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Gametogenetic cycle and reproductive effort assessed by two methods in 3 age classes of Pacific oysters, *Crassostrea gigas*, reared in Normandy

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Abstract:

Two methods were used to estimate the reproductive output of female Pacific oysters reared in Normandy: histology with image analysis and ELISA (Enzyme-Linked ImmunoSorbent Assay) which allowed the quantification of egg protein. Condition indices, gonad area and gametogenetic stages of the oysters were determined in the entire population (males and females) between May and October 2005. All investigations were performed in 3 age classes: oysters in their first, second or third years (corresponding to spat, half-grown and market-sized oysters, respectively). Both quantitative histology and ELISA provided similar results in terms of reproductive effort (illustrated by the gonado-somatic index, GSI) except during the GSI drop, corresponding to spawning, which was less marked with the ELISA method. Growth depended on oyster age, the sex ratio was well balanced and the reproductive cycle was synchronized in all age classes. In the 3 age classes, most of the oysters were ripe and ready to spawn on August 8, and ten days after the post-spawning stage was observed in 40% of spat oysters and 70% of half-grown and market-sized bivalves. The major difference between age classes was observed in the reproductive investment, with spat having a lower reproductive output. For example, in males and females, the gonad area reached 78–79% in the median animal section at full maturity (August 8) in half-grown and marketable oysters while it attained only 59% in spat. At the same time, GSI in females was, respectively in spat and the 2 oldest age classes, 33% (quantitative histology)–36% (ELISA) and 55% (quantitative histology)–60% (ELISA). The mean assessed gonad weight and fecundities increased with the age of the oysters: 1.3 g and 12 million eggs, 7.8 g and 135 million eggs, and 11.5 g and 146 million eggs in spat, half-grown and market-sized oysters, respectively. Marked differences thus appear between 2 and 3-year-old oysters and spat. As early as their first reproductive cycle, the young oysters not only showed the reproductive features of the species in Normandy, but also a pronounced lower reproductive effort. This lower energy demand could explain their higher survival rate.

Keywords: Condition index; *Crassostrea gigas*; ELISA; Gametogenetic cycle; Image analysis; Reproductive effort

Introduction

The Pacific oyster, Crassostrea gigas (Thunberg), was introduced into France in 1967 (Grizel and Héral, 1991). In the Bay of Veys (Normandy), ~130 ha of the eastern side of the bay were reserved for oyster farming where annual production reaches10,200 tons. This region is well known for the rapid growth of oysters which can be marketed after only 2 or 3 years of rearing. In Normandy, as in North West France (e.g. Brittany), spawning and/or larval survival are not sufficient to allow the maintenance of sizeable natural populations. Nevertheless, the oysters reared in the Bay of Veys appear to invest a relatively high proportion of the net energy in reproduction and show high condition indices allowing them to be classified in the best commercial category known as “special” (Costil et al., 2005). In this region, the reproductive effort (i.e., part of net production allocated to gonad production) seems to be very important but no quantitative data are yet available. The investment in reproduction (vs somatic growth) in oysters is not easy to evaluate because the gonad is diffuse and numerous gonadal tubules develop within connective tissue. In C. gigas, the reproductive effort was estimated 1) by measuring the difference in weight just prior to and after spawning (Deslous-Paoli and Héral, 1988; Pouvreau et al., 2000), 2) by counting or weighing the number of gametes released during spawning induction (Massapina et al., 1999) and 3) by counting the number of eggs from histological preparations of the gonad (Dinamani, 1987; Lango-Reynoso et al., 2000). The reproduction effort can also be estimated

via determination of the gonad area proportion using quantitative image analysis applied to histological slides of oyster sections (Heffernan and Walker, 1989; Enriquez-Diaz, 2004). A more recent alternative method consists in quantifying the protein using the egg protein-specific antibody in an enzyme-linked immunosorbent assay (ELISA) (Choi et al., 1993; Kang et al., 2003; Park and Choi, 2004; Park et al., 2005; Ngo et al., 2006).

Mass mortalities have affected juvenile and adult oysters sporadically along the French coasts since the 1980s (Maurer et al., 1986; Soletchnik et al., 1999) but no causative agent was detected. The published literature suggests that many of the mortalities occurring in Pacific oysters are the result of multiple factors or stresses (including pathogens, elevated temperature, low dissolved oxygen, xenobiotic stress, high productivity), and mortalities coincide with the period of maximum gonad condition for spawning (Beattie et al., 1980; Cheney et al., 2000). The stress of reproduction could therefore be highly implicated in the mass mortality events and is thus worth studying. In the Bay of Veys, such mortalities have occurred since 1994 (Goyard, 1996) and “adult” losses reached up to 58% (in summer 2001 which was disastrous for oyster farming) (Costil et al., 2005). Compared with other French shellfish basins, the Bay of Veys originality is due to the fact that oyster mortality generally occurs late in the season and juveniles are less affected than older animals. It can be hypothesized that the differential mortality between age classes is due to differences in the course of gametogenesis (critical maturation stages not occurring simultaneously with mortality environmental factors) and/or to differential reproductive effort.

We conducted experiments during the period of oyster sexual maturity, between May and October 2005, with the following main objectives: (1) to determine the condition indices and the gametogenetic stages reached by oysters during this period; (2) to measure the reproductive effort of female oysters by two different methods and compare the resulting

data; 3) to compare the gametogenetic cycle and reproductive effort of three age classes (spat, half-grown oysters and marketable oysters) and (4) to discuss oyster reproduction in relation to summer mass mortalities.

2. Material and methods

2.1. Study area and reared oysters

The research program was conducted on the French coast of the English Channel, in the Bay of Veys (Normandy) located in the western Bay of Seine, between Utah Beach and Omaha Beach. This bay (with an agricultural catchment basin of 3,400 km^2) comprises 37 km^2 of intertidal area covered mainly by fine sediments (Costil et al., 2005). The estuary is macrotidal with a tidal range up to 8 m and inside the bay seawater dominates freshwater. The study station, located in the Gefosse area (1°06.052'W; 49°23.111'N), showed a height above sea level of 2.2 m (mean time of oyster emersion of about 28%). Water salinity depends on various parameters such as tidal regime and climatic conditions. At the time of heavy rainfall, low salinities (such as 6 p.p.t.) can be recorded at the beginning of the floodtide; when rainfall is low, minimal salinity decreases to no less than ~ 20 p.p.t. (Costil et al., 2005). Most of the time, water salinity is about 30-33 p.p.t. (Ropert, pers. comm.). Mean temperature at high tide ± 1 hour is generally minimal in February-March (5-8°C depending on year) and maximal in August (19-20°C) (Costil et al., 2005; Ropert, pers. comm.).

In the Bay of Veys, Pacific oysters are cultivated off-bottom in culture bags which are placed onto iron tables. The animals studied belonged to three age classes: oysters in their first, second or third years, corresponding respectively to spat, half-grown and market-sized oysters (Table 1). They originated from a hatchery or natural spatfall. All spat and marketable oysters were settled in the Bay of Veys whereas the half-grown oysters were raised in another

shellfish area (Meuvaines, Normandy) during their first year and then transplanted in the Bay of Veys in February 2005. The original density of each bag (50 x 100 cm) was about 150-200 oysters depending on age classes, spat being placed into half bags during part of the study.

2.2. Sampling design

In 2005, oysters were sampled seven times (from May) or six times (from June) for biometric analyses and reproductive effort using image analysis, and the ELISA method, respectively (Table 2). Because of technical constraints it was not possible to sample as many half-grown oysters as spat and marketable ones. At each study date, the oysters were sampled randomly and immediately brought to the laboratory for analysis. At the same study station, a survey of oyster mortality was conducted by the Laboratoire Environnement Ressources de Normandie (L.E.R.N.; IFREMER Port-en-Bessin).

2.3. Biometric parameters of oysters

In the laboratory, oysters were cleaned, scrubbed to remove any attached epifauna and weighed in order to determine the whole weight (WW). Each oyster was then opened and shell valves were weighed (DSW) after drying in an oven for 24h. Wet soft tissue was retained for 15 min on a sloped plane covered with absorbent tissue paper and superficially dried tissues were then weighed (WMW). Oysters for measuring reproductive effort using the oyster egg protein-specific antibody in ELISA were frozen and then dry tissue weight (DMW) was determined after freeze-drying for a minimum of 48h.

The condition index MI (as Meat Index), commonly used by French scientists and oyster farmers, was calculated using the following equation: (WMW x 100) / WW. MI allows the

classification of oysters into 3 categories used for the oyster marketing in France: “special”: MI > 10.5, “fine”: 6.5 < MI < 10.5 and “non-classified”: MI < 6.5 (Fleury et al., 2005).

2.4. Gametogenetic stages and histology-image analysis method

Except for spat which was too small, from May to July oysters were dissected in order to remove the greatest amount of somatic tissue as possible (mantle, labial palps, gills, muscle, heart). The remaining “visceral mass” was weighed (VMW) and then cut across in three equal slices which were fixed in Davidson’s fluid. These were then routinely processed for histology and 5-µm paraffin-imbedded sections were stained according to the trichrome protocol of Prenant Gabe (Gabe, 1968). Sex of the oysters was determined and each individual was classified into distinct phases of gonadal maturation based on microscopic analysis and according to Lubet’s modified classification (1959) (see Costil et al., 2005 for details). Briefly, Stage I corresponds to gonial multiplication in tubules which are poorly developed (IR differing from I by the presence of at least 50% of residual gametes of the previous reproductive cycle). During stage II gonadal tubules highly develop while vitellogenesis occurs in females and various categories of sexual cells can be observed in males. Stage III is associated with maximum gonad size and includes 3 sub-stages: IIIA (stalk oocytes and oocytes still in the process of vitellogenesis / all male cell categories including spermatozoa), IIIB (oysters are ready to spawn or only limited spawning has occurred) and IIID (highly or totally spent; post-spawning and resorption stage).

Slides of the histological preparations of *C. gigas* tissues were scanned with an Epson Perfection 3200 Photo® Scan Digitizing Table. The images were then analyzed using Imaq Vision Builder software (Texas Instrument®). The analysis included the main stages of RGB (Red, Green and Blue) extraction, image mask from ROI (Region Of Interest), threshold and particle analysis. This program allows determining: 1) area fractions of gonadal tissue and 2)

the whole oyster surface of the 3 sections. The following parameters were calculated: percentage of gonadal surface area (GA_1; with the central slide only, for both males and females); gonadal weight (GW = GA_3 x VMW; GA_3 corresponding to the mean percentage of gonadal area determined with the three sections) and gonado-somatic index (GSI = GW/WMW), both parameters for females only. From the histological slides of oysters at full maturity, oocyte diameter was also determined by measuring ~ 300 oocytes per age class (with Imaq Vision Builder software).

2.5. Quantification of oyster eggs using ELISA

Quantity of egg protein and subsequently the amount of eggs in an individual oyster was determined using indirect ELISA with *C. gigas* egg-specific antibody as the primary antibody and goat anti-rabbit IgG alkaline phosphatase-conjugated as the secondary antibody (Kang et al., 2003). For analysis, 10 mg of powdered female oyster tissue containing eggs was dissolved in 2 mL phosphate buffer saline (0.15M NaCl, pH-7.4, PBS) and homogenized using an ultrasonifier. The oyster homogenate was diluted up to 1000-fold and a 100µL aliquot of diluted homogenate was added to the 96 wells on a polystyrene micro-plate and incubated at 4°C overnight. After incubation, the plate was washed four times with PBS containing 0.05% Triton X-100 (PBST), and 150µL of 1% bovine serum albumin was added to each well as a blocking agent. The plate was incubated for 1h at room temperature and washed four times with PBST-100. After washing, 100µL of the rabbit anti oyster egg IgG was added to each well and the plate was incubated 1h and washed again. Goat antirabbit IgG alkaline phosphatase conjugate (1:1000 diluted, SIGMA®) was added in 100µL aliquot to each well and the plate was incubated again for 1 h and washed. Finally, 100 µl of ρ-nitrophenylphosphate (ρ-NPP) substrate dissolved in diethanolamine buffer (SIGMA®) was added as a coloring agent. Optical density of each well in the plate was measured at 405 nm

using a microplate reader. Each ELISA plate included oyster egg standard and oyster samples; three replicates were made for each sample and the quantity of eggs (expressed in dry weight) was estimated from a standard curve determined using the absorbance of various concentration of oyster egg included in the plate. The gonado-somatic index (GSI) was calculated as in the histology-image analysis method but in this case the gonadal weight was related to dry meat weight (and not wet meat weight as in histology-image analysis). Moreover, fecundity was estimated as the number of eggs produced from each female oyster during a spawning period from the ratio of total egg weight measured by ELISA and the previously estimated weight of an individual egg (22 ng).

2.6. Data treatment

To test for significant differences of each biological parameter among age classes, several methods were used depending on the type of variable (measures or frequencies) and the normality of data sets (Scherrer, 1984). One way ANOVAs were applied to the data (transformed or not) having met the assumptions of the test, otherwise non-parametric tests (Kruskal-Wallis) were used. In both cases, multiple comparison tests (Student Newman Keuls) were used to distinguish different groups. Chi2 tests and Fisher's tests were performed to compare frequencies. These analyses were conducted with Statgraphics Plus 4.0 software.

3. Results

Mortalities were less than 5% during the study period, and 2005 was the year with the lowest mortality since the beginning of the survey in 2000 (Ropert, pers. comm.).

3.1. Growth performances and condition indices

From the beginning to the end of the study, growth was higher for spat (with a factor of 15.2) compared to half-grown (3.5) and marketable oysters (1.2), the whole weight reaching 34.9, 81.7 and 84.05 g respectively (Table 3). The shell weight (corresponding to 55.5 - 66.8% of the whole weight) followed the same pattern. Wet and dry meat weights were highly correlated ($p < 0.001$) and both allowed the determination of the spawning period. The latter, characterized by a change in the oyster meat appearance, was illustrated by a marked drop in both meat weight and meat index which occurred between 8 - 18 August (Fig. 1a & b) for the three age classes. Before this period a slight drop was observed between June 21 and the July 20 for spat and half-grown oysters which showed significantly lower and higher values of the meat index, respectively (ANOVAs, $p < 0.001$). Nevertheless, the oysters studied could be classified into the “special” category, whatever the date and age class.

3.2. Sex ratio and gametogenetic stages

Histological analysis allows the determination of oyster sex and a more accurate determination of the sexual maturation stage than biometric parameters and visual observations. Considering the whole study period or each of the 4 main periods of the reproductive cycle (maturation, beginning of spawning, spawning peak and resumption of a new cycle), it appears that the sex ratio was well balanced (1:1) for each of the 3 age classes (spat: n = 253; half-grown: n = 122; marketable: n = 146).

There were no significant differences in maturation stage between age classes (χ^2 tests and Fisher’s tests; $p > 0.05$). Thus the reproductive cycle appeared synchronous whatever the oyster age. At the beginning of the study (May or June depending upon age classes), a majority of oysters were in stage II corresponding to gonadal tubule development and sexual cell differentiation (Fig. 2). In the 3 age classes, the gonad showed its maximum volume on

July 20 (stages IIIA or IIIB) and almost all the bivalves were ripe and ready to spawn on August 8. Ten days later, the post-spawning stage (IIID) was observed in 40% of spat and 70% of half-grown and marketable oysters. In early September, a large proportion of the animals (especially half-grown oysters) were still ready to spawn (IIIB) while 10% of spat and market-sized oysters began a new reproductive cycle (stage I). In mid October, the main stage observed was stage I (I and IR) with a proportion of 95% for spat and 80% for half-grown oysters.

3.3. Reproductive effort of both male and female oysters (assessed by quantitative histology)

On May 9 the oyster gonad occupied a small proportion of the whole surface area with an occupation percentage of 2% in spat and 10% in half-grown oysters (Fig. 3). The gonad area then sharply increased until July 20. From late July to early August the gonad surface increase was regular before the marked decrease due to spawning events. On September 5 the gonad area had developed again in half-grown oysters but remained the same in spat and slightly decreased in marketable bivalves. In mid October the gonad surface reverted to the proportion noted in May for all age classes. Concerning these, significant differences were observed between spat and the 2 others classes from May to early August, with a greater investment in reproduction in the oldest oysters (Table 4). During and after the spawning period no significant differences in gonadal surface area were noticed, except for half-grown oysters on September 5.

3.4. Reproductive effort assessed by the 2 methods and fecundity in female oysters

Considering the individual or the mean values (Fig. 4), the estimation of the gonad area of females only and of both males and females led to similar results and no significant

differences in reproductive effort were found between the 2 sexes whatever the age class ($p < 0.01$). In fully ripe females (August 8), the assessed gonad ranged from 1.27 g (± 0.78) to 11.51 g (± 2.39) in spat and marketable oysters, respectively (Table 5).

With the gonado-somatic index (GSI expressed in mg wet gonad/mg wet tissue in quantitative histology, and in mg dry egg/mg dry tissue in ELISA) the animal size was not taken into account and data were standardized. Similar trends were observed with the 2 methods used which both illustrated clearly maturation and spawning-resorption processes (Fig. 5a & b). Moreover, the values of GSI were similar and significantly correlated for spat and half-grown oysters (both $r = 0.91$, $p < 0.05$); for marketable animals, the correlation was also high ($r = 0.89$) but not significant due to the low number of values ($n = 4$). Considering the 3 age classes together, the correlation was highly significant ($r = 0.92$, $n = 14$, $p < 0.001$). However, a main difference was noticed in the 3 age classes: the drop of GSI following spawning was less apparent with the ELISA method. Indeed spawning in spat, half-grown and marketable oysters was respectively 27.9%, 39.9% and 37.4% higher with quantitative histology than with ELISA.

Enzyme-linked immunosorbent assay (ELISA) allowed the estimation of female fecundity which also depended greatly on oyster age: the average fecundity was only 12.2 million eggs (± 7.31) for spat and reached 135.1 (± 4.60) and 146 million eggs (45.4) respectively, in half-grown and marketable oysters (Fig. 6). The corresponding maximum values (on August 8) were 24.37, 139.94 and 234.26 million eggs (Table 5). Female fecundity was strongly correlated to dry meat weight in spat and market-sized oysters ($p < 0.001$) (the slight variation in both fecundity and meat weight in half-grown oysters not allowing conclusions). No significant differences in oocyte size at full maturity (about 40 µm on average) were found among the 3 age classes (ANOVA, $p = 0.054$).

4. Discussion

4.1. Comparison of the 2 methods used to assess reproductive effort of female oysters

Stereological methods coupling histology and image analysis have been widely employed in studies of marine bivalves reproduction, including mussels (e.g. Lowe et al., 1982), clams (Morvan and Ansell, 1988; Heffernan et al., 1989), scallops (Sundet and Lee, 1984) and oysters (O'Beirn et al., 1996; Enriquez Diaz, 2004). Some studies focus on the dynamics of maturation processes considering the percentage of gonad occupied by oocytes and spermatogenic stages, while other investigations estimate the reproductive effort by assessment of the gonad area compared with whole tissues. With image analysis applied to histological slides, the operator interacts with the screen *via* the keyboard command and this interaction is important because of the staining quality variation (Hefferman and Walker, 1989). Indeed, a similar histological protocol (performed by the same technician) leads nevertheless to colour variations of the histological slides (according to sex and maturation stages), and the operator's implication remains necessary. In the present work, a single operator realized the image analysis in order to minimize the skew due to the relative subjectivity of the method. For female oysters, from 3 levels of the gonad area (GA_3) the entire gonadal weight was calculated, and this estimation was considered accurate by Enriquez-Diaz (2004) for the description of the gametogenetic cycle and the detection of differences in reproduction effort among oyster stocks. Heffernan and Walker (1989) considered that the histological-image analysis method provides accurate information as did Morvan and Ansell (1988) who determined the fecundity and oocyte dynamics of the clam, *Tapes rhomboides*, using 6 sections.

In the present study, gonado-somatic index (GSI) values estimated by ELISA were somewhat lower than percentage gonad area values (both illustrating reproductive output of females) because the first parameter relates to whole weight of the oysters whereas the second refers to

328 the “visceral mass” (excluding weight of adductor muscle, mantle, gills and labial palps).
329 According to Kang et al. (2003), who developed the enzyme-linked immunosorbent assay
330 (ELISA) in order to the estimate the reproductive effort in *C. gigas*, this method is sensitive
331 enough to measure a small quantity of eggs and considered a choice method to study bivalve
332 reproduction. Using polyclonal antibodies developed from eggs and sperm of the American
333 oyster *Crassostrea virginica*, Choi et al. (1993) successfully quantified eggs or sperm in this
334 species. A major result of the present study is that the GSI showed similar profiles with both
335 methods, although GSI curves differed at the moment of spawning. The drop in the GSI curve
336 was less pronounced with ELISA. Differences concerning spawning are difficult to interpret
337 unless we hypothesize that egg protein concentrations vary with the maturation stage of
338 oocytes (and are especially abundant in immature eggs not released during the main
339 spawning). We cannot exclude differences between the two methods due to inter-individual
340 variation in spawning which was shown to be important (Costil, unpublished data). Random
341 effects may also play a role when sample size is small (6 or 10 individuals on August 18). The
342 potential bias in quantitative histology may concern the wet weights (WMW and VMW)
343 which more than dry weights could show slight seasonal variations. With ELISA, it could be
344 hypothesized that the antibody linked to other compounds than egg proteins (i.e., cross-
345 reaction). Indeed, both *C. gigas* and *C. virginica* eggs share some peptides with somatic
346 tissues causing the cross-reaction during ELISA (Choi et al., 1993; Kang et al., 2003).
347 Nevertheless, the rabbit antibody was verified and after treating the antiserum with the
348 immunoabsorbent, Kang et al. (2003) demonstrated (with an indirect immunofluorescence
349 assay) a highly specific interaction between the antibody and the egg proteins. Both methods
350 entail advantages and disadvantages. Those of quantitative histology are, respectively, a rather
351 easy implementation and low cost, but a time-consuming procedure. Moreover, the GSI
352 calculation requires a dissection to obtain the VMW, which is impossible in small individuals
353 (early spat). The ELISA method is more complicated, requiring production of the oyster egg-

specific antibody, and at present antibody for only females is available in *C. gigas*. On the other hand, this method is sensitive enough to measure a small quantity of eggs (<10 eggs per assay), which is especially useful when analyzing early spat or triploid oysters.

4.2. Age-related growth performances

Oyster growth was especially rapid during the 6 months of the present study, above all in spat and to a lesser extent in half-grown bivalves. A slowdown in growth in relation to the age of the animals is a common biological phenomenon previously recorded for Pacific oysters (Quayle, 1988). Nevertheless, spat growth appeared notably fast because by October spat reached a whole weight (34.92 g) higher than that of half-grown (6 months older) oysters at the beginning of the study (23.19 g). This could be due to better environmental conditions in the Bay of Veys compared to Meuvaines, the other Norman shellfish area from which the half-grown oysters originated, as reported by the REMORA network which studies growth and quality of Pacific oysters distributed among various French oyster-growing areas (Fleury et al., 2005). Another explanation would be that higher water temperatures in 2005 compared to 2004 (when half-grown oysters were spat) stimulated oyster growth. The Bay of Veys is well known for its high productivity (Goyard, 1996) which results in high meat weights and condition indices (Costil et al., 2005; Fleury et al., 2005); because of their high quality, oysters are classified in the best commercial category (“special”). In particular, half-grown oysters were distinguished from the 2 other classes by their exceptionally high meat index (up to 29.34 ± 3.73). Very few studies were devoted to the effect of age on the biological performance of marine bivalves, since this requires simultaneous observations of different age classes. In multi-annual surveys, differences observed between years are attributable both to inter-annual variability in environmental conditions and to the age effect (Cigarria, 1999). In a 2-year survey carried out at 2 sites located in the Arcachon basin (South France), Maurer and

Borel (1986) obtained different growth curves (whole weight) in 1-year old and 2-year old oysters which were studied simultaneously: the younger oysters grew faster and continuously from March to November whereas the older bivalves grew slower and stopped growing in summer. According to Quayle (1988) the slowdown of shell growth (in length, width and thickness) in August in Canada was not completely explained but was associated with spawning.

4.3. Age-related reproductive traits

Changes in meat weight in marine bivalves are related to the reproductive cycle and patterns of energy storage and mobilization (Worrall and Widdows, 1984; Cigarria, 1999). Sexual maturation can be illustrated by a tissue weight increase, as was observed in the Bay of Veys during spring concomitantly to a phytoplankton bloom, and spawning is associated with an important and abrupt decrease of tissue weight and consequently of condition indices (Costil et al., 2005); after a maturation period throughout spring, the gonad acquired its maximal volume in June and the first oysters ready to spawn were observed in July in all age classes. In this study, the slight drop noticed between the June 21 and the July 20 could correspond to minor partial spawning (not observed with GSI), whereas major (but also partial) spawning occurred in mid August. According to Mann (1979), spawning occurs when water temperature exceeds 18°C (which is generally attained in the Bay of Veys), but temperature also determines the extent of spawning and gamete resorption. The main spawning event, synchronous in males and females, was likely followed by minor events of gamete emission and gonad resorption. In her experimental study, Enriquez-Diaz (2004) showed that gamete emission was achieved with difficulty when food quantity was high, which is the case in the Bay of Veys. A majority of half-grown oysters were again in stage IIIB, showing an increased gonad area on September 5 (after the main spawning); this was not the case in the 2 other age

406 classes. A similar trend was observed in half-grown oysters in previous years (Costil et al.,
407 2005). An exceptionally fast maturation of gonial cells can be hypothesized, but in female
408 oysters it could also be due to maturation of oocytes completing vitellogenesis, because in the
409 Bay of Veys generally certain gametes are not fully mature when spawning begins. This is
410 consistent with a greater gonad area increase in females compared with the whole sample
411 (females and males) on September 5. According to Soniat and Ray (1985), re-maturation
412 phenomenon in summer is combined with higher food availability which the half-grown
413 oysters may be more capable to take advantage of. Despite the differences mentioned above,
414 no significant differences were found between age classes in oocyte size, sex ratio and
415 gametogenic stage course. On average, the oocyte diameter ranged from 39.9 µm in half-
416 grown bivalves to 41.0 µm in spat. These values are slightly higher than those reported for the
417 same species by Lango-Reynoso et al. (2000): mean diameter of 36.1 µm in Brittany and 34.9
418 µm in Marennes-Oléron (French Atlantic coast). Establishment of the sex ratio requires
419 examination of a great number of animals. In the present study, sex ratio was determined in a
420 total of 276 spat, 132 half-grown individuals and 145 market-sized oysters. The well balanced
421 sex ratio in the 3 age classes are quite surprising, especially for spat because it has been
422 reported that *C. gigas* is a successive protandric hermaphrodite (Buroker, 1983). In young-of-
423 the-year classes, there are generally more males than females, whereas there is a
424 preponderance of females at the beginning of the second year (Dinamani, 1987). Whatever
425 the age class, the reproductive cycle was synchronous, and spat (10 months old) did not show
426 a delay in sexual differentiation and spawning during its first cycle. In *C. gigas* reared in New
427 Zealand, Dinamani (1987) observed sexual differentiation at 6 months and spawning within
428 the first year. By contrast, Maurer and Borel (1986) reported that in the Arcachon area, 2-
429 year-old oysters consistently matured and spawned, whereas in 1-year-old animals spawning
430 did not occur throughout the entire population because of inter-individual differences in stage
431 of sexual maturation. The reproductive cycle of Pacific oyster appears very plastic depending

on rearing locations and hydro-climatic conditions. In *C. virginica*, O'Beirn et al. (1996) also observed spawning events varying according to locations (in a Georgia sound), year and age class: minor spawning events or gradual resorption of the gametes tended to occur in "adults", whereas young-of-the-year oysters spawned more massively.

The physiological status of oysters and mussels plays a major role in the occurrence of summer mass mortalities and the role of the reproductive cycle is therefore emphasized (Mori, 1979, Perdue et al., 1981, Goulletquer et al., 1998). In the Bay of Veys, summer mortalities occurred when both temperature exceeded 19°C and oysters showed the maximum or declining values of meat weight and condition index. The maturity stage at the time of mass mortalities was III (especially, B or D), corresponding to sexual ripeness and spawning (Costil et al., 2005). In this bay, summer mortalities especially affect the oldest age classes, whereas spat are generally spared (Royer et al., 2006). During the study in 2005, no mass mortalities were observed and the relation of mortality to reproduction is thus difficult to establish. Since the reproductive cycle was shown to be synchronous among all age classes every year, it is likely that quantitative rather than qualitative aspects of reproduction explain the differences in mortality noticed among age classes.

4.4. Age-related reproductive effort

Female fecundity was correlated with dry meat weight (r = 0.91-0.99), as previously reported by Kang et al (2003) for *C. gigas* reared in Korea (r = 0.86). Fecundity was obtained by dividing total egg weight measured using ELISA by the individual weight of a single egg, which was previously estimated at 22 ng. Kang et al. (2003) estimated a weight of 13 ng per egg; this weight was also reported for *C. virginica* (Lee and Heffernan, 1991; Choi et al., 1993). In *C. gigas*, the number of eggs produced by a market-sized individual has been estimated to be 50-100 million (Quayle, 1988). In the present study, all half-grown and

458 marketable oysters (except one individual) contained over 110 million gametes and up to 234
459 million eggs. Kang et al. (2003), also using ELISA, reported fecundity ranging from 4 to 196
460 million eggs in 2-3 year-old oysters reared in Korea. In the same survey (2000) using the
461 same method, Ngo et al. (2006) calculated mean fecundities between 1.6 (± 0.7)-106.5 (±
462 50.7) and 4.9 (± 2.4)-99.1 (± 61.9) million eggs at the top (0-2 m) and bottom (3-5 m) of a
463 long-line suspended culture, respectively. The review in Kang et al. (2003) reported
464 fecundities ranging from 0.12 to 100 million eggs (with a value up to 300 million by
465 computer simulation) for *C. virginica*, and from 30 to 148 million eggs for *C. gigas*. The
466 fecundities calculated in the present study are similar to or higher than those reported in the
467 literature. Hofmann et al. (1994) suggested that oysters reared at higher latitudes (as in the
468 Bay of Veys) produce more eggs compared to oysters living in higher water temperatures
469 where the animals expend more energy for respiration. Kang et al. (2003) calculated a
470 maximum mean value of GSI of 42.3% in 2-3 year-old *C. gigas*, the highest individual value
471 reaching 66.7%. This survey also revealed a maximum mean of 49.5% in oysters reared at the
472 surface (Ngo et al., 2006). Using the ELISA method, it was reported that *C. virginica*
473 produced ~ 20% of their body weight as eggs and, the maximum GSI was 42% (Choi et al.,
474 1993). These GSI values are lower than those found here using the same method in half-
475 grown (61%) and marketable (59.6%) oysters (with the highest individual value of 79%); they
476 are close to GSI obtained by quantitative histology for half-grown (55.7%) and marketable
477 (54.2%) oysters. All these results emphasize the high reproductive effort of female oysters in
478 the Bay of Veys. The average gonadal areas of both males and females (using the median
479 section) were 79.6% in marketable oysters and 78% in half-grown oysters. These results are
480 consistent with the large gonad surfaces (~ 75%) found in half-grown oysters reared in the
481 Bay of Veys in 2002 (Enriquez Diaz, 2004). In early September, the gonad proportion was
482 27% - 57% depending on age class. Enriquez Diaz (2004), who also noticed partial spawning,
483 indicated a percentage of ~ 30% in September. Enriquez Diaz (2004) emphasized the high

reproductive effort of oysters settled in the Bay of Veys in comparison to oysters reared in Marennes-Oléron Bay. Maximum annual values of wet gonad weight and reproductive effort were 1.9 g (± 0.7) and 53% in Marennes-Oléron, and 6.1 g (± 1.5) and 86% in the Bay of Veys. The 6.1 g value is consistent with the wet gonad weight estimated for equivalent size (half-grown) oysters in this study (7.8 g ± 2.9). The differences in the reproductive effort of oysters reared at Marennes-Oléron and in the Bay of Veys were attributed to lower phytoplankton concentrations in the former area; the positive correlation between the amount of food available and the reproductive effort was experimentally demonstrated by Enriquez Diaz (2004). Food availability (rather than water temperature) is the main determinant of the quantity and quality of oyster reproductive output (Hofmann et al., 1992; Kang et al., 2000). Depending on location and year, O'Beirn et al. (1996) observed that the percentage of area occupied by gonads was as high as 65% in females and 80% in males in young-of-the-year *C. virginica*. Considering the weight loss during spawning in *C. gigas*, Deslous-Paoli and Héral (1988) reported a reproductive effort of 17.8% (of dry weight) in 1-year-old males and 43.1% in 1-year-old females with values reaching 55.9% for males and 61.9% for females in 3-year-old animals. The proportion of total production expended on gamete output rose from 18% in 1-year-old oysters to 84% in "adults" (Héral, 1989). In the present study, differences in reproductive output between sexes were not obvious but those between age classes were. In the Bay of Veys, whatever the method of reproductive effort assessment employed, the reproductive effort of spat was less compared to the 2 older classes (maximum gonad area: 59.5% in both males and females spat and 64.9% in females; GSI: 36% with ELISA and 33.2% with quantitative histology). This difference could partially explain the lower mortality in this age class which is not observed in all French shellfish areas. In *Mytilus edulis*, Worrall and Widdows (1984) indicated that highest mortalities were recorded in the medium to large size classes which had the highest reproductive effort. Greater losses of 2-year-old mussels could be explained by higher reproductive output (Emmett et al., 1987). Tremblay et al.

(1998) and Myrand et al. (2000) also linked high reproductive effort and mortality in *M. edulis*. Mori (1979) observed a close relationship between the degree of soft-body growth and mass mortality in *C. gigas*. Mori (1979) and Kang et al. (2000) reported that in eutrophic areas gonad hypertrophy is accompanied by physiological disturbances. Shpigel et al. (1992) compared biological performances of 1-year-old diploid and triploid Pacific oysters and concluded to possible linkage between reproduction and summer mortality. Individuals with a high reproductive effort show high metabolic and energy demands (possibly combined with depletion of carbohydrate reserves) and, they are thus more susceptible to mortality if other risk factors occur.

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References

Beattie, J.H., Chew, K.K., Hersberger, W.K., 1980. Differential survival of selected strains of pacific oysters (*Crassostrea gigas*) during summer mortality. Proc. Nat. Shellfish. Assoc. 70, 184-189.

535 Buroker, N.E., 1983. Sexuality with respect to shell length and group size in the Japanese
536 oyster *C. gigas*. Malacologia 23, 271-279.
537 Cheney, D.P., Macdonald, B.F., Elston, R.A., 2000. Summer mortality of Pacific oysters,
538 *Crassostrea gigas* (Thunberg): Initial findings on multiple environmental stressors in Puget
539 Sound, Washington, 1998. J. Shellfish Res. 19, 353-359.
540 Choi, K.S., Lewis, D.H., Powell, E.N., Ray, S.M., 1993. Quantitative measurement of
541 reproductive output in the American oyster, *Crassostrea virginica* (Gmelin), using an
542 enzyme-linked immunosorbent assay (ELISA). Aquac. Fish. Manage. 24, 375-398.
543 Cigarria, J., 1999. Effects of age, size, and season on growth of soft tissue in the oyster
544 *Crassostrea gigas* (Thunberg, 1793). J. Shellfish Res. 18, 127-131.
545 Costil, K., Royer, J., Ropert, M., Soletchnik, P., Mathieu, M., 2005. Spatio-temporal
546 variations in biological performances and summer mortality of the Pacific oyster *Crassostrea*
547 *gigas* in Normandy (France). Helgoland Mar. Res. 59, 286-300.
548 Deslous-Paoli, J.M., Héral, M., 1988. Biochemical composition and energy value of
549 *Crassostrea gigas* (Thunberg) cultured in the Bay of Marennes-Oléron. Aquat. Living Resour.
550 1, 239-249.
551 Dinamani, P. 1987. Gametogenic patterns in populations of pacific oyster, *Crassostrea gigas*,
552 in Northland, New Zealand. Aquaculture 64, 65-76.
553 Emmett, B., Thompson, K., Popham, J. D., 1987. The reproductive and energy storage cycle
554 of two populations of *Mytilus edulis* from British Columbia. J. Shelfish Res. 6, 29-36.
555 Enriquez-Diaz, M.R., 2004. Variabilité et bioénergétique de la reproduction chez l'huître
556 creuse *Crassostrea gigas*. Thèse de l'Université de Bretagne Occidentale, 189 pp
557 (http://www.ifremer.fr/docelec/doc/2004/these-1095.pdf).
558 Fleury, P.G., Simonne, C., Claude, S., Palvadeau, H., Guilpain, P., d'Amico, F., Le Gall, P.,
559 Vercelli, C., Pien, S., 2005b. Résultats des stations nationales REMORA (suivi de la

560 croissance de l'huître creuse sur les côtes françaises), année 2004. IFREMER, 48 pp
561 (http://www.ifremer.fr/remora/pdf/Remora04_Integ.pdf).
562 Gabe, M. 1968. Techniques histologiques. Masson and Compagnie, Paris, 1113 pp.
563 Goulletquer, P., Soletchnik, P., Le Moine, O., Razet, D., Geairon, P., Faury, N., Taillade, S.,
564 1998. Summer mortality of the Pacific cupped oyster *Crassostrea gigas* in the Bay of
565 Marennes-Oléron (France), ICES, Lisbon CM. CC/14, 14-20.
566 Goyard, E., 1996. Bilan national de croissance de l'huître creuse de 1993 à 1885. Report
567 IFREMER, La Trinité-sur-mer, 34 pp.
568 Grizel, H., Héral, M., 1991. Introduction into France of Japanese oyster (*Crassostrea gigas*).
569 J. Cons. Perm. Int. Explor. Mer 47, 399-403.
570 Heffernan, P.B., Walker, R.L., 1989. Quantitative image analysis methods for use in
571 histological studies of bivalve reproduction. J. Moll. Stud. 55, 135-137.
572 Heffernan, P.B., Walker R.L. Carr J.L., 1989. Gametogenic cycles of three bivalves in
573 Wassaw sound, Georgia: I. *Mercenaria mercenaria* (Linnaeus, 1758). J. Shellfish Res. 8, 51-
574 60.
575 Héral, M., 1989. Traditional oyster culture in France: Ellis Horwood Limited. 342-387 pp.
576 Hofmann, E.E., Klick, J.M., Powell, E.N., Boyles, S. Ellis, M., 1994. Modeling oyster
577 population: II. Adult size and reproductive effort. J. Shellfish Res. 13, 165-182.
578 Kang, S.G., Choi K.S., Bulgakov, A.A., Kim, Y., Kim, S.Y., 2003. Enzyme-linked
579 immunosorbent assay (ELISA) used in quantification of reproductive output in the pacific
580 oyster, *Crassostrea gigas*, in Korea. J. Exp. Mar. Biol. Ecol. 282, 1-21.
581 Lango-Reynoso, F., Chavez-Villalba J.C., Cochard J.C., Le Pennec, M., 2000. Oocyte size, a
582 means to evaluate the gametogenic development of the Pacific oyster, *Crassostrea gigas*
583 (Thunberg). Aquaculture 190, 183-199.

584 Lee, R.F. Heffernan, P.B., 1991. Lipids and proteins in eggs of eastern oysters *Crassostrea*
585 *virginica* (Gmelin) and northern quahogs *Mercenaria mercenaria* (Linneaus, 1785). J.
586 Shellfish Res. 10, 203-206.
587 Lowe, D.M., Moore, M.N., Bayne, B.L., 1982. Aspects of gametogenesis in the marine
588 mussel *Mytilus edulis* L. J. Mar. Biol. Assoc. U.K. 62, 133-145.
589 Lubet, P., 1959. Recherches sur le cycle sexuel et l'émission des gamètes chez les Mytilidés et
590 les Pectén idés. Revue des Travaux de l'I.S.T.P.M. 23, 387-548.
591 Mann, R., 1979. Some biochemical and physiological aspects of growth and gametogenesis in
592 *Crassostrea gigas* and *Ostrea edulis* grown at sustained elevated temperatures. J. Mar. Biol.
593 Assoc. U.K. 59, 95-110.
594 Massapina, C., Joaquim, S., Matias, D., Devauchelle, N., 1999. Oocyte and embryo quality in
595 *Crassostrea gigas* (Portuguese strain) during a spawning period in Algarve, South Portugal.
596 Aquat. Living Resour. 12, 327-333.
597 Maurer, D., Borel, M., 1986. Croissance, engraissement et cycle sexuel de *Crassostrea gigas*
598 dans le bassin d'Arcachon: comparaison des huîtres âgées de 1 et 2 ans. Haliotis 15, 125-134.
599 Maurer, D., Comps, M., His, E., 1986. Caractéristiques des mortalités estivales de l'huître
600 *Crassostrea gigas* dans le bassin d'Arcachon. Haliotis 15, 309-317.
601 Mori, K., 1979. Effect of artificial eutrophication on the metabolism of the japanese oyster
602 *Crassostrea gigas*. Bull. Eur. Ass. Fish. Pathol. 12, 11-13.
603 Morvan, C., Ansell, A.D., 1988. Stereological methods applied to reproductive cycle of *Tapes*
604 *rhomboides*. Mar. Biol. 97, 355-364.
605 Myrand, B., Guderley, H., Himmelman, J.H., 2000. Reproduction and summer mortality of
606 blue mussels *Mytilus edulis* in the Magdalen Islands, southern Gulf of St. Lawrence. Mar.
607 Ecol. Prog. Ser. 197, 193-207.

608 Ngo, T.T.T., Kang, S.G., Kang, D.H., Sorgeloos, P., Choi, K.S., 2006. Effect of culture depth
609 on the proximate composition and reproduction of the Pacific oyster, *Crassostrea gigas* from
610 Gosung Bay, Korea. Aquaculture 253, 712-720.
611 O'Beirn, F.X., Heffernan, P.B., Walker, R.L., Jansen, M.L., 1996. Young-of-the-year oyster,
612 *Crassostrea virginica*, reproduction in coastal Georgia. Estuaries 19, 651-658.
613 Park, K.I, Choi, K.S., 2004. Application of Enzyme-Linked Immunosorbent Assay (ELISA)
614 for studying of reproduction in the Manila clam *Ruditapes philippinarum* (Mollusca:
615 Bivalvia): I. Quantifying eggs. Aquaculture 241, 667-687.
616 Park, K.-I., Choi, J.-W., Choi, K.-S., 2005. Development of a *Saxidomus purpuratus*
617 (Mollusca: Bivalvia) egg-specific antibody for the quantification of eggs using an enzyme-
618 linked immunosorbent assay. J. Shellfish Res. 24, 1079-1086.
619 Perdue, J.A., Beattie, J.H., Chew, K.K., 1981. Some relationships between gametogenic cycle
620 and summer mortality phenomenon in the pacific oyster (*Crassostrea gigas*) in Washington
621 State. J. Shellfish Res. 1, 9-16.
622 Pouvreau, S., Gangnery, A., Tiapari, J., Lagarde, F., Garnier, M., Bodoy, A., 2000.
623 Gametogenic cycle and reproductive effort of the tropical blacklip pearl oyster, *Pinctada*
624 *margaritifera* (Bivalvia: Pteriidae), cultivated in Takapoto atoll (French Polynesia). Aquat.
625 Living Res. 13, 37-48.
626 Quayle, D.B., 1988. Pacific oyster culture in British Columbia. Department of Fisheries and
627 Oceans, Ottawa. 241 pp.
628 Royer, J., Ropert, M., Mathieu, M., Costil, K. 2006. Presence of spionid worms and other
629 epibionts in Pacific oysters (*Crassostrea gigas*) cultured in Normandy, France. Aquaculture
630 253, 461-474.
631 Scherrer, B., 1984. Biostatistique. Gaëtan Morin, Montreal, Canada. 250 pp.

632 Shpigel, M., Barber, B.J., Mann, R., 1992. Effects of elevated temperature on growth,
633 gametogenesis, physiology, and biochemical composition in diploid and triploid pacific
634 oysters, *Crassostrea gigas* Thunberg. J. Exp. Mar. Biol. Ecol. 161, 15-25.
635 Soletchnik, P., Le Moine, O., Faury, N., Razet, D., Geairon, P., Goulletquer, P., 1999.
636 Mortalité de l'huître *Crassostrea gigas* dans le bassin de Marennes-Oléron: étude de la
637 variabilité spatiale de son environnement et de sa biologie par un système d'informations
638 géographiques (SIG). Aquat. Living Resour. 12, 131-143.
639 Soniat, T.M., Ray, S.M., 1985. Relationships between possible available food and the
640 composition, condition and reproductive state of oysters from Galveston Bay, Texas. Contrib.
641 Mar. Sci. 28, 109-121.
642 Sundet, J.H., Lee, J.B., 1984. Seasonal variations in gamete development in the Iceland
643 scallop *Chlamys islanda*. J. Mar. Biol. Assoc. U.K. 64, 411-416.
644 Tremblay, R., Myrand, B., Sevigny, J.M., Blier, P., Guderley, H., 1998. Bioenergetics and
645 genetic parameters in relation to susceptibility of blue mussels, *Mytilus edulis* (L.) to summer
646 mortality. J. Exp. Mar. Biol. Ecol. 221, 27-58.
647 Worrall, C.M., Widdows, J., 1984. Investigation of factors influencing mortality in *Mytilus*
648 *edulis* L. Mar. Biol. Lett. 5, 85-97.

649 Table 1

650 Characteristics of the batches corresponding to the 3 age classes studied at the beginning of
651 the study (mean ± SD) and date of oyster transfer to study sites

652

Features	Spat	Half-grown	Marketable
Age	10 months	22 months	39 months
Whole weight (g)	2.29 ± 1.41	23.19 ± 5.09	69.78 ± 17.55
Shell length (mm)	26.83 ± 6.77	72.19 ± 7.44	86.43 ± 10.15
Origin	Hatcheries	Natural spatfall	Hatcheries
Date of oyster transfer to study sites	2005 March	2005 February	2003 June

653

654 Table 2

655 Number of sampled oysters at each date and for each age class. First and second numbers
656 correspond to the numbers of oysters of which reproductive effort was studied by,
657 respectively, histology - image analysis and quantification of oyster eggs using ELISA

Sampling date	Spat	Half-grown	Marketable
05-09-05	24 + 0	12 + 0	0 + 0
06-21-05	20 + 25	10 + 10	20 + 19
07-20-05	20 + 21	10 + 10	20 + 17
08-08-05	20 + 19	10 + 10	20 + 19
08-18-05	20 + 24	10 + 10	10 + 10*
09-05-05	20 + 20	10 + 10	10 + 0*
10-17-05	20 + 23	10 + 10	0 + 0

658 * The number of experimented oysters was reduced because of a lack of individuals

659 Table 3

660 Mean growth (± SD) observed in the 3 age classes during the study period and, correlation
661 calculated between the dry meat weight and the wet meat weight

	Spat oysters		Half-grown oysters		Marketable oysters	
	Start of the study (05-09-05)	End of the study (10-17-05)	Start of the study (05-09-05)	End of the study (10-17-05)	Start of the study (06-21-05)	End of the study (09-05-05)
Whole weight (g)	2.29±1.41	34.92±7.88	23.19±5.09	81.67±16.18	69.78±17.55	84.05±18.21
Shell weight (g)	1.53±0.91	19.93±3.9	12.87±2.57	50.66±10.81	44.49±11.29	51.01±11.64
Correlation dry-wet meat weight	r=0.94 ; N=129		r=0.76 ; N=50		r=0.98 ; N=53	

662

663 Table 4

664 Results of ANOVAs for the comparisons of gonad surface area studied in 2005 in the 3 age
665 classes: S (Spat), H (Half-grown oysters) and M (Marketable oysters)

Date	Df within groups	F-Ratio	p-value	Homogeneous groups
May 5	26	28.21	< 0.0001	S; H
June 21	45	34.43	< 0.0001	S; H/M
July 20	43	27.30	< 0.0001	S; H/M
August 8	44	19.92	< 0.0001	S; H/M
August 18	37	2.46	0.0992	S/H/M
September 5	37	8.89	< 0.001	S/M; H
October 17	28	0.30	0.5852	S/H

666

667 Table 5

668 Biological features (including reproduction parameters) in Pacific oysters belonging to 3 age
669 classes at full maturity (on August 8 2005) in the Bay of Veys. Mean values (± SD): Shell
670 length (SL) in cm, wet weight (WMW) and dry tissue weight (DMW) in grams

	Spat	Half-grown oysters	Marketable oysters
SL	5.52 (0.69)	7.6 (0.28)	8.86 (1.04)
WMW	3.68 (1.29)	15.14 (1.27)	21.95 (4.76)
DMW	0.68 (0.23)	3.74 (0.22)	5.95 (1.53)
DMW/WMW	0.185	0.247	0.271
Gametogenesis stage	IIIB	IIIB	IIIB
Mean diameter of oocytes (µm)	41.0 (5.4)	39.9 (6.4)	40.2 (5.7)
Oocyte diameter range (µm)	25.8-56.1	24.4-58.4	26.7-56.7
Estimated gonad wet weight (g)	1.27 (0.78)	7.79 (2.91)	11.51 (2.39)
GSI (mg gonad/mg wet tissue)	0.33	0.557	0.542
GSI (mg egg/mg dry tissue)	0.36	0.61	0.596
Fecundity mean (million eggs/female)	12.2(7.3)	135 (4.6)	146 (45.4)
Fecundity range (million eggs/female)	2.6-24.4	129-140	111-234

671

672 **Figure captions**

673

674 Fig. 1. Variations in wet meat weight (a) and meat index (b) of spat, half-grown and
675 marketable oysters from May to October 2005.

676 Fig. 2. Temporal variation in proportion of sexual maturity stages for spat (S), half-grown (H)
677 and marketable (M) oysters from May to October 2005. For maturity stage description see
678 text and Costil et al. (2005).

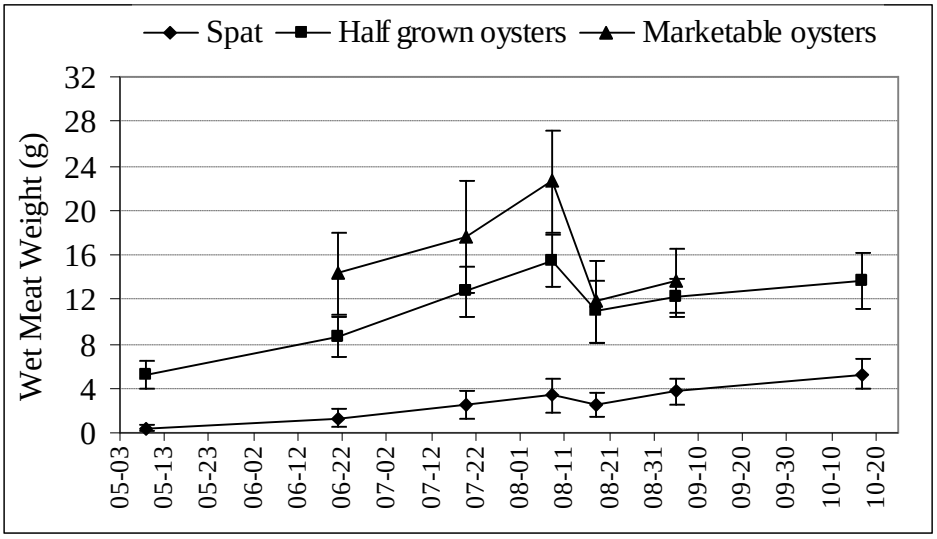
679 Fig. 3. Temporal variation in proportion of gonad area (GA_1), estimated by histology and
680 image analysis for spat, half-grown and marketable oysters from May to October 2005.

681 Fig. 4. Correlation between gonad areas estimated from one section (y) in both males and
682 females and from 3 sections (x, y, z) in females only for the 3 age classes (n = 17). Spat: n =
683 6, $y = 1.05x + 0.02$, $R^2 = 0.94$; Half-grown oysters: n = 6, $y = 0.96x + 0.08$, $R^2 = 0.98$;
684 Marketable oysters: n = 5, $y = 1.01x + 0.02$, $R^2 = 0.98$.

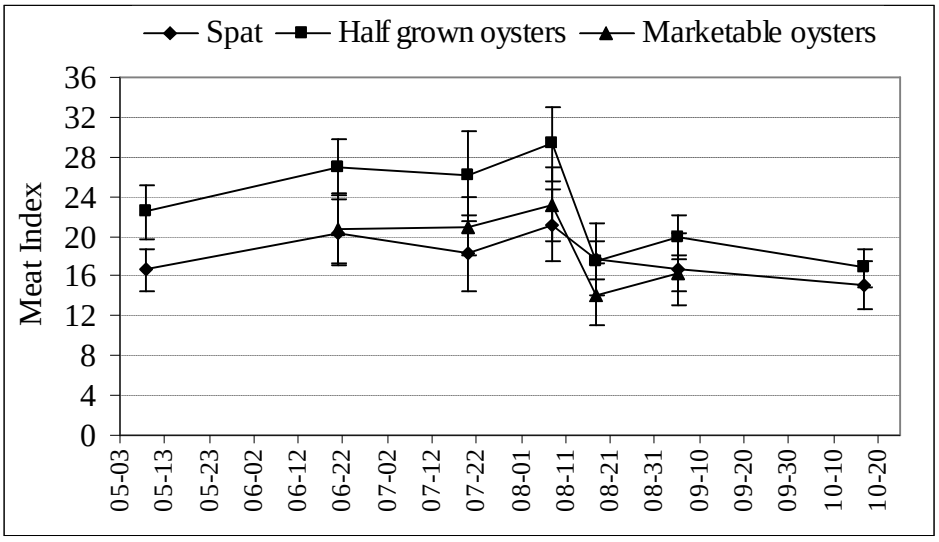
685 Fig. 5. Temporal variation of the gonado-somatic index (GSI) estimated by a) histology and
686 image analysis and b) ELISA technique for spat, half –grown and marketable female oysters
687 from May to October 2005.

688 Fig. 6. Correlation between oyster dry tissue weight (g) and fecundity (million eggs / female)
689 estimated from ELISA, for spat (a) and half-grown and marketable (b) oysters on August 8
690 2005 (n = 19). Spat: n = 9, $y = 1.05x + 0.02$, $R^2 = 0.94$; Half-grown oysters: n = 4, $y = 0.96x +$
691 0.08, $R^2 = 0.98$; Marketable oysters: n = 10, $y = 1.01x + 0.02$, $R^2 = 0.98$.

692



693
694 Figure 1a
695
696



697
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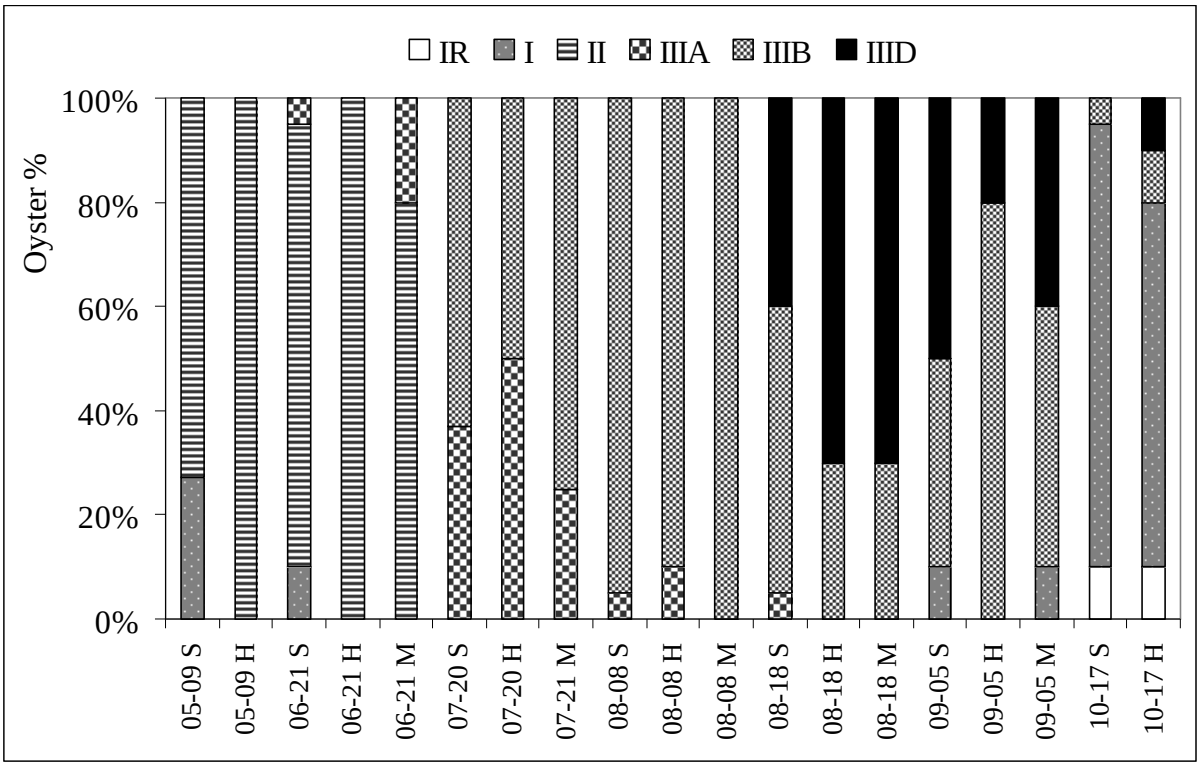
699 Figure 1b.



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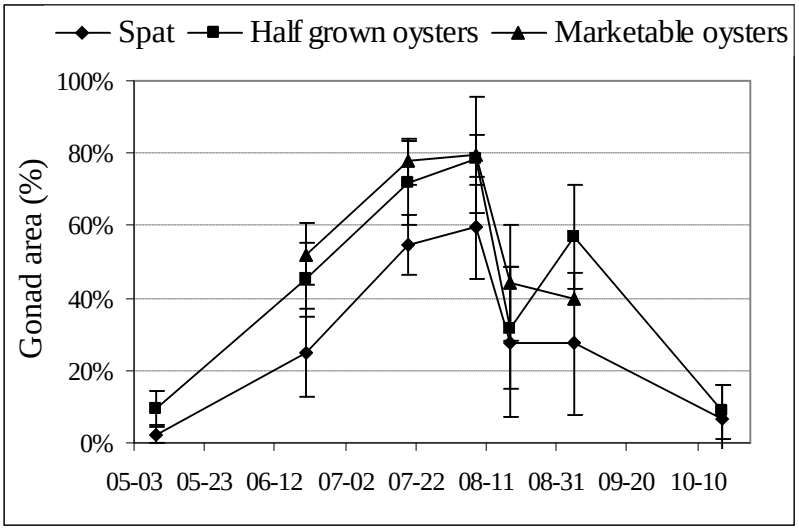

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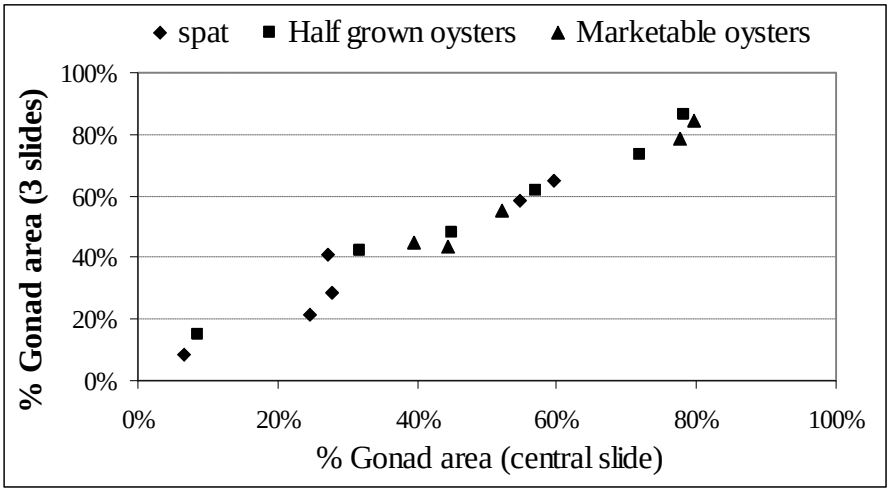

Figure 4.

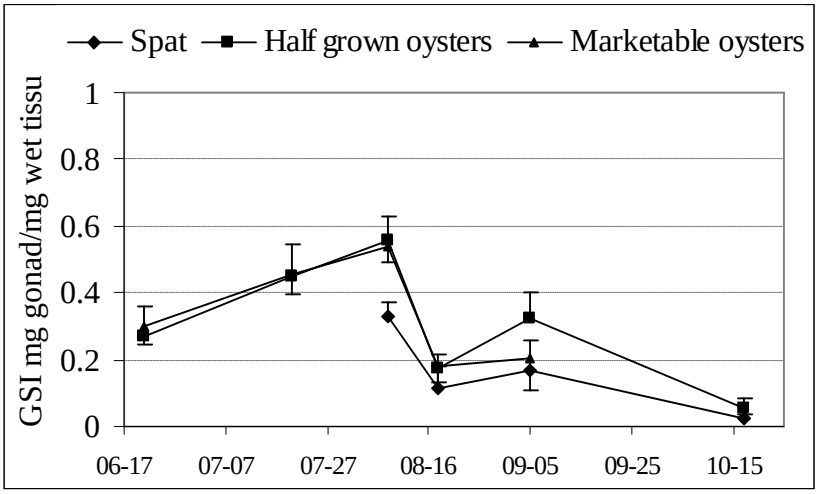

Figure 5a.

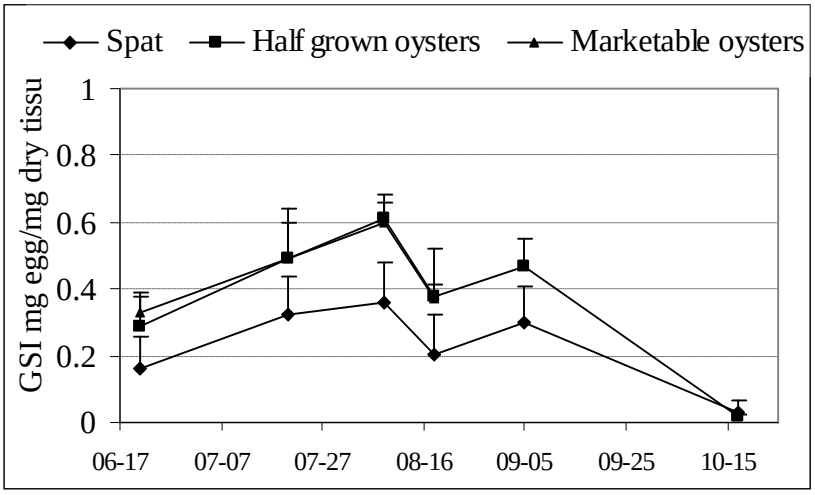

Figure 5b.

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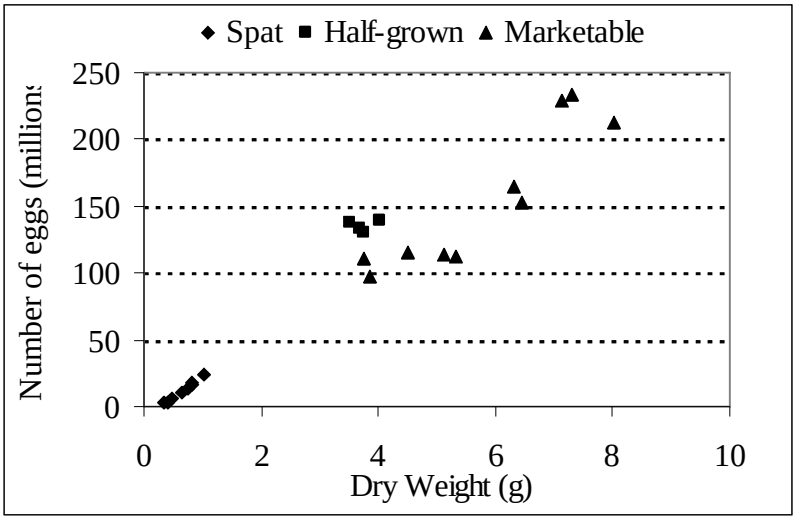

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727 Figure 6.

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