Feeding ethology and surface sediment reworking by the ampharetid polychaete Melinna palmata Grube, 1870: Effects on sediment characteristics and aerobic bacterial community composition

Cécile Massé, Frédéric Garabetian, Bruno Deflandre, Olivier Maire, Laurence Costes, Nathalie Mesmer-Dudons, Jean-Claude Duchêne, Guillaume Bernard, Antoine Grémare, Aurélie Ciutat

Feeding ethology and surface sediment reworking by the ampharetid polychaete *Melinna palmata* Grube, 1870: effects on sediment characteristics and aerobic bacterial community composition

MASSÉ Cécile[1, *], GARABETIAN Frédéric[1], DEFLANDRE Bruno[1], MAIRE Olivier[1], COSTES Laurence[2], MESMER-DUDONS Nathalie[2], DUCHÊNE Jean-Claude[2], BERNARD Guillaume[2], GRÉMARE Antoine[1], CIUTAT Aurélie[2]

[1] Université de Bordeaux, EPOC, UMR 5805, F-33615 Pessac, France.

[2] CNRS, EPOC, UMR 5805, F-33615 Pessac, France.

*Corresponding author: c.masse.epoc@gmail.com

ABSTRACT

The present study was aiming at describing the feeding ethology and assessing surface sediment reworking together with associated effects on sediment characteristics and aerobic bacterial community composition by the ampharetid polychaete *Melinna palmata*, which exhibits very dense populations in the Arcachon Bay (French Atlantic coast). There was a plasticity in the main aspects (i.e., positioning of the tube at the sediment-water interface, stretching of the worms outside their tubes while feeding, location and mechanism of faeces production) constitutive of the current knowledge regarding feeding ethology in ampharetids. On average, worms dedicated 84.6% of their time to the prospection of surface sediments and produced faeces exclusively at the water sediment interface, which resulted in surface sediment reworking and induced the zonation of surface sediment in three distinct areas, namely: undisturbed sediment, prospected and faecal mound areas. Average individual surface prospected area and surface sediment reworking rate were 28 cm^2 and 6.8 $mm^3.h^{-1}$, respectively. Surface sediments were coarser and their bulk organic contents were lower in prospected areas, intermediate in faecal mound areas and higher in undisturbed sediments. Oxygen penetrated deeper in the sediment column in faecal mound areas. Aerobic bacterial community composition associated with surface oxygenated sediments within these three areas also significantly differed, which suggests that sediment reworking *per se* (i.e., irrespective of changes in redox conditions) do have an effect on those compositions. These results are discussed in terms of potential food limitation in the very dense populations of *M. palmata* present in the Arcachon Bay. It is suggested that the high

31 densities of these populations result from both hydrosedimentary fluxes due to tidal currents and from
32 the enhancement of particle sedimentation in *Zostera noltei* meadows.
33
34 Key words: Ampharetids, *Melinna palmata*, Feeding ethology, Surface sediment reworking, Sediment
35 characteristics, Aerobic bacterial community composition.
36

1. INTRODUCTION

Once sedimented, particulate organic matter undergoes early diagenesis (i.e., a sequence of mineralization reactions taking place in the top sediment column). This process is mainly achieved by bacterial communities featuring different metabolic capacities in relation with the availability of a series of final electron acceptors. In the absence of benthic macrofauna, these receptors show a typical vertical zonation (Froelich et al., 1979). By modifying this redox sequence and by creating microenvironments submitted to oscillatory conditions, bioturbation by benthic macrofauna disturbs oxidation processes taking place in the top sediment column (Burdige, 1993). Bioturbation activities include the mixing and spatial redistribution of both sediment particles (i.e., sediment reworking), and pore-water solutes (i.e., bioirrigation; Rhoads, 1974; Kristensen et al., 2012). They directly affect the three components cuing the efficiency of mineralization processes taking place in the top sediment column, namely the spatial distributions of: (1) particulate organic matter; (2) final electron acceptors; and (3) bacterial community biomass (Aller and Yingst, 1985; Reichardt, 1988), composition (Bertics and Ziebis, 2009; Laverock et al., 2010) and biogeochemical functions (Bertics et al., 2010; Gilbertson et al., 2012; Yazdani Foshtoni et al., 2015). By doing so, bioturbation strongly influences the biogeochemical processes taking place at the sediment-water interface (Aller, 1994; Aller and Aller, 1998; Lohrer et al., 2005; Furukawa, 2005; Aller, 2014; Braeckman et al., 2014).

Sediment reworking is typically a 3D process (Rosenberg et al., 2008). Its quantification is however usually achieved based on a reduced number of dimensions due to the difficulty in penetrating the sediment matrix. Because of the interaction with the vertical zonation of biogeochemical processes (see above) and due to the importance of the problematic of carbon burial, most attention has been devoted to the vertical dimension (see for example Maire et al., 2008). Classically, the assessment of sediment reworking is based on the coupling between: (1) the assessment of a vertical profile of tracer concentrations, and (2) the modelling of this profile. This whole process usually results in the computation of a vertical biodiffusion coefficient (Db) and/or non-local exchange functions, which account for the intensity of vertical sediment reworking through biodiffusion and non-local transport. Conversely, the quantification of horizontal sediment reworking

has received much less attention although some early modelling studies have suggested that this component may be quantitatively as important as the vertical one (Wheatcroft et al., 1990; Wheatcroft, 1991) and that surface mixing is often considered as an ecological trait in studies assessing the relationship between species diversity and ecosystem functioning (e.g. Hewitt et al., 2008). The use of thin aquaria and luminophores (i.e., sediment particles coated with a fluorescent paint) coupled with sophisticated image acquisition and analysis techniques has only been recently introduced allowing for the 2D (i.e., vertical and horizontal) assessments of sediment reworking (e.g. Gilbert et al., 2003; Maire et al., 2007b, 2007c, 2010; Bernard et al., 2012).

The analysis of the effects of bioturbation on bacterial community composition and function has also favored the assessment of the vertical component due to the tight interactions between these communities and available final electron acceptors. Gallery biodiffusors (*sensu* Kristensen et al., 2012) have been mostly used to tackle this question. Through the ventilation of their burrow, these organisms enhance oxygen penetration deep in the sediment column (e.g. Papaspyrou et al., 2006; Laverock et al., 2010; Pischedda et al., 2011) and thereby induce a patchy distribution of bacterial assemblages (e.g. Laverock et al., 2010). Moreover, and because their activity is often discontinuous, they induce short-term temporal changes in bacterial assemblage functionalities, which results in a non-equilibrium dynamics of mineralization processes (e.g. Wenzhöfer and Glud, 2004; Pischedda et al., 2008). One difficulty when studying the effect of sediment reworking on bacterial assemblages and functions is the unraveling between the effect of sediment reworking *per se* and those related with changes in redox conditions (e.g. Laverock et al., 2010). A possibility to tackle this difficulty is to focus on benthic macrofauna performing sediment reworking without altering the oxygenation of the sediment. This is potentially the case of “Surface deposit feeding Sessile Tentaculate” worms (SST, Fauchald and Jumars, 1979), which collect a very thin layer of particles at the sediment-water interface. Sediment reworking by these organisms typically creates distinct areas at the sediment surface with a clear distinction between undisturbed sediments, and prospected and faecal mound areas (e.g. Nowell et al., 1984). This allows for the establishment of a stratified sampling strategy of surface oxygenated sediment to assess the effect of sediment reworking on sediment characteristics

and associated aerobic bacterial community composition (see Warwick et al. (1986) and Olafsson et al. (1990) for a similar approach regarding meiofauna composition).

During the present study, we used the ampharetid polychaete *Melinna palmata* as a biological model. This species is abundant in the *Zostera noltei* meadows and the bare intertidal mudflats of the Arcachon Bay (France) where it has been suggested that its dense populations inhibit vertical sediment reworking (Bernard et al., 2014). Although, *M. palmata* clearly belongs to SST (Fauchald and Jumars, 1979; Jumars et al., 2015), its feeding ethology has not been deeply investigated yet. The present study therefore aimed at: (1) describing the anatomy of feeding organs; (2) establishing a typology of the different behaviors and assessing the time allocation pattern between these behaviors; (3) quantifying surface sediment reworking, and (4) assessing the effects of sediment reworking on surface sediment main characteristics, oxygen penetration and bacterial community composition. These objectives were tackled using an *ex situ* experimental approach. Due to the practical/technical difficulties in achieving replication (e.g. Maire et al., 2007a), this resulted in three series of experiments dedicated to one or combinations of the above mentioned specific aspects.

2. MATERIAL AND METHODS

2.1. Worm collection and maintenance

Melinna palmata is an ampharetid worm with a large boreo-mediterranean distribution (Grehan, 1991). It is abundant along the Atlantic coasts from Norway to Morocco (Guillou and Hily, 1983; Grehan, 1991; Cacabelos et al., 2011), in the Black Sea, the Sea of Azov, the Persian Gulf and the Mediterranean Sea (Holthe, 1986; Zaabi and Alfi, 2006). It has been recorded all along the metropolitan French coast (http://resomar.cnrs.fr/bases/index.php; Dauvin et al., 2003). Individual worms are typically between 15 and 50 mm in length and between 2 and 3 mm in width. They bear 16 thoracic and about 60 abdominal segments. The base of their two sets of four gills is implanted on the dorsal part of their first segment (Fauvel, 1927; Rouse and Pleijel, 2001). The life span is between 2

and 2.5 years in inner Galway Bay (Grehan, 1991) and secondary production in Southampton waters is 0.42 g $C^{-2}yr^{-1}$ (Oyenekan, 1988).

For the present study, adult worms were collected during April 2012 (anatomy of feeding organs, 1st and 2nd series of experiments) and December 2013 (3rd series of experiments and tentacles number) in the Arcachon Bay (French Atlantic Coast) at the "Germanan" site (44°42'726''N, 1°07'940''W) where extremely dense populations of *M. palmata* have been reported in *Zostera noltei* intertidal seagrass meadows (Blanchet et al., 2004; Bernard et al., 2014). Back at the laboratory, tubes were isolated from the sediment by gently sieving on a 1 mm square mesh, and collected by hand. Worms were then acclimatized (>10 days) in aquaria containing sieved (1 mm mesh to discard other macrofauna; e.g. Queiros et al., 2015) sediment from the collection site and fuelled with a continuous flow of filtered seawater from the Arcachon Bay before any subsequent manipulations/experimentations.

2.2. Anatomy of feeding organs

The tentacles of 10 worms collected in December 2013 were counted under a Nikon® SMZ25 stereomicroscope. The anatomy of the feeding organs was investigated using Scanning Electron Microscopy (SEM). Fifteen worms collected in April 2012 were left for one hour in filtered (0.22 µm) and sterilized seawater to ensure that their tentacles were deployed. Heads and tentacles were then dissected and fixed in 2.5 % glutaraldehyde with 0.4 M cacodylate buffer and 7 % NaCl for 24 h. Samples were rinsed 3 × 30 min in 0.4 M cacodylate and 4 % NaCl before being post-fixed with 2 % osmium tetroxide in 0.4 M cacodylate and 10 % NaCl. They were then dehydrated through a series of incubations in increasing alcohol concentrations (10 min in 50 %, 10 min in 70 %, 10 min in 90 %, 10 min in 95 %, 2 × 10 min in absolute and 15 min in propylene oxide). After critical-point-drying, samples were coated with gold and observed using a Quanta 200 SEM (FEI Company) at the Bordeaux Imaging Centre (University of Bordeaux, France).

142 **2.3. Experiments**

143 Three series of experiments were carried out during April 2012, November 2012 and February
144 2014 (Fig. 1). Before each experiment, worms were carefully removed from their tube and examined
145 under a binocular microscope to check for physical integrity. All experiments were achieved on single
146 individual worms, which were introduced at the center of a parallelepiped (11.5×17.5×6.5 cm,
147 corresponding to a density of 50 ind.m^{-2}) aquarium filled with a 6 cm layer of sediment from the
148 collection site (sieved on 1 mm-mesh to remove other macrofauna and previously stabilized for 10
149 days) and placed under a continuous flow of filtered seawater from the Arcachon Bay for 10 days.
150 This time period proved sufficient for worms to build a new tube and to efficiently burrow in the
151 sediment.
152

153 *2.3.1.First series of experiments*

154 The aims of the first series of experiments (Fig. 1A) were to: (1) describe feeding ethology, (2)
155 characterize worm behaviors, (3) assess time allocation pattern, (4) quantify the surface of sediment
156 reworked areas, and (5) assess the effect of surface sediment reworking on main sediment
157 characteristics and aerobic bacterial community composition. Thirteen worms were studied during this
158 experiment series (temperature: 15.9 ± 2.0 °C; salinity: 30.5 ± 1.7).
159

160 *2.3.1.1. Feeding ethology, typology of behaviors and time allocation pattern*

161 A computer piloted IDS µeye UI-1580SE-C-HQ video sensor (Stemmer Imaging) was
162 positioned 30 cm straight above the aquarium sediment surface, which was illuminated with infrared
163 light. This system allowed for the collection of images of the sediment surface during 24 hours at a
164 frequency of 0.1 Hz. Collected images were assembled in an AVI film, which was then visually
165 analyzed to assess: (1) feeding ethology, (2) different types of behavior, and (3) time allocation
166 patterns between these behaviors.
167

2.3.1.2. Surface sediment reworked areas

Additional images of the sediment surface were collected under ambient light on days 10, 15, 20, 25 and 30 (starting from the beginning of the acclimation period) using a Nikon ® D7000 Reflex 16.2 Mpixels camera fitted with a 18-105 mm lens. A special care was taken to insure that the image plan was strictly parallel to the sediment surface. For all worms, a zonation of the sediment surface became rapidly visible. This included: (1) a faecal mound area near the tube opening, (2) a prospected area devoid of surface particles, and (3) undisturbed sediment (Fig. 2A and B). The faecal mound and the total affected areas were manually drawn on each collected image and their surface was assessed using the Image J® software (USA National Institutes of Health) after appropriate calibration. The surfaces of prospected areas were computed as the difference between the surfaces of total affected and faecal mound areas.

2.3.1.3. Surface sediment sampling

At the end of the 20 d experiment period, the surface sediments of: (1) faecal mound areas, (2) prospected areas, and (3) undisturbed sediments were sampled using a truncated (to allow the passage of the largest particles) pipette tip fitted to the needle port of a 10 mL syringe. All the surface sediment of each three areas was sucked by gently pulling the syringe plunger while the truncated tip was moved to sample the very top (i.e., about 2 mm) surface sediment layer. Each sample was homogenized and divided in two for the assessments of: (1) main sediment characteristics, and (2) aerobic bacterial community composition.

2.3.1.4. Main sediment characteristics

Sediment granulometry was assessed using a Malvern® Master Sizer laser microgranulometer and expressed as median diameter (D50) and mud content (i.e., the volume % of particles less than 63 µm in size). Particulate organic carbon (POC) and nitrogen (PON) were measured on 10 mgDW (dry weight) freeze dried decarbonated (with 0.2N HCl according to Kennedy et al., 2005) sediment

194 samples using a ThermoFinnigan® Flash Elemental Analyser Series 1112. Chlorophyll *a* and
195 phaeophytin *a* were assessed on a 6 mL 90 % acetone extracts of 400 mg WW (wet weight) sediment
196 samples using a Perkin Elmer® spectrofluorometer (Neveux and Lantoine, 1993). Depending on the
197 amount of available sediment, these measurements were achieved on 1 to 3 replicates.
198

199 *2.3.1.5. Aerobic bacterial community composition*

200 Aerobic bacterial community composition was characterized by Automated Ribosomal
201 Intergenic Spacer Analysis (ARISA), a PCR-based whole-community fingerprinting method (Fisher
202 and Triplett, 1999). Within two hours after collection, 0.465 ± 0.003 g WW (Wet Weight) of sediment
203 from each zone was placed in preservative buffer (100 mM Tris-HCl [pH 8.0], 10 0 mM EDTA [pH
204 8.0], 1.5 NaCl and 1 % [wt/vol] cetyltrimethylammonium bromide) (Zhou et al. 1996). Samples were
205 stored at -80°C until analysis. DNA extraction was achieved using 700 µL of homogenized thawed
206 sediment sample. DNA was extracted and purified coupling a bead beating method (Lysing matrix E
207 tubes) and Fast Prep (MP Biomedicals): two runs at 5.5 m s^{-1} during 30 s with the use of an extraction
208 kit UltraClean® Soil DNA Isolation Kits (MO BIO Laboratories Inc.). The amount of extracted and
209 purified DNA was quantified by spectrophotometry with 2 µL of DNA solution, using an Epoch
210 microplate spectrophotometer (Biotek instruments). PCR amplification of the 16S-23S rDNA
211 intergenic spacer was carried out using 5'FAM labelled S-D-Bact-1522-B-S-20 (5'-TGC GGC TGG
212 ATC CCC TCC TT-3') and L-D-Bact-132-a-A-18 primers (5'-CCG GGT TTC CCC ATT CGG-3')
213 (Normand et al., 1996). The final reaction mix (25 µL) consisted of 1X PCR buffer (Promega),
214 1.5 mM $MgCl_2$, 0.3 mg mL^{-1} bovine serum albumin (BSA), 5 % Dimethyl sulfoxide (DMSO),
215 200 µM of each deoxynucleoside triphosphate (Invitrogen), 0.5 µM of each primer (Invitrogen),
216 0.25 U of Taq polymerase (Promega) and 10 ng of template DNA at about 1 ng μL^{-1}. Amplification
217 was performed with a Thermocycler (Eppendorf AG). After an initial denaturation at 94ºC for 5 min,
218 35 cycles of denaturation (94ºC, 1 min), annealing (55ºC, 1 min) and extension (72ºC, 1 min) were
219 performed, followed by a final extension (72ºC, 10 min). For each extracted DNA sample, triplicate
220 PCR assays were performed using 3 x 10 ng of template DNA. Amplification products of the three

assays were pooled and purified using QIAquick PCR Purification Kit (QIAgen). Purified amplification products were then quantified using the spectrophotometric method previously described. Finally, 1.5 µL of amplification product adjusted by dilution to about 10 ng μL^{-1} were mixed with 0.1 µL GeneScan 1200 LIZ internal size standard (Applied Biosystems) and 10 µL Hi-Di formamide (Applied Biosystems). The mixture was denatured at 94°C for 4 min and fragments were discriminated using an ABI 3730XL automated sequencer (Applied Biosystems®) operated by the *Plateforme Genome-Transcriptome Pierroton* (a joined facility of INRA and University of Bordeaux). Resulting electrophoregrams were analysed using the Applied Biosystems® Peak Scanner software. Peak sizes inferior to 200 bp and superior to 1200 bp were considered as background noise and eliminated. Then, an "optimal divisor" (Od) was determined to remove fluorescence background within remaining peaks (Osborne et al., 2006). Peaks contributing less than 0.1% (i.e. Od value) of the total amplified DNA (as determined by relative fluorescence intensity) were indistinguishable from baseline noise and eliminated. Binning was carried out under the R software (available on http://cran.r-project.org) using the algorithm "Interactive binner" (available on http://www.ecology-research.com – Ramette, 2009). This allowed for the assessment of the relative abundance of each Operational Taxonomic Unit (OTU) in each sediment replicate. The ARISA fingerprinting method is based on 16S–23S ITS size. Since bacterial species have various numbers and types of ribosomic operons, there is no simple relationship between the occurrences of a bacterial species and the number and types of retrieved OTU (Hill et al., 2003). Moreover, PCR biases may distort OTU relative abundances (Wintzingerode et al., 1997). It is nevertheless assumed that OTU richness and composition realistically reflect bacterial taxonomic diversity (Forney et al. 2004) and support diversity pattern analyses (Ramette, 2007). It is important to underline that ARISA only account for changes in OTU relative abundances.

245 *2.3.2.Second series of experiments*

246 The aim of the second series of experiments (Fig. 1B) was to assess the effect of surface
247 sediment reworking on oxygen penetration within the sediment column. Oxygen microprofiles were
248 achieved on five aquaria containing individual worms after an incubation period of 20 days
249 (temperature: 16.0 ±1.6 °C; salinity: 32.5 ±0.8).
250 Four Unisense® A/S Clark-type microelectrodes (Revsbech, 1989; 100 µm sensor-tip
251 diameter, 90 % response time < 7 s, stirring sensitivity < 1) were positioned on a common holder
252 (forming a 2.5 cm side square) fixed on a MC-232, Unisense ® motorized micromanipulator. These
253 microelectrodes were connected to a high-sensitivity Unisense® picoammeter. The whole system was
254 connected to a portable computer and controlled by the SensorTrace® PRO v3.0 software. Oxygen
255 profiles were performed with a vertical resolution of 100 µm and an equilibration time of 7 s down to
256 a 1 cm depth in the sediment column. Two to four sets of 4 profiles were achieved in each aquarium.
257 Overall, 13 micro-profiles were located in faecal mound areas, 18 in prospected areas and 21 in
258 undisturbed areas. Oxygen micro profiles were processed using the PRO2FLUX software (Deflandre
259 and Duchêne, 2010) to assess oxygen penetration depths.
260

261 *2.3.3.Third series of experiments*

262 The aims of the third series of experiments (Fig. 1C) were to further assess worm behaviors,
263 feeding ethology and time allocation patterns, and to quantify surface sediment reworking. Thirteen
264 worms were studied during this series (temperature: 13.5 ± 0.9 °C; salinity: 28.9 ± 1.8).
265 Methodologies for the assessments of feeding ethology, worm behaviors and time allocation patterns
266 were strictly similar to those used during the first series of experiments.
267
268 Surface sediment reworking rates were assessed for 5 individual worms (15.2 ± 5.0 mgWW)
269 through laser telemetry (Maire et al., 2008). This technique allowed for successive microtopography
270 mapping of the sediment surface, which were later compared to assess the volume of reworked

sediment during the time interval between two consecutive scans. Experimental aquaria were placed under a set of motorized cross tables (401XR Parker® Hannifin precision linear positioners with 5 mm ball screw) connected to Vix500 Microstepper Indexer Drives with XL-PSU power supplies. These tables were computer controlled, which allowed for precise (i.e., ±1.5 µm) positioning (Duchêne, 2012). A laser telemeter (Sick OD80) was attached to the lower Y table. The raw data (in volts) generated by the telemeter were converted in linear distances using an appropriate calibration (Maire et al., 2007b). The whole system was used for assessing the microtopography of 5 cm side square surfaces centered on tube openings and including faecal mound areas. Each scan was achieved within a 20 min time period and with a 15 µm vertical resolution. Surface sediment reworking rates were assessed by summing the differences in microtopography between two consecutive scans of the faecal mound area divided by the time duration between the beginnings of these two scans. Positive differences corresponded to an elevation, whereas negative ones corresponded to a digging of the sediment-water interface. Overall, nine couples (1-3 for each worm) of consecutive (time interval between 15 and 74.5 h) scans were achieved during this experiment.

2.4. Data processing

2.4.1.Time allocation patterns.

Time periods allocated to the different types of behaviors were assessed during the first and the third series of experiments. A Kolmogorov-Smirnov test was performed to check for possible differences in time allocation patterns between these two series.

2.4.2.*Main sediment characteristics.*

Changes in main sediment characteristics within undisturbed sediment, faecal mound and prospected areas were described using a non-metric Multi Dimensional Scaling (nMDS; Clarke and Warwick, 2001) based on untransformed data (i.e., median grain size, POC, PON, chlorophyll *a* and phaeophytin *a*) and using Euclidean distance. Mud content was not used for this analysis because of its

296 strong redundancy with D50. The sediment characteristics mainly responsible for the difference
297 between areas were identified using the SIMiliarity PERcentage analysis (SIMPER) procedure (Clarke
298 and Warwick, 2001). Differences in the values of each characteristic between areas were looked for
299 using univariate Friedman ANOVAs for paired samples.

300 2.4.3.*Aerobic bacterial community composition.*

301 Aerobic bacterial community composition was described using nMDS (Clarke and Warwick,
302 2001) based on untransformed data and Bray-Curtis dissimilarities. Significant differences between
303 community composition were looked for using a multivariate One-Way PERMANOVA (Anderson,
304 2001; McArdle and Anderson, 2001) with the three above-mentioned “sediment areas” as fixed factor.
305 Bacterial diversity was assessed using the complementary of the Simpson index (1-D) and associated
306 equitability ($E_{1/D}$) with:

307 $$D = \sum_{i=1}^{i=S} {p_i}^2$$

308 $$E_{1/D} = \frac{1/D}{S}$$

309 where p_i is the relative abundance of OTU i and S is OTU richness

310 Differences in the values of D and $E_{1/D}$ between areas were looked for using univariate
311 Friedman ANOVAs for paired samples.

312 2.4.4.*Oxygen*

313 Significant differences between oxygen penetration depths between the three considered areas
314 were looked for using a Friedman ANOVA for paired samples.

315 All statistics, except for Friedman ANOVAs (Excel®), were performed using the PRIMER® 6
316 package (Clarke and Warwick, 2001) and its PERMANOVA add on (Anderson, 2001).

3. RESULTS

3.1. Anatomy of feeding organs and feeding ethology

Examined worms bore from 11 to 15 tentacles (12.6 ± 1.3 mean ± sd). Tentacles were implanted side by side around the upper lip of the mouth (Fig. 3A and B). On the inner side of each tentacle, a groove was densely covered with cilia (Fig. 3C and D). Tentacle outer side consisted in an accordion shaped structure with only a few dispersed clumps of cilia (Fig. 3E). A particular structure without cilia was present at the tip of each tentacle (Fig. 3F).

The position and the elevation of the anterior part of the tube varied between worms. On some occasions, the anterior part of the tube was vertical and only slightly emerging from the sediment surface. On some others, the anterior part of the tube was lying horizontal at the sediment surface. None of the observed worms left their tube during our experiments. Moreover, worms did not always stretch their anterior part out of the tube while feeding (Fig. 4A and B) but they occasionally did (Fig 4C). While feeding tentacle tips prospected the sediment surface. For worms 2.0 ± 0.2 cm in length, everted tentacles stretched up to 3.9 ± 0.2 cm over the sediment surface and active tentacles could be almost four times longer than resting ones. We did not notice any evidence of particle-size selection on tentacles. Conversely, we observed that worms were able to detach mineral fragments of several millimeters in size from the sediment and to transport them along the ciliary grooves of their tentacles to their mouth. We only occasionally observed active tentacle retractions toward tube openings. We also observed the presence of an area where large mineral particles were deposited near tube openings (see the tip of the red arrow on Fig. 2B). In spite of numerous attempts using thin aquaria and/or transparent substrates, we never observed the production of faeces within the sediment column (CM personal observation). Faeces were violently expulsed from the tube and catapulted on the sediment surface ca 1 cm away from tube openings (Fig. 4D-F). This defecation mode resulted in the creation of ca 1 cm high faecal mounds where we never observed prospection by tentacles while worms were feeding.

343

344 *3.2. Typology of behaviors and time allocation pattern*

345 Seven types of behaviors were identified: (1) *Prospection* when tentacles explored the
346 sediment surface to collect particles and gills are visible outside the tube; (2) *Gill movements* when gill
347 waving could be seen outside the tube opening and when no tentacle is stretched; (3) *Head movements*
348 when head could be seen quickly entering or exiting the tube; (4) *Maintenance* when worms
349 agglomerated particles at the opening of the tube using their tentacles; (5) *Immobility* when gills were
350 visible outside the tube but remained immobile; (6) *Hidden in the tube* when worms were not visible at
351 all at the sediment surface; and (7) *Faeces egestion* which occurred concomitantly with the six above-
352 mentioned behaviors.

353 Time allocation patterns between behaviors did not significantly differ between the first and
354 the third series of experiments (Kolmogorov-Smirnov test, $p > 0.05$) allowing for the pooling of the
355 results of these two series. Overall, monitored individuals were active (i.e., all behavior types but
356 *Immobility* and *Hidden in the tube*) 89.5 ± 2.4 % of the time (Fig. 5). Otherwise, they were either
357 immobile (7.0 ± 8.9 % of the time) or hidden in their tube (3.5 ± 3.6 % of the time). During activity
358 phases, *Prospection* was by far the most dominant behavior (84.6 ± 10.1 % of the time); followed by
359 *Gill movements* (3.3 ± 3.8 % of the time); Maintenance (1.2 ± 1.2 % of the time); and *Head*
360 *movements* (0.4 ± 0.8 % of the time). *Faeces egestion* occurred on average every 45.4 ± 3.9 min.

361 *3.3. Surface sediment reworking*

362 During the first series of experiments, average total surface sediment reworked area increased
363 from 37 to 49 cm² between the beginning and the end of the "experiment periods" (Friedman ANOVA
364 for paired samples, $p<0.01$). During the same time, average faecal mound surface increased from 9 to
365 22 cm² (Friedman ANOVA for paired samples, $p<0.01$). Conversely, the average surface of the
366 prospected area remained stable at 28 cm² (Friedman ANOVA for paired samples $p > 0.10$). Changes
367 recorded for individual worms globally showed the same pattern with one exception characterized by a
368 transitory decline in total and prospected areas (Fig. 6).

An example of a microtopography scan taken during the third series of experiments is shown in Fig.7. One can distinguish the faecal mound, which reaches 11 mm in height and a prospected area, which extends down to 4 mm in depth relative to the undisturbed area. The average surface sediment reworking rate ($+4.9\ 10^{-6} \pm 1.3\ 10^{-5}$ $mm^3\ h^{-1}$) and the mean change in the height of the undisturbed area ($3.3\ 10^{-7}$ mm) were negligible. Average surface sediment reworking rates were -4.6 ± 7.1 $mm^3\ h^{-1}$ and 6.8 ± 5.1 $mm^3\ h^{-1}$ in the prospected and the faecal mound areas, respectively. In the prospected area, surface sediment reworking induced a sediment excavation of 1.9 ± 0.2 mm in average. Conversely, it resulted in a mean elevation of the faecal mound area of 10.5 ± 1.9 mm. Considering a simple surface sediment transport between the prospected and the faecal mound areas, and the fact that the whole prospected areas may not have been covered in totality by our scans, the average surface sediment reworking rate of *M. palmata* was estimated to be 6.8 $mm^3.h^{-1}$.

3.4. Sediment characteristics and oxygen penetration depths

Overall, the granulometrical and main biochemical characteristics of surface sediments significantly differed between the undisturbed sediment and the prospected and faecal mound areas (multivariate One-Way PERMANOVA, $p < 0.05$; Fig. 8). The SIMPER analysis showed that POC accounted for 59.2 % of the dissimilarity between faecal mound and prospected areas, to 62.8 % of the dissimilarity between faecal mound areas and undisturbed sediments, and to 64.3 % of the dissimilarity between prospected areas and undisturbed sediments. However, all characteristics significantly differed between areas (Friedman ANOVA for paired samples, $p < 0.01$; Table 1). Undisturbed sediments showed the finest mean D50 (13.8 ± 2.0 µm), the second highest mud content (83.8 ± 4.8 %), the highest mean POC and PON contents (33.5 ± 4.0 mg g^{-1} DW and 3.2 ± 0.8 mg g^{-1} DW, respectively) and the highest mean pigments contents (1.4 ± 0.5 µg g^{-1}DW in chlorophyll *a* and 6.8 ± 2.3 mg g^{-1} DW in phaeophytin *a*). Conversely, prospected areas were characterized by the coarsest sediment particles (D50 = 17.1 ± 2.2 µm, mud content = 76.6 ± 3.9 %) the lowest mean POC and PON contents (27.0 ± 5.4 mg g^{-1} DW and 2.2 ± 0.7 mg g^{-1} DW, respectively) and the lowest mean pigment contents (1.2 ± 0.8 µg g^{-1}DW of chlorophyll *a* (not significantly different from the faecal

mound area) and 4.0 ± 2.3 µg g^{-1}DW of phaeophytin *a*, respectively). Faecal mound areas showed intermediate D50 (14.5 ± 2.3 µm), highest mud content (84.3 ± 4.6 %), mean POC and PON contents (30.0 ± 3.6 mg g^{-1} DW and 2.7 ± 0.6 mg g^{-1} DW, respectively), and mean pigment contents (1.0 ± 0.4 µg g^{-1}DW of chlorophyll *a* and 6.7 ± 1.4 µg g^{-1}DW of phaeophytin *a*). Overall, there was a clear opposition between D50 on one-side and POC and PON contents on the other side. This opposition segregated most the three considered spatial areas. Changes in pigment contents were largely independent and did not clearly differentiate these areas (Fig. 8).

Oxygen penetration depths significantly differed between areas (Friedman ANOVA for paired samples, $p<0.01$). Mean oxygen penetration depths were shallower in prospected areas (4.0 ± 0.1 mm) than in undisturbed sediments (4.9 ± 0.2 mm) and in faecal mound areas (7.2 ± 0.4 mm).

3.5. Aerobic bacterial community composition

Overall, 296 OTUs were found, ranging from 200 to 1166 bp. In undisturbed sediments, we recorded 209 OTUs ranging from 200 to 1032 bp, versus 218 ranging from 200 to 1032 bp in faecal mound areas and, 238 ranging from 200 to 1166 bp in prospected areas. Mean values of 1-D significantly differed between areas (Friedman ANOVA for paired samples, $p<0.01$). They were lower in undisturbed sediments (0.909 ± 0.043) than in faecal mound (0.938 ± 0.012) and prospected (0.948 ± 0.012) areas. $E_{1/D}$ also significantly differed between areas (Friedman ANOVA for paired samples, $p<0.05$) with lower values in undisturbed sediments (0.177 ± 0.049) than in prospected (0.220 ± 0.078) and faecal mound (0.234 ± 0.041) areas. Aerobic bacterial community composition significantly differed between the three areas (multivariate One-Way PERMANOVA, $p < 0.05$ – Fig. 9). Within-group average Bray-Curtis similarity was 61.2 % in undisturbed sediments, 66.2 % in faecal mound and 66.0 % in prospected areas. These values were clearly higher than between-groups average similarities (i.e., 48.9 % between undisturbed sediments and faecal mound areas, 48.3 % between undisturbed sediments and prospected areas, and 52.9 % between faecal mounds and prospected areas).

4. Discussion

4.1. Anatomy of feeding organs and feeding ethology

The observations achieved during the present study allow refining the current knowledge regarding the anatomy of the feeding organs and the feeding ethology in ampharetid polychaetes. Our morphological observations confirm that the upper lip of *Melinna palmata*, which bears the tentacles is eversible as already described in *Melinna pacifica* (Zhadan and Tzetlin, 2002). Conversely to what had been previously observed for the terebellid *Eupolymnia nebulosa* (Grémare 1988), they showed: (1) the accordion structure of the tentacle, and (2) the existence of a particular structure near the tip of each tentacle that could possibly play the role of an adhesive cup.

Bacescu (1972) pictured the positioning of *M. palmata* while feeding. He described a tube, with a posterior part vertically penetrating within the sediment column and an anterior part largely emerging from the sediment surface, which is fastened obliquely by the worm when feeding at the sediment-water interface. Jumars et al. (2015) described a more general pattern for tube positioning in ampharetids with the posterior part of the tube curving down in the sediment and the anterior part usually lying parallel to the sediment surface allowing for a horizontal posture of the worm with its ventral side down. According to Bacescu (1972), while feeding, *M. palmata* "stretches out of the tube spreading the tentaculate palate over the substratum". Our own observations clearly show that there is not a unique pattern of tube positioning. Most often, tube openings remained vertical only slightly emerging from the sediment surface with worms positioned head up. On some occasions, however, we observed anterior parts of the tube lying on the sediment surface. Interestingly, Buchanan (1963) already reported vertical positioning of tube openings in dense populations of *Melinna cristata* and Fauchald and Jumars (1979) related this positioning with the scarcity of available food. The lack of a unique general pattern is true as well for the extension of worm outside of their tubes while feeding since most of the time, only a few tentacles and the very extremities of the gills were visible at the sediment surface. When everted, tentacles stretched up to ca 4 cm (for 2 cm long worms), which is rather limited as is the case in ampharetids compared to terebellids (Warwick et al., 1986; Grémare, 1988; Jumars et al., 2015). While extended, tentacle tips prospected the sediment surface to collect

448 particles, which fully supports that ampharetids use their retractable and ciliated tentacles to pick up
449 food particles on the sediment surface (Fauchald and Jumars, 1979). Overall, and besides differences
450 in their number and extension, the functioning of the tentacles appeared very similar to the one
451 described for *Eupolymnia nebulosa* (Grémare, 1988; Maire et al., 2007a). However, an important
452 difference is that on some occasions we observed a combination of ciliary entrainments along and
453 muscular contractions of the tentacles to convey particles to the mouth as already reported for
454 *Hobsonia florida* (Taghon, 1982). Conversely to this author, we were, however, not able to observe
455 the retraction of mucous coated tentacles into the mouth. During our experiments, worms only
456 produced faeces at the water sediment interface, which has already been observed for several other
457 ampharetids (e.g. Nowell et al., 1984), including *M. palmata* (Olafsson et al., 1990). Faeces were
458 violently expulsed from tubes, which resulted in the deposition of fresh faeces ca one centimeter away
459 from tube openings. Nowell et al. (1984) observed a similar pattern in the deep-sea ampharetid
460 *Amphicteis scaphobranchiata*. These authors described the expulsion mechanism, which includes
461 gills, mucous and body binding, and takes much less than 10 s (i.e., the time lag between the
462 acquisitions of two consecutive images during our own experiments). We were not able to depict this
463 process for *M. palmata* based on the experiments described in the present paper. However, other video
464 recordings show that worms bind their body prior egestion so that their pygidia are located close to
465 tube openings and directly expelled faeces (CM personal observation). According to Nowell et al.
466 (1984), faeces expulsion constitutes an adaptation to maintain a feeding pit, which enhances new
467 particle deposition within feeding area of individual *A. scaphobranchiata* living in the deep sea (see
468 also Taghon, 1982). This hypothesis is not necessarily appropriate for shallower ampharetids due to
469 stronger hydrodynamism. Moreover, in *M. Palmata* faecal pellets were expulsed within the ca 4 cm
470 radius of prospected areas even though worms never actively transported particles from their faecal
471 mound area (see also below).
472

473 *4.2. Evidence for and quantification of surface sediment reworking*

474 Queiros et al. (2015) stated that *M. palmata* "is a tube-dweller exhibiting conveyor-belt transport
475 of particulates". During our own experiments, we observed that faeces egestion only occurred at the
476 sediment-water interface. Together with the collection of surface particles by the tentacles, and the
477 lack of motility in ampharetids (Jumars et al., 2015; confirmed in the case of *M. palmata* by our own
478 observations), this is likely to result in mostly horizontal particle displacements at the sediment-water
479 interface and thus in surface sediment reworking.

480 To our knowledge, vertical sediment reworking by *M. palmata* has only been experimentally
481 quantified once through *ex situ* luminophore experiments and associated image analysis techniques
482 (Queiros et al., 2015). These authors reported vertical biodiffusion coefficients (Dbs) less than $1cm^2.y^{-1}$
483 with maximal penetration depth typically less than 2cm. Among the 4 species studied by Queiros et
484 al. (2015), *M. palmata* was clearly the one with the lowest Dbs. Moreover, *M. palmata* was also the
485 only species not featuring any marked seasonal changes in Dbs, thereby suggesting that its vertical
486 sediment reworking intensity is not cued by seasonally changing parameters but rather limited in itself.
487 Although caution should be taken when comparing Dbs derived from different studies since their
488 assessments can be greatly affected both by technological (e.g. incubation duration, vertical
489 resolution…) and environmental parameters (e.g. temperature, food availability, animal density…),
490 this interpretation is further supported by the analysis of literature data regarding Dbs derived from
491 luminophore experiments (see for example Maire et al., 2007c). The Dbs found for *M. palmata*
492 (Queiros et al., 2015) are clearly among the lowest ever reported. They are for example much lower
493 than those reported for the deposit-feeding bivalve *Abra ovata* during summertime (i.e., up to ca
494 31 $cm^2.y^{-1}$ in the absence of food addition) and equivalent to those reported during wintertime when
495 this bivalve is considered almost totally inactive due to low temperature (Maire et al., 2007c; Fig.7).
496 Bernard et al. (2014) carried out a series of *in situ* luminophore experiments in both seagrass beds and
497 adjacent bare sediments within the Arcachon Bay where *M. palmata* is present. They reported a
498 significant negative correlation between *M. palmata* abundances and Dbs and attributed this effect to
499 the stabilization of the sediment induced by dense populations of this species (Brenchley, 1982). Here

again, this supports weak vertical sediment reworking by *M. palmata*. Moreover, while running out these experiments, Bernard (personal communication) observed the penetration of luminophores 2 to 4 cm deep in the sediment immediately following their introduction at the sediment surface. This was attributed to passive transfers within the tubes of *M. palmata*. Similar transfers also likely occurred within siphonal galleries during the *A. ovata* experiments mentioned above and probably partly accounted for the decrease in Dbs with incubation duration reported by Maire et al. (2007c) during their wintertime experiments. Anyhow, this type of potential artefactual luminophore penetration may clearly contribute to an overestimation of (already weak) Dbs in *M. palmata*. Based on all this set of rationale and our own observations, our conclusion is that *M. palmata* does not belong to any existing functional group of sediment reworking. Indeed, the unique characteristics of the particle mixing of *M. palmata*, conveying particles only on the sediment surface, led us to propose a new functional group of sediment reworking: the surface conveyors. Further studies would validate this hypothesis.

Such a sediment reworking mode results in the zonation of the surface sediment in three distinct areas: (1) faecal mounds resulting from faeces accumulation, (2) prospected areas that may be conversely depressed relative to the general sediment surface (Nowell et al., 1984), and (3) undisturbed sediments. Such a zonation has already been observed for several surface tentaculate deposit-feeders (eg Nowell et al., 1984; Warwick et al., 1986) including *M. palmata* (Olafsson et al., 1990). During the present study, we observed a radius of ca 4 cm (for 2 cm long worms) for prospected areas, which is much higher than the 2 cm reported by Gibbs et al. (1981) for 25-35 mm long worms. Conversely, the distances between tube openings and the areas of faeces deposition were smaller during our experiments (i.e., typically close to 1cm) than the 4-5 cm reported by Olafsson et al. (1990, Fig. 1) based on *in situ* observations of a Scottish *M. palmata* population. To our knowledge, the only assessment of surface sediment excavation by an ampharetid is qualitative (i.e., several mm) for *Amphicteis scaphobranchiata* (Taghon, 1982), which is fully compatible with the 1.9 mm reported during the present study for time-limited experiments.

The use of laser telemetry to infer surface sediment reworking rates has only been introduced recently (Maire et al., 2007b; Duchêne, 2012), and to our knowledge the only comparable surface sediment reworking rates to ours are therefore those measured by Maire et al. (2007b) in the deposit-

528 feeding bivalve *Abra ovata*. These authors reported major short-term temporal changes in surface
529 sediment reworking rates in relation with the intensity of inhalant siphon activity. Nevertheless,
530 average values over 27 h time periods were equal or superior to 50 $mm^3.h^{-1}$, which is about 8 times the
531 average value (i.e., 6.8 $mm^3.h^{-1}$) found during the present study. Irrespective of differences in
532 experimental conditions that we did not control for, this suggests that the aspiration by sediment
533 siphons is a more efficient mechanism of surface deposit feeding than the use of tentacles. This is
534 supported by the comparison of the average proportions of time allocated to feeding in: (1) *M. palmata*
535 (84.6%, present study), (2) another surface tentaculate feeder the terebellidae *Eupolymnia nebulosa*
536 (74.2 %, Maire et al., 2007a), (3) *Abra ovata* (40.5 %, Grémare et al., 2004), and (4) *Abra nitida*
537 (54.6 %, Grémare et al., 2004). Duchêne and Rosenberg (2001) quantified the activity of *Melinna*
538 *cristata* at the surface of a large sediment core incubated *ex situ*. They also concluded to the
539 investment of a large proportion of time in (feeding) activity at the sediment-water interface by this
540 species. All these results/observations suggest that the meeting of nutritional requirements required
541 less time allocation in siphon than in tentaculate surface deposit-feeders although additional studies
542 are clearly required to further tackle this point.
543

544 *4.3. Effect on surface sediment granulometry biochemical characteristics and oxygen penetration*
545 *depth*

546 Sediment reworking by *Melinna palmata* induced an increase in the median size diameter (D50)
547 of surface sediment in prospected areas. It is not fully clear, however, whether this resulted from a
548 selection for finer particles or from the simple depletion of finer surface particles and then the
549 ingestion of coarser particles initially located slightly deeper in the sediment column. Positive
550 selection for finer particles by deposit-feeders has classically been put in relation with the optimal
551 foraging strategy (Taghon et al., 1978) and the fact that these particles are the one featuring the highest
552 organic matter content due to their higher surface/volume ratio (Mayer et al., 2004). Although, this
553 paradigm is currently under debate for deposit-feeders as a whole (see Jumars et al., 2015 for a
554 review), it is still generally accepted that surface tentaculate deposit feeders do indeed tend to

preferentially feed on particles smaller than the available median grain size (Jumars et al., 2015). Moreover, it has been suggested that in this feeding guild, particle size selection is mostly mechanical and results from three opposite processes taking place at the tips and along the tentacles (Jumars et al., 1982), namely: (1) particle encounter by the tentacles, which would lead to a positive selection toward larger particles according to the De Lesse's principle; (2) detachment of particles from the sediment, which would result in a selection toward smaller particles because of their higher surface/volume ratio, which better counteracts gravity forces; and (3) differential loss during the transfer of particles within the ciliary groove to the mouth, which here again favors the selection of finer particles due to their higher surface/volume ratio (Jumars et al., 1982) and to their quicker speed of displacement along the tentacles (Maire et al., 2007a). This theoretical mechanical model of particle selection has been tested on the terebellid *Eupolymnia nebulosa* (Grémare, 1988; Maire et al., 2007a). Based on: (1) direct observations of natural particles transiting along the tentacles, and (2) comparison of particle size-selection during feeding (with a positive selection for smaller particles) and tube building (with a positive selection for larger particles), these authors concluded that tentacles are likely not the only organs involved in particle-size selection and that another selection process probably occurs at the level of the mouth as already observed in the spionid *Streblospio benedicti* (Kihslinger and Woodin, 2000). During our own experiments, we did not notice any direct evidence of particle-size selection on the tentacles, which proved able to transport particles several millimeters in size. Conversely, (1) the presence of a structure that may potentially constitute an adhesive cup near the tip of each tentacle may contribute to reduce the mechanical positive selection for finer particles during their detachment from the sediment surface, (2) the limited maximal extension of the tentacles may also contribute to limit the preferential loss of larger particles during their transit along the tentacles so as occasional retraction of tentacles toward the mouth. Moreover, the occurrence of intermediate (i.e., between the unaffected sediment and the prospected area) D50 in faecal mound areas suggests that the size of the particles ingested by *M. palmata* may more rely on their availability at the sediment surface than on their size. Conversely, the observation of the deposition of large mineral particles at the immediate vicinity of tube openings supports the occurrence of a selection process after the transit of particles along the tentacles. Indeed such accumulation probably does not result from the preferential egestion

583 of large inorganic particles (Jumars et al., 2015) since, during our experiments, faecal pellets were
584 catapulted away from tube openings (see above).

585 During the present study, we assessed two bulk characteristics of sedimentary organics, namely
586 POC and PON. Both parameters correlated negatively with D50, which at least partly reflects the
587 general negative relationship linking sediment grain size and associated organic matter concentrations
588 (Mayer et al., 2004; see above). Chlorophyll *a* and phaeophytin *a* are derived from (fresh or degraded,
589 respectively) plant materials and are therefore not representative of bulk sedimentary organics. During
590 the present study, changes in their concentrations were largely independent of those of D50, POC and
591 PON, which may reflect the fact that these concentrations result largely from the presence of
592 individual particles (e.g. diatoms and or plant-derived detritus) rather than from the coating of organic
593 matter to the surface of sediment grains. We identified POC as the parameter contributing most to
594 differences between undisturbed sediments, prospected and faecal mound areas. Chlorophyll *a* and
595 phaeophytin *a* concentrations contributed much less to these differences. Our conclusion is thus that
596 the main effect of *M. palmata* on the biochemical characteristics of surface sediment is quantitative
597 and probably mainly result from changes in sediment granulometry.

598 During the present study, mean oxygen penetration depths were between 4.0 (prospected areas)
599 and 7.2 mm (faecal mound areas). These values are slightly higher than those (i.e., typically between 2
600 and 4 mm) measured both *ex* and *in situ* at the Germanan site (Delgard, 2013; Rigaud et al., 2018).
601 Besides biological activity, the two main factors affecting oxygen penetration within the sediment
602 column are: (1) sediment granulometry, which largely controls sediment porosity and sediment
603 organic content, and (2) organic mineralization processes, which largely control oxygen consumption
604 in the sediment column. During the present study, deeper oxygen penetration were recorded in faecal
605 mound areas despite the fact that they presented intermediate D50 and bulk (i.e., POC and PON)
606 organic content. This may partly result from the fact that faecal mound areas are the ones showing the
607 lowest concentrations of chlorophyll *a*, which is representative of a highly labile component of
608 sedimented POM. Moreover, faecal mound areas are indeed basically constituted by large particle
609 aggregates (i.e., faecal pellets at different stages of dislocation; see Fig. 2 and 4), which are destroyed
610 during microgranulometrical analyses. Sediment microgranulometry measurements therefore probably

not constitute a sound proxy of sediment porosity in this particular case. Unfortunately, sediment porosity was not directly assessed during the present study. A clear possibility is thus that sediment porisity is especially high in faecal mound areas, which would also contribute to enhance oxygen penetration within the sediment column.

4.4. Potential intraspecific interactions and food limitation in field populations

In the English Channel and the Celtic sea, the density of subtidal *Melinna palmata* populations is typically only several hundred of individuals per meter square or less (Gage, 1972; Oyenekan, 1988; Olafsson et al., 1990; Grehan, 1991; Dauvin et al., 2007). To our knowledge, the highest recorded density was 1613 ind.m^{-2} in the Bay of Morlaix (Ibanez and Dauvin, 1988). As for the Bay of Biscay, the highest recorded density, except for the Arcachon Bay, is apparently 1000 ind.m^{-2} (Cacabelos et al., 2011). Several studies suggest that *M. palmata* populations positively respond to an enrichment in fine particles and/or organic matter. Some populations have apparently benefited from the vicinity of a local sewage output in the Bay of Brest (Guillou and Hily, 1983) and from an enrichment of the Bay of Seine in fine particles (Dauvin et al., 2007). At a larger scale, the spread and the increase of *M. palmata* during the 1970s in Romanian waters was attributed to an increase in the frequency of phytoplanktonic blooms due to eutrophication (Gomoiu, 1982 cited by Grehan, 1991).

During the present study, worms were collected from a very dense (i.e., up to 6745 ind.m^{-2} during spring; Bernard et al., 2014) intertidal population associated with a *Zotera noltei* meadow. These authors also reported high (i.e., up to 2000 ind.m^{-2} during spring) densities in adjacent bare sediments. To our knowledge, these densities are the highest ever reported for *M. palmata*. They respectively correspond ca 1.5 and 5.0 cm^2 surfaces available per individual worm, respectively. These values are much smaller than those of individual prospected areas measured during the present study (i.e., mean value of 28 cm^2), which suggests the occurrence of intraspecific interactions in the populations of the Arcachon Bay. Massé (2014) assessed the effects of worm density on the feeding ethology and time allocation patterns of *M. palmata* using the same methodologies as in the present study. She reported that at high (i.e., >1184 ind.m^{-2}) densities, worms did not only forage in

638 prospected areas but also in neighboring faecal mound areas. Together with the occurrence of lower
639 bulk sedimentary organics in prospected areas (see above), this change in feeding behavior tends to
640 suggest that food limitation may occur in field populations. Ampharetids are classified as discretely
641 motile by Jumars et al. (2015). However, the main mechanism involved in motility is tube elongation,
642 which apparently cannot take place in dense populations when tube anterior parts are vertical
643 (Fauchald and Jumars, 1979) as was most often the case during the present study and in the Germanan
644 *Z. noltei* meadow (Bernard, personal observations). In such cases, taking into account sediment
645 transport to feeding (i.e., prospected) areas is essential when elaborating foraging theories (Nowell et
646 al., 1984). In this context, it is worthwhile to notice that the two populations of the Arcachon Bay are
647 both intertidal, and that tidal currents probably contribute to sedimentary movements between
648 undisturbed sediments, prospected and faecal mounds areas thereby allowing for the continuous
649 renewal of the food resources available for *M. palmata*. Along the same line, the difference in
650 densities between the *Z. noltei* and the bare sediment populations may result from the enhancement of
651 particle trapping by seagrass meadows (Gacia et al., 1999; Gacia and Duarte, 2001; Hendricks et al.,
652 2008).

653 *4.5. Effect on aerobic bacterial community composition*

654 Many studies have assessed the effect of macrobenthic bioturbators on sediment bacteria (see
655 review in Table 2). These studies have concerned a large variety of macrobenthic species belonging to
656 different phylla. They have been carried out either *ex* or *in situ* and for some of them have involved
657 experimental incubations. They also differ by the parameters used to assess bacterial responses. Most
658 studies have dealt with abundance, biomass or viability assessments. As far as ampharetids are
659 concerned, the only available study is the one by Aller and Aller (1986) on *Amphicteis* sp. It has been
660 achieved *in situ* by sampling surface sediments around a single worm at a 4827m depth and has shown
661 an increase in surface sediment bacterial abundances from the prospected area to the tube insertion at
662 the sediment-water interface.

663 Studies assessing bacterial community composition have been mostly carried out *in situ* and
664 have involved samplings at different depths within the sediment column. Observed differences in

665 bacterial community composition thus potentially resulted from changes in oxygen and other electron
666 acceptors availabilities. Only several studies have tackled the effect of macrobenthic bioturbators on
667 bacterial community composition in surface oxygenated sediments. Most of them were however based
668 on the comparison of bacterial community composition in field stations (Bertics and Ziebis, 2009) or
669 experimental enclosures (Laverock et al., 2010) with different bioturbator densities but not from the
670 direct sampling of reworked and non-reworked surface sediments. Wilde and Plante (2002) directly
671 compared bacterial community composition in the faecal mounds of *Balaglonossus aurantiacus* and
672 ambient surface sediments. Based on functional parameters, they reported qualitative differences that
673 were transitory since microbial assemblage composition of degrading faeces rapidly converged with
674 those of ambient surface sediments. These patterns were interpreted as resulting from: (1) the
675 differential digestion of ingested bacteria, and (2) the stimulation/injection of non culturable bacteria
676 during gut passage.

677 Our own results also show that aerobic bacterial community composition differ in undisturbed
678 sediments, faecal mound and prospected areas by *Melinna palmata*. In this sense, they are in good
679 agreement with previous works based on spatial assessments of biogeochemical processes (Reichardt,
680 1988; Bertics and Ziebis, 2010; Bertics et al., 2010, 2012). As mentioned above, changes in sediment
681 bacterial community composition are often related to changes in oxygenation. Sediment reworking is
682 not the only activity of benthic macrofauna that can produce such changes. Hydraulic activities (i.e.,
683 the induction of water transports within biogenic structures and sediment interstices by benthic
684 macrofauna) could as well be involved (Woodin et al., 2010, 2016; Volkenborn et al., 2010).
685 However, several rationale suggest that this is probably not the case in *Melinna palmata*. As stated
686 above, the gills are most of the time out of the tube, and no ventilation behavior of the tube was
687 observed during the present experiments. Second, dedicated experiments using fluoresceine (Pascal et
688 al., 2016) have shown that bioirrigation rates are negligible (Massé, 2014). Third, the transfers of
689 water and inorganic solutes across the mucous lining of the tubes of *Melinna cristata* are clearly
690 inhibited (Hannides et al., 2005). Overall, our interpretation is that surface sediment reworking is thus
691 indeed responsible of the differences in aerobic bacterial community composition recorded during the
692 present study.

693 During our experiments, aerobic bacterial community composition seemed to differ most
694 between undisturbed sediments and prospected areas and were intermediate in faecal mound areas.
695 Diversity and equitability also tended to be lower in undisturbed sediments than in prospected and
696 faecal mound areas. This last result is in contradiction with those of Wilde and Plante (2002) who
697 reported a lower diversity in faecal mounds than in ambient sediments. Discrepancies between the two
698 studies may clearly result from differences in the methodological approaches used to assess bacterial
699 community composition. As far as the present study is concerned, higher bacterial diversity in faecal
700 mounds may result from: (1) the egestion of resident (i.e., enteric) bacteria (Harris, 1993), (2) quick
701 changes in faeces bacterial community composition in faeces as suggested by Wilde and Plante
702 (2002), which would result in the presence of different bacterial communities in faecal mounds, and
703 (3) the possible ingestion (and then the further egestion) of bacteria associated with the surface
704 sediment of prospected areas after excavation (see also the section of the discussion regarding the
705 effect on sediment granulometry). The occurrence of higher diversity in prospected areas than in
706 undisturbed sediments may result from the continuous disturbance experienced by the former due to
707 feeding by *M. palmata*. The intermediate disturbance hypothesis (IDH; Grime, 1973; Connell, 1978)
708 indeed predicts higher biodiversity in areas submitted to intermediate frequency of discrete disturbing
709 events. In prospected areas, the periodic removal of bacteria attached to organic particles from the
710 sediment surface might constitute such events. In the theoretical framework of IDH, the species
711 richness peak results from co-occurrence of K- and r-strategists (Mac Arthur and Wilson, 1967). These
712 concepts are still in debate for micro-organisms (Sousa, 1979), with special respect to general trends in
713 bacterial carbon use in ready biodegradability tests (Vásquez-Rodriguez et al., 2007) or soil
714 community (Fierer et al., 2007). For bacteria, K-strategist species might be slow growing, specialized,
715 exoenzyme producing so-called oligotrophic bacteria while r-strategist species might be fast growing,
716 generalist, opportunist so-called copiotrophic bacteria (Fierer et al., 2007). Accordingly, by removing
717 part of the bacterial biomass, *M. palmata* would promote an optimal degradation of the sediment
718 organic matter in the oxic layer. Further studies could take advantage in testing this hypothesis by
719 addressing community level substrate utilization in prospected areas.
720

AKNOWLEGMENTS

Cécile Massé was supported by a doctoral fellowship from French *Ministère de l'Enseignement Supérieur et de la Recherche.* We thank Isabelle Svahn from the Bordeaux Imaging Center (BIC) – Electronic Microscopy Pole of the University of Bordeaux. We also thank Franck Salin, head of *Plateforme Genome-Transcriptome Pierroton* (INRA, Bordeaux, France) for ARISA analyses.

728 **REFERENCES**

729 Aller, R.C. 1994. Bioturbation and remineralization of sedimentary organic matter: effects of redox
730 oscillation. Chem. Geol. 114: 331-345

731 Aller, R.C., 2014. Sedimenary diagenesis, depositional environments, and benthic fluxes. In: Holland,
732 H.D. and Turekian K.K. (eds.) Treatise on Geochemistry, Second edition, vol. 8, pp. 293-334

733 Aller, J.Y., Aller, R.C., 1986. Evidence for localized enhancement of biological activity associated
734 with tube and burrow structures in deep-sea sediments at the HEBBLE site, western North Atlantic.
735 Deep-sea res. 33(6): 755:790

736 Aller, R.C., Aller, J.Y., 1998. The effect of biogenic irrigation intensity and solute exchange on
737 diagenetic reaction rates in marine sediments. J. Mar. Res. 56: 905-936

738 Aller, R.C., Yingst, J.Y., 1985. Effects of marine deposit feeders *Heteromastus filiformis* (Polychaeta),
739 *Macoma balthica* (Bivalvia) and *Tellina texana* (Bivalvia) on averaged sedimentary solute transport,
740 reaction rates, and microbial distributions. J. Mar. Res. 43: 615-645

741 Alongi, D.M., 1985. Microbes, meiofauna, and bacterial productivity on tubes constructed by the
742 polychaete *Capitella capitata*. Mar. Ecol. Prog. Ser. 23: 207-208

743 Andresen, M., Kristensen, E., 2002. The importance of bacteria and microalgae in the diet of the
744 deposit-feeding polychaete *Arenicola marina*. Ophelia. 56 (3): 179-196

745 Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Austral.
746 Ecol. 26: 32-46

747 Bacescu, M.C., 1972. Substratum-Animals. In Marine Ecology, Vol. 1, part 3, edited by O. Kinne,
748 Wiley Interscience, New-York, 1291-1322

749 Bernard, G., Grémare, A., Maire, O., Lecroart, P., Meysman, F.J.R., Ciutat, A., Deflandre, B.,
750 Duchêne, J.C., 2012. Experimental assessment of particle mixing fingerprints in the deposit-feeding
751 bivalve *Abra alba* (Wood). J. Mar. Res. 70 : 689-718

752 Bernard, G., Delgard, M.L., Maire, O., Ciutat, A., Lecroart, P. Deflandre, B., Duchêne, J.C., Grémare,
753 A., 2014. A comparative study of sediment particles mixing in a *Zostera noltei* meadow and a bare
754 sediment mudflat. Mar. Ecol. Prog. Ser. 514: 71-86

755 Bertics, V.J., Ziebis, W., 2009. Biodiversity of benthic microbial communities in bioturbated coastal
756 sediments is controlled by geochemical microniches. ISME J. 3: 1269-1285

757 Bertics, V.J., Ziebis, W., 2010. Bioturbation and the role of microniches for sulfate reduction in
758 coastal marine sediments. Environ. Microbiol. 12(11): 3022-3034

759 Bertics, V.J., Sohm, J.A., Treude, T., Chow, C.E.T., Capone, D.G., Fuhrman, J.A., Ziebis, W., 2010.
760 Burrowing deeper into benthic nitrogen cycling: the impact of bioturbation on nitrogen fixation
761 coupled to sulfate reduction. Mar. Ecol. Prog. Ser. 409: 1-15

762 Bertics, V.J., Sohm, J.A., Magnabosco, C., Ziebis, W., 2012. Denitrification and nitrogen fixation
763 dynamics in the area surrounding an individual ghost shrimp (*Neotrypaea californiensis*) burrow
764 system. Appl. Environ. Microbiol. 78(11): 3864-3872

765 Bird, F.L., Boon, P.I., Nichols, P.D., 2000. Physicochemical and microbial properties of burrows of
766 the deposit-feeding Thalassinidean ghost shrimp *Biffarius arenosus* (Decapoda: Callianassidae).
767 Estuar. Coastal Shelf S. 51: 279-291

768 Blanchet, H., de Montaudouin, X., Lucas, A., Chardy, P., 2004. Heterogeneity of macrozoobenthic
769 assemblages within a *Zostera noltii* seagrass bed: diversity, abundance, biomass and structuring
770 factors. Estuar. Coastal Shelf S. 61: 111-123

771 Braeckman, U., Yazdani Foshtomi, M., Van Gansbeke, D., Meysman, F., Soetaert, K., Vincx, M.,
772 Vanaverbeke, J., 2014. Variable importance of macrofaunal functional biodiversity for
773 biogeochemical cycling in temperate coastal sediments. Ecosystems. 17: 720-737

774 Branch, G.M., Pringle, A., 1987. The impact of the sand prawn *Callianassa kraussi* Stebbing on
775 sediment turnover and on bacteria, meiofauna, and benthic microflora. J. Exp. Mar. Biol. Ecol. 107:
776 219-235

777 Brenchley, G.A., 1982. Mechanisms of spatial competition in marine soft-bottom communities. J. Exp.
778 Mar. Biol. Ecol. 60: 17-33

779 Buchanan, J.B., 1963. The bottom fauna communities and their sediment relationship off the coast of
780 Northumberland. Oikos. 14: 154-175

781 Burdige, D.J., 1993. The biogeochemistry of manganese and iron reduction in marine sediments.
782 Earth-Sci. Rev. 35: 249-284

783 Bussmann, I., Reichardt, W., 1991. Sulfate-reducing bacteria in temporarily oxic sediments with
784 bivalves. Mar. Ecol. Prog. Ser. 78: 97-102

785 Cacabelos, E., Moreira, J., Lourido, A., Troncoso, J.S., 2011. Ecological features of Terebellida fauna
786 (Annelida, Polychaeta) from Ensenada de San Simon (NW Spain). Animal biodiversity and
787 conservation. 34(1): 141-150

788 Clarke, K.R., Warwick, R.M., 2001. Change in marine communities: An approach to statistical
789 analysis and interpretation, 2nd edition, PRIMER-E, Plymouth

790 Connell, J.H., 1978. Diversity in tropical rain forests and coral reefs. Science. 4355(199): 1302-1310

791 Cuny, P., Mirailles, G., Cornet-Barthaux, V., Acquaviva, M., Stora, G., Grossi, V., Gilbert, F., 2007.
792 Influence of bioturbation by the polychaete *Nereis diversicolor* on the structure of bacterial
793 communities in oil contaminated coastal sediments. Mar. Pollut. Bull. 54: 452-459

794 Dauvin, J.C., Dewarumez, J.M., Gentil, F., 2003. An up to date list of polychaetous annelids from the
795 English Channel. Cah. Biol. Mar. 44: 67-95

796 Dauvin, J.C., Ruellet, T., Thiebaut, E., Gentil, F., Desroy, N., Janson, A.L., Duhamel, S., Jourde, J.,
797 Simon, S., 2007. The presence of *Melinna palmata* (Annelida: Polychaeta) and *Ensis directus*
798 (Mollusca: Bivalvia) related to sedimentary changes in the Bay of Seine (English Channel, France).
799 Cah. Biol. Mar. 48: 391-401

800 Deflandre, B., Duchêne, J.C., 2010. PRO2FLUX - A software program for profile quantification and
801 diffusive O2 flux calculations. Environ. Modell. Softw. 25: 1059-1061

802 Delgard, M.L., 2013. Etude des effets cet du rôle des herbiers à *Zostera noltii* sur la biogéochimie des
803 sédiments intertidaux. Thèse de Doctorat, Université de Bordeaux 213pp

804 Dobbs, F.C., Guckert, J.B., 1988a. *Callianassa trilobata* (Crustacea: Thalassinidea) influences
805 abundance of meiofauna and biomass, composition, and physiologic state of microbial communities
806 within its burrow. Mar. Ecol. Prog. Ser. 45: 69-79

807 Dobbs, F.C., Guckert, J.B., 1988b. Microbial food resources of the macrofaunal deposit feeder
808 *Ptychodera bahamensis* (Hemichordata: Enteropneusta). Mar. Ecol. Prog. Ser. 45: 127-136

809 Duchêne, J.C., 2012. Hydroid and serpulid recruitment patterns using a new laser microtopography
810 technique. J. Exp. Mar. Biol. Ecol. 412: 27-36

811 Duchêne, J.C., Rosenberg, R., 2001. Marine benthic faunal activity patterns on a sediment surface
812 assessed by video numerical tracking. Mar. Ecol. Prog. Ser. 223: 113-119

813 Fauchald, K., Jumars, P.A. 1979. The diet of worms: a study of polychaete feeding guilds. Oceanogr.
814 Mar. Biol. Ann. Rev. 17: 193-284

815 Fauvel, P., 1927. Polychètes sédentaires, Faune de France, ed. Lechevalier, Paris, 494p

816 Fierer, N., Bradford, M., Jackson, R., 2007. Toward an ecological classification of soil bacteria.
817 Ecology. 88: 1354-1364

818 Fisher, M.M., Triplett, E.W., 1999. Automated approach for ribosomal intergenic spacer analysis of
819 microbial diversity and its application to freshwater bacterial communities. Appl. Environ.
820 Microbiol. 65: 4630-4636

821 Forney, L.J., Zhou, X., Brown, C.J., 2004. Molecular microbial ecology: land of the one-eyed king.
822 Curr. Opin. Microbiol. 7(3): 210-220

823 Froelich, P.N., Klinkhammer, G.P., Bender, M.L., Luedtke, N.A., Heath, G.R., Cullen, D., Dauphin,
824 P., Hammond, D., Hartman, B., Maynard, V., 1979. Early oxydation of organic matter in pelagic
825 sediments of the eastern equatorial Atlantic: suboxic diagenesis. Geochim. Cosmochim. Ac. 43:
826 1075-1090

827 Furukawa, Y., 2005. Biogeochemical consequences of infaunal activities. In Interactions between
828 macro-and microorganisms in marine sediment, Coastal and Estuarine Studies 60. American
829 Geophysical Union, Washington DC. 390p

830 Gacia, E., Granata, T.C., Duarte, C.M., 1999. An approach to measurement of particle flux and
831 sediment retention within seagrass (*Posidonia oceanica*) meadows. Aquat. Bot. 35: 255-268

832 Gacia, E., Duarte, C.M., 2001. Sediment retention by a Mediterranean *Posidonia oceanica* meadow:
833 the balance between deposition and resuspension. Estuar. Coast. Shelf Sci. 52: 505-514

834 Gage, J., 1972. Community structure of the benthos in Scottish sea-lochs. I. Introduction and species
835 diversity. Mar. Biol. 14: 281-297

836 Gibbs, P.E., Bryan, G.W., Ryan, K.P., 1981. Copper accumulation by the polychaete *Melinna*
837 *palmata*: an antipredation mechanism? J. Mar. Biol. Assoc. UK. 61: 707-722

838 Gilbert, F., Hulth, S., Strömberg, N., Ringdahl, K., Poggiale, J.C., 2003. 2-D optical quantification of
839 particle reworking activities in marine surface sediments. J. Exp. Mar. Biol. Ecol. 285-286: 251-263

840 Gilbertson, W.W., Solan, M., Prosser, J.I., 2012. Differential effects of microorganism-invertebrate
841 interactions on benthic nitrogen cycling. FEMS Microbio. Ecol. 82: 11-22

842 Gomoiu, M.T., 1982. On the populations of *Melinna palmata* Grube at the Romanian littoral of the
843 Black Sea. Cercetari mar. 15: 115-131

844 Goñi-Urriza, M., de Montaudouin, X., Guyoneaud, R., Bachelet, G., de Wit, R., 1999. Effect of
845 macrofaunal bioturbation on bacterial distribution in marine sandy sediments, with special reference
846 to sulphur-oxidising bacteria. J. Sea. Res. 41: 269-279

847 Grehan, A.J., 1991. Demography and reproductive biology of *Melinna palmata* (Ampharetidae:
848 Polychaeta) in inner Galway Bay on the west coast of Ireland. Mar. Biol. 109: 459-467

849 Grémare, A., 1988. Feeding, Tube-building and particle-size selection in the terebellid *Eupolymnia*
850 *nebulosa*. Mar. Biol. 97: 243-252

851 Grémare, A., Duchêne, J.C., Rosenberg, R., David, E., Desmalades, M., 2004. Feeding behavior and
852 functional response of *Abra ovata* and *A. nitida* compared by image analysis. Mar. Ecol. Prog. Ser.
853 267: 195-208

854 Grime, J.P., 1973. Competitive exclusion in Herbaceous vegetation. Nature. 242(5396): 344-347

855 Grossman, S., Reichardt, W., 1991. Impact of *Arenicola marina* on bacteria in intertidal sediments.
856 Mar. Ecol. Prog. Ser. 77: 85-93

857 Guillou, M., Hilly, C., 1983. Dynamics and biological cycle of *Melinna palmata* (Ampharetidae)
858 population during the recolonisation of a dredged area in the vicinity of the harbour of Brest
859 (France). Mar. Biol. 73: 43-50

860 Hannides, A.K., Dunn, S.M., Aller, R.C., 2005. Diffusion of organic and inorganic solutes through
861 macrofaunal mucus secretions and tube linings in marine sediments. J. Mar. Res. 63: 975-981

862 Hansen, K., King, G.M., Kristensen, E., 1996. Impact of the soft-shell clam *Mya arenaria* on sulfate
863 reduction in an intertidal sediment. Aquat. Microb. Ecol. 10: 181-194

864 Harris, J.M., 1993. The presence, nature, and role of gut microflora in aquatic invertebrates: a
865 synthesis. Microb. Ecol. 25: 195-231

866 Hendricks, I.E., Sintes, T., Bouma, T.J., Duarte, C.M., 2008. Experimental assessment and modeling
867 evaluation of the effects of the seagrass *Posidonia oceanica* on flow and particle trapping. Mar. Ecol.
868 Prog. Ser. 356: 163-173

869 Hewitt, J.E., Trush, S.F., Dayton, P.D., 2008. Habitat variation, species diversity and ecological
870 functioning in a marine system. J. Exp. Mar. Biol. Ecol. 366: 116-122.

871 Hill, T.C., Walsh, K.A., Harris, J.A., Moffett, B.F., 2003. Using ecological diversity measures with
872 bacterial communities. FEMS. Microbiol. Ecol. 43: 1-11

873 Holthe, T., 1986. Polychaeta Terebellomorpha. Marine invertebrates of Scandinavia 7.
874 Universitetsforlaget, Oslo. 194p

875 Ibanez, F., Dauvin, J.C., 1988. Long-term changes (1977 to 1987) in a muddy fine sand *Abra alba* –
876 *Melinna palmata* community from the Western English Channel: multivariate time series analysis.
877 Mar. Ecol. Prog. Ser. 49: 65-81

878 Jumars, P.A., Self, R.F.L., Nowell, A.R.M., 1982. Mechanics of particle selection by tentaculate
879 deposit feeders. J. Exp. Mar. Biol. Ecol. 64: 47-70

880 Jumars, P.A., Dorgan, K.M., Lindsay, S.M., 2015. Diet of worms emended: an update of Polychaete
881 feeding guilds. Annu. Rev. Mar. Sci. 7: 497-520

882 Kennedy, P., Kennedy, H., Papadimitriou, S., 2005. The effects of acidification on the determination
883 of organic carbon, total nitrogen and their stable isotopic composition in algae and marine sediment.
884 Rapid Commun. Mass. Spectrom. 19: 1063-1068

885 Kinoshita, K., Wada, M., Kogure, K., Furota, T., 2008. Microbial activity and accumulation of organic
886 matter in the burrow of the mud shrimp, *Upogebia major* (Crustacea: Thalassinidea). Mar. Biol.
887 153:277-283

888 Kihslinger, R.L., Woodin, S.A., 2000. Food patches and a surface deposit feeding spionid polychaete.
889 Mar. Ecol. Prog. Ser. 201: 233-239

890 Kristensen, E., Penha-Lopes, G., Delefosse, M., Valdemarsen, T., Quintana, C.O., Banta G.T., 2012.
891 What is bioturbation? The need for a precise definition for fauna in aquatic sciences. Mar. Ecol.
892 Prog. Ser. 446: 285-302

893 Laverock, B., Smith, C.J., Tait, K., Osborn, A.M., Widdicombe S., Gilbert, J.A., 2010. Bioturbating
894 shrimp alter the structure and diversity of bacterial communities in coastal marine sediments. ISME
895 J. 4: 1531-1544

896 Laverock, B., Kitidis, V., Tait, K., Gilbert, J.A., Osborn, A.M., Widdicombe, S., 2013. Bioturbation
897 determines the response of benthic ammonia-oxidizing miccroorganisms to ocean acidification. Phil.
898 Trans. R. Soc. B. 368: 20120441

899 Laverock, B., Tait, K., Gilbert, J.A., Osborn, A.M., Widdicombe, S. 2014. Impacts of bioturbation on
900 temporal variation in bacterial and archaeal nitrogen-cycling gene abundance in coastal sediments.
901 Environ. Microb. Rep. 6(1): 113-121

902 Lohrer, A.M., Trush, S.F., Hunt, L., Hancock, N., Lundquist, C., 2005. Rapid reworking of subtidal
903 sediments by burrowing spatangoid urchins. J. Exp. Mar. Biol. Ecol. 321: 155-169

904 Mac Arthur, R., Wilson, E.O., 1967. The theory of Island biogeography. Princeton University press.

905 Maire, O., Duchêne, J.C., Amouroux, J.M., Grémare, A., 2007a. Activity patterns in the terebellid
906 polychaete *Eupolymnia nebulosa* assessed using a new image analysis system. Mar. Biol. 151: 737-
907 749

908 Maire, O., Duchêne, J.C., Bigot, L., Grémare, A., 2007b. Linking feeding activity and sediment
909 reworking in the deposit-feeding bivalve *Abra ovata* with image analysis, laser telemetry and
910 luminophore tracers. Mar. Ecol. Prog. Ser. 351: 139-150

911 Maire, O., Duchêne, J.C., Grémare, A., Malyuga, V.S., Meysman, F.J.R., 2007c. A comparison of
912 sediment reworking rates by the surface deposit-feeding bivalve *Abra ovata* during summertime and
913 wintertime, with a comparison between two models of sediment reworking. J. Exp. Mar. Biol. Ecol.
914 343: 21-36

915 Maire, O., Lecroart, P., Meysman, F., Rosenberg, R., Duchêne, J.C., Grémare, A., 2008.
916 Quantification of sediment reworking rates in bioturbation research: a review. Aquat. Biol. 2: 219-
917 238

918 Maire, O., Merchant, J.N., Bulling, M., Teal, L.R., Grémare, A., Duchêne, J.C., Solan, M., 2010.
919 Indirect effects of non-lethal predation on bivalve activity and sediment reworking. J. Exp. Mar.
920 Biol. Ecol. 395: 30-36

921 Massé, C., 2014. Effets de la bioturbation sur la diversité des communautés bactériennes du sédiment.
922 Approches expérimentales et *in-situ*. De *Melinna palmata* aux communautés de la vasière Ouest-
923 Gironde. PhD thesis, university of Bordeaux, 268pp

924 Matsui, G.Y., Ringelberg, D.B., Lovell, C.R., 2004. Sulfate-reducing bacteria in tubes constructed by
925 the marine infaunal polychaete *Diopatra cuprea*. Appl. Environ. Microb. 70(12): 7053-7065

926 Mayer, L.M., Schick, L.L., Hardy, K.R., Wagai, R., McCarthy, J., 2004. Organic matter in small
927 mesopores in sediments and soils. Geochim. Cosmochim. Ac. 68(19): 3863-3872

928 McArdle, B.H., Anderson, M.J., 2001. Fitting multivariate models to community data: a comment on
929 distance-based redundancy analysis. Ecology. 82(1): 290-297

930 Mermillod-Blondin, F., Rosenberg, R., François-Carcaillet, F., Norling, K., Mauclaire, L., 2004.
931 Influence of bioturbation by three benthic infaunal species on microbial communities and
932 biogeochemical processes in marine sediment. Aquat. Microb. Ecol. 36: 271-284

933 Mermillod-Blondin, F., François-Carcaillet, F., Rosenberg, R., 2005. Biodiversity of benthic
934 invertebrates and organic matter processing in shallow marine sediments: an experimental study. J.
935 Exp. Mar. Biol. Ecol. 315: 187-209

936 Michaud, E., Desrosiers, G., Aller, R.C., Mermillod-Blondin, F., Sundby, B., Stora, G., 2009. Spatial
937 interactions in the *Macoma balthica* community control biogeochemical fluxes at the sediment
938 surface and microbial abundances. J. Mar. Res. 67: 43-70

939 Neveux, J., Lantoine, F., 1993. Spectrofluorimetric assay of chlorophylls and phaeopigments using the
940 least squares approximation technique. Deep sea research 40(1): 1747-1765

941 Normand, P., Ponsonnet, C., Nesme, X., Neyra, M., Simonet, P., 1996. ITS analysis of prokaryotes. In
942 Molecular Microbial Ecology Manual. Section 3.4.5, pp1-12. Edited by Akkermans, A.D.L., van
943 Elsas, J.D., and De Bruijn, F.J. Dordrecht: Kluwer.

944 Nowell, A.R.M., Jumars, P.A., Fauchald, K., 1984. The foraging strategy of a subtidal and deep-sea
945 deposit feeder. Limnol. Oceanogr. 29(3): 645-649

946 Olafsson, E., Moore, C.G., Bett, B.J. 1990. The impact of *Melinna palmat*a Grube, a tube-building
947 polychaete, on meiofaunal community structure in a soft-bottom subtidal habitat. Estuar. Coast.
948 Shelf. Sci. 31: 883-893

949 Osborne, C.A., Rees, G.N., Bernstein, Y., Janssen, P.H., 2006. New threshold and confidence
950 estimates for terminal restriction length polymorphism analysis of complex bacterial communities.
951 Appl. Environ. Microbiol. 72(2): 1270-1278

952 Oyenekan, J.A., 1988. Population dynamics and secondary production in *Melinna palmata*
953 (Polychaeta: Ampharetidae). Mar. Biol. 98: 247-251

954 Papaspyrou, S., Gregersen, T., Cox, R.P., Thessalou-Legaki, M., Kristensen E., 2005. Sediment
955 properties and bacterial community in burrows of the ghost shrimp *Pestarella tyrrhena* (Decapoda:
956 Thalassinidea). Aquat. Microb. Ecol. 38: 181-190

957 Papaspyrou, S., Gregersen, T., Kristensen, E., Christensen, B., Cox, R.P., 2006. Microbial reaction
958 rates and bacterial communities in sediment surrounding burrows of two nereidid polychaetes
959 (*Nereis diversicolor and N. virens*). Mar. Biol. 148: 541-550

960 Pascal, L., Maire, O., Volkenborn, N., Lecroart, P., Bichon, S., de Montaudouin, X., Grémare A.,
961 Deflandre, B., 2016. Influence of the mud shrimp *Upogebia pusilla* (Decapoda: Gebiidea) on solute
962 and porewater exchanges in an intertidal seagrass (*Zostera noltei*) meadow of Arcachon Bay: an
963 experimental assessment. J. Exp. Mar. Biol. Ecol. 477: 69-79

964 Pischedda, L., Poggiale, J.C., Cuny, P., Gilbert, F., 2008. Imaging oxygen distribution in marine
965 sediments. The importance of bioturbation and sediment heterogeneity. Acta Biotheor. 56: 123-135

966 Pischedda, L., Militon, C., Gilbert, F., Cuny, P., 2011. Characterization of specificity of bacterial
967 community structure within the burrow environment of the marine polychaete *Hediste (Nereis)*
968 *diversicolor*. Res. Microbiol. 162: 1033-1042

969 Queiros, A.M., Stephens, N., Cook, R., Ravaglioli, C., Nunes, J., Dashfield, S., Harris, C., Tilstone,
970 G.H., Fishwik, J., Braeckman, U., Somerfield, P.J., Widdicombe, S., 2015. Can benthic community
971 structure be used to predict process of bioturbation in real ecosystems? Prog. Oceanogr.137: 559-569

972 Ramette, A., 2007. Multivariate analyses in microbial ecology. FEMS. Microbiol. Ecol. 62(2): 142-
973 160

974 Ramette, A., 2009. Quantitative community fingerprinting methods for estimating the abundance of
975 operational taxonomic units in natural microbial communities. Appl. Environ. Microb.75: 2495-2505

976 Reichardt, W., 1988. Impact of bioturbation by *Arenicola marina* on microbiological parameters in
977 intertidal sediments. Mar. Ecol. Prog. Ser. 44: 149-158

978 Revsbech, N.P., 1989. An oxygen microsensor with a guard cathode. Limnol. Oceanogr. 34(2): 474-
979 478

980 Rhoads, D.C., 1974. Organism-sediment relations on the muddy sea floor. Oceanogr. Mar. Biol. Annu.
981 Rev. 12: 263-300

982 Rigaud, S., Deflandre, B., Maire, O., Bernard, G., Duchêne, J.C., Poirier, D., Anschutz, P., 2018.
983 Transient biogeochemistry in intertidal sediments: New insights from tidal pools in *Zostera noltei*
984 meadows of Arcachon Bay (France). Mar. Chem. 200: 1-13

985 Rosenberg, R., Grémare, A., Duchêne, J.C., Davey, E., Franck, M., 2008. 3D visualization and
986 quantification of marine benthic biogenic structures and particle transport utilizing computer-aided
987 tomography. Mar. Ecol. Prog. Ser. 363: 171-182

988 Rouse, G., Pleijel, F., 2001. Polychaetes. Oxford University Press, Oxford. 354p

989 Shen, H., Jiang, G., Wan, X., Li, H., Qiao, Y., Trush, S., He, P., 2017. Response of the microbial
990 community to bioturbation by benthic macrofauna on intertidal flats. J. Exp. Mar. Biol. Ecol. 488:
991 44-55

992 Sousa, W.P., 1979. Disturbance in marine intertidal boulder fields: the nonequilibrium maintenance of
993 species diversity. Ecology. 60: 1225-1239

994 Steward, C.C., Nold, S.C., Ringelberg, D.B., White, D.C., Lovell, C.R., 1996. Microbial biomass and
995 community structures in the burrows of bromophenol producing and non-producing marine worms
996 and surrounding sediments. Mar. Ecol. Prog. Ser. 133: 149-165

997 Taghon, G.L., Self, R.F.L., Jumars, P.A., 1978. Predicting particle selection by deposit feeders: a
998 model and its implications. Limnol. Oceanogr. 23(4): 752-759

999 Taghon, G.L., 1982. Optimal foraging by deposit-feeding invertebrates: roles of particle size and
1000 organic coating. Oecologia. 52: 295-304

1001 Vázquez-Rodríguez, G.A., Garabetian, F., Rols, J.L., 2007. Inocula from activated sludge for ready
1002 biodegradability testing: homogenization by preconditioning. Chemosphere. 68(8): 1447-1454

1003 Volkenborn, N., Polerecky, L., Wethey, D.S., Woodin, S.A., 2010. Oscillatory porewater bioadvection
1004 in marine sediments induced by hydraulic activities of *Arenicola marina*. Limnol. Oceanogr. 55(3):
1005 1231-1247

1006 Wada, M., Urakawa, T., Tamaki, A., 2016. Dynamics of bacterial community structure on intertidal
1007 sandflat inhabited by the ghost shrimp *Nihonotrypaea harmandi* (Decapoda: Axiidae:
1008 Callianassidae) in Tomioka Bay, Amakusa, Japan. Gene 576: 657-666

1009 Warwick, R.M., Gee, J.M., Berge, J.A., Ambrose, W.J., 1986. Effects of the feeding activity of the
1010 polychaete *Streblosoma bairdi* (Malmgren) on meiofaunal abundance and community structure.
1011 Sarsia 71(1): 11-16

1012 Wenzhöfer, F., Glud, R.N., 2004. Small-scale spatial and temporal variability in coastal benthic O2
1013 dynamics: Effects of fauna activity. Limnol. Oceanogr. 49(5): 1471-1481

1014 Wheatcroft, R.A., Jumars, P.A., Smith, C.R., Nowell, A.R.M., 1990. A mechanistic view of the
1015 particulate biodiffusion coefficient: Step lengths, rest periods and transport directions. J. Mar. Res.
1016 48: 177-207

1017 Wheatcroft, R.A., 1991. Conservative tracer study of horizontal sediment mixing rates in a bathyal
1018 basin, California borderland. J. Mar. Res. 49: 565-588

1019 Wilde, S.B., Plante, C.J., 2002. Spatial heterogeneity of bacterial assemblages in marine sediments:
1020 the influence of deposit feeding by *Balaglonossus aurantiacus*. Estuar. Coast Shelf S. 55: 97-107

1021 Wintzingerode von F., Göbel, U.B., Stackebrandt, E., 1997. Determination of microbial diversity in
1022 environmental samples: pitfalls of PCR-based rRNA analysis. FEMS. Microbiol. Rev. 21(3): 213-
1023 229

1024 Woodin, S.A., Wethey, D.S., Volkenborn, N., 2010. Infaunal hydraulic ecosystem engineers: cast of
1025 characters and impacts. Integr. Comp. Biol. 50(2): 176-187

1026 Woodin, S.A., Volkenborn, N., Pilditch, C.A., Lohrer, A.M., Wethey, D.S., Hewitt, J.E., Trush, S.F.,
1027 2016. Same pattern, different mechanism: locking onto the role of key species in seafloor ecosystem
1028 process. Sci. Rep. 6:26678

1029 Yazdani Foshtomi, M., Braeckman, U., Derycke, S., Sapp, M., Van Gansbeke, D., Sabbe, K.,
1030 Willems, A., Vincx, M., Vanaverbeke, J., 2015. The link between microbial diversity and nitrogen
1031 cycling in marine sediments is modulated by macrofaunal bioturbation. PLOS One 10(6): e0130116.
1032 doi:10.1371/journal.pone.0130116

1033 Zaabi, S., Alfi, A., 2006. Significations écologiques de la variabilité des polychaetes dans le golfe de
1034 Tunis. Bull. Inst. Natu. Scien. Tech. Mer de Salammbô. 33: 29-36

1035 Zhadan, A.E., Tzetlin, A.B., 2002. Comparative morphology of the feeding apparatus in the
1036 Terebellida (Annelida: Polychaeta). Cah. Biol. Mar. 43: 149-164

1037 Zhou, S., Bruns, M.A., Tiedje, J.M., 1996. DNA recovery from soils of diverse composition. Appl.
1038 Environ. Microbiol. 62: 316-322

1039

Figure legends:

Fig. 1: General flowcharts of the three series of experiments carried out during the present study.

Fig. 2: *First series of experiments*. Top views of the sediment surface after reworking by *Melinna palmata*; A & B differ by the location of the faecal mound relative to the tube opening. FM: Faecal Mound area; PA: Prospected Area; TO: Tube Opening; US: Undisturbed Sediment. The red arrow shows large the accumulation of large mineral particles near the tube opening.

Fig. 3: Picture of the buccal cavity and the tentacles of *Melinna palmata* obtained by stereomicroscopy (A) and scanning electron microscopy (B - F). Gi: gills; T: tentacles; L: lip; Bc: buccal cavity; As: Accordion structure; Gr: ciliary groove; Tip: tip of the tentacle; Ci: cilia.

Fig. 4: *First and third series of experiments*. Examples of images showing the different positions of the tube and the tentacles at the sediment-water interface (A-C) and sequence of 3 consecutive images picturing defaecation (D-F). A: The tube is vertical and only the tube opening can be seen; B-C: The anterior part of the tube is lying on the sediment surface, the gills of the worm are visible outside the tube in C; D: Positioning of the worm just before defecation; E: Expulsion of the faeces (yellow arrow) from the tube; F: Location of the newly deposited faeces (yellow arrow) away from the tube opening. CF: Catapulted Faeces, F: Faeces, G: Gills, NDF: Newly Deposited Faeces, T: Tube, TO: Tube Opening. Red dots indicate the particle movements detected along the tentacles.

Fig. 5: *First and third series of experiments*. Time allocation pattern of *Melinna palmata* between its different behaviors. Vertical bars are standard deviations.

Fig. 6: *First series of experiments*. Temporal changes in the: (A) total surface of reworked sediment, (B) surface of the faecal mound area, and (C) surface of the prospected area by individual worms(see text for details). Each symbol corresponds to an individual worm.

Fig. 7: *Third series of experiments*. Example of a microtopography scan. FM: Faecal Mound, PA: Prospected Area.

Fig. 8: *First series of experiments*. nMDS plot based the granulometrical and biogeochemical characteristics of surface sediments (see text for details).

Fig. 9: *First series of experiments*. nMDS plot based on aerobic bacterial community composition (see text for details).

Fig. 1

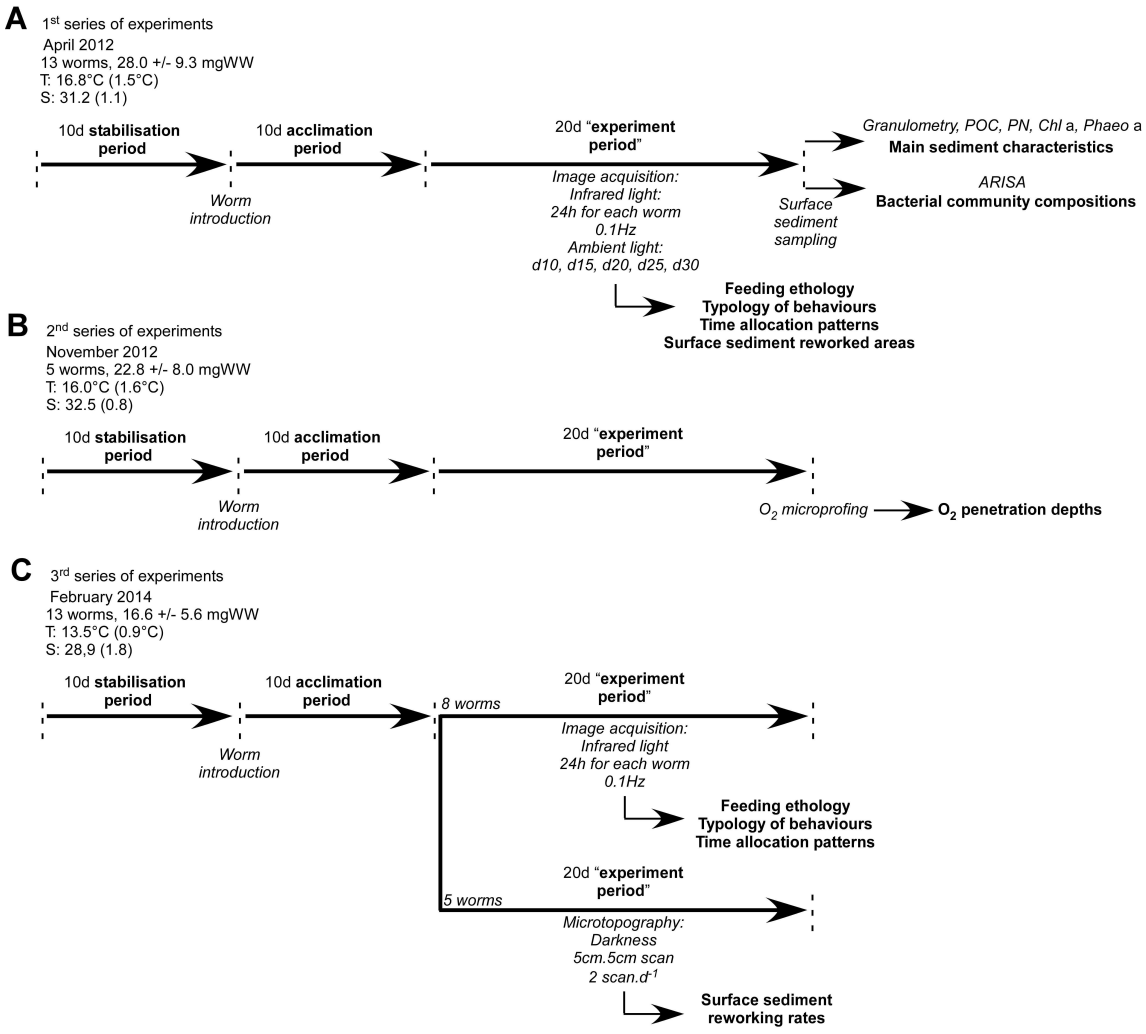

A
1st series of experiments
April 2012
13 worms, 28.0 +/- 9.3 mgWW
T: 16.8°C (1.5°C)
S: 31.2 (1.1)
10d stabilisation period
10d acclimation period
20d "experiment period"
Worm introduction
Image acquisition:
Infrared light:
24h for each worm
0.1Hz
Ambient light:
d10, d15, d20, d25, d30
Surface sediment sampling
Granulometry, POC, PN, Chl a, Phaeo a
Main sediment characteristics
ARISA
Bacterial community compositions
Feeding ethology
Typology of behaviours
Time allocation patterns
Surface sediment reworked areas
B
2nd series of experiments
November 2012
5 worms, 22.8 +/- 8.0 mgWW
T: 16.0°C (1.6°C)
S: 32.5 (0.8)
10d stabilisation period
10d acclimation period
20d "experiment period"
Worm introduction
O2 microprofing
O2 penetration depths
C
3rd series of experiments
February 2014
13 worms, 16.6 +/- 5.6 mgWW
T: 13.5°C (0.9°C)
S: 28,9 (1.8)
10d stabilisation period
10d acclimation period
8 worms
20d "experiment period"
Worm introduction
Image acquisition:
Infrared light
24h for each worm
0.1Hz
Feeding ethology
Typology of behaviours
Time allocation patterns
5 worms
20d "experiment period"
Microtopography:
Darkness
5cm.5cm scan
2 scan.d-1
Surface sediment reworking rates

Fig. 2

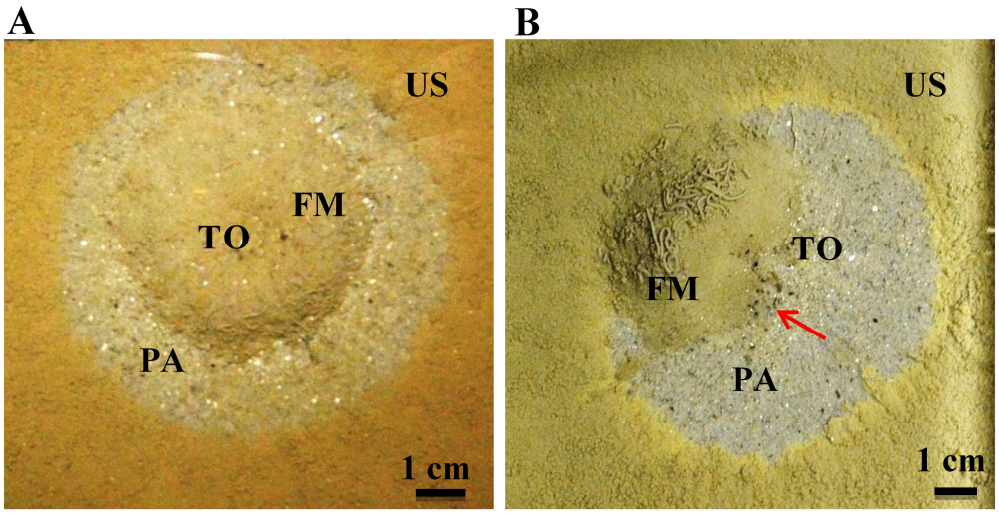
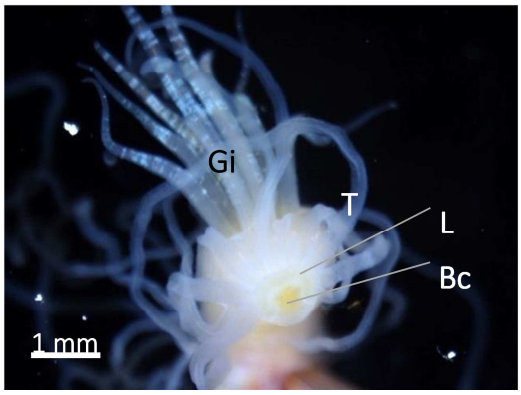
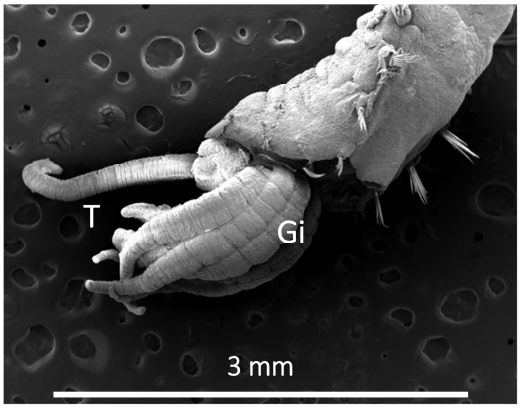


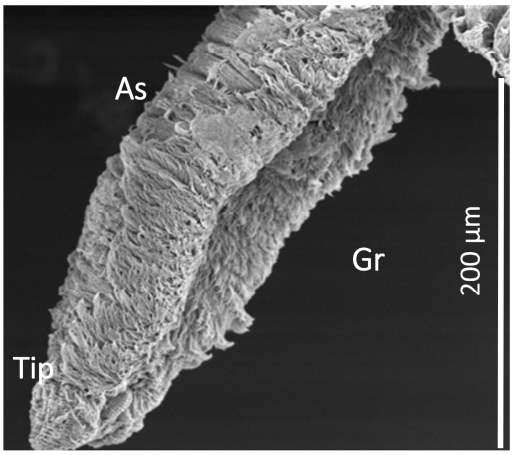
Fig. 3



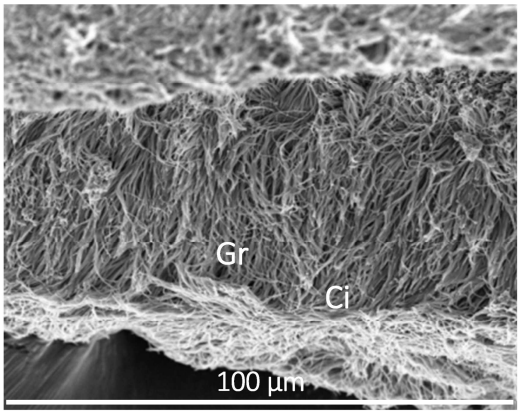
***A**: Mouth with tentacles and gill crown in background*



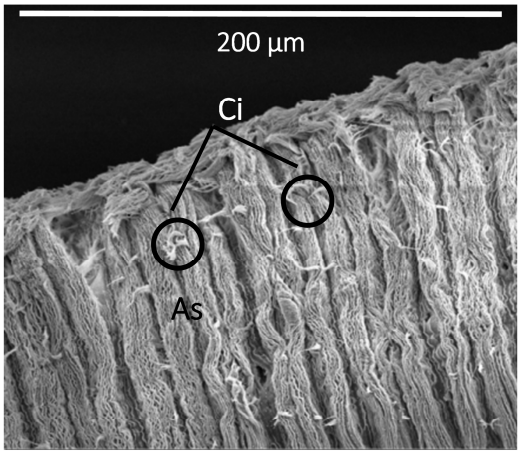
***B**: Head on lateral view with gills and a tentacle*



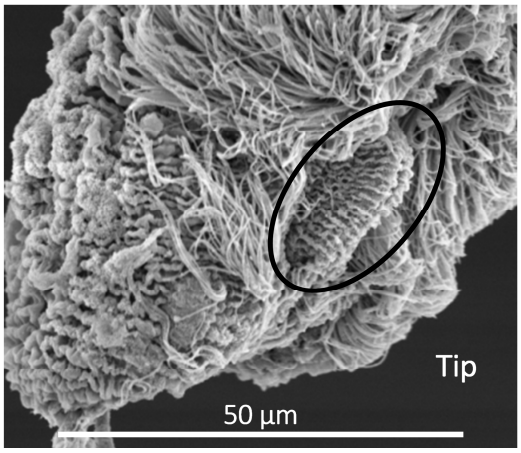
***C**: Tip of a tentacle showing the ciliary groove and the accordion structure*



***D**: Ciliary groove on the inner side of a tentacle*



***E**: Accordion structure on the outer side of a tentacle*



***F**: Particular structure close to the tip of a tentacle*

Fig. 4

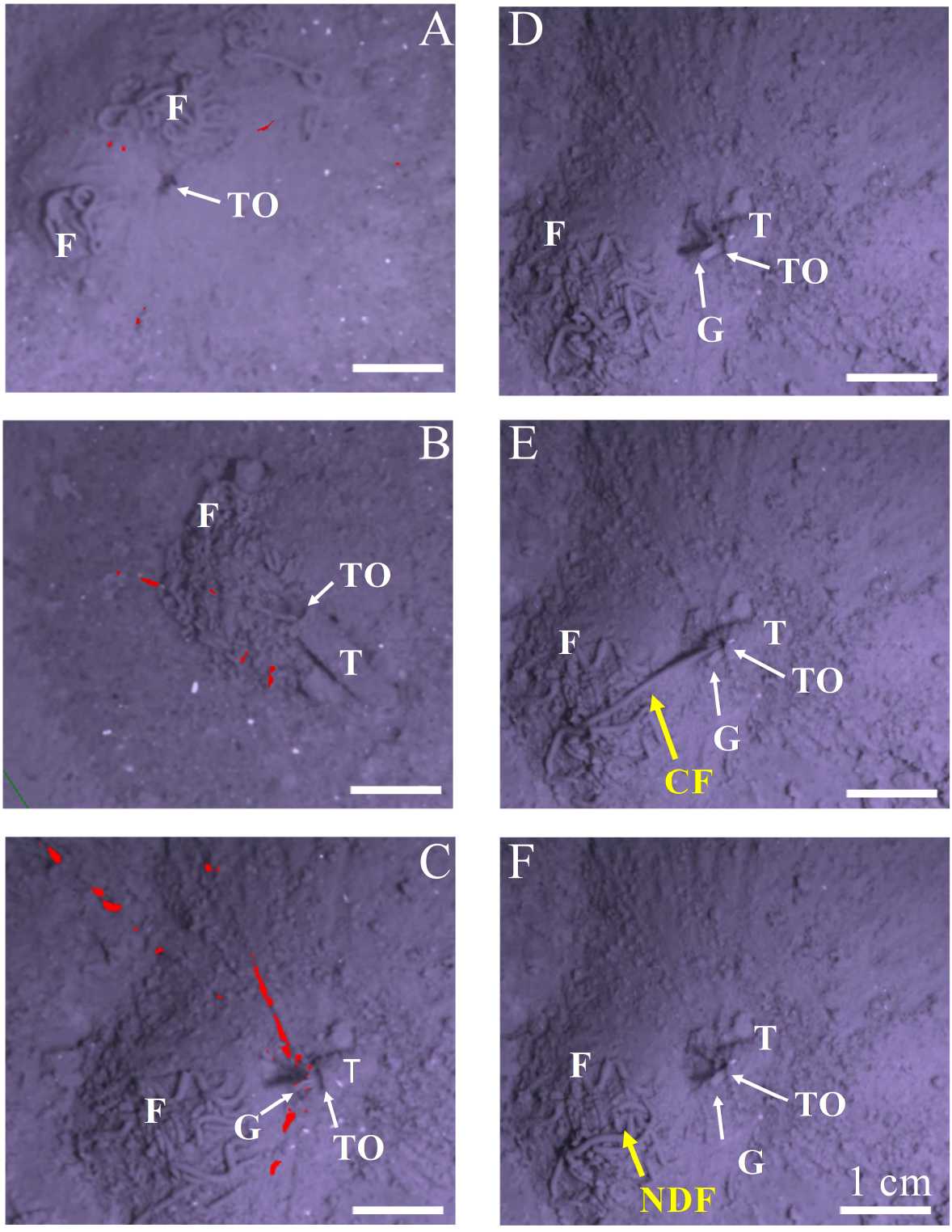


Fig. 5

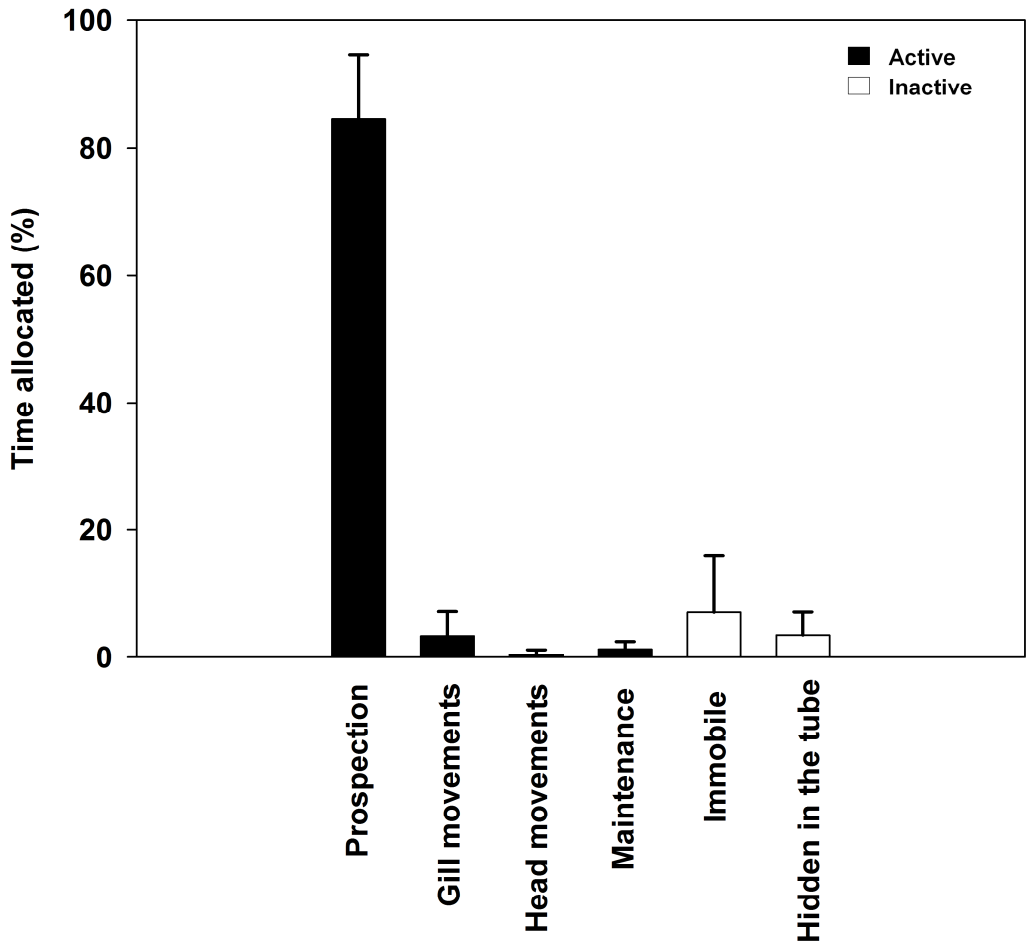

100
80
60
40
20
0
Time allocated (%)
Active
Inactive
Prospection
Gill movements
Head movements
Maintenance
Immobile
Hidden in the tube

Fig. 6

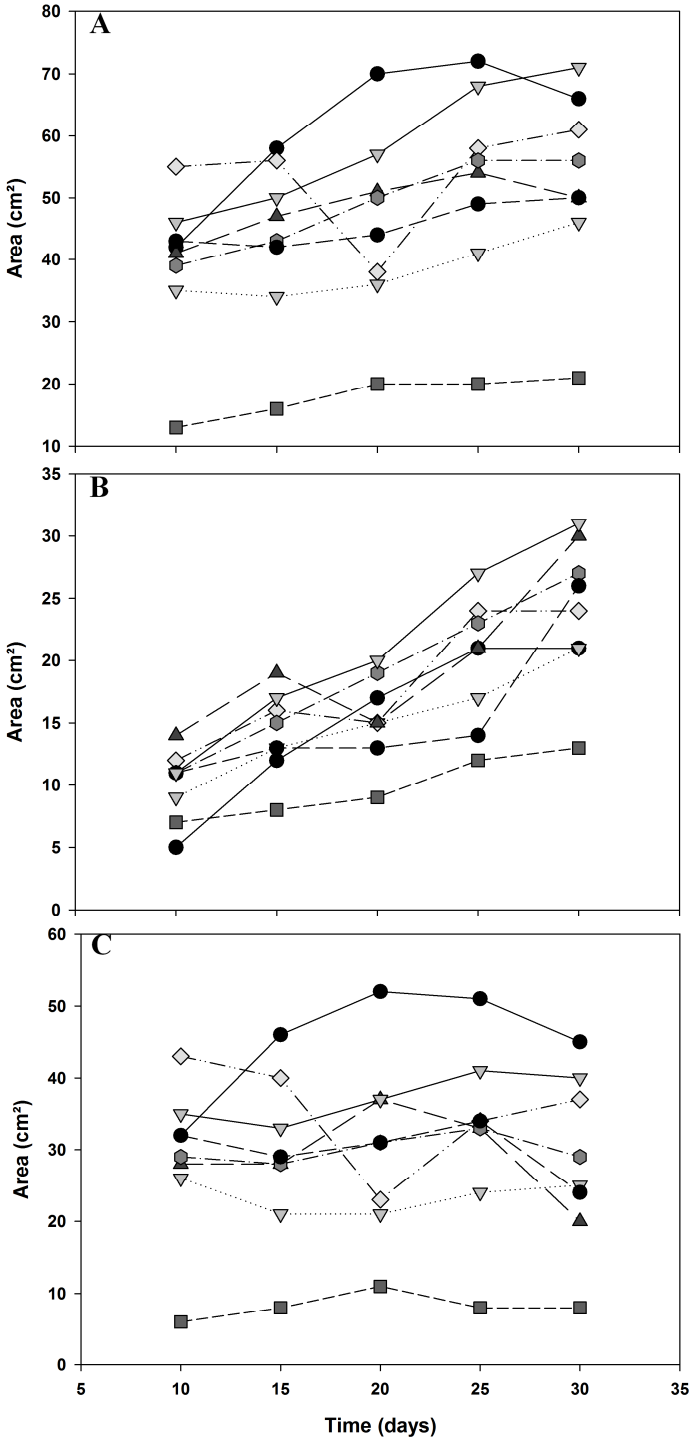


Fig. 7

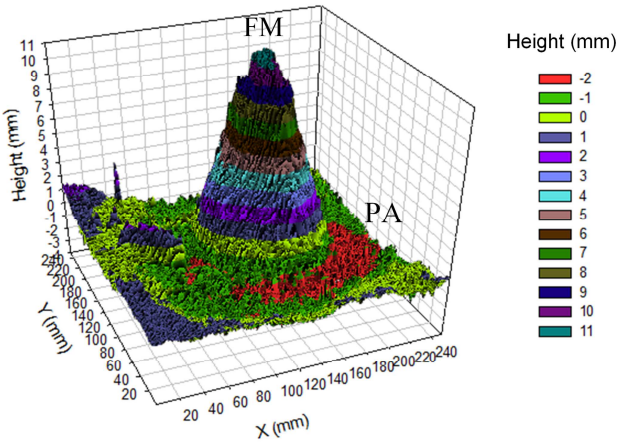


Fig. 8

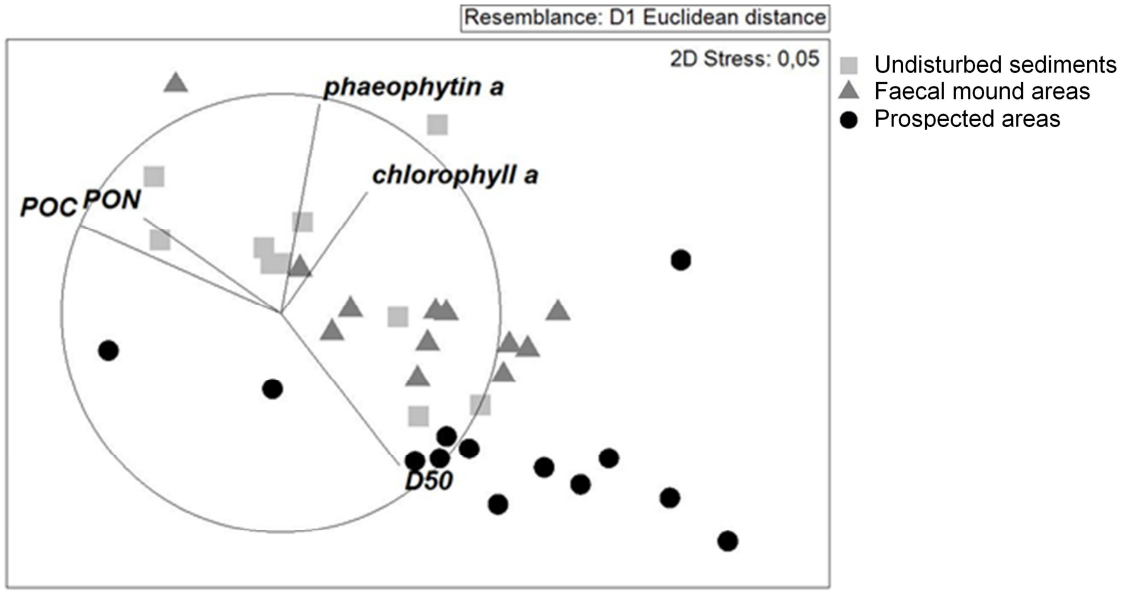

Resemblance: D1 Euclidean distance
2D Stress: 0,05
phaeophytin a
chlorophyll a
POC
PON
D50
Undisturbed sediments
Faecal mound areas
Prospected areas

Fig.9

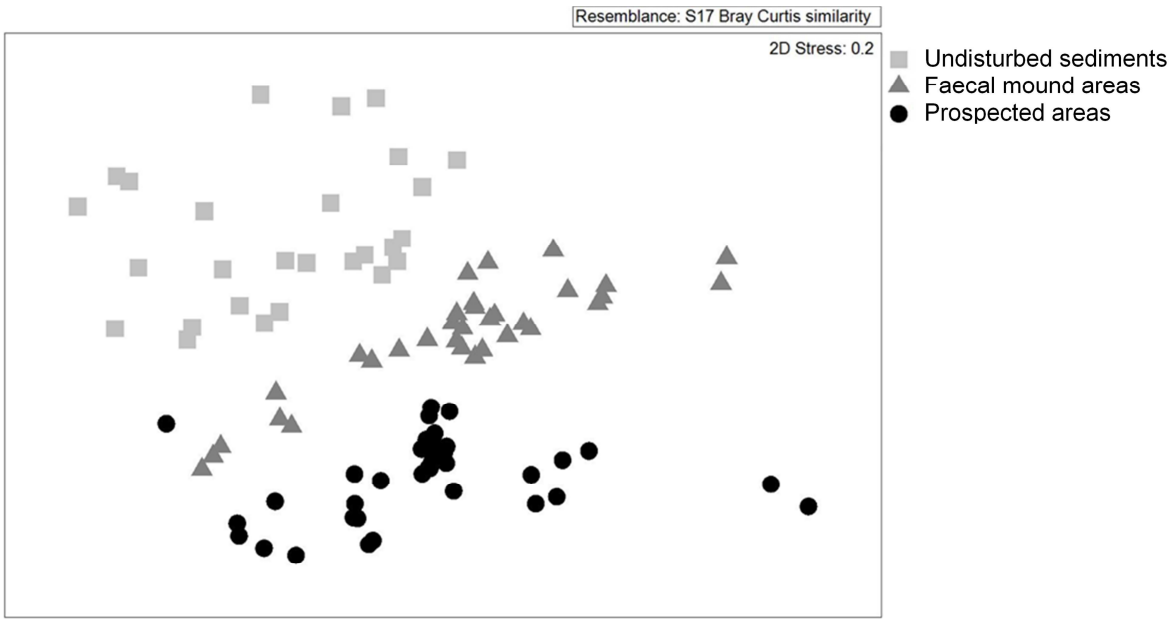

Resemblance: S17 Bray Curtis similarity
2D Stress: 0.2
Undisturbed sediments
Faecal mound areas
Prospected areas

Table legends:

Table 1: Mean ± Standard deviation of several parameters measured in the three areas defined by the sediment reworking of *Melinna palmata*: median grain size (D50), mud content, chlorophyll and phaeophytin *a*, particulate organic carbon (POC) and nitrogen (PON) and oxygen penetration depth

Table 2: Review of studies on effects of macrofaunal biotubation on bacterial abundance, biomass, activity or composition with the bioturbator used and the study type. Each number refers to a reference

TABLE 1

	Series of experiments	Mean ± Std. Dev. Undisturbed sediments	Prospected areas	Faecal mound areas
D50 (µm)	1	13.8±2.0	17.1±2.2	14.5±2.3
Mud content (% volume)	1	83.8±4.8	76.6±3.9	84.3±4.6
Chlorophyll *a* (µg/g DW)	1	1.4±0.5	1.2±0.8	1.0±0.4
Phaeophytin *a* (µg/g DW)	1	6.8±2.3	4.0±2.3	6.7±1.4
POC (mg/g DW)	1	33.5±4.0	27.0±5.4	30.0±3.6
PON (mg/g DW)	1	3.2±0.8	2.2±0.7	2.7±0.6
Oxygen penetration depth (mm)	2	4.9 ± 0.2	4.0 ± 0.1	7.2 ± 0.4

TABLE 2

Bioturbators	Study type		Effect on bacteria				Studied layers	
	Ex-situ (Experimental)	***In-situ***	**Abundance Biomass Viability**	**Production**	**Activity**	**Composition**	**Depth** (gradient, burrow vs ambient)	**Surface** (horizontal gradient)
Polychaeta								
Alitta virens	14	12	12, 14			12	12, 14	
Amphicteis sp.		3	3				3	3
Arenicola marina		4, 5, 7, 11	4, 5, 7, 11		4		4, 5, 7, 11	4, 11
Branchyoasicus americana		6	6			6	6	
Capitella capitata	2		2	2			Tubes (2)	
Diopatra cuprea		9	9			9	Tubes (9)	
Hediste diversicolor	8, 10, 13	12, 15	8,10, 12		8, 10	12, 13, 15	8, 10, 12, 13, 15	
Heteromastus filiformis	1		1				1	
Notomastus lobatus		6	6			6	6	
Perinereis aibuhitensis		33	33			33	33	
Bivalvia								
Arctica atlantica	16		16				16	
Cerastoderma edule	8, 10	7	7, 8, 10		8, 10		7, 8, 10	
Macoma balthica	1, 14		1, 14				1, 14	
Meretrix meretrix		33	33			33	33	
Mya arenaria	14	17	14, 17		17		14, 17	
Tellina texana	1		1				1	
Crustacea								
Biffarius arenosus		26	26		26	26	26	
Callianassa kraussi								
Callianassa subterranea	19					19	19	19
Callianassa trilobata		28	28			28	28	
Corophium volutator	8, 10		8, 10		8, 10		8, 10	
Neotrypaea californiensis	23, 24, 25	22, 24, 25	22		23, 24, 25	22, 24	22	22, 23, 24, 25
Nihonotrypaea harmandi		32	32			32	32	
Pestarella tyrrhena		18	18			18	18	
Uca crenulata		22	22			22	22	22
Upogebia deltaura	21	20	20, 21		21	19	20, 21	19
Upogebia major	28	28	28		28		28	
Hemichordata								
Balaglonossus aurantiacus		6, 30	6, 30		30	6	6	30
Ptychodera bahamensis		31	31			31		31

1: Aller and Yingst, 1985; 2: Alongi, 1985; 3: Aller and Aller, 1986; 4: Reichardt, 1988; 5: Grossman and Reichardt, 1991; 6: Steward et al., 1996; 7: Goñi-Urriza et al., 1999; 8: Mermillod-Blondin et al., 2004; 9: Matsui et al., 2004; 10: Mermillod-Blondin et al., 2005; 11: Andresen and Kristensen, 2002; 12: Papaspyrou et al., 2006; 13: Cuny et al., 2007; 14: Michaud et al., 2009; 15: Pischedda et al., 2011; 16: Bussmann and Reichardt, 1991; 17: Hansen et al., 1996; 19: Papaspyrou et al., 2005; 19: Laverock et al., 2010; 20: Laverock et al., 2014; 21: Laverock et al., 2013; 22: Bertics and Ziebis, 2009; 23: Bertics and Ziebis 2010; 24: Bertics et al., 2010; 25: Bertics et al., 2012; 26: Bird et al., 2000; 27: Dobbs and Guckert 1988a; 28: Kinoshita et al., 2003; 29: Branch and Pringle, 1987; 30: Wilde and Plante, 2002; 31: Dobbs and Guckert, 1988b; 32: Wada et al., 2016; 33: Shen et al., 2017