as food, is 0.6 to 2.0×10^6 μm^3 ml^{-1} (Table 6). The two estimates overlap but the first estimate, based on five dinoflagellate foods, is higher than the second based on the experiments with *H. triquetra*. This discrepancy may be due to variations in the average cell size among dinoflagellate cultures of a species, variations in the response of *Favella* sp. to different dinoflagellate species, and variations in the physiological state of *Favella* sp. among experiments.

The volume of suitably-sized dinoflagellate at the start and at the end of each caging experiment was calculated (Table 3). Dinoflagellate volume was suboptimal for *Favella* sp. (less than 0.6×10^6 μm^3 ml^{-1}) during six of the seven experiments, but this alone does not appear to explain the low net growth rate of *Favella* sp. in two of the field experiments (Fig. 6). In the five caging experiments in which the net growth constants for *Favella* sp. were positive, the field growth constants are roughly comparable with laboratory results at similar food volumes.

All suitably-sized dinoflagellates may not be equally good foods on a per volume basis. Macrozooplankton are known to avoid dense concentrations of the dinoflagellates *Gymnodium flavum* Kofoid and Swezy (Huntley, 1982) and *G. splendens* Lebour (Fiedler, 1982). Stoecker *et al.*, (1981) showed that *Amphidinium carterae* Hulburt and *Prorocentrum marielebouriae* (Parke and Ballantine) Loeblich III are poor foods for *Favella* sp. The presence of the flagellate *Olisthodiscus luteus* Carter is known to cause reductions in the growth rates of tintinnids (Verity and Stoecker, 1982). None of the above algae were present during the caging experiments.

Other factors, perhaps more important, are the changes in dinoflagellate abundance and species composition that occurred in the cages during the incubations (Table 3).

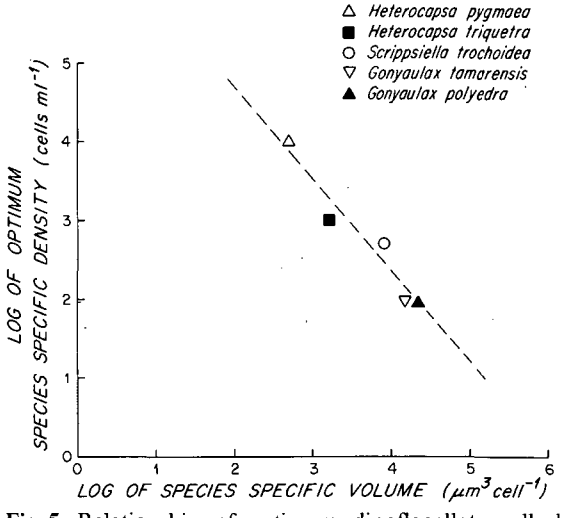

Fig. 5. Relationship of optimum dinoflagellate cell density as food for *Favella* sp. with the cell volume of 5 dinoflagellate species ($r = -0.9694$; $P < 0.01$). Data from Stoecker *et al.* (1981)

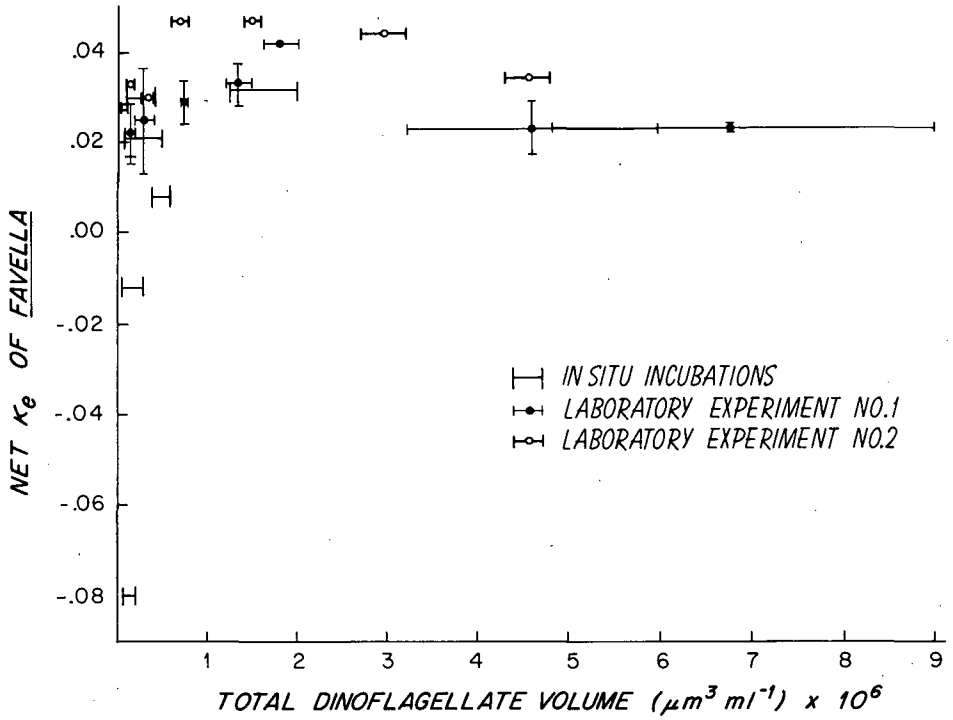

Fig. 6. *Favella* sp. Net growth constants (K_e) as a function of food availability (dinoflagellate μm^3 ml^{-1}) *in-situ* and in culture. Vertical bars are standard deviations for the mean net growth constants. Horizontal bars are the estimated range of food availability

Dinoflagellate density decreased during the *in-situ* incubations; the decreases were usually greater in the unfiltered than in the filtered treatments (Table 3). Grazing by *Favella* sp. and other large microzooplankters may account for most of the decreases in the unfiltered treatments. In the filtered treatments, grazing by microzooplankton can partially account for the decreases in dinoflagellate densities as some *Favella* sp. passed through the nitex mesh and *Balanion* sp. was not removed by the mesh. Physical manipulation may have injured dinoflagellate cells in both unfiltered and filtered treatments and thus have caused algal population declines as well.

Favella sp. can grow fast even in the absence of high dinoflagellate densities when alternate prey are available. In an earlier caging experiment done in June, 1981 in Salt Pond, Eastham, Massachusetts, USA, we observed a high growth rate for *Favella* sp. ($K_e = 0.051$), at relatively low dinoflagellate (mostly *Heterocapsa triquetra*) densities (67 ml^{-1}) but at high densities of smaller ciliates. During this experiment *Favella* sp. preyed on other ciliates, including *Balanion* sp., as well as on dinoflagellates; laboratory experiments have confirmed these observations of carnivory (Stoecker and Evans, submitted).

Two related factors, which probably influence division rate in *Favella* sp., are mating and cyst formation, both of which occurred during the caging experiments but not during the laboratory experiments. Typically, these phenomena occur near the end of *Favella* sp. blooms as well as in old laboratory cultures. Little is known about the effects of sexuality on tintinnid population dynamics but Coats and Heinbokel (1982) found that sexuality could induce phased division in *Eutintinnus pectinis*. Mating may be necessary for the continued viability of tintinnids (Gold, 1970, 1971). Cyst formation in tintinnids is assumed to be a mechanism for repopulation after unfavorable conditions (Paranjape, 1980) and may be particularly important for *Favella* sp., which depends on seasonally abundant dinoflagellates as its primary food. In culture, cyst formation is usually associated with a decline in growth rate (Stoecker, unpublished data). Thus the physiological state of *Favella* sp. as well as immediate food availability may have caused a reduction in growth rate.

Mortality due to parasitism by *Duboscquella* sp. (Fig. 2) may have been an important factor in determining net growth rates. This dinoflagellate is a specific parasite of tintinnids (Chatton, 1920; Hofke, 1931; Cachon, 1964), and we have previously observed outbreaks of it near the end of *Favella* sp. blooms. The significance of *Duboscquella* sp. as a mortality factor is difficult to evaluate. The parasite appears to become abundant when *Favella* sp. populations are stressed, for example by low food concentrations.

Another potential mortality factor in the *in-situ* incubations was injury due to physical manipulation. Pumping, filteration, and filling of the cages may have been injurous to some phytoplankton and microzooplankton species (Verity, personal communication).

The naked ciliate *Balanion* sp. increased in the cages only during the June 3, 82 experiment although it was present at the beginning of three *in-situ* experiments (Table 4). *Balanion* sp. associated with *Heterocapsa triquetra* blooms and grows well with this dinoflagellate as its sole algal food (Stoecker and Evans, submitted); *Gonyaulax spinifera* and *Scrippsiella trochoidea* are probably too large to be suitable food items for *Balanion* sp. (Stoecker, unpublished data). Until June 3, 82, *H. triquetra* densities

were probably too low to support the growth of *Balanion* sp. (Table 3). In this last caging experiment, *Balanion* sp. had an average net growth constant of 0.065, (generation time = 10.7 h), a growth rate comparable to that observed in culture at similar *H. triquetra* densities (Table 7). Temperature probably was not an important factor limiting the growth of *Balanion* sp., as the *Balanion* sp. strain (CILY II) isolated from this bloom grows as well or better at 20° than at 15 °C. However, another *Balanion* sp. strain, (CILY I), which was isolated during a winter *H. triquetra* bloom, when the water temperature was 4 °C, grew at 15° but not at 20 °C (Stoecker, unpublished data). *Balanion* sp. grows well at salinities ranging from 18 to 32‰ s (Trowbridge and Stoecker, unpublished data), therefore salinity did not limit its growth in the field experiments. Because *Favella* sp. preys on *Balanion* sp. and can limit its populations (Stoecker and Evans, submitted), *Favella* sp. density must also have affected the net growth rate of *Balanion* sp.

The results of the caging experiments for the other abundant tintinnids, *Eutintinnus pectinis* and *Tintinnopsis kofoidi* (Table 4), are difficult to interpret because we know less about their growth requirements and susceptibility to predation by other microzooplankton. *E. pectinis* grows well when cultured with mixtures of small haptophytes and prasinophytes as food (Heinbokel, 1978; Stoecker, unpublished data) and thus appears to be adapted to different algal foods than *Favella* sp. Heinbokel (1978) found that at optimum food densities, *E. pectinis* achieved a growth rate equivalent to 0.05 to 0.06 K_e and thus can grow faster than we observed in the caging experiments (Table 4). *E. pectinis,* like *Favella* sp., was parasitized by *Dubosquella* sp. (Table 4), which may have limited its net growth. We have not succeeded in culturing *T. kofoidi* and know even less about its growth requirements.

Robertson (1983) found that the larger, more southern *Favella* sp., *F. panamensis,* preys on a smaller tintinnid, *Tintinnopsis tubulosa.* In our microscopic observations, we did not observe that other tintinnids were ingested by *Favella* sp., but it is possible this happens. The higher net growth rates of the two smaller tintinnid species in the filtered compared with the unfiltered treatments (Table 4) suggests that predation or competition by larger forms may limit their population growth.

We believe that, under certain circumstances, caging can be a useful tool for studying microzooplankton populations at close to *in-situ* conditions: unconcentrated microplankton populations can be enclosed in cages allowing water exchange with the environment. However, enclosure, itself, alters the environment for plankton populations, which may explain some of the changes we observed between the beginning and end of the incubations. At the end of our incubations, caged ciliate populations had usually increased. This was probably due in part to the absence of copepod predators (Robertson, 1983; Turner and Anderson, submitted; Sanders and Stoecker, submitted). Enclosure, by definition, prevents sinking of particles and migration of the enclosed populations. For example, heavily parasitized tintinnids, which swim poorly, could not sink out of the experiment and this may have increased the infection rate. Most importantly, the cages prevent migration, which must influence the microdistribution of both the microzooplankton and their food throughout the diurnal cycle (Stoecker and Anderson, in preparation). Because we did the experiments with water from the depths of maximum *Favella* sp. abundance and because the cages restricted dinoflagellate migration, the algae were kept in contact with high populations of grazers for 24 h. This may have been responsible for the decreases in food availability during the incubation. Microdistribution also has an important influence on the feeding rate of *Favella* (Stoecker and Guillard, 1982), which should affect growth rates.

Microplankton caging experiments such as those described herein can probably be done only in eutrophic waters where population densities are sufficiently high so that small cages are useful. Further, the cage membranes are delicate so cages cannot be used in rough water. Data on growth requirements from controlled laboratory studies are necessary for interpreting cage experiments. When such information is available, caging can be useful in the study of microplankton population dynamics.

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