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Influence of environment factors on bacterial ingestion rate of the deposit-feeder *Hydrobia ulvae* and comparison with meiofauna

Pierre-Yves Pascal[1, *], Christine Dupuy[1], Pierre Richard[1], Anne-Gaelle Haubois[1], Nathalie Niquil[1]

[1] Littoral, Environnement et SociétéS (LIENSS) UMR 6250 CNRS – Université de La Rochelle, 2 Rue Olympe de Gouges, 17042 La Rochelle cedex, France

*: Corresponding author : Pascal P. Y., email address : pypascal@univ-lr.fr

Abstract:

Deposit feeders are able to process a considerable volume of sediment, containing large quantities of associated bacteria. However, conclusions concerning the trophic role played by benthic bacteria in marine sediments are still not fully elucidated. This study deals with bacterivory by the gastropod *Hydrobia ulvae*, one of the most abundant deposit-feeding species in intertidal mudflats in Western Europe. Ingestion rates of bacteria were determined during grazing experiments using ^{15}N pre-enriched bacteria. Grazing experiments were performed in order to measure effects of abiotic (temperature, salinity and luminosity) and biotic (bacterial and algal abundances) factors on ingestion rates of bacteria by *H. ulvae* of an intertidal mudflat (Brouage, Marennes-Oléron, France). The mean ingestion rate of bacteria by *H. ulvae* was 1149 ng C ind^{-1} h^{-1}. The general trend showed a temperature effect with an optimum around 30 °C, and the assimilation rate was significantly lower at 5 °C. Bacterial assimilation did not significantly differ between salinity 18 and salinity 31. Ingestion was the same in light and in dark conditions. Results were compared with those of other grazing experiments conducted simultaneously in similar conditions with two other grazers with different size and feeding modes: the foraminifera *Ammonia tepida* and a nematode community from the superficial sediment of the Brouage mudflat. *H. ulvae* and nematodes presented a feeding behavior less influenced by environmental changes than *A. tepida*. *H. ulvae* ingested bacteria at a higher rate than smaller meiofaunal grazers and seemed to have a lower ability to selectively ingest diatoms than meiofaunal grazers.

Keywords: Deposit-feeding, Hydrobia ulvae, Gastropod, Bacteria, Grazing, Environmental factors, Ammonia tepida, Nematodes, Mudflat

Introduction

In pelagic environments, bacteria are heavily grazed and consequently play a major role in food webs (Azam et al., 1983). In the benthic environment, bacteria are generally 1000 times more abundant than in pelagic systems, reaching abundances of about 109 cells cm3 34 (Schmidt et al., 1998). However, microbial food web research in sediment is in its infancy and the trophic significance of benthic bacteria remains elusive (Review in Kemp, 1986). The deposit-feeder Hydrobia ulvae is one of the most abundant species of macrofauna inhabiting intertidal mudflats in Western Europe (Bachelet and Yacine-Kassab, 1987; Barnes, 1990; Sola, 1996). Deposit feeders typically process at least one body weight of sediment daily (Lopez and Levinton, 1987). This sediment includes highly digestible and nutritious microphytobenthos and bacteria, less digestible plant debris and completely indigestible refractory detritus (Rice and Rhoads, 1989). Diatoms have been found to be a major source of nutrition for H. ulvae (Fenchel et al., 1975; Jensen and Siegismund, 1980; Lopez and Cheng, 1983a; Bianchi and Levinton, 1984; Haubois et al., 2005a). However bacteria have also been found as food for Hydrobia species (Cammen, 1980; Jensen and Siegismund, 1980; Bianchi and Levinton, 1981; Levinton and Bianchi, 1981). Due to high abundances of H. ulvae in intertidal mudflats, carbon flow from bacteria to snails may be a significant pathway in this type of environment. The objective of the present study was to quantify the bacterial ingestion rate of H. ulvae and to investigate how this rate varies with abiotic (temperature, salinity and luminosity) and

biotic (bacterial and algal abundance) factors. Intertidal mudflats are subject to large and quick changes in many environmental factors at short time scales (circadian and tidal cycles) (Guarini et al., 1997) and these variations may significantly influence snail feeding behaviour. Bacterivory of *H. ulvae* was then compared to that of other grazers of different sizes and feeding modes, the foraminifera *Ammonia tepida* and a nematode community from surficial sediment of an intertidal mudflat (Brouage-Marennes Oléron-France). Bacterial ingestion of both grazers have been previously described (Pascal et al., 2008b; Pascal et al., In press). All grazing experiments were performed simultaneously in similar conditions using stable isotope enriched prey (^{13}C enriched algae and ^{15}N enriched bacteria).

Experimental procedure

Study site

The Brouage intertidal mudflat is located in the eastern part of the Marennes-Oléron Bay (Atlantic coast of France). Meteorological conditions exhibit a strong seasonality typical of a temperate climate. Range of temperature and salinity in emerged sediments are more extreme during summer tidal cycles (Guarini et al., 1997). Minimum and maximum mud temperatures are 5°C and 34°C respectively. The maximum daily range of mud temperature due to emersion and immersion cycle reaches 18°C (Guarini et al., 1997). Salinity of overlaying water is controlled by the Charente River freshwater input, ranging from 25 to 35 over the year (Héral et al., 1982). Salinity of the upper layers of sediment may also decrease with rainfall. The sediment surface irradiance shifts from dark during submersion and night emersions to high levels of incident light during daytime emersions. This irradiance can reach 2000 µM of photons m^{-2} s^{-1} (Underwood and Kromkamp, 2000). Details of numerous benthic organisms and processes are available concerning this intertidal zone (gathered in Leguerrier et al., 2003; Leguerrier et al., 2004; Degré et al., 2006).

Preparation of ^{15}N enriched bacteria

Superficial sediment (1 cm depth) was collected on the Brouage mudflat (45,55,074 N; 1,06,086 W). One cm^3 of the collected sediment was added to 20 cm^3 of bacterial liquid culture medium and kept in darkness for 24 hours at 13°C. The composition of this culture medium was previously described in Pascal et al. (2008a). This primary culture was then subcultured for 24 hours under the same conditions to get approximately 2×10^9 cells cm^3. Finally, bacteria were collected in 0.2 µm filtered seawater after 3 centrifugations (3500 g, 10 mn, 20°C), frozen in liquid nitrogen and kept frozen at -80°C until grazing experiments.

Preparation of ^{13}C enriched algae

An axenic clone of the diatom *Navicula phyllepta* (CCY 9804, Netherlands Institute of Ecology NIOO-KNAW, Yerseke, The Netherlands), the most abundant diatom species in the study area (Haubois et al., 2005b), was cultured in medium described by Antia and Cheng (1970) and containing $NaH^{13}CO_3$ (4 mM). Diatoms were concentrated by centrifugation (1500 g, 10 mn, 20 °C), washed three times to remove the ^{13}C-bicarbonate, and freeze-dried.

Quantification of bacteria and algae abundance

In order to determine the ratio between enriched and non-enriched preys in microcosms, abundances of bacteria and algae were assessed. To separate bacteria from sediment particles, incubation in pyrophosphate (0.01M for at least 30 min) and sonication (60 W) were performed. Bacteria from both sediment and culture were labelled using 4.6-diamidino-2-phenylindole dihydrochloride (DAPI) (2500 µg l^{-1}), filtered onto 0.2 µm Nucleopore black filter (Porter and Feig, 1980) and then counted by microscopy. We verified the absence of ciliates and flagellates in the bacterial culture during this microscope observation step. The abundance of diatoms in the sediment was assessed using Chl *a* as a proxy, measured using fluorometry (Lorenzen, 1966).

Grazing experiments

100 The top centimeter of sediment was collected during ebb tide from the same study area at
101 midday on March 13, 2006. It was sieved on 500 µm, 200 µm and 50 µm in order to
102 concentrate respectively *H. ulvae*, *A. tepida* and nematodes. Before sieving, snails were
103 placed on natural sediment and kept for 24 h at 20°C in the dark. Time between sieving and
104 the start of the grazing experiment never exceeded two hours in order to avoid starvation bias
105 in feeding behavior (Calow, 1975). Seventeen handpicked specimens of *H. ulvae* were placed
106 in polypropylene Petri dishes (ø = 9 cm). This density was chosen in order to avoid a density-
107 dependence effect on the individual ingestion due to space limitation (Blanchard et al., 2000).
108 A fraction of the sediment passing through the 50 µm mesh was mixed with the ^{15}N enriched
109 bacteria. This slurry contained 10.5×10^8 bacteria cm^3, ^{15}N enriched bacteria being twice as
110 abundant as non-enriched ones. Four cm^3 of this slurry were put into each microcosm. Each
111 experiment was carried out in triplicate, along with at least one control. Control samples were
112 frozen (-80°C) in order to kill any grazers.
113 The calculation of bacterial ingestion rate relies on the assumption that enriched preys
114 accumulate in snail's gut at a constant rate, and that no egestion of labelled materials occurs
115 during incubation time. A kinetic study was run for 1 to 12 hours including the 2 hour run that
116 was used for all other experiments. Incubations were made under the following standardized
117 conditions that were close to the mean values recorded on the study site: temperature (20°C),
118 salinity (31), luminosity (darkness), bacterial abundance (10.5×10^8 bacterial cells cm^3) and
119 algal abundance (15 µgChla g dry $sediment^{-1}$). For each experiment to determine the effects
120 of environmental factors, only one incubation factor was modified so as to determine its effect
121 on *H. ulvae*'s grazing activity. After the sieving step, snails were transferred without
122 acclimation into different microcosms to simulate short-term changes of environmental
123 factors. To test the effect of temperature, the snails were placed at 5°C, 15°C, 30°C and 40°C:
124 these temperatures are in the range of those measured in the study area (Guarini et al., 1997).

The effect of salinity was investigated by placing *Hydrobia* in microcosms with a salinity of 18. To decrease salinity, cultured bacteria were rinsed with 0.2 µm filtered-sea-water diluted with 0.2 µm filtered distilled water. Such decrease in salinity can occur in field conditions when sediment is exposed to heavy rainfall. The light effect was tested with a light intensity of 83 µM of photons m^{-2} s^{-1}. Bacterial abundance was modified adding various quantities of bacteria enriched in ^{15}N. Bacterial abundances (total enriched and non-enriched) tested were 4, 7 and 17 cells cm^3 wt sed^{-1} with respectively the following ratio between abundance of total and enriched bacteria: 6.1, 2.0 and 1.3. Algal abundance was modified by adding various quantities of cultured *N. phyllepta* enriched in ^{13}C while bacterial abundances (total enriched and non-enriched) were kept constant at 10×10^8 cells cm^3. Algal abundance (total enriched and non-enriched) were 26, 64 and 114 µgChla g dry sed^{-1} with respectively the following ratio between abundance of total and enriched algae: 2.4, 1.3 and 1.2.

Incubations were stopped by freezing microcosms at -80°C. Samples were thawed and *H. ulvae* were separated by hand from their shell and the 17 specimens of each microcosm were pooled and homogenized using a Potter-Eveljhem.

Isotope analysis and calculations

$\delta^{15}N$ and $\delta^{13}C$ of prey (bacteria and algae) and grazers were measured using an EA-IRMS (Isoprime, Micromass, UK). Nitrogen isotope composition is expressed in the delta notation ($\delta^{15}N$) relative to air N_2: $\delta^{15}N = [((^{15}N/^{14}N)_{sample} / (^{15}N/^{14}N)_{reference})-1] \times 1000$. Carbon isotope composition is expressed in the delta notation ($\delta^{13}C$) relative to Vienna Pee Dee Belemnite (VPDB): $\delta^{13}C = [((^{13}C/^{12}C)_{sample} / (^{13}C/^{12}C)_{reference})-1] \times 1000$.

Incorporation of ^{15}N is defined as excess above background ^{15}N (control experiment) and is expressed in terms of specific uptake (*I*). *I* was calculated as the product of excess ^{15}N (*E*) and biomass of N per grazer. *I* was converted to bacterial carbon grazed using the C/N ratio of bacteria. *E* is the difference between the background ($F_{background}$) and the sample (F_{sample}) ^{15}N

fraction: $E = F_{sample} - F_{background}$, with $F = {}^{15}N / ({}^{15}N + {}^{14}N) = R / (R + 2)$ and R = the nitrogen isotope ratio. For the $F_{background}$, we used control values measured with killed grazers (frozen). For *H. ulvae* we used the highest value measured in control ($\delta^{15}N = 12.42$ and $\delta^{13}C = -13.72$). R was derived from the measured $\delta^{15}N$ values as: $R = ((\delta^{15}N/1000)+1) \times R\text{air}N_2$ where $R\text{air}N_2 = 7.35293 \times 10^{-3}$ (Mariotti, 1982). The uptake of bacteria was calculated as Uptake = ($I \times$ (% $C_{enriched\ bacteria}$ / % $N_{enriched\ bacteria}$)) / ($F_{enriched\ bacteria} \times$ incubation time). This uptake was multiplied by the ratio between the abundance of total and enriched bacteria determined by DAPI counts.

Incorporation of ^{13}C was calculated analogously, with $F = {}^{13}C / ({}^{13}C + {}^{12}C) = R / (R + 1)$, $R\text{air}N_2$ is replaced by $R_{VPDB} = 0.0112372$ and Uptake = I / ($F_{enriched\ bacteria} \times$ incubation time). The uptake measured was multiplied by the ratio between the abundance of total and enriched diatom, determined from fluorometrical measurements.

Enriched *N. phyllepta* carbon consisted of 22.95 ± 0.54% ^{13}C. The C/N ratio of enriched bacteria was 3.49 and bacterial nitrogen consisted of 2.88 ± 0.03% ^{15}N. The individual average weight of *H. ulvae* was 0.54 ± 0.08 mg and each specimen was composed on average of 184 ± 19 µgC and 43 ± 4 µgN (N = 72 samples of at least 17 specimens each). Ingestion rate as $gC_{bacteria}\ gC_{H.\ ulvae}^{-1}\ h^{-1}$ was obtained by dividing ingestion rate of bacteria ($gC\ ind^{-1}\ h^{-1}$) by *H. ulvae* mean weight ($gC\ ind^{-1}$).

Variations of uptake rates according to salinity and irradiance were tested using bilateral independent-samples two-tailed tests. One-way analyses of variance (ANOVA) were used in order to test the impact of temperature and algal and bacterial abundance on uptake rates of bacteria and algae. The Tukey test was used for post-hoc comparisons.

Results

The kinetic experiment showed that accumulation of bacteria in *H. ulvae* increased linearly during the first two hours of incubation and then levelled off (Fig. 1). The linear regression

slope for the first two hours indicated an uptake rate of 1149 ngC ind^{-1} h^{-1} equivalent to 6.43 10^{-3} $gC_{bacteria}$ $gC_{\textit{H. ulvae}}^{-1}$ h^{-1} (r^2 = 0.98). The linear regression slope between five and twelve hours was more than seven times lower than for the two first hours and indicated an uptake rate of 145 µgC ind^{-1} h^{-1} equivalent to 0.81 10^{-3} $gC_{bacteria}$ $gC_{\textit{H. ulvae}}^{-1}$ h^{-1} (r^2 = 0.98).

Ingestion of bacteria increased from 462 to 1277 ngC ind^{-1} h^{-1} when temperature increased from 5°C to 30°C, and then decreased reaching 1059 µgC ind^{-1} h^{-1} at 40°C (Fig. 2). Ingestion rate of bacteria by *H. ulvae* was significantly decreased at 5°C (F = 10; p<0.01), but ingestion rates observed at 10, 20, 30 and 40°C were not significantly different.

The ingestion rate measured for a salinity of 31 (1149 ± 285 ngC ind^{-1} h^{-1}) was similar to the one measured for a salinity of 18 (1085 ± 58 ngC ind^{-1} h^{-1}) (two-tailed test, p = 0.20).

The ingestion rate observed under light conditions (1478 ± 246 ngC ind^{-1} h^{-1}) was similar to the one observed in darkness (1149 ± 285 ngC ind^{-1} h^{-1}) (two-tailed test, p = 0.72).

Ingestion rates of bacteria were significantly linked to bacterial abundance in microcosms (F = 38; p<0.001) (Fig. 3). Ingestion rate increased linearly from 38 ± 13 to 1117 ± 93 ngC ind^{-1} h^{-1}) when bacterial concentrations increased from 4 to 10 × 10^8 cells cm^3 and increased, though not significantly to 1604 ± 366 ngC ind^{-1} h^{-1} for a bacterial concentration of 17 × 10^8 cells cm^3.

Dual labeling of prey allowed simultaneous assessment of the ingestion of bacteria and algae. When algal concentration increased from 15 to 114 µgChla g^{-1} with constant bacterial abundance (10.5 × 10^8 cells cm^3), the ingestion rate of algae remained constant (F = 3.3; p = 0.11) (Fig. 4). However, ingestion rate of bacteria remained constant for algal concentration in the sediment between 15 and 64 µgChla g dry wt sed^{-1} but significantly decreased at the highest algal concentration of 114 µgChla g dry wt sed^{-1} (F = 4.4; p < 0.05). As a result, when algal abundance increased, the fraction of algae in the diet of *H. ulvae* increased.

Grazing experiments were simultaneously performed with two other grazers: the foraminifera *A. tepida* and a nematode community. Feeding behaviour of *A. tepida* (Pascal et al., 2008b) and nematodes (Pascal et al., In press) has been previously described. All grazing incubations were conducted at the same time and in similar conditions, making comparisons between grazers possible. The effects of environmental factors (temperature, salinity and luminosity) on ingestion rates of bacteria are summarized in Table 1. Among the different grazers tested, *A. tepida* was the most affected by salinity and temperature. Light only affected nematodes and increased their feeding activity. Classification of grazers according to their maximal ingestion rates of bacteria and algae reported per grazer weight gave the following list arranged in ascending order: *A. tepida*, nematode community and *H. ulvae* (Table 1). For the dual labelling experiment, ratios between algae and bacteria ingested were measured for each grazer, at each algal concentration. Comparison of those ratios between grazers reflected their respective ability to discriminate between food sources (*i.e.* algae and bacteria). At the three algal concentrations tested, *A. tepida* and nematodes showed a higher ratio of algae:bacteria ingested than *H. ulvae*, suggesting a better ability to preferentially ingest algae over bacteria compared to the gastropod (Fig. 5).

Discussion

Kinetic experiment

During the 12 hour incubation, *H. ulvae* first accumulated the enriched bacteria linearly over 2 hours (Fig. 1). This suggests that ingestion rate of bacteria was constant and that no egestion of recently ingested labelled material occurred during this period. After 4 hours of incubation, the accumulation rate of bacteria by *H. ulvae* decreased. This may have two nonexclusive origins: egestion of labelled bacteria and a decrease in feeding activity over time. As all other grazing experiments were performed during two hours, they consequently reflected the ingestion rates of *H. ulvae*.

Linear accumulation of labelled diatom or bacteria by *H. ulvae* had been previously recorded from 45 min (Fenchel et al., 1975) to 2 hours (Blanchard et al., 2000; Haubois et al., 2005a). Molluscs have complex digestive tracts allowing partitioning of food particles within the gut. Relatively indigestible material passes quickly to the intestine and is subjected to extracellular digestion. More nutritious material like bacteria and algae is diverted to the digestive gland where it undergoes intracellular digestion. For *Hydrobia totteni*, gut residence time is 30-40 min (Lopez and Cheng, 1983b) whereas digestive gland residence time is 5 hours (Kofoed et al., 1989). Assuming a similar situation for *H. ulvae*, the absence of egestion during the two hour long incubations would mean that all bacteria are diverted to the digestive gland. In their grazing experiment with enriched diatoms, Sokolowski et al. (2005) observed that during the first 4 h phase of experiment, accumulation rate was 3 times higher than during the last 12 h phase. We observed exactly the same ratio between accumulation rates found during the grazing periods 0-2 h and 0-12 h. Those similar results suggest that digestive processes for bacteria and algae may be similar.

Range of ingestion rates

To our knowledge, there is no data dealing with the ingestion rate of bacteria by *Hydrobia* to compare with our values. However, concerning algal ingestion our data are consistent with those previously measured. In the present study, ingestion rates fluctuated between 1.2-1.8 $\mu gC\ ind^{-1}\ h^{-1}$. In the literature, ingestion rates of snails fed with diatoms are 0.5-2.9 $\mu gC\ ind^{-1}\ h^{-1}$ for *Hydrobia truncata* (Forbes and Lopez, 1989) and 1.2 (Sokolowski et al., 2005), 1.12-1.33 (Blanchard et al., 2000) and 0.04-2.08 (Haubois et al., 2005a) for *H. ulvae*.

The maximal ingestion rates of algae and bacteria by *Hydrobia ulvae* were higher than meiofaunal rates. Nevertheless, individual weight of *H. ulvae* is more than one hundred and one thousand times higher than *A. tepida* and nematodes respectively (Table 1). Body size is an important determinant of many physiological processes and maximal ingestion rate is

generally inversely correlated to body size (e. g. Moloney and Field, 1989). The present study focuses only on two potential prey, bacteria and algae, although other food sources are available in sediment. For instance Dissolved Organic Material may constitute an important food supply for nematodes (Lopez et al., 1979; Meyer-Reil and Faubel, 1980; Montagna, 1984; Jensen, 1987) and foraminifera (Schwab and Hofer, 1979). Nematodes and foraminifera may be principally dependent on those other resources and present consequently low ingestion rates of bacteria and algae.

Effect of abiotic factors on bacterial ingestion rate

The general trend showed a temperature effect with an optimum around 30°C. However, except at the lowest temperature (5°C), differences between feeding rates of *H. ulvae* observed in the present study were not significant, indicating a limited influence of temperature. In a similar manner, Barnes (2006) did not detect changes in feeding activity of *H. ulvae* during *in situ* experiments with the same range of temperature. Ingestion rates observed at salinities of 18 and 31 were not significantly different in the present study. Grudeno & André (2001) also observed that shell growth of juvenile. *H. ulvae* was unaffected by salinity in the range of 15-30. Light did not affect the ingestion of bacteria by *H. ulvae*. The literature gives conflicting results concerning the effect of light. Barnes (1986) found that crawling activity of snails was higher in darkness whereas Orvain & Sauriau (2002) observed an increase of *H. ulvae* crawling activity with light. However in Orvain & Sauriau's experiment, light may have induced formation of algal biofilm, affecting microphytobenthic distribution and thus indirectly affecting snail activity levels.

In intertidal mudflats, the surficial centimeter of sediment is subjected to fast and large environmental variations. The ability of a grazer to sustain feeding activity when environmental conditions fluctuate can be interpreted as an adaptation to this habitat. All compared grazers in the present study came from the top centimeter of sediment of the same

study area and grazing incubations were performed in similar conditions. Compared to other grazers, the feeding response of the foraminifera *A. tepida* presented the largest ranges of variation indicating that they may present a low adaptation to rapid environmental changes (Table 1). Nevertheless, *A. tepida* is considered as one of the most tolerant species of foraminifera to temperature and salinity variations (Bradshaw, 1961; Walton and Sloan, 1990) and more generally to environmental changes (Samir, 2000; Armynot du Chatelet et al., 2004; Bouchet et al., 2007). *A. tepida* may be able to survive starvation when the environment is unfavourable and may await optimal conditions to feed and develop. An alternate explanation could be that when conditions are hostile, foraminifera move from the top layers of sediment to deeper layers (Severin and Erskian, 1981; Severin, 1987; Groß, 2002). Feeding activity of nematodes and *H. ulvae* appeared to be more independent of environmental variables. The nematode community was mainly composed of three species, *Chromadora macrolaima*, *Daptonema oxycerca* and *Ptycholaimellus jacobi* (Pascal et al., In press), known to dwell and feed in surface sediment. Moreover, *H. ulvae* is considered the most environmentally tolerant of Northwest European *Hydrobia* (Hylleberg, 1975; Lassen and Kristensen, 1978). However caution must be taken in interpretation of our results because the effects of each environmental factor on feeding behaviour of snails were studied independently whereas in natural environment, all these factors covary. Moreover, seasonal acclimatising capacity was not taken into account (Barnes, 2006).

Feeding response to bacterial and algal abundances

In fine grained environments, *Hydrobia* ingests mouthfuls of sediment containing organic food source, including bacteria and microalgae (Kofoed, 1975; Lopez and Cheng, 1983b; Levinton et al., 1984). Due to high size and feeding mode of *H. ulvae*, the snail probably has a very low ability to ingest selectively very small preys such as bacterial cells. In opposition,

298 larger preys such as diatom cells can be selectively ingested from sediment by *Hydrobia*
299 (Fenchel, 1975; Lopez and Levinton, 1978; Lopez and Kofoed, 1980).

300 Indiscriminant ingestion of bacteria by *H. ulvae* implies that bacterial ingestion is
301 exclusively dependent on ingestion rate of sediment and the concentration of bacteria in
302 sediment. This type of feeding indicates that relationships between bacterial ingestion and
303 bacterial concentration in sediment can be described by (i) a power law relation, (ii) a linear
304 increase or (iii) null or decreasing relation. Those relations mean that when prey concentration
305 increases, ingestion rate of sediment respectively (i) increases, (ii) remains constant or (iii)
306 decreases. In the present study, the ingestion rate of bacteria first increased linearly with
307 bacterial concentration and then levelled off at the highest concentration (Fig. 3). This relation
308 may involve a constant ingestion rate of sediment at the lowest concentration of bacteria and a
309 decrease of ingested sediment at the highest bacterial concentration.

310 Ingestion of algae by snails was not influenced by algal concentration in the range of
311 concentration tested (Fig. 4). Contrarily, *Hydrobia* had been found previously to have
312 increasing algal ingestion rate when algal concentration increased in a similar range of values
313 (Forbes and Lopez, 1986; Forbes and Lopez, 1989; Haubois et al., 2005a). The ability of a
314 deposit feeder to alter its ingestion rate depends on its sensory capacity to recognize food
315 quality (Taghon, 1982). This perception may differ between the freeze-dried diatoms in the
316 present study and live diatoms in other studies, which could explain the different responses
317 observed.

318 The ratio between algae and bacteria ingested denotes grazer ability to select diatoms from
319 the sediment/bacteria aggregate. At each algal concentration, this ratio was always higher for
320 *A. tepida* and nematodes, indicating higher algal selection efficiency. The nematode
321 community was composed mainly of epigrowth feeders (75%) and non selective deposit
322 feeders (21%). Epigrowth feeders puncture diatom cells with their teeth to ingest cell

323 contents. Consequently, they are mainly dependant on algal resources (Jensen, 1987). *A.*
324 *tepida* uses a network of pseudopodia to gather and ingest food particles. This feeding mode
325 allows foraminifera to be highly selective in ingested food (Lee et al., 1966; Lee and Muller,
326 1973). *Ammonia* may also greatly depend on algal resources, as this foraminifera was found
327 to ingest rapidly and with high efficiency fresh algal deposits (Moodley et al., 2000).
328 Montagna and Yoon (1991) also observed that nematodes demonstrate a high efficiency in
329 selective ingestion of algae in comparison with other meiofaunal groups. *H. ulvae* appeared
330 less proficient in algal selection than meiofaunal grazers. *N. phyllepta,* the algal species used
331 in the present study may have been too small (<30 μm) to allow selective ingestion by *H.*
332 *ulvae,* but this hypothesis can reasonably be rejected as *Hydrobia* is not able to select diatoms
333 according to the cell size (Levinton, 1987; Haubois et al., 2005a). In sandy sediment,
334 *Hydrobia* presents epipsammic browsing activity by taking particles into the buccal cavity,
335 scraping off attached microorganisms and then spitting out the particles (Lopez and Kofoed,
336 1980). As a result, gut contents and even fecal pellets of *Hydrobia* can contain more diatoms
337 than the offered sandy sediment (Fenchel et al., 1975; Lopez and Levinton, 1978).
338 Conversely, in fine grained sediment, *Hydrobia* ingests mouthfuls of sediment containing
339 organic food, including microalgae (Kofoed, 1975; Lopez and Cheng, 1983b; Levinton et al.,
340 1984). Results of the present study also suggest that *H. ulvae* feeding on muddy sediment
341 present a limited ability to discriminate between algae and the sediment/bacteria aggregate.
342 Taghon and Jumars (1984) pointed out that for animals having limited particle selection
343 ability, foraging strategies are mainly a function of ingestion and digestion processes. Indeed,
344 in the present study *H. ulvae* appeared to decrease the rate of ingested sediment at high algal
345 and bacterial concentrations. In the present study, labelled prey were distributed
346 homogeneously in sediment. Feeding rates and feeding behaviour of *H. ulvae* may be
347 different when algae are condensed in biofilm.

In the Brouage mudflat, *H. ulvae* and meiofauna are present, on average, in similar biomasses throughout the year (Degré et al., 2006). The present study suggests that *H. ulvae* ingests bacteria at a higher rate than meiofaunal do. In the study area, benthic bacteria would therefore be grazed to a higher extent by macrofauna than by meiofauna.

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527
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Figure captions

Figure 1. Bacteria uptake by *H. ulvae* (mean ± SD, N =3) as function of incubation time (h).

Figure 2. Bacterial ingestion rate by *H. ulvae* (mean ± SD, N =3) as function of temperature (°C). Different letters above bars indicate significant differences between incubation conditions (ANOVA; Tukey test).

Figure 3. Bacterial ingestion rate by *H. ulvae* (mean ± SD, N =3) as function of bacterial abundance (10^8 cells cm^3). Different letters above bars indicate significant differences between incubation conditions (ANOVA; Tukey test).

Figure 4. Ingestion rates of algal carbon ○ (mean ± SD) and bacterial carbon ● (mean ± SD) by *H. ulvae* (ngC ind^{-1} h^{-1}) as a function of algal abundance (μgChla g dry wt sed^{-1}). Bacterial abundance was kept constant (1.05×10^9 cells cm^3). * above bars indicate significant differences between incubation conditions (ANOVA; Tukey test).

Figure 5. Ratio between algae and bacteria taken up by three different grazers (the foraminifera *A. tepida*, Brouage mudflat nematode community and the gastropod *H. ulvae*) as a function of algal abundance (μgChla g dry wt sed^{-1}). Bacterial abundance was kept constant (1.05×10^9 cells cm^3).

Table captions

Table 1. Comparison of the feeding activity of three different grazers (the foraminifera *A. tepida*, the Brouage mudflat nematode community and the gastropod *H. ulvae*).

	Meiofauna		Macrofauna
	Ammonia tepida	**Nematode community**	***Hydrobia ulvae***
Individual weight ($gC_{organic}$ ind^{-1})	**1.0×10^{-6}**	**1.3×10^{-7}**	**1.8×10^{-4}**
	Effect of environmental factors on ingestion rate of bacteria		
Ratio between ingestion rates of bacteria at 30 and 10°C	**3.6**	**1.6**	**1.5**
Effect of salinity (18 against 31 ‰)	**Negative**	**None**	**None**
Effect of luminosity (Darkness against 83 µM of photons m^{-2} s^{-1})	**None**	**Positive**	**None**
	Maximal ingestion rates of bacteria and algae		
Maximal ingestion rate of bacteria (10^{-3} $gC_{bacteria}$ gC_{grazer}^{-1} h^{-1})	**0.06**	**0.92**	**7.45**
Maximal ingestion rate of algae (10^{-3} gC_{algae} gC_{grazer}^{-1} h^{-1})	**0.94**	**5.08**	**9.56**

Table 1

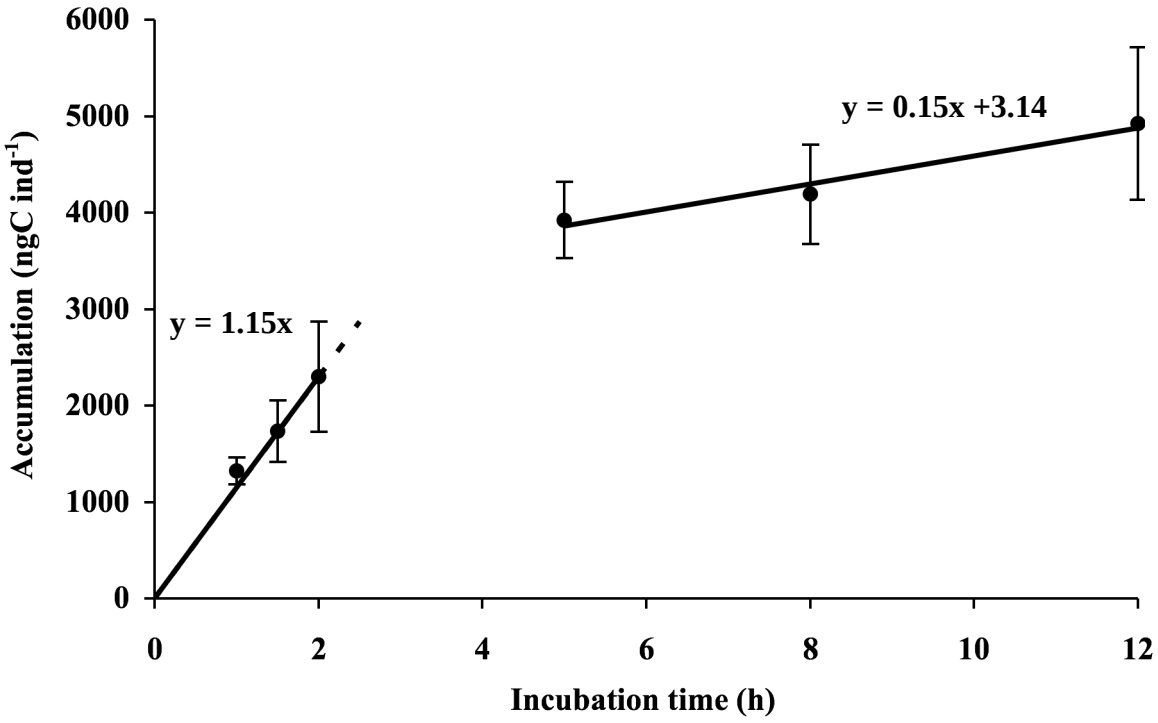
6000
5000
4000
3000
2000
1000
0
Accumulation (ngC ind-1)
y = 1.15x
y = 0.15x +3.14
0
2
4
6
8
10
12
Incubation time (h)

Fig. 1

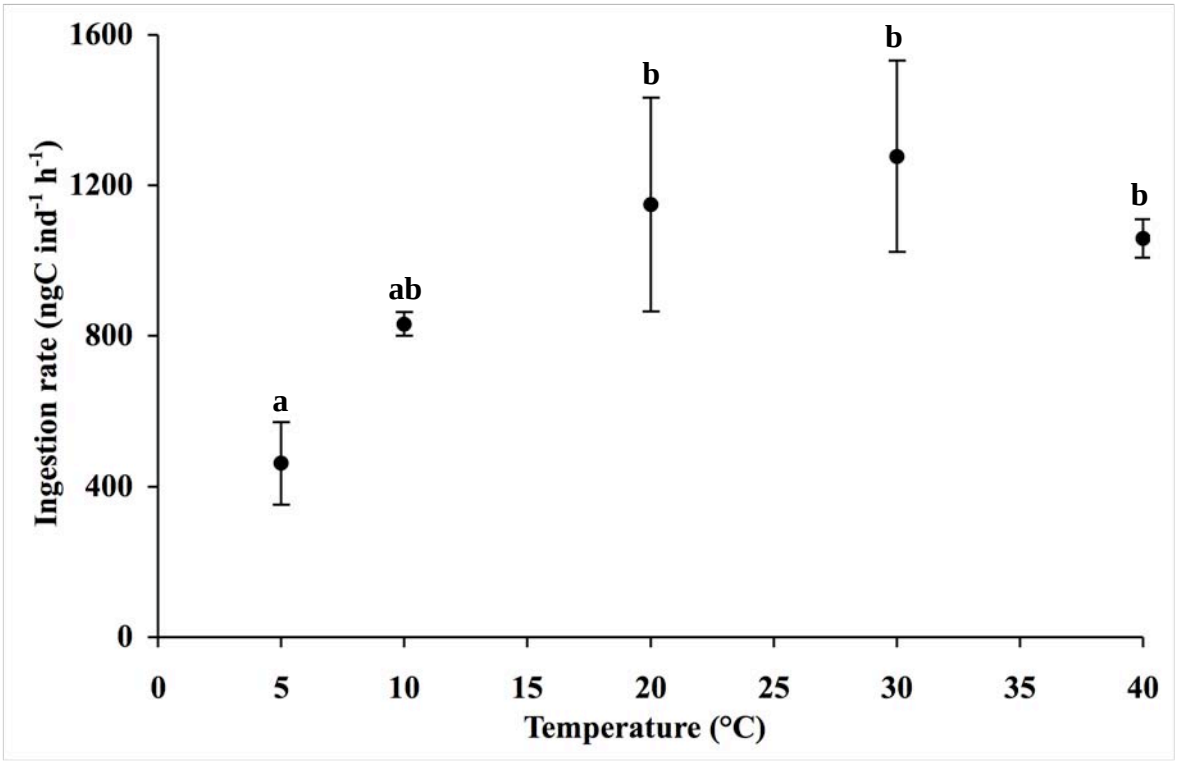
1600
1200
800
400
0
Ingestion rate (ngC ind-1 h-1)
a
ab
b
b
b
0
5
10
15
20
25
30
35
40
Temperature (°C)

Fig. 2

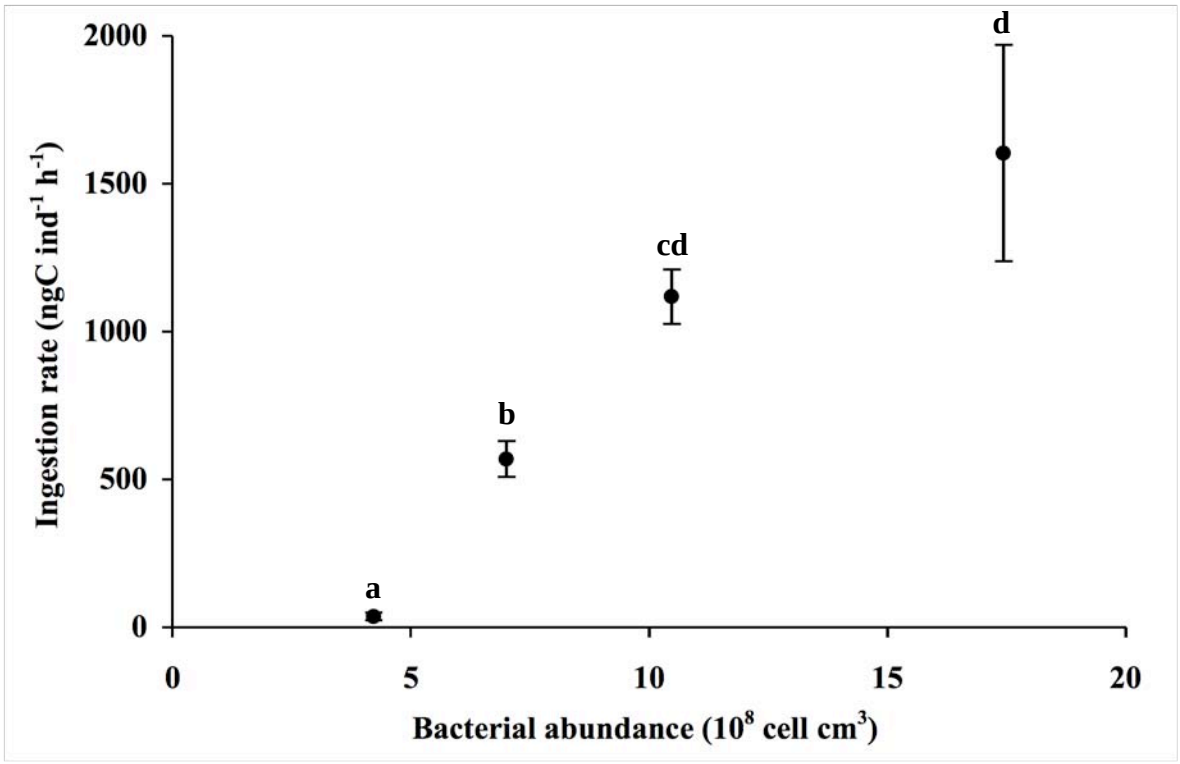
Ingestion rate (ngC ind-1 h-1)
2000
1500
1000
500
0
a
b
cd
d
0
5
10
15
20
Bacterial abundance (10^8 cell cm^3)

Fig. 3

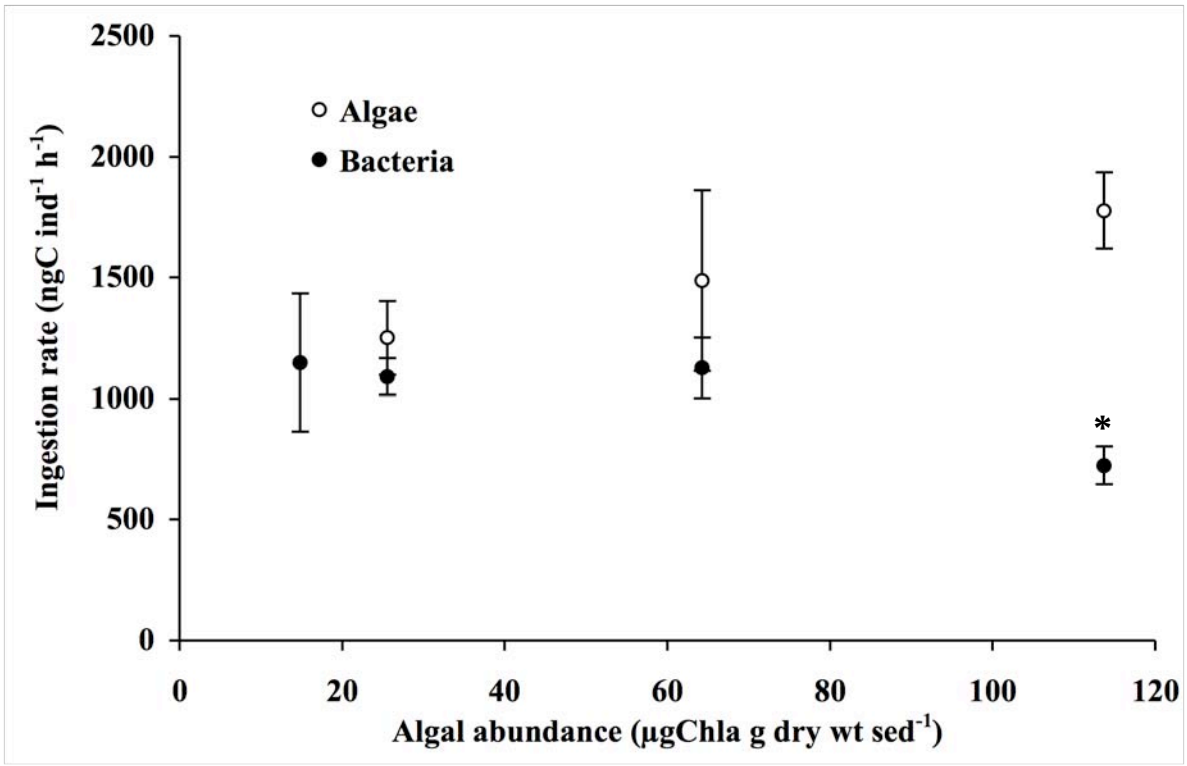
Ingestion rate (ngC ind-1 h-1)
2500
2000
1500
1000
500
0
Algae
Bacteria
*
0
20
40
60
80
100
120
Algal abundance (µgChla g dry wt sed-1)

Fig. 4

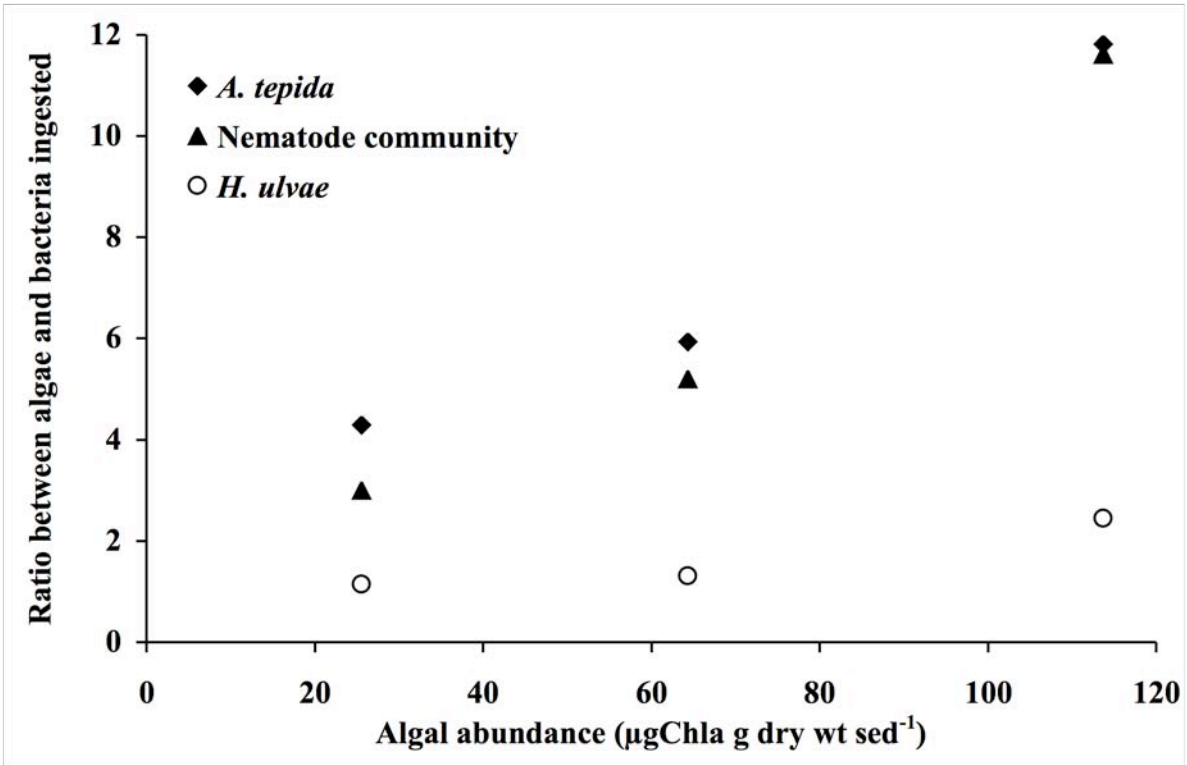
Ratio between algae and bacteria ingested
12
10
8
6
4
2
0
A. tepida
Nematode community
H. ulvae
0
20
40
60
80
100
120
Algal abundance (µgChla g dry wt sed-1)

Fig. 5