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Bacterivory in the common foraminifer *Ammonia tepida*: Isotope tracer experiment and the controlling factors

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Abstract:

The majority of sediment dweller foraminifera are deposit feeders. They use their pseudopodia to gather sediment with associated algae, organic detritus and bacteria. Uptake of bacteria by foraminifera have been observed but rarely quantified. We measured uptake of bacteria by the common foraminifera Ammonia tepida using ^{15}N pre-enriched bacteria as tracers. In intertidal flats, seasonal, tidal and circadian cycles induce strong variations in environmental parameters. Grazing experiments were performed in order to measure effects of abiotic (temperature, salinity and irradiance) and biotic (bacterial and algal abundances) factors on uptake rates of bacteria. In mean conditions, A. tepida grazed 78 pgC ind^{-1} h^{-1} during the first eight hours of incubation, after which this uptake rate decreased. Uptake of bacteria was optimal at 30°C, decreased with salinity and was unaffected by light. Above 7 x 10^8 bacteria ml wt sed^{-1}, uptake of bacteria remained unchanged when bacterial abundance increased. Algal abundance strongly affected algal uptake but did not affect uptake of bacteria. As uptake of bacteria represented 8 to 19% of microbes (algae plus bacteria) uptake, Ammonia seemed to be mainly dependant on algal resource.

Keywords: Bacteria; Environmental factor; Foraminifera; Grazing; Mudflat; Prey abundance; Trophoecology

Introduction

Benthic foraminifera are heterotrophic protozoa that have the morphological characteristics of pseudopodia and a test with one or more chambers. Since the Cambrian era, they have been present in a wide range of environments, from shallow brackish waters to deepest oceans. They are used as proxies for paleoecological studies because they are wide spread, numerous and well preserved. In recent times, foraminifera increasingly appeared as dominant members of benthic communities in both shallow and deep-sea environments (Snider et al. 1984; Alongi 1992; Gooday et al. 1992; Moodley et al. 1998; Moodley et al. 2000), suggesting that they may play an important role in food webs (Altenbach 1992; Linke et al. 1995).

Foraminifera exhibit a wide range of trophic behaviours: dissolved organic matter (DOM) uptake, herbivory, carnivory, suspension feeding and most commonly, deposit feeding (Lipps 1983). Deposit feeders are omnivorous, using their pseudopodia to gather fine-grained sediment with associated bacteria, organic detritus and, if available, algal cells. As a large part of organic detritus is indigestible, it must be cycled by bacteria before becoming available to deposit feeders (Levinton 1979). Benthic bacteria are highly abundant and productive in benthic sediments. Due to their high nutritional value they are suspected to be an important resource for sediment dwelling fauna.

Bacteria could play a major role or be an obligatory item in foraminiferal nutrition. Several littoral benthic foraminifera require bacteria to reproduce (Muller and Lee 1969) and have been shown to selectively ingest bacteria according to strain (Lee et al. 1966; Lee and Muller 1973). Some epiphytic foraminifera show a farming strategy. They produce nutrient-rich substrate for bacteria and then ingest cultured bacteria (Langer and Gehring 1993). Foraminifera are also able to feed actively on bacterial biofilms (Bernhard and Bowser 1992). Bacteria may also play a symbiotic role in bathial species of foraminifera (Bernhard 2003). Uptake of bacteria by *Ammonia* has been displayed using direct food vacuole observation

(Goldstein and Corliss 1994) and bacteria labelled with fluorescent dyes (Langezaal et al. 2005). Nevertheless, those studies do not give access to quantitative data concerning the uptake rate of bacteria, and the precise role that bacteria play in foraminiferal nutrition remains elusive. Assessing grazing rate on bacteria remains a major point that must be documented to determine the role that foraminifera play in benthic food webs.

Ammonia is one of the most common genera of benthic foraminifera with a worldwide distribution in inner shelf, estuarine, and salt marsh environments (Murray 1991). One remarkable characteristic of this genus is its ability to survive over a broad range of temperatures, salinities, and seasonal regimes (Bradshaw 1961; Walton and Sloan 1990).

The aim of this study is to assess experimentally in different controlled conditions uptake rates of bacteria by *Ammonia* from an intertidal mudflat habitat (Marennes-Oléron, France). ^{15}N enriched bacteria were used as tracers to determine uptake rate of bacteria (Pascal et al. 2008). This habitat is subject to large and quick changes in many environmental features. Three relevant time scales drive these environmental variations: long-term (seasonal cycle), medium-term (lunar cycle) and short-term (solar and tidal cycles) (Guarini et al. 1997). Since these variations may influence foraminiferal feeding behaviour, grazing experiments were performed in order to evaluate effects of abiotic (temperature, salinity and irradiance) and biotic (bacterial and algal abundances) factors on uptake rates of 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. Temperature and salinity of 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 river Charente 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 ml of the collected sediment was added to 20 ml of bacterial liquid culture medium and kept in darkness during 24 hours at 13°C. The composition of this culture medium were previously described in Pascal et al. (2008). This primary culture was then subcultured during 24 hours under the same conditions to get approximately 2×10^9 cells ml^{-1}. 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. 2005), 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 during 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 check the absence of ciliate and flagellate in bacterial culture during this microscope observation step. Abundance of diatom in sediment was assessed using Chl *a* as a proxy, measured using fluorometry (Lorenzen 1966).

Grazing experiments

Incubation of enriched bacteria and algae with foraminifera were performed in Petri dishes (4.5 cm diameter). Experiments were done in standardized condition similar to field ones: temperature (20°C), irradiance (darkness), salinity (31‰), bacterial abundance (10.5×10^8 cells ml wt sed^{-1}) and algal abundance (15 µgChla g dry sed^{-1}). For each type of experiment, one environmental incubation factor was modified in order to determine its impact on foraminifera grazing activity. Each experiment was carried out in triplicate, along with triplicate controls. Control samples were frozen (-80°C) in order to kill foraminifera.

115 During the ebb tide of 13th of March 2006, one sample of the first centimetre of sediment
116 was collected from the Brouage intertidal mudflat (France). First, the sediment was sieved on
117 a 500 µm mesh in order to remove macrofauna. Then, it was sieved on a 200 µm mesh to
118 extract large foraminifera. One ml of the sediment remaining on the mesh was put in each
119 microcosm. Sediment that passed through the 200 µm mesh was sieved through a 50 µm
120 mesh. Fraction passing through the mesh was mixed with ^{15}N enriched bacteria. This slurry
121 contained 10.5×10^8 cells ml of wet sediment^{-1} with a ratio of total and enriched bacteria of
122 1.5. Four ml of this slurry were put in each microcosm.
123 First, for the calculation of grazing rates, a kinetic study was realised to validate the linear
124 or hyperbolic uptake kinetics. Incubations for this kinetic study were run during variable
125 times (1 to 12 hours). As all other experiments were run for 5 hours, this first step is necessary
126 to check the linear uptake during the first five hours of incubation.
127 For each type of experiment one environmental incubation factor was modified. Light
128 effect was tested with one irradiance (83 µM of photons m^{-2} s^{-1}). In order to decrease salinity,
129 cultured bacteria were rinsed with 0.2 µm filtered sea-water diluted with 0.2 µm filtered fresh
130 water (final salinity of 18‰). Bacterial abundance was modified adding various quantities of
131 bacteria enriched in ^{15}N. Bacterial abundances (total enriched and non-enriched) tested were
132 4, 7 and 17 cells ml wt sed^{-1} with respectively the following ratio between abundance of total
133 and enriched bacteria: 6.1, 2.0 and 1.3. Algal abundance was modified adding various
134 quantities of cultured *N. phyllepta* enriched in ^{13}C while bacterial abundances (total enriched
135 and non-enriched) were kept constant at 10×10^8 cell ml^{-1}. Algal abundance (total enriched
136 and non-enriched) were 26, 64 and 114 µgChla g dry sed $^{-1}$ with respectively the following
137 ratio between abundance of total and enriched algae: 2.4, 1.3 and 1.2.
138 Incubations were stopped by freezing the microcosms at -80°C. Samples were thawed and
139 stained with rose Bengal in order to identify freshly dead foraminifera. For each sample, 150

specimens of the species *A. tepida* were picked up individually and cleaned of any adhering particles. Samples from experiments with ^{13}C enriched *N. phyllepta* were processed with HCl 0.1 M in silver boats to remove any inorganic carbon.

Isotope analysis and calculations

$\delta^{15}N$ and $\delta^{13}C$ of prey (bacteria and algae) and grazers (*A. tepida*) 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 in bacterial carbon grazed using 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). R was derived from the measured $\delta^{15}N$ values as: $R = ((\delta^{15}N/1000)+1) \times RairN_2$ where $RairN_2 = 7.35293 \times 10^{-3}$ (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 $F = {}^{13}C / ({}^{13}C + {}^{12}C) = R / (R + 1)$, $RairN_2$ 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 average weight of *A. tepida* used was 18.1 ± 3 µg DW (n = 115 samples of 150 specimens each). Decalcified specimens of *A. tepida* were composed on average of 1.03 ± 0.23 µg of C and 0.15 ± 0.03 µg of N. Uptake expressed as $gC_{bacteria}\ h^{-1}\ gC_{Ammonia}^{-1}$ was obtained by dividing uptake of bacteria ($gC\ ind^{-1}\ h^{-1}$) by *A. tepida* decalcified mean weight ($gC\ ind^{-1}$).

Variations of uptake rates according to salinity and irradiance were tested using bilateral independent-samples t-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

Foraminiferal isotopic compositions and rates of bacterial and algal uptakes rates are presented in Table 1.

During the kinetic experiment, uptake of bacteria by *A. tepida* increased linearly during the first eight hours of incubation and then levelled off (Fig. 1). The linear regression slope for the first eight hours suggested an uptake rate of 78 $pgC\ ind^{-1}\ h^{-1}$ equivalent to 75×10^{-6} $gC_{bacteria}\ gC_{Ammonia}^{-1}\ h^{-1}$ ($r^2 = 0.99$). The linear regression slope between eight and twelve hours was more than five times lower than for the first eight hours and suggested an uptake rate of 14 $pgC\ ind^{-1}\ h^{-1}$.

Temperature had a significant effect on *Ammonia* uptake rate of bacteria ($F = 27$; $p<0.001$). Temperatures tested fluctuated between 5 and 40°C and were in the range of those found in the study area (Guarini et al. 1997). Uptake of bacteria was almost null at 5°C, then increased with temperature. It reached an optimum at around 30°C and then decreased (Fig. 2). Uptake rates measured at 10, 20 and 40 °C were not significantly different. Maximum

188 uptake rate of bacteria (30°C) reached 113 pgC ind^{-1} h^{-1} and was significantly different from
189 others.

190 Uptake rate of bacteria by *Ammonia* decreased significantly from 67 to 32 pgC ind^{-1} h^{-1}
191 when salinity dropped down from 31 to 18‰ (bilateral t-test; $p<0.05$) (Fig. 3). In the study
192 area, salinity of overlaying water fluctuates between 25 to 35‰ (Héral et al. 1982) but salinity
193 of sediment can be reduced by rainfall.

194 The sediment surface irradiance shifts from dark during submersion and night emersions to
195 high levels of incident light during daytime emersions. Irradiance tested (83 µM of photons
196 m^{-2} s^{-1}) corresponds to a low irradiance. Light did not affect foraminifera feeding activity:
197 uptake rates of bacteria were similar under light and darkness (bilateral t-test; $p = 0.71$) (Fig.
198 3).

199 Ingestion of bacteria was significantly linked with abundance of bacteria in microcosm (F
200 $= 32$; $p<0.001$) (Fig. 4). Four different bacterial concentrations were tested: 4, 7, 10 and $17 \times$
201 10^8 cells ml wt sed^{-1}. There was no uptake when the bacterial abundance was 4×10^8 cells ml
202 wt sed^{-1} (Fig. 5), uptake rate of bacteria remained constant around 67 pgC ind^{-1} h^{-1} when
203 bacterial concentrations increased from 7 to 17×10^8 cells ml wt sed^{-1} (Fig. 4).

204 When algal concentration increased from 15 to 114 µgChla g dry sed^{-1} with constant
205 bacterial abundance (10.5×10^8 cell ml^{-1}), the uptake rate of bacteria remained constant ($F =$
206 1.4; $p = 0.29$) (Fig. 5). The uptake rate of algae increased from 329 to 971 pgC ind^{-1} h^{-1}
207 linearly when algal abundance increased ($r^2 = 0.99$; $p<0.001$) (Fig. 7). When algal abundance
208 increased, the fraction of algae in the diet of foraminifera increased. The fraction of bacteria
209 decreased from 18.8 to 14.4 and 7.8% of microbes (algae plus bacteria) taken up when algal
210 concentration was equal to 25.6, 64.3 and 113.7 µgChla g dry sed^{-1} respectively.

Discussion

Experimental procedure

Like all various methods previously developed and applied to measure bacterivory, the method used in the present study presents methodological shortcomings that make interpretation of the resulting problematic. For instance, sieving the sediment changes the bacterial availability for foraminifera, bacteria being not attached to particle as in natural situation. Foraminifera are known to selectively graze different bacterial strains (Lee et al. 1966; Bernhard and Bowser 1992). As grazing experiments are based on the hypothesis that grazers take up ^{15}N enriched bacteria and natural sediment bacteria at the same rate, the cultured bacteria community has to present characteristics roughly similar to the natural one. Despite the fact that culture modified the specific composition of the natural bacterial community, characteristics of size, activity and diversity of the cultured bacterial consortium in our experiments would be more representative of the natural community than in most previous grazing experiments (Pascal et al. 2008). As enriched algae (monospecific and freeze-dried) may present characteristics different from natural algal community, bias due to selective ingestion of algae may exist. Control experiments were always performed in similar conditions to assess bias due to bacterial or algal cell adhesion on foraminiferal test. ^{13}C enriched freeze dried algae are potential source of enriched DOM and transfer to bacteria drive to formation of ^{13}C enriched bacteria but as incubations were short-term, we consider as negligible this bias due to recycling.

The kinetics of bacterial uptake

Foraminifera use pseudopodia in order to form a long and extensive network for trapping food particles (Travis and Bowser 1991). Actively feeding specimens are characterized by feeding cysts. Aggregates of particles are firmly attached around the test apertures and they may encompass the entire test. Collected material is partitioned into small fractions before

ingestion. The possibility of extracellular digestion (Meyer-Reil and Köster 1991) and reticulopodial digestion (Lee et al. 1991) has been suggested. Food vacuoles contain large amounts of sediment, organic detritus, algal cells and bacteria (Goldstein and Corliss 1994). Those vacuoles are most abundant in the terminal chamber, but occur throughout the last four chambers as well. The digestion of bacteria seems to occur in the terminal chamber of *Ammonia* (Goldstein and Corliss 1994; Langezaal et al. 2005). This genus ingests bacteria and readily digests them, implying that bacteria are more probably used as food source than as symbionts (Langezaal et al. 2005).

Langezaal et al. (2005) found that *Ammonia beccarii* grazed 90 bacteria during a 20 h period. Converting their uptake rate into bacterial biomass (Norland et al. 1995), gives a grazing rate of 1.7 pgC ind^{-1} h^{-1}. This rate is lower than the rate found in the present study (78 pgC ind^{-1} h^{-1}). This may be linked to the bacterial concentration used by Langezaal et al. (2005) in their microcosms (1.4×10^3 cells ml^{-1}), which was substantially lower than benthic bacterial abundance in natural environments (c.a. 10^9 cells ml^{-1}) and in the present study.

In present experiments, uptake of bacteria by *Ammonia* was rapid and detectable after 2 hours of incubation (Fig. 1). This is in accordance with Moodley et al. (2000) who observed a detectable uptake of algal carbon by *Ammonia* after three hours of incubation. Uptake of bacteria increased linearly during the first eight hours and then levelled off (Fig. 1). A similar pattern was observed for uptake of algae by *Ammonia* (Moodley et al. 2000). This levelling off may reflect satiation or more likely excretion, effective after eight hours. Foraminifera have been reported to assimilate ingested algal carbon within 12 h (Rivkin and De Laca 1990) and *Ammonia* was found to assimilate carbon from phytodetritus in 12 h (Moodley et al. 2000). A simplified energy budget can be calculated with following parameters, $C = A + FU$ (C = food uptake, A = assimilation of metabolisable energy, FU = loss by faeces and urinary wastes) (Klekowski et al. 1979; Schiemer 1982). We suggest that during the first eight hours

of incubation, *Ammonia* takes up and assimilates bacteria. Then, after eight hours, uptake and assimilation still occur but excretion begins. By assuming, that uptake is constant during all the grazing experiments, assimilation and excretion rates can be assessed. The first slope from the origin to eight hours would correspond to the uptake rate while the second slope would correspond to the assimilation rate. *Ammonia* retains five times more tracer during the first eight hours than after. As a result, 17% of ingested bacteria would be assimilated and 83% would be rejected. This result is disputable because the uptake rate constancy was not determined and the uptake rate after eight hours is determined from only two data points, however, this result fits well with assimilation rate of bacteria by nematodes (25%) (Herman and Vranken 1988) and polychaetes (26%) (Clough and Lopez 1993).

Effects of abiotic factors

Studies on the influence of environmental factors on *Ammonia* are limited. However, Bradshaw (1957; 1961) determined the influence of temperature and salinity on *Ammonia* reproductive activity, growth rate and survival under experimental conditions.

In the present experiments, temperature had a similar effect on uptake rate of bacteria to the one shown by Bradshaw on growth and reproductive rates. We found no uptake of bacteria at 5°C (Fig. 2), in accordance with Bradshaw, showing that foraminifera metabolism is very low at low temperatures. Under a temperature less than 10°C, *Ammonia* fail to grow and reproduce and individuals appear to live an indefinitely long period (Bradshaw 1957). The optimal grazing temperature appeared at 30°C (Fig. 2) as in Bradshaw's experiments (Bradshaw 1961). When temperature exceeded 30°C in our microcosms, grazing rate decreased. Similarly, no growth was observed in Bradshaw's experiments, and specimens lived less than one day at 35°C (Bradshaw 1957). Those physiological characteristics have implications for foraminiferal abundances at the seasonal time scale. Limited activity during winter prevents reproduction and limits abundance, while in summer, high temperature can

lead to mortality, in particular in the intertidal habitat subject to a wide range of rhythmically and rapidly varying temperature due to tidal cycles.

Uptake rate of bacteria declined when salinity was reduced to 18‰ (Fig. 3). This result is also in accordance with those of Bradshaw (1957; 1961). According to this author, normal growth and reproduction of *Ammonia* occur when salinity fluctuates between 20 and 40‰, and *Ammonia* failed to grow below 13‰. *Ammonia* is an euryhaline genus found from brackish (Debenay and Guillou 2002) to hypersaline environments (Almogi-Labin et al. 1992). However, conditions of brackish environments would not be optimal for *Ammonia*. Foraminifera use a network of pseudopoda to gather and ingest food particles. Each pseudopoda contains an elongated cytoskeleton primarily composed of microtubules. Modifications of salinity induce a decrease of the number of pseudopodal microtubules (Koury et al. 1985), that may lead to a lower pseudopodal efficiency. This could explain the lower uptake rate observed at low salinity, considering that sediment salinity is under control of tidal cycles and weather conditions, like rainfall, which induces a strong decrease of salinity during low tide.

Light did not affect uptake rate of *Ammonia* (Fig. 3). Although foraminifera frequently form symbiosis with algae, *Ammonia* is not known as an algal-bearing foraminifera (Lee and Anderson 1991). Consequently, irradiance would not influence feeding behaviour of *Ammonia*. This is confirmed by our grazing experiment results with a low irradiance rate. Owing to this, *Ammonia* seems not influenced by nycthemeral cycles and seems able to graze in superficial sediment exposed to sun in the temperature range that allows grazing. This suggests that *Ammonia* is able to feed actively in environment affected by irradiance variations.

Effects of biotic factors

Sedimented organic carbon from the photic zone can represent a major food source for deep-sea benthic foraminifera (Gooday 1988). Many studies report that the abundance of benthic foraminifera assemblages is strongly correlated with surface ocean productivity (e. g. Altenbach et al. 1999; Fontanier et al. 2002). Quick uptake of phytodetritus was observed within the deep sea but also within shallow water dwellers (Middelburg et al. 2000; Moodley et al. 2000; Moodley et al. 2002; Moodley et al. 2005; Nomaki et al. 2005a; Nomaki et al. 2005b). This high reactivity to food pulses may imply that uptake rates are strongly linked with food abundance and type of food.

Theoretically, food uptake by a grazer increases with abundance of food. However, above a threshold value of prey concentration, uptake rate remains constant (Holling's prey-dependent type II functional response (Holling 1959)). Uptake of bacteria is not detectable at the lowest bacterial concentration of 4.2×10^8 cells ml wt sed^{-1} (Fig. 4). This lack of uptake may occur for different reasons. One possibility could be that foraminifera does not feed at low bacterial concentrations. Most probably, the ratio between enriched and non-enriched bacteria used in our experiments was not high enough to allow uptake detection at low concentration. Above 7×10^8 cells ml wt sed^{-1}, the rate of uptake of bacteria remained constant, despite the increased of bacterial abundance (Fig. 5). The threshold value of prey abundance may have been overshot, which would mean that uptake by *Ammonia* would seldom be higher than those measured. Bacterial abundance in superficial marine sediment is relatively constant around 10^9 cells ml wt sed^{-1} and seldom lower than 7×10^8 cells ml wt sed^{-1} (Schmidt et al. 1998). According to these data, bacterial abundance in natural environment would always satisfy the *Ammonia* optimal uptake rate and would never be limiting factor for uptake.

Algal uptake rate increased linearly with algal abundance reaching 971 pgC ind^{-1} h^{-1} without levelling off for the tested values (Fig. 5). Algal abundances used during this experiment (15 to 114 µgChla g dry sed^{-1}) are not high enough to reach the maximum algal uptake rate; indeed *Ammonia* was found to graze at a higher rate on *Chlorella* (2180 pgC ind^{-1} h^{-1}) (Moodley et al. 2000). In natural conditions, chlorophyll *a* content of the first centimetre of sediment varies between 0 and 50 µgChla g dry sed^{-1} (review in MacIntyre et al. 1996). However, through vertical migration, benthic microalgae concentrate near the surface during diurnal low tides producing a biofilm. In this algal mat, concentration of chlorophyll *a* can reach 150 µgChla g dry sed^{-1} (Serôdio et al. 1997) and even 300 µgChla g dry sed^{-1} (Kelly et al. 2001). *Ammonia* feeding on the algal biofilm would then present a higher uptake rate than in the present study.

The use of differential labelling of bacterial food (^{15}N) and algal food (^{13}C) allows to access simultaneous uptake rates of bacteria and algae, thus permitting to determine the preferred item according to their availability. While algal uptake increased with algal abundance, uptake of bacteria remained constant (Fig. 5). *Ammonia* still ingested bacteria when other food resources were available. Bacteria might be a source of essential compounds for deposit feeders (Lopez and Levinton 1987). This assumption is in accordance with Muller and Lee (1969) who suggested that some foraminifera reproduce only when bacteria are present as food source. Then, uptake of bacteria would be essential for *Ammonia*.

Uptake of bacteria by *Ammonia* never represented more than 19% of microbial biomass (bacteria plus algae) taken up. This low contribution of bacteria to food uptake was also observed with algal concentrations comparable with sediment natural conditions of 25 µg Chla g dry sed^{-1} (MacIntyre et al. 1996). Muller (1975) suggest that shallow water dwelling species mainly depend on algal resources. In their study, van Oevelen et al. (2006) found that

357 bacterial carbon constitutes only 9% of total needs of hard-shelled foraminifera of an
358 intertidal mudflat community.

359 In intertidal areas, algal abundances vary seasonally (e. g. Haubois et al. 2005). In addition,
360 benthic microalgae of intertidal sediments vertically migrate with rhythms associated with
361 diurnal and tidal cycles (Blanchard et al. 2001). During day time, at low tide, algal cells
362 concentrate near the surface of sediment and form a mat (Herlory et al. 2004). According to
363 our results, *Ammonia* seems to depend principally on algal feeding resource. For this reason,
364 this species may feed on the mat of microphybenthos when it is formed in order to maximize
365 its rate of energy gain. This feeding behaviour would imply that *Ammonia* dwells at the
366 surface of the sediment during low tide. Thus, *Ammonia* would be subject to all of the fast and
367 large environmental variations that are typical of the intertidal habitat, especially at the air-
368 sediment interface during low tide. Though *Ammonia* is considered as one of the most tolerant
369 genus of foraminifera to temperature and salinity variations (Bradshaw 1961; Walton and
370 Sloan 1990), we showed that variations of these parameters influence uptake of bacteria (Fig.
371 2 and 3). Vertical migration from the food-rich surface into deeper layers is a possible
372 mechanism for foraminifera to avoid unfavourable conditions (Groß 2002). In this deeper
373 layer, bacteria would constitute a large part of the diet of *Ammonia*. When temperature and
374 salinity allow *Ammonia* to migrate to the surface sediment, *Ammonia* would principally graze
375 on the microphytobenthic mat.

376 In conclusion, bacteria appeared to be quantitatively of minor importance in the nutrition
377 of foraminifera compared to algae. The present work demonstrates that, at the tidal scale,
378 grazing rate of bacteria is affected by abiotic (temperature and salinity) whereas it would not
379 be affected by biotic (algal and bacterial abundances) factors. *A. tepida* may further respond
380 to environmental changes at a seasonal scale, by physiological adjustment and shifting of its

optimum conditions. However, the present study does not permit to evaluate this acclimation capacity and more efforts need to be made to take it into account.

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Table and figure captions

Table 1. Foraminiferal isotopic compositions ($\Delta^{15}N$ and $\Delta^{13}C$ means ± SD, N = 3) and bacterial and algal uptake rates calculated.

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

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

Figure 3a & 3b. Bacteria uptake rate (mean ± SD, N = 3) under low versus high salinity (a) and dark versus light incubation (b). * indicate significant difference (t-test).

Figure 4. Bacteria uptake rate (mean ± SD, N = 3) as function of bacteria abundance (10^8 cell ml wt sed^{-1}). Different letters above bars indicate significant differences between incubation conditions (ANOVA; Tukey test).

Figure 5. Algae uptake rate ○ (mean ± SD, N = 3) and bacteria uptake rate ● (mean ± SD, N = 3) as function of algal abundance (μgChla g dry wt sed^{-1}) under constant bacteria abundance (10.5×10^8 cell ml wt sed^{-1}).

Figure

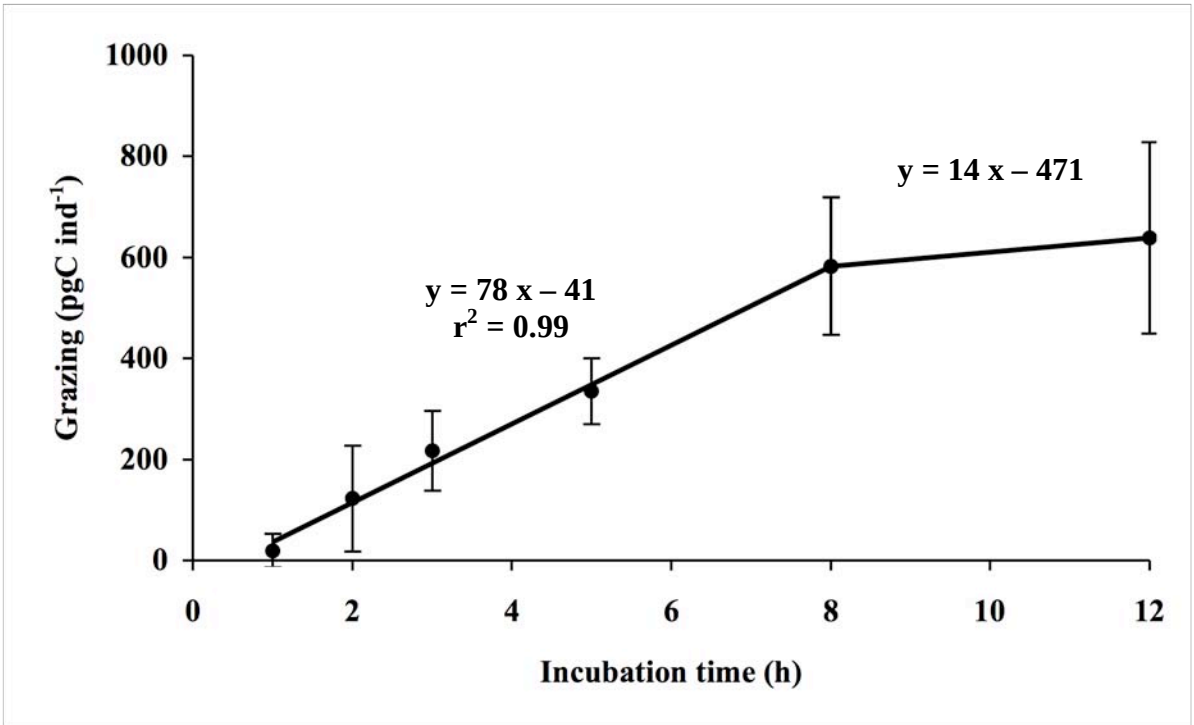


Fig. 1

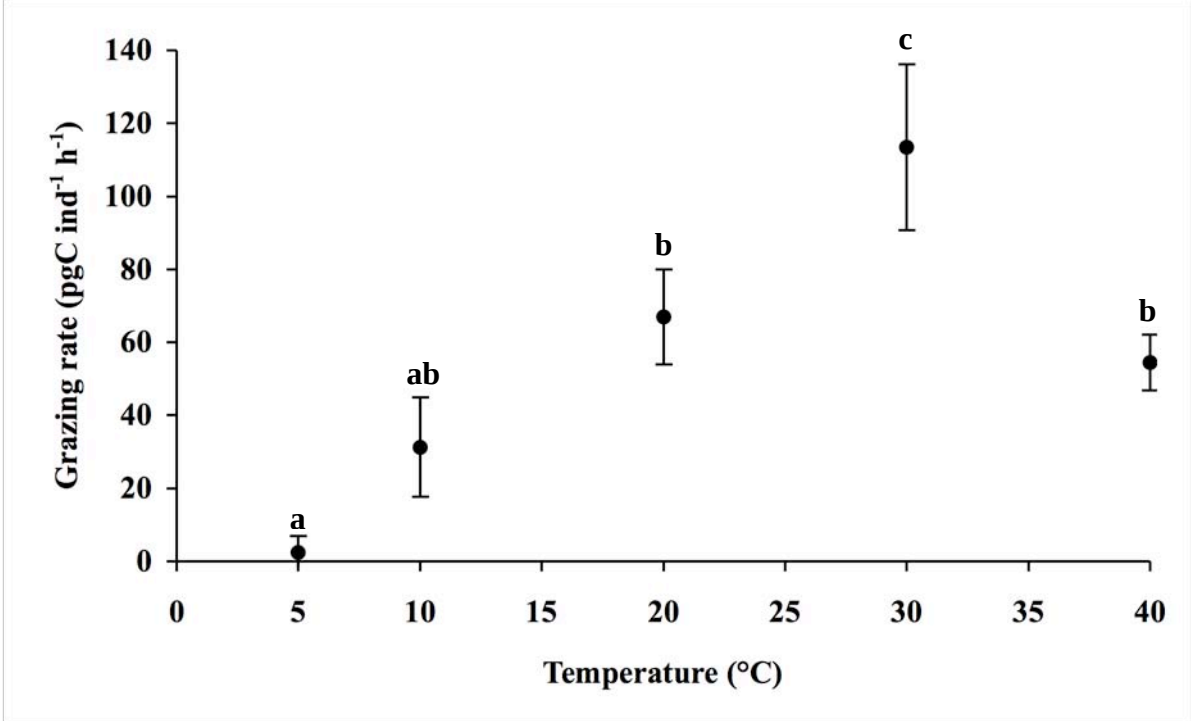
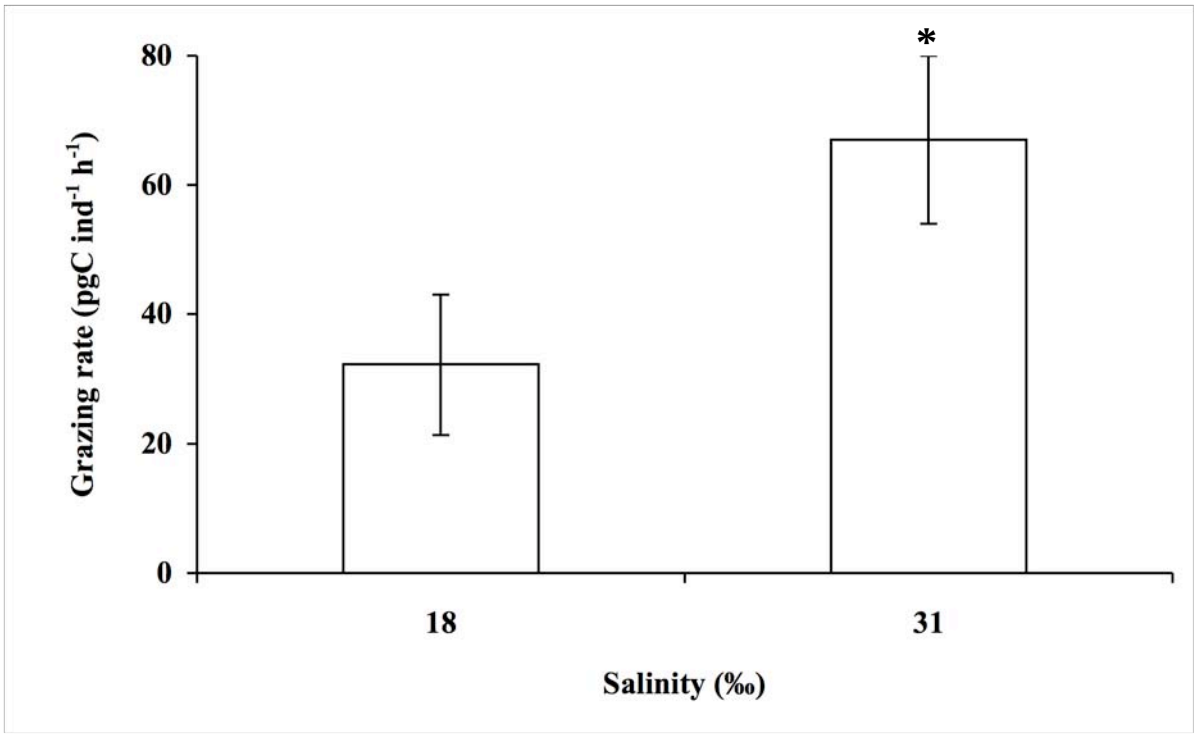


Fig. 2


Grazing rate (pgC ind-1 h-1)
80
60
40
20
0
*
18
31
Salinity (‰)

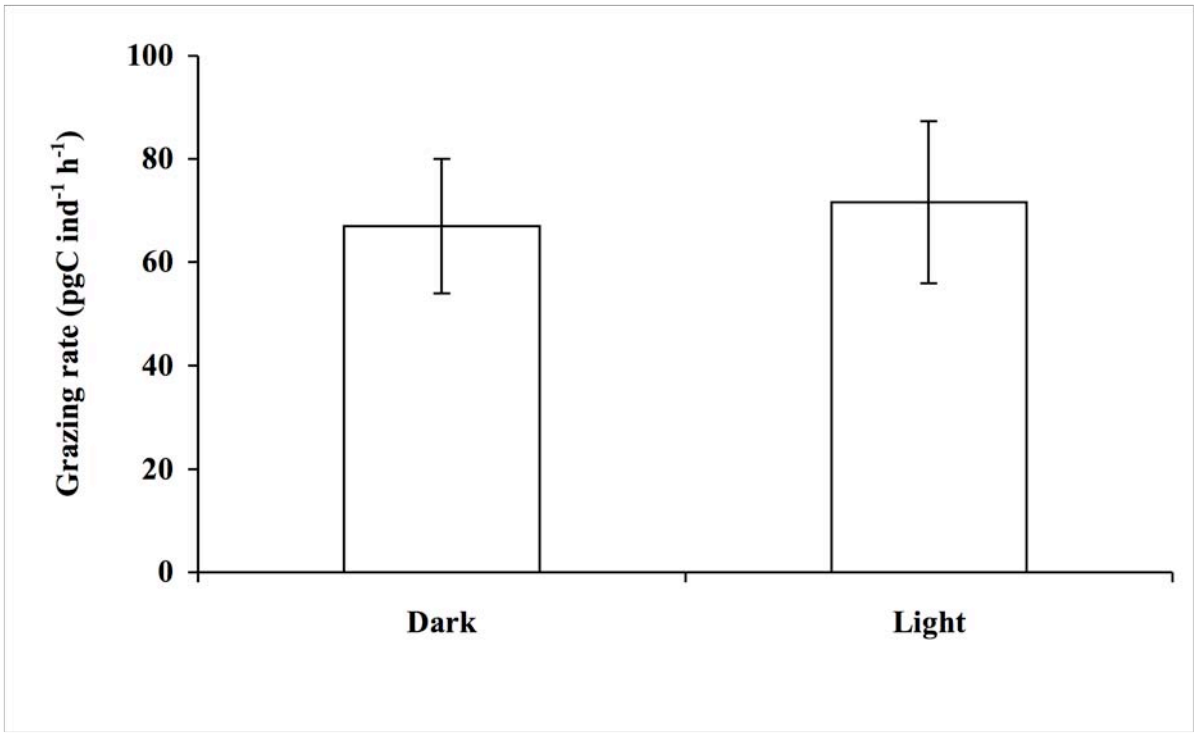

Grazing rate (pgC ind-1 h-1)
100
80
60
40
20
0
Dark
Light

Fig. 3a & 3b

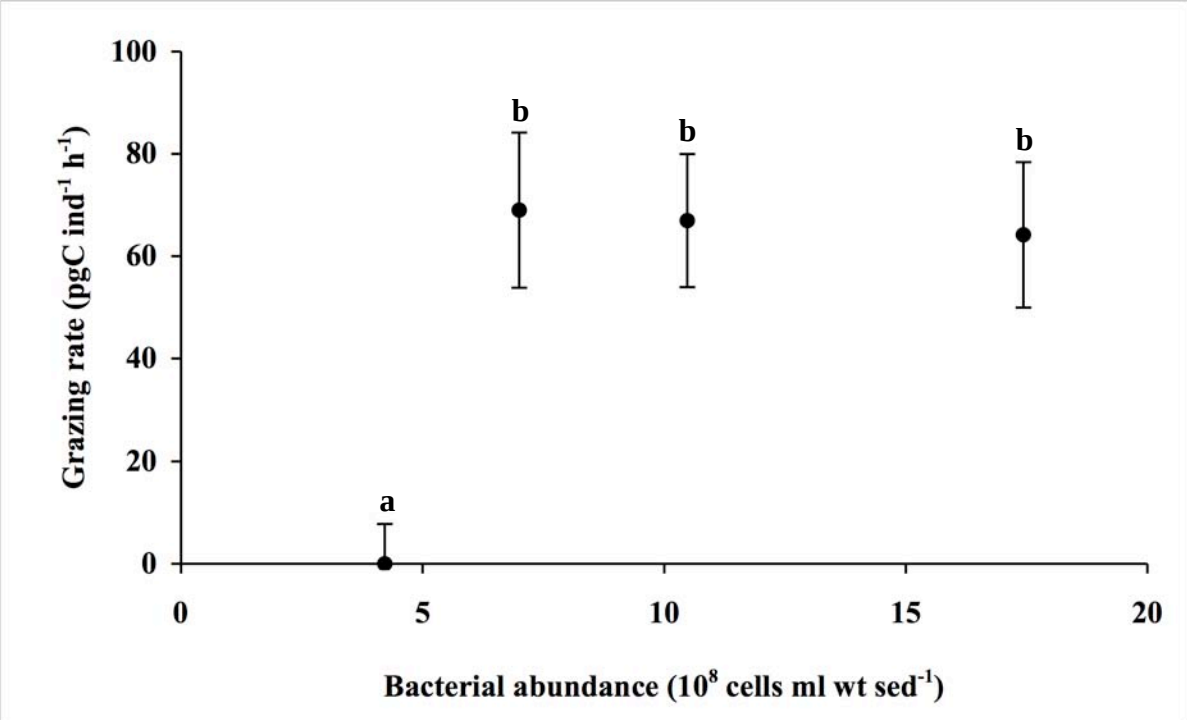

Grazing rate (pgC ind-1 h-1)
100
80
60
40
20
0
b
b
b
a
0
5
10
15
20
Bacterial abundance (10^8 cells ml wt sed-1)

Fig. 4

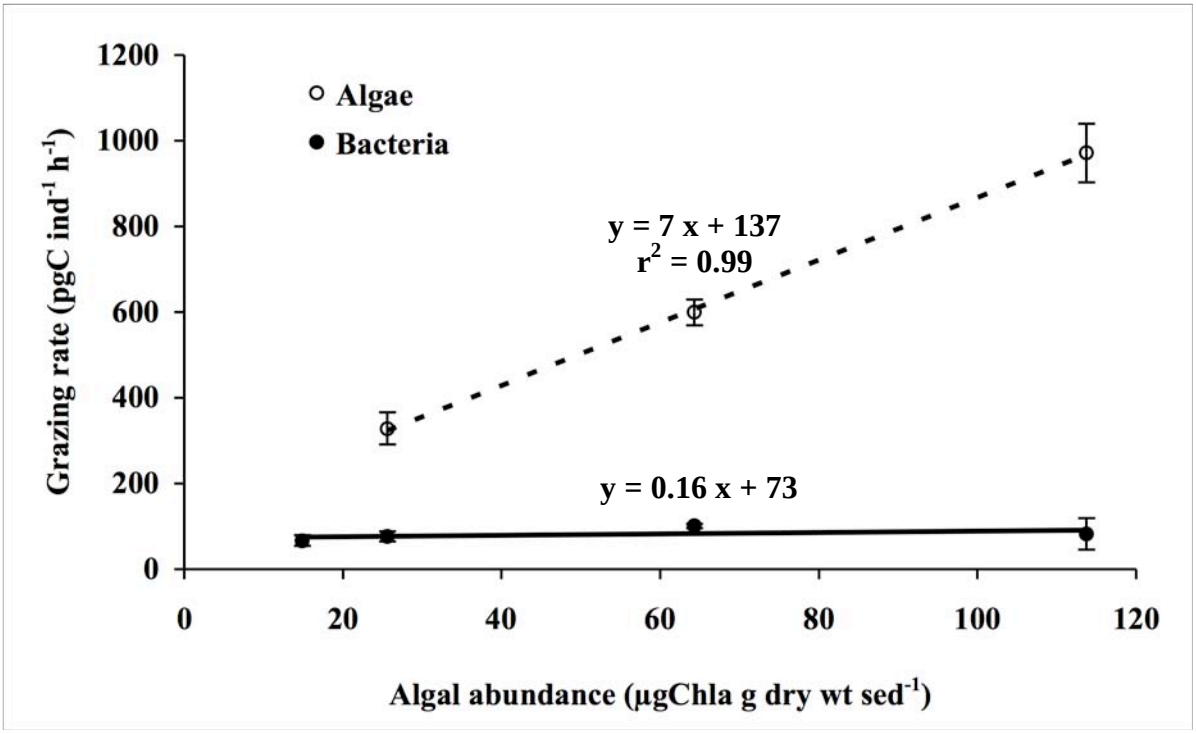

Grazing rate (pgC ind-1 h-1)
1200
1000
800
600
400
200
0
Algae
Bacteria
y = 7 x + 137
r2 = 0.99
y = 0.16 x + 73
0
20
40
60
80
100
120
Algal abundance (µgChla g dry wt sed-1)

Fig. 5

Table

	$\Delta^{15}N$		Bacteria uptake	$\Delta^{13}C$		Algae uptake
	Control	Normal	(pg C ind^{-1} h^{-1})	Control	Normal	(pg C ind^{-1} h^{-1})
Kinetics (hours)						
1	13.21 ± 0.33	13.40 ± 0.33	19.07 ± 33.03			
2	13.27 ± 0.18	14.50 ± 1.06	61.29 ± 52.57			
3	14.83 ± 0.12	17.02 ± 0.79	72.35 ± 26.24			
5	16.70 ± 0.19	20.08 ± 0.66	66.96 ± 13.00			
8	16.45 ± 0.21	22.33 ± 1.37	72.83 ± 16.97			
12	17.23 ± 0.48	23.67 ± 1.91	53.20 ± 15.80			
Temperature (°C)						
5	15.89 ± 0.45	16.01 ± 0.23	2.44 ± 4.54			
10	15.11 ± 0.34	16.68 ± 0.69	31.24 ± 13.63			
30	16.71 ± 0.63	22.44 ± 1.15	113.44 ± 22.77			
40	18.53 ± 0.24	21.28 ± 0.39	54.42 ± 7.65			
Irradiance						
Light	18.07 ± 0.19	21.69 ± 0.79	71.65 ± 15.73			
Salinity						
18	16.24 ± 0.48	17.87 ± 0.55	32.22 ± 10.82			
Bacterial abundance (10^8 cells ml wt sed^{-1})						
4.2	12.83 ± 0.10	12.82 ± 0.11	0.84 ± 7.78			
7.0	14.10 ± 0.10	16.70 ± 0.57	69.00 ± 15.16			
17.4	20.96 ± 1.67	24.86 ± 0.86	64.17 ± 14.20			
Algal abundance (µg Chla g dry sed^{-1})						
25.6	16.70 ± 0.19	20.55 ± 0.57	76.29 ± 11.35	-3.63 ± 1.99	9.86 ± 1.55	328,80 ± 37.77
64.3	16.94 ± 0.08	22.03 ± 0.29	100.97 ± 5.73	-11.37 ± 0.27	36.31 ± 2.39	598,90 ± 30.05
113.7	17.48 ± 0.60	21.63 ± 1.84	82.15 ± 36.55	-8.21 ± 0.50	75.73 ± 5.93	971.45 ± 68.58

Tab. 1