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ORIGINAL ARTICLE

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Spatio-temporal variations in biological performances and summer mortality of the Pacific oyster *Crassostrea gigas* in Normandy (France)

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Abstract Mortality and biological performances of half-grown *Crassostrea gigas* were studied from spring 2000 to autumn 2001 at six instrumented stations located in two areas (Gefosse and Grandcamp) of the Bay of Veys (Normandy). Shell and meat growth, condition indexes and a macroscopic maturity index were determined on oysters deployed at the six stations in order to assess spatial variability in the influence of environmental conditions. In addition, histological and biochemical analyses were performed in order to determine the sex and establish the reproductive cycle (at all six sites) and the biochemical composition (at four stations). The data set including monthly mean temperatures and data provided by examination of 2,837 oysters were analysed by Principal Component Analysis and a Hierarchical Ascending Clustering which resulted in the formation of four clusters. The highest station on the shoreline belonged to a cluster characterized notably by low total weight due to a short immersion/feeding period, whereas all other stations belonged to another single cluster. Nevertheless, various biological differences were found between these stations, e.g. the reproductive cycle was generally synchronized throughout the bay but some differences relative to spawning occurrence were observed. In 2000, oyster mortality was higher at Gefosse than at Grandcamp, the latter being a more marine area. In 2001, oyster mortalities were significantly higher and all stations were strongly affected. In the Bay of Veys, oyster biological performances and mortality thus showed spatio-temporal variations which were worthy to be discussed.

Keywords *Crassostrea gigas* · Mortality · Reproduction · Growth · Condition index · Biochemical composition

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Introduction

The Pacific oyster, *Crassostrea gigas* (Thunberg), was introduced into France in 1967 (Grizel and Héral 1991). In the Bay of Veys (Normandy), oyster farming began in 1968, and in 2000, 160 ha of the eastern side of the bay were reserved for shellfish farming including 130 ha for oyster farming (10,200 tons per year). In France, 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 northwest France (e.g. Brittany), spawning and/or larval survival are not sufficient to allow the maintenance of sizeable natural populations. Despite the economic importance, limited data is available concerning the biological performances (growth and reproduction) of oysters in the Bay of Veys, and oysters have not been the subject of a sustained study considering spatio-temporal variability.

Mass mortalities of Pacific oysters have occurred since the 1950s in different countries including Japan and the United States (Glude 1975; Mori 1979; Lipovsky and Chew 1972; Perdue et al. 1981) but no causative agent was elucidated. Mortalities were associated with areas of high productivity, high nutrient levels, and water temperature exceeding 20°C and coincided with the period of maximum gonad condition for spawning (Beattie et al. 1980). In France, summer mortality affected juvenile and adult oysters sporadically along the French coasts since the 1980s (Maurer et al. 1986;

Bodoy et al. 1990; Soletchnik et al. 1999). Mass mortalities have been recorded in the Bay of Veys since 1994 (Goyard 1996) and adult losses reached 35% in 1997 and 51% in 1998 (Fleury et al. 1999). The Bay of Veys originality is due to the fact that oyster mortality is generally not synchronous with that in other French shellfish basins, and that juveniles are less affected than older oysters. All these mortalities threaten commercial production and require investigation.

We conducted experiments between February 2000 and August 2001 with the following objectives: (1) to study the spatio-temporal variability of adult oyster mortalities and biological performance on a 130 ha oyster bank in the Bay of Veys; (2) to provide a preliminary assessment of the influence of environmental factors (particularly temperature) on oyster mortality and biological performance.

Material and methods

Study area and site selection

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 (Fig. 1). Four rivers lead into the Bay of Veys and this bay, with an agricultural catchment basin of 3,400 km^2, comprises 37 km^2 of intertidal area covered mainly by fine sediments. The estuary is macrotidal with a tidal range up to 8 m and inside the bay seawater dominates freshwater (Colin 1999).

Six study stations distributed throughout the bay were chosen in order to assess the spatial variability of environmental conditions and oyster losses (Fig. 1). Stations GR1 and GR2 were located in the oyster growing area named Grandcamp while station CB was set in the centre of the oyster bed, and stations GE1, GE2, and GE3 were in the zone named Gefosse. Stations GR1 and GR2 were farthest from the estuary and least affected by river water (Table 1). At the beginning of flood tide, water enters the shellfish culture area through two "filling channels" which are filled with a mix of seawater and freshwater, even if the latter is relatively insignificant. In contrast to other stations, GR1 and GR2 were influenced only by seawater from the open sea. The substrata were heterogeneous, including rocks, at GR1 and GR2, and mud and fine sands at the other four stations.

Reared oysters

In the Bay of Veys, Pacific oysters are off-bottom cultivated in culture bags placed onto iron tables. These oysters, originating from natural spat, had initially been reared on the west coast of the Cotentin peninsula (Normandy) and were then deployed in the Bay of Veys in February 2000, at half-grown stage and at a mean whole weight of 30 g. At the beginning of the study, oysters were weighed and shared out in order to constitute homogeneous batches (ANOVA; $P = 0.09$). At each station, 1,595 oysters were deployed ($N_{total} = 9{,}570$), 185 oysters per bag except for three half-bags with 100 individuals which were devoted to the study of mortality.

Field assessments

At CB and GE2, YSI oxygen-temperature-salinity and pH meters were used to measure continuously these water quality parameters, and a data set with a frequency of 15 min was obtained. At each station, water and air temperature were recorded continuously using a Micrel temperature-salinity and pressure probe; a reading was taken every 10 min.

At each station, the progression of oyster mortalities was followed by means of semi-monthly to monthly field observations in triplicate half-bags ($N = 3{\times}100$). The original density of each half-bag was maintained by replacing the animals removed with others stockpiled in nearby bags, planted at similar densities.

Mean daily mortalities (MDM) were assessed using the formula: $(N_d/N_t){\times}100/(t2-t1)$ where N_d represents the number of dead or moribund oysters, N_t the number of (live) oysters at the beginning of the interval and $(t2-t1)$ the interval between two successive countings. Cumulative mortalities (CM) were calculated by the following equation: [1−(survival rate at $t2$×survival rate at $t1$)]×100.

Biological parameters monitored in the laboratory

At each study station, 30 oysters were randomly sampled monthly in 2000; in 2001, only six study dates were retained from January to August. In the laboratory, oysters were cleaned, weighed to the nearest 0.01 g, scratched to remove any attached epifauna and reweighed in order to determine the whole weight (WW) and, by subtraction, the fouling weight (FW). Shell length (L), width (w), and thickness (T) were measured to the nearest 0.01 mm using a vernier caliper. The thickness coefficient (TC) was determined with the following equation: $TC = 2T/(w+L){\times}100$. Each oyster was then opened and the soft tissue was examined for the allocation of a macroscopic Index of Sexual Maturity (SMI). Stage 0 was attributed to thin oysters, 0.5 to partially thin oysters (with digestive gland partially visible), 1 to fleshy individuals (digestive gland hidden by the gonad), 2 to ripe oysters (which seem "veined"), and 1.5 to post-spawning oysters or oysters in the process of gonadal resorption (tissues appearing heterogeneous). Wet soft tissue was retained for 15 min on a sloped plane covered with absorbent tissue paper. Superficially dried tissues were weighed and frozen and then dry

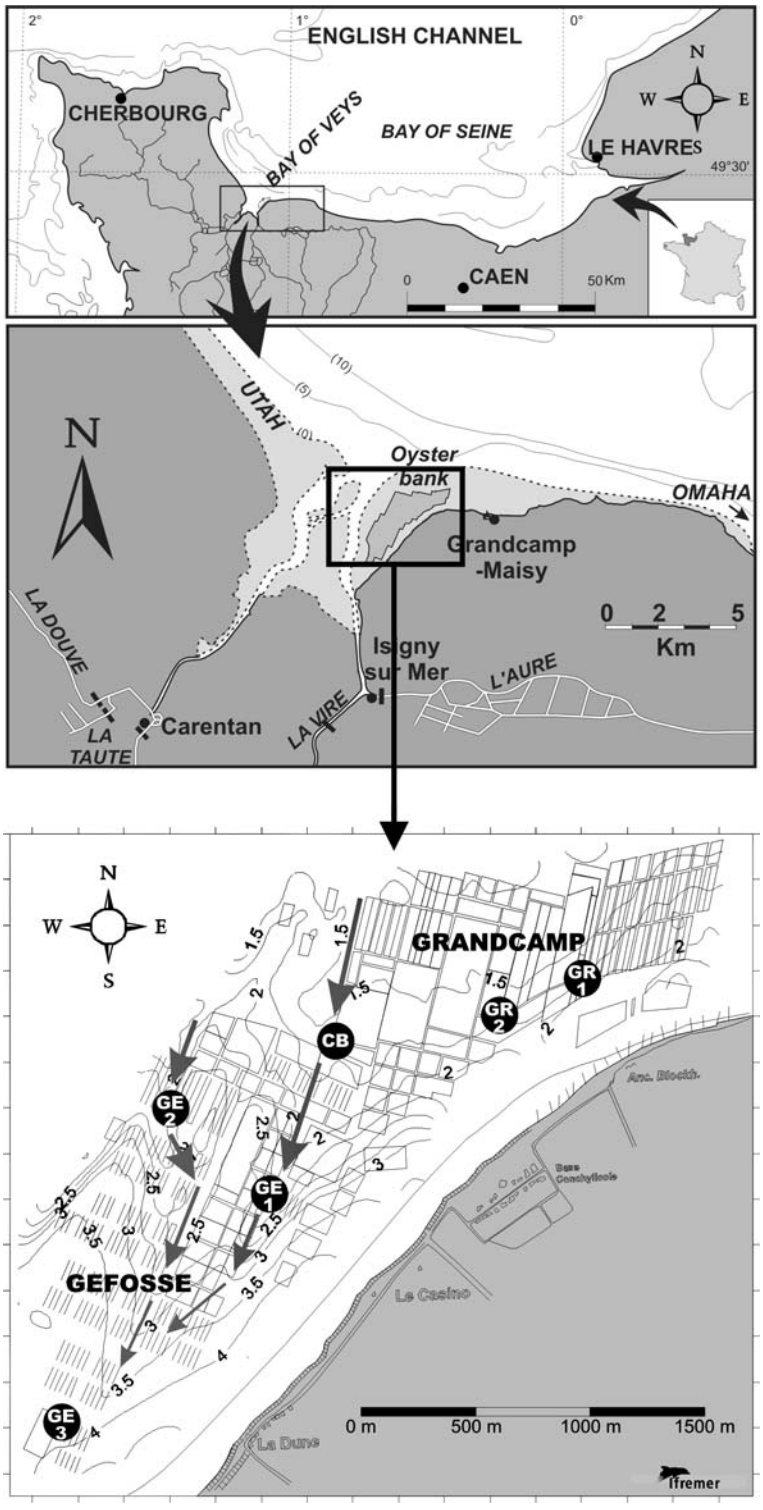

Fig. 1 Geographical localization of the study area and the six sampling stations in the Bay of Veys. *Arrows* indicate the two "filling channels" (one near GE2 and the other close to CB and GE1) through which water comes to the shellfish culture area at the beginning of flood tide

tissue weight was determined after freeze-drying for a minimum of 48 h. Shell valves were weighed after drying in a furnacle for 24 h.

For both shell and soft tissue, growth rates between the sampling dates were calculated using the general formula: $(\ln Wt2\text{-}\ln Wt1)\times 100/(t2-t1)$ where W represented weight and $(t2-t1)$ the interval between the two samples considered. Two condition indexes were used to determine the quality or "fatness" of oysters. Firstly, a Condition Index (CI) was calculated from the dry meat weight and shell weight according to the formula CI = dry meat weight (mg)/dry shell weight (g) (Walne and Mann 1975). Another condition index (MI or Meat Index), commonly used by French scientists and oyster

Table 1 Geographical coordinates and environmental characteristics of the six study stations in the Bay of Veys

Study stations	GR1	GR2	CB	GE1	GE2	GE3
Geographical coordinates	49°23.290′N 1°04.507′W	49°23.237′N 1°05.089′W	49°23.184′N 1°05.466′W	49°22.566′N 1°05.589′W	49°23.076′N 1°06.218′W	49°22.237′N 1°06.394′W
Height above sea level (m)	1.75	1.70	1.65	2.20	2.20	3.90
Mean time of oyster emersion (%)	22	21	21	28	28	49
Origin of the "first water" when the tide comes in	Seawater (tide wave)	Seawater (tide wave)	Mixed water from eastern channel	Mixed water from eastern channel	Mixed water from western channel	Mixed water from the two channels

farmers, was calculated using the following equation: (wet meat weight×100)/whole weight. MI allows the classification of oysters into three categories: "special": MI > 10.5, "fine": 6.5 < MI < 10.5 and "non-classified": MI < 6.5.

Thirty individuals from each sample (five per site) were used for microscopic histological examination. Cross-sections from oysters were cut behind the labial palps and 5-mm-thick sections were fixed in Bouin's solution. 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). The oysters were classified into distinct phases of gonadal maturation based on microscopic analysis and according to Lubet's (1959) modified classification (Table 2).

For each sample collected from March 2000 to March 2001 ($N = 30$) at stations GR1, CB, GE2, and GE3, the dry tissues of 10 oysters were pooled and homogenized in triplicate. Each biochemical component was determined by a colorimetric method. Protein was analysed by the method of Bradford using bovine albumin as a standard. Carbohydrate and glycogen were extracted in 15% trichloroacetic acid and precipitated with 100% ethanol (standards being, respectively, D glucose and oyster glycogen) and determined using the phenol-sulfuric acid method as described by Dubois et al. (1956). Extraction for total lipid was performed in a mixture of chloroform and methanol (Bligh and Dyer 1959) and lipid content was analysed using the method of Marsh and Weinstein (1966) with tripalmitate as a standard.

Data treatment

To test for significant differences of each biological parameter among sites, 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. If the assumptions were not met, nonparametric tests (Kruskal–Wallis) were used. In both cases, multiple comparison tests (Student Newman Keuls) were then undertaken to distinguish different groups. Chi^2 tests were performed to compare frequencies. These analyses were conducted with Statgraphics Plus 4.0 software.

Multivariate descriptive techniques were used: (1) to provide a reduced description of the large data set ($N = 2{,}837$), (2) to analyse relationships between variables, (3) to construct clusters of individuals, (4) to characterize these clusters by explanatory (quantitative) variables, and (5) to illustrate the clusters with additional qualitative variables (Escofier and Pages 1990). As a first step, a Principal Component Analysis (PCA), which is based on a geometric representation of the data, was performed. Subsequently, a Hierarchical Ascending Clustering (HAC) using Ward's criterion was carried out

Table 2 Characterization of distinct phases of bivalve gonadal maturity based on microscopic analysis, modified after Lubet (1959)

Stages	Gonad	Gonadal maturity	Follicle maturity in male	Follicle maturity in female
0	Reduced; follicles collapsed and very unobtrusive	Inactive; connective tissue very abundant	Very regressed tubules with a single gonial cell layer	Very regressed tubules with a single gonial cell layer
I	Developing	Early active; connective tissue abundant	Phase of gonial mitoses; follicles containing small spermatogonia	Phase of gonial mitoses; follicles containing quite large oogonia
IR	Developing	As I but residual gametes present and occupying more than 50% of the gonadal surface	As I	As I
II	Developing	Fully active	Spermatogonia evolving into spermatocytes and spermatids	Beginning of vitellogenesis
IIIA	Maximal volume; partially ripe	Fully active; connective tissue greatly reduced	Follicles containing all the sexual cell categories including spermatozoa	Presence of stalk oocytes and oocytes still in the process of vitellogenesis
IIIB	Maximal volume; ready to spawn	Fully ripe; connective tissue greatly reduced	Tubules full of mature spermatozoa oriented with tails towards the lumen	Free mature oocytes occupying centre of lumen in the follicle
IIID	Volume in the process of reduction	Partially or totally spent; post-spawning and resorption	Most follicles empty or partially so	Residual oocytes in the process of cytolysis; presence of phagocytes

on main factorial coordinates. These analyses were performed using SPAD.N software (CISIA, 1996).

Results

Environmental Monitoring

In the Bay of Veys, no obvious hypoxia was observed during the study, and turbidity was characterized by a very high variation depending on tidal regime and hydrodynamics. Turbidity regularly increased up to 100 NTU and sometimes exceeded 200–250 NTU.

Water temperature showed strong seasonal variations (Fig 2). During the study period, mean temperature at high tide ± 1 h was minimal in March (8.4°C in 2000 and 6.7°C in 2001) and maximal in August (19.7°C and 20.3°C, in 2000 and 2001, respectively). Water salinity depends on various parameters such as tidal regime and climatic conditions. Figure 3 illustrates water salinity during two periods chosen as an example: at the time of heavy rainfall in November (Fig. 3a) and during a period of low rainfall in July (Fig. 3b). In the first case, large salinity variations (from 5 to 33 ppt) were observed in the highest site GE3, and these variations increased with tidal range. The lowest salinities were recorded at Gefosse, i.e. 6 ppt at GE1, while at Grandcamp no salinities below 15 ppt were recorded. When rainfall was low, minimal salinity was not less than 21 ppt, and the salinity reduction was most important at GE1 compared with CB and above all GR1. Even if all the stations were not situated at the same bathymetric level (Table 1), it appeared that the more southern area (Gefosse; GE1, GE2, and GE3) corresponded more to estuarine conditions than Grandcamp (GR1 and GR2), which constituted a relatively more marine environment.

Shellfish mortalities

Substantial mortalities of Pacific oysters occurred at all six stations, the highest daily mortality rates being observed during the summer period: in 2000 from July to October (with a maximum value of 0.49% day^{-1}), and in 2001 from July to the end of the study in November (with a highest value of 1.74% day^{-1}) (Fig. 4a). Mortality was lower in 2000 than in 2001. No significant differences were found in daily mortality rates between the stations (Kruskal–Wallis; $P = 0.89$), while cumulative mortalities (Fig. 4b) showed significant differences among sites in both years (ANOVA; $P < 0.001$). In 2000, oysters from CB and GE1 had a significantly higher mortality than those from GR1 and GR2 (SNK test) (Table 3). A different result was obtained in 2001 as two groups could be distinguished: GR1, CB, and GE2 on the one hand and the three other sites on the other hand. At the end of the study, the most important losses had occurred at GE1 and GE3 and the lowest mortalities at GR1 (SNK).

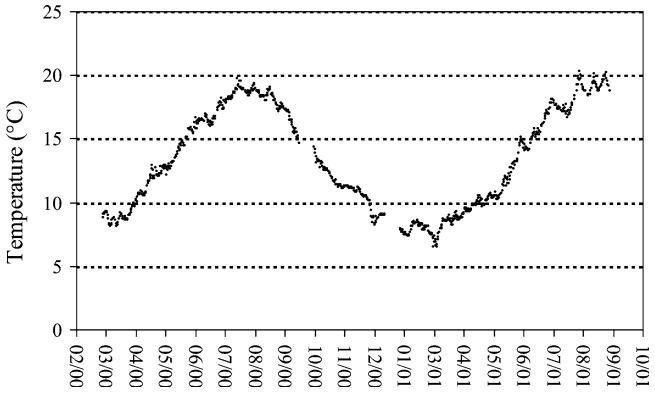

Fig. 2 Mean water temperature (°C) at high tide (± 1 h) at station CB (located in the centre of the Bay of Veys) from March 2000 to October 2001

Biological parameters relative to shell measurements

The whole weight of oysters deployed in Bay of Veys varied according to the stations and reached a maximum of 78.5 g (GR1) after 1-year study and a maximum of 95.6 g (GE2) at the end of the study (Table 3; Fig. 5a). Whole weight correlated well with shell measures: length (0.84), width (0.78) and thickness (0.74). The whole weight was composed of 60.1% shell, 17.3% tissue and 22.7% water contained inside the shell; its dynamics were similar to that of the shell weight (Fig. 5a). Barring a few decreases due to sampling hazards, the shell grew all year, with the highest growth rates (> 0.3% per day) recorded from June to September 2000. The shells of the oysters located at GE3 were significantly lighter than those of the oysters from five other study sites (Table 3) and another significant difference in shell growth was calculated between the oysters at GE1 and GR1 (ANOVA; $P < 0.001$ and SNK). The shape of oysters at GE3 differed from the others, showing a significantly smaller length and a significantly higher thickness coefficient (Table 3). Epifauna (especially barnacles) represented, on average, 10.1% of the whole weight, the lowest and highest fouling rates being observed at GR1 and GE2, respectively (Table 3).

Biological parameters relative to soft tissue and reproduction

Wet and dry soft tissue weights showed a high correlation of 0.85, and mean meat water content ranged from 75.6% at site GE3 to 77.8% at CB (average: 76.6% ± 0.9). Dry meat weight showed seasonal changes according to the life cycle of the oyster, with an important increase during spring and the beginning of summer followed by a decrease more or less marked depending on sites in summer and autumn, and finally a

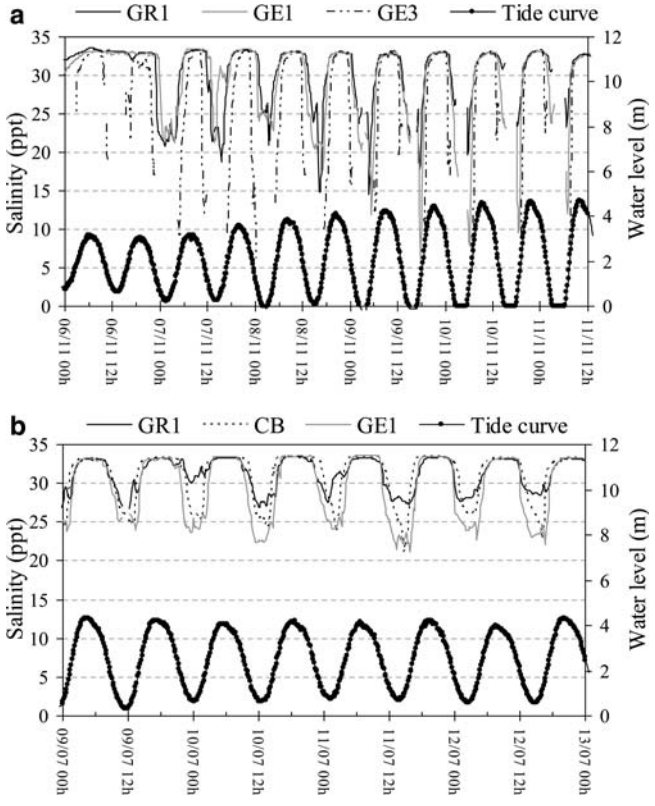

Fig. 3 Tide curves and salinity (ppt) recorded **a** in November 2000 at stations GR1, GE1, and GE3 and **b** in July 2000 at stations GR1, GE1, and CB

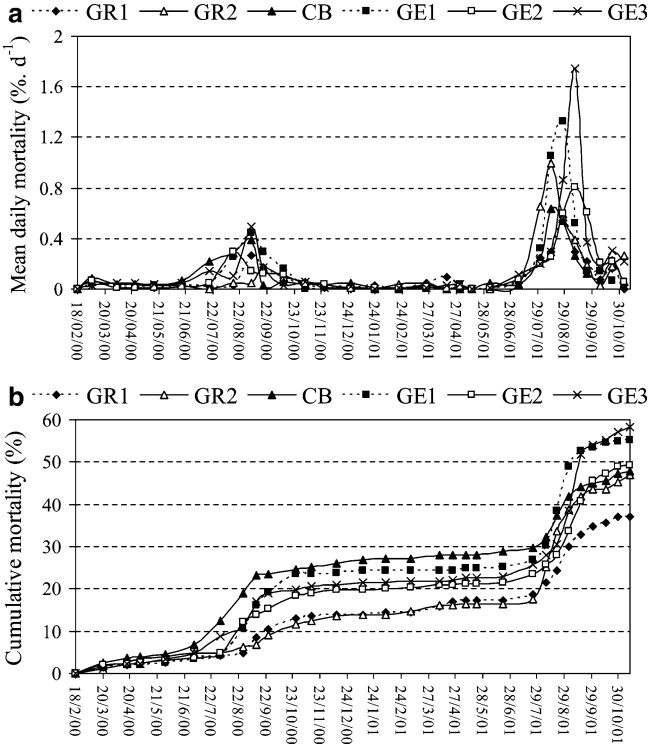

Fig. 4 Seasonal changes in *C. gigas* **a** mean daily mortality (% day^{-1}) and **b** cumulative mortality (%) at the six stations from March 2000 to August 2001

Table 3 Various parameters obtained from 2,837 Pacific oysters reared at six stations (GR1 to GE3) in the Bay of Veys from March 2000 to August 2001

	GR1	GR2	CB	GE1	GE2	GE3
CM during "summer 2000" (%)	9.6	7.1	19.2	20.8	15.4	15.7
CM during "summer 2001" (%)	22.7	35.9	25.6	38.6	33.7	43.9
WW after 1-year study (g)	78.48 ± 10.70	65.82 ± 12.44	78.23 ± 18.14	76.93 ± 14.36	73.09 ± 15.54	48.91 ± 9.08
WW at the end of the study (g)	75.79 ± 18.09	84.45 ± 20.02	80.58 ± 22.91	94.14 ± 27.66	95.65 ± 25.55	66.77 ± 11.1
Shell MDGR (% day^{-1})	0.16	0.17	0.23	0.22	0.22	0.13
ML of oyster shell (mm)	99.39 ± 11.04	102.27 ± 9.77	99.79 ± 12.48	102.33 ± 14.37	103.58 ± 10.13	88.06 ± 7.01
Mean TC	39.31 ± 5.68	40.06 ± 8.02	40.32 ± 9.48	39.71 ± 5.43	40.44 ± 5.25	42.64 ± 5.38
Fouling (% of WW including fouling)	7.1	11.0	9.1	6.9	14.2	12.3
Meat MDGR (% day^{-1})	0.36	0.32	0.35	0.45	0.49	0.32
Mean MI	16.6 ± 2.68	17.2 ± 2.57	16.9 ± 2.52	17.3 ± 2.73	18.5 ± 2.87	17.1 ± 2.48
Maximum MI	33.3	34.7	31.5	31.7	37.0	33.8
Glycogen (%)	16.68	–	24.87	–	29.48	24.81
Protein (%)	35.56	–	29.51	–	26.95	27.66
Lipid (%)	9.90	–	8.76	–	9.04	10.06

Average is accompanied by standard deviation. For index calculations, see text
CM cumulative mortality; *WW* whole weight; *MDGR* mean daily growth rate; *ML* mean length; *TC* thickness coefficient; *MI* meat index

stagnation or slight decrease during winter (Fig. 5b). Soft tissue growth differed significantly between stations (ANOVA; $P < 0.001$), except for sites GR1, GR2, and CB (SNK test). Mean daily growth rates (including both decreases and increases in tissue weight) varied from 0.32% per day at GR2 and GE3 to 0.49% per day at GE2 (Table 3). The condition index (CI) increased drastically during spring 2000 and 2001 and reached nearly 120 in July 2000 at both GE1 and GE2 (Fig. 5c). Except at GR1 and GR2, this index decreased considerably during August and after a slight gain in September remained low during winter. In 2001, the sampling regime did not allow resolution of the expected peak between May and August. There were significant differences in CI between stations (ANOVA; $P < 0.001$; SNK) and three groups of homogeneous sites could be distinguished: CB–GR2, GR2–GR1–GE1–GE3, and GE2 where the oysters showed the highest values of CI. The meat index (MI) showed a similar trend, especially the decrease noticed in August for four of the six stations, GR1 and GR2 in this case not being concerned. The SNK test for MI permitted the distinction of only two groups, GE2 on the one hand and the five other sites on the other; at GE2 the maximum value (37) and the highest average of MI (18.5) were calculated at GE2 (Table 3).

Oyster reproduction can be evaluated by a visual examination of the soft tissues and the determination of a macroscopic SMI for each oyster. The maximum SMI (2) was attained at the beginning of July 2000 for almost all of the oysters and only a very slight decrease, probably corresponding to partial spawning, was observed in August. During the winter, the oysters appeared quite fat (and not almost transparent as often observed in other areas), and mean SMI remained close to 1 at all of the stations. Microscopic analysis allowed determination of sex, and over the whole study period the sex ratio was well-balanced (Fig. 6a), and there were no significant differences between stations. Considering each study date, it appeared that the sex ratio was unbalanced on three dates in 2001 (January, March, and June) and above all that the sex ratio was well-balanced before the mass mortality period (17/04/2000 to 04/07/2000) and subsequently unbalanced towards females (11/12/2000 to 07/03/2001) (χ^2 test ; $P < 0.005$). Even if the individuals which could not be sexed (6.02% in all) were considered as males, the sex ratio remained unbalanced towards females ($P < 0.01$). Hermaphrodites made up 2.55% of the whole batch. During the whole study, it was noteworthy that only a few oysters were reproductively inactive (Fig. 6b). At the beginning of the study, all oysters were in the stage of gonial multiplication (stage I) and, from mid-April to the end of May 2000, all animals were in gonadal development. Stage-III animals, corresponding to maximum gonadal development, were observed from the beginning of July. Some oysters went straight from IIIA to IIID, which was the majority stage in August and September when spawning and gonadal resorption occurred. During the period of complete maturity (August and September), there were significant differences among the study stations (χ^2 test; $P < 0.005$) with two clusters: GR1 and GR2 where the majority stage was IIIB, and the four other sites where stage IIID dominated. From the end of October to mid-November, oyster reproduction differed significantly at CB by comparison with sites GR1, GE3 (χ^2 test; $P < 0.005$) and GR2 (χ^2 test; $P < 0.05$): at CB, oysters reinitiated more precociously a new reproductive cycle.

The contents of glycogen and carbohydrate were not significantly different (ANOVA; from $P = 0.51$ at CB to $P = 0.81$ at GE2), indicating that glycogen was the dominant carbohydrate. Over the study period glycogen, proteins and lipids in total represented between 62.53% of the sample weight at GE3 and 65.47% at GE2. Proteins were slightly (CB and GE3) or more clearly (GR1) in the majority, except for GE2 where the major com-

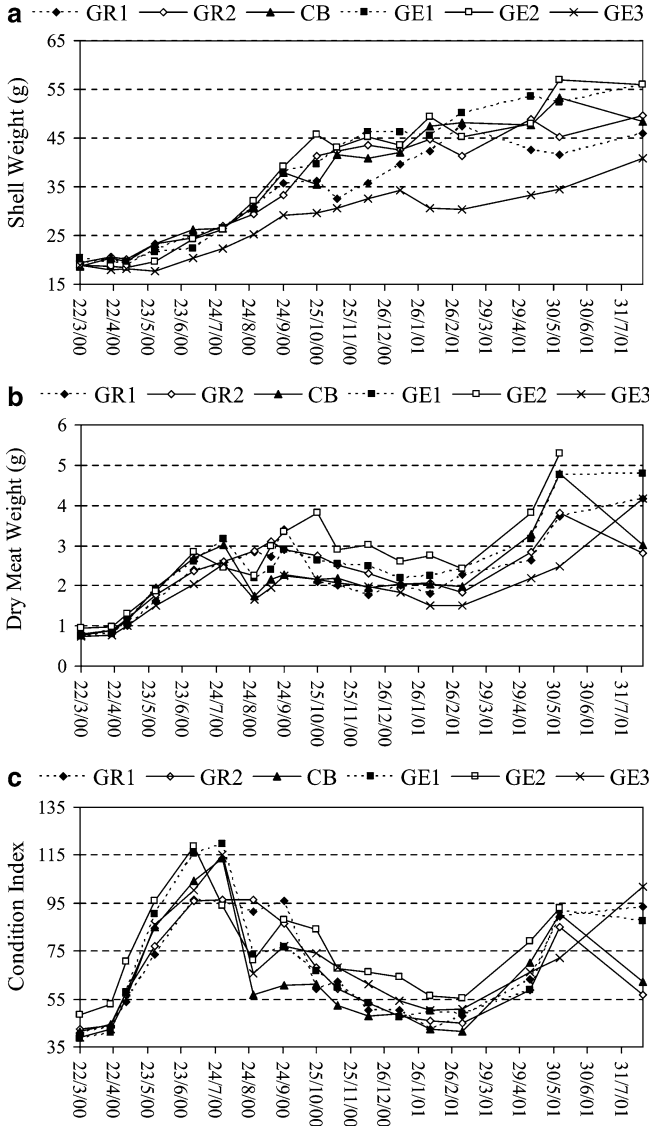

Fig. 5 Growth performances in *C. gigas* at the six stations from March 2000 to August 2001: mean shell weight (g) (**a**) mean dry meat weight (g) (**b**), and mean condition index (Walne and Mann 1975) (**c**)

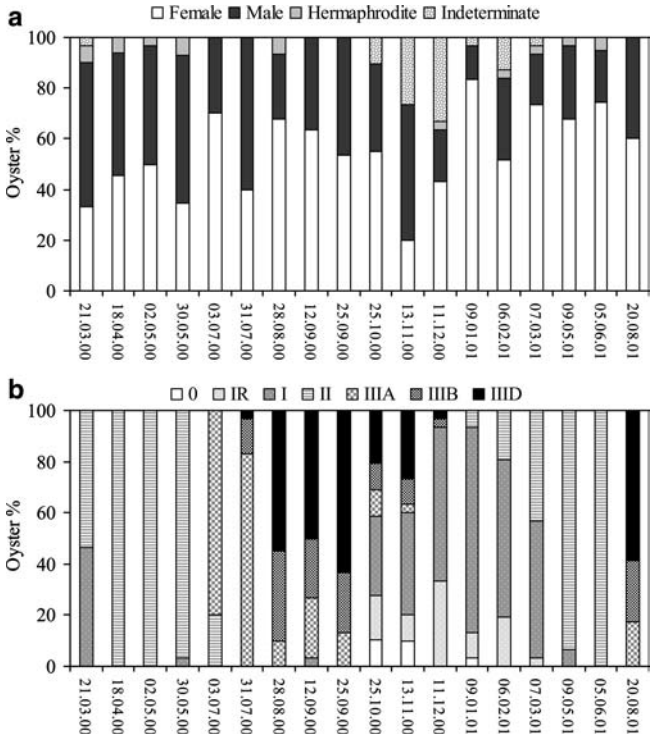

Fig. 6 Seasonal variation in sex ratio (**a**) and proportion of Pacific oyster sexual maturity stages (**b**) at the six stations from March 2000 to August 2001. For maturity stage description see Table 2

ponent was glycogen (Table 3). There were significant differences between the stations in oyster protein expressed as percentage or quantity per animal (ANOVA; $P < 0.001$). The oysters deployed at GR1 and GE2 were characterized by the highest and lowest percentages of proteins, respectively (Fig. 7a), and an opposite trend was observed for glycogen (Fig. 7b). Considering the content of lipids, oysters at GE3 did not differ significantly from oysters at CB in terms of amount per animal and from those at GR1 on a percentage basis (ANOVA and SNK tests). Over the whole study, the percentages of proteins remained relatively constant, most of the values calculated at CB, GE2, and GE3 ranging from 25 to 30% (except at the end of the study); at GR1, the percentages ranged from 35 to 40% from June to December 2000. Glycogen percentage curves showed important variations according to the station. Similar trends but different in their extent, were observed for the oysters at CB and GE3 (Fig. 7b). At GE2, a slight decrease occurred from the beginning of May, and subsequently (from the end of August) the glycogen percentages were always above 30%. For the oysters at GR1, three periods could be distinguished: spring with values over 20%, summer with a reduction to around 10%, and autumn with percentages close to 15%. It is important to focus on lipids because they constitute a reproduction index (e.g. Deslous-Paoli et al. 1982). The amount of lipids per individual strongly increased at all stations from April to the beginning (GE2) or end of July (CB and GE3), or even to the end of September (GR1) (Fig. 7c). In terms of percentages, lipids ranged from 7.0 to 11.7%, and the most notable result was the stabilization of lipid content at GR1 in comparison with the three other sites (Fig 7d).

A synthesis based on a multivariate analysis

The data set obtained by the biometric analysis of 2,837 individuals (providing ten variables) and mean monthly water temperature was used as a matrix for a PCA followed by an HAC in order to analyse relationships between quantitative variables and to determine clusters of individuals. The two first factors appear relevant because they represent 77.88% of the total variance (axis 1:

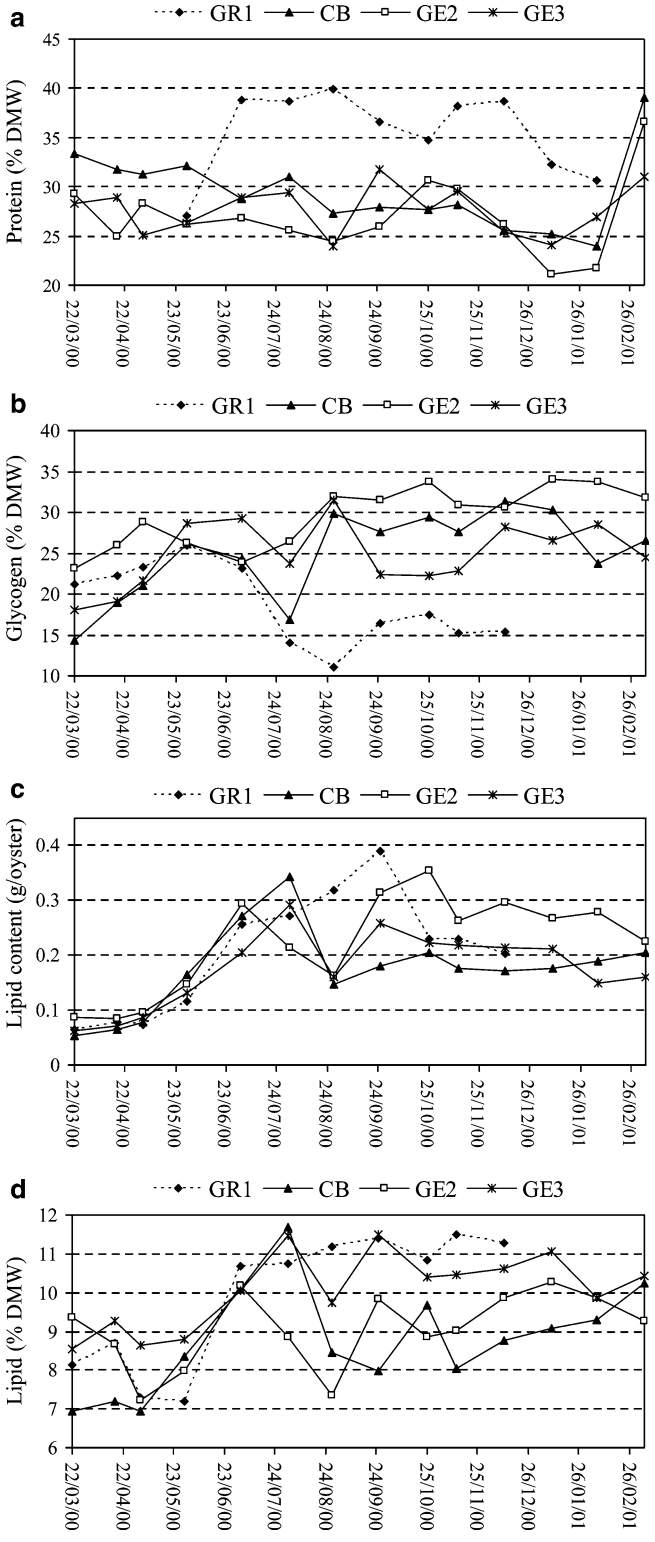

Fig. 7 Biochemical composition of *C. gigas* at four stations (GR1, CB, GE2, and GE3) from March 2000 to March 2001: seasonal variation in **a** protein and **b** glycogen content as percentage of dry meat weight (DMW); mean amount of lipid per oyster (g) (**c**) and variation in lipid content as percentage of DMW (**d**)

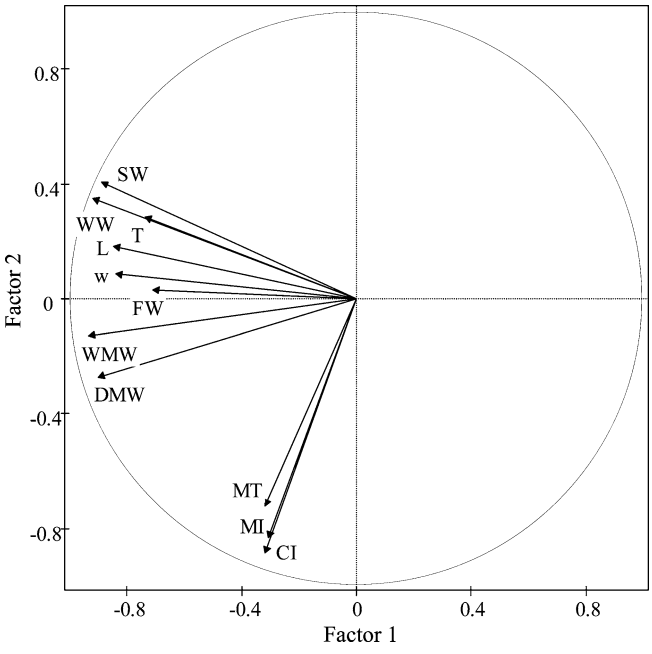

Fig. 8 Representation of the active variables on the two first factorial axes (factor 1 = 55.2% and factor 2 = 22.7% of whole inertia) obtained with the PCA carried out on all studied oysters (2,837 individuals). This shows the relationships between the 11 variables (10 biometric variables and temperature). *SW* shell weight; *WW* whole weight; *L* length; *w* width; *T* thickness; *FW* fouling weight; *WMW* wet meat weight; *DMW* dry meat weight; *MI* meat index; *CI* condition index; *MT* mean monthly temperature

55.21% and axis 2: 22.67%). F1 shows a "size effect" because of the high correlation between all of the variables. Axis 2 splits the variables into two: variables relative to soft tissue (negative coordinates) and variables relative to shell and whole weight (positive coordinates) (Fig. 8). Axis 1 is mainly explained by wet meat weight (−0.93), whole weight (−0.92), dry meat weight (−0.90) and shell weight (−0.89), whereas axis 2 is best explained by condition indexes (−0.88 and −0.83 for CI and MI, respectively) and mean temperature (−0.72). Moreover, temperature was mainly correlated to CI (0.64). In the principal plane of the PCA, the individual representation includes no aberrant points and illustrates a continuous distribution of quantitative variables characterizing these individuals. The HAC leads to the formation of four clusters with a high value of the ratio (inter-cluster inertia/total inertia) reaching 0.74. The barycentre (corresponding to the mean individual) of each cluster and each additional qualitative variable are illustrated on the principal plane in Fig. 9 and the most representative variable modalities of the four clusters are indicated in Table 4. The study chronology is easily distinguishable on the first two axes since it follows shell and meat growth. Months are associated with different sexual maturity stages. On the principal plane, a gradient of sexual maturity stages is observed from stage 0 to stage 2. Stage 1.5, corresponding to follicle resorption, occupies an offset position along this gradient. Along

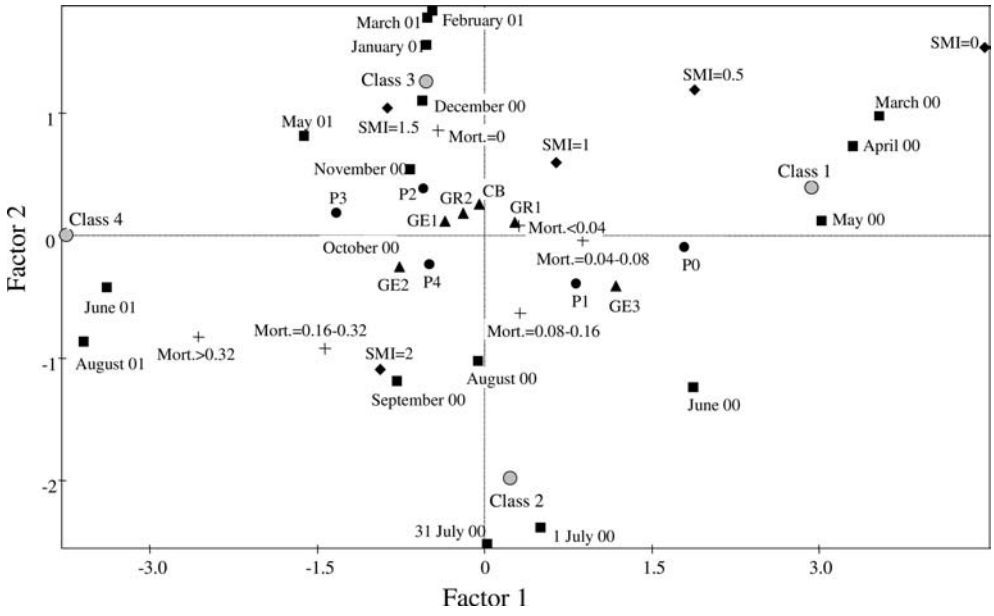

Fig. 9 Representation of the illustrative variable modalities and cluster barycentres (Classes 1–4) on the two first factorial axes obtained with the successive multivariate analysis (PCA and HAC). *SMI* sexual maturation index; *Mort.* mortality class (expressed by mean daily rates)

axis 2, null and low mortalities have positive coordinates by contrast to high mortalities, and the highest mortalities belong to cluster 4. With regard to study stations, GE3 belongs to cluster 1, whereas the other sites are included in cluster 3, the latter consisting of the highest number of individuals (996 oysters) (Table 4). Nevertheless, within cluster 3, the five stations occupy different positions and show particular characteristics which are worthy of discussion.

Discussion

Biological performances

Data for oyster growth in the Bay of Veys can be compared with that of other French oyster farming areas studied within the framework of the French monitoring network "REMORA" initiated by IFREMER in 1993. This network studies the growth and the quality of two batches of cupped oysters (one-year old and two-year old) distributed among various French oyster-growing areas. In terms of whole weight and the highly correlated variables, shell weight and size, oyster growth in the Bay of Veys was close to (e.g. in 1997 and 2000) or superior to (e.g. in 1998 and 2001) the national mean (Fleury et al. 1999, 2002). A part of the studied batch was reared in the south of the Marennes-Oléron Bay (the Ronce-Perquis oyster bed), located on the Atlantic coastline, for a specific comparison with the Bay of Veys (Faury et al. 2001). During the common study period (March 2000–January 2001), oyster growth was slower in Marennes-Oléron than in the Bay of Veys: as much as −50% for the shell weight and −150% for the meat weight. In 2000, highest growth rates in the Bay of Veys were recorded from June to September, the period of high growth generally reported by the REMORA network. In the Bay of Veys, oyster growth varied according to the station and, after 1 year of the study (7 March 2001), the maximum growth was attained by oysters reared at stations CB and GR1. This high value of total weight recorded at GR1 (Table 3) was probably due to an overestimation of oyster weight. The relatively slow growth of oysters at GE3 was shown on the principal plane of the PCA. This station was the most southern, but it was above all the highest on the foreshore, and hence oysters at this station had a shorter time to filter-feed (45% versus 66% at GE1 and GE2

Table 4 Most representative variable modalities of the four clusters determined by Hierarchical Ascending Clustering (HAC) carried out on main factorial coordinates of Principal Component Analysis (PCA)

	Class 1	Class 2	Class 3	Class 4
Numbers of individuals	457	726	996	658
Condition and meat indices	Intermediate	High	Low	Intermediate
Whole weight (∼ shell weight)	Low	Intermediate	Intermediate	High
Period	March to May 2000	June to August 2000	September 2000 to May 2001	June and August 2001
Sexual maturity stage	0 and 0.5	2	1 and 1.5	–
Stations	GE3	–	GR1, GR2, CB, GE1, and GE2	–
Mortality class	–	0; < 0.04; 0.04–0.08	0.08–0.16; 0.16–0.32	> 0.32

and 72–77% at the three other sites). Comparable phenomena have been observed for the whole weight of *C. gigas* deployed at three bathymetric levels (Goulletquer et al. 1987) and for oysters located in sites at the head versus the mouth of a bay (Garcia-Esquivel et al. 2000). The shells of oysters at GE3 were more compact with a significantly higher TC than at other stations, probably due to more intense hydrodynamics at the shoreline level.

The REMORA network indicates that the oysters reared in the Bay of Veys generally show high biological performances in terms of meat weight and condition indexes. The Bay of Veys appears then as an area characterized by high productivity (Goyard 1996), and the oysters belong to the best commercial category (''special''), with often the highest values of MI (Fleury et al. 1999, 2002). Within the Bay of Veys, differences were recorded between Grandcamp (including GR1 and GR2) and Gefosse (GE1, GE2, and GE3), MI reaching, for example, 12.0 and 14.1, respectively (national mean: 8.9) in 1998, and 11.5 and 16.1 (national mean: 9.4) in 2000. During the peak in summer 2000, the CI attained 93 and 119 at Grandcamp and Gefosse, respectively. During the same period and for the same population deployed in the south of the Marennes Oléron Bay, the CI did not exceed 70, indicating the lower quality of the trophic environment in this area. In *C. gigas* reared in Portugal, the maximum CI reached 85 and 103 in two studied estuarine stations (Almeida et al. 1999). In a medium growth Canadian site, CI values of cupped oysters reared in suspended nets declined from a maximum greater than 130 to a low of 49.2 (Brown and Hartwick 1988). The minimum CI values in the Bay of the Veys were comparable and ranged from 55.1 at GE2 to 41.4 at CB, the seasonal variations being thus generally of lesser magnitude. Even though comparisons of oyster biological performances between studies performed in various countries or regions are sometimes difficult, notably because of different farming methods and calculation of different condition indexes, it appears that the Bay of Veys, and especially Gefosse (as opposed to Grandcamp), is a productive oyster area.

Brown and Hartwick (1988) attributed differences among some locations in the growth of shell height and dry meat (but not whole and shell weight) to an overall lower phytoplankton biomass. In the Bay of Veys, as in the English Channel, two phytoplankton blooms are generally observed, the most important in spring and the other in autumn. Excluding station GE3, differences noticed between Grandcamp and Gefosse in terms of shell and soft tissue growth and, to a lesser extent, in condition indexes, might be related to differences in water quality. Gefosse was more influenced by estuarine waters which might provide a higher quantity and/or quality of trophic resources, but this has not as yet been demonstrated. Moreover, the environmental impact was probably the result of various factors, as in a Mediterranean Sea area where condition indices were correlated mainly with temperature and, to a lesser extent, with dissolved oxygen, chlorophyll *a*, and suspended matter (Cano et al. 1997).

Before the beginning of the study, oysters were transferred from the west coast of the Cotentin peninsular to the Bay of Veys, the latter probably being richer in terms of trophic resources. It cannot therefore be excluded that some changes recorded in biological parameters in the beginning of the study were due to this transfer. Nevertheless, in marine bivalves, changes in meat weight are especially associated with the reproductive cycle and patterns of energy storage and mobilization (Barber and Blake 1981; Worrall and Widdows 1984; Cigarria 1999). Sexual maturation can be illustrated by a tissue weight increase, as was observed during spring in the Bay of Veys, and spawning is associated with an important and sudden decrease of tissue weight and consequently of condition indices. In 2000, such a drop was recorded between 4 July and 28 August at GE2, between 31 July and 28 August at CB, GE1, and GE3, and between 25 September and 25 October at GR1. This suggested a slightly more precocious spawning at GE2 and a very late spawning or absence of spawning (replaced by resorption) at GR1. The absence of a sudden decrease at GR2 may be interpreted as an absence of spawning. These observations were reinforced by oyster lipid content which increased during sexual maturity from mid-April and then decreased simultaneously with meat weight and condition indices. This decrease can be considered a spawning marker since gametes are particularly rich in lipids. It is difficult to determine accurately the reasons for such differences in the timing of spawning, but environmental parameters including water quality may influence this process. Lipids appeared to drop simultaneously with spawning at all stations except GR1, followed by an increase. Lipid percentage subsequently remained rather stable, even during winter, all values being above 8%. This is a surprising result given the low quantity of gametes held in the follicles. In the Bay of Veys, reserves might thus include an important amount of lipid as well as glycogen. The same population reared in the Marennes Oléron Bay showed a similar lipid profile with an increase up to 14% before spawning (but only 0.15 g per oyster) and lipid content dropped to 9% (and 0.10 g per oyster) after spawning. Slight variations in lipids (8–12%) or more important variations (1–8.5%) have been recorded in *C. gigas* according to ecosystem (Almeida et al. 1999).

Concerning biochemical composition, it is important to underline that monthly sampling cannot detect rapid variations of *C. gigas* energy reserves. Maximum protein content never exceeded 40%, a relatively low value by comparison with those reported in the literature (e.g. Almeida et al. 1999; Garcia-Esquivel et al. 2000; Deslous-Paoli and Héral 1988). Our low values might be due to the use of a potentially inappropriate standard (bovine albumin). Nevertheless, the major results obtained were the general richness in glycogen in oysters culti-

vated in the Bay of Veys (review in Deslous-Paoli and Héral 1988), and the relative richness in glycogen and paucity in protein contained in oysters reared at GE2 in comparison with those at GR1. Glycogen constitutes an important component of reserves in many bivalves (Gabbott 1975; Ruiz et al. 1992; Mathieu and Lubet 1993) and storage energy (mainly as glycogen) is linked with the quality of food available during phytoplanktonic blooms in spring and autumn (Deslous-Paoli et al. 1988). Differences within the Bay of Veys could suggest better trophic conditions at Gefosse in relation to Grandcamp. In the same population deployed in the Marennes Oléron Bay, glycogen content decreased from 9 to 1% (before spawning), when values ranged from 10 to 30% in the Bay of Veys, indicating both the eutrophic nature of the Bay of Veys and the limited capacity of the Marennes Oléron Bay (Heral et al. 1988; Soletchnik et al. 1999). It was remarkable that in the Bay of Veys the carbohydrate reserve was composed only of glycogen. Various authors have reported lower values of glycogen percentage in carbohydrates: 48% (Deslous-Paoli and Héral 1988), 56% (Lee et al. 1975), 79.5% (Whyte and Englar 1982), and 65–100% (Maurer and Borel 1986). A pattern corresponding to alternation of storage and mobilization of energetic reserves in association with the reproductive cycle has been reported by various authors (Gabbott 1975; Bayne 1976; Berthelin et al. 2000). The latter authors explained, for *C. gigas* cultivated on the western Atlantic coast of France, that glycogen and lipids were stored during the winter period and that gametogenesis in spring and summer was associated with an increase in lipid and protein contents taking place at the expense of glycogen reserves. Even though the present biochemical data cannot definitely refute the existence of such a cycle, especially during the first part of the study and at GR1, this cycle was not obvious from September because little variation was observed. According to Bayne (1976), two kinds of bivalve species can be distinguished: ''opportunistic species'' use the energy obtained from food directly for gonad development, and reserve storage and gamete production cycles may overlap temporarily, whereas ''conservative species'' use previously stored energy reserves. In two Korean bays, *C. gigas* was considered an opportunistic species because gametogenesis started simultaneously with reserve accumulation and proceeded until gonad maturity was reached (Kang et al. 2000). In the present study, *C. gigas* seems also to behave as an opportunistic animal notably because the intense reproductive activity was accompanied by an increase in glycogen content during spring 2000.

In the present study, the reproductive cycle of oysters cultivated in the Bay of Veys was determined for the first time. After a maturation period throughout spring, gonads acquired their maximal volume at the beginning of July and the first oysters ready to spawn were observed at the end of July. At Gefosse and at CB, slight decreases in the macroscopic index suggested partial spawning which was confirmed by microscopic maturity stages, and the majority of oyster gonads contained remaining gametes, even until November. At an Irish site, ripe females developed in July and males reached the ripe stage in September, but spawning occurred synchronous in males and females; at another station, oysters matured but did not spawn and the lack of spawning was not due to temperature or chlorophyll *a* content in the water but probably to TBT contamination (Steele and Mulcahy 1999). Oysters which fail to spawn or spawn partially can resorb gametes and reconvert them to glycogen (Quayle 1988). During the wintering period 2000–2001 in the Bay of Veys, the oysters appeared quite ''fat'' (but not veined) and the mean macroscopic index remained close to 1 at all stations. Even if no gametes remained in the follicles, the presence of abundant connective tissues might induce this visual ''fatness''. Differences observed between Grandcamp and Gefosse in terms of soft tissue weight, condition indexes, and biochemical composition, were also corroborated by microscopic studies showing that during the period of complete maturity (August and September), the majority stage at GR1 and GR2 was IIIB whereas at the four other stations it corresponded to post-spawning and/or resorption. It may be hypothesized that these biological differences were due to environmental differences as already reported by Deslous-Paoli et al. (1982): the inter-annual differences recorded in the use of reserves resulted from a deficiency of food and from temperature effects on oyster physiology, particularly on gametogenesis and spawning which can be delayed. Such differences in spring–summer oyster production were also reported in oyster populations deployed at 15 sites in the Ronce-Perquis oyster bank (Marennes Oléron Bay) in 1997 (Soletchnik et al. 1999). During this experiment, slight differences at the micro-spatial scale had important effects on growth performances in this trophically limited ecosystem. Spawning period and effort seemed to be more homogeneous, however, than in the comparable area of oyster bank in the Bay of Veys. Quale (1988) observed that in exceptionally cool summers oysters failed to spawn and resorbed gametes. In *Crassostrea gigas* spawning is dependent on temperature exceeding 18°C (Mann 1979). This threshold was attained at all stations in the Bay of Veys which, however, showed a certain thermal heterogeneity (in addition to that due to differences in bathymetric level). Indeed, around high tide, thermal differences sometimes occurred between Gefosse and Grandcamp and this might contribute to the explanation of spawning differences in oysters reared at equivalent bathymetric level (e.g. GR1, GR2, and CB). Compared with the more estuarine Gefosse area, Grandcamp could also be less favourable for oyster spawning because of lesser variations in environmental factors known to act as a stimulus for spawning. Another difference was noticed in that oysters deployed at CB reinitiated a new reproductive cycle more precociously than animals reared at Grandcamp (GR1 and GR2) and at the highest station, GE3. The parallel be-

tween GE3 and the Grandcamp stations may be explained by similar trophic conditions because even if GE3 was situated within the Gefosse area, the oysters at this station had a reduced time to feed. No significant differences in sex ratio were found between stations. The sex ratio was well-balanced before the mass mortality period (from April to the beginning of July), but was subsequently unbalanced in favour of females (from December to March).

Oyster mortalities

A differential mortality according to sex was not noticed in *C. gigas* reared in the Arcachon basin (Maurer et al. 1986). Perdue et al. (1981) reported that mortalities affected females of *C. gigas* to a greater extent than the males. In the present study, however, significantly greater losses were recorded for males than for females, but these results must be considered with caution because the proportion of males to females in the samples of 30 individuals each, could be quite variable from one date to the next. Further data are necessary to determine whether there is a differential mortality with respect to sex in the Bay of Veys.

In the Bay of Veys, oyster mortality showed a strong seasonal variation, and the highest rates were recorded from July to October in both years. In 2001, mortalities were markedly higher than in 2000, but it cannot be concluded that mortality affected the 1-year-old oysters to a greater extent because the 2001 losses were particularly important for all studied oyster batches and most of the professional stocks. The possible occurrence of causative pathogens was investigated by the IFREMER network REPAMO, but no obvious infestation was detected (Arzul, personal communication). On the principal plane of the PCA, highest mortalities are close to the maximum Sexual Macroscopic Index (SMI = 2) and have negative coordinates along axis 2 which is best explained by condition indexes (and mean monthly temperature). The physiological status of oysters and mussels play a major role in the occurrence of mortality and the implication of the reproductive cycle is especially emphasized. The advanced maturation process is generally accompanied by important energy expenditure and the scope for growth can be thus nearly zero, while it is positive during early maturation stages as well as the post-spawning stage (Soletchnik et al. 1997). In *Mytilus edulis*, spring mortality was correlated with spawning rather than with temperature and food supply; highest mortalities were recorded in the medium to large size classes which have the highest reproductive effort (Worrall and Widdows 1984). A close relationship has been observed between the degree of soft-body growth and mass mortality in *C. gigas* (Mori 1979). In the present study, reproduction was analysed by a histological method which was qualitative and therefore did not allow calculation of the reproductive effort. Quantitative histological techniques have been employed in our more recent studies. In the Bay of Veys, "summer mortalities" occurred when oysters showed their maximum CI and during the drop of meat weight and condition indexes; the maturity stage was III (A, B or D) and corresponded to sexual ripeness and spawning. Summer mortality in 2000 was lower than in 2001, but more information on the maturation process was available in 2000. Oysters at sites GR1 and GR2 had both the lowest mortality rate in summer 2000 (7–10%) and a delayed hypothetical spawning in September–October (or no spawning). In contrast, oysters at the other sites spawned in August and showed a more intensive peak of mortality in August–September. In the Bay of Veys, the greatest mortality events therefore occurred mainly during the spawning period, whereas in the Marennes Oléron Bay mortality occurred more often during the intensive maturation process and before spawning. This suggests that reproduction (and especially spawning) cannot alone be implicated in triggering mortality. In 2000, for the same batch reared in the Marennes Oléron Bay, mortality occurred mostly in July (~37%) and spawning at the beginning of August. Moreover, after 1 year of rearing, mean cumulated mortality was lower than 20% in the Bay of Veys and 50% in the south of the Marennes Oléron area for the same oyster population. Such a result is not a constant, and in some years oysters are more affected in the Bay of Veys than in Marennes-Oléron Bay. The results of 2001 were more difficult to interpret because of long sampling intervals, but on 20 August, spawning (at least partial) had already occurred at GR2 and CB (Fig. 6) and high mortality rates were recorded at Gefosse and also at Grandcamp stations. Such results corroborated the hypothesis of a negative impact of spawning on oyster survival, and suggest the spawning event to correspond to a critical period during which tissue restructuring and important metabolic changes occur. Nevertheless, in a study performed on *C. gigas* and *C. virginica* deployed in the Marennes-Oléron Basin, no positive correlation could be established between weight loss due to spawning and mortality; the only significant mortality peak for *C. gigas* occurred after spawning (Soletchnik et al. 2002). According to Mori (1979), over-maturation, "a pathological phenomenon caused by a long residence of ripe oocytes or sperms in the gonad", seemed even to accelerate oyster mass mortality.

An influence of environmental factors (Gefosse versus Grandcamp, and 2000 versus 2001), on mortality could not be excluded. This influence might be direct or indirect via reproduction, and such a hypothesis is plausible because many authors have already reported effects of the environment on bivalve mass mortality. For instance in an experimental study, oyster mortality was four to five times greater in nutrient-enriched seawater (Lipovsky and Chew 1972). Mortality in triploid juvenile *C. gigas* and *C. virginica* depended on the salinity regime (Calvo et al. 1999). Higher rates of mortality tended to occur at or immediately after neap tides when dissolved oxygen was lowest, and during

periods of elevated air and water temperature (Cheney et al. 2000). In 2000, "more marine stations" located in the Grandcamp area were less affected by oyster mortality, potentially due to higher salinity and/or comparably nutrient-poor waters. However, differences in substratum nature or other unexplored environmental factors could also be important because mass mortalities were recorded in the whole bay in 2001. Slightly higher mean monthly temperatures in 2001 may be considered as one of the explicative hypotheses: 17.0°C and 17.8°C in July, 18.8°C and 19.2°C in August, 2000 and 2001, respectively. 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, and the physiological stress associated with reproduction (Cheney et al. 2000). In both Japan and the United States, mortalities were associated with areas of high productivity, high nutrient levels, and water temperature exceeding 20°C, and coincided with periods of maximum gonad condition for spawning (Beattie et al. 1980). In *Mytilus edulis*, Tremblay et al. (1998) suggested that the irregular outbreaks of summer mortality were the result of a synergistic interaction involving dietary deficiencies, temperature, a possible post-spawning stress, and the genetic characteristics of the stock. All of these hypotheses are worthy of future investigation.

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