Seasonal variation in consumption of benthic bacteria by meio- and macrofauna in an intertidal mudflat

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Abstract

The trophic fate of benthic bacteria in an intertidal mudflat (Brouage, Marennes-Oléron, France) was evaluated in situ, and environmental parameters that potentially influence the consumption of bacteria by the most abundant organisms of the meio- and macrofauna were identified. Variations in grazing rates were estimated using ^{15}N pre-enriched bacteria at different temporal and spatial scales on a cross-shore transect over the mudflat. Grazing incubations were performed in microcosms with freshly collected grazers. Environmental factors varied more by season than by day or sampling station. Bacterial uptake by grazers did not appear to be strongly influenced by abiotic factors and was not linked to bacterial abundance. Algal abundance was negatively correlated with bacterivory in both the nematode community and the foraminifer *Ammonia tepida*, suggesting that bacteria constitute an alternative resource that is consumed when algae are less abundant. Bacteria were mainly ingested by the mudsnail *Hydrobia ulvae* and secondarily by nematodes with grazing rates of copepods and *A. tepida* being considerably lower. The estimated grazing in the upper and middle part of the mudflat represented 7% and 28% of bacterial production, respectively. In the lower mudflat, daily grazing never represented >3% of bacterial production throughout the year. Consequently, grazing appears to be a minor factor in the regulation of bacterial production. Bacterivory did not vary clearly according to season; consequently, the fate of bacteria in this benthic food web is poorly structured by season.

Bacteria constitute the foundation of all ecosystems on earth through the degradation and regeneration of nutrients. However, bacteria can also play a major role in food webs and establishing this role remains a challenge in marine microbial ecology. There is now evidence that a substantial proportion of bacterial production is grazed in pelagic systems (Sherr et al. 1987). Although bacterial abundance in marine soft sediment is generally 1000 times higher than in pelagic systems (Schmidt et al. 1998), studies on the trophic fate of bacteria are less common in sediments than in pelagic environments (Kemp 1990), mainly due to technical difficulties. By analogy to pelagic systems, it has often been hypothesized that grazing is one of the most important fates of benthic bacteria (Hondeveld et al. 1994).

Pelagic bacteria are principally consumed by small nanozooplankton grazers (<20 μm; Sherr and Sherr 2000). In benthic systems, sediment bacteria are consumed by microfauna (ciliates and flagellates) as well as by larger grazers from the meio- and macrofauna (Kemp 1990). One of the simplest approaches to determine the trophic links between benthic bacteria and grazers is to compare their respective abundances in natural environments. However, interpretation of these results is complex because grazers can affect bacterial dynamics through processes other than grazing, such as bioturbation and mucus secretion.

Moreover, both positive and negative relationships between grazer and bacterial biomass can be due to a strong trophic link between grazers and bacteria. These two types of organisms have very different generation times (hours for bacteria and weeks for grazers). As a result, food webs should be considered as a system of nested processes with different response times. An alternative approach is to measure bacterial grazing rates and bacterial stock simultaneously, but studies employing this approach have reached contradictory conclusions, with some authors finding a negligible percentage of bacterial production removed by grazers (Epstein and Shiaris 1992) and others concluding that meiofauna alone can consume the entire bacterial community (Montagna 1984). Epstein (1997) suggested that discrepancies among studies may be due to grazing rates that are highly time-dependent: if only one or two seasons are sampled, the results cannot be generalized and are not representative of long-term trends. To our knowledge, the trophic fate of bacteria has never been analyzed throughout the year in intertidal mudflat sediments.

The goal of the present study was to determine the trophic fate of benthic bacteria in the Brouage mudflat (Marennes-Oléron Bay, France) on significant temporal and spatial scales. Grazing rates of the most abundant grazers in the meiofauna (i.e., the foraminifer *Ammonia tepida*, nematodes, and copepods) and the macrofauna (i.e., the deposit feeder *Hydrobia ulvae*) were measured. [15]N pre-enriched bacteria were used as tracers in grazing experi-

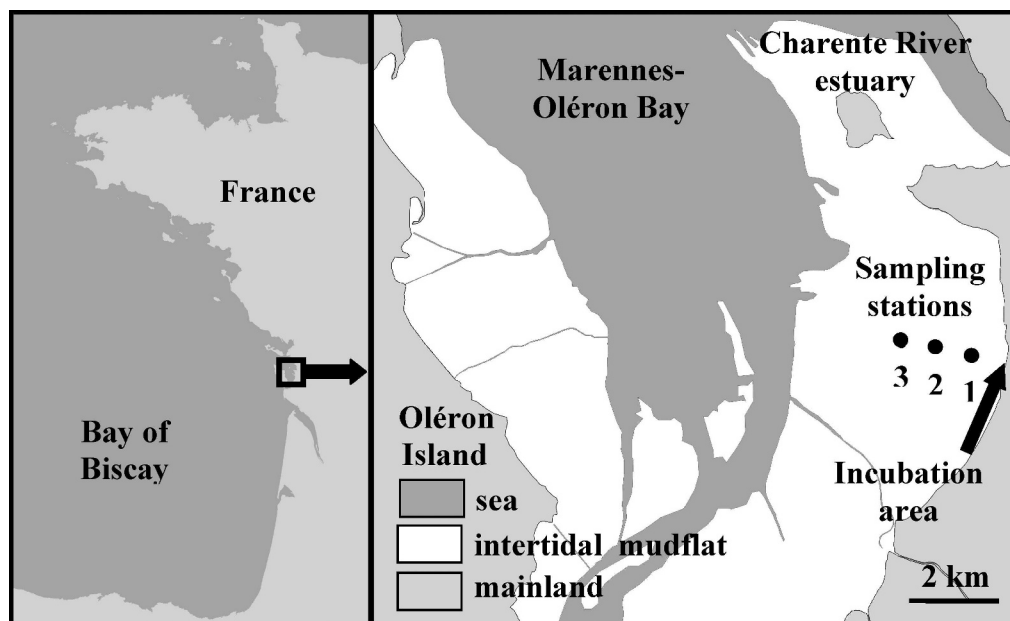* Corresponding author: ppascal@lsu.edu

Fig. 1.   Map of the study area and sampling site.

ments performed in microcosms with freshly collected grazers and incubated in near-natural conditions.

Methods

Study site—Sampling was performed in Marennes-Oléron Bay, located between the mainland Atlantic coast of France and Oléron Island. This macrotidal system is influenced by continental inputs mainly from the Charente River and occasionally from the Gironde River. It covers 170 km² of which 60 km² are intertidal mudflats. Brouage mudflat is >4 km wide and the sediment consists of silt and clay particles (95% <63 μm). Samples were collected along a cross-shore transect, encompassing the main geomorphological units (Haubois et al. 2002), with the three sampled stations (Sta. 1, Sta. 2, and Sta. 3 in Fig. 1) located at 0.7, 1.6, and 2.2 km from the upper shoreline, respectively.

Bacterial enrichment—15N enrichment of bacteria was done with the method of Pascal et al. (2008b). Briefly, superficial sediment was sampled to a depth of 1 cm during ebb tide in Sta. 3. Bacteria from superficial sediment were subcultured in a liquid bacterial culture medium containing 1 g L⁻¹ of $^{15}NH_4Cl$ (99% ^{15}N-enriched NH_4Cl; Cortec-Net). Cultured bacteria were rinsed from their medium by centrifugation and then frozen in liquid nitrogen until use in grazing experiments. Bacteria were thawed 20 min before the beginning of each grazing experiment. Enriched bacteria abundance was 10⁹ bacteria mL⁻¹ and bacteria-produced nitrogen was 3.85% ^{15}N.

Sampling—Sta. 3 was regularly sampled in 2006 (31 Jan; 13 Mar; 25, 26, and 27 Apr; 24 May; 28 Jun; 26 Jul; 21 Sep; 22 Nov) and a single sample was taken in 2007 (20 Feb). The two other stations were sampled on 27 April 2006. All thirteen sampling events took place during the daylight ebb

tide during spring tides. During each sampling event and in each station, the top centimeter of sediment over a 1-m² surface was collected in three areas each separated by 2 m. The potential hydrogen (pH) of the sediment was measured in situ before sampling. The triplicate sediment samples were independently homogenized and used to perform grazing experiments and to measure biotic parameters. Abiotic parameters were measured on one of the triplicates. In order to compare grazing rates both in surficial and deeper sediment, a thicker layer (1–5 cm) was also sampled in April 2006 using the same protocol in all three stations of the transect.

Grazing experiments—Grazing experiments were based on the assumption that grazers show inertia in their feeding behavior and, consequently, that grazing rates remain unchanged between the natural environment and the microcosms incubated in situ (Fig. 1). For each sample, 100 mL of sediment was sieved on a 63-μm mesh to remove fine particles and bacteria. Macrofauna was removed by hand. The sediment remaining on the mesh was then mixed with enriched bacteria and placed in 100-mL beakers taped with cellophane, thereby forming a microcosm. To measure mudsnail bacterivory, five specimens of *Hydrobia ulvae* were added to each microcosm. For each sampling event, one control experiment using five specimens of *H. ulvae*, was performed in similar conditions where the sediment with mudsnails was first frozen in liquid nitrogen for ≥20 min to kill off grazers. To incubate microcosms in conditions as close as possible to natural conditions and to avoid working in unsuitable places, beaker microcosms were inserted in the muddy sediment along the beach (Fig. 1). Incubations lasted 5 h and the time between sediment sampling and the start of the incubation never exceeded 1 h. Incubations were stopped by sieving sediment on 63-μm mesh and freezing the remaining sediment

in liquid nitrogen. When the samples were thawed, nematodes and copepods were mixed with Ludox HS40 and extracted from sediment by centrifugation (Heip et al. 1985). For each sample, $\geq$700 nematode and 130 copepod specimens were picked individually at random. After centrifugation, the pellet fraction was stained with Rose Bengal in order to identify freshly dead foraminifers. *Ammonia tepida* and *Haynesina germanica* were picked separately and cleaned of any adhering particles. It was not possible to assess daily variations in bacterivory by *A. tepida* because samples were lost. Nor was it possible to assess foraminiferal bacterivory at different spatial scales because foraminifer biomass was too low. Biomass of *H. germanica* was high enough for isotopic analyses only for three sampling events over the year of study. *H. ulvae* were separated from their shell by hand and all specimens from the same microcosm were pooled and homogenized using a Potter–Elvehjem homogenizer. September samples of *H. ulvae* were spoiled; thus, snail grazing-rate data were not available for this period.

Isotopic analysis and calculations—δ^{15}N and δ^{13}C of bacteria and grazers were measured using an Elemental Analyser–Isotopic Ratio Mass Spectrometer (Isoprime; Micromass). Natural nitrogen isotopic composition is expressed in the delta notation (δ^{15}N) relative to air N_2: $\delta^{15}N = [(^{15}N / ^{14}N)_{sample} / (^{15}N / ^{14}N)_{reference}) - 1] \times 1000$. Carbon isotopic composition is expressed in the delta notation (δ^{13}C) relative to Vienna Pee Dee Belemnite: $\delta^{13}C = [(^{13}C / ^{12}C)_{sample} / (^{13}C / ^{12}C)_{reference}) - 1] \times 1000$. Carbon and nitrogen contents were obtained along with isotopic ratios during isotopic analysis.

Incorporation of ^{15}N is defined as excess (above background) ^{15}N and is expressed in terms of specific uptake (I) calculated as the product of excess ^{15}N (E) and biomass of N per grazer. E is the difference between the background ($F_{background}$) and the sample (F_{sample}) ^{15}N fraction: $E = F_{sample} - F_{background}$, where $F = ^{15}N / (^{15}N + ^{14}N) = R / (R + 2)$ and R is the nitrogen isotopic ratio. For $F_{background}$, we used values measured with the control grazers (frozen). R was derived from the measured δ^{15}N values: $R = [(\delta^{15}N / 1000) + 1] \times R_{air}N_2$ where $R_{air}N_2 = 7.35293 \times 10^{-3}$. The uptake of bacteria was calculated as $[I \times (\% C_{enriched\ bacteria} / \% N_{enriched\ bacteria}) / (F_{enriched\ bacteria} \times incubation\ time)]$. In preliminary experiments, we determined that bacterial abundance in 63-μm sieved sediment did not exceed 2.9×10^7 cells mL^{-1}. Because this represented <3% of the abundance of isotopically enriched bacteria added to the microcosm, we considered that the fraction of natural bacteria remaining in sieved sediment was negligible. Consequently, calculations of grazing rates were based on the assumption that grazers only took up isotopically enriched bacteria.

Abiotic factors—The median grain size of sediment was characterized using a Malvern Mastersizer 2000 (size range = 0.02–2000 μm). Elemental analysis (carbon and nitrogen) of sediment was determined with a FlashEA 1112 Elemental Analyzer (Thermo). The fraction of mineral C contained in carbonate was determined using a Bernard

calcimeter. C abundance used for the calculation of the C : N ratio was total C content minus C contained in carbonate. Water content was estimated by weight loss after freeze-drying 100 mL of sediment. Organic matter content was estimated by weight loss after combustion of 1 g of freeze-dried sediment at 450°C for 24 h. Carbohydrate concentration and particulate protein content were determined using standard methods (Dubois et al. 1956; Lowry et al. 1951). Temperature was measured in microcosms regularly every 30 min during the 5-h incubation and a mean temperature was calculated for the entire incubation period.

Biotic factors—Nanoflagellates were extracted from sediment using a solution of Percoll-sorbitol (Price et al. 1978), stained with 4,6-diamidino-2-phenylindole dihydrochloride (DAPI; 2500 μg L^{-1}), filtered onto a 3-μm Nucleopore black filter. They were then counted and individual length (L) and width (2r) was measured under microscope. Biovolume (V) of individuals was calculated as (4/3) π(L/2) r^2. Biomass (carbon values) was calculated from cell volumes and concentrations using a carbon content of 200 fg C μm^{-3} (Bak and Nieuwland 1989).

Copepods and nematodes were extracted using Ludox HS40. To estimate abundance, a Motoda-box was used to split samples and obtain aliquots containing $\geq$500 individuals. At each sampling event, a large number of specimens were extracted and weighed for isotopic analyses of grazing experiments. From this analysis preparation, we determined mean individual weight and carbon content for copepods and nematodes. Total weight of the foraminifer *A. tepida* was determined in the same way and a conversion factor was used to determine the organic fraction of foraminifers (organic carbon = 5.8% of total weight in this area).

For each sampling event, triplicates of 400 mL of collected sediment were sieved on 500-μm mesh to determine the abundance of *H. ulvae* and to recover snails for grazing experiments. Carbon content of snails was determined from isotopic analyses.

Algal biomass in sediment was assessed using chlorophyll *a* as a proxy and measured using fluorometry. Carbon algal biomass was estimated using a carbon : chlorophyll ratio of 45 (Jonge 1980). To determine bacterial abundance, bacteria were extracted from sediment particles by incubation in sodium pyrophosphate (0.01 mol L^{-1} for $\geq$30 min) and sonication. Bacteria were stained using DAPI (2500 μg L^{-1}), filtered onto 0.2-μm Nucleopore black filters and then counted under an epifluorescence microscope. Individual length (L) and width (2r) were determined by computer-assisted image analysis (Axio Vision Release 4.3) with an epifluorescence microscope (Axioskop 2 mot plus; Zeiss) equipped with a charge-coupled device camera (AxioCam MRc5; Zeiss). For each sampling event, biovolume (V) of $\geq$500 bacterial cells was calculated as πr^2 (L − 2/3r; Fuhrman 1981). Biovolume was converted to carbon biomass using a ratio of 220 fg C μm^{-3} (Bratbak and Dundas 1984).

Bacterial production was estimated by tritiated thymidine incorporation (Garet and Moriarty 1996). Thymidine

incorporation was converted to numbers of bacterial cells using a ratio of 5×10^{17} cells per mol of thymidine.

Data analysis—To analyze the variability of environmental factors that potentially influence bacterivory, principal component analysis (PCA) was performed using pH, temperature, water content, median grain size, carbohydrate, protein, C:N, organic matter, and algal and bacterial biomass data from all surficial sediment samples ($n = 13$). Variation in grazing rates of nematodes according to depth was tested using t-tests. One-way analysis of variance (ANOVA) was used to test for variation in grazing rates of meiofauna among different sampling events. The Tukey test was used for post hoc comparisons. Spearman rank (r_s) correlations were performed to investigate the relationships between grazing rates of meiofauna and environmental parameters. PCA and statistical analyses were run using the statistical software Excel Stat Pro© (Addinsoft).

Results

Abiotic variables measured throughout the year at Sta. 3 are presented in Table 1 and biotic variables are presented in Table 2. The PCA performed on the environmental factors that potentially influence bacterivory (Fig. 2), showed that spatial and daily variation appeared to be lower than seasonal variation. The F1 and the F2 axes together explained 54% of observed variability (Fig. 2). Data points clustered according to seasons: a summer–autumn group (comprising samples from Jul, Sep, and Nov 06), a winter group (comprising samples of Jan and Mar 06 and Feb 07) and a spring group (comprising samples of Apr, May, and Jun 07). In April 2006, spatial and daily variation was considered. The three sampled stations (Apr P1, Apr P2, and Apr P3) as well as the three consecutive days for Sta. 3 (Apr P3a, Apr P3b, and Apr P3c) formed a tightly clustered group. The summer–autumn period was characterized by high biomass and bacterial production and by low water content, algal biomass, and organic matter (Fig. 2). The winter period was characterized by elevated median grain size and high carbohydrate content in sediments and low pH, temperature, bacterial production, and C:N. Furthermore, January and March 2006 samples showed high algal biomass and high water content. The spring period was characterized by high values of pH and C:N and low values of median grain size and carbohydrates.

At Sta. 3, the annual mean bacterial and algal biomasses were 1.26 ± 0.27 and 3.54 ± 1.55 g C m^{-2} respectively, and mean flagellate biomass was 0.44 ± 0.29 μg C m^{-2}. Nematodes dominated the meiofauna in biomass throughout the year in surficial sediment at Sta. 3 (0.24 ± 0.05 g C m^{-2}). Mean biomass of copepods and foraminifers were 29 ± 25 mg C m^{-2} and 9.8 ± 8.4 mg C m^{-2} respectively (Table 2). During September and November 2006 and February 2007, the foraminifer community was dominated by *H. germanica*. During the rest of the year, *A. tepida* represented $92 \pm 5\%$ of total foraminiferal biomass. The mudsnail *H. ulvae* had an annual mean biomass of $86 \pm$ 70 mg C m^{-2}. In April 2006, Sta. 1 was characterized by high nematode biomass (1.52 ± 0.55 g C m^{-2}), whereas Sta. 2 was characterized by high biomass of *H. ulvae* (795 ± 234 mg C m^{-2}).

During April 2006, a deeper layer (2–5 cm) of sediment was sampled in the three stations of the transect. At Sta. 1, 2, and 3, bacterial biomass of this deeper layer represented, respectively, 5.54 ± 1.35 g C m^{-2}, 5.67 ± 0.26 mg C m^{-2}, and 5.93 ± 0.30 mg C m^{-2}, whereas nematode biomasses were 88 ± 31 mg C m^{-2}, 65 ± 18 mg C m^{-2}, and 18 ± 3 mg C m^{-2}.

In the lower part of the mudflat, the range of seasonal fluctuation of grazing rates measured in pg C ind^{-1} h^{-1} was 8–36 for nematodes, 44–228 for copepods, 35–608 for *A. tepida*, 24–123 for *H. germanica* (Figs. 3A and B) and 883–2389 for *H. ulvae* (Fig. 3C). Over the year of study, there were significant differences in grazing rates of nematodes ($F_7 = 5.3$, $p < 0.01$), copepods ($F_7 = 5.8$, $p < 0.001$) and *A. tepida* ($F_7 = 6.7$, $p < 0.01$). Variation in bacterivory by *A. tepida* at daily and spatial scales could not be estimated. On a daily scale, there were no differences in bacterivory by nematodes ($F_2 = 1.7$, nonsignificant [n.s.]) or copepods ($F_2 = 7.1$, n.s.; Fig. 3A). Nematode grazing rates did not differ significantly among stations ($F_2 = 2.6$, n.s.; Table 3). In contrast, the copepod grazing rate was significantly higher at Sta. 1 than at Sta. 2 or 3 ($F_2 = 9.5$, $p < 0.05$).

Due to the low biomass of copepods and foraminifers in the deeper layers of sediment, their grazing rates could not be measured. Individual grazing rates of nematodes on bacteria did not differ significantly between surficial (0–1 cm) and deeper (1–5 cm) layers of sediment at Sta. 1 ($t = 0.8$, n.s., df = 4) and 2 ($t = 0.8$, n.s., df = 4). At Sta. 3, nematodes dwelling in deeper sediment grazed bacteria at a higher rate than in surficial sediments ($t = 3.7$, $p < 0.05$, df = 4; Table 3).

Meiofaunal grazing was poorly correlated with abiotic factors (Table 4), with the exception of nematode grazing, which was negatively influenced by the water content of the sediment and positively by median grain size; however, the p-values of these correlations were not significant. The grazing rate of *A. tepida* was also positively correlated with bacterial production, and nematode grazing rate was negatively correlated with flagellate biomass, but the p-values of these correlations were also not significant. There was a strong and significant negative relationship between nematode and *A. tepida* grazing rates and algal biomass. None of the grazers were affected by the grazing activity of other grazers. Ratios between the biomass of grazers and bacterial biomass they consumed provide an estimate of the importance of bacteria in their diets (Table 5). The lower the ratio is, the greater the importance of bacteria is in grazer diets. Observations were similar at each station of the mudflat: bacteria were more important in the diet of the macrofaunal grazer *H. ulvae* than in the diet of other meiofaunal grazers.

In the spatial comparisons, bacteria removed by grazers in Sta. 2 reached 332 mg C m^{-2} d^{-1}, representing 18.9% of bacterial stock and 28.6% of bacterial production (Table 6). In the deeper layer of sediment, the fraction of

Table 1. Values of abiotic factors measured in surficial sediment through the year at Sta. 3.

	2006								2007
	Jan	Mar	Apr	May	Jun	Jul	Sep	Nov	Feb
pH	7.9	8.0	8.1	8.4	8.4	7.7	7.4	8.1	7.9
Salinity	31.4	28.4	27.2	29.8	28.5	29.9	30.0	27.9	27.0
Temperature (°C)	7.2	19.8	19.8	19.3	32.9	33.7	23.6	13.8	13.2
Water content (%)	62.8	61.6	65.1	58.4	57.8	59.4	52.0	60.2	56.3
Median grain size (μm)	12.3	8.8	12.1	9.2	12.8	17.2	16.7	9.4	12.9
Carbohydrate (mg g dry sed^{-1})	2.0	1.8	1.0	1.8	1.8	2.1	1.7	6.8	9.1
Protein (mg g dry sed^{-1})	39.5	46.8	60.5	53.2	62.7	51.0	59.1	16.9	22.6
C : N	2.3	7.3	8.6	9.3	7.7	8.3	10.9	11.9	3.7
Organic matter (% dry sed^{-1})	10.6	11.5	11.3	9.8	10.8	10.2	8.4	8.6	9.1

bacteria removed by nematodes represented 0.003%, 0.002%, and 0.001% of bacterial stock at Sta. 1, 2, and 3, respectively.

In the lower part of the mudflat, the range of fluctuation over the year of study was 0.93–1.76 gC m^{-2} for bacterial biomass and 0.47–2.29 gC m^{-2} d^{-1} for bacterial production (Fig. 4A). In this area, the amount of bacteria removed by grazers throughout the year fluctuated between 1.8 mg C m^{-2} d^{-1} and 34.6 mg C m^{-2} d^{-1}. On average, macrofauna removed 17.3 ± 16.7 times more bacteria than meiofauna did. This daily grazing pressure represented 0.17–2.66% of bacterial biomass and 0.18–2.95% of bacterial production. Except in April, *H. ulvae* grazed at a higher rate than meiofauna, with values ranging from 0.9 mg C m^{-2} d^{-1} to 34.0 mg C m^{-2} d^{-1} compared to ranges of 0.4 mg C m^{-2} d^{-1} to 1.9 mg C m^{-2} d^{-1} for meiofauna (Fig. 4B and C). Nematodes were the main meiofaunal grazers. Bacteria and bacterivore feeding behaviors did not present clear variations seasonally (Fig. 4); consequently, the fate of bacteria in this benthic food web appeared poorly structured over time.

Discussion

Methodological considerations—We assumed that environmental conditions affecting grazers vary primarily in season. The sampling strategy was thus set up to explore seasonal rather than spatial variation. Our results corroborated this assumption as the PCA showed greater separation of sampling events by season than by spatial location or by specific day. Our sampling strategy should, therefore, cover a large range of environmental conditions.

Among the grazers, the mudsnail *H. ulvae* is highly mobile on the studied mudflat (Haubois et al. 2002). This mobility implies that snails are not necessarily adapted to the local conditions where they are collected. Consequently, we did not study the effects of environmental factors on grazing rates of *H. ulvae*.

For the other studied grazers, 15 variables were examined and correlations were tested for statistical significance at the 5% level (Table 4). This procedure tends to deem too many tests significant; to compensate, probability values could be adjusted for the number of simultaneous tests using a Bonferroni correction. However,

this correction has the major disadvantage of reducing the power to detect more than one false null hypothesis (Miller 1981). Consequently Bonferroni correction was not applied and the correlations with high *p*-values presented in Table 4 should be interpreted with caution.

The variability of bacterivory by grazers appeared heterogeneous among sampling events. The present study is based on the assumption that variability is real and arises from variation in the feeding behavior of grazers. Another potential source of variability may be related to the experimental methods, such as isotopic contamination during sample preparation. Natural causes of variability could not be discriminated from artificial ones and, although everything was done to minimize it, this potential bias should be kept in mind. Concerning the feeding behaviors of the nematode and copepod communities, caution must also be taken in interpreting their response because their estimated grazing activity reflects the action of the most abundant taxa and can consequently mask species-specific patterns (Buffan-Dubau and Carman 2000). Moreover, during each sampling event, grazers were collected and rapidly put in contact with ^{15}N labeled bacteria in microcosms. If natural and isotopically enriched bacteria communities are dissimilar, selective ingestion by grazers can render the evaluation of their bacterivory unrealistic. In a previous paper, we determined that the diversity of labeled bacteria changed slightly after being cultured, but that their size and activity were roughly similar to those of the natural bacterial community (Pascal et al. 2008b). Despite this change in community composition, the bacteria used for the grazing experiment showed characteristics that were more representative of the natural community than usually noted in other grazing studies. In spite of potential biases, experiments were based on the assumption that grazers took up enriched bacteria in the same way as natural bacteria.

A first way to compare our results with those from the literature is to compare individual grazing rates of bacteria. Bacterivory measured using the ^{15}N prelabeled bacteria method in experimental conditions is comparable with that reported in the literature (Pascal et al. 2008b). Moreover, the grazing rates found in this previous experimental study were within the range of those found in the present work: 27 vs. 8–36 pg C ind^{-1} h^{-1} for nematodes, 67 vs. 35–

Table 2. Values of biotic factors measured in surficial sediment through the year at Sta. 3 (means ± SD).

| | 2006 | | | | | | | | 2007 |
	Jan	Mar	Apr	May	Jun	Jul	Sep	Nov	Feb
Algal biomass (g C m^{-2})	6.85 (±0.42)	5.55 (±0.18)	3.07 (±0.11)	2.80 (±0.53)	2.94 (±0.14)	2.74 (±0.13)	2.78 (±0.23)	2.92 (±0.20)	2.33 (±0.36)
Flagellate biomass (mg C m^{-2})	1.07 (±0.25)	0.53 (±0.12)	0.80 (±0.71)	0.28 (±0.05)	0.13 (±0.10)	0.35 (±0.12)	0.12 (±0.06)	0.54 (±0.13)	0.34 (±0.21)
Nematode biomass (g C m^{-2})	0.31 (±0.04)	0.16 (±0.04)	0.23 (±0.02)	0.24 (±0.04)	0.20 (±0.02)	0.20 (±0.06)	0.27 (±0.02)	0.22 (±0.06)	0.29 (±0.01)
Copepod biomass (mg C m^{-2})	21.4 (±1.7)	22.9 (±1.3)	19.3 (±1.4)	93.2 (±13.2)	21.6 (±5.8)	12.7 (±2.2)	19.0 (±10.1)	30.4 (±7.6)	16.5 (±2.2)
A. tepida biomass (mg C m^{-2})	3.9 (±0.3)	20.9 (±5.6)	23.9 (±2.8)	7.3 (±2.2)	2.3 (±0.8)	7.4 (±7.5)	2.3 (±0.2)	3.8 (±0.2)	16.8 (±14.7)
H. ulvae biomass (mg C m^{-2})	154 (±55)	31 (±53)	4 (±7)	117 (±32)	202 (±50)	113 (±59)	22 (±25)	111 (±40)	19 (±9)
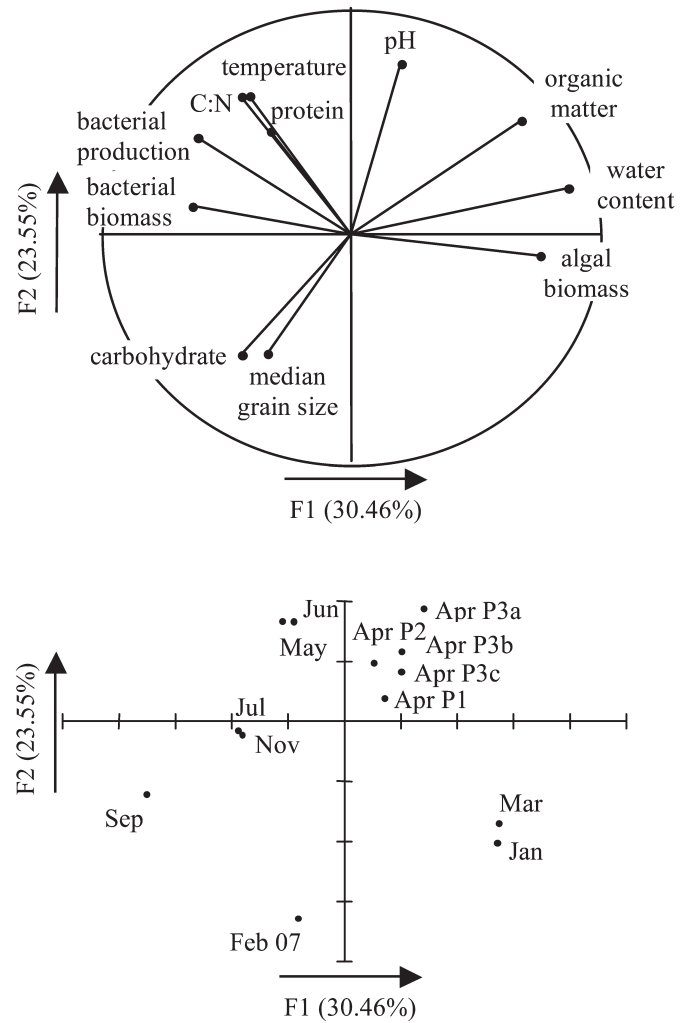
Fig. 2. PCA calculated using 13 observations (samples of surficial sediment) and 11 variables (8 abiotic and 3 biotic variables). For each variable, the circle of correlation is reported. Sampling events are represented by the abbreviation of their months: Apr P1, 2, and 3 represent samples taken in Sta. 1, 2, and 3 and Apr P3a, b, and c represent sampling performed on 25, 26, and 27 April 2006 at Sta. 3, respectively.

608 pg C ind^{-1} h^{-1} for *A. tepida*, and 1149 vs. 883–2389 ng C ind^{-1} h^{-1} for *H. ulvae*. Further, these values were remarkably similar despite slight differences in methodology: in the laboratory experimental study, grazers were allowed to graze on a slurry of natural and enriched bacteria, whereas in the present study, they grazed only on enriched bacteria.

A second way to compare our results with those from the literature is to evaluate grazers' effect on natural bacterial stock. In the present study, meiofauna grazed 0.04–0.34% of bacterial standing stock per day in the 1-cm-deep surficial mudflat sediment. Using a method where tracers can be directly ingested in sediment to label bacteria as they are grazed, the proportion of bacterial stock grazed per day was 24% in a shallow estuary (Montagna and Yoon 1991) and reached 81% in a salt marsh (principally due to polychaetes; Montagna 1984). Using fluorescent prelabeled
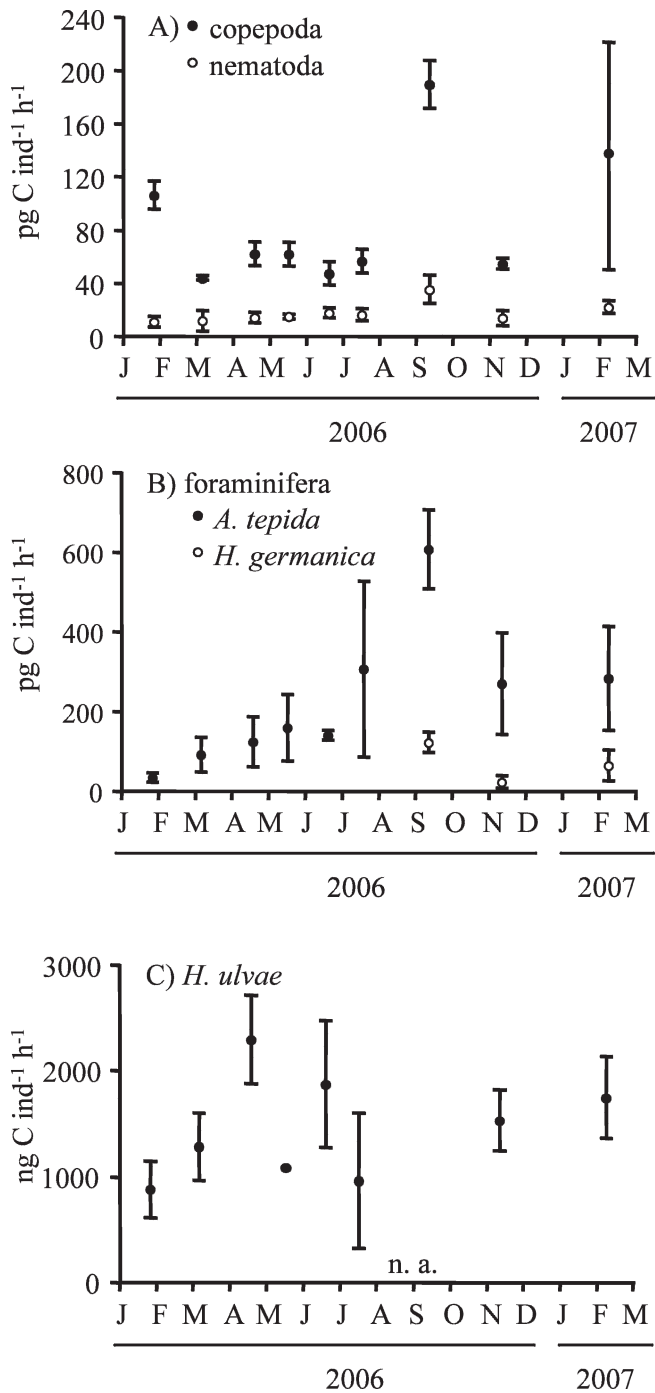
Fig. 3. Individual grazing rates (pg C ind^{-1} h^{-1}) of (A) copepods and nematodes, (B) foraminifers (*A. tepida* and *H. germanica*), (C) and *H. ulvae* (ng C ind^{-1} h^{-1}) through the year at Sta. 3 (means ± SD; n. a. = not available).

bacteria, one study reported a rate of 0.03% in intertidal mudflats (Epstein and Shiaris 1992). Another study, using integrated modeling and in situ isotopic-tracers method, found 3% in an intertidal mudflat (Van Oevelen et al. 2006). Differences observed between studies may be due to the different locations (i.e., the low grazing effect in our study may be characteristic of the Brouage mudflat). Differences may also be attributed to the different

techniques. Direct addition of labels for estimating grazing rates has several shortcomings, including direct ingestion of free labels and absorption into epicuticular bacteria associated with the surface tissues of grazers (Carman 1990). Another potential bias rarely taken into account is the direct ingestion of substances released by labeled prey. Sediment bacteria produce copious amounts of extracellular polymeric secretions (Decho 1990), which are assimilated by grazers with high efficiency (Decho and Moriarty 1990) and may represent a more important carbon source than bacterial cells themselves in grazers' diets (Hall and Meyer 1998).

Evaluation of the trophic fate of bacteria necessitates assessment of bacterial consumption by the main grazers. In the present study, bacteria were principally grazed by macrofauna. However, conclusions concerning the general effect of macrofauna must be made with caution because not all potential grazers could be taken into consideration. Bivalves are less abundant than gastropods, but they represent the dominant fraction of macrofaunal biomass in the mudflat (Bocher et al. 2006). According to their natural isotopic signature, two of these dominant bivalves (*Scrobicularia plana* and *Macoma balthica*) feed preferentially on a mixture of benthic diatoms and marine phytoplankton (Riera et al. 1999). However, these bivalves may also influence bacterial biomass through their deposit feeding activity.

Grazing experiments were performed over a 5-h period. This time duration may allow the studied grazers to assimilate rather than only ingest food. Ingestion rate of nematodes would be four times higher assuming an assimilation of 25% (Somerfield et al. 2005). In a previous study, the principal bacterivore *H. ulvae* was experimentally shown to graze at a rate 30% lower during 5-h incubation than during 2-h incubation (Pascal et al. 2008*b*). Consequently, such incubation periods would lead to an underestimation of grazing rates on bacteria.

Evaluation of the trophic fate of bacteria also necessitates assessment of bacterial production. The estimation of this production from incorporation of thymidine into DNA has uncertainties common to all benthic studies (Moriarty and Pollard 1990), particularly in sediment rich in clay (Garet and Moriarty 1996).

Effects of abiotic and biotic factors on bacterivory— During this study, incubations were performed at in situ temperatures ranging from 7.2°C to 33.7°C and in situ salinity ranging from 27.0 to 31.4. Despite these fluctuations, meiofaunal grazing was not significantly affected by temperature or salinity (Table 4). Contrasting results were found in a previous experimental study where temperature affected the grazing rates of the foraminifer *A. tepida* as well as nematodes, and decreased salinity affected the grazing rates of *A. tepida* (Pascal et al. 2008*a*). However, in these experiments, which were performed in controlled conditions, grazers were not acclimated at each tested temperature and salinity. Consequently, the grazing response after rapidly transferring grazers to different abiotic conditions demonstrates the result of short-term environmental variation. In the present in situ study, grazers were

Table 3. Grazing rates of meiofauna and macrofauna in surficial sediment and grazing rates of nematodes in the deeper layer of sediment along the cross-shore transect (27 Apr 06; mean $\pm$ SD; n. a. = not available).

Grazing rates	Sampling station		
	1	2	3
Nematode in surface (pg C ind^{-1} h^{-1})	11.7 ($\pm$3.3)	7.6 ($\pm$1.5)	18.2 ($\pm$5.3)
Nematode in deep layer (pg C ind^{-1} h^{-1})	13.8 ($\pm$3.3)	9.4 ($\pm$3.6)	30.1 ($\pm$7.1)
Copepod (pg C ind^{-1} h^{-1})	227.6 ($\pm$84.4)	59.9 ($\pm$8.5)	73.0 ($\pm$5.1)
Foraminifera (pg C ind^{-1} h^{-1})	n. a.	n. a.	125.2 ($\pm$62.8)
H. ulvae (ng C ind^{-1} h^{-1})	n. a.	n. a.	2297 ($\pm$418)

incubated with their prey under conditions that closely matched their natural environmental conditions. Thus, our results do not contradict the experimental studies: while meiofauna may be affected by very short-term variation in temperature and salinity, they may acclimate and adjust to local environmental conditions throughout the year, reducing the effect of seasonal variation.

Nematode grazing decreased when sediment had high water content and low median grain size, but caution must be taken in the interpretation of these results, because the correlations were not significant (Table 4). Consequently, the bacterivory of meiofauna appeared to be only weakly influenced by variation in the abiotic factors of the sediment in the study area.

Grazing activity can also be limited by biotic factors such as resource limitation or competition between grazers. No relationship was observed between grazing rates of bacteria and bacterial abundance. If meiofauna had been food-limited by bacteria, we should have observed increased feeding rates when bacterial biomass increased. Additionally, the absence of negative interactions between the grazing activities of each type of grazer may indicate that there was no competition for the bacterial resource. For these two reasons, bacteria may not constitute a

limiting resource in this study area. Ratios between grazer biomass and grazed bacterial biomass also suggest that bacteria were less important in meiofaunal diets than in macrofaunal diets (Table 5).

Copepods appeared to ingest bacteria independently of algal abundance (Table 4). It is difficult to elaborate on this result because the composition and diet of this community from the Brouage mudflat has been poorly studied. However, the finding that feeding behavior was unrelated to algal resources contradicts studies on copepods in other locations (Buffan-Dubau and Carman 2000). On the contrary, bacterial grazing rates of nematodes and the foraminifer *A. tepida* decreased with increasing algal abundance, suggesting that algae are preferentially ingested over bacteria and that bacteria are consumed when algal abundances are low. The same result was found in experiments performed on grazers from the same mudflat using dual-labeled prey (Pascal et al. 2008*a*). Changes in food preference in response to variation in algal abundance were observed both on short time scales in controlled conditions (Pascal et al. 2008*a*) and on long time scales in situ (present study). This observation is in accordance with stable isotopic analysis suggesting that microphytobenthos dominates the diet of the Brouage nematode community

Table 4. Correlation coefficients (r_s, Spearman rank) on the grazing rates of meiofauna and abotic or biotic parameters of the environment ($n = 13$).

	Bacterial grazers		
	Nematoda	Copepoda	*A. tepida*
pH	0.099	−0.391	−0.175
Salinity	−0.119	0.140	−0.119
Temperature	0.320	−0.122	0.450
Sediment water content	−0.599*	−0.390	−0.475
Median grain size	0.566*	−0.104	0.265
Organic matter	−0.456	−0.209	−0.431
C:N	0.275	0.022	0.425
Carbohydrate	0.016	0.253	0.182
Protein	0.390	0.203	−0.055
Algal biomass	−0.731**	−0.143	−0.829***
Flagellate biomass	−0.560*	−0.214	−0.525
Bacterial biomass	0.247	0.050	0.470
Bacterial production	0.209	−0.170	0.569*
Bacterial grazing by *A. tepida*	0.547	0.083	
Bacterial grazing by copepods	0.143		0.083
Bacterial grazing by nematodes		0.143	0.547

* $p<0.05$; ** $p<0.01$; *** $p<0.001$.

Table 5. Ratios between grazer biomasses (mg C m^{-2}) and bacteria consumed (mg C m^{-2} d^{-1}) by those grazers in surficial and deeper layers of sediment along the cross-shore transect (27 Apr 06).

Ratio between grazers biomass and bacterial biomass grazed	Sampling station		
	1	2	3
Nematode in surficial sediment	526	831	367
Nematode in deeper sediment	488	594	216
Copepods	28	88	138
*A. tepida**	333	283	395
*H. ulvae**	5	2	5

* Data estimated with grazing rates measured at Sta. 3.

(Riera et al. 1996) and that this community is dominated by epigrowth feeders (i.e., diatom consumers [Rzeznik-Orignac et al. 2003]).This food preference was also suggested by natural isotopic composition of meiofauna in other locations; low algal abundances imply a shift in meiofaunal diets and ingestion of other food sources (Carman and Fry 2002). Epstein's (1997) study also suggests that there are alternative states in microbial food-web dynamics, and that ingestion rates of algae and bacteria are inversely correlated.

Why are bacteria less attractive than algae to grazers? There are several possible explanations. First, bacteria are generally less abundant than algae in temperate intertidal sediments (Rublee 1982). Second, bacteria may lack essential components, such as fatty acids, that are present in diatoms (Zhukova and Kharlamenko 1999). Finally, bacteria and algae differ in their spatial distribution. Benthic microalgae are concentrated at the air–sediment interface during diurnal low tides. Grazers are known to feed on this algal biofilm (Buffan-Dubau and Carman 2000), limiting the energy spent selecting food particles and/ or rejecting nondigestible material. Given the more homogenous vertical distribution of bacteria (Joint et al. 1982), grazing on bacteria would be less efficient than on algae.

Determining the role bacteria play in the diet of benthic grazers constitutes a paired ecological question of the trophic fate of bacterial production. In the present study, the ingestion of all food sources was not quantified for each grazer. Nevertheless, the ratio between grazer biomass and

Table 6. Bacteria taken up by grazers in surficial and deeper layers of sediment along the cross-shore transect (27 Apr 06).

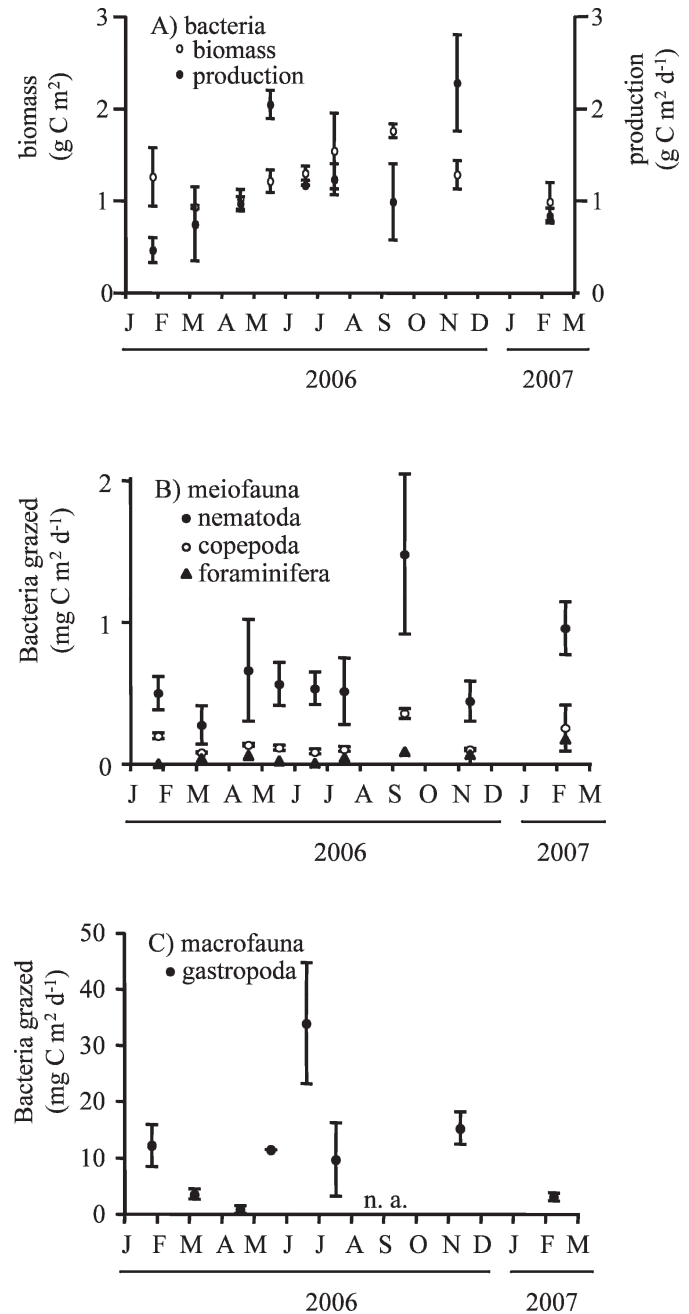| Bacterial biomass grazed by:	Sampling point		
	1	2	3
Nematodes in surficial sediment (mg C m^{-2} d^{-1})	2.89	0.79	0.66
Nematodes in deeper sediment (mg C m^{-2} d^{-1})	0.18	0.11	0.08
Copepods (mg C m^{-2} d^{-1})	0.43	0.11	0.14
*A. tepida** (μg C m^{-2} d^{-1})	3.08	2.60	60.44
*H. ulvae** (mg C m^{-2} d^{-1})	59.94	331.69	2.67

* Data estimated with grazing rates measured at Sta. 3.

Fig. 4. (A) Bacterial biomass (gC m^{-2}) and production (gC m^{-2} d^{-1}) and bacteria grazed (mg C m^{-2} d^{-1}) by (B) meiofauna and (C) *H. ulvae* in surficial sediment at Sta. 3 through the year (means ± SD; n. a. = not available).

grazed bacterial biomass provides an approximation of the role played by bacteria in each grazer's diet. Bacteria appear to be comparatively more important in the *H. ulvae* diet than in meiofaunal diets (Table 5). This mudsnail shows lower efficiency in algal selection in comparison with meiofaunal grazers from the Brouage mudflat (Pascal et al. 2008a). Benthic organisms with the lowest bacterial diet would consequently present the highest ability to select algae, suggesting that bacteria do not constitute a preferentially ingested resource.

Bacteria and bacterivore feeding behaviors did not vary in a systematic way over time (Fig. 4). In sandflats (Epstein 1997) and in silty and sandy sediments (Hamels et al. 2001), the highest rates of bacterivory were observed during autumn and winter. In contrast, Montagna (1984) did not observe significant variations in bacterivory between summer and winter within the finest sediment from a salt marsh. Seasonality of benthic bacterivory may be grain-size dependant; however, more studies are needed to clarify this relationship. To our knowledge, this study is the first to focus on the trophic fate of bacteria across different seasons in mudflat sediment.

Trophic fate of bacteria—The present study suggests that bacterial abundance and production were not negatively influenced by grazer activity. Consequently, whether grazing constitutes a significant fate of bacterial production should be considered.

In general, pelagic bacteria are grazed principally by protozoa (Sherr and Sherr 2000). Benthic systems differ from pelagic systems in that protozoa are proportionally less abundant relative to bacteria, but ingestion rates may be comparable (Kemp 1990). Ciliates favor interstitial volumes (Hamels et al. 2004) and low organic-matter content (Bak and Nieuwland 1989). As a result, highest abundance of ciliates is found in fine sands and the lowest in muddy sediments (Alongi 1986). We did not measure ciliate abundance in the present study, because they are relatively scarce and have limited grazing activity in intertidal mudflat sediments (Epstein and Shiaris 1992). In his review, Kemp (1990) attests that flagellates can ingest a large fraction of bacterial production, but only when their relative abundance is in the order of 1 flagellate to 1000 bacteria or more. Indeed, more recent studies confirmed that in silty sediments this ratio was lower than $1:10^4$ and flagellates never grazed $>1\%$ of bacterial stock per day (Hamels et al. 2001). In muddy sediments, with a ratio of $6.8:10^4$, flagellates removed 0.2% of bacterial stock per day (Epstein and Shiaris 1992). In the present study, this ratio never exceeded $1.3:10^5$, suggesting that flagellate grazing has a limited effect on bacteria.

The grazing rate of nematodes on bacteria was higher in deeper than in surficial sediment in the lower part of the mudflat (Sta. 3). Conversely, at the two other stations, there were no vertical differences in feeding rates. It is generally accepted that the proportion of epigrowth feeding decreases with sediment depth (Heip et al. 1985). Consequently, grazing on algae decreases with depth. Furthermore, the existence of two distinct food webs segregated by depth was suggested by Rudnick (1989). The present study suggests that this segregation is not systematic and may depend on shore elevation. Alongi (1989) and Kemp (1990) consider that the fraction of bacteria grazed declines roughly in proportion to the decrease of predator abundance with increasing depth. Our results do not conflict with this and, in the three stations studied, the fraction of bacterial stock grazed was >200 times higher in surficial than in deeper sediment. This result demonstrates that meiofaunal grazing has limited effect on bacteria in both surficial and in deeper sediments.

In the lower part of the Brouage mudflat, bacteria were grazed by macrofauna at, on average, a rate 12 times higher than by meiofauna. Similarly van Oevelen et al. (2006) conclude that macrofauna graze bacteria at a rate eight times higher than meiofauna. Extensive literature exists on the role of bacteria as food for macrofauna (review in Lopez and Levinton 1987) but control of bacteria by macrofauna has received less attention. In his review, Kemp (1990) concludes that macrofauna have not been shown to reduce bacterial abundance, except when exceptionally high densities of macrofauna are present. In the mudflat studied here, *H. ulvae* was mainly distributed in the upper half of the mudflat (Bocher et al. 2006; Haubois et al. 2002). Patterns of distribution found in the present study were similar, with the highest snail biomass in Sta. 1 and 2 and the lowest in Sta. 3. Snails grazed 6%, 19%, and 0.3% of bacterial stock per day in Sta. 1, 2, and 3, respectively. Results of the present study consequently corroborate Kemp's conclusions (1990) and *H. ulvae* showed the greatest effect in the upper part of the mudflat where snail abundances were high. Bacterial stock grazed by *H. ulvae* fluctuated greatly during the year (Fig. 4C). These variations were mainly due to large fluctuations in snail density, which has also been observed by Haubois et al. (2002).

In the upper and middle part of the mudflat, grazing estimates represented respectively 7% and 28% of bacterial production and, over the year of study, never represented $>3\%$ of bacterial production in the lower part of the mudflat. Even in light of the potential biases discussed above, grazing is a secondary fate of bacterial production in the upper Brouage mudflat and a minor fate in the lower part of the mudflat. This general conclusion is in accordance with other studies conducted in intertidal mudflats (Epstein and Shiaris 1992; Van Oevelen et al. 2006). If consumption of bacteria is low, the fate of unconsumed bacterial production remains unexplained and poorly documented (Meyer-Reil 1984). Benthic bacteria can potentially enhance planktonic biomass when the top few centimeters of sediment are resuspended (Blanchard et al. 1997). Bacterial mortality can also be induced by viral lysis and programmed cell death. After cell death, bacterial carbon joins the organic carbon pool in the sediment and has two potential fates: recycling by degradation and burial. Veuger et al. (2006) concluded that burial would not be a major fate of bacterial carbon in intertidal mudflats and Novitsky (1986) came to a similar conclusion in tropical marine beach sediments. According to Alongi (1989), the major part of bacterial production remains ungrazed but instead lyses and the lysate is recycled back into the bacterial community and participates in biogeochemical cycles maintaining the cycling of essential compounds.

In the sediment of the Brouage mudflat, grazing is a secondary or even minor fate of bacterial production and bacteria appear to be grazed principally by the large macrofaunal grazers *H. ulvae* (>500 μm). The highest bacterivory appeared to be due to *H. ulvae*, although this mudsnail has a lower capacity to select benthic diatoms than *A. tepida* and the nematode community (Pascal et al. 2008a).

Feeding behavior of the latter grazers appeared to be more influenced by algal resources than by bacteria, suggesting that algae constitute a preferential food source over bacteria. Bacterivory did not vary according to season; consequently, the fate of bacteria in this benthic food web is poorly structured in the Brouage mudflat over the seasons.

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