



HAL
open science

N-REMINERALIZATION IN PLANKTONIC PROTOZOA

C Ferrierpages, F Rassoulzadegan

► **To cite this version:**

C Ferrierpages, F Rassoulzadegan. N-REMINERALIZATION IN PLANKTONIC PROTOZOA. Limnology and Oceanography, 1994, 39 (2), pp.411-418. 10.4319/lo.1994.39.2.0411 . hal-03504909

HAL Id: hal-03504909

<https://hal.science/hal-03504909v1>

Submitted on 26 Sep 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

- gopecten irradians*), p. 511–541. In Novel phytoplankton blooms. Coastal Estuarine Stud. V. 35. Springer.
- GERRITSEN, J., AND K. G. PORTER. 1982. The role of surface chemistry in filter feeding by zooplankton. *Science* **216**: 1225–1227.
- HART, M. W. 1990. Manipulating external Ca^{2+} inhibits particle capture by planktotrophic echinoderm larvae. *Can. J. Zool.* **68**: 2610–2615.
- . 1991. Particle captures and the method of suspension feeding by echinoderm larvae. *Biol. Bull.* **180**: 12–27.
- HARVEY, H. W. 1937. Note on selective feeding by *Calanus*. *J. Mar. Biol. Assoc. U.K.* **22**: 97–100.
- JØRGENSEN, B. C. 1966. Biology of suspension feeding. Pergamon.
- KIEFER, D. A., AND J. BERWALD. 1992. A random encounter model for the microbial planktonic community. *Limnol. Oceanogr.* **37**: 457–467.
- KOEHL, M. A. R., AND J. R. STRICKLER. 1981. Copepod feeding currents: Food capture at low Reynolds number. *Limnol. Oceanogr.* **26**: 1062–1073.
- LABARBERA, M. 1978. Particle capture by a pacific brittle star: Experimental test of the aerosol suspension feeding model. *Science* **201**: 1147–1149.
- . 1984. Feeding currents and particle capture mechanisms in suspension feeding animals. *Am. Zool.* **24**: 71–84.
- RASSOULZADEGAN, F., AND L. FENAUX. 1979. Grazing of echinoderm larvae (*Paracentrotus lividus* and *Arbacia lixula*) on naturally occurring particulate matter. *J. Plankton Res.* **1**: 215–223.
- , ———, AND R. R. STRATHMANN. 1984. Effect of flavor and size on selection of food by suspension-feeding plutei. *Limnol. Oceanogr.* **29**: 357–361.
- SHIMETA, J., AND P. A. JUMARS. 1991. Physical mechanisms and rates of particle capture by suspension-feeders. *Oceanogr. Mar. Biol. Annu. Rev.* **29**: 191–257.
- SOKAL, R. R., AND F. J. ROHLF. 1981. Biometry, 2nd ed. Freeman.
- STRATHMANN, R. R. 1971. The feeding behavior of planktotrophic echinoderm larvae: Mechanisms, regulation, and rates of suspension-feeding. *J. Exp. Mar. Biol. Ecol.* **6**: 109–160.
- , L. FENAUX, AND M. F. STRATHMANN. 1992. Heterochronic developmental plasticity in larval sea urchins and its implications for evolution of nonfeeding larvae. *Evolution* **46**: 972–986.
- , T. L. JAHN, AND J. R. C. FONSECA. 1972. Suspension feeding by marine invertebrate larvae: Clearance of particles by ciliated bands of a rotifer, pluteus, and trochophore. *Biol. Bull.* **142**: 505–519.
- WERNER, B. 1959. Das Prinzip des endlosen Schleimfilters beim Nahrungserwerb wirbelloser Meerestiere. *Int. Rev. Gesamten Hydrobiol.* **44**: 181–215.

Submitted: 16 March 1993

Accepted: 19 May 1993

Amended: 8 July 1993

Limnol. Oceanogr., 39(2), 1994, 411–419

© 1994, by the American Society of Limnology and Oceanography, Inc.

N remineralization in planktonic protozoa

Abstract—Ammonium excretion rates of two marine planktonic protozoa (an oligotrichous ciliate, *Strombidium sulcatum*, and an aplastidic flagellate, *Pseudobodo* sp.) both isolated from oligotrophic Mediterranean waters were quantified with heat-killed bacteria as the food source for the protozoans. Ammonium excretion rates ranged from 0.25 to 2 $\mu\text{g N mg DW}^{-1} \text{ h}^{-1}$ for the ciliate and from 2.8 to 8.5 for the phagotrophic flagellate. Maximum excretion rates took place during the early exponential growth phase, suggesting possible covariation of excretion rates with either protozoan body size or their growth rates or

metabolic state. Comparison of these excretion and regeneration rates with those obtained in situ for oligotrophic waters ($\sim 0\text{--}37 \text{ nmol liter}^{-1} \text{ h}^{-1}$) indicates that protozoa may account for >50% of the nutrient recycling in these waters.

The association of bacteria and protozoa play a significant role in nutrient recycling in marine environments (Johannes 1965; Fenchel and Harrison 1976). Since the paper by Eppley and Peterson (1979), there is increasing evidence that primary production in the ocean relies mostly on nutrients recycled in the euphotic zone by protozoa rather than bacteria (Glibert 1982; Wheeler and Kirchman 1986; Caron et al. 1988; Ferrier and Rassoulzadegan 1991). The importance of the microbial consortia in recycling processes likely increases as systems approach oligotrophy (Harrison 1980).

Acknowledgments

We thank David A. Caron and John Dolan as well as three anonymous reviewers for comments.

As a part of the Ph.D. program of C.F., this research is a contribution of the Ecologie du microzooplancton team and was supported by CNRS (URA 716) and the E.E.C. MAST II program Targeted on the Mediterranean (sub-project MEDIPELAGOS) grants.

Most studies of nutrient recycling have mainly concerned community aspects of nutrient flux through pelagic ecosystems. There are few data enabling us to differentiate the role of functional groups through consideration of key species of protozoa (Caron and Goldman 1990 and references therein). Here, we report on attempts to quantify ammonium excretion rates in an oligotrichous ciliate and in a population of aplastidic flagellates. Excretion rates and regeneration efficiency were measured during the whole growth phase of the protozoan populations.

For ammonium excretion experiments, we used the oligotrichous ciliate, *Strombidium sulcatum*, and a naturally occurring population of aplastidic flagellates isolated from the north-west Mediterranean Sea and grown on the same planktonic bacterial cultures. Bacterial cultures were obtained according to Rivier et al. (1985). Seawater was taken from the surface and filtered on a 0.4- μm Nuclepore membrane. A few drops of the filtrate were transferred into 1 liter of seawater passed through a 0.22- μm Sterivex GS Millipore filter containing sterile wheat grains to promote growth of the natural bacterial assemblage. Ciliates were introduced into these bacterial cultures containing $\sim 10^8$ bacteria ml^{-1} . Cultures were subsequently incubated in the dark at 12°C. Cultures of aplastidic flagellates were obtained by inoculating ~ 10 drops of 5- μm Nuclepore membrane filtrate (gravity) into the bacterial cultures.

We used heat-killed bacteria (Sherr et al. 1987) as the food source for the protozoa. Bacterial cultures were prepared as described above. In the stationary growth phase, cultures were separated from the wheat grains and heated for 2 h at 60°C (Sherr et al. 1987). They were subsequently passed through a 4- μm Nuclepore membrane to remove bacterial aggregates and stirred for 10 min. To check the nonviability of the resulting heat-killed bacterial suspension, we tested changes in the concentration of bacteria daily in control bottles during experiments. Only insignificant changes occurred for bacterial biomass. These suspensions were then used as a food source at a final concentration of $\sim 2 \times 10^7$ cells ml^{-1} . Two sets of bacterial suspensions were used in these experiments: heat-killed (experimental) and live (control) bacterial stocks prepared identically. Before their introduction in these me-

dia, ciliates were washed several times in 0.22- μm Sterivex Millipore-filtered seawater and inoculated into the experimental and control media to obtain a final ciliate concentration of ~ 1 cell ml^{-1} . In separate containers, aplastidic flagellates were added, at final concentrations of $\sim 10^3$ cells ml^{-1} , to both bacterial media. These flagellates were already grown on heat-killed bacteria to avoid transferring live bacteria in the heat-killed bacterial culture. To obtain an estimate of ammonium release due to the bacteria alone required two additional sets of heat-killed and live bacterial cultures, without protozoa.

All cultures were run in triplicate with a single sample taken daily from each culture to determine protozoan and bacterial abundance as well as ammonium concentrations. Samples were taken daily for 12 d. Ciliates were fixed with a 2% vol/vol final volume concentration of alkaline Lugol's solution and counted with an inverted Zeiss microscope. Heterotrophic bacteria and flagellates were preserved with formaldehyde (0.3% final concn), stained with DAPI (Porter and Feig 1980), and counted with an epifluorescence Zeiss microscope. Bacterial N content was measured in a series of heat-killed and live bacterial cultures by placing 200 ml of the bacterial cultures on precombusted GF/F filters. These filters were then analyzed with a CHN autoanalyzer (W.C. Heraeus; A.I.E.A.), calibrated with acetanilide and corrected for blank; bacterial concentrations were measured before and after filtration on GF/F filters to determine the number of bacteria retained by the filter.

Specific growth rates (μ) of the protozoa were measured in both experimental and control media (3 replicates for each) as follows:

$$\mu = (\ln C_1 - \ln C_0)/(t_1 - t_0). \quad (1)$$

C_0 and C_1 are the concentrations of ciliates or flagellates in the beginning (t_0) and at the end (t_1) of the incubation time.

Simultaneously, changes in ammonium concentrations were measured with a Technicon AutoAnalyzer following the method of Solórzano (1969). Specific protozoan ammonium excretion rates (E_R , $\mu\text{g-atoms N protozoa}^{-1} \text{h}^{-1}$) were estimated for bottles containing heat-killed or live bacteria as follows:

$$E_R = (N_1 - N_0)/[C(t_1 - t_0)]. \quad (2)$$

Table 1. Carbon and nitrogen content in the heat-killed and live bacteria measured with a CHN autoanalyzer.

Total content on the filter				
Bacterial concn (cells)	N (mg)	C (mg)	C:N	N cell ⁻¹ (pg)
Live bacteria				
3.1 × 10 ⁹	0.048	0.228	4.748	0.016
3.0 × 10 ⁹	0.047	0.230	4.992	0.015
4.5 × 10 ⁹	0.069	0.323	4.653	0.015
Heat-killed bacteria				
2.6 × 10 ⁹	0.039	0.197	5.058	0.015
2.4 × 10 ⁹	0.040	0.196	4.921	0.018
3.9 × 10 ⁹	0.065	0.324	4.992	0.017

In the live-bacteria vessels, protozoan excretion rates (E_{RL}) represented "net" excretion rates (i.e. the difference between protozoan excretion and bacterial uptake). In the heat-killed bacteria, these rates (E_{RHK}) should represent actual protozoan excretion rates. N_0 and N_1 are the ammonium concentrations ($\mu\text{g-atoms N ml}^{-1}$) at the beginning and end of the incubation time (h^{-1}). The theoretical average concentration of protozoa during incubation (C , cells ml^{-1}) was calculated assuming an exponential growth of cells:

$$C = (C_1 - C_0) / (\ln C_1 - \ln C_0). \quad (3)$$

The protozoan specific nitrogen ingestion rates were calculated as follows:

$$I_N = Q_N \cdot (B_0 - B_1) / [C \cdot (t_1 - t_0)]. \quad (4)$$

I_N is the particulate organic nitrogen ingestion rate ($\mu\text{g-atoms N protozoa}^{-1} \text{h}^{-1}$); Q_N is the bacterial nitrogen cell quota; B_0 and B_1 are the bacterial concentrations (cells ml^{-1}) at the beginning and in the end of the incubation time (h^{-1}).

The ciliate or flagellate nitrogen excretion efficiency (R in %) was then estimated as

$$R = (E_R / I_N) \times 100. \quad (5)$$

The N content of the live and heat-killed bacteria varied between 1.14 and 1.43×10^{-9} $\mu\text{g-atoms N cell}^{-1}$ respectively (Table 1). GF/F filters were quite efficient in retaining bacteria since only 1.5% of the total amount of bacteria was in the filtrate. In bacterial cultures without protozoa, there was no significant ammonium excretion (Table 2). In live bacterial cultures, the amount of ammonium decreased rapidly, whereas a slight increase was observed in 6-d-

Table 2. Ammonium concentrations ($\mu\text{g-atoms liter}^{-1}$) in heat-killed and live bacterial cultures (without ciliates) during a 10-d incubation.

Incubation time (d)	Ammonium concn	
	Heat-killed	Live
1	1.2	1.5
2	1.2	1.5
3	1.3	1.4
4	1.2	1.2
5	1.5	1.2
6	1.8	0.6
7	1.9	0.5
8	1.8	0.6
9	1.8	0.2
10	1.8	0.2

old heat-killed bacterial media. However, this increase was small relative to the changes observed in the experiments with protozoans and represented ~5% of the amount of ammonium in the protozoan cultures.

In excretion experiments, ciliates fed either live or heat-killed bacteria exhibited growth curves with a lag phase of 4 d, an exponential growth phase of 5 d, and a stationary phase of 3 d (Fig. 1). However, ciliate concentrations were higher in the live bacterial cultures (max, ~130 ciliates ml^{-1}) than in the media with heat-killed bacteria (~65 ciliates ml^{-1}). Decreases in bacterial abundance were directly proportional to the development of ciliates. However, the decrease in bacterial concentration was sharper in heat-killed than in live bacterial cultures. During the exponential phase, ciliates were growing at rates from 0.01 to 0.06 h^{-1} and 0.01 to 0.1 h^{-1} on heat-killed and on live bacteria, respectively. The amount of ammonium excreted covaried with the development of ciliates. A maximum of ~15 $\mu\text{g-atoms liter}^{-1}$ of ammonium was observed at the stationary phase of the ciliates in both media. Estimates of particulate N ingestion rates were obtained from Eq. 3 and are given in Table 3. They varied from 0.1 to 63×10^{-6} $\mu\text{g-atoms N cell}^{-1} \text{h}^{-1}$ (4×10^3 to 8×10^3 bacteria ciliate⁻¹ h^{-1}). Maximum values (6.30×10^{-5} $\mu\text{g-atoms N cell}^{-1} \text{h}^{-1}$) occurred during the lag and early exponential phase in heat-killed bacterial media. These rates thereafter decreased and were negligible during stationary phase.

Ammonium excretion rates in *S. sulcatum* at the beginning of the exponential phase (Fig.

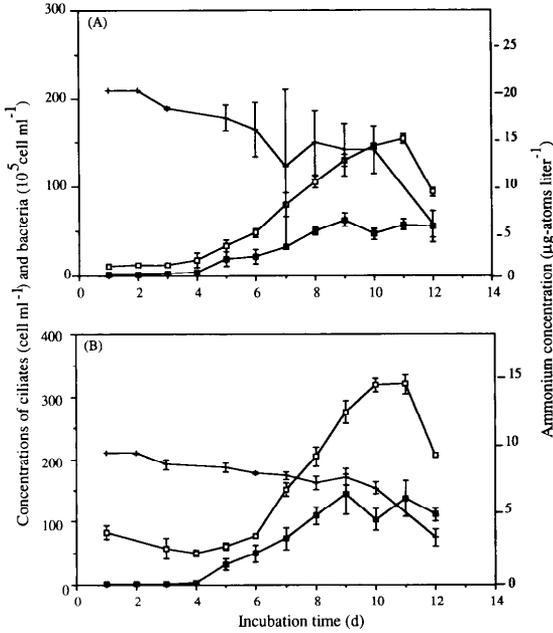


Fig. 1. Changes in concentrations of ciliates (■), bacteria (+), and ammonium (□) during a 12-d incubation. A. Heat-killed bacterial cultures. B. Live bacterial cultures. Error bars represent standard deviations.

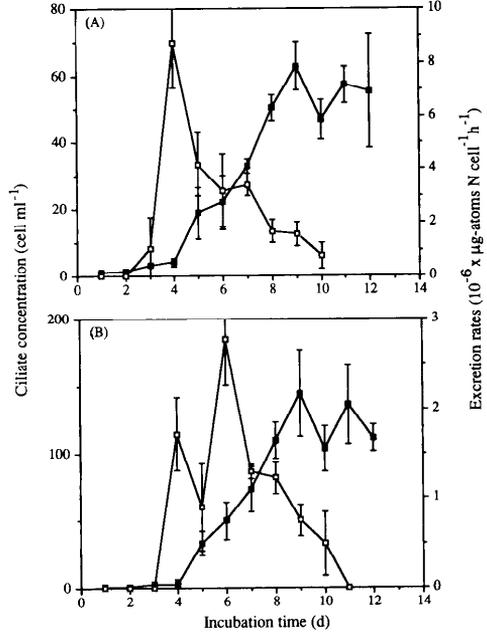


Fig. 2. Changes in ciliate concentrations (■) and in specific excretion rates (□) during a 12-d incubation. A. Heat-killed bacterial cultures. B. Live bacterial cultures. Error bars represent standard deviations.

2) reached values of 2.72 and $8.72 \times 10^{-6} \mu\text{g-atoms N ciliate}^{-1} \text{ h}^{-1}$ for ciliates fed on live and heat-killed media respectively. In heat-killed media, values decreased slightly during exponential phase and became negligible at the stationary phase; in live bacterial cultures, ammonium release rates remained high during the exponential phase and dropped off dramatically at the stationary phase. Regeneration efficiency values were low during the la-

tency and early exponential phase (<30%, Table 3). They increased at the end of the exponential phase (>100%) and during the stationary phase (between 30 and 100%).

Flagellates exhibited growth patterns similar to those obtained for ciliates (Fig. 3). The length of the different growth phases varied with the food (heat-killed or living bacteria) used. The exponential and stationary phases lasted 3 and 5 d when fed on live bacteria and 6 and 1 d

Table 3. Ciliate and phagotrophic flagellate nitrogen ingestion rates (I , $\mu\text{g-atoms N cell}^{-1} \text{ h}^{-1}$) and regeneration efficiency (Rn in %) during a 12-d incubation (in the heat-killed bacterial cultures). ND—not detectable.

Incubation time (d)	Ciliate exp.		Phagotrophic flagellate exp.	
	$I \times 10^6$	Rn	$I \times 10^{-9}$	Rn
1-2	ND	ND	ND	ND
2-3	63.40 ± 2.20	2.65 ± 0.95	0.00 ± 0.00	0.00 ± 0.00
3-4	14.38 ± 4.55	8.99 ± 4.71	422.00 ± 75.00	0.67 ± 0.18
4-5	7.42 ± 3.91	32.12 ± 26.23	33.10 ± 14.40	25.02 ± 5.51
5-6	7.47 ± 4.70	> 100	1.96 ± 1.06	40.80 ± 9.50
6-7	1.58 ± 0.97	> 100	0.42 ± 0.06	33.30 ± 7.14
7-8	1.50 ± 1.11	> 100	1.60 ± 1.50	76.88 ± 19.19
8-9	0.15 ± 0.11	> 100	2.14 ± 0.88	58.05 ± 29.73
9-10	0.33 ± 0.17	> 100	0.02 ± 0.01	43.07 ± 39.83
10-11	6.25 ± 2.63	30.28 ± 16.04	0.07 ± 0.05	> 100
11-12	3.55 ± 0.58	20.46 ± 09.15	0.10 ± 0.07	> 100
12-13	—	—	0.02 ± 0.00	> 100

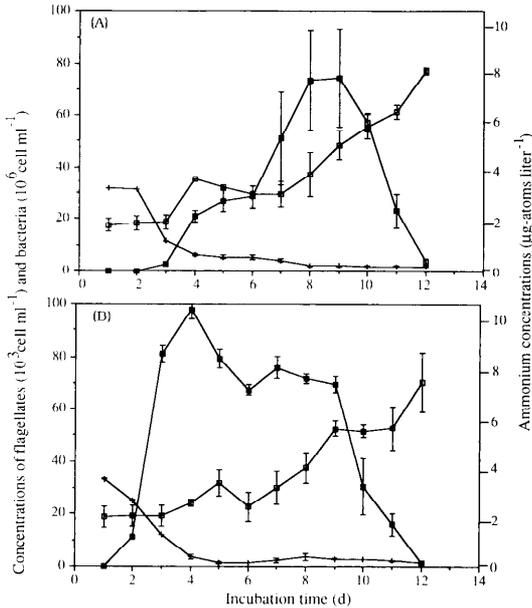


Fig. 3. Changes in concentrations of aplastidic flagellates (■), bacteria (+), and ammonium (□) during a 12-d incubation. A. Heat-killed bacterial cultures. B. Live bacterial cultures. Error bars represent standard deviations.

when fed with heat-killed bacteria. Furthermore, flagellates fed on live bacteria reached concentrations ($1 \times 10^5 \text{ cells ml}^{-1}$) higher than those obtained with killed bacteria (max, $7 \times 10^4 \text{ cells ml}^{-1}$). Growth rates were $\sim 0.08 \text{ h}^{-1}$ at exponential phase in both bacterial media. The ammonium concentration increased in both cultures with incubation time and reached $8 \mu\text{g-atoms N liter}^{-1}$ at the end of the stationary phase (Fig. 3). Particulate N ingestion rates (Table 3) reached maximum values at the beginning of the exponential phase ($4.21 \times 10^{-7} \mu\text{g-atoms N cell}^{-1} \text{ h}^{-1}$). Ingestion rates were ~ 20 bacteria flagellate $^{-1} \text{ h}^{-1}$ at earlier incubation times (Table 3).

Changes in the flagellate ammonium excretion rates (Fig. 4) were identical to those obtained for the ciliate, with high values at the beginning of the exponential phase (2.78 and $7.72 \times 10^{-9} \mu\text{g-atoms N cell}^{-1} \text{ h}^{-1}$ for live and heat-killed media). Excretion rates decreased sharply, but remained measurable during the stationary phase in the live bacterial cultures (avg, $0.5 \times 10^{-9} \mu\text{g-atoms N cell}^{-1} \text{ h}^{-1}$). Regeneration efficiency values presented the same pattern as for the ciliates (Table 3): low during latency and the early exponential phase

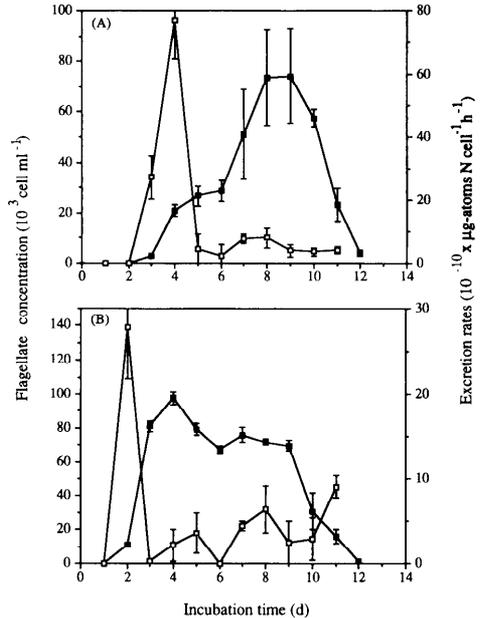


Fig. 4. Changes in phagotrophic flagellate concentrations (■) and in specific excretion rates (□) during a 12-d incubation. A. Heat-killed bacterial cultures. B. Live bacterial cultures. Error bars represent standard deviations.

(<40%), then increasing during the stationary (43–76%) and senescent phases (>100%).

Flagellates have been considered major consumers of bacterial production in aquatic ecosystems (Fenchel 1982b; Sieburth 1984; Rassoulzadegan and Sheldon 1986). However, recent studies have shown that ciliates are also able to consume a large fraction of picoplankton populations (Rivier et al. 1985; Rassoulzadegan et al. 1988). The consumption of bacteria constitutes a major portion of the nitrogen regeneration in the upper oceans. Protists, because of their abundance and their relatively high weight-specific excretion rates, probably account for a large portion of nitrogen regeneration in a variety of marine systems (Goldman et al. 1985; Verity 1985; Caron and Goldman 1990).

Planktonic protozoa constitute an important part of microzooplankton populations in Mediterranean waters where cell densities can be as high as $10\text{--}15$ ciliates ml^{-1} and 10^3 flagellates ml^{-1} (Rassoulzadegan 1975). Oligotrichous planktonic ciliates are often the dominant group of microzooplankton (Rassoulzadegan 1977; Sherr et al. 1989). The ge-

nus *Strombidium* (Claparède and Lachman) or the family Strombiidae include most species of oligotrichous ciliates and represent >50% of the total concentration of ciliates inhabiting these waters (Sherr et al. 1989). The phagotrophic flagellate used in this study is similar to one described by Fenchel (1982a) as *Pseudobodo* sp. The cell is 4–5 µm in diameter and has two flagella (with a pronounced asymmetry). Because of their abundance in the waters studied (Rassoulzadegan et al. 1988), these flagellates likely play a significant role in nutrient recycling processes.

Earlier field studies on nutrient regeneration in the ocean (Johannes 1965) have generally ignored the precise contribution of each microbial component to nitrogen regeneration. To quantify protozoan nitrogen excretion rates, it was important to separate excretion by predators (ciliates or flagellates) from the ammonium uptake by bacterial prey (Wheeler and Kirchman 1986). Other investigators have used procaryotic inhibitors (Sherr et al. 1986). However, we did not use this method because of the fragility of the oligotrichous ciliates studied (Tremaine and Mills 1987). Other studies used different indirect methods (Lucas et al. 1987; Goldman et al. 1985; Caron et al. 1988) which did not explicitly avoid ammonium uptake by prey organisms. In this work, we used heat-killed bacteria, whose C:N ratio is not substantially different from those for live cultures (Table 1 and Caron et al. 1990) and is consistent with ranges given for live bacteria from natural assemblages (Bratbak 1985). The comparison of protist feeding on heat-killed and live bacterial cultures allowed us to measure grazing rates on nongrowing prey, to estimate protozoan excretion rates, to calculate protozoan regeneration efficiency, and to estimate ammonium uptake by live bacteria.

Protozoan-specific growth rates during the exponential growth phase were comparable in heat-killed and live bacterial media and are in agreement with the literature (Fenchel 1982b; Sherr et al. 1983; Stoecker and Evans 1985). This result suggests that the chemical properties of the bacteria are not dramatically affected by heating and implies that ammonium excretion rates were realistic.

Results obtained here suggested that, relative to bacterial activity, the bulk of ammonium regeneration is performed by protozoa.

Indeed, there was no significant N regeneration in protozoa-free bacterial culture. Moreover, maximal ammonium excretion rates obtained in the heat-killed bacterial cultures (ciliate or flagellate experiments) were higher than those obtained in the live bacterial culture. This difference in excretion rates is likely due to the uptake of ammonium by bacteria. This nutrient uptake could be estimated as

$$B_{un} = [(E_{RHK,max} \times C_{HK}) - (E_{RL,max} \times C_L)]/B. \quad (6)$$

B_{un} is the ammonium uptake rate by bacteria ($\mu\text{g-atoms N cell}^{-1} \text{ h}^{-1}$), $E_{RHK,max}$ and $E_{RL,max}$ are the maximal excretion rates in heat-killed and live bacteria respectively ($\mu\text{g-atoms N protozoa}^{-1} \text{ h}^{-1}$), C_{HK} and C_L are the protozoan concentrations at $E_{R,max}$ in heat-killed and live bacteria respectively, and B is the average concentration of live bacteria at $E_{R,max}$. Calculated ammonium uptake rates by bacteria are equal to 3.5 and $2.8 \times 10^{-12} \mu\text{g-atoms N cell}^{-1} \text{ h}^{-1}$ in ciliate and flagellate cultures.

Ammonium weight-specific (dry weight, DW) excretion rates obtained for the two protozoa are within the ranges reported in the literature (Table 4). Studies on excretion rates by individual species of protozoa are relatively rare. Our experiment represents the first values of excretion rates in a planktonic oligotrichous ciliate fed on bacteria and in a planktonic applastidic flagellate isolated from the Mediterranean Sea—two species different from those studied elsewhere (Table 4). Moreover, this study describes the changes in ammonium excretion rates of protozoa as they go from exponential growth to senescence. These weight-specific excretion rates were calculated assuming mean values for ciliate or flagellate cell sizes. Recent studies (Ohman and Snyder 1991) showed that changes in protozoan cell size can be significant during growth, with smaller cell sizes characteristic of the beginning of the exponential phase or during the stationary phase. In this case, our highest weight-specific excretion rates could be even higher, because they were obtained at the beginning of the exponential growth phase when protozoan cell sizes could have been relatively small. However, size variations were not obvious during our experiments, and we decided to use mean size values.

Ciliates exhibited lower weight-specific ex-

Table 4. Recent rates of weight-specific ammonium regeneration [$\mu\text{g N (mg DW)}^{-1} \text{h}^{-1}$] estimated for protozoa.

Reference	Species	Food	Wt-specific excretion rates
Ciliates			
Berman et al. 1987	<i>Cyclidium</i> sp. (scuticociliate)	Bacterial and algal food	48–178
Gast and Horstmann 1983	<i>Euplotes vannus</i> (scuticociliate)	Algal and bacterial food	3
Verity 1985	<i>Tintinnopsis</i> sp. (Tintinnida)	Algal food	1.5–3
This work	<i>Strombidium sulcatum</i> (oligotrichous ciliate)	Bacterial food	0.25–2
Flagellates			
Andersson et al. 1985	<i>Ochromonas</i> sp. (chrysoomonads)	Bacterial food	28
Goldman et al. 1985	<i>Paraphysomonas imperforata</i> (chrysoomonads)	Bacterial and algal food	3–20
Sherr et al. 1983	<i>Monas</i> sp. (chrysoomonads)	Bacterial food	6–51
This work	<i>Pseudobodo</i> type	Bacterial food	2.8–8.5

cretion rates than flagellates, probably because of the decrease in metabolic rates with increasing cell volume. Rates obtained for planktonic protozoa are considerably higher than those reported for most macroplankton species (Caron and Goldman 1990). Excretion rates also depend on protozoan specific growth rates (Caron and Goldman 1990) and on a possible change in the metabolism in a given species (proteic/lipidic metabolism). They vary with ingestion rates, which are also very high at the beginning of the exponential growth phase when bacteria are not limiting. The ingestion rates (~ 20 bacteria flagellate $^{-1} \text{h}^{-1}$ and 6×10^3 bacteria ciliate $^{-1} \text{h}^{-1}$) we obtained are in agreement with the literature (Lucas et al. 1987; Rivier et al. 1985). When prey concentrations became limiting, ingestion rates decreased sharply.

We also found that during the exponential growth phase, protozoa seem to conserve N. Indeed, in both flagellate and ciliate experiments, N regeneration efficiency (Rn) remained quite low ($< 50\%$) during the lag and most of the exponential phases (Table 3) where N is mostly used to form protozoan biomass. Low Rn values during the exponential phase are not surprising because protozoan gross growth efficiency can reach values as high as 50–70% (Fenchel 1982b). However, during the stationary and decreasing phases in both experiments, Rn values quickly increased ($> 100\%$). Similar results were obtained by Goldman et al. (1985) for the aplastidic flagellate *Paraphysomonas imperforata*. Rela-

tively high regeneration efficiency as observed during the senescent phase likely can be achieved only when protozoa go through a starvation phase (Table 3).

In our experiment, we investigated two component relationships, but in nature, all the components of the microbial food web (Azam et al. 1983) are present simultaneously, with complex interactions. However, our results could be applicable to natural systems when protozoa are well fed. According to our data, 10^3 flagellates can excrete $8 \times 10^{-3} \text{ nmol h}^{-1}$. This result is comparable with in situ estimates of N regeneration based on ^{15}N size-fractionated studies ($1\text{--}15 \times 10^{-3} \text{ nmol h}^{-1}$ per 10^3 flagellates, Lucas et al. 1987). In the Mediterranean Sea, average concentrations of flagellates and ciliates are 10^3 and 1 cell ml^{-1} , respectively. With the maximum excretion rates measured in this work, we obtained N regeneration rates of 8 and $9 \text{ nmol N h}^{-1} \text{ liter}^{-1}$ for flagellates and ciliates respectively, which give a total value of $17 \text{ nmol N h}^{-1} \text{ liter}^{-1}$ for planktonic protozoan assemblages in these oligotrophic waters. Nitrogen regeneration rates measured in situ in oligotrophic environments ranged from 1 to $36 \text{ nmol liter}^{-1} \text{ h}^{-1}$ for the Sargasso Sea, 0 to 37 for the Gulf Stream and 0.1 to 0.6 for the North Pacific Ocean (Glibert et al. 1982; Wheeler and Kirchman 1986; Eppley and Peterson 1979). Comparing these values with those in our experimental model ($17 \text{ nmol cell}^{-1} \text{ h}^{-1}$) suggests that protozoa could account for 50% of the recycled N. Even if maximum excretion rates are not specifically

indicative of oligotrophic waters, representatives of groups studied here may account for most of the regeneration.

There are still few data available on nutrient excretion rates by individual species of protozoa (Goldman et al. 1985; Gast and Horstmann 1983; Verity 1985). However, these data help us understand protozoan interactions in more complex microbial communities. Furthermore, the nutrient regeneration was measured throughout the whole growth cycle of the species and not solely during exponential growth. Rates and magnitude of nutrient regeneration during a whole cycle are still unknown but are necessary when a whole community is studied because, in situ, protozoa can be in any physiological stage.

C. Ferrier-Pagès
F. Rassoulzadegan

URA—CNRS 716
Station Zoologique, BP 28
06230 Villefranche-sur-mer, France

References

- ANDERSSON, A., C. LEE, F. AZAM, AND A. HAGSTRÖM. 1985. Release of amino acids and inorganic nutrients by heterotrophic marine microflagellates. *Mar. Ecol. Prog. Ser.* **23**: 99–106.
- AZAM, F., AND OTHERS. 1983. The ecological role of water column microbes in the sea. *Mar. Ecol. Prog. Ser.* **10**: 257–263.
- BERMAN, T., M. NAWROCKI, G. T. TAYLOR, AND D. M. KARL. 1987. Nutrient flux between bacteria, bacterivorous nanoplanktonic protists and algae. *Mar. Microb. Food Webs* **2**: 69–82.
- BRATBAK, G. 1985. Bacterial biovolume and biomass estimations. *Appl. Environ. Microbiol.* **49**: 1488–1493.
- CARON, D. A., AND J. C. GOLDMAN. 1990. Protozoan nutrient regeneration, p. 283–306. *In* G. M. Capriulo [ed.], *Ecology of marine protozoa*. Oxford.
- , AND M. R. DENNETT. 1988. Experimental demonstration of the roles of bacteria and bacterivorous protozoa in plankton nutrient cycles. *Hydrobiologia* **159**: 27–40.
- , K. G. PORTER, AND R. W. SANDERS. 1990. Carbon, nitrogen, and phosphorus budgets for the mixotrophic phytoflagellate *Poterioochromonas malhamensis* (Chrysophyceae) during bacterial ingestion. *Limnol. Oceanogr.* **35**: 433–443.
- EPPLEY, R. W., AND B. J. PETERSON. 1979. Particulate organic matter flux and planktonic new production in the deep ocean. *Nature* **282**: 677–680.
- FENCHEL, T. 1982a, b. Ecology of heterotrophic microflagellates. 1. Some important forms and their functional morphology. 4. Quantitative occurrence and importance as bacterial consumers. *Mar. Ecol. Prog. Ser.* **8**: 211–233; **9**: 35–42.
- , AND P. HARRISON. 1976. The significance of bacterial grazing and mineral cycling for the decomposition of particulate detritus, p. 285–299. *In* J. M. Anderson and A. Macfadyen [eds.], *The role of terrestrial and aquatic organisms in decomposition processes*. Blackwell.
- FERRIER, C., AND F. RASSOULZADEGAN. 1991. Density-dependent effects of protozoans on specific growth rates in pico- and nanoplanktonic assemblages. *Limnol. Oceanogr.* **36**: 657–669.
- GAST, V., AND U. HORSTMANN. 1983. N-remineralization of phyto- and bacterioplankton by the marine ciliate *Euplotes vannus*. *Mar. Ecol. Prog. Ser.* **13**: 55–60.
- GLIBERT, P. M. 1982. Regional studies of daily, seasonal, and size fraction variability in ammonium remineralization. *Mar. Biol.* **70**: 209–222.
- , J. C. GOLDMAN, AND E. J. CARPENTER. 1982. Seasonal variations in the utilization of ammonium and nitrate by phytoplankton in Vineyard Sound, Massachusetts, USA. *Mar. Biol.* **70**: 237–249.
- GOLDMAN, J. C., D. A. CARON, D. A. ANDERSEN, AND M. R. DENNETT. 1985. Nutrient cycling in a microflagellate food chain; 1. Nitrogen dynamics. *Mar. Ecol. Prog. Ser.* **24**: 231–242.
- HARRISON, W. G. 1980. Nutrient regeneration and primary production in the sea, p. 433–460. *In* *Primary productivity in the sea*. Brookhaven Symp. Biol. 31. Plenum.
- JOHANNES, R. E. 1965. Influence of marine protozoa on nutrient regeneration. *Limnol. Oceanogr.* **10**: 434–442.
- LUCAS, M. I., T. A. PROBYN, AND S. J. PAINTING. 1987. An experimental study of microflagellate bacterivory: Further evidence for the importance and complexity of microplanktonic interactions. *S. Afr. J. Mar. Sci.* **5**: 791–808.
- OHMAN, M. D., AND R. A. SNYDER. 1991. Growth kinetics of the omnivorous oligotrich ciliate *Strombidium* sp. *Limnol. Oceanogr.* **36**: 922–935.
- PORTER, K., AND Y. S. FEIG. 1980. The use of DAPI for identifying and counting aquatic microflora. *Limnol. Oceanogr.* **25**: 943–948.
- RASSOULZADEGAN, F. 1975. *Ecologie et relations trophiques du microzooplancton dans un écosystème néritique*. These, Univ. P. and M. Curie (Paris). 158 p.
- . 1977. Evolution annuelle des ciliés pélagiques en Méditerranée nord-occidentale: Ciliés Oligotriches “non tintinnides” (Oligotrichina). *Ann. Inst. Oceanogr. Paris* **53**: 125–134.
- , M. LAVAL-PEUTO, AND R. W. SHELDON. 1988. Partitioning of the food ration of marine ciliates between pico- and nanoplankton. *Hydrobiologia* **159**: 75–88.
- , AND R. W. SHELDON. 1986. Predator-prey interactions of nanoplankton and bacteria in an oligotrophic marine environment. *Limnol. Oceanogr.* **31**: 1010–1021.
- RIVIER, A., D. C. BROWNLEE, R. W. SHELDON, AND F. RASSOULZADEGAN. 1985. Growth of microzooplankton: A comparative study of bacterivorous zooflagellates and ciliates. *Mar. Microb. Food Webs* **1**: 51–60.
- SHERR, B. F., E. B. SHERR, T. L. ANDREW, R. D. FALLON, AND S. Y. NEWELL. 1986. Trophic interactions be-

- tween heterotrophic protozoa and bacterioplankton in estuarine water analyzed with selective metabolic inhibitors. *Mar. Ecol. Prog. Ser.* **32**: 169–179.
- , ———, AND T. BERMAN. 1983. Grazing, growth, and ammonium excretion by a heterotrophic microflagellate fed with four species of bacteria. *Appl. Environ. Microbiol.* **45**: 1196–1201.
- , ———, AND R. D. FALLON. 1987. Use of mono-dispersed fluorescently labelled bacteria to estimate in situ protozoan bacterivory. *Appl. Environ. Microbiol.* **53**: 958–965.
- SHERR, E. B., F. RASSOULZADEGAN, AND B. F. SHERR. 1989. Bacterivory by pelagic ciliates in coastal waters of the NW Mediterranean Sea. *Mar. Ecol. Prog. Ser.* **55**: 235–240.
- SIEBURTH, J. MCN. 1984. Protozoan bacterivory in pelagic marine waters, p. 405–444. *In* J. E. Hobbie and P. J. leB. Williams [eds.], *Heterotrophic activity in the sea*. Plenum.
- SOLÓRZANO, L. 1969. Determination of ammonia in natural waters by the phenylhypochlorite method. *Limnol. Oceanogr.* **14**: 799–801.
- STOECKER, D. K., AND G. T. EVANS. 1985. Effects of protozoan herbivory and carnivory in a microplankton food web. *Mar. Ecol. Prog. Ser.* **25**: 159–167.
- TREMAINE, S. C., AND A. L. MILLS. 1987. Inadequacy of the eucaryote inhibitor cycloheximide in studies of protozoan grazing on bacteria at the freshwater–sediment interface. *Appl. Environ. Microbiol.* **53**: 1969–1972.
- VERITY, P. G. 1985. Grazing, respiration, excretion, and growth rates of tintinnids. *Limnol. Oceanogr.* **30**: 1268–1282.
- WHEELER, P. A., AND D. L. KIRCHMAN. 1986. Utilization of inorganic and organic nitrogen by bacteria in marine systems. *Limnol. Oceanogr.* **31**: 998–1009.

Submitted: 19 August 1992

Accepted: 21 July 1993

Amended: 12 August 1993

Limnol. Oceanogr., 39(2), 1994, 419–424

© 1994, by the American Society of Limnology and Oceanography, Inc.

Cladoceran body size and vulnerability to copepod predation

Abstract—We examined species-related and age-related mortality caused by a predaceous copepod (*Acanthocyclops robustus*) in five closely related cladocerans representing a wide spectrum of body size. Animals of all five species from clonal cultures were exposed for 1, 2, or 4 d in medium from a copepod-infested enclosure that either did or did not contain natural densities of *Acanthocyclops* adults and copepodites (40–50 copepods liter⁻¹). In each of the five species, the 1-d-old neonates were most, and adults least, vulnerable to the copepods. Small-bodied *Daphnia cucullata* and *Ceriodaphnia reticulata* remained vulnerable until maturation, but the susceptibility of large *Daphnia magna* drastically decreased in 3- and 5-d-old animals, and 7-d-old adults were immune to copepod predation.

Size-selective predation by fish and invertebrates has long been considered to play an important role in structuring zooplankton populations and communities by selective removal of large-bodied or small-bodied prey. Although the structuring role of fish predation has been confirmed by many field and exper-

imental data (see Hall et al. 1976; Bengtsson 1987), the role of invertebrate predation has remained in question since it was first suggested by Dodson (1974). Most experimental evidence for the importance of invertebrate predation is less compelling than it is for fish (see Zaret 1980).

Of many different morphological, behavioral, and life-history features, body size seems to be the most important factor responsible for the vulnerability of zooplankton prey to predation. This notion is particularly clear in respect to fish predation, which seems evident from each major review of the subject (Zaret 1980; Kerfoot and Sih 1987; Lazarro 1987). It is less clear, however, when predation by invertebrates is reviewed, because other morphological and behavioral features of prey are often suggested to be more important than prey body size to various invertebrate predators, including predaceous copepods.

Although some field and laboratory experiments show that prey body size may be important to calanoid copepods such as *Diaptomus shoshone* (Anderson 1970; Dodson 1974) and cyclopoid copepods such as *Acanthocyclops viridis* (Smyly 1970) or *Mesocyclops edax* (Confer 1971; Brandl and Fernando 1975;

Acknowledgments

We thank Winfried Lampert, Stephen Wickham, and two anonymous reviewers for comments on an earlier version of the manuscript. Maren Volquardsen provided technical assistance.