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1 **Failure of bivalve foundation species recruitment related to trophic changes during an**  
2 **extreme heat wave event**

3

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20

21 **ABSTRACT**

22 Bivalves are regulators of coastal lagoons and provide a wide range of ecosystem services.  
23 However, coastal lagoons are sensitive to climate change. Our objective was to describe the  
24 drivers of the cascade of ecological events that occurred during a summer heat wave and resulted  
25 in recruitment failure of the oyster *Crassostrea gigas*. Results showed elevated temperature and  
26 salinity caused a shift in planktonic food availability toward smaller taxa. These trophic changes  
27 did not affect food accumulation by oyster larvae or their fatty acid composition but did affect  
28 post-metamorphosis success, as their gill development was not adapted to these small particles.  
29 This resulted in the failure of oyster recruitment and stimulated the development of annelids, a  
30 trophic and spatial competitor that can better ingest small particles. This knowledge suggests  
31 that in the context of marine heat waves, the ecological limits of oyster larvae are narrower than  
32 their physiological limits.

33

34 **KEYWORDS**

35 Climate change, Phenology, Extreme Heat Wave, Bivalves, Pacific Oyster, *Crassostrea gigas*,  
36 Reproduction, Larval Ecology, Cascade of Environmental Effects, Trophic Changes.

37

## 1. INTRODUCTION

Coastal lagoons provide a wide range of ecosystem services (Chapman 2012, Villamagna et al. 2013, Kermagoret et al. 2019), associated with biodiversity, including bivalves which are of great ecological interest and high commercial value for some of them. Bivalves also have an important regulatory functions in the ecosystem thanks to their capacity to extract particles, to regenerate and store nutrients and to form hard biogenic structures (Smaal et al. 2019). However, because coastal lagoons are shallow and have limited exchange with the ocean, they are highly sensitive to eutrophication, heat waves, hypoxia and acidification, as well as to the effects of global climate change (Lloret et al. 2008, Lu et al. 2018, Thomas et al. 2018). An atmospheric heat wave is defined as five consecutive days with a maximum temperature 5°C above the 1976-2005 normal (Jouzel et al. 2014). Summer 2019 was characterized by two heat waves of exceptional intensity over France, including the Thau Basin, one from June 24 to July 7, the other from July 21 to 27. The absolute heat record for France (46 °C) was measured in Vérargues in the Hérault administrative department (Météo-France 2019), which includes the Thau basin. Marine heatwaves (MHW) are extreme events defined as abrupt but prolonged periods of high sea surface temperatures that can occur anywhere, at any time (Scannell et al. 2016, Schlegel et al. 2017, Hobday et al. 2018). High water temperatures increase the metabolic requirements of bivalves (Filgueira et al. 2016, Thomas & Bacher 2018). Even if temperatures remain within the species' thermal range, high temperatures combined with salinity and/or food variations, can negatively impact the life cycle of bivalves (Filgueira et al. 2016).

Several studies suggest that global changes are disrupting plankton communities and their nutritional values by affecting the abundance, size and diversity of primary producers

61 (Klauschies et al. 2012, Sommer et al. 2012, Trombetta et al. 2019). Generally, elevated  
62 temperatures affect phytoplankton cell size with a shift from larger to smaller species (Bec et  
63 al. 2005, Trombetta et al. 2019). Adult bivalves can assimilate small phytoplanktonic particles  
64 (Sonier et al. 2016). However, the efficiency of the capture is regulated by the morphology of  
65 their gills, and is generally low when small particles as picoplankton are present (Rosa et al.  
66 2018). Larvae feed through a less selective velum (Bower & Meyer 1990). Marine  
67 phytoplankton species are major producers of long-chain polyunsaturated essential fatty acids  
68 (EFA) but are predicted to decrease due to ocean warming (Hixson & Arts 2016, Colombo et  
69 al. 2017). The fatty acids docosahexaenoic acid (22:6 $\omega$ 3; DHA), eicosapentaenoic acid (20:5 $\omega$ 3;  
70 EPA) and arachidonic acid (AA) are essential for the growth and survival of marine  
71 invertebrates, particularly during their metamorphosis from pelagic larvae to benthic juveniles  
72 and ultimately, their recruitment success (Gagné et al. 2010, Bassim et al. 2015). Since EFAs  
73 are poorly biosynthesized by marine animals, their intake depends on their food (Glencross  
74 2009, Da Costa et al. 2015). Thus, both the right size and the right fatty acid composition of  
75 larval food are essential for the recruitment success of bivalves.

76 The aim of this study was to identify the environmental factors and trophic conditions  
77 (Supplementary material table1 & table 2) associated with the recruitment failure of the Pacific  
78 oyster, *Crassostrea gigas*, during a heat wave. We compared two contrasted years (2017 no heat  
79 wave and 2019 heat wave) in four sites in the Thau lagoon, France (Fig. 1). We hypothesize that  
80 the heat waves, characterized by high temperatures and high salinity, have a negative impact on  
81 oyster recruitment due to poor larval feeding conditions caused by changes in plankton diversity.

82

## 83 **2. MATERIALS AND METHODS**

84 **2.1 Experimental design**

85 Oyster recruitment was monitored from July 24 to August 21, 2017, and from July 2 to 29, 2019,  
86 at four experimental sites in the Thau lagoon (southern France; Fig 1.). The average depth of  
87 the lagoon is 4 m. The lagoon covers an area of 7 500 ha (19 km x 4.5 km) of which 20% is  
88 used for shellfish culture (oysters and mussels). The lagoon is connected to the Mediterranean  
89 Sea via a network of channels through Sète Harbor(Fiandrino et al. 2017). Two experimental  
90 sites were located inside the shellfish farming areas (Marseillan and Bouzigues) while two  
91 others were located outside the shellfish farming areas (Meze and Listel) (Fig 1.).

92

93 **2.2 Oyster analyses**

94 Three sets of oyster collectors were submerged vertically 2 m below the surface at each of the  
95 four study sites in the Thau lagoon to collect young settlers (pediveligers settled on collectors,  
96 metamorphosed juveniles, and newly metamorphosed juveniles). The collectors were installed  
97 once the oyster's larval supply reached a density of 10 000 larvae/m<sup>3</sup> (VELYGER network<sup>44</sup>).  
98 The collectors located inside the shellfish culture areas were suspended from existing farming  
99 structures. Those outside the area were suspended using a mooring system (Lagarde et al. 2017,  
100 2019). Each collector was made of 44 white PVC plastic plates (15 cm diameter; surface area  
101 of 250 cm<sup>2</sup>) stacked on a 110 cm tube. Two weeks after their immersion, three plates per  
102 collector were harvested [at the top (i.e., the 5<sup>th</sup> from the surface), in the middle (the 22<sup>nd</sup>) and  
103 at the bottom (the 39<sup>th</sup>)] and data were pooled to assess the abundance of young settlers and fatty  
104 acid (FA) content (µg larva<sup>-1</sup>). A similar sampling procedure was used four weeks after the  
105 collectors were immersed to assess the abundance of juveniles.

106 The abundance of young settlers and juveniles was assessed on the upper surface of each plate  
107 using standard 15 cm<sup>2</sup> sub-units. Depending on the abundance, 3 to 12 sub-units were randomly  
108 selected for counting and the resulting replicates were averaged to obtain the total number of  
109 individuals per plate. Recruitment was evaluated from the abundance of juveniles and  
110 metamorphosis from the ratio of juvenile to young-settler abundances. Size at metamorphosis  
111 was estimated by measuring the prodissoconch II (PII) (Martel et al. 1995). A maximum of 60  
112 spats were removed from each plate sampled after the fourth week after immersion, and placed  
113 on a plasticine flange fixed on a microscope blade. Observations were made under the wide-  
114 range zoom lens of a high-resolution digital microscope Keyence (VHX 2000E, 1 μm resolution,  
115 HDR images), and the maximum dorsoventral axis was measured. This measurement  
116 corresponds to the distance between the umbo and the most distant part of the clear demarcation  
117 formed by a growth line delimiting the PII from the dissoconch shell.

118

119 The fatty acid (FA) composition of young settlers was determined using a pool of 77 to 212  
120 individuals per replicate (2-3 replicates per site depending on pediveliger abundances). Samples  
121 were preserved in vials with 3 mL of dichloromethane methanol (CH<sub>2</sub>Cl<sub>2</sub>:MeOH, 2:1 v:v),  
122 closed with a Teflon-lined cap under nitrogen atmosphere and stored at -80 °C until analysis.  
123 Lipids were extracted by grinding in dichloromethane methanol using a modified Folch  
124 procedure (Parrish 1999). Fatty acid methyl esters (FAME) were prepared using sulfuric acid  
125 and methanol (2:98 v:v) at 100 °C for 10 min and using 19:0 as internal standard (Lepage &  
126 Roy 1984). Samples were purified on an activated silica gel with 1 mL of hexane ethyl acetate  
127 (v:v) to eliminate free sterols. FAME were analyzed in the full scan mode (ionic range: 50–650  
128 m:z) on a Polaris Q ion trap coupled to a Trace GC Ultra gas chromatograph (Thermo Scientific)

129 equipped with a TriPlus autosampler, a PTV injector and an ITQ900 mass detector (Thermo  
130 Scientific). An Omegawax 250 (Supelco) capillary column was used for separation using high  
131 purity helium. Xcalibur v.2.1 software (Thermo Scientific) was used for FAME identification  
132 and quantification with the standards (Supelco 37 Component FAME Mix and Supelco  
133 menhaden oil). Unknown peaks were identified according to their mass spectra with emphasis  
134 on FA trophic makers.

135

### 136 **2.3 Environmental measurements**

137 Environmental factors were measured once a week (supplementary files table 1 and table 2) just  
138 after immersion of the collectors until the plates were harvested, i.e., a total of five weeks.  
139 Temperature ( $^{\circ}\text{C}$ ), salinity (PSU) and dissolved oxygen concentrations ( $\text{mg L}^{-1}$ ) were measured  
140 at a depth of 1 m and at the bottom of the water column with an Oxi1970i WTW oximeter and  
141 an LF 197-S WTW conductivity meter.

142

143 Potential food for oysters is expressed as the concentration of total suspended particulate matter  
144 varying in size from 0.7 and 20  $\mu\text{m}$  ( $\text{TPM}_{0.7-20\mu\text{m}}$ ,  $\text{mg L}^{-1}$ ). It consisted of inorganic ( $\text{PIM}_{0.7-20\mu\text{m}}$ ,  
145  $\text{mg L}^{-1}$ ) and organic particulate matter ( $\text{POM}_{0.7-20\mu\text{m}}$ ,  $\text{mg L}^{-1}$ ). Once a week, three replicate water  
146 samples were collected at a depth of 1 m using a Ruttner Standard Water Sampler (Hydro-Bios  
147 Apparatebau) and stored at 4  $^{\circ}\text{C}$  for less than 2 hours before filtration for the measurement of  
148 the concentrations ( $\text{mg mL}^{-1}$ ) of pico and nano-seston. In 2017, 500-mL subsamples of 1-L  
149 samples were used for filtration, while 1-L subsamples of 2-L samples were used in 2019. Water  
150 samples were first filtered by gravity through a Nuclepore membrane (20  $\mu\text{m}$  pore size).  
151 Fractioned water samples were then filtered using a vacuum pressure pump (0.3 bar) on pre-



152 weighed (Mettler Toledo XP6 microbalance) pre-combusted (at 500 °C) Whatman 25 mm GF/F  
153 filters (0.7 µm pore size). The GF/F filters were rinsed with an isotonic seawater solution of  
154 ammonium formate (38 g L<sup>-1</sup> distilled water) to eliminate salt deposits and stored in Millipore™  
155 PetriSlide™ containers at - 25°C. The filters were dried at 70 °C for 24 h, weighed and the  
156 concentration of total particulate matter TPM<sub>0.7-20µm</sub> was determined. The filters were then  
157 combusted at 500 °C for 5 h and reweighed to determine the concentration of particulate  
158 inorganic matter (PIM<sub>0.7-20µm</sub>, mg L<sup>-1</sup>). The concentration of particulate organic matter (POM<sub>0.7-  
159 20µm</sub>, mg L<sup>-1</sup>) was the difference in weight between the dried and the combusted filter. To  
160 determine the FA content of the pico- and nano-seston (µg.mg TPM<sub>0.7-20µm</sub><sup>-1</sup>), 1-L water samples  
161 collected in 2017 and 2-L water samples collected in 2019 were filtered as described above  
162 without addition of ammonium formate solution. GF/F filters were stored in 3 ml of  
163 CH<sub>2</sub>Cl<sub>2</sub>:MeOH (2:1 v:v) under a nitrogen atmosphere in vials with a Teflon-lined cap and stored  
164 at -80 °C. The mass of total fatty acids in the seston (MTFA; µg mg<sup>-1</sup> POM) and its composition  
165 (% fatty acids) were obtained as already described for oysters, with lipid extraction carried out  
166 by sonification rather than grinding.

167

168 Plankton diversity was collected in 1-L samples in 2017 and in 2-L samples in 2019 collected  
169 weekly with a Ruttner Standard Water Sampler (Hydro-Bios Apparatebau) at each sampling  
170 site. This sampling strategy provided 40 observations (4 sites x 5 weeks x 2 years).  
171 Phytoplankton was characterized using the standard Utermöhl method NF-EN-152014, 2006 in  
172 10 mL seawater samples. Abundances are expressed as the number of individuals per liter in 52  
173 diatom taxa and 38 dinoflagellate taxa. Chlorophyll *a* (Chl-*a*), *b* (Chl-*b*) and *c* (Chl-*c*) biomasses  
174 were evaluated in 200 ml seawater samples filtered (Bec et al. 2005, 2011) on Whatman GF/F

175 membranes (0.7  $\mu\text{m}$  pore size) with a vacuum pressure pump ( $<10$  cm Hg). Filters were stored  
176 in glass tubes at  $-20$   $^{\circ}\text{C}$  until analysis. To determine the contribution of picophytoplankton ( $<3$   
177  $\mu\text{m}$ ), nanophytoplankton (3 to 20  $\mu\text{m}$ ) and microphytoplankton ( $>20$   $\mu\text{m}$ ), two out of three  
178 samples were size-fractionated beforehand by gravity through Nuclepore membranes (3 and 20  
179  $\mu\text{m}$  pore size). Filters were ground in acetone (90%) and extracted at 4  $^{\circ}\text{C}$  for 24 h in the dark.  
180 Pigment contents were measured with a spectrofluorometer (Perkin-Elmer LS50b) (Neveux &  
181 Lantoiné 1993) and are expressed in  $\mu\text{g chl } a \text{ L}^{-1}$ . Concentrations of picocyanobacteria ( $<1$   $\mu\text{m}$ ),  
182 autotrophic picoeukaryotes ( $<3$   $\mu\text{m}$ ), nanophytoplankton (3-20  $\mu\text{m}$ ) and bacteria were estimated  
183 using a FACSCalibur flow cytometer Becton Dickinson methods (Marie et al. 1997, Bec et al.  
184 2011). Seawater samples (1-ml) were analyzed; abundances are expressed in cells per liter. Total  
185 picophytoplankton abundances were assessed by summing picocyanobacteria and  
186 photosynthetic picoeukaryote abundances. Fluorescent beads (0.94  $\mu\text{m}$ ; 2 and 3  $\mu\text{m}$ ,  
187 Polysciences) were added to each sample. To measure bacterial abundances, seawater samples  
188 were fixed with prefiltered (0.2  $\mu\text{m}$ ) buffered formaldehyde (2% final concentration) and stored  
189 in liquid nitrogen. The procedure was slightly modified as higher concentrations of  
190 fluorochromes (SYBR Green I) were used (Bouvy et al. 2016). The fixed samples were  
191 incubated with SYBR Green I (Molecular Probes) at a final concentration of 1/375 at 4  $^{\circ}\text{C}$  for  
192 15 min in the dark. Stained bacterial cells excited at 488 nm were determined according to their  
193 side-scattered light and green fluorescence collected using a 530/30 nm filter. Fluorescent beads  
194 (0.94  $\mu\text{m}$ ; Polysciences) were added to each sample.

195 Protozooplankton (heterotrophic flagellates) abundances were determined using the standard  
196 2006 Utermöhl method NF-EN-152014, and are expressed in cells per liter. Until used for  
197 heterotrophic flagellate analysis, 30-ml seawater samples were preserved with 2.5-ml of

198 prefiltered (0.2  $\mu\text{m}$ ) formaldehyde and kept at 4 °C in the dark. Before counting, 10 ml  
199 subsamples were stained with 4',6-diamidino-2-phenylindole (DAPI) for a final concentration  
200 of 2.5  $\mu\text{g ml}^{-1}$ . Heterotrophic flagellates were counted by size class (2-5  $\mu\text{m}$ , 5-10  $\mu\text{m}$  and >10  
201  $\mu\text{m}$ ) under an epifluorescence microscope (Olympus AX70) with UV illumination (Sherr et al.  
202 1993).

203

#### 204 **2.4 Territorial competition**

205 Percent cover of tubeworm (*Ficopomatus enigmaticus*) on plates sampled in the fourth week  
206 after immersion (6 plates per site) was estimated to assess territorial competition with oyster  
207 juveniles, but only during the 2019 sampling season, as no tubeworms were observed in 2017.  
208 Photographs of each plate were taken with a GoPro HERO4 Silver camera equipped with a  
209 macro pro filter (San Mateo, CA, USA) and the % of tubeworms recovered on the plate was  
210 estimated using Image-Pro Insight 9.1 software (MediaCybernetics, Rockville, MD, USA).

211

#### 212 **2.5 Statistical analyses**

213 All PERMANOVA analyses were performed with Primer 7 and Permanova+1 (version 7.0.13)  
214 software. A two-way PERMANOVA (n perm.: 9999) was conducted using a Euclidian distance  
215 matrix to test the effect of year (2 fixed levels) and sampling site (4 fixed levels) on size at  
216 metamorphosis, total and essential fatty acid contents in young settlers and on all the  
217 environmental variables measured, except the oxygen level, which was added as a third factor  
218 (depth) in the analysis. Homogeneity was evaluated using the permutation analysis of  
219 multivariate dispersion (PERMDISP) routine. When significant PERMANOVAs were  
220 observed, post hoc multiple comparison tests were carried out. Multivariate analyses of total

221 FA composition in young settlers and in seston, including *a posteriori* pairwise comparison,  
222 were done using distance-based permutational multivariate analysis of variance  
223 (PERMANOVA, 9999 permutations) based on Euclidian dissimilarities with year (2 fixed  
224 levels) and sampling site (4 fixed levels) as sources of variation. Variations in FA composition,  
225 expressed in percentages, were visualized using non-metric multidimensional scaling (n-MDS).  
226 The similarity percentages (SIMPER) procedure was performed on untransformed data to  
227 identify the FAs that explained the most dissimilarity between significant different levels.

228

### 229 **3. RESULTS**

#### 230 **3.1 Oyster recruitment**

231 Recruitment numbers showed dramatic annual variability with great success at some sites in  
232 2017 but an overall near-zero recruitment level at all sites in 2019 (Fig. 2a, b). In 2017, the  
233 metamorphosis survival rate, expressed as the ratio of juvenile to young settler abundances per  
234 plate, also showed marked spatial variability. The ratio of juvenile ( $123 \pm 9$  ind. plate<sup>-1</sup>) to  
235 young-settler abundances per plate ( $49 \pm 6$  ind. plate<sup>-1</sup>) was 2.5 in Bouzigues, suggesting up to  
236 100% successful metamorphosis by competent larvae and the arrival of competent larvae from  
237 elsewhere. However, in the other sites, recruitment level decreased by 24% ( $94 \pm 16$  juveniles  
238 plate<sup>-1</sup>) in Meze, 90% ( $13 \pm 2$  juveniles plate<sup>-1</sup>) in Listel, and 97% ( $4 \pm 2$  juveniles plate<sup>-1</sup>) in  
239 Marseillan. A poorer supply of larvae ( $6 \pm 2$  young-settlers plate<sup>-1</sup>) was observed in Marseillan,  
240 but the metamorphosis survival rate was 0.6. However, in Meze and Listel, the low recruitment  
241 rates were not linked to the supply of larvae, as young settler abundances were higher in Meze  
242 ( $328 \pm 71$  young settler plate<sup>-1</sup>, with a metamorphosis survival rate of 0.3) and in Listel ( $670 \pm$   
243  $65$  young settler plate<sup>-1</sup>, with a metamorphosis survival rate of 0.02) than in Bouzigues. Failure

244 characterized the 2019 oyster recruitment season: low abundances of young settlers were  
245 observed in Meze ( $116 \pm 5$  ind. plate<sup>-1</sup>) and in Listel ( $31 \pm 2$  ind. plate<sup>-1</sup>), with almost 3 and 22  
246 times fewer individuals than in 2017, respectively. This trend was not observed in Bouzigues  
247 ( $84 \pm 9$  ind. plate<sup>-1</sup>) or in Marseillan ( $45 \pm 3$  ind. plate<sup>-1</sup>) in 2019. Instead, young settlers were  
248 respectively 2 and 7 times higher in 2019 than in 2017. However, two weeks later, almost no  
249 juveniles were observed on the plates (average  $0.14 \pm 0.06$ ), regardless of the sites, pointing to  
250 a general oyster recruitment failure in 2019.

251 The size of juveniles at metamorphosis (PII length) was established in all samples, except  
252 samples from Bouzigues in 2019 (Fig 2c, d), in which no metamorphosis of young settlers to  
253 juveniles was observed. PII individuals sampled in 2019 were 5.1% smaller (mean  $262 \pm 1$   $\mu$ m)  
254 than those sampled in 2017 (mean  $276 \pm 1$   $\mu$ m). Differences among sites were only observed in  
255 2017, when PII sizes in Bouzigues were 2.7% smaller than those in Meze ( $p = 0.02$ ), Listel ( $p$   
256  $= 0.01$ ) and Marseillan ( $p = 0.03$ ).

257 No differences in total fatty acid (TFA) contents were observed in young settlers in the four  
258 sites and the two years. The overall TFA average was  $51 \pm 19$  ng larvae<sup>-1</sup> ( $p > 0.06$ ). The sum of  
259 essential fatty acids (EFA) corresponded to about 10% of TFA with an effect of year  $\times$  site (df=3  
260 and 19, pseudo- $F=6.47$ ,  $p=0.007$ ), as individuals in Listel ( $p=0.02$ ) and Marseillan ( $p=0.006$ )  
261 had 5 times lower TFA contents in 2017 than in 2019. The fatty acid composition of young  
262 settlers varied with the year  $\times$  site interaction (df=3 and 19, pseudo- $F=2.34$ ,  $p=0.017$ ), as  
263 individuals sampled in Listel ( $p=0.047$ ) and Marseillan ( $p=0.044$ ) had different profiles in the  
264 two years (Supplementary material Fig. 1). According to SIMPER analysis, the interannual  
265 differences observed at these two sites were linked to DHA (22:6n3), EPA (20:5n3), AA  
266 (20:4n6), 18:2n6, 18:0 and 16:0 explained more than 83% of the average dissimilarity in the

267 fatty acid profiles. DHA, EPA and AA levels in young settlers sampled in 2019 were twice  
268 higher than in 2017, while the levels of 18:2n6 were five times lower in 2019 than in 2017,  
269 except for the Meze and Bouzigues sites ( $p > 0.09$ ).

270

### 271 **3.2 Physico-chemical parameters**

272 Average water temperatures were 2.6°C higher and salinity was 0.34 S higher in 2019 than in  
273 2017 (Fig 3a, b, Supplementary Table 3 and Supplementary Table 4). A site effect was also  
274 observed for salinity in the Thau lagoon. Salinity increased from east to west: the mean value at  
275 Marseillan was 0.68 S higher than at Bouzigues. Conversely, no effect of site on temperature  
276 was observed. There was a site  $\times$  year effect on oxygen concentration (Supplementary Table 5).  
277 No difference was observed among sites in 2017 (c). The lowest values were observed in  
278 Bouzigues in 2019 ( $p = 0.001$ ) near the bottom of the lagoon (21.8% lower than in 2017).  
279 Oxygen concentrations varied with water depth, lower values generally being observed near the  
280 bottom (Fig. 3c).

281

### 282 **3.3 Potential food for oyster larvae**

283 Concentrations of TPM<sub>0.7-20</sub> (), PIM<sub>0.7-20</sub> () and POM<sub>0.7-20</sub> () were more than twice higher in 2019  
284 than in 2017 (Fig. a, b, c, Supplementary Table 6, 7 and 8). Significant differences among the  
285 four sites were only observed in POM<sub>0.7-20</sub> concentrations. In both years, POM<sub>0.7-20</sub>  
286 concentrations in Marseillan were 0.7 and 0.8 times lower than in Listel and Meze ( $p = 0.01$  and  
287 0.03 respectively). An effect of year  $\times$  chl-*a* biomasses fraction was observed (Supplementary  
288 Table 9). Mean nanophytoplankton and picophytoplankton biomasses ( $p = 0.0001$  and  
289  $p = 0.0004$  respectively) were 3 times higher in 2019 (Fig.4d, e) than in 2017. A site  $\times$  year

290 effect was also observed, chl-*a* biomass values were 45% lower in Bouzigues than in Listel  
291 ( $p=0.01$ ) and Meze ( $p = 0.004$ ) in 2017. In 2019, biomasses in Marseillan were 62% lower than  
292 at the other sites ( $p < 0.02$ ). Interannual variability in chl-*a* was only found in Bouzigues with 3  
293 times more biomass in 2019 ( $p = 0.0007$ ) than in 2017. Similar patterns were observed for chl-  
294 *b* and chl -*c* biomass, with twice as much chl-*b* in the samples collected 2019 samples than in  
295 the samples collected in 2017 ( $0.069 \mu\text{g L}^{-1}$  versus  $0.026 \mu\text{g L}^{-1}$ ;  $p=0.0001$ ), and a more than  
296 two-fold increase in chl-*c* ( $0.103 \text{ ug L}^{-1}$  versus  $0.046 \text{ ug L}^{-1}$ ), particularly in Listel ( $p=0.039$ )  
297 and Bouzigues ( $p=0.0003$ ).

298 Flow cytometry data showed an effect of the year factor on cells smaller than  $3 \mu\text{m}$  (Fig. ).  
299 Abundances of picoeukaryotes ( $<3 \mu\text{m}$ ) (Supplementary Table 10), picocyanobacteria ( $<1 \mu\text{m}$ )  
300 (Supplementary Table 11 and 12) and bacteria (Supplementary Table 14) were higher in 2019  
301 than in 2017. However, nanophytoplankton ( $3\text{-}20 \mu\text{m}$ ) abundances decreased by 39% in 2019  
302 (Supplementary Table 13). The abundance of total heterotrophic flagellates did not vary  
303 significantly among sites or between years, mean value  $2\ 866 \pm 291 \text{ cell mL}^{-1}$ . Dinoflagellate  
304 and diatom abundances were affected by the year factor ( $df=1$  and  $35$ , pseudo- $F=5.64$ ,  $p=0.023$ ),  
305 total values decreased by 60% in 2019 compared to 2017. These variations were linked to a 93%  
306 decrease in *Chaetoceros* abundance from  $184\ 715 \pm 66\ 846$  to  $12\ 483 \pm 3\ 540 \text{ cells L}^{-1}$  (Simper  
307 contribution: 77%,  $df=1$  and  $35$ , pseudo- $F=8.73$ ,  $p=0.0001$ ) and a decrease that led to the  
308 disappearance of *Skeletonema* in Listel and Meze between 2017 and 2019. Diatom taxa were  
309 fewer in number at all sites sampled in 2019 with a maximum of 13 compared to 21 taxa  
310 identified in 2017. A marked increase in *Pseudo-nitzschia* ( $19\ 920 \pm 10\ 513$  to  $50\ 562 \pm 13\ 652$   
311  $\text{cells L}^{-1}$ ) with a Simper contribution of 8% and ( $df=1$  and  $35$ , pseudo- $F=8.73$ ,  $p=0.0001$ ),  
312 *Leptocylindrus* (Simper contribution 7%), *Thalassionema*, and *Cylindrotheca* ( $1\ 837 \pm 222$  to

313 18 712 ± 12 010 cells L<sup>-1</sup>) was observed in 2019 compared to 2017. This trend is especially  
314 expressed in Bouzigues (Fig. 6). This result also reflects the higher diversity of dinoflagellate  
315 taxa observed in 2019 (16 taxa) than in 2017 (12 taxa).

316 TFA contents in the TPM<sub>0.7-20</sub> samples were twice higher in 2019 (19.2 µg mg TPM<sub>0.7-20</sub><sup>-1</sup>) than  
317 in 2017 (9.9 µg mg TPM<sub>0.7-20</sub><sup>-1</sup>; df=1 and 61, pseudo-*F*=17.1, p=0.0002) with no differences  
318 among sites and year × site effects. The fatty acid composition of the TPM<sub>0.7-20</sub> samples differed  
319 between years (df=3 and 76, pseudo-*F*=3.08, p=0.0001; Fig. S2) and, as determined by the  
320 SIMPER analysis, explained 97% of the differences in the levels of 18:1n9, 18:0, 16:1, 18:2n6,  
321 16:0, 14:0, 20:5n3 and 22:6n3. Twenty-six percent of the difference observed between years  
322 was related to 18:1n9, a FA that was twice as abundant in 2017 (up to 24.1% of the TFA)  
323 compared to 2019. The dissimilarity in the FA profiles observed between years was also  
324 explained by higher values of 18:2n6 (representing up to 10.8% of TFA), and EPA (7%) in  
325 2017. 18:2n6 and EPA were, respectively, 11.3% and 5% higher in 2017 than in 2019. The most  
326 abundant FAs in the TPM<sub>0.7-20</sub> samples in 2019 were 16:1 and DHA which explained,  
327 respectively, 13% and 4.3% of the dissimilarity shown by the SIMPER analysis.

328

### 329 **3.4 Territorial competition by worms**

330 The percent cover of tubeworms (*Ficopomatus enigmaticus*) on plates in 2019 showed a marked  
331 increase in this species. Differences were observed among the sites (df=3 and 33, pseudo-  
332 *F*=157, p=0.0001). Results showed similar cover of tubeworms (93.6 ± 1.5%) in Listel and  
333 Bouzigues and a lower cover in Meze (83.2 ± 2.6%) (p < 0.032) and in Marseillan (23.6 ± 3.7%)  
334 (p < 0.0001).

335



#### 4. DISCUSSION

The aim of this study was to identify the environmental and trophic drivers of the decline in the recruitment of the Pacific oyster, *Crassostrea gigas*, in association with a heat wave. Our hypothesis that a heat wave has a negative effect on oyster recruitment by altering plankton diversity was confirmed. While oyster recruitment was normal in 2017, an unprecedented failure was observed in summer 2019 in the Mediterranean Thau lagoon. The atmospheric conditions during a heat wave have a strong direct effects on marine and lagoon environments that supply a variety of ecosystem services and valuable host species (Sarà et al. 2021). Temperature and salinity conditions are key ecological and physiological factors for *Crassostrea* larvae (His et al. 1989b, Baldwin & Newell 1995a, Devakie & Ali 2000, Troost et al. 2009). In controlled experimental settings, the entire larval life of *C. gigas*, including metamorphosis, showed a high tolerance to temperatures ranging from 17 °C to 32 °C at a salinity level of 34, with low mortality ( $\leq 10\%$ ) and the maximum growth rate at 32 °C (Rico-Villa et al. 2009). The physiological limits of temperature tolerance were therefore not reached in our experimental conditions and temperature was not the origin of the failure in this case. Salinity did not drop below 38 in either the 2017 or 2019 recruitment season, and intermittently reached more than 40 in 2019. *Crassostrea gigas* is an estuarine organism that tolerates a wide range of salinity (Nell & Holliday 1988), but no information is available in the literature on the upper salinity tolerance of the larval stage in real conditions. The high salinity in 2019 could represent the physiological salinity threshold for oyster larvae. Our results showed that the larval shell (prodissoconch) at the time of metamorphosis (PII) was smaller in 2019, suggesting a reduction in larval growth or faster achievement of metamorphosis competence in high salinity years. In agreement with Nell and Holliday (1988) who reported an optimal salinity range for larval

359 growth up to 27 and a very marked growth reductions at 31-39 (Nell & Holliday 1988), the  
360 smaller observed\_PII size could be related to growth limitation under high salinity. Interestingly,  
361 these authors reported no significant effect of salinity on larval survival between 19 and 39 but  
362 the growth rate of larvae decreased markedly from 30 S (Helm & Millican 1977). Upper  
363 tolerance limits of oysters to high salinity ranging from 35 S to 45 S should thus be further tested  
364 in the laboratory including interactions with high temperature and different nutritional inputs  
365 (His et al. 1989a).

366 The heat wave that occurred in 2019 resulted in large quantities of particulate matter and  
367 chlorophyll biomass, but their quality appeared to be unfavorable for oyster recruitment. The  
368 failure of oyster recruitment in 2019 could thus be linked to the change in the phytoplankton  
369 community with low abundance of forage diatoms and high abundance of picoplanktonic  
370 prokaryotes and eukaryotes, flagellates, and of the diatoms *Pseudo-Nitzschia* and  
371 *Cylindrotheca*. However, the trophic environment was not characterized by a planktonic  
372 community poor in fatty acids, and it was in fact richer than in 2017. Pediveliger larvae  
373 accumulated the same quantity of fatty acids in 2019 as in 2017, but metamorphosis failures  
374 were observed at all sites. We suggest that this failure may be linked to inappropriate trophic  
375 conditions, which in turn, are mainly linked to the size of picoplankton species. These species  
376 are poorly retained by the newly developed gills of young metamorphosed juveniles. Except for  
377 larvae, the retention efficiency of bivalves for particles < 3-4  $\mu\text{m}$  is low (Baldwin & Newell  
378 1995b, Rosa et al. 2018). Our results suggest that the overabundance of small particles  
379 (picoplanktonic prokaryotes and eukaryotes) could be critical for larval settlement and  
380 metamorphosis. Higher chlorophyll biomass was observed in the nanophytoplankton fraction  
381 during the heat wave of 2019 than in 2017, indicating changes in the phytoplankton community.

382 The heat wave was characterized by the increasing abundances of picocyanobacteria (Bec et al.  
383 2005, Collos et al. 2009, Derolez et al. 2020b) and decreasing abundances of  
384 nanophytoplankton. The Thau lagoon began an oligotrophication trajectory in the early 2000s  
385 (Collos et al. 2009, Derolez et al. 2020a). This process caused a community shift due to a  
386 reduction in nutrient loads since the 1970s thanks to improved wastewater treatment in the  
387 watershed aimed at halting eutrophication (EC 1991a b, 2000). The reduction in nutrient loads  
388 has been amplified by a decrease in total rainfall since the 2000s due to climate change (Derolez  
389 et al. 2020a). Our results corroborate evidence that the proportion of small taxa, like  
390 picoplankton, in the phytoplankton community, is increasing in coastal, marine and freshwater  
391 ecosystems in response to global warming (Daufresne et al. 2009, Mousing et al. 2014, Pinckney  
392 et al. 2015). Small phytoplankton cells have been observed to dominate in oligotrophic  
393 environments (Irwin et al. 2006).

394

395 The high temperatures and high salinity in 2019 had a negative impact on trophic conditions for  
396 larvae. However, recruitment failure appeared to be more linked to the ecological limitations of  
397 the larvae at the time of their metamorphosis than to their physiological state. At the same time,  
398 high temperatures and high salinity stimulated the development of the annelid *Ficopomatus*  
399 *enigmaticus*, triggering a shift in community composition that is destructive for oyster  
400 recruitment. We consequently hypothesize that these annelids are important territorial  
401 competitors (Heiman & Micheli 2010, McQuaid & Griffiths 2014, Peria & Pernet 2019) and  
402 trophic competitors of oyster larvae (Davies et al. 1989, Bruschetti et al. 2008, 2018, Pan &  
403 Marcoval 2014) in shallow water and brackish habitats.

404 This study demonstrates, for the first time, an ecological process leading to the recruitment  
405 failure of the Pacific oysters due to an extreme heat wave. The oligotrophication trajectory of  
406 our study site combined with the effects of high water temperatures promoted variations in the  
407 phytoplankton communities that benefit picophytoplankton including cyanobacteria, that are  
408 likely unfavorable for the successful larval development of oysters until their juvenile  
409 metamorphosis (Lagarde et al. 2017). The present study thus reveals the ecological limits of the  
410 reproductive process of the Pacific oyster in the context of a heat wave in a Mediterranean  
411 lagoon. The heat wave phenomenon observed in 2019 severely disrupted the reproductive cycle  
412 of oysters in the Thau lagoon. In this context, the oyster nursery function within an oyster  
413 farming ecosystem can only be achieved or maintained when pico-, nano- and  
414 microphytoplankton communities are present and abundant and oysters can find favorable areas  
415 for larval development and optimize their recruitment. This study provides evidence that, in the  
416 conditions caused by a heat wave, the ecological limits of Pacific oyster larvae are narrower  
417 than their physiological limits. The effects of climate change, particularly the warming of waters  
418 in semi-enclosed basins, will certainly lead to problems in larval harvesting in the near futures.  
419 The information presented in this paper should help adapt oyster aquaculture, including  
420 husbandry practices, to a future marked by climate change.

## 421 **5. Data and code availability**

422 All data used in the current study and scripts used in our analysis are publicly available or were  
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427

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598

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614

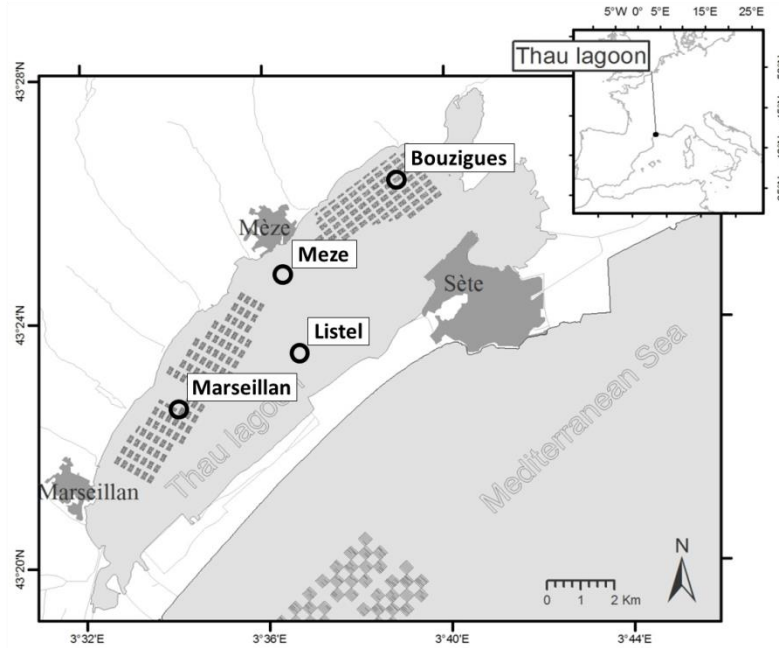
## 615 **8. AUTHOR CONTRIBUTIONS**

616 A.C.M. was involved in investigation, methodology, writing, data curation, formal analysis, and  
617 visualization. R.T. and F.L. were involved in conceptualization, funding acquisition,  
618 investigation, methodology, writing, data curation, formal analysis, visualization, and project  
619 administration. S.P. was involved in conceptualization, funding acquisition, investigation,  
620 methodology, writing and project administration. B.B was involved in conceptualization,  
621 funding acquisition, investigation, methodology, writing, data curation, formal analysis, and  
622 visualization. C.R. contributed to funding acquisition, methodology, writing, data curation and  
623 formal analysis. A.A and A.G. contributed to writing and interpretation. G.M. contributed to  
624 funding acquisition, investigation, methodology, writing and formal analysis. M.R., M.Ho,  
625 M.Ha. and T.M. contributed to conceptualization, investigation, methodology and writing.

## 626 **9. COMPETING INTERESTS**

627 The authors declare no competing interests.

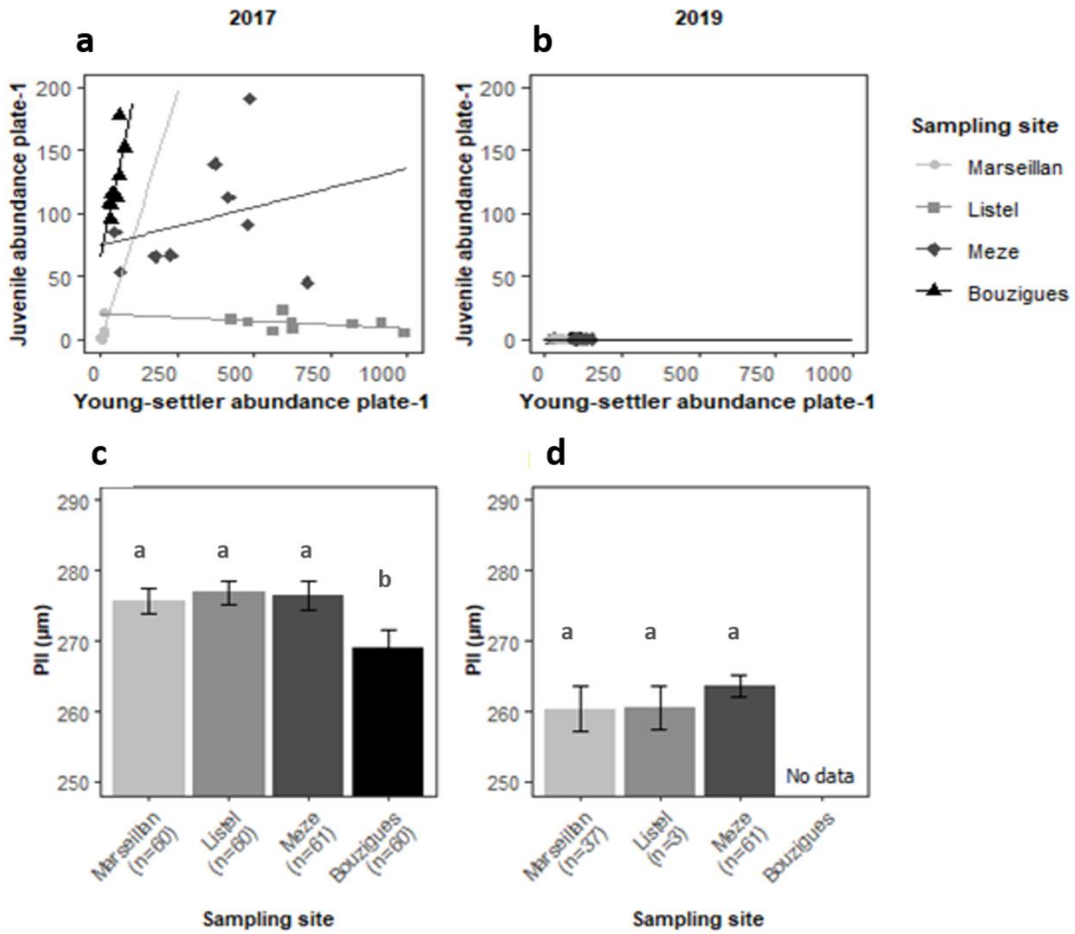
628



629

630 Fig. 1. The four sampling sites in the Thau lagoon. Marseillan and Bouzigues are located in the  
 631 shellfish farming area; shaded areas indicate the location of shellfish culture areas. Meze and  
 632 Listel are located outside the aquaculture area.

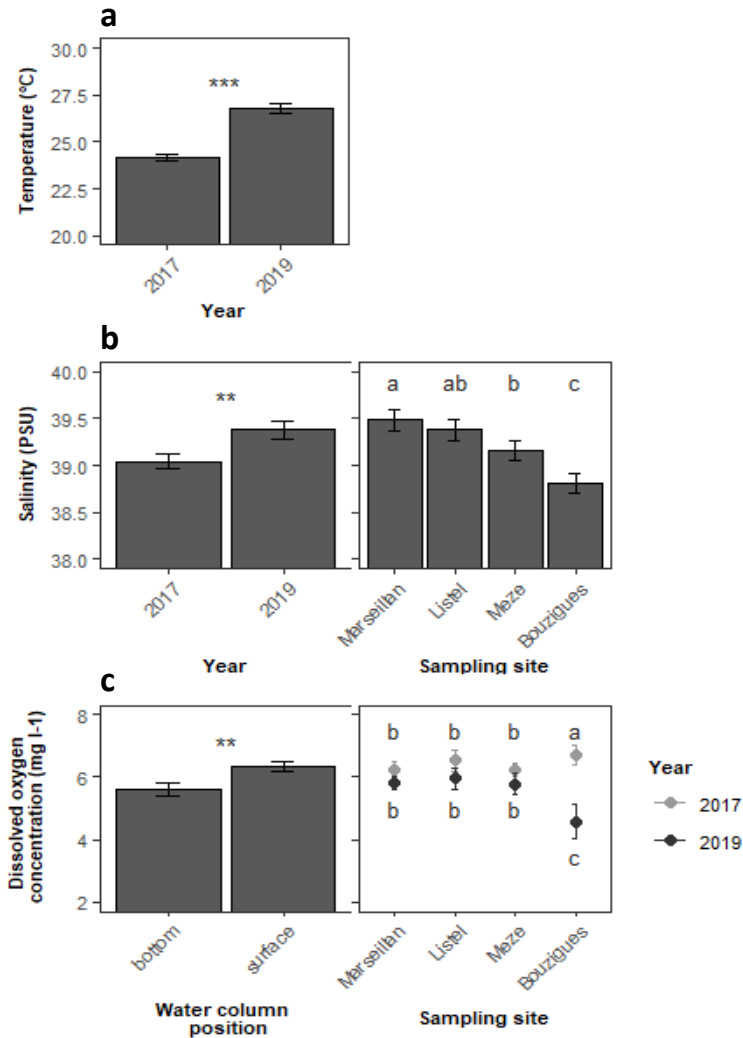
633



634

635 Fig. 2. Variability of oyster recruitment and prodissoconch II size according to the years 2017  
 636 (no heat wave) and 2019 (heat wave). *Crassostrea gigas* recruitment performance with young  
 637 settlers (pediveligers + post-larvae) and juvenile abundance per collector plate observed at the  
 638 four sampling sites during the summer recruitment events in (a) 2017 and in (b) 2019. Size at  
 639 metamorphosis was estimated by the length of prodissoconch II shell (PII,  $\mu\text{m} \pm \text{SE}$ ) of juveniles  
 640 sampled in (c) 2017 and (d) 2019. Different letters indicate significant differences between sites  
 641 according to post-hoc multiple comparison tests after PERMANOVA.

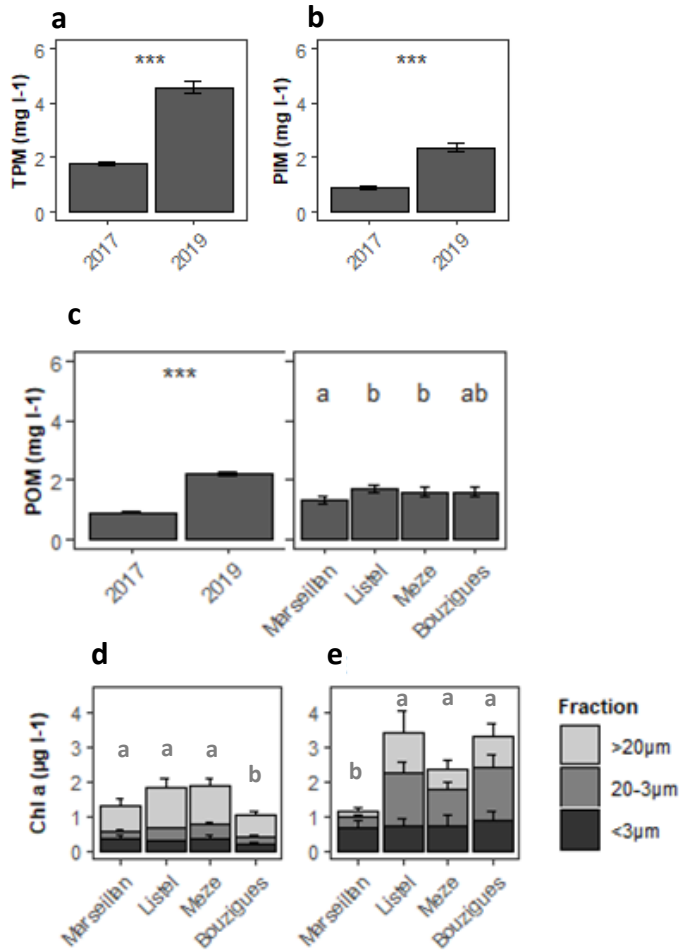
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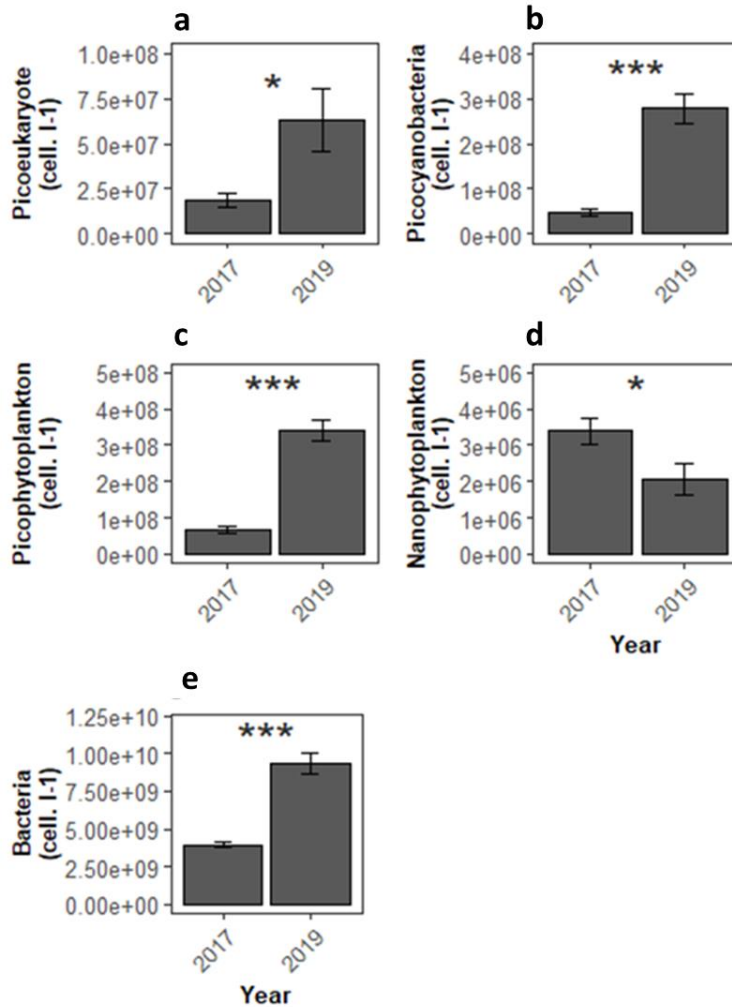
644 Fig. 3. Physico-chemical monitoring in 2017 (no heat wave) and 2019 (heat wave). (a) Mean  
 645 temperature ( $^{\circ}\text{C} \pm \text{SE}$ ) per year ( $n = 40$ ), (b) mean salinity ( $\text{PSU} \pm \text{SE}$ ) per year ( $n = 40$ ) and per  
 646 sampling site ( $n = 20$ ) and (c) mean dissolved oxygen concentration ( $\text{mg L}^{-1} \pm \text{SE}$ ) according to  
 647 the position of the sample in the water column ( $n = 40$ ) and per year and sampling site ( $n = 10$ ).  
 648 Stars indicate significant differences in parameter average per year (\*  $p \leq 0.05$ , \*\*  $p \leq 0.01$ , \*\*\*  
 649  $p \leq 0.001$ ). Different letters indicate significant differences between sites according to post hoc  
 650 multiple comparison tests after PERMANOVA.





651

652 Fig. 4. Hydrobiological monitoring in 2017 (no heat wave) and 2019 (heat wave). Mean  
 653 concentrations of (a) total particulate matter (TPM, mg L<sup>-1</sup> ± SE), (b) particulate inorganic  
 654 matter (PIM, mg L<sup>-1</sup> ± SE) and (c) particulate organic matter (POM, mg L<sup>-1</sup> ± SE) per year and  
 655 sampling site (n = 5 per sampling site and year). Mean concentrations of chlorophyll-a (d, 2017  
 656 and e; 2019; µg L<sup>-1</sup> ± SE), found in the picophytoplankton fraction (< 3 µm), the  
 657 nanophytoplankton fraction (3 to 20 µm) and the microphytoplankton fraction (> 20 µm) per  
 658 year and sampling site (n = 5 per sampling site, year and phytoplankton fraction). Stars indicate  
 659 significant differences according to parameter average by year (\* p ≤ 0.05, \*\* p ≤ 0.01, \*\*\* p ≤  
 660 0.001). Different letters indicate significant differences between sites according to post-hoc  
 661 multiple comparison tests after PERMANOVA.



663

664 Fig. 5: Monitoring of picophytoplankton population in 2017 (no heat wave) and 2019 (heat

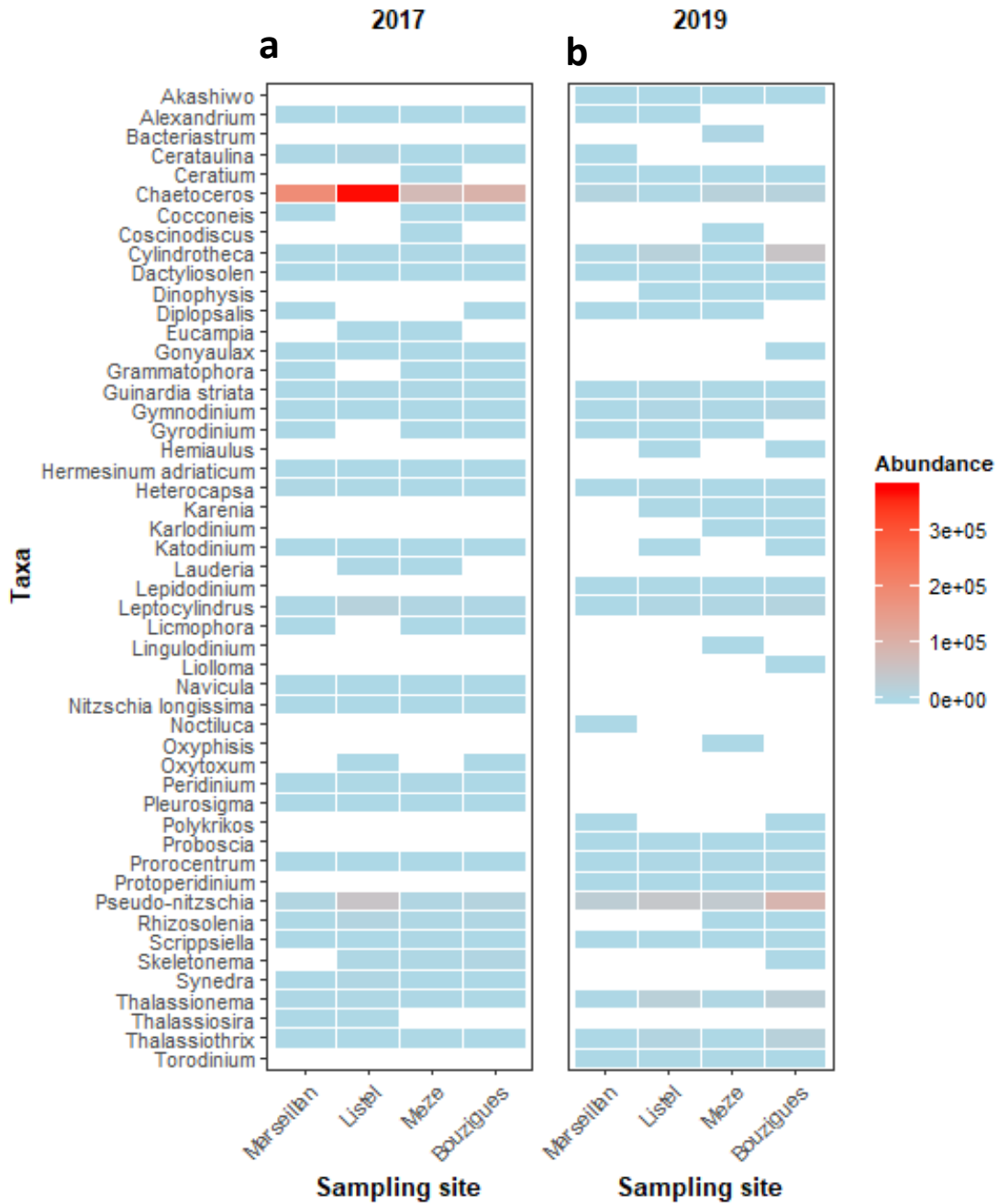
665 wave). Average abundances for all sites of (a) photosynthetic picoeukaryotes, (b)

666 picocyanobacteria, (c) picophytoplankton, (d) nanophytoplankton and (e) bacteria (cells L<sup>-1</sup> ±

667 SE) per year (n=20). Stars indicate significant differences according to parameter average by

668 year (\* p ≤ 0.05, \*\* p ≤ 0.01, \*\*\* p ≤ 0.001).

669



670

671 Fig. 6: Heatmap of microphytoplankton genera with changes in abundances in 2017 (no heat  
 672 wave) and 2019 (heat wave). Average phytoplankton abundance (cells L<sup>-1</sup>) per taxon and  
 673 sampling site in (a) 2017 (n = 5) and (b) in 2019 (n = 4).

674

| <b>Variables</b>                          | <b>Description</b>  | <b>Unit of measure</b> | <b>Abbreviation</b>   |
|---|---|------------------------|-----------------------|
| <b><i>Oyster variables</i></b>            |   |                        |                       |
| <i>Oyster pediveligers</i>                | <i>Abundance on collector plates</i>  | <i>ind. plate-1</i>    | <i>pediveligers</i>   |
| <i>Newly metamorphosed juveniles</i>      | <i>Abundance on collector plates</i>  | <i>ind. plate-1</i>    | <i>postlarvae</i>     |
| <i>Young settlers</i>                     | <i>Abundance of pediveligers+ newly metamorphosed juveniles on collector plates</i>   | <i>ind. plate-1</i>    | <i>Young settlers</i> |
| <i>Prodissoconch II size</i>              | <i>Measurement of prodissoconch maximum shell height along maximal dorsoventral axis of larvae or juvenile Pacific oysters</i>  | <i>mm</i>              | <i>PII size</i>       |
| <i>Total fatty acid in young settlers</i> | <i>Total fatty acid contents in larvae (young settlers)</i>   | <i>ng larvae-1</i>     | <i>TFA</i>            |
| <i>Essential fatty acids</i>              | <i>Sum of essential fatty acids in larvae (docosahexaenoic acid (22:6<math>\omega</math>3; DHA), eicosapentaenoic acid (20:5<math>\omega</math>3; EPA) and arachidonic acid (AA))</i> | <i>ng larvae-1</i>     | <i>EFA</i>            |

| <b>Variables</b>                                 | <b>Description</b>   | <b>Unity</b>                              | <b>Abbreviation</b>     |
|--|--|---|-------------------------|
| <b>Environmental variables</b>                   |  |   |                         |
| Temperature                                      | Discrete measure   | °C  | -                       |
| Salinity   | Discrete measure   | No unit                                   | -                       |
| Oxygen concentration                             | Discrete measure   | mg l <sup>-1</sup>                        | -                       |
| Total particulate matter <sub>0.7-20µm</sub>     | Total particular pelagic material in the 0.7-20µm fraction               | mg l <sup>-1</sup>                        | TPM <sub>0.7-20µm</sub> |
| Particulate organic matter <sub>0.7-20µm</sub>   | Particulate pelagic material in fraction the 0.7-20µm fraction           | mg l <sup>-1</sup>                        | POM <sub>0.7-20µm</sub> |
| Particulate inorganic matter <sub>0.7-20µm</sub> | Particulate inorganic pelagic material in the fraction 0.7-20µm fraction | mg l <sup>-1</sup>                        | PIM <sub>0.7-20µm</sub> |
| TFA content in TPM <sub>0.7-20</sub>             | TFA content in TPM <sub>0.7-20</sub>                                     | µg mg TPM <sub>0.7-20</sub> <sup>-1</sup> |                         |
| Total chlorophyll a                              | Total chlorophyll a biomass  | µgChla l <sup>-1</sup>                    | chloa                   |
| Total chlorophyll b                              | Total chlorophyll b biomass  | µgChlb l <sup>-1</sup>                    | chl ob                  |
| Total chlorophyll c                              | Total chlorophyll c biomass  | µgChlc l <sup>-1</sup>                    | chl oc                  |
| Picophytoplankton biomass                        | Chlorophyll a biomass in the <3µm fraction (picoeukaryotes)              | µgChla l <sup>-1</sup>                    | pico_chloa              |
| Nanophytoplankton biomass                        | Chlorophyll a biomass in the 3-20µm fraction (nanoeukaryotes)            | µgChla l <sup>-1</sup>                    | nano_chloa              |
| Picophytoplankton+nanophytoplankton              | Biomass  | µgChla l <sup>-1</sup>                    | nano_total_chloa        |
| Microphytoplankton > 20µm                        | Biomass (microeukaryotes)  | µgChla l <sup>-1</sup>                    | micro_chloa             |
| Bacteria   | Abundance of picocyanobacteria (<1 µm)                                   | 10 <sup>6</sup> cell. l <sup>-1</sup>     | bacteria                |
| Total picoeukaryotes                             | Abundance  | 10 <sup>6</sup> cell. l <sup>-1</sup>     | peuk_tot                |
| picoeukaryotes+cyanophyceae                      | Abundance  | 10 <sup>6</sup> cell. l <sup>-1</sup>     | pico_tot                |
| Nanophytoplankton                                | Abundance  | 10 <sup>6</sup> cell. l <sup>-1</sup>     | nano                    |
| cryptophyceae                                    | Abundance  | 10 <sup>6</sup> cell. l <sup>-1</sup>     | crypto                  |
| Nanophytoplankton + cryptophyceae                | Abundance  | 10 <sup>6</sup> cell. l <sup>-1</sup>     | nano_tot                |
| Heterotrophic flagellates                        | Abundance  | cell l <sup>-1</sup>                      | HF                      |
| Ciliates   | Abundance  | cell l <sup>-1</sup>                      | ciliates                |
| Tintinnidae                                      | Abundance  | cell l <sup>-1</sup>                      | tinti                   |
| Diatoms  | Abundance  | cell l <sup>-1</sup>                      | diatom                  |
| Dinoflagellates                                  | Abundance  | cell l <sup>-1</sup>                      | Dinoflagellate          |
| <b>Territorial competition by worms</b>          |  |   |                         |
| Worm coverage                                    | Percent cover of tubeworms ( <i>Ficopomatus enigmaticus</i> ) on plates  | %   | -                       |

680 *Supplementary Table 3: multivariate PERMANOVA investigating site and year effect for Temperature*

| Source             | df | SS     | MS       | Pseudo-F | P(perm) | Unique perms | P(MC)  |
|--------------------|----|--------|----------|----------|---------|--------------|--------|
| site               | 3  | 7,087  | 2,3623   | 1,158    | 0,3305  | 9951         | 0,3335 |
| year               | 1  | 135,72 | 135,72   | 66,53    | 0,0001  | 9825         | 0,0001 |
| position           | 1  | 3,2    | 3,2      | 1,5686   | 0,2085  | 9805         | 0,217  |
| sitexyear          | 3  | 0,3865 | 0,12883  | 0,063154 | 0,9764  | 9951         | 0,9754 |
| sitexposition      | 3  | 2,573  | 0,85767  | 0,42042  | 0,7357  | 9950         | 0,7371 |
| yearxposition      | 1  | 1,1045 | 1,1045   | 0,54142  | 0,4681  | 9828         | 0,473  |
| sitexyearxposition | 3  | 0,0865 | 0,028833 | 0,014134 | 0,9977  | 9955         | 0,9977 |
| Res                | 64 | 130,56 | 2,04     |          |         |              |        |
| Total              | 79 | 280,72 |          |          |         |              |        |

693 *Supplementary Table 4: multivariate PERMANOVA investigating site, depth and year effect for salinity*

| Source             | df | SS     | MS       | Pseudo-F | P(perm) | Unique perms | P(MC)  |
|--------------------|----|--------|----------|----------|---------|--------------|--------|
| Site               | 3  | 5,331  | 1,777    | 7,5677   | 0,0002  | 9962         | 0,0004 |
| Year               | 1  | 2,2445 | 2,2445   | 9,5587   | 0,0031  | 9805         | 0,0034 |
| position           | 1  | 0,072  | 0,072    | 0,30663  | 0,5764  | 9733         | 0,5827 |
| sitexyear          | 3  | 0,5245 | 0,17483  | 0,74457  | 0,5286  | 9960         | 0,5323 |
| sitexposition      | 3  | 0,059  | 0,019667 | 0,083755 | 0,9666  | 9945         | 0,9679 |
| yearxposition      | 1  | 0,1125 | 0,1125   | 0,47911  | 0,4824  | 9806         | 0,4966 |
| sitexyearxposition | 3  | 0,0805 | 0,026833 | 0,11428  | 0,9545  | 9942         | 0,9503 |
| Res                | 64 | 15,028 | 0,23481  |          |         |              |        |
| Total              | 79 | 23,452 |          |          |         |              |        |

706 *Supplementary Table 5: multivariate PERMANOVA investigating site, depth and year effect for oxygen*

| Source             | df | SS     | MS      | Pseudo-F | P(perm) | Unique perms | P(MC)  |
|--------------------|----|--------|---------|----------|---------|--------------|--------|
| site               | 3  | 3,8333 | 1,2778  | 1,3099   | 0,2739  | 9944         | 0,27   |
| year               | 1  | 15,878 | 15,878  | 16,277   | 0,0004  | 9825         | 0,0001 |
| position           | 1  | 10,039 | 10,039  | 10,292   | 0,002   | 9854         | 0,0018 |
| sitexyear          | 3  | 10,01  | 3,3366  | 3,4205   | 0,0215  | 9947         | 0,0217 |
| sitexposition      | 3  | 3,8499 | 1,2833  | 1,3156   | 0,2758  | 9955         | 0,2805 |
| yearxposition      | 1  | 3,3048 | 3,3048  | 3,388    | 0,0708  | 9812         | 0,0682 |
| sitexyearxposition | 3  | 1,7959 | 0,59865 | 0,6137   | 0,6012  | 9955         | 0,5985 |
| Res                | 64 | 62,43  | 0,97547 |          |         |              |        |
| Total              | 79 | 111,14 |         |          |         |              |        |

719 *Supplementary Table 6: multivariate PERMANOVA investigating site and year effect for TPM*

| Source    | df  | SS     | MS      | Pseudo-F | P(perm) | Unique perms | P(MC)  |
|-----------|-----|--------|---------|----------|---------|--------------|--------|
| site      | 3   | 1,493  | 0,49767 | 0,28089  | 0,8424  | 9962         | 0,8364 |
| year      | 1   | 207,48 | 207,48  | 117,1    | 0,0001  | 9839         | 0,0001 |
| sitexyear | 3   | 2,0244 | 0,67479 | 0,38085  | 0,7691  | 9958         | 0,7708 |
| Res       | 100 | 177,18 | 1,7718  |          |         |              |        |
| Total     | 107 | 388,6  |         |          |         |              |        |

728 *Supplementary Table 7: multivariate PERMANOVA investigating site and year effect for PIM*

| Source    | df  | SS      | MS       | Pseudo-F | P(perm) | Unique perms | P(MC)  |
|-----------|-----|---------|----------|----------|---------|--------------|--------|
| site      | 3   | 0,11747 | 0,039156 | 0,039431 | 0,9901  | 9949         | 0,9904 |
| year      | 1   | 54,939  | 54,939   | 55,325   | 0,0001  | 9814         | 0,0001 |
| sitexyear | 3   | 0,33001 | 0,11     | 0,11077  | 0,957   | 9958         | 0,9508 |
| Res       | 100 | 99,303  | 0,99303  |          |         |              |        |
| Total     | 107 | 154,73  |          |          |         |              |        |

736

737 *Supplementary Table 8: multivariate PERMANOVA investigating site and year effect for POM*

| Source    | df  | SS     | MS      | Pseudo-F | P(perm) | Unique perms | P(MC)  |
|-----------|-----|--------|---------|----------|---------|--------------|--------|
| site      | 3   | 1,4638 | 0,48793 | 2,796    | 0,0429  | 9952         | 0,0407 |
| year      | 1   | 48,888 | 48,888  | 280,15   | 0,0001  | 9824         | 0,0001 |
| sitexyear | 3   | 1,193  | 0,39765 | 2,2787   | 0,0834  | 9952         | 0,0832 |
| Res       | 100 | 17,451 | 0,17451 |          |         |              |        |
| Total     | 107 | 69,327 |         |          |         |              |        |

745

746 *Supplementary Table 9: multivariate PERMANOVA investigating site, size and year effect for CHLOA*

| Source         | df  | SS      | MS      | Pseudo-F | P(perm) | Unique perms | P(MC)  |
|----------------|-----|---------|---------|----------|---------|--------------|--------|
| site           | 3   | 3,35    | 1,1167  | 3,9887   | 0,0088  | 9958         | 0,0088 |
| year           | 1   | 3,6519  | 3,6519  | 13,045   | 0,0003  | 9848         | 0,0007 |
| taille         | 2   | 1,8257  | 0,91286 | 3,2608   | 0,0401  | 9953         | 0,0456 |
| sitexyear      | 3   | 2,9083  | 0,96945 | 3,4629   | 0,0167  | 9953         | 0,0175 |
| sitexsize      | 6   | 1,984   | 0,33066 | 1,1811   | 0,3199  | 9933         | 0,3246 |
| yearxsize      | 2   | 5,0665  | 2,5333  | 9,0488   | 0,0004  | 9951         | 0,0004 |
| sitexyearxsize | 6   | 0,84964 | 0,14161 | 0,50582  | 0,8156  | 9949         | 0,8092 |
| Res            | 96  | 26,876  | 0,27995 |          |         |              |        |
| Total          | 119 | 46,512  |         |          |         |              |        |

758

759 *Supplementary Table 10: multivariate PERMANOVA investigating site and year effect for PEUK\_TOT*

| Source    | df | SS         | MS         | Pseudo-F | P(perm) | Unique perms | P(MC)  |
|-----------|----|------------|------------|----------|---------|--------------|--------|
| site      | 3  | 1,2885E+16 | 4,2951E+15 | 1,3441   | 0,2784  | 9945         | 0,2768 |
| year      | 1  | 1,959E+16  | 1,959E+16  | 6,1306   | 0,0155  | 9835         | 0,0187 |
| sitexyear | 3  | 2,6684E+15 | 8,8948E+14 | 0,27835  | 0,8512  | 9952         | 0,8401 |
| Res       | 32 | 1,0226E+17 | 3,1955E+15 |          |         |              |        |
| Total     | 39 | 1,374E+17  |            |          |         |              |        |

767

768 *Supplementary Table 11: multivariate PERMANOVA investigating site, size and year effect for CYAN*

| Source    | df | SS         | MS         | Pseudo-F | P(perm) | Unique perms | P(MC)  |
|-----------|----|------------|------------|----------|---------|--------------|--------|
| site      | 3  | 2,552E+16  | 8,5068E+15 | 0,7044   | 0,5664  | 9949         | 0,5635 |
| year      | 1  | 5,3384E+17 | 5,3384E+17 | 44,205   | 0,0001  | 9851         | 0,0001 |
| sitexyear | 3  | 1,2146E+16 | 4,0486E+15 | 0,33524  | 0,8082  | 9953         | 0,797  |
| Res       | 32 | 3,8645E+17 | 1,2077E+16 |          |         |              |        |
| Total     | 39 | 9,5796E+17 |            |          |         |              |        |

776

777 *Supplementary Table 12: multivariate PERMANOVA investigating site, size and year effect for PICO*

| Source    | df | SS         | MS         | Pseudo-F | P(perm) | Unique perms | P(MC)  |
|-----------|----|------------|------------|----------|---------|--------------|--------|
| site      | 3  | 2,3685E+15 | 7,8951E+14 | 0,083154 | 0,9729  | 9939         | 0,9697 |
| year      | 1  | 7,5797E+17 | 7,5797E+17 | 79,832   | 0,0001  | 9841         | 0,0001 |
| sitexyear | 3  | 3,7254E+15 | 1,2418E+15 | 0,13079  | 0,9431  | 9944         | 0,938  |
| Res       | 32 | 3,0383E+17 | 9,4946E+15 |          |         |              |        |
| Total     | 39 | 1,0679E+18 |            |          |         |              |        |

785

786

*Supplementary Table 13 : multivariate PERMANOVA investigating site, size and year effect for NANO*

787

| Source    | df | SS         | MS         | Pseudo-F | P(perm) | Unique perms | P(MC)  |
|-----------|----|------------|------------|----------|---------|--------------|--------|
| site      | 3  | 1,2396E+12 | 4,1319E+11 | 0,13497  | 0,9421  | 9944         | 0,9377 |
| year      | 1  | 1,7765E+13 | 1,7765E+13 | 5,8032   | 0,0196  | 9837         | 0,0175 |
| sitexyear | 3  | 2,0028E+13 | 6,6759E+12 | 2,1807   | 0,1051  | 9950         | 0,1082 |
| Res       | 32 | 9,7961E+13 | 3,0613E+12 |          |         |              |        |
| Total     | 39 | 1,3699E+14 |            |          |         |              |        |

794

795

*Supplementary Table 14 : multivariate PERMANOVA investigating site, size and year effect for BACT\_TOT*

796

| Source    | df | SS         | MS         | Pseudo-F | P(perm) | Unique perms | P(MC)  |
|-----------|----|------------|------------|----------|---------|--------------|--------|
| site      | 3  | 1,622E+19  | 5,4066E+18 | 0,93657  | 0,4508  | 9949         | 0,4387 |
| year      | 1  | 2,909E+20  | 2,909E+20  | 50,392   | 0,0001  | 9839         | 0,0001 |
| sitexyear | 3  | 1,0607E+19 | 3,5358E+18 | 0,61249  | 0,6213  | 9957         | 0,6151 |
| Res       | 32 | 1,8473E+20 | 5,7728E+18 |          |         |              |        |
| Total     | 39 | 5,0246E+20 |            |          |         |              |        |

803

804



Non-metric MDS



805

806 *Supplementary Figure 1. Non-metric multi-dimensional scaling of the Euclidean similarity matrix based on the relative*  
807 *abundance of fatty acid profiles measured in young settlers larvae collected in 2017 and 2019 at each sampling sites in the*  
808 *Thau lagoon.*

809