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ROLE OF BACTERIA AS FOOD FOR ZOOPLANKTON IN A EUTROPHIC TROPICAL POND (IVORY COAST)

MARC BOUVY*, ROBERT ARFI, DANIEL GUIRAL, MARC PAGANO and LUCIEN SAINT-JEAN

KEYWORDS: bacteria; zooplankton; grazing; tropical pond; eutrophic.

ABSTRACT

The aim of the study was to determine whether bacteria could be a substantial source of carbon for zooplankton and whether the grazing pressure of these metazoan filter-feeders could influence the fate of bacterial production. Eight grazing experiments using natural bacteria labelled with ^3H thymidine were conducted in a tropical pond (Ivory Coast) during various phases of biological colonization (rotifer-dominated and copepod-dominated phases of the colonization). Higher grazing and clearance rates were observed with rotifers *(Brachionus plicatilis* and *Hexarthra intermedia*), while very low values were obtained when the cyclopoid copepod *Apocyclops panamensis* was dominant. Less than 1% of the bacterial production was harvested when copepods were dominant, while *B. plicatilis* consumed up to 36% of this production. However, this consumption of bacteria appeared to contribute only to an insignificant proportion of the daily carbon intake (*e.g.* 0.9 to 7.1% of body carbon for rotifers). The low contribution of bacteria in the nutrition of zooplankton is discussed in terms of their cell size and their relative abundance in the total amount of seston available.

INTRODUCTION

The fate of bacterial production in aquatic ecosystems remains a problematic question because microorganisms generally present high growth rates coupled with low variations of abundance. The various loss factors (grazing, sedimentation, lysis, viruses, antibiotics; see PACE, 1988) may approximately equal bacterial growth (RIEMANN, 1985; GUDE, 1986; SCAVIA and LAIRD, 1987; TORRETON, 1991). Protozoa, mainly heterotrophic microflagellates (SHERR and SHERR, 1984), are often reported to be responsible for the control of bacterial numbers. Direct ingestion by various metazoans such as rotifers, appendicularia, salpa, cladocera and copepods has also been demonstrated in several studies (POURRIOT, 1977; MULLIN, 1983; PACE *et al.*, 1983; RIEMANN, 1985; GUDE, 1989; BOON and SHIEL, 1990; TURNER and TESTER, 1992). However, the trophic role of bacterioplankton remains unclear, especially their contribution to the diet of suspension-feeding zooplankters. Studies tend to conclude that bacterioplankton can be important as food (*e.g.* STARKWEATHER *et al.*, 1979; CAUMETTE *et al.*, 1983; BERN, 1987; WYLIE and CURRIE, 1991) or insignificant (*e.g.* FORSYTH and JAMES, 1984; SEAMAN *et al.*, 1986). Furthermore, only a few studies have adressed relationships between bacteria and filter-feeders in tropical or subtropical ecosystems (CAUMETTE *et al.*, 1983; HART and JARVIS, 1993).

In this study, we report the results of zooplankton grazing experiments based on native bacteria labelled with ^3H-thymidine. These experiments were performed for the different zooplanktonic organisms found during the progressive colonization of a tropical aquaculture pond. This colonization was characterized, as in other cases known (ARFI *et al.*, 1991; GUIRAL *et al.*, 1994), by the successive dominance of protozoans, rotifers and crustaceans. When rotifers are a major component

of plankton, they may likely exert significant grazing pressure on bacterial populations (*e.g.* RIEMANN, 1985; BOON and SHIEL, 1990). It is clear that bacterial cells (excluding viruses) represent the lowest size of edible biological material but all zooplanktonic organisms have not the ability to remove these particles. Our objectives were to evaluate (1) the validity of the ^{3}H-thymidine method to assess zooplankton ingestion rates on bacteria (2) the contribution of bacteria as food resource for zooplankton, and (3) the importance of grazing in the fate of bacterial production in a eutrophic tropical pond.

MATERIAL AND METHODS

The experiments were carried out in April 1990 at the experimental aquaculture station of Layo, near the Ebrié lagoon (Ivory Coast; 5°N, 5°W), where ponds are used for fish rearing (BONOU, 1990; LEGENDRE *et al.*, 1987). The pond used in this study (around 600 m^2, 0.5 m to 1 m depth) was emptied, limed, and progressively self-filled with oligohaline ground water and rainfall (GUIRAL *et al.*, 1994).

Eight grazing experiments were performed during the subsequent colonization of the pond, which was characterized by the successive appearance and dominance of two rotifers, *Brachionus plicatilis* and *Hexarthra intermedia*, and a cyclopoid copepod, *Apocyclops panamensis* (see GUIRAL *et al.*, 1994). Thus, these different taxa were investigated more or less separately in the successive experiments (see experimental schedule in Table 1). The species, *B. plicatilis*, was largely dominant (>90% of the total number) during experiments 1 and 2, while both rotifer species were present in almost similar proportions during experiments 3 and 4. The copepod, *A. panamensis*, was dominant in experiments 5 and 6, with a predominance of the youngest stage (55%) in experiment 5. In experiment 6, a pre-sieving of natural subsamples with a 200 µm mesh was conducted in order to select adult stages. After filtration, the zooplanktonic community was represented by 99% of adults. In the experiments 7 and 8, a mixed distribution of rotifers and copepods (mainly young stages) occurred.

Labeling of bacterioplankton.

Subsurface water samples were collected with sterile bottles. In order to remove grazers, water was immediately filtered through a 3 µm pore-size Nuclepore membrane. Tritiated methylthymidine (118 Ci mmole^{-1} specific activity, CEA) was added to a final concentration of 5 nM. Samples were incubated for 2 h in the dark at 30°C (close to pond temperature).

Table 1. Total density (ind l^{-1}) of zooplanktonic taxa and their relative densities (%) in experimental jars and concentration factors (CF = ratio between density in the jar and density in the pond). Bp= *Brachionus plicatilis*, Hi= *Hexarthra intermedia* and N, C, Ad.= nauplii, copepodites and adults of *Apocyclops panamensis*, respectively.

Exp.	Date	Total density	Rotifers		*Apocyclops panamensis*			CF
			Bp	Hi	N	C	Ad	
1	09/04	53020	99.4	0.5	0.1	0.0	0.0	4.1
2	11/04	70280	91.2	8.5	0.2	0.1	0.0	3.0
3	13/04	126750	38.2	60.4	0.8	0.5	0.1	5.4
4	13/04	201370	47.8	50.2	1.7	0.2	0.0	7.3
5	18/04	145410	14.5	30.1	42.3	13.1	0.0	14.5
6	21/04	27030	0.5	0.0	0.0	1.0	98.5	10.3
7	24/04	124380	60.0	0.1	27.9	9.6	2.3	23.5
8	24/04	46560	54.6	0.3	36.9	5.7	2.5	0.6

Incubation with zooplankton

Zooplankton was collected using a bucket, concentrated with a 63 µm sieve and then rinsed with 0.22 µm filtered pond water. One subsample was preserved with buffered formalin (5%) for enumeration of the organisms. A second subsample was collected on a Whatman GF/F filter for subsequent carbon analysis (CHN LECO analyzer) in order to estimate the mean individual carbon weight of the zooplankton (Wi). A third subsample (3 ml) was transferred into each experimental jar (200 ml) containing 50 ml of 0.22 µm filtered pond water. After a dark acclimation for 30 min, the labelled bacterioplankton (15 ml) was put into experimental jars and the feeding experiments were started. Bacterial densities in the jars were diluted by a factor of 0.22 (ratio between volume of bacterioplankton and total volume) comparatively with abundances in the pond. Densities of zooplankton in the jars ranged between 3 and 15 times the natural abundance (Table 1) except for experiment 7 (24 times). Although some studies have demonstrated that a 20-40 fold concentration of zooplankton can reduce grazing rates by a factor of 2 to 3 (GRIFFITH and CAPERON 1979; BJORNSEN *et al.*, 1986), RIEMANN and BOSSELMANN (1984) assumed that grazing rates were not modified by the experimental zooplankton concentration.

For each experiment, incubations were performed for 0, 10, 20 and 60 min at 30°C on a rotating apparatus (50 r.p.m.), which prevented sedimentation of particles. Time-zero incubations were used as controls. Incubations were stopped

by filtering the water through a 63 µm mesh net to retain the grazers, which were then collected on GF/F filters. These filters were immediately transferred to scintillation vials and organisms retained on filters were dissolved for at least 48 h at 50°C by adding a tissue solubilizer (0.5 ml; Soluene, Packard). Aliquots of the filtrate were filtered on 0.22 µm cellulose nitrate filters (Whatman) in order to estimate the ^3H activity in intact bacterial cells. Filters were rinsed with 0.22 µm filtered pond water and placed in scintillation vials. All the labelled samples (*i.e.* zooplankton and bacteria) were radioassayed after addition of scintillation cocktail (10 ml, Ready Safe, Beckman). Before counting, the vials were stored for at least 24 h in the dark to allow decay of chemoluminescence caused by the alkaline solubilizer. During five grazing experiments, some incubations were performed in duplicates and thus we have 9 duplicates among the 24 measurements (3 grazing periods and 8 experiments). Variability of the duplicate measurements varied between 0.5% and 12.8% demonstrating coherent variations between them.

Several assumptions and conditions (see details in GUDE, 1986; BJORNSEN *et al.*, 1986) were necessary to obtain valid grazing rates (see below) . Advantage of the method using labeling with ^3H thymidine for estimating ingestion on natural bacteria is its specificity for bacteria under some conditions (*e.g.* label concentration, incubation time). These conditions were satisfied in our experiments and it is likely that only bacteria are labelled (FUHRMAN and AZAM, 1982). An other assumption is based on the incorporation of labelled thymidine into macromolecules and then the radioactivity might be metabolically conservative. It is the case in our study with incubations stopped by dilution with non-labelled thymidine to a final concentration of 10 mM. No change in label incorporation rate was detected in bacterial assemblages during incubation with zooplankton (unpublished data). An other condition is the active uptake by grazers of labelled bacterial cells. Incubations with CO_2-narcotized zooplankton were performed once to estimate the passive absorption of label by animals. No significant increase of radioactivity was detected (+4% between t_0 and t_{60}). Thus, incorporation of radioactivity was due only to feeding on bacteria since no uptake of soluble radioactive thymidine or passive absorption by zooplankton occurred.

Clearance and ingestion rates

Clearance rates, defined as ml of water cleared of bacterioplankton per individual per hour, were calculated as the clearance of ^3H labelled bacterioplankton from the formula:

$$CR = (DPM\ Z_t - DPM\ Z_0)/(DPM\ B * n * t)$$

where:
DPM Z_t is zooplankton activity after incubation time
DPM Z_0 is zooplankton activity at t_0
DPM B is activity of bacterioplankton per ml
n is number of grazers
t is incubation time (h).

Ingestion rates of zooplankton (expressed as µgC µgC^{-1} h^{-1}) were calculated from clearance rates (CR) using the formula:

$$I = (CR * Cb)/(Wi * 0.22)$$

where:
Cb is carbon biomass of bacteria in the jars,
Wi is individual weight of zooplankton (µg C ind^{-1})
0.22 is dilution factor (see above).

We also expressed the ingestion rate as percentage body carbon per day by multiplying the result by 24 and by 100.

The population ingestion rate expressed as µg C l^{-1} h^{-1} was obtained by multiplying the ingestion rate by the zooplanktonic biomass in the pond.

Bacterial biomass and production, sestonic biomass

For each incubation period, bacterial concentrations were estimated from subsamples fixed with buffered formalin (5% final concentration). Enumeration of total and free-living bacteria was made by epifluorescence microscopy after staining with DAPI (PORTER and FEIG, 1980). Biomasses were estimated from biovolume determinations (up to 100 cells) made from photographic slides and a digitizing table. Biovolumes were calculated assuming a spherical form for cocci and a cylindrical form for all the other cell types. Biovolumes were converted to biomasses using a factor of 0.2 pg C µm^{-3} (SIMON and AZAM, 1989).

Thymidine incorporation was measured from *in situ* water samples (TORRETON and BOUVY, 1991) according to the procedure described by FUHRMAN and AZAM (1982). Thymidine incorporation (nM h^{-1}) was converted into bacterial production (µg C l^{-1} h^{-1}) using a factor of $0.65*10^9$ cells nmole^{-1} obtained from our own dilution-growth experiments and using a carbon/volume factor of 0.2 pg C µm^{-3} (SIMON and AZAM, 1989).

Sestonic biomass was estimated for each experiment from carbon analyses (CHN LECO analyzer) of 50 ml subsamples collected on precombusted (550°C) GF/F filters.

Table 2. Partition of ^3H label in the grazing experiments after incubation. Radioactivity expressed in k DPM per incubation jar. Radioactivity in zooplankton (ZP). Recovery (%) by zooplankton. Clearance rate expressed in ml per zooplankton community per jar per hour. *: final concentration of labelled Thymidine = 10 nM.

EXP.	Initial k DPM	Time min	Final k DPM	Init-Final k DPM	ZP k DPM	Recovery %	Clearance ml jar^{-1} h^{-1}
1	2282	10	2224	58	35.1	60.3	10.31
		20	2018	264	74.3	28.0	10.90
		60	1714	568	158.5	27.8	7.75
2*	16200	10	15369	831	90.3	10.8	3.40
		20	13716	2484	188.4	7.6	3.55
		60	11727	4473	277.6	6.2	1.74
3	3764	10	3309	456	35.1	7.7	4.30
		20	3141	623	74.2	11.9	4.54
		60	2400	1364	194 1	14.2	3.97
4	3359	20	2959	400	66.4	16.5	4.01
		60	2692	667	148.7	22.2	3.00
5	2925	20	2788	137	0.6	0.5	0.04
		60	2767	158	1.3	0.8	0.03
6	971	10	869	102	0.6	0.6	0.25
		20	900	71	1.2	1.7	0.25
		60	908	63	2.2	3.5	0.15
7	3200	10	3112	88	10.5	11.9	1.34
		20	3071	129	21.9	17.0	1.40
		60	3000	200	47.8	23.9	1.02
8	2557	10	2530	27	4.9	18.1	0.78
		20	2507	50	7.3	14.6	0.58
		60	2433	124	14.8	11.9	0.39

RESULTS AND DISCUSSION

Validity of the grazing rate estimations

In order to evaluate the loss of label not due to grazing, the recovery of label was calculated for each experiments (Table 2). Theoretically, radioactivity measured in grazers should equal the difference in radioactivity between the initial and final suspensions. This recovery was low in some cases, but was not linked to the clearance rate. Several reasons have been proposed by NAGATA and OKAMOTO (1988) for this low recovery, of which the self absorption of beta-emittance by animal bodies was thought to be the most important.

Time courses were conducted for each experiment in order to determine appropriate incubation periods. Clearance rates were calculated for each incubation time (Table 2). In most cases, label was linearly incorporated up to 20 min incubation time, meaning theoretically that the ^3H label was neither defaecated nor respired by the grazers. Between 20 and 60 min, the label uptake showed always a decrease. Thus, clearance rates were calculated from radioactivity determined at 20 min. In two experiments (3 and 7), linearity was not observed and clearance rates were calculated as the mean radioactivity in the 10 and 20 minute experiments. Loss of label was also minimized by processing the grazers immediately after the incubation.

Table 3. Mean bacterial cell volume (MCV) and equivalent spherical diameter (ESD), density of bacteria and carbon biomass of bacteria (Cb) and sestonic particles (Cp) in the jars, and Cb/Cp-ratio. Bacterial densities and biomass in the pond can be obtained by dividing the data in this table by 0.22 (dilution factor in experimental jars; see methods).

Exp	MCV µm^3	ESD µm	Density 10^6 ml^{-1}	Cb µgC ml^{-1}	Cp µgC ml^{-1}	Cb/Cp %
1	0.034	0.40	7.51	0.051	1.28	4
2	0.035	0.41	6.31	0.044	0.51	9
3	0.058	0.48	2.79	0.032	0.37	9
4	0.052	0.46	2.83	0.029	0.40	7
5	0.064	0.50	4.30	0.055	0.74	7
6	0.050	0.46	3.11	0.031	2.22	1
7	0.042	0.43	2.32	0.019	1.64	1
8	0.061	0.49	4.01	0.049	0.99	5

Several authors (*e.g.* GUDE, 1989) have stated that a bacterial threshold at concentrations close to 10^6 cells ml^{-1} is necessary to obtain a grazing effect. In our feeding experiments, bacterial numbers ranged between $2.32 \cdot 10^6$ and $7.51 \cdot 10^6$ cells ml^{-1} (Table 3), and were thus sufficient for detecting a grazing effect.

Despite the low recovery of label which seems to be typical of grazing experiments with thymidine, the short incubation times and the consequent relative stability in the availability of labelled food in the experimental jars can be considered as favourable conditions under which it is possible to make accurate estimates of clearance and ingestion rates. Thus, these rates were considered sufficient for comparing the feeding behaviour of zooplankton at the different colonization stages in the pond.

Clearance and ingestion rates

The highest clearance and ingestion rates were found when rotifers were dominant (experiments 1 to 4), the lowest rates when copepods were dominant (experiments 5 and 6; Table 4). Intermediate values were found for mixed assemblages. *B. plicatilis* displayed higher rates than *H. intermedia* (Table 4). The clearance rates for *B. plicatilis* (see Table 4) indicated that individuals ingested more in experiment 1 than in experiment 2. This fact can be related to the demographic characteristics. In experiment 1 there was a fast growing and a

Table 4. Clearance and ingestion rates of zooplankton in experimental jars, *in situ* bacterial production and estimation of the percentage of bacterial production removed by zooplankton grazing. After the experiment number the main taxa are indicated in parenthesis (symbols as in Table 1).

Experiment	individual weight µg C ind^{-1}	clearance rate µl ind^{-1} h^{-1}	specific ingestion %body C d^{-1}	population ingestion µgC l^{-1} h^{-1}	bacterial production µgC l^{-1} h^{-1}	product. grazed %
1 (Bp)	0.178	2.02	7.08	6.86	19.0	36.10
2 (Bp)	0.192	0.70	1.39	2.64	20.0	13.20
3 (Hi+Bp)	0.117	0.53	1.59	1.82	21.1	8.60
4 (Hi+Bp)	0.106	0.29	0.88	1.07	20.3	5.30
5 (N+C)	0.107	0.00	0.02	0.01	36.5	0.03
6 (Ad)	0.532	0.14	0.09	0.05	30.6	0.17
7 (Bp+N+C)	0.131	0.16	0.26	0.07	22.9	0.32
8 (Bp+N+C)	0.123	0.25	1.07	0.26	30.1	0.88

parthenogenetic population (1 to 3 eggs female^{-1}) with then high food carbon requirements, while in experiment 2, the population grew more slowly (0.1 egg ind^{-1}), with a mixture of parthenogenetic and sexual individuals (unpubl. data).

The clearance rates observed for *B. plicatilis* alone or mixed with *H. intermedia* (0.3 to 2 µl ind^{-1} h^{-1}) are comparable with values reported for other rotifer species fed on bacteria. STARKWEATHER *et al.* (1979) reported filtering rates of 0.1 to 1 µl ind^{-1} h^{-1} for *B. calyciflorus* fed on a pure culture of bacteria or on mixtures of bacteria and yeast. In a eutrophic lake, SANDERS *et al.* (1989) reported clearance rates of 0.06 to 0.29 µl ind^{-1} h^{-1} for *Hexarthra sp.* With *Keratella cochlearis*, BOGDAN and GILBERT (1982) reported values of around 1 µl ind^{-1} h^{-1} whereas BJORNSEN *et al.* (1986) reported higher rates (4 µl ind^{-1} h^{-1}). Our results were also similar to the lowest values reported for *Brachionus* species fed on algae (1.5 to 2.5 µl ind^{-1} h^{-1}) at concentrations higher than 5 µg C ml^{-1} (from NAGATA, 1989, ROTHHAUPT, 1990). Thus, filtering rates calculated here for *B. plicatilis* at low bacterial biomasses (0.03 to 0.5 µg C ml^{-1}) were comparable with those found for rotifers fed with either algae at high concentrations or bacteria over a large range of concentrations. These observations agree with the general tendency for rotifers to diminish their clearance rates with increasing concentrations, or with decreasing sizes of food particles, or both (STARKWEATHER *et al.*, 1979; ROTHHAUPT, 1990). In this scheme, bacteria, which represent the lowest size of edible particles, are grazed at a low and constant clearance rate. In contrast, TURNER and TESTER (1992) found very high clearance rates (32 to 418 µl ind^{-1} h^{-1}) for B. *plicatilis* fed on fluorescently labelled bacteria (*E. coli*). However, their values were perhaps atypically high, due to the large bacterial cell volume (0.7 µm^3).

The values found for *A. panamensis* (*c.* 0 and 0.14 µl ind^{-1} h^{-1}; Table 4) are lower than those reported by PEDROS-ALIO and BROCK (1983) for cyclopoid copepods (copepodites and adults) fed on natural bacteria (0.1 to 1.1 µl ind^{-1} h^{-1}). These low values confirm that freshwater copepods are generally inefficient bacteria feeders, as stated by several authors (PEDROS-ALIO and BROCK, 1983; NAGATA and OKAMOTO, 1988; SANDERS *et al.*, 1989; WYLIE and CURRIE, 1991).

Role of bacteria as food for zooplankton

Since the clearance rates of rotifers on bacteria appeared fairly constant regardless of the bacterial biomass available (see STARKWEATHER *et al.*, 1979 and discussion above), we assumed that the experimental values (Table 4) gave good estimates of *in situ* clearance rates. We made the same assumption for copepods. On this basis, we calculated *in situ* ingestion rates from the experimental clearance rates and from *in situ* bacterial biomass (see methods). The daily ingestion rates (0.02 to 7.08% of body carbon; Table 4) were too low to insure at least their energy requirement for respiration (20 to 65% for rotifers cited in POURRIOT *et al.*, 1982). Thus bacteria did not appear to be a significant source of carbon for the zooplankton in our study. This result differs from several other studies which have shown that free living bacteria could act as a significant source of carbon for metazoans. GLIWICZ (1969) estimated that natural bacteria could represent 20 to 70% of the diet of zooplanktonic communities from various lakes. WYLIE and CURRIE (1991) also observed that natural bacteria could play sometimes a significant trophic role as food in

zooplankton dominated by cladocerans. Other studies have shown that pure bacterial cultures could be used successfully for rearing rotifers in experimental conditions (STARKWEATHER *et al.*, 1979; SEAMAN *et al.*, 1986), indicating that bacteria have a potentially high nutritive value for zooplankton.

Whatever the complexity of the behavioural phenomena involved in the collection of food particles (see STARKWEATHER *et al.*, 1979; ROTHHAUPT, 1990), two main reasons can be advanced to explain the poor trophic contribution of bacteria in our study. Firstly, their small size (0.4-0.5 μm Equivalent Spherical Diameter) and the high percentage of free-living bacteria (close to 70% of the total bacterial number) probably led to low collecting efficiencies by rotifers and copepods, as discussed by STARKWEATHER *et al.* (1979) and NIVAL and NIVAL (1976), respectively. Secondly, the bacterial biomass (0.02 to 0.5 μg C ml^{-1}; Table 3) was low compared with other potential food sources (*i.e.* organic seston available in the pond). In absolute terms, the bacterial biomass was considerably lower than the concentration required to obtain substantial ingestion of bacterial cultures by rotifers (STARKWEATHER *et al.*, 1979; SEAMAN *et al.*, 1986). Furthermore, bacterial biomass as used here represented only 1 to 9% of the total sestonic material in the experimental jars (Table 3). This proportion was even lower in the pond than in the jars because seston size was limited to 0.9-3 μm size fractions (see methods). WYLIE and CURRIE (1991) considered that bacteria can only be a significant source of food for crustacean zooplankton (cladocerans) if their density was similar to that of the algal biomass. But recently, HART and JARVIS (1993) reported a very low contribution (<3%) of bacteria in the total carbon intake of cladocerans in a hypertrophic (large concentration of edible algal particles) subtropical reservoir. In the pond, bacterial biomass only equalled 10% of the algal biomass, and, therefore, zooplankton could have covered their daily energy requirement from the larger sestonic components (*i.e.* protozoa and phytoplankton). Bacterioplankton, which was less accessible to filter feeders, played only a minor role in the nutrition of metazoa. Thus, as discussed by WYLIE and CURRIE (1991), one important question to be investigated in the context of the trophic significance of bacteria to zooplankton concerns the definition of ecological situations (*i.e.* biotope, trophic level, temporal evolution, etc.) which lead to the co-occurrence of high densities of bacteria and efficient bacteria-feeders (*i.e.* rotifers and cladocerans).

Fate of in situ bacterial production

When comparing the zooplanktonic population ingestion rates to the rates of *in situ* bacterial production, it was obvious that bacterial production was only controlled to a significant extent by grazing when *B. plicatilis* was dominant in experiments 1 and 2 (36.1% and 13.2%, respectively). In enclosures with fish, RIEMANN (1985) demonstrated that rotifers may become a major component in the zooplanktonic community and this author concluded that these small grazers were responsible for the disappearance of more than 50% of the bacterial production. When *A. panamensis* dominated the zooplankton community (experiments 5 and 6), the percentage of bacterial production grazed by metazoan zooplankton was very low (0.03 to 0.17%). In Lake Mendota, PEDROS-ALIO and BROCK (1983) also observed that a low percentage (1-10%) of the bacterial production was grazed by zooplankton. They suggested that most of the bacterial biomass was controlled through sedimentation of attached cells. In our study, this type of control could explain also a part of the fate of bacterial production due to the proportion of attached bacteria (close to 30%; unpubl. data). Grazing by heterotrophic microorganisms (ciliates >10 μm), which are dominant bacterioplankton consumers, was an other important factor contributing to the loss of bacteria. A peak of ciliate abundance was noted in our investigation just before the rotifer-dominated phase of colonization (GUIRAL *et al.*, 1994). Indeed, some studies in freshwater environments have demonstrated that protozoa (flagellates and ciliates) could be the major grazers of bacteria (BEAVER and CRISMAN, 1989; SANDERS *et al.*, 1989). Thus, during the early stages of the pond colonization, it appears likely that bacterial production would have been consumed indirectly by zooplankton via the flux of carbon through heterotrophic protozoa and the microbial loop. Thus it is possible that the relevance of bacterial production to zooplankton is probably far greater than indicated by our grazing study. This hypothesis supports the idea that ciliates act as a link between bacterioplankton and higher trophic levels. Future research will be focused on these relations in order to quantify interactions between all the trophic levels within the pond.

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Address of the authors.

Centre de Recherches Océanologiques, ORSTOM, 29 rue des Pêcheurs, BP V18, Abidjan, COTE D'IVOIRE.

* Corresponding author; present address: ORSTOM/HEA, B.P. 5045, 34032 Montpellier Cedex 1, France.