

# Functional structure of microbial food web in the Senegal River Estuary (West Africa): impact of metazooplankton

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*We studied the impact of various metazooplanktonic predators (two calanoid copepods and barnacle larvae) on microbial plankton isolated from the Senegal River Estuary. Experiments performed in microcosms were based on size-class fractionation (3, 12, 20 and 60  $\mu\text{m}$  size classes) of the microbial community in order to obtain different assemblages, including bacteria, heterotrophic nanoflagellates (HNF), ciliates, pico- and nanophytoplankton. Removal of bacterial predators  $>3 \mu\text{m}$  increased the net growth rate of heterotrophic bacteria from  $0.437 \text{ day}^{-1}$  in the presence of all predators (fraction  $<60 \mu\text{m}$ ) to  $0.682 \text{ day}^{-1}$  (fraction  $<3 \mu\text{m}$ ). Removal of protozoa  $>12 \mu\text{m}$  caused an increased net growth rate of flagellates from  $0.102 \text{ day}^{-1}$  (fraction  $<60 \mu\text{m}$ ) to  $0.332 \text{ day}^{-1}$  (fraction  $<12 \mu\text{m}$ ). The same results were also observed on protozoa dynamics in the presence of *Temora stylifera* ( $0.498 \text{ day}^{-1}$ ), suggesting a trophic control of the protozoa  $>12 \mu\text{m}$  by this copepod, allowing an increase in HNF growth rates through indirect effect. The highest ingestion rate over 60 h of experiment was recorded for the copepod *Temora* ( $61.1 \mu\text{m}^3 \text{ ngC}^{-1} \text{ h}^{-1}$ ) and the lowest value for barnacle larvae ( $25.6 \mu\text{m}^3 \text{ ngC}^{-1} \text{ h}^{-1}$ ). An intermediate value ( $47.5 \mu\text{m}^3 \text{ ngC}^{-1} \text{ h}^{-1}$ ) was recorded for the copepod *Acartia clausi*. All these ingestion rates were mainly channeled in the size classes between  $<3$  and  $15\text{--}18 \mu\text{m}$  equivalent spherical diameter (ESD) ( $6$  and  $2435 \mu\text{m}^3$ , respectively), with the highest values noted between  $<3$  and  $6\text{--}9 \mu\text{m}$  classes. In the presence of *Temora*, the cascading effects can explain both the decreases in mean volume and in growth rate for bacteria, due to the filtration of ciliates by the zooplanktonic predator, allowing the development of the HNF. The presence of a large size class of bacteria (among the five classes identified by flow cytometry) and a high abundance of HNF suggest an efficient heterotrophic pathway within the microbial loop of this sub-saharan estuary.*

## INTRODUCTION

Planktonic food webs can be dominated by herbivorous, multivorous or microbial webs according to the relative abundance and trophic status of their components (Legendre and Rassoulzadegan, 1995). The microbial components of aquatic food webs can be divided into two main size classes: (i) picoplankton ( $<2 \mu\text{m}$ ) including autotrophic (prokaryotic and eukaryotic phytoplanktonic cells) and heterotrophic micro-organisms (bacteria and small flagellates), and (ii) nanoplankton (between  $2$  and  $20 \mu\text{m}$ ) with nanophytoplankton, large flagellates and small ciliates. Flagellates and ciliates are the most important picoplanktivorous

protozoa in most aquatic environments (McManus and Fuhrman, 1988; Sanders *et al.*, 1992), and they may affect the size structure of bacterial assemblages (Jürgens and Güde, 1994; Jürgens and Matz, 2002). On the other hand, ciliates are a major cause of mortality for bacterivorous flagellates (Azam *et al.*, 1983; Ducklow, 1983). Zooplankton are an important link in herbivorous and multivorous webs, grazing on and controlling plankton species ( $<20 \mu\text{m}$ ) and being in turn the major food sources of the zooplanktivorous fish in the upper trophic level (Stibor *et al.*, 2004). Thus, the protozoa community can be considered as a major trophic link between bacteria and larger zooplankton in many aquatic food webs.

Some studies indicated that there is a tightly size-structured predator–prey coupling within the nano-planktonic food chain with at least three trophic levels (Wikner and Hagström, 1988; Sherr *et al.*, 1992). Direct predation limitation has been defined as the degree to which the *per capita* growth rate is decreased by predation (Osenberg and Mittelbach, 1995). Indirect effect of predation can be defined as trophic cascades, based on predation limitation on several trophic levels (Paine, 1980). However, many studies demonstrate that such interactions seem to be lacking (Güde, 1988; Sanders *et al.*, 1992; Calbet *et al.*, 2001) because of two main reasons: (i) many preys are resistant to predators, and thus predators are unable to reduce the abundance of the preys enough for a cascade to occur, (ii) the existence of omnivory, with a grazing pressure on more than one trophic level by the same predator (Polis and Strong, 1996). To demonstrate the occurrence of trophic cascades, it may therefore be necessary to perform experiments in which all trophic levels are studied, not only within the microbial food web but also including the upper trophic levels such as zooplanktonic predators. Indeed, many authors have recently considered copepods as a switch between alternative trophic cascades along different food chains in marine pelagic systems (Stibor *et al.*, 2004; Calbet and Saiz, 2005).

In previous papers (Troussellier *et al.*, 2004, 2005), we showed that the Senegal River estuarine waters were characterized by a very abundant and active bacterial community and a large abundance of picophytoplanktonic cells. Concomitant studies on zooplankton community revealed a dominance by two calanoid copepods (*Acartia clausi* and *Temora*, mainly *Temora stylifera*) and Cirripedia larvae (barnacle) belonging to the genus *Balanus* (Pagano *et al.* in press; Champalbert, personal communication). Because of their particular feeding modes, these zooplanktonic species may produce different effects on the microbial food web by consuming simultaneously or/and alternatively algae and ciliates.

In this study, we aimed to identify the main food links between autotrophic and heterotrophic micro-organisms in the Senegal River Estuary (West Africa), and thus we assembled experimentally artificial food webs with typical representatives of the estuarine planktonic community. On the basis of fractionation by size classes of the different groups of the microbial food web (bacteria, flagellates, ciliates, pico- and nanophytoplankton), the specific goals of this paper were (i) to study the direct predator–prey relationships inside the microbial food web, (ii) to identify competitive interactions within three trophic food chains (depending on the metazooplanktonic predator) and (iii) to examine possible indirect effects of predation such as trophic cascades.

## METHODS

The sampled area of the Senegal River Estuary is situated between 16°02'N and 15°54'N (Troussellier *et al.*, 2004, 2005). Experiments were conducted on 21 May 2002 at the end of the dry season, simultaneously with the experiments based on bacterioplankton dynamics using a mesocosm approach (Troussellier *et al.*, 2005). An integrated water sample issued from a mesocosm filled with estuarine water was collected and transported to the laboratory. The water was filtered immediately through 60 and 20 µm mesh-sized sieves, and 12 and 3 µm Nuclepore polycarbonate filters with a sterilized Nalgene filtering device. The <60, <20 and <12 µm fractions were filtered by gravity, and the <3 µm fraction was filtered with a vacuum <2 cm Hg (bottles B1–B4). Zooplanktonic organisms were directly collected from the mesocosm with a 200 µm plankton net. Then copepods and barnacle larvae were identified, counted under a dissecting microscope and poured at the beginning of the experiment into the bottles B5, B6 and B7 containing <60 µm filtered water.

Seven experimental bottles (500 mL, acid cleaned and rinsed) were incubated for 60 h in controlled conditions (temperature close to *in situ*: 24°C; light–dark cycle 12:12 h in attenuated light conditions: 10 µmol m<sup>-2</sup> s<sup>-2</sup>) and placed on a rotating wheel (1 rpm). The light value used for incubation corresponded to the *in situ* value measured at 5 m depth (using a Li COR 193 spherical quantum sensor) where metazooplankton was mostly concentrated during the daylight because of its diel vertical migration. Zooplanktonic predators and expected microbial components were disposed according the experimental procedure:

- (i) B1: water filtered under 3 µm (bacteria and small flagellates; picophytoplankton)
- (ii) B2: water filtered under 12 µm (bacteria and all flagellates; pico- and nanophytoplankton)
- (iii) B3: water filtered under 20 µm (bacteria and flagellates and small ciliates; phytoplankton)
- (iv) B4: water filtered under 60 µm (bacteria, flagellates and all ciliates; phytoplankton)
- (v) B5: water filtered under 60 µm + *T. stylifera* (calanoid copepod)
- (vi) B6: water filtered under 60 µm + *A. clausi* (calanoid copepod)
- (vii) B7: water filtered under 60 µm + barnacle larvae (Cirripedia)

At each sampling time (0, 12, 24, 36, 48 and 60 h), subsamples in triplicate were preserved for further analyses.

By standard techniques using the fluochrome DAPI and epifluorescence microscopy, we have determined abundance and biomass of heterotrophic nanoflagellates (HNF) and bacterial biovolume. Bacterial abundance

was determined by flow cytometry. Mean bacterial and flagellate volumes were estimated by measurement of up to 100 cells using an Olympus DP 50 camera mounted on an Olympus BX 60 epifluorescence microscope equipped with a Plan Neofluar  $\times 100/1.3$  oil-immersion objective. Images were acquired from the camera (ANALYSYS software, Soft Imaging System, GmbH) housed in a computer. Bacteria were considered as basically shaped like cylinders with hemispheric ends, and their biovolume was estimated according to the formula described by Blackburn *et al.* (1998). Flagellate cell volumes were estimated by the equation of an ellipsoid. Ciliates were determined from Lugol's fixed samples by inverted microscopy at  $\times 200$ – $400$  magnification using the Utermöhl technique (1958). Biovolume of each identified taxon was estimated by assuming standard geometric shapes. Biomass of the different organisms was calculated on the basis of published conversion factors:  $200 \text{ fgC } \mu\text{m}^{-3}$  for bacteria (Simon and Azam, 1989);  $220 \text{ fgC } \mu\text{m}^{-3}$  for HNF (Borsheim and Bratbak, 1987); and  $190 \text{ fgC } \mu\text{m}^{-3}$  for ciliates (Putt and Stoecker, 1989).

Bacterial cells were enumerated using SYBR Green staining and flow cytometry according to the method described by Marie *et al.* (1997). Briefly, 1 mL formaldehyde-fixed sub-samples were directly incubated with SYBR Green (Molecular Probes, Eugene, OR, USA) at a final concentration of 2.5% (v/v) for 10–15 min at room temperature in the dark. For each sub-sample, three replicate counts were performed with a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA, USA) equipped with an air-cooled argon laser (488 nm, 15 mW). Stained bacterial cells, excited at 488 nm, were enumerated according to their right-angle light scatter (RALS) and green fluorescence (FL1) collected at 530/30 nm. These cell parameters were recorded on a four-decade logarithmic scale mapped onto 1024 channels. Fluorescent beads (0.94  $\mu\text{m}$ , Polysciences Inc., Warrington, PA, USA) were systematically added to each sample. Standardized RALS and FL1 values (cell RALS and FL1 divided by 0.94  $\mu\text{m}$  beads RALS and FL1, respectively) were used as an estimation of the relative size and nucleic acid content of bacterial cells, respectively (Troussellier *et al.*, 1999). Five bacterial cell populations (from the smallest size noted Bact-A to the largest noted Bact-E) were discriminated on the basis of their apparent size and nucleic acid content.

Phytoplankton counts were performed with the same cytometer. Cells excited at 488 nm were detected and enumerated according to their RALS properties, and their orange (585/42 nm) and red fluorescence (>650 nm) emissions related to phycoerythrin and chlorophyll pigments, respectively. Fluorescent beads (0.94 and 2  $\mu\text{m}$ ) were also systematically added to each sample. Cell

concentrations were calculated as for bacterioplankton. Two different phytoplanktonic groups (P1 and P2) were discriminated according to their fluorescence and scatter characteristics as described previously (Troussellier *et al.*, 1993). Acquisition and preliminary analysis of flow cytometric data were done using CellQuest software (Becton Dickinson).

To estimate the type of material (quantity and size of particles) filtered by metazooplankton, subsamples were collected and fixed with glutaraldehyde (2% final concentration) at the end of the experiment. Particle volume and size spectra determinations were performed with a Coulter Counter Multisizer II equipped with 70  $\mu\text{m}$  aperture tube. For each sample, twelve 0.5 mL replicates were analysed. Data above 25  $\mu\text{m}$  ESD were excluded because the variability between replicates was too high (CV% > 25%).

Metazooplankton organisms from each experimental bottle were transferred into 5% buffered formalin solution for subsequent enumeration and measurements to calculate the zooplankton carbon biomass, using length–carbon weight relationships from the literature (Mauchline, 1998 for copepods; Uye, 1982 for cirriped larvae). For copepods, adult, copepodite and nauplii stages were counted. For *Balanus* sp., nauplii and cypris larval stages were counted. Ingestion rates were calculated for each 3  $\mu\text{m}$  size fraction from the difference in particle volume (Coulter Counter measurement) between control (B4) and experimental (B5, B6 and B7) bottles at the end of the experiment. Ingestion rates were expressed as  $\mu\text{m}^3 \text{ ngC}^{-1} \text{ h}^{-1}$  knowing the individual carbon weight of organisms and also as  $\mu\text{gC L}^{-1} \text{ h}^{-1}$  using the sestonic carbon:volume ratio of 393  $\text{fgC } \mu\text{m}^{-3}$  obtained from LECO CHN analyser and Coulter counter measurements (data not shown).

Exponential growth was assumed for the different groups of micro-organisms, and the net growth rate ( $\mu$  expressed in  $\text{day}^{-1}$ ) was calculated according to the equation  $\mu = (\ln N_t - \ln N_i)/(t_t - t_i)$ , where  $N_t$  and  $N_i$  are the total abundance of cells at  $t_t$  and  $t_i$ , respectively. Grazing rates were calculated assuming that the initial increase of net growth rate, due to predator removal, was equal to the grazing rate in the free water mass. The initial growth rate was tested by linear regression (Sigma Stat). The non-parametric Kruskal–Wallis test was used for analysing differences in growth rates.

## RESULTS

### Initial distribution of organisms

At time zero, total bacterial abundance did not vary between the filtrations with a mean of  $1.57 \times 10^7$  cells  $\text{mL}^{-1}$  (CV = 4.2 %). Different types of bacteria in relation with their size (from SSC analyses obtained by flow

cytometry) were distinguished and followed along the experiment (Table I). The greatest proportion of cells was formed by the Bact-A class (~64% of the total number of cells) with the smallest estimated size. The Bact-B class was ~3% of total counts, while the class Bact-C was ~20%. The largest bacteria (size classes D and E) represented low percentage ~3–4% of total counts (Table I). However, bacterial number was reduced to 13% in the <3 μm treatment. Mean bacterial volume did not differ significantly ( $P < 0.01$ ) between treatments, with a value of  $0.099 \mu\text{m}^3$  ( $n = 554$ ,  $\text{CV} = 10.7\%$ ; Table II). Abundance of HNF was high, with a mean value of  $1.68 \times 10^5$  cells  $\text{mL}^{-1}$  ( $\text{CV} = 8.4\%$ ) for the >3 μm filtrations. In the <3 μm treatment, HNF abundance was reduced to 32%. HNF community was represented by organisms with a <5 μm maximum length. Their mean volume ranged between 0.95 and  $10.01 \mu\text{m}^3$  (mean =  $3.63 \mu\text{m}^3$ ;  $n = 76$ ,  $\text{CV} = 64.1\%$ ). Three distinct populations can be discerned following their size distribution; however, two main populations dominated the community. The smallest HNF formed a population, characterized by a mean volume of  $1.45 \mu\text{m}^3$  (min–max =  $0.95\text{--}2.07 \mu\text{m}^3$ ;  $\text{SD} = 0.33$ ), representing 33% of total

abundance. A medium-sized population, characterized by a mean volume of  $3.32 \mu\text{m}^3$  (min–max =  $2.15\text{--}5.68 \mu\text{m}^3$ ,  $\text{SD} = 0.86$ ), represented 49% of total abundance. These two populations, representing 82% of total abundance, were both characterized by a normal size distribution (Kolmogorov–Smirnov test). A third population, composed by the largest cells ( $>5.68 \mu\text{m}^3$ ), represented a smaller part of the total HNF community (18%).

Ciliates were totally excluded in the 3 μm filtration and reduced to 47% in the <12 μm filtration. A total of 39 cells  $\text{mL}^{-1}$  were raised for the >20 μm filtrations (Table I). Ciliates were represented by large organisms with a mean volume of  $4697 \mu\text{m}^3$  ( $n = 44$ , min–max =  $457\text{--}29\,452 \mu\text{m}^3$ ). The taxonomical composition was not detailed in this study, but the community was mainly dominated by aloricate oligotrich ciliates (*Strobilidium*, *Strombidium* and *Strombidiopsis*) and tintinnids (*Tintinnopsis*).

Phytoplankton abundance, as enumerated by cytometry, amounted to  $1.67 \times 10^5$  cells  $\text{mL}^{-1}$  in the <60 μm fraction and  $7.93 \times 10^4$  cells  $\text{mL}^{-1}$  in the <3 μm fraction, demonstrating a large proportion of small algal cells (Table I). Flow cytometry analysis of phytoplankton allowed us to

Table I: Initial distribution of the different types of micro-organisms in the fractions <3 μm, <12 μm, <20 μm, <60 μm, <60 μm + *Temora* (Z1), <60 μm + *Acartia* (Z2) and <60 μm + barnacle larvae (Z3)

	Total bacteria					Total phytoplankton				Ciliates	
	(cells $\text{mL}^{-1}$ )	Bact-A (%)	Bact-B (%)	Bact-C (%)	Bact-D (%)	Bact-E (%)	(cells $\text{mL}^{-1}$ )	P1 (%)	P2 (%)	HNF (cells $\text{mL}^{-1}$ )	(cells $\text{mL}^{-1}$ )
Z1	$1.63 \times 10^7$	63.9	2.9	20.2	2.8	0.4	$1.67 \times 10^5$	84.4	15.6	$1.74 \times 10^5$	39
Z2	$1.63 \times 10^7$	63.9	2.9	20.2	2.8	0.4	$1.67 \times 10^5$	84.4	15.6	$1.71 \times 10^5$	39
Z3	$1.63 \times 10^7$	63.9	2.9	20.2	2.8	0.4	$1.67 \times 10^5$	84.4	15.6	$1.84 \times 10^5$	39
60 μm	$1.63 \times 10^7$	63.9	2.9	20.2	2.8	0.4	$1.67 \times 10^5$	84.4	15.6	$1.84 \times 10^5$	39
20 μm	$1.68 \times 10^7$	61.2	5.4	19.8	3.6	0.4	$1.71 \times 10^5$	84.4	15.6	$1.56 \times 10^5$	38
12 μm	$1.45 \times 10^7$	74.6	4.1	23.1	3.3	0.4	$1.71 \times 10^5$	87.7	12.3	$1.45 \times 10^5$	20
3 μm	$1.45 \times 10^7$	69.8	3.5	17.6	1.7	0.0	$7.93 \times 10^4$	98.6	1.4	$1.16 \times 10^5$	0

Total bacteria, total phytoplankton, heterotrophic flagellates (HNF) and ciliates are reported for each fraction. Bacterial populations are discriminated by flow cytometry (Bact-A, Bact-B, Bact-C, Bact-D and Bact-E) and expressed in percentages of the total bacteria (cells  $\text{mL}^{-1}$ ). Phytoplanktonic populations are discriminated by flow cytometry (P1 as picophytoplankton and P2 as nanophytoplankton) and expressed in percentages of the total phytoplankton (cells  $\text{mL}^{-1}$ ).

Table II: Mean of bacterial volumes of each fraction at  $T_0$  and after 60 h

Time (h)	Fraction	3 μm	12 μm	20 μm	60 μm	Z1	Z2	Z3
0	mean	0.092	0.117	0.096	0.098	0.102	0.084	0.107
	n	121	75	74	54	86	57	87
60	mean	0.089	0.061	0.085	0.106	0.066	0.055	0.103
	n	41	48	46	72	86	53	37
<i>P</i> (t-test)		0.755	<b>0.011</b>	0.985	0.572	<b>0.008</b>	0.590	0.758

*n* is the number of measured bacterial cells. All values are expressed in  $\mu\text{m}^3$ . Fractions: <3 μm, <12 μm, <20 μm, <60 μm, <60 μm + *Temora* (Z1), <60 μm + *Acartia* (Z2) and <60 μm + barnacle larvae (Z3). Values in bold are <0.05 and are thus significant.

discriminate two main size classes (P1 and P2) according to their RALS and FL3 values. Algal cells belonging to group P1, whose abundance was not significantly affected by 3  $\mu\text{m}$  filtration, were considered as <3  $\mu\text{m}$  cells and defined as picophytoplankton. They represented 85% of total density in the <60  $\mu\text{m}$  fraction and 99% in the <3  $\mu\text{m}$  fraction. The P2 class, which was not detected in the <3  $\mu\text{m}$  fraction, represented a small percentage of total phytoplankton abundance (~15% for the fractions <20  $\mu\text{m}$ ) and was defined as nanophytoplankton.

The metazooplanktonic predators added to bottles (500 mL) at the beginning of the experiment were: 24 adults of *Temora* in B5; 26 adults of *A. clausi* in B6; 73 cirriped larvae in B7, representing 48, 52 and 146 ind.  $\text{L}^{-1}$ , respectively. Thus, zooplankton carbon biomass represented 338, 258 and 284  $\mu\text{C L}^{-1}$ , respectively, for *Temora*, *A. clausi* and cirriped larva (Table IV). These concentrations were comparable with the biomass recorded during daytime in the deepest layer near the bottom, where metazoans concentrate due to diel vertical migration (130–545  $\mu\text{C L}^{-1}$ ; Pagano, unpublished results).

### Growth and grazing rates of micro-organisms

Estuarine water was filtered to obtain four size groups of micro-organisms without zooplanktonic predators (B1–B4). These experiments, based on differential filtration, were performed to calculate the grazing rates of each component, assuming that the initial increase of net growth rate, due to predator removal, was equal to the grazing rate in the free water mass. The response period was determined as the initial period with an exponential growth of each component. Thus, growth rates were calculated over the first 24 h for heterotrophic bacteria, over the first 48 h for HNF and over the first 60 h for ciliates. An example of time series was illustrated from HNF abundance, demonstrating the choice of the 48 h incubation time for calculation of their growth rates (Fig. 1). For the phytoplankton, the computation was made over the first 12 h, because after this period all the growth rates decreased. Food web linkages in the microbial component of the plankton community are deduced from the comparison of the net growth rates of components (bacteria, flagellates, ciliates and phytoplankton) in the different treatments (Fig. 2).

#### Bacteria

The removal of large bacterivorous predators (fraction <3  $\mu\text{m}$ ) increased the growth rate of heterotrophic bacteria (0.682  $\text{day}^{-1}$ ; *t*-test,  $P = 0.05$ ) compared with the growth rates in the presence of all predators (fraction <60  $\mu\text{m}$ ; 0.437  $\text{day}^{-1}$ ). When ciliates and large flagellates were removed (12 and 20  $\mu\text{m}$  filtration), the

net growth rates were 0.540 and 0.454  $\text{day}^{-1}$ , respectively. The bacteria showed the same dynamics in the presence (Z1, Z2 and Z3) or absence (<60  $\mu\text{m}$ ) of zooplanktonic predators. From measurements obtained by epifluorescence microscopy at the end of the incubation time, statistically significant lower mean bacterial biovolume was observed in the fraction <12  $\mu\text{m}$  ( $P = 0.011$ ) in the presence of flagellates and in the presence of *Temora* as metazooplankton predators ( $P = 0.008$ ) (Table II).

Among the different bacterial populations, following their size obtained by flow cytometry, the class Bact-B (initially ~3% of total counts) showed the highest positive growth rates especially in the presence of predators >20  $\mu\text{m}$ . Lower positive growth rates were observed in the fractions <12 and <20  $\mu\text{m}$  in the presence of potential bacterial predators. Negative growth rates were observed for the class Bact-C (initially ~20% of total counts) with a proportion of cells decreasing to 6% of the total counts in the fraction <20  $\mu\text{m}$ . For the largest bacteria, class Bact-D showed positive and stable growth rates between treatments, whereas the rates of class Bact-E (with the lowest initial proportion, 0.4% of total counts) decreased in most treatments (Table III).

#### Heterotrophic nanoflagellates

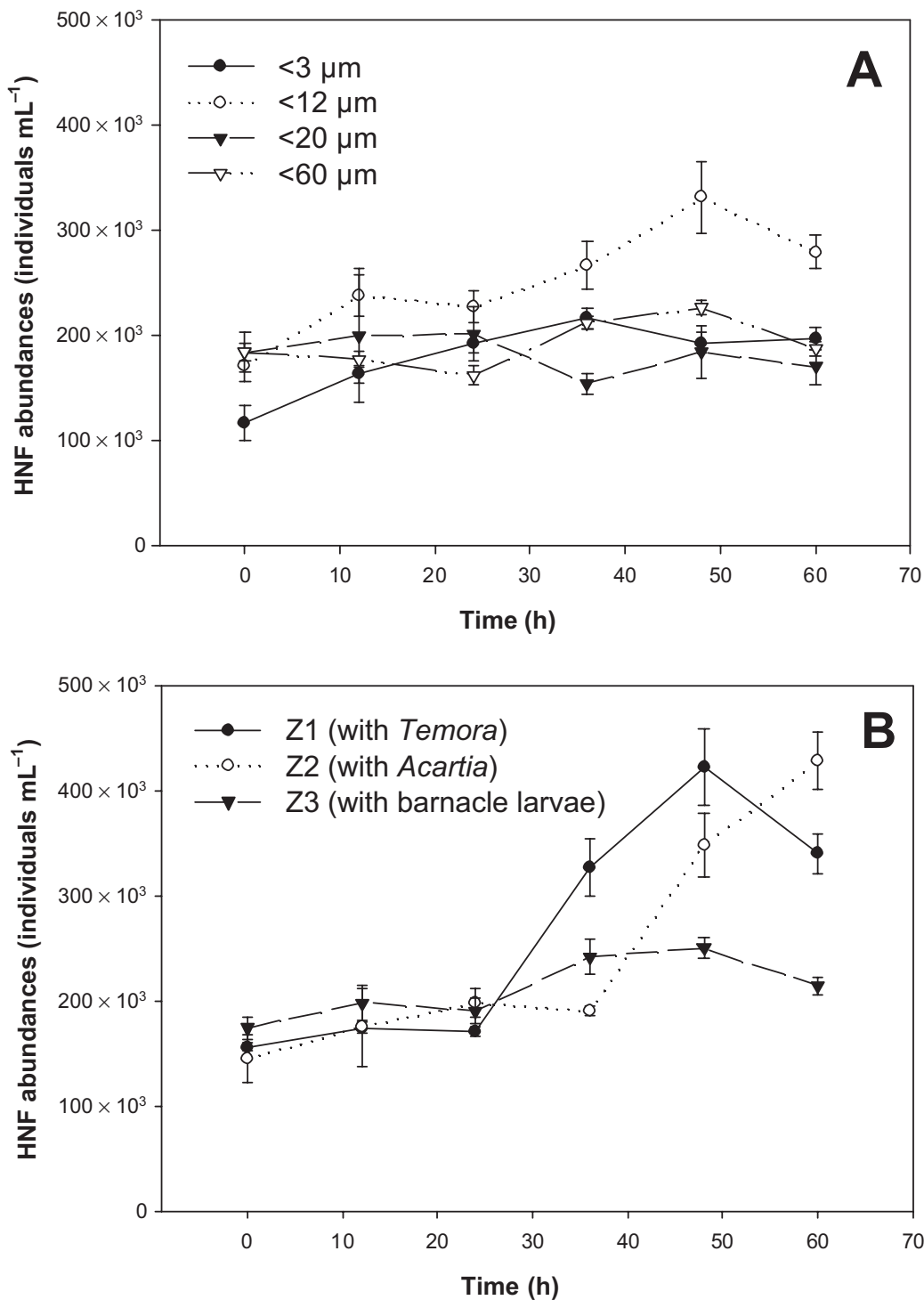
Removal of protozoa >12  $\mu\text{m}$  caused an increased net growth rate of HNF from 0.102  $\text{day}^{-1}$  (fraction <60  $\mu\text{m}$  with all protozoa) to 0.332  $\text{day}^{-1}$  (*t*-test,  $P = 0.03$ ). A growth rate increase was also observed in the presence of *Temora* (0.498  $\text{day}^{-1}$ ) or *Acartia* (0.437  $\text{day}^{-1}$ ), suggesting a removal of the protozoa >12  $\mu\text{m}$  by these two copepods (Fig. 2). However, in the fraction <20  $\mu\text{m}$  without large ciliates, growth rates were zero, suggesting that both growth and grazing rates were balanced by a substantial predation on HNF.

#### Ciliates

Positive net growth rates of ciliates were observed only in the presence of *Acartia* (0.191  $\text{day}^{-1}$ ) and barnacle larvae (0.051  $\text{day}^{-1}$ ), indicating a low direct impact of these predators on this microbial component (Fig. 2). However, in the presence of the calanoid copepod *Temora*, negative growth rate was noted (–0.290  $\text{day}^{-1}$ ), indicating that the growth of ciliates was highly limited by predation and grazed intensively by this type of metazooplankton predator (Fig. 2).

#### Phytoplankton

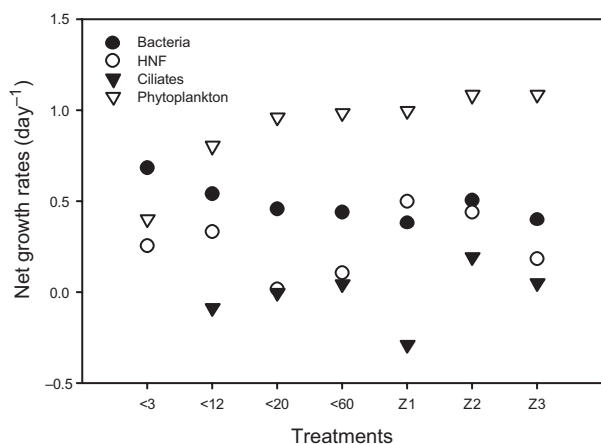
Phytoplankton abundance analysed by flow cytometry did not show significant statistical difference between the treatments (except the <3  $\mu\text{m}$  fraction; Table I), with the highest positive growth rates occurring during the first 12 h (Fig. 2). Indeed, lower growth rates were observed in the fraction <3  $\mu\text{m}$  (0.402  $\text{day}^{-1}$ ), contrasting with



**Fig. 1.** Time series of heterotrophic flagellate abundances (individuals mL<sup>-1</sup>) during the incubation period. **(A)** Abundances in the fractions <3, <12, <20 and <60 μm. **(B)** Abundances in the presence of metazooplankton: *Acartia*, *Temora* and barnacle larvae. Vertical bars show standard errors of three replicates per treatment.

the other rates (between 0.803 and 1.087 day<sup>-1</sup>). Positive growth rates of the main size class (P1) confirmed the results obtained for the total counts. The larger class

(P2) represented only a small percentage of total counts (between 12.3 and 15.6 % for the initial concentration). Negative growth rates in this class P2 occurred for all the



**Fig. 2.** Effects of removal of different size classes of predators on net growth rates of heterotrophic bacteria, heterotrophic flagellates, ciliates and phytoplankton in Senegal River Estuary. Treatments: <3  $\mu\text{m}$ , <12  $\mu\text{m}$ , <20  $\mu\text{m}$ , <60  $\mu\text{m}$ , <60  $\mu\text{m}$  + *Temora* (Z1), <60  $\mu\text{m}$  + *Acartia* (Z2) and <60  $\mu\text{m}$  + barnacle larvae (Z3).

treatments (except for the fraction <3  $\mu\text{m}$ ), and the lowest rates were observed in the presence of *Temora* identified as the main predator. No obvious negative growth rates were observed for smaller size class (P1; Table III).

**Particle filtration and ingestion rates by metazooplankton**

Concentrations of particle volume at the end of the experiment ranged between the highest value observed in the control without metazooplankton ( $8.8 \times 10^9 \mu\text{m}^3 \text{L}^{-1}$ ) and the lowest one measured in the presence of

*Temora* ( $7 \times 10^9 \mu\text{m}^3 \text{L}^{-1}$ , Table IV). In all treatments, small particles (ESD <9  $\mu\text{m}$ ) were dominant and represented 67% of the total seston volume in the control. Ingestion rates along the incubation time (60 h) were different in relation with the type of metazooplankton (Table IV). The highest value was recorded for the copepod *Temora* ( $61.1 \mu\text{m}^3 \text{ngC}^{-1} \text{h}^{-1}$ ) and the lowest one for barnacle larvae ( $25.6 \mu\text{m}^3 \text{ngC}^{-1} \text{h}^{-1}$ ). These rates were mainly channeled between the <3 and the 15–18  $\mu\text{m}$  ESD classes (6 and  $2435 \mu\text{m}^3$ , respectively). Higher values were noted between <3 and 6–9  $\mu\text{m}$  size classes (e.g.  $201 \mu\text{m}^3$ ) for all treatments.

**DISCUSSION**

**Methodological considerations**

The size fractionation approach allows the separation of different functional fractions of the microbial community. On the basis of the link between size and functional characteristics of organisms, this method was successfully used in many aquatic systems to elucidate trophic links among microbial components (Samuelsson and Andersson, 2003; Vaqué *et al.*, 2004). However, the fractionation method may cause cell damage and may then increase the amount of dissolved organic matter (Gasol and Moran, 1999), inducing a possible increase of bacterial growth (Vaqué *et al.*, 1994). In our study, the use of 3  $\mu\text{m}$  filtration has been chosen to avoid the elimination of large bacterial cells which were previously observed in Senegal River estuarine waters (Troussellier *et al.*, 2005) and to eliminate a maximum of bacterial predators. However,

*Table III: Effects of removal of different size classes of predators on net growth rates of the different class size of bacterial and phytoplanktonic populations discerned by flow cytometry (Methods), and of heterotrophic nanoflagellates (HNF) and ciliates*

Treatment	3 $\mu\text{m}$	12 $\mu\text{m}$	20 $\mu\text{m}$	60 $\mu\text{m}$	Z1	Z2	Z3
<b>Bacteria</b>							
Bact-A	1.050	0.745	0.986	1.074	0.910	1.145	1.121
Bact-B	1.523	0.702	0.474	1.952	1.144	1.513	1.316
Bact-C	<b>-0.531</b>	<b>-0.429</b>	<b>-0.746</b>	<b>-0.671</b>	<b>-0.506</b>	<b>-0.410</b>	<b>-0.541</b>
Bact-D	0.513	0.405	0.074	0.813	0.510	0.658	0.463
Bact-E	0.548	<b>-0.301</b>	<b>-0.370</b>	<b>-0.181</b>	<b>-0.287</b>	0.064	<b>-0.175</b>
<b>Phytoplankton</b>							
Small (P1)	0.412	0.898	1.110	1.178	1.197	1.271	1.278
Large (P2)	0.244	<b>-0.222</b>	<b>-0.232</b>	<b>-0.713</b>	<b>-0.728</b>	<b>-0.481</b>	<b>-0.493</b>
HNF	0.254	0.331	0.000	0.102	0.498	0.437	0.180
Ciliates	ND	<b>-0.089</b>	<b>-0.005</b>	0.043	<b>-0.290</b>	0.191	0.051

Treatments: <3  $\mu\text{m}$ , <12  $\mu\text{m}$ , <20  $\mu\text{m}$ , <60  $\mu\text{m}$ , <60  $\mu\text{m}$  + *Temora* (Z1), <60  $\mu\text{m}$  + *Acartia* (Z2) and <60  $\mu\text{m}$  + barnacle larvae (Z3). Values in bold represented negative growth rates. ND, not determined. All values are expressed in  $\text{day}^{-1}$ .

Table IV: Feeding experiments: zooplankton density and carbon biomass during the experiment

	Units	<60 $\mu\text{m}$	<60 $\mu\text{m}$ <i>Temora</i>	<60 $\mu\text{m}$ <i>Acartia</i>	<60 $\mu\text{m}$ <i>Cirripedia</i>
Zooplankton density	ind. $\text{L}^{-1}$	0	48	52	146
Zooplankton carbon biomass	$\mu\text{C L}^{-1}$	0	338	258	284
Particle volume concentration	$\times 10^9 \mu\text{m}^3 \text{L}^{-1}$	8.8	7.0	7.5	8.2
Ingestion rates by class size ( $\mu\text{m}$ ESD)	$\mu\text{m}^3 \text{ngC}^{-1} \text{h}^{-1}$				
<3			5.54	4.34	0.37
3–6			24.93	17.80	15.42
6–9			12.89	11.13	7.87
9–12			6.44	4.50	1.94
12–15			6.48	4.97	0.00
15–18			4.79	4.72	0.00
18–21			0.00	0.00	0.00
Total ingestion rates	$\mu\text{m}^3 \text{ngC}^{-1} \text{h}^{-1}$		61.1	47.5	25.6

Food concentrations are expressed as particle volume concentration in control (<60  $\mu\text{m}$ ) and experience jars. Ingestion rates of particles are expressed by class size and in total for each type of metazooplankton predator.

in this fraction, bacterial growth rates ( $\mu = 0.682 \text{ day}^{-1}$ ) may be underestimated due to the presence of small heterotrophic flagellates presumably preying upon bacteria. Nevertheless, this value is close to the net growth rate of bacterial community ( $\mu = 0.760 \text{ day}^{-1}$ ) obtained from another experiment based on dilution technique of estuarine water (Troussellier *et al.*, 2005).

As a result of bottle effects, zooplankton grazing rates can be underestimated and misinterpreted by this technique. During the incubation, bacteria and phytoplankton can grow faster in the experimental bottles where nutrients can be regenerated by the ingestion and excretory activity of zooplankton (Roman and Rublee, 1980). Flagellates probably grow faster than diatoms where ammonia is excreted by the copepods. However, this process might also occur in natural conditions. Bottle effects in plankton rate measurements have been studied, and short-time incubations or large containers have been recommended by many authors to minimize their effects. Although certain physical parameters such as light and temperature can be controlled, factors such as diffusion, interface area and substrate level may change immediately upon enclosure and over the incubation time. These ‘bottle effects’ on rate measurements have been investigated for bacteria and phytoplankton (Sheldon *et al.*, 1973) and zooplankton (Mullin, 1963). Therefore, the response period to compute the growth rate was determined as the initial period with an exponential growth of each component. Thus, growth rates were calculated over the first 24 h for heterotrophic bacteria and phytoplankton) and over the first 48 h for HNF, as suggested by Froneman (2002) and Sipura *et al.* (2003).

### Direct effects of consumers inside the microbial food web

Food web organization in the Senegal River Estuary appears uncommon due to the relatively small size of heterotrophic flagellates. From our results, it is clear that the removal of consumers >3  $\mu\text{m}$  had a significant positive effect on the net growth rate of bacteria, while removing consumers >12  $\mu\text{m}$  had a significant positive effect on the net growth rate of HNF. The bacterial class Bact-B showed its lowest growth rates in the presence of HNF and small ciliates (treatments <12 and <20  $\mu\text{m}$ ), confirming that these heterotrophic organisms are the most important grazers of bacteria in aquatic ecosystems (e.g. Sanders *et al.*, 1989). Higher bacterial growth rates were observed in the presence of predators of size >20  $\mu\text{m}$  (treatment <60  $\mu\text{m}$ ), suggesting predation control on bacterivorous predators (HNF) by large ciliates. The smallest bacterial cells (class Bact-A) showed the same growth rate trends as class Bact-B for the different treatments, suggesting that there was no escaping from predation by being small. The larger cells belonging to the class Bact-E, often described as grazing-resistant bacteria as reported by Jürgens *et al.* (1999), appeared not to resist the grazing exerted by heterotrophic flagellates and were partly consumed, as revealed by the low or negative growth rates in most treatments (Table III). Thus, bacterial predators were able to graze upon the different bacterial size classes. This result did not confirm our conclusions obtained from experiments in Selingué reservoir (Mali, West Africa), with a proportion of bacterial cells being able to escape HNF grazing pressure (Bouvy *et al.*, 2004).

Phytoplankton community did not show any response to predator removal, except for the pico-sized autotrophs (P1), with a decrease of their growth rate in the presence

of predators of size  $<3 \mu\text{m}$  ( $0.412 \text{ day}^{-1}$ , Table III). Nanophytoplankton (P2) did not show any modification of their growth rates to predator exclusion, indicating that they were not predation-limited, as demonstrated in an oligotrophic system by Samuelsson and Andersson (2003). Removing consumers  $>3 \mu\text{m}$  had a significant negative effect on the net growth of picophytoplankton (P1), demonstrating that small flagellates can pass through the  $3 \mu\text{m}$  membrane and may have a direct grazing impact on the picophytoplankton. Indeed, two main distinct HNF populations (representing 82% of the total abundance) were characterized in relation with their size (mean volume of  $1.45$  and  $3.32 \mu\text{m}^3$ ), and it is obvious that bacteria and picophytoplankton are the two types of prey for these HNF populations. The majority of pelagic HNF are omnivorous feeders, but generally algae are less abundant than bacteria and therefore the probability to capture algae is much lower compared with the probability to capture bacteria (Boenigk and Arndt, 2002). However, the phytoplankton community of the Senegal River Estuary was characterized by very abundant picophytoplanktonic cells representing 85% of total density in the  $<60 \mu\text{m}$  fraction, corroborating our previous results (Troussellier *et al.*, 2004). Thus, in the Senegal River Estuary, we can conclude that the picoflagellates ingest more bacteria than picophytoplankton (diameter  $\sim 1.5 \mu\text{m}$ , data not shown) while the nanoflagellates ingest more picophytoplankton than bacteria. Sherr and Sherr (1991) suggested that flagellates  $<5 \mu\text{m}$  are the main consumers of  $<1 \mu\text{m}$ -sized cells, while  $5\text{--}20 \mu\text{m}$  flagellates select larger prey ( $1\text{--}10 \mu\text{m}$ ).

Our results suggest strong trophic linkages, at the basis of the food web in the Senegal River Estuary, between bacteria, flagellates and ciliates, and confirm a very active microbial loop in terms of carbon transfer towards the upper trophic levels, as suggested by Troussellier *et al.* (2004). Despite the low nutrient concentration observed *in situ*, inducing no responses of phytoplankton community except the pico-sized autotrophs, microheterotrophs could maintain high growth rates by retrieving nutrients via the active microbial loop, as also demonstrated in an oligotrophic aquatic system by Samuelsson and Andersson (2003). Conventionally, the predator–prey size ratio is fixed to 10, as proposed for the microbial loop by Azam *et al.* (1983). If there was a factor of 10 by linear dimension, bacteria of  $0.57 \mu\text{m}$  (as ESD) observed in our study would be consumed by flagellates in the  $5\text{--}6 \mu\text{m}$  range, and they would be in turn preyed upon by protistan of  $50\text{--}60 \mu\text{m}$  size. However, our results revealed a better agreement with a predator–prey (flagellates–bacteria) size ratio of 3–4, as summarized by Hansen *et al.* (1994) and Calbet and

Landry (1999). With a mean size of small flagellates ranging between  $0.95$  and  $2.07 \mu\text{m}$ , the predator–prey (flagellate–bacteria) size ratio ranged between 1.66 and 3.63, corroborating the results described above. On another hand, a more conventional predator–prey size ratio  $\sim 10$  was observed between ciliates and flagellates (mean ESD of  $20.03$  and  $3.63 \mu\text{m}$ , respectively). However, in our study, the ciliates did not respond to the removal of predators (low growth rates in the  $60 \mu\text{m}$  fraction) and appeared to be limited by prey availability, as demonstrated by Samuelsson and Andersson (2003).

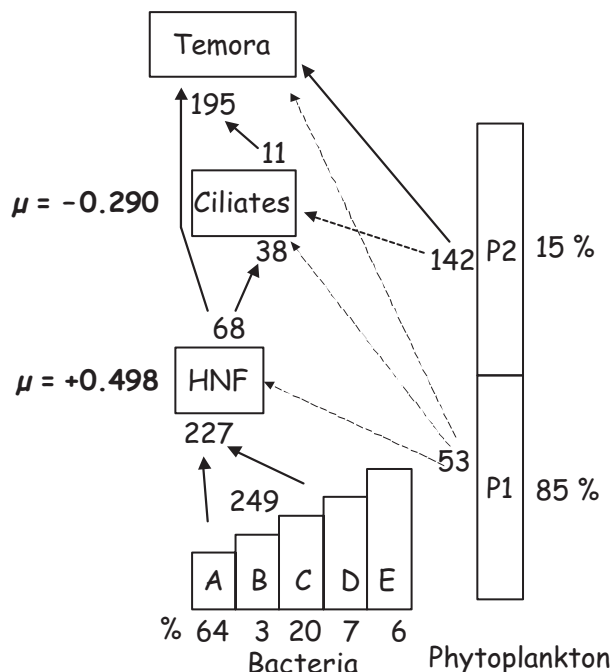
### Indirect effects of metazooplankton

As recently demonstrated by Calbet and Landry (1999) and Sipura *et al.* (2003), large metazooplankton can potentially influence the dynamics of organisms at lower level of the marine food web via cascading impacts on trophic intermediates. Especially, small crustaceans like copepods may serve as an important link between the microbial and classical food webs (Turner and Roff, 1993). Calanoid copepods are mostly omnivorous with varying tendencies to herbivory or carnivory (Burns and Gilbert, 1993). Zooplanktonic communities in the Senegal River Estuary are dominated by Cirripedia larvae and calanoid copepods (mainly *T. stylifera*, accompanied by *A. clausi*, and *Paracalanus* spp.), which represent a biomass of  $3\text{--}26 \mu\text{gC L}^{-1}$  (Pagano *et al.*, in press).

Our results highlighted the impact of bacterivorous flagellates on the bacterial community, especially on the largest cell sizes (Fig. 3). In the  $<12 \mu\text{m}$  fraction, the absence of ciliates allowed the development of flagellates which in turn consumed directly the bacterial cells. In the  $<60 \mu\text{m}$  fraction with the presence of *Temora*, only the trophic cascading effects can explain the decrease of the mean of bacterial volume and the low growth rates observed, due to the consumption of ciliates by the metazooplanktonic predator, allowing the development of the heterotrophic flagellates. Thus, our results indicate that metazooplankton predation on ciliates is a key mechanism for the regulation of bacterioplankton density and community structure, as demonstrated by Jürgens *et al.* (1994). However, such a cascading effect was not observed in the presence of *Acartia* or cirriped. This lack of cascading effect is also compatible with other studies on microbial plankton dynamics, with no change in HNF numbers observed with *Acartia tonsa* as predators in estuarine waters (Sipura *et al.*, 2003). These authors suggested cross-linkages between flagellate predators (ciliates and copepods) leading to zero net effect.

### Food size selectivity of the three metazooplanktonic predators

The collection and ingestion of particles vary between organisms, according to the abundance and the nature



**Fig. 3.** Trophic interactions between the metazooplankton predator *Temora* and the components of the microbial food web from an experiment based on differential fractionation of water from the Senegal River Estuary. Growth rates of ciliates and HNF ( $\mu$ ) were indicated and expressed in  $\text{day}^{-1}$ . All the values of carbon production, carbon requirements and carbon loss by predation according to the type of calculations (see the text) are expressed as  $\mu\text{gC L}^{-1} \text{day}^{-1}$ . See also values reported in Table V.

of the nutritive particles (size, form, nature, taste, relative abundance, etc.). Specific ingestion rates ( $25\text{--}60 \mu\text{m}^3 \text{ngC}^{-1} \text{h}^{-1}$ ) were lower than those reported by Pagano *et al.* (2003) for calanoid copepods in Ebrié lagoon (range between  $47.1$  and  $216.8 \mu\text{m}^3 \text{ngC}^{-1} \text{h}^{-1}$ ), but were within the range of values summarized by Mauchline (1998). It appeared that  $<3 \mu\text{m}$  particles were poorly selected by the two calanoids and not selected by the barnacle larvae. It is clear that picoplankton (bacteria and picophytoplankton) seems to be poorly edible prey for the main zooplanktonic organisms of the Senegal River Estuary. Natural bacteria could sometimes play a significant trophic role as food for zooplankton dominated by cladocerans (Wylie and Currie, 1991; Modenutti *et al.*, 2003) or for younger stages of copepods such as nauplii (Turner and Tester, 1992). From grazing experiments carried out in a tropical lagoon, Pagano *et al.* (2003) clearly demonstrated that *A. clausi* shows null or very low values of particle-size selectivity for the small particles  $<3 \mu\text{m}$ . Although some authors have mentioned the lowest size limit for ingestion  $\sim 3 \mu\text{m}$  for *Acartia* species (Nival and Nival, 1976; Poulet, 1978), others have reported distinctly lower values, as for example  $1.5 \mu\text{m}$

for *Acartia tonsa* (Gaudy and Pagano, 1987). The highest size classes for ingestion were  $\sim 15\text{--}18 \mu\text{m}$  ESD for the two calanoids and  $9\text{--}12 \mu\text{m}$  ESD for the barnacle larvae. These upper size limits for ingestion are lower than those reported by Pagano *et al.* (2003) for *A. clausi* ( $\sim 36 \mu\text{m}$  ESD). Copepods generally feed preferentially on particles  $>5\text{--}10 \mu\text{m}$  ESD (Bautista *et al.*, 1992; references therein). Our results confirm these observations, and it seems that the three metazooplanktonic predators seek preferentially the intermediate particles (size range between  $3$  and  $9 \mu\text{m}$  ESD). Nevertheless, the impact of the calanoid copepods on larger particle (between  $9$  and  $18 \mu\text{m}$  ESD) is also obvious, corresponding to the size class of ciliates and larger phytoplankton (P2; Fig. 2). The negative growth rates of ciliates in the presence of *Temora* show that ciliates are actively consumed by this predator. Little information concerning the size selectivity and filtration rate of barnacle larvae is available in the literature. The impact of diatom food species (*Chaetoceros calcitrans* and *Skeletonema costatum*) on the larval development of *Balanus amphitrite* was evaluated by Desai and Anil (2004), who reported high grazing rate with *C. calcitrans* with a cell size of four micrometre diameter ( $\sim 17 \mu\text{m}^3$ ). In the present investigation, maximum ingestion rates of barnacle larvae were noted in the  $3\text{--}6 \mu\text{m}$  size class (Table IV), corroborating the data obtained by Desai and Anil (2004). However, grazing impact by the barnacle larvae on all prey categories was generally low ( $1.3\text{--}9.2\%$ ) in Disko Bay, West Greenland (Turner *et al.*, 2001).

### Carbon budget with *T. stylifera*

From the results obtained on particle ingestion by size class (see above) and the obvious data obtained from growth rates in the presence of *Temora*, it is possible to establish predatory interactions from *Temora* to bacteria along the food chain (Fig. 3; Table V). Carbon requirements for *Temora* population was estimated as  $195 \mu\text{gC L}^{-1} \text{day}^{-1}$  from total ingestion rates (Table IV). Variations in growth rates after different filtration treatments were used to calculate grazing rates and to identify trophic links (Table V). Calculations indicated a negative growth rate of ciliates in the presence of *Temora* (apparent growth rate  $\mu_a = -0.290 \text{ day}^{-1}$ ) and a positive growth rate without predators in the  $60 \mu\text{m}$  fraction (net growth rate  $\mu_n = +0.043 \text{ day}^{-1}$ ). Thus, the minimum grazing rate ( $G = \mu_n - \mu_a$ ) on ciliates was of  $0.333 \text{ day}^{-1}$ , corresponding to  $11.3 \mu\text{gC L}^{-1} \text{day}^{-1}$  and this value was assumed to be the minimal carbon production of ciliates (Table V). Carbon demand for this minimal ciliate production can be estimated as  $38 \mu\text{gC L}^{-1} \text{day}^{-1}$ , assuming a 30% growth efficiency. For HNF, calculations indicated a high positive growth rate

Table V: Different parameters allowing to draw up the carbon budget with *Temora* as zooplankton predator

Units	$\mu_a$ (day <sup>-1</sup> )	$\mu_n$ (day <sup>-1</sup> )	G (day <sup>-1</sup> )	Density (cells L <sup>-1</sup> )	Volume ( $\mu\text{m}^3$ )	Carbon production ( $\mu\text{gC L}^{-1} \text{ day}^{-1}$ )	Carbon demand ( $\mu\text{gC L}^{-1} \text{ day}^{-1}$ )
Bacteria	ND	0.682	ND	$1.63 \times 10^7$	0.102	249	ND
Phytoplankton							
Small (P1)	ND	0.412	ND	$1.45 \times 10^5$	0.89 <sup>a</sup>	53	ND
Large (P2)	-0.728	0.244	0.972	$2.60 \times 10^4$	5.55 <sup>a</sup>	142	ND
HNF	0.498	0.498	0	$1.71 \times 10^5$	3.63	68	227
Ciliates	-0.290	0.043	0.333	$3.80 \times 10^4$	4697	11	38

Apparent growth rates ( $\mu_a$ ), net growth rates ( $\mu_n$ ) and grazing rates ( $G$ ) of bacteria, phytoplankton, HNF and ciliates are presented. Carbon production and demand are also presented using density, and biovolume of each component. ND, not determined.

<sup>a</sup>carbon; cell ratio according to the cell size (Smayda, 1978).

( $\mu_a = 0.498 \text{ day}^{-1}$ ) in the presence of *Temora*, higher than the growth rates obtained without potential predators in the fraction  $<12 \mu\text{m}$  (Table III). Thus, if we consider a mean volume of  $3.63 \mu\text{m}^3$ , we can assume that the ciliate growth rate is equal to its minimal grazing rate on HNF, corresponding to  $68 \mu\text{gC L}^{-1} \text{ day}^{-1}$ . Therefore, a large part of the HNF production (56%) was channeled to ciliates, while the rest (44%) could be consumed directly by *Temora* (Fig. 3). Carbon demand for the HNF production can be estimated as  $227 \mu\text{gC L}^{-1} \text{ day}^{-1}$ , assuming a 30% growth efficiency. Net bacterial production can be estimated from grazing rates of the total community obtained elsewhere by a dilution method (Troussellier *et al.*, 2005). With a growth rate of  $0.682 \text{ day}^{-1}$  and a mean biovolume of  $0.102 \mu\text{m}^3$  (Table II), bacterial production amounted to  $249 \mu\text{gC L}^{-1} \text{ day}^{-1}$  and could support the carbon demand of HNF ( $210 \mu\text{gC L}^{-1} \text{ day}^{-1}$ ). Nanophytoplankton representing 15% of total counts was characterized by a negative growth rate ( $\mu = -0.728 \text{ day}^{-1}$ ) in the presence of *Temora* and a positive growth rate ( $\mu = 0.244 \text{ day}^{-1}$ ) in absence of predators (fraction  $<3 \mu\text{m}$ ). Thus, the minimum grazing rate  $G$  on nanophytoplankton was of  $0.972 \text{ day}^{-1}$ , corresponding to a carbon loss of  $142 \mu\text{gC L}^{-1} \text{ day}^{-1}$ , assuming a conversion factor of  $5.55 \text{ pgC}$  per cell (Smayda, 1978). Picophytoplankton ( $<2 \mu\text{m}$  size class), representing 85% of the total counts, was characterized by higher positive growth rates in absence of predators, and thus we could only calculate the carbon production in the fraction  $<3 \mu\text{m}$  ( $53 \mu\text{gC L}^{-1} \text{ day}^{-1}$ ), assuming a conversion factor of  $0.89 \text{ pgC}$  per cell (Smayda, 1978). Finally, the carbon requirements of *Temora* necessary for the total particle ingestion during the experiment could be supported by the different microbial components, especially by nanophytoplankton (72.8%), heterotrophic flagellates (12.3%) and ciliates (5.6%), as illustrated in Fig. 3. We can also apply a 30% loss factor to ciliate

contribution for compensating the effects of the Lugol fixatives (Calbet and Saiz, 2005); thus, the contribution of ciliates to *Temora* diet would rise to 7.3%, representing nevertheless only a small part of the carbon demand of this copepod. Calbet and Saiz (2005) concluded that the relative importance of ciliate consumption by copepods (generally  $\sim 30\%$ ) clearly depends on the trophic state of the system. While phytoplankton community of Senegal River Estuary was represented by 85% of small cells, the copepod *Temora* must ingest a large part of nanophytoplankton with the ciliate contribution to complete their diet.

Information from trophic cascades have previously been used to analyse the microbial food web structure (Wikner and Hagström, 1988; Dolan and Gallegos, 1991; Calbet and Landry, 1999; Modenutti *et al.*, 2003). Quick responses of  $<1$  day were generally measured in these studies when removing the potential predators of a prey. In our study, all the microbial components responded to the removal of predators and appeared to be limited by top down factors (predation limited). In contrast, there was no response during the first 24 h in experiments performed in the Pacific Ocean (Samuelsson and Andersson, 2003), suggesting that the microbial responses depend on the initial level of bacteria (Calbet *et al.*, 2001). Only one of the calanoid copepods present in the estuarine water (*Temora*) was able to feed upon ciliates. We can thus suggest that the high ratio between bacterial and flagellate abundances observed in Senegal River Estuary (Troussellier *et al.*, 2004) is linked to the cascading effect exerted by *Temora*. HNF responded positively to the decline by grazing impact of the upper trophic component constituted by the calanoid *Temora*. This is the first report of a distinct trophic cascade involving copepods, ciliates, bacterivorous flagellates and bacteria in West African estuarine waters. This study clearly indicates that predation on ciliates by the preponderant metazooplankton, identified

as *Temora*, plays a major role on the regulation of the microbial structure in this tropical estuary. As reviewed by Turner and Roff (1993), our results show evidence for considerable intertwining of the ‘microbial’ and ‘classical’ food webs in Senegal River Estuary.

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