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# Experimental estimation of assimilation rates of meiofauna feeding on $^{14}\text{C}$ -labelled benthic diatoms

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## ABSTRACT

Knowledge of the trophic position of meiofauna in marine sediments is still contradictory primarily due to the scarcity of experimental data. The present study analyses diatom-feeders among the meiofauna. The assimilation rate of carbon by meiofauna was determined through a grazing experiment using in situ collected diatoms pre-labelled with  $^{14}\text{C}$ . The assimilation rates of diatom carbon were estimated for the bulk meiofaunal community ( $0.84 \text{ ng C.ind}^{-1}.\text{h}^{-1}$ ), for the bulk harpacticoid copepods ( $1.92 \text{ ng C.ind}^{-1}.\text{h}^{-1}$ ) and for two dominant nematode species (*Metachromadora remanei*  $1.26 \text{ ng C.ind}^{-1}.\text{h}^{-1}$ , *Daptonema oxycerca*  $1.35 \text{ ng C.ind}^{-1}.\text{h}^{-1}$ ). Experimental results were extrapolated to compute the consumption of meiofauna in situ that would attain up to 12% of primary benthic production in the Brouage mudflat (Marennes-Oléron Bay, southwest Atlantic coast).

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## 1. Introduction

The Marennes-Oléron Bay is dominated by intertidal mudflats and characterised by high biological productivity due to intense microalgal and bacterial production (Hamels et al., 2001). In such systems, microphytobenthos, which is mainly composed of motile diatoms, is the main source of primary production (Colijn and de Jonge, 1984) and constitutes an essential source of food for numerous benthic species (Herman et al., 2000). Therefore, quantitative data regarding the relationship between epipelagic diatoms and meiofauna are essential to understand its influence on energy fluxes in the benthic ecosystem.

Meiofauna constitutes an inconspicuous component of the benthic fauna, and although they are small organisms (40  $\mu\text{m}$ –1 mm), they are the most abundant and diverse metazoan taxa in marine sediments. Meiofauna can contribute up to 30% of the living biomass in sediment, and it is assumed that their contribution to the food of deposit-feeding macrofauna is of similar magnitude (Coull, 1990, 1999; Degré et al., 2006; Gerlach, 1978; Leguerrier et al., 2003). Meiofauna feed on bacteria, diatoms and protozoa as well as other small organisms. Although, numerous studies document meiofauna feeding on benthic diatoms (Admiraal et al., 1983; Blanchard, 1991; Bouwman et al., 1984; Middelburg et al., 2000; Montagna et al., 1995; Pascal et al., 2008; Riera et al., 1996), the results on their relative importance in the

food web are contradictory; some studies indicate that meiofauna has a grazing rate high enough to control microphytobenthic biomass and production (Blanchard, 1991; Montagna, 1995; Montagna et al., 1995), whereas other studies suggest that meiofauna uses only a small share of carbon from primary production (Middelburg et al., 2000; Moens et al., 2002; van Oevelen et al., 2006).

This work focuses on experimentally assessing the rate of diatom carbon uptake by meiofauna from an intertidal mudflat of the Brouage (Marennes-Oléron Bay, the southwest Atlantic coast of France). The primary aim of this work was to assess the quantity of carbon assimilated by meiofauna. The secondary aim was to evaluate whether the assimilation rate varies among different taxa. For this purpose, an experiment using  $^{14}\text{C}$  as a tracer was conducted in microcosms to measure the assimilation rate of carbon originating from diatoms in meiofauna and in some dominant taxa in the meiofaunal community such as copepods and nematodes (*Metachromadora remanei* and *Daptonema oxycerca*).

## 2. Materials and methods

### 2.1. Study site

The study was conducted in the Brouage mudflat, which is an intertidal area (40  $\text{km}^2$ ) in the Bay of Marennes-Oléron, located on the southwestern Atlantic coast of France (46°15' N, 1°10' W) (Fig. 1). The Brouage mudflat has flat bottom slope (1:1000) and a 4.5 km wide large tidal area (4.5 km wide) (Goulet et al., 2000). This site is known for its shellfish culture (Gouletquer and Héral, 1997), its role as nursery for juvenile fish (Le Pape et al., 2003), and as a feeding ground for shorebirds

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Fig. 1. Position of sampling site in Brouage Mudflat (Bay of Marennes-Oléron).

(Triplet et al., 2001). The Brouage mudflat has become the best-studied mudflat of Marennes-Oléron Bay and an important experimental site due to its exceptional morpho-sedimentological features (Bassoullet et al., 2000) and high primary productivity (Guarini et al., 1997). Most of the components of the Brouage ecosystem have been studied: the abundances and diversity of macrofauna, meiofauna and flora (Cariou-Le Gall and Blanchard, 1995; Rzeznik-Orignac et al., 2003; Sauriau et al., 1989) and numerous data are available on its benthic ecology (Degré et al., 2006; Leguerrier et al., 2003; Riera and Richard, 1996).

## 2.2. Sediment characteristics

To characterise the chlorophyll *a* content in the sediment, the top 2 cm of five replicated cores (2.6 cm of diameter) were sampled and deep frozen at  $-80^{\circ}\text{C}$  for later analysis. Chlorophyll was extracted from the sediment by agitation for 12 h in the dark with 90% acetone. Chlorophyll *a* content was measured by a fluorimetric method (Lorenzen, 1966).

## 2.3. Meiofaunal composition

Meiofaunal density (individuals  $10\text{ cm}^{-2}$ ) and taxonomic composition in situ were obtained from five replicated cores taken at random within a  $50 \times 50\text{ cm}$  area using a plastic syringe corer ( $11.3\text{ cm}^2$  surface area). The top 2 cm of sediment from each core were preserved in 4% formaldehyde solution. Meiofauna was extracted from the sediment using Ludox TM (Heip et al., 1985). A motoda-box sample splitter was used to collect a subsample containing at least 500–800 individuals for abundance estimations of nematodes and other taxa. From each subsample, 100 nematodes were randomly removed, mounted on slides and examined microscopically for species identification. Feeding types and species of the nematodes were determined according to the classification of Wieser (1953, 1960) and Platt and Warwick (1983, 1988), respectively.

## 2.4. Grazing experiment

The method used in this study was previously described by Blanchard et al. (2000) and is based on the addition of pre-labelled

epipellic diatoms ( $^{14}\text{C}$ ) in the sediment containing meiofauna. The assimilation rate of carbon by meiofauna was first computed according to assumptions that are explained in the discussion and then converted into an ingestion rate. This experiment was performed in the laboratory in microcosms and conducted according to following steps (Fig. 2):

- Step 1. Meiofaunal sampling. The sediment containing meiofauna was collected by scraping off the top 2 cm of sediment at low tide within a 50 cm  $\times$  50 cm area.
- Step 2. Diatom sampling. Epipellic diatoms were collected in the same area by scraping the top 2 cm of sediment. In the laboratory, diatoms were isolated from the mud using their upward migration (Paterson and Crawford, 1986). The sediment was evenly distributed into a tray to form a 1-cm-thick layer and was covered with three 200- $\mu\text{m}$  nets. Trays were set at ambient temperature and exposed under natural light. The following day, diatoms had migrated upwards, and the uppermost net was removed and rinsed with GF/F filtered seawater to collect motile microalgae.
- Step 3 A. Diatom labelling with  $^{14}\text{C}$  and setting up of microcosms. To label the isolated diatoms,  $\text{NaH}^{14}\text{CO}_3$  (40–50 mmol $^{-1}$  stock solution) was added to a 500-ml suspension to reach a final concentration of approximately 0.4  $\mu\text{Ci ml}^{-1}$ . The suspension was then placed under saturating light condition for 2 h, with continuous mixing using a magnetic stirrer. After the incubation period, the microalgal suspension was centrifuged (1000 rpm for 5 min) to concentrate the diatoms; the supernatant containing unused  $^{14}\text{C}$ -bicarbonate was discarded. The remaining diatoms were rinsed with filtered seawater and centrifuged again; this step was repeated twice.

- Step 3 B. Control of conservative behaviour of  $^{14}\text{C}$  in the diatoms. The method assumed a conservative behaviour of the  $^{14}\text{C}$  label in diatoms, i.e., no loss of radioactivity from pre-labelled diatoms due to respiration or excretion during incubation with animals. If the radioactivity was lost from the diatoms through respiration or extraction during the 4-h period following labelling, then the amount of radioactivity on GF/F filters was expected to decrease as a function of time. To check for a conservative behaviour of the label, a suspension of labelled diatoms was prepared according to step 3A. Subsequently, 1-ml replicates of the rinsed labelled diatoms were filtered on GF/F fibre glass filters every hour until 4 h after the end of the labelling process.
- Step 4. Addition of labelled diatoms to the microcosms. Labelled diatoms were added to 1 litre of sediment containing the meiofauna. The sediment was homogenised and split into equal parts in four microcosms composed of plastic boxes (15 cm  $\times$  25 cm). At the beginning of the experiment, 4 sediment replicates of 1 ml each were sampled to measure the specific activity of diatoms (dpm.unit Chl  $a^{-1}$ ) and their total biomass. Afterwards, microcosms were immediately placed in the dark to avoid division of diatoms.
- Step 5. Computing the conversion ratio. Before the addition of labelled diatoms, the microcosms already contained non-labelled diatoms. Meiofauna thus ingested a mix of labelled and non-labelled diatoms. Assuming meiofauna cannot select its food according to radioactivity, we could use the average radioactivity of diatoms to compute conversion ratio. Before the start of the experiment, 4 sediment replicates of 1 ml each were sampled to measure the amount of radioactivity (dpm.ml $^{-1}$ ) and Chl  $a$  (ng Chl  $a$ .ml $^{-1}$ ). The Chl  $a$ /radioactivity ratio can be thus calculated (ng Chl  $a$ .dpm $^{-1}$ ). Chlorophyll  $a$  was measured fluorometrically (Lorenzen, 1966). This constituted the start of the experiment ( $T_0$ ). The Chl  $a$  was transformed into a quantity of carbon according to de Jonge (1980): carbon/chlorophyll  $a = 45$ .
- Step 6 A. The 2-h grazing experiment. The grazing experiment lasted 2 h. To monitor the kinetics of radioactive accumulation in meiofauna, each microcosm was sampled (50 ml of sediment) at the following times:  $T_0$  (0 min),  $T_1$  (20 min),  $T_2$  (40 min),  $T_3$  (60 min),  $T_4$  (80 min), and  $T_5$  (120 min). For each sample, the incubation period was stopped by immediate centrifugation (4500 rev. mn $^{-1}$  during 7 min) to extract meiofauna from the sediment. Extracted meiofauna were frozen at  $-20^\circ\text{C}$ . The sorting of nematodes (*M. remanei*, *D. oxycerca*), bulk of both copepods and meiofauna was completed within 5 days. Animals were handpicked with a fine needle under a dissecting microscope, rinsed in sterile artificial sea water to remove adhering particles, and placed into scintillation vials (300 individuals per vials) containing a drop of distilled water. Afterwards, 1 ml of Soluene-350 (Pacard) was added to the vials containing animals, and the vials were incubated at  $60^\circ\text{C}$  for 72 h to solubilise the animal tissue. Before the radioactivity measurements, 8 ml of Hionic-fluor (Pacard) was added to each vial. The radioactivity of the samples was measured in a Pacard Tri-Crab scintillation counter.
- Step 6 B. Controls of non-grazing processes. The measured label uptakes represent the sum of two labels; one due to the feeding on diatoms and the other due to passive  $^{14}\text{C}$  absorption or adsorption by meiofauna. To evaluate passive label uptake, killed-controls were used according to the method described by Montagna (1983). At the beginning of the grazing experiment, sediment samples were immediately frozen at  $-20^\circ\text{C}$  ( $T_0$ ), and the kinetics of the radioactivity of animal organisms was monitored every 20 min for 120 min. Thus, the measured  $^{14}\text{C}$  uptake was due to non-grazing and non-living

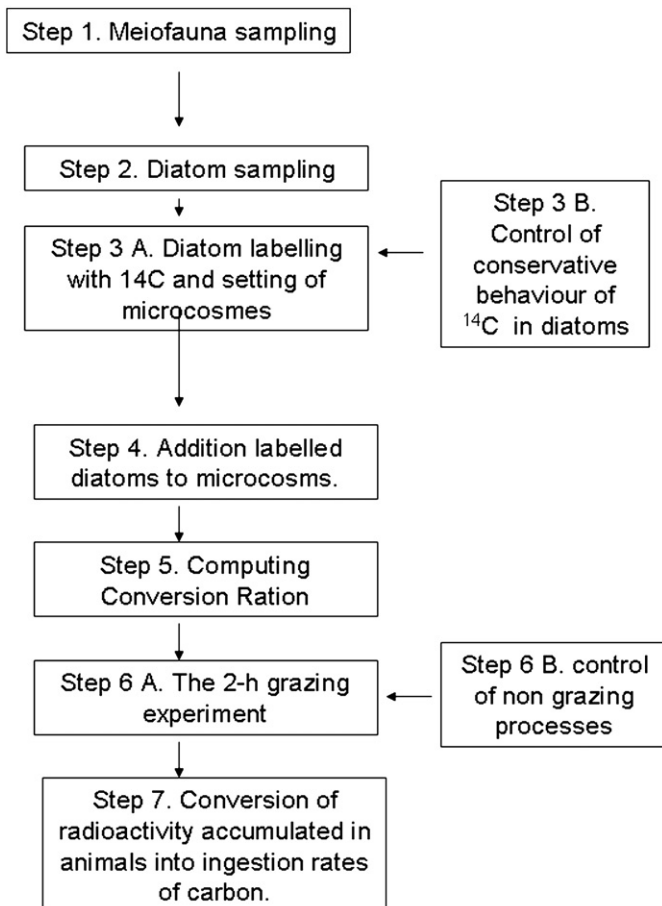


Fig. 2. Main steps in the experimental protocol.

processes, i.e., adsorption or absorption. The label uptake due to the feeding on diatoms was computed as the difference between the radioactivity measured in microcosms and the radioactivity fixed in frozen-killed controls.

Step 7. Conversion of radioactivity uptake due to feeding into carbon uptake rate. All results were expressed as disintegration per minute (DPM) per 300 organisms per time unit. A linear regression of label uptake due to feeding per time was used to retrieve the slope coefficient from which the uptake rate was computed. The uptake rate of meiofauna (URM) was determined from the amount of radioactivity ingested by meiofauna per hour (IR). As non-feeding processes were accounted for in the previous steps, any label uptake can be related to preying on diatoms. The estimated conversion ratio ( $CR_{Chla}$ ) was used to convert radioactivity into its equivalent of Chla:  $URM \text{ (ng Chla/meiofauna} \times \text{hour)} = IR \text{ (dpm/meiofauna} \times \text{hour)} \times CR_{Chla} \text{ (ng Chla/dpm)}$ .

The computation of the assimilation rate of animals is based on assumptions that are explained in the discussion. The conversion ratio ( $CR_c$ ) of carbon to radioactivity were calculated and expressed in  $\text{ng C.dpm}^{-1}$  (step 5A).  $CR$  allows converting the measured animal radioactivity into the carbon that is present in the animals that is from diatoms.

The p-values were computed to check the significance of the coefficient of linear regression. The level of 5% was assumed to represent a significant difference.  $R^2$  describes the quantity of variation of the values (y) explained by the predictor (x).

### 3. Results

#### 3.1. Composition of meiofauna

Six meiofaunal taxa were identified in the samples: Nematoda (dominance 95%), Harpacticoid Copepoda (2%), Turbellaria (1%), Ostracoda (1%), Oligochaeta (0.5%) and Polychaeta (0.5%). The mean density was 2112 ( $\pm 1250$ ) individuals per  $10 \text{ cm}^2$ . Six nematode species represented 75% of meiofaunal abundance, including epigrowth feeders (2A) – *M. remanei* – (30%), *Ptycholaimellus jacobii* (8%) and *Chromadora macrolaima* (8%), a selective deposit feeder (1A) – *Terschellingia longicaudata* (13%), non-selective deposit feeders (1B) – *D. oxycerca* (9%) and *Sabatieria pulchra* (7%). With regard to nematode trophic type, the community was dominated by diatom feeders (59%). Deposit feeders (26%) and omnivore/predators (5%) exhibited marginal abundances in the community studied (Rzeznik-Orignac et al., 2003).

#### 3.2. Control parameters

The parameters of radioactivity and chlorophyll *a* concentration in the sediment measured in microcosms are presented in Table 1. Label uptake in control samples (non-grazing processes) was assessed to be a maximum of 10% of final radioactivity. The amount of  $^{14}\text{C}$  accumulated in labelled diatoms did not change during the 4 h following labelling.

#### 3.3. Uptake rate in meiofauna

The linear model for radioactivity uptake was satisfactory, explaining from 51% to 84% of the variance. The assimilation rate of carbon by the bulk of meiofauna was  $0.84 \text{ ng C.ind}^{-1}.\text{h}^{-1}$  (Fig. 3). The assimilation rate of *M. remanei*  $1.26 \text{ ng C.ind}^{-1}.\text{h}^{-1}$  (Fig. 5) was similar to that of *D. oxycerca*,  $1.35 \text{ ng C.ind}^{-1}.\text{h}^{-1}$  (Fig. 4). The relationships observed were statistically significant (regression:  $F=9.52$   $p=0.013$ ,  $t=3.09$   $p=0.013$  for *M. remanei* and; regression:  $F=13$   $p=0.05$ ,  $t=3.60$   $p=0.005$  for *D. oxycerca*). The relationship observed was statistically significant (regression:  $F=36.96$   $p=0.001$ ,  $t=10.8$   $p=0.000$ ). The assimilation rate of the bulk of copepods (Fig. 6) was higher than that of two

**Table 1**

The parameters measured in the experiments (mean  $\pm$  SE): radioactivity of mud, concentrations of chlorophyll *a*, conversion ratio ( $CR$ ), and label uptake measured for non-grazing process (controls).

Taxa	Radioactivity (dpm.ml <sup>-1</sup> )	Chlorophyll (µg.ml <sup>-1</sup> )	CR (µgC.dpm <sup>-1</sup> )	Control (dpm.ind. <sup>-1</sup> )
Bulk of meiofauna	204,625 $\pm$ 39,950	17.48 $\pm$ 1.7	0.00384	0.040 $\pm$ 0.001
<i>D. oxycerca</i> ,	100,675 $\pm$ 28,437	20.00 $\pm$ 7.3	0.00912	0.010 $\pm$ 0.003
<i>M. remanei</i> ,				0.069 $\pm$ 0.04
Bulk of copepods				

species of nematodes, *M. remanei* and *D. oxycerca*, reaching  $1.92 \text{ ng C.ind.}^{-1} \text{ h}^{-1}$ . The relationship observed was statistically significant (regression:  $F=14.79$   $p=0.009$ ,  $t=-3.85$   $p=0.009$ ).

### 4. Discussion

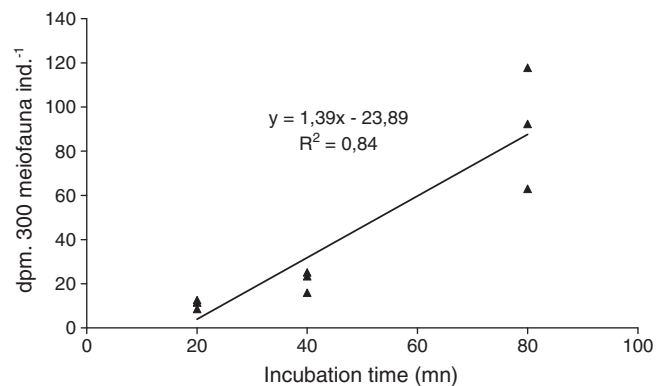
#### 4.1. Meiofaunal composition

Meiofauna is always very abundant in all types of marine sediment with abundances reaching many millions of individuals per  $\text{m}^2$ . The mean abundance of meiofauna in the Brouage mudflat was estimated to be  $2112 \times 10^3 \text{ ind.m}^{-2}$ , and nematodes represented approximately 95% of the total meiofauna. Similar abundances have already been observed by Montagna et al. (1995) in the Marennes Oléron Bay and in other European estuaries such as the Lynher, the Tagus and the Westerschelde (Warwick and Price, 1979; Soetaert et al., 1995). The high abundances of meiofauna correspond roughly to 1–2 g of biomass per  $\text{m}^2$  and represent 10%–20% of total macrofaunal biomass in the Marennes–Oléron Bay (Leguerrier et al., 2003). Although meiofauna represents 10% of total macrofaunal biomass, its throughput and production could be equal or even higher than that of macrofaunal production (Leguerrier et al., 2003). Similar conclusions were drawn in other ecosystems such as the Wadden Sea (Kuipers et al., 1981), the Bay of Saint Brieuc (Chardy et al., 1993), and Morlaix Bay (Chardy and Dauvin, 1992).

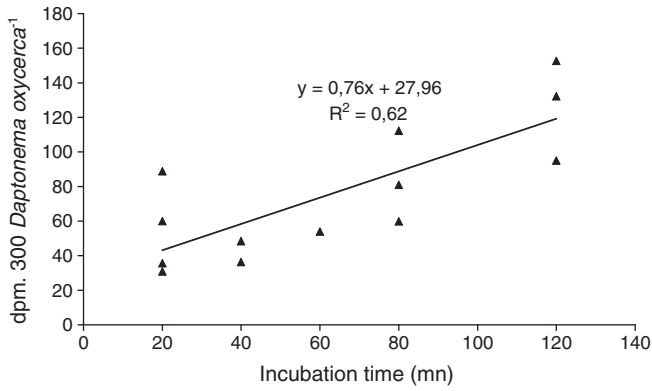
To assess exchanges between the trophic levels “meiofauna” and “primary producers” in Marennes–Oléron Bay, we performed a grazing experiment using all meiofaunal taxa. We also repeated grazing measurements at the species level for two dominant nematode species (*M. remanei* and *D. oxycerca*) and for harpacticoid copepods.

#### 4.2. Radioactivity uptake in meiofauna

The data from the present study suggest an accumulation of radioactive material in meiofauna proportional to the feeding time (experimental incubation time,  $t_0$   $t_1$   $t_2$  ...):  $^{14}\text{C}$  accumulations in meiofauna

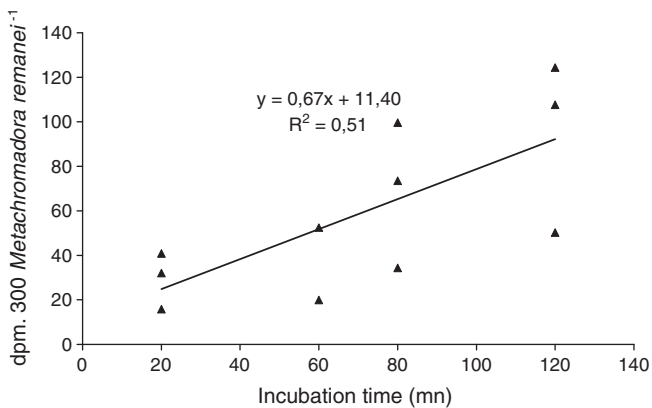


**Fig. 3.** Accumulation of radioactivity in bulk of meiofauna feeding on labelled diatoms ( $\text{dpm. 300 ind}^{-1}$ ) as a function of time. The duration of experiment was 80 min. The slope of the linear accumulation ( $\text{dpm.ind}^{-1}.\text{h}^{-1}$ ) is related to the mean assimilation rate.

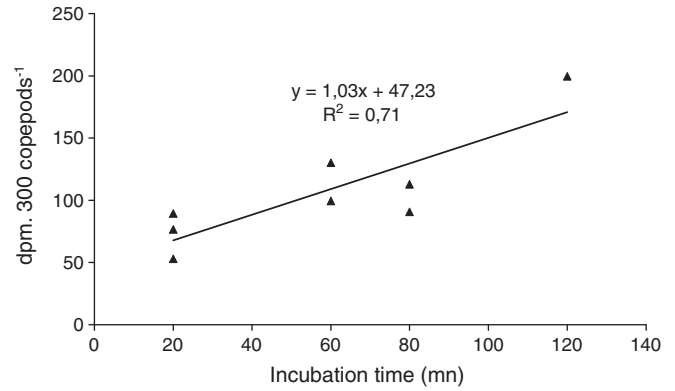


**Fig. 4.** Accumulation of radioactivity in *Daptonema oxycerca* feeding on labelled diatoms (dpm. 300 ind<sup>-1</sup>) as a function of time. The duration of experiment was 120 min. The slope of the linear accumulation (dpm.ind<sup>-1</sup>.h<sup>-1</sup>) is related to the mean assimilation rate.

were linear for a 2-h incubation period. The constant accumulation of labelled food had previously been observed with comparable incubation times (Herman and Vranken, 1988; Moens et al., 1999; Pascal et al., 2008). The interpretation of the results in terms of biological processes (ingestion, assimilation, respiration...) was not obvious. In previous studies, Schiemer (1987) and Moens et al. (1999) suggested that many data regarding the meiofaunal feeding rate on microflora and measurements of the absorption of labelled food provide an estimate of the assimilation rate rather than an ingestion rate. We investigated this question through a review of the available literature on the different processes involved (ingestion, assimilation, defecation) and their time scales. In a majority of studies (Admiraal et al., 1983; Tietjen and Lee, 1977a, 1977b), the experimental duration was approximately an hour. The intervals of defecation for nematodes and copepods (e.g., the time that passed between ingestion and the appearance of defecation) extend from a few minutes to about half an hour (Crofton, 1966; Croll et al., 1977; Duncan et al., 1974; Mapes, 1965; Moens et al., 1999). Defecation takes place if the experimental duration exceeds that of intestinal transit. Because nematode defecation intervals are very short (Duncan et al., 1974; Thomas, 1989), the gut contents would be renewed every few minutes. For these reasons, it has been suggested in the present study, the linear accumulation of label indicates assimilation rather than ingestion. As all other grazing experiments were run for 2 h or longer, they provided the assimilation rate of food. Assuming an assimilation rate of 25% (Herman and Vranken, 1988; Somerfield et al., 2005), the ingestion rate would be four times higher.



**Fig. 5.** Accumulation of radioactivity in *Metachromadora remanei* feeding on labelled diatoms (dpm. 300 ind<sup>-1</sup>) as a function of time. The duration of experiment was 120 min. The slope of the linear accumulation (dpm.ind<sup>-1</sup>.h<sup>-1</sup>) is related to the mean assimilation rate.



**Fig. 6.** Accumulation of radioactivity in copepods feeding on labelled diatoms (dpm. 300 ind<sup>-1</sup>) as a function of time. The duration of experiment was 120 min. The slope of the linear accumulation (dpm.ind<sup>-1</sup>.h<sup>-1</sup>) is related to the mean assimilation rate.

#### 4.3. Diatom consumption by meiofauna

The assimilation rate obtained for the meiofaunal community was 0.84 ng C.ind<sup>-1</sup> h<sup>-1</sup>. With 2112 × 10<sup>3</sup> ind.m<sup>-2</sup>, the assimilation rate of the community could thus be extrapolated to 42.57 mg C.m<sup>-2</sup> per day. This value was of a similar order of magnitude as that obtained by Montagna et al. (1995) by in situ tracing (<sup>14</sup>C) diatoms in Marennes-Oléron Bay. They showed that the meiofaunal community assimilated between 58 and 189 mg C.m<sup>-2</sup> per day. However, the two different experimental approaches i.e., the experimental microcosms (present study) and in situ radioactive-tracing (Montagna et al., 1995), supplied similar results for the meiofaunal assimilation rate of microphytobenthic carbon in the Marennes-Oléron Bay.

Furthermore, the grazing experiment provided results at the species level. It is interesting to compare results at the species level with those of the group level (all meiofauna). The sum of assimilation rates of two nematode species and copepods reach up to 53% of the meiofaunal assimilation rate measured directly. This result demonstrates that other taxa composing the local meiofauna feed on benthic diatoms, accounting for the missing 47%. In the mudflat of Brouage, the relative abundance of epistrate-feeders can fluctuate seasonally from 20 to 70% (Rzeznik-Orignac et al., 2003). The variation in abundance of trophic types in the meiofauna, in particular, diatom-feeder abundance, can influence the amount of microphytobenthos consumption.

As for the assimilation rates at the species level, similar results were obtained in different studies (Table 2). It appears that assimilation rates obtained for *M. remanei* and *D. oxycerca* are of similar magnitude to other herbivorous nematodes (Table 2) or copepods. For example, in an adjacent mudflat of the Marennes-Oléron, similar results for harpacticoid copepods were obtained from the in situ <sup>14</sup>C tracing experiment, e.g., incubation of a radioactive tracer in the sediment (Blanchard, 1991; Montagna et al., 1995) leading to assimilation rates of copepods that were 40% higher than those of herbivorous nematodes (Blanchard, 1991). This finding confirms that copepods have a slightly higher assimilation rate than nematodes.

#### 4.4. Estimation of amount of ingested diatoms by meiofauna

The microphytobenthic production in the mudflat of Brouage was estimated at 400 g C.m<sup>-2</sup>.y<sup>-1</sup> or about 1 g C.m<sup>-2</sup>.d<sup>-1</sup> (Guarini, 1998). Assuming that the meiofaunal feeding rate is constant during the year and represents 4 times the assimilation rate (Herman and Vranken, 1988), the meiofauna in the Brouage Mudflat would consume 128 mg C m<sup>-2</sup> d<sup>-1</sup> or 47 g of C m<sup>-2</sup> y<sup>-1</sup>. This amount corresponds to 13% of the microphytobenthic primary production. The value is comparable to that obtained by an inverse analysis realised for the trophic web of the Brouage Mudflat (12%, Leguerrier et al., 2003). Meiofaunal consumption of microphytobenthic carbon (10–20%) is higher that of

macrofauna (3–5%) despite the biomass of the latter being five times higher than that of meiofauna (Haubois et al., 2005; Leguerrier et al., 2003).

In comparison, in an adjacent mudflat in the Marenne-Oléron Bay, it was estimated (Blanchard, 1991) that ingestion of benthic microalgae by meiofauna, e.g.,  $33.94 \mu\text{g C} \cdot 10 \text{ cm}^{-2} \cdot \text{h}^{-1}$  ( $814.6 \text{ mg C} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$ ) could be easily sustained by an equal amount recorded for microphytobenthic production. In the Ems-Dollard estuary (Admiraal et al., 1983), it was estimated that the meiofauna consumed  $300 \text{ mg C} \cdot \text{d}^{-1} \cdot \text{m}^{-2}$ , which corresponds to only 10% of the benthic primary production. This trend is also common in a salt-water marsh in South Carolina where high levels of meiofaunal grazing on the microphytobenthos was observed (Montagna, 1984). Meiofauna may also be dependent on other food sources such as bacteria, detritus, protozoa, oligochaetes or nematodes (Moens and Vincx, 1997), but in the Brouage Mudflat, as herbivorous nematodes represent approximately 59% of the total meiofauna (Rzeznik-Orignac et al., 2003); undoubtedly, these nematodes are primarily responsible for the carbon flow from the microphytobenthos to the meiofauna compartment. Our results are in accordance with the conclusion of Pascal et al. (2008) who found that algal assimilation by nematodes represented more than three times the bacterial assimilation in the Brouage mudflat.

The potential annual ingestion rate of the meiofaunal community in the Brouage mudflat relies on the extrapolation of a measured assimilation rate to the mean meiofaunal abundance for all hours of the day and all the seasons of the year. These assumptions need to be examined. Nematodes may respond to environmental changes on a seasonal scale by physiologically adjusting and shifting their optimum conditions (Gee, 1985). Moreover, the grazing rate is obviously controlled by different environmental conditions, both biotic (i.e., bacterial, algal abundances) and abiotic (i.e., temperature, salinity, luminosity). The intertidal mudflat of Brouage is subject to a wide range of environmentally varying factors. 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). Although the Brouage mudflat undergoes drastic environmental changes, such as widely varying minimum and maximum mud temperature ( $5^{\circ}$ – $34^{\circ} \text{C}$ ), high salinity (18–35 PSU), and irradiance reaching  $2000 \mu\text{M}$  of photons  $\text{m}^{-2} \text{ s}^{-1}$  (Underwood and Kromkamp, 2000), the nematode assemblage appears to have adapted to these highly fluctuating conditions and their feeding activity is little affected (Pascal et al., 2008). In

contrast, seasonal changes in the abundance or diversity of meiofaunal taxa would alter the results of the extrapolation of food uptake.

#### 4.5. Methodological considerations

Most of the previous literature describes experiments carried out in laboratory conditions, for example, the use of an artificial medium such as agar and the isolation of species from sediment or selected food sources (Admiraal et al., 1983; Tietjen and Lee, 1977a, 1977b). Another method, the injection of a radioactive tracer ( $^{14}\text{C}$ ) directly into sediment cores (Blanchard, 1991; Montagna, 1984; Montagna et al., 1995), was carried out in situ. However, the various pathways of absorption of the tracer by the organisms make it difficult to interpret the results in situ. The protocol used in the present study relies on microcosm experiment in the laboratory. The animals were not starved before the experiment. They were not isolated from sediment in order to preserve the natural feeding behaviour on a wide range of food sources naturally present in the sediment, such as protists, bacteria, diatoms, detritus and others organisms. These experimental conditions seemed appropriate for investigating the ingestion rate of meiofauna.

In the grazing experiment, we assume that grazing is not label dependent. The underlying assumption is that migrating species of labelled diatoms are representative of the species in the sediment. To assure that the conditions in the experimental microcosms were as close to natural conditions as possible, the substrate consisted of sampled sediments containing a natural assemblage of meiofauna and diatoms. Despite the fact that the addition and mixing of labelled diatoms to natural sediment in the microcosm disrupted the microbial–meiofaunal spatial relationships, the size and diversity of diatom assemblages used in the present study would be more representative of natural assemblages than those in most previous experiments.

#### 4.6. Conclusions

The experiment carried out at the species level allowed us to confirm that fluxes of carbon from diatoms to herbivorous meiofauna take place. This study improves our knowledge of the role played by meiofauna in the mudflat system and allows us to better understanding the fate of carbon from the microphytobenthos in the food web. The meiofauna had a small biomass but constituted a very active trophic level compared to the macrofauna in the Brouage mudflat. It highlights how the specific structure of the meiofauna could play a key role in the control of energy fluxes from primary production to the meiofaunal trophic level and that the trophic diversity of meiofauna should be fully integrated into food web studies.

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**Table 2**

Assimilation rates of nematodes and various copepods obtained in the present study and in others studies on nematodes belonging to the same trophic group or copepods. Assimilation from others sources was calculated as 25% of ingestion rate. (DOM = dissolved organic matter).

Trophic group	Species	Food	Assimilation rate (ngC/ind/h)	Authors
Epistrate-feeders (2A)	<i>Metachromadora remanei</i>	Diatoms	1.26	Present study
	<i>Eudioplogaster paramatus</i>	Diatoms	0.42	Admiraal et al. (1983)
	<i>Chromadora germanica</i>	Diatoms	1	Tietjen and Lee (1977b)
	<i>Chromadora macrolaimoides</i>	Diatoms	9	Tietjen (1980)
Deposit-feeders (1A and 1B)	<i>Daptonema oxycerca</i>	Diatoms	1.35	Present study
	<i>Rhabditis marina</i>	Bacteria, DOM	0.9–1.6	Tietjen and Lee (1977a)
	<i>Monhystera denticulate</i>	Bacteria, DOM	0.1	Tietjen and Lee (1977a)
Copepods	Various species	Diatoms	1.92	Present study
		Diatoms	0.2–2.8	Montagna et al. (1995)

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