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Linking behaviours, sediment reworking, bioirrigation and oxygen dynamics in a soft-bottom ecosystem engineer: the mud shrimp *Upogebia pusilla* (Petagna 1792)

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Highlights:

Behaviours control bioturbation processes

Sediment reworking is induced by “Burrowing”

About 60 % of the volume of reworked sediment occurs around burrow walls

Bioirrigation and oxygen dynamics are induced by “Ventilating”

“Ventilating” is induced by a 12-25% O₂ saturation and indirectly cued by “Burrowing”

Abstract

Despite the role of bioturbation in controlling biogeochemical processes taking place at the sediment-water interface, little is still known about how animal behaviours influence sedimentary biogeochemical processes. In the present work, we used the mud shrimp *Upogebia pusilla* as model organism to assess the effect of behaviour types/intensities on sediment reworking, bioirrigation and sediment oxygen dynamics based on three sets of seasonal experiments. We identified four main behaviours: “Resting”, “Walking”, “Ventilating” and “Burrowing”. Mud shrimp were much more active (*i.e.*, not “Resting”) during summer than during winter and to a lesser extent spring due to elevated temperature. There were three distinct components of sediment reworking. The first one consisted in the expulsion of sediment particles from the burrow to the sediment-water interface, the second one to the transfer of sediment downward within the burrow and the third one to the transport and compaction of sediment across burrow walls. Temporal changes in the intensities of all components correlated tightly since they all resulted from “Burrowing”. The temporal dynamics of porewater exchange and oxygen dynamics were clearly discontinuous and in direct relation with “Ventilating”. However, other behaviours, and especially “Burrowing” were also involved in the control of this dynamics through the modulation of oxygen availability within the burrow, which is cuing the onset of ventilation at a 12-25 % saturation threshold. These results underline the importance of taking into account changes in animal behaviour types/intensities at the appropriate temporal scales when assessing the impact of bioturbation on biogeochemical processes taking place at the sediment-water interface.

Keywords:

Behavior, Seasonal changes, Bioturbation, Sediment reworking, Bioirrigation, Oxygen dynamic

1. Introduction

In marine soft-sediment, organic matter mineralization is controlled by the balance between redox reactions, organic matter sedimentation and transport (*i.e.*, advection/diffusion) processes (Aller, 2014). In the absence of macrofauna, this leads to a vertically stratified dominance of specific organic matter mineralization pathways (typically following the sequence of O₂, NO₃⁻, Mn- and Fe-oxides, SO₄²⁻ and CO₂, Froelich et al., 1979) controlled by diffusive transport. A large majority of the uppermost layer of marine sediments is however inhabited by burrow dwelling infauna which profoundly disrupt this balance through bioturbation (*sensu* Kristensen et al., 2012). Bioturbation encompasses two distinct processes, namely sediment reworking (*i.e.*, the movements of sediment particles and associated organic matter) and burrow ventilation, which causes bioirrigation (*i.e.*, the exchange of water and solute between the overlying water column and porewater). The effect of sediment reworking on organic matter mineralization is highly dependent on sediment reworking types. Downward conveyor-belt feeders can for example quickly transfer particulate organic matter deep in the sediment column where mineralization is less efficient. Conversely, upward conveyor-belt feeders counteract the burying of particulate organic matter by repeatedly bringing it back to the sediment-water interface. Through bioirrigation, bioturbating infauna increases the transport of the most energetically favorable electron acceptor (*i.e.*, oxygen) in deep and otherwise anoxic sediment layers enhancing aerobic mineralization of organic matter. Additionally, oxygen oxidizes reduced inorganic metabolites produced during anaerobic processes lowering their porewater concentration and generating a reservoir of potential oxidants, both improving conditions for the anaerobic mineralization of organic matter as well (Volkenborn et al., 2016).

A lot of *in-* and *ex-situ* experiments have been conducted to assess the functional consequences of bioturbation (Fanjul et al., 2015; Mermillod-Blondin et al., 2004;

Papaspyrou et al., 2007; Pascal et al., 2016b). Overall, their results suggest that bioturbation increases the sediment capacity for organic matter mineralization and thus enhances carbon and nutrient cycling (Aller, 1994; Kristensen et al., 2012; Lohrer et al., 2004; Mermillod-Blondin and Rosenberg, 2006; Meysman et al., 2006). For most of them these studies are based on: (1) the comparison between bioturbated and unbioturbated sediment, and (2) relatively short term (typically several days or weeks) experiments. They thus establish an operational link between the presence of bioturbating species and their functional consequences but fail to provide relevant information on the processes at play and on the spatial and temporal scales at which they operate.

The elementary processes constitutive of bioturbation usually take place at small (*i.e.*, typically less than a few cm and s) spatial and temporal scales. From a spatial standpoint, bioturbation generates microenvironments where the nature and the intensity of biogeochemical processes are modified (Kristensen et al., 2012; Meysman et al., 2006). From a temporal standpoint, it induces short-term oscillations in redox conditions affecting the microbial communities responsible for organic matter mineralization (Aller, 2014). Such oscillations result from the fact that bioturbation is caused by intermittent activities associated with different behaviours of benthic macrofauna. This qualitative link has been acknowledged for a long time (*e.g.*, Kristensen et al., 2012; Meysman et al., 2008). However, direct quantitative assessments have been achieved only recently due to technological difficulties in “penetrating” the sediment column. Corresponding studies have mostly dealt with bioirrigation in relation with several technological developments such as image analysis (Grémare et al., 2004; Hollertz and Duchêne, 2001; Jordana et al., 2000), pressure captors (Wethey et al., 2008; Wethey and Woodin, 2005; Woodin et al., 2016) and planar optodes (Jovanovic et al., 2014; Przeslawski et al., 2009; Timmermann et al., 2006) allowing for the simultaneous high-frequency recording of behaviours, hydraulic activities and associated

consequences in terms of oxygenation (Volkenborn et al., 2012a, 2012b). Studies are clearly less advanced regarding sediment reworking. To our knowledge, the only available study quantitatively linking sediment reworking and feeding activity in benthic macrofauna is the one by Maire et al. (2007) carried out on the deposit-feeding bivalve *Abra ovata*. This particular species induces sediment reworking within only the top few centimeters of the sediment column and mostly through a biodiffusive mode resulting from its feeding activity (Bernard et al., 2016; Maire et al., 2007). There is thus still a clear need to carry out similar studies on different kinds of species transporting sediments over larger spatial scales (*e.g.* through non-local transport) and presenting a larger variety of behaviours potentially generating bioturbation. The overall aim of the present study was therefore to establish a qualitative and quantitative link between the behaviour, bioturbation processes and sediment biogeochemical processes in a large bioturbating species.

We selected a mud shrimp as model organism. Mud shrimp are considered among the most influential ecosystem engineers in marine soft-bottom ecosystems (Atkinson and Taylor, 2005; Cadée, 2001; Pillay and Branch, 2011). They dig large and complex burrows which extend deep (*i.e.*, down to 2 m) within the sediment column and connect to the overlying water by several distant openings (Coelho et al., 2000; Kinoshita, 2002; Nickell and Atkinson, 1995). The maintenance and ventilation of their burrows result in strong sediment reworking and bioirrigation that profoundly affect the physical (*e.g.*, granulometry, porosity and permeability), chemical (*e.g.*, organic carbon content, oxygen concentration) and biological (*e.g.*, structure and diversity of both prokaryotic and eukaryotic communities) properties of the sediment (Aller and Dodge, 1974; Berkenbusch et al., 2000; Bird et al., 2000; Laverock et al., 2011; Pillay et al., 2007). By doing so, mud shrimp deeply affect the biogeochemical functioning of benthic ecosystems (D'Andrea and DeWitt, 2009; Pascal et al., 2016b; Pillay et al., 2007; Ziebis et al., 1996). Numerous studies have already been

dedicated to the autecology of mud shrimp including the assessments of their behaviours and time allocation patterns between behaviours (*e.g.*, “Wander”, “Ventilate”, “Burrow”, “Rest”; Stamhuis et al., 1996), sediment reworking, and bioirrigation rates (Berkenbusch and Rowden, 1999; Branch and Pringle, 1987; Dworschak, 1981; Dworschak et al., 2006; Pascal et al., 2016b; Rowden et al., 1998; Stamhuis et al., 1997), and effects on spatio-temporal changes in the oxygenation of their burrows and surrounding sediments (Forster and Graf, 1995; Volkenborn et al., 2012b). These studies have however been mainly carried out on deposit-feeding mud shrimp even though one cannot exclude that feeding types may have a significant effect on how time is allocated between behaviours (Stamhuis et al., 1996). Moreover, they have mostly focused on the direct relationship between burrow ventilation and bioirrigation and largely ignored the assessment of the relationship between behaviour types and sediment reworking. In addition, the assessments of sediment reworking rates have been mostly restricted to the quantification of the expulsion of sediment at the sediment-water interface and have largely neglected the component of sediment reworking taking place below sediment surface, around burrow walls. To our knowledge, these studies (but see Pascal et al., 2016b) have also largely ignored seasonal changes in time allocation patterns, and bioirrigation rates.

The present study aimed at filling these gaps by focusing on a suspension-feeding mud shrimp and investigating how behaviour (types and time allocation) affects (1) sediment reworking, (2) bioirrigation and (3) oxygen dynamics within its burrow. This first required to achieve comprehensive descriptions of the behaviours, between-behaviour time allocation pattern and sediment reworking of our biological model: *Upogebia pusilla* (Petagna 1792). This suspension-feeding mud shrimp occurs all along the East Atlantic coast from Norway to Mauritania (De Saint Laurent and Le Boeuf, 1979; Hayward and Ryland, 1990) and in the Mediterranean Sea (Dworschak, 1987a). It presents dense populations in the Arcachon Bay

(i.e., our study site; Pascal et al., 2016a) where it significantly affects benthic biogeochemical processes (Pascal et al., 2016b).

2. Material and methods

2.1. Collection and maintenance of animals and sediment

Three sets of controlled laboratory experiments were carried out in winter (January), spring (April) and summer (August) 2014. During each season, 30 adult specimens of the mud shrimp *Upogebia pusilla* were collected at low tide in an intertidal *Zostera noltei* meadow (44° 40.782' N, 1° 08.321' W) of the Arcachon Bay (Southwest coast of France) using a bait piston pump. Mud shrimp were immediately isolated in individual containers. Back at the laboratory, they were measured (Total Length from the tip of the rostrum to the tip of the telson, ± 1 mm) and weighted (Blotted Wet Weight, ± 0.1 mg). Twenty undamaged mud shrimp (4.6 ± 0.4 cm in TL and 1.9 ± 0.3 g in BW) were selected. They were then individually introduced in (1) 10 thin aquaria (25 (L) \times 1.9 (W) \times 50 (H) cm) for the assessments of burrow characteristics, behaviour, sediment reworking and oxygen dynamics, and (2) 10 sediment cores (9.4 cm inner diameter, 40 cm long) for the assessment of bioirrigation. Ten other cores were kept without mud shrimp and used as controls. Thin aquaria and sediment cores were previously filled with a 30 cm layer of sieved (500 μ m mesh) muddy sand sediment collected at the sampling site at each season (consistently D_{50} : 159 μ m, organic carbon: 0.35 % DW). Mud shrimp were not fed during experiments. Experiments started 48 h after mud shrimp introduction, which allowed for the construction of a whole (i.e., open-ended) burrow during all seasons. All aquaria and sediment cores were kept in a dark thermo-regulated room (at 12, 16 and 22 °C in January, April and August, respectively, corresponding to the measured seawater temperature during field sampling) during the whole experiment durations (a month). All the aquaria and sediment cores were

fuelled with a continuous natural seawater flow ($\sim 0.25 \text{ L min}^{-1}$, same temperature as above) to ensure a good oxygenation of experimental enclosures.

The use of thin aquaria clearly constitute a compromise between the necessity of getting a visual access to the lumen of the burrows in view of assessing mud shrimp behaviour, and possible artefacts due to space limitation. There are nevertheless several rationale suggesting that these artefacts remain limited in the case of the present study: (1) care was taken to use small mud shrimp that are likely less affected by space limitation, (2) at the beginning of the experiment (i.e., 48h after mud shrimp addition) burrows shapes and characteristics were fully consistent with those previously reported for *U. pusilla* (Dworschak, 1983), (3) those characteristics were similar to those assessed through resin casting for individual burrows from the sampled field population (see Table 1 and supplementary material Table S3), and (4) at the end of the winter and to a lesser extent spring experiments significant portions of the burrow lumens remained hidden thereby suggesting that aquarium width probably does not constitute a limiting factor.

2.2. Sediment reworking and burrow characteristics

Sediment reworking was first quantified during a 30 days period using luminophores (i.e., inert particle tracers fluorescing under UV light). Previous tests have shown that luminophores stay strictly at the sediment surface in the absence of mud shrimp. Sediment reworking by meiofauna or other small organisms was thus negligible. Fifteen gDW of luminophores (100 - 160 μm size range) were homogeneously spread at the sediment surface of each thin aquarium. Both sides of each aquarium were then photographed with a digital camera (D100, Nikon ®, 3008×2000 pixels) under UV light ($\lambda = 380 \text{ nm}$) immediately following luminophore input (D0) and after 3, 6, 12, 24, 48, 72, 96, 120, 144, 168, 192, 216, 240, 288, 336, 408, 480 and 720 h. Two vertically adjacent pictures ($27 \times 18 \text{ cm}$ each) were

194 taken for each side of the aquarium and assembled afterwards as a single image (27×35 cm
 195 in size, $92 \mu\text{m}^2 \text{ pixel}^{-1}$ in resolution). Composite images were saved in JPEG format and
 196 analysed as described in Maire et al. (2006), allowing for the assessment of 1D luminophore
 197 vertical concentration profiles. Vertical biodiffusion (D_{bv}) and bioadvection (V_{bv}) coefficients
 198 were then estimated by fitting a biodiffusion-bioadvection transport model to these vertical
 199 profiles (Gérino et al., 1998; Meysman et al., 2003):

$$\frac{\partial^2 C}{\partial t} = D_{bv} \frac{\partial^2 C}{\partial z^2} - v_{bv} \frac{\partial C}{\partial z} \quad (1)$$

200 where C is the luminophore concentration (%), t is the time (year) and z is the vertical depth
 201 in the sediment column (cm). D_{bv} and V_{bv} were estimated by convergent iterations and
 202 weighted least-squared regressions of model prediction on observed luminophore profiles
 203 (see Maire et al., 2006 for further details).

204 The volume of sediment expelled at the sediment-water interface (V_{ES} , cm^3) was
 205 estimated by measuring the average height of sediment over the original sediment-water
 206 interface (\bar{H} , Fig. 1) and using the following equation:

$$V_{ES} = \bar{H} \times L \times W \quad (2)$$

208 where L and W are the length of and the width of the aquarium, respectively.

209 The average thickness of the reworked layer surrounding burrows (T_{RS} , cm) was
 210 estimated by measuring the distance between the burrow wall and the most distant
 211 luminophore in five different sections randomly distributed within the burrow (Fig. 1). The
 212 average volume of reworked sediment surrounding the burrow within the aquarium (B_{RS} ,
 213 cm^3) was then computed using the following equation:

$$B_{RS} = \frac{[L_b \times \pi(r_b + T_{RS})^2] - [L_b \times \pi r_b^2]}{2} \quad (3)$$

where L_b is the total burrow length (cm), r_b is the mean burrow radius (cm) and T_{RS} is the mean thickness of the reworked sediment layer surrounding the burrow (cm).

The diffusion coefficient accounting for the component of sediment reworking occurring perpendicularly to burrow wall (D_{bw}) was modelled after 4 days of incubation (*i.e.*, when T_{RS} reaches its maximum value; see discussion for more details) by using a simple bi-diffusive model (Cochran, 1985) to fit the profile of luminophore concentration perpendicular to burrow wall:

$$\frac{\partial^2 C}{\partial t} = D_{bw} \frac{\partial^2 C}{\partial x^2} \quad (4)$$

Where C is the luminophore concentration (%), t is the time (year) and x is the orthogonal distance from the burrow wall (cm). This was achieved on three aquaria per season. Selected aquaria were those with the longest proportion of visible burrow wall and thus those allowing for the best assessments of decrease in luminophore concentration with increasing orthogonal distances from burrow wall.

Burrow lengths and maximal depths within the sediment column as well as the numbers of openings and chambers (*i.e.*, dilatations of the burrow tube that serve mainly as turning chambers) (Fig. 1) were also measured on thin aquaria pictures at D0, and after 4 (D4) and 30 days (D30) of experiments using ImageJ software.

2.3. Behaviour

Mud shrimp behaviours were recorded within thin aquaria for a 3-day time period starting one day after luminophore input. Behaviours were recorded using an automated image acquisition system composed of a video sensor (μ Eye UI-1480SE, IDS Imaging®, 2560 × 1920 pixels) connected to a microcomputer and driven by the AviExplore software (Romero-Ramirez et al., 2016). This sensor was positioned in front of the aquarium side displaying the

longest visible part of the burrow lumen. Images were acquired in total darkness under infrared light ($\lambda > 700$ nm) during 72 h with an acquisition frequency of 0.1 Hz. Collected images with a surface resolution of $98 \mu\text{m}^2 \text{pixel}^{-1}$ were assembled in a time-lapse AVI video file. Time-lapse sequences were then visually inspected, which allowed for the identification of the main behaviours and the assessments of: (1) the proportion of time allocated to each behaviour, (2) the average bout-duration of each behaviour, and (3) the average time between two bouts of the same behaviour (interval-duration). Despite the small thickness width of our aquaria, mud shrimp could sometimes remain hidden in parts of their burrow, which was covered by a thin layer of sediment. In order not to bias the estimates of the relative proportions of time devoted to the different behaviours (Stamhuis et al., 1996), only records during which mud shrimp were visible more than 90 % of the time were considered for analysis. Accordingly, the behaviours of only 4, 5 and 5 mud shrimps were investigated during winter, spring and summer, respectively. Despite this relatively low replication, interindividual variability in time allocation pattern within a same season was quite low (Fig. 2). Overall, this corresponded to 840 h of monitoring.

2.4. Oxygen dynamics

The O_2 saturation within the burrow was recorded during a 72 h time-period starting immediately after the end of the sediment reworking experiment. By the end of sediment reworking experiments during winter and spring, mud shrimp were not visible enough to allow accurate behavioural observations. Oxygen dynamics study was thus achieved only during summer on the 5 thin aquaria within which mud shrimp were visible more than 90 % of the time. O_2 saturations were measured at a frequency of 1 Hz, simultaneously with a second sequence of behaviour monitoring as described above. A micro-optode (OXF1100, Pyroscience) was positioned using a micromanipulator in the lumen of the “U” part of the

burrow (*i.e.*, ~10 cm deep in the sediment column; 2 mm from the burrow wall). A mini-optode (OXROB10, Pyroscience) was placed in the overlying water ~10 cm above the sediment surface to check for the full oxygenation of the water column. Both sensors were connected to a Firesting oxygen meter (Pyroscience) driven by the Firesting logger software (Ver. 2.364). A two-point linear calibration of both sensors was initially achieved between the O₂ concentration of the overlying water measured by Winkler titration (Grasshoff et al., 1999) and a zero O₂ concentration in a sodium ascorbate solution. After 72 h of continuous recording, the micro-optode was carefully removed from the sediment and the aquarium was hermetically sealed with a PVC lid. The mini-optode was then inserted into the lid and repositioned in the overlying water ~10 cm above the sediment surface. The overlying water was continuously stirred with a Teflon-coated magnetic disc at ~100 rpm during incubations. Measurements were stopped as soon as the O₂ level in the overlying water fell below 70 % of its initial value.

2.5.Porewater exchange

Porewater exchange rates were assessed as a proxy of bioirrigation and were quantified following Pascal et al. (2016b). A fluororimetric probe (Cyclops ©-7, Turner Design, $\lambda_{\text{excitation}}=490\text{nm}$; $\lambda_{\text{measured}}=514\text{nm}$) was positioned in the overlying water of each sediment core. Uranine (sodium fluorescein, C₂₀H₁₀Na₂O₅) was used as transport tracer to quantify the volumetric exchange of water and dissolved solutes across the sediment–water interface (Na et al., 2008). At the start of each experiment a solution of uranine was added in the overlying water to reach an initial concentration of 10 µg L⁻¹. Constant aeration and homogenization of the tracer in the overlying water were insured by air bubbling. The decrease in uranine concentration was then continuously (1 Hz) measured during 36 h. The porewater exchange rate (Q in mL h⁻¹) was estimated by fitting a simplified version of the

285 mathematical model developed by Meysman et al. (2007) to the moving average (*i.e.*, over 1
286 min) of the tracer concentration:

$$287 \quad C_{ow}(t) = \frac{A}{B} C_{ow}^0 + \left(1 - \frac{A}{B}\right) C_{ow}^0 e^{-Bt} \quad (5)$$

$$288 \quad A = \frac{Q}{V_{pw} + M_s K_{ad}}, B = \frac{Q}{V_{ow}} \left(1 + \frac{V_{ow}}{V_{pw} + M_s K_{ad}}\right) \quad (6)$$

289 where C_{ow} is the concentration of the dissolved tracer in overlying water ($\mu\text{g L}^{-1}$), V_{ow} and
290 V_{pw} the volumes (mL) of overlying and of porewaters, M_s the sediment wet weight (gWW)
291 and K_{ad} (mL gDW^{-1}) the mean adsorption rate of uranine onto sediment particles (see Pascal
292 et al., 2016b for further details). The first 30 min of the experiments were not considered for
293 the calculation of porewater exchange rate since the decrease during this time period was
294 mostly due to (1) the mixing of overlying and burrow water and (2) the rapid adsorption of
295 uranine onto sediment organic particles (Pascal et al., 2016b).

296 **2.6.Data analysis**

297 All data in the main text are expressed as average \pm standard error. For each
298 behaviour, the significance of between seasons differences in: (1) proportions of allocated
299 time, (2) bout-durations, and (3) interval-durations were assessed using One-Way
300 PERmutation Multivariate ANalyses Of VAriances (PERMANOVAs). Since all records
301 lasted exactly the same time, these analyses were performed on raw durations. The effect of
302 seasons on the component of sediment reworking occurring perpendicularly to the burrow
303 wall (as indicated by D_{bw}) was assessed using a One-Way PERMANOVA. The effects of
304 experiment durations and seasons on burrow characteristics and sediment reworking
305 parameters were assessed using Two-Way PERMANOVA. The design consisted in two fixed
306 factors (Experiment duration and Season) with a third random factor (Replicate) nested
307 within Season. In case of significant interaction between fixed factors, pairwise tests were

performed to characterise their modalities. The effect of seasons on porewater exchange rate (Q) was assessed using a Two-Way PERMANOVA. The effects of the tested factors on the dispersion (*i.e.*, among experiment variability) of each variable were checked using the PERMDISP procedure (Anderson, 2006) (same design as described above). All PERMANOVAs and associated tests were performed on untransformed data using Euclidean distance and the PRIMER v6 package with the PERMANOVA+ add-on software (Anderson et al., 2008; Clarke and Warwick, 2001).

The relationships between: (1) reworked sediment surrounding the burrow (B_{RS}) and the volume of sediment expelled at the sediment-water interface (V_{ES}), (2) the durations of the time period preceding each ventilation event and the magnitudes of the decrease in O_2 concentration in the overlying water column during ventilation (summer experiments), and (3) the O_2 saturations in the burrow at the onset of ventilation and the distances of the mud shrimp from the micro-optode (summer experiments) were tested using simple linear regression models. The relationships between the (1) different types of activity, and (2) total volume of reworked sediment was assessed using a (forward stepwise) multiple linear regression model. All regression analyses were performed using the SIGMAPLOT, v12.5 software.

3. Results

3.1. Burrow characteristics

Mud shrimp built a complete open-ended burrow within less than 24 hours during winter and less than 12 hours during spring and summer. All initial U-shape burrows progressively evolved into Y-shape burrows during spring and summer *versus* only 30 % during winter. Mud shrimp burrows were on average between 34.5 ± 7.3 and 96.7 ± 27.5 cm

long with an average maximal depth between 14.0 ± 3.3 and 26.7 ± 8.9 cm within the sediment column (Table 1). They had 2 to 3 openings and a rather constant diameter close to 1.5 cm with on average 2.2 ± 0.5 to 5.1 ± 1.2 chambers located 10 to 20 cm apart (Table 1). Burrow depth and the number of openings significantly differed between experiment durations but not between seasons (Table 2). Both parameters tended to increase with experiment durations (Table 1). Burrow length and the number of chambers significantly differed between seasons and experiment durations with a significant interaction between these two factors (Table 2) due to a more pronounced increase in the values of these two parameters with experiment durations during summer and a less marked one during winter (Table 1).

3.2.Behaviours

Four main behaviours were identified, namely “Walking”, “Ventilating”, “Burrowing” and “Resting” (Table 3). During “Walking”, mud shrimp alternatively walked and turned in their burrow without transporting sediment. During “Ventilating”, mud shrimp generated a unidirectional water flow by beating their four pairs of pleopods. “Ventilating” behaviour was further divided into two subclasses, namely: (1) “Flushing” during which mud shrimp quickly (*i.e.*, < 1 min on average) renewed the water of their burrow, usually immediately following a period of intense burrowing; and (2) “Pumping” which consisted in a regular beating movement of pleopods inducing a constant water flow through the burrow, without sediment transport towards the sediment-water interface. These two behaviours could easily be discriminated based on: (1) the pleopod beating frequency, and (2) the ejection of unconsolidated sediment in the overlying water. “Burrowing” consisted in digging and carrying sediment. During “Resting”, no visible movement was observed, although mud shrimp periodically bended their abdomen under their cephalothorax.

For each behaviour, the proportions of allocated time significantly differed between seasons (Fig. 2, One-Way PERMANOVA, $p < 0.001$ in all cases). Mud shrimp significantly spent more time both “Ventilating” and “Burrowing” during summer than during winter (43.0 ± 2.5 vs. 14.1 ± 1.3 % and 36.7 ± 0.7 vs. 25.3 ± 2.7 %, respectively) whereas the time allocated to “Resting” was reduced (18.7 ± 1.9 vs. 55.7 ± 2.3 %). Moreover, the way mud shrimp seasonally changed their time allocation differed between behaviours. Changes in the proportion of time allocated to “Ventilating” mainly resulted from changes in the mean interval-duration between successive ventilation bouts, whereas the mean duration of each ventilation bout did not significantly differ between seasons (Table 3). Conversely, changes in the proportions of time allocated to “Burrowing” resulted from significant changes in both bout- and interval-durations (Table 3). Changes in the proportion of time allocated to “Resting” resulted from significant differences between bout-durations whereas interval-durations did not significantly differ between seasons mostly due to a high variability during spring (Table 3).

3.3. *Sediment reworking*

The thickness of the reworked sediment layer surrounding the burrow (T_{RS}), the volume of reworked sediment surrounding the burrow (B_{RS}) and the volume of sediment expelled at the sediment-water interface (V_{ES}) all significantly differed between experiment durations and seasons with significant interactions between these two factors (Table 2). T_{RS} sharply increased during the first 4 days of the experiments (*i.e.*, until D4) and then remained relatively constant until the end of the experiment (Fig. 3A). This initial increase was more pronounced during summer than during spring and winter. B_{RS} also sharply increased until D4 but did not stabilize afterward due to continuous increases in burrow lengths (Fig. 3B). Here again the initial increase was more pronounced during summer than during spring and

winter. Conversely, V_{ES} tended to increase constantly with experiment durations but at a higher rate during summer versus an intermediate and a lower one during spring and winter, respectively (Fig. 3C). At D4, B_{RS} and V_{ES} correlated positively (simple linear regression, $F = 61.554$, $p < 0.001$; Fig. 3D). B_{RS} then represented 66.0, 56.4 and 56.6 % of total cumulative reworked sediment (*i.e.*, $B_{RS} + V_{ES}$) during winter, spring and summer, respectively. Overall, changes in the total volume of reworked sediment were best described by the amount of time allocated to “Burrowing” (multiple linear regression, $F = 75.372$, $p < 0.001$; supplementary material Fig. S1). Associated model was:

$$y = 0.00617x - 2.433 \quad (5)$$

where y represents the total volume of reworked sediment and x the amount of time (min) allocated to “Burrowing”.

Vertical biodiffusion (D_{bv}) and bioadvection (V_{bv}) coefficients significantly differed between experiment durations and seasons (Table 2) without significant interactions between these two factors. Changes in D_{bv} with experiment durations showed similar patterns during all 3 seasons (Fig. 4A). D_{bv} increased during the first days of the experiment to reach maximal values (90.2 ± 44.0 , 347.7 ± 114.1 and $1181.7 \pm 189.8 \text{ cm}^2 \text{ y}^{-1}$, during winter, spring and summer, respectively) around D4. D_{bv} then consistently decreased until the end of the experiment. The lack of interaction between seasons and experimental durations in affecting V_{bv} likely resulted from the very high variability in V_{bv} during the very first days of the experiments. Indeed, the relationships between V_{bv} and experiment durations seemed to show different patterns between seasons (Fig. 4B). During summer, V_{bv} increased during the first days of the experiment to reach a maximal value of $234.7 \pm 111.8 \text{ cm y}^{-1}$ at D3. V_{bv} then decreased to $144.5 \pm 10.3 \text{ cm y}^{-1}$ at D17 before remaining almost constant until the end of the experiment. In spring, V_{bv} reached its maximal value ($101.2 \pm 10.1 \text{ cm y}^{-1}$) only at D17 and then slightly decreased until the end of the experiment. In winter, V_{bv} remained nil until D10.

It then steadily increased to reach its maximal value ($46.6 \pm 18.5 \text{ cm y}^{-1}$) at D30. Overall, mean D_{bv} and V_{bv} were always higher in summer than in spring and in spring than in winter.

Biodiffusion coefficients associated with the component of sediment reworking occurring around burrow wall (D_{bw}) significantly differed between seasons (One-Way PERMANOVA, $F = 38.745$, $p = 0.004$). Average D_{bw} were higher during summer, intermediate during spring and lower during winter (7.9 ± 1.4 , 3.6 ± 0.9 and $1.1 \pm 0.0 \text{ cm}^2 \text{ y}^{-1}$, respectively).

3.4.Porewater exchange

Porewater exchange rates (Q) significantly differed in the presence and the absence of the mud shrimp and between seasons with a significant interaction between these two factors (Two-way PERMANOVA, $p < 0.001$). Porewater exchange rates in the absence of the mud shrimp were low (1.2 ± 0.2 , 1.3 ± 0.2 and $1.4 \pm 0.2 \text{ mL ind.}^{-1} \text{ h}^{-1}$ during winter, spring and summer, respectively) and did not significantly differed between seasons. Porewater exchange rates were significantly higher in the presence than in the absence of the mud shrimp. In the presence of the mud shrimp porewater exchange rates significantly differed between seasons with lower values during winter than during spring and during spring than during summer (5.7 ± 2.3 , 8.1 ± 1.6 and $21.2 \pm 5.6 \text{ mL ind.}^{-1} \text{ h}^{-1}$, respectively). Representative examples of temporal changes in uranine concentration recorded in the overlying water during core incubations in the presence of a mud shrimp are presented in supplementary material Fig. S2. Uranine concentration decreased non-linearly over time with a succession of periods of low and rather constant decrease lasting for 47.3 ± 19.6 , 17.4 ± 5.0 and $9.5 \pm 2.0 \text{ min}$ on average during winter, spring and summer, respectively, interspersed by sudden drops in uranine concentration.

3.5. Oxygen dynamics

In summer, all temporal changes in O₂ saturation in the overlying water column showed a typical pattern in stair-shape (Fig. 5). Stair-shape curves consisted in a succession of periods of relatively low oxygen decline ($-0.08 \pm 0.04 \text{ \% min}^{-1}$) interspersed by periods of relatively rapid oxygen decline ($-0.33 \pm 0.09 \text{ \% min}^{-1}$). Periods of relatively low decline lasted on average $11.8 \pm 5.2 \text{ min}$ during which mud shrimp were either “Burrowing”, “Walking” or “Resting” while periods of rapid decline lasted on average $2.9 \pm 1.3 \text{ min}$ during which mud shrimp were always “Ventilating”. Ventilation bouts sometimes lasted longer than the period of oxygen declines *per se* as can be seen on the third and the sixth steps in Fig. 5.

Oxygen saturations in the burrow lumen were between 95 and 10 % (Fig. 6A, B and C). The directions and magnitudes of temporal changes in O₂ saturations were tightly related to the behaviours of mud shrimp (Fig. 6A, B and C). Slow decreases ($-0.9 \pm 0.3 \text{ \% min}^{-1}$) in O₂ saturation were detected when mud shrimp were “Resting” or “Walking” (Fig. 6A, C). Rapid O₂ saturation decreases ($-6.7 \pm 3.4 \text{ \% min}^{-1}$) were detected when mud shrimp were “Burrowing” (Fig. 6B, C). Conversely, sharp increases in O₂ saturation ($27.4 \pm 4.3 \text{ \% min}^{-1}$) were always associated with “Ventilating” (Fig. 6A, B and C). The mean ventilation duration necessary for O₂ saturation to reach its maximum value during a single ventilation event was $2.9 \pm 0.7 \text{ min}$. There was a significant positive linear relationship ($F = 165.364, p < 0.001$) between the distance of the mud shrimp from the sensor and the values of O₂ saturation at the onset of ventilation (Fig. 6D). O₂ saturation inducing the onset of “Ventilating” (*i.e.*, measured when mud shrimp were in the immediate vicinity of the optical sensor) was consistently between 12 and 25 %.

4. Discussion

4.1. Behaviours and time allocation pattern

Previous studies regarding mud shrimp behaviours have mostly been carried out on deposit-feeding species whereas *Upogebia pusilla* is a suspension-feeder (Dworschak, 1987b). The four behaviours identified during the present study were nevertheless similar to those described for the deposit feeding mud shrimp *Calocaris templemani* (Gagnon et al. 2013) and *Callianassa subterranea* (Stamhuis et al., 1996). The average proportion of time allocated to active behaviours (*i.e.*, “Burrowing”, “Ventilating” and “Walking”) by *U. pusilla* were 43, 60 and 82% during winter, spring and summer, respectively. This is quite similar to the 47 and 66% reported during winter for *C. templemani* (Gagnon et al., 2013) and during spring for *C. subterranea* (Stamhuis et al., 1996), respectively. This tends to suggest that the overall level of activity in mud shrimp is not related to feeding modes. There were however differences in time allocation between *U. pusilla* and the two deposit-feeding mud shrimp. Those differences were mainly associated to “Ventilating” and “Walking”. During winter, *U. pusilla* spent 14.1 % of its time ventilating *versus* only 5.0 % for *C. Templemani*. Accordingly, during Spring *U. pusilla* spent 26.6% of its time ventilating *versus* only 9.9% for *C. subterranea*. Conversely, and during the same seasons, *U. pusilla* spent only 4.9 % of its time “Walking” *versus* 27.3 % for *C. templemani* and 2.6% *versus* 14.8% for *C. subterranea*. These differences probably relate to trophic modes since: (1) “Ventilating” may occur more frequently in suspension feeders because it is related both to respiration and nutrition, and (2) deposit-feeders rely on “Walking” to forage for food within their burrows (Pillay and Branch, 2011).

To our knowledge, this study is the first one assessing seasonal changes in between-behaviour time allocation in a mud shrimp. Between winter and summer, the proportion of

time allocated by *U. pusilla* to an active behaviour increased by a factor 2.5. More specifically, the proportions of time allocated to “Burrowing” and “Ventilating” increased by a factor 1.4 and 3, respectively. These increases both resulted from marked decreases in interval-durations. In the case of “Burrowing”, a counter effect was however associated with the reduction of bout duration during summer. This is consistent with an indirect control of temperature on time allocation pattern. During summertime, oxygen consumptions by *U. pusilla* and associated microbial communities indeed increased by a factor 3.8 (Pascal et al., 2016b). Since our results also suggest that the level of O₂ saturation triggering ventilation in *U. pusilla* is constant (between 12 and 25 %; Fig. 6D) and assuming this level constant over seasons, elevated temperature during summer contributes to: (1) the reduction in the time of “Burrowing” required to reach this threshold and thereby to the diminution of the duration of “Burrowing” bouts, and (2) the increase in the frequency of “Ventilating” bouts. Overall, our results therefore clearly support the major role of temperature in controlling seasonal changes in between-behaviour time allocation pattern by *U. pusilla*. In this sense, they are in good agreement with previous observations by Berkenbusch and Rowden (1999) who reported a strong positive correlation between sediment turnover (*i.e.* the ejection of deep material from the burrow forming mounds at the sediment surface) rate by *Callinassa filholi* and seawater temperature. It should however be stressed that the influence of temperature on between behaviour time allocation is not necessarily direct (*i.e.* acting on mud shrimp metabolism), but may also be indirect (*i.e.*, through enhancement of organic matter mineralization) since both pathways contribute to limit oxygen availability within the burrows.

4.2. Sediment reworking and “Burrowing”

4.2.1. The different components of sediment reworking and their relative importance

There are three distinct patterns in the sediment reworking induced by *Upogebia pusilla* (see Fig. 1 for visual description). Individual of this species rework sediment: (1) outside their burrows by expelling excavated sediment at the sediment-water interface (P1), (2) below the sediment surface by introducing and then moving downward sediment particles within their burrows (P2), and (3) by compacting sediment against the walls of their burrow (P3). P1 and P2 are mostly vertical, whereas P3 occurs perpendicularly to burrow walls (*i.e.*, mostly horizontally due to the general morphology of the burrows, Fig. 1). Previous studies assessing sediment reworking by mud shrimp have only considered P1 through the assessment of sediment turnover rates (*e.g.*, Berkenbusch and Rowden, 1999; Pillay et al., 2012; Rowden et al., 1998; Suchanek, 1983). During the present study, we also assessed: (1) P1 by measuring the volume of sediment expelled at the sediment-water interface (V_{ES}), (2) the resultant of P1 and P2 by using a 1D vertical biodiffusion-bioadvection transport model (characterized by a biodiffusion and a bioadvection coefficient: D_{bv} and V_{bv} , respectively), and (2) P3 by experimentally measuring the volume of reworked sediment surrounding the burrow (B_{RS}) and by using a simple biodiffusion model (characterized by a biodiffusion coefficient: D_{bw}).

The relative importance of P1 and P3 can be assessed based on the comparison of sediment reworked volumes (*i.e.*, V_{ES} and B_{RS}). During all three seasons the thickness of the reworked sediment layer surrounding the burrow (T_{RS}) stabilized after *ca.* 4-5 days. We thus decided to limit our comparisons to the periods of initial increases in T_{RS} (*i.e.*, until D4). V_{ES} then accounted on average for about 40 % of the total (*i.e.*, $V_{ES} + B_{RS}$) volume of reworked sediment. By comparing the volumes of the burrows and expelled sediments, Dworschak

(1983) estimated this proportion to be 50 % in natural environment. These two values clearly underline the importance of sediment reworking occurring below the sediment surface and the necessity of considering this component when assessing sediment reworking in *U. pusilla*. This statement is likely to hold for other mud shrimp irrespective of their feeding mode and the associated pattern of extension of their burrow (Dworschak, 1983; Nickell and Atkinson, 1995). Although less straightforward, due to the interactions between P1, P2 and P3 and the fact that two different models were used, the comparison between D_{bv} and D_{bw} also provides information on the relative intensity of vertical (*i.e.*, mostly P2 and to a lesser extent P1) and horizontal (*i.e.*, mostly P3) sediment reworking intensities, respectively. After four days (*i.e.*, when T_{RS} reached their maximum values), D_{bw} were about 100-folds lower than D_{bv} . This is due to the fact that, although affecting larger volumes of sediment, sediment compaction against the burrow wall (*i.e.*, P3) transports particles over small distances, which results in a thin (typically 5 mm) layer of sediment around burrow wall and thus a low D_{bw} . This is coherent with the fact that in thalassinid the maintenance of the burrow is typically insured by sediment compaction (Dworschak, 1983). P1 and *a fortiori* P2 induce particle displacements over a much larger (typically several tenth of cm) spatial scale in association with upward non-local transport (mostly P1) and enhanced downward biodiffusion (mostly P2). Overall, P1, P2 and P3 all constitute important but highly different components of sediment reworking.

4.2.2. *Evidences for the induction of sediment reworking by “Burrowing”*

There are several lines of evidence suggesting that the different component of sediment reworking tightly correlate between each other. First, all proxies of the different components (*i.e.*, V_{ES} for P1, D_{bv} for P2, B_{RS} and D_{bw} for P3) of sediment reworking showed a similar seasonal pattern with higher values during summer, intermediate ones during spring

and lower ones during winter. Second, at D4 there was a significant correlation between V_{ES} and B_{RS} . Third, during summer (*i.e.*, when sediment reworking was highest), D_{bv} and V_{bv} showed similar temporal changes during the whole experiment duration. This suggests that all three components are probably controlled by the same behaviour of the mud shrimp. Even though cross correlation effects cannot be absolutely excluded, their probability of occurrence can indeed be considered low. Based on the multiple linear regression model linking the total volumes (*i.e.*, $V_{ES} + B_{RS}$) of reworked sediment at D4 and the proportions of time allocated to the 4 different behaviours, our results suggest that “Burrowing” is the behaviour that is mostly inducing sediment reworking. This statement is further supported by the similarity of the seasonal changes in the proportion of time allocated to “Burrowing” and all the proxies of the different components of sediment reworking. This is in good agreement with the mechanisms inducing P1 (*i.e.*, sediment excavation) and P3 (*i.e.*, sediment compaction along burrow walls) but a little less clear for P2 (*i.e.*, sediment introduction within the burrow), which could *a priori* result from a wider set of mechanisms/activities including “Burrowing”.

4.3. Porewater exchange, oxygen dynamics and behaviours

4.3.1. Evidences for the direct effect of “Ventilating” on porewater exchange and oxygen dynamics

During the present study, porewater exchange rates were assessed based on the monitoring of the temporal dynamics of uranine concentrations in the overlying water. This dynamics clearly presented a cyclical pattern with alternation between relatively long periods of low decrease and short events of sharp decrease in concentration. This is clearly indicative of the discontinuous nature of porewater exchange. The mean time duration of low decrease (*i.e.*, low porewater exchange) periods differed between seasons. They were longer during winter and shorter during summer (47.3 vs. 9.5 min, respectively). Moreover, during all three

seasons, they tended to be similar to the time intervals between two consecutive ventilation bouts (47.3 vs. 42.5 min, 17.4 vs. 17.7 min, and 9.5 vs. 9.5 min, during winter, spring and summer, respectively). This suggests that, similarly to the Polychaete *Marenzelleria viridis* (Quintana et al., 2011), in *U. pusilla*, “Ventilating” is the behaviour inducing porewater exchange.

This is further supported by the analysis of the dynamics of O₂ saturation in the overlying water of the thin aquaria during summer experiments. Oxygen saturation also showed a cyclical pattern superimposed to an overall decreasing trend quite similar to uranine. The mean time interval between two consecutive drops was 11.8 minutes and the duration of each drop was 2.9 minutes. These values are consistent with those available for *U. pusilla* (Dworschak, 1981) and other mud shrimp (Forster and Graf, 1995; Stamhuis et al., 1996; Volkenborn et al., 2012b). During the present study, the average duration of an individual cycle of O₂ saturation in the overlying water was thus 14.7 minutes, which is fully compatible with the mean length of a ventilation cycle (*i.e.*, 16.4 minutes) considering associated variability. Moreover, the direct examination of the two time-series further supports that temporal changes in the O₂ saturation of the overlying water are clearly controlled by the “Ventilating” behaviour of the mud shrimp since drops in O₂ saturation of overlying waters were always associated with ventilation events.

4.3.2. Evidences for the effect of “Burrowing” on the onset of “ventilating”

During summer experiment, oxygen saturation within the burrow showed a cyclical pattern as already observed for other mud shrimp such as *C. subterranea* (Forster and Graf, 1995) and *N. californiensis* (Volkenborn et al., 2012b) and for the polychaete *Allita succinea* (Murphy and Reidenbach, 2016). Temporal changes were clearly related with the behaviour of the mud shrimp. Oxygen decreases were very low when mud shrimp were “Resting” or

“Walking”. Since thalassinid mud shrimp show low basal metabolic rates (Atkinson and Taylor, 2005), microbial community respiration and diffusive transport of O₂ through the burrow wall probably account for the main part of oxygen consumption during these time periods. Conversely, O₂ saturation dropped quickly when mud shrimp were “Burrowing” due to: (1) the increase in mud shrimp metabolism and (2) the release of reduced compounds previously trapped within the sediment column, which are quickly re-oxidized through a series of coupled oxidation-reduction reactions that ultimately consume oxygen (Aller et al., 1983; Kristensen, 2000; Kristensen and Kostka, 2005).

We also found a significant positive relationship between O₂ saturation measured by the optode within the burrow and the distances of the mud shrimp to this sensor at the onset of ventilation events. Given that mud shrimp initiate a ventilation event at a given O₂ saturation, this suggests that the spatial distribution of O₂ saturation within the burrow is not uniform. More specifically, this result supports the fact that O₂ saturation would be minimal close to the mud shrimp and would increase with increasing distance from the mud shrimp. There are some rationales to support this assumption: (1) the mud shrimp is respiring and thus consuming O₂ (Forster and Graf, 1995; Koike and Mukai, 1983), and (2) its activity contribute to release reduced compounds, which are readily oxidized in the oxygenated burrow water (Aller et al., 1983; Kristensen, 2000; Kristensen and Kostka, 2005). According to these hypotheses, the O₂ saturation threshold at which *U. pusilla* initiates ventilation would be between 12 and 25%, which is fully coherent with the minimal O₂ saturation levels observed within the burrow water of *C. japonica* (12 %, Koike and Mukai, 1983), *N. californiensis* (20 %, Volkenborn et al., 2012b) and *U. major* (30 %, Koike and Mukai, 1983). No data on oxygen dynamics were available for winter and spring. However, the existence of an O₂ saturation threshold triggering burrow ventilation together with behavioural data suggest that the oscillation frequency in oxygen saturation within the burrow

would be the lowest during winter and intermediate during spring. Here again there are some rationales supporting this assumption: (1) a lower oxygen consumption by the sediment at low temperature (Epping and Helder, 1997; Hall et al., 1989; Thamdrup et al., 1998), and (2) as observed during this study and similarly to what has been observed for other thalassinids (Stanzel and Finelli, 2004), an increase in ventilation and burrowing event frequencies with temperature.

5. Conclusion

We assessed how different behaviours by the mud shrimp *Upogebia pusilla* affect sediment reworking, bioirrigation and oxygen dynamics at and below the sediment-water interface. Our results show that *U. pusilla* is an efficient bioturbating species causing both high sediment reworking and porewater exchange rates. There were three components of sediment reworking: (1) the expulsion of excavated sediment at the sediment-water interface (2) the introduction and then the downward transport of surface sediment particles within the burrow, and (3) the compaction of sediment around burrow walls. The intensities of all three components correlated positively so that sediment reworking as a whole appeared mainly induced by the “Burrowing” behaviour. In summer, porewater exchange and oxygen dynamics were directly cued by the “Ventilating” which is initiated by an oxygen saturation level between 12 and 25%. “Ventilating” was thus also indirectly cued by “Burrowing”, which, conversely to “Walking” and “Resting” induces marked decreases in O₂ saturation within the burrow. This underlines the importance of taking into account changes in animal behaviour types/intensities at the appropriate temporal scales when assessing the impact of bioturbation on biogeochemical processes taking place at the sediment-water interface.

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892 **Table 1** Seasonal changes in the mean (\pm SE), minimum (Min) and maximum (Max) values of main burrow characteristics (length, depth, number of
893 openings and number of chambers) recorded immediately after luminophore input (D0), at day 4 (D4) and at day 30 (D30).

Time / Season	<i>n</i>	Length			Depth			Number of openings			Number of chambers		
		Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
D0													
Winter	10	34.5 ± 7.3	24.6	49.0	14.0 ± 3.3	9.6	18.7	2.0 ± 0.0	2	2	2.2 ± 0.5	2	3
Spring	10	39.9 ± 13.4	19.8	61.0	16.5 ± 5.9	6.5	24.5	2.0 ± 0.0	2	2	2.9 ± 0.5	2	4
Summer	10	42.3 ± 13.1	16.4	58.4	15.7 ± 4.9	8.4	24.7	2.0 ± 0.0	2	2	2.8 ± 0.8	2	4
D4													
Winter	10	35.9 ± 9.4	24.6	57.1	14.3 ± 3.3	10.0	18.7	2.0 ± 0.0	2	2	2.2 ± 0.5	2	3
Spring	10	44.5 ± 17.6	21.6	81.3	17.9 ± 7.3	7.0	26.1	2.0 ± 0.0	2	2	2.9 ± 0.5	2	4
Summer	10	52.3 ± 9.9	35.6	66.1	19.3 ± 5.4	13.9	27.3	2.0 ± 0.0	2	2	2.8 ± 0.8	2	4
D30													
Winter	10	47.1 ± 16.8	28.3	81.8	15.5 ± 3.8	12.9	23.5	2.6 ± 0.6	2	3	3.8 ± 1.5	2	6
Spring	10	76.8 ± 26.0	48.3	127.0	22.4 ± 8.2	14.2	29.8	2.5 ± 0.5	2	3	4.4 ± 1.3	3	7
Summer	10	96.7 ± 27.5	66.6	154.9	26.7 ± 8.9	16.1	31.0	2.3 ± 0.5	2	3	5.1 ± 1.2	4	8

895 **Table 2** Results of the PERMANOVA and PERMDISP analyses for assessing differences in burrow length, burrow depth, number of burrow openings,
896 number of chambers, mean thickness of the reworked sediment layer surrounding the burrow (T_{RS}), volume of reworked sediment surrounding the burrow
897 (B_{RS}) and volume of sediment expelled at the sediment-water interface (V_{ES}), between Seasons and (experiment) Durations. p values in bold indicate
898 significant factor effect. *: PERMDISP, p < 0.05

Factors		Burrow length	Burrow depth	Number of burrow openings	Number of chambers	T _{RS}	B _{RS}	V _{ES}	D _{bv}	V _{bv}
<i>Seasons</i>	df	2	2	2	2	2	2	2	2	2
	MS	4478.8	234.63	0.0333	6.7111	0.184	32969	83391	1.618E7	6.832E5
	Pseudo-F	7.047*	2.727*	0.443	4.387	43.979	42.573*	54.447*	78.580	12.052*
	p(perm)	0.005	0.097	0.883	0.023	0.001	0.001	0.001	0.001	0.001
<i>Durations</i>	df	2	2	2	2	15	15	15	15	15
	MS	10595	287.09	0.9	22.411	2.285	121490	410850	2.208E5	24467
	Pseudo-F	82.991*	29.399*	11.951*	42.315*	1192.700*	255.160*	296.570*	2.182	1.942*
	p(perm)	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.013	0.034
<i>Replicate(Seasons)</i>	df	27	27	27	27	27	27	27	27	27
	MS	635.52	86.038	0.0753	1.530	0.004	774.43	1531.6	2.0479E5	56379
	Pseudo-F	4.978*	8.811*	1.000*	2.888*	2.184	1.627	1.106	2.024	4.475
	p(perm)	0.001	0.001	0.483	0.003	0.003	0.046	0.378	0.004	0.001
<i>Season x Durations</i>	df	4	4	4	4	30	30	30	30	30
	MS	1408.2	61.061	0.033	1.478	0.0462	49489	49748	1.115E5	14616
	Pseudo-F	11.030*	6.253*	0.443*	2.790	24.131*	25.985*	35.911*	1.102	1.160
	p(perm)	0.001	0.002	0.8	0.049	0.001	0.001	0.001	0.338	0.274
<i>Residuals</i>	df	54	54	54	54	300	300	300	300	300
	MS	127.7	9.7651	0.0753	0.5296	0.0019	476.14	1385.3	1.012E5	12599

901 **Table 3** Description, average bout-duration (minutes \pm SE and interval-duration (*i.e.*, average time duration between two bouts of the same behaviour;
902 minutes \pm SD) of each behaviour during winter (n = 4), spring (n = 5) and summer (n = 5) experiments. * indicates significant between seasons differences
903 (one-way PERMANOVA, p < 0.05)

Behaviour	Description		Bout-duration (minutes)			Interval-duration (minutes)		
			Winter	Spring	Summer	Winter	Spring	Summer
Walking ^a	Walking or turning through the burrow without sediment transport	*	1.8 \pm 0.1	2.0 \pm 0.2	1.0 \pm 0.2	42.5 \pm 9.4	80.4 \pm 11.9	80.4 \pm 16.7
Ventilating ^b	Movements of the four pairs of pleopods		5.7 \pm 1.1	7.9 \pm 2.0	6.9 \pm 0.7	* 42.4 \pm 12.2	17.7 \pm 2.3	9.5 \pm 1.0
- Flushing	<i>Strong ventilation causing the ejection of sediment out of the burrow</i>	*	0.7 \pm 0.1	0.8 \pm 0.0	0.9 \pm 0.0	512.6 \pm 222.5	159.4 \pm 64.0	69.8 \pm 14.3
-Pumping	<i>Movement of the pleopods without sediment transport</i>		6.2 \pm 1.1	9.9 \pm 2.7	8.3 \pm 0.7	* 46.9 \pm 12.4	23.1 \pm 3.4	12.1 \pm 1.4
Burrowing ^c	Sediment transport	*	12.7 \pm 1.5	9.2 \pm 1.4	7.1 \pm 0.6	* 46.1 \pm 14.3	21.9 \pm 4.3	12.7 \pm 1.3
Resting ^d	Stay stationary	*	16.9 \pm 2.6	17.1 \pm 2.5	7.7 \pm 0.8	18.6 \pm 1.9	28.8 \pm 6.0	31.8 \pm 2.6

904 Corresponding terminology in Stamhuis et al. (1996) ^aWander, ^bVentilate, ^cBurrow, ^dRest
905 Corresponding terminology in Gagnon et al. (2013) ^aOther activities, ^bIrrigation of burrow, ^cSediment to surface, Transport and digging + manipulation, ^dIn
906 sight inactive

Figure captions:

Fig. 1 Burrow: Schematic view of a representative burrow. Green line: Former sediment-water interface as evidenced by the remaining of the luminophore layer, dotted line: burrow length, T_{RS} : average thickness of the reworked sediment layer surrounding the burrow, \bar{H} : average height of the sediment expelled at the sediment water-interface. Red dotted arrows represent the three identified patterns of sediment reworking (P1, P2 and P3; see text for details).

Fig. 2 Behaviour: Seasonal changes in the proportions of time allocated to the different behaviours. Vertical bars are standard errors. Different letters indicate significant (PERMANOVA and pair-wise test, $p < 0.05$) differences between seasons.

Fig. 3 Sediment reworking: Seasonal changes in the relationships between experiment duration and: (A) the thickness of reworked sediment surrounding the burrow (T_{RS}), (B) the volume of reworked sediment surrounding the burrow (B_{RS}), and (C) the volume of sediment expelled at the sediment-water interface (V_{ES}). Relationship between B_{RS} and V_{ES} at day 4 (D).

Fig. 4 Sediment reworking modelling: Seasonal changes in the relationships between experiment duration and: (A) average vertical biodiffusion (D_{bv}), and (B) vertical bioadvection (V_{bv}) coefficients. Vertical bars are standard errors.

Fig. 5 Oxygen dynamics in the overlying water during core incubations: Representative example of the effect of *Upogebia pusilla* activity on temporal changes of O_2 saturation in the overlying water. Red, blue, green and black colours depict changes during “Burrowing”, “Ventilating”, “Walking” and “Resting”, respectively.

Fig. 6 Oxygen dynamics in the burrow water: Examples of temporal changes in O_2 saturation when the mud shrimp was (A and B) far and (C) close from the micro-optode. Red, blue, green and black colours depict changes during “Burrowing”, “Ventilating”, “Walking” and “Resting”, respectively. Relationship between O_2 saturation at the onset of a ventilation event and the mud shrimp distance from the sensor (D). See text for details.











