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AN EXPERIMENTAL STUDY OF NANOFLAGELLATE BACTERIVORY

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SHORT COMMUNICATION

ABSTRACT

Heterotrophic nanoflagellate *Pseudobodo tremulans* (4.8 to 7.0 μm) and heterotrophic bacteria, isolated from coastal waters in Ubatuba, SP, Brazil, were used in experiments to analyze quantitatively the relationships between bacteria and nanoflagellates. The meaning of these results for the role of heterotrophic nanoflagellates in the Ubatuba coastal ecosystem is discussed.

Keys words: nanoflagellate, heterotrophic bacteria, coastal ecosystem, bacterivory

It has been widely accepted that a highly dynamic microbial loop consisting of pelagic bacteria, autotrophic pico- and nanoplankton, heterotrophic nanoflagelattes and microciliates is an integral part of planktonic food webs (3). Protozoa in the size range between 2 - 20μm (nanoplankton) are the major consumers of free-living pelagic bacteria in the sea (15). Several investigations on the dynamics of microbial loop have used a combination of laboratory and field methods (2, 12). The present investigation aims to quantify the bacterivory by a heterotrophic nanoplanktonic flagellate in "in vitro" conditions in order to get some indication of the importance of bacteria and nanoflagelattes in the Ubatuba coastal water ecosystem.

Surface seawater samples were collected in Ubatuba coastal region (southeast of Brazilian coast - 23°S 25°W). The system is considered to be meso-oligotrophic and is characterized by temperatures ranging from 14 to 24°C and salinities between 35 and 36‰. The primary production is low and nitrogen has been considered to be its main limiting factor (1).

Water samples were enriched with sterile rice grains and incubated at 20°C during 10 days before use. A heterotrophic nanoplanktonic flagellate (4.8 to 7.0 μm) identified as *Pseudobodo tremulans* was isolated from these enrichment cultures by micropipeting and a heterotrophic bacteria was isolated from the same enrichment by direct plating onto rice agar (9). The isolated microorganisms were maintained in stock cultures using a broth culture media (9) prepared with natural seawater and sterile rice grains, in the dark at 20°C (ambient water temperature at sampling time).

The experiments were carried out in duplicate and represented two different situations. In the first experimental procedure, the two microorganisms were grown without Cycloheximide, whereas in the second, microorganisms were grown in the presence

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of Cycloheximide used to inhibit the growth of nanoflagellates and reduce or eliminate predators in the system. Aliquots (5 ml) of BACTERIA + NANOFLAGELLATE stock cultures in the exponential growth phase (bacteria $10^8 ml^{-1}$ and nanoflagellate $10^5 ml^{-1}$) were transferred to filtered rice media and filtered rice media plus Cicloheximide (200mg l^{-1}). The cultures were maintained in the dark at 20°C. During 13 days at different time intervals 5ml aliquots were taken from each culture flask and fixed with formaldehyde (5% final concentration).

Bacterial abundance was estimated in preserved samples by epifluorescence microscopy using the Acridine Orange direct count method (AODC) (11). Nanoflagellates were quantified by epifluorecence microscopy according to the procedures described by Fenchel (7). Bacteria and nanoflagellate growth curves were done using the average values of the microorganism densities obtained from the duplicates of the experiments. Nanoflagellate biovolume was estimated by measuring the diameters of approximately 50 individual cells in different samples taken during the bacterivory experiments and assuming a spherical morphology (the equation used was $4/3\pi R^3$). The nanoflagellate carbon conversion factor used was 0.08 pg C mm^{-3} (16). An estimated biovolume of 0.10 μm^3 was used for bacteria and a carbon conversion factor of 0.22 pg C μm^{-3}, according to Bratbak and Dundas (4).

Microbial dynamics in the experiments were described according to a modified Lotka-Volterra type predator-prey model (2). The data used were obtained from growth curves of flagellate and bacteria in both situations (with and without Cycloheximide) at the first bacterial peak.

Fig. 1A represents the growth curves of bacteria and nanoflagellate populations during the experiment without inhibitor (BACTERIA + NANOFLAGELLATE) showing the predator-prey nature of the populations involved. In these experiment, bacterial density increased during the first 71h (maximum 1.75 x 10^8 ml^{-1}) and then decreased, reaching constant numbers after a time lapse of 167h (Fig. 1A). Approximately 96h after, the bacterial maximum nanoflagellates reached a peak of 5.00 x 10^5 ml^{-1} and then declined.

Fig. 1B shows the growth curves of both bacteria and nanoflagellate populations in presence of Cycloheximide. Cycloheximide inhibited both nanoflagellate growth and bacterivory for a period of about 168h. After a time interval of 239h, the nanoflagellates reappeared probably because the

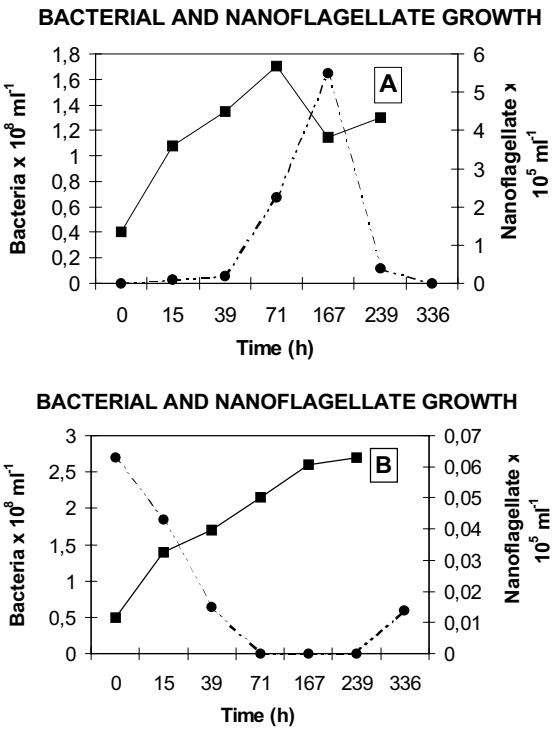

Figure 1. Growth of bacterial (■—■) and nanoflagellate (●—●) populations in the experiments without Cycloheximide (**A**) and with Cycloheximide (**B**).

Cycloheximide was metabolized by these microorganisms (6). At this time, protozoan grazing was repressed and bacterial abundance increased during the first 167h, reaching a value of 2.75 x $10^8 ml^{-1}$.

Table 1 shows the arithmetical means of the grazing data for experiments without and with Cycloheximide, prey and predator densities, growth rates, grazing rates, clearance rates and gross growth efficiency. The average bacterial growth rate in the grazing system was 0.102 divisions h^{-1} and the nanoflagellate one was 0.079 divisions h^{-1}. In the experiment without Cycloheximide each nanoflagellate consumed 82 bacteria per hour. The estimated average clearance rate obtained for one nanoflagellate was 4.6 x 10^{-7} ml^{-1} h^{-1}. The nanoflagellate gross growth efficiency was 39%. The extrapolation of the clearance rate obtained without Cycloheximide to the nanoheterotrophic population densities (mainly nanoflagellates) found in the Ubatuba coastal waters (8, 13) revealed that between 1 and 16% of the water column is cleared of bacteria per day by nanoflagellate populations.

In the experiment with inhibitor, Cycloheximide

Table I. Avarege values of grazing parameters obtained in the first bacterial peak. Experiment 1 was done without inhibitors and experiment 2 in the presence of Cycloheximide.

Cycloheximide	x	y	u(x)	u(y)	f(x)	f(x)/x	Y	Y
	Bacteria x 10^{-8} ml^{-1}	*Flagellates* x 10^{-5} ml^{-1}	*(d h^{-1})*	*(d h^{-1})*	*Bacteria* flagellate^{-1} h^{-1}	*ml flagellate^{-1} h^{-1}*	*Flagellate* bacteria^{-1}	*%*
absent	1.75	2.2	0.102	0.079	82	4.6 x 10^{-7}	1.0 x 10^{-3}	39
present	2.75	0	0.104	0	0	0	0	0

u(x) = bacterial growth rate
u(y) = nanoflagellate growth rate
f(x) = grazing rate
f(x)/x = clearance rate
Y = Yield (gross growth efficiency)

inhibited nanoflagellate growth and bacterivory and therefore the clearance rate. Comparatively to the experiment without Cycloheximide, the average value found for bacterial densities in this experiment was higher, in spite of the bacterial growth rate being similar (0.104 divisions h^{-1}) (Table 1).

The nanoflagellate growth rate obtained in this study is within the range of values of 0.01 to 0.25 d h^{-1} reported by Sherr and Sherr (15) and is very similar to that reported by Parslow *et al*. (14) for *Pseudobodo* sp (0.083 d h^{-1}) growing on *Micromonas pusilla*. In the absence of grazing, when nanoflagellate growth was inhibited by Cycloheximide, the bacterial population reached higher densities showing that the functional and numerical responses of the protozoa are probably adequate to control the size of bacterial populations (7).

The nanoflagellate grazing rate obtained in the abscence of Cycloheximide is close to those observed by several other authors (2) specially by Fenchel (7) for *Pseudobodo tremulans* (84 bacteria flagellate^{-1} h^{-1}). However, the clearance rate obtained was lower than those reported in literature. Since clearance is inversely proportional to prey densities and cellular volume (6), the high bacterial densities and bacterial volume observed in this work may be responsible for the low clearance rate obtained. Although low, this rate was equivalent to that obtained by Fenchel (8.0 x 10^{-7} ml flagellate^{-1} h^{-1}) (7) using hydrodynamic mathematical models.

The extrapolation of the nanoflagellate clearance rate to "in situ" bacterial and nanoheterotrophic densities in Ubatuba coastal waters showed that the nanoflagellate populations cleared a significant portion of bacteria in water column. This value, although significant, is not as high as those obtained by other authors (2, 12). According to Fenchel (7) and Lucas *et al*. (12), the nanoflagellate grazing rates and the clearance rates depend upon predator and prey species and their densities, relative sizes and the feeding mode of the predator. These factors explain the wide range noted in the literature of grazing and clearance rate values obtained in "in vitro" conditions.

The gross growth efficiency found in the present work is in the range of values reported by Fenchel (7) and Caron *et al*. (5) and is very close to that obtained by Lucas *et al*. (12) for *Pseudobodo* sp. A gross growth efficiency of 39% may indicate either a highly efficient transference of organic matter to higher trophic levels or a high nutrient regeneration rate. The amount of organic matter that could be transferred to higher trophic levels should depend upon the nanoflellate respiration and excretion rates (10). If the nanoflagellate gross growth efficiency is high and respiration and excretion rates are low, the protozoa will have an important role in transference of organic matter to higher trophic levels. On the other hand, if the nanoflagellate gross growth efficiency is high but respiration and excretion rates are also higher, nanoflagellates will be more important in the nutrient regeneration processes.

The results obtained in this work suggest that heterotrophic nanoflagellates and bacteria occupy an important role in planktonic food web of Ubatuba coastal ecosystem. Therefore, studies on nanoflagellate metabolism and nutrient regeneration by these organisms are needed to provide a better understanding of the role of nanoflagellates in this ecosystem either as a source of biomass for higher trophic levels or as organic matter mineralizers.

RESUMO

Um estudo experimental da bacterivoria por nanoflagelados

O nanoflagelado heterotrófico de dimensões entre 4,8 e 7,0 µm (*Pseudobodo tremulans*) e uma bactéria heterotrófica, isolados das águas costeiras de Ubatuba, SP, Brasil, foram utilizados em experimentos com o objetivo de analisar quantitativamente as relações entre bactérias e nanoflagelados. O significado dos resultados obtidos em relação ao papel dos nanoflagelados heterotróficos no ecossistema costeiro de Ubatuba é discutido.

Palavras-chave: nanoflagelados, bactérias heterotróficas, ecossistema costeiro, bacterivoria

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