

# N-remineralization of phyto- and bacterioplankton by the marine ciliate *Euplotes vannus*

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**ABSTRACT:** Ammonia excretion by the marine ciliate *Euplotes vannus* was investigated with the aid of feeding experiments involving algal food organisms (*Dunaliella tertiolecta*) and a bacterial diet (*Vibrio* sp.). The ciliates displayed a typical growth curve with a generation time of 6.2 to 6.9 h at 25 °C. With increasing consumption of the preferred food organisms by ciliates a steady increase in ammonia content of the culture medium was observed. During the logarithmic growth phase *Euplotes vannus* excreted  $0.22 \times 10^{-5}$  and  $0.41 \times 10^{-5}$   $\mu\text{mol N ciliate}^{-1} \text{h}^{-1}$ , utilizing algal and bacterial nourishment, respectively. The role of ciliates in the regeneration of inorganic nutrients in water is discussed.

## INTRODUCTION

Continuous primary production in the euphotic zone of aquatic systems without nutrient input depends on regeneration of inorganic phosphorus and nitrogen compounds. Together with bacteria, zooplankton are important in the remineralization on plant-derived nutrients. There are numerous studies of excretion of inorganic nutrients by larger zooplankton (Harries, 1959; Conover and Corner, 1968; Butler et al., 1969; Conover and Mayzaud, 1976; Quetin et al., 1980). The largest constituent of excreted nitrogen is ammonia, comprising over 70 % of the total N-excretion (Corner and Newell, 1967; Butler et al., 1970).

In contrast, little is known of N-remineralization by microzooplankton, especially protozoans. Harrison (1978) indicates that microzooplankton of less than 183  $\mu\text{m}$  in diameter may be the dominant remineralization group. Johannes (1965) studied the influence of marine protozoans on P-regeneration and concluded that, although protozoans represent only a small proportion of the faunal biomass, they are the predominant source of input of animal-derived nutrients.

Our experiments were principally concerned with the excretion of ammonia by ciliates fed bacteria and algae. In this way we hoped to further the understanding of the capability of a microzooplanktonic organism for regenerating inorganic nitrogen derived from a phytoplanktonic and bacterioplanktonic biomass.

## MATERIALS AND METHODS

Experiments on excretion were conducted employing the hypotrich ciliated protozoan *Euplotes vannus* (80 to 100  $\times$  35 to 40  $\mu\text{m}$ ), originating from the North Sea and isolated by Dr. G. Uhlig. The marine bacterial strain *Vibrio* sp.<sup>1</sup>, and the green algae *Dunaliella tertiolecta*<sup>2</sup>, served as food organisms.

Ciliates were maintained in aged, particle-free, filtered (0.2  $\mu\text{m}$  Sartorius membrane filter) North Sea water diluted with distilled water to a salinity of 30 ‰. The starting culture of ciliates was provided with *Vibrio* sp. on the 1st, 3rd and 4th day of cultivation and was employed for experimental purposes on the 5th day. To remove uningested bacteria and excretory products the starting culture was repeatedly filtered. This was accomplished through careful siphoning of culture solution through a 20  $\mu\text{m}$  mesh until only 1/3 of the initial volume remained. The original volume was restored through the addition of a particle-free, isotonic solution.

In order to enrich the bacterial culture, a seawater medium of 5 g yeast-extract, 1 g peptone, 750 ml aged North Sea water and 250 ml distilled water was employed (autoclaved for 20 min at 120 °C). Prior to feeding, the bacteria were washed by repeated cen-

<sup>1</sup> Supplied by Dr. M. Rieper

<sup>2</sup> Supplied by Dr. E. Hagmeier

trifuging and resuspension in particle-free, isotonic North Sea water.

The 6 d old starting culture of the algae *Dunaliella tertiolecta* was maintained in a F/10 medium (Guillard and Ryther, 1962) 30‰ S and placed so as to receive light from the northern sky. The ammonia content of the algal culture was negligibly small at the start of the experiment so that purifying could be waived.

To test algal metabolic influence on the N-content of the water, a portion of the algal food suspension was irradiated with UV-light (Philips MLU 300 WE) for 2 h in a shallow dish. Inactivation of algae treated in this manner resulted in rapid reduction in primary productivity ( $^{14}\text{C}$ -method) to a level of only 2% of that of untreated algae (= 100%).

For the study of ciliate ammonia excretion, 7 parallel experiments were conducted under utmost sterile conditions in 4 l round flasks (Table 1).

Table 1. Experimental design

Experi- mental vessel	Ciliates <i>Euplotes</i> <i>vannus</i> (50 ml)	Algae <i>Dunaliella t.</i> untreated (400 ml)	Algae <i>Dunaliella t.</i> UV- irradiated (400 ml)	Bacteria <i>Vibrio sp.</i> (50 ml)
1 Control	+			
2	+			+
3 Control				+
4	+	+		
5	+		+	
6 Control		+		
7 Control			+	

The flasks were filled with particle-free, aged, 30‰ S North Sea water to a volume of 2 l and, thereafter, incubated in the dark at 25°C. After shaking, flasks were sampled about every 3 h for the first 24 h. A few further samples were drawn until termination of the

experiment after 67 h. Algal and bacterial numbers were assessed microscopically using acridine orange fluorescence (Zimmermann et al., 1978).

Ciliates were isolated by filtration through 0.4 µm Unipore filter, stained for 10 min with 2% Erythrosine in a 5% aqueous phenol solution, then counted under the stereo-microscope. Ammonia content ( $\mu\text{mol N l}^{-1}$ ) was determined according to Graßhoff (1968).

## RESULTS

### Ciliate growth

*Euplotes vannus* nourished on bacteria as well as on algae produced a characteristic growth curve (Fig. 1a, b).

With algal food (*Dunaliella*, UV-*Dunaliella*) an 18 h lag-phase was observed, with an adjustment by the ciliates (previously cultivated with bacteria) to the new food organisms. Thereafter, the ciliate population steadily increased and after 35 h attained 112 ciliates  $\text{ml}^{-1}$  with *Dunaliella tertiolecta* as food. With UV-*Dunaliella*, accompanied by considerable bacterial growth (see below), there were 199 ciliates  $\text{ml}^{-1}$  after 44 h. Following a barely recognizable stationary phase a distinct drop in the number of ciliates occurred (death phase).

With bacterial diet (*Vibrio sp.*) the ciliates, already adapted to this form of nourishment, grew somewhat irregularly, but without lag period. Towards the end of this logarithmic growth the ciliates reached a maximum of 153 cells  $\text{ml}^{-1}$ .

In control flasks with un nourished protozoans, the number of individuals did not exceed 20  $\text{ml}^{-1}$ . When bacterial or algal foodstuff was present, generation time during logarithmic growth was 6.2 to 6.9 h at 25°C.

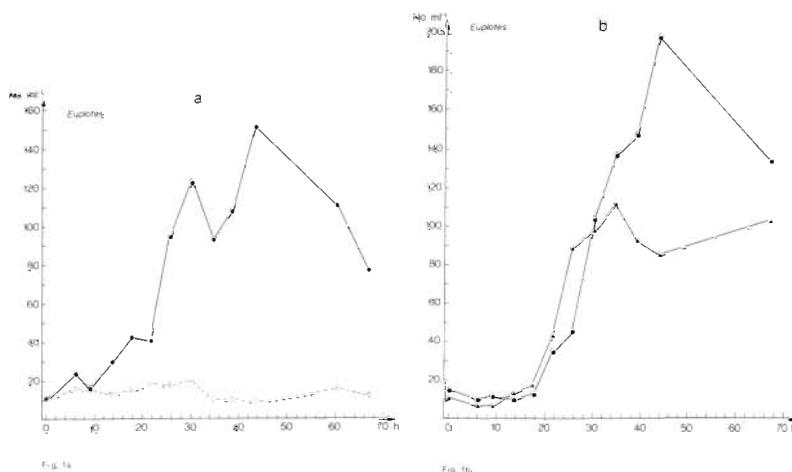


Fig. 1. *Euplotes vannus*. Population growth during feeding experiments (a) with bacteria *Vibrio sp.* (filled rhombs), without bacteria as control (open rhombs); (b) with algae *Dunaliella tertiolecta* (filled triangles) and UV-treated *D. tertiolecta* (filled circles)

### Food consumption

Changes in concentration of the food organisms offered (*Vibrio* sp., *Dunaliella*, UV-*Dunaliella*) were directly proportional to the development of ciliates (*Euplotes vannus*). The development of bacterial and algal numbers is presented in Fig. 2. Since the *Dunaliella* culture contained accompanying bacteria the latter were continually held under close surveillance, although they only represent a small contribution to the whole biomass (14.5% based on volume) (Fig. 2b, c).

With a purely bacterial diet (*Vibrio* sp.) a progressive reduction in the *Vibrio* population was strongly correlated with an increase in ciliates. In the absence of ciliates, bacterial counts remained more or less constant during the experiment (Fig. 2a).

Although somewhat temporarily prolonged, a similar 'predator-prey' relationship is displayed by untreated chlorophyceae. Whereas *Dunaliella* cells were still capable of division during the first 10 h of the experiment, the ciliates were in a lag phase and consequently the number of algal cells initially increased slightly. When the protozoans proliferated rapidly, sharp decline in phytoplankton commenced, finally

resulting in their disappearance. The number of bacteria and their total biomass accompanying the algal food organisms remain constantly low in this system and, therefore, can hardly be of significance for the nourishment of *Euplotes vannus* (Fig. 2b).

A different picture resulted with *Euplotes vannus* fed on UV-*Dunaliella* (Fig. 2c). Through intensive UV-irradiation ca. 80% of the algal cells were lysed. The organic and inorganic compounds (see below) subsequently released were utilized by the bacteria contained within the culture leading to an enormous bacterial production ( $33 \times 10^6$  bacteria  $\text{ml}^{-1}$ , after 67 h, Fig. 2c). However, in the presence of ciliates these accompanying bacteria were steadily consumed, with the remaining UV-*Dunaliella*; hence the bacterial population was held at a low level ( $4 \times 10^6$  bacteria  $\text{ml}^{-1}$ ). In this case the conversion of algal biomass into ciliate biomass occurred mainly via the bacteria.

### NH<sub>4</sub>-excretion by ciliates

Decomposing organic compounds (especially proteins) derived from food, the ciliates released considerable quantities of ammonia into the water (Fig. 3a, b).

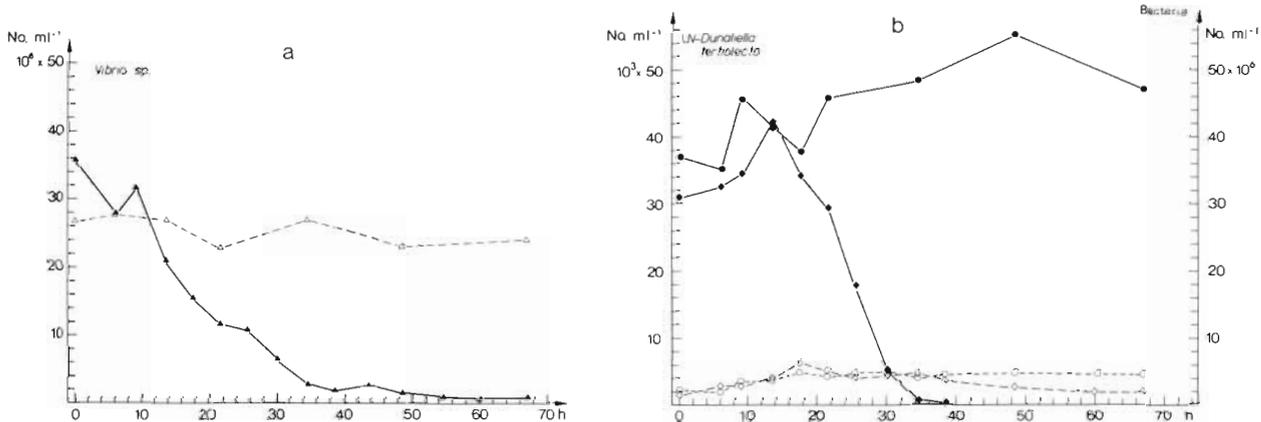
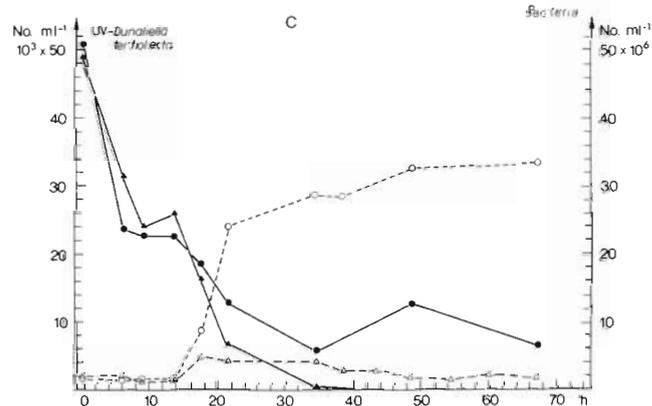


Fig. 2. *Euplotes vannus*. Decline of food organisms when feeding on (a) bacteria *Vibrio* sp. (filled triangles), bacteria control without ciliates (open triangles); (b) untreated algae *Dunaliella tertiolecta* (filled rhombs) and its accompanying bacteria (open rhombs), and *D. tertiolecta* without ciliates as control (filled circles), including accompanying bacteria (open circles); (c) UV-treated *D. tertiolecta* (filled triangles), and UV-*D. tertiolecta* without ciliates as control (filled circles), and accompanying bacteria (open circles)



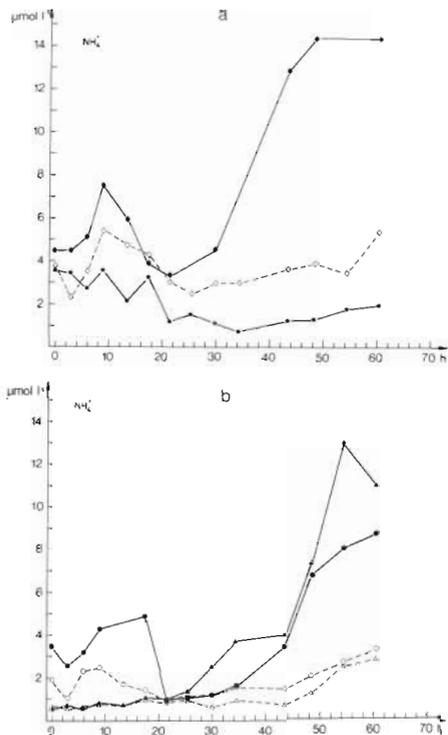


Fig. 3. *Euplotes vannus*. Alterations of dissolved ammonia during feeding. (a) with bacteria *Vibrio* sp. (filled rhombs), bacteria without ciliates (open rhombs), and unfed ciliates (asterisks) as controls; (b) with algae *Dunaliella tertiolecta* (filled triangles), *D. tertiolecta* without ciliates as control (open triangles), an UV-treated *D. tertiolecta* (filled circles), as well as UV-treated *D. tertiolecta* without ciliates (open circles) as control

Nevertheless, processes other than excretion by ciliates also produced recognizable alterations in the ammonia content of the culture medium.

When bacteria (*Vibrio* sp.) were offered as food, a small  $\text{NH}_4$ -peak was observed after 9.5 h. This peak was presumably not the result of grazing, but seems directly attributable to the food organisms themselves, as suggested by the graph obtained from the ciliate-free control (Fig. 3a).

Only through distinct proliferation of ciliates did the sudden rise in the  $\text{NH}_4$ -concentration of the culture medium commence. Under these conditions a maximal excretion of  $11.03 \mu\text{mol N l}^{-1}$  was attained by *Euplotes vannus* fed with bacteria.

With UV-irradiated algae it was noted that indeterminate quantities of inorganic and organic compounds, which influenced the ammonia content of the water as well as the development of bacteria, were released by lysis of algal cells. N-remineralization by ciliates, however, was of considerably greater consequence.

The untreated algal cells exhibit no measurable influence on  $\text{NH}_4$ -concentration in the water for the

first 20 h (Fig. 3b). Incubation in the dark apparently prevented N-consumption by *Dunaliella tertiolecta*. After ingestion of algal food by *Euplotes vannus*, excretion of ammonia was evident. The release of ammonia continued during the stationary phase of ciliate growth. Maximal concentration, achieved by *E. vannus* nourished on untreated *D. tertiolecta*, amounted to  $12.45 \mu\text{mol N l}^{-1}$ .

## DISCUSSION

Considerable amounts of ammonia in the form of excretory products were released during utilization of algal and bacterial biomasses for ciliate growth. Excretion rate, an indication of the extent of remineralization, was expressed as  $\mu\text{mol nitrogen excreted by 1 ciliate cell h}^{-1}$ ; it was determined during logarithmic growth by using the following equation:

$$e = \frac{a_2 - a_1}{x \cdot t}$$

where  $e$  = rate of excretion;  $a_2 - a_1$  = amount of ammonia excreted between 2 successive samplings;  $t$  = time between 2 successive samplings;  $x$  = number of ciliates at  $t/2$ . During logarithmic growth of *Euplotes vannus* the rate was  $0.22 \times 10^{-5} \mu\text{mol N ciliate}^{-1} \text{ h}^{-1}$  using *Dunaliella tertiolecta* as nourishment, and  $0.41 \times 10^{-5} \mu\text{mol N ciliate}^{-1} \text{ h}^{-1}$  with a bacterial diet. Dependence on the food organisms offered was probably due to differing availabilities of the respective food biomasses.

The small size of ciliates is closely associated with a high turnover rate and short generation time. For *Euplotes vannus* we determined a generation time of 6.2 to 6.9 h at 25 °C. For marine benthic ciliates, Fenchel (1968) calculated generation times ranging from 2.4 h (at 20 °C for *Uronema marinum*) to 46 h (at 20 °C for *Condylostoma patulum*). The relatively high degree of metabolic activity of ciliates, as opposed to that of marine crustaceans, permits an interesting comparison of the excretory rates of these 2 groups. Comparison with macrozooplankton, however, is complicated by the dissimilarity of experimental conditions reported in numerous publications. Nevertheless, reference to Quetin et al. (1980) may in this case be helpful. The authors investigated 19 planktonic species of crustaceans. The test animals were individually transferred into nutrient-free, filtered seawater a few hours following sorting and isolation. They were then maintained for several hours at 5.5 °C; at the beginning and end of the experiment the ammonia content in their respective containers was measured. In this manner excretory rates of between  $1.4 \mu\text{g N g}^{-1}$  wet weight  $\text{h}^{-1}$  and  $0.02 \mu\text{g N g}^{-1}$  wet weight  $\text{h}^{-1}$  were

obtained. An average value of  $0.3 \mu\text{mol N g}^{-1}$  wet weight  $\text{h}^{-1}$  for the 19 species was calculated. Since biological reactions increase in rate by a factor of 2 to 3 with a corresponding increase in temperature of  $10^\circ\text{C}$  (e. g. McLaren, 1963), the corrected rate of excretion for these crustaceans can be assumed to be about  $1.9 \mu\text{mol N g}^{-1}$  wet weight  $\text{h}^{-1}$ . If we also consider the excretory rates obtained in our study in terms of the animals' wet weight\*, an average value of  $40 \mu\text{mol N g}^{-1}$  wet weight  $\text{h}^{-1}$  is observed for *E. vannus* at  $25^\circ\text{C}$ . According to this calculation ammonia regeneration by *E. vannus* is about 20 times faster than by marine crustaceans. A greater remineralizing ability of ciliates than of marine crustaceans was similarly documented for P-generation; Johannes (1965): 'Per unit weight marine protozoa excrete dissolved phosphorus 1 to 2 orders of magnitude faster than marine macrofauna.' Barsdate et al. (1974) studied the phosphorus cycle in decomposing plant material and bacteria with and without bacterial grazers. They pointed out that phosphorus cycling is more rapid in systems with protozoans, although excretion by protozoans is minor, with the increase being mainly due to stimulating bacterial activity. On the other hand, Taylor and Lean (1981) doubt whether microzooplankton with their relatively small biomass can regenerate dissolved phosphorus much more rapidly than larger zooplanktonic crustaceans. Smetacek (1981) compared protozooplanktonic and metazooplanktonic biomasses and concluded that during periods of major development in spring and autumn protozooplankton of the Kiel Bight attain biomass values approaching those characteristic of metazooplankton in summer ( $0.5 \text{ g C m}^{-2}$ ). Nevertheless, employing size fractionation, Harrison (1978) has indicated that in coastal water bodies microplankton ( $< 183 \mu\text{m}$ ) may well be the primary remineralizers of nitrogen. Furthermore, as reported by Harrison (1978), Johansen (1976) has shown that specific  $\text{NH}_4$ -excretory rates for tintinnids are 1 or 2 orders of magnitude higher than rates for macrozooplankton. However, assessments of preserved samples tend to underestimate protozooplankton biomass (Scharf, 1979).

Our investigations and those reported in literature indicate that, on the basis of their high metabolic activities, ciliates can contribute significantly and very quickly to the regeneration of nutrients. Of course, along with excretion a series of other processes are involved in nutrient regeneration. Decomposition of organic compounds through bacterioplankton (von Brand et al., 1937; Goltermann, 1964; and others) and autolysis of planktonic cells leads to the release of a certain amount of inorganic nutrients (Hoffmann,

1956). Furthermore, nutrient release from the sediment directly influences the production of phytoplankton (von Bodungen et al., 1976).

The interaction between ciliates and bacteria appears complicated. On the one hand, bacteria serve as nourishment for the ciliates, thereby promoting ciliate production. On the other hand, development of the bacterial population is halted at a stage of 'physiological youth' due to grazing and, consequently, the attainment of a self-restricting cell-number is never realized. This phenomenon in turn promotes a bacterially effected remineralization. Further studies must be conducted in order to obtain information concerning the extent to which microzooplankton contribute to the total cycle of the regeneration of inorganic nutrients.

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\* Calculated from cellular volume as determined by the light and electron microscopical examination of 200 ciliates

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