
Rising water temperatures, reproduction and recruitment of an invasive oyster, *Crassostrea gigas*, on the French Atlantic coast

Mickaël Dutertre^{a,*}, Peter G. Beninger^a, Laurent Barillé^a, Mathias Papin^b and Joël Haure^b

^a Université de Nantes, Nantes Atlantique Universités, Équipe Mer-Molécules-Santé EA 2160, Faculté des Sciences et des Techniques, 2 rue de la Houssinière, BP 92208, Nantes 44322, France

^b IFREMER, Laboratoire Conchylicole des Pays de la Loire, Polder des Champs, Bouin 85230, France

*: Corresponding author : M. Dutertre, Tel.: +33 02 51 12 56 91; fax: +33 02 51 12 56 68, email address :

Mickael.Dutertre@univ-nantes.fr

Abstract:

The recent appearance and invasion of feral oysters (*Crassostrea gigas*) along the northern European Atlantic coast, underscores the necessity to investigate the relationship between environmental variables, reproductive physiology, larval development and recruitment. We studied these relationships at both high (HT) and intermediate (IT) – turbidity sites, through historical data on water temperatures, multi-parameter environmental probes, histological analyses, and field collections of planktonic larvae and settled post-larvae in 2005 and 2006. A progressive warming trend was observed, especially since 1995, when oyster proliferation first became severe. Threshold temperatures for oocyte growth, larval development and settlement were achieved in both 2005 and 2006. The HT site showed greater numbers of larvae and post-larvae than the IT site for both years, with the highest numbers of post-larvae observed at both sites during the warmer summer of 2006. These results suggest that increased temperatures in northern European waters allow successful reproduction, larval development, and recruitment of *C. gigas*. High turbidity conditions further enhance this success.

Keywords: Aquaculture; Climate warming; Coastal waters; *Crassostrea gigas*; Reproductive cycle; Oyster larvae; Settlement; Turbidity

1. Introduction

Suspension-feeding bivalves are coastal ecosystem engineers that regulate matter and energy fluxes by coupling pelagic and benthic processes (see Dame and

43 Olenin, 2005 for reviews). In the twentieth century, over-exploitation, pollution, and
44 disease led to a worldwide decline in native oyster populations, accompanied by
45 economic losses and ecological changes (Newell, 1988; Quayle, 1988; Ruesink et
46 al., 2005). The Pacific cupped oyster, *Crassostrea gigas*, was voluntarily introduced
47 in several new coastal areas around the world for aquaculture purposes, because of
48 its rapid growth rate, high tolerance to environmental variations and low susceptibility
49 to oyster diseases (Coleman, 1986; Smith et al., 1986; Grizel and Héral, 1991).

50 Although successful introductions of *C. gigas* occurred particularly in northern
51 temperate countries of Europe and North America, water temperatures precluded
52 substantial larval recruitment in the northernmost regions (Le Borgne et al., 1973;
53 Gruet et al., 1976; Gouletquer, 1995; Drinkwaard, 1999). France is a particularly
54 interesting case, since the occurrence of massive and regular feral *C. gigas*
55 recruitments in the southern Atlantic regions, but not in the northern ones, suggested
56 that the limit for the successful larval development was situated south of Bourgneuf
57 Bay (Gouletquer and Héral, 1991; Robert and Gérard, 1999). However, in the past
58 decade, feral oysters have proliferated on northern European Atlantic coasts,
59 unrelated to new introductions, and *C. gigas* is now considered to be an invasive
60 organism from Spain to the North Sea (Reise et al., 1999; Wehrmann et al., 2000;
61 Cognie et al., 2006; Brandt et al., 2008). This phenomenon is particularly visible in
62 northern French turbid bays, where the feral *C. gigas* build long-lasting reefs and
63 colonize racks on which are attached farmed *C. gigas* bags (Martin et al., 2004;
64 2005). In these areas, trophic competition with feral oysters has been suggested to
65 explain the decline in farmed oyster growth performance in the last ten years (Cognie
66 et al., 2006). Although reproduction performances of farmed adult oysters are being

67 elucidated (Dutertre et al., in revision), field studies on natural recruitment are also
68 necessary to understand the recent feral oyster invasion (Underwood and
69 Fairweather, 1989; Grosberg and Levitan, 1992; Smaal et al., 2005).

70 Optimal larval development of *C. gigas* requires a water temperature higher
71 than 22°C during at least two weeks (Arakawa, 1990; Shatkin et al., 1997; Rico-Villa
72 et al., 2008) and oyster larvae are affected by food quality and quantity (Baldwin and
73 Newell, 1995; Powell et al., 2002; Rico-Villa et al., 2008). As larval survival is the
74 determining element for the settlement of feral oyster populations (Gosling, 2003),
75 environmental influences on larval development need to be clarified by field studies,
76 especially in turbid coastal waters which are characterized by seasonal and short-
77 term variations of the environmental conditions (Mann, 1982).

78 In an attempt to determine the causes of the recent invasion of feral oysters, in
79 northern cold temperate ecosystems, the present study analyzed the larval
80 development and post-larval recruitment of *C. gigas* at the southern and northern
81 geographic extremes of Bourgneuf Bay in relation to real-time monitored
82 environmental factors.

83

84 **2. Materials and methods**

85 **2.1. Adult oyster sampling and tissue fixation**

86 Feral and farmed oysters were collected at two oyster-farming sites in
87 Bourgneuf Bay, between February 2005 and July 2006 (Fig. 1, Haure and Baud,
88 1995). The northern site, La Coupelasse (47°1' 34.7"N, 2°1' 55.9"W) , is a high -

89 turbidity mudflat compared to the southern sandy-muddy bottom site, Gresseloup
90 (46°57' 2.6"N, 2°7' 53.4"W) .

91 At the beginning of the study, in February 2005, adult farmed oysters (shell
92 length = 69.2 ± 4.9 SD mm, originating from 18-month hatchery-born spat,) were
93 installed, at both the northern and southern sites, in 1.0 × 0.5 m plastic, 20 mm mesh
94 bags and tied to oyster racks (3.0 × 1.0 m) at 0.6 m above the bottom. Each bag
95 contained 280 individuals, corresponding to 5 - 10 kg of oysters. At each site, 15
96 farmed and 15 feral oysters were then sampled once monthly until March 2006 and
97 then twice monthly until July 2006. Feral oysters, in the same range of shell length
98 as farmed ones, were collected near oyster racks on fixed substrata. For each oyster,
99 shell dimensions (length, width and height) were measured with a caliper and whole
100 mass was determined before shucking. Soft tissues were then immediately fixed in
101 cold aqueous Bouin's solution (approx. 5 x animal volume) for at least two weeks
102 (Beninger et al., 2001).

103

104 **2.2. Histological preparation**

105 After fixation, a 0.5 mm-thick slice of the visceral mass was removed from the
106 region along the line connecting the left and right palp-gill junctions (Morales-Alamo
107 and Mann, 1989). The tissue was rinsed under running water overnight to eliminate
108 excess Bouin's solution, dehydrated and prepared for paraffin embedding.
109 Embedded tissues were sliced with a microtome to obtain 10 histological sections per
110 individual, 7 µm in thickness. These sections were rehydrated, stained with modified
111 Masson's trichrome and dehydrated before being mounted on glass slides in

112 mounting medium (Beninger et al., 2001). Mounted histological sections were then
113 dried at 60°C for at least one week.

114

115 **2.3. Microscopic determinations and oocyte size measurements**

116 Oyster larvae are characterized by an initial endotrophic stage (utilization of
117 stored oocyte reserves), followed by a mixotrophic stage (oocyte reserves + ingested
118 particles) and finally an exotrophic stage (ingested particles only - Lucas et al., 1986;
119 His and Seaman, 1992; Cannuel and Beninger, 2005). Oocytes increase in size
120 during vitellogenesis, so the relative amount of oocyte reserves was therefore
121 estimated by measuring oocyte sizes in histological sections of female oysters,
122 observed on a computer screen connected to a video camera (Nikon DXM 1200F)
123 and optical microscope (Olympus AX70). LUCIA 4.80 software (Image Analysis
124 Systems) was used to measure oocyte area showing sections passing through both
125 the clear nucleus and a least one nucleolus (n = 30 per oyster). Oocyte diameter was
126 then calculated as follows:

$$127 \text{ Oocyte diameter } (\mu\text{m}) = 2 \times \sqrt{(\text{oocyte area } (\mu\text{m}^2) / \pi)}$$

128 Monthly means (\pm 95 % confidence intervals, CI) of oocyte diameters were
129 calculated and used to determine the gonadal development stage (Lango-Reynoso et
130 al., 2000): early gametogenesis (3.0 to 12.0 μm), growing (12.1 to 30.0 μm), mature
131 (30.1 to 41.0 μm) or atretic (41.1 to 60 μm).

132

133

134 **2.4. Determinations of D-larva and post-larval densities**

135 Unambiguous identification of *C. gigas* larvae was achieved through a
136 preliminary inventory of larval bivalve species at the two study sites, followed by
137 identification using literature data (Rees, 1950; Le Pennec, 1978; His, 1991) and
138 computer image analysis of key shell characteristics (Lucia G 4.80 software).

139 During known oyster spawning periods, plankton samples were collected at the
140 northern and southern sites using a boat-mounted pump provided with a flowmeter.
141 Each plankton sample (1.5 m³) was fixed in 10% formaldehyde – seawater solution
142 and prepared for analysis as in Auby et al. (2002). Each sample was manually
143 homogenized with a loop-ended glass stick to avoid damage to larvae, and two 0.5
144 ml aliquots were transferred to Sedgewick-Rafter counting cells and observed using
145 a light microscope (Olympus AX70) in order to determine D-larva densities (shell
146 height = 57 to 105 µm; Rees, 1950; Le Pennec, 1978; His, 1991).

147 At each site, clusters of 10 striated tubular collectors (commonly used as
148 substrata for oyster spat settlement in the field; length = 120 cm, diameter = 2 cm)
149 were tied side by side to oyster racks (3.0 × 1.0 m) at 0.6 m from the bottom. The
150 clusters were rolled up at the end of each tidal cycle to count the post-larval (i.e.
151 recently-settled spats) density, and replaced with new clusters for the next tidal cycle.

152

153 **2.5. Environmental monitoring**

154 Multi-parameter water quality probes (YSI 6600) were fixed to oyster racks
155 installed at each sampling site, to record temperature (°C), salinity (from
156 conductivity), suspended particulate matter (SPM) concentration (nephelometry,

157 NTU) and chlorophyll-*a* (chl-*a*) concentration (fluorometry, %) every hour. The
158 corresponding monthly means were plotted with their 95 % confidence intervals (n =
159 720).

160 Food is implicitly defined as ingestible matter. Since we do not know what,
161 precisely, the oysters are ingesting, we can only use indicators of available food.
162 These must take into account:

163 Quantity

164 Suspended Particulate Matter – the total amount of particles, quality not specified

165 Particulate Organic Matter (POM) – potentially digestible particles, but quality not
166 specified

167 Chlorophyll-*a* – amount of available phytoplankton, proportion of the available
168 particulate matter not specified

169 Quality

170 POM:SPM – an indication of the organic content of the SPM (dilution of POM)

171 Chl-*a*:POM – an indication of the quality of the organic matter

172 In order to characterize potential food amount, availability, and quality, field
173 calibrations for suspended matter were performed simultaneously from both probe
174 records and natural seawater samples collected at each oyster sampling site over
175 two tidal cycles. Some seawater samples (n = 17) were dried at 60°C for 48 h and
176 then ashed at 450°C for 4 h (Barillé-Boyer et al., 2003) to obtain SPM and POM
177 concentrations (mg.l⁻¹) respectively, while other samples (n = 16) were analyzed by
178 spectrophotometry after extraction with acetone (Lorenzen, 1967) to determine chl-*a*

179 concentrations ($\mu\text{g.l}^{-1}$). Linear regressions obtained from field samples were used to
180 transform hourly probe records into concentrations as follows:

181 $\text{SPM (mg.l}^{-1}\text{)} = 1.44 \times \text{turbidity (NTU)} + 12.92, n = 17, r^2 = 0.93$

182 $\text{POM (mg.l}^{-1}\text{)} = 0.18 \times \text{turbidity (NTU)} + 3.42, n = 17, r^2 = 0.94$

183 $\text{Chl-a (}\mu\text{g.l}^{-1}\text{)} = 4.63 \times \text{fluorometry (\%)} + 1.65, n = 16, r^2 = 0.92$

184 Food dilution and quality, herein estimated as percent organic content of SPM
185 (POM:SPM ratio) and percent chl-a content of POM (chl-a:POM ratio) respectively,
186 were calculated as follows:

187 $\text{POM:SPM (\%)} = (\text{POM (mg.l}^{-1}\text{)} / \text{SPM (mg.l}^{-1}\text{)}) \times 100$

188 $\text{Chl-a:POM (\%)} = (\text{chl-a (mg.l}^{-1}\text{)} / \text{POM (mg.l}^{-1}\text{)}) \times 100$

189

190 **2.6. Historical data of water temperature**

191 Daily water temperatures (WT) in Bourgneuf Bay were calculated between
192 January 1970 and December 2006, using the following regression (Haure and Baud,
193 1995):

194 $\text{WT} = 0.8703 \times \text{AT} + 0.036 \times \text{TC} - 0.0969$

195 Daily atmospheric temperatures (AT) were obtained from Météo-France's
196 Climathèque database (Noirmoutier station, $2^{\circ}15'24'$ 'W, $47^{\circ}00'18''$ N) and tidal
197 coefficients (TC) using the Marées dans le monde 2.02© software.

198

199 **2.7. Statistical analysis**

200 Sigmastat 3.1 (Systat software) was used to check the normality and
201 heteroscedasticity of data distributions and then to perform statistical analyses.
202 Temporal and spatial variations of environmental factors were compared by Student
203 t-tests or two-way parametric ANOVA, while correlation between them was
204 determined by Spearman correlation tests. Analyses of data from histological
205 determinations, as well as larval and post-larval cumulative densities, were first
206 performed with two-way parametric ANOVA within each reproductive cycle, and a
207 *posteriori* by Student-Newman-Keuls (SNK) tests.

208

209 **3. Results**

210 **3.1. Environmental variations**

211 *3.1.1. Seston quantity and quality*

212 Over the sampling period, SPM, POM and chl-a concentrations were always
213 higher at the HT site compared to the IT site (Fig. 2A, B and C - two-way ANOVA, $p <$
214 0.01). The monthly mean POM:SPM ratio, used to estimate potential food dilution,
215 was lower at the HT site in 2005 and 2006 (Fig. 3A, t-test, $p < 0.01$). On the other
216 hand, the monthly mean chl-a:POM ratio, used to estimate food quality, was higher at
217 the HT site in 2005 (Fig. 3B, t-test, $p < 0.01$), while no significant difference between
218 sites was reported in 2006 (Fig. 3B, t-test, $p = 0.98$).

219

220 *3.1.2. Fine-scale variations of water temperature and salinity: 2005 - 2006*

221 Monthly mean water temperatures, largely typical of a northern temperate
222 nearshore ecosystem, were not significantly different between the sites in 2005 and
223 2006 (Fig. 2D, Student t-test, $p = 0.95$ and $p = 0.99$, respectively). However, the
224 summer period was warmer in 2006 vs. 2005, especially in July, where the mean
225 water temperature was 1.5°C higher (Fig. 2D). The daily amplitude of water
226 temperature was higher at the HT site (Table 1, two-way ANOVA, $p < 0.01$). Monthly
227 mean salinity, ranging from 29.0 to 35.3, was not significantly different between the
228 sites in 2005 and 2006 (Student t-test, $p = 0.93$ and $p = 0.10$, respectively).

229

230 *3.1.3 Historical variations in water temperature*

231 Historical annual mean and warmest-month calculated mean water
232 temperatures are presented for Bourgneuf Bay from 1970 – 2006 (Fig. 4). For the 17-
233 year period from 1970 – 1987, annual means were higher than the annual medians
234 for only 2 years (11.8%), versus 15 years (83.3%) for the 18-year period from 1988 –
235 2006. This situation prevailed in 10 of 11 years (91%) from 1995 – 2006. Similarly,
236 the warmest month means over the 17-year period from 1970 – 1987 were above the
237 threshold temperature for successful reproduction (20°C – Chávez-Villalba et al.,
238 2002; Rico-Villa et al., 2008) only 2 years (11.8%), whereas over the 18-year period
239 from 1988 – 2006, this situation prevailed in 9 years (50%).

240

241 **3.2. Microscopic determinations and oocyte size**

242 Variations in oocyte size allowed identification of two distinct seasonal
243 reproductive cycles in 2005 and 2006 (Fig. 5). In 2005, no significant difference was

244 observed for mean oocyte diameter in intra-site (two-way ANOVA, $p = 0.85$) and
245 inter-site (two-way ANOVA, $p = 0.81$) comparisons. Similarly, in 2006, no significant
246 difference was observed for mean oocyte diameter in intra-site (two-way ANOVA, $p =$
247 0.84) and inter-site (two-way ANOVA, $p = 0.94$) comparisons. In both years, the
248 oocyte growth stage began in the same periods (end of March – beginning of April).
249 However, the mature stage, corresponding to the dominance of ready-to-spawn post-
250 vitellogenetic oocytes in the gonads, was reached more quickly in 2006 than in 2005
251 (two vs. three months). Gonads entered a degenerating stage (evidence of atresia in
252 unspawned oocytes such as cell size increase and clearer cytoplasm – Dutertre et
253 al., in revision), more prematurely in 2006 than in 2005 (July vs. August,
254 respectively).

255

256 **3.3. D-larva and post-larval densities**

257 Cumulative D-larva densities showed significant differences related to both year
258 and site (Figs. 6 and 7, two-way ANOVA, $p < 0.05$ and $p < 0.01$, respectively).
259 Cumulative D-larva densities were higher at the HT site for both years (SNK-tests, p
260 < 0.05 for 2005 and $p < 0.01$ for 2006). For the IT site, cumulative D-larva densities
261 were higher in 2006 compared to 2005 (SNK-test, $p < 0.05$), while, for the HT site, no
262 significant differences were observed between the two years (SNK-test, $p = 0.30$). At
263 both sites, D-larvae appeared at the same periods: over two months in 2005, from
264 the beginning of July to the beginning of September, with a marked increase of the
265 planktonic larva densities observed at the end of August at the HT site. In 2006, the
266 HT site showed two main peaks of planktonic larva densities, at the end of July and

267 at the beginning of August, while, at the same periods, two smaller peaks of D-larva
268 densities were recorded at the IT site.

269 Cumulative natural post-larval recruitment also showed significant differences
270 related to both year and site (Figs. 6 and 7, two-way ANOVA, $p < 0.01$). Natural
271 recruitment was much higher in 2006 compared to 2005 at both sites (SNK-tests, $p <$
272 0.001 for the HT site and $p < 0.05$ for the IT site). Inter-site differences in the monthly
273 post-larval counts were also evident, with proportionately higher counts at the HT site
274 in both 2005 and 2006.

275

276 **4. Discussion**

277 **4.1. Water temperature and recent oyster invasion**

278 At approximately 28000 t, the feral oyster stock in Bourgneuf Bay equals 70%
279 of the annual farmed oyster production (Cognie et al., 2004; Martin et al., 2004;
280 2005). Water temperature variations, since the introduction of *C. gigas* for
281 aquaculture, clearly show that the onset of the feral oyster invasion coincided with a
282 marked water warming (Fig. 4). Indeed, between 1970 and 1995, when annual mean
283 water temperature was usually lower than the median temperature (13.4°C),
284 cumulative feral oyster recruitment was very low (Le Borgne et al., 1973; Gruet et al.,
285 1976; Gouletquer, 1995). Massive recruitment of feral oysters, observed since 1995
286 (Cognie et al., 2006) corresponded to the beginning of the period where summer
287 months often showed water temperature higher than 20°C, which is required for
288 successful *C. gigas* larval development in hatcheries (Arakawa, 1990; Shatkin et al.,
289 1997; Chávez-Villalba et al., 2002; Rico-Villa et al., 2008). These quantitative

290 historical data thus support earlier hypotheses of a relationship between *C. gigas*
291 proliferation in cool temperate European ecosystems and global warming (Diederich
292 et al., 2005; Ruesink et al., 2005; Smaal et al., 2005). Among the temperature-
293 related variables which could contribute to this proliferation are those which chiefly
294 affect larval survival and subsequent recruitment of feral oysters: oocyte reserves,
295 spawning period and seston conditions (Baldwin and Newell, 1995; Powell et al.,
296 2002; Chávez-Villalba et al., 2003; Rico-Villa et al., 2008).

297

298 **4.2. Oocyte fate timed by water temperature thresholds**

299 During two successive reproductive cycles of *C. gigas*, oocyte diameter
300 variations showed that the field reproductive cycle was timed by discrete water
301 temperature thresholds (Dutertre et al., in revision). The oocyte growing stage,
302 characterized by both an increase in size and in vitellin reserves (see Gosling, 2003
303 for recent review), began when spring water temperature reached 8-10°C. The
304 mature stage was reached more quickly in 2006 than in 2005 (two vs. three months),
305 corresponding to a reduced daily amplitude of water temperatures in 2006, and also
306 to a greater energy level of breeders due to higher spring food quality (chl-a:POM
307 ratio) and/or recovery of energy from the large amount of reabsorbed atretic oocytes
308 at the end of summer 2005 (Dutertre et al., in revision). Although early partial spawns
309 could be detected when daily variations in water temperature briefly exceeded 18°C,
310 water temperatures of 15 – 18°C cause mature, unspawned oocytes to enter atresia
311 (Dutertre et al. in revision). Major spawning activities were recorded when summer
312 water temperature, higher than 20°C, could efficiently sustain *C. gigas* larval
313 development (Chávez-Villalba et al., 2002; Rico-Villa et al., 2008).

314 Similar oocyte size in farmed and feral oysters at both sites indicated that future
315 fertilized eggs would contain equivalent amounts of vitellus for the endotrophic and
316 mixotrophic larval stages (Lucas et al., 1986; His and Seaman, 1992; Cannuel and
317 Beninger, 2005). This result is in agreement with the observation that variations in
318 reproductive effort in *C. gigas* reflect variations in gamete quantity rather than quality
319 (Caers et al., 2002; Chávez-Villalba et al. 2003; Cannuel and Beninger, 2005).

320

321 **4.3. Planktonic larval life**

322 Maximal planktonic larva densities in the water column were observed during
323 defined summer periods in which high oyster fecundity was synchronized with a
324 water temperature higher than 20 - 22°C. D-larva densities corresponded to the
325 patterns of breeder spawning strategy at both sites in 2005 and 2006, but early
326 spawns, which occurred when water temperature was below the threshold allowing
327 an optimal larval development (Dutertre et al., in revision), were not accompanied by
328 larval presence. Moreover, although IT oysters had more pronounced spawns
329 compared to HT oysters in both years (Dutertre et al., in revision), D-larva densities
330 were higher at the HT site, which presented slightly, but non-statistically significant,
331 higher summer temperatures. This may also be due to the significantly higher level of
332 chlorophyll-*a* at the HT site; in other words, the poor food quality at the HT site was
333 amply compensated by the sheer amount of food available for the larvae.

334

335 **4.4. Feral oyster recruitment**

336 Natural post-larval recruitment at both HT and IT sites was much higher in 2006
337 (4540 and 1489 annual settled post-larvae. m⁻², respectively) compared to 2005 (45

338 and 4 annual settled post-larvae.m⁻², respectively). Although 2006 could be
339 considered an exceptionally favorable year for oyster reproduction and post-larval
340 recruitment in relation to the warmer summer temperatures, natural recruitment in
341 Bourgneuf Bay remained very low compared to more southern coastal ecosystems.
342 Indeed, the best natural recruitments in Arcachon Bay over the past twenty years
343 were reported in 2003 and 2006 with more than 60 000 settled post-larvae.m⁻² of
344 limed tiles (Auby et al., 2006). Environmental conditions at the HT site, notably the
345 chlorophyll-a levels, regardless of the organic matter dilution, appear to promote local
346 feral oyster recruitment. This is confirmed by oyster-farmer practices over the past
347 several years, which preferentially use the HT site to install artificial spat collectors
348 (Marion Petit, Section Régionale Conchylicole, Bouin, France, pers. com.). Once
349 established, large feral oyster reefs can disrupt flow, limit larval dispersal, and offer
350 substrate for settlement, enhancing local post-larval recruitment at the HT site.

351

352 **5. Conclusion**

353 The historical data presented here clearly show that *C. gigas* proliferation in
354 the Bourgneuf Bay ecosystem corresponds to warmer water temperatures,
355 particularly since 1995. A similar evolution in water temperatures has been recorded
356 at more northerly sites (Wadden Sea), also corresponding to increasing *C. gigas*
357 recruitment (Diederich et al, 2005). The underlying processes of reproduction and
358 development are acutely sensitive to such warming through the threshold
359 temperatures of oocyte growth and larval development, and ultimately greater
360 recruitment of post-larval feral oysters, as shown by the recent fine-scale temporal
361 data of the present study. A continuation of the warming trend in water temperatures

362 should thus produce an intensification of this proliferation, and a range extension
363 northward in shallow European bays, including those used for oyster farming. Given
364 the now near-ubiquitous distribution of *C. gigas* in temperate coastal habitats, these
365 observations should serve to alert the marine environment research community to
366 potentially similar situations worldwide.

367

368 **Acknowledgements**

369 We thank David Lecossois for the installation of the animals used in this study in
370 oyster bags at his farm, and Odile Aumaille for technical assistance in histological
371 analyses. Research funding was provided by the Syndicat Mixte pour le
372 Développement de l'Aquaculture et de la Pêche (SMIDAP) de la Région des Pays de
373 la Loire, and M. Dutertre was supported by a PhD scholarship from the Ministère
374 Français de la Recherche et de l'Enseignement Supérieur.

375

376 **References**

- 377 Arakawa, K.Y., 1990. Natural spat collecting in the Pacific oyster *Crassostrea gigas*
378 (Thunberg). *Marine Behavior and Physiology* 17, 95-128.
- 379 Auby, I., Maurer, D., Valvason, M.L., Gueguen, C., Guillard, F., 2002. Mise au point
380 d'une nouvelle méthode de suivi de la reproduction de l'huître creuse:
381 Comparaison avec la méthode traditionnelle. Rapport Direction de
382 l'Environnement et de l'Aménagement du Littoral. IFREMER
383 RST.DEL/02.01/ARCACHON.
- 384 Auby, I., Maurer, D., Cassam-Chenai, Y., Tournaire, M.-P., Neaud-Masson, N.,
385 Rumèbe, M., Cantin, C., Debort, H., Germain, J.-M., Navarro, R. 2006.
386 Reproduction de l'huître creuse dans le Bassin d'Arcachon: Année 2006.
387 Rapport Direction de l'Environnement et de l'Aménagement du Littoral.
388 IFREMER RST/LER/AR/06-006.
- 389 Baldwin, B.S., Newell, R.I.E., 1995. Feeding rate responses of oyster larvae
390 (*Crassostrea virginica*) to seston quantity and composition. *Journal of*
391 *Experimental Marine Biology and Ecology* 189, 77-91.
- 392 Barillé, L., Haure, J., Cognie, B., Leroy, A., 2000. Variations in pallial organ and
393 eulatero-frontal cirri in response to high particulate matter concentrations in the
394 oyster *Crassostrea gigas*. *Canadian Journal of Fisheries and Aquatic Sciences*
395 57, 837-843.
- 396 Barillé-Boyer, A.-L., Barillé, L., Massé, H., Razet, D., Héral, M., 2003. Correction for
397 particulate organic matter as estimated by loss on ignition in estuarine
398 ecosystems. *Estuarine, Coastal and Shelf Science* 58, 147-153.

399 Beninger, P.G., Cannuel, R., Blin, J.-L., Pien, S., Richard, O., 2001. Reproductive
400 characteristics of the archaeogastropod *Megathura crenulata*. Journal of
401 Shellfish Research 20, 301-307.

402 Brandt, G., Wehrmann, A., Wirtz, K.W., 2008. Rapid invasion of *Crassostrea gigas*
403 into the German Wadden Sea dominated by larval supply. Journal of Sea
404 Research 59, 279-296.

405 Caers, M., Utting, S.D., Coutteau, P., Millican, P.F., Sorgeloos, P., 2002. Impact of
406 the supplementation of a docosahexaenoic acid-rich emulsion on the
407 reproductive output of oyster broodstock, *Crassostrea gigas*. Marine Biology
408 140, 1157-1166.

409 Cannuel, R., Beninger, P.G., 2005. Is oyster broodstock feeding always necessary?
410 A study using oocyte quality predictors and validators in *Crassostrea gigas*.
411 Aquatic Living Resources 18, 35-43.

412 Chávez-Villalba, J., Barret, J., Mingant, C., Cochard, J.C., Le Penneec, M., 2002.
413 Autumn conditioning of the oyster *Crassostrea gigas*: A new approach.
414 Aquaculture 210, 171-186.

415 Chávez-Villalba, J., Cochard, J.C., Le Penneec, M., Barret, J., Enríquez-Díaz, M.,
416 Cáceres-Martínez, C., 2003. Effects of temperature and feeding regimes on
417 gametogenesis and larval production in the oyster *Crassostrea gigas*. Journal of
418 Shellfish Research 22(3), 721-731.

419 Cognie, B., Barillé, L., Carrié, C., Rosa, P., 2004. Estimation des stocks d'huîtres
420 sauvages en baie de Bourgneuf (partie Loire-Atlantique). Rapport de contrat
421 Région des Pays de la Loire, arrêté n°02-5923-0 du 19 mars 2002, 19 pp.

422 Cognie, B., Haure, J., Barillé, L., 2006. Spatial distribution in a temperate coastal
423 ecosystem of the wild stock of the farmed oyster *Crassostrea gigas* (Thunberg).
424 *Aquaculture* 259, 249-259.

425 Coleman, N., 1986. A review of introductions of the Pacific oyster (*Crassostrea gigas*)
426 around the world and a discussion of the possible ecological consequences of
427 introducing the species into Victoria, Australia. Marine Resources Man and
428 Wildlife Service, Department of Conservation, Forests and Lands,
429 Queescliff, Victoria, Australia, 39 pp.

430 Dame, R.F., Olenin, S., 2005. The comparative roles of suspension-feeders in
431 ecosystems. NATO Science Series. IV. Earth and Environmental Sciences vol.
432 47, Springer, 359 pp.

433 Diederich, S., Nehls, G., van Beusekom, J.E.E., Reise, K., 2005. Introduced Pacific
434 oysters (*Crassostrea gigas*) in the northern Wadden Sea: invasion accelerated
435 by warm summers? *Helgoland Marine Research* 59, 97-106.

436 Drinkwaard, A.C., 1999. Introductions and developments of oysters in the North Sea
437 area: a review. *Helgoland Meeresunters* 52, 301-308.

438 Dutertre, M., Beninger, P.G., Barillé, L., Papin, M., Rosa, P., Barillé, A.-L., Haure, J.,
439 in revision. Temperature and seston quantity and quality effects on field
440 reproduction of farmed oysters, *Crassostrea gigas*, in Bourgneuf Bay, France.
441 *Aquatic Living Resources*

442 Gosling, E., 2003. Reproduction, Settlement and Recruitment. In: *Bivalve molluscs:*
443 *biology, ecology and culture*. Gosling E. (ed.), Fishing News Books, Blackwell
444 Science, Oxford, 131-168.

445 Gouletquer, P., 1995. Cycle de reproduction naturelle de l'huître creuse *Crassostrea*
446 *gigas*. Groupe de travail sur la Reproduction des Mollusques Bivalves
447 d'Aquaculture Marine, IFREMER, Nantes, France.

448 Gouletquer, P., Héral, M., 1991. Aquaculture of *Crassostrea gigas* in France. The
449 ecology of *C. gigas* in Australia, New Zealand, France and Washington State.
450 Oyster Ecology Workshop, Annapolis, USA, 12-19.

451 Grizel, H., Héral, M., 1991. Introduction into France of the Japanese oyster
452 (*Crassostrea gigas*). Journal du Conseil International pour l'Exploration de la
453 Mer, 47, 399-403.

454 Grosberg, R.K., Levitan, D.R., 1992. For adults only? Supply-side ecology and the
455 history of larval biology. Trends in Ecology and Evolution 7(4), 130-133.

456 Gruet, Y., Héral, M., Robert, J.M., 1976. Premières observations sur l'introduction de
457 la faune associée au naissain d'huîtres japonaises *Crassostrea gigas*
458 (Thunberg), importé sur la côte atlantique française. Cahiers de Biologie Marine
459 17, 173-184.

460 Haure, J., Baud, J.P., 1995. Approche de la capacité trophique dans un bassin
461 ostréicole (baie de Bourgneuf). Rapport Direction Ressources Vivantes
462 IFREMER RIDVR-95-96/RA-BOUIN.

463 His, E., 1991. Biologie et écotoxicologie des véligères de *Crassostrea gigas*
464 (Thunberg) dans le Bassin d'Arcachon. PhD thesis, University of Bordeaux I,
465 France.

466 His, E., Seaman, M.N.L., 1992. Effects of temporary starvation on the survival, and
467 on subsequent feeding and growth, of oyster (*Crassostrea gigas*) larvae. Marine
468 Biology 114, 277-279.

469 Lango-Reynoso, F., Chávez-Villalba, J., Cochard, J.-C., Le Pennec, M., 2000.
470 Oocyte size, a means to evaluate the gametogenic development of the Pacific
471 oyster, *Crassostrea gigas* (Thunberg). *Aquaculture* 190, 183-199.

472 Le Borgne, M., Gras, M.P., Comps, M., Carruesco, G., Razet, D., 1973. Observations
473 sur la reproduction des huîtres dans la Seudre (Bassin de Marennes-Oléron) en
474 1972. 5 pp.

475 Le Pennec, M., 1978. Genèse de la coquille larvaire et postlarvaire chez divers
476 bivalves marins. PhD thesis, University of Bretagne Occidentale, France.

477 Lorenzen, C.J., 1967. Determination of chlorophyll and pheopigments:
478 Spectrophotometric equations. *Limnology and Oceanography* 12, 343-346.

479 Lucas, A., Chebab-Chalabi, L., Aldana Aranda, D., 1986. Passage de l'endotrophie à
480 l'exotrophie chez les larves de *Mytilus edulis*. *Oceanologica Acta* 9(1), 97-103.

481 Mann, K.H., 1982. Ecology of coastal waters: a systems approach. *Studies in*
482 *ecology*, vol. 8, Univ. California Press, Berkeley, 322 pp.

483 Martin, J.L., Haure, J., Dupuy, B., Papin, M., Palvadeau H., Nourry, M., Penisson, C.,
484 Thouard, E. 2004. Estimation des stocks d'huîtres sauvages sur les zones
485 concédées de la partie vendéenne de la baie de Bourgneuf en 2003. Rapport
486 IFREMER, DRV/RA/LCPL/2004-05, 21 pp.

487 Martin J.L., Haure J., Dupuy, B., Papin, M., Palvadeau, H., Nourry, M., Penisson, C.,
488 Thouard, E., 2005. Estimation des stocks d'huîtres sauvages sur les zones non
489 concédées de la partie vendéenne de la baie de Bourgneuf. Rapport IFREMER,
490 AGS/LGP/Bouin/2005-01, 17pp.

491 Morales-Alamo, R., Mann, R., 1989. Anatomical features in histological sections of
492 *Crassostrea virginica* (Gmelin, 1791) as an aid in measurements of gonad area
493 for reproductive assessment. *Journal of Shellfish Research* 8, 71-82.

494 Newell, R.I.E., 1988. Ecological changes in Chesapeake Bay: are they the results of
495 overharvesting the American oyster, *Crassostrea virginica*? Chesapeake
496 Research Consortium Publications 129, 536-546.

497 Powell, E.N., Bochenek, E.A., Klinck, J.M., Hofmann, E.E., 2002. Influence of food
498 quality and quantity on the growth and development of *Crassostrea gigas*
499 larvae: a modelling approach. *Aquaculture* 210, 89-117.

500 Quayle, D.B., 1988. Pacific oyster culture in British Columbia. *Canadian Bulletin of*
501 *Fisheries and Aquatic Sciences* 218, 1-24.

502 Reise, K., Gollasch, S., Wolff, W., 1999. Introduced marine species of the North Sea
503 coasts. *Helgoland Marine Research* 52, 219–234.

504 Rees, C.B., 1950. The identification and classification of lamellibranch larvae. *Hull*
505 *Bulletins of Marine Ecology* 3(19), pp. 73-104.

506 Rico-Villa, B., Pouvreau, S., Robert, R., 2008. Influence of food density and
507 temperature on ingestion, growth and settlement of Pacific oyster larvae,
508 *Crassostrea gigas*. *Aquaculture* 287, 395-401.

509 Robert, R., Gérard, A., 1999. Bivalve hatchery technology: the current situation for
510 the Pacific oyster *Crassostrea gigas* and the scallop *Pecten maximus* in France.
511 *Aquatic Living Resources* 12, 121-130.

512 Ruesink, J.L., Lenihan, H.S., Trimble, A.C., Heiman, K.W., Micheli, F., Byers, J.E.,
513 Kay, M.C., 2005. Introduction of non-native oysters: ecosystem effects and
514 restoration implications. *Annual Review of Ecology, Evolution and Systematics*
515 36, 643-689.

516 Shatkin, G., Shumway, S.E., Hawes, R., 1997. Considerations regarding the possible
517 introduction of the Pacific oyster (*Crassostrea gigas*) to the Gulf of Maine: a
518 review of global experience. *Journal of Shellfish Research* 16, 463-477.

519 Smaal, A., van Stralen, M., Craeymeersch, J., 2005. Does the introduction of the
520 Pacific oyster *Crassostrea gigas* lead to species shifts in the wadden sea? In:
521 *The Comparative Roles of Suspension-Feeders in Ecosystems* / Dame, R.F.,
522 Olenin, S., - Dordrecht, Kluwer (NATO Science Series IV. Earth &
523 Environmental Sciences Vol. 47), pp. 277 - 289.

524 Smith, P.J., Ozaki, H., Fujio, Y., 1986. No evidence for reduced genetic variation in
525 the accidentally introduced oyster *Crassostrea gigas* in New Zealand. *New*
526 *Zealand Journal of Marine and Freshwater Research* 20, 569-574.

527 Underwood, A.J., Fairweather, P.G., 1989. Supply-side ecology and benthic marine
528 assemblages. *Trends in Ecology and Evolution* 4(1), 16-20.

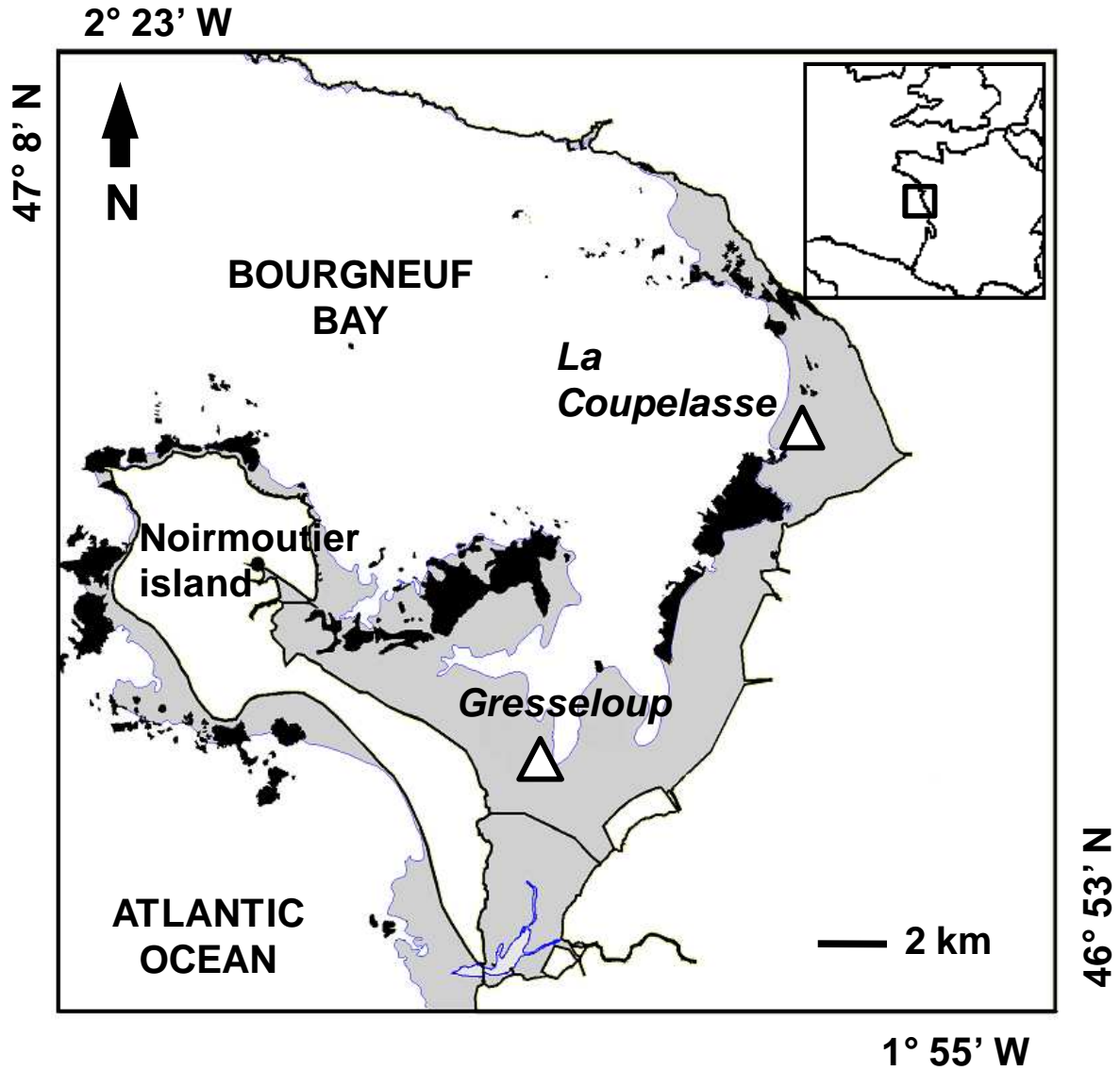
529 Wehrmann, A., Herlyn, M., Bungenstock, F., Hertweck, G., Millat, G., 2000. The
530 distribution gap is closed-first record of naturally settled Pacific oysters
531 (*Crassostrea gigas*) in the East Frisian Wadden Sea, North Sea.
532 *Senckenbergiana Maritima* 30, 153-160.

533 Table 1. Mean daily amplitudes (\pm SD) of water temperature at the northern high
534 turbidity (HT, La Coupelasse) and southern intermediate turbidity (IT, Gresseloup)
535 sites of Bourgneuf Bay in 2005 and 2006.

	Year	n (days)	Amplitude of water temperature ($^{\circ}$ C)
HT site	2005	278	3.43 \pm 2.01
	2006	349	2.23 \pm 1.40
IT site	2005	251	2.39 \pm 1.63
	2006	347	1.78 \pm 1.29

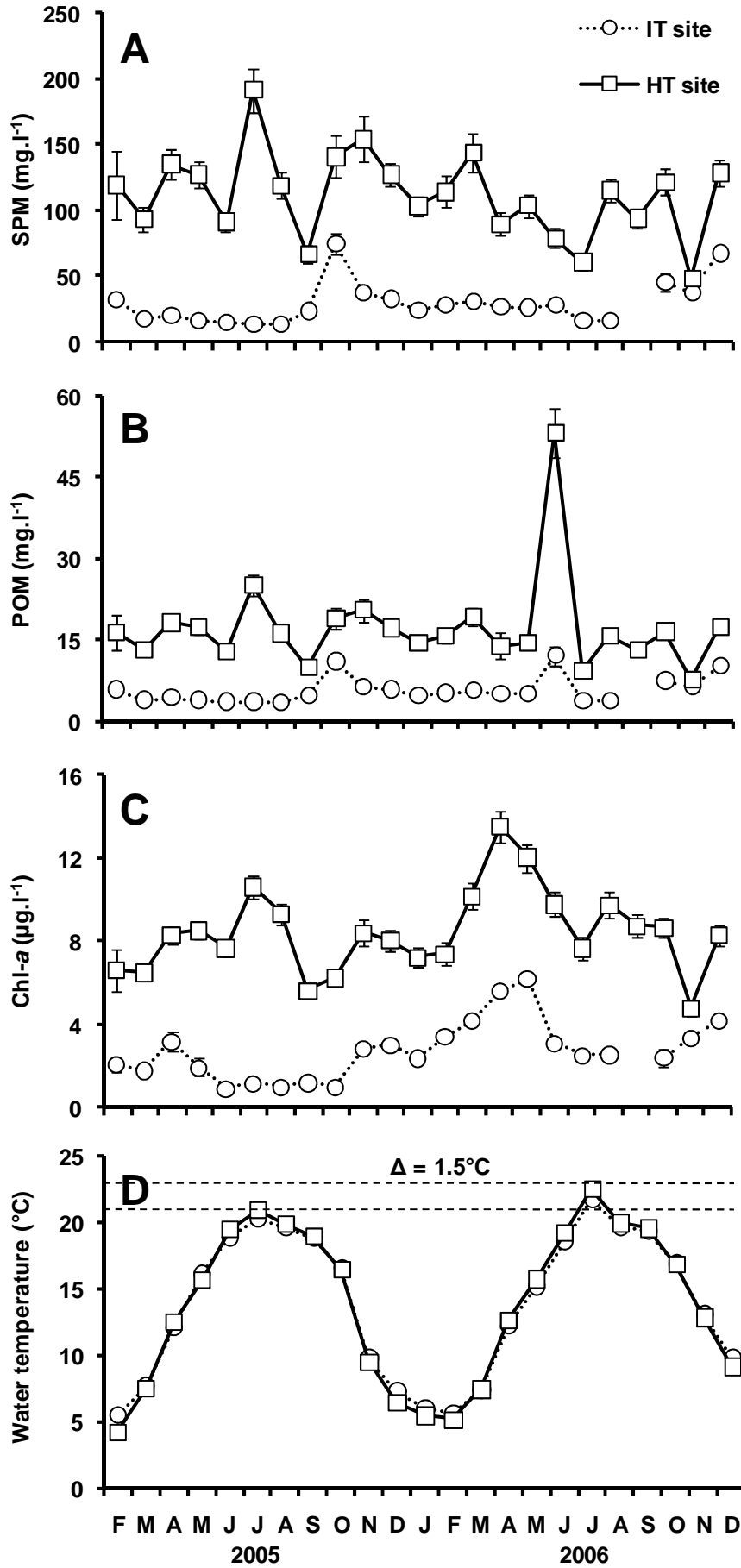
536

537

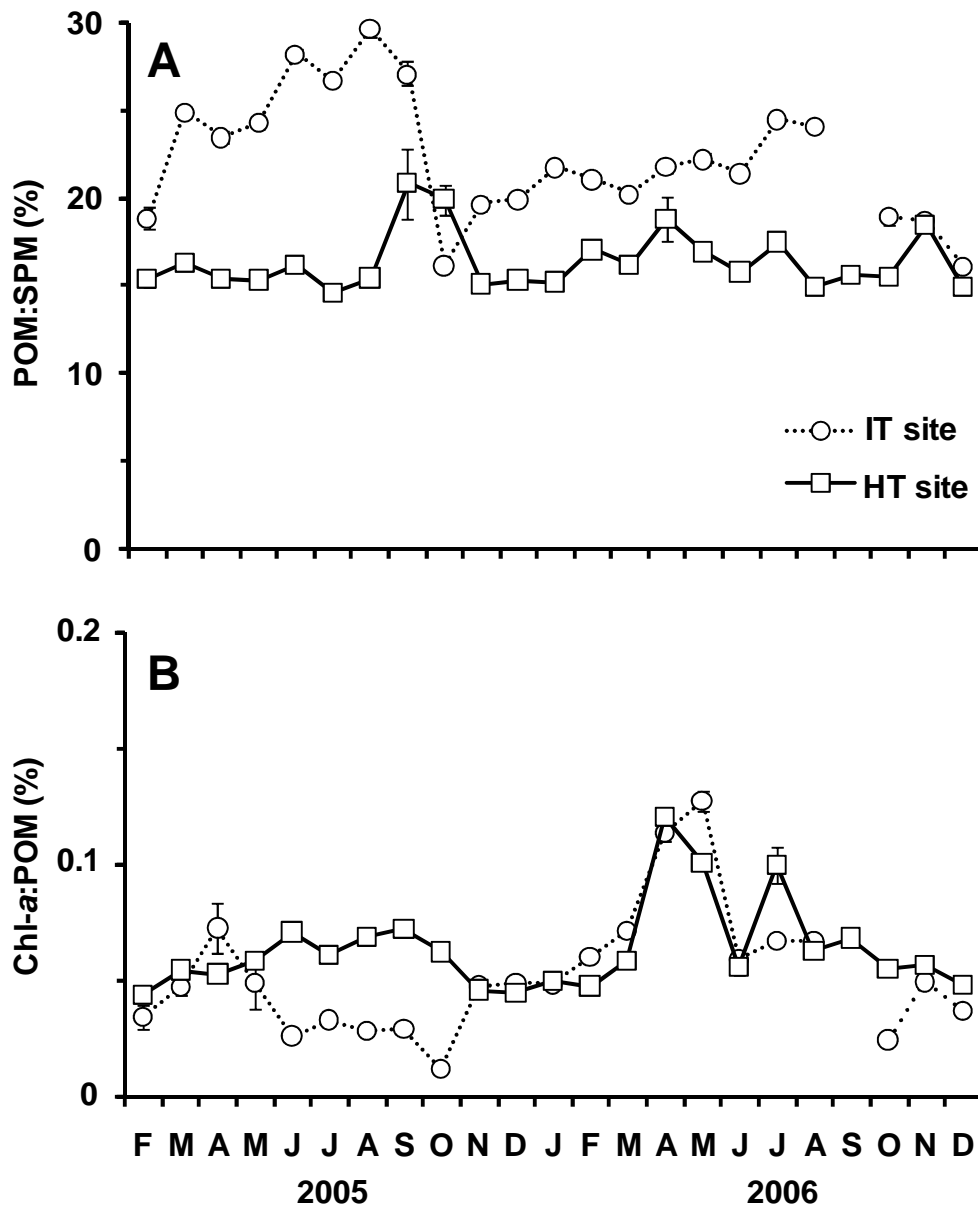


538

539 **Fig. 1.** Location of northern (La Coupelasse) and southern (Gresseloup) oyster
 540 sampling sites (Δ) in Bourgneuf Bay. The intertidal zone and rocks are represented
 541 by gray and black areas respectively (modified from Barillé et al., 2000).

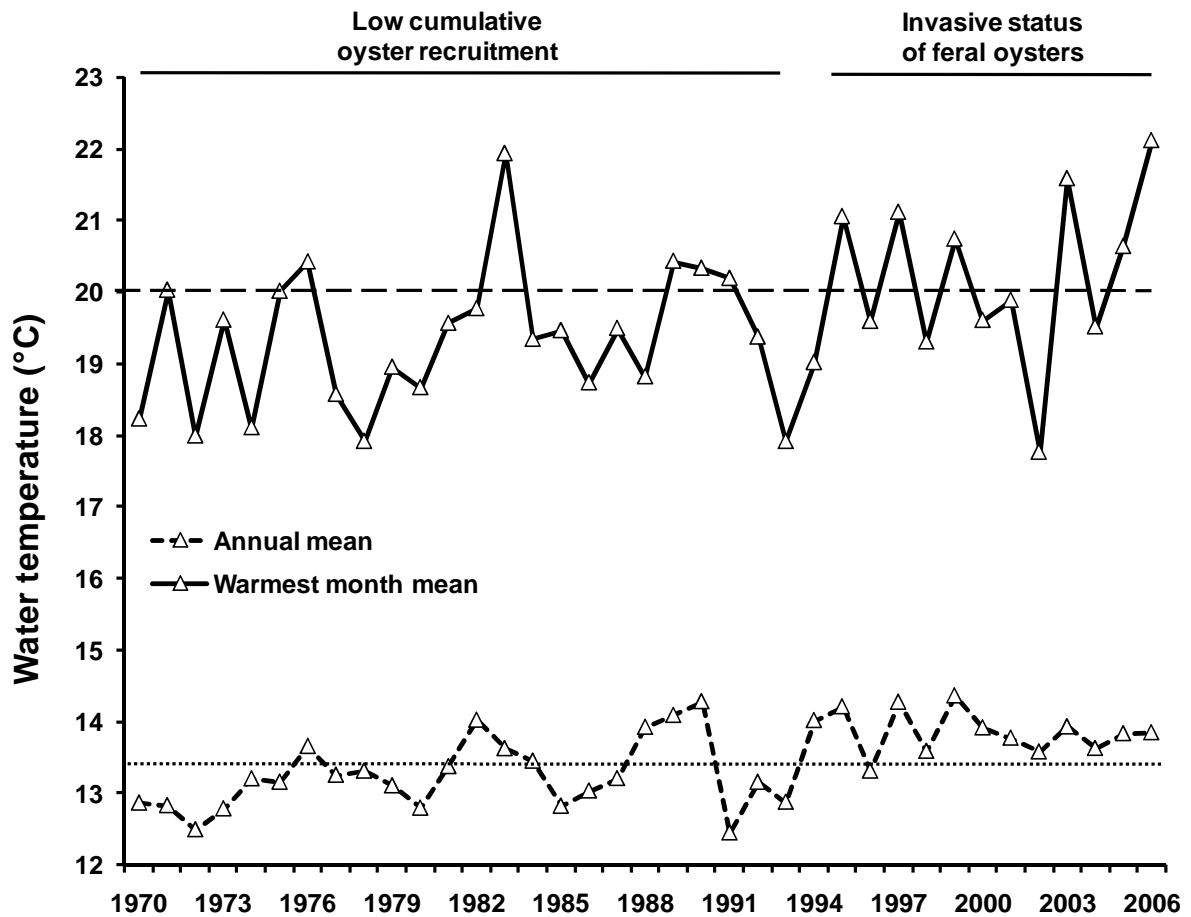


543 **Fig. 2.** Variations of suspended particulate matter (SPM, A), particulate organic
544 matter (POM, B) and chlorophyll-*a* (chl-*a*, C) concentrations, and water temperature
545 (D) at the northern high turbidity (HT) and southern intermediate turbidity (IT) sites of
546 Bourgneuf Bay, in 2005 and 2006. Means \pm 95 % confidence intervals.



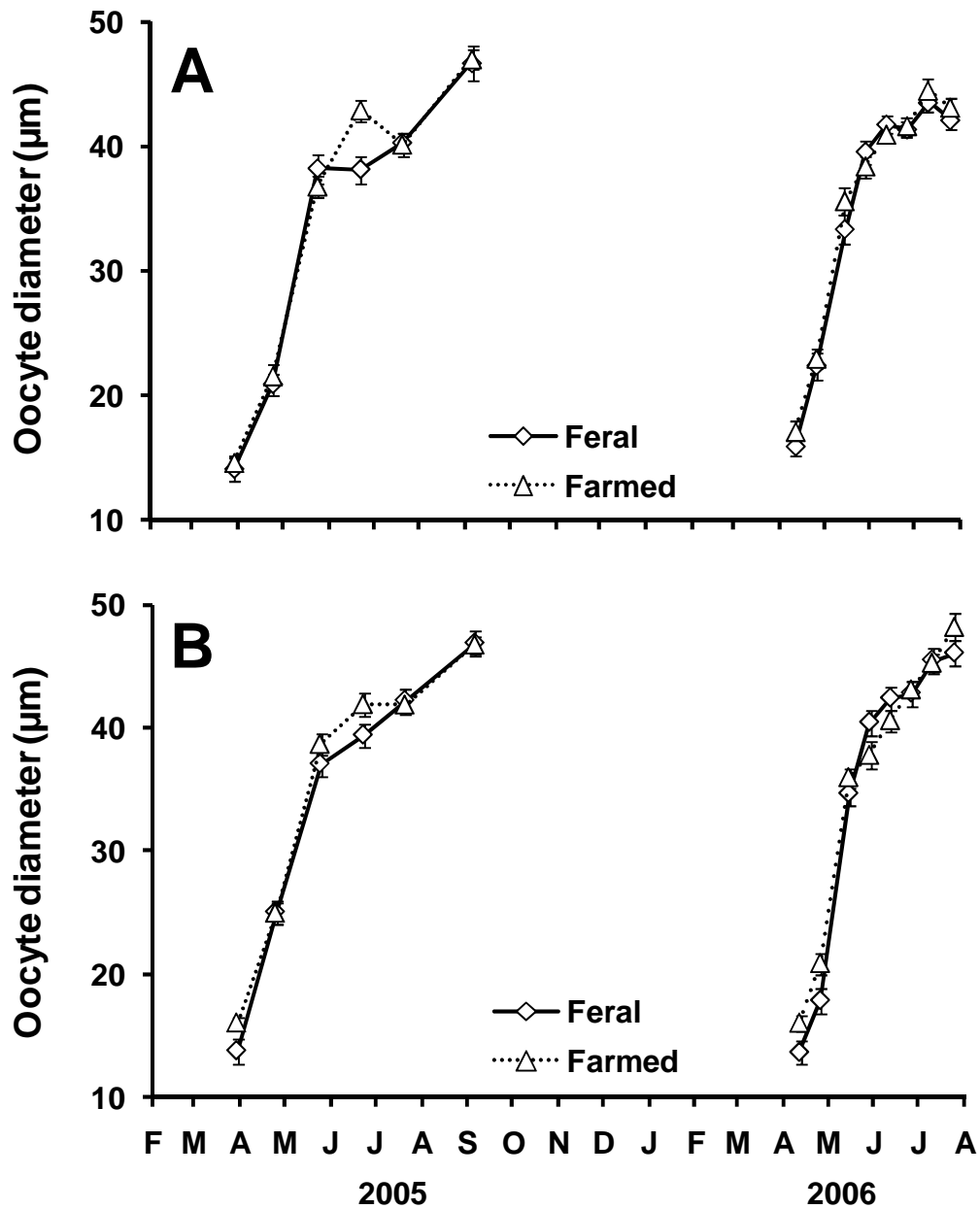
547

548 **Fig. 3.** Dilution (POM:SPM, A) and quality (chl-a:POM, B) of organic particles at
 549 northern high turbidity (HT) and southern intermediate turbidity (IT) sites of
 550 Bourgneuf Bay in 2005 and 2006. Chl-a: chlorophyll-a, POM: particulate organic
 551 matter, SPM: suspended particulate matter. Means \pm 95 % confidence intervals.



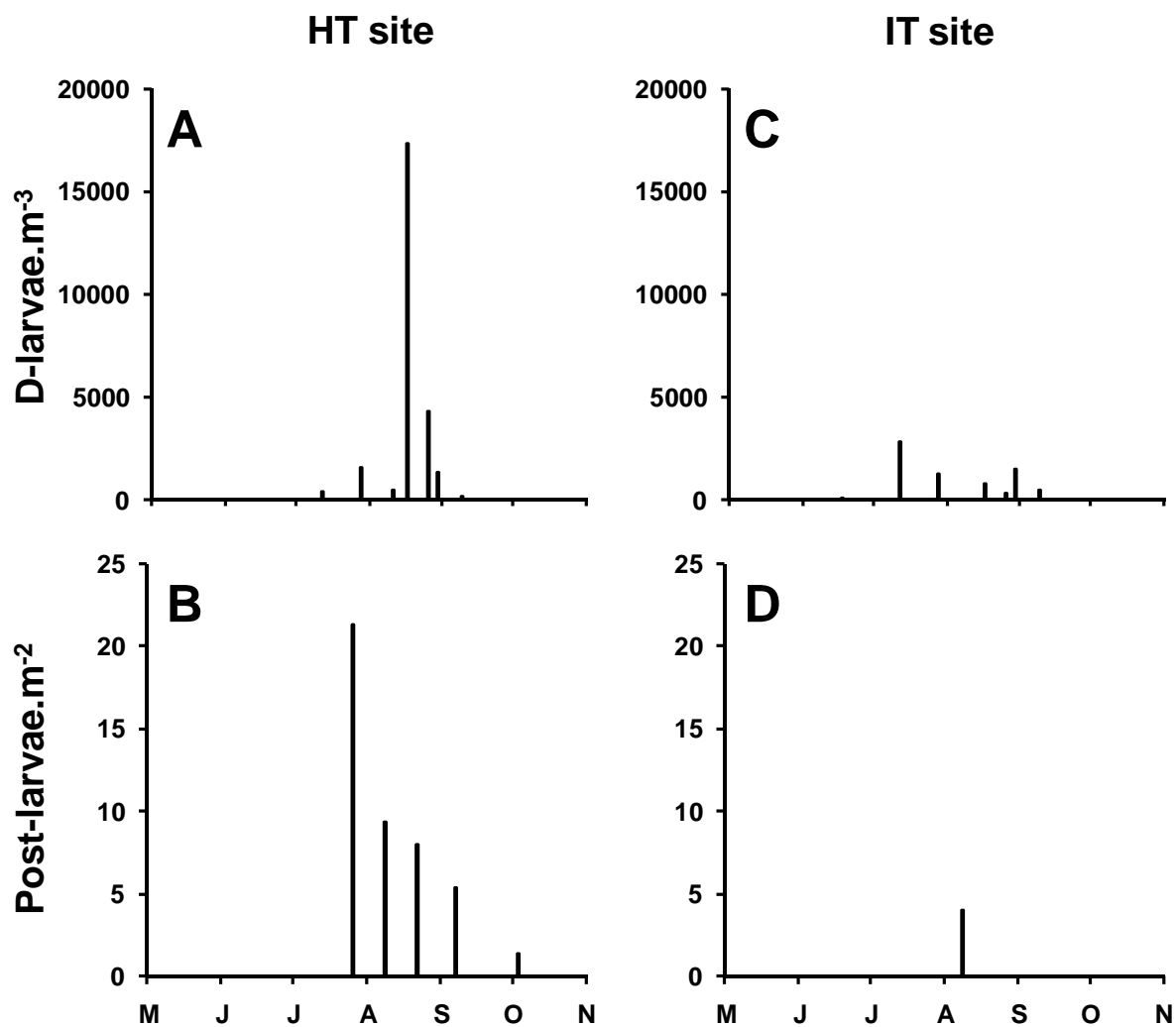
552

553 **Fig. 4.** Annual mean and warmest month mean of water temperature since the
 554 introduction of *Crassostrea gigas* in the 1970's (Météo-France, Climathèque
 555 database, Noirmoutier, 2007), and relationship with the natural recruitment of feral
 556 oysters in Bourgneuf Bay (Gouletquer, 1995; Cognie et al., 2004; 2006; Martin et al.,
 557 2004; 2005). Dotted line corresponds to the median temperature (13.4°C) of annual
 558 mean water temperature, dashed line corresponds to the minimal threshold (20°C)
 559 for optimal *C. gigas* larval development.



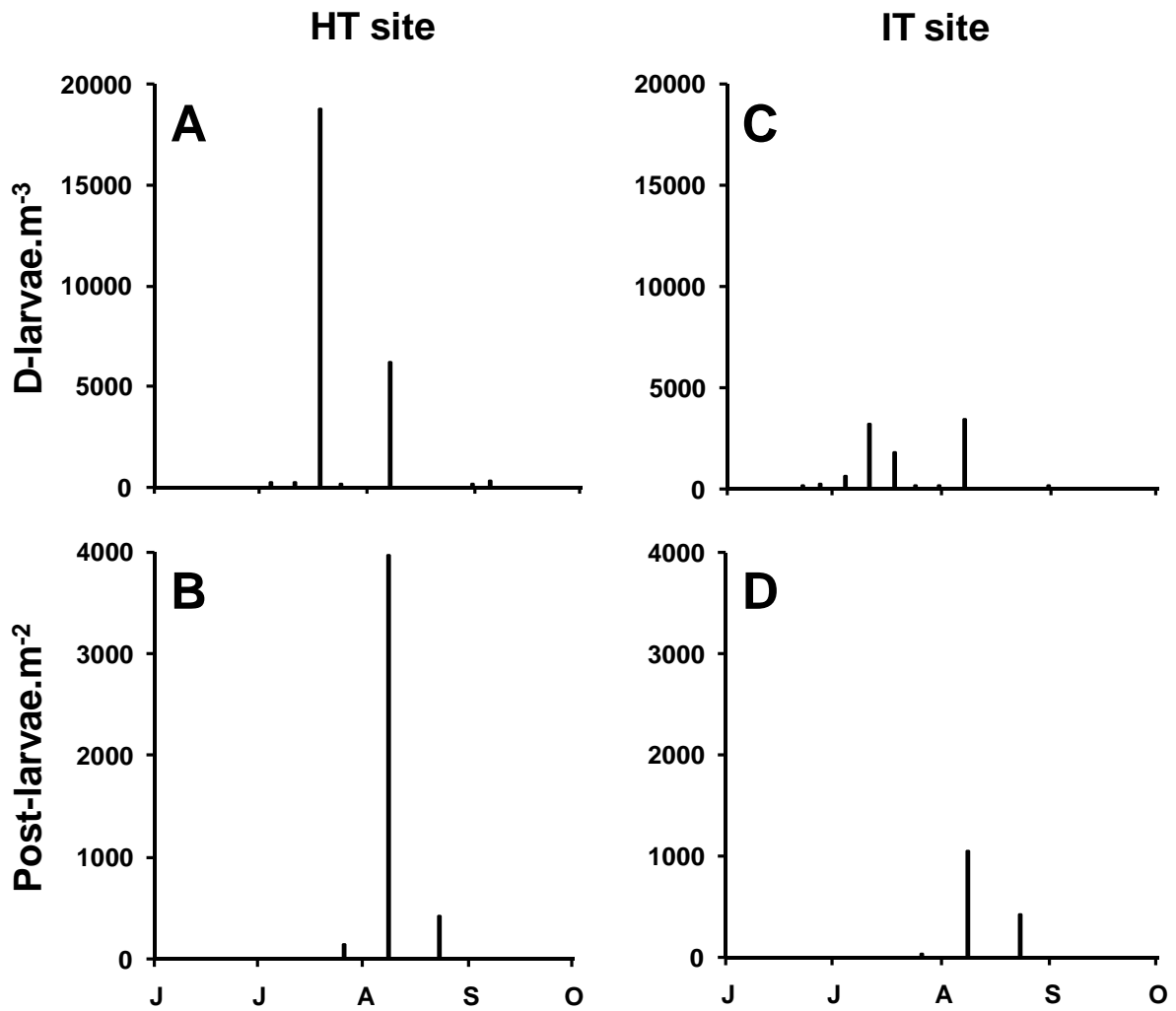
560

561 **Fig. 5.** Mean oocyte diameters (\pm 95% IC) for feral and farmed oysters, *Crassostrea*
 562 *gigas*, at northern high turbidity (HT, A) and southern intermediate turbidity (IT, B)
 563 sites of Bourgneuf Bay in 2005 and 2006.



564

565 **Fig. 6.** D-larva (A, C) and post-larval (B, D) densities at the northern high turbidity
 566 (HT) and southern intermediate turbidity (IT) sites of Bourgneuf Bay for the year
 567 2005.



568

569 **Fig. 7.** D-larva (A, C) and post-larval (B, D) densities at the northern high turbidity
 570 (HT) and southern intermediate turbidity (IT) sites of Bourgneuf Bay for the year
 571 2006.

572