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Influence of oyster culture on biogeochemistry and bacterial community structure at the sediment-water interface

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Abstract:

Bacterial community structure and some biogeochemical parameters were studied in the sediment of two Pacific oyster farming sites, Aber Benoît (AB) and Rivière d'Auray (RA) in Brittany (France), to examine the ecological impact of oysters and to evaluate the emission of sulfide and ammonia from sediment. At AB, the organic matter accumulated in the sediment beneath the oyster tables was rapidly mineralized, with strong fluxes of ammonia and sulfide that reached 1014 µmol m^{-2} h^{-1} - and 215 µmol m^{-2} h^{-1}, respectively, in June 2007. At RA, the fluxes were about half as strong on average and better distributed through the year. The ammonia and sulfide concentrations in the overlying water never reached levels that would be toxic to oysters in either site, nor did hypoxia occur.

Total culturable bacteria (TCB) varied greatly according to the temperature: from 1.6 10^4 to 9.4 10^7.cell g^{-1} sediment. Inversely, the bacterial community structure remained surprising stable through the seasons, marginally influenced by the presence of oysters and by temperature. Bacterial communities appeared to be characteristic of the sites, with only one common phylotype, *Vibrio aestuarianus* a potential oyster pathogen. These data refine the hypothesis of seawater toxicity to oysters due to ammonia and sulfide fluxes and show that the measured environmental factors only had a weak influence on bacterial community structure.

Keywords : sediment ; biogeochemical parameters ; fluxes ; bacterial structure ; *Crassostrea gigas*

Introduction

Since the 1990s, culture of the oyster *Crassostrea gigas* in France has been subject to mortality episodes, which generally occur in June as soon as the temperature reaches 19 °C. Over the period since they began, these mortality episodes have increased dramatically. Since 2008, they have been seen to start at temperatures around 16 °C, due to the appearance of a new type of OsHv1 herpes virus that is much more virulent than the previously known strain (Segarra *et al.*, 2010). Before this development, a multidisciplinary research project known as Morest (Samain & McCombie, 2008) was conducted to study the original mortality syndrome. The conclusions of Morest underlined the involvement of multiple factors, including the genetic and physiological status of the oysters, occurrence of pathogens and environmental factors including temperature, pollutants, food availability and sediment proximity. For this last factor it was demonstrated that mortality rate increased with the closeness to the sediment at which the oysters were reared. Indeed, mortality of oysters reared directly on or a few centimeters above the sediment was significantly higher than those reared on oyster racks 50-70 cm above it. The beginning of mortality episodes appeared to be concomitant with the release of hydrogen sulfide and ammonia from the sediment in June, as a result of mineralization of organic matter − especially that generated by oyster farming itself. Before the mortality, a growth deficit was generally recorded for oysters grown directly on bottom, suggesting that chronic toxicity was caused by a factor from the sediment (Soletchnik *et al.*, 2005; Samain & McCombie, 2008). As early as the 1950s, a study showed how the intensive oyster culture could modify the surface sediment through oyster biodeposition and how, in turn, this modified sediment could disturb oyster growth and survival. (Susumu & Takeo, 1955). At Katsurashina, an oyster farming area in Japan, the sediment was found to contain high levels of sulfide and ammonia, which are known to be toxic to macroorganisms (Van Sprang *et al.*, 2000). In the Etang de Thau (south of France),

where there is semi-intensive culture of oysters attached to ropes, the benthic flux of ammonia and oxygen demand at the water-sediment interface were respectively 1-5 and 1.8-3 times higher underneath the suspended oysters than outside the culture area (Mazouni *et al.*, 1996). It is also well known that bacteria are the main actors in the degradation of organic matter in the aquatic environment (Kemp, 1990), leading to the production of reduced compounds including methane, the reduced forms of Mn and Fe, ammonia and hydrogen sulfide. Bacterial abundance tends to increase noticeably in sediment under intensive fish farming (Rajendran & Nagatomo, 1999, Caruso *et al.*, 2003). This increase is accompanied by a shift in the bacterial community composition and activities (Vezzulli *et al.*, 2002). For oysters, two studies, one done in the USA (Richardson *et al.*, 2008) and the other in Japan (Asami *et al.*, 2005), have also reported a substantial change in microbial communities and an increase of sulfide production underneath cultured oysters.

The present study was undertaken to verify whether the presence of oysters could significantly modify the biotic and abiotic characteristics of sediment by measuring some biogeochemical parameters. Ammonia, sulfide, nitrate and phosphate were measured in both overlying and pore waters in order to evaluate diffusive fluxes, and high resolution measurements of oxygen profiles were performed in order to determine the oxygen penetration depth. In addition, organic matter was measured in the solid phase. Bacterial community structure and composition were studied to determine which biogeochemical parameters influenced it and to identify the potential functional groups and any oyster pathogens present.

Materials and methods

87 **Site descriptions**

88 Sampling was performed in two estuarine areas of France where oyster farming is highly
89 developed: (1) the downstream part of Aber Benoît (4°36'W and 48°36'N) and (2) an oyster
90 culture site (Fort-Espagnol) near the mouth of Rivière d'Auray, (2°58'W and 47°36'N).
91 Aber Benoît (Fig.1, Table 1) is an estuary 31 km in length, with a catchment area of 140 km²,
92 situated in the northwest of Finistère (Brittany). The average water flow in spring is 0.418 m^3
93 s^{-1}, but this site is subjected to strong seawater currents (up to 3.5 m s^{-1}) due to the high tidal
94 amplitude, and salinity ranges from 2.4 to 3.4‰. Anthropic activities, which mainly center on
95 animal husbandry, lead to moderate discharges of organic matter, nitrate-rich fertilizer and
96 sometimes pesticides into Aber Benoît. However, the dilution due to high tide (5 m of tidal
97 amplitude) and currents prevent serious pollution. The oyster-growing area (250 ha) is located
98 both within and between Aber Benoît and Aber Wrac'h. Before 2008, no summer mortality
99 occurred and the temperatures never reached 19 °C.
100 The estuarine area of Auray (Fig. 1, Table 1) is 56.4 km in length and represents the western
101 part of the Gulf of Morbihan in south Brittany. It is influenced by anthropic activities, mainly
102 agriculture, on a catchment area of 800 km² around the two principal rivers, the Loch and the
103 Sal. These rivers flow into Rivière d'Auray, with an average flow of 2.99 m^3s^{-1} for the Loch
104 and about half this for the Sal. The tidal flux allows a renewal of 50 % of the Gulf's water
105 every 10 days (20 tides) and produces a current of up to 4 m s^{-1}. In the downstream part of
106 Rivière d'Auray, which is used for oyster farming, salinity can vary from 2.7 to 3.5 %. Oyster
107 mortality has occurred almost every year since the phenomenon began, notably because the
108 temperatures exceed 19 °C in summer.
109 The sediment of Aber Benoît is sandy-mud with a deep grey color, while Rivière d'Auray
110 sediment is black muddy-sand. Sediments at both sites have high mean organic carbon
111 contents (Fig. 3).

112 **Sample collection**

113 Sediment samples were collected on a monthly basis from June 2007 to September 2007. In
114 2008, samples were taken in February, March, May and June. At each site, 6 bags containing
115 either 100 or 250 15-month-old oysters were attached to oyster tables, 50 cm above the
116 sediment, in April 2007. The mean size of the oysters was 6.2 ± 0.9 cm, for a mean weight of
117 13.8 ± 1.9 g. Sediment samples were subsequently collected just beneath the experimental
118 oyster bags ("Oyster" stations) and at 30 m away from them ("Reference" stations). Samples
119 for this study were all collected under similar hydrodynamic conditions (moderate spring tide,
120 ebb tide). For biogeochemical parameter analysis, 3 replicate sediment samples were
121 collected from 20 cm depth with 15 cm of overlying water, using PVC tubes of 40 cm length
122 and 9 cm diameter, as described in Mudroch and Azcue (1995). Any disturbance of the
123 sediment water-interface was carefully avoided.

124 For bacterial counts and bacterial community structure analysis, samples were taken to 5 cm
125 depth at 3 randomly selected points spaced about 1 m apart, using Falcon tubes (50 mL
126 volume, 3 cm diameter) severed at 6 cm. In 2008, 4 replicate sediment samples were
127 collected. The quantity of sediment collected per sample to be used for bacterial analyses was
128 the same as that used in most other microbiological studies (Caruso *et al.*, 2003; Richardson
129 *et al.*, 2008). The relative similarity of samples from a single station in terms of counts and
130 bacterial community structure demonstrated *a posteriori* that they were representative of the
131 stations.

132 For bacterial counting, 5 g of each sediment sample were suspended in 10 ml of sterile
133 filtered seawater and homogenized by vortexing for 5 min. The supernatants were then serial
134 tenfold diluted in sterile seawater and plated on marine agar (Difco) for total culturable
135 bacteria counts (TBC), and on TCBS for vibrios. As in previous studies examining the impact
136 of fish farming or temperature on bacteria in the sediment (Gonzalez-Acosta, *et al.*, 2006;

137 Castine *et al.*, 2009), culturable bacteria counting was chosen in preference to direct counting
138 of bacteria stained with orange acridine or DAPI because this method is more sensitive to
139 changes in physico-chemical parameters such as organic matter and temperature.
140 *Vibrionacaea,* notably those that can grow on TCBS, are important for marine farming
141 because this family includes most pathogens of farmed marine animals, especially those of
142 *Crassostrea gigas* (Gay *et al.*, 2004; Garnier *et al.*, 2007). The remainder of each of the
143 sediment samples was stored at -80 °C until DNA extraction.
144
145 **Sediment treatments**
146 All overlying and pore water treatments were performed at the sampling sites after collection
147 of the sediment cores. An aliquot of overlying water was collected immediately for further
148 nutrient and sulfide analyses. High resolution vertical profiling of dissolved O_2 was then
149 carried out both on the overlying water and on the sediment core, using miniaturized Clark-
150 type oxygen sensors (Unisense OX500: http://www.unisense.com) coupled with a
151 picoammeter (Unisense PA 2000) and a micromanipulator (Unisense MM33) according to
152 Duchemin *et al.* (2005). These microsensors (tip diameter: 500 µm) are well-suited to
153 performing high resolution measurements of O_2 profiles and determining O_2 penetration depth
154 without disturbing the sediment (Revsbech et al., 1980). Subsequently, within less than 30
155 min, one core from each station was sliced into eight horizontal layers down to a total depth
156 of 8 cm (4 slices of 0.5 cm from the top down to 2 cm depth, 2 slices of 1 cm down to 4 cm,
157 and 2 slices of 2 cm down to 8 cm). In July, each core was sliced down to 8 cm: whereas, on
158 the other dates, only one core was sliced down to 8 cm − the two other cores only being sliced
159 down to 1 cm (slices: 0-0.5 cm and 0.5-1 cm). For every level, a sub-sample was centrifuged
160 at 3000 rpm and 4 °C for 20 min in a Whatman VectaSpin 20TM centrifuge tube filter
161 (0.45 µm) in an inert atmosphere (N_2) in order to collect pore waters, which were then stored

at -20 °C. Finally, a sub-sample of wet sediment was frozen for subsequent organic C analysis.

The pore water was acidified to pH ~ 2 and an aliquot frozen for later analysis of phosphate, nitrate and ammonia. Another aliquot was diluted fiftyfold and preserved by adding zinc chloride to precipitate the sulfide as zinc sulfide.

Biogeochemical parameter analyses and flux determination

After thawing, an aliquot of the pore water was diluted tenfold before nutrient analysis. Ammonia (NH_4^+), nitrate (NO_3^-) and phosphate (PO_4^{3-}) were analysed by segmented flow analysis (Aminot *et al.*, 2009). Hydrogen sulfide (H_2S, HS^-, S^{2-}) was measured using the colorimetric methylene blue method according to Fonselius *et al.*(1999).

Organic carbon was measured using a vario EL-III CN elemental analyser after decarbonation.

Diffusive fluxes were calculated using the Fick's first law adapted for sediments (Berner & Honjo, 1981):

$$Fd = -\Phi \times D_s \left(\frac{dC}{dz}\right) \quad (1),$$

where Fd is the rate of efflux (µmol m^{-2} d^{-1}), Φ is the sediment porosity (dimensionless) of the upper sediment sample, Ds is the bulk diffusion coefficient (m^{-2} d^{-1}) and dC/dz is the concentration gradient at the sediment-water interface (µmol m^{-4}). For ammonia, nitrate and phosphate, dC/dz was calculated from linear regression on the concentration values of water at the bottom and in the sediment just below the interface. In the case of HS^-, the concentration gradient was calculated for the depth interval with the greatest concentration gradient change, as was the corresponding porosity (Sahling *et al.*, 2002). Therefore, the sulfide flux represented the maximum of the ascendant flux observed locally in the sediment,

187 rather than the flux across the sediment water interface. Consequently, ammonia and sulfide
188 fluxes could not be directly compared. Ds was corrected for tortuosity, *i.e.*, $D_s = \frac{D_0}{\theta^2}$, where
189 θ is the tortuosity (dimensionless) and D_0 is the diffusion coefficient in water for PO_4^{3-}, NH_4^+
190 or HS^- ($m^2\ d^{-1}$). The diffusion coefficient in water (D_0) is corrected for the *in situ* bottom
191 water temperatures (Li & Gregory, 1974) and the value of θ is assumed to be equal to
192 $\sqrt{1-2\times\ln\varphi}$ (Boudreau, 1996).
193
194 **DNA extraction**
195 DNA was extracted from 5 g of sediment according to the SDS-based lysis method of Zhou *et*
196 *al.* (1996), and was suspended in 500 µl of sterile deionised water. Finally, the DNA was
197 purified with the Wizard® DNA Clean-Up System (Promega, Madison), according to the
198 manufacturer's instructions. After quantification by optical density, the DNA was diluted to
199 10 ng μL^{-1} for PCR amplification.
200
201 **PCR amplification and DGGE analysis**
202 As in most microbial analyses on intertidal sediment, bacteria were studied to gain an overall
203 view of the microbial community. Archeae were not considered because these
204 microorganisms are more specialized in specific functions such as methanogenesis and
205 ammonium oxidation, parameters that were not taken in account in this study. The primers
206 chosen are universal for the amplification of any bacterium, without selection of a particular
207 group. The primers 341f-GC (5'-GC-clamp-CCTTACGGGAGGCAGCA-3') and 518r (5'-
208 ATTACCGCGGCTGCTGG-3') (Muyzer *et al.*, 1993) were used to amplify a 230-bp
209 fragment of the V3 region of the bacterial 16S rRNA. A 40-bp GC-clamp
210 (CGCCCGCCGCGCGCGGCGGGCGGGGGCACGGGGGG) was attached to the 5' end of

211 the forward primer (Muyzer, *et al.*, 1993). The reaction mixture contained, 20 ng DNA, 1 µM
212 of each primer, 2 mM $MgCl_2$, 0.2 mM of each deoxynucleotide triphosphate and 1 U
213 AmpliTaq DNA polymerase (Invitrogen, life technologies) with 1× its buffer in milliQ water,
214 to give a final volume of 25 µl.
215 A PCR was carried out in a thermocycler (My Cycler TM thermal cycler, Biorad, USA) using
216 the touch-down program described in Murray *et al.* (1996), with slight modifications: Initial
217 denaturation at 94 °C for 3 min, followed by 10 cycles with denaturation at 94 °C for 30 s and
218 touchdown annealing from 65 °C to 56 °C for 30 s. The annealing temperature was decreased
219 1 °C for each cycle, and was then set at 55 °C for 20 cycles, with primer extension at 72 °C
220 for 30 s. The final extension was run at 72 °C for 30 min to achieve fragment elongation
221 according to Syvyk *et al.* (2008), prior to cooling at 4 °C.
222 PCR products were loaded onto 8 % (w/v) polyacrylamide gels (Acrylamide/bis-acrylamide
223 37.5:1 Bio-Rad laboratories, France) cast in 1× TAE (40 mM Tris, 20 mM acetic acid, 1mM
224 EDTA, pH 8.0). These gels were made with a denaturing gradient ranging from 40 to 80 %
225 (100 % denaturing mixture contained 7 M urea and 40 % deionized formamide). DGGE was
226 carried out with the D-code DGGE system (Bio-Rad laboratories, France). Electrophoresis
227 was conducted in 1×TAE buffer at a constant voltage of 80 V and a temperature of 60 °C for
228 18 h. After electrophoresis, gels were stained with Sybr-gold diluted at 1/10 000 for 30 min
229 and rinsed with 0.5×TAE for 15 min. The gels were read using a fluorimager (typhoon 9400,
230 Amersham) with fluorescence at 540 nm, followed by image analysis.
231
232 **Sequencing and phylogenetic analysis of DGGE bands**
233 Interesting DGGE bands (17 in Aber Benoît and 18 in Rivière d'Auray) were chosen
234 according to their intensity and presence/absence criteria and excised from the gels. These
235 were reamplified using the usual primers (341f with GC-clamp and 518r). The bands were

236 excised from a second gel and reamplified with 341f without the GC-clamp and 518r. The
237 amplified products were then cloned into PCR II plasmids using a TA cloning kit, and
238 transformed into the *Escherichia coli* DH5α (Fisher Scientific SA, France) according to the
239 manufacturer's instructions. Four positive clones per band were randomly chosen for
240 sequencing. The sequencing was done using the SP6 promoter primer and a 16-capillary
241 ABI3130 XL sequencer. The sequences were then analysed using Bioedit software and
242 compared to the GenBank database using its online software and the Basic Local Alignment
243 Search Tool algorithm (BLAST) (Altschul *et al.*, 1997). The sequences were deposited in
244 Genbank, where they were assigned the numbers FN555177 to FN555194 and FN555208 to
245 FN555229.
246
247 **DGGE and Statistical analysis**
248 DGGE banding profiles from all sediment samples were analyzed using Gel ComparII
249 software (Applied Maths, Kortrijk, Belgium) to obtain a matrix consisting of the relative
250 intensity and position of each DNA band. Although GelCompar normalization was performed
251 using external ladders, gel effect was significant. To avoid misinterpretation due to
252 superimposition of gel and treatment effects, analyses were only performed on individual gels
253 or between gels without significant gel effects. For each site, the 2007 profiles were analyzed
254 separately from those of 2008, because the samples taken in these two years had been loaded
255 onto separate gels. The DGGE data matrix was used to generate a triangular similarity matrix
256 based on the Bray-Curtis coefficient, using PRIMER software (PRIMER-E, Plymouth, UK).
257 Multi-dimensional scaling (MDS) (Kruskal & Wish, 1978), representing the similarity ranks
258 of community structure between samples, were then derived from the matrices. The resulting
259 MDS map shows every band pattern as a point, so relative changes among different DGGE
260 patterns can be visualized and interpreted as the distances between the points. The more

similar the DGGE banding patterns, the smaller the inter-point distance. The analysis of similarity (ANOSIM) routine was used to examine the statistical significance (significance data reported as p-values) of differences between the DGGE profiles from Oyster station samples and Reference station samples, and differences according to temporal variation. The Spearman correlation coefficient and associated *p* significance level (obtained by a permutation test using 5000 permutations) were computed to quantify the correlation between the rank similarity matrices obtained for environmental variables (using Euclidean distance) on one hand and genetic structure (using Bray-Curtis dissimilarity index) on the other (Clarke & Ainsworth, 1993).

Results

Biogeochemical parameters of overlying water and sediment

The overlying water temperature in Aber Benoît varied between 14.7 and 18.1 °C over the spring, summer and autumn (Table 2). In Rivière d'Auray, over the same seasons, it ranged from 14.7 to 21.8 °C (Table 3). In February and March 2008, it varied between 9 and 11 °C in both sites.

NH_4^+ and PO_4^{3-} concentrations in overlying waters at Aber Benoît were always lower than 3.5 µmol l^{-1} and 1.5 µmol l^{-1}, respectively, at both Oyster and Reference stations. NO_3^- concentrations varied from 29 µmol l^{-1} (Oyster in July) to 66 µmol l^{-1} (Reference station in June). In Rivière d'Auray, NH_4^+ and PO_4^{3-} concentrations never exceeded 6 and 1 µmol l^{-1}, respectively, whereas NO_3^- concentrations varied from about 1 µmol l^{-1} (July) to 70 µmol l^{-1} (March) both at Oyster and Reference stations. Sulfide was never detected in the overlying waters at either site. Abnormally low dissolved oxygen concentrations (*i.e.*, hypoxia) were

285 never measured at either the Oyster or Reference stations; oxygen concentrations varied from
286 165 µmol l^{-1} to 300 µmol l^{-1}.
287 At both sites, according to the oxygen profiles, sediments were always anoxic below 2-3 mm
288 depth and no significant differences between Oyster and Reference stations could be
289 observed; Fig. 2 shows the example of May 2008.
290 The other biogeochemical parameters varied according to site, season and station (Tables 2
291 and 3, Fig. 3, 4, 5).
292 In Aber Benoît, a minor enrichment in organic carbon (OC) generally occurred beneath the
293 oyster tables, making the level 1.2 times higher on average (949 µmol g^{-1}, 1.14 % dry
294 sediment) than at the Reference station, with a peak in May 2008 (2072 µmol g^{-1}, 2.49 % dry
295 sediment). During June 2007 and June 2008, peaks of NH_4^+ fluxes (6 times higher than the
296 other months) were recorded at the Oyster station. Ascendant sulfide fluxes (Fig. 5)
297 significantly increased at the Oyster station in May, July and August 2008 (up to 215 µmol m^{-2}
298 h^{-1}), while they were always close to zero at the Reference station. Phosphate fluxes were
299 weak and almost all values of nitrate flux were negative, indicating that strong redox occurred
300 in the sediment. Positive correlations were found between temperature and nitrate flux ($R = 0.713$, $p < 0.05$) and between phosphate and ammonia fluxes ($R = 0.808$, $p < 0.05$).
302 In Rivière d'Auray, organic carbon (OC) ranged from 959 to 2300 µmol g^{-1} (1.15 to 2.76 %
303 dry sediment), with an average of 2.07 % dry sediment at Reference and 1.71 % at the Oyster
304 station. The OC values at Reference remained high in winter but were more variable at the
305 Oyster station. The ammonia flux (Fig. 4) was half as much in Reference as Oyster. In
306 contrast, the ascendant sulfide flux, concentrated in 2 peaks in September 2007 and May
307 2008, was 5 times higher in Reference than in Oyster.
308 Phosphate flux reached 2 to 19.2 µmol g^{-1} for Oyster, while the nitrate flux was negative,
309 reflecting the anoxia of the sediment. OC and ammonia flux were only positively correlated in

310 Rivière d'Auray underneath the oyster tables ($R = 0.939$ $p < 0.05$), and phosphate and
311 ammonia fluxes showed the same pattern ($R = 0.639$, $p < 0.05$).
312
313 **Bacterial counts**
314 Total culturable bacteria (Marine agar) and vibrios (TCBS) counts varied according to the
315 season. Their numbers, expressed in log10 (Fig. 6A and B), were positively correlated with
316 the temperature (R ranged from 0.641 to 0.877, $p < 0.05$), but not with OC or nutrient fluxes.
317 Total culturable bacteria (TCB) reached only 3.10^6 CFU g^{-1} in Aber Benoît but reached up to
318 10^8 CFU g^{-1} in Rivière d'Auray. However, the vibrios/THB ratio was 7 times higher on
319 average in Aber Benoit than in Rivière d'Auray. The TCB and vibrio concentrations in Oyster
320 and Reference stations for both sites were of the same order, but significantly different in
321 June, July and August 2007.
322
323 **DGGE profiles and phylogenetic analysis**
324 Amplification and DGGE analysis of the 16S rDNA fragments from Rivière d'Auray and
325 Aber Benoît showed different banding patterns (Fig. 7), but appeared to be little different
326 between replicates, stations or sampling dates within a single site.
327 In Aber Benoît, the total number of bands detected was 24.1 (± 2.9) for Oyster, with the
328 lowest number in February and the highest in September. For Aber Benoît Reference, the total
329 number of detected bands was 23.5 (± 2.6), with the lowest number in June 2008 and the
330 highest in September 2007.
331 In Rivière d'Auray, the number of bands ranged from 15 to 24 for Oyster and from 20 to 28
332 for Reference. The lowest number of bands occurred in September at both sampling stations,
333 and the highest numbers were observed in 2008 during February and March for Oyster, and
334 February and June for Reference.

335 In Aber Benoît, no band was found to be station-specific and each band was detected at least
336 once. The same pattern occurred in Rivière d'Auray except in February 2008, where 3 bands
337 (27, 30, 34) were specific to Oyster and 2 (40, 42) to Reference.
338 At each site, 4 bands (n° 1, 4, 9, and 15 in Aber Benoît and 20, 21, 25 and 33 in Rivière
339 d'Auray) were frequently present at both stations throughout the survey, although these varied
340 widely in strength. The DNA was successfully sequenced from 18 bands for Rivière d'Auray
341 and 17 for Aber Benoît. Most bands corresponded to a unique sequence (Tables 4 and 5),
342 except some that contained several different sequences. These sequences were affiliated with
343 seven major phylogenetic groups including Acidobacter, Bacilli, Bacteroidetes,
344 Verrucomicrobia, α-, δ-, γ-Proteobacteria and one unclassified environmental strain.The
345 members of γ-Proteobacteria were the most numerous, with many uncultured bacteria and
346 some vibrios, such as *Vibrio aestuarianus*, *Vibrio anguillarum* or *V. ordalii.* Two sequences
347 corresponded to the functional group of sulfate-reducing bacteria (bands 1 and 28).
348
349 **Bacterial community structure**
350 First, replicates from the same sampling station were compared to check for similarity. In AB
351 the MDS analyses of the 2007 profiles (Fig. 8) showed that the replicates of both stations for
352 the same date were clustered together or were in close relation to one another. This implied
353 that there was a variation between successive months but that oyster tables only had a
354 significant influence in June, as assessed by the ANOSIM analyses.
355 In June and July 2007 in Rivière d'Auray, the three replicates from Oyster and three from
356 Reference grouped separately (Fig. 9), but these became mixed in the following months.
357 There were, however, some extreme points at Reference in July and August 2007. The
358 ANOSIM analysis revealed variation between the consecutive months of June and July for

359 both stations, August and September, for Oyster and also revealed a significant effect of
360 oyster bags on bacterial community structure for all sampling dates except July.
361 In 2008, all replicates from Aber Benoît were dispersed and only influenced by date between
362 February and March at Reference, and by oyster bag in February.
363 Most of the profiles from Rivière d'Auray were grouped together, except those from
364 Reference in February and March and those from Oyster in March, which formed separate
365 clusters. This grouping indicates an effect of date and oyster bag on the bacterial community
366 structure at the beginning of the year only, confirmed by ANOSIM analyses, which was not
367 observed later on.
368 Using Spearman correlation rank analysis, temperature showed a significant relation with the
369 bacterial profiles of Aber Benoît in June-July 2007, August-September 2007, and in 2008. In
370 Rivière d'Auray, however, temperature was only significantly related to the profiles in June-
371 July (Table 6). The nitrate fluxes were also significantly related to the bacterial community
372 structure in June-July at Aber Benoît, and in August-September in Rivière d'Auray.
373 Phosphate flux also had a significant correlation with community structure in August-
374 September.
375
376 **Discussion**
377 Some previous studies have demonstrated that oysters on tables induce a long-term
378 accumulation of organic matter in the sediment beneath them (Mazouni *et al.*, 1996), due to
379 feces and pseudo-feces deposits they produced. In contrast, other studies have shown no
380 measurable impact of oyster cultures on benthic sediment biogeochemistry, due to a highly
381 dynamic environment and low oyster production intensity (Crawford *et al.*, 2003; Mallet *et*
382 *al.*, 2006). In this present case, the impact of oysters on organic matter was only seasonal. In
383 Aber Benoît, organic matter accumulated beneath the oyster tables from March to May, but

384 decreased quickly in the May-June period. For Rivière d'Auray, two peaks of organic matter
385 were measured underneath the oyster tables in May and in July, but they decreased
386 progressively in the following months. The sediment of the Rivière d'Auray Reference station
387 contained 1.2 times more organic matter and, therefore, appeared atypical; this was probably
388 due to the accumulation and degradation of stranded algae (macrophytes) regularly observed
389 there.
390 In Aber Benoît, fluxes of ammonia at the sediment-water interface and maxima of the
391 ascendant sulfide fluxes in the sediment resulting from organic matter (OM) mineralization
392 were much higher on average at the Oyster station than at the Reference station; 13 and 653
393 fold, respectively. This suggests an efficient and rapid degradation of OM underneath oyster
394 tables. For Rivière d'Auray, the fluxes of the Oyster sediment were better distributed through
395 the year, with a lower mean level (1.8 times less for ammonia and 10 times less for sulfide)
396 than at Aber Benoît. The difference between Oyster and Reference stations at Rivière d'Auray
397 was also less marked for the ammonia flux (only 2.2 times higher in average in Oyster) than
398 for the sulfide flux (4 times lower). These fluxes did not induce a high ammonia concentration
399 in the overlying seawater, which never reached a level toxic to oysters. Neither sulfide nor
400 anoxia was detected, despite low oxygen penetration depths into the sediment. This means
401 that the high ascendant sulfide flux did not result in a high efflux from the sediment, and
402 highlights the differing behavior of ammonia and sulfide fluxes. Most of the ascendant sulfide
403 flux would have been intercepted in the superficial sediment through precipitation with Fe^{2+}
404 (from FeS) or oxidation by aerobic chemotrophic sulfide oxidizers or phototrophic sulfide
405 oxidizers. Unfortunately, DGGE band sequencing did not reveal clear specialist chemotrophic
406 or phototrophic sulfide oxidizers, although some Rhodobacter strains possess capacities to
407 oxidize sulfide. Therefore, the effects of the changes in sediment biogeochemistry on oyster
408 health, previously reported in the Morest project (Samain & McCombie, 2008), could not

409 have been caused by ammonia and sulfide fluxes such as these, unless considerable quantities
410 of these nutrients are suddenly released by a sediment resuspension event.
411 The intensity of mineralization reflected by these fluxes may depend on the nature of newly
412 deposited OM, which is probably mainly feces and pseudofeces from oysters (Asami *et al.*,
413 2005), although no analysis was made in the present study to confirm this point. OM appeared
414 more easily degradable in Aber Benoît than in Rivière d'Auray, even though the mean
415 temperature was 2 °C lower. Otherwise, the transport of feces and pseudofeces from the
416 Oyster station to the Reference station appeared limited in Aber Benoît, since
417 biomineralization remained weak at Reference despite the small distance between the stations.
418 The current generated by high tides may disperse the feces and pseudofeces from oysters
419 across large area and thus dilute them.
420 Abundance of TCB and vibrios was not greatly influenced by OM or correlated with the
421 ammonia and sulfide fluxes, which may reflect the activity of these microorganisms. At the
422 Oyster station of Aber Benoît in May, when the peak of OM appeared, TCB reached only 3.4
423 $\times 10^5$ cell g^{-1}. The organic matter decreased to 705 µmol g^{-1} in the following month, while
424 TCB continued to increase up to 1.4×10^6 cell g^{-1}. Thus, the relationship between organic
425 matter and culturable bacteria seems more complex than that described by Vezzulli, *et al.*
426 (2002), Vezzulli & Fabiano (2006) and Richardson *et al.* (2006). In these previous studies,
427 abundance of TCB in the sediment increased with the same magnitude as organic matter
428 content. It is possible that the most active bacteria were not culturable and/or that their
429 activity was not directly related to their number. The relationship between abundance and
430 bacterial activity in sediment has not yet been investigated in any depth. Overall, the main
431 factor explaining the fluctuation of bacterial concentrations was temperature.
432 There was, however, a temperature difference of just 1.3 °C less in June 2008 than in June
433 2007 in AB, which cannot completely explain why the bacterial concentrations were lower in

June 2008. In addition, these lower bacterial concentrations coincided with a metabolic slowdown of bacterial activity, revealed by the weaker fluxes of ammonia and sulfide. A large dispersion of bacterial profiles also occurred in 2008, as shown by the MDS analyses, while in 2007 they were grouped by sampling date. The nature of newly-deposited OM and its distribution within stations could induce a decrease in growth of culturable bacteria compared with June 2007, and a greater diversity of bacterial populations.

At Rivière d'Auray the bacterial concentrations (TCB) reached similar levels in June 2008 as in June 2007, although the temperature was 2.3°C less in 2008. The bacterial profiles from the two stations became similar from May onwards, whereas the replicates clustered separately the year before. This difference coincided with a higher amount of organic matter in the sediment, which was apparently degraded slowly. It could be the nature and distribution of newly deposited OM that stimulates the bacterial growth and homogeneity of profiles between stations in this case. Nevertheless factors modifying the bacterial community structure can remain unknown (Boer *et al.,* 2009)

The fact that vibrios were more abundant in AB (1 % TCB in average) than in RA (0.16 %) may be due to a better degradability of organic matter at AB, since these bacteria are particularly organotrophic. Among these vibrios, *Vibrio aestuarianus*, which is pathogenic to *C. gigas*, was frequently detected; however, it is difficult to estimate the true risk it posed to the oysters in terms of weakening effects or as a direct cause of mortality. To examine these issues, it would be necessary to prove that the *Vibrio aestuananus* strains that infected the oysters came from the sediment. It has been shown that some strains collected in a previous study (Azandegbe *et al.*, 2010) and later identified (results not shown) were slightly different from the virulent strains isolated in oysters.

The bacterial community structure of Aber Benoît sediment is significantly affected by the temperature. The predominant influence of this factor on bacterial communities agrees with

459 previous studies (Gonzalez-Acosta *et al*; 2006; Tabuchi*, et al.*, 2010). Its impact on the
460 bacterial community structure was less noticeable in Rivière d'Auray, where there was only a
461 significant effect in June and July 2007. Inversely, the effect of oysters was more often
462 significant in Rivière d'Auray than in Aber Benoît, with 5 out of 7 dates showing a significant
463 inter-station difference rather than only two. The correlations with the other biogeochemical
464 parameters, including ammonia, phosphate and nitrate, were very scattered.
465 The majority of the DGGE bands were affiliated to the γ-Proteobacteria. Several studies have
466 already shown the importance of this bacterial class in marine sediments. δ-Proteobacteria,
467 which is generally in second position, was less represented in the present study (Gray &
468 Herwig, 1996; Urakawa*, et al.*, 1999; Bowman*, et al.*, 2003; Bowman & McCuaig, 2003;
469 Bissett *et al.*, 2006).
470 Interestingly, the second bacterial class in Rivière d'Auray was the Bacilli. This group is
471 generally scarce in aquatic environments except in polluted areas, notably those contaminated
472 by hydrocarbons (Cavallo *et al.*, 1999; Chikere *et al.*, 2009). In addition, one strain of
473 *Bacillus* found (band n° 34) is closely related to a *Bacillus* sp. of terrestrial origin and may,
474 therefore, be an indicator of river input. Two strains that are members of the
475 *Desulfobacterium* genus, found at the Oyster station of Aber Benoît (band n° 1) and Rivière
476 d'Auray (band n° 28), were only ones that could be attributed to a specific metabolism.
477 Phylogenetic groups were similar between the two sites, except for *Bacilli* (RA) and
478 *Verrucomicrobia* (AB). In contrast, the phylotypes within each group were different, except
479 for *V. aestuarianus*. Finally the sediment of each site harbored its own bacterial community
480 regardless of the influence of oyster culture. In a recent study, van der Zaan *et al.*, (2010)
481 reported a degree of stability in terms of abundance and functional groups in sediment
482 bacterial flora exposed to pollutants. Consequently, as suggested by Bissett *et al.* (2006), the

483 sediment may have the ability to maintain diverse communities despite the disruption caused
484 by changes in organic matter input.
485 In conclusion, the oyster tables in Aber Benoît were probably the source of the greater
486 quantity of organic matter measured underneath them. This organic matter was more rapidly
487 degraded than in Rivière d'Auray, despite the temperature being 2 °C lower on average. The
488 substantial ammonia and sulfide fluxes, which may result from this degradation, were
489 insufficient to induce seawater toxicity, except maybe in the event of sediment resuspension.
490 These flux variations did not coincide with any change in bacterial community structure that
491 were little influenced by the temperature and the input of organic matter. The identified
492 bacteria belonged to bacterial groups that are common in sediment. Among the vibrios, *V.*
493 *aestuarianus* could represent a threat for oysters as it is pathogenic to them.
494 One reason why the bacterial community structures were found to be so static is perhaps
495 because the methods were not sensitive enough to reveal metabolically active bacteria.
496 In future study, the metabolically active bacteria would probably be easier to detect if
497 bacterial profiles could be determined from direct RNA extraction including rRNA (Nimnoi,
498 *et al.*, 2011), rather from template DNA. In addition, future work would need to focus on
499 particular functional groups using specific primers and real time PCR in order to monitor their
500 abundances (van der Zaan, *et al.*, 2010).
501 To confirm that the ammonia and sulfide do not reach the threshold of toxicity, they could be
502 monitored with specific electrodes, as used by Borum, *et al* (2005) and Berner et al. (1963).
503 The physiological status of oysters could be regularly examined by measuring the expression
504 of some genes or enzymes involved in stress responses, as already performed in some
505 previous studies (Le Moullac *et al.*, 2007; Kawabe & Yokoyama, 2012).
506
507

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648

Table 1. Main characteristics of Aber Benoît and Rivière d'Auray

	Aber Benoît	**Rivière dôAuray**
Location	North-west Brittany 4°36'W and 48°36'N	In the gulf of Morbihan South Brittany 2°58'W and 47°36'N
Catchment area	140 km²	800 km²
Average spring water flow	0.418 $m^3 s^{-1}$	2.99 $m^3 s^{-1}$ for the Loch river about 1.5 $m^3 s^{-1}$ for the Sal river
Anthropic influences	+	++
Oyster culture **Annual production/ area**	1500 t/ 38 ha	4500 t/ 1635 ha
Summer mortality of oyster before 2008	No	Yes
Depth at zero tide	14 m	20 m
Mean tidal range	6 m	5 m
Seawater current	up to 3.5 m s^{-1}	up to 4 m s^{-1}
Sediment	Sandy-mud	Muddy-sand
Salinity (percentage)	2.4 to 3.4	2.7 to 3.5.
Temperature range (this study)	11.1 to 18.1°C	10.8 to 21.8 °C

Table 2. Some biogeochemical characteristics of Aber Benoît sediment. Values of NO_3^-, PO_4^{3-} are means ± (SD) of calculated fluxes based on 3 replicates.

Sampling months	Temperature in °C	NO_3^-(µmol m^{-2} h^{-1})		PO_4^{3-}(µmol m^{-2} h^{-1})	
		Reference station	Oyster station	Reference station	Oyster station
June 2007	16.8	-39 (2)	-30 (2)	0 (0)	34 (6)
July 2007	18.1	-35 (0)	-22 (4)	0.1 (0)	0.7(0.1)
Aug 2007	16.6	-28 (9)	-25 (7)	1 (0)	2 (1)
Sept 2007	15.1	-36 (2)	-27 (2)	1.3(0.3)	0.6(0.1)
March 2008	11.1	-60 (12)	-52 (1)	1 (0)	1 (0)
May 2008	14.7	-31 (4)	-30 (8)	1 (1)	3 (2)
June 2008	15.5	-43 (8)	-36 (8)	2 (0)	45 (40)

Table 3. Some biogeochemical characteristics of the Rivière d'Auray sediment. Values of NO_3^-, PO_4^{3-} are means ± (SD) of calculated fluxes based on 3 replicates.

Sampling months	Temperature in °C	NO_3^-(µmol m^{-2} h^{-1})		PO_4^{3-}(µmol m^{-2} h^{-1})	
		Reference station	Oyster station	Reference station	Oyster station
June 2007	20.6	-2(0)	-1(0)	2(1)	9(2)
July 2007	21.8	-21(36)	-1(1)	3.7(1.6)	2.6(0.1)
Aug 2007	19.6	2(2)	0(0)	2(2)	12(2)
Sept 2007	14.7	2(2)	0(0)	1.6(0.6)	19.2(20.4)
March 2008	10.8	-74(8)	-77(13)	1(1)	1.8(0.2)
May 2008	18.2	-33(4)	-45(7)	6(4)	17(15)
June 2008	18.3	-5(8)	-3(5)	2(1)	10(2)

Table 4. Sequence analysis of bands excised from DGGE gels of Aber Benoît sediment. Letters correspond to clones of the same band.

Band n°	Phylum or class (family)	Accession number	Most related species in Genbank	Homology
1	*Deltaproteobacteria* (*Desulfobacteriaceae*)	FN555177	Sulphate-reducing bacterium JHA1	96 %
4a	*Gammaproteobacteria*	FN555178	Uncultured bacterium	96 %
5	*Acidobacteria*	FN555179	Uncultured bacterium clone C08P3MbH	94 %
6	*Gammaproteobacteria*	FN555180	Uncultured bacterium clone AV19F59b	98 %
7	*Flavobacteria*	FN555181	*Winogradskyella* sp. K7-7	98 %
8	*Flavobacteria*	FN555182	Uncultured bacterium clone Mn3b-B11	98 %
9	*Gammaproteobacteria*	FN555183	Uncultured bacterium clone AN05aug-063	98 %
10	*Gammaproteobacteria*	FN555184	Uncultured bacterium clone NY06dec-099	97 %
11-a	*Verrucomicrobia*	FN555185	Uncultured *verrucomicrobium* bacterium clone LD1-PB2	95 %
11-bc	*Deltaproteobacteria (Desulfobacteraceae)*	FN555186	Uncultured *Desulfobacterium* sp clone S57	98 %
12-acd	*Gammaproteobacteria* (*Vibrionaceae*)	FN555188	*Vibrio aestuarianus* 01/064	99 %
12-b	*Gammaproteobacteria*	FN555187	Uncultured *gammaproteobacterium* clone B05_RAMPDAY15	97 %
13	*Alphaproteobacteria; (Rhodobacteraceae)*	FN555189	*Roseovarius aestuarii*	97 %
14	*Gammaproteobacteria*	FN555190	Uncultured bacterium Ld1-1	98 %
15	*Deltaproteobacteria*	FN555191	Uncultured *deltaproteobacterium* clone YS-UMF5_122	98 %
16	*Alphaproteobacteria*	FN555192	Uncultured *alphaproteobacterium* clone CL33-G06	98 %
17	*Alphaproteobacteria* (*Rhodospirillaceae*)	FN555193	Uncultured bacterium clone C15cm.A08	97 %
18	*Verrucomicrobia*	FN555194	Uncultured bacterium clone L2-B01	98 %

Table 5. Sequence analysis of bands excised from DGGE gels of Rivière d'Auray sediment. Letters correspond to clones.

Band n°	Phylum or class *(family)*	Accession number	Most related species in Genbank	Homology
22	*Gammaproteobacteria (Vibrionaceae)*	FN555208	*Vibrio anguillarum* *Vibrio ordalii*	98 % 98 %
23	*Gammaproteobacteria*	FN555209	Uncultured bacterium isolate JH12_C45	98 %
24	*Gammaproteobacteria*	FN555210	Uncultured bacterium clone NY04sep-017	97 %
27	*Gammaproteobacteria*	FN555211	Uncultured bacterium clone OS02-TRNA-74 16S	98 %
28	*Deltaproteobacteria*	FN555212	*Desulfobacterium* sp AN05aug-096	97 %
29-ab	*Gammaproteobacteria (Vibrionaceae)*	FN555213	*Vibrio aestuarianus* strain 01/031	99 %
29-c	*Alphaproteobacteria*	FN555214	Uncultured *Rhodobacteraceae* bacterium clone Q8-C10	98 %
30	*Gammaproteobacteria*	FN555215	Uncultured *gammaproteobacterium* clone SIMO-4102	96 %
31	*Flavobacteria*	FN555216	Unculture *Bacteroidetes* bacterium clone CL18-D02	92 %
33	*Gammaproteobacteria*	FN555217	*Microbulbifer thermotolerans*	97 %
34	*Bacilli* (*Bacillaceae*)	FN555218	*Bacillus* sp. GN-M06-10	97 %
35	*Deltaproteobacteria*	FN555219	Uncultured *Deltaproteobacterium* clone YS-UMF5_80	98 %
36-a	*Bacilli* (*Paenibacillaceae*)	FN555220	*Brevibacillus laterosporus* strain 42S9	96 %
36-b	*Bacilli (Bacillaceae*)	FN555221	*Pontibacillus sp. R527*	96 %
37	*Gammaproteobacteria*	FN555222	*Microbulbifer thermotolerans*	96 %
38	*Gammaproteobacteria*	FN555223	*Marinobacter taiwanensis* strain GI-1701	98 %
39	*Bacilli (Bacillaceae*)	FN555224	Uncultured bacterium clone MFC-10	98 %
40	*Bacilli* (*Bacillaceae*)	FN555225	bacterium enrichment culture clone heteroA8_4W	96 %
41-ab	*Acidobacteria*	FN555226	Uncultured *Acidobacteriaceae* bacterium clone NdGal159	97 %
41-c	*Unclassified bacterium*	FN555227	Uncultured bacterium clone G2-01	93 %
41-d	*Gammaproteobacteria*	FN555228	*Marinobacter sp.*	98 %
42	*Bacilli* (*Bacillaceae)*	FN555229	Bacterium enrichment culture clone 080213-ABM-Febex	96 %

Table 6. Statistical significance of bacterial structure changes as a function of different parameters. Spearman correlation was used to test for the correlation between bacterial structures and environmental parameters. Values in brackets are Rho and p.

Parameter	**Aber Benoît**	**Rivière dôAuray**
Temperature	June-July 2007 (0.325, 0.018) August-September 2007 (0.413, 0.03) All months in 2008 (0.139, 0.06)	June-July 2007 (0.398, 0.01)
Nitrate flux	June-July (0.198, 0.083)	June-July (0.412, 0.032) August-September (0.307, 0.066).
Phosphate flux		August-September (0.213, 0.046).

Fig. 1. Map showing (A) Aber Benoît and (B) Rivière d'Auray in the gulf of Morbihan. Locations of the sampling sites are indicated by arrows.

Fig. 2. Oxygen microprofiles as a function of depth (mm) at oyster and reference sites in Aber Benoît and Rivière d'Auray (May 2008). The X axis, represents the sediment water interface. O_2 is expressed both in mg l^{-1} and µmol l^{-1}**.**

Fig. 3. Organic Carbon (OC) values in Aber Benoît and Rivière d'Auray. Values are means (± SD) based on 3 replicates.

Fig. 4. Histogram of NH_4^+ values in Aber Benoît and Rivière d'Auray. Values are means (± SD) of calculated fluxes based on 3 replicates.

Fig. 5. Histogram of sulfide values in Aber Benoît and Rivière d'Auray. Values are the calculated fluxes from one sediment sample.

Fig. 6. Culturable bacterial counts in Aber Benoît and Rivière d'Auray: (A) Total culturable bacteria (Marine Agar) and (B) vibrios (TCBS), according to dates.

Fig.7. DGGE profiles of sediments sampled (A) in Aber Benoît in 2007 and (B) in Rivière d'Auray in 2008. The sequenced bands are numbered.

Fig. 8. MDS maps derived from DGGE band profiles of all 24 samples taken in 2007 and all 32 samples taken in 2008, showing spatial and temporal variations in bacterial diversity in the sediment of Aber Benoît. The ANOSIM were performed to test for significant differences between dates and between sites with oysters (O) or without (R). Influence of oyster tables in June 2007 (R = 0.956, 10 %) and February 2008 (R = 0.656, 2.9 %). Variation between successive months between June-July 2007 (Reference: R = 0.926, 10 %; Oyster: R = 1, 10 %) and between February-March 2008 (Oyster: R = 0.771, 2.9 %).

Fig. 9. MDS maps derived from DGGE band profiles of all 24 samples taken in 2007 and all 32 samples taken in 2008, showing spatial and temporal variations of bacterial diversity in the sediment of Rivière d'Auray. The ANOSIM were performed to test for significant differences between dates and between sites with oysters (O) or without (R). Influence of oyster tables in June 2007 (R = 0.815, 10 %), August 2007 (R = 0.593, 10 %), September 2007 (R = 1, 10 %), February 2008 (R = 1, 10 %) and March 2008 (R=1, 0.1%). Variation between successive months between June-July (Oyster: R = 1, 10 %; Reference: R = 0.519, 10 %) and between August- September (Oyster: R = 0.667, 2.9 %).

Fig.1

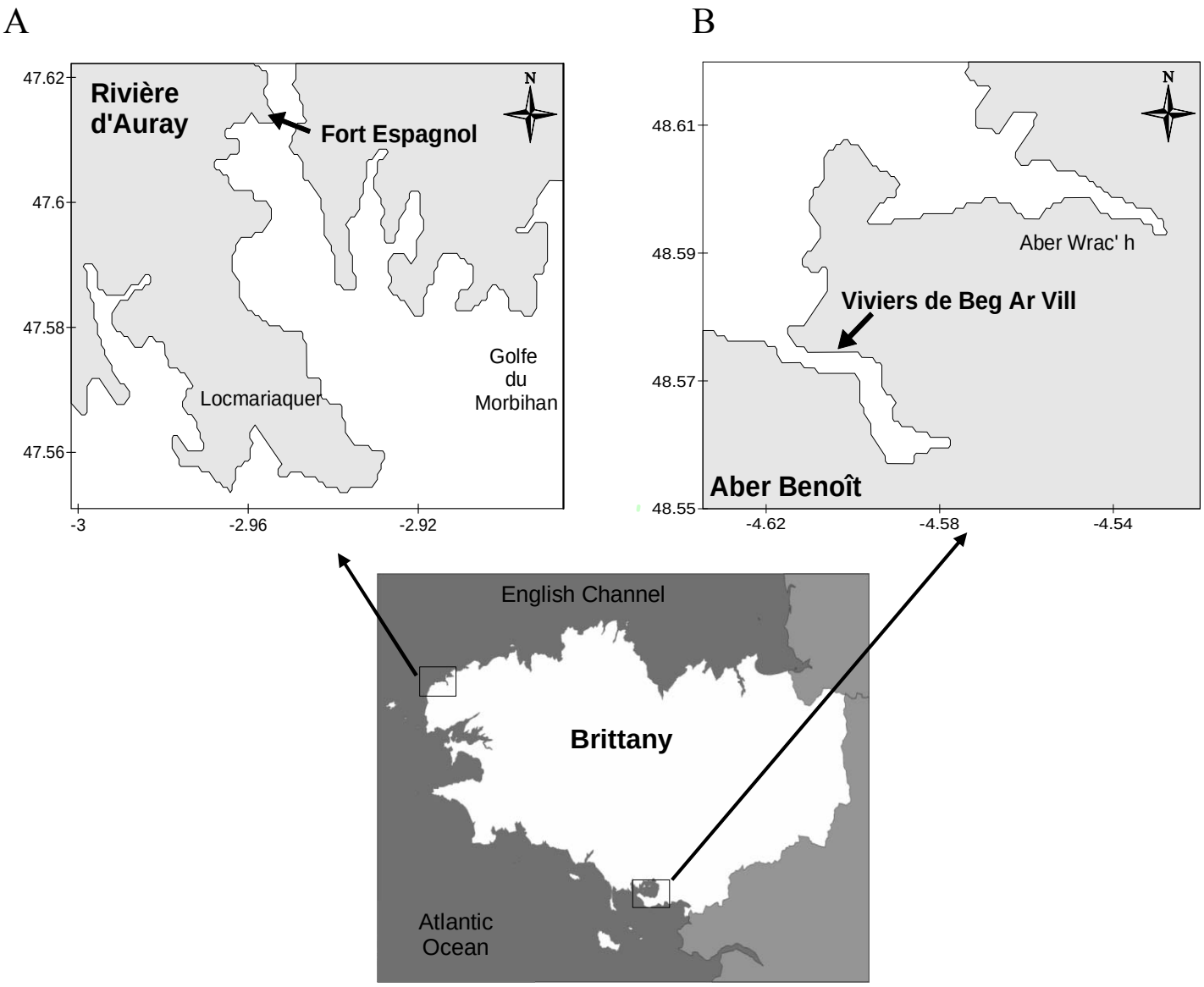

A
B
Rivière
d'Auray
Fort Espagnol
Locmariaquer
Golfe
du
Morbihan
47.62
47.6
47.58
47.56
-3
-2.96
-2.92
N
Aber Wrac' h
Viviers de Beg Ar Vill
Aber Benoît
48.61
48.59
48.57
48.55
-4.62
-4.58
-4.54
English Channel
Brittany
Atlantic
Ocean

Fig. 2

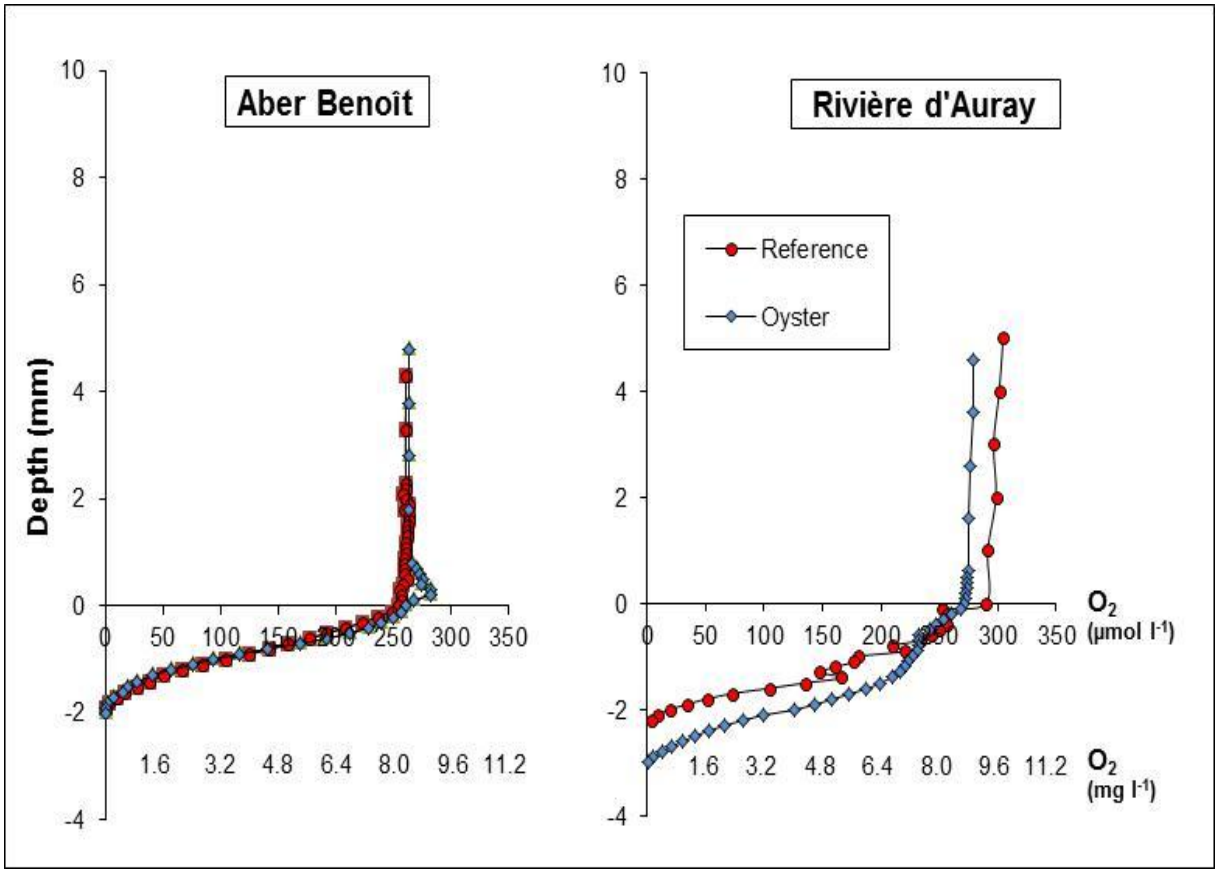

Aber Benoît
Rivière d'Auray
Depth (mm)
Reference
Oyster
10
8
6
4
2
0
-2
-4
0 50 100 150 200 250 300 350
1.6 3.2 4.8 6.4 8.0 9.6 11.2
O2 (µmol l-1)
O2 (mg l-1)

Fig. 3

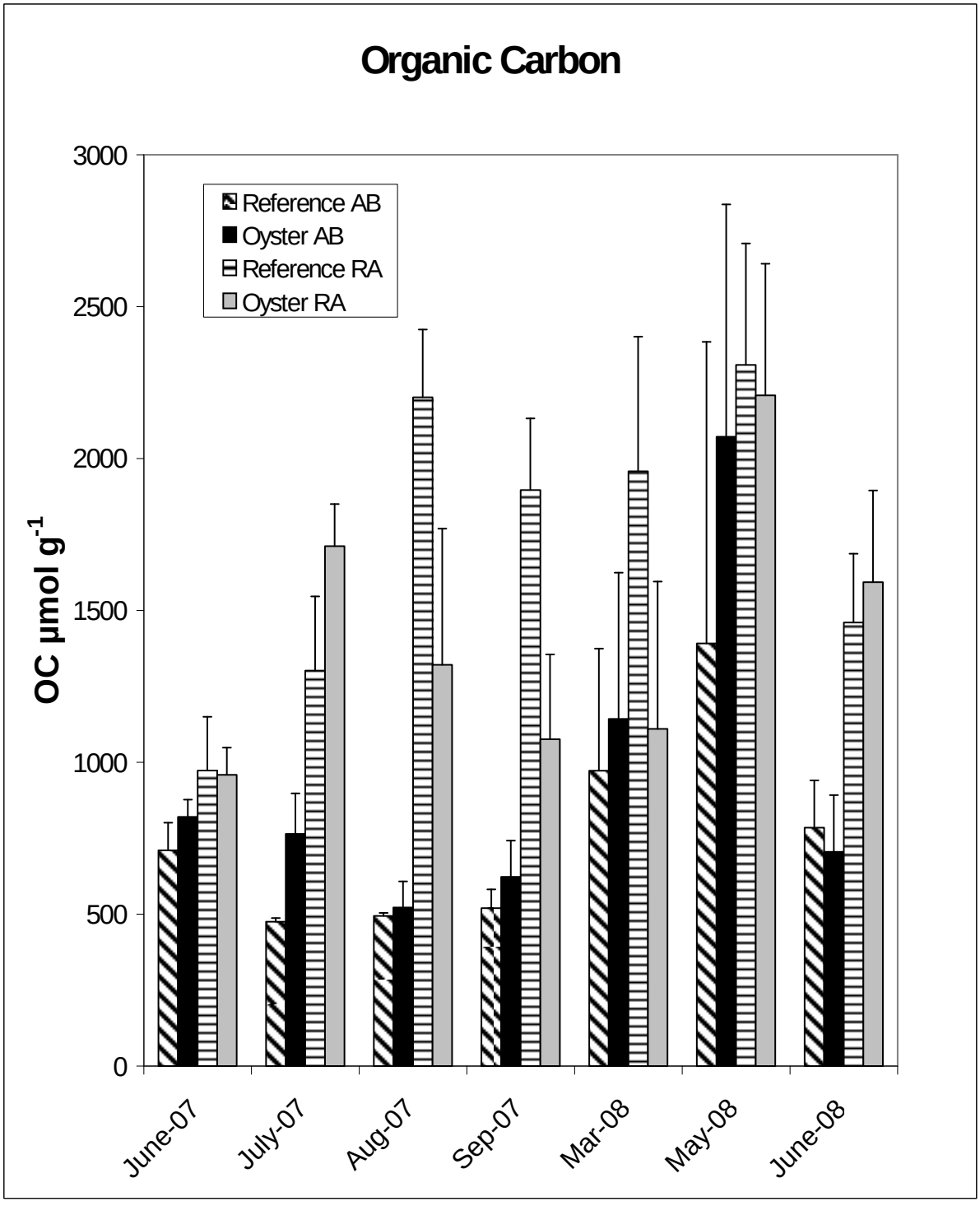

Organic Carbon
OC µmol g-1
3000
2500
2000
1500
1000
500
0
Reference AB
Oyster AB
Reference RA
Oyster RA
June-07
July-07
Aug-07
Sep-07
Mar-08
May-08
June-08

Fig. 4

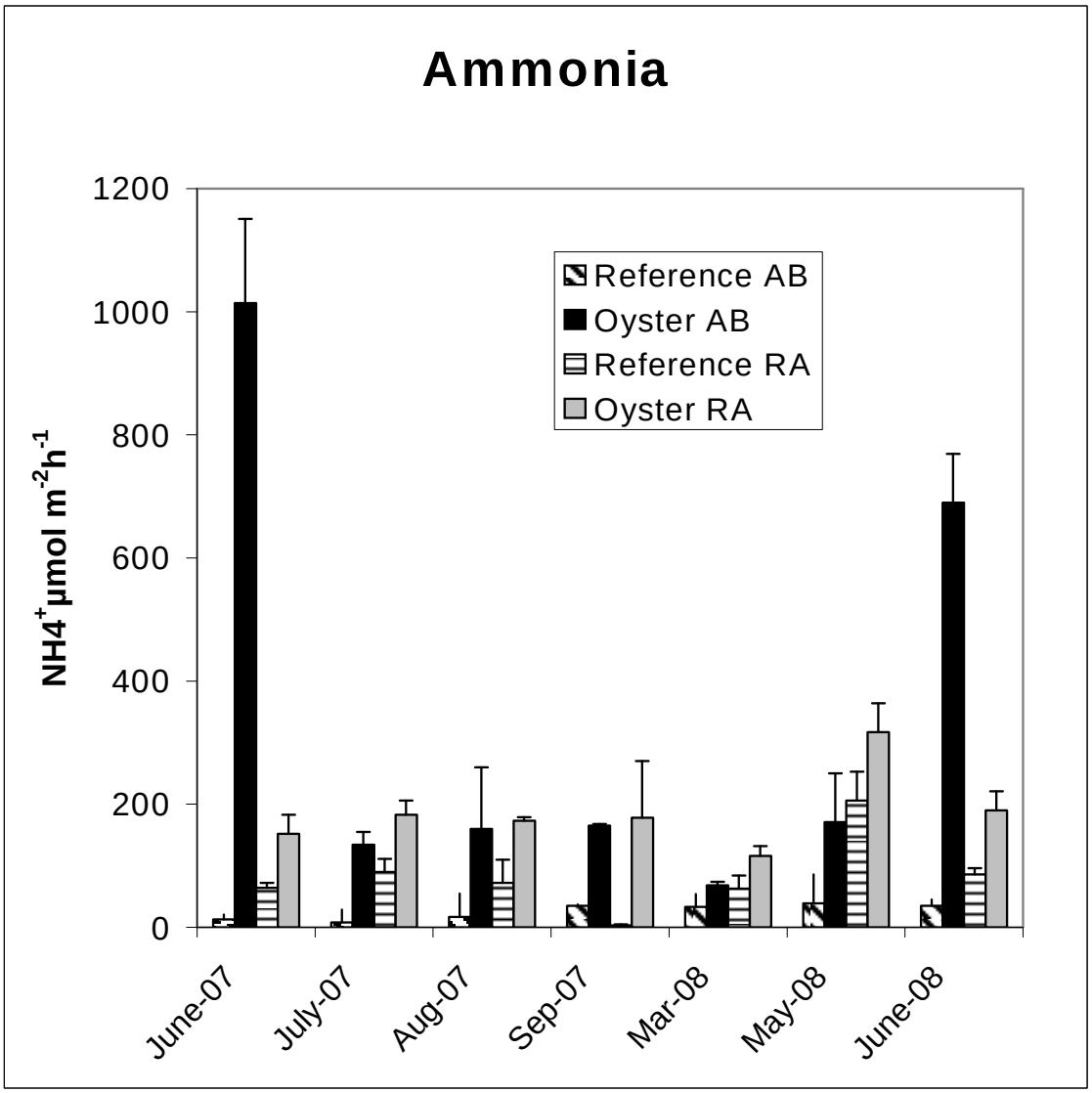

Ammonia
1200
1000
800
600
400
200
0
NH4+µmol m-2h-1
Reference AB
Oyster AB
Reference RA
Oyster RA
June-07
July-07
Aug-07
Sep-07
Mar-08
May-08
June-08

Fig. 5

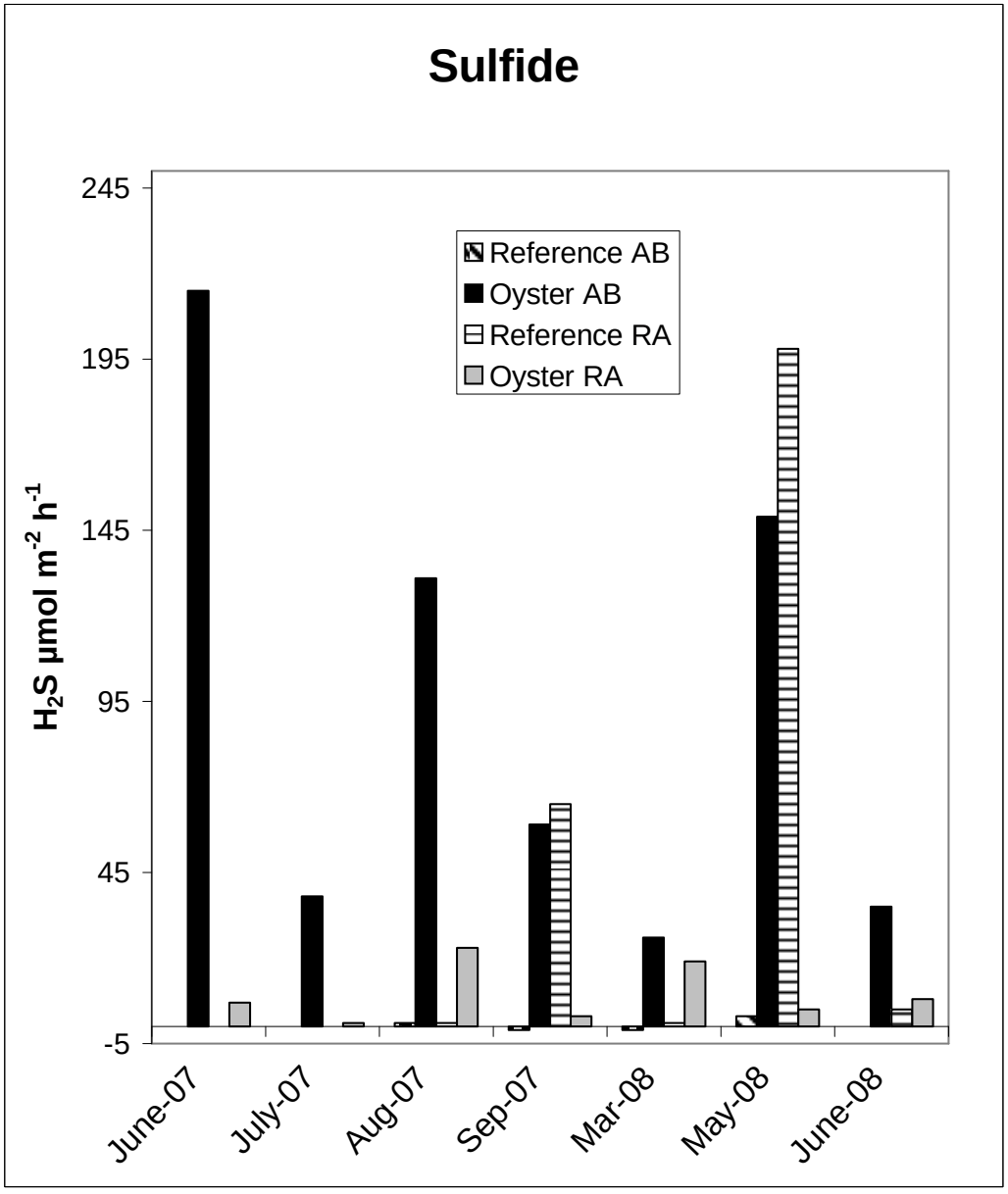
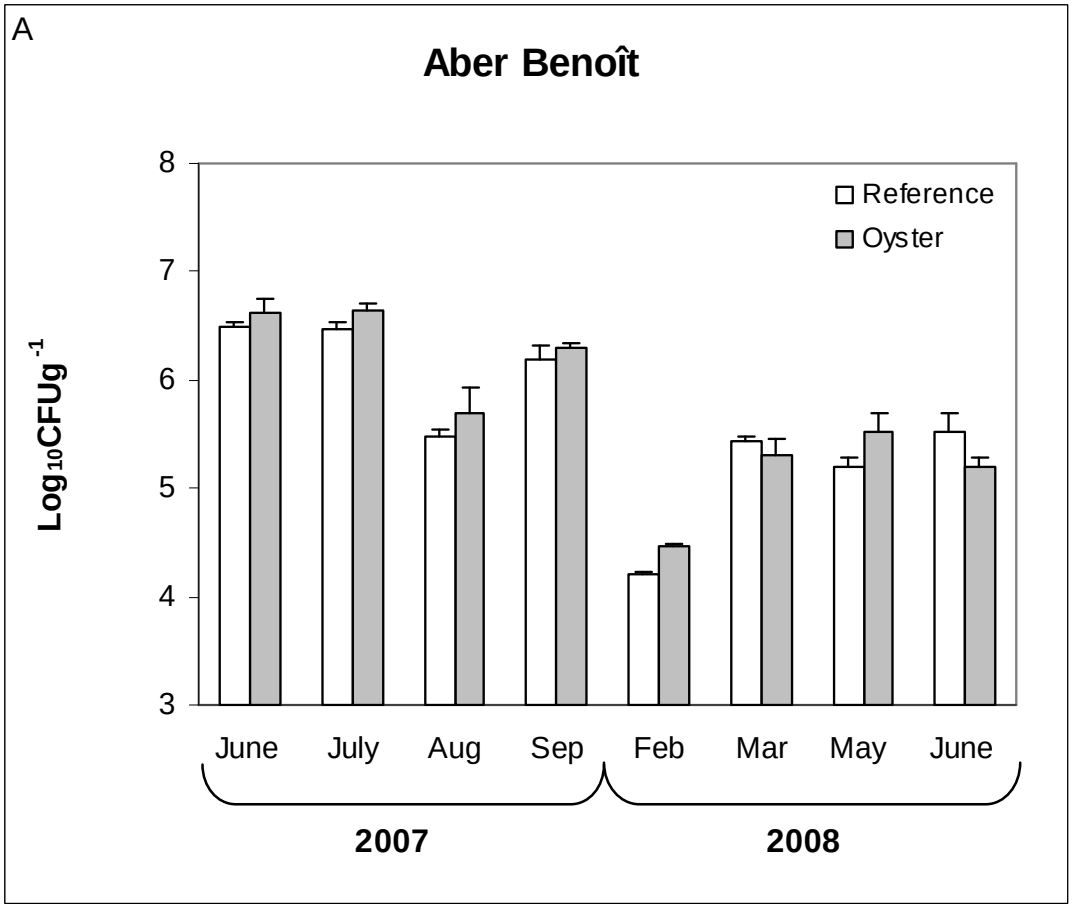
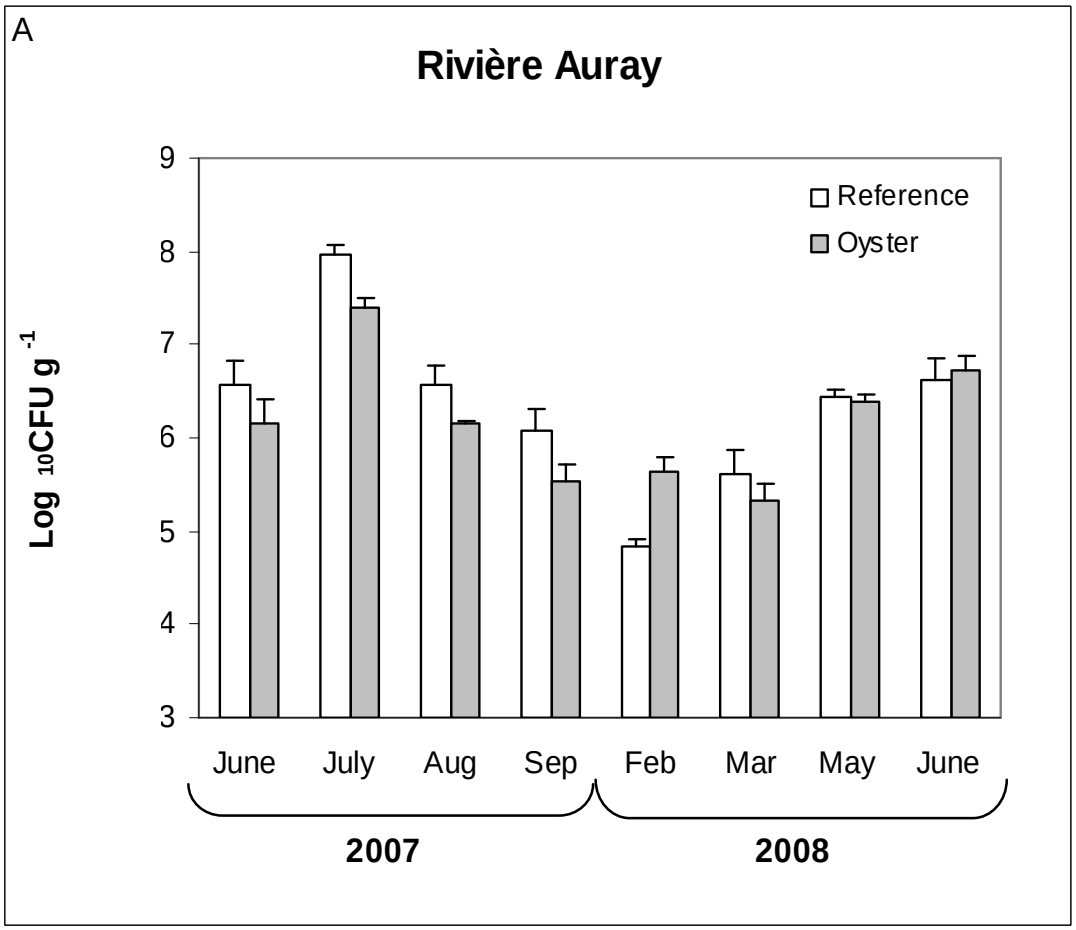
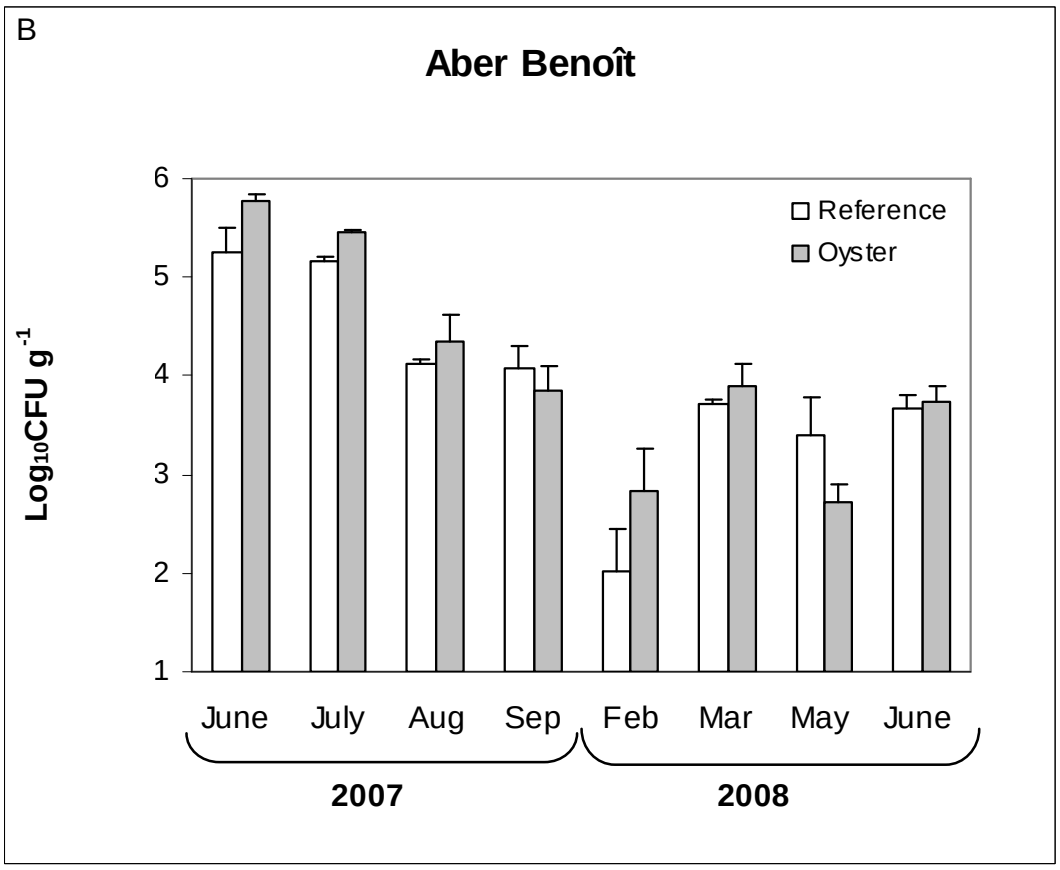

Sulfide
245
195
145
95
45
-5
H2S µmol m-2 h-1
Reference AB
Oyster AB
Reference RA
Oyster RA
June-07
July-07
Aug-07
Sep-07
Mar-08
May-08
June-08

Fig. 6


A
Aber Benoît
Reference
Oyster
Log10CFUg-1
8
7
6
5
4
3
June
July
Aug
Sep
Feb
Mar
May
June
2007
2008


A
Rivière Auray
Reference
Oyster
Log 10CFU g-1
9
8
7
6
5
4
3
June
July
Aug
Sep
Feb
Mar
May
June
2007
2008


B
Aber Benoît
Log10CFU g-1
6
5
4
3
2
1
Reference
Oyster
June
July
Aug
Sep
Feb
Mar
May
June
2007
2008

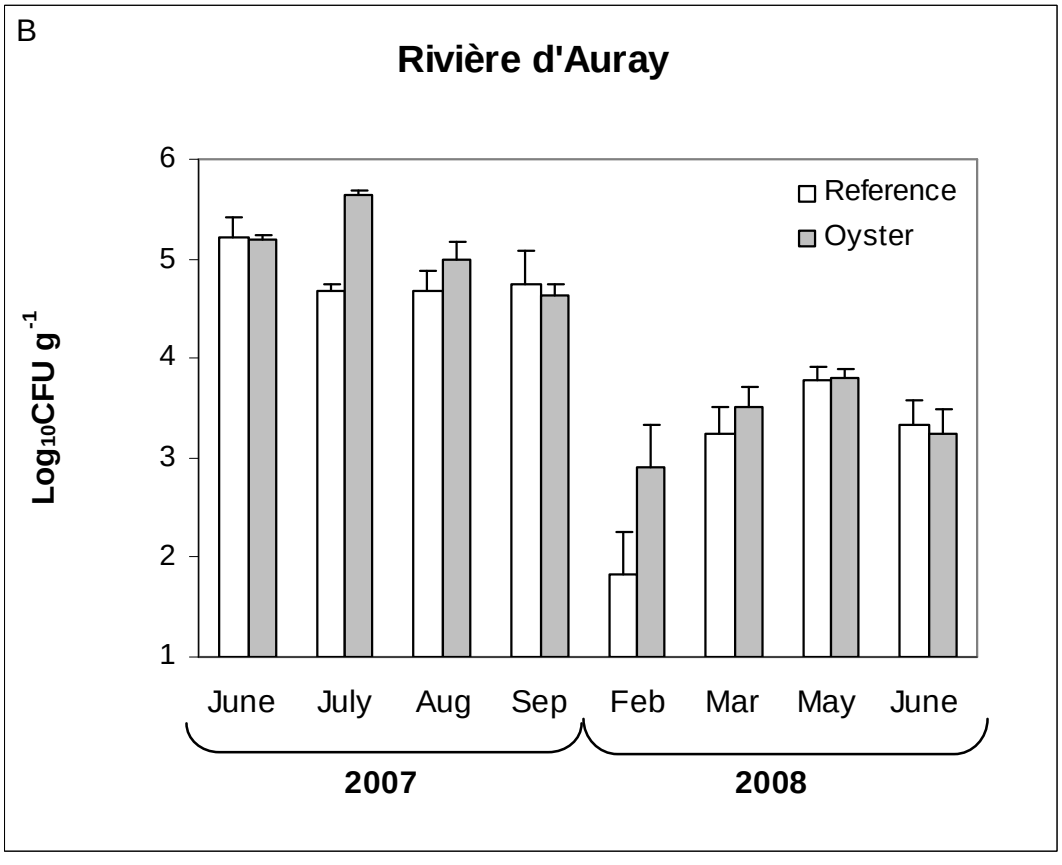

B
Rivière d'Auray
Log10CFU g-1
6
5
4
3
2
1
Reference
Oyster
June
July
Aug
Sep
Feb
Mar
May
June
2007
2008

Fig. 7

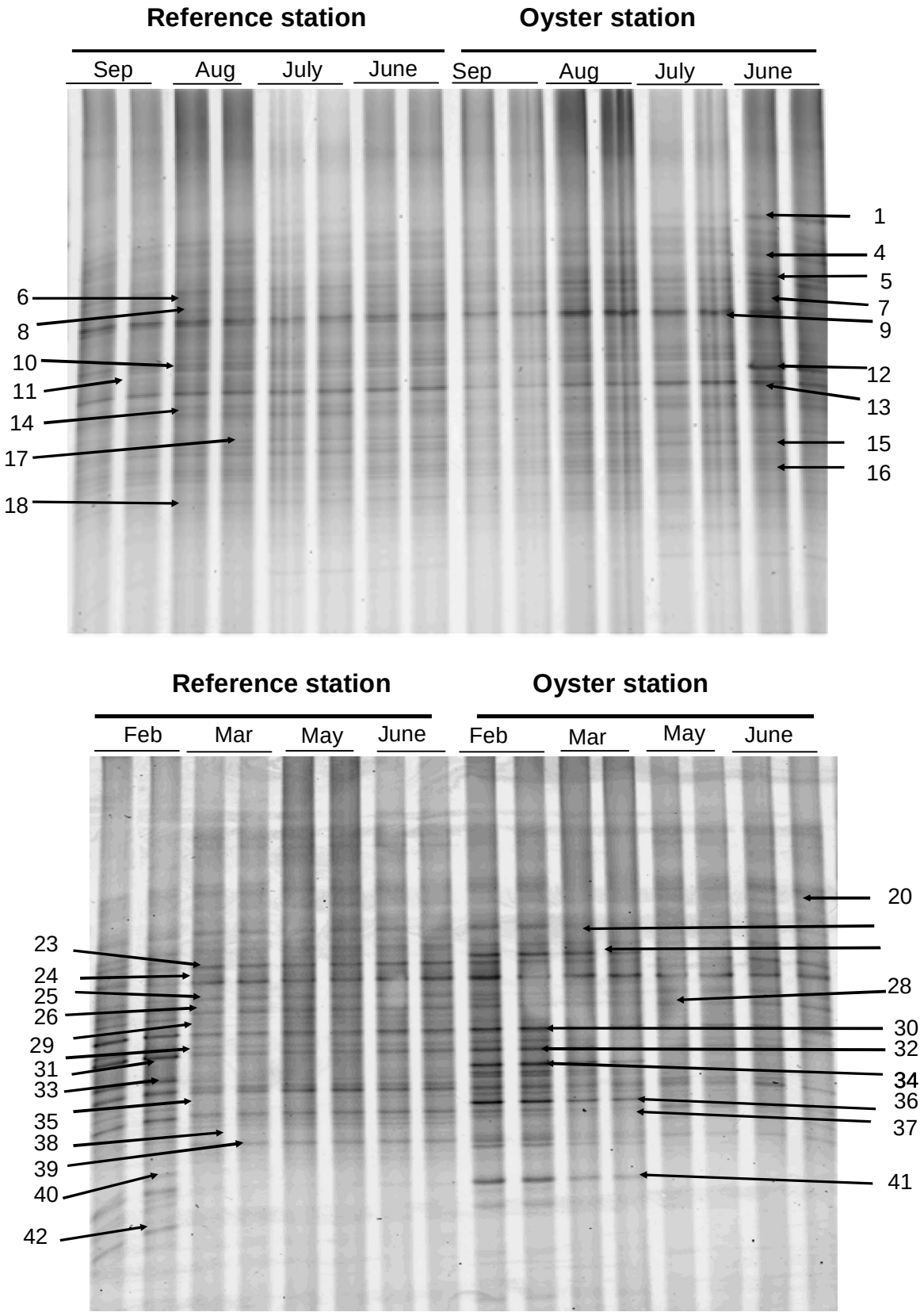
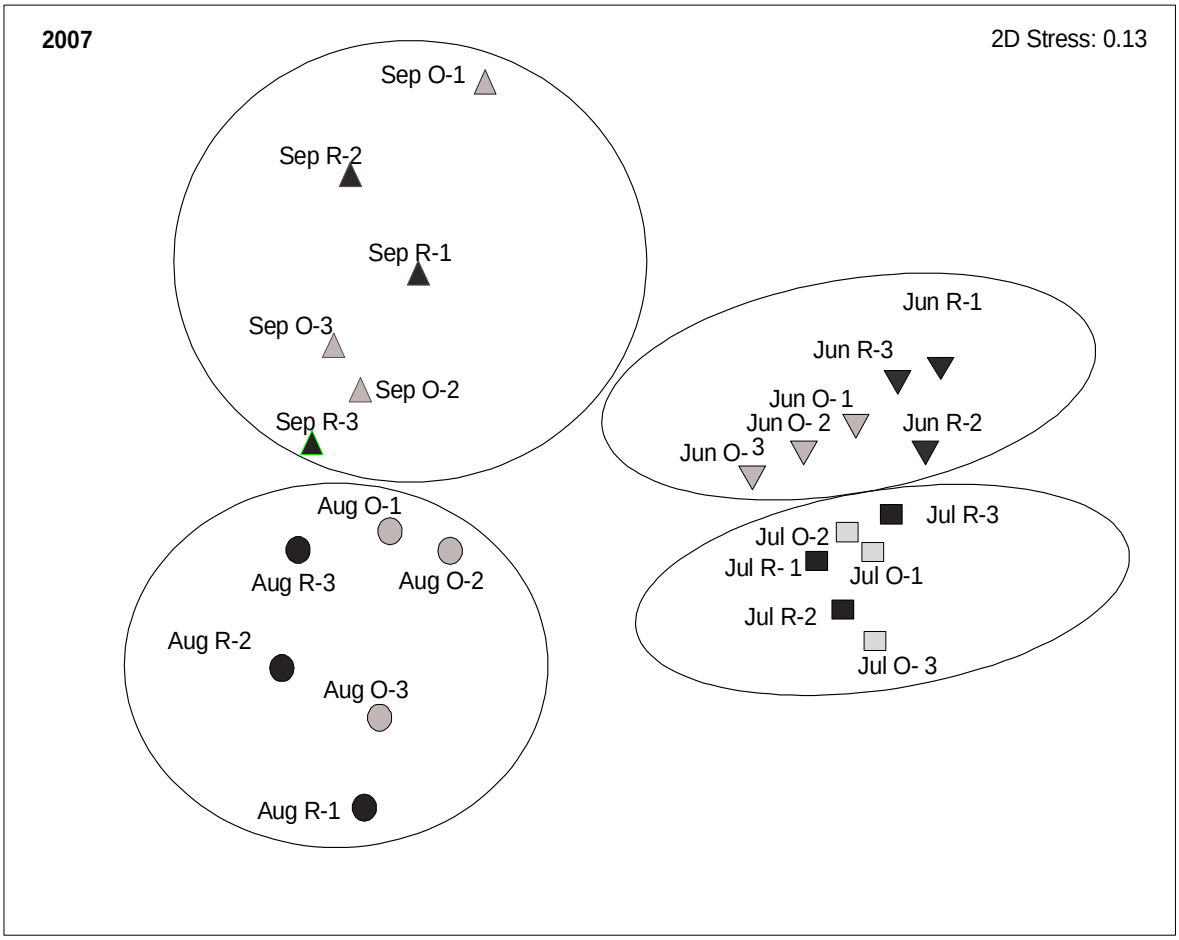

Reference station
Oyster station
Sep
Aug
July
June
Sep
Aug
July
June
1
4
5
7
9
12
13
15
16
6
8
10
11
14
17
18
Reference station
Oyster station
Feb
Mar
May
June
Feb
Mar
May
June
20
28
30
32
34
36
37
41
23
24
25
26
29
31
33
35
38
39
40
42

Fig. 8


2007
2D Stress: 0.13
Sep O-1
Sep R-2
Sep R-1
Sep O-3
Sep O-2
Sep R-3
Jun R-1
Jun R-3
Jun O- 1
Jun O- 2
Jun R-2
Jun O- 3
Aug O-1
Aug R-3
Aug O-2
Aug R-2
Aug O-3
Aug R-1
Jul R-3
Jul O-2
Jul R- 1
Jul O-1
Jul R-2
Jul O- 3

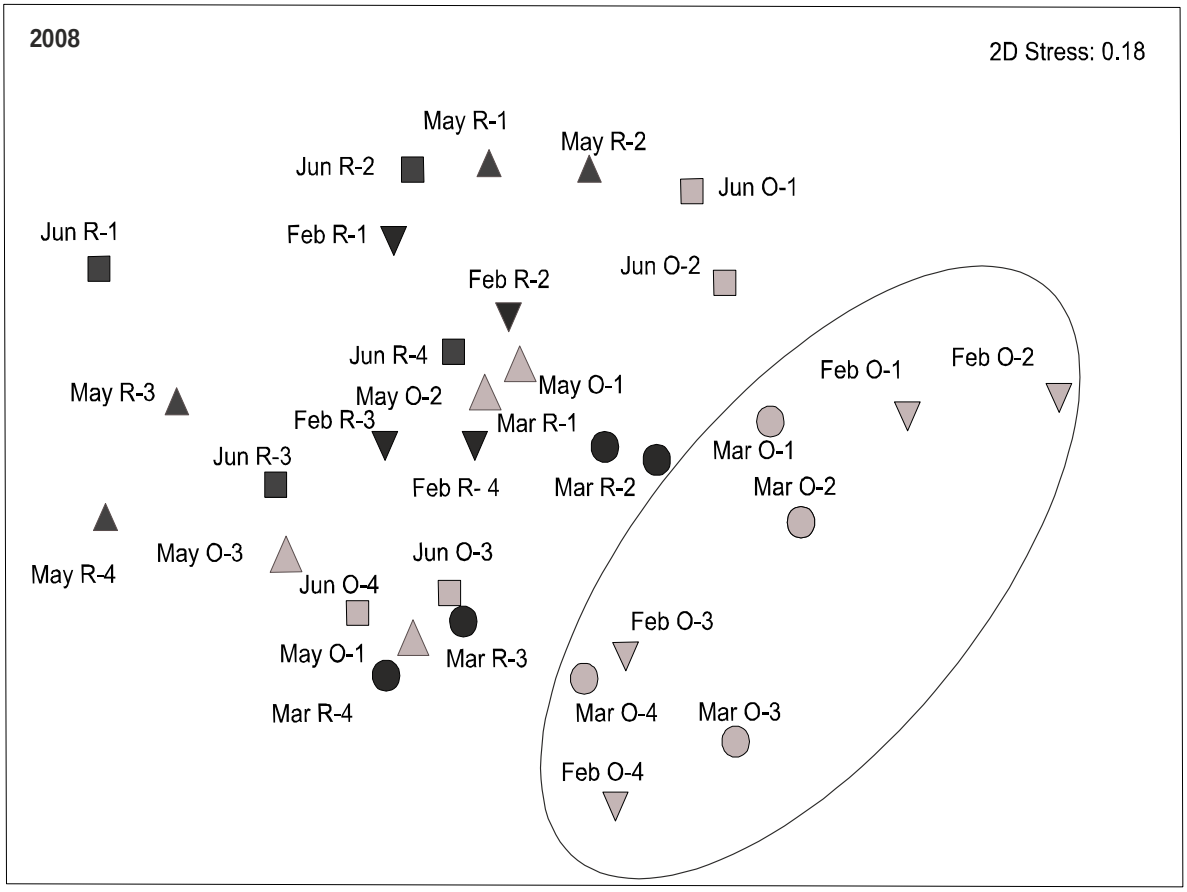
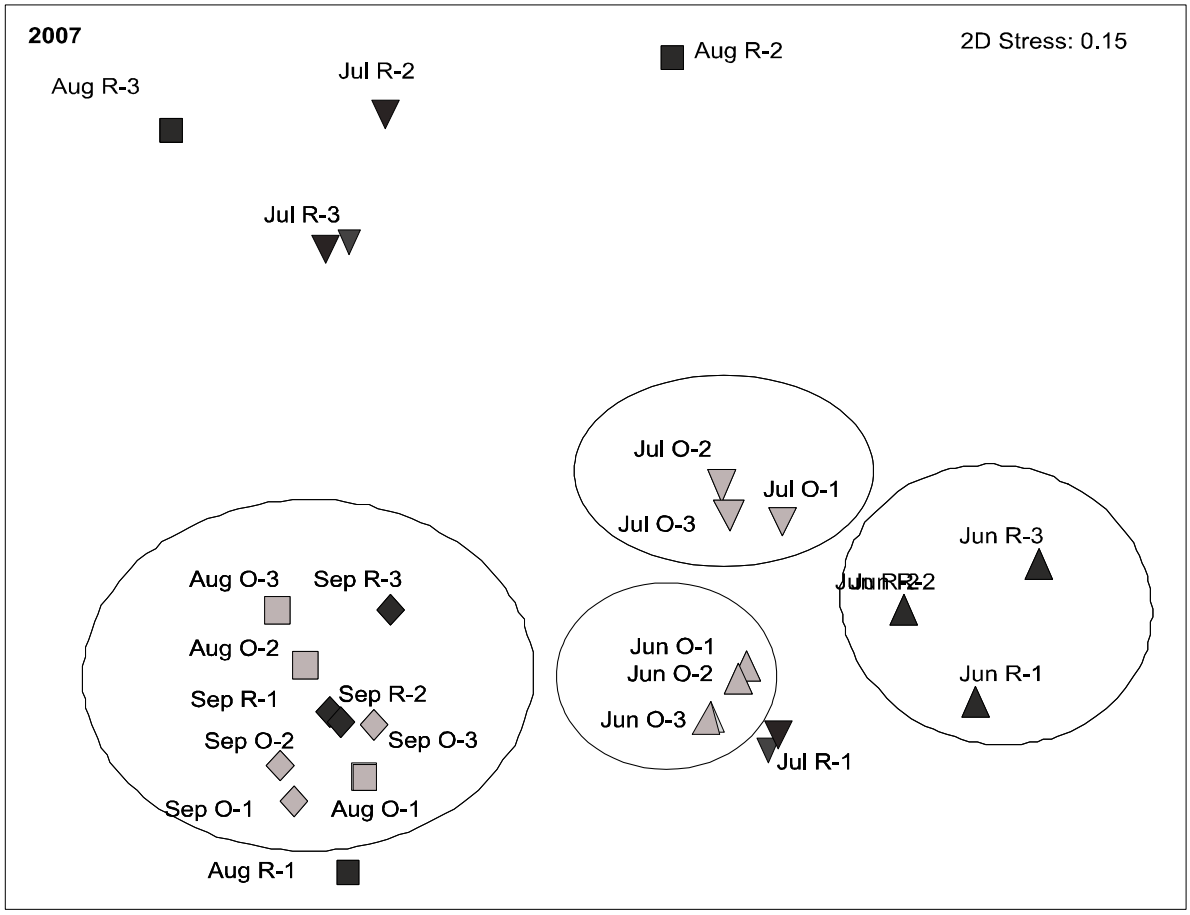
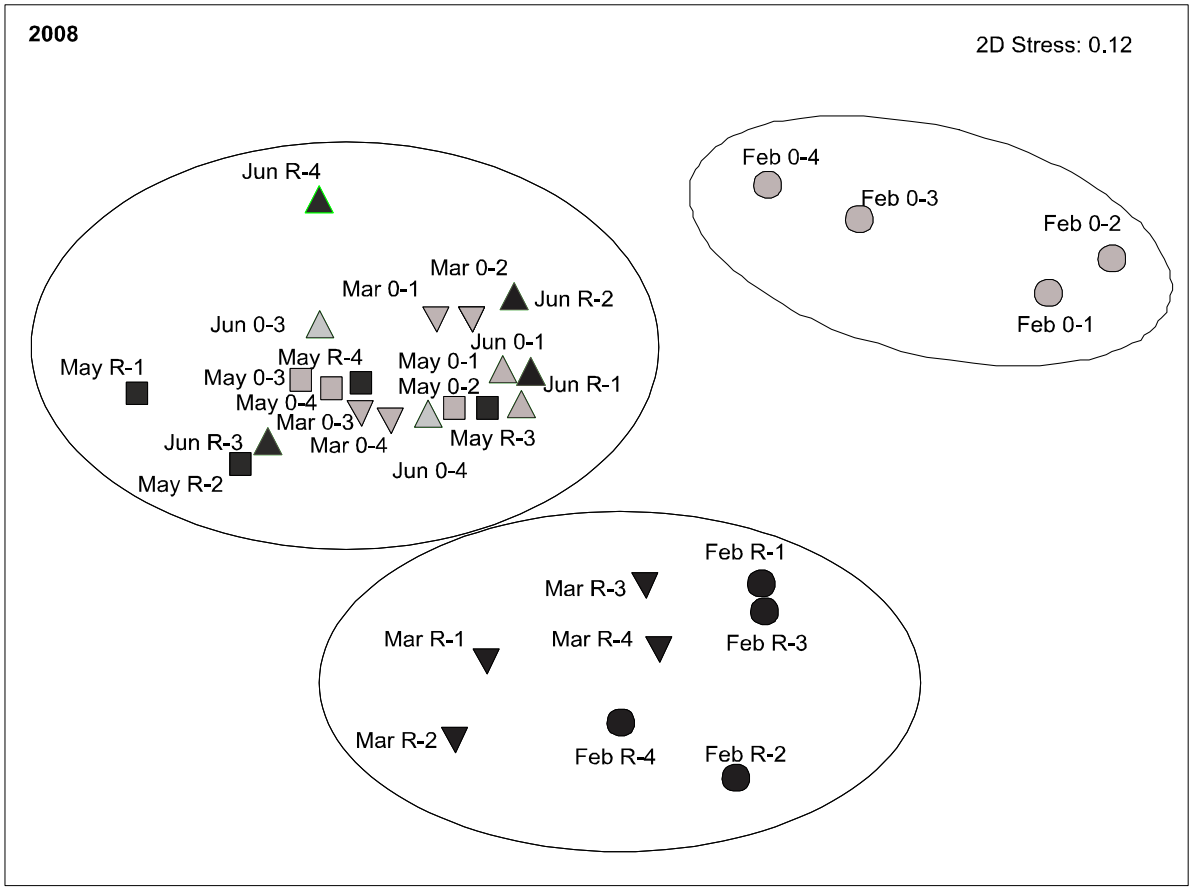

2008
2D Stress: 0.18
May R-1
May R-2
Jun R-2
Jun O-1
Jun R-1
Feb R-1
Jun O-2
Feb R-2
Jun R-4
May O-1
May R-3
May O-2
Feb R-3
Mar R-1
Jun R-3
Feb R- 4
Mar R-2
Feb O-1
Feb O-2
Mar O-1
Mar O-2
May R-4
May O-3
Jun O-3
Jun O-4
Feb O-3
May O-1
Mar R-3
Mar R-4
Mar O-4
Mar O-3
Feb O-4

Fig. 9


2007
2D Stress: 0.15
Aug R-2
Aug R-3
Jul R-2
Jul R-3
Jul O-2
Jul O-1
Jul O-3
Jun R-3
Jun R-2
Jun R-1
Jun O-1
Jun O-2
Jun O-3
Jul R-1
Aug O-3
Sep R-3
Aug O-2
Sep R-1
Sep R-2
Sep O-2
Sep O-3
Sep O-1
Aug O-1
Aug R-1


2008
2D Stress: 0.12
Jun R-4
Mar 0-2
Mar 0-1
Jun R-2
Jun 0-3
Jun 0-1
May R-4
May 0-1
May R-1
May 0-3
May 0-2
Jun R-1
May 0-4
Mar 0-3
May R-3
Jun R-3
Mar 0-4
Jun 0-4
May R-2
Feb 0-4
Feb 0-3
Feb 0-2
Feb 0-1
Feb R-1
Mar R-3
Mar R-1
Mar R-4
Feb R-3
Mar R-2
Feb R-4
Feb R-2