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Summer Mortality of the Pacific cupped oyster *Crassostrea gigas* in the Bay of Marennes-Oléron (France)

by

P. Goulletquer, P. Soletchnik, O. Le Moine, D. Razet, P. Geairon, N. Faury, S. Taillade,
IFREMER Shellfish Aquaculture Research Laboratory of Poitou-Charentes
B.P. 133, 17390 La Tremblade, France.

Abstract : A comprehensive research program has been developed since 1995 to focus *on C. gigas* abnormal mortality rates occurring regularly during the summer period in the Bay of Marennes Oleron (France). Several subprojects were carried out including in-vitro ecophysiological studies to asses 1) the relationship between reproductive status, scope for growth and mortality, 2) effects of stress temperature on physiological functions. Meanwhile, field studies concerned monitoring surveys of calibrated oyster populations at various emersion time and geographic sites. A database was developed and incorporated into a geographic information system (GIS) to overlay several layers of information and establish relationship among environmental parameters and mortality rates. Concomitantly, seawater quality monitoring surveys were developed to assess carrying capacity and overall environmental conditions affecting oysters. Temperature probes were deployed at the near vicinity of oysters as well as a multiparameter probe to record, temperature, salinity, turbidity, pH and O2 concentration on a continuous basis. Moreover, a valvometer recording continuously the oyster valve closure activity was deployed near the previous probe to establish relationship with environmental conditions. *C. gigas* summer mortality rates will be discussed and a general hypothesis will be proposed to explain mortality occurrence.

Keywords: *C. gigas*, summer mortality, environmental conditions, stress, ecophysiology, in-situ continuous monitoring

Résumé : Un large programme de recherches a été développé depuis 1995 sur les mortalités anormales de *C. gigas* affectant régulièrement les populations en période estivale dans le Bassin de Marennes Oléron. Plusieurs sous projets ont été réalisés comprenant des études écophysiologiques in vitro afin d'estimer 1) les corrélations entre maturation sexuelle, potentiel de production et taux de mortalité, 2) les effets de stress thermique sur les fonctions physiologiques. En parallèle, les expérimentations terrain ont concerné les suivis de populations d'huîtres calibrées en fonction des niveaux d'émersion et de distribution spatiale. Une base de données a été développée et incorporée dans un système d'information géographique (SIG) afin de superposer différentes couches d'information et d'établir des corrélations entre les paramètres environmentaux et les taux de mortalité. Simultanément, des suivis hydrobiologiques ont été effectués afin d'estimer la capacité trophique du secteuret d'enregistrer les conditions environmentales qui peuvent affecter les populations d'huîtres. Des sondes température ont été mises en place à proximité des huîtres ainsi qu'une sonde multiparamétrique afin d'enregister en continu lees température, salinité, turbidité, pH et teneur en oxygène. De plus, un valvomètre enregistrant en continu l'activité valvaire des huîtres fut utilisé à proximité de la sonde multiparamétrique afin d'établir des corrélations entre les conditions environnementales. Une discussion sur les mortalités estivales de *C. gigas* sera effectuée et une hypothèse générale explicative proposée.

Mots clés: *C. gigas*, mortalité estivale, conditions environnementales, stress, écophysiologie, suivis environnementaux in-situ en continu

Introduction

Summer mortalities of Pacific cupped oysters *Crassostrea gigas* have become a recurring obstacle for the industry since the 1950s in several countries, including Japan and the United States (Mori, 1979; Beattie et al., 1980; Perdue et al., 1981; Friedman et al., 1991; Glude, 1975; Ventilla, 1984). Besides the Pacific oyster, mostly affected by summer mortality events, other species including the blue mussel *M. edulis* have been recently concerned (Myrand et Bergeron, 1991; Myrand and Gaudreault, 1995; Mallet et al., 1990). Several biological factors as well as hydrobiological conditions have been related to summer mortality which can reach more than 50% mortality rate : elevated seawater temperatures, salinity stress, reproductive stress, and pathogens (Lipowsky and Chew, 1972; Friedman et al., 1991; Mori, 1979; Mori et al., 1965; Perdue, 1983 ; Beattie et al., 1988; Meyers and Short, 1990; Glude, 1975; Koganezawa, 1975; Imai et al., 1965). In most cases, summer mortality showed similar patterns, affecting adult oysters characterized by a fast growing rate, rapid metabolism and/or gonad formation under high nutrients and warm water conditions. Overall conditions were resumed to develop the physiological stress theory (Glude, 1975; Ventilla, 1984). More recently, protozoan (Hexamita sp.) and bacterial (Nocardia sp.) infections were also associated with summer mortality events, respectively by Meyers and Short (1990) and Friedman et al. (1991). Although likely contributing to increased mortality rates, these infections were mainly considered as secondary invaders and not as the causative mortality agent. Koganezawa (1975) reported that oyster mass mortality arose in 1945 when the hanging culture technique was developed, which brought rapid fattening, growth, and extraordinary maturation of gonads under the nutritionally rich and high temperature conditions. To address the issue and therefore to limit summer oyster mortality, Japanese farmers adopted two cultural practices, including seed hardening and/or rearing oysters in poor nutritional areas during the gonad-maturing period and moving to nutritionnally rich places in late summer where they fatten during fall and winter. Both methods aimed to prevent and postpone heavy gonad formation (Koganezawa, 1975; Ventilla, 1984).

In the USA, Glude (1975) concluded a comprehensive 6 year study on summer mortality by identifying locations, where high potential of summer mortalities can occur, mainly the near head of highly productive estuaries, while low mortality areas were characterized as having less turbidity, lower productivity and slow growing thin oysters. The critical contribution of environmental conditions to summer mortality was therefore obviously recognized. Meanwhile, the seed source study did not lead to any genetic effect, neither resistant strain to oyster summer mortality. Although several parasites were found over the time experiment, no strong relationship was established with summer mortality rates. However, a possible bacterial infection was not rejected as an explanation while no virological study was carried out (Glude, 1975). Failure to find a causative organism tend to strengthen the physiological stress theory. Mass mortalities were obtained at the laboratory by holding oysters in water warmer than 18°C and increasing the nutrients (Lipowsky and Chew, 1972). Based on those results, a selective breeding program was developed in the Washington State (Beattie et al., 1980). It was assumed for the purposes of the selection, that summer mortalities and laboratory mortalities were of similar etiology, and that the selected parameter was conservative. Since the main field criterion correlated with mortality was elevated temperature, selection was performed by a temperature challenge at 21°C, then juveniles were produced and challenged again prior to production for the next generation. However, offspring survival did not appear to be directly related to the level of parent survival. Although, improved survival rate for these families appeared promising (Beattie et al., 1988), the program eventually faded since poor growth rates and limited condition were observed (D. Cheney, pers. com.). However, the study confirmed the relationship noted by the Japanese researchers between summer mortalities and reproductive physiology aspects of *C. gigas* (Perdue et al., 1981), while environmental characteristics such as long periods of exposure, warm temperatures, dinoflagellate blooms where recognized as possible "trigger" for the mortality among animals already in a stressed state. More recently, research programs have been developed to assess whether induced thermotolerance can result in increased oyster survival during summer mortality (Clegg et al., 1998; Shamseldin et al., 1997).

Since the massive introduction of the Pacific oyster *C. gigas* in France during the 1970s' (Grizel et Héral, 1991), similar summer mortalities have been regularly observed mainly in the Bay of Arcachon where naturall spatfall occurs on a yearly basis (Maurer and Comps, 1984; Maurer et al., 1986). Besides adult oysters, summer mortality rates have affected chronically juveniles oysters since the early 1990s and have been associated with an Herpes virus like occurrence and environmental stressful conditions (Renault et al., 1994, 1995). Since the 1980s, summer mortality have affected adult oysters sporadically along the French coastline (e.g., Southern Brittany, Marennes Oleron Bay). By way of example, a mass mortality of *C. gigas* occurred in 1988 in the Southern part of the Bay of Marennes-Oleron, leading eventually to the loss of 7800 metric tons (41% mortality rate) (Bodoy et al., 1990). Since no pathogen neither infection and pollution was detected in spite of intensive monitoring surveys, mortality rates were correlated with stressful environmental conditions. Since then, summer mortality occurrence is reported on a yearly basis on this part of the Marennes Oleron Bay, without any pathogen occurrence.

This study aims to review the comprehensive research program developed since 1995 focusing on the summer mortality of the Pacific cupped oyster *C. gigas* in the Marennes Oleron Bay. Most of the previously cited studies focused on the oyster population and failed to explore quantitatively the effects and interactions between factors likely to induce environmental disturbance, and therefore shellfish stress. For example, although considered as a critical factor, temperature stress has not yet been really quantitatively and in-situ evaluated. In contrast, this program has developed several projects to assess quantitatively the environmental conditions effects and their interactions on the oysters so as to provide insights on relationships between summer mortality occurrence and those conditions. Besides field studies, in vitro experiments were developed to verify in-situ hypothesis and strengthen summer mortality explanation.

I. Materials & Methods

I.1 In-Situ Field studies

The research program was conducted on Ronce Les Bains, an oyster bank located within the intertidal area in the southern part of the Bay of Marennes Oleron (Fig.1). The experimental site concerned 1,600 oyster leasing grounds covering 175ha, producing around 10,000 metric tons per year. Although variable according to topography, emersion time is one of the largest in the Bay reaching about 1/3 on a yearly basis. Two types of oyster culture are practiced without spatial overlapping : on-bottom culture, by seeding directly on muddy bottom seed or adult oysters, and off-bottom culture by using iron tables onto which oyster bags are deployed. Mortality rates affect mostly on-bottom cultured adult oysters with an apparent randomly patchiness distribution.

To analyze the etiology of the Pacific cupped oyster summer mortality and establish relationships between environmental conditions and mortality rates, several field projects have been developed (Table 1). The critical aspect for a better understanding of these mortality rates was to develop a multifactorial approach considering spatial and temporal monitoring surveys. This concerned oyster population characteristics as well as environmental

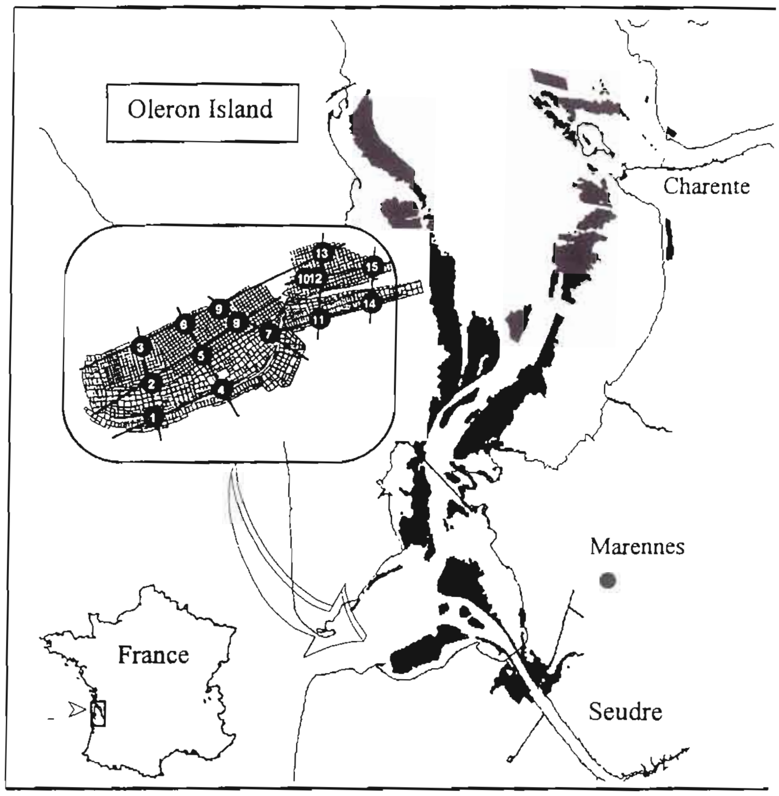


Fig.1 : Geographic distribution of the experimental site, the Ronce oyster bank located in the southern part of the Marennes Oleron Bay (France).

data. Actually, when environmental disturbances each affect a shellfish population, it appears critical to assess their joint impact and obtain knowledge of whether disturbances act independently. In the absence of independence, interaction among factors implies that one factor effect is dependent on the levels of others (Lenihan and Peterson, 1998).
In 1996, to study the 'origin', 'spatial distribution' and 'cultural practices' factors, 48 professional oyster populations were studied over a 4 month period (Lodato, 1997). Besides historical data on each oyster batch (i.e., age, origin : hatchery/natural spatfall, settlement origin, location and process for each rearing stage), parameters monitored every month included : rearing density, culture type (i.e., bags or on-bottom, single oyster or cluster), seeding time, bottom type, geographic location. Oyster biometry, condition index, maturation stage, biochemical composition (i.e., proteins, carbohydrates, glycogen, lipids) were estimated as well as mortality rates by counting dead oyster shell in bags and by sampling $0{,}5m^2$ on bottom. All the methods were previously described by Goulletquer et al. (1996), Lodato (1997) and Soletchnik et al. (1997, 1998). When abnormal mortality was observed (>30%), oysters were sampled for pathological study including PCR analysis for Herpes virus. In the succeeding years, oyster production was studied using similar parameters while a single (3 year old) calibrated population was equally deployed on experimental sites with common rearing characteristics (Fig.2). Since no spatial overlapping of off- and on-bottom culture was commonly observed, both cultural practices were tested simultaneously on each experimental site. Oyster stock assessment was performed by using both an aerial photographic coverage (1/4000) and by a stratified cluster sampling strategy (Cochran, 1977; Bacher et al., 1986). With regard to environmental conditions, sediment characteristics were analyzed according to Razet et al. (1996) and the following probes-data logger were deployed for temperature, GO TinyTags®; temperature-salinity, Suber®, and the multiparameter (T°C, Salinity, NTU, pH,

Experimental Site and sampling population :oysters cultured on the leasing grounds located on the Ronce Les Bains Oyster bank (175ha, 1600 leases, 10,000metric tons/year)						
Year	Monitoring surveys*	Site (n)	Scale	Frequency	Parameters	Comments-Objectives
1995	Oyster Production	1	Temporal	3 months	Biometry-Growth – Biochemical composition – Mortality rate	Basic survey carried out on 5 sites in the Marennes Oléron Bay (off bottom culture)
	Hydrobiology	1	Temporal	Continuous	Temperature-Salinity	Evaluation of stress on oysters (off-bottom culture)
1996	Oyster Production	48	Spatial & Temporal	1 month	Biometry – Growth Biochemical composition – Mortality rate	Assessment of oyster origin, cultural practices (e.g., density, biomass, rearing type) effects on production – mortality. Multifactorial approach on farmers' oyster samples
	Stock Assessment	all	Spatial	1	Rearing density & % exploitation	Global evaluation of the stocking biomass and current exploitation
	Temperature		Spatial & Temporal	Continuous	Temperature per site on & off bottom	Stress comparison per site between off- and on-bottom cultured oysters
	Hydrobiology 1	1	Temporal	Continuous	Temperature – Salinity	Evaluation of stress on oysters (off-bottom culture)
	Valvometer	1	Temporal	Continuous	Valve closure activity	Simultaneous evaluation of environmental conditions effects on oyster valve activity-relationship between oyster growth –mortality and environmental conditions- Environmental stress on oyster activity (e.g., O_2 depletion)
	Hydrobiology 2	1	Temporal	Continuous	Multiparameter probe: T°C, Salinity, pH, NTU, O_2 concentration	
1997	Oyster Production	15	Spatial & Temporal	2 weeks	Biometry-Growth – Biochemical composition – Mortality rate	Assessment of the spatial & temporal variability of oyster production per site using a single calibrated population equally deployed (cte density). Site selection using a 3x5 factorial plan (bathymetric&geographic). Evaluation of temperature stress per site and culture type. Establish relationships between sediment characteristics and mortality occurrence per site
	Temperature	15	Spatial & Temporal	Continuous	Temperature per site on & off bottom	
	Sediment	15	Spatial & Temporal	2 weeks	Eh, Organic content, bacteria, nutrients,	
	Valvometer	1	Temporal	Continuous	Valve closure activity	Simultaneous evaluation of environmental conditions effects on oyster valve activity-relationship between oyster growth –mortality and environmental conditions- Environmental stress on oyster activity (e.g., O_2 depletion)
	Hydrobiology 2	1	Temporal	Continuous	Multiparameter probe: T°C, Salinity, pH, NTU, O_2 concentration	
1998	Oyster production	4	Spatial & Temporal	2 weeks	Biometry – Growth - Biochemical composition-Mortality rate	Assessment of the spatial & temporal variability of oyster production per site using a single calibrated population equally deployed (cte density). Site selection using a bathymetric & geographic gradient. Evaluation of temperature stress per site and culture type.
	Temperature	8	Spatial & Temporal	Continuous	Temperature per site on & off bottom	
	Valvometer	1	Temporal	Continuous	Valve closure activity	Simultaneous evaluation of environmental conditions effects on oyster valve activity-relationship between oyster growth –mortality and environmental conditions- Assessment of environmental stress on oyster activity (e.g., O_2 depletion). Spatial approach by using a 2nd probe connected to a depth recorder, GPS positioning and the GIS inboard the research vessel (spatial transects tracking abnormal seawater quality).
	Hydrobiology 2	>2	Spatial & Temporal	Continuous	Multiparameter probe: T°C, Salinity, pH, NTU, O_2 concentration	

*Additional monitoring networks: hydrobiology (1977-), phytoplankton survey, oyster production (1985-), bathymetry (1970 & 1994), leasing grounds numeric database.

Table 1: Monitoring surveys carried out since 1995 o analyze oyster *C. gigas* summer mortality etiology in the Marennes Oléron Bay.

O_2, Pressure) Solomat®. While the temperature probes were deployed per site, on- and off-bottom, the multiparameter probe was deployed at the water column-sediment interface at the near vicinity of the valvometer Micrel®, which was monitoring individually and continuously 8 oysters' valve activity. The valvometer is considered as a biological early warning system which might detect abnormal or/and stressful conditions for shellfish (e.g., pollutants, hypoxic conditions) (Baldwin and Kramer, 1992).

Besides assessing statistically the joint impact of environmental disturbances by using experimental design, knowledge is also required on factors showing various temporal scales (e.g., long- & short-term variability). For example, topography will change on a long term basis by siltation on oyster beds, therefore affecting shellfish population (e.g., reduced filtration time). Global warming may effect similarly shellfish population. Knowledge on topography and long term changes were obtained by using available environmental database (DDE, unpublished data; Meteo France; Soletchnik et al., 1998).

I.2. In-vitro Laboratory Studies

Several experiments were carried out to specify the status of oyster population according to their reproductive development stage since maturation has been considered as a critical physiological process during summer mortalities (Glude, 1975; Perdue et al., 1981; Ventilla, 1984). The Scope for Growth (SFG) is an integrated physiological parameter reflecting the energy balance between acquisition (feeding-absorption) and catabolism, mainly due to respiration. This parameter has been widely used to assess environmental quality and characterize the physiological status of shellfish population (Bayne et al., 1985; Widdows et al., 1990). Several experiments were developed to estimate physiological functions, including a reevaluation of the allometric relationship for the respiration model, and SFG values with respect to the reproductive stage for various age classes (Soletchnik et al., 1997). The latter parameter was considered since summer mortalities affect preferentially adult oysters and reproductive effort is increasing with age for the Pacific oyster *C. gigas* (Héral, 1986 ; Soletchnik et al., 1997). Methodologies were presented in Soletchnik et al. (1996). Moreover, experiments were carried out to assess the temperature range effecting the oyster physiology with regard to shell size and thickness. Obviously, the thermal inertia due to shell thickness differs significantly between adult and juveniles oysters, therefore likely affecting variously oysters during the summer low tides. The stress temperature effect requires a quantitative assessment and a model development. Thermal challenges (Δ23°C in 2 hours) were based on in-situ records and carried out in a controlled physiological chamber where oysters were equipped with an internal temperature probe to assess inertia for various shell size. Valve activity was concomitantly recorded using a valvometer.

I.3 Statistical Data treatment

All the spatialized data were incorporated into a Geographic Information System using ArcView ® software to facilitate data treatment and comparisons. Two basic layers of information concerned a numeric database of the leasing grounds, geographically calibrated using Lambert II coordinates (Affaires Maritimes MO, unpublished data), and a topographic database (DDE, unpublished data). All the statistical data treatment including ANOVA, ANCOVA, time-series and multifactorial analysis PCA, were performed using Statgraphics ® software.

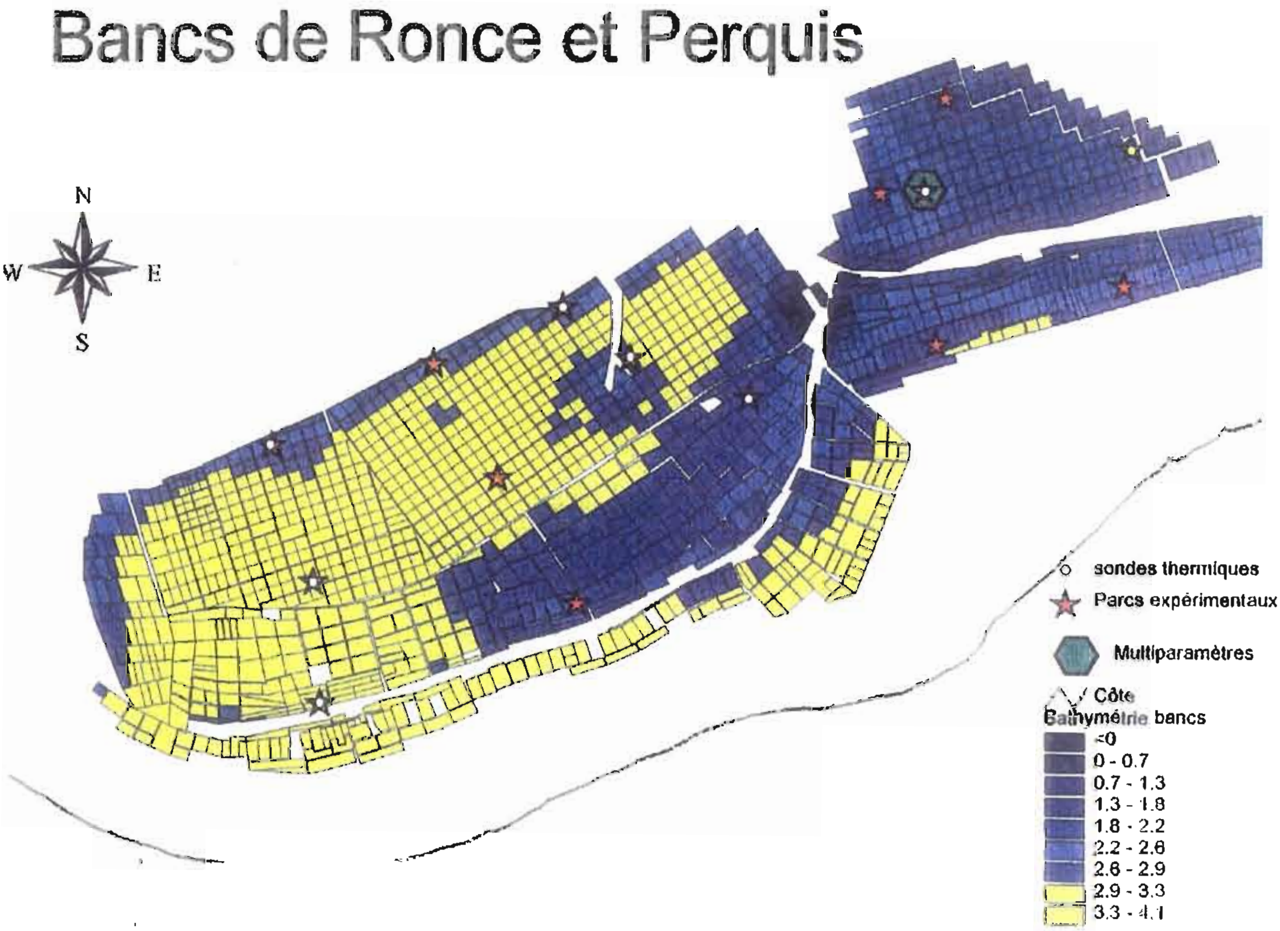


Figure N°2: Experimental design in 1997: site distribution based on a depth gradient and W-E geographic directions for the deployment of the oyster population, temperature probes and the multiparameter probe.

II. Results and Discussion

II.1 Long term environmental changes

Hydrobiological parameters

Among all the hydrobiological parameters monitored since 1977, temperature and ammonia (N-NH4+) showed a significant trend change over the years, and concerning the Ronce Les Bains oyster bank. A global warming significant trend has been described by time-series analysis : the mean yearly seawater temperature has averaged 13.5°C until 1987, then reached 14°C between 1998 and 1993, and peaked at 15°C since 1995 (Fig.3) (Soletchnik et al., 1998). Meanwhile, seawater temperature increase was higher in the southern part of the Bay of Marennes Oleron. Such a trend is highly significant in term of oyster pressure on carrying capacity : a 4% filtration rate increase results from a 1.5°C temperature increase (Bougrier et al., 1995; Soletchnik et al., 1997).

The ammonia concentration has shown a distinctive pattern in the southern part of the Bay since 1988 reaching significantly greater values than in other parts of the Bay (Fig.3). While ammonia concentration varied between 1 to 3µmoles/l in the Bay of Marennes Oléron, a 4 µmoles/l has been noted in the southern part of the Bay, therefore representing a seawater deterioration (Fig.3).

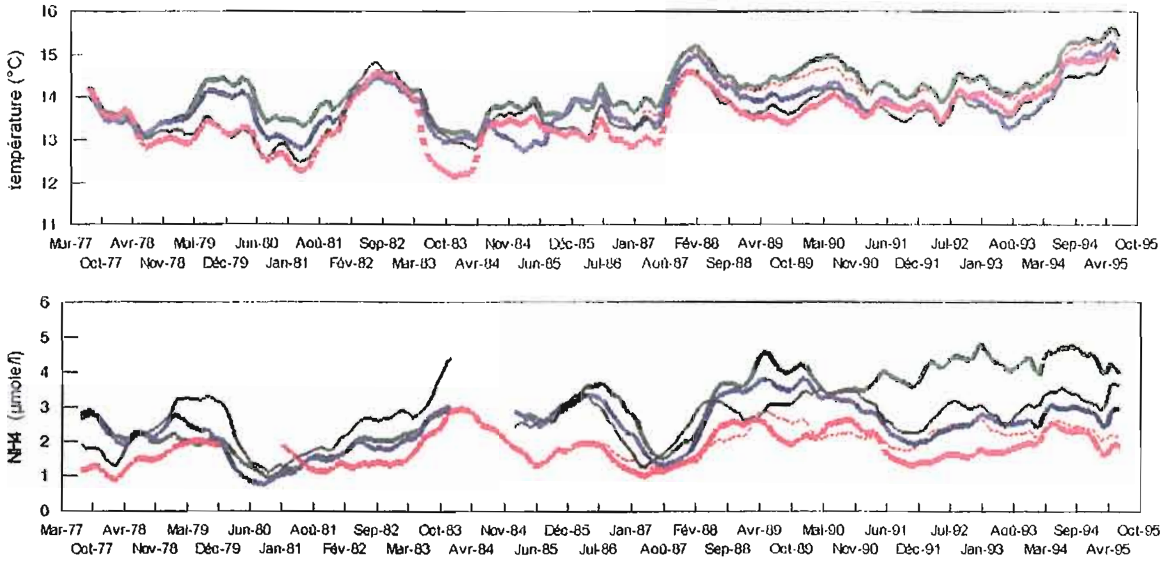


Figure N°3: Historical trend (1977-) of seawater temperature and ammonia (N-NH4+) at 5 stations in the Bay of Marennes Oleron (green line=hydrological station at the near vicinity of Ronce bank).

Topography trends

The topography changes have had several consequences over a 25 year period : around the Maumusson sound, these changes induced a 7% and 2% decrease of seawater exchange with the ocean between 1970-1985 and 1985-1990 respectively; therefore limiting the seawater renewal in the southern part of the bay, and likely affecting the seawater residence time over the oyster beds. Considering oyster stocking biomass and seawater volumes, increased siltation rate led to increasing pressure on the carrying capacity in the southern part of the Bay: presently the ratio is $145kg/10^3m^3$ and $360kg/10^3m^3$, oyster biomass vs seawater volume, in the northern and southern part of the Bay respectively (factor 2.5).

Besides the overall seawater volume, topography has changed due to preferential siltation over the leasing grounds, leading to 0,5m rise in several leasing grounds (Fig.4). While the average depth is 1.81m (above the marine chart zero) in the northern part of the Bay, the mean value reaches 2.4m in the southern part, inducing unbalanced emersion time for oysters with 16% and 31% on a yearly basis respectively. Simulations carried out using the tidal model showed that an 0.5 m bottom rise induced up to 22% filtration time decrease (and concomitant exposure increase to stress at low tide) for oyster population located on several leases from the Ronce Les Bains bank (Fig.4).

II.2 Oyster population monitorings (1995-1998)

Several results were obtained based on the 3 year monitoring study. By way of example, the 1997 data are presented on figures 5-8 to assess the overall growth rate, meat condition, sexual maturation and concomitant mortality rate based on depth, geographic distribution and rearing type (on- & off bottom). At the study completion, mortality rates and economic yield were estimated (Fig.9&10). Although a significant and positive relationship between growth rate, meat condition and depth site location was reported, the relationship with mortality was limited. Similarly to previous years, abnormal mortality rates were first observed mid-June and increased progressively until mid-July to finally reach between 23-33% and 8-19% for on- and off-bottom culture respectively. The mortality rate discrepancy between on- & off bottom culture was systematically observed since 1995 whatever the mortality rate intensity.

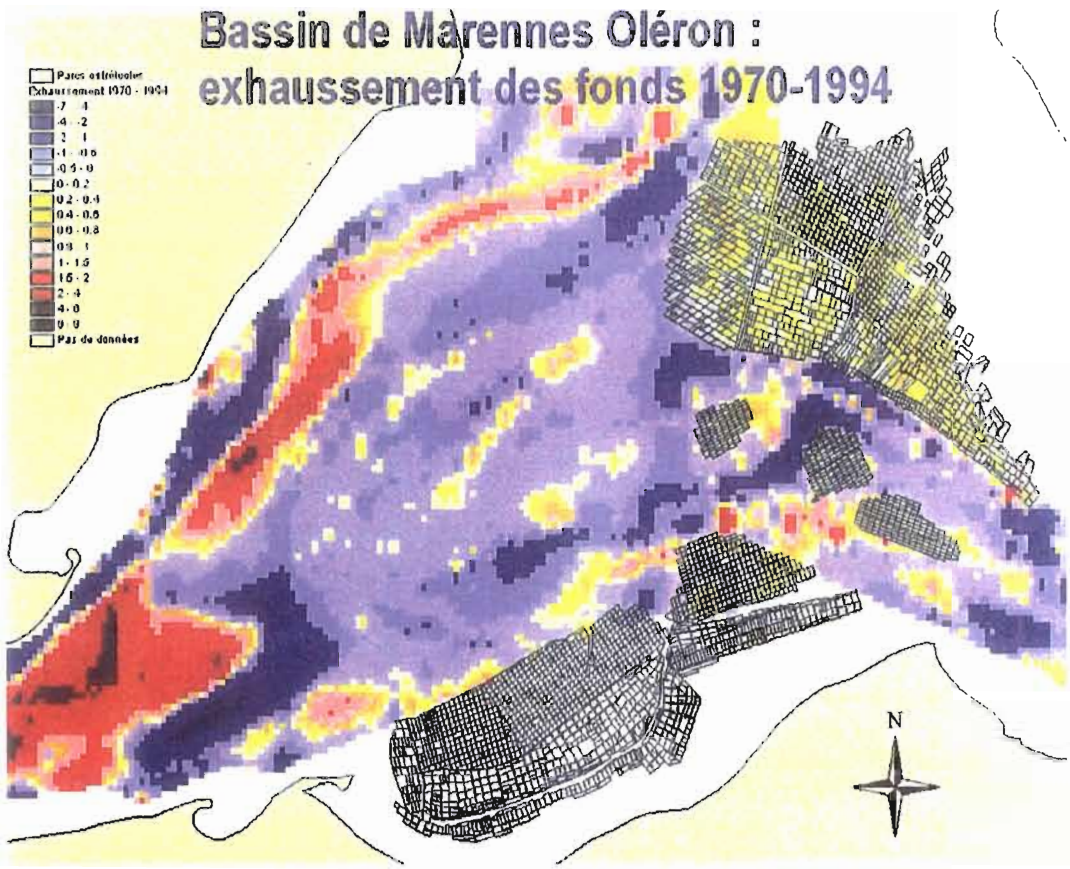


Figure N°4 : Bathymetric changes (1970-1994) in the southern part of the Bay of Marennes-Oleron and simulation of oyster emersion time, and therefore filtration time decrease (%) for a 0.5m bottom rise.

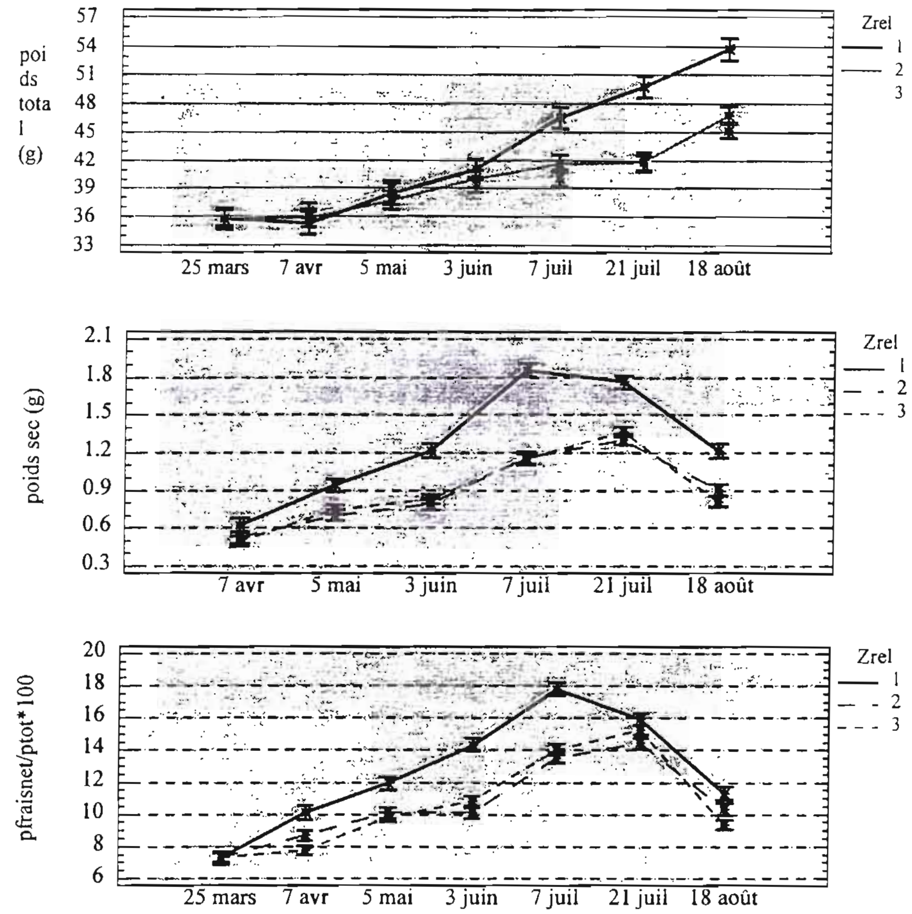
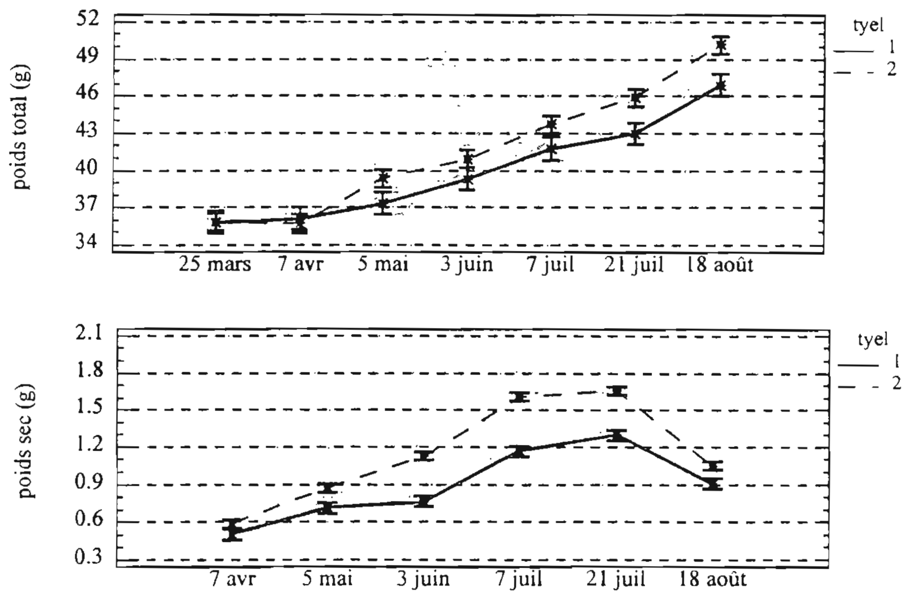


Figure N°5: ANOVA results of the total weight (A), dry meat weight (B), condition index (100*fresh meat weight/total weight) (C) related to site bathymetry (Z).



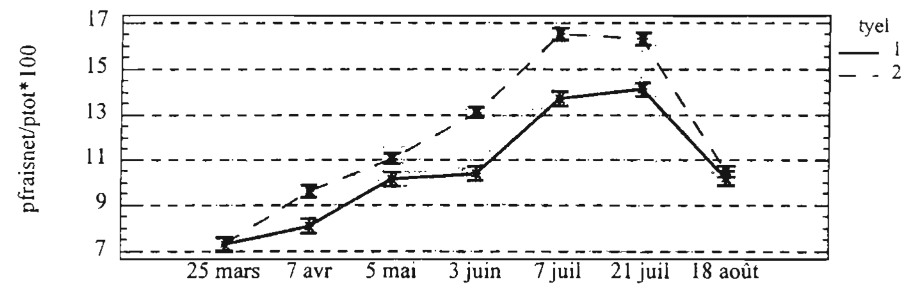


Figure N°6: ANOVA results of the total weight (D), dry meat weight (E), condition index (100*fresh meat weight/total weight) (F) related to rearing type (tyel) (off =1 & 2=on-bottom).

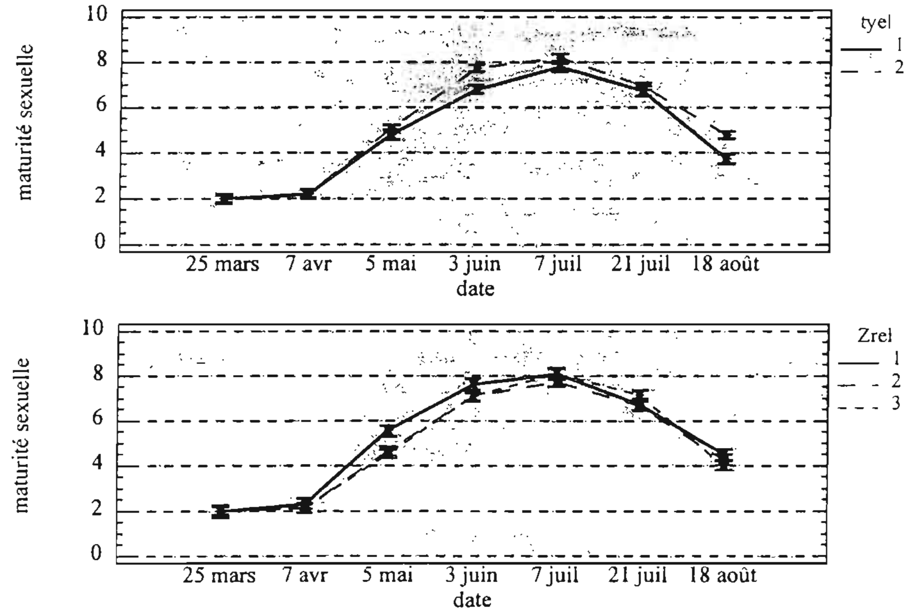


Figure N°7: ANCOVA results for the maturation index (SMS) related to trend and site bathymetry (Zrel) *(1: 1,7-2,2 m; 2: 2,3-2,7 m; 3: 2,8-3,7 m)*.

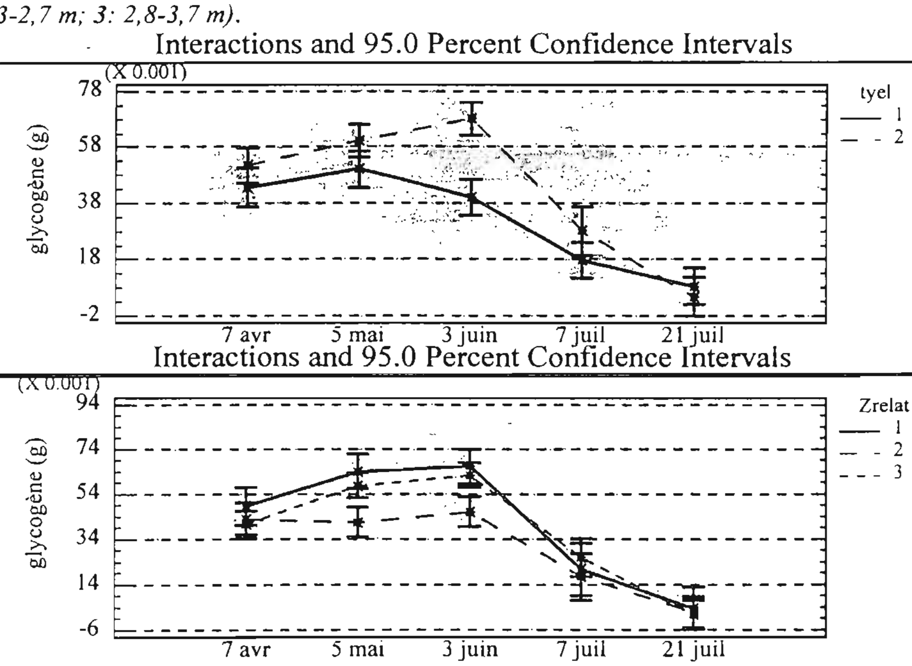


Figure N°8: ANCOVA results for oyster meat glycogen concentration for various rearing type and at various depths *(1: 1,7-2,2 m; 2: 2,3-2,7 m; 3: 2,8-3,7 m)*.

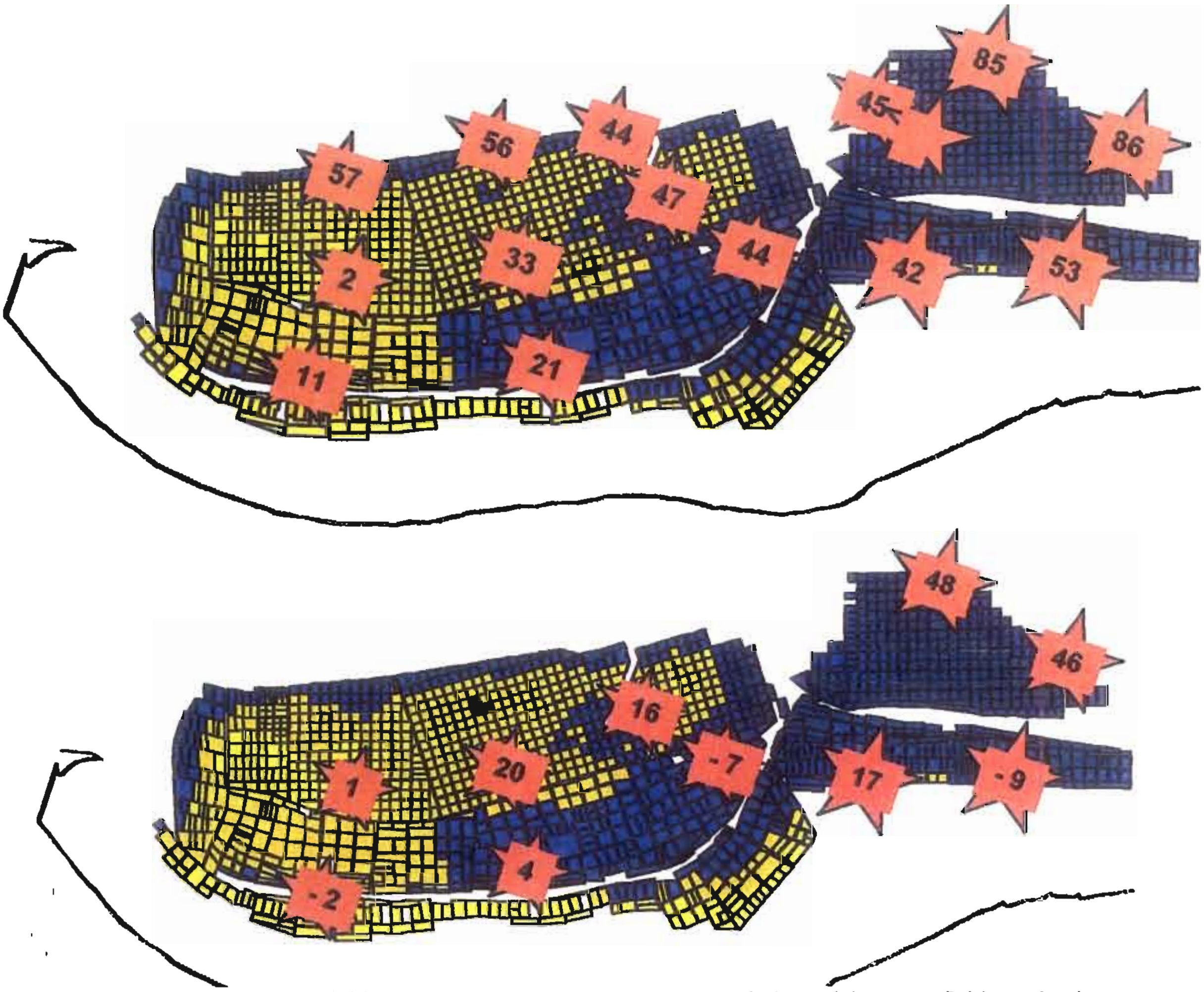


Figure N°9: Oyster economic yield (+-n% initial biomass) at the completion of the 1997 field monitoring survey for off- (A) & on-(B) bottom culture (lease colors represent the depth gradient).

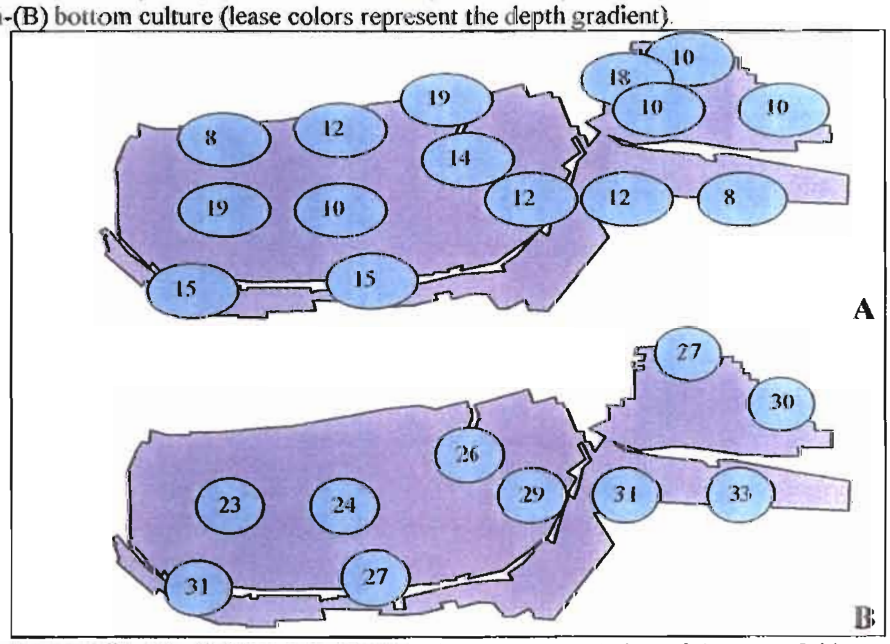


Figure N°10: Oyster mortality rate (%) per experimental site at the completion of the 1997 field monitoring survey for off-bottom (A) and on-bottom (B) culture.

In contrast to mortality rate, we should note that overall production was systematically greater for oysters located off-bottom compared to on-bottom, in spite of a reduced filtration time. It should be correlated to food availability (e.g., water column compared to interface water-sediment) as well as environmental quality at the interface (e.g., turbidity, temperature, oxygen concentration). In spite of several parameters monitored for each site at the sediment level (i.e., bacteria, Eh, organic matter, nutrients) no statistical spatial relationship has been established with the mortality rate. All the production results showed a large spatial variability although the lease acreage concerned 175ha (Fig.9). Every year, mortality occurred concomitantly to a peak in oyster meat weight, a minimum glycogen concentration and a maximal sexual maturity (Fig.5-8). Besides the significant and systematic difference between on & off bottom culture, no density effect on mortality rate was significant in 1997, and likely resulted from a physical constraint (i.e., oyster stability) in spite of its large variability (i.e., 3 to 24kg/m^2) (Lodato, 1997). Similarly, the 3 various natural spat origins used by the oyster farmers did not show any significant effect on mortality rate. Comparison of cultural practices, particularly the single or clustered oysters, showed a significant pattern with reduced mortality rate in the later case (23% vs 17%).

These results confirmed the fact that abnormal mortality rates cannot be explained by only one parameter but by a combined and synergetic effect of environmental conditions on a critical oyster physiological status. Particularly, the systematic discrepancy of mortality rate per site between on & off bottom culture prompted us to focus on specific environmental survey at the near vicinity of the sediment.

Continuous environmental monitorings

One of the critical aspect of the environmental survey between on- and off-bottom culture is presented on figure 11. On-bottom oysters were regularly affected by a significant temperature stress reaching Δ30°C in less than 1h30 between low and high tide in July and august 1996. In contrast, off-bottom cultured oysters were in a more stable environment with maximal temperature reaching 35°C and therefore resulting in a limited 15°C temperature stress. This was likely due to the heat absorption by the muddy grey sediment under the sunshine at low tide, around mid-day for this geographic latitude.

Using a multiparameter probe, concomitantly to a valvometer, provided more information about the environmental impact on oyster physiology (Fig.12). During the two last weeks of June, an irregular pattern of O_2 concentration was recorded during the neap tides: O_2 saturation varied from >120% to less than 50% between day and night, representing a stress for oysters who showed an hyperactivity and no valve closure. Moreover, this event was concomitant to seawater temperature record high (>20°C). The O_2 concentration range resulted from the phytoplanktonic activity showing a bloom from early to mid-June. However, following the strong O_2 variability period, a continuous saturation decrease down to 60% occurred which was not restored during the following spring tides. Meanwhile the phytoplankton concentration and therefore the carrying capacity decreased. Abnormal mortality rates were firstly observed at that time.

In vitro ecophysiological studies

The field studies have quantitatively estimated the temperature stress affecting oysters during the tidal cycle in summer. Therefore, in-vitro experiments provided insights how the oyster reacted to these stress (Fig.13-15). First of all, we should note that oyster shell inertia has a drastic effect on when and how long the living animal will be affected by the thermal stress (Fig.13). The results demonstrated that juvenile oysters (14g total weight) reach the external temperature (40°C) in less than 30', while it requires 60' for 2 year old oysters (90g). Noticeably, 3 year old oysters (150g) never reached the equilibrium at 40°C following a 3 hours exposure time. Therefore, the potential thermal stress is highly variable depending on

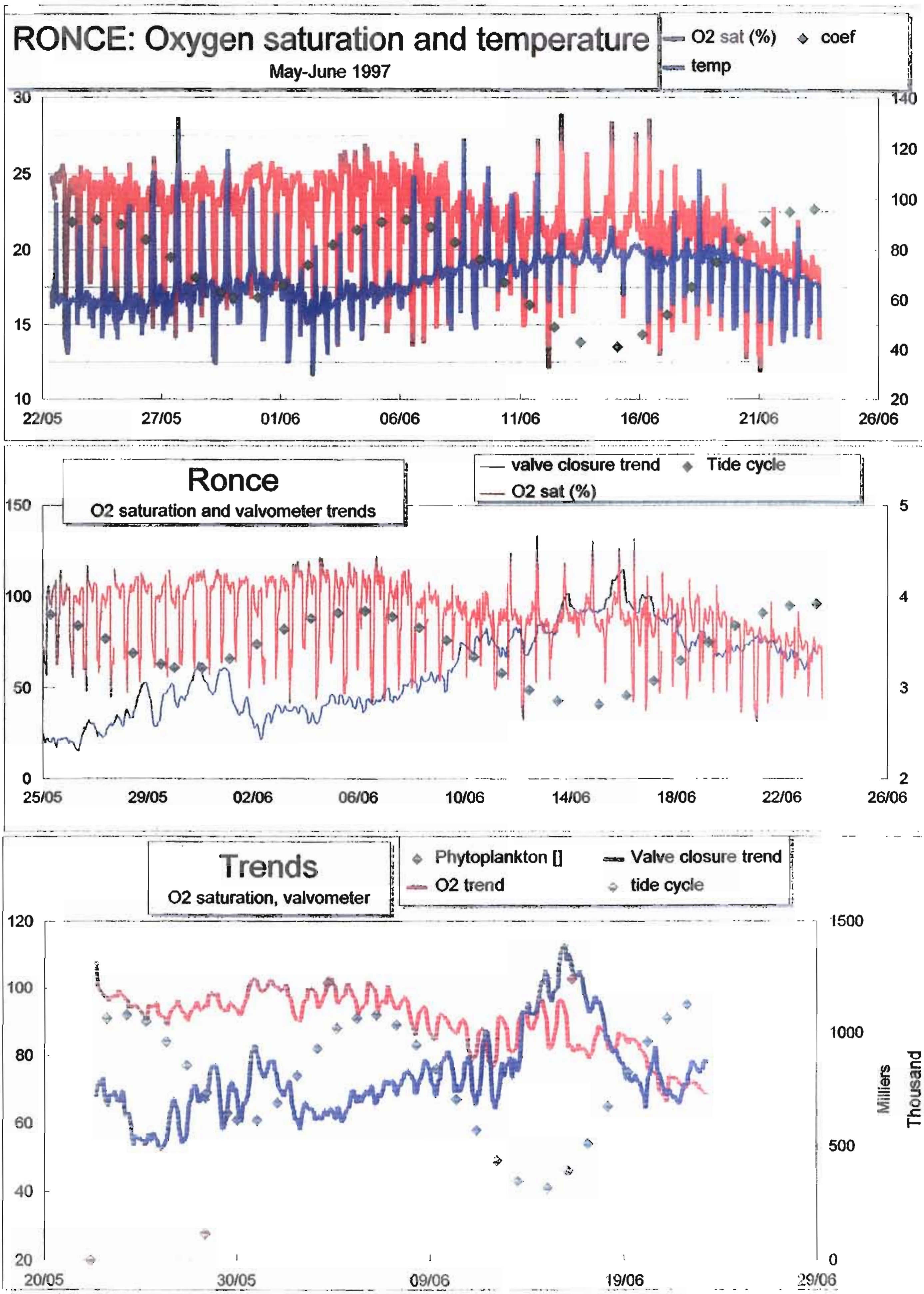


Fig. 12: Environmental continuous monitoring related to oyster valve activity and tide cycle

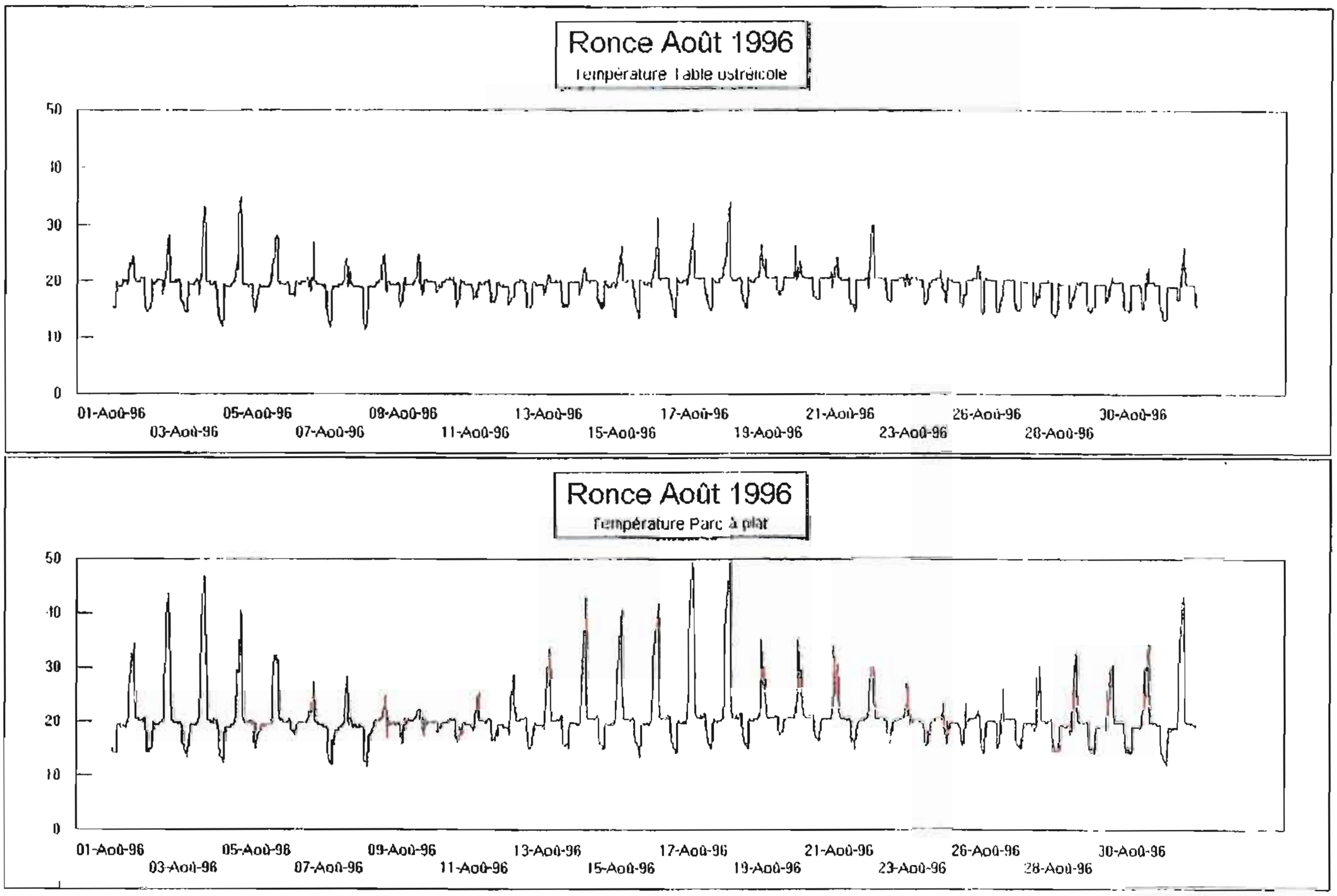


Figure N°11: Continuous in-situ monitoring of temperature at the near vicinity of oysters cultured off- (A) and on-bottom (B) culture in August 1996.

location within the intertidal area (i.e., low tide time exposure), and oyster age. Since oyster populations deployed on Ronce oyster bank by the farmers were usually 2 year old oysters, the thermal stress was likely to affect oyster physiology in about one hour of exposure. Thermal stress had a significant impact (at 1%) on oyster respiration rates (Fig.14) with a 15% increase following the stress, 0.81mg and 0.96mg O2/h respectively. Similarly, concomitant respiration increase was observed with sexual maturation stage (Fig.15) (Lagarde, 1997). These results should be correlated with the negative scope for growth for mature oysters reported by Soletchnik et al. (1997). The negative energy budget during the high maturation stage resulted from a reduced absorption efficiency and increased respiration rates (Fig.16).

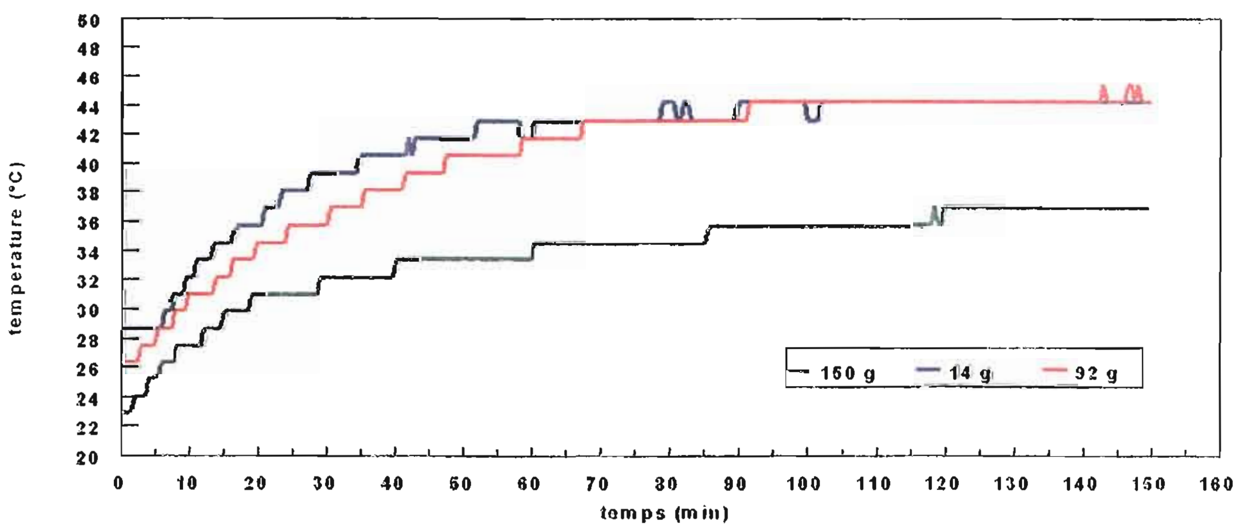


Fig. N°13: Estimation of the shell thermal inertia. Time exposure for the living animal to reach the 40°C external temperature according to age and size (3y=150g., 2y=92, 1y=14g).

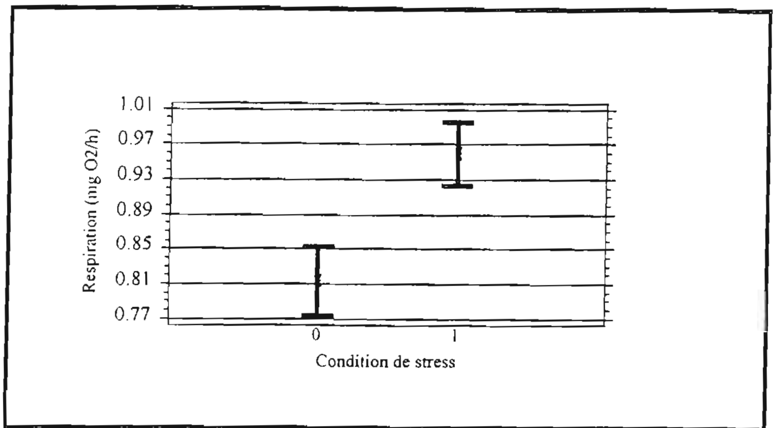


Fig. N°14: ANOVA results of the mean (+-SD) oyster respiration rates function of stress (1) and control (0).

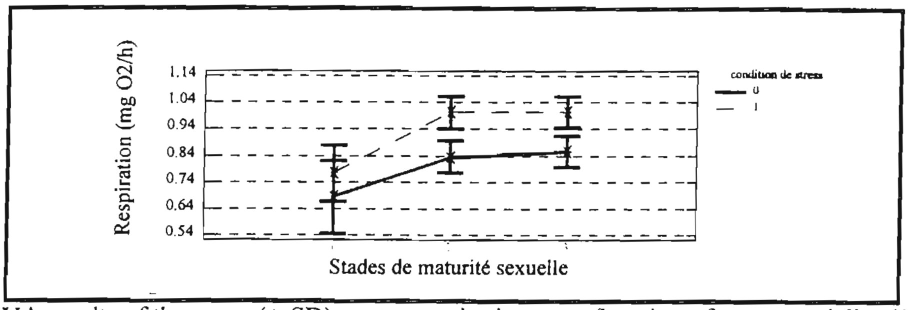


Fig. N°15: ANOVA results of the mean (+-SD) oyster respiration rates function of stress modality (1=stress, 0=control) and sexual maturation (1, immature; 2, mature; 3=prespawning).

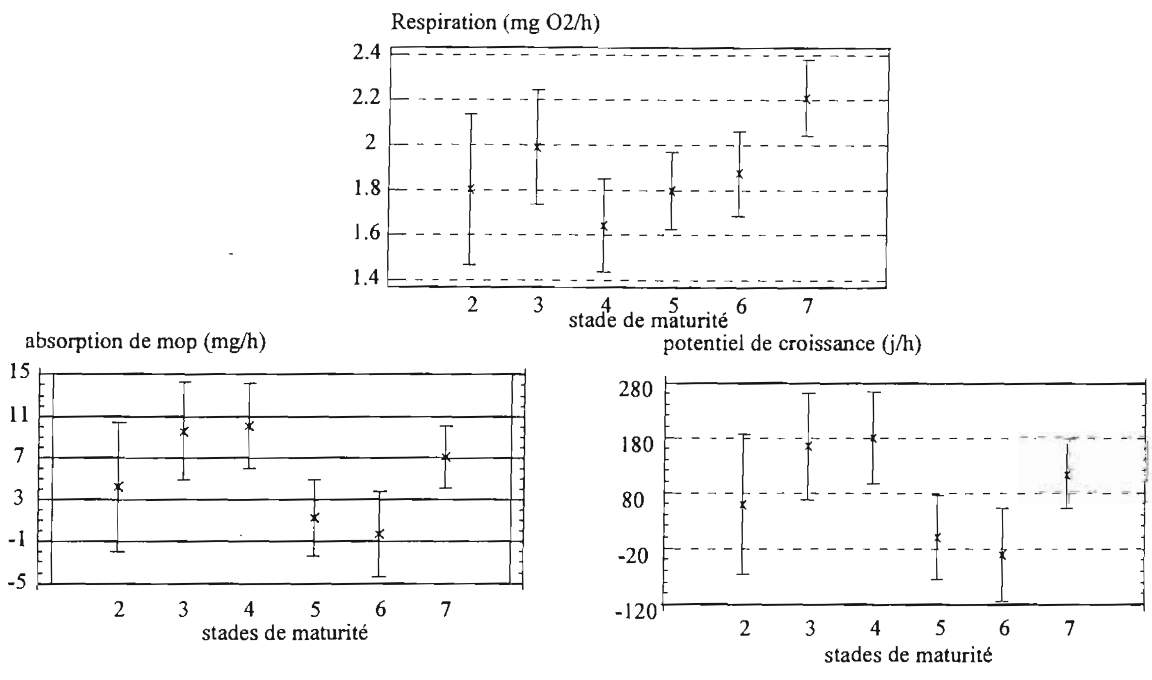


Fig. N°16: Mean (+-SD) estimates for the 2 year old oyster respiration (mgO2/h), absorption rates (mg POM/h) and Scope for Growth (SFG in J/h) function of the sexual maturation stage (2, early maturation; 3-6, increasing maturation; 7, post-spawning).

III. Conclusions

The results of our large scale field experiments on the Ronce oyster bank and the concomitant in-vitro physiological studies, provided significant knowledge to further explain how the abnormality mortality rates might occur. All the typical characteristics of summer oyster mortality were fulfilled in the case of Ronce oyster Bank. All the experiments carried out confirmed the physiological stress theory proposed by Glude (1975) and Ventilla (1984) as well as the critical effects of reproductive status and temperature stress (Perdue et al., 1981). A further step in oyster mortality understanding was obtained by 1) assessing in-situ quantitatively the environmental and stressful conditions and 2) by testing their effects in-vitro, as well as by 3) specifying the successive events leading to mortality.

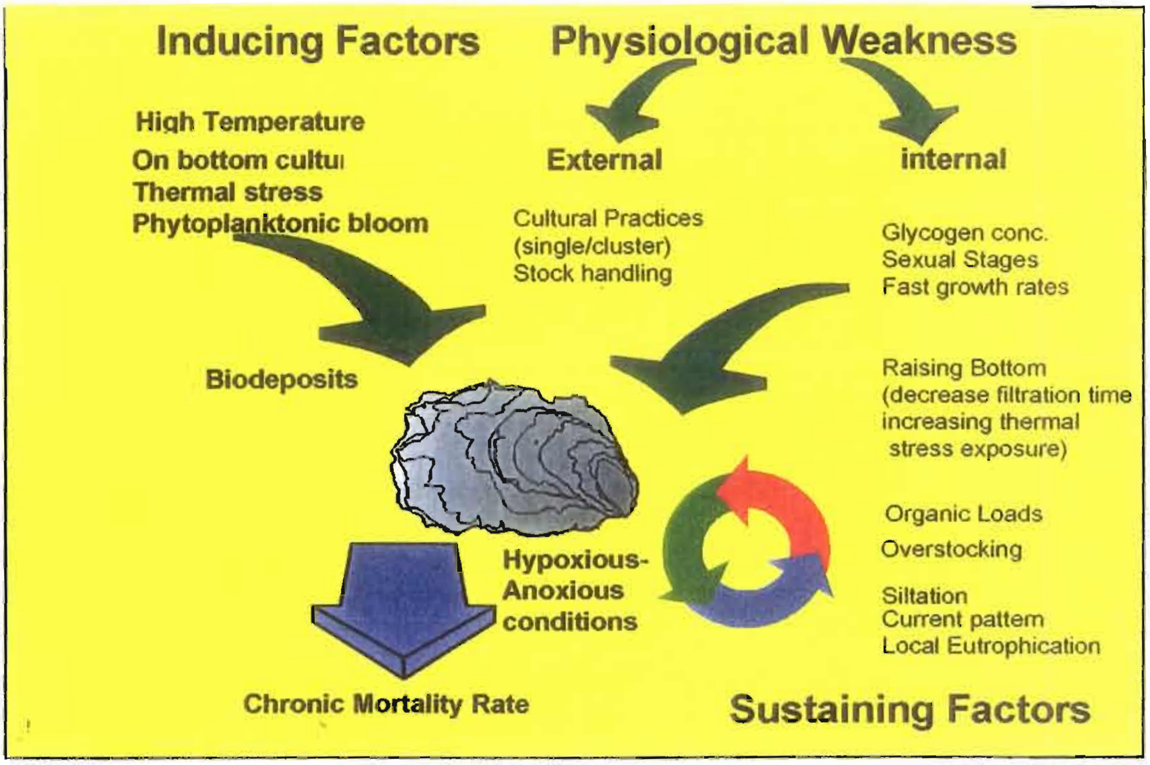


Fig. N°17: Global hypothesis for abnormal summer mortality of the Pacific cupped oyster C. gigas in Ronce oyster Bank (Marennes Oleron Bay) (LCPC, 1998)

Moreover, to address the mortality issue, it appears critical to emphasize the need for a global approach by considering the main factors and their interactions likely to affect oyster survival rates. The key aspect remains the assessment of environmental conditions varying at different time scales and concomitantly contributing to mortality rates. We have describe long term and global ecosystem trends which make sensitize the oyster population to punctual stress. The global warming trend, 1.5°C seawater increase in 20 years, is likely to contribute to the present and future mortality rates affecting this species in the Bay of Marennes-Oleron as well as along the Atlantic coast. This strain was recently and massively introduced into French waters during the 1970's to sustain the oyster industry affected by diseases on the Portuguese *C. angulata*. The broodstock and spat were introduced from British Columbia (Canada) and Japan (Miyagi) respectively, however in both cases originating from the latter where seawater temperature peaked at 25°C and salinity below 33ppt in summer (Marteil and Barrau, 1972). Higher seawater temperature are regularly observed in summer in the Marennes-Oleron Bay. Moreover, Shamseldin et al. (1997) reported a LT50 at 42.3°C (1h exposure) for *C. gigas*, temperature commonly reached for oysters directly cultured on-bottom in contrast to off-bottom. Similarly Maurer and Borel (1986) in the southern Bay of Arcachon where oysters are more exposed to temperature stress, concluded that juveniles Pacific cupped oyster were not fully adapted to these conditions. Although, successful in terms of reproduction and natural spatfall, the Marennes-Oleron Bay oyster population is likely more sensitized to the increased seawater temperature trend. Besides the temperature variable, the ammonia concentration increasing trend represents an indicator of seawater quality deterioration which impact on oysters is not yet determined. The natural siltation aggravated locally by cultural practices represents a long term change which impact is obvious and therefore requires appropriate management. By way of example, the oyster farmers operating on the Ronce oyster bank have no legal obligation to remove their cultural equipment (e.g., tables) once in a year in contrast to other oyster banks, therefore aggravating siltation rates.

Besides the long term ecosystem changes, we should note that this oyster bank is one of the most productive in the Marennes Oleron Bay as demonstrated by the national monitoring network REMORA (Goyard et al., 1997). This highly productivity is due to the nutrients inputs coming from the southern Gironde estuary through the Maumusson sound. The mixing between those and the Marennes-Oleron waters induce local phytoplanktonic blooms which are then largely exploited by oyster biomass and not homogeneously and spatially distributed over the Ronce Bank, explaining the spatial variability of oyster production.

Based on our results, a global hypothesis to explain abnormal mortality rates and the 'triggers' inducing these events can be proposed (Fig.17): following the phytoplanktonic blooms and the resulting fast growth rate and accelerated oyster sexual maturation, the ecosystem appears unbalanced mainly by oxygen depletion (hypoxia) at the near vicinity of on-bottom culture, while seawater temperature rises to maximum values during neap tides. Meanwhile the carrying capacity is decreasing and no oxygen recovery is observed during the succeeding spring tides when emersion time induces record high stressful temperature conditions. The fast growth rate oysters present a critical physiological status with an advanced maturation stage, an hyperactivity as demonstrated by valve activity, and an increased respiration rate induced by the previous factor and by the thermal stress (>30°C) from emersion. Although Shamseldin et al. (1997) demonstrated that sublethal thermal shock followed by a recovery period, induced a thermotolerance lasting at least 10 days, the overall physiological oyster condition, the absolute temperature values, their duration and the lack of recovery period are likely to limit oyster withstanding to temperature shocks. Meanwhile, those oysters have exhausted their energetic reserves (i.e., glycogen concentration near zero) critically required for anaerobiosis at low tide (Zwaan, 1977; Gäde, 1983; Zaandee et al., 1986; Wu and Lam, 1997). Besides the lack of energy reserves, their glycogen storage capacity is particularly limited in summer (Mathieu et al., 1998). Those combined conditions are likely lethal as demonstrated by their negative impacts during in vitro experiments.

The hypoxia event was clearly demonstrated locally by in-situ continuous monitoring. However, further investigations are required to assess spatially the distribution of hypoxic waters and their relationships with abnormal mortality rates. This could be addressed by two complementary approaches : 1) an environmental monitoring of hypoxic conditions using the multiparameter probe, connected onboard to the geographic information system, therefore facilitating a systematic spatial coverage, and 2) at the oyster physiological level for various populations with the glucose 6 phosphate deshydrogenase analysis, considered as a biomarker for hypoxia by Wu and Lam (1997). Moreover, another biomarker, adenylate energy charges (AEC) in oysters showed reduced values between May and July in the Marennes Oleron Bay (Moal et al., 1989). The continuous decrease in AEC reflects the highest energy demand at that time. Without abnormal mortality rates, these authors reported that animal, from the same location, subjected to different amounts of emersion adapt to maintain their energy charge. A lack of adaptation due to overall physiological status might also contribute to abnormal mortality. Therefore, all these likely appropriate biomarkers (i.e., HSP-70, AECs, deshydrogenase) for stress in oysters are likely of particular interest to establish relationship with mortality occurrence, and therefore, will be monitored over time during the next summer mortality field study.

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