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Bacterivory of a mudflat nematode community under different environmental conditions

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Abstract

The fate of the benthic bacterial biomass is a topic of major importance in understanding how soft-bottom environments function. Because of their high abundance, production and nutritional value, benthic bacteria may constitute an important food resource for benthic fauna. The trophic role of bacteria for a nematode community on the Brouage mudflat (Marennes-Oléron-France), dominated by three species: *Chromadora macrolaima* (64% of the abundance), *Daptonema oxycerca* (15%) and *Ptycholaimellus jacobii* (8%), was determined in grazing experiments using ¹⁵N pre-enriched bacteria. On intertidal flats, seasonal, tidal and circadian cycles induce strong variations in environmental conditions. Grazing experiments were performed in order to measure the effects of abiotic (temperature, salinity and luminosity) and biotic (bacterial and algal abundances) factors on assimilation rates of bacteria by nematodes. In order to assess simultaneously bacterial and algal assimilation rates, algal abundances were modified adding ¹³C pre-enriched *Navicula phyllepta*. Assimilation rate was significantly lower at 5°C, moreover general trend show a prominent temperature effect with an optimum around 30°C. Assimilation at salinity 18 was

25 not significantly different from the assimilation at salinity 31. Assimilation was higher under
26 light condition than in the dark. Above 10^9 bacteria ml^{-1} , assimilation of bacteria remained
27 unaffected by bacterial abundance. However, assimilation of algae increased with the algal
28 concentration. Nematode kept feeding under conditions of stress which are typical of the
29 surficial sediment habitat and they appeared to be principally dependent on the algal resource.

30 **Keywords**

31 Nematode, bacteria, grazing, environmental factor, mudflat

32 **Introduction**

33 Bacteria play a major role in cycling organic matter in marine sediments through the
34 remineralization of nutrients and organic matter and production of particulate and dissolved
35 carbon (e.g. Legendre and Rassoulzadegan 1996; Rivkin et al. 1996). Although bacterial
36 abundance remains stable, around 10^9 cells ml^{-1} (review in Schmidt et al. 1998), bacterial
37 production rates vary greatly (Sander and Kalff 1993). This discrepancy between abundance
38 and production may be induced by bottom-up, biochemical and top-down control (van
39 Oevelen et al. 2006a). In the top-down control situation, bacterial carbon is assumed to be
40 regulated by higher trophic levels of benthic food webs.

41 The majority of animals feeding on sedimentary deposits are more dependent on attached
42 bacteria than on nonliving organic debris (e. g. Fenchel 1972). Models of benthic ecosystems
43 emphasize the role of bacteria and their immediate grazers as a major route through which
44 organic material is processed (e. g. Kuipers et al. 1981). Consequently, quantitative data
45 dealing with the link between bacteria and benthic fauna are essential for understanding the
46 extent to which this trophic link structures energy and material fluxes in the communities.

47 Results concerning the impact of meiofaunal grazing on bacteria are conflicting (Kemp
48 1990). Montagna (1984b) suggested that meiofaunal grazing pressure (principally through

49 polychaetes) represents a significant stimulatory effect on the microbial community and may
50 be important in sandy sediments (Montagna and Bauer 1988). However, quantitative studies
51 on meiobenthos bacterivory are sparse.

52 Although they are small and inconspicuous, nematodes are consistently the most abundant
53 meiobenthic taxon in mudflat sediments. Their average densities of 10^6 ind m^{-2} represents a
54 biomass of roughly 0.2 to 2 gC m^{-2} and some authors have suggested that their ecological
55 significance is great in terms of food-web relationships (review in Platt and Warwick 1980;
56 Heip et al. 1985).

57 Benthic bacteria can constitute a significant food source for nematodes (Lopez et al. 1979;
58 Tietjen 1980; Montagna 1984b; Montagna 1995; Moens et al. 1999b). According to Wieser
59 (1960), there are four different feeding groups of nematodes: selective deposit feeders (1A),
60 non-selective deposit feeders (1B), epigrowth feeders (2A) and omnivore-predators (2B).
61 Nematodes of each feeding groups are potential bacterivores, even predacious may benefit
62 directly from bacterial carbon (Moens et al. 1999b). Deposit and epigrowth feeders feed on
63 bacteria and unicellular eukaryotes in different ways. Deposit-feeding species have no teeth
64 and generally swallow the food whole and undamaged. They feed predominantly on bacteria
65 associated with detritus. The epigrowth feeding species puncture the cell membrane with their
66 teeth and ingest only the cell contents (juice feeders). Diatoms and other benthic microalgae
67 are known to be important trophic sources for many epigrowth feeders but the importance of
68 bacteria as a food source remains poorly documented (Moens and Vincx 1997). The aim of
69 this study was to experimentally assess rates of bacteria uptake by a nematode community
70 from an intertidal mudflat using ^{15}N enriched bacteria as tracers. The intertidal habitat studied
71 is subject to a wide range of environmental varying factors. Three relevant time scales drive
72 these environmental variations: long-term (seasonal cycle), medium-term (lunar cycle) and
73 short-term (solar and tidal cycles) (Guarini et al. 1997). Variations concern both biotic (i.e.

74 temperature, salinity and luminosity) and abiotic factors (i.e. bacterial and algal abundances).
75 As those variations may influence the feeding behavior of nematodes, one aim of the present
76 study is to determine if nematodes bacterivory is constant in the mudflat or influenced by
77 environmental factors. Other aim is to describe feeding behavior of nematodes when an
78 alternative algal resource is available.

79 For this purpose, a mudflat nematode community from surficial sediment was put in
80 microcosms, in contact with labeled preys: a bacterial community and one algal species.
81 Grazing experiments were performed in order to evaluate effects of abiotic (temperature,
82 salinity and luminosity) and biotic (bacterial and algal abundances) factors on rates of prey
83 uptake. We focused on the surficial mudflat sediment nematode community because the
84 surficial sediment (i) has high bacterial production, (ii) contains the highest nematodes
85 densities and (iii) undergoes faster and more wide-ranging changes in environmental factors
86 than do the deeper layers.

87 **Material and methods**

88 *Study site*

89 The Brouage intertidal mudflat is located in the eastern part of the Marennes-Oléron Bay
90 (Atlantic coast of France). Meteorological conditions exhibit a strong seasonality typical of a
91 temperate climate. Temperature and salinity of emerged sediments are more extreme during
92 summer tidal cycles (Guarini et al. 1997). Minimum and maximum mud temperatures are 5°C
93 and 34°C respectively. The maximum daily range of mud temperature due to emersion and
94 immersion cycle reaches 18°C (Guarini et al. 1997). Salinity of overlaying water is controlled
95 by the river Charente freshwater input, ranging from 25 to 35 over the year (Héral et al.
96 1982). Salinity of the upper layers of sediment may also decrease with rainfall. The sediment
97 surface irradiance shifts from dark during submersion and night emersions to high levels of
98 incident light during daytime emersions. This irradiance can reach 2000 μM of photons $\text{m}^{-2} \text{s}^{-1}$

99 (Underwood and Kromkamp 2000). Details of numerous benthic organisms and processes are
100 available concerning this intertidal zone (gathered in Leguerrier et al. 2003; Leguerrier et al.
101 2004; Degré et al. 2006).

102 *¹⁵N enriched bacteria and ¹³C enriched algae as tracer*

103 The method used was described in Pascal et al. (2008). This method is based on the
104 assumption that grazers ingest unselectively enriched and natural bacteria. Briefly, one
105 centimeter-depth of surficial sediment was sampled during ebb tide in the Brouage mudflat
106 (45°55N, 1°06W) (Fig. 1). Bacteria from surficial sediment were cultured in a liquid bacterial
107 culture medium containing $^{15}\text{NH}_4\text{Cl}$ 1 g l⁻¹ (99% ¹⁵N-enriched NH_4Cl CortecNet), rinsed by
108 centrifugation and frozen until the grazing experiments. An axenic clone of the *Navicula*
109 *phyllepta* diatom (CCY 9804, Netherlands Institute of Ecology NIOO-KNAW, Yerseke, The
110 Netherlands) was cultured in a liquid medium containing $\text{NaH}^{13}\text{CO}_3$, then rinsed and freeze-
111 dried until the grazing experiments. Isotopic composition of enriched prey was assessed using
112 mass spectrometer. For these experiments, labeled preys were mixed with sediment from the
113 Brouage mudflat that had been previously sieved through a 50 µm mesh. The abundance of
114 bacteria and algae in the slurry was estimated in order to determine the ratio between enriched
115 and unenriched prey.

116 *Grazing experiments*

117 The top centimeter of sediment was collected on March 13, 2006. At the time of sampling,
118 sediment presented a temperature of 7°C and a salinity of 29. Sediment sampled was first
119 sieved through a 500 µm mesh to remove macrofauna, then through a 50 µm mesh to extract
120 meiofauna. One ml of the sediment remaining in the mesh was put into each microcosm
121 (Pyrex beakers, $\varnothing = 4.5$ cm) and the fraction which passed through the mesh was mixed with
122 ¹⁵N enriched bacteria. This slurry contained 10.5×10^8 bacteria ml⁻¹, with the ¹⁵N enriched
123 bacteria being twice as abundant as unenriched ones. Four ml of this slurry were put into each

124 microcosm. Each experiment was carried out in triplicate. Control samples were frozen (-
125 80°C) in order to kill any nematodes.

126 A kinetic study was performed to validate its linear or hyperbolic shape in order to
127 calculate the grazing rate. Incubations for this kinetic study were run for 1 to 12 hours,
128 including the 3 hours run that was used for all other experiments. Incubations were made
129 under the following standardized conditions that were close to the year-round mean values
130 recorded on the study site: temperature (20°C), salinity (31), luminosity (darkness), bacterial
131 abundance (10.5×10^8 bacterial cell ml⁻¹) and algal abundance (15 µgChla g⁻¹).

132 For each other experiment one environmental incubation factor was modified. In order to
133 decrease salinity, cultured bacteria were rinsed with 0.2 µm filtered-sea-water diluted with 0.2
134 µm filtered water (final salinity of 18). The light effect was tested with a light intensity of 83
135 µM photons m⁻² s⁻¹. Bacterial abundances (total enriched and non-enriched) tested were 4, 7
136 and 17 cells ml wt sed⁻¹ with respectively the following ratio between abundance of total and
137 enriched bacteria: 6.1, 2.0 and 1.3. Algal abundance was modified by adding various
138 quantities of cultured *N. phyllepta* enriched in ¹³C while bacterial abundances (total enriched
139 and non-enriched) were kept constant at 10×10^8 cells ml⁻¹. Using two isotopes for labeling
140 bacterial (¹⁵N) and algal food (¹³C) offers the opportunity to assess bacterial and algal
141 ingestion rates simultaneously. Algal abundance (total enriched and non-enriched) were 26,
142 64 and 114 µgChla g dry sed⁻¹ with respectively the following ratio between abundance of
143 total and enriched algae: 2.4, 1.3 and 1.2.

144 Incubations were halted by freezing the microcosms at -80°C. Samples were thawed and
145 nematodes were extracted from the sediment using Ludox TM (Heip et al. 1985). For each
146 sample, at least 700 nematode specimens were picked up randomly and individually with
147 Pasteur pipette, rinsed twice in Milli-Q water to remove adhering particle and transferred in
148 aluminium cup.

149 *Isotope analysis and calculations*

150 $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of prey (bacteria and algae) and grazers (nematodes) were measured using
 151 an EA-IRMS (Isoprime, Micromass, UK). Nitrogen isotope composition is expressed in the
 152 delta notation ($\delta^{15}\text{N}$) relative to air N_2 : $\delta^{15}\text{N} = [({}^{15}\text{N}/{}^{14}\text{N})_{\text{sample}} / ({}^{15}\text{N}/{}^{14}\text{N})_{\text{reference}}] - 1] \times 1000$.
 153 Carbon isotope composition is expressed in the delta notation ($\delta^{13}\text{C}$) relative to Vienna Pee
 154 Dee Belemnite (VPDB): $\delta^{13}\text{C} = [({}^{13}\text{C}/{}^{12}\text{C})_{\text{sample}} / ({}^{13}\text{C}/{}^{12}\text{C})_{\text{reference}}] - 1] \times 1000$.

155 The ^{15}N incorporated was defined as excess (above the background level) ^{15}N and is
 156 expressed in terms of specific uptake (I). I was calculated as the product of excess ^{15}N (E) and
 157 the biomass of N per grazer. E is the difference between the background ($F_{\text{background}}$) and the
 158 sample (F_{sample}) ^{15}N fraction: $E = F_{\text{sample}} - F_{\text{background}}$, with $F = {}^{15}\text{N} / ({}^{15}\text{N} + {}^{14}\text{N}) = R / (R + 2)$
 159 and $R =$ the nitrogen isotope ratio. For the $F_{\text{background}}$, we used highest control values ($\Delta^{15}\text{N} =$
 160 11.78 and $\Delta^{13}\text{C} = -16.34$) measured using killed (frozen) grazers. R was derived from the
 161 measured $\delta^{15}\text{N}$ values as: $R = ((\delta^{15}\text{N}/1000) + 1) \times R_{\text{airN}_2}$ where $R_{\text{airN}_2} = 7.35293 \times 10^{-3}$
 162 (Mariotti 1982). The uptake of bacterial carbon was calculated as $\text{Uptake} = (I \times (\% C_{\text{enriched}}$
 163 $\text{bacteria} / \% N_{\text{enriched bacteria}}) / (F_{\text{enriched bacteria}} \times \text{incubation time})$. This uptake was multiplied by
 164 the ratio between the abundance of total and enriched bacteria determined by DAPI counts.

165 Incorporation of ^{13}C was calculated analogously, with $F = {}^{13}\text{C} / ({}^{13}\text{C} + {}^{12}\text{C}) = R / (R + 1)$,
 166 R_{airN_2} is replaced by $R_{\text{VPDB}} = 0.0112372$ and $\text{Uptake} = I / (F_{\text{enriched bacteria}} \times \text{incubation time})$.
 167 The uptake measured was multiplied by the ratio between the abundance of total and enriched
 168 diatom, determined from fluorometrical measurements.

169 Enriched *N. phyllepta*-produced carbon consisted of $22.95 \pm 0.54\%$ ^{13}C . The C/N ratio of
 170 enriched bacteria was 3.49 and bacterial nitrogen consisted of $2.88 \pm 0.03\%$ ^{15}N . The average
 171 weight of nematodes was $0.33 \pm 0.18 \mu\text{g DW}$ and each nematode was composed on average
 172 of $0.11 \pm 0.05 \mu\text{gC}$ and $22.28 \pm 5.82 \text{ ngN}$ ($N = 72$ samples of at least 700 specimens each).

173 Uptake expressed as $\text{gC}_{\text{bacteria}} \text{gC}_{\text{nematode}}^{-1} \text{h}^{-1}$ was obtained by dividing the uptake of bacteria
174 ($\text{gC ind}^{-1} \text{h}^{-1}$) by the mean nematode weight (gC ind^{-1}).

175 Variations of assimilation rates with respect to the salinity and luminosity were tested
176 using two-tailed test. One-way analysis of variance (ANOVA) was used in order to test the
177 impact of temperature and algal and bacterial abundance on the uptake rates of bacteria and
178 algae. The Tukey test was used for post-hoc comparisons.

179 *Nematode community composition*

180 Nematode communities used in the grazing experiments were extracted from sediment
181 with Ludox TM (Heip et al. 1985). In order to determine the taxonomic composition of the
182 community studied, 303 nematodes were collected at random, determined to species or
183 generic level under the microscope and sorted by feeding group as indicated by Wieser (1953;
184 1960).

185 **Results**

186 *Composition of the nematode community*

187 In the sample collected for the taxonomy of the nematode community, 19 species
188 belonging to 18 genera were observed (Tab. 1). Three species made up more than 87 % of the
189 community: *Chromadora macrolaima* (64%), *Daptonema oxycerca* (15%) and
190 *Ptycholaimellus jacobii* (8%). The other species were much less abundant, 11 representing less
191 than 1 %.

192 The community was dominated by epigrowth feeders 2A (75%) due to high abundances of
193 *C. macrolaima* and *P. jacobii*. Non-selective deposit feeders 1B (21%) were the second most
194 abundant trophic group due to high abundance of *D. oxycerca*. Selective deposit feeders 1A
195 (2%) and omnivores/predators 2B (1%) exhibited marginal abundances in the community
196 studied.

197 *Uptake of microbes by nematodes*

198 Nematodes isotopic compositions and rates of bacterial and algal uptakes are presented in
199 Table 2.

200 The kinetic experiment showed that bacterial uptake by the nematode community
201 increased linearly during the twelve hours of incubation (Fig. 2). The linear regression slope
202 suggested an assimilation rate of bacteria of $32 \text{ pgC ind}^{-1} \text{ h}^{-1}$ equivalent to $25 \times 10^{-5} \text{ gC}_{\text{bacteria}}$
203 $\text{gC}_{\text{nematode}}^{-1} \text{ h}^{-1}$ ($r^2 = 0.98$).

204 Temperature had a significant effect on the assimilation rate of bacteria ($F = 7.5$, $p < 0.005$).
205 Temperature tested fluctuated between 5 and 40°C and were in the range of those found in the
206 study area (Guarini et al. 1997). Uptake of bacteria was reduced at 5°C, then increased with
207 temperature to reach an optimum at around 30°C and then decreased (Fig. 3). This rate
208 increased from $3 \text{ pgC ind}^{-1} \text{ h}^{-1}$ to $25 \text{ pgC ind}^{-1} \text{ h}^{-1}$ when the temperature rose from 5°C to
209 30°C and then decreased, reaching $18 \text{ pgC ind}^{-1} \text{ h}^{-1}$ at 40°C. Overall trend is showing a
210 prominent temperature effect but assimilation rates observed at 10, 20, 30 and 40°C were
211 nevertheless not significantly different from each other but were significantly different from
212 those observed at 5°C.

213 The assimilation rate at a salinity of 31 ($19 \pm 2 \text{ pgC ind}^{-1} \text{ h}^{-1}$) was similar (two-tailed test, p
214 $= 0.72$) to that found for a salinity of 18 ($18 \pm 4 \text{ pgC ind}^{-1} \text{ h}^{-1}$) (data not shown).

215 Light significantly affects the feeding activity of nematodes (two-tailed test, $p < 0.05$).
216 Assimilation rates were more than twice as high ($41 \pm 11 \text{ pgC ind}^{-1} \text{ h}^{-1}$) under light ($83 \mu\text{M}$ of
217 photons $\text{m}^{-2} \text{ s}^{-1}$) than in darkness ($19 \pm 2 \text{ pgC ind}^{-1} \text{ h}^{-1}$) (data not shown).

218 Assimilation rates were significantly linked with bacterial abundance ($F = 52$, $p < 0.001$)
219 (Fig. 4). Its value was null for the lowest tested abundance ($4 \times 10^8 \text{ cells ml}^{-1}$), was amplified
220 more than fourfold when abundance increased from $7 \times 10^8 \text{ cells ml}^{-1}$ to $10 \times 10^8 \text{ cells ml}^{-1}$
221 and remained stable between this last value and $17 \times 10^8 \text{ cells ml}^{-1}$.

222 Using two isotopes to label bacterial (^{15}N) and algal food (^{13}C) offers the opportunity to
223 assess bacterial and algal uptake rates simultaneously. Assimilation of algae increased linearly
224 when algal abundance increased ($r^2 = 0.99$, $p < 0.05$). Bacteria represented 25, 16 and 8 % of
225 algal plus bacterial intake when algal concentrations were respectively 26, 64 and 114 μgChla
226 g^{-1} , with bacterial abundance remaining constant (Fig. 6). Plotted data indicated that
227 assimilation of bacteria declines when the algal concentration is high (114 $\mu\text{gChla g}^{-1}$) and the
228 bacterial concentration remains constant (Fig. 5). Nevertheless, these differences were not
229 significant and assimilation of bacteria was unaffected by algal abundance ($F = 3.24$, $p =$
230 0.11).

231 Discussion

232 *Nematode community*

233 Rzeznik-Orignac *et al.* (2003) studied the Brouage mudflat nematode community and
234 showed that in the lower part of the mudflat and over four seasons (2000-2001), six dominant
235 species represented 45% of the community: *Metachromadoroides remanei* (11.5%),
236 *Terschellingia longicaudata* (11.2%), *P. jacobii* (8.6%), *C. macrolaima* (8.6%), *Sabatiera*
237 *pulchra* (5.2%) and *D. oxycerca* (0.7%). In our study, performed in March 2006, only three
238 species, although all mentioned in the Rzeznik-Orignac *et al.* study, represented 87% of the
239 community (Tab. 1). As the sampling site was the same, difference in nematode community
240 composition may be due to inter-seasons or inter-years fluctuations. More probably, major
241 difference has to be put into relation with the sampling method used in each of these studies.
242 In the present one, only the top centimeter of sediment was sampled, whereas the top 5 cm
243 were sampled in the Rzeznik-Orignac *et al.* (2003) study. As nematodes exhibit a strong
244 vertical distribution of species in sediment (e. g. Platt 1977; Steyaert *et al.* 2001), the
245 community in the present study is not representative of the total Brouage mudflat nematode
246 community. Moreover, the three major species of the community under study belong to the

247 *Daptonema* and *Ptycholaimellus* genera that were mainly found in the 5 mm surficial layer of
248 sediment (Steyaert et al. 2003) and the third one, belonging to the family Chromadoridae is a
249 typical surface-dwelling epigrowth feeder (Platt 1977).

250 Feeding habits on diatoms of some genera close to *Chromadara* are known from culture
251 experiments: *Chromadorita tenuis* (Jensen 1987) and *Chromadora macrolaimoides* (Tietjen
252 and Lee 1973). *Chromadora macrolaima* and *Ptycholaimellus jacobi* break or pierce the
253 frustule of diatoms to suck out their contents whereas *Daptonema oxycerca* swallow the
254 whole diatom cell (Wieser 1953; Wieser 1960). Our nematode community is mainly
255 composed by three dominant species known or suspected to feed predominantly on diatoms.
256 The entire nematode community presents however higher abundances of bacterial grazers
257 (Rzeznik-Orignac et al. 2003). As a result, extrapolation of the present grazing results to the
258 rest of the community or to other communities must be realized with caution.

259 *Grazing experiments*

260 Like all various methods previously developed and applied to measure bacterivory, the
261 method used in the present study presents methodological shortcomings that make
262 interpretation of the resulting problematic. For instance, sieving the sediment changes the
263 bacterial availability, bacteria being not attached to particle as in natural situation. Indeed,
264 disruption of microbial-meiofaunal spatial relationships is known to affect grazing rate with
265 slurry method (Carman et al. 1989). Nematode grazing can be highly dependent on their
266 bacterial prey's activity, size and species (Tietjen et al. 1970; Tietjen and Lee 1973; Romeyn
267 and Bouwman 1983; Grewal and Wright 1992; Moens 1999; Moens et al. 1999a). One major
268 hypothesis in grazing experiments is that grazers did not select for or against the added
269 labeled bacteria over the bacteria present in the sediments. Consequently, the cultured bacteria
270 community must present characteristics that are roughly similar to the natural one. Despite the
271 fact that our culturing process modified the specific composition of the natural bacterial

272 community, the size, activity and diversity of the bacterial consortium used in the present
273 study would be more representative of the natural community than in most previous grazing
274 experiments (Pascal et al. 2008).

275 *Bacterial and algal uptake*

276 ^{15}N accumulation in nematodes was linear over the 12-hour incubation period (Fig. 2). The
277 constant accumulation of labeled food in nematodes had already been observed with
278 comparable incubation times (Herman and Vranken 1988; Moens et al. 1999c). Since the
279 nematode defecation intervals are very short (e. g. Thomas 1989) and since the gut is emptied
280 completely with each defecation (Duncan et al. 1974), the gut contents would thus be renewed
281 every few minutes. For these reasons, it has been suggested that during long incubation times,
282 the linear accumulation of the label indicates assimilation rather than ingestion (Schiemer
283 1987; Moens et al. 1999c). The slope of this linear curve gives an assimilation rate of 32 pgC
284 $\text{ind}^{-1} \text{h}^{-1}$. Assuming an assimilation rate of 25% (Herman and Vranken 1988; Somerfield et al.
285 2005), the ingestion rate would be four times higher, at 128 pgC $\text{ind}^{-1} \text{h}^{-1}$. As all other grazing
286 experiments were run for 3 hours, they provided the assimilation rate of food.

287 *Abiotic factors*

288 Behavioral responses of plant-parasites and terrestrial nematodes to several stimuli such as
289 electrical, mechanical and chemical stimuli and physical factors such as temperature and light
290 have been well described (review in Croll 1970). However, similar studies concerning the
291 effect of environmental conditions on the feeding behavior of marine or brackish-water
292 nematodes are scarce and limited in scope.

293 Temperature has received attention as a factor influencing the growth and reproduction of
294 estuarine and marine nematodes (review in Heip et al. 1985). The influence of temperature on
295 feeding has only been studied in the predacious nematode *Enoploides* (Moens et al. 2000), the
296 epigrowth feeder *Chromadora macrolaimoides* (Tietjen and Lee 1973) and in two brackish-

297 water bacterivorous species: *Pellioiditis marina* and *Diplolaimelloides meyli* (Moens et al.
298 1996; Moens and Vincx 2000). In the present study, nematodes exhibited a classical response
299 which was more or less related to a bell-shaped function also observed by Moens and Vincx
300 (2000). The assimilation rate of bacteria rose when the temperature increased and reached its
301 upper value at 30°C before declining. As assimilation rates recorded for the tested
302 temperatures were statistically different at 5°C, it may be suggested that the nematode grazing
303 rate is lower in winter when the temperature drops to under 5°C. However, nematodes may
304 adjust their optimum temperature conditions along seasons (Gee 1985). Studied nematodes
305 were probably adapted to low temperature as sediment temperature was 7°C at the time of the
306 sampling. Montagna (1984b) did not observe different grazing rates between winter and
307 summer, but the temperature range in his study area was small (18 and 23°C).

308 Salinity plays a major role in determining the spatial structure of the nematode community
309 along permanent gradients (Soetaert et al. 1995) or in its seasonal variations (Chatterji et al.
310 1995). However nematodes can also be affected by short-term variations in salinity (Forster
311 1998) and intertidal habitats are subject to major changes in interstitial salinity over short time
312 periods. During low tide, raindrops implode into fine sediments, disturbing and mixing the
313 surface sediment of mudflats. Moreover, freshwater run-off passively diffuses into the
314 interstitial pores of the sediment. In their review of marine nematode ecology, Heip et al.
315 (1985) compiled an extensive list of marine and estuarine species with their salinity
316 tolerances. *C. macrolaima*, the most abundant species in the present study, is found in areas
317 with salinities ranging from 35 to 24 whereas *D. oxycerca*, the second most abundant species,
318 is found in area ranging from 35 to 0.9. This species seemed to be able to tolerate gradual
319 changes in salinity but not rapid ones. After exposure to salinity of 3.33 for 10 min and 48 h,
320 Forster (1998) recorded adult mortalities of 10-35% and 70% respectively. This species from
321 the lower level of the intertidal zone is able to osmoregulate but unable to sustain water

322 regulation over long periods (Forster 1998). In the present study, assimilation at salinity 18
323 was not significantly different from the assimilation at salinity 31. Since the nematodes were
324 not acclimated until the grazing experiment, they seemed to be able to cope with rapid, but
325 limited, osmotic stress. Our conclusions concerning the minor role of salinity in bacterial
326 assimilation by nematodes are in accordance with Moens and Vincx's (2000) results. Among
327 the three factors they investigated (temperature, salinity and food abundance) affecting *P.*
328 *marina* and *D. meyli* food intake, salinity played a minor role in bacterial assimilation by
329 nematodes.

330 When exposed to air at low tide, benthic organisms at the sediment's surface are subject to
331 the highest solar and UVB radiation that can be experienced by marine organisms. There are
332 few investigations assessing the effects of luminosity on nematodes. The predacious
333 nematode *Enoploides* caught approximately twice as many prey nematodes in the dark as in
334 light (Moens et al. 2000). On the other hand, Sundbäck et al. (1996a) concluded that ambient
335 UVBs did not exert any strong selective pressure on the meiofaunal community of a muddy
336 microtidal area. Nozais et al. (1999) observed a deleterious effect of UVBs on the nauplia
337 stages of harpacticoid copepods from a tidal mudflat. However, they did not observe any
338 effect on nematode abundance. In our study, light may not present damaging effect as
339 nematodes fed actively, moreover bacterial assimilation was enhanced when light increased.
340 Uptake rates of bacteria by foraminifera (Pascal et al. In press) and the gastropoda *Hydrobia*
341 *ulvae* (Pascal et al., in prep) that were obtained by grazing experiments performed under the
342 same conditions as in present study did not demonstrate any effect of light. Consequently, the
343 response we observed should not be due to experimental bias and seems to be specific to
344 nematodes. Montagna et al. (1995) observed a significant correlation between algal
345 production and the grazing rate of a nematode community from the Brouage mudflat. In their
346 experience, algal production was increased by increasing the light intensity above

347 microcosms. They concluded that nematode's ingestion rate increases with algal production.
348 In intertidal mudflats, benthic diatoms migrate in the surface sediment along the diurnal cycle
349 (e. g. Serôdio et al. 1997). The nematode community under study was dominated by
350 epigrowth feeders (Tab. 1), with a diet mainly composed of benthic diatoms. Those
351 nematodes may graze when luminosity is high during low tide in order to graze in algal
352 biofilm and maximize their algal intake. This feeding behavior has not been documented for
353 nematodes, but harpacticoid copepods were shown to graze at a higher rate just after the
354 mudflat became exposed (Decho 1988). Buffan-Dubau and Carman (2000) also observed a
355 midday feeding peak by ostracods and harpacticoid copepods. This result suggests that
356 nematodes would have a feeding behavior principally controlled by algae and that bacteria
357 may be taken up accidentally with algae.

358 *Biotic factors*

359 For nematodes, the effects of bacterial concentration on growth, fecundity, population
360 development and feeding rates have been studied (Nicholas et al. 1973; Schiemer et al. 1980;
361 Schiemer 1982a; Schiemer 1982b; Schiemer 1983; Vranken et al. 1988; Moens and Vincx
362 2000). Nematode ingestion rates are generally proportional to food availability (Nicholas et
363 al. 1973; Schiemer et al. 1980; Moens et al. 1996). However, the relationship between
364 assimilation rate and food concentration is not linear and have been described by a Michaelis-
365 Menten function (Schiemer 1982b). This function is consistent with Holling's prey-dependent
366 type II functional response (Holling 1959). Assimilation rates depend on the amount of
367 ingested food and efficiency with which the material is assimilated. The assimilation
368 efficiency have been found to decrease when food concentration increases: at a high food
369 concentration *Plectus palustris* presented a low assimilation efficiency (12%) (Duncan et al.
370 1974) whereas at a food concentration one order of magnitude lower, the assimilation
371 efficiency reached 57% (Schiemer et al. 1980). A similar conclusion was drawn by Moens et

372 al. (2006) on the marine bacterivore *Pellioiditis marina*. Low efficiencies at high
373 concentrations appear to result from short gut retention times, with the gut transit being too
374 fast for effective digestion (Taghon et al. 1978). In the present study, it is not possible to
375 determine if assimilation rates fluctuations are due to variations of ingestion rates or
376 assimilation efficiencies. The number of bacterial concentrations tested was limited, however
377 the response observed could be described by a sigmoid function. This function is consistent
378 with Holling's prey-dependent type III functional response (Holling 1959). The threshold
379 value for significant assimilation appeared to fall between 4 and 7×10^8 cells ml^{-1} and the
380 threshold value for constant assimilation appeared to be between 7 and 10×10^8 cells ml^{-1} .
381 Those values are in the range of data provided by literature for fast growing opportunistic
382 bacterivores nematodes typical of plant material or root systems: an optimal grazing rate was
383 obtained for *Caenorhabditis briggsae* (Nicholas et al. 1973) and *P. marina* and *D. meyli*
384 (Moens and Vincx 2000) at a bacterial concentration of 5 , 25 and 5×10^8 cells ml^{-1}
385 respectively. Our study, dealing with tidal flat microalgal grazers community, suggests that
386 the optimal level of bacterial foraging is also reached at high bacterial density, when bacterial
387 concentrations attain 1×10^9 cells ml^{-1} . As bacterial concentrations are rarely lower in marine
388 sediments, (Hondeveld et al. 1992; Schmidt et al. 1998; Hamels et al. 2004), bacterial
389 assimilation rates would seldom be lower than $18 \text{ pgC ind}^{-1} \text{ h}^{-1}$.

390 The relationship between the assimilation rate of algae and the algal concentration is
391 poorly documented. Montagna et al. (1995) observed a proportional rise in the algal grazing
392 rate with increasing algal concentration. In the present study, algal assimilation increased
393 linearly without reaching a plateau. This function may be consistent with Holling's prey-
394 dependent type I functional response (Holling 1959). Thus, the algal concentration threshold
395 was not reached and maximal algal assimilation may be higher than those we obtained. Under
396 natural conditions, the chlorophyll a content of the first centimeter of sediment varies between

397 0 and 50 $\mu\text{gChla g}^{-1}$ (review in MacIntyre et al. 1996). However, through vertical migration,
398 benthic microalgae were concentrated near the surface during diurnal low tides and produced
399 a biofilm with an average thickness of 50 μm (Herlory et al. 2004). In this thin layer of algal
400 mat, the concentration of chlorophyll a can reach 150 $\mu\text{gChla g}^{-1}$ (Serôdio et al. 1997) and
401 even 300 $\mu\text{gChla g}^{-1}$ (Kelly et al. 2001). Thus, the higher algae abundance used in the present
402 study (114 $\mu\text{gChla g}^{-1}$) is not representative of conditions occurring in the natural
403 environment and the nematode community feeding on the algal biofilm could thus present a
404 higher assimilation rate of algae.

405 In our experiment, algal assimilation by nematodes always represented more than three
406 times the bacterial assimilation. Nematodes are able to select potential food items.
407 *Chromadora macrolaimoides* have a preference for diatoms and chlorophytes whereas they
408 assimilate fewer bacteria (Tietjen and Lee 1973). *D. oxycerca* is able to swallow particles of
409 different sizes and large frustules of diatoms (Boucher 1974). The three dominant species of
410 the community studied, i.e. *C. macrolaima*, *D. oxycerca* and *P. jacobi*, were found to
411 dominate the Brouage nematode community during spring diatom blooms (Rzeznik-Orignac
412 et al. 2003) indicating that they are highly dependent on algal resources. The use of natural
413 stable isotopic analyses led to a similar conclusion elsewhere, that the microphytobenthos
414 constitutes the main food source for the nematode community dwelling in the surficial
415 centimeter of mud in the study area (Riera et al. 1996). Montagna et al. (1984b), looking at
416 the grazing rates of a nematode community from a saltmarsh, found that diatoms are selected
417 14 more times than bacteria. Algal carbon accounted on average for more than 90% of carbon
418 grazed by a nematode community from a microtidal sandy sediment (Sundbäck et al. 1996b).
419 In the present study, nematodes also ingested algae at a higher rate than bacteria. Depending
420 on the algal concentration, the nematode community assimilates 3 to 11 times more diatoms
421 than bacteria.

422 Nematode production can be estimated on the basis of the P/B ratio, bearing in mind that
423 the choice of this ratio may be inaccurate (Heip et al. 1982). With an individual biomass of
424 $0.11 \mu\text{gC ind}^{-1}$, the estimation of production yielded $0.11 \text{ ngC ind}^{-1} \text{ h}^{-1}$, given a P/B ratio of 9
425 as often advocated (Gerlach 1971; Warwick and Price 1979; Bouvy 1988). Using a 10%
426 factor for energy conversion efficiency (Bouvy 1988), a nematode carbon demand of 1.13
427 $\text{ngC ind}^{-1} \text{ h}^{-1}$ can be calculated. In the present study, the maximum ingestion rate of bacteria
428 measured would represent 4% of this energy demand. On the other hand, the maximum algal
429 ingestion measured would correspond to 15% of this demand. To get 100% of their energy
430 needs, nematodes may graze at a higher rate on the algal compartment, as discussed above.
431 Nematodes may also be dependent on other food sources such as detritus, protozoa,
432 oligochaetes or nematodes (Moens and Vincx 1997). The role of Dissolved Organic Matter in
433 nematode nutrition also remains elusive, although it is likely to be highly relevant (Lopez et
434 al. 1979; Meyer-Reil and Faubel 1980; Montagna 1984a; Jensen 1987). Bacterial carbon was
435 found to constitute 6% of the total carbon requirement of a mudflat nematode community
436 (van Oevelen et al. 2006b). On the other hand, using inverse modeling, Van Oevelen et al.
437 (2006c) suggested that mudflat community of nematodes relied for 50 % on algae and 39 %
438 on bacteria. Variations between studies can be due to differences in methodology and to
439 nematode community composition.

440 In the brouage mudflat, nematode present a mean densities of $2112 \text{ ind } 10 \text{ cm}^{-2}$ (Rzeznik-
441 Orignac et al. 2003) whereas biomasses of benthic bacteria represent 0.846 gC m^{-2} (Degré et
442 al. 2006). If nematodes grazing rates measured in the present study are representative of those
443 of total community, it would imply that 0.02 % of bacterial biomass is assimilated by
444 nematodes each day. This extrapolation is debatable, as studied nematodes feed
445 predominantly on diatoms whereas total community may be more dependent on bacterial
446 resource, consequently grazing of bacteria is probably underestimated.

447 **Conclusion**

448 Caution must be taken in interpreting our results, since the impact of each environmental
449 factor on the feeding behavior of the nematode community was studied separately, whereas in
450 natural environment all these factors covary greatly. The combination of temperature and
451 salinity factors was found to have a higher impact than each factor taken alone (Tietjen and
452 Lee 1972; Tietjen and Lee 1977). Moreover, nematodes may respond to environmental
453 changes at a seasonal scale, by physiological adjustment and shifting of their optimum
454 conditions (Gee 1985). However, the nematode community studied appeared to have adapted
455 to the highly variable environment constituted by the surficial sediment of intertidal mudflats,
456 except at low temperatures (5°C), and their feeding activity is only slightly decreased by
457 temperature, salinity or light stress. Due to high abundance of bacteria in the marine sediment,
458 nematodes may never be food limited with bacteria. Nematodes kept feeding under conditions
459 of stress which were typical of the surficial sediment habitat, moreover they appeared to be
460 principally dependent on the algal resource. Consequently, the community of nematodes
461 dwelling in the top centimeter of the Brouage mudflat may also have a feeding strategy which
462 is strongly linked to the formation of algal biofilm during the diurnal ebb.

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693

694 **Table and Figures captions**

695

696 **Table 1.** List of species ranked by dominance (feeding types according to Wieser (1953;
697 1960)). 1A: selective deposit feeders; 1B: non-selective deposit feeders; 2A: epigrowth
698 feeders; 2B: omnivores/predators.

699 **Table 2.** Nematodes isotopic composition ($\Delta^{15}\text{N}$ and $\Delta^{13}\text{C}$ mean \pm SD, N = 3) and bacterial
700 and algal uptake rates calculated.

701 **Figure 1.** Map of the study site.

702 **Figure 2.** Assimilation of bacterial carbon (mean \pm SD, N =3) as function of incubation time
703 (h).

704 **Figure 3.** Assimilation rate of bacterial carbon (mean \pm SD, N =3) as function of temperature
705 ($^{\circ}\text{C}$). Different letters above bars indicate significant differences between incubation
706 conditions (ANOVA; Tukey test).

707 **Figure 4.** Assimilation rate of bacterial carbon (mean \pm SD) as function of bacterial
708 abundance (10^8 cell.ml $^{-1}$). Different letters above bars indicate significant differences between
709 incubation conditions (ANOVA; Tukey test).

710 **Figure 5.** Assimilation rate of bacterial carbon (mean \pm SD) as function of algal abundance
711 ($\mu\text{gChla.g}^{-1}$).

712 **Figure 6.** Uptake rate of algal carbon \circ (mean \pm SD) and bacterial carbon \bullet (mean \pm SD) as
713 function of algal abundance ($\mu\text{gChla g}^{-1}$). Bacterial abundance was constant (10.5×10^8 cells
714 ml $^{-1}$).

Table
Table 1

Genera species	Feeding type	Abundance relative (%)
<i>Chromadora macrolaima</i>	2A	64.2
<i>Daptonema oxycerca</i>	1B	15.2
<i>Ptycholaimellus jacobi</i>	2A	7.9
<i>Sabatieria pulchra</i>	1B	2.6
<i>Axonolaimus paraspinosus</i>	1B	1.7
<i>Praeacanthonchus punctatus</i>	2A	1.7
<i>Halalaimus sp.</i>	1A	1.0
<i>Aegialoalaimus sp.</i>	1A	1.0
<i>Sphaerolaimus gracilis</i>	2B	0.7
<i>Spilophorella sp.</i>	2A	0.7
<i>Metachromadora sp.</i>	2A	0.7
<i>Theristus sp.</i>	1B	0.3
<i>Parodontophora marina</i>	1B	0.3
<i>Tripyloides marinus</i>	1B	0.3
<i>Eleutherolaimus sp.</i>	1B	0.3
<i>Desmolaimus zeelandicus</i>	1B	0.3
<i>Daptonema hirsutum</i>	1B	0.3
<i>Terschellingia sp.</i>	1A	0.3
<i>Viscosia sp.</i>	2B	0.3

Table 2

	$\Delta^{15}\text{N}$		Bacteria uptake ($\mu\text{g C ind}^{-1} \text{h}^{-1}$)	$\Delta^{13}\text{C}$		Algae uptake ($\mu\text{g C ind}^{-1} \text{h}^{-1}$)
	Control	Normal		Control	Normal	
Kinetics (hours)						
1		12.01 \pm 0.25	3.67 \pm 4.06			
2		14.24 \pm 0.86	20.00 \pm 7.03			
3	11.78 \pm 0.23	15.20 \pm 0.38	18.51 \pm 2.05	-15.4 \pm 1.32		
5		20.63 \pm 1.49	28.80 \pm 4.84			
8		27.72 \pm 1.55	32.41 \pm 3.15			
12	11.56 \pm 0.87	32.66 \pm 4.91	28.31 \pm 6.65	-15.8 \pm 0.77		
Temperature ($^{\circ}\text{C}$)						
5		12.41 \pm 0.53	3.38 \pm 2.87			
10		14.60 \pm 0.50	15.26 \pm 2.70			
30		16.38 \pm 0.79	24.92 \pm 4.27			
40		15.18 \pm 1.73	18.42 \pm 9.38			
Irradiance						
Light		19.42 \pm 2.01	41.43 \pm 10.88			
Salinity						
18		15.03 \pm 0.66	17.62 \pm 3.57			
Bacterial abundance (10^8 cells ml wt sed $^{-1}$)						
4.2		11.75 \pm 0.20	-0.65 \pm 4.39			
7.0		12.25 \pm 0.40	3.34 \pm 2.93			
17.4		15.86 \pm 0.10	18.34 \pm 0.45			
Algal abundance ($\mu\text{g Chla g dry sed}^{-1}$)						
25.6		15.39 \pm 0.59	19.58 \pm 3.19	-4.44 \pm 6.53		58.94 \pm 32.35
64.3		15.58 \pm 0.51	20.59 \pm 2.78	25.60 \pm 4.79		107.11 \pm 12.24
113.7	11.50 \pm 0.17	14.34 \pm 0.61	13.87 \pm 3.33	-16.34 \pm 0.53	52.14 \pm 33.29	161.20 \pm 78.30

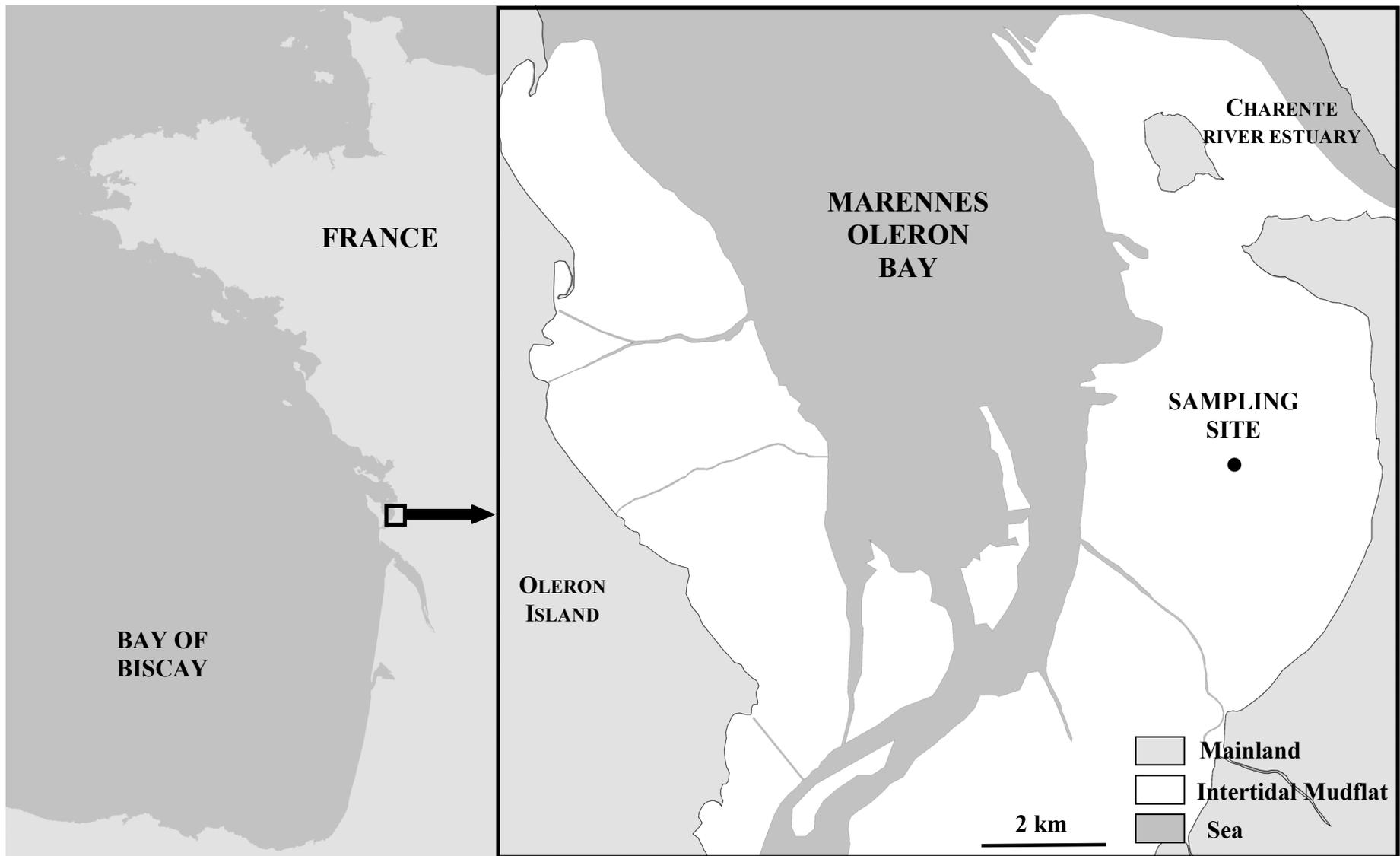


Fig. 1

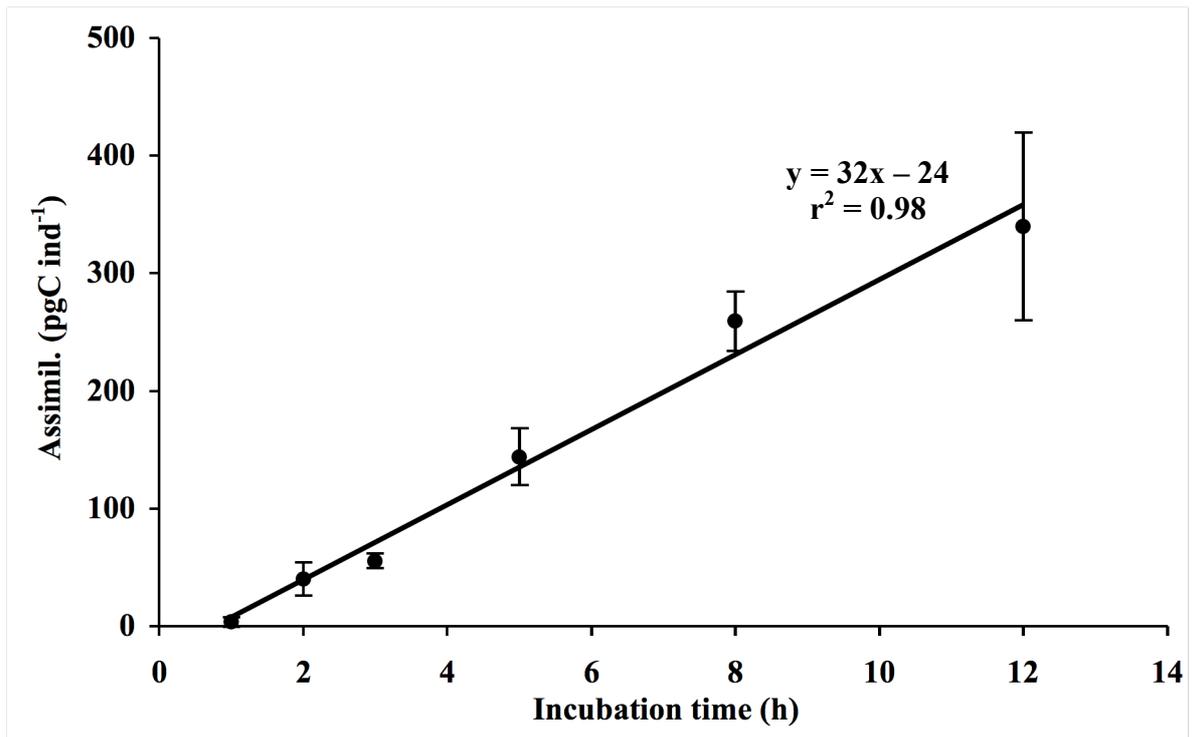


Fig. 2

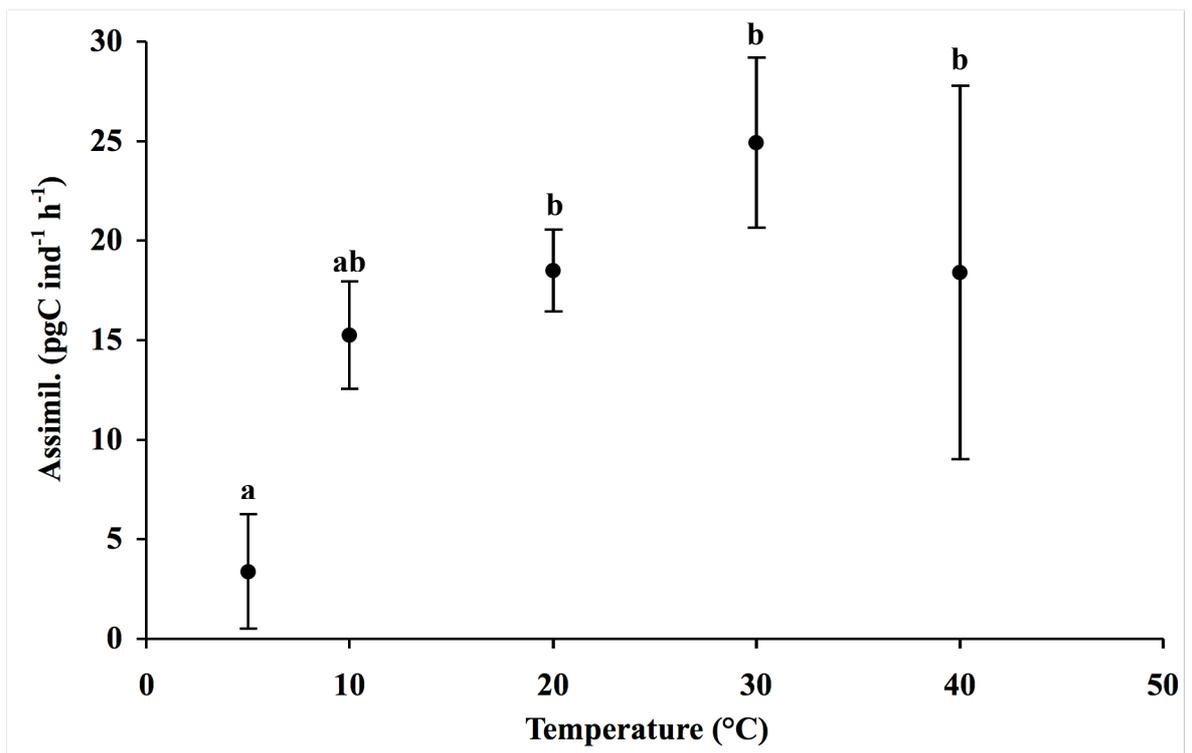


Fig. 3

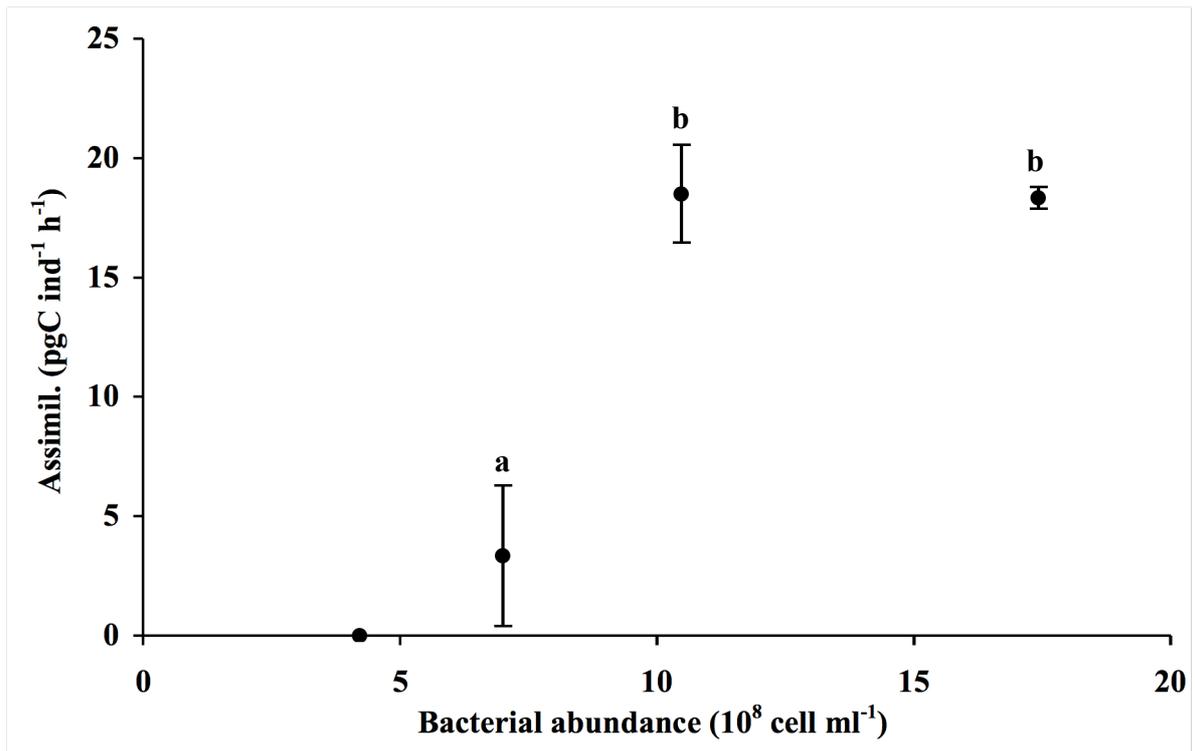


Fig. 4

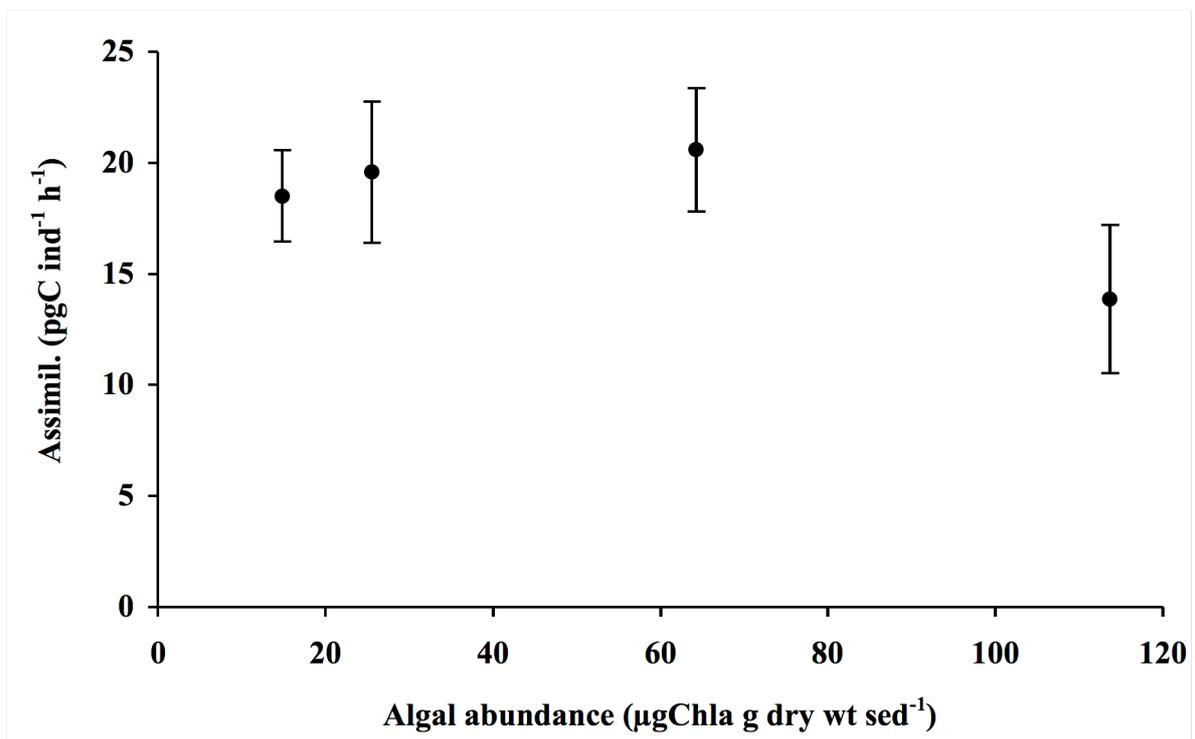


Fig. 5

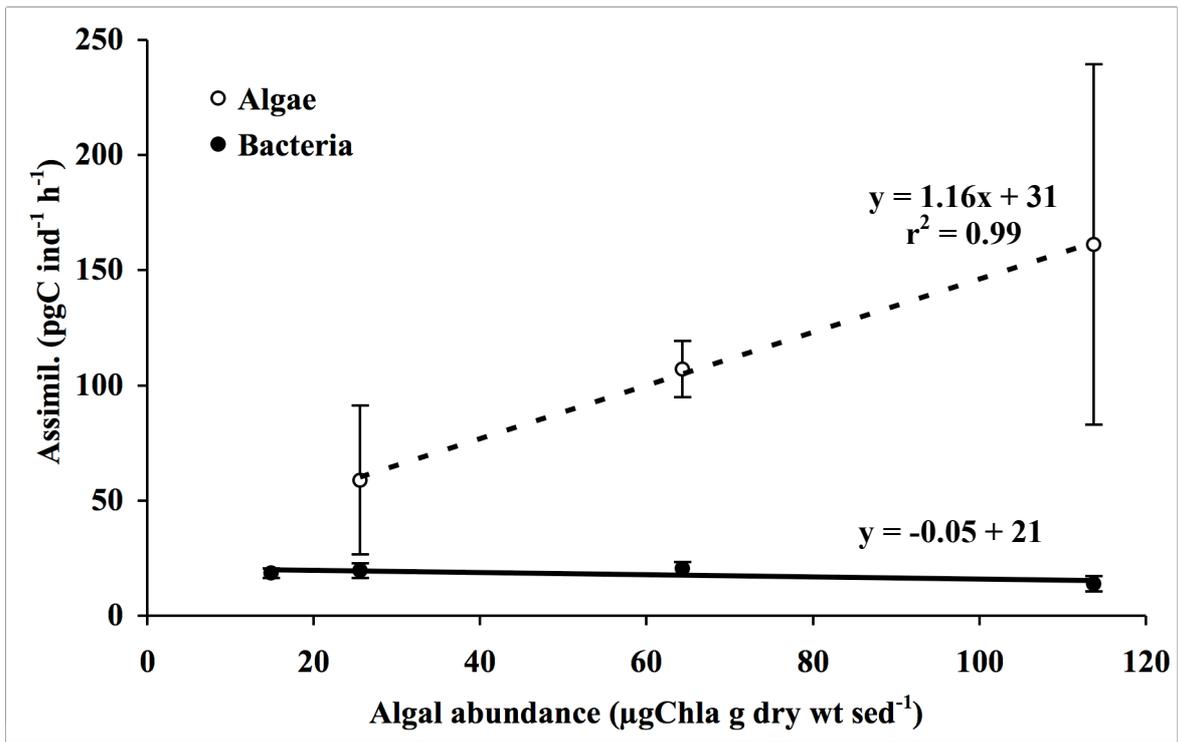


Fig. 6