室内飼育実験で観察された繊毛虫Eutintinnus lususundaeとFavella taraikaensisの成長速度

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Growth Rates of Ciliate *Eutintinnus lususundae* and *Favella taraikaensis* Observed in the Laboratory Culture Experiments[1),2)]

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Abstract

Tintinnid ciliate *Eutintinnus lususundae* and *Favella taraikaensis* were cultured by providing a mixture of *Isochrysis galbana*, *Prorocentrum minimum* and *Rhodomonas* sp. for supporting their growth. In the culture experiments, *F. taraikaensis* was observed to have a phenotype, *coxliella* form, as previously discovered in the congeneric species *F. ehrenbergii*. *F. taraikaensis* revealed the conjugational capability between *favella* form and *coxliella* form. Mean generation times during the log phase were 17.8-21.2 h for *E. lususundae* and 17.6-28.4 for *F. taraikaensis*. The minimum generation times for the respective species were 6.6-13.5 h and 10.0-26.9 h. It is likely that the interspecific competition between these two species occurs at the density over 10-20 indiv·ml^{-1}.

Tintinnid ciliates are commonly distributed over cold and warm, and neritic and oceanic water masses of the world oceans. Some modern planktologists emphasize the importance of so-called microzooplankton in marine ecosystems because of their remarkably high metabolic and growth rates as well as large standing biomass. Effective nutrient regenerator, energy transferrer from nanoplankton to net zooplankton and regulator of the nanoplankton abundance are considered as their peculiar roles in nature. One of the representative groups of such microzooplankton is the tintinnids (e.g., JOHANNES 1964, BEERS & STEWART 1967, 1971, HEINBOKEL 1978, CAPRIULO & CARPENTER 1980, HARGRAVES 1981, RASSOULZADEGAN & ETIENNE 1981, PAASCHE & KRISTIANSEN 1982).

We have observed rapid growth ability of two tintinnids, *Eutintinnus lususundae* KOFOID & CAMPBELL and *Favella taraikaensis* HADA, which are common in Japanese coastal waters, by the laboratory culture experiments. Striking morphological variation of the latter species was also observed in producing loricae of both *favella* form and *coxliella* form as found in a congeneric species *F. ehrenbergii* by LAVAL-PEUTO (1977, 1981). Prior to these experiments the availability of some unicellular algal species for tintinnid growth was preliminarily determined.

Materials and Methods

Preliminary Experiments

Healthy specimens of *E. lususundae* and *F. taraikaensis* were sorted from the fresh plankton

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samples collected with a fine net (40 μm in mesh size) in Sendai Harbor on the coast of the northeastern Japan. In preliminary cultures each bunch of ten specimens was inoculated into 10 or 30 ml sterilized filtered water of the Harbor and provided with single or mixed suspension of algal species at an order of 10^4 cells·ml^{-1}. The cultures were carried out in test tubes or conical beakers and gently stirred on a shaker. The temperature was adjusted to that of the harbor water at respective sampling times. To determine the availability of the algal species for growth, tintinnid density in the culture vessels was monitored with a magnifying lens at proper intervals for a few weeks. The algae which had supported the tintinnid growth were then employed in the following growth experiments.

Growth Experiments

Growth experiments were conducted in 2 series. The first consisted of 3 successive cultures of single species of *F. taraikaensis* (Exps. 1A–C). The second consisted of 2 successive mixed cultures of *F. taraikaensis* and *E. lususundae* (Exps. 2A–B). Each culture was run in large volume of filtered water (500–600 ml) in a 1 liter conical flask, and gently airated to supply oxygen and to stir the media. The air was prefiltered through a membrane filter of 0.45 μm in pore size.

Exp. 1A originated from 32 natural specimens of *favella* form of *F. taraikaensis* collected on June 20, 1981. After increase of tintinnid density was seen with a magnifying lens, precise cell countings were started on the 5th day (June 25) of culture and lasted till the 41st day (July 31). The countings were done under an inverted microscope for 10 ml aliquots pippeted from the cultures. Exp. 1B originated from 106 specimens of *favella* form sorted from Exp. 1A at log phase (7 days old), and Exp. 1C from a 10 ml aliquot of Exp. 1B at stationary phase (24 days old) containing both *favella* and *coxliella* forms. Cell countings lasted from the 2nd day to the 34th day in Exp. 1B and from the 4th day to the 10th day in Exp. 1C. Densities at the start of these experiments were 0.054, 0.154 and 0.490 indiv·ml^{-1}, respectively.

Exps. 2A–B were done in essentially the same manner. Exp. 2A originated from the mixed populations of 19 *E. lususundae* and 3 *favella* forms of *F. taraikaensis* collected on July 10, 1981. Exp. 2B was started by inoculating a 10 ml aliquot of Exp. 2A at log phase (5 days old). The initial densities of *E. lususundae* and *F. taraikaensis* were 0.032 and 0.005 indiv·ml^{-1} in Exp. 2A and 0.012 indiv·ml^{-1} (both species) in Exp. 2B. These inocula of *F. taraikaensis* contained no *coxliella* form.

About 10 ml of dense mixture of food algae, *Isochrysis galbana*, *Prorocentrum minimum* and *Rhodomonas* sp. which had been selected in the preliminary experiments (see p. 35), were supplied every 2–3 days in early stage and twice a day in later stage of the cultures. By these supplies the food concentration was kept around 10^4 cells·ml^{-1} and the subsampled volume for cell countings was roughly compensated throughout the cultures.

Intact specimens, empty loricae and naked animalcules were counted separately for *Eutintinnus*. Intact specimens and empty loricae of each *favella* form or *coxliella* form and naked animalcules were counted for *Favella*. The naked animalcules of both species could be distinguished from each other by their size and shape: those of *Eutintinnus* are cylindrical in

shape, small in cell diameter and then pale in color but those of *Favella* are large and dark spherical or conical bodies.

Although the empty loricae appeared in significant number, their proportion to total counts was small. To simplify the figures, their number is not illustrated in Figs. 1A-C and 2A-B. Difference between total number and sum of the rest indicates the empty loricae. By the same reason, naked animalcules of *Eutintinnus* and *Favella* counted in Exps. 2A-B were included respectively into the counts of *Eutintinnus* and *favella* in Fig. 2A-B.

Calculation of Growth Rates

Growth rates of tintinnids are indicated by the generation time. The generation time (T) of the protozoa which grows by the binary fission could be calculated by the following formula:

$$T=\frac{t_2-t_1}{\log_2 N_2-\log_2 N_1}$$

where N_1 and N_2 are densities of living tintinnids (indiv$\cdot$ml^{-1}) at time t_1 and t_2, respectively. Mean generation time (T_{mean}: hours) was calculated from the initial density N_1 at the inoculation (t_1=0 h) and the increased density N_2 at the end of the log phase (t_2). The minimum generation time (T_{min}) was calculated from a successive data set obtained at the beginning and the end of an interval when the most rapid growth was observed.

Technical Error

Extent of technical error in the present methods was determined on a mixed culture of *F. taraikaensis* and *F. ehrenbergii* in 1982. Five replicates of 10 ml subsample were taken from the culture and cell countings were done. Coefficient of variation (CV) of counted number in five subsamples would indicate the extent of the technical error, while those of individual errors which arose from each procedure such as subsampling, fixation, counting, etc. could not be separately determined. Calculated CV values were 12 % (total count=85, n=5) for living *F. taraikaensis* and 11 % (total count=566, n=5) for living *F. ehrenbergii*. The value for the empty loricae exceeded 30 % in both species.

Results

Preliminary Experiments

In the preliminary cultures, single species or mixed species of monocultures of *Chaetoceros gracilis*, *Chlorella* sp., *Dunaliella tertiolecta*, *Isochrysis galbana*, *Heterosigma* sp., *Prorocentrum minimum*, *Rhodomonas* sp. and *Thalassiosira decipiens* (solitary form) were supplied. Neither tintinnid could survive in cultures with *C. gracilis*, *Chlorella* sp., *D. tertiolecta*, *Heterosigma* sp., and *T. decipiens*. Mucous excreta of two diatoms, *C. gracilis* and *T. decipiens*, tended to catch tintinnids. Monoculture of *Rhodomonas* sp., probably because of the large sinking rate, was an inferior diet. *I. galbana*, as its cell size seemed too small for the present tintinnids, was not solely tested. While *P. minimum* was a fairly good diet among algae tested in the monocultures, a mixture of *P. minimum* with *I. galbana* and *Rhodomonas* sp. was the best diet especially in long term cultures. Therefore, the mixed suspension of these three

algal cultures was used in the following growth experiments.

Growth Experiment Series 1

Fig. 1A-C shows growth curves of *F. taraikaensis* observed in three successive cultures. Rapid growth was evident during the first one week or the log phase. Mean generation time (T_{mean}) during the log phase calculated for a batch of *favella* and *coxliella* forms and naked animalcules was 23.5-28.4 h, and T_{min} 12.6-26.9 h. The growth rate of the populations originated from the natural specimens and younger culture was higher (Exps. 1A, 1B) than that from aged culture (Exp. 1C) (Table 1).

Although Exps. 1A and 1B started with the inoculation of only *favella* form, both *favella*

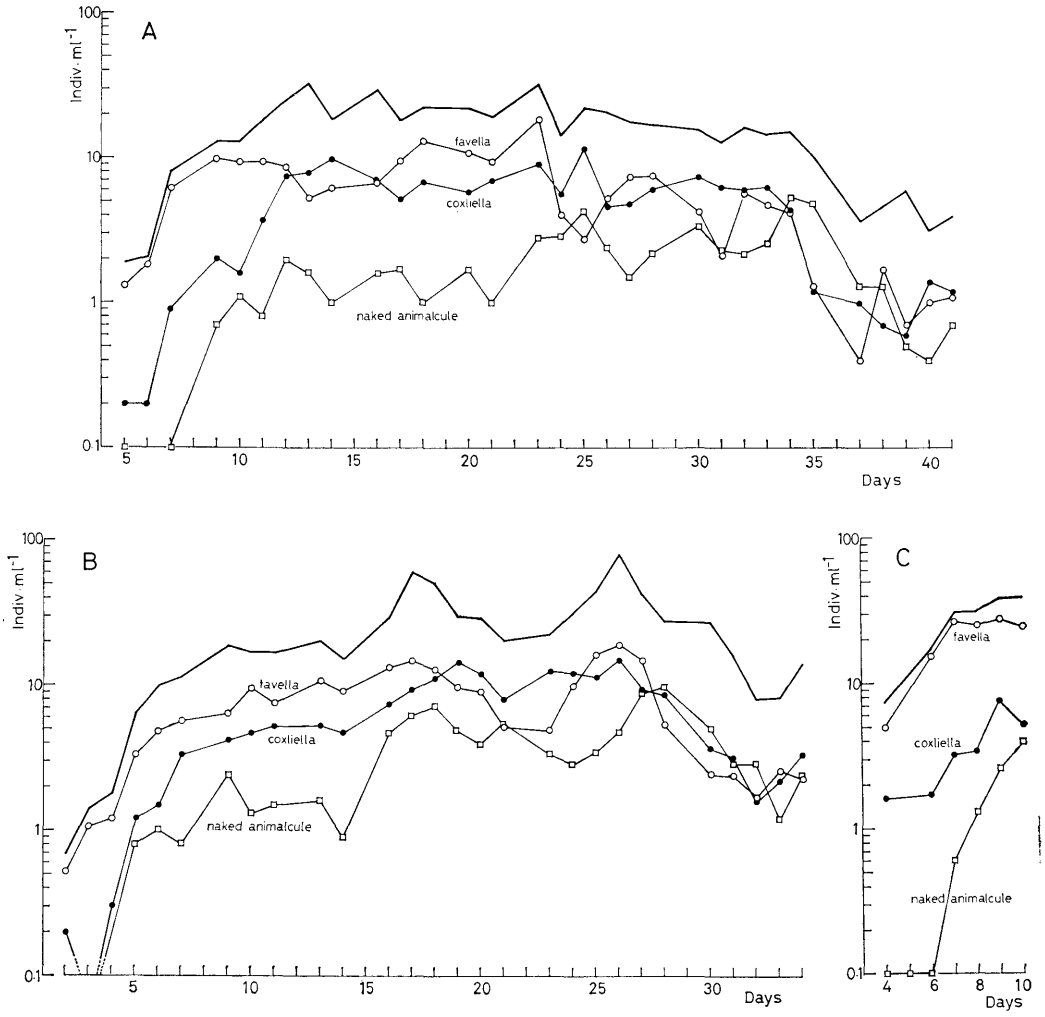

Fig. 1A-C. Growth curves of *Favella taraikaensis*. The curves are drawn separately for *favella* and *coxliella* forms and naked animalcules as well as the total number including empty loricae (bold line). A, B and C denote the experiment number 1A, 1B and 1C (see text).

TABLE 1. MEAN AND MINIMUM GENERATION TIMES OF *Entintinnus lususundae* AND *Favella taraikaensis* OBSERVED IN THE LABORATORY CULTURE EXPERIMENTS

	Exp. 1A	Exp. 1B	Exp. 1C	Exp. 2A		Exp. 2B	
Species[1)]	F	F	F	E	F	E	F
Duration of log phase (d)	7	5	7	10	7	7	7
Mean generation time (T_{mean}) (h)	23.9	23.5	28.4	21.2	17.6	17.8	17.8
Minimum generation time (T_{min}) (h)	12.6	14.6	26.9	6.6	10.0	13.5	12.3

[1)] F: *Favella taraikaensis*, E: *Eutintinnus lususundae*.

and *coxliella* forms appeared. In Exp. 1B more than 1/4 of the population were occupied by *coxliella* form by the second day (Fig. 1B). On the other hand, despite of the prevalence of *coxliella* form in the inoculum (55 %) in Exp. 1C, it was defeated by *favella* form within first 4 days (Fig. 1C). During long term cultures, occurrence of *coxliella* form generally increased with culture age. Following *coxliella* form naked animalcules also increased with the age. With progress of the cultures, occurrences of three forms were likely to be equilibrated around equal proportions and then the cultures declined into senescent phase (Fig. 1A–B).

There seems to be an antagonism between *favella* and *coxliella* forms in Exp. 1A, but it was not evinced in Exp. 1B. Conjugating pairs of *favella* form with *coxliella* form occurred at comparable frequency to those of *favella* forms or *coxliella* forms. Encystment of *favella*

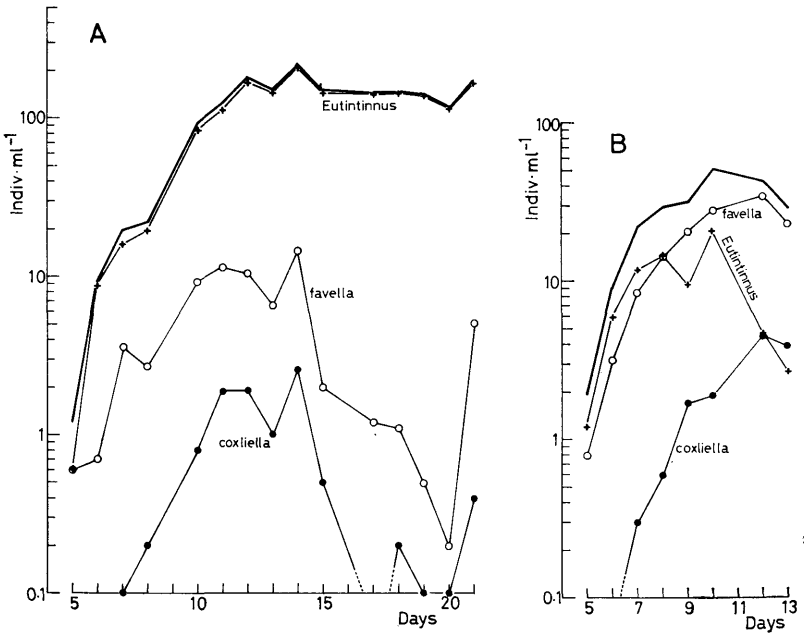

Fig. 2A–B. Growth curves of the mixed populations of *Eutintinnus lususundae* and *Favella taraikaensis*. The curves are illustrated separately for *coxliella* form and *favella* form (including the naked animalcules) of *F. taraikaensis*, and for *E. lususundae* (including its naked animalcules). Bold line indicates the total number including empty loricae of both species. A and B denote the experiment number 2A and 2B (see text).

form was often observed but no *coxliella* cyst was found (TANIGUCHI & KAWAKAMI, unpublished).

The maximum densities in total number of Exps. 1A, 1B and 1C were 33, 80 and 39 indiv•ml^{-1} respectively. Those in living number excluding empty loricae were 30, 39 and 38 indiv•ml^{-1} (Fig. 1A-C).

Growth Experiment Series 2

During the course of Exp. 2A, *Eutintinnus* of which dominancy in the inoculum was very high (more than 86 %) was prevailing over *Favella*. Although initial growth of the latter was rapid enough to attain the same density with *Eutintinnus* within first 5 days, its maximum density summing up *favella* and *coxliella* forms was less than 10 % of that of *Eutintinnus*. Period of stationary phase of *favella* and *coxliella* forms was very short. During senescent phase, occurrences of *favella*, *coxliella* and naked animalcules of *Favella* (the latter was included in *favella* count in Fig. 2A-B) were roughly equilibrated as observed in Exps. 1A and 1B (Fig. 2A). In Exp. 2B, which started with the mixed *Eutintinnus* and *Favella* populations of equal density in Exp. 2A, *Favella* gradually prevailed over *Eutintinnus*. No apparent stationary phase was observed for the latter. Lesser abundance of *coxliella* form than *favella* form in the growth phase was evident (Fig. 2B).

T_{mean} and T_{min} during the log phase of two experiments were 17.8-21.2 h and 6.6-13.5 h for *Eutintinnus* including naked animalcules. Those for *Favella*, including *favella* and *coxliella* forms and naked animalcules, were 17.6-17.8 h and 10.0-12.3 h, respectively (Table 1). No conjugating pairs or cysts were found throughout Exps. 2A and 2B.

Discussion

Coxliella form of *F. taraikaensis* was produced from the inocula which had contained only *favella* form and increased with culture age (Figs. 1A-C, 2A-B). This fact demonstrates that *F. taraikaensis* also have a phenotype, *coxliella* form, as discovered in the congeneric species *F. ehrenbergii* by LAVAL-PEUTO (1977). Moreover, the conjugation between *favella* form and *coxliella* form was newly revealed in this work. These marked morphological variabilities of this species along with those of *Eutintinnus* will be described elsewhere.

Although Exps. 2A and 2B were not designed to elucidate the interspecific competition between *Eutintinnus* and *Favella*, the results obtained could intimate that the competition possibly occurs between the species. While *Eutintinnus* predominated over *Favella* in the inoculum, the higher initial growth rate (T_{mean}) of *Favella* made densities of both species the same level by 5th day. However, the growth rate of *Favella* began to decrease when *Eutintinnus* grew to ca. 20 indiv•ml^{-1} and then dominancy of the latter was recovered. *Favella* dwindled after *Eutintinnus* exceeded ca. 100 indiv•ml^{-1} (Fig. 2A). When the densities of both species at the start were at the same level, their initial growth rates were also equivalent. After both species exceeded 10 indiv•ml^{-1}, *Eutintinnus* seemed to surrendered to *Favella* (Fig. 2B). These facts suggest that difference in the size of seeding populations is one of the determinative factors in the interspecific competition, and the competition hardly occurs until the

TABLE 2. MEAN AND MINIMUM GENERATION TIMES OF VARIOUS TINTINNID SPECIES

Species	Temperature (°C)	Generation time (h)	Reference
Eutintinnus lususundae	17	6.6–13.5 (T_{min}) 17.8–21.2 (T_{mean} for 7–10 days)	This work
E. pectinis	18	15.7 (T_{min})	HEINBOKEL (1978)
Favella ehrenbergii	20	>22.1 (T_{mean} for 4 days)	STOECKER et al. (1981)
F. taraikaensis	17	10.0–26.9 (T_{min}) 17.6–28.4 (T_{mean}) for 5–7 days)	This work
Helicostomella subulata	12–14	6–14 (T_{min})	PARANJAPE (1980)
	18	28.6 (T_{min})[1)]	HEINBOKEL (1978)
Tintinnopsis acuminata (?)	18	16.1 (T_{min})[1)]	HEINBOKEL (1978)
Tps. beroidea	12.5	19.2–26.4 (T_{min})	GOLD & POLLINGHER (1971)
	18	17.5 (T_{min})[1)]	HEINBOKEL (1978)

[1)] Estimated from Figs. 1–3 of HEINBOKEL (1978) by the present authors.

tintinnid populations attain fairly high density which is unusual in the natural conditions. One hundred individuals per milliliter of a small-sized tintinnid *Helicostomella subulata* in Bedford Basin, Canada (PARANJAPE 1980) might be the highest record in nature. Even in the eutrophic neritic waters, densities over a few tens indiv·ml^{-1} are not common. It is most likely that low density less than 1 indiv·ml^{-1} in the subtropical oceanic areas can not suggest the competition among tintinnid species in such areas (cf. TANIGUCHI 1977).

Growth ability of tintinnids, though variable with quality and quantity of food (GOLD 1971, HEINBOKEL 1978, STOECKER et al. 1981), should be very high (FENCHEL 1974). Table 1 shows the calculated values of T_{mean} and T_{min} in this work. In this calculation we assumed that the logarithmic growth began just after the inoculation. Therefore, if there had been the lag phase in the present cultures, as reported by PARANJAPE (1980) for *Helicostomella*, the T_{mean} values in Table 1 should be considered to be overestimated. Nevertheless, most T_{mean} values were shorter than 24 h. T_{mean} was prolonged in a culture the inoculum of which originated from the aged population (Exp. 1C). T_{min}, which indicates the most rapid growth ability, was sometimes reduced to less than 12 h in both species: 6.6 h and 10.0 h were recorded for the T_{min} of *Eutintinnus* and *Favella*, respectively (Table 1).

Table 2 summarizes the reported values of the generation time of various tintinnids obtained by the laboratory culture experiments. While the experimental conditions were very diverse, it can be seen, as a whole, that the average generation time of the growing populations ranges from 12 h for young cultures to 48 h for aged cultures and the minimum generation time is shortened to 6 h. These values are comparable to growth rates of most phytoplankters observed under the optimal conditions (cf. IWASAKI 1975). This partially supports the arguments on the peculiar roles of microzooplankton in nature previously mentioned in the introduction. Confirmation of the arguments, however, needs further studies on the standing biomass, feeding capability and metabolic activity of the microzooplankton including tintinnids.

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