

Evaluation of the oyster farming potential of the Cintra bay (southern Morocco)

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**Abstract:** The bay of Cintra is a marine ecosystem located in the southern Moroccan Atlantic known for its biological richness and therefore for its high fishery productivity. This bay was chosen as a new destination for the development of aquaculture in the southern regions of Morocco. To highlight its potential in terms of oyster farming, a first rearing trial of triploid cupped oysters (*Crassostrea gigas*) as well as parallel monitoring of the phytoplankton population and ecological parameters were undertaken. The results obtained showed that the oysters adapt well to the conditions of the new environment where mortality was negligible, or even absent after a month of launching. In terms of biological performance, the growth of individuals is continuous during the annual cycle and after eight months the weight could reach 30.5 g which is a minimum weight for marketing. The AFNOR and Lawrence and Scott indices showed a good physiological state and a better commercial quality of the oysters. The filling rate of these oysters, with an average weight of 39.7g when lifting, is very high according to the Lawrence and Scott index (126.3) and the average AFNOR index (around 21.8) rank them in the “Special” category.

**Key words:** potential, rearing, *Crassostrea gigas*, bay of Cintra, Morocco

**Introduction**

Aquaculture is a part of fishery sector that is used to control food production (animals and aquatic plants) in order to obtain a better supply for consumption (FAO, 2015). In the Dakhla-Oued Eddahab region, the shellfish farming has already shown success in Dakhla Bay and has become one of the economic pillars, thanks to high biological productivity of its marine ecosystems. The shellfish production of the oyster farms in the bay of Dakhla was estimated at 430 tonnes in 2008 (Délégation des Pêches Maritimes-Dakhla, 2009). In addition and according to the aquaculture development plan established in 2013, the target shellfish production is around 26,054 T / year (Agence Nationale du Développement d’Aquaculture, 2016). By 2020, the Dakhla Oued Eddahab region will contribute to 30% (or 30,000 T / year) of aquaculture production (all products included) (Department of Studies and Financial Forecasts, 2016).

The strong demand on the bay of Dakhla, already exploited since 2003, as well as the requirements of the new strategy of the Halieutis plan, launched in 2009 (Cour des comptes, 2018), necessitated the search for another marine ecosystem meeting the needs of aquaculture development in the region. In this regard, the bay of Cintra was chosen and to highlight its farming oyster potential, a first trial of triploid cupped oyster breeding (*Crassostrea gigas*) was undertaken. The choice of triploid cupped oysters was based on the fact that the sterility of these organisms allows better growth and survival of the individuals as well as a marketing spread over the whole year compared to diploids (Brenda Paulina et al., 2017; Haure et al., 2003; Hawkins et al., 2000; Hand et al., 1998a; Nell et al., 1994; Allen and Downing, 1986). As a result of this better growth, these individuals tend to quickly reach commercial size, which reduces the time of exposure to possible contamination that can cause their mortality (Barber & Mann, 1991). They are, also, characterized by better shape (Walton et al., 2013) and show elevated condition indices (Matthiessen & Davis, 1992; Nell, 2002; Shpigel et al., 1992; Wadsworth, 2018). From an economic point of view, the use of triploid cupped oysters reduces rearing costs and increases the production rate (Dunham, 2011; Catt, 1998). In the present breeding trial, the biometry of the spat of the triploid cupped oysters as well as the physicochemical (temperature, salinity, dissolved oxygen, etc.) and biological (phytoplankton and chlorophyll a) parameters of the environment, often involved in the process of growth and reproduction (Thomas et al., 2016; Park et al., 2013; Ruiz et al., 1992), were monitored monthly. The commercial quality and physiological (health) status of the oysters were assessed by calculating the condition indices commonly used for this type of study (Lucas and Beningeri, 1985), The commercial quality and physiological (health) state of oysters were evaluated by calculating the condition indices

commonly used for this kind of study (Lucas and Beningeri, 1985), namely the Lawrence and Scott index and the AFNOR index.

## Materials and methods

### 1. Study site

The bay of Cintra is located south of the city of Dakhla by 160 km (Fig. 1). It is a semi-closed marine ecosystem characterized by a wide opening (20 km) on the Atlantic Ocean with an estimated area of 173 km<sup>2</sup>.

### 2. Choice of the rearing area

The choice of areas for carrying out the farming triploid oyster project on tables (technique used in this trial) must meet certain conditions (Doiron and al., 2008), including a bathymetry not exceeding 10 m and slightly agitated water (sheltered from swells) (Orbi et al. 1999, Makaoui et al, 2016). Thus, an area of the northern part of the bay of Cintra was chosen and delimited (Fig. 2) in order to install the rearing structures.

### 3. Biological and technical rearing equipment

The cultivated species is the Japanese oyster *Crassostrea gigas*. This one comes from a French hatchery and the spat are triploids and are introduced at a size of 6 mm and a caliber weight 0.1g. The period of introduction chosen is summer because it corresponds to the best growth in cupped oysters reared in the region (bay of Dakhla). These spat are reared in "ostreophilic" bags of 4 mm mesh fixed on iron oyster tables. These latter are raised so that the immersion time for the mollusks is respected; in fact a prolonged immersion time has a beneficial effect on the growth of oysters (Gouletquer et al., 1987). A set of three tables (10 bags/tables) was prepared and placed north of the bay and the initial density was 1000 individuals per bag. When the shell size of the individuals becomes larger, a doubling of the bags is carried out to reduce the quantity of oysters, thus maximizing growth and improving quality. Due to the remoteness of the rearing site (160 km from the National Fisheries Research Institute), cleaning of the bags and maintenance of the rearing structures was only possible once a month.

### 4. Environmental monitoring

It is carried out every month by sampling surface water. The following parameters were monitored annually from August 2015 to September 2016:

- Phytoplankton and chlorophyll a

The concentration of micro-algae in the rearing environment was controlled in two ways. The first is counting using a Leica type inverted phase contrast microscope, after decanting the samples in 10 ml cuvettes for 24 hours (Uthermohl method, 1958) and the results are expressed in numbers of cells per liter. The second was the chlorophyll a assay where a known volume (1l) was filtered through Whatman (GF/F) type glass micro-fibril filters with a diameter of 47 mm. Chlorophyll a, extracted in acetone was then determined by spectrophotometer (UVmini 1240, Shimadzu) according to the method of Lorenzen (1967). The results were expressed in mg/m<sup>3</sup>.

- Suspended Matter (SM)

The suspended matter content (seston) was measured according to the method described by Aminot and Chaussépied (1983) and expressed in (mg/l).

- Dissolved oxygen

Winkler's method (Aminot and Chaussepied, 1983) has made it possible to determine the dissolved oxygen of the environment and the oxygen contents are expressed in ml/l. These contents are then used in the equation determined by Aminot and Chaussepied (1983) to calculate the oxygenation rate, which is expressed as a percentage (%).

- Temperature and salinity

These two parameters were measured *in situ* on the water surface. The temperature was measured with a standard thermometer and expressed in °C and the salinity was measured with a refractometer and expressed in PSU (or ‰).

## 5. Monitoring of individuals

It is carried out monthly at the rearing point for mortality counting and in the laboratory for biometrics.

### 5.1 Mortality

For mortality monitoring, a sample of 450 to 500 individuals was taken from all the bags placed on the tables installed in the selected rearing area. The counting of dead and live individuals was carried out on site and an estimate of the total mortality was made for all the tables.

### 5.2 Biometrics

#### - MONITORING OF WEIGHT AND LINEAR GROWTH

Individual growth was monitored by weighing and measuring 30 oysters taken at random. Each month the oysters were weighed using a balance type AND (EK-600H) accurate to 0.01 grams and the biometry was determined using an electronic calliper type Vogel with a maximum of 200mm.

#### - THE QUALITY INDICES OF ADULTS

Biometric analyses of oyster samples from the minimum marketing weight and at the harvest in September 2016 allowed the calculation of various quality indexes, such as:

- Afnor (1985) filling index :  $I = (\text{Fresh weight} / \text{Total weight}) \times 100$

This index classifies merchant oysters into two categories (L'Officiel de la conchyliculture, 2000):

- $6.5 < I < 10.5$  : "Thin" oyster
- $I > 10.5$  "Special" oyster

- Lawrence and Scott's index (1982) :

$$I1 = (\text{Dry weight of flesh} / (\text{Total weight} - \text{Shell weight})) \times 1000$$

This index is a commonly used quality and fill indicator.

## Results

### 1. Environmental monitoring

At the rearing point located north of the Bay of Cintra, the oxygenation rate was very high throughout the study and the percentages recorded ranged from 81 to 170%. Water was characterized by a high turbidity governed by the tidal and hydrodynamic regime where the suspended matter contents varied between 80 mg/l as the maximum recorded in August 2016 and 14 mg/l as the lowest value measured in December 2015 (Fig. 3). There was a seasonal variation in temperature, with the lowest values (17-18°C) characterizing the winter season and the highest values recorded during the summer and early autumn (between 22 and 24°C) (Fig. 3). Similarly, salinity within the rearing site varied seasonally and values ranged from 36.5 to 36.9 ‰ (Fig. 3). The total concentrations of phytoplankton cells ( $> 10\mu\text{m}$ ) belonging to the Diatoms group (responsible for the growth of bivalves) and recorded at the rearing site in the Bay of Cintra, also vary seasonally between  $1.2 \times 10^4$  and  $6.8 \times 10^5$  cell/l (Fig.3). The first phytoplankton blooms took place at the end of November until early January 2016 and then concentrations declined rapidly until early February 2016. The second blooms began in early April 2016 and cell concentrations remained almost constant until early May 2016 when a decline began to mark the rest of the study cycle. These phytoplankton blooms were dominated by Diatoms of the genera *Chaetoceros*, *Leptocylindrus*, *Nitzschia*, *Navicula*, *Pseudo-nitzschia*, *Grammatophora* and *Lioloma* (Fig.3). The latter genus was detected only once during the present study and its name needs to be confirmed.

Chlorophyll *a* contents varied between 0.1 and 0.9 mg/m<sup>3</sup>, depending on the season (Fig.4). Fluctuations were marked by three peaks of chlorophyll *a*, the first of the order of 0.85 mg/m<sup>3</sup> in February 2016 and the two others reached 0.53 and 0.48 mg/m<sup>3</sup> in April and May 2016, successively. In fact, the chlorophyll contents recorded in this study are underestimated due to the use of the UV-VIS Spectrophotometer which is less sensitive to chlorophyll pigments. Normally, during the oceanographic surveys carried out between 2015 and 2016, these contents (using the Fluorimeter apparatus) can exceed 4mg/m<sup>3</sup> during the fall (October 2015) and do not reach 1mg/m<sup>3</sup> during the spring (May 2016) (Makaoui et al., 2016). It should be noted that the peaks of Chl. *a* coincided with the phytoplankton blooms observed at the rearing point during the monitoring period (Fig. 4).

## 2. Spat growth

The monthly average whole weight of oyster spat rearing in the Cintra Bay varies seasonally and reaches a maximum of 39.7g (a weight gain of 39.6g) at the end of the rearing cycle.

The weight evolution curve shows different spat growth rates (Fig. 5). The most remarkable were the growth recorded between August and September 2015, where the average whole weight increased from 0.1 to 1.5g (15 times more than the starting weight), and that of the following period (between September and October 2015) where this average whole weight doubled 11 times and reached 16.7g. Between October and December 2015, the evolution was very slow and the average whole weight did not exceed 18.6g. On the contrary, from December 2015 to March 2016, a rapid increase in this weight was observed and the maximum value recorded is 30.5g. After this remarkable increase, the evolution stagnated until June 2016 to regain its accelerated rhythm until the end of the cycle (September 2016) where the average whole weight recorded was around 39.7g.

Monitoring of spat size was also carried out and showed that all three biological parameters (length, width and thickness) are in continuous growth throughout the rearing period. The shape of the curves (Fig.6) shows that this size is also subject to seasonal fluctuations. Thus, initially this growth is rapid during the warm period between August (23.5°C) and October 2015 (22°C) (Fig.4). When the temperature starts to decrease, between October 2015 and June 2016 (Fig.4), the growth rate becomes a little slower and then restarts its rapid increase from June 2016 (20.8°C).

## 3. Condition Indices

The evolution of the average Lawrence and Scott (1982) index calculated from March, when the weight of the individuals reached the marketing size (set at 30.5g), until the end of the experiment is shown in Figure 7. The average index decreased significantly between March and July 2016, then increased rapidly the following month, and the average recorded at the time of the harvest was  $126.3 \pm 50$ . For the average AFNOR index, the calculation at the harvest showed an average reaching  $22 \pm 6$ .

## 4. Mortality rate

Mortality rates during the first four weeks after spat release ranged from 26 to 58.3%. From the end of the first month of rearing until the third month (October 2015) the rate did not exceed 2%. For the rest of the rearing period, mortality was almost negligible with rates of around 0.1%.

## Discussion

The growth of bivalves is mainly regulated by water temperature and the quality and quantity of phytoplankton available in the environment (Karine Grangeré et al., 2010; Cognie et al., 2001; Numaguchi 1995; Albentosa et al., 1993; Cano, Porstique and Rocamora 1997, Foster-Smith, 1975; Bayne, 1991). Other factors that may intervene and influence the growth and condition of bivalve mollusks include density, age, salinity, pollutants, flow velocity, polyculture with other species, shoreline height, and air exposure (Richard et al. 2015; Hildreth and Stickle, 1980; Summer, 1981; Gillmor, 1982; Whyte and Englar, 1982; Brown, 1988; Pridmor, Peterson and Allen 1996; Sara and Mazzola 1997).

Monitoring of the biological parameters of triploid oysters, reared for the first time in Cintra Bay, gave satisfactory results in terms of growth performance. Thus, the three parameters length, width and thickness of the individuals showed rapid growth as soon as they were put in the water, then slowed down while maintaining a positive evolution until the end of rearing. The same evolution was observed in the average total weight where individuals reached commercial size after only eight months (>30g minimum marketable weight, CNC, 2017; Saint-Feux et al., 1983). It should be noted that in the Bay of Veys (France), considered as an ecosystem with a very high trophic capacity, triploid cupped oysters only reach this size after twelve months of rearing (Gouletquer et al., 1996). The growth performance of triploids, observed in this study, is probably due to two factors: one is related to the biological character of the oysters themselves and the other is related to the environmental conditions of the chosen growing environment.

Thus, from a biological point of view, oysters have the power to regulate the amount of food ingested by adapting the filtration rate and particle retention efficiency (Gerdes 1983; Ward, Sanford, Newell and MacDonald 1998). Moreover, the performance of triploids is mainly related to their reduced reproduction (Haure et al., 2003; Hawkins et al., 1998; Hand et al., 1998a). Indeed, the energy used for reproduction for diploid oysters appears to be available for growth and survival for triploids (Allen and Downing, 1986; Barber and Mann, 1991; Hawkins et al., 1994; Hand et al., 1998a). Slower growth in weight and height can be explained by a decrease in the amount of food in the environment, variation in temperature and/or lysis of the genitals for triploids (Bather and Baud, 1992; Allen & Dovming, 1986, Gérard et al., 1997).

As for the environmental conditions, the bay of Cintra has very important potentialities such as:

- Phytoplankton richness (monthly total flora between  $1.2 \times 10^4$  and  $6.8 \times 10^5$  cells/l), especially diatoms. In fact, coastal areas offer more cellular concentrations of plankton (Ducrotoy et al., 2000) essential for bivalve feeding (Kang et al., 2006; Grant, 1996; Jorgensen, 1975), particularly diatoms having a direct impact on oyster tissue growth and gonadal development (Dupuy et al., 2000b, Kanget et al., 2006). In addition, this richness is characterized by a diversity of algal species constituting a mixed diet favoring the good growth, survival and metamorphosis of oysters (Albentosa et al., 1993);
- Temperature (between 17 and 24°C) favorable for growth, exceeding that of the lower critical limit between 17 and 20°C for gametogenesis and oviposition for oysters in general (Héral & Deslous-Paoli, 1991, Giese, 1959; Sastry, 1975; Chung, Seo & Park 1998; Ren et al., 2003). Indeed, *C. gigas* tolerates a wide range of temperatures (OSPAR, 2009);
- Oxygenation (very high rates reaching 170%) for a better survival of individuals (Cheney et al. 2000; Morton et al., 1957);
- Strong water current (Hilimi et al., 2017) allowing the oysters to grow better (Valero, 2006).

In general, the Bay of Cintra is subject to the influence of oceanic waters (Hilmi et al., 2017) which, through the buffering role they exert, moderate environmental variations (Maurer, 1989). This relative stability would explain the best growths obtained on this type of site as well as their regularity (Maurer, 1989). Similarly, this site is part of the upwelling area of southern Morocco known for its nutrient richness and relatively warm temperatures (Makaoui et al., 2005; Saad et al., 2013; Makaoui et al., 2017). These characteristics allow this type of area to favor the production of triploid oysters with substantially higher growth rates (Brake et al., 2004). Finally, all these marine conditions characterising Cintra Bay seem to be preferred by the two species of *Crassostrea gigas*, di and triploids (Laing et al., 2005).

Monitoring of triploid mortality throughout the rearing cycle in Cintra Bay has shown that these organisms resist well to various stresses. This power of resistance under stressful conditions has also been reported by Hawkins and Day (1996) and Hawkins (1996) and they linked it to sterility and higher heterozygosity in triploids. However, the high mortality rates recorded only occurred in the first month following spat release. Similar studies suggest that many oyster mortalities have been linked to multiple factors or stresses, including pathogens, high temperature, low dissolved oxygen, xenobiotic stress and physiological stress associated with reproduction (Costil et al., 2005; Sotelnik et al., 2006; Cheney et al., 2000).



For the present study and given that the oysters are sterile, certified before transportation and the rearing environment is highly oxygenated, the temperature may be the cause of mortality marking the beginning of the rearing cycle in the summer period (23.5°C). The transfer of spat from a hatchery (controlled environment) in France to the Bay of Cintra (natural environment) in southern Morocco cannot be without effect on the biological state of the spat (Costil et al., 2005) and may also be a stress factor for these small organisms (6mm) leading to sudden mortality due to the change of environment. In the same context, Pernet et al. (2010) found that the transfer of oysters from the sea to the Thau lagoon generated a rapid accumulation and utilization of sugars which may be an effect of the increase in the temperature of the environment during the transfer or an effect of the change in the nutritional quality of the environment hence the spat mortality recorded.

The AFNOR (1985) and Lawrence and Scott (1982) indices provide data on the commercial quality and physiological (health) status of bivalve mollusks (Dridi et al., 2007; Haure et al., 2003) and are commonly used to assess the effects of the surrounding environment on these organisms (Davis, 1988; Rheault and Rice, 1996; Gasmi et al., 2017). Thus, the results obtained from the calculation of these indices indicate that oysters reared in Cintra Bay generally show high biological performance in terms of whole weight and condition indices. This bay seems to be, therefore, a very favorable environment for shellfish farming and the oysters produced are of the best commercial category. According to several authors, the condition index varies greatly with dry weight (Maurer, 1989; Lucas & Beninger 1985; Mann, 1978; Lawrence & Scott, 1982) and its fluctuations are managed, in particular, by modification of the food supply and in adult individuals by gametogenesis and gamete release (Li et al. 2009; Soletchnik et al., 2006; Yildiz et al., 2011; Shpigel et al., 1992; Davis, 1989; Vercelli et al., 2000). Hydro-climatic conditions and habitat type have also been indicated as contributing factors to changes in this index (Gasmi et al., 2017; Dutertre et al., 2009; Jourdain 1996; Maguire et al., 1994b). In the present case, the AFNOR index calculated at the harvest time reached 21.8 showing, thus, that the commercial quality of oysters is "Special". The monthly average Lawrence and Scott (1982) indices recorded exceeded 85 (generally values over 80 indicate the good physiological condition of the oyster; Barillé et al., 2000) and are better at the beginning of maturation and at harvest time (between 125 and 126.5). These good indices are probably the result of the reduced gonadal development (Beaumont and Fairbrother, 1991; Maguire and Kent, 1990) and the reduction and/or inhibition of gametogenesis in adult triploids (Allen and Downing, 1990; Tabarini, 1984; Allen et al., 1986). However, the remarkable decreasing in these average indices between March and July 2016 can be attributed to several factors, in particular, the increase in turbidity (high suspended matter reaching 63 mg/l) leading, therefore, to the clogging of rearing bags (cleaned once a month) and, consequently, to the reduction in the level of food available for individuals. Added to this, the strong current that overturns (each time) the rearing tables and causes the silting of the bags, which disrupted the normal growth of the individuals during this period. The lysis of sexual products in mature triploids (considered sterile) and the reallocation of energy for shell growth instead of flesh are also factors contributing to the decrease of this condition index (Normand et al., 2008; Vercelli et al., 2000; Landau and Guo, 1999 and Scarpa et al., 1996).

## Conclusion

In this study, conducted for the first time in the Bay of Cintra, the results of the monitoring of physico-chemical conditions and primary productivity show that this site is very suitable for oyster farming. Triploid oysters show a better adaptation and biological performance. The mortality rate is almost non-existent after the first month of launching. The growth of the individuals is continuous throughout the rearing cycle and the condition indices classify the triploid oysters in the best commercial category (Special) and indicate their good physiological condition. From a technical point of view, rearing on tables seems to be a technique not recommended because of the strong water circulation that continually destabilizes these tables and leads to a disruption of the normal growth of the individuals. Therefore, thinking about an adapted and less costly technique (less manpower, logistics, maintenance frequency, etc.) will contribute to a rapid and economic evolution of this practice in the Bay of Cintra.

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Figures

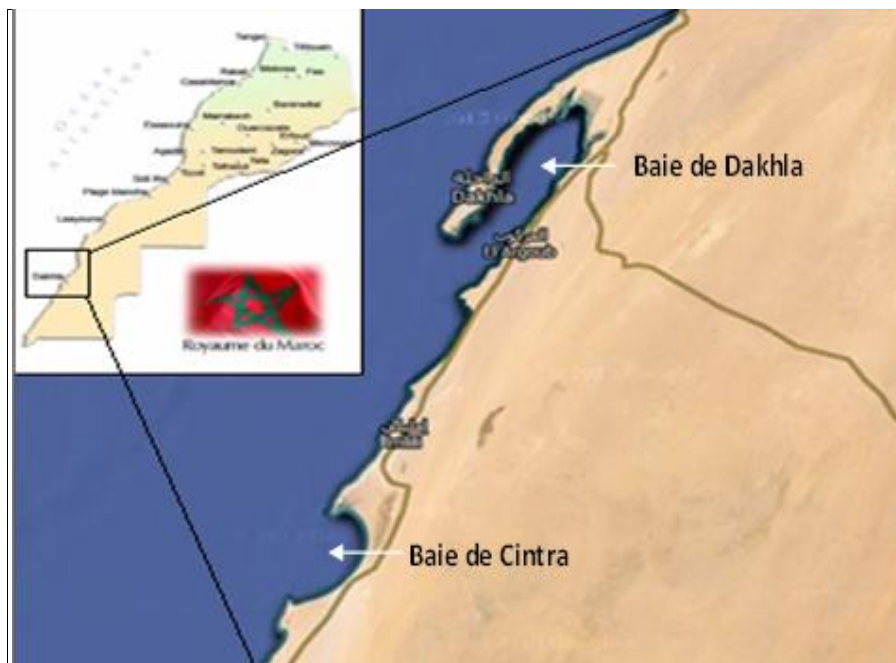
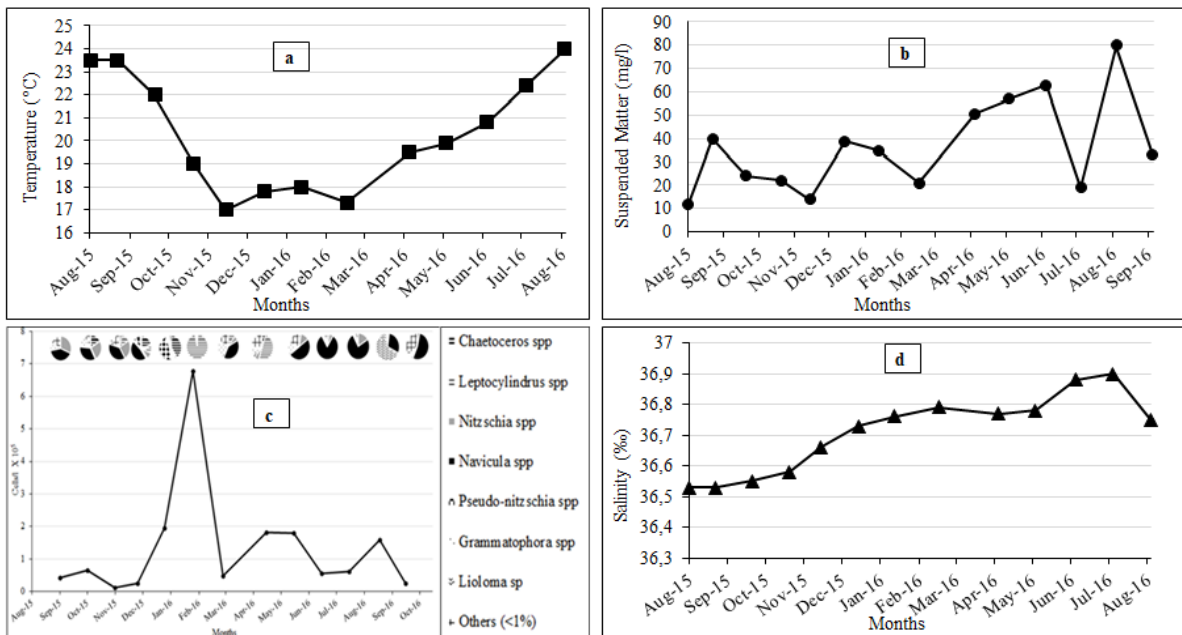


Figure 1 : Geographical location of the bay of Cintra.



Figure 2: Chosen area for oyster farming in the bay of Cintra.

Figure 3: Evolution of a) temperature, b) suspended matter concentration, c) total diatom cell concentrations and



d) salinity in the Cintra rearing site during the period August 2015-September 2016

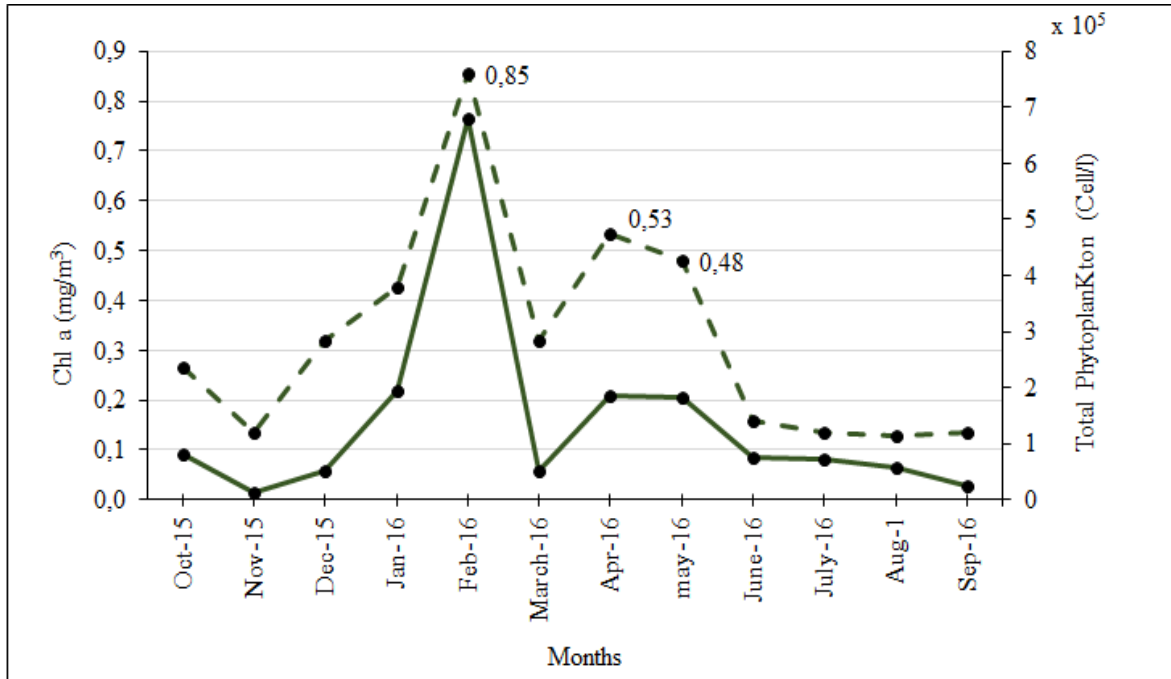


Figure 4 : Evolution of chlorophyll a and total phytoplankton concentration in the Cintra rearing site during the period October 2015-September 2016.

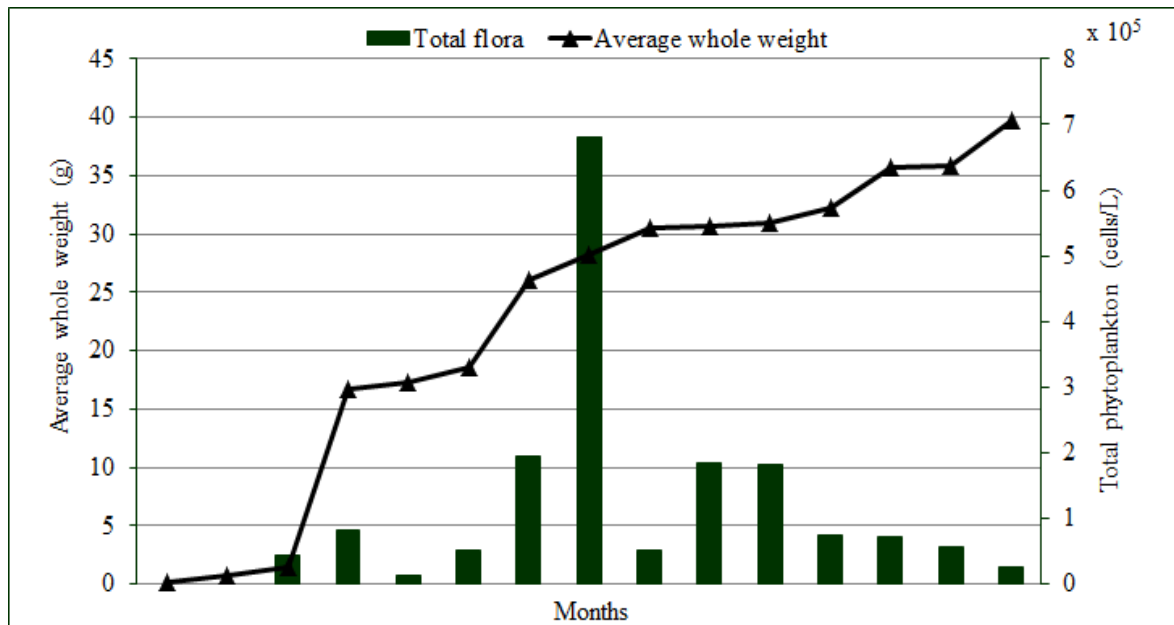


Figure 5 : Evolution of the total cell concentration of phytoplankton\* and the average whole weight of triploid cupped oyster spat in Cintra bay during the period August 2015-September 2016.

\* Le suivi des concentrations cellulaires du phytoplancton n'était possible qu'à partir du mois de septembre 2015.

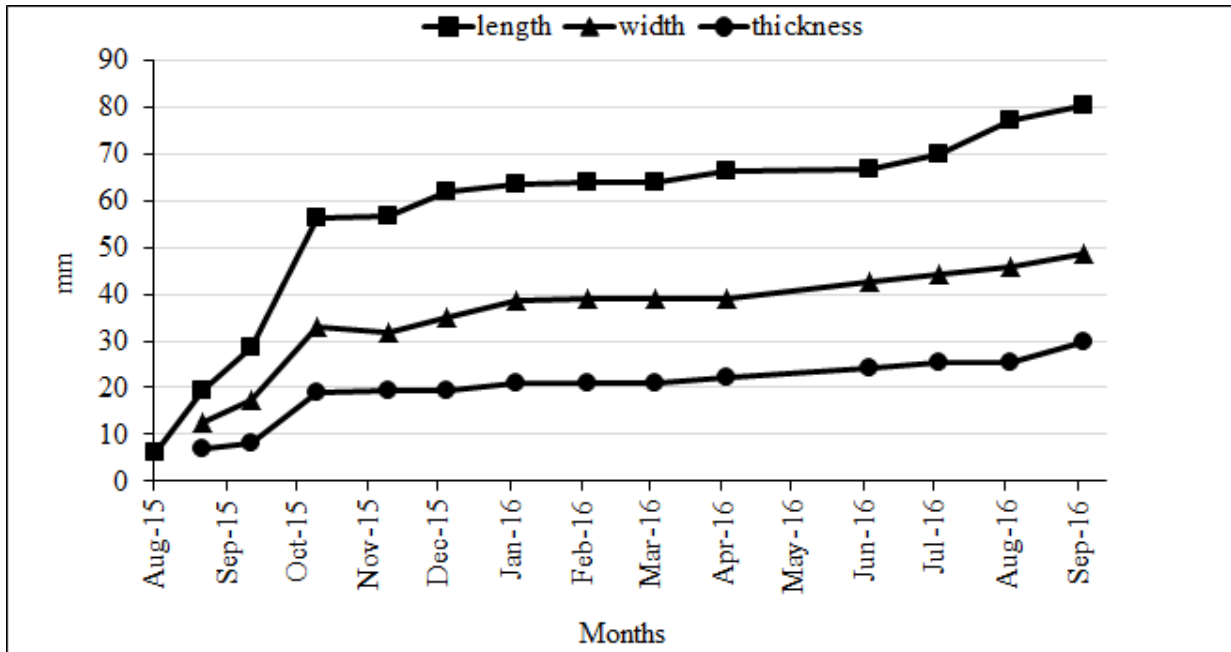


Figure 6 : Evolution of allometric parameters of triploid cupped oyster spat in Cintra bay during the period August 2015-September 2016.

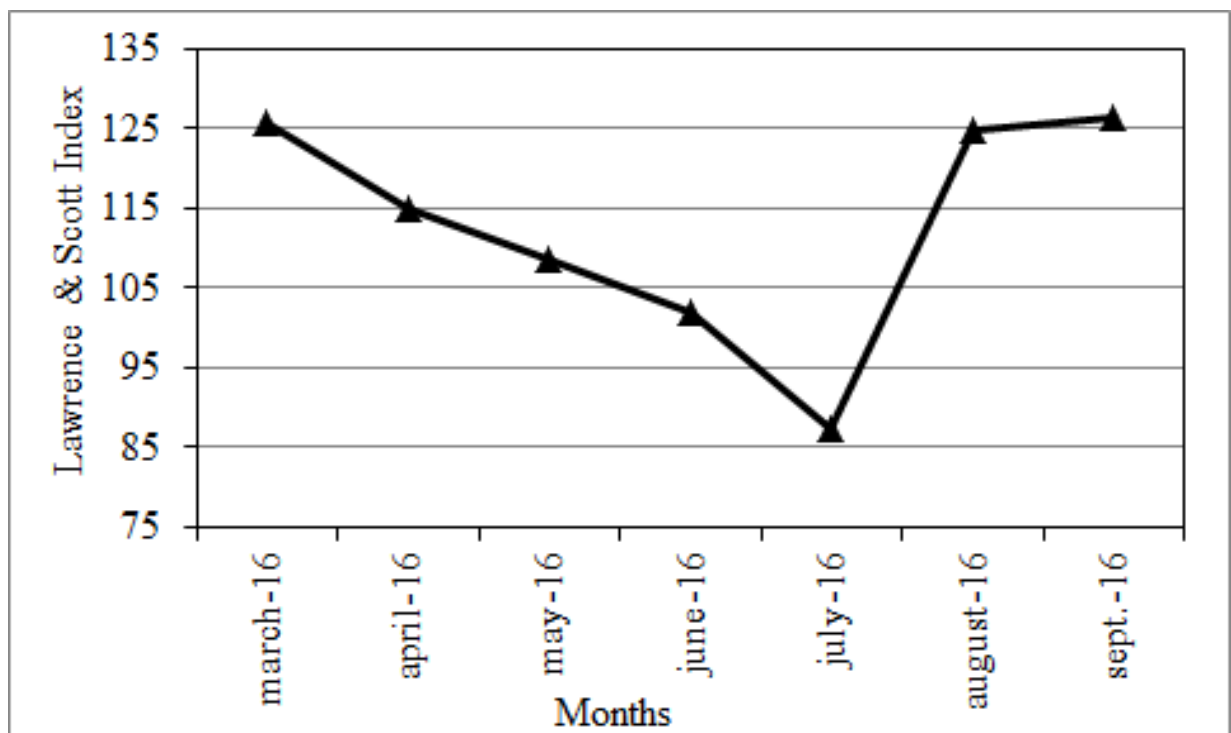


Figure 7: Evolution of the average condition indices of cupped triploid oysters exceeding >30.5g in Cintra bay between March and September 2016.