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Gametogenesis, reproductive investment, and spawning behavior of the Pacific giant oyster *Crassostrea gigas*: evidence of an environment-dependent strategy

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Abstract:

The progress of gametogenesis was studied in oysters *Crassostrea gigas* having the same origin (Tremblade), but cultured during 1 year in two distinctive French marine areas, the Baie des Veys and Marennes-Oléron. We assessed seasonal changes in the reproduction cycle on the basis of stereological techniques to estimate reproductive investment and measurement of gonad evolution area by quantitative histology. From a qualitative point of view, both oyster groups presented typical reproductive stages, but showed differential timing, in particular during the sequence of spawning and duration of the re-absorption stage. Oysters in Baie des Veys had a single partial spawning in August and a re-absorption stage that extended until winter. Oysters in Merennes-Oléron had a partial spawning in July and massive release of gametes during August. Spawnings in both the areas were related to maximum temperature (19°C). The quantitative analysis showed, on an annual basis, a higher reproductive investment by oysters from Baie des Veys, 86% against 53% in the other group. Larger gonads, higher gamete production, and more intensive spawning were the characteristics of oysters in Baie des Veys. Recently, the reproduction pattern and investment has been related with summer mortalities; therefore, a quantitative understanding of reproductive processes becomes necessary for *C. gigas*. Environmental conditions at each site may explain differences in the progress and intensity of gametogenesis. While temperature regulated the time and speed of gametogenesis, results suggest that the intensity was influenced by the quantity of available food but may need further research. However, nutrient recycling from unreleased gametes in the gonads of oysters from Baie des Vey is a factor to be considered in the results of this study.

Keywords: *Crassostrea gigas* - Gametogenesis - Image analysis - Pacific oysters - Reproductive investment - Stereology

1. Introduction

The Pacific oyster *Crassostrea gigas* (Thunberg 1793) is the most important commercial oyster species cultivated in the world (FAO 2003). This species was introduced into France in 1969 after the mass mortality that decimated the native and farmed species, *Crassostrea angulata* (Deslous-Paoli 1982; Grizel and Héral 1991). The production of the Pacific oyster, more than 128 metric tons in 2003, has become a major industry in French aquaculture. Among the different production areas in France, the Baie des Veys in Normandy (Kopp et al. 1997) and Marennes-Oléron in Charente-Maritime (Soletchnik et al. 1998) are the most important. Summer mortality, affecting juveniles and adults of diploid and triploid variants, is one of the major problems affecting production of *C. gigas* since the early 1980s (Dégremont et al. 2005). The cause of die-offs is not well known, but complex interactions between environment, oysters, and pathogens are indicated (Cheney et al. 2000). It has been suggested that die-offs of *C. gigas* result from biotic and abiotic synergic processes that are related to critical stages, especially those linked to reproduction processes; end of maturity, spawning, and gamete re-absorption (Goulletquer et al. 1998). It seems that during these phases, there are physiological and/or metabolic disturbances associated with the maturation of gonads (Maurer et al. 1986). Therefore, a profound understanding of reproductive processes is needed for *C. gigas*.

Reproduction is one of the most important physiological processes in the life cycle of any bivalve species. The annual reproductive cycle of *C. gigas* has been widely studied at different locations worldwide (Yakovlev 1977; Mann 1979; Dinamani 1987; Barber 1996; Lango-Reynoso et al. 2006; Chávez-Villalba et al. 2007). In France, the Pacific oyster exhibits a seasonal reproductive cycle with (1) initiation of gametogenesis in winter; (2) active phase of gametogenesis (growing stage) in spring; (3) maturity and spawning in summer; (4) and a re-absorption period in autumn (degenerating stage) (Fabioux et al. 2005). This pattern has been described with the aid of a variety of techniques that range from dissection and biometry to accurate histological description, image analysis, and biochemical composition. All these methods provide either qualitative or quantitative information. The qualitative methods are generally based on developmental stage criteria used for macroscopic (Mason 1958) or microscopic observations (Quayle 1969; Yakovlev 1977), which often result in a subjective classification. In an effort to overcome subjectivity of these former methods and to avoid information losses, several quantitative methods have been proposed; assessment of oocyte number and size (Kennedy and Battle 1964; Newell et al. 1982; Murankana and Lannan 1984; Lango-Reynoso et al. 2000) and measurement of the gonad areas (Brousseau 1978; Lannan et al. 1980; Barber and Blake 1983; Heffernan and Walker 1989). However, quantitative approaches, such as gonad development intensity and energy cost of gametes maintenance are scarce (Ruiz et al. 1992). Barillé et al. (1997) determined the scope for growth of *C. gigas*, indicating that the model they proposed identified storage and gametogenesis periods and predicted spawning intensity. New methods are in progress, such as NMR (nuclear magnetic resonance) and imaging (MRI), which represent promising tools to achieve a deeper understanding of the physiology of oysters (Pouvreau et al. 2006). However, deterministic relationships between environment and reproduction processes are still poorly known. As a consequence, the cost of reproduction in bivalves remains virtually unknown (Honkoop 2003).

This study presents information about the reproductive investment, determined by a quantitative approach, of giant Pacific oysters *Crassostrea gigas* having the same origin, but cultivated in different zones. Gametogenesis development was followed and related to hydrological conditions in Baie des Veys and Marenne-Oléron. These zones exhibited strong differences in the trophic conditions between them. Observations on sex change and on summer mortality are discussed.

2. Materials and methods

2.1. Study sites, sampling, and biometry

This study was performed on individuals from a single population; spat were produced in an IFREMER shellfish hatchery (La Tremblade, France) in February 2001 and reared for one year in the IFREMER shellfish nursery (Bouin, France). In February 2002, the oysters were divided into two groups; the first one was set in the eastern part of the Baie des Veys and the second group was placed in the southern part of the Marennes-Oléron (Fig. 1). These two sites are dissimilar due to differences in their hydrobiological conditions. Baie des Veys, situated in the south-eastern part of the English Channel, is an area of high oyster productivity, with growth and fattening rates near the highest observed in France (Ropert 1998). The Marennes-Oléron area located on the Atlantic coast, north of Bordeaux, is used for spat collection and rearing of adults (Soletchnik et al. 1998). This bay is characterized by a limited carrying capacity due to the large biomass (about a thousand tons) present in its waters.

The reproductive cycle of *C. gigas* at both sites was followed for one year, from March 2002 to February 2003, through a systematic sampling scheme. Sampling was performed monthly from December to April, and twice a month from May to November. Each time, ten oysters were collected from each site by IFREMER coastal laboratory personnel: LCPC in Charente-Maritime and LCN in Normandy. A total of 180 oysters from Baie des Veys and 188 oysters from Marennes-Oléron were examined. After field collection, the samples were sent to the laboratory for measurement, dissection, and histological analysis. Environmental parameters as temperature and chlorophyll *a* were recorded at in the IFREMER stations by Daniel (2002) and Razet (pers. comm.) during each sampling period at both sites, but information for Baie des Veys was available only for May through October 2002.

For all specimens, shell length (antero-posterior axis) was measured to the nearest mm prior to dissection. All soft tissue was then removed from the shell, drained for 5 min on absorbent paper and weighted to the nearest g. After flesh dissection, individual visceral mass (digestive gland + gonad) was weighted and prepared for histological analysis.

The gonad of *C. gigas* is fused with digestive gland and mixed with storage tissue. Consequently, reproductive and non-reproductive tissues cannot be properly separated (Heffernan and Walker 1989; Urban and Riascos 2002); therefore, quantitative parameters such as gonado-somatic index and biochemical composition are roughly assessed. This led us to develop a specific quantitative method to estimate gonad development from histological preparations and quantitative image analysis.

2.2. Histological analysis

For each oyster, the length of the visceral mass was measured and then divided into three equal portions. These three subsamples were fixed in Davidson's solution (Shaw and Battle 1957) and dehydrated in an ascending alcohol series for embedding in wax. Sections of 5 µm were cut at representative body levels from each portion; the first one, or anterior section, was performed just behind the labial palps, whereas the next one, or middle section, was cut at the mid-visceral level, and the last one, or posterior section, just before the pericardial cavity (Fig. 2). These tissue samples mainly contained outer epithelial, connective and storage tissues, and gonadal and digestive tissue layers. The three sections were mounted and stained in Harris hematoxylin and counter-stained in eosin Y following the standard method (Martoja and Martoja-Pierson 1967).

2.3. Qualitative analyses

Each slide was first examined under light microscope for sex determination. Presence of sperm, oocytes, or missing gametes defined individuals as male (M), female (F), hermaphrodite (HP), or sexually undifferentiated (U). Each oyster was assigned a stage of gonadal development on the basis of descriptions made by Kennedy and Battle (1964) and Mann (1979), as follows:
Stage 0 (resting stage): There is no trace of sexuality; follicles are non-existent or elongated and consist of undifferentiated germinal epithelium;
Stage I (early growth stage): Follicles are small and isolated with numerous spermatogonia or oogonia;
Stage II (late growth stage): Follicles are actively developing with primary gametes and some free (secondary) oocytes and spermatozoa;
Stage III (maturation stage): Near ripe or ripe follicles are densely packed with maturing gametes; presence of oocytes with distinct nucleus and nucleolus, spermatozoa are oriented with tails toward the follicle lumen; and
Stage IV (spawning and reabsorbing stages): Follicles are distended and some are broken; however, numerous gametes may still remain. In some cases, redevelopment takes place with increased number of primary oocytes and spermatocytes. In other cases, gametes are refractory, re-development is not obvious, and phagocytes are present.

2.4. Quantitative analyses

Percentages of gonad and digestive gland areas (GA and DGA, respectively) were determined on each histological section. Slides were digitally scanned (HP Scanjet 7400c). Scanned images were saved and stored (TIFF format). Tissue areas were measured by using image analysis software (Imaq Vision Builder, National Instruments).
The gonadal biomass (GB) was determined by extrapolation (stereology principle described by Versterby 1993) as follows: $GB = (W_{vm} \times GA)/100$, where GB is the gonadal biomass in g (wet weight); GA is the mean percentage of gonadal area (in % of the mean of the three sections for each individual), and W_{vm} is the visceral mass (gonad + digestive gland) in g (wet weight).
Total soft tissue production (STp, in g) and gonad production (Gp, in g) were calculated on a temporal basis (between t and t-1), as follows: $STp = W(t) – W(t-1)$ and $Gp = GB(t) – GB(t-1)$. The reproductive investment (RI, %), *i.e.* the part of production allocated to gonad, was then calculated as follows: $RI = Gp/STp$.
Spawning intensity was determined by analyzing samples just before (ts-1) and after the spawning event (ts). Gamete spawning (Gsp, g) was estimated from gonad wet weight losses, as follows: $Gsp = GB(ts) – GB(ts-1)$. The comparison between Gp and Gsp allowed us to describe the spawning event as being partial or complete.

2.5. Statistical analyses

Sex ratios were tested with Wilks' G^2 test and Z test. The percentage of individuals in the five developmental stages throughout the annual cycle was also tested with Wilks' G^2 test among the sites on each sampling data. Finally, the gonadal biomass values were analyzed with the Mann-Whitney test. Statistical significance was set at $P = 0.05$.

3. Results

Temperature at the study sites was higher at Marennes-Oléron than in Baie des Veys throughout the study period (Fig. 3). A peak of 18 °C was detected in mid-July in Marennes-Oléron; at Baie des Veys, the temperature at this same time was around 14 °C. Nevertheless, the maximum value in both areas was 19 °C, but two weeks later in

September at Baie des Veys. After the peak, a similar decreasing temperature curve occurred until November. Chlorophyll records showed different patterns in the two areas (Fig. 3). In Marennes-Oléron, low concentrations occurred in winter; there were two peaks, one in July and one in August. In Baie des Veys, low concentrations occurred in May and September; a single peak occurred in July. The maximum value for Marennes-Oléron (13 µg/L) was detected in August, but this record was about the half of that detected in Baie des Veys (25 µg/L).

3.1. Somatic growth

The weight of oysters of Baie des Veys and Marennes-Oléron rose during the year, increasing from 13 g (March 2002) to approximately 43 g and 70 g (February 2003), respectively (Fig. 4). Growth (weight) was significantly different from July 2002 to the end of the study, with higher values in oysters from Baie des Veys. The rate of increase in weight was ~4.7 g.month^{-1} or 57.0 g. y^{-1} in this bay against 2.5 g month^{-1} or 30.0 g y^{-1} in Marennes-Oléron. Flesh wet weight was always higher in oysters from Baie des Veys than oysters from Marennes-Oléron throughout the year, with the highest values in summer (13.6 g), but also in autumn (11.5 g) (Fig. 4). In contrast, the flesh wet weight in specimens of Marennes-Oléron remained relative constant during the year (4.7 g). The differences between the two groups were significant from March 2002 to February 2003. Shell growth pattern was similar in both areas, with more mass in oysters from Baie des Veys; significant differences were present from July to September and in November. Oysters from Baie des Veys reached an average shell length of 83 mm and those from Marennes-Oléron reached 68.3 mm by the end of the study period (Fig. 4).

3.2. Sex ratio

At Baie des Veys, 55 of the 180 oysters (31%), were sexually undifferentiated, 46 (25%) were female, and 78 (43%) were male. The general sex ratio was 1 female:1.69 male. At Marennes-Oléron, 80 of the 188 oysters (43%) were sexually undifferentiated, 48 (25%) were female, and 59 (31%) were male. The general sex ratio was ~1 female: 1.22 male. Difference in sex ratios between the two bays was not significant on an annual basis ($p = 0.116$, $z = 1.198$, Z-test).
Sex proportions were different at each site (Fig. 5). Oysters in Marennes-Oléron had more females during the reproduction period, but with not present after September 2002. At Baie des Veys, females were present from March to December 2002. Differences between both sites were significant for the female population, especially at the end of the summer and in autumn ($p = 0.009$, Wilks' G^2 test). Males were present throughout the study period at both sites, but there was a higher proportion of males from April to October at Baie des Veys and during two peaks occurring in April and July at Marennes-Oléron. Undifferentiated oysters were more abundant at Marennes-Oléron and were present for longer periods compared to Baie des Veys. Significant differences were noted in early spring and summer ($p = 0.001$, Wilks' G^2 test). Undifferentiated animals predominated during the winter period at both sites. Incidence of hermaphrodites was generally low (<1%). Only one hermaphrodite was found at each site in spring during the seasonal sex change period. Based on sex determination, it appeared that the gametogenic cycle began earlier at Marennes-Oléron. At this site, most of the oysters were sexually differentiated during spring, while most oysters at Baie des Veys were still undifferentiated (Fig. 5).

3.3. Gametogenic cycle: qualitative approach

Gametogenic activity was evident most of the year (especially for male individuals) with a well-defined seasonal reproductive cycle at both sites. Analysis of histological changes in gonads showed the five typical stages in the gametogenic cycle (Fig. 6). At Baie des Veys, 80% were undifferentiated in March (stage 0). Stages I and II occurred from March to July

and, in July and August, the population reached stage III. Individuals in spawning condition (stage IV) began to appear in August and were observed until late November. Ripe and late spawning oysters were occasionally observed after this time. Re-absorption and resting stages, characterized by unspawned gametes, were found in the gonads throughout winter (Fig. 6).
At Marennes-Oléron, there was very low activity in early spring and gonads showed only well-developed inter-follicular storage tissue (stage 0). Stages I and II were mainly observed in May when the gonad tissue contained numerous spermatocytes and oocytes. The maturation stage, characterized by maximum development of acini (stage III), lasted until early August. The spawning stage began partly present in early July and lasted until August, when massive and synchronized spawning occurred. Re-absorption of gametes took place from September to November, and after that, oysters re-initiated gametogenesis, but the sexes were indistinguishable (Fig. 6). Comparing reproductive cycles, oysters from Marennes-Oléron exhibited low gametogenic activity compared to specimens from Baie des Veys. Higher proportions of all gametic categories were found in Baie des Veys oysters. This was most evident during spawning and reabsorbing stages in the northern oysters, which extended from summer until the end of the study (Fig. 6).

3.4. Gametogenic cycle: quantitative approach

Gonad area (GA, %) and digestive gland area (DGA, %) had similar general patterns during the study period at both sites (Fig. 7). At Marennes-Oléron, GA started to increase in April 2002, and a month earlier at Baie des Veys. GA increased faster in May and June (~30 to 70%), but with significant temporal differences between the two sites. At Marennes-Oléron, GA accounted for about 74-75% of the visceral mass at the beginning of July, while the Baie des Veys specimens reached the same maximum value more than a month later. In July, a reduction of ~10% at Marennes-Oléron, possibly from a partial and unsynchronized gametes discharge (early July). The decrease in GA (early July) did not occur at Baie des Veys. At the beginning of August, a second and very rapid decrease in GA was observed at Marennes-Oléron that was the result of a massive spawning. This rapid decrease in GA was delayed by two weeks at Baie des Veys and was not as intensive. Significant differences ($p < 0.035$, Mann-Whitney test) in GA between oysters from both sites (2% at Marennes-Oléron and 30% at Baie des Veys) were apparent in September, indicating massive spawning at Marennes-Oléron and partial spawning at Baie des Veys. Differences of GA between the sites were recorded until autumn, when there was a slow decline of GA in oysters from Baie des Veys, reaching a minimum (~2%) in February. A few ripe oysters (GA > 10%) were still found in December. GA in oysters from Marennes-Oléron reached a minimum (GA < 1%) in September and remained low through February (Fig. 7).
The digestive gland area (DGA, %) had seasonal development, with significant differences ($p < 0.021$, Mann-Whitney test) between the two oysters groups (Fig. 7). During spring, the area decreased in oysters from both sites to approximately 5% at the end of summer. In autumn, DGA rose in both oysters groups to 30% in the Marennes-Oléron oysters and 20% in the Baie des Veys oysters. Differences between both sites were significant. In winter, both groups had a slight increase of the DGA, from 23 to 27% in the Marennes-Oléron group and 13 to 16% in Baie des Veys group (Fig. 7).
There were striking differences in the evolution of the estimated gonad biomass (GB) between the two populations (Fig. 8). GB was similar in oysters from both sites from March to June; but in July, the GB Marennes-Oléron oysters stopped increasing and remained at ~1.9 ± 0.7 g until the main spawning event in August (a small, partial spawning occurred in early July). In contrast, GB continued to increase in Baie des Veys oysters, reaching ~6.1 ± 1.5 g in July just before the spawning event in August. Differences in GB between both sites were statistically significant ($p < 0.025$, Mann-Whitney test) from July to December. In the case of the digestive gland biomass (DGB), a similar seasonal pattern occurred in samples from both sites (Fig. 8). In both groups, DGB increased from March until May, but decreased in summer from 0.73 to 0.47 g at Baie des Veys and from 0.47 to 0.26 g at Marennes-Oléron.

DGB was greater at Baie des Veys, with significant differences ($p < 0.01$, Mann-Whitney test) in 11 of the 17 samples.
These data led to estimates of gamete release (Gsp), expressed in g. At Marennes-Oléron during the main spawning event in August, Gsp mean value was ~1.8 g and at Baie des Veys it was ~4.5 g. By September, the gonads of oysters from Marennes-Oléron were completely empty, whereas approximately 28% of gametes remained in the gonads of oysters from Baie des Veys. This pattern suggests that spawning at Baie des Veys was not complete not synchronized. We calculated the reproductive investment (RI, %) on an annual basis, finding that RI was higher in oysters from Baie Des Veys (86%) than in oysters from Marennes-Oléron (53%).

4. Discussion

The pattern of gametogenic development of *Crassostrea gigas* in oysters from both areas were similar to those reported by Berthelin et al. (2000). The reproductive cycle showed a clearly defined seasonal pattern, including five distinctive stages. Nevertheless, qualitative data from histological tissue examination showed that the gametogenic cycle started sooner and was faster in oysters from Marennes-Oléron, especially for the growing, maturing, and spawning stages. Previous studies had detected differences in the initiation or completion of gametogenesis between oysters (*C. gigas*) from northern and southern sites (Chavez-Villalba et al. 2003). However, in that investigation, oysters from northern sites (Baie des Veys and Aber Benoît) initiated gonadal growth, achieved maximal gonadal development, and began spawning about one month earlier than oysters from Marennes-Oléron. This is an opposite behavior to the pattern recorded in this study. This situation seems to be related to higher temperatures at Marennes-Oléron during spring, with a peak of 18.5 °C in mid-June that produced the partial spawning. It is known that gamete maturation and spawning in *C. gigas* is a function of temperature (Muranaka and Lannan 1984). We effectively detected that the main spawning observed at Baie des Veys and the second but massive at Marennes-Oléron were associated with maximum temperatures (19.2 °C at Baie des Veys and 19.4 °C at Marennes-Oléron). However, individuals in spawning condition were observed until late November in Baie des Veys.
The quantitative data shows strong differences in gametogenesis between the groups in terms of gamete production. For reproductive effort, defined as gamete production over total production for a defined period, oysters from Baie des Veys dedicated more than 86% of their growth to gametes compared to 53% in oysters from Marennes-Oléron. These observations confirm previous findings of higher proportions of mature oocytes in oysters from Baie des Veys, conditioned in winter, spring, and early summer, than in oysters coming from Marennes-Oléron and four other sites along the Atlantic coast of France (Chavez-Villalba et al. 2003). These authors claimed that better performances relied on the availability of stored reserves, suggesting that Baie des Veys oysters are located in zones with accessibility to better food that favored accumulation of nutrients. This could account for the results of this study for the reproduction period. The level of chlorophyll *a* (phytoplanktonic bloom) was significantly higher during June-July at Baie des Veys (25 $\mu g\ l^{-1}$) compared to the level (13 $\mu g\ l^{-1}$) detected at Marennes-Oléron. Although chlorophyll *a* may not always fully represent food availability for oysters, the difference between sites is presumably in the same order for other components of suspended organic matter considered potential food for oysters (protozoa, bacteria, zooplankton, and detritus). We think that the differences in the reproductive cycles were associated with differences in availability of food, as suggested by other authors (Maurer and Borel 1986; Ruiz et al. 1992; Chávez-Villalba et al. 2003).
Perdue et al. (1981) found that the area represented by the gonad could vary from 0 and 65% of the visceral mass. We found similar results in specimens from both sites, reaching ~69% in Baie des Veys specimens during the maximum reproductive period in August. Gonads in specimens from Marennes-Oléron were larger in June (67%) than in August (55%), before the massive spawning. These variations in the extent of proliferation of the

gonad seem to be typical of marine bivalves where the gonad is diffused through the visceral mass tissue, and undergoes seasonal changes (Morvan and Ansell 1988). Seasonal variation is related to the number of oocytes produced by an individual per spawning. Chávez-Villalba et al. (2003) found that the average number of oocytes produced by *C. gigas* at different times was always significantly higher in northern locations (ranging from 4×10^6 in winter and 50×10^6 in summer), compared to quantities in southern groups (2×10^6 in winter to 25×10^6 in summer). Even though we calculated gamete production from biomass assessment, our results agree with previous observations. On average, oysters in Baie des Veys produced more gametes than those from Marennes-Oléron (6.1 versus 1.9 g). The spawning intensity also differed; oysters from Baie des Veys displayed partial and/or asynchronous gametes losses (~4.5 g) in contrast to one massive and synchronous spawning (~1.8 g) by the oysters from Marennes-Oléron. Differences in spawning behavior occurred during the re-absorption period, which was comparatively longer and in higher proportions in oysters from Baie des Veys. This pattern seems to occur in populations located in the north of France. For example, at Aber Benoît in Brittany, oysters have an incomplete spawning and residual oocytes are slowly re-absorbed from September to January (Chávez-Villalba et al. 2001). Gamete recycling has been suggested as a source of nutrients for gametogenesis in the Pacific oyster (Lango-Reynoso et al. 2006). Reserves in *C. gigas* are constituted during the autumn and the winter and these reserves are used later in gametogenesis (Berthelin et al. 2000). This could indicate that in Baie des Veys oysters, apart from the ambient food, there are nutrients coming from the re-absorption of oocytes that were used later for gametogenesis. It is possible that the additional energy provided by recycling is directed to build more gametes in the oysters at Baie des Veys than those at Marennes-Oléron.

Females were present nearly all year at Baie des Veys whereas females at Marennes-Oléron were present from March through September. The proportion of females on a seasonal or monthly basis seems to be related to metabolic processes. Females are more metabolically active than males and require more nutrients to mature. Since more energy was available for gametogenesis in Baie des Veys oysters, this could explain difference in timing of females between the two sites. Still, the relationship of females to phytoplankton blooms was evident in Marennes-Oléron, as was reported by Lango-Reynoso et al. (2006) for this area. They found that hermaphrodites are most common in spring because they represent an alteration from male to female. Hermaphrodites were also detected in spring at both sites but represented <1% and were considered isolated cases, as reported in other studies (Paniagua-Chávez and Acosta-Ruiz 1995; Steele and Mulcahy 1999).

Dégremont et al. (2005) illustrated the difficulty of predicting the timing of mortality events in three oyster-raising sites in France. One of the objectives of this study was to describe the relationship of reproduction to die-offs. However, no die-off occurred were detected; mortality was within the normal range: 11% at Baie des Veys and 7% at Marennes-Oléron. For many authors, summer die-offs originate in the expensive effort required for the reproduction process. Indeed, over this study period, metabolic demands for reproduction, growth, and maintenance are at their maximum, and consequently, many oysters may lack the energetic reserves needed to defend themselves against pathogenic or environmental disturbances (Goulletquer et al. 1998; Myrand et al. 2000; Berthelin et al. 2000). Recently, Delaporte et al. (2006) demonstrated that hemocyte functions in *C. gigas* may be perturbed by high reproductive effort. However, very little is known about the physiological cost of reproduction in bivalves (Honkoop 2003) or the trade-off between reproduction and mortality. Our observations permitted detection of significant differences in terms of reproductive investment between oysters having the same site of origin, but cultivated in areas with different trophic conditions. The methods used in this study could be useful for providing relevant information about the relationship of reproduction and mortality events in future studies.

In conclusion, we demonstrated that specific stock of oysters placed in two different environments at the start of a new gametogenic cycle (early spring) developed very different reproductive strategies. Gametogenesis was strongly affected by environmental conditions

with water temperature regulating its time and speed and the quantity of available food influenced its intensity. Gamete recycling appeared to be an additional source of nutrients in oysters at the northern site, which displayed a higher reproductive investment than oysters at the southern site. Previous observations lead to the conclusion that *C. gigas* has high phenotypic reproductive plasticity. The environment-dependent strategy is presumably an adaptation mechanism to succeed in different habitats.

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Figures

Fig. 1

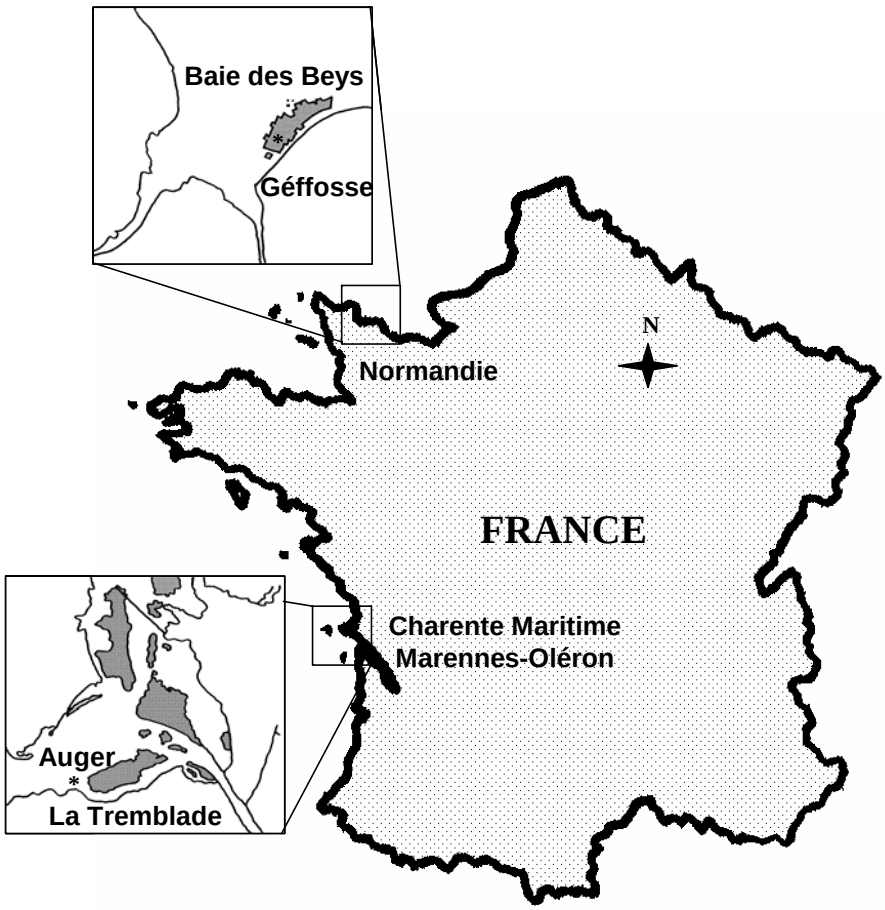

Fig. 1 Location of the field sites at Baie des Veys and Marennes-Oléron, France

Fig. 2

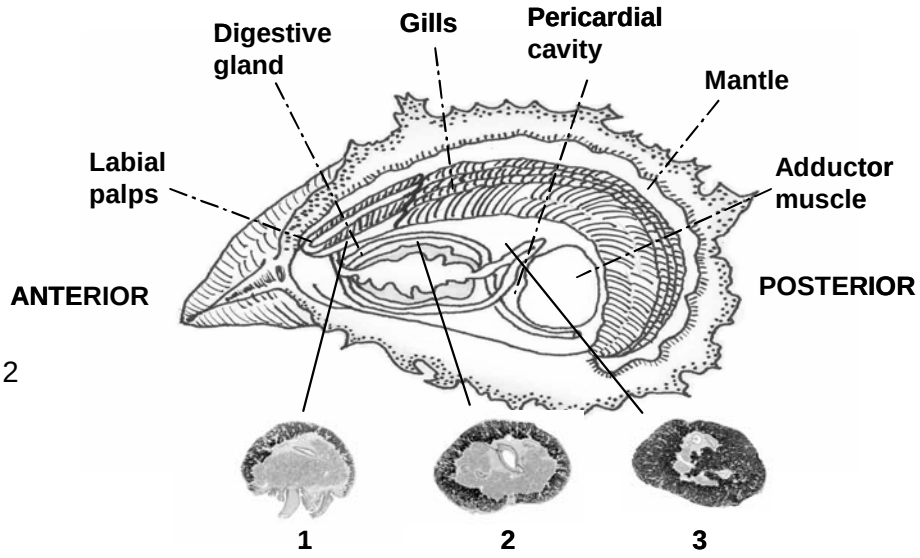

Fig. 2 Drawing of *Crassostrea gigas* (adapted from Barillé et al., 1997), showing the three levels in which the cuts were done for image analysis. (1) Anterior section; (2) Middle section; and (3) Posterior section. Directional body axes based on the comparative anatomy approach proposed by Stasek (1963)

Fig. 3

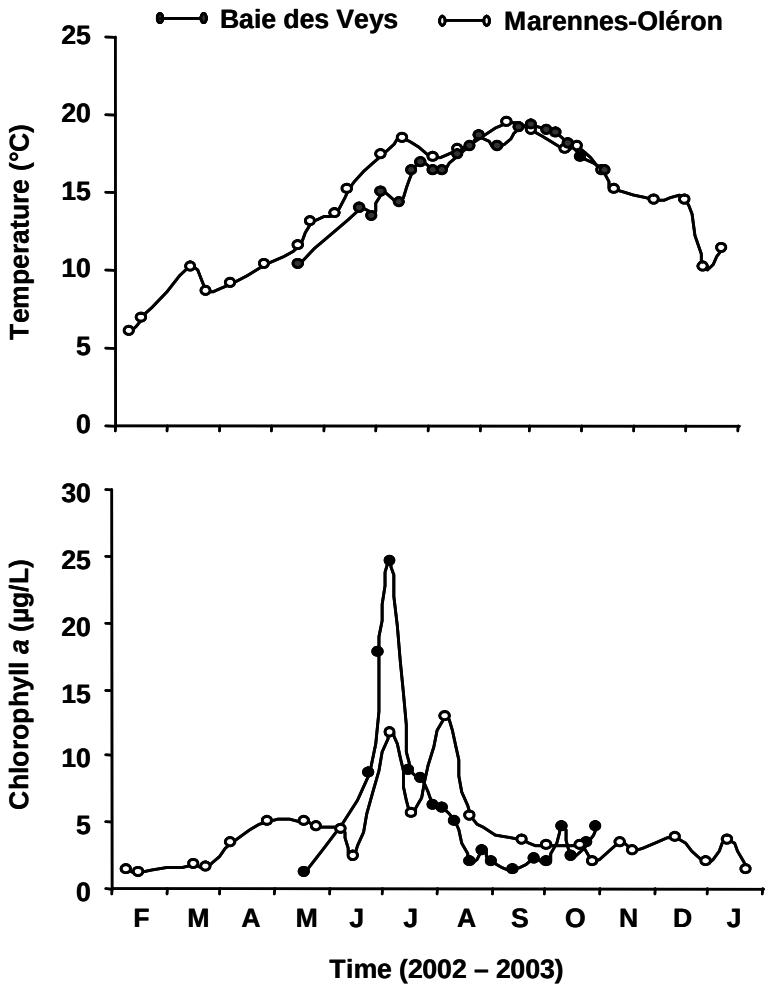

Fig. 3 Temperature and chlorophyll *a* records at the study sites of Baie des Veys and Marennes-Oléron

Fig. 4

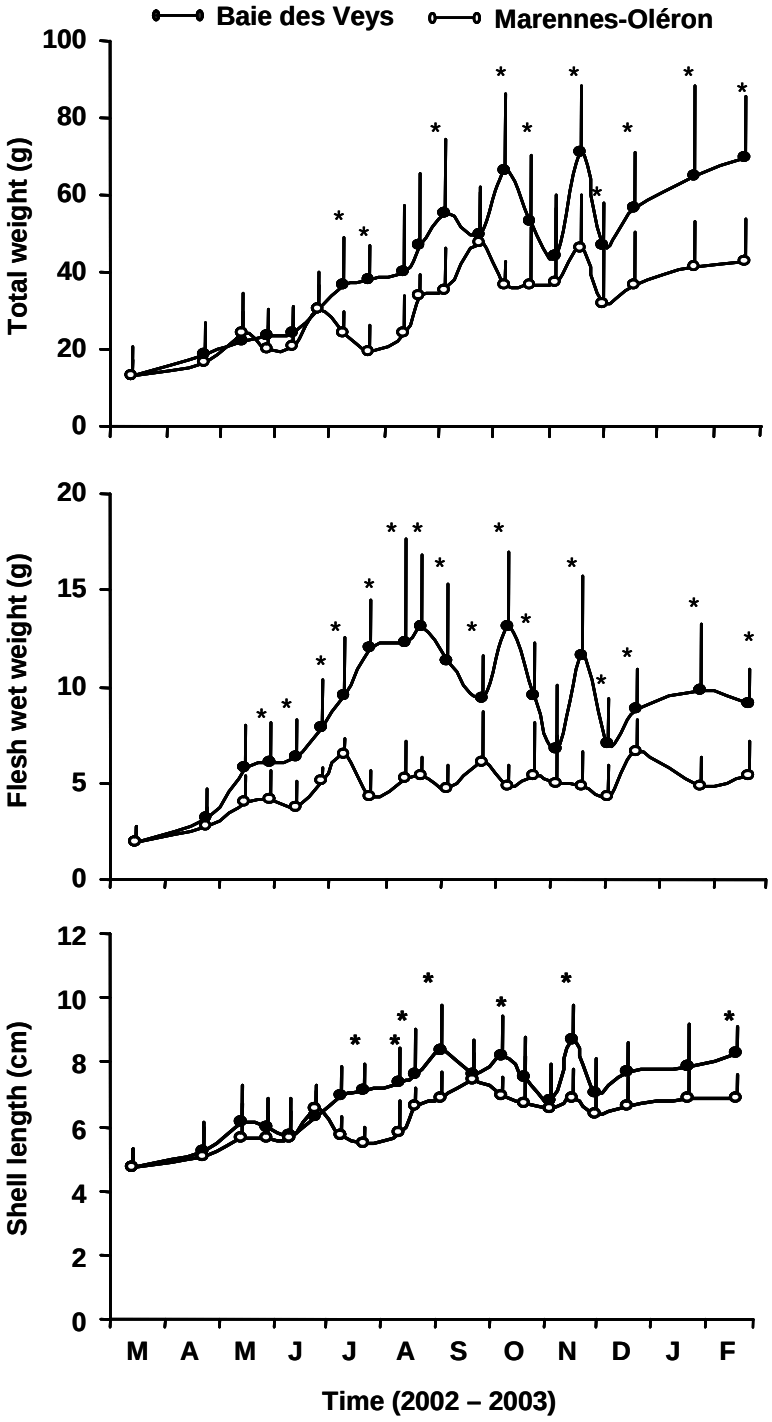

Fig. 4 Growth of *Crassostrea gigas* (mean ± SD) in terms of total weight (shell and flesh), flesh wet weight, and shell length (anterior-posterior axis). * = Significant differences present

Fig. 5

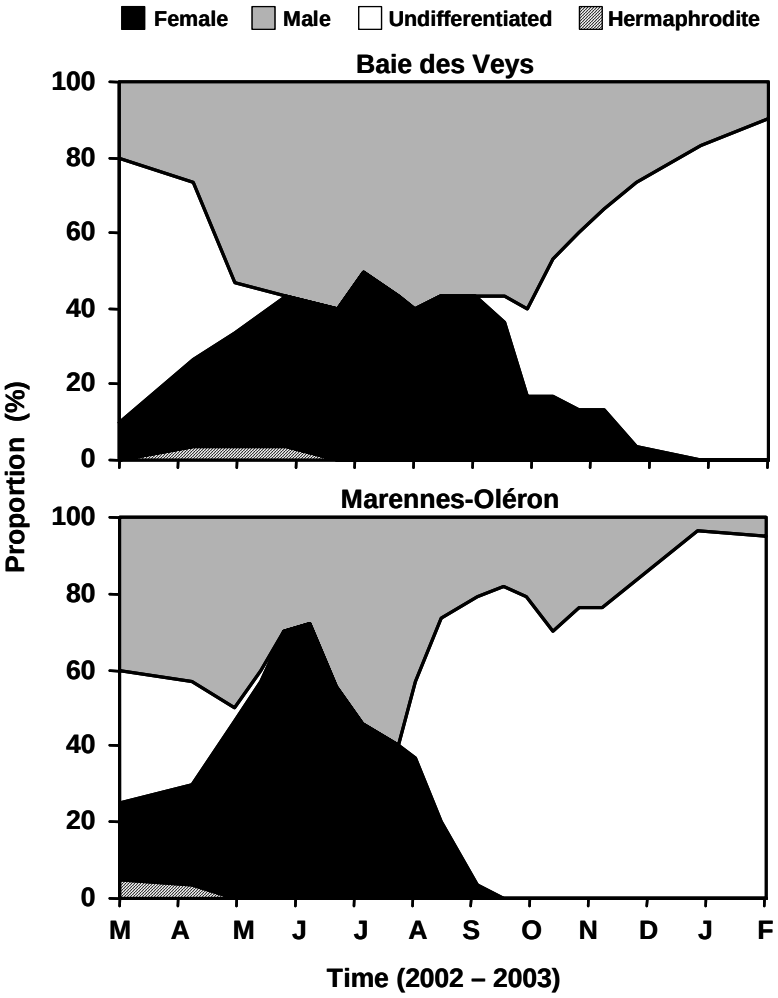

Fig. 5 Proportion of females, males, undifferentiated, and hermaphrodite *Crassostrea gigas* oysters during the study period

Fig. 6

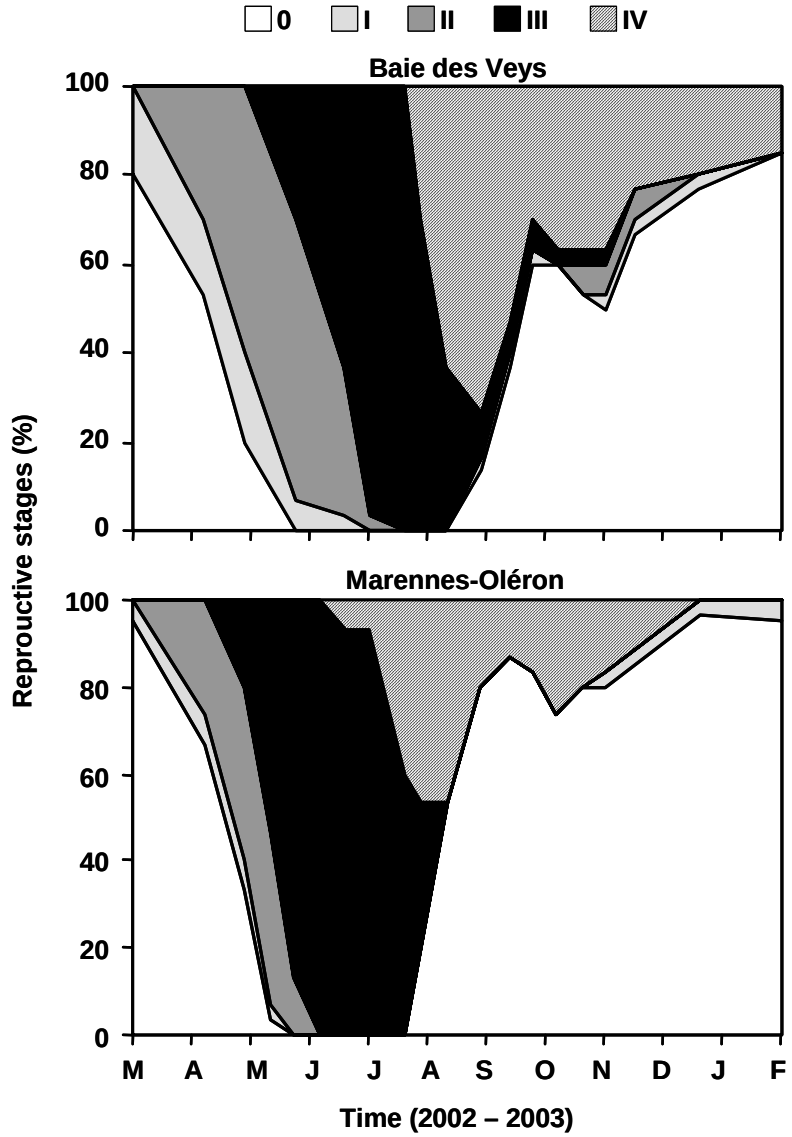

Fig. 6 Percentage of reproductive stages of female and male Pacific oysters *Crassostrea gigas* during the study period. 0 = Resting stage, I = Early growth stage, II = Late growth stage, III = Maturation stage, and IV = Spawning and reabsorbing stages

Fig. 7

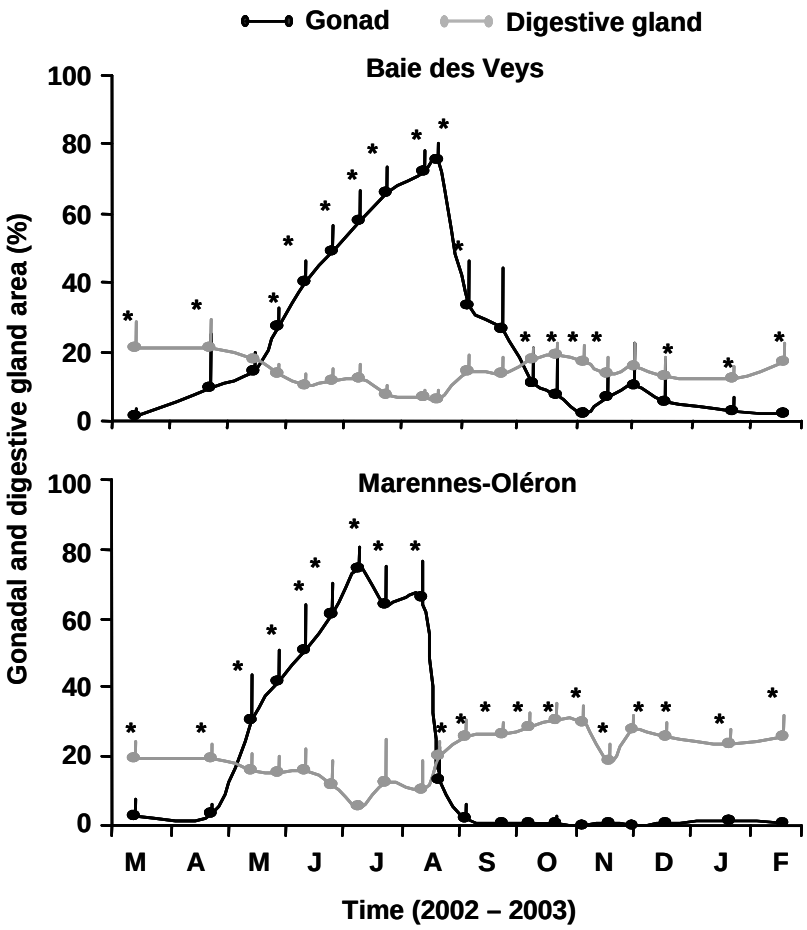

Fig. 7 Seasonal variations of mean (± SD) of gonad and digestive gland areas in relation to the visceral mass of Pacific oysters *Crassostrea gigas* cultured at different sites. * = Significant differences present

Fig. 8

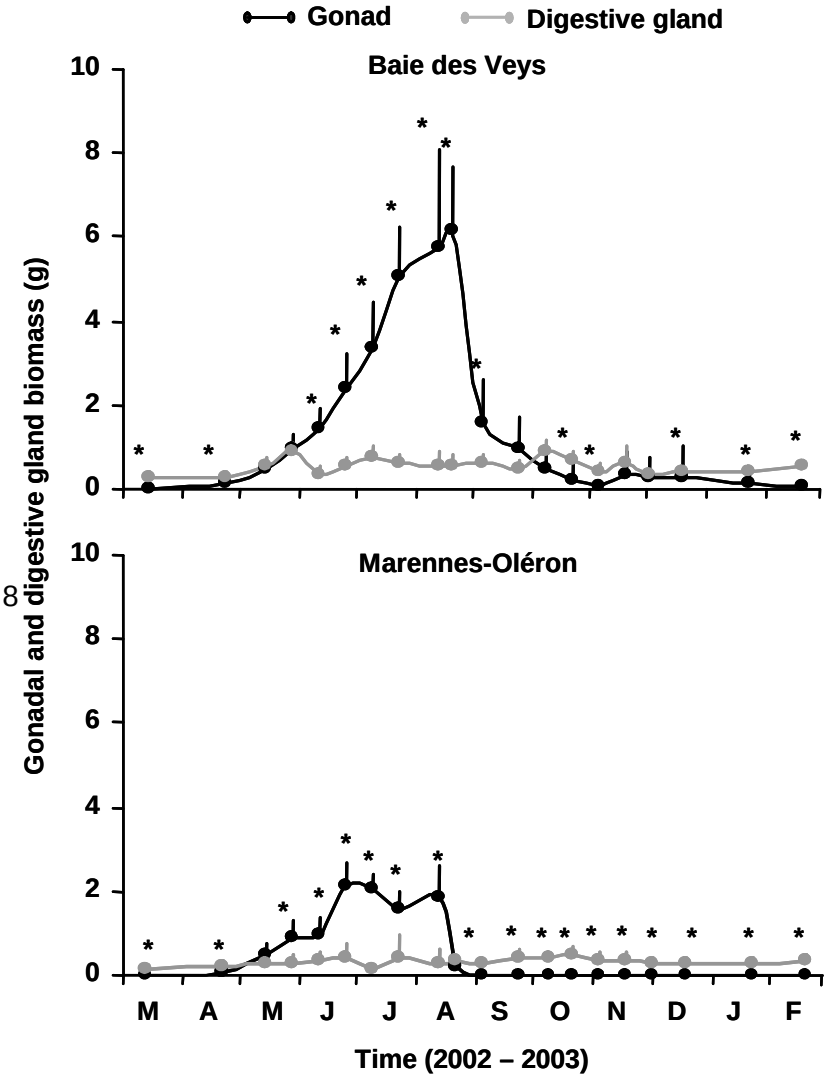

Fig. 8 Seasonal variations of mean (± SD) of gonad and digestive gland biomass of Pacific oysters *Crassostrea gigas* cultured at different sites. * = Significant differences present