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Disentangling effects of resuspension and mixing with
offshore waters above an intertidal mudflat

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1 **Running title: Microorganism dynamics in a mudflat during rising tide**

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4 **Microorganism dynamics during a rising tide: Disentangling effects of resuspension and**
5 **mixing with offshore waters above an intertidal mudflat**

6

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28 **Abstract**

29 Resuspension of microphytobenthic biomass that builds up during low tide has been
30 acknowledged as a major driver of the highly productive food web of intertidal mudflats. Yet,
31 little is known about the contribution to pelagic food web of the resuspension of other
32 microorganisms such as viruses, picoeukaryotes, cyanobacteria, bacteria, nanoflagellates, and
33 ciliates, living in biofilms associated with microphytobenthos and surficial sediment. In the
34 present study, a novel approach that involves simultaneous Lagrangian and Eulerian surveys
35 enabled to disentangle the effects of resuspension and mixing with offshore waters on the
36 dynamics of water column microorganisms during a rising tide in the presence of waves.
37 Temporal changes in the concentration of microorganisms present in the water column were
38 recorded along a 3 km cross-shore transect and at a fixed subtidal location. In both surveys,
39 physical and biological processes were separated by comparing the time-evolution of
40 sedimentary particles and microorganisms concentrations. During a rising tide, sediment
41 erosion under waves action occurred over the lower and upper part of the mudflat, where
42 erodibility was highest. Although erosion was expected to enrich the water column with the
43 most abundant benthic microorganisms, such as diatoms, bacteria and viruses, enrichment
44 was only observed for nanoflagellates and ciliates. Grazing probably overwhelmed erosion
45 transfer for diatoms and bacteria, while adsorption on clayed particles may have masked the
46 expected water column enrichment in free viruses due to resuspension. Ciliate enrichment
47 could not be attributed to resuspension as those organisms were absent from the sediment.
48 Wave agitation during the water flow on the mudflat likely dispersed gregarious ciliates over
49 the entire water column. During the rising tide, offshore waters imported more autotrophic,
50 mainly cyanobacteria genus *Synechococcus* sp. than heterotrophic microorganisms, but this
51 import was also heavily grazed. Finally, the water column became a less heterotrophic
52 structure in the subtidal part of the semi-enclosed bay, where mixing with offshore waters
53 occurs (50% decrease), compared to the intertidal mudflat, when resuspension occurs. The

54 present study suggests that this differential evolution resulted predominantly from dilution
55 with offshore waters less rich in heterotrophic microorganisms. Indeed, any input of
56 microorganisms accompanying physical transfers due to bed erosion or offshore waters
57 mixing was immediately buffered, probably to the benefit of grazers.

58

59 **1. Introduction**

60 The productivity of coastal systems, especially intertidal mudflats, and their capacity
61 to enrich adjacent terrestrial and marine zones through trophic pathways (i.e. export by mobile
62 consumers) and hydrodynamic pathways (i.e. waves, wave-generated currents, estuarine
63 currents and tides) is now common knowledge. The biological productivity of intertidal
64 mudflats is due to the intense activity of benthic microorganism communities. During
65 emersion, epipellic diatoms (microphytobenthos, MPB) form a biofilm (up to 20 mg
66 chlorophyll *a* m²) in the top centimeter layer of a mud surface (Blanchard and Cariou-Le Gall
67 1994; Blanchard et al. 1997; Herlory et al. 2004). Prokaryote communities are associated with
68 this biofilm, and bacterial production (secondary production) can be as high or even higher
69 than MPB production (primary production) (Cammen 1991; Garet 1996; Van Duyl and Kop
70 1994). Bacterial concentration is generally about 10⁹ cells per cm³ in a mudflat (Schmidt et al.
71 1998). Nanoflagellate concentrations range from 100 to several million cells per mL of
72 sediment (Gasol 1993), with greater concentrations in surficial sediment (Alongi 1991).
73 Conversely, ciliates are more abundant in fine sand (Fenchel 1969; Kemp 1988; Epstein
74 1997) compared to muddy sediment enriched with organic matter (Giere 1993). Viruses are
75 also abundant in marine sediments (Danovaro et al. 2008).

76 Resuspension of microorganisms living either in the pore water of surficial sediment
77 or attached to surficial sedimentary particles have been reported under tidal currents at
78 subtidal sites (Shimeta et al. 2002). Large tidal currents in macrotidal bays are likely to induce
79 unconsolidated sediment resuspension (Mehta et al. 1989). Yet, resuspension of sediment

80 across intertidal mudflats, where recurrent desiccation promoted sediment consolidation
81 (Anderson and Howel 1984), generally requires higher shear stress than those induced by tidal
82 current and has been mainly attributed to wave action (Bassoulet et al. 2000; French et al.
83 2008). However, sediment erodibility thresholds may be significantly reduced (bed friction
84 velocity below 3 cm s^{-1}) due to macrofaunal bioturbation activity (Orvain et al. 2007).

85 Resuspended microorganisms may greatly affect pelagic and benthic food webs.
86 Resuspended diatoms and autotrophic nanoflagellates may alter phytoplankton community
87 structure, enhance phytoplankton biomass and modify the size structure of primary producers,
88 ultimately modifying microbial food web function (Marquis et al. 2007; Ory et al. 2010). In
89 addition, resuspended heterotrophic cells, such as nanoflagellates, prokaryotes (bacteria and
90 archaea) and viruses, can affect the function of the food web, and favour the microbial loop or
91 the viral shunt (Wainright 1987; Garstecki et al. 2002; Seymour et al. 2007). Furthermore,
92 some of these resuspended microorganisms may be used as food resources for
93 mesozooplankton and benthic suspension feeders (Carlson et al. 1984), such as oysters
94 (Dupuy et al. 2000) and bivalve mollusks (*Scrobicularia plana*) (Hughes 1969).

95 Many studies have investigated the dynamics of the MPB biomass, including
96 resuspension of these microorganisms (Lucas et al. 2000; Shimeta et al. 2002; Guarini et al.
97 2008). Some studies have qualitatively and quantitatively evaluated the resuspension of other
98 microorganisms present in the intertidal mudflat. Protist and bacteria resuspension thresholds
99 have been quantified at a subtidal coastal site with *in situ* flumes and sampling of the benthic
100 boundary layer during tidal accelerations (Shimeta and Sisson 1999; Shimeta et al. 2002).
101 Shimeta et al. (2003) studied the resuspension of benthic protists at subtidal coastal sites with
102 differing sediment compositions. Other studies have explored the effects of sediment
103 resuspension on a coastal planktonic microbial food web, either experimentally (Garstecki et
104 al. 2002; Pusceddu et al. 2005; Wu et al. 2007) or in the field (Grémare et al. 2003). However,
105 in field studies, resuspension is often accompanied by other physical processes, such as river

106 flooding and tidal rise, which require adapted sampling strategies to separate the contribution
107 of each process.

108 In the current study, we applied a novel approach based on two simultaneous
109 Lagrangian and Eulerian field surveys to disentangle the effect of resuspension and mixing
110 with offshore waters on the dynamics of water column microorganisms during a tidal flow.
111 The time-evolution of microorganisms, including viruses, autotrophic protists, heterotrophic
112 protists and prokaryotes present in the water column, were carried out at one fixed location
113 (Eulerian) and one mobile station (Lagrangian) in the Marennes-Oleron bay (French Atlantic
114 coast). In both surveys, physical and biological processes were separated by comparing the
115 time-evolution of suspended sediment particles and microorganisms concentrations.

116

117 **2. Methods**

118 *2.1 Study site and sampling strategy*

119 The study was carried out during the afternoon rising tide (tidal amplitude of 3.8 m) in
120 the Bay of Marennes-Oléron (BMO) on 24 July, 2008. Located between the mainland French
121 Atlantic coast and Oléron Island, BMO is a macrotidal bay with a tidal range up to 6 m during
122 spring tides. This macrotidal system is influenced by continental inputs, mainly from the
123 Charente River to the north of the BMO (monthly average discharge was $30 \text{ m}^3 \text{ s}^{-1}$ in July
124 2008, slightly less than the median value of $40 \text{ m}^3 \text{ s}^{-1}$ over the last ten years). The BMO
125 covers 170 km^2 , of which 60 km^2 are intertidal mudflats. The Brouage mudflat is $>4 \text{ km}$ wide,
126 and its sediment consists of silt and clay particles (95% of $<63 \mu\text{m}$, median grain size $d_{50} = 10$
127 μm). Triplicate samples of the first 1 cm layer of sediment were taken at the end of low tide in
128 the upper region of the mudflat (Fig. 1). Only concentrations of bacteria and viruses were
129 assessed in surficial sediment. Two simultaneous field surveys (Lagrangian and Eulerian)
130 were carried out to separate the effect on the dynamics of water column microorganisms of
131 resuspension and mixing with offshore waters during tidal flow. Adopting a Lagrangian

132 strategy avoided transport flux gradients that occur in an Eulerian survey. Such transport flux
133 gradients occur as the water level changes in an Eulerian survey and pelagic concentrations
134 are generally expected to be mixed (diluted or enriched) by offshore waters in proportion to
135 water depth. Conversely, no mixing (especially dilution) is expected in a Lagrangian survey,
136 where the water level remains constant. In both types of surveys, temporal changes in
137 concentrations of a group of organisms, or particulate or dissolved matter that depart from
138 these expectations indicate an imbalance between the many biogeochemical processes that
139 potentially affect this group (Fig. 2). For particulate matter or living low-motility cells, an
140 overall increase in concentration indicates that either benthic resuspension or population
141 growth dominate, whereas a decrease indicates that either sedimentation mediated by sorption
142 on matters or population decay (e.g. grazing) dominates.

143 Before organizing the field measurements, a 2D barotropic model was used to compute
144 using a backward procedure the trajectory of a drifter reaching the shore in the northern part
145 of the Brouage mudflat (Fig. 1) at the end of the rising tide (Nicolle and Karpytchev 2007).
146 The model used a high resolution, finite element grid and TELEMAC software to solve the
147 depth integrated equations of Saint Venant (Hervouet and Van Haren 1994; Hervouet 2007).
148 The Eulerian subtidal station was located at the origin of the drifter trajectory for the case of a
149 no-wind tidal circulation in the BMO (Fig. 1).

150 The Lagrangian survey consisted of tracking a submerged buoy following the tidal front
151 during the first 2 h 45 min of the rising tide over the intertidal Brouage mudflat, between
152 15:45 h and 18:30 h local time (Fig. 1). The tidal front travelled roughly at a speed of 30 cm s⁻¹.
153 Cylindrical drifters (46 cm in diameter and 50 cm in height) were used to track the
154 advancing tidal currents into the BMO. Drifter walls were made of plastic film wrapped
155 around a thin metal rod armature and a 10 cm plastic spherical buoy was fixed on its top. The
156 drifter was completely immersed to be directed by surface currents and the buoyant sphere
157 which emerged from water was used to keep track of the drifter position. The drifter was

158 tracked using a flatboat which engine was stopped and left drifting for at least 5 min before
159 sampling in order to avoid bottom resuspension artefacts. Water samples were obtained at five
160 different stations along the 3 km cross-shore transect (Fig. 1) on 24 July, 2008. Due to rapid
161 drifting when the engine was stopped before sampling, first Lagrangian station was already
162 located a 100 m away from the Eulerian station. Water depth varied little during the
163 Lagrangian survey, ranging from 40 to 70 cm. One sample was collected in the middle of the
164 water column at each station using a 1 L plastic bottle attached to a graduated stick. Short
165 wave agitation combined with limited water depth during the Lagrangian survey did not allow
166 sampling with an open-ended Nisking bottle, so a smaller closed-bottom bottle was used.
167 However, sampling bias due to accumulation of settling particles in a closed-bottom bottle
168 was reduced by a fast sampling lasting less than a few seconds and by introducing the bottle
169 upside down. Since wave length was greater than water depth, short wave agitation ensured
170 sufficient mixing over the entire water depth, making it safe to assume no stratification for all
171 sampled microorganisms (except very close to the bottom) occurred during the Lagrangian
172 survey. Each individual sample was divided into multiple aliquots to perform replicate counts.
173 Immediately before preparation of aliquots, each sample was gently agitated and subsampling
174 was performed very quickly to prevent sedimentation.

175 The Eulerian survey consisted of sampling the water column with horizontal 3 L Niskin
176 bottles at the same subtidal spot during the rising tide, starting at low tide (Fig. 1). Water
177 depth at the sampling station increased from 1.10 m at the beginning of the survey to 3.1 m at
178 the end of sampling. Three water samples were taken 0.5 m below the surface and 0.5 m
179 above the bottom of the water column during the first 2 h 45 min of the rising tide on 24 July,
180 2008. Aliquots of each sample were prepared as described above.

181

182 Mean and standard deviation of all variables at the beginning of the rising tide were
183 computed from the three samples taken at time zero of the Eulerian and Lagrangian surveys.

184 An 80% confidence interval for this initial measurement was computed using a Student law
185 ($t_{n-1}(1-\alpha)=1.886$, $\alpha=10\%$). Evolution of the concentration of particulate inorganic matter
186 (PIM) and each microorganism at the Eulerian site were predicted using on a simple mixing
187 model based on water depth evolution, assuming that the (1) water column was well-mixed at
188 the Eulerian site and (2) concentration of PIM or each microorganism in incoming waters
189 could be estimated from the average concentration measured to the north of the BMO basin
190 taken before the experiment then 15 days after. The average concentration PIM or each
191 microorganism expected after mixing with offshore waters during tidal flow, C_t , was
192 calculated during the Eulerian survey for sampling times after 1h15min and 2h45min as
193 follows:

$$C_t = C_0 \frac{h_0}{h_t} + C_{ext} \left[1 - \frac{h_0}{h_t} \right]$$

194
195 where C_0 is the average concentration at time zero of the Eulerian survey, h_0 is the water depth
196 at this time, h_t is the water depth at time t and C_{ext} is the average offshore concentration. An
197 80% confidence interval for this predicted value was build applying a Student law ($n=3$) using
198 standard deviation of C_0 and C_{ext} . Backward trajectories reaching the Eulerian survey
199 sampling station after 1h15min and 2h45min were also computed for the the case of no-wind
200 tidal circulation in the BMO with the 2D barotropic model and indicated that offshore waters
201 most probably came from the northern entrance of the BMO, west of the Charente river
202 mouth (Fig. 1). In the absence of river flooding over the summer period, the impact of the
203 Charente river on the pelagic ecosystem was limited at this location (Stanisière, personal
204 communication), allowing us to assume that the concentration in the north of the BMO was
205 spatially uniform (Ory et al. 2010). Offshore concentrations at 0.5 m below the surface were
206 measured on July 12 and 29, 2008, at a subtidal station roughly 15 km northwest of the BMO
207 (water depth = 17 m, 46.1153°N, 01.4139°Wr). Values for C_{ext} on July 24 were interpolated
208 by taking the mean between offshore concentrations at the two dates (see Table 1) and

209 uncertainty on offshore concentrations on the day of the survey was accounted for using
210 standard deviation between the two dates. Variability estimate based on those two days are
211 similar to within day variability (H. Montanié, personal communication) and to within
212 summer variability (Ory et al., 2010) in the north of BMO basin.

213

214 *2.2 Physical forcings*

215 Hydrological parameters, such as temperature and salinity, were recorded on board
216 with multi-parameter probes (YSI 6600EDS-M) during the Eulerian survey. Average wind
217 speed and direction were measured every hour by Météo-France at the La Rochelle
218 Aéroport meteorological station (46.1733°N, 1.1883°W, roughly 20 km north of the BMO).
219 A single-point Nortek acoustic Doppler velocimeter (ADV) was used to measure the 3D
220 velocity at 15.6 cm a.b. (above bottom, outside the wave boundary layer which thickness
221 yielded 3 cm at maximum, Fredsoe and Deigaard,1992) and pressure as soon as the tide level
222 was higher than 50 cm at the location 45.9161°N, 1.0890° W (Fig. 1). Thus, ADV data
223 collection was interrupted during low tide from 12:45 h to 18:15 h on 24 July, 2008.
224 Measurements consisted of 2 min 30 s time series recorded at a frequency of 32 Hz every 15
225 min for the three velocity components and pressure. Turbulent Reynolds shear stress outside
226 the wave boundary layer were computed as the covariance of horizontal and vertical velocity
227 deviations from the mean flow after removing wave-induced velocities. Wave-induced
228 velocities were computed by applying a 0.5 Hz low pass filtering on raw velocity
229 measurements (Guizien et al. 2010). Assuming a logarithmic boundary layer, bed shear stress
230 associated with tidal current was then linearly extrapolated to the bed using Reynolds shear
231 stress measured at 15.6 cm a.b. and zero Reynolds shear stress at the free surface.

232 Wave density spectra were computed from pressure time series sampled at 4 Hz and
233 used to derive the wave parameters (i.e. mean spectral period, T_m , and significant height, H_s)
234 over the Brouage mudflat. Assuming that the wave field was uniform over the mudflat, wave

235 orbital velocities at 40 cm a.b. were derived from T_m and H_s , according to the linear wave
236 theory for varying water depths, D , at the Eulerian survey site and for a constant water depth
237 of 40 cm during the Lagrangian survey. Time-dependent bed shear stress associated with a
238 sine wave with these orbital velocities are described by their maximum value and average
239 value over a wave period (half the maximum value) using Guizien and Temperville (1999)
240 parameterization. Bed roughness was assumed to be 0.5 cm based on the bioroughness height,
241 which could be visually estimated on the mudflat. By definition, bed friction velocity (u^*)
242 either due to waves or to the tidal current is the square root of the bed shear stress divided by
243 seawater density.

244

245 *2.3 Particulate inorganic matter (PIM)*

246 Total particulate matter was measured as previously described by Aminot and
247 Chaussepied (1983). Filters were combusted at 490°C for 2 h to eliminate organic carbon
248 content and then weighed. A water sample (from 300 to 1000 mL) was filtered onto a
249 Whatman GF/C (47 mm in diameter) under <10 mm Hg vacuum pressure. After sample
250 filtration, each filter was rinsed twice with MilliQ water to remove salt, dried at 60°C for 12 h
251 then weighed to measure total particulate matter. Filters were combusted at 490°C for 2 h to
252 eliminate organic carbon content and then weighed to determine content of particulate
253 inorganic matter (PIM).

254

255 *2.4 Enumeration of microorganisms*

256 Water samples (up to 50 mL) were stained with 1% alkaline Lugol's solution to
257 visualise microplanktonic cells, such as diatoms, dinoflagellates and ciliates, ranging from 20
258 to 200 μm . Microphytoplanktonic cells were counted using an Utermöhl settling chamber
259 (Hydro-Bios® combined plate chambers) under an inverted microscope. Taxonomic
260 determination was carried out in accordance with systematics literature (Nezan, 1996; Ricard,

261 1987; Sournia, 1986), and benthic species were differentiated from planktonic species based
262 on knowledge of the habitat.

263 For nanoplanktonic cells (3 to 15 μm), water samples (up to 20 mL) were fixed using
264 1% paraformaldehyde then stained with DAPI (4',6'-diamidino-2-phénylindole). Autotrophic
265 nanoflagellates (ANF) and heterotrophic nanoflagellates (HNF) were counted as previously
266 described by Dupuy et al. (1999), which was a modified methodology from Haas (1982),
267 Caron (1986), and Sherr et al. (1994).

268 For picoeukaryotes (or picophytoeukaryotes) and *Synechococcus* (1 to 2 μm), water
269 samples (3 mL) were fixed using 2% formaldehyde, frozen in liquid N_2 , and counted using a
270 FACSCan flow cytometer (Bd-Bioscience) as previously described by Marie et al. 2000.

271 For viruses (20 nm), triplicate water samples (3 mL) were fixed in filtered 2%
272 formaldehyde and stored for less than a day at 4°C. Samples were then filtered through 0.02
273 μm Anodiscs (25 mm, Whatman) and counted using epifluorescence microscopy after
274 staining for 30 min with Sybr Green I (Noble and Fuhrman, 1998). Viruses were counted in at
275 least 15 random fields of view under blue excitation (Zeiss Axioskop 1000x). Only free
276 viruses were enumerated.

277 For bacteria (1 to 2 μm), triplicate water samples (3 mL) were fixed with filtered 2%
278 formaldehyde and stored for less than a day at 4°C. Samples were filtered through 0.02 μm
279 Anodiscs (25mm, Whatman). Samples were enumerated using epifluorescence microscopy
280 after staining for 30 min with Sybr Green I (Noble and Fuhrman 1998). Bacteria were counted
281 in at least 15 random fields of view under blue excitation (Zeiss Axioskop 1000x). Free
282 bacteria were counted with a fixed focalisation on the filter surface while attached bacteria on
283 aggregates deposited on the filter were screened by varying focal distance.

284 For each group of autotrophic or heterotrophic organisms, biomass was calculated
285 using conversion factor for cell to carbon content (Table 2) multiplied by abundance.

286

287 **3. Results**

288 *3.1 Physical forcings*

289 On 24 July, a thermal wind in the morning turned from the southeast (land) to the west
290 (sea), reaching a maximum speed of 8.7 m s^{-1} around low tide at 15:00 h (Fig. 3a). Short
291 period waves, with a mean period ranging from 2 to 4 s, started growing under wind action
292 after 11:00 h during ebb tide. At 12:30 h, when the ADV emerged, significant wave height
293 was already 0.15 m and had increased up to 0.2 m at 18:30 h when ADV was submerged
294 again at the end of the rising tide (Fig. 3a). Given the slight decrease in wind speed after
295 15:00 h and based on personal observation, wave height was at least 0.2 m during the survey
296 period from 15:45 h to 18:30 h. Therefore, a wave height of 0.2 m was used to estimate bed
297 friction velocity caused by waves. Maximum and mean bed friction velocity due to waves
298 were at least 4.5 cm s^{-1} and 3.2 cm s^{-1} , respectively, during the Lagrangian survey under 0.4 m
299 water depth. In contrast, maximum and mean bed friction velocities due to waves were less
300 than 2 cm s^{-1} and 1.4 cm s^{-1} , respectively, when the Eulerian survey started at low tide. These
301 values continuously decreased to 0.5 cm s^{-1} and 0.35 cm s^{-1} , respectively, as the water level
302 rose (Fig. 3b). Bed friction velocity induced by the tidal current reached a maximum value of
303 1.3 cm s^{-1} when the tidal flow arrived at the ADV location (water depth of 0.5 m) and rapidly
304 decreased to less than 0.5 cm s^{-1} during the rest of the rising tide over the intertidal mudflat.

305

306 *3.2 Changes in the concentration of particulate inorganic matter during the Eulerian* 307 *and Lagrangian surveys*

308 PIM concentration displayed a large spatial variability at the beginning of the rising tide
309 surveys (Fig. 4), with a value that was twice as high in the subtidal site (300 mg L^{-1})
310 compared to the first Lagrangian sampling station (140 mg L^{-1}). However, at the end of the
311 Lagrangian survey, the PIM concentration had increased more than five-fold, up to 1180 mg
312 L^{-1} , largely overpassing the 80% confidence interval around the initial average concentration.

313 Moreover, PIM concentration steadily increased at an average rate of $0.11 \text{ mg L}^{-1} \text{ s}^{-1}$ as tidal
314 flow progressed over the mudflat (Fig. 4). Meanwhile, the rate of increase in the PIM
315 concentration was not constant throughout the survey, reaching $0.18 \text{ mg L}^{-1} \text{ s}^{-1}$ during the first
316 hour, falling to $0.02 \text{ mg L}^{-1} \text{ s}^{-1}$ during the next 40 min and again increasing to $0.12 \text{ mg L}^{-1} \text{ s}^{-1}$
317 for 30 min before falling again to $0.05 \text{ mg L}^{-1} \text{ s}^{-1}$ during the last 30 min. Assuming a uniform
318 vertical PIM concentration over the 0.4 m water depth due to strong wave agitation, erosion
319 rates decreased from $460 \text{ mg m}^{-2} \text{ s}^{-1}$ in the lower part of the mudflat during the first hour to 60
320 $\text{mg m}^{-2} \text{ s}^{-1}$ in the middle part of the mudflat during the next 40 min and increased again to 300
321 $\text{mg m}^{-2} \text{ s}^{-1}$ during the next 30 min. In contrast, in the Eulerian survey, PIM concentration
322 decreased, with values remaining within the 80% confidence interval of predicted dilution by
323 offshore waters (Fig. 4).

324

325 *3.3 Changes in the concentration of autotrophic microorganisms during the Eulerian* 326 *and Lagrangian surveys*

327 The dynamics of the various taxa of autotrophic microorganisms counted during the
328 Lagrangian and Eulerian surveys was compared to initial concentration and predicted changes
329 by mixing with offshore waters, respectively. At the beginning of both surveys, two third of
330 diatoms were benthic species. This proportion increased during the Lagrangian survey to
331 nearly 80%. In the Eulerian survey, this proportion decreased to less than 20%, reflecting the
332 differential effect of resuspension and mixing with offshore waters on water column
333 microorganism dynamics. Although changes in benthic diatom concentration during the
334 Lagrangian survey suggested alternate phases of gain in the lower and upper parts of the
335 mudflat, where erosion was the greatest, and loss in the middle regions of the mudflat, none of
336 these changes were beyond the 80% confidence interval of the initial concentrations
337 determined at the beginning of the survey (Fig. 5a). However, the decrease in benthic diatom
338 concentration was not statistically significant. In contrast, a predicted significant increase in

339 pelagic diatoms was observed during the Eulerian survey. During the Lagrangian survey,
340 changes in pelagic diatoms concentration were again not statistically significant given the
341 large variability observed at the beginning of survey (Fig. 5b).

342 Smallest autotrophs were mainly comprised of ANF (0.6 to 1×10^4 cells mL^{-1}) in both
343 surveys, whereas picophytoeukaryotes and *Synechococcus* sp. (e.g. cyanobacteria) displayed
344 very low concentrations (Fig. 5b-d). During the Lagrangian survey, ANF concentration nearly
345 doubled during the first 1h15 min, departing from 80% confidence interval around value
346 when surveys started, and then decreased during the next hour (Fig. 5b). During the Eulerian
347 survey, ANF concentration displayed opposite dynamics at the surface and at the bottom. In
348 any case, by the end of the survey, values at the surface and at the bottom overpassed the 80%
349 confidence interval around values predicted from dilution with offshore waters.

350 Picoeukaryotes concentration steadily decreased during the Lagrangian survey.
351 However, this trend was not statistically significant given the large variation coefficient at the
352 beginning of survey (Fig. 5c). During the Eulerian survey, picoeukaryote concentration
353 displayed opposite dynamics at the surface and at the bottom, increasing at the surface and
354 decreasing at the bottom (Fig. 5c). However, values were never outside the large 80%
355 confidence interval around values predicted from mixing with offshore waters.

356 The concentration of *Synechococcus* sp. remained fairly constant (4.5×10^3 cells mL^{-1})
357 during both surveys (Fig. 5d), with values lower than the 80% confidence interval of
358 predicted mixing by offshore waters.

359

360 *3.4 Changes in the concentration of heterotrophic microorganisms during the*
361 *Eulerian and Lagrangian surveys*

362 During the Lagrangian survey, free virus concentration steadily decreased from $1.2 \times$
363 10^7 particles mL^{-1} to 7×10^6 particles mL^{-1} (Fig. 6a). However, this decrease was not
364 statistically significant. During the Eulerian survey, free virus concentration at the surface

365 steadily decreased within the 80% confidence interval of predicted dilution by offshore
366 waters. Conversely, at the bottom, free virus concentration significantly increased during the
367 first 1h15 min, reaching a value of 1.8×10^7 viruses mL^{-1} . This value was out of the 80%
368 confidence interval around values predicted from dilution by offshore waters and even out of
369 the 80% confidence interval around value when survey started. However, free virus
370 concentration at the bottom decreased during the next 1h30 min, reaching a value of 4×10^6
371 viruses mL^{-1} , which was within the 80% confidence interval around values predicted from
372 dilution by offshore waters.

373 Free bacteria dynamics were similar to free virus dynamics during both surveys (Fig.
374 6b). For attached bacteria concentration, no significant change was observed during the
375 Lagrangian survey ($6\text{--}8 \times 10^6$ cells mL^{-1}), while concentration decreased during the Eulerian
376 survey by one order of magnitude, which was still within the 80% confidence interval of
377 predicted dilution with offshore waters (Fig. 6c).

378 The dynamics of HNF was similar to the ANF dynamics in the both surveys. During the
379 Lagrangian survey, HNF concentration increased steadily over the first 2 h10 min to almost
380 twice the concentration measured at the start of the survey (3×10^3 cells mL^{-1}), but then
381 decreased during the last 30 min (Fig. 6d). During the Eulerian survey, HNF concentration
382 displayed opposite dynamics at the bottom and at the surface, but both values remained within
383 the 80% confidence interval around surveys initial value and the large 80% confidence
384 interval around values predicted from mixing with offshore waters (1.3×10^3 cells mL^{-1}).

385 During the Lagrangian survey, ciliate concentration doubled along the transect to values
386 greater than the 80% confidence interval estimated when the survey began (Fig. 6e). During
387 the Eulerian survey, ciliate concentration decreased at the bottom and at the surface more than
388 expected from dilution with offshore waters at the bottom (i.e. lower than the 80% confidence
389 interval).

390

391 In summary, the water column only became significantly enriched with nanoflagellates
392 (autotrophs and heterotrophs) and ciliates during the Lagrangian survey over the mudflat,
393 where resuspension occurred. In contrast, mixing (typically leading to dilution) with offshore
394 waters was observed at the Eulerian site, except for free viruses and free bacteria at the
395 bottom (gain), ANF (gain) and *Synechococcus* and ciliates (loss). No significant change was
396 detected in autotroph biomass in any of the surveys (Fig. 7a) or in heterotroph biomass during
397 the Lagrangian survey (Fig. 7b). In contrast, heterotroph biomass was decreased by 50% at
398 the end of the Eulerian survey as expected from dilution with less heterotrophic offshore
399 waters (Fig. 7b). Regardless, heterotroph biomass remained greater than autotroph biomass
400 throughout both surveys.

401

402 **4. Discussion**

403 In the present study, we aimed at testing in situ whether and how the pelagic system
404 structure was modified during the rising tide by physical transfer of autotrophic and
405 heterotrophic microorganisms. Physical transfer included mixing with offshore waters and
406 sediment erosion and was evidenced by temporal changes in particulate inorganic matter
407 concentration.

408 *4.1 Decrease of resuspended microorganisms by grazing predators*

409 Over the mudflat, significant sediment erosion occurred under wave action, but at
410 different rates across the intertidal area. These changes were reflected by subsequent changes
411 in erodibility across the mudflat as bed friction velocity was kept constant during the
412 Lagrangian survey. A very low erosion rate in the middle region of the Brouage mudflat with
413 large bed friction velocities was indicative of low erodibility of the consolidated sediment,
414 possibly due to desiccation during tidal emersion (Anderson and Howell 1984; Paterson et al.
415 1990). On July 24, water content in the upper layer of mud fell from 55 to 46% during the 4 h
416 low tide. Such periods of desiccation occurred on many days throughout the particularly dry,

417 windy and hot month of July 2008 (Dupuy et al., personal communication). Variability in
418 erosion rates across the mudflat may also be attributed to changes in bioturbation intensity
419 (Widdows et al. 1998). Specifically, more active bioturbators in the Brouage mudflat, such as
420 *Scorbicularia plana* (Orvain 2005), are generally more abundant in the upper mudflat
421 (Sauriau et al. 1989; Bocher et al. 2007; Orvain et al. 2007). Moreover, erodibility increase
422 due to bioturbation activity should also be enhanced by low tide duration: the upper in the
423 mudflat, the longer the low tide and hence, the higher the bioturbation pressure. Surprisingly,
424 the input of taxon that are generally abundant in the sediment, such as diatoms, bacteria and
425 nanoflagellates (Paterson et al. 2009), was not observed during sediment erosion. Differences
426 in resuspension thresholds for benthic microorganisms may have been due to cell size,
427 specific gravity, behaviour, or association with particles (Shimeta et al. 2002). However, bed
428 friction velocities reaching an average of 3.2 cm s^{-1} due to waves prevented sedimentation of
429 particles with settling velocities less than 4 cm s^{-1} (i.e. particles with diameters smaller than
430 $320 \text{ }\mu\text{m}$) (Fredsoe and Deigaard 1992). Thus, considering the much lower density of organic
431 matter compared to sedimentary matter and suspension thresholds determined by Shimeta et
432 al. (2002), suspension thresholds were reached for all microorganisms included in the present
433 study. Moreover, wave vertical velocities were undoubtedly greater than the swimming speed
434 of any of these microorganisms ($<1 \text{ mm s}^{-1}$, Bauerfeind et al. 1986), precluding any migration
435 process. In addition, all microorganisms counted in the present study were found at high
436 densities in the Brouage muddy sediment before the rising tide, except for ciliates. As such, a
437 significant increase in microorganism concentrations was expected to occur along with
438 erosion of the muddy bed. Conversely, a stagnation or a decrease in the concentration of any
439 microorganism during the Lagrangian survey therefore most likely indicated consumption by
440 grazers.

441 Guarini et al. (2008) developed a model that simulates the dynamics of microalgal
442 biomass in semi-enclosed littoral ecosystems and suggested that resuspension of the MPB

443 occurs at the beginning of the rising tide, even in the absence of simultaneous sediment
444 erosion. Such recurrent MPB resuspension is consistent with the high proportion of benthic
445 diatom species observed at the beginning of both surveys in the present study. Meanwhile,
446 enrichment in benthic diatoms species was not significant in our survey. However, benthic
447 diatom concentration simultaneously increased with mud erosion in the lower and upper part
448 of the mudflat. Of the smaller autotrophs, only ANF significantly increased in the lower part
449 of the mudflat, where sediment erosion was greatest, during the first hour of the Lagrangian
450 survey. This significant increase in ANF concentration in the water column suggests that ANF
451 concentrations in eroded sediment during the survey was at least 10^6 cell mL⁻¹, which is a
452 hundred times greater than previously reported average values in the first top cm of the BMO
453 sediment (10^4 cell mL⁻¹, C. Dupuy personal communication). Yet, ANF most probably
454 accumulated at the very surface of sediment in a layer of 1-2 cells thickness, just like diatoms
455 does, to photosynthesize (Guarini et al., 2000). These findings support routine measurement
456 of the detailed vertical distribution of microorganisms for estimation of erosion fluxes.
457 *Synechococcus* concentrations remained low, and picophytoeukaryote concentration
458 decreased during the same period. We therefore suggest that the erosion flux of benthic
459 diatoms and ANF, largely present at the mud surface, was immediately overwhelmed by a
460 grazing flux due to heterotrophic protists (HNF, ciliates), micrometazoan and mesometazoans
461 planktonic organisms (Sherr et al. 1986; Leakey et al. 1992; Calbet et al. 2008) or benthic
462 suspension feeders (Hughes 1969; Carlson et al. 1984).

463 Free virus concentration decreased during the Lagrangian survey, while it was expected
464 to increase with resuspension as these microorganisms are also largely present in mud (10^{10}
465 cell mL⁻¹ sediment; Hewson et al. 2001, and the present study). Again, this suggests that virus
466 resuspension flux during the rising tide due to waves was compensated by a loss process.
467 However, adsorption of free viruses onto clay particles may also occur in addition to or as an
468 alternative to grazing (Malits and Weinbauer 2009). Reversible sorption and hydrophobic

469 effects are linked to the ionic strength of the given environment, most notably to the
470 concentration of cations like Na^+ , which may change between bed sediment and water (Gerba
471 1984). Finally, water agitation has been recently shown to enhance viral sorption to clay
472 (Syngouna and Chrysikopoulos 2010). Therefore, together with wave agitation, availability of
473 sorption sites on the clayed mud of the BMO (Helton et al. 2006), salt water may favour virus
474 adsorption onto sedimentary particles. Similar to viruses, electrostatic properties, pH,
475 temperature, and salinity are thought to govern the sorption of bacterial cells onto clay
476 minerals (Jiang et al. 2007). As such, bacteria adsorption onto resuspended sedimentary
477 particles should not be excluded during the Lagrangian survey. Free bacteria concentration
478 remained constant while an increase was expected as these organisms are abundant in the mud
479 ($5 \cdot 10^8$ cell mL^{-1} , Garet 1996 and 10^9 cell mL^{-1} in the present study) and were undoubtedly
480 resuspended. However, no significant enrichment in attached bacteria was observed during
481 the Lagrangian survey despite that a thousand-fold enrichment was expected from the large
482 PIM increase (ten-fold) and the high concentration of bacteria in bed sediment (10^9 cell mL^{-1})
483 compared to water (10^6 cell mL^{-1}). As free virus concentration did not strongly increase,
484 significant enhancement of virally mediated bacterial mortality was unlikely. Thus, attached
485 and free bacteria were most likely heavily grazed immediately after resuspension during the
486 Lagrangian survey.

487 Significant HNF concentration increase during the Lagrangian survey is consistent with
488 expected resuspension of benthic HNF given the bed friction velocities (due to waves) that
489 largely exceeded thresholds for HNF resuspension (Shimeta et al. 2002; $0.25\text{-}0.80$ cm s^{-1}). In
490 contrast, HNF production (secondary production) could not explain concentration doubling,
491 given nanoplankton growth rate ranging from 7 h (Dupuy et al, 2007) to 20 h (Calbet et al.
492 2008; Liu et al. 2009). Thus, doubling of HNF concentration in the water column suggest that
493 HNF concentration in eroded sediment during the survey was roughly 10^5 cell mL^{-1} , which is
494 a hundred times greater than previously reported average values found in the first top cm of

495 the BMO sediment (10^3 cell mL^{-1} , C. Dupuy personal communication). This result suggests
496 that HNF accumulated at the very surface of sediment during the present study and pinpoints
497 again the importance of accounting for vertical distribution of microorganisms when
498 estimating erosion fluxes. Similar zonation of nanoflagellates at the surface of sediment
499 compared to deeper layers have been previously reported during summer periods in the North
500 Sea (Hondeveld et al. 1994) and also in the BMO in July 2008 (Dupuy et al., submitted). In
501 any case, after 2 h, the decrease in HNF concentration during the Lagrangian survey indicated
502 grazing on HNF by ciliates or micrometazoan and mesometazoan planktonic organisms
503 (Sherr et al. 1986; Hartmann et al. 1993; Calbet et al. 2008) also buffered resuspension flux.

504 In contrast, a significant increase in ciliate concentration during the Lagrangian survey
505 could not be attributed to benthic transfer that accompanies mud erosion. Although some
506 ciliate genera can be found in sediment, such as scuticociliates (*Uronema* sp.), ciliate
507 concentration is generally very low in mud sediments (Giere 1993), with concentrations lower
508 than 20 cell mL^{-1} in the BMO surficial sediment (C. Dupuy, personal communication).
509 Simulated erosion of sediment cores taken in the BMO during the same period as the present
510 study confirmed ciliates erosion was insignificant (C. Dupuy, personal communication).
511 Besides, ciliates taxa found in the present study were Order Oligotrichida (*Strombidium* spp.)
512 and Order Tintinnida (*Tintinnopsis* spp.), which are generally part of the suprabenthos due to
513 behavioural adaptations leading to depth zonation above the sediment-water interface after
514 vertical migration when vertical flow motion is small. Compared to other groups of
515 microorganisms analysed in the present study, ciliates are relatively more motile organisms
516 (velocities up to 0.5 mm s^{-1}) that can control their position in the water column at low
517 turbulence levels (Jonsson 1989). Ciliate concentration increase during the Lagrangian survey
518 may be attributed to redistribution by waves vertical stirring of ciliates accumulated at a
519 particular depth during slack tide.

520

521 *4.2 Mixing with offshore waters*

522 Mixing (dilution or enrichment) with offshore waters was expected during the Eulerian
523 survey, where water depth varied. It should be noted that the 80% confidence interval around
524 prediction from mixing with offshore waters reduces when offshore waters are less
525 concentrated than inner basin waters (heterotrophic microorganisms) while it is reversed when
526 offshore are more concentrated than inner basin waters (autotrophic microorganisms). Thus,
527 detecting deviation from dilution prediction should be more accurate than detecting deviation
528 from import prediction.

529 The PIM concentration evolved as predicted by dilution during the Eulerian survey,
530 indicating that no significant erosion or sedimentation occurred. Thus, settling velocity of
531 suspended particles should be comparable to mean wave bed friction velocities, ranging from
532 1.4 to 0.5 cm s⁻¹, while bed sediment erosion threshold should be larger than 1.4 cm s⁻¹. In
533 contrast, during the Lagrangian survey, a strong increase in PIM concentration indicated that
534 bed friction velocities were high enough to erode the mud. Bed friction velocity due to the
535 tidal current (1.3 cm s⁻¹) was lower than the largest bed friction velocity observed during the
536 Eulerian survey which did not cause significant erosion. Thus, mud erosion during the
537 Lagrangian survey was more likely driven by bed friction velocities due to waves (average
538 velocity of 3.2 cm s⁻¹ and 4.5 cm s⁻¹ maximum) as suggested by Bassoullet et al. (2000).
539 However, the current study only indicates that the erosion threshold for the fine-grained mud
540 sediment of the Brouage mudflat (median grain size was 10 to 12 µm) lies between 1.4 cm s⁻¹
541 (largest value during Eulerian survey during which erosion was not observed) and 4.5 cm s⁻¹
542 (largest value during Lagrangian survey during which erosion was observed) in terms of bed
543 friction velocity.

544

545 During the Eulerian survey, no significant change in diatom concentration was detected
546 reflecting similar order of magnitude of concentration in the inner basin and in the north of

547 the basin. However, a total community change occurred with replacement of benthic diatoms
548 by pelagic diatoms in proportion of water depth change during the rising tide. No significant
549 primary production of diatoms was expected given the short duration of the Eulerian survey
550 compared to the generation time for microphytoplankton (1 division d^{-1} , Calbet and Landry
551 2004 to 2 divisions d^{-1} , Dupuy et al. 2007) and water turbidity (Struski and Bacher 2006;
552 Bouman et al. 2010). Thus, mixing was the most likely dominant process.

553 Significantly lower than expected concentrations (based on mixing with offshore
554 waters) of low motility *Synechococcus* (at the surface and at the bottom) suggest that grazing
555 pressure was strong during tidal flow due to microzooplankton, mesozooplankton and/or
556 bivalve mollusks (*Macoma balthica*, *Scrobicularia plana*) (Hugues 1969; Hartmann et al.
557 1993; Dupuy et al. 1999). Ciliates also exhibited lower than expected concentrations at the
558 bottom. However, for these motile organisms, the assumption of a vertically homogeneous
559 distribution was probably no longer true as turbulence intensity decayed during the Eulerian
560 survey. Thus, ciliates losses may not be attributed to grazing only. Motility may also explain
561 the opposite dynamics of nanoflagellates during the Eulerian survey between surface and
562 bottom, given their swimming speed can reach 0.3 to 0.5 $mm\ s^{-1}$ and yield a transport of 1 to 2
563 m after one hour (Bauerfeind et al. 1986). However, an overall significant increase in ANF
564 concentration at the end of the Eulerian survey suggested that primary production started.

565 Conversely, during the first portion of the Eulerian survey, free virus and free bacteria
566 concentration significantly increased at the surface compared to the offshore water dilution
567 curve, suggesting that secondary production or desorption dominated over grazing. This
568 synchronous increase was congruent with the synchronous virus and bacteria dynamics
569 usually observed in BMO at the monthly scale in summer (Ory et al. 2010, 2011).

570

571 4.3 Strengths and limitations of the sampling strategy

572 Adapting sampling strategy to disentangle mixing with offshore waters and
573 resuspension and separating physical processes from biotic processes using PIM as a
574 reference lead to the conclusion that grazing pressure must be intense during rising tide.
575 However, temporal changes in concentrations that follow the dilution curve in an Eulerian
576 survey or remain constant in a Lagrangian survey simply mean that loss and gain processes
577 balance each other out. In addition, conclusive demonstration of loss or gain processes
578 requires that temporal changes in surveys are larger than uncertainties on initial conditions. In
579 the present study, uncertainties in PIM, diatoms, picoeukaryotes and attached bacteria
580 concentrations at the beginning of surveys reached 100%, which prevented the detection of
581 any significant decrease. Yet, significant increases could be detected when values more than
582 doubled, as seen for PIM. These large uncertainties have different origins. For PIM,
583 uncertainties were mainly due to large differences between the Lagrangian and Eulerian
584 samples taken when surveys started and advocate for increasing sampling effort and
585 localization precision in the intertidal area where large horizontal spatial gradients may exist.
586 For diatoms, attached bacteria and picoeukaryotes, large uncertainties also came from large
587 difference between the Eulerian samples and points out the low precision on concentration
588 determination for some microorganisms. Reducing these uncertainties requires increased
589 sample volume and superior enumeration efforts.

590

591 **5. Conclusions**

592 During a rising tide, expected water column enrichment of benthic microorganisms
593 (small to large autotrophs and heterotrophs) from sediment resuspension was largely
594 overwhelmed by loss processes, except for nanoflagellates (ANF and HNF). The dominant
595 loss process was likely grazing. However, adsorption onto clayed particles may have also
596 masked enrichment for free viruses and bacteria . A combination of resuspension and
597 grazing/adsorption processes led to non-significant changes in both heterotroph and autotroph

598 total biomass during the rising tide in the nearshore area. In the meanwhile, offshore waters
599 imported autotrophic organisms, mainly *Synechococcus*, while heterotrophic microorganisms
600 were diluted. However, autotrophic organisms import was significantly grazed. As a result,
601 combination of offshore waters import and grazing led to stability of autotrophs biomass in
602 the deeper areas of the BMO, while heterotrophs biomass was reduced by 50%. Lastly, when
603 resuspension occurred over a tidal flat during a rising tide, the water column evolved to a less
604 heterotrophic structure over the mudflat in the deepest part of the semi-enclosed bay
605 compared to nearshore. Thus, the present study suggests that this differential evolution mainly
606 reflects dilution with low concentrated offshore waters, as grazing pressure erased any
607 microorganisms inputs accompanying physical transfers due to bed erosion or offshore waters
608 mixing.

609

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624 **References**

625

626 Alongi, D. M. 1991. Flagellates of benthic communities: characteristics and methods of study,
627 p. 57–75. In D. J. Patterson [ed.], *The Biology of Free-living Heterotrophic Flagellates*.
628 Clarendon Press, Oxford.

629 Aminot, A., and M. Chaussepied. 1983. *Manuel des analyses chimiques en milieu marin*.
630 CNEXO, Brest.

631 Anderson, F. E., and B. A. Howell. 1984. Dewatering of an unvegetated muddy tidal flat
632 during exposure – dessication or drainage? *Estuaries* **7**: 225-232.

633 Bassoulet, P., P. Le Hir, D. Gouleau, and S. Robert. 2000. Sediment transport over an
634 intertidal mudflat: field investigations and estimation of fluxes within the `Baie de
635 Marennes-Oleron (France). *Cont. Shelf Res.* **20**: 1635-1653.

636 Bauerfeind, E., Elbrächter, M., Steiner, R. and J. Thronden. 1986. Application of Laser
637 Doppler Spectroscopy (LDS) in determining swimming velocities of motile
638 phytoplankton. *Mar Biol* **93**: 323-327.

639 Blanchard, G.F, and V. Cariou-Le Gall. 1994. Photosynthetic characteristics of
640 microphytobenthos in Marennes-Oleron Bay, France : Preliminary results. *J. Exp. Mar.*
641 *Biol. Ecol.* **182**: 1–14.

642 Blanchard, G.F., J. M. Guarini, P. Richard, and P. Gros. 1997. Seasonal effect on the
643 relationship between the photosynthetic capacity of intertidal microphytobenthos and
644 short-term temperature changes. *J. Phycol.* **33**: 723-728.

645 Blanchot, J., and M. Rodier. 1996. Picophytoplankton abundance and biomass in the western
646 tropical Pacific Ocean during the 1992 El Nino year: results from flow cytometry. *Deep*
647 *Sea Res Part I: Oceanogr. Res. Papers* **43**: 877-895.

648 Bocher, P., T. Piersma, A. Dekinga, C. Kraan, M. G. Yates, T. Guyot, E. O. Folmer, and G.
649 Radenac. 2007. Site- and species-specific distribution patterns of mollusks at five

650 intertidal soft-sediment areas in northwest Europe during a single winter. *Mar. Biol.*
651 **151**:577-594.

652 Bouman, H. A. , T. Nakane, K. Oka, K. Nakata, K. Kurita, S. Sathyendranath, and T. Platt.
653 2010. Environmental controls on phytoplankton production in coastal ecosystems: A
654 case study from Tokyo Bay. *Est. Coast. Shelf. Sci.* **87**: 63-72.

655 Calbet, A., and M. R. Landry. 2004. Phytoplankton growth, microzooplankton grazing, and
656 carbon cycling in marine systems. *Limnol. Oceanogr.* **49**:51-57.

657 Calbet, A., I. Trepát, R. Almeda, V. Saló, E. Saiz, J. I. Movilla, M. Alcaraz, L. Yebra, and R.
658 Simó. 2008. Impact of micro- and nanograzers on phytoplankton assessed by standard
659 and size-fractionated dilution grazing experiments. *Aquat. Microb. Ecol.* **50**: 145-156.

660 Cammen, L.M. 1991. Annual bacterial production in relation to benthic microalgal production
661 and sediment oxygen uptake in an intertidal sandflat and an intertidal mudflat. *Mar.*
662 *Ecol. Prog. Ser.* **71**: 13-25.

663 Caron, D. A. 1983. Technique for enumeration of heterotrophic and phototrophic
664 nanoplankton, using epifluorescence microscopy, and comparison with other
665 procedures. *Appl. Environ. Microbiol.* **46**: 491-498.

666 Carlson, D. Townsend, D. W., Hilyard, A. L., Eaton J. F. 1984. Effect of an Intertidal Mudflat
667 on Plankton of the Overlying Water column. *Can. J. Fish. Aquat. Sci.*, 41, 1523-1528.

668 Danovaro, R., C. Corinadelsi, M. Filippini, U. R. Fisher, M. O. Gessner, S. Jacquet, M.
669 Magagnini, and B. Velimirov. 2008. Viriobenthos in freshwater and marine sediments:
670 a review. *Fresh. Biol.* **53**: 1186-1213.

671 Dupuy, C., S. Le Gall, H. J. Hartmann, and M. Bréret. 1999. Retention of ciliates and
672 flagellates by the oyster *Crassostrea gigas* in French Atlantic coastal ponds: protists as
673 a trophic link between bacterioplankton and benthic suspension-feeders. *Mar. Ecol.*
674 *Prog. Ser.* **177**: 165-175.

675 Dupuy, C., A. Pastoureaud, M. Ryckaert, P. G. Sauriau, and H. Montanié. 2000. Impact of the
676 oyster *Crassostrea gigas* on the microbial community in Atlantic coastal ponds near La
677 Rochelle. *Aquat. Microb. Ecol.* **22**: 227-242.

678 Dupuy, C., M. Ryckaert, S. Le Gall, and H. J. Hartmann. 2007. Seasonal variations of
679 planktonic communities in Atlantic Coastal pond: importance of nanoflagellates.
680 *Microb. Ecol.* **53**: 537-548.

681 Epstein, S. S. 1997. Microbial food webs in marine sediments. I. Trophic interactions and
682 grazing rates in two tidal flat communities. *Microb. Ecol.* **34**:188-198.

683 Fenchel, T. 1969. The ecology of marine microbenthos. IV. Structure and function of the
684 benthic ecosystem, its chemical and physical factors and the microfauna communities
685 with special reference to the ciliated protozoa. *Ophelia* **5**:1-182.

686 Fredsoe, J., and R. Deigaard. 1992. Mechanics of coastal sediment transport. 369 p. In World
687 Scientific [ed.], *Advanced Series on Ocean Engineering* 3.

688 French, J.R., H. Burningham, and T. Benson. 2008. Tidal and Meteorological Forcing of
689 Suspended Sediment Flux in a Muddy Mesotidal Estuary. *Estuaries Coasts* **31** : 843-
690 859.

691 Fournier, J., Dupuy, C., Bouvy, B., Courrodon-Real, M., Charpy, L., Pouvreau, S., Le
692 Moullac, G., Le Pennec, M., Cochard, J.C. 2012 Pearl oysters *Pinctada margaritifera*
693 grazing on natural plankton in Ahe atoll lagoon (Tuamotu archipelago, French
694 Polynesia). *Marine Pollution Bulletin* **65** : 490-499

695 Garet, M. J. 1996. Transformation bactérienne de la matière organique dans les sédiments
696 côtiers. Relation entre les métabolismes respiratoires et les activités exoprotéolytiques
697 bactériennes, PhD Microbiologie : Univ. Bordeaux 2.

698 Garstecki, T., S. A. Wickham, and H. Arndt. 2002. Effects of experimental sediment resus-
699 pension on a coastal planktonic microbial food web. *Est. Coast. Shelf. Sci.* **55**: 751-762.

- 700 Gasol, J. M. 1993. Benthic flagellates and ciliates in fine freshwater sediments: calibration of
701 a live counting procedure and estimation of their abundances. *Microb. Ecol.* **25**:247-
702 262.
- 703 Gerba, C. P. 1984. Applied and theoretical aspects of virus adsorption to surfaces, 30: 133-
704 168. In *Advances in Applied microbiology* [ed.], Academic press, Inc.
- 705 Giere, O. 1993. *Meiobenthology. The microscopic fauna in aquatic sediments*, 328 pp.
706 Springer-Verlag, Berlin.
- 707 Grémare, A., Amouroux, J.M., Cauwet, G., Charles, F., Courties, C., deBovée, F., Dinet, A.,
708 Devenon, J.L., Durrieu de Madron, X., Ferré, B., Fraunié, P., Joux, F., Lantoine, F.,
709 Lebaron, P., Naudin, J.J., Palanques, A., Pujo-Pay, M., Zudaire, L. 2003. The effects of
710 a strong winter storm on physical and biological variables at a shelf site in the
711 Mediterranean. *Oceanologica Acta*, 26(4), 407-419.
- 712 Guarini, J. M., Blanchard, G.F., Gros, Ph., Gouleau, D., Bacher, C. 2000. Dynamic model of
713 the short-term variability of microphytobenthic biomass on temperate intertidal
714 mudflats. *Mar. Ecol. Prog. Ser.* **195**: 291-303.
- 715 Guarini, J. M., N. Sari, and C. Moritz. 2008. Modelling the dynamics of the microalgal
716 biomass in semi-enclosed shallow-water ecosystems. *Ecol. Modeling* **211**: 267-278.
- 717 Guizien, K., F. Charles, D. Hurther, and H. Michallet. 2010. Spatial redistribution of *Ditrupa*
718 *arietina* (soft bottom Mediterranean epifauna) during a moderate swell event: evidence
719 and implications for biotic quality indices. *Cont. Shelf Res.* **30**: 239-251.
- 720 Guizien, K., and A. Temperville. 1999. Frottement de fond sous une houle irrégulière. *C. R.*
721 *Acad. Sci. Paris*, t. 327, Série Iib: 1375-1378.
- 722 Haas, L.W. 1982. Improved epifluorescence microscopy for observing planktonic
723 microorganisms. *Ann. Inst. Oceanogr.* **58**: 261-266.

- 724 Hartmann, H. J., H. Taleb, L. Aleya, and N. Lair. 1993. Predation on ciliates by the
725 suspension-feeding calanoid copepod *Acanthodiaptornus denticornis*. Can. J. Fish.
726 Aquat. Sci. Paris. **50**:1382-1393.
- 727 Hervouet, J. M., and L. Van Haren. 1994. TELEMAC-2D Principle Note (Electricité de
728 France, Technical Report HE- 43/94/051/B).
- 729 Hervouet, J. M. 2007. Hydrodynamics of Free Surface Flows: Modelling With the Finite
730 Element Method, Wiley-Blackwell, 360 pp.
- 731 Hewson, I., J. M. O'Neil, C. Heil, G. Bratbak, and D. Dennison. 2001. Effects of concentrated
732 viral communities on photosynthesis and community composition of co-occurring
733 benthic microalgae and phytoplankton. Aquat. Microb. Ecol. **25**: 1-10.
- 734 Herlory, O., J. M. Guarini, P. Richard, and J. F. Blanchard. 2004. Microstructure of
735 microphytobenthic biofilm and its spatio-temporal dynamics in an intertidal mudflat
736 (Aiguillon Bay, France). Mar. Ecol. Prog. Ser. **282**: 33-44.
- 737 Hondeveld B.J.M., Nieuwland G., van Duyl F.C., Bak R.P.M. 1994. Temporal and spatial
738 variations in heterotrophic nanoflagellate abundance in North Sea sediment. Mar. Ecol.
739 Prog. Ser. **109**: 235-243.
- 740 Hughes, R.N. 1969. A study of feeding in *Scrobicularia plana*. J. Mar. Biol. Ass. U. K. **49**:
741 805-823.
- 742 Jiang, D., Q. Huang, P. Cai, X. Rong, and W. Chen. 2007. Adsorption of *Pseudomonas*
743 *putida* on clay minerals and iron oxide. Colloids and surfacesB : Biointerfaces **54**: 217-
744 221.
- 745 Jonsson P.R. 1989. Vertical distribution of planktonic ciliates – an experimental ciliates : an
746 experimental analysis of swimming behaviour. Mar. Ecol. Prog. Ser. **52**: 39-53.
- 747 Kemp, P. F. 1988. Bacterivory by benthic ciliates: significance as a carbon source and impact
748 on sediment bacteria. Mar. Ecol. Prog. Ser. **49**:163-169.

- 749 Koroleff, F., 1969. Direct determination of ammonia as indophenol blue. International
750 Council for the Exploration of the Sea, 1969/C:9, Hydrol Commun, pp 19-22.
- 751 Labry, C., A. Herbland, and D. Delmas. 2002. The role of phosphorus on planktonic
752 production of the Gironde plume waters in the Bay of Biscay. *J. of Plankton Res.* **24**:
753 97-117.
- 754 Leakey, R. J. G., P. H. Burlull, and M. A. Sleight. 1992. Planktonic ciliates in Southampton
755 Water: abundance, biomass, production, and role of pelagic carbon flow. *Mar. Biol.* **14**:
756 67-83.
- 757 Liu, H., K. Suzuki, J. Nishioka, R. Sohrin, and T. Nakatsuka. 2009. Phytoplankton growth
758 and microzooplankton grazing in the Sea of Okhotsk during late summer of 2006.
759 *Deep-Sea Res. I.* **56**: 561-570.
- 760 Lucas, C.H., J. Widdows, M. D. Brinsley, P. N. Salkeld, and P. M. J. Herman. 2000. Benthic-
761 pelagic exchange of microalgae at a tidal flat. 1. Pigment analysis. *Mar. Ecol. Prog. Ser.*
762 **196**: 59-73.
- 763 Malits, A., and M. G. Weinbauer. 2009. Effect of turbulence and viruses on prokaryotic cell
764 size, production and diversity. *Aquat. Microb. Ecol.* **54**: 243-254.
- 765 Marie, D., F. Partensky, N. Simon, L. Guillou, and D. Vaultot. 2000. Flow cytometry analysis
766 of marine picoplankton. In: Diamond RA, DeMaggio S [ed.], *In living color. Protocols*
767 *in flow cytometry and cell sorting.* Springer-Verlag, Berlin.
- 768 Marquis, E., N. Niquil, D. Delmas, H. J. Hartmann, D. Bonnet, F. Carlotti, A. Herbland, C.
769 Labry, B. Sautour, P. Laborde, and C. Dupuy. 2007. Planktonic food web dynamics
770 related to phytoplankton bloom development on the continental shelf of the Bay of
771 Biscay, French coast. *Est. Coast. Shelf. Sci.* **73**: 223-235.
- 772 Mehta, J.A., E. J. Hayter, W. R. Parker, R. B. Krone, and A. M. Teeter. 1989. Cohesive
773 sediment transport. I. Process description. *J. Hydraulic Eng.* **115**:1076-1093.

- 774 Nezan, E., 1996. Surveillance du Phytoplankton marin : manuel illustré adapté à la formation
775 des analystes. (IFREMER, Eds.). Brest.
- 776 Nicolle, A., and M. Karpytchev. 2007. Evidence for spatially variable friction from tidal
777 amplification and asymmetry in the Pertuis Breton (France). Cont. Shelf Res. **27**: 2346-
778 2356.
- 779 Noble, R.T., and J. A. Fuhrman. 1998. Use of SYBR Green I for rapid epifluorescence counts
780 of marine viruses and bacteria. Aquat. Microb. Ecol. **14**: 113-118.
- 781 Orvain, F. 2005. A model of sediment transport under the influence of bioturbation activities:
782 generalisation to a key-species *Scrobicularia plana*. Mar. Ecol. Progr. Ser. **286**: 43-56.
- 783 Orvain F., P. G. Sauriau, A. Sygut, L. Joassard, and P. Le Hir. 2004. Roles of *Hydrobia ulvae*
784 bioturbation and the physiological stage of microphytobenthic mats in resuspended
785 sediment and pigment fluxes. Mar. Ecol. Prog. Ser. **278**: 205-223.
- 786 Ory, P., H. J. Hartmann, F. Jude, C. Dupuy, Y. Del Amo, P. Catala, F. Mornet, V. Huet, B.
787 Juan, D. Vincent, B. Sautour, and H. Montanié. 2010. Pelagic food web patterns: do
788 they modulate virus and nanoflagellate effects on picoplankton during the
789 phytoplankton spring bloom? Environ. Microbiol., **12** : 2755-2772
- 790 Ory, P., Palesse, S., Delmas ,D. and Montanié H., 2011 In situ structuring of virioplancton
791 through bacterial exoenzymatic activity ; interaction with phytoplankton. Aquatic
792 microbial ecology, **64**: 233-252.
- 793 Paterson, D.M., R. M. Crawford, and C. Little. 1990. Subaerial exposure and changes in the
794 stability of intertidal estuarine sediments. Est. Coast. Shelf. Sci. **30**:541-556.
- 795 Paterson, D. M., R. Aspden, and K. S. Black. 2009. Intertidal flats: Ecosystem functioning of
796 soft sediments systems. In: Perillo GME, Wolanski E, Cahoon DR, Brinson MM [ed.],
797 Coastal Wetlands: An Integrated Ecosystem Approach. Elsevier.

- 798 Pelegri, S. P., J. R. Dolan, and F. Rassoulzadegan. 1999. Use of high temperature catalytic
799 oxidation (HTCO) to measure carbon content of microorganisms. *Aquat. Microb. Ecol.*
800 **16**: 273-280.
- 801 Pusceddu, A., C. Fiordelmondo, and R. Danovaro. 2005. Effects on the Benthic Microbial
802 Loop in Experimental Microcosms. *Microb. Ecol.* **50**: 602-613.
- 803 Ricard, M., 1987. Atlas du phytoplancton marin, vol. 2. (CNRS, Eds.). Paris.
- 804 Schmidt, J. L., J. W. Deming, P. A. Jumars, and R. G. Keil. 1998. Constancy of bacterial
805 abundance in surficial marine sediments. *Limnol. Oceanogr.* **43**: 976-982.
- 806 Seymour, J. R., L. Seuront, and J. G. Mitchell. 2007. Microscale gradients of planktonic
807 microbial communities above the sediment surface in a mangrove estuary. *Est. Coast.*
808 *Shelf. Sci.* **73**: 651-666
- 809 Sherr, E., B. F. Sherr, and G. A. Paffenhofer. 1986. Phagotrophic protozoa as food for
810 metazoans: a "missing" trophic link in marine pelagic food webs? *Mar. Microb. Food*
811 *Webs* **1**:61-80.
- 812 Sherr, E. B., D. A. Caron, and B. F. Sherr. 1994. Staining of heterotrophic protists for
813 visualisation via epifluorescence microscopy. In: Kemp, PF, Sherr, BF, Sherr, EB, Cole,
814 JJ [ed.], 213-227. *Handbook of Methods in Aquatic Microbial Ecology*. Lewis
815 Publishers, Boca Raton, FL.
- 816 Shimeta, J., and J. Sisson. 1999. Taxon-specific tidal resuspension of protists into the subtidal
817 benthic boundary layer of a coastal embayment. *Mar. Ecol. Prog. Ser.* **177**: 51-62.
- 818 Shimeta, J, C. L. Amos, S. E. Beaulieu, and O. M. Ashiru. 2002. Sequential resuspension of
819 protists by accelerating tidal flow: Implications for community structure in the benthic
820 boundary layer. *Limnol. Oceanogr.* **47**: 1152-1164.

- 821 Shimeta, J., C. L. Amos, S. E. Beaulieu, and S. L. Katz. 2003. Resuspension of benthic
822 protists at subtidal coastal sites with differing sediment composition. *Mar. Ecol. Prog.*
823 *Ser.* **259**: 103-115.
- 824 Sournia, A., 1986. *Atlas du phytoplancton marin*, vol. 1. (CNRS, Eds.). Paris.
- 825 Struski, C., and C. Bacher. 2006. Preliminary estimate of primary production by phytoplank-
826 ton in Marennes-Oleron Bay, France. *Est. Coast. Shelf. Sci.* **66**: 323-334.
- 827 Syngouna, V.I. and Chrysikopoulos, C.V. 2010. Interaction between viruses and clays in stat-
828 ic and dynamic bacth system. *Environ Sci Technol* 44: 4539-4544
- 829 Van Duyl, F.C., and A. J. Kop. 1994. Bacterial production in North Sea sediments: clues to
830 seasonal and spatial variations. *Mar. Biol.* **235**: 323-327.
- 831 Widdows, J., M. D. Brinsley, and M. Elliott. 1998. Use of in situ flume to quantify particle
832 flux. 139:85-97, In: Black K.S., Paterson D.M., Cramp A. [ed.], *Sedimentary processes*
833 *in the Intertidal Zone*. Geol. Soc. London, Special publication.
- 834 Wu, Q. L., Y. Chen, K. Xu, Z. Liu, and M. W. Hahn. 2007. Intra-habitat heterogeneity of mi-
835 crobial food web structure under the regime of eutrophication and sediment resuspen-
836 sion in the large subtropical shallow Lake Taihu, China. *Hydrobiologia* **581**: 241-254.
- 837
- 838

Figure 1: Map of the Marennes-Oléron Bay in Europe. The grey area indicates land and the contour labeled 0 delimites the largest extent of the intertidal area (lowest sea level during the highest tidal coefficient). Open circles indicate the successive locations of the Lagrangian survey stations, the open square indicates the Eulerian survey location and the filled triangle indicates the ADV location. Trajectories simulated with the Telemac model are displayed: thin lines figure trajectories reaching the Eulerian survey location at 17:20 h (solid) and 18:40 h (dashed) and the thick line figures trajectory leaving the Eulerian survey location at 16:00 h.

Figure 2: Expected temporal changes in concentrations during a Lagrangian survey (black line) and during a Eulerian survey (gray line) of the water column.

Figure 3: (a) Wind speed at La Rochelle Aéroport and significant wave height (H_s) on the intertidal flat during high tide (light gray) on 24 July, 2008. (b) Measured bed friction velocities associated with the tidal current (open triangles) and computed bed friction velocities associated with waves in a constant 40 cm water depth during the Lagrangian survey (filled circles for the maximum, open circles for the average) and for water depth increasing from 1.1 to 3.1 m during the Eulerian survey (filled squares for the maximum, open squares for the average).

Figure 4: Temporal changes in concentration of particulate inorganic matter (PIM) in the middle of the water column during the Lagrangian survey (white bar) and during the Eulerian survey (square) at the surface (gray) and at the bottom (white) of the water column. The black vertical bar represents the average concentration in the middle of the water column in the lower part of the mudflat when surveys started with its 80% confidence interval. Lines display the 80% confidence interval of concentrations calculated assuming only mixing with

incoming offshore waters when tide rose during the Eulerian survey (see formula in the Methods section).

Figure 5: Temporal changes in the abundances of autotrophic organisms: benthic diatoms (a), pelagic diatoms (b), autotrophic nanoflagellates (ANF, c), Picophytoeukaryots (d) and *Synechococcus* (e) in the middle of the water column during the Lagrangian survey (white bar) and during the Eulerian survey (square) at the surface (gray) and at the bottom (white) of the water column. The black vertical bar represents the average concentration in the middle of the water column in the lower part of the mudflat when surveys started with its 80% confidence interval. Lines display the 80% confidence interval of concentrations calculated assuming only mixing with incoming offshore waters when tide rose during the Eulerian survey (see formula in the Methods section).

Figure 6: Temporal changes in the abundances of heterotrophic organisms: free viruses (a), free bacteria (b), attached bacteria (c), heterotrophic nanoflagellates (HNF, d) and ciliates (e) in the middle of the water column during the Lagrangian survey (white bar) and during the Eulerian survey (square) at the surface (gray) and at the bottom (white) of the water column. The black vertical bar represents the average concentration in the middle of the water column in the lower part of the mudflat when surveys started with its 80% confidence interval. Lines display the 80% confidence interval of concentrations calculated assuming only mixing with incoming offshore waters when tide rose during the Eulerian survey (see formula in the Methods section).

Figure 7: Temporal trends in the autotrophs (a) and heterotrophs (b) biomass in the middle of the water column during the Lagrangian survey (white bar) and during the Eulerian survey (square) at the surface (gray) and at the bottom (white) of the water column. The black

vertical bar represents the average concentration in the middle of the water column in the lower part of the mudflat when surveys started with its 80% confidence interval.

Table 1: Concentrations of parameters at the north station on July 12 and 29, 2008, used as offshore value C_{ext} in equation (1).

	July 12 / July 29
PIM	18.2 / 6.67 mg L ⁻¹
Pelagic diatoms	112.7 / 52.8 cell mL ⁻¹
Benthic diatoms	17.3 / 4.2 cell mL ⁻¹
ANF	1.8 / 1.0 10 ³ cell mL ⁻¹
Picoeukaryots	5.5 / 2.88 10 ³ cell mL ⁻¹
<i>Synechococcus</i> sp.	1.44 / 2.25 10 ⁴ cell mL ⁻¹
Free virus	3.8 / 2.34 10 ⁶ cell mL ⁻¹
Free bacteria	2.65 / 2.48 10 ⁶ cell mL ⁻¹
Attached bacteria	5.3 / 7.5 10 ³ cell mL ⁻¹
HNF	1.5 / 0.6 10 ³ cell mL ⁻¹
Ciliates	3.66 / 4.58 cell mL ⁻¹

Table 2: Conversion factors and their corresponding literature reference used to convert abundance to biomass of carbon for each type of plankton organism.

Cells or organism	Conversion factor (pg C/cell)	Reference
Bacteria	0.016	Labry et al. (2002)
Synechococcus	0.104	Blanchot and Rodier (1996)
Picoeukaryotes	0.104	Blanchot and Rodier (1996)
Nanoflagellates	3.14	Pelegri et al. (1999)
Ciliates	3.14	Pelegri et al. (1999)
Diatoms	225	Fournier et al. (2012)

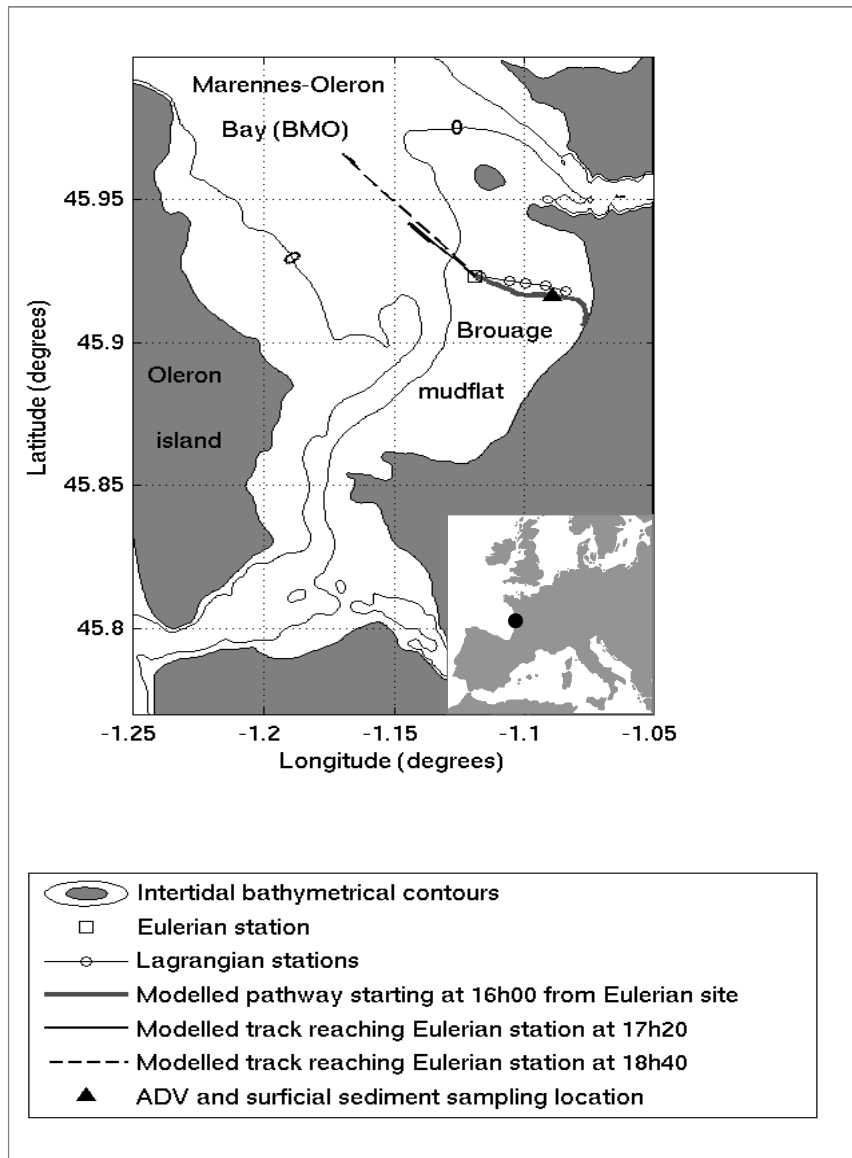


Figure 1

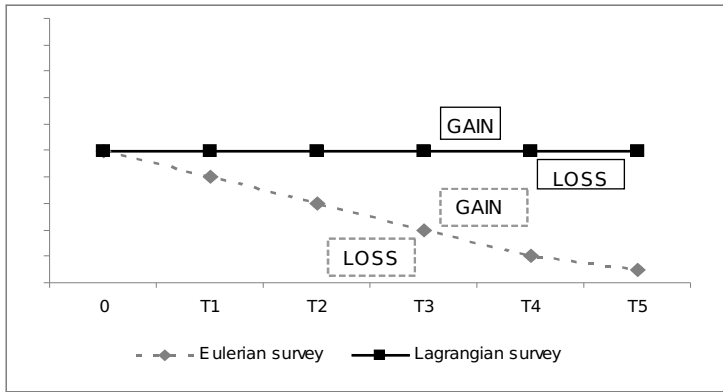


Figure 2

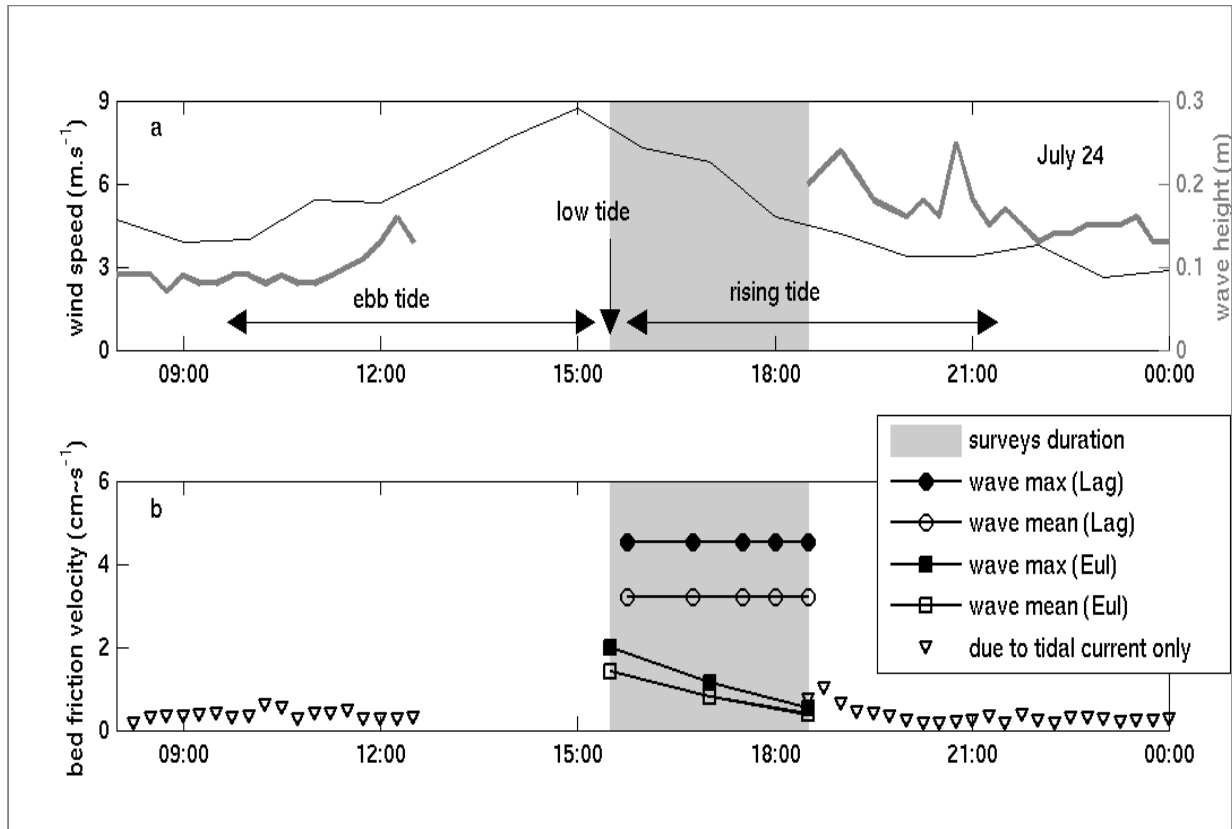


Figure 3

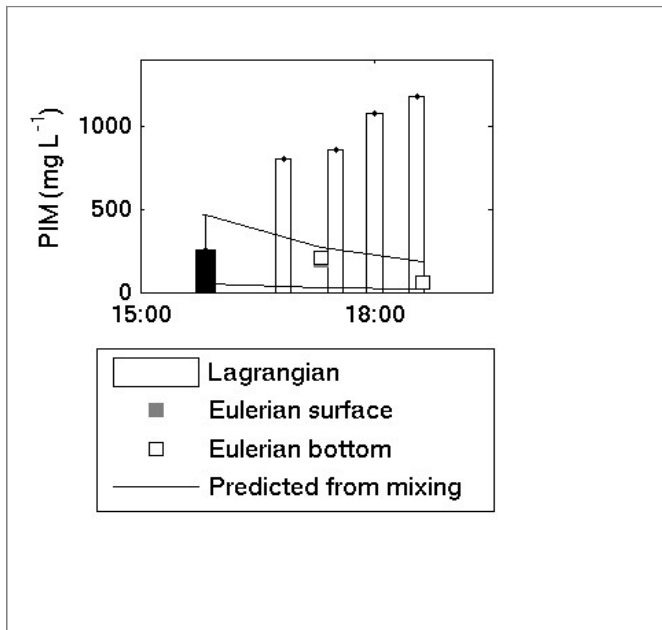


Figure 4

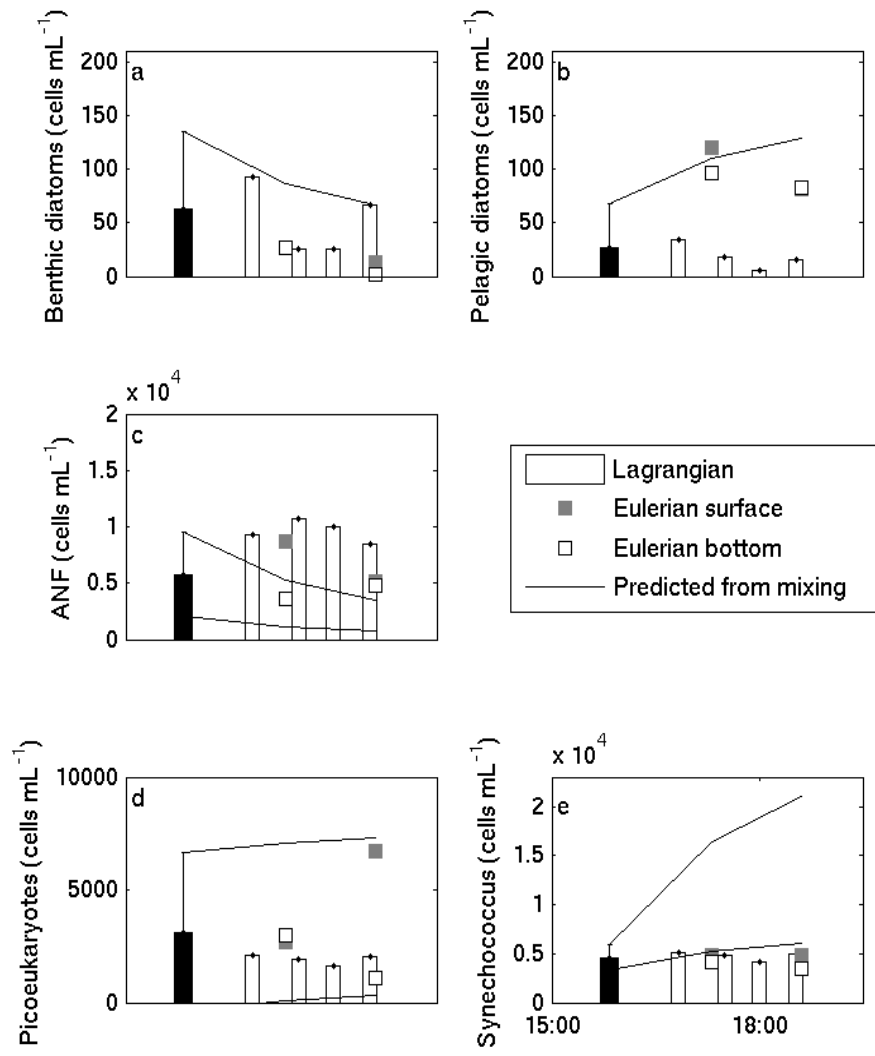


Figure 5

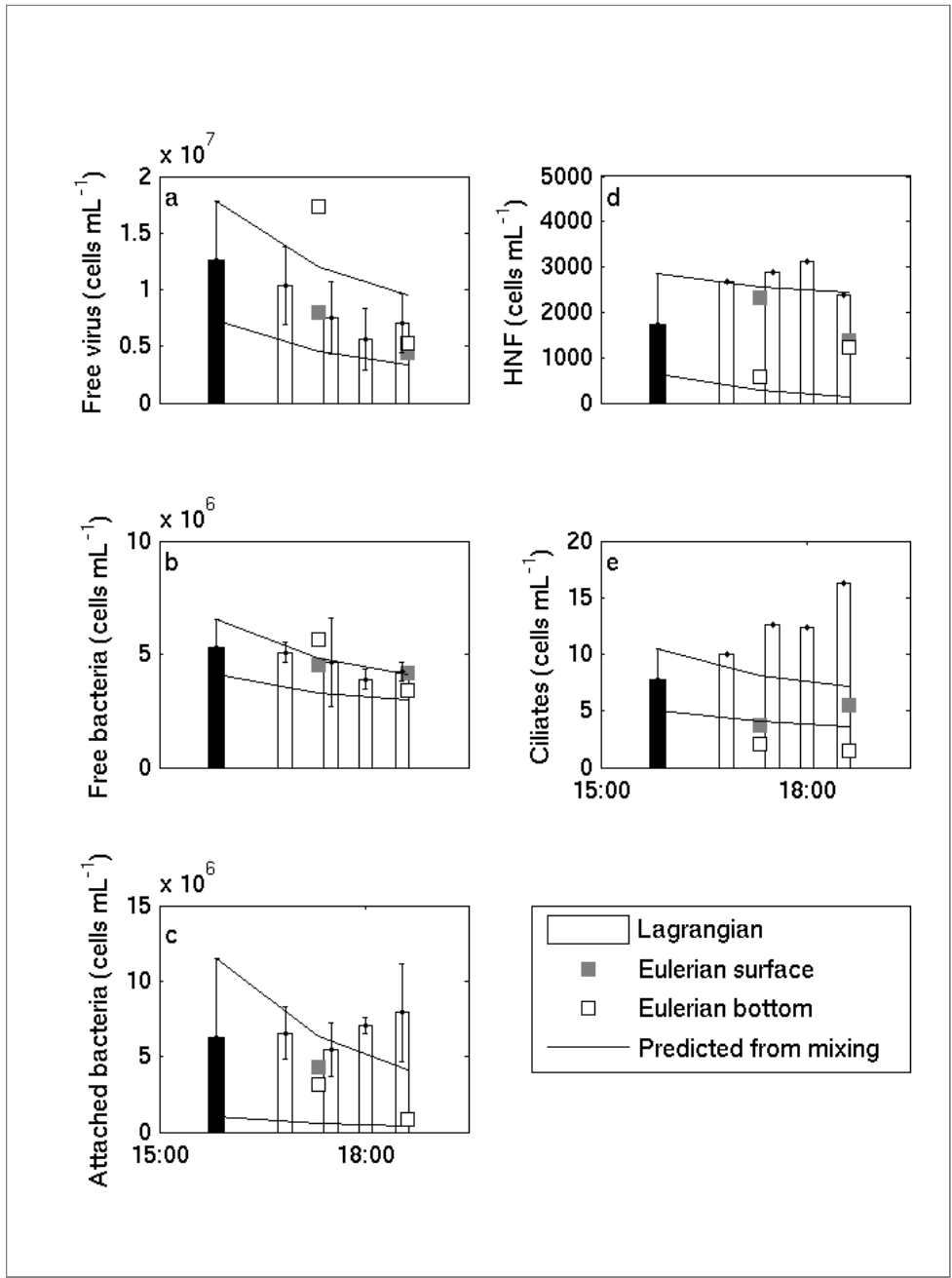


Figure 6

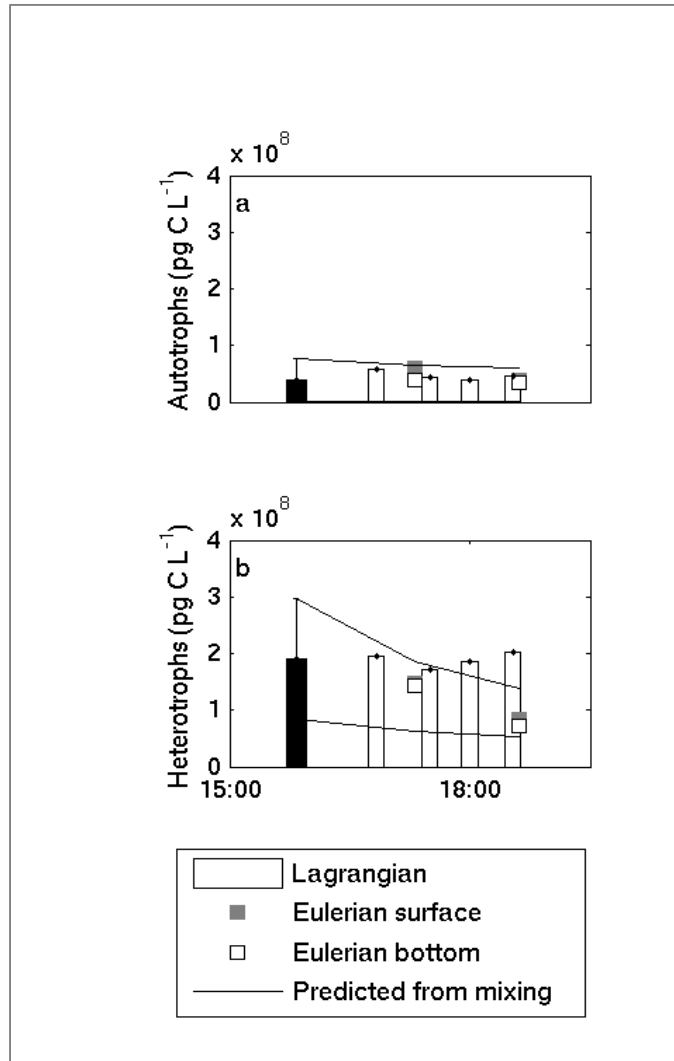


Figure 7