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Patrick Soletchnik, Christophe Lambert, Katherine Costil. Summer mortality of *Crassostrea gigas* (Thunberg) in relation to environmental rearing conditions. *Journal of Shellfish Research*, 2005, 24, pp.197-207. hal-00616796

**HAL Id: hal-00616796**

**<https://hal.science/hal-00616796>**

Submitted on 24 Aug 2011

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## SUMMER MORTALITY OF *CRASSOSTREA GIGAS* (THUNBERG) IN RELATION TO ENVIRONMENTAL REARING CONDITIONS

PATRICK SOLETCHNIK,<sup>1\*</sup> CHRISTOPHE LAMBERT<sup>2</sup> AND KATHERINE COSTIL<sup>3</sup>

1 : IFREMER: Laboratoire Environnement Ressources Poitou-Charentes (LERPC). B.P. 133, 17 390 La Tremblade, France;

2 : Laboratoire des Sciences de l'Environnement Marin, Institut Universitaire Européen de la Mer, Université de Bretagne Occidentale. Place Copernic, technopole Brest-Iroise. 29280 Plouzané, France;

3 : Laboratoire de Biologie et Biotechnologies Marines (UMR IFREMER Physiologie et Ecophysiologie des Mollusques Marins), Université de Caen Basse-Normandie, Esplanade de la Paix, 14 032 Caen cedex, France

**ABSTRACT :** The purpose of this study is to investigate summer mortality of the cupped oyster, *Crassostrea gigas*, in relation to culture practices in the traditional oyster production region of Marennes-Oléron (France). Four oyster rearing conditions, varying culture location ("on-" or "off-bottom"), and site depth (65% to 80% daily immersion termed "deep" or 45% to 65%, termed "shallow") were studied to compare biologic performance and maturation status of oysters, in relation to sediment and water column parameters. The most severe mortality occurred in June to July in "on-bottom" reared oysters (25%), as compared with 10% mortality in "off-bottom" cultured oysters. Oysters (shell and meat) grew significantly better when reared "off-bottom" than "on-bottom." Reproductive effort was almost double in "off-bottom" reared oysters, compared with those "on-bottom"; thus, reproduction cannot be directly related to mortality in this summer mortality event. Low glycogen content recorded for both "on" and "off-bottom" reared oysters in summer, confirmed the probable lack of food and/or the overstocking in the Marennes-Oléron Bay, but did not discriminate among culture conditions. Whatever the immersion depth ("deep" or "shallow" conditions), "on-bottom" cultured oysters were adversely affected in growth, reproductive effort, and survival suggesting a direct effect of the mud (the so called "mud effect") on the biologic performance of oysters cultured on the bottom. Data from monitoring of sediment redox potential, organic content, and ammonium release did not support hypotheses that these parameters were alone responsible for the observed differences in mortality events.

**KEY WORDS:** *Crassostrea gigas*, Marennes-Oléron Bay, marine ecosystem, sediment, hydrology, summer mortality.

### INTRODUCTION

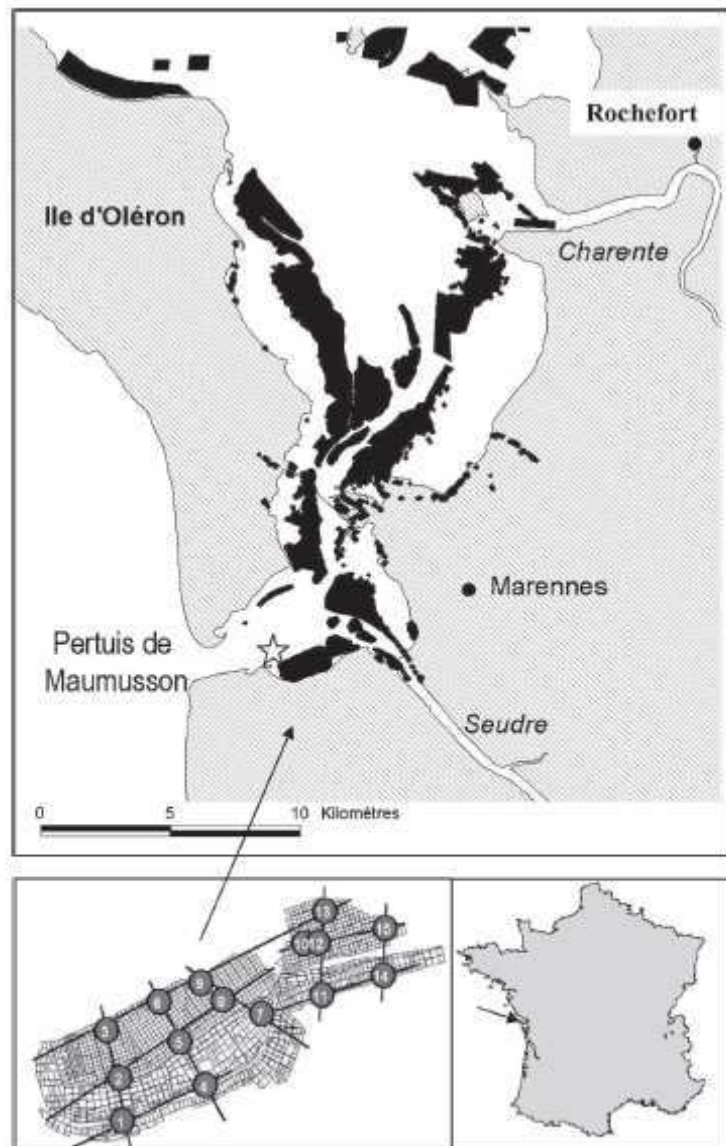
The Marennes-Oléron Bay, Europe's largest production area for cupped oysters (*Crassostrea gigas*, Thunberg), is situated on the French Atlantic coast, between La Rochelle and Marennes (1°10'W, 45°48'N; Fig. 1). It is bordered in the north by the Charente estuary, in the south by that of the Sèvre and in the west by Oléron Island. Of the two rivers flowing into the bay, the Charente is the largest, with an output of 10–400 m<sup>3</sup> s<sup>-1</sup>, whereas the Sèvre has flow rates of only 1–40 m<sup>3</sup> s<sup>-1</sup> (Vouvé 2000). In the Marennes-Oléron Bay, *C. gigas* standing stock reaches an average of 110,000 tons over an area of 2,600 hectares. The annual production, 30,000–50,000 tons, represents 25% to 40% of the total production in France, according to the specific year. The area is active in the collection of juveniles, partly reared on site and partly sold to other production areas. During the last 2 decades (1980–2000), the bay has experienced a significant increase in biomass of suspension-feeding species. From a previous stock of filter-feeders around 10,000 tons in 1971, it was estimated at more than 86,000 tons in 1984 to 1985. Finally during a third period (1993 to 1995) the biomass has been estimated to be more than 124,000 tons (Héral 1989, Sauriau 1992). Moreover, today, carrying capacity of the bay and economic productivity can be affected by anthropogenic pressure and global change (Soletchnik et al. 1998, Soletchnik 2001).

Since the beginning of the 1960s, abnormal episodes of *C. gigas* mortality have increased in the world, as reported in Japan by Imai et al. (1965), Mori et al. (1965) and in the United States by Beattie et al. (1980), Perdue (1983), Farley (1992), and Cheney et al. (2000 and 2001). In Europe, where the Pacific cupped oyster *C. gigas* was introduced from Japan around 1970 to replace *C. angulata*, summer mortality events were also reported. Significant mortality events (>30%) occurred in 1976 to 1977 (Parache 1989) and then in the 1980s on various oyster-rearing sites: (1) Arcachon Bay, south-west in 1982 to 1983 (Maurer et al. 1986); (2) Marennes-Oléron Sound in 1988 and 1993 (Lodato 1997); and (3) Brittany (west) and Normandy (north-west) in 1994 to 1995 (Gouilletquer et al. 1998, Fleury et al. 2001).

Moreover, in the Marennes-Oléron Bay, oysters reared "onbottom" using traditional culture methods are more severely affected by mortality than "off-bottom" cultures (Soletchnik et al. 1999). According to Cheney et al. (2000), mortalities occurring in *C. gigas* are the result of multiple factors or stressors including elevated temperature, low dissolved oxygen, xenobiotic stress, and physiologic stress associated with reproduction. Summer mortalities of oysters coincide with the gonad maturation period (Mori 1979, Perdue et al. 1981, Maurer et al. 1986, Soletchnik et al. 1997). For example, Japanese authors (Mori et al. 1965, Tamate et al. 1965) compared Onagawa Bay, where summer mortality was negligible, to Matsushima Bay, where summer mortality events were important, and they concluded that an over-maturation of oocytes in the eutrophic Matsushima Bay leads to "physiological disorder and metabolic disturbance" of oysters.

The aim of this study is to investigate if rearing conditions, including both culture techniques ("on" and "off-bottom" condition) and two depths, might affect the rearing performance of *C. gigas*. These two factors ("rearing method" and "depth") were crossed to create four rearing conditions. Biologic parameters, including mortality, shell growth, meat growth, and biochemical contents were monitored and analyzed for possible relationships with environmental conditions (both sediment and water column) to explore which parameters may contribute to differences in oysters reared under the varied conditions during the spring and summer period. Unlike classic studies focusing on single parameters, our

combining of biologic performance with hydrologic and sedimentary parameters permitted us to test whether proximity to the sediment and depth of rearing have a direct (e.g., due to physico- chemical quality of sediment) or indirect (e.g., by access to trophic resources) effect on the survival and biology of oysters and therefore on shellfish productivity.



**Figure 1.** Experimental sites on “Ronce-Perquis” oyster bank in the southern part of the Marennes-Oléron Bay (French Atlantic Coast). Black point surface are the 250 ha of breeding oysters in the Marennes-Oléron Bay. “Star point” face to the oysterbank was the station for hydrological samplings.

## MATERIALS AND METHODS

### *Animals and Experimental Site*

Adult oysters reared in the Marennes-Oléron Bay were used in this study. They were <2 y old at the beginning of the experiment. The experimental area (Ronc Perquis 175 ha), situated in the southern part of the bay, has been used since 1996 for oysterfarming studies (Lodato 1997). Fifteen sites (Fig. 1) were chosen to form a survey covering the entire area. Each site included an “on” and “off-bottom” set of cultured oysters. For “on-bottom”, an enclosure of about 15 square meters was sampled. For the “off-bottom” condition, oysters were reared in traditional plastic bags (0.5 m × 1.0 m) supported on an “iron culture table” 50-cm high. Among the 15 sampled sites, 8 sites represented the shallow water (S) condition (n 1, 2, 5, 7, 8, 10, 11, 12; Fig. 1) with 50% to 65% immersion time, and seven (n 3, 4, 6, 9, 13, 14, 15; Fig. 1) were identified as the deep water (D) condition, with an immersion time ranged from 65% to 80%. Accordingly, 4 experimental conditions were defined:

- D on: oysters reared on deep sites (D) and “on-bottom” culture condition (65% to 80% immersion)
- D off: oysters reared on deep sites (D) and “off-bottom” culture condition (62% to 76% immersion)
- S on: oysters reared on shallow sites (S) and “on-bottom” culture condition (48% to 65% immersion)
- S off: oysters reared on shallow sites (S) and “off-bottom” culture condition (45% to 60% immersion)

### *Biologic Parameters*

All the following parameters were measured monthly from March to August. At each sampling date and for both rearing conditions (“on” and “off-bottom”), results are expressed as the mean value obtained in the eight deep water and in the seven shallow water-sampling sites (Fig. 1).

#### **Mortality**

For “on-bottom” oysters, dead and live animals were counted inside a wooden frame with an area of about 0.5 m<sup>2</sup> (0.7 m × 0.7 m), as described by Lodato (1997). For “off-bottom” oysters, mortality was monitored by counting dead oysters in 7 to 8 oyster bags, containing an initial population of 200 live oysters. The daily mortality rate was calculated for each period:  $([\text{initial number} - \text{final number}] / [\text{final number} + \text{initial number}] / 2) / \text{number of days} \times 100$ .

Biometry was recorded from 30 individuals per sampling date (an additional sample was being done in October) and site. Weighings (in g) were recorded to the nearest 0.01 g. Total live weight (shell + meat) was immediately recorded when oysters were brought from the shore to the laboratory. Dry shell weight was measured after 24 h drying in an oven at 60°C, and dry meat weight was recorded after freeze-drying for 36 h.

#### **Biochemical Analyses**

All biochemical analyses were performed on 3 pools of 10 oysters. Each biochemical component was determined by a spectrophotometric method. Protein content was evaluated using the Lowry et al. (1951) method after extraction with 1N sodium hydroxide. Lipids were extracted and purified according to the protocol of Bligh and Dyer (1959), and the analytical procedure was from Marsh and Weinstein (1966). Carbohydrate and glycogen (precipitated with absolute ethanol) were quantified using the phenol-sulfuric acid method as described by Dubois et al. (1956). Results are expressed as mean of lipid, carbohydrate, and glycogen content in mg per oyster.

#### **Environmental Parameters**

Sediment and hydrologic measurements were made to better understand how environment could affect the biologic performances of the oysters reared under the four experimental conditions.

#### **Sediment**

For sediment analysis, results were expressed as the mean value obtained in the 8 shallow sites (n 1, 2, 5, 7, 8, 10, 11, 12) and in the 7 deep sites (n 3, 4, 6, 9, 13, 14, 15). Samplings were done twice per month from April to August: redox potential (Eh, in mVolt) is a chemical indicator for the quality of superficial layer of sediment. High positive values suggest good oxidative processing of organic material (recycling) and positive photosynthetic activity; whereas, negative values suggest a reduced environment and the lack of oxygen in the superficial mud layer. In this study, Eh was measured in situ in the upper 2 cm of the sediment using a potentiometer and adapted hydrogen probe. “Eh reference” (Ehref) was used to correct Eh readings (as cited in Hussenot & Martin 1995):  $Eh = E - E_{\text{href}}$ . Carbon content was measured with a carbon analyzer (CHNS/O 2400); 25 mg dry mud aliquot was assayed, and values were converted to mg of carbon per gram of dry mud. Ammonium, nitrites, nitrates, silicates, and phosphates contents of the mud were measured in the laboratory by colorimetric methods with a flux analyzer (auto analyzer: Skalar); interstitial water was obtained by centrifugation of one Liter of a 2-cm depth mud sample. Results were expressed as mM of the various components per L of mud.

#### **Water Column**

Seasonal changes in seawater composition were monitored fortnightly from February to September 1997 at a station facing the oyster bank (marked by > on Fig. 1). Data were obtained from 2 samplings: 50 cm under the surface and 50 cm above the bottom. Methods have been presented in a previous hydrologic study covering a 1977 to 1985 period (Soletchnik et al. 1998) including: physical measurements: temperature and dissolved oxygen; nutrients: ammonium, nitrates, silicates, phosphates; chlorophyll a.

#### **Statistical Analysis**

Biometric measures are presented as: mean ( $120 < n < 210$ ) ± SD. To test significant differences of environmental or biologic parameters according to rearing conditions, parametric (ANOVA or MANOVA) and non parametric (Kruskal-Wallis test) methods were used (Scherrer 1984). In cases of percentage or ratio data, variables were normalized using the “Arcsin square root” transformation. The Least significant difference (LSD) test (Fisher

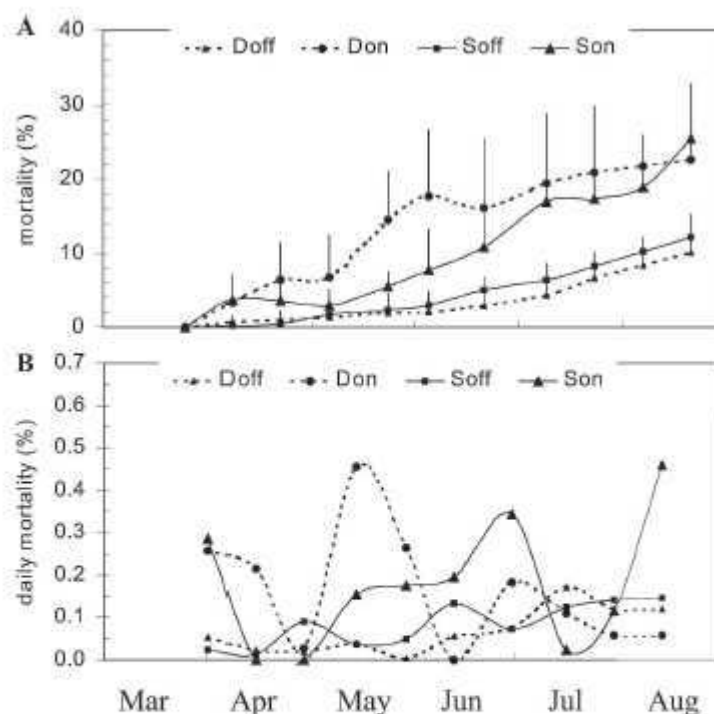
algorithm procedure) was performed in ANOVA to test homogeneous groups of responses. Statistical analyses were performed using STAGRAPHS Plus 5.1 software.

## RESULTS

### Biologic Parameters

#### Mortality

In mid July, cumulative mortality reached 15% for “on-bottom” culture and less than 5% for the “off-bottom” culture (Fig. 2a). In August, differences continued between “off” and “on-bottom” culture, and mortality reached 25% and 10%, respectively, for “on” and “off-bottom” cultures. Despite differences between “deep on-bottom” (Don) and “shallow on-bottom” (Son) cultures in May–June, with an earlier mortality event in May for the oysters reared Don, final mortality was similar for the different groups of oysters reared with more or less immersion time. A multifactor analysis of variance (MANOVA) performed on August mortality results shows there were no significant differences in mortality between deep (D) and shallow (S) conditions ( $P = 0.2152$ ). In contrast, a significantly higher mortality ( $P = 0.0000$ ) was observed for oysters reared “on-bottom” as compared with those reared on tables (“off-bottom”). No significant interactions were found between “culture type” and “depth” of culture ( $P > 0.05$ ). The “daily mortality rate” variable allowed us to clarify the occurrence of mortality in time (Fig. 2b). Oysters reared “on-bottom” in the deeper condition (Don) were affected by a sudden mortality in May, when in shallow water (Son), mortality looked more chronic. The daily mortality rate never exceeded 0.2% in both “off-bottom” conditions.



**Figure 2.** Variation during the studied period (March to August 1997) of (a) mean cumulative mortality (in % + SD,  $n = 4$  to 8) and (b) mean daily mortality (in % per day + SD,  $n = 4$  to 8) of *Crassostrea gigas* oysters reared in 4 conditions: Don = deep sites and “on-bottom” culture condition (65% to 80% immersion); Doff = deep sites and “off-bottom” culture condition (62% to 76% immersion); Son = shallow sites and “on-bottom” culture condition (48% to 65% immersion); Soff = on shallow sites and “off-bottom” culture condition (45% to 60% immersion).

#### Growth Performance

The MANOVA tests conducted on growth variables show that the 3 factors (time, type of rearing, and depth of rearing) all had a significant ( $P < 0.05$ ) effect on total weight, shell weight, and dry meat weight (Table 1). For these 3 variables, the interaction “time” and “type of rearing” was significant ( $P < 0.001$ ), indicating that the effect on growth of rearing “on-bottom” or “off-bottom” was not stable during the period of oyster maturation. Growth performance was compared in October, after oysters recovered from their spawning period. Within 7 m, in the deep water condition, the weight gain of oysters reared “on-bottom” was +56%, compared with +70% for “off-bottom” reared oysters. In the shallow

water condition, weight gain was +58% and +72% for “on” and “off-bottom” reared oysters, respectively (Fig. 3a). The same trend was observed in shell growth (Fig. 3b): in deep water, +67% “on-bottom” +80% on tables and in shallow water, +46% “on-bottom” +57% on tables. At the end of the study, both “off-bottom” culture as well as “deeper” conditions (longer immersion time) allowed a significantly better growth of the shell (LSD test;  $P < 0.0000$ ). Oysters reared “on-bottom” had more irregular shell growth patterns: there was a 1 m delay in growth initiation for oysters in the deep site (Don) and nearly 4 m for the shallow site (Son). As for shell growth, at the end of the experiment, the analysis of variance applied to dry meat weight allowed us to discriminate ( $P < 0.0000$ ) all the experimental conditions (Fig. 3c). A gain of around 16% and 19% was observed when comparing growth of dry meat weight of oysters reared on tables, versus “on-bottom.” Similarly, an improvement of dry meat weight of about 30% was observed, comparing oysters reared in deep water condition versus shallow water. Thus, similarly to shell growth, growth of dry meat was improved by “on table” culture conditions and increased immersion time. Increase of dry meat weight from March to July reflected the maturation processing. The weight decrease at the beginning of August coincided with the release of gametes during spawning for all conditions, except oysters reared on tables in the deep site (Doff), for which spawning had taken place about 15 days earlier. These results are in accordance to the oyster larvae survey program (Piquet, pers. comm.) showing two main peaks of oyster larvae in the south part of the basin, one in the middle of July and one during the first week of August. Measures of the loss of dry meat weight in summer (Fig. 3c) allowed us to estimate the spawning effort (gametes released): oysters reared “off-bottom” showed almost twice the spawning effort (0.67–0.75 g) obtained “on-bottom” (0.33–0.36g), regardless of the depth condition.

TABLE 1.

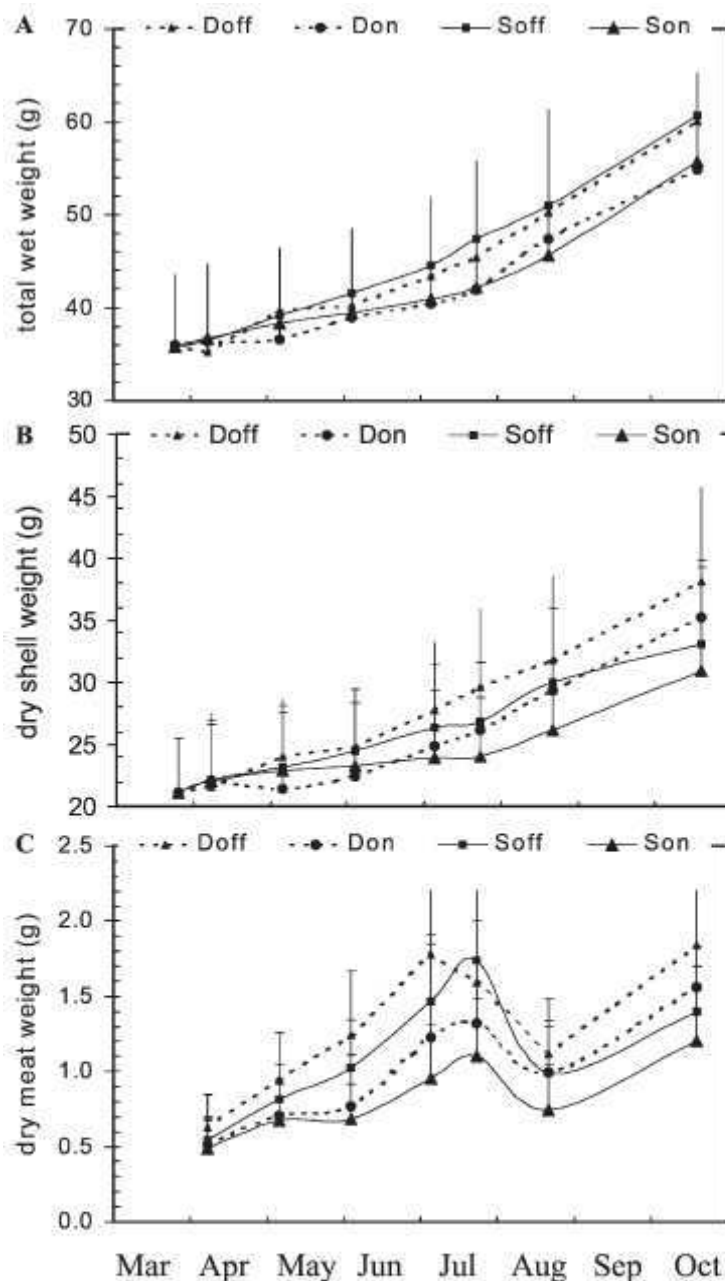
Analyses of variance for total weight, dry shell and meat weight, versus factors: time (T), rearing conditions (“on” bottom or “off” bottom rearing) (R), and depth (shallow or deep sites) (D), and their interactions.

Variable	Factor	df	MS	Fisher Test	P
Total wet weight (g)	Time (T)	7	36954.7	481.63	<0.001
	Rearing (R)	1	8762.2	114.20	<0.001
	Depth (D)	1	445.9	5.81	<0.05
	T × R	7	819.2	10.68	<0.001
	T × D	7	55.0	0.72	ns
	R × D	1	106.3	1.39	ns
	Residual		6053	76.7	
Dry shell weight (g)	Time (T)	7	13509.7	442.7	<0.001
	Rearing (R)	1	4705.3	154.2	<0.001
	Depth (D)	1	2145.2	70.3	<0.001
	T × R	7	314.7	10.3	<0.001
	T × D	7	598.2	19.6	<0.001
	R × D	1	75.2	2.5	ns
	Residual		6082	30.5	
Dry meat weight (g)	Time (T)	6	92.1	499.8	<0.001
	Rearing (R)	1	111.3	604.1	<0.001
	Depth (D)	1	36.3	197.2	<0.001
	T × R	6	5.4	29.2	<0.001
	T × D	6	3.7	19.9	<0.001
	R × D	1	0.05	0.27	ns
	Residual		5300	0.18	

#### Biochemical Analysis

The MANOVA tests carried out on the biochemical components of the meat show that, like for the growth data, the 3 factors, time, type of rearing, and depth of rearing, and the interaction of the latter two factors with time, were highly significant ( $P < 0.001$ ) (Table 2). The lipid content of the dry meat, reflecting gametogenesis, confirmed the observation made on dry meat weight, with a drop in July (Doff) or beginning August (Soff) from ~240 mg to ~100 mg lipid per animal, for oysters reared on tables, and from ~150 mg to ~90 mg in the beginning of August for oysters cultured “on-bottom” (Fig. 4a). Contrasting biochemical patterns appeared for both carbohydrates and glycogen content, between “on” and “off-bottom” rearing conditions (Fig. 4b, 4c): carbohydrates increased from 20 mg per oyster in March, to 120 mg and 70 mg in May for “off” and “on-bottom” rearing conditions, respectively. Thereafter, carbohydrate content dropped to 20–30 mg per oyster in the second part of July, just before spawning and during the last period of carbohydrate storage conversion into lipids. These biochemical changes show that this period is intensively costly, in

terms of energy. Glycogen comprised 33% to 80% of carbohydrates according to rearing conditions and sampling dates. Glycogen contents of the “on-bottom” culture oysters decreased slightly from below 50 mg to about 10 mg per oyster from the beginning of May to the middle July; whereas, glycogen content of “off-bottom” cultured oysters showed a delayed decrease, 75–20 mg, from the end of May to July (Fig. 4c). For both “Son” and “Soff” rearing conditions in July, glycogen content decreased to 9 mg per oyster, representing only 2% to 3% of the dry body weight. For lipid profiles, differences between oysters reared in deep sites and those in shallow were probably attributable to time of spawning, and the main biochemical differences were also related to the culture conditions, “on” or “off-bottom.” The amount per oyster in lipid or carbohydrate, almost double for “off-bottom” culture compared with “on-bottom” culture, is in agreement with the growth and quantities of gametes release in these two conditions. In contrast to the three other conditions, oysters reared in deep water and “on table” showed a precocious start in spawning in July, when the main spawn occurred between the end of July and the beginning of August.



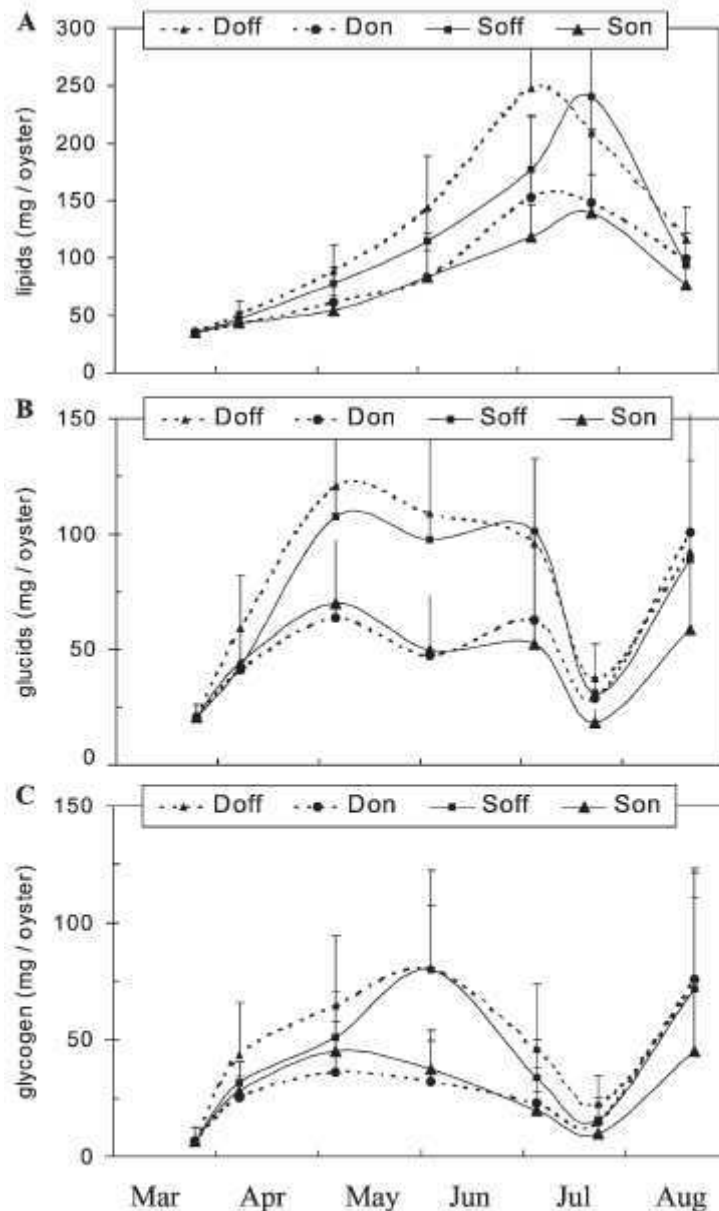
**Figure 3.** Variation during the studied period (March to October 1997) in g + SD ( $n = 30$ ) of (a) mean total wet weight; (b) dry shell weight, (c) dry meat weight of oysters reared in 4 conditions: Don = deep sites and “on-bottom” culture condition (65% to 80% immersion); Doff = deep sites and “off-bottom” culture condition (62% to 76% immersion); Son = shallow sites and “on-bottom” culture condition (48% to 65% immersion); Soff = on shallow sites and “off-bottom” culture condition (45% to 60% immersion).

TABLE 2.

Analyses of variance for dry meat biochemical content (lipids, carbohydrates and glycogen content per oyster), versus factors: time (T), rearing conditions ("on" bottom or "off" bottom rearing) (R), and depth (shallow or deep sites) (D), and their interactions.

Variable	Factor	df	MS	Fisher test	P
Dry meat lipids content (mg)	Time (T)	6	216571	92.37	<0.001
	Rearing (R)	1	212675	90.71	<0.001
	Depth (D)	1	47215	20.14	<0.001
	T × R	6	15791	6.74	<0.001
	T × D	6	11530	4.92	<0.001
	R × D	1	1114	0.48	ns
	Residual	520	2344		
Dry meat carbohydrates content (mg)	Time (T)	6	438008	199.85	<0.001
	Rearing (R)	1	138353	63.13	<0.001
	Depth (D)	1	72718	33.18	<0.001
	T × R	6	9878	4.51	<0.001
	T × D	6	33502	15.29	<0.001
	R × D	1	49	0.02	ns
	Residual	498	2191		
Dry meat glycogen content (mg)	Time (T)	6	535631	247.67	<0.001
	Rearing (R)	1	81401	37.64	<0.001
	Depth (D)	1	43493	20.11	<0.001
	T × R	6	12412	5.74	<0.001
	T × D	6	24967	11.54	<0.001
	R × D	1	583	0.27	ns
	Residual	487	2163		





**Figure 4.** Variation during the studied period (March to August 1997) of biochemical composition of the dry meat in mg per oyster + SD ( $n = 12-24$ ) (a) lipids, (b) carbohydrates, (c) glycogen of oysters reared in 4 conditions: Don = deep sites and “on-bottom” culture condition (65% to 80% immersion); Doff = deep sites and “off-bottom” culture condition (62% to 76% immersion); Son = shallow sites and “on-bottom” culture condition (48% to 65% immersion); Soff = on shallow sites and “off-bottom” culture condition (45% to 60% immersion).

### Environmental Parameters

#### Sediment

Redox potential of the mud had a seasonal cycle that ranged from  $-80$  to  $+50$  mV and  $-20$  to  $+50$  mV for “shallow” and “deep” sites, respectively, and curves appeared to be bimodal (Fig. 5a). After increasing Eh values in April for both conditions, a decrease was observed during late spring and reduced conditions in the mud started earlier at depth, compared with shallower sites. No significant differences were revealed from the overall Eh measures (Table 3). Carbon content per gram of dry mud increased from 17–22 mg between April and June for “shallow” sites and from 14–19 mg between April and late July, for “deep” sites (Fig. 5b). “Shallow” sites always showed more carbon than deeper sites (Table 3). In interstitial water from the mud, nitrogen components ranged from 0–30 mmol L<sup>-1</sup> of nitrates, and from 40–160 mmol L<sup>-1</sup> of ammonium (Fig. 5c, 5d). No significant differences were found between the nitrate content in shallow and deep sites (until July); whereas, deep sites showed a significant higher ammonium concentration, 84.4 mmol L<sup>-1</sup> mean for the overall period compared with shallow sites, 57.0 mmol L<sup>-1</sup> (Table 3). Silicate contents were also

significantly different between “deep” and “shallow” conditions, with again a higher value (149 mmol L<sup>-1</sup>) for the deeper condition (Fig. 5e; Table 3). The silicate concentrations increased from 80–100 mmol L<sup>-1</sup> to 180–240 mmol L<sup>-1</sup> between March– April to July–August, showing progressive silicate regeneration (recycled) in the interstitial water. Phosphates also increased in the interstitial water, but later and suddenly at the end of July, from 2 mmol L<sup>-1</sup> to 10 mmol L<sup>-1</sup> (Fig. 5f). This increase was more marked at the deeper sites where phosphate levels in the mud were significantly higher than at the shallower sites over the whole study period (Table 3). This difference was greatest in August.

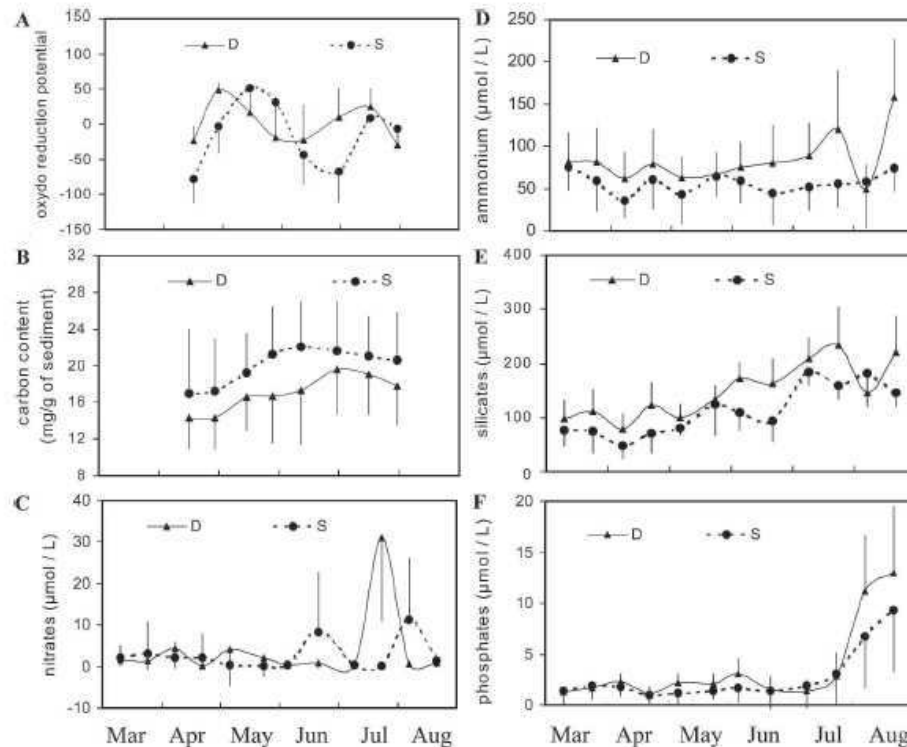


Figure 5. Variation during the studied period (March to August 1997), for D = deep sites (62% to 80% immersion time) and S = shallow sites (45% to 65% immersion time), of: (a) the mean oxido-reduction potential in mV (+SD for D; -SD for S;  $n = 5-8$ ), and (b) the mean carbon content in mg per g of sediment (+SD for S; -SD for D;  $n = 5$  to 8) in the two first centimetres of sediment, and the mean nutrient content in  $\mu\text{mol per L}$  of sediment (2-cm depth); ( $n = 7-9$ ): (c) nitrates (+SD for S; -SD for D), (d) ammonium (+SD for D; -SD for S), (e) silicates (+SD for D; -SD for S), (f) phosphates, (+SD for D; -SD for S).

TABLE 3.

Non parametric Kruskal-Wallis tests for (1) Redox potential (mV), (2) carbon content in the sediment ( $\text{mg g}^{-1}$ ) and (3) nutrients content in interstitial seawater ( $\mu\text{mol L}^{-1}$ ) (nitrates, ammonium, silicates and phosphates), compared between the shallow and deep sites conditions.

Variable	H	P
Redox potential	1.207	0.2719 (n.s.)
Carbon content ( $\text{mg g}^{-1}$ )	9.768	0.0017 (**)
Nitrates ( $\mu\text{mol L}^{-1}$ )	1.295	0.2551 (n.s.)
Ammonium ( $\mu\text{mol L}^{-1}$ )	16.101	<0.0001 (***)
Silicates ( $\mu\text{mol L}^{-1}$ )	10.472	0.0012 (**)
Phosphates ( $\mu\text{mol L}^{-1}$ )	6.406	0.0114 (**)

#### Seawater Column

Water temperature varied from 9°C in February to 22°C in August (Fig. 6a). After an initial increase from ~15°C to ~20°C during May to the middle of June, a cooling period was observed at the end of June, followed by a new increase until the maximum in August. Dissolved oxygen ranged 80% to 107% with the highest values in June (Fig. 6b) related to a peak of chlorophyll a ( $12 \text{ mg L}^{-1}$ ) (Fig. 6c). Heterogeneity in water column (comparing bottom and surface sampling) was seen in the middle of July, with 80% values of dissolved oxygen for “surface” measurement (Fig. 6b). Chlorophyll a showed a very inconsistent pattern (disrupting events), with monthly alternation in values ranging 7–15 mg

L<sup>-1</sup> (approximate middle of the month) and values ranging 1–3 mg L<sup>-1</sup> (around the end of each month) with a good correlation between “bottom” and “surface” (Fig. 6c). The main blooms, in June, July, and August occurred some days after a low tide coefficient level (neap tide period). High chlorophyll a concentrations (about 14 mg L<sup>-1</sup>) were also recorded in September. Nutrient concentrations in the water column depend on water inflow, primary production, and sediment exchanges. Phosphates (0.1–0.5 mmol L<sup>-1</sup>) increased in the water column starting in August (Fig. 6d), when they also increased in the interstitial water of the mud, but to a greater extent (Fig. 5f). Silicate concentrations in the water were ~13–17 mmol L<sup>-1</sup> in February to March and September (Fig. 6e). Values oscillated during spring to reach the lowest values (~2 mmol L<sup>-1</sup>) at the end of June. Water-column silicates increased again during summer, as well as in the sediment interstitial water (Fig. 5e). Nitrates decreased from 30 mmol L<sup>-1</sup> in February to March to ~3 at the end of summer (Fig. 6f), whereas ammonium rose slightly from 1 mmol L<sup>-1</sup> to 6 mmol L<sup>-1</sup> in surface samples, and 3.5 mmol L<sup>-1</sup> in bottom samples (Fig. 6g). Differences in ammonium concentration only occurred in summer, with slightly more ammonium detected in the surface of the water column. If nitrates were low in the interstitial water (Fig. 5c), then ammonium was trapped in the sediment and maintained a high concentration (Fig. 5d), with little release to the water column (Fig. 6g), in contrast to nitrates during spring (Fig. 6f).

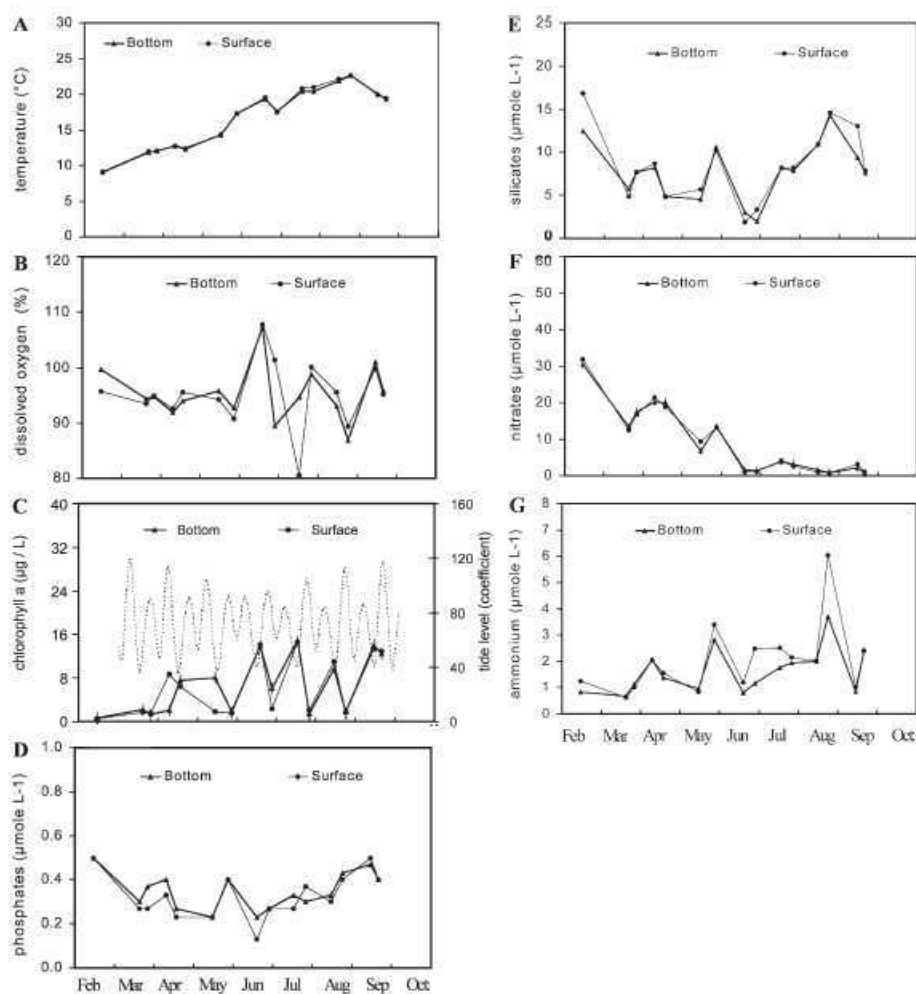


Figure 6. Hydrological parameters: (a) temperature, (b) dissolved oxygen, (c) chlorophyll a, (d) phosphates, (e) silicates, (f) nitrates, (g) ammonium in the water column in the south of Marenne-Oléron Bay from February to September 1997. “Bottom”: sampling 50 cm above the bottom; “Surface”: samplings 50 cm below the surface.

## DISCUSSION

Comparison of the four rearing conditions tested in the present study revealed first that oyster growth performance (shell and meat weight) was poorer in shallow water sites than in deeper sites. These results are in accordance with those of Gouletquer et al. (1987) and Garcia-Esquivel et al. (2000), who reported on the whole weight of *C. gigas* deployed in 3 bathymetric levels or on oysters located at the head versus the mouth of a bay, respectively.

The second clear finding of this study was the drastic effect of nearness to the bottom on oyster survival. This effect has already been reported by Soletchnik et al. (1999). Moreover, the combined effect of culture methods, “off-” and “on-bottom” conditions, and immersion time; deep or shallow site, on biologic performance was demonstrated in this study. Indeed, better performance, as shell and meat growth, were obtained for oysters reared on tables located at deeper sites (Doff condition); whereas, the lowest performance was obtained with oysters reared “on-bottom” in shallower waters (Son condition). Intermediate conditions (Don and Soff conditions) showed similar results in late August, but were dissimilar in the earlier period, better performance seen in oysters reared at lower depth (Soff). Surprisingly, a reverse comparison was observed in October, with a clear advantage for the oysters reared at greater depth, directly on the sediment. This reversed result between “spring-early summer” period and October, contrasting “deep water” and “on-bottom” conditions is more pronounced than the “shallow water” and “off-bottom” contrast. We hypothesize that these contrasts may be attributable to food availability differences; during early fall, the time of access to food (immersion) was more limiting than the culture condition and inversely in spring and early summer.

Third, concerning the reproductive cycle of oysters, two interesting results were obtained in this study: Gametes releasing (estimated by dry meat weight loss during spawning) appeared independent of the immersion time, but strongly related to culture conditions: culture “on-bottom” reduced the reproductive effort by a factor two, as well as biochemical components related to energy storage and metabolic processes involved in reproduction. The reproductive effort was inversely proportional to oyster mortality rate: lower reproductive effort was, thus, related to the higher mortality rate for oysters reared “on-bottom”. Recent literature emphasized the role of the physiologic status of marine bivalves and particularly the reproductive status in mortality occurrence. In *Ensis arcuatus*, mortality was explained as a postspawning phenomenon and larger razor clams were described as more susceptible to mortality from undetermined causes (Fahy et al. 2002). In the mussel, *Mytilus edulis*, reared in Canada, summer mortality began in late July because the major spawning event was ending (Myrand et al. 2000). In the same species, Worrall and Widdows (1984) reported that highest mortalities were associated with highest reproductive effort. According to Beattie et al. (1980), *C. gigas* mortalities were coincident with the period of maximum gonad condition for spawning. In this study, highest mortalities arose before spawning, and oysters in conditions with higher mortalities did not show the highest reproductive effort. It thus appears that reproductive processes, which were potentially involved in mortality occurrence, did not act in a same way and/or were not a major contributor to mortality in this study.

Independent of mortality events, differences were observed in spawning occurrence. For example, oysters reared in deep water and “off-bottom” showed a precocious spawning, which may indicate better maturation of oysters reared on iron tables than those reared on-bottom. An earlier gamete release seems to reduce basic metabolic and energetic costs of ripe gonads (Soletchnik et al. 1997, Honkoop 2003). Accordingly, earlier spawning diminishes the chance of food limitation later in the season and thus an energy deficit during the spring critical period when trophic resources fluctuate widely in the Bay (Vouve 2000).

Biochemical components of oyster meat are often analyzed to assess culture conditions and biologic status of the animals (Maurer et al. 1986, Deslous-Paoli & Heral 1988, Ruiz et al. 1992, Garcia-Esquivel et al. 2000, Kang et al. 2000). Glycogen plays a central role in the energetic reserves of many marine bivalves (Gabbot 1975, Ruiz et al. 1992, Mathieu & Lubet 1993). In the present study, the ratio of glycogen/total carbohydrates showed similar trends in the four rearing conditions and was highly variable (33% to 80%). A decrease between two consecutive sampling dates can suggest first, that glycogen had been mobilized from reserves to supply energy for gametogenesis, or second, that an important amount of free carbohydrates had been assimilated from the environment by oysters. Neither of these two hypothesis can be excluded, however, during this study, concomitant chlorophyll peaks in the water column (April and June to July), and decreasing glycogen/carbohydrate ratios seem to reinforce the second hypothesis, because tissue glycogen content is often related to food source availability (Deslous-Paoli et al. 1981, Kang et al. 2000). In Marennes-Oléron Bay, 1% glycogen in the dry meat weight of oysters had been interpreted as a sign of overstocking (Deslous-Paoli & Heral 1988). In this study, low glycogen content of 10 mg per oyster, that is to say <1% of the dry body weight, recorded in oysters reared in shallow water conditions in the end of July, suggests that food resource was scarce at this time and in this environment. Further, food limitation was coincident with the increased energetic cost to reach sexual ripeness. If a low level of glycogen was sometimes related to mortality events (Mori 1979, Perdue et al. 1981, Allen & Downing 1986, Maurer et al. 1986), this may not be the only cause of mortality observed in this study where “off-bottom” bred oysters were not as severely affected by mortality as “on-bottom” reared oysters with almost the same low glycogen content.

Spring and summer are maturation seasons for oysters in the Marennes-Oléron Bay (Deslous-Paoli et al. 1981, Soletchnik et al. 1997, Soletchnik et al. 2002). Type of culture and rearing depth both interacted in affecting oyster lipid profiles, which reflect the maturation pattern. Deep-water culture supported higher lipid contents in oyster than shallow water culture, and it could be interesting to investigate more precisely the reproductive effort according to immersion time in further studies. Nevertheless, the main difference in lipid content, as for the other biochemical components, arose from the difference between “on” and “off-bottom” culture. Spawning did not depend on immersion time, but it did change with the type of culture. Thus, reproductive investment was double for “on table” rearing condition, compared with “on-bottom” rearing, independent of the immersion time. Significant differences between “on” and “off-bottom” reared oysters were also recorded for carbohydrate and glycogen contents, showing the inefficiency of “on-bottom” oysters in storing sugar from surrounding food resources. According to the trends in the evolution of lipid content and growth in terms of

dry weight, it appears that maturation investment and somatic gains observed either in shallow water (45% immersion) or in deeper water (70% immersion) were similar during spring and early summer (until spawning). It seems, therefore, that oysters reared on tables experienced more favorable environmental conditions compared with those reared on the sediment surface. Even though the length of the period of access to food was shorter for oysters reared on tables, this parameter was not primordial during maturation and spawning. However, this factor became more important during the postspawning period, because the growth performances in late-summer and early-autumn were linked with the duration of immersion. Thus, finally, independent of immersion time, “on-bottom” cultured oysters had poorer growth, reproductive effort, and survival. These results suggested the hypothesis that one factor (or more), which could be called “mud effect”, resulting from the bottom nearness, was able to affect the growth, reproduction, and survival capacity of oysters reared “on-bottom.”

In this study, the risk of chemical toxic effect from the sediment was investigated particularly through redox potential and ammonium changing in the interstitial mud water. Redox potential is a good indicator for sediment “health,” including its capacity to serve as a habitat for benthic fauna (Hussenot & Martin 1995). Intensive culture in shallow water bays is known to reduce the redox potential of the surface sediment. During this study, redox potential in the upper 2 cm of the mud gave values as low as  $-50$  to  $-70$  mV in June on top of the oyster bank; according to Farias and Salamanca (1990),  $-100$  mV represents a reduced sediment. High positive values, ranged  $150$ – $300$  mV, were found only in the mudflat in the northern part of the Marennes-Oléron Bay in a 1996 to 1998 study (Vouve 2000). In the southern part of the bay, the low Eh of the sediment may indicate a stress to “on-bottom” oysters, even though the sediments were not actually anoxic. In the present study, dissolved oxygen in the water column ranged from 91% to 102%, excluding strong hypoxic events. Concerning nitrogen components, direct toxic effects on oysters of  $\text{NH}_4^+$  released from the mudflat was improbable. In fact, during this study the maximum values of measured ammonium and nitrate in the water column did not exceed 6 and 30  $\text{mmol L}^{-1}$  respectively, and Epifanio and Srna (1975) showed that nitrate concentrations as high as 10,000 to 100,000  $\text{mmol L}^{-1}$  are necessary to induce 50% mortality on bivalves. However, the risk may increase under basic conditions of pH which transforms the  $\text{NH}_4^+$  form to the much more toxic  $\text{NH}_3$  form, and these conditions could be found during phytoplanktonic blooms through photosynthetic activity. However, this remains to be demonstrated as a real environmental risk for oyster culture in the south of Marennes-Oléron Bay.

Temperature, and particularly its variation, could affect oysters, particularly in shallow waters. Nevertheless, higher mortality rates in shallow sites were not recorded, and results only underlined the major effect of bottom proximity. The maximum temperature was recorded after the main mortality events (except in “Son condition”) had already occurred. Nevertheless, for a short period, at the surface of the mud, temperature in spring is able to reach much higher values than in the water column (Guarini et al. 1997). Sediment, which is a very active biologic compartment, may also play a role in the primary production by trapping and releasing nutrients. This could significantly modify the cycle of nutrients in the top of the mudflat and consequently the availability of food for oysters reared “on-bottom.” Oxygen plays an important role in this microecosystem, producing an oxidative layer as suitable mudflat habitat for oysters. Nutrients are trapped in this layer and continuously exchanged with the overlying free water. Interstitial water ammonium concentration ranging from 40–160  $\text{mmol L}^{-1}$  in spring and summer confirmed an excess in nutrient for primary production in the mudflat as already reported by Vouve (2000). Lower values of ammonium and silicate concentrations at the upper sites in spring and summer may be related to the nutrient uptake for primary production, especially microphytobenthic, directly related to the immersion time (Guarini et al. 1997, Thornton et al. 1999). This would suggest more available food for oysters reared at upper level on the oyster bank, but this is in contradiction with maturation performances during spring and summer for these oysters. The relative trophic richness of shallow sites might not therefore compensate for the shorter filtration time experienced by oysters placed at this level. Phosphate concentration in the sediment showed a “flat profile” from March to July and increased suddenly during July, suggesting biologic (recycling) and chemical processes. According to Feuillet-Girard et al. (1997), decreasing values in the water column from March to June suggested an uptake process, when increasing values from June to September suggested a releasing process. In this study, increasing concentration in phosphates, silicates, and ammonium in the water column from the end of June to August, suggest an increasing release of these nutrients from the sediment. Finally, the nutrient level in mud interstitial water does not explain the observed differences in oyster performances according to bottom nearness.

## CONCLUSION

The two main effects identified in this study were “immersion time” and the factor called “mud effect.” Immersion time clearly allowed improved oyster growth performance from spring to early autumn, in relation to increasing food access when immersion increased. But this immersion time effect is considerably less in light of spawning investment or mortality, with both under control of the “mud effect.” From “sediment study” either redox potential or ammonium monitoring did not reveal a real risk for oyster cultures, regardless of depth. Other potential risks have to be considered. Lower nutrient concentration in the interstitial water of sediment higher up the shoreline suggests increased primary production at this level, which could partially compensate in spring for the reduced immersion time.

That study pointed out how two sites spaced by only few decimeters in water column height, may constitute contrasted ecosystems able to induce significant differences in the south of the Marennes-Oléron Bay. Oysters, overstocked in this part of the bay, may contribute to this fragile balance. The absence of growth during May for “on-bottom” reared oysters show how sensitive this micro environment can be during spring and reveals (bioindicator action) an environmental stress, which remains to be investigated. As sediment also traps toxicants, research must also investigate the “toxicological point of view.” Special attention must be paid to ammonium release, which can be toxic for benthic fauna under basic (pH) conditions, but probably also to other environmental stressors, such as PCB, HAP, or pesticides.

## ACKNOWLEDGMENTS

The author thanks the LCPC team (Laboratoire Conchylicole du Poitou Charente) for realizing technical work, and sampling oysters, water column, and sediment during experiment and making laboratory analysis and biochemical measurements in 1997. The authors also thank Dr Gary Wikfors for his expert linguistic guidance and Drs Benoit Veron and Pascal Claquin for critical reading. This study was supported by the French program on summer mortality of oysters: MOREST, managed by Jean François Samain (UMR Ifremer PE2M).

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