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Oocyte size, a means to evaluate the gametogenic development of the Pacific oyster, *Crassostrea gigas* (Thunberg)

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Abstract

This study reports on the results of 38,100 measurements of the oocyte diameters of the Pacific oyster, *Crassostrea gigas*, in three populations living along the French Atlantic shoreline. The analysis of oocytes' diameters and histological examinations of the gonads indicated that *C. gigas* individuals collected in 1996, 1997 and 1998 in the Bay of Brest (Anse du Roz and Pointe du Château) and Marennes-Oléron showed a clearly defined pattern of seasonal gametogenic development. Comparisons between reproductive stages, sites and years did not reveal significant differences in oocyte diameter ($^{*}P > 0.05$). Considerations from qualitative and quantitative characteristics of this species allowed us to propose a reproductive scale based on oocyte diameter and consisting of four stages: (1) ''Early gametogenesis'' with an oocyte diameter mean of 8.47 ± 4.6 μm, (2) ''Growing stage'' with a diameter mean of 21.4 ± 8.4 μm, (3) ''Mature stage'' with a mean of 36 ± 4.4 μm and (4) ''Degenerating stage'' with 46 ± 7.3 μm in mean diameter. This scale was generated from a modal distribution analysis (interactive program for fitting mixtures of distributions) and microscopic descriptions of gonad characteristics. The use of this scale for reproductive studies in *C. gigas* is discussed.

Keywords: Reproduction; Oocyte; Gametogenesis; *Crassostrea gigas*; Oyster

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1. Introduction

Since its introduction to France in 1969, the Pacific oyster *Crassostrea gigas* has been widely farmed along the French coasts (Chevalier et al., 1975). Its production from aquaculture has become a major industry activity producing 150,000 t in 1994 (Dauvin, 1997). Today national and regional resource management programs are directed at determining important reproductive factors for this species (Thouard, 1997). Within this scope, techniques to permit standardized and accurate quantitative estimations of gametogenic development, and make comparisons with similar studies, become of primary importance.

There are several means of assessing gamete development in bivalves. They mainly consist of: (i) visual observation in relation to the relative size, shape and color of the gonads (Mason, 1958); (ii) gonad index, the relative weight of the gonad to body weight (wet or dry) (Hughes-Games, 1977; Grant and Tyler, 1983a; Bodoy et al., 1986); (iii) mean oocyte diameter (Kennedy and Battle, 1964; Muranaka and Lannan, 1984); and (iv) developmental stages based on certain cytological characteristics (histology) (Quayle, 1969; Yakovlev, 1977; Steele, 1998). Histological techniques provide general information on gonad development. For example, the seasonal variations in the different stages of gametogenesis have been followed through histological examination allowing also the identification of any phenomenon liable to affect reproductive activity in bivalves (Paulet, 1990). Several researchers have correlated the developmental stages to certain cytological features generally recognized in all bivalve mollusks. Unfortunately, the determination of these stages is often subjective and there is little agreement as to criteria to establish the number of stages that should be included in the classification scale (Barber and Blake, 1991).

The methods used to evaluate gametogenesis in bivalves have advantages and drawbacks. Barber and Blake (1991) mentioned that the most complete approach would consist in applying at least two methods, each of them being either quantitative or qualitative; they also underlined that histology is always required to verify reproductive events concerning gamete development. However, at the present time there is neither a reproductive scale nor classification relying on the use of both methods: all the scales describing the gametogenesis are only based on qualitative features (Table 1).

This study aimed at describing the gametogenic development of *C. gigas* populations along the Atlantic shoreline of France from oocyte diameter with the help of image analysis and histology. This led us to propose a qualitative and quantitative reproductive scale.

2. Material and methods

2.1. Sampling

This study was performed with cultivated individuals sampled over 3 years (1996, 1997 and 1998) in three different places in Western France, the Bay of Brest (Anse du Roz and Pointe du Château) and the region of Marennes-Oléron, respectively (Fig. 1).

Table 1
Gametogenic development stages proposed for the genus *Crassostrea*

Species of genus *Crassostrea*	Developmental stages	Author(s)
C. gigas	Stages 0–5. Empty gonad that corresponds to sexual repose or the final period of gamete release Stage 1. Early activity of gametogenesis, gonia multiplication. Stage 2. Gonad well developed, but difficult gamete dissociation. Stage 3P. Great number of gametes with easy dissociation. Gonad begins to fill in. Stage 3H. Totally full gonad covering visceral tissue-abundant gametes. Stage 4. Release of gametes. Gonad becomes smaller, colorless and watery in appearance.	Marteil (1976), Le Dantec (1957, 1968)
	Stage 0. Undifferentiated. Stage 1. Developing-early activity. Stage 2. Developing-late activity. Stage 3. Ripped. Stage 4. Partially spent. Stage 5. Totally spent. Stage 6. Post-spawning. Stage 7. Resorption.	Mann (1979), Dinamani (1987), Steele (1998)
	Stage 1. Post-spawning. Stage 2. Reduction. Stage 3. Growth-and-maturation. Stage 4. Pre-spawning.	Yakovlev (1977)
	Stage 1. Small oocytes next to the wall of the follicles and stained in dark purple. Stage 2. Growing club-shaped oocytes partially filling the lumen of the follicle. Stage 3. Ova fill the lumen of the follicle.	Robinson (1992)
C. virginica	Inactive Developing and pre-spawning Partially spawned Advanced post-spawning Indifferent	Loosanoff (1942), Sakuda (1966)
	Stage 0. Follicles are nonexistent or elongated, with walls consisting of undifferentiated germinal epithelium. Stage 1 (Early active). Follicles contain oogonia or spermatogonia and primary oocytes or spermatocytes or no free oocytes or spermatozoa. Stage 2 (Late active). Secondary (free) oocytes and spermatocytes predominate in the follicles; there are some spermatozoa. Stage 3 (Mature). Mature gametes(ova or spermatozoa) totally filling the follicles; presence of ova	Kennedy and Battle (1964), Barber (1996)

(continued on next page)

Table 1 (*continued*)

Species of genus *Crassostrea*	Developmental stages	Author(s)
C. virginica	with distinct nucleus and nucleolus, spermatozoa oriented with tails toward the follicle lumen. Stage 4 (Spawned). Follicles contain species devoid of gametes, although numerous gametes may still remain, follicles walls may be broken. Redevelopment as evidenced by increased number of primary oocytes or spermatocytes. Stage 5 (Reabsorbing). Follicles have a shrunken appearance and contain numerous phagocytes and products of reabsorption. Gametes are refractory and development is not evident. Indifferent/inactive. Oysters with little or no follicular material present, making difficult sex determination in some cases. Early Developing. The expansion of the follicle and the appearance of well-defined spermatogonia or oogonia along the follicle wall. A central lumen is present in each follicle. Late Developing. The maturation of gametes is evident. Some ripe gametes appear in the central lumen. Ripe. Female with many mature, spherical oocytes, 45-50 μm in diameter, that appear to be free within the follicular lumen. Male oysters having spermatids radiating toward the center of the follicle where they arrange themselves in radial columns Spawning. Reduced numbers of mature gametes in the follicles. Spent. Oysters with ruptured follicles and residual gametes. Reabsorption of the gametes, and reinvasion of the follicles by follicular cells ensues.	Brousseau (1987, 1995)
C. echinata	Stage I (Resting or inactive). Sex-indeterminate follicles degenerate; they are empty or contain occasional residual gametes. Stage II (Developing). from early phases when follicles contain gonial stages to later ones when interfollicular connective tissue disappears and follicles contain all gametogenic stages, including some sperm in males and free oocytes in females. Stage III (Morphological ripe). Male follicles distended with sperm which may form a plug in the lumen, sometimes with a narrow band of earlier spermatogenetic stages on wall; female follicles packed with free oocytes in the lumen and some stalked oocytes on wall. Stage IV (Spawning). Follicles with emptied lumen, gametes present, connective tissue reappears between follicles.	Kennedy (1977), Lopez and Gomez (1982)

Table 1 (*continued*)

Species of genus *Crassostrea*	Developmental stages	Author(s)
C. echinata	Stage IVa (Early gametogenic). Stages on follicles walls, residual gametes in lumen, follicles generally do not occupy as much space as in developing gonads. Stage V (Spent). Collapsed follicles, may contain pycnotic gametes, absence of gonia indicates return to resting stage. Stage 0. Resting or spent gonad, inactive, considerable connective tissue, presence of amoebocytes. Stage 1. Beginning gametogenesis; oogonia, spermatogonia present from no ripe gametes to first ripe gametes, amoebocytes still present. Stage 2. Increase in gonad mass up to 1/2 or 2/3 fully ripe condition; follicles with about equal proportion of the ripe and developing gametes. Stage 3. Up to fully ripe condition, early stage of gametogenesis reduced, when full oval polygonal and compacted, or sperm ripe with distend follicles. Stage 2r. Post-spawning-regressive, partially emptied gonad, residual gametes left, amoebocytes may be present, interfollicular connective tissue beginning to reappear. Stage 1r. Post-spawning advanced regressive, few residual gametes remained, amoebocytes present and considerable connective tissue.	Braley (1984)
C. iridescens	I. Inactivity. II. Pre-reproductive or maturation. III. Reproductive or spawned (partial or advanced spawning).	Ruiz-Durá (1974)
C. rhizophorae	Stage I. Indeterminate. Mo. Male or female. V. Virginal. D. Considerable connective tissue and shrunken follicles. M. Mature. Ep. Partial spawning. Ec. Complete spawning.	Vélez (1976, 1977)
C. cucullata	Stage I. Recovering/regression. Stage II. Early gametogenesis/early growing. Stage III. Late growing/mature. Stage IV. Recently spent.	Nagabhushanam and Bildarkar (1977)
C. glomerata	Early gametogenic phase (d1–d3). Indifferent gonia mostly, few definitive oocytes 1, 2 and 3. Phase of maturation of gonad (d4–d5). Oocytes 3 mostly, few mature ova and mature ova.	Asif (1979)

(continued on next page)

Table 1 (*continued*)

Species of genus *Crassostrea*	Developmental stages	Author(s)
C. glomerata	Regression or spawning phases (r1–r2). Partially empty follicles with mature ova and oocytes 3. Indifferent phase (i). Occasionalova.	
C. madrasensis	D. Developing[a] R. Regression[a] I. Indeterminate	Joseph and Madhyastha (1984)

[a] D or R are in different grades of gonadal development or regression (grades; 1, 2, 3 and 4).

The sampling procedure was conducted every 15 days over 2 years in Anse du Roz (February 1996 to December 1997), and every 30 days for the oysters from Marennes-Oléron (March 1997 to July 1998) and Pointe du Château (March 1997 to December 1998). Each time, 10 female oysters were collected per site, fixed in Bouin's solution and processed for histological examination. A standard section of the visceral mass was taken at the intersection of the mantle and gonad, and was dehydrated through an increasing ethanol concentration series. Dehydrated samples were cleared and embedded in paraffin following a standardized procedure. Sections 5 μm thick were cut, mounted on glass slides and stained with a solution of Groat's Hematoxylin and Eosin Y (Martoja and Martoja-Pierson, 1967). The specimens were examined under a microscope for determining oocyte size/frequency and gametogenic activity, then the recorded images were processed by digital image analysis.

Several samples from six populations along the French Atlantic shoreline; Arcachon, Aber Benoît, Baie des Veys, La Trinité, Bouin and La Tremblade (Fig. 1), were collected in December 1998, February and April 1999; they were treated according to the procedure described above. The mean oocyte diameters were compared with those of the three cultured populations.

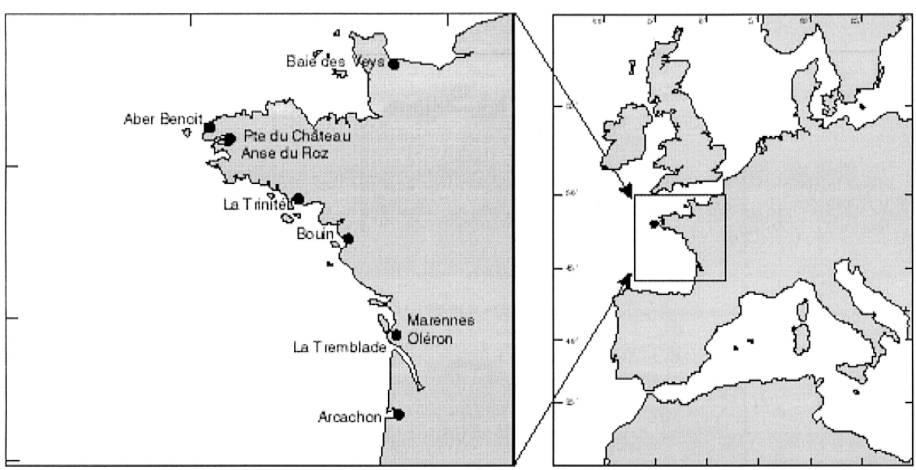

Fig. 1. Sampling sites during this study of *C. gigas.*

2.2. Measurements

The histological slides were observed under a Leica microscope at two magnifications (40× and 100×) depending on the oocyte size. A code was assigned to each image recorded with a video camera (Sony), then processed by image analysis (Silicon Graphics station; Software Visilog 5.1). The surface of each oocyte was measured by drawing its perimeter on the computer screen to calculate an area in «pixels» (s) that was transformed into a theoretical diameter (D) expressed in μm using the relation ($D_{\text{theoretical}}\sqrt{4S/\pi}$) established from a previous calibration. The measurements were conducted only in the oocytes that displayed a well-defined germinal vesicle to ensure

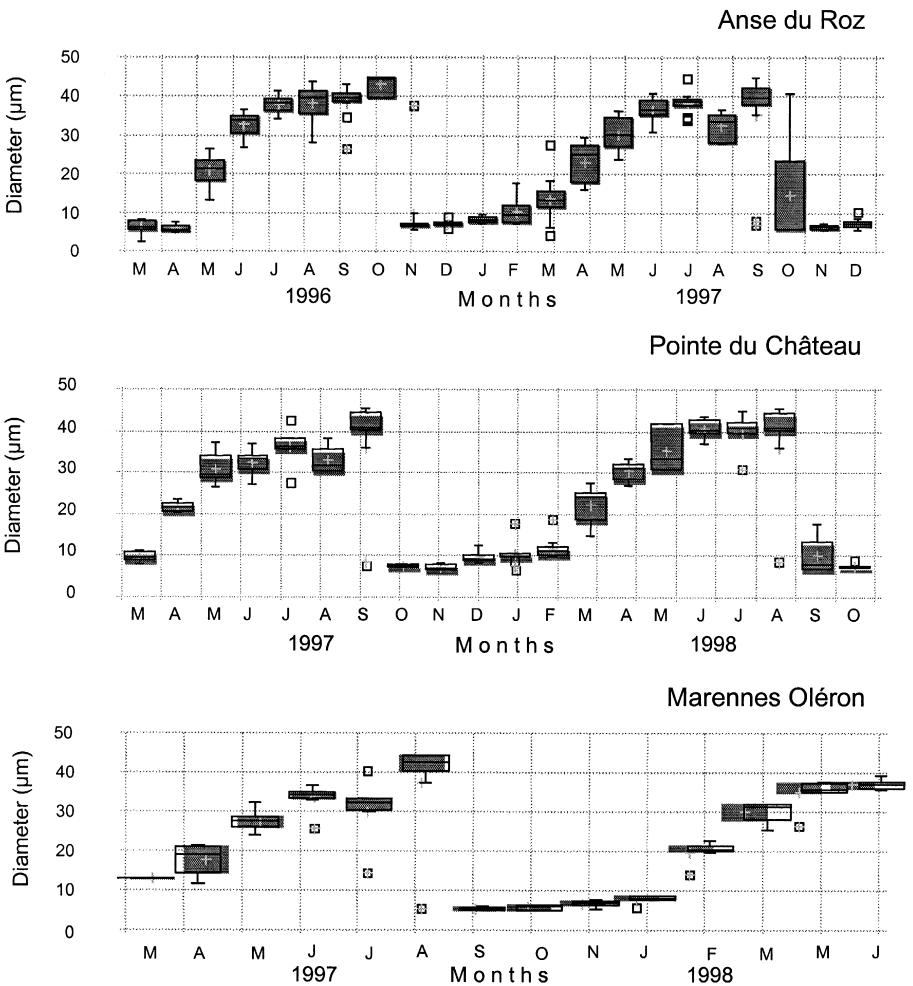

Fig. 2. *C. gigas*. Seasonal variations of oocyte diameter (mean ± S.D.). Oysters sampled at Anse du Roz (1996–1997) and at Pointe du Château and Marennes Oléron (1997–1998).

that each section passed through the center of the gamete. This operation was carried out on 100 randomly chosen oocytes per sample.

2.3. Statistical analysis

For each individual a frequency histogram was computed and analyzed by running an interactive program for fitting mixtures of distributions (Macdonald and Green, 1988) to identify the different percentages in oocyte diameter within a given organism (modal analysis).

The mean oocyte diameter and standard deviation were computed for each organism. Then, the sample means per month and the standard deviation of means about the sample means were calculated (Grant and Tyler, 1983b). Using one-way ANOVA ($^{*}P < 0.05$), the mean oocyte diameters of each sample were compared for each gametogenesis stage and site.

This analysis allowed us to identify different stages of oocyte development vs. time. From these results, we made a four-reproductive-stage classification for *C. gigas* in relation to oocyte diameter. To assess this scale, an F_{α} test ($^{*}P < 0.01$, $^{*}P < 0.05$) was performed comparing the mean oocyte diameters of the oysters from the six natural populations previously mentioned.

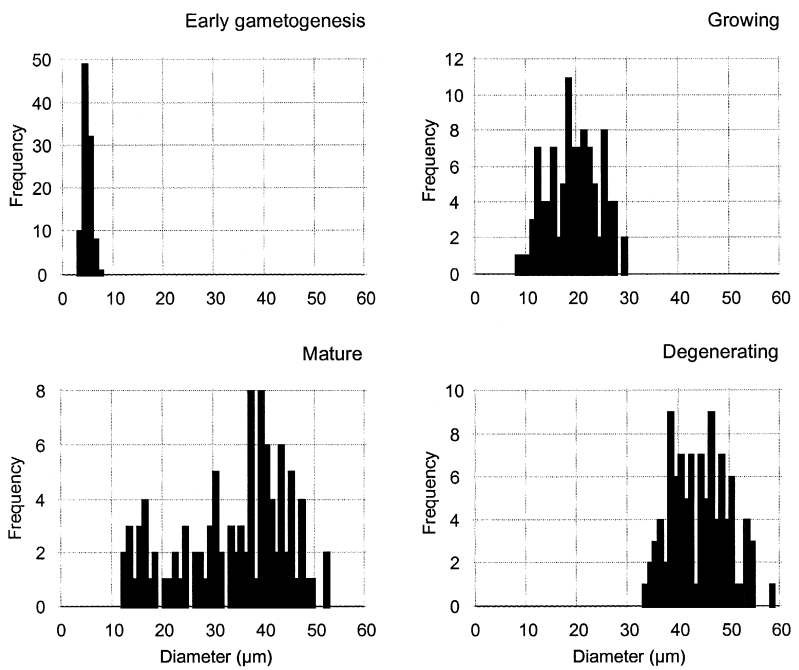

Fig. 3. *C. gigas*. Individual temporal changes in size-frequency distributions of the oocyte diameters characterizing the four stages of reproduction discerned in this study.

Table 2
C. gigas. Modal analysis of the oocyte size-frequency from the three populations studied

Modal Class (MC)	Oocyte percentage in MC	$X \pm$S.D. (μm)[a]	*n*
Early gametogenesis	100	6.5 ± 1.19	12,200
Growing	50	21 ± 1.08	9300
	50	34 ± 1.62	
Mature	90	40 ± 0.04	12,000
	10	19 ± 4.6	
Degenerating	70	5.5 ± 1.0	4000
	30	46 ± 3.6	

[a] Mean ($\pm$S.D.) per modal class.

2.4. Histological analysis

Reproductive scales established by Kennedy and Battle (1964) for *C. virginica* and Mann (1979) for *C. gigas* (see Table 1), were used to summarize the histological characteristics observed at each reproductive stage found in this study.

3. Results

3.1. Oocyte diameters

A total of 38,100 measurements of the oocyte diameter of *C. gigas* from the three populations studied were carried out; they corresponded to oysters from Anse du Roz (18,600), Pointe du Château (12,100) and Marennes-Oléron (7400). The mean oocyte sizes for each population are presented in Fig. 2. The oysters from the three sites followed the same diameter distribution. The proliferation of primary oocytes over the 2 years of study started in November and continued until March; the mean oocyte diameter in this period for the three populations was 8.5 ± 4.6 μm ($n = 12{,}200$). Oocyte

Table 3
C. gigas. Reproductive scale based on the oocyte diameter

Stage	Stage interval (μm)	Histological description
Early gametogenesis	3.0–12.0	Follicles are elongated and often isolated in the abundant connective tissue, with walls consisting of primary oocytes of homogeneous size.
Growing	12.1–30.0	Start of growth, a large interval of different oocyte sizes in all gametogenic stages can be observed including some free oocytes. Interfollicular connective tissue disappears.
Mature	30.1–41.0	Follicles completely filled with mature oocytes with distinct nucleus (relatively homogeneous size).
Degenerating	41.1–60	Follicles containing degenerating oocytes, often elongated in shape, sometimes broken. Obvious redevelopment indicated by increased number of primary oocytes.

growth was observed from March–April to June and the mean oocyte diameter was then 21.4 ± 8.4 μm ($n = 9\,300$). Between July and September at Anse du Roz and Pointe du Château, there were mature oocytes with a mean diameter of 36.1 ± 4.4 μm ($n = 12\,000$). After this period, degenerating oocytes with a mean diameter of 46 ± 7.3 μm

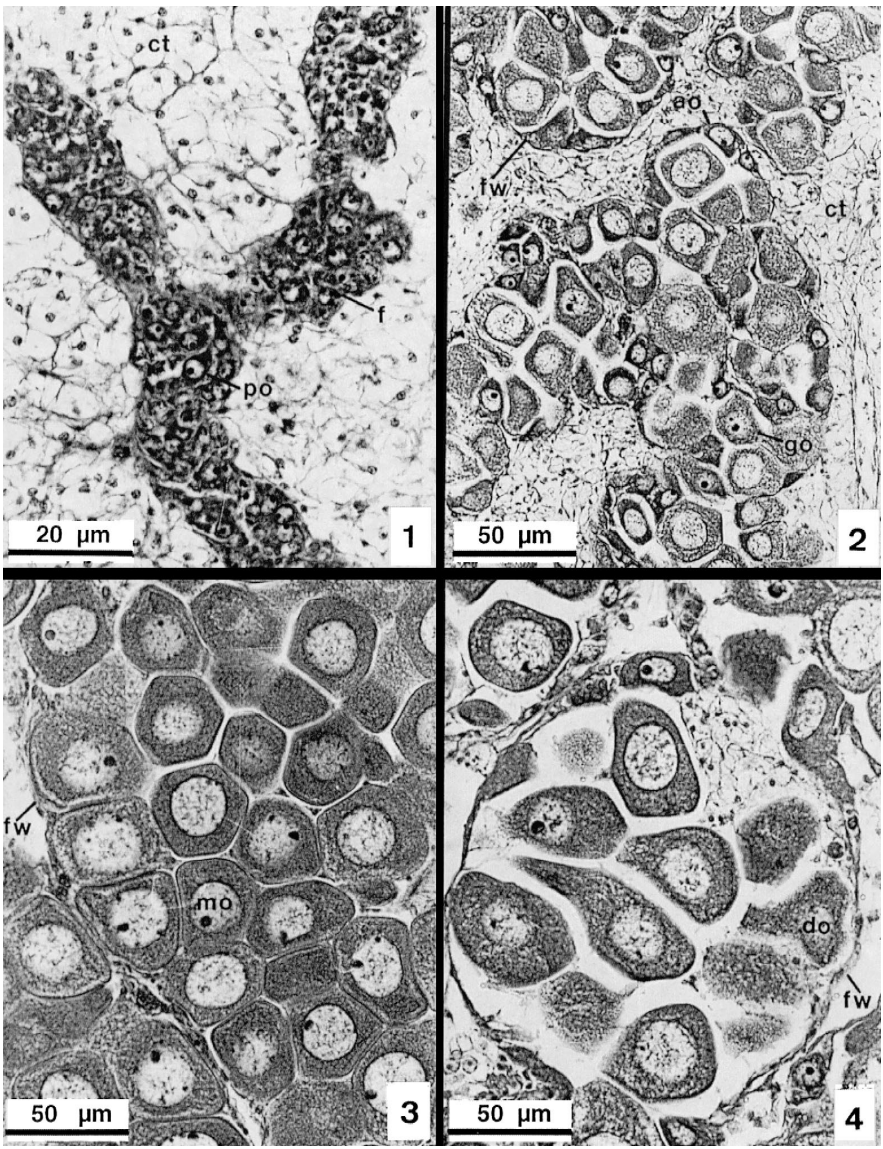

Fig. 4. *C. gigas*. Histological sections showing the reproductive stages described in this study; (1) Early gametogenesis stage (100×), (2) Growing stage (40×), (3) Mature stage (40×) and (4) Degenerating stage (40×). Adhering oocyte (ao), connective tissue (ct), degenerating oocyte (do), follicle (f), follicle wall (fw), growing oocyte (go), mature oocyte (mo), primary oocyte (po).

($n = 4\ 600$) were observed in oysters from Anse du Roz in September–October 1996 and 1997, in September 1997 and August 1998 at Pointe du Château, and from Marennes-Oléron in July–August 1997.

Temporal changes in the size frequency distribution of the oocyte diameters for each individual revealed four distinctive distributions throughout the gametogenic development of this species. Fig. 3 shows an example of each characteristic distribution associated with the results of modal analysis.

3.2. Modal analysis

A modal analysis of the oocyte size-frequency in the studied populations was performed; the modal classes obtained are presented with their respective percentages in Table 2. The first class corresponded to ''early gametogenesis'' with a single mean of 6.5 ± 1.2 μm. In the second class, i.e. ''growing stage'', two groups in a 1:1 ratio were found with means of 21 ± 1.1 and 34 ± 1.6 μm, respectively. The third and fourth classes also showed two groups: in the ''mature stage'' the mean for 90% of the oocytes was 40 ± 0.0 and 19 ± 5 μm for the remaining 10% of the cells, and in the last class (''degenerating stage'') there was a 7:3 ratio with means of 5.5 ± 1.0 to 46 ± 3.6 μm, respectively.

Once the characteristics of each group had been identified in relation to temporal changes and modal analysis, a gametogenesis scale for *C. gigas* was established (Table 3). Fig. 4 describes the principal characteristics observed by microscopy in the gonad for each stage.

3.3. Tested populations

To corroborate whether this classification may permit one to correctly evaluate the reproductive stage in natural populations, the proposed scale was tested on data collected

Table 4
C. gigas. Classification of six populations from the French Atlantic coasts according to the reproductive scale based on the oocyte diameter. Number of individuals per reproductive stage; E — Early gametogenesis, G — Growing, M — Mature and D — Degenerating

Populations	Sampling periods											
	December 1998				February 1999				April 1999			
	E	G	M	D	E	G	M	D	E	G	M	D
Aber Benoît	1	4	1	1	6	1				5		
Arcachon	8				7				1	6		
Baie des Veys	7	1			9					4		
Bouin	9				7				1	7		
La Tremblade	10				4					4		
La Trinité	8	1			8				1	3		
Mean (μm)	4.6	21.8	36.0	41.2	5.9	13.5			5.12	15.3		
Oocytes measured	4300	600	100	100	4100	100			300	2900		

from the six natural populations previously mentioned. A reproduction stage was assigned to each individual on the basis of their size-frequency distribution and modal analysis (Table 4). Then, an ANOVA test was performed to compare the oocyte mean for each animal vs. the characterized mean of each stage of the proposed scale. No significant differences ($^{*}P < 0.05$) were observed between the two sets of data.

4. Discussion

The analysis of female developing stages requires considerations from both qualitative and quantitative data, i.e. histology is necessary to describe the reproductive events pertaining to gamete development, and quantitative estimates are important because they both eliminate the subjectivity and semantic problems associated with description and tend to provide ecologically meaningful information (Barber and Blake, 1991). Several investigators have used stage characterization as additional information to quantitative measurements. Histological examinations, which include quantitative measurements of gonadal matter, should be a part of any study on the reproduction of bivalve mollusks because they provide detailed information not available otherwise (Dinamani, 1987; Morales-Alamo and Mann, 1989).

In relation to the qualitative histological characteristics of the female gonad (as well as the male developing features observed in this study), we found that they are similar to those reported in previous studies dealing with the same species, in particular, they are similar to those made by Quayle (1969) in the waters of British Columbia (Canada), Yakovlev (1977) in the sea of Japan, and Steele (1998) in Ireland. So, further descriptions are not needed.

Sastry (1979) working on Pectinidae has correlated the average oocyte size with the stages of gametogenic cycles in *Argopecten irradians*. Ruiz-Durá (1974), Morales-Alamo and Mann (1989) and Frias-Espericueta et al. (1997) made a reproductive scale for *Ostrea corteziensis*, *C. virginica* and *C. iridescens*; it was based on two categories, maturation and spawning in relation with the oocyte diameter. A similar classification was performed by Lubet and Allarakh (1994) for *Saccostrea cucullata*, but this scale was based on the age of the oocyte and considered that young oocytes had a diameter below 50 μm, whereas old oocytes were generally bigger ($^{*}P > 50$ μm).

The results of oocytes diameter analysis and histological gonad examinations from a previous study had indicated that *C. gigas* samples collected at Anse du Roz between March 1996 and December 1997 showed a clearly defined seasonal reproductive cycle (Lango-Reynoso et al., 1999). In the study reported here, the populations from Pointe du Château and Marennes-Oléron had the same reproductive pattern over the same period, the entire cycle of this species can be split into four well-distinctive stages from the temporal changes of the oocyte diameter; i.e. early gametogenesis, growing, mature and degenerating stages.

In the early gametogenesis stage, the whole oocyte diameter population had a homogeneous modal class (6.5 ± 1.2 μm). Unfortunately, most published data do not show the minimum oocyte diameter for comparison purposes; indeed, there is no information available dealing with Ostreidae for oocyte sizes from reproductive cycles,

these are reported from the pre-reproductive or mature stage only. In the present study, the oocyte diameter was similar to that reported by Beninger and Le Pennec (1991) in a previous study where they showed that stem cells of several Pectinidae rising into primary oogonia had a mean diameter of 8–10 μm.

Early gametogenesis stage was observed from October/November to March for the three populations. This corresponds to winter months when water temperature is low. According to our observations it is the time when germinal cells produce young oocytes that remain in a ''stand-by'' stage (homogeneous mean) until the temperature increase and greater availability of food at the beginning of spring. These observations are similar to those made in several temperate Pectinidae like, for example, in *A. irradians* in which the primary germ cells and gonial cells are developing in winter and early spring (Sastry, 1970).

The Growing stage was observed in April–June for the three populations studied; this corresponds to the highest gametogenic activity. In this stage, there was a re-activation of the young oocytes development. At the beginning of this period, oocyte size started to increase, but in a heterogeneous way, and so oocytes of very different sizes occur at the same time. This stage showed two modal classes (50% each) 21 ± 1.1 and 34 ± 1.6 μm. In this respect, Heffernan et al., (1989) mentioned that measurements of oocyte diameter were of limited use when dealing with early gametogenic development as there can be large variation in oocyte diameter in individual of the same gametogenic stage. Nevertheless, the modal analysis exercised in this study allowed the identification of overshadowed modes of the population frequency distribution. So, it was possible to determine reliable parameters (mean, standard deviation and percentage of oocyte diameter) to establish the intervals proposed in this study.

As mentioned before, only a few reports deal with oocyte diameter in bivalves for the first stages of reproduction. In terms of comparison, *O. edulis* produces oocytes between 40 and 70 μm during Growing stage (Román, 1992), but it is clear that there are important differences between species since *O. edulis* generates larger oocytes than *C. gigas* through the gametogenic cycle.

Although in the growing stage the oocyte diameter shows high heterogeneity, there is a trend to oocyte size standardization when oysters reach the mature stage. At the beginning of this period, 90% of oocyte population presented a modal class of 40 ± 0.0 μm, the rest can be ranged in a second mode of 19 ± 4.6 μm. This standardization trend seems to focus onto spawning synchronization purposes. However, this phenomenon is not exclusive of *C. gigas*. Toba et al. (1993) and Laruelle et al. (1994) have noticed that individuals of Veneridae species, *Ruditapes decussatus* and *R. philippinarum*, generally seem synchronized during the first maturation, certainly due to temperature increase. This synchrony is lost during spawning because some clams are ripe while others have already spawned.

Burlot et al. (1998) mentioned that maximum oocyte diameters for *C. gigas* in Marennes-Oléron are between 60 and 70 μm. For this same location and for the mature stage we determined a mean diameter of 34.9 ± 9.8 μm with a maximum of 61.4 μm for 3.3% of population. These differences are not easily comparable since these authors did not report on the means employed for assessing gametogenesis. In another study of *C. gigas* in the sea of Japan, Yakovlev (1977) has reported that at later stages of

gametogenesis, some oocytes became pear-shaped and reached 80 μm in length. The larger oocytes observed by this author were probably due to environmental and genetic differences between populations which could have resulted in variations of gametogenic process for the same species.

The degenerating stage was observed from the end of August or the beginning of September until the end of October; there were two modal classes, 5.5 ± 1.0 and 46 ± 3.6 μm, which correspond to 70% and 30% of the oocyte population, respectively. In the degenerating stage we noticed that, after the spawning period, the gonad displayed two oocyte types, i.e. unreleased cells and the cells resulting from oogonia multiplication (new oocyte generation). Resting oocytes are reabsorbed within the gonad by a lysis process usually described as oocyte atresia. This process has been detailed in several bivalve species (Lubet et al., 1987; Lubet and Mann, 1987; Pipe, 1987; Dorange and Le Pennec, 1989a; Dorange and Le Pennec, 1989b; Beninger and Le Pennec, 1991; Steele, 1998).

The tests used to asses our scale in relation to the different population studied, showed that an oyster classification into one of the four reproductive stages described here was possible from the mean and frequency distribution. This result was also confirmed by the modal analysis that even allowed determination of the modal percentage distribution. During the study periods reported here (December 1998, February and April 1999), the oocyte distributions for each individual presented a mode tendency that could be assigned to the corresponding reproduction stage. It was clear that within a population there were different tendencies at the same time that did not allow their classification in group, requiring always an individual analysis. For example, during December the oocyte distributions from the Aber Benoît population corresponded to the four reproductive stages reported in this study (Table 4). This particular case can be related to alterations in the reproductive cycle in this geographical area. Indeed, it seems that during the reproductive season these oysters do not spawn; they apparently retain their gametes, which are reabsorbed afterwards within the gonad. Unfortunately, until now there is no available information about this phenomenon. Nevertheless, almost all oysters from other populations presented an oocyte distribution that corresponded to a single reproductive stage according with the natural season variation.

Even if the methods reported here may be considered labor-intensive, we believe this study presents valuable methodology to investigate the gametogenic development of *Crassostrea* species and potentially other bivalves. The scale proposed constitutes an approach that combines both qualitative and quantitative attributes not found in others studies. These characteristics allow an individual evaluation of reproductive stages in *C. gigas*, and quantitative comparisons with similar studies.

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