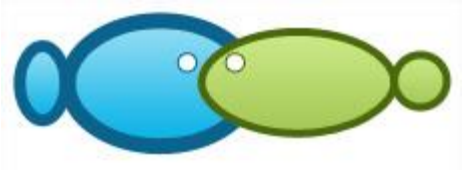

Evaluation of the growth potential and contamination of the European clam (*Ruditapes decussatus*), raised using the suspension culture technique at the Oualidia lagoon in Morocco

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Abstract. This work, carried out in the lagoon of Oualidia (Morocco), aims to study the growth potential of the European clam (*Ruditapes decussatus*) raised according to the suspension culture technique, as well as to compare the accumulation of chemical contaminants (cadmium , lead and mercury) and bacteria (*Escherichia coli*) in clams raised in suspension and those buried in sediment. The growth of the *R. decussatus* clam was monitored by assessing the growth curves and survival under different rearing conditions. Chemical contamination was determined by the analysis of Cd, Pb (ICP-MS) and Hg Direct Mercury Analyzer (DMA), and bacteriological contamination was estimated by determining the MPN of *E. coli* β-galactosidase (β-gal) in live shellfish. The hydrological conditions were monitored, and the results of the rearing showed that the system ensures a better survival rate and faster growth of the species in suspension ($p<0.05$). Results for chemical contaminants (Cd, Pb and Hg) showed no significant effect between clams in the water column and in the sediment ($p>0.05$). For bacteriological contamination (*E. coli*), it has been found that decontamination of clams in the water column is more rapid and extensive than in the sediment, likewise contamination occurs more rapidly at higher concentrations in suspended clams.
Key Words: accumulation, bacteriological, chemical, clams, rearing system.

Introduction. The clam *Ruditapes decussatus* is a burrowing bivalve mollusc which commercial interest resides in being a consequent source of proteins, its gustative qualities and notable robustness allow this shell to be shipped all over the world at any time of year (Joly 1982; Gervasoni 2009) allowing the small-scale fishermen to substantially increase their income. It lives buried a few centimeters deep in soft bottoms (sandy, sandy-muddy substrates) of the infralittoral level and in coastal lagoons (Gallois 1973; Maitre-Allain 1983; FAO 2006; Gervasoni 2009). It particularly appreciates sheltered coastal areas such as brackish water environments communicating with the sea. The life of the clam depends a lot on environmental conditions: the ecological limits of these molluscs vary between 5 to 30°C for temperature, and 15 to 40‰ for salinity (Cépralmar 1987; Besançon et al 2013).

The shape of its shell ranges from oval to quadrangular, the growth ridges are apparent, and the coloration is variable depending on the individuals and the environment in which they are found. The length reaches 80 mm for older individuals (Gallois 1973; Mazouni 1999; FAO 2006). Growth of the European clam is sustained, and individuals reach the commercial size of 35 mm in about 3.5 to 4 years (Arnal & Pato 1978; Maitre-Allain 1983; Lavaud 2014). Clam growth is discontinuous: important in autumn, it stops in winter due to the decrease of temperature and is followed by an increase in growth in spring due to warming waters and the proliferation of

phytoplankton (Besançon et al 2013). *R. decussatus* has a wide geographical distribution; it is recorded from Denmark to Congo and is widely distributed along the Mediterranean and Red Sea coasts (Parache 1982; CNEXO 1983).

In Morocco, natural stocks are present in semi-sheltered coastal areas: mouths of rivers and lagoons. The most important stocks in Morocco are: Oued Tahaddart, the lagoon of Moulay Bousselham, the mouth of Bouregreg, the lagoon of Sidi Moussa, the lagoon of Oualidia and the lagoon of Nador (Labbardi 2006). In recent years, stock abundance and density in these sites have declined due to various factors including environmental variations, predation and mainly pressure from intensive fishing (Kamara et al 2008). Hence the need for measures to protect the species and develop aquaculture activity.

Present directly on the substrate (sand or mud), the clam buries itself using its foot. It is a very accessible prey for predators and its production and/or harvest cannot be considered without thinking about the health of the consumer, since the substrate in which the clam develops is a reservoir of external pollution. The rearing of *R. decussatus* is now only a marginal activity. In fact, this specie is cultivated by a few farmers as a complement to oysters (Bordeyne et al 2009)

Growing of *R. decussatus* is normally done on the substrate (after pre-growing up to 12-13 mm (T8), the clams are sown in the sediment): in fact many authors affirm that clams become deformed when they are reared above ground (Boglino et al 2009; Gervasoni 2009; Besançon et al 2013). In 2015, Cépralmar tested a method of monitoring clams in "suspended tanks containing sediment", the results were very satisfactory, the only negative point of the test carried out was the loss of a few individuals, without any trace of shell (Cepralmar 2016). The study carried out by Devic (2010) on chains in the Thau pond shows very encouraging growth results of the European clam (*R. decussatus*), the size has reached 22 mm and the author proposes to finish the growth of the animals up to commercial size in the sediment (Devic 2010).

The challenge of this study is to be able to continue the growth of this species in the water column and to harvest individuals that can be marketed without health risks. Indeed, studies have shown that the consumption of contaminated shellfish is a major public health risk (Plusquellec et al 1986; Valentin et al 2000). The way in which bivalves are fed using seawater filtration inevitably leads to their contamination with bacterias, viruses, toxins and toxic chemicals present in their environment (Oliveira et al 2011; Lavaud 2014).

Microbiological contamination may involve the appearance of a certain number of microorganisms, pathogenic or not, in coastal waters: most often, these are fecal germs, adapted to the human or animal organisms, such as viruses and enteric bacteria (Hervio-Heath et al 2012). The best known and most studied is the *E. coli* bacteria*,* several strains of which have been identified as causing serious diseases (Allocati et al 2013; Damayanti et al 2019). In the aqueous medium, the bacteria adsorb on the particles suspended in the water and sediment along with them. Many studies agree that the bacterial load is higher in the sediment compartment (and particularly at the surface (biofilm) than in the overlying water column (Alzieu 1999; Derrien 2004; Sigwald et al 2012).

Trace metal elements (TME), known for their toxicity and contamination of aquatic environments, are among the substances which concentrations must be measured to assess their levels of contamination and thus their potential ecological impact (Klassen et al 2010). Unlike organic substances, trace metals are infinitely persistent and their form in the environment is highly variable, which influences their environmental properties (Papp 2011; Mejjad et al 2018). Cadmium, lead, and mercury have been identified as "priority hazardous substances" (Papp 2011). They bioaccumulate in the bodies of aquatic organisms and pose a risk to human health (Yap et al 2016; Wang & Lu 2017; Puspitasari et al 2020). As a matter of fact, the accumulation of lead and cadmium in the human body affects kidney's function (Hartanto & Tjahjono 2020).

To estimate the health risk to consumers, shellfish growing environments are subject to a health classification based on the count of the fecal contamination indicator

E. coli and the concentration of trace metal elements (lead, cadmium, and mercury) in shellfish tissues.

The objective of this study is to test for the first time in the lagoon of Oualidia, a reputed site for oyster farming in Morocco, a system for rearing clams (*R. decussatus*) suspended in the water column, a suitable system capable of ensuring the survival and normal growth of the species beyond the commercial size of 35 mm and to evaluate the effect of the rearing technique on the accumulation of contaminants.

Material and Method

Biological material. In March 2017, 8000 individuals of clam *(R. decussatus)* with an average size of 20 mm and an average weight of 2 grams, collected from the natural population in Oualidia lagoon (Figure 1), were used for the trial installation.

Choice of study site. The experimental study was carried out in a semi-enclosed environment of the Oualidia lagoon in Morocco (Figure 1). The Oualidia lagoon (32°45'N-9°30'W), located on the Moroccan Atlantic coast between El Jadida and Safi, is a site considered with biological and ecological interests on the RAMSAR list (Ramsar 2016). In the lagoon of Oualidia aquaculture is practiced in five parks that exploit 1/6th of the surface of the wetland (Zbiry 2018; Lakhlalki et al 2020), consisting mainly in oyster farming, which has been practiced since 1970. It runs parallel to the coast over approximately 7.5 km long and 0.5 km wide. The average depth of the main channel is 4 m in some places and almost half (53%) of the lagoon surface is exposed at low tide (Karim et al 2017). Tidal currents are very intense upstream of the lagoon, with maximum intensities of about 138 cm s^{-1} observed in October-November (Makaoui et al 2018). The driving force behind the hydrological circulation of this lagoon is the tide, which is semi-diurnal (Sarf 1999; Kamara et al 2005; Karim et al 2017).

Figure 1. Map of the study area showing the location of the installation of the clam rearing system in the Oualidia lagoon.

The point of installation of the system was chosen because of its hydrological and hydrodynamic conditions. The upstream part of the lagoon where tidal currents are very intense was avoided (Makaoui et al 2018). Other additional conditions were considered, namely: access to the rearing point and proximity to a shellfish farm.

Rearing system design. The choice of bags was inspired by the oyster farming technique practiced on the same site chosen for this study. In March 2017, 8 suspended bags, 100 cm long and 50 cm wide with a 10 mm mesh (Figure 2), were fixed by metal supports sunk vertically into the sediment.

The laying strata of the rearing bags were fixed 60 cm apart to cover the entire water column. To compare the results with natural conditions, a pocket was buried in the sediment (Figure 3). In total, the system is composed of four levels, namely:

- Level 1 (N1): Bags are fled into the sediment to return to natural conditions.
- Level 2 (N2): Bags are deposited on the sediment; individuals are between the two environments, sediment, and water column.
- Level 3 (N3): Bags are 60 cm from the bottom at low tide, in the water column, individuals in this level are always submerged at high and low tide.
- Level 4 (N4): Bags are 120 cm from the bottom at low tide, individuals at this level are submerged at high tide and exposed at low tide.

Two bags are placed for each level. One for monitoring growth (bag A), and the other for monitoring mortality and deformation (bag B).

Figure 2. Rearing bags.

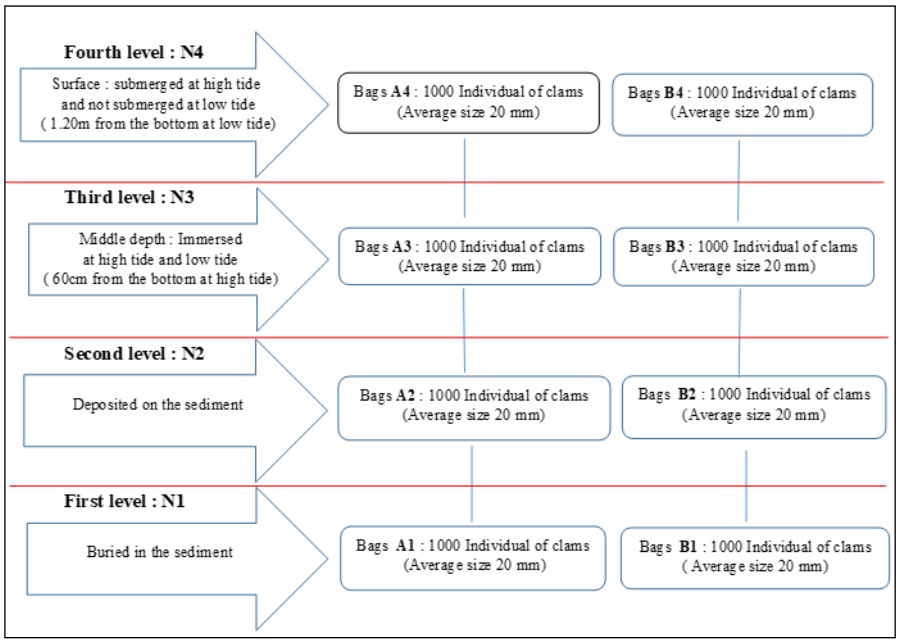

Figure 3. Clam farming protocol in the Oualidia lagoon.

The density applied is 1000 clams in each bag. Which is a density of 2000 individuals per m^2. Throughout the duration of the study, each month at low tide, hydrological parameters (temperature, salinity, pH, dissolved oxygen, and suspended matter) were monitored, both at the surface and near the bottom. The parameters (temperature, salinity, pH) were measured *in-situ* using a WTW-type probe. Dissolved oxygen was

measured using Winkler's chemical method. Suspended matter was determined by filtration and double weighing (Aminot & Kerouel 2004).

Monitoring the growth of individuals. Thirty individuals were taken from the bags (A1, A2, A3 and A4) each month to study:

- Individual size: Length, Height and Thickness for each level; measurements are taken with a caliper.
- The total individual weight recorded using an electronic scale.
- The individual fresh weight of the clam meat recorded using an electronic scale.
- The individual dry weight of the clam meat after freeze-drying, taken using an electronic scale.
- The individual dry weight of the clam shell recorded using an electronic scale, after drying at 60°C in an oven for 48 hours.

Monitoring the Condition Index. The Clam Condition Index is regularly analyzed to determine significant phenomena such as growth or reproduction that are characterized by seasonal fluctuations. The condition index used is that of Waln and Mann (1975) defined according to the following formula:

$$CI = (\text{Dry weight of the flesh} / \text{Dry weight of the shell}) \times 1000$$

Monitoring of mortality and deformation. Dead and living individuals are counted each month, in the bags (B1, B2, B3 and B4) and dead individuals are removed. This makes it possible to determine, for each level, two mortality rates (Fleury et al 2011):

- The instantaneous mortality rate (IM), observed at time t:

$$\text{Instantaneous mortality}_{(t)} = \text{Number dead}_{(t)} / \text{Number dead}_{(t)} + \text{Number alive}_{(t)}$$

- The cumulative mortality rate (CM) at time t :

$$\text{Cumulative mortality}_{(t)} = 1 - [(1 - CM_{(t-1)}) \times (1 - IM_{(t)})] = CM_{(t)}$$

- The deformation rate: counting, each month, in the bags (B1, B2, B3 and B4) of all bulging individuals (deformed).

Metallic contamination. The flesh of individuals clams taken from the two levels N1 and N3 (Bags A1 and A3) for growth monitoring is then crushed and stored in decontaminated flasks until analysis.

0.25 grams of dry flesh was mineralized in a microwave oven according to standards NM EN 13805 (2015) and NM EN 13804 (2015) using a mixture of 6 ml of nitric acid HNO3 (65%) and 2 ml of ultrapure deionized water in Teflon flasks. The minerals obtained are recovered in a conical tube and the final volume is adjusted to 50 ml with deionized water. The determination of cadmium (Cd) and lead (Pb) contents was carried out by an ICP-MS (Inductively Coupled Plasma Mass Spectrometer - Thermo iCAP Q Series) according to NM EN 15763 (2012), while the total mercury (Hg) contents were determined by atomic absorption spectrophotometry using a Milestone DMA-80 direct mercury analyzer (EPA 2007).

For quality control and validation of the results, the samples were analyzed in triplicate. The mean and standard deviation of the three measurements is reported as the result in mg kg^{-1} dry mass. A blank and a certified reference material (Nist-2976) were used to assess the accuracy and precision of the procedure.

Bacteriological contamination. Each month ten individual clams of level N1 and N3 (Bags A1 and A3) were collected in sterile plastic bags and transported in a cooler as quickly as possible to the laboratory and analyzed without delay according to standard NM ISO 16649-3 (2017): After preparation under aseptic conditions of a mother

suspension diluted to 1/3, the determination of the MPN of *E. coli* β G+ in live shellfish was carried out in two steps (Presumptive test and confirmatory test).

Statistical analysis. The data was compiled, and graphs were plotted using Microsoft Excel to visually assess the growth and survival of clams at different levels of rearing. A comparison between the effect of the different levels and their interactions on each of the parameters (condition index, growth, mortality, and chemical contamination) was carried out using an analysis of variance (ANOVA). The conditions of homogeneity of the variances were verified with the Levene test and the normality of the residues with the Shapiro-Wilk test. When the conditions for the application of the analysis of variance are not met, the data are subjected to a transformation to the normal distribution. When $p<0.05$ the results are significantly different. The Tukey test was used to identify the best breeding level. Statistical processing was carried out using R software.

Results and Discussion

Hydrological parameters. The hydrological parameters measured are temperature, salinity, pH, dissolved oxygen, and suspended matter. Knowing that the identified study point is 1.20 m deep at low tide, these parameters were measured at the surface and at bottom.

The water temperature values recorded *in situ* during this study allowed us to illustrate temporal variations in this parameter (Figure 4). The mean values are 20.64°C at the surface and 20.95°C at the bottom, respectively. This small variation between surface and bottom temperatures is due to the shallow depth which facilitates air-water exchanges. A minimum of 15.01°C was recorded in January 2018 and a maximum of 27.60°C in August 2017. The results obtained are in perfect agreement with previous work on the lagoon of Oualidia with an average temperature that varies between 14.5°C and 27.0°C, according to two distinct periods: winter with lower temperatures and summer marked with high temperatures (INRH 2015).

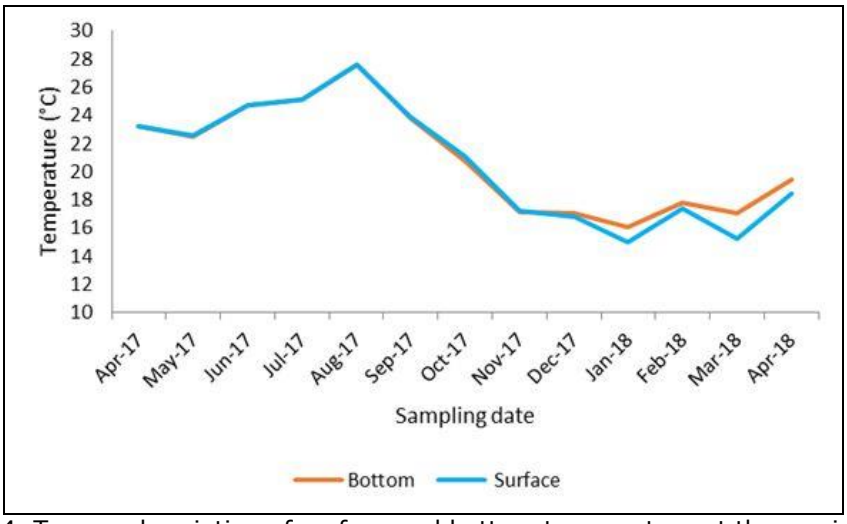

Figure 4. Temporal variation of surface and bottom temperature at the rearing site.

Figure 5 shows the variation of salinity of the study period with values ranging from 23.0 to 35.8 psu on the surface, and 23.3 to 35.5 psu on the bottom, with an average of 29.3 psu. In the open sea, the lagoon is more influenced by the flooding of water masses with marine characteristics and at low tide salinity is reduced upstream of the lagoon, due to several underground infiltrations of fresh water into the lagoon. According to the study conducted by Makaoui et al (2018), the salinity of the lagoon varies between 20 and 35 ‰ at low tide, while at high tide it reaches 30 at 36 ‰ during all seasons.

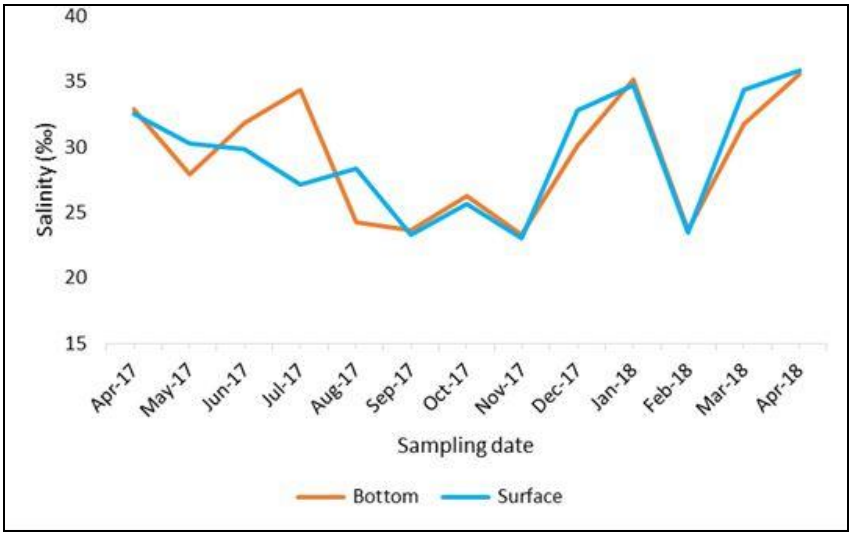

Figure 5. Temporal variation in surface and bottom salinity at the rearing site.

The pH of seawater generally ranges from 7.5 to 8.5 and is influenced by temperature and the photosynthesis and respiration activities of microorganisms (Harvey 1955). Collected pH data show values ranging from 7.1 to 8.7 with a mean of 8.1 (Figure 6). This parameter is related to the growth of macroalgae; during periods of algal growth, there is a release of CO_2 which tends to increase the pH, whereas during periods of algal mortality (summer period), the bacteriological decomposition of algae induces a relatively low pH environment (Elmanama et al 2006). The results found are generally like those highlighted by INRH (2015) which are between 7.5 and 8.5.

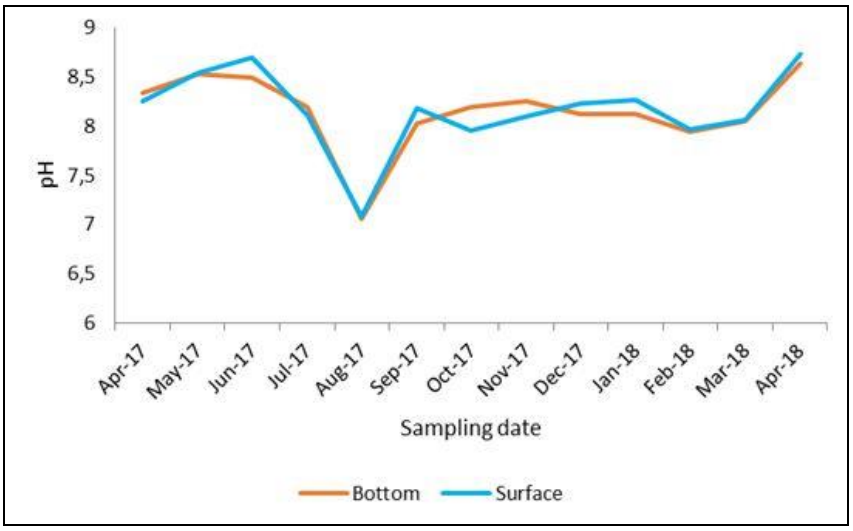

Figure 6. Temporal variation of surface and bottom pH at the rearing site.

During the study period, good oxygenation was observed throughout the year. Oxygen levels ranged from 6.26 to 8.83 mg l^{-1} with a mean value of 7.68 mg l^{-1} (Figure 7). The variation, observed between the surface and bottom values recorded during the months of May and June, is due to the algal bloom that the site is experiencing at that time. The high oxygen values observed from January onwards are mainly due, on the one hand, to the strong currents upstream of the lagoon (Karim et al 2017)**,** on the other hand, to the winds that agitate the surface waters. These results corroborate those highlighted by Damsiri et al (2014) and Makaoui et al (2018) who found that the waters of the Oualidia lagoon are well oxygenated with an average of 7.15 mg l^{-1}.

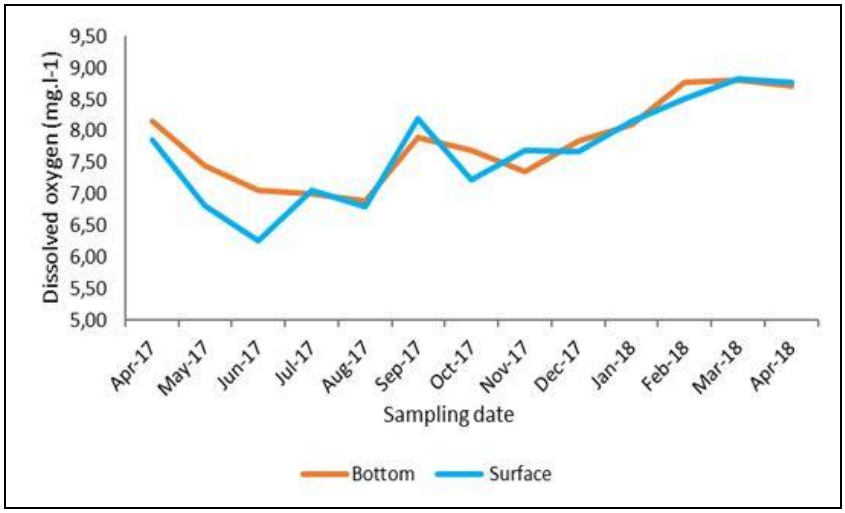

Figure 7. Temporal variation of dissolved oxygen at the surface and bottom of the rearing site.

The average concentration of suspended matter is around 22.1 mg l^{-1} at the surface and 25.4 mg l^{-1} at the bottom, higher values are noted in summer and winter, a maximum of 60.6 mg l^{-1} was recorded in January (Figure 8). INRH (2015) reports concentrations of suspended matter in the lagoon between 20 and 40 mg l^{-1}. These variations seem to be governed by several factors, notably the summer development of phytoplankton and the wind regime, given the shallow depth of the rearing site, especially at low tide. According to Castaings (2008), swells and currents cause surface sediments to be lifted and re-suspended.

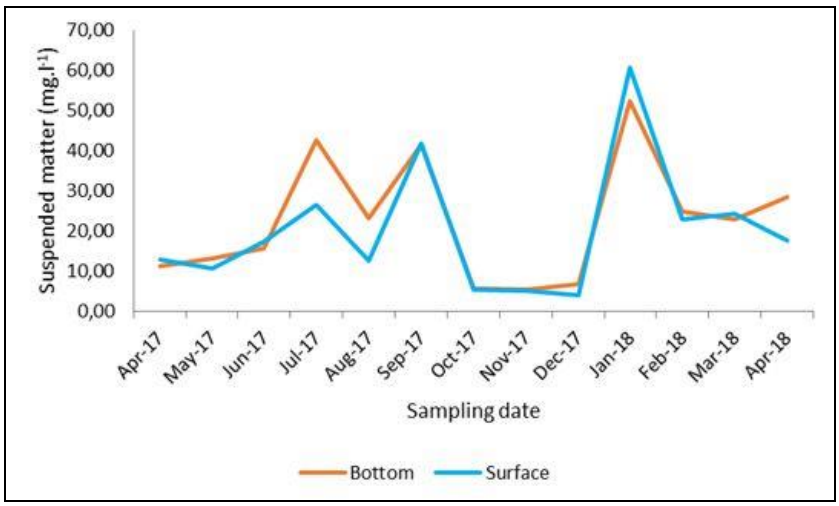

Figure 8. Temporal variation of suspended matter on the surface and bottom on the rearing site.

Growth monitoring. Figures 9 and 10 successively illustrate the mean temporal variation in the length and total weight of individuals on the different rearing levels. The curves show that the growth of *R. decussatus* on the four levels N1, N2, N3 and N4 is linear and continuous over the period of the study. At the start of the study, the average clam length was 20 mm, and the average total weight was 2 g. After 13 months of culture, clams reached a maximum average length of 37.07 mm for N1, 39.84 mm for N2, 39.18 mm for N3 and 40.08 mm for N4 (Figure 9), and maximum average total weight of 12.7 grams for N1, 14.7 grams for N2, 15.1 grams for N3 and 15.2 grams for N4 (Figure 10).

The growth results of this study show a gain in length of 17.1 mm for N1, 19.8 mm for N2, 19.2 mm for N3 and 20.1 mm for N4. The total weight of cultured clams increased by 10.7 grams for N1, 12.7 grams for N2, 13.1 grams for N3 and 13.2 grams for N4. A gain in length and total weight in all levels between July and September was noted. According to Besançon et al (2013), in a bibliographic study carried out in the

Thau lagoon, the increase in the growth of the *R. decussatus* clam in spring is due to the warming of the water and the proliferation of phytoplankton.

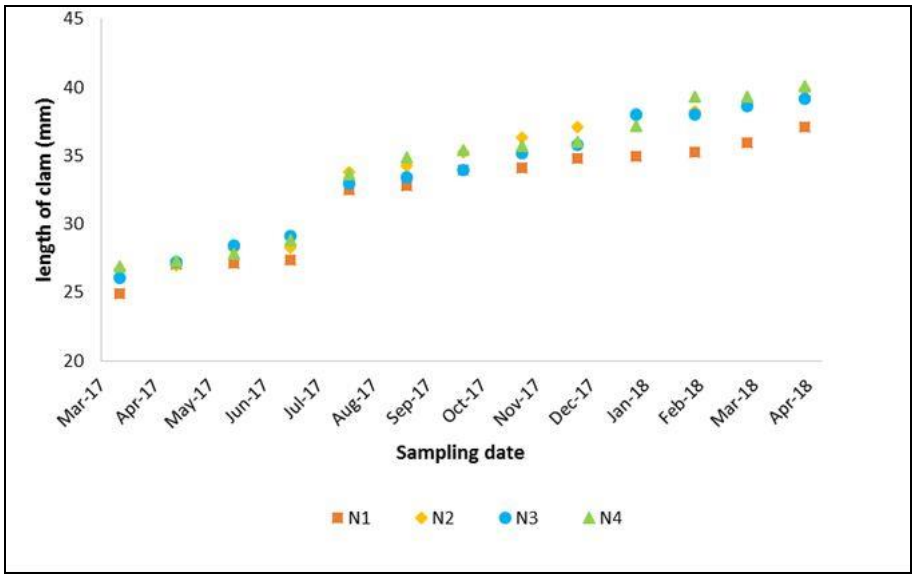

Figure 9. Temporal development of the average length of clam *(R. decussatus*).

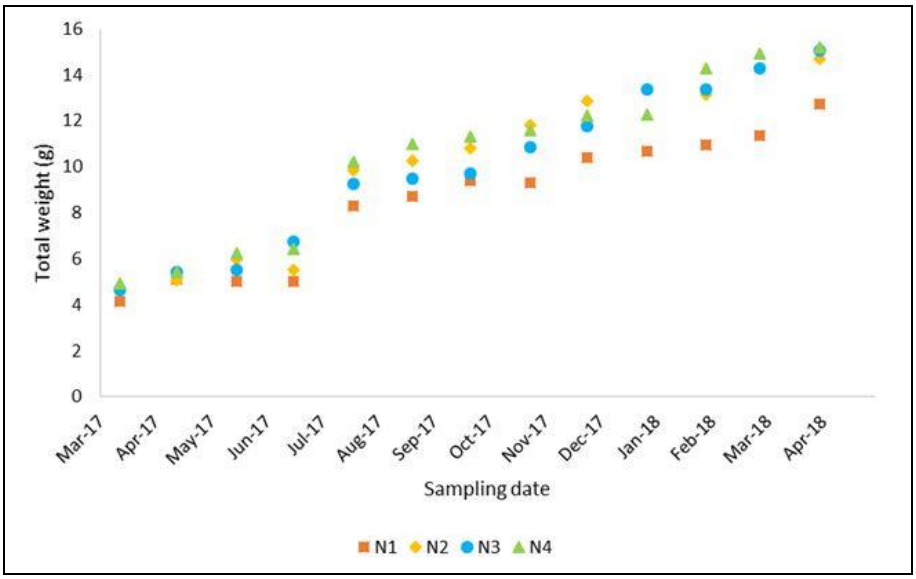

Figure 10. Temporal development of the average total weight of clam (*R. decussatus*).

These results were analyzed by statistical ANOVA tests carried out on the mean length and mean total weight (Table 1) of the individuals in the different levels (N1, N2, N3 and N4) which shows a significant effect ($P<0.05$) of the levels of the clam culture bags on these two parameters. In addition, it has been noted that growth in the water column is faster than in the sediment. A size of 35 mm (commercial size) is reached in the water column three to four months before it is reached in clams buried in the sediment. The results highlighted by Gras et al (1981) show that clams sown in open fields between May and July 1978 take 12 mm and 1.35 grams on average. According to the study carried out by the National Centre Oceans Exploitation (CNEXO 1983), the clam *R. decussatus* can reach, in its natural environment, a size close to 30 mm in 3 years and 50 mm in 6 years. Growth is faster for small sizes: the percentage growth of the initial length decreases from 70 % to 30 % when the size increases from 17 mm to 25 mm. The increase in length and total weight was accompanied by an increase in fresh weight, the results of which are shown in (Figure 11). The curves show a linear and continuous

growth in fresh weight, with a significant variation between the different breeding levels ($p < 0.05$) (Table 1).

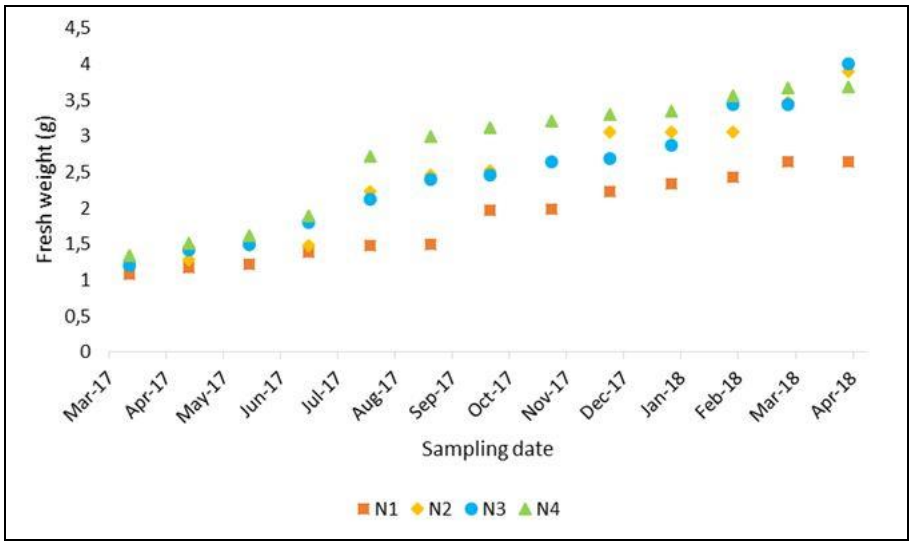

Figure 11. Temporal development of the average fresh weight of clam (*R. decussatus*).

Table 1

Fixed-factor ANOVA evaluating the effect of culture technique on clam growth

	Statistics			
	Residuals	*Df*	*F-value*	*p*
Length (mm)	1556	3	18.09	< 0.05
Total weight (g)	1556	3	24.52	< 0.05
Fresh weight (g)	1556	3	82.78	< 0.05

Monitoring the Condition Index. Increasing values of the condition index are interpreted as the beginning of gonadal development and falling values as expulsion of genital products (Paulet et al 1988). For the three levels N1, N2 and N3, the condition index gradually decreased from April 2017 to November 2017. The values vary between 40.2 and 103.5 for N1, 53.2 and 99.5 for N2, 48.6 and 98.8 for N3. Then from December 2017, we again observe an increase in the values of this index, while for the N4 level the fluctuation was less pronounced with values ranging from 71.9 to 102.6, a weaker oviposition was nevertheless observed (Figure 12). From April and until November, the condition index for the N1, N2 and N3 levels decreases due to the gradual gonadal emission over a long period of time. Between November and December, the increase in the condition index corresponds to a period of gonad restoration, as environmental conditions become favorable. In fact, in the Oualidia lagoon, the emission of gametes for the clam is progressive during the summer and increases from September to December depending on environmental conditions.: autumn phytoplankton flowering (Rharbi et al 2003) and low temperatures linked to upwelling currents, which are very frequent in the region in autumn (Rharbi 2000). For level N4, the behavior of individuals differs from individuals at levels N1, N2 and N3. This may be due to stress caused by the effects of tides on this level. Indeed, at low tide the clam at this level is not emergent and is in direct contact with the ambient air unlike the individuals at the other levels.

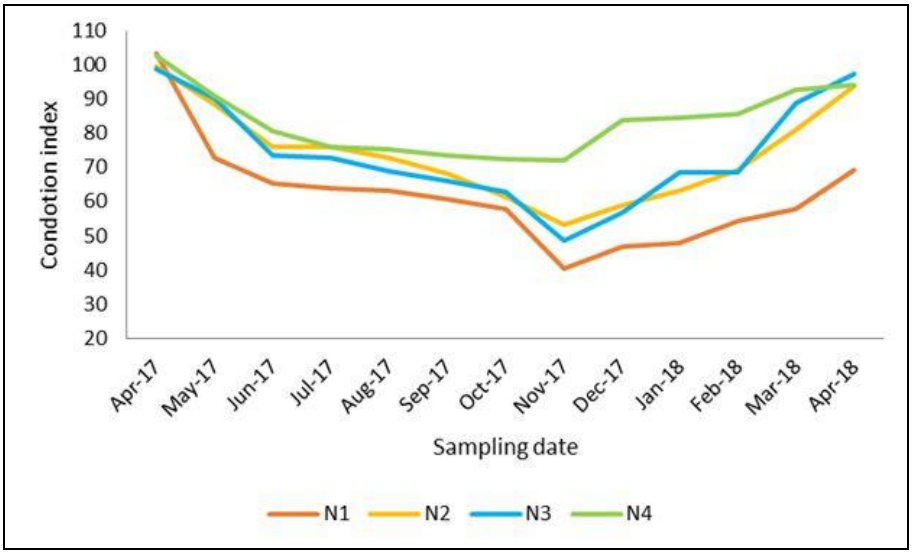

Figure 12. Temporal variation of the condition index of the clam (*R. decussatus)* in the different levels of rearing.

Mortality and deformation. Figure 13 shows the evolution of the intensity of the cumulative mortality rates observed and highlights the disparities in the intensity of mortality by level. Thus, comparable cumulative mortality rates are observed for the three levels N2, N3 and N4 in the water column, which differ from those obtained for level N1 in the sediment, which has experienced mortality affecting almost its entire population, which may be linked to predation. Indeed, examination and findings of the dead individuals revealed that the shells showed signs of predation. For the other levels in the water column, the cumulative mortality rate was low throughout the study period and varied between 14.9 % and 23.9 %.

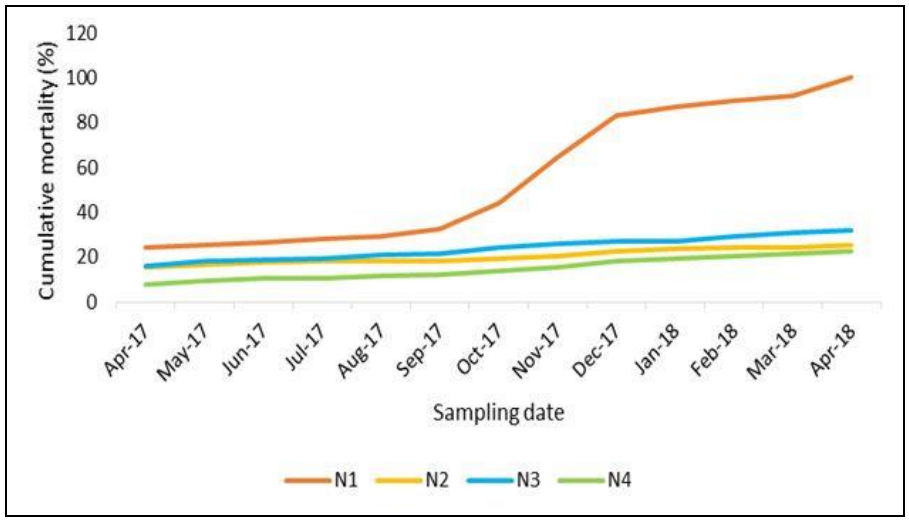

Figure 13. Variation of the intensity of the cumulative mortality during the rearing period.

A particularly good correlation (R^2) between total weight and length was noted for individuals of the three levels N2 (0.9955), N3 (0.9871) and N4 (0.9934) with a low mortality rate, as opposed to individuals of level N1 (0.9798) with a high mortality rate (Figure 14).

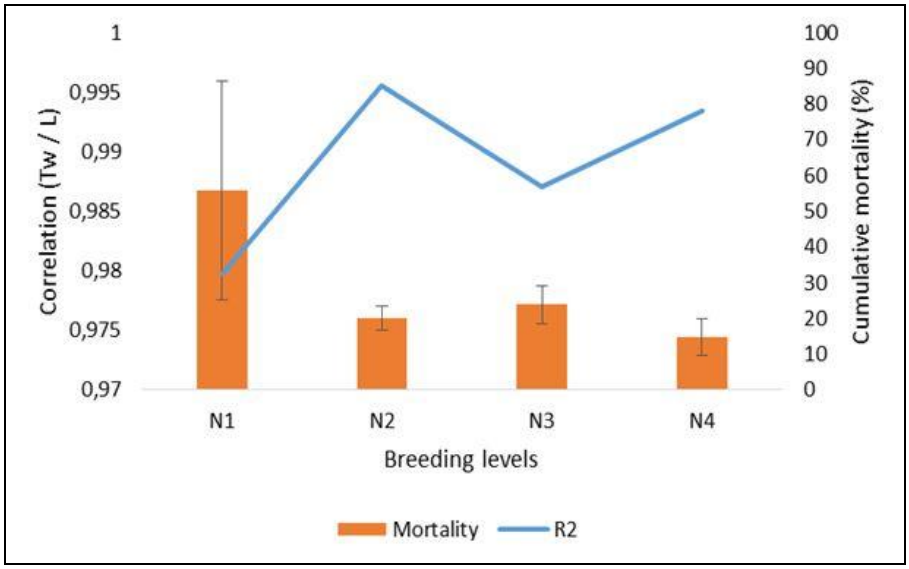

Figure 14. Correlation (TW-L) and mean cumulative mortality in breeding levels.

During the study, shell deformations were observed on a few individuals, and these were noted from the first month of follow-up. The percentages of deformation for all levels were too low and reached respectively 0.2% for N1, 0.5% for N2, 0.7% for N3 and 1.2% for N4. If the deformation rates are low, this would be related to the hydrodynamic conditions of the point selected for the installation of the rearing system.

Evaluation of system efficiency by the Tukey test. A post-hoc Tukey test was used to identify the best level of rearing. The results of this test (Table 2) show that individuals at level N2 and N3 had the best growth rate in length, total weight, and fresh weight. Both levels showed a variation in condition index comparable to normal conditions and a low mortality rate. Level N4 had the best growth rate in length, total weight, and low mortality. The N1 level had less growth in length, total weight, and fresh weight than the other levels and a high mortality rate. This test allows us to optimize the system by selecting levels N2 and N3 as the best levels for clam rearing.

Table 2

Results of the Tukey post hoc test conducted on length, total weight, fresh weight, condition index and mortality to assess the best rearing level (N1, N2, N3 and N4) of *R. decussatus*

Source of variability	*N1*	*N2*	*N3*	*N4*
Length	b	a	a	a
Total weight	b	a	a	a
Fresh weight	c	a	a	b
Condition Index	a	ab	ab	b
Mortality	b	a	a	a

Monitoring of contamination levels. The monitoring of contaminants focused on the parameters retained in the sanitary classification of shellfish production areas, namely Cd, Pb and Hg for chemical contaminants and *E. coli* for bacteriological contaminants.

Figures 15, 16 and 17 show the temporal variations in the concentration of Cd, Pb and Hg in the rearing levels N1 and N3 of the clam (*R. decussatus*). For Cd, the concentrations in the clam reared in sediment N1 range from 0.20 to 0.50 mg kg^{-1} and in the clam reared in the water column N3 range from 0.24 to 0.47 mg kg^{-1}. For Pb, the same variation was noted for the two levels N1 and N3 with values ranging from 0.07 to 0.14 mg kg^{-1}. For Hg, the concentrations are almost similar and vary between 0.04 to 0.09 mg kg^{-1} for N1 and 0.05 to 0.10 mg kg^{-1} for N3.

Clam rearing conditions in both N1 and N3 have no significant effect ($p>0.05$) on the accumulation of trace elements (Cd, Pb and Hg). However, the concentrations recorded in clams throughout the study period do not exceed the values recorded in oysters (*Crassostrea gigas*) reared on the same site (INRH 2015).

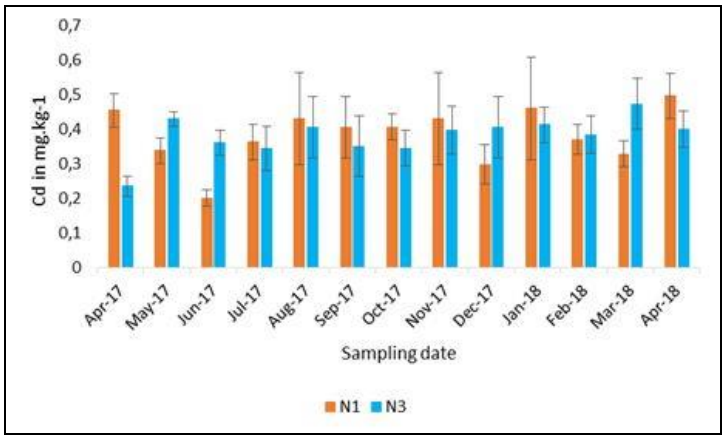

Figure 15. Temporal variation in the concentration of Cd of the clam (*R. decussatus*).

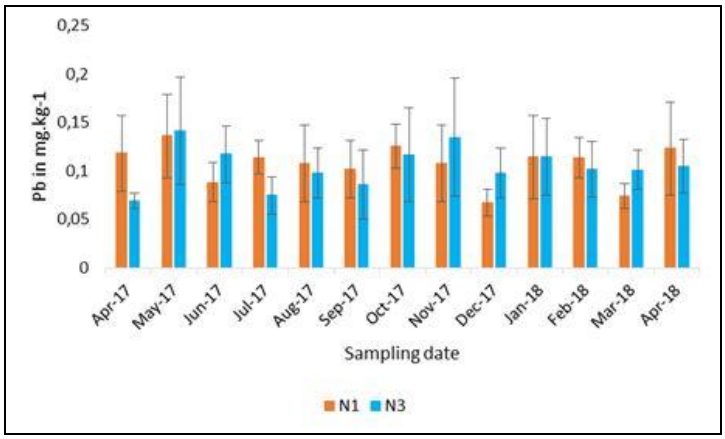

Figure 16. Temporal variation in the concentration of Pb of the clam (*R. decussatus*).

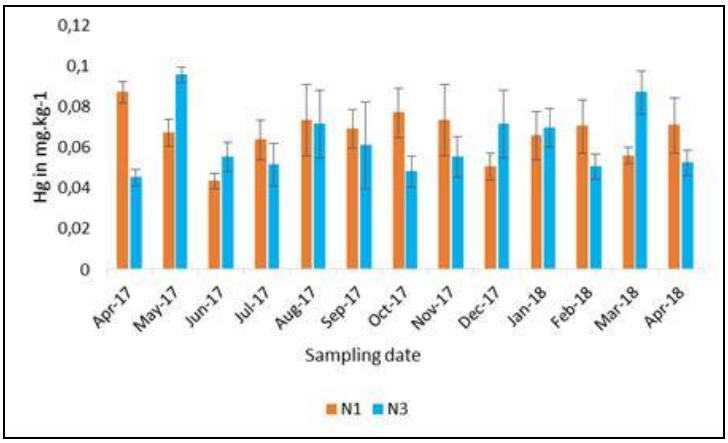

Figure 17. Temporal variation in the concentration of Hg of the clam (*R. decussatus*).

Figure 18 shows the contamination of the clam (*R. decussatus*) by *E. coli* in the two levels studied, N1 and N3. The results recorded for the two levels N1 and N3 vary between 110 and 490 of *E. coli*/100 grams for N1 and between 0 and 490 of *E. coli*/100 grams for N3. The spatial evolution of bacteriological contamination is not the same in sediment and water column clams, mainly from July onwards. Concerning the clam in the water column we note that the decontamination of the clam is faster and more important

than in the sediment, it starts in July to reach 0 *E. coli*/100 grams in November, contrary to the clam in the sediment the decontamination only starts in August and it reaches a minimum of 130 *E. coli* /100 grams in November. This can be explained by the fact that bacteria can survive more in the sediment than in the water column. It is generally accepted that coliforms die very quickly in saline environments and that their presence in the environment reflects recent contamination (Sigwald et al 2012). On the other hand, starting in December we see a faster and more significant increase in contamination in the clam in the water column, which may be due to leaching and infiltration of agricultural practices around the lagoon in the rainy season that occurs with the first rains. The pollution peaks observed in winter could correspond to point source inputs from the watersheds (Derrien 2004). However, this contamination does not exceed 500 *E. coli*/100 grams.

According to the results of the present study, bacteriological contamination of the environment can be detected more quickly in clams in the water column than in clams in the sediment.

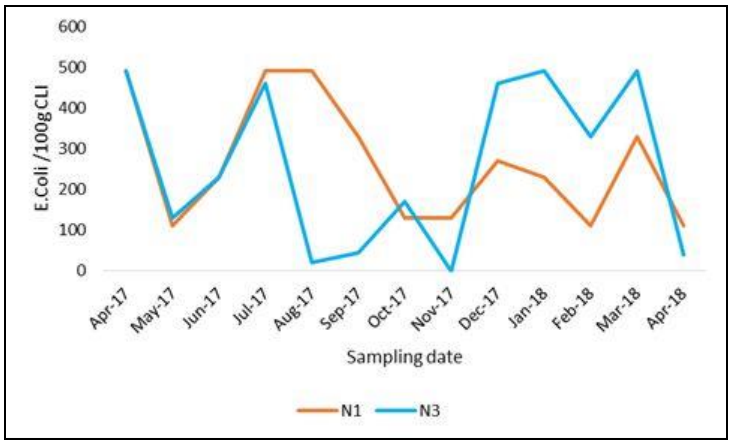

Figure 18. Temporal variation in the concentration of *E. coli* of the clam (*R. decussatus*).

Conclusions. The present study developed a suspended culture system for the European clam (*R. decussatus*) which evaluated the survival and growth performance of the species, and a comparison of the accumulation of chemical (cadmium, lead and mercury) and bacteriological (*Escherichia coli)* contaminants in the clam reared in suspension and the clam buried in the sediment.

In conclusion, this study has shown the main advantages of suspension farming, namely:

- Better growth with low mortality rates in the water column than in the sediment.
- Lighter shell coloring, and cleaner, white flesh that does not contain sand grains.

For the sanitary quality of the clam, the results of the study show no significant effect for the accumulation of chemical contaminants between the clam raised in the water column and the sediment. However, bacteriological contamination may occur more rapidly at higher concentrations in the clam in the water column than in the sediment.

Ultimately the designed farming system could be used for clam farming at potential shellfish sites with suitable environmental characteristics.

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References

Allocati N., Masulli M., Alexeyev M. F., Di Ilio C., 2013 *Escherichia coli* in Europe: an overview. International journal of environmental research and public health 10(12):6235-6254.

Alzieu C., 1999 [Dredging and Marine Environment - State of Knowledge]. Ifremer Center of Nantes [in French].
Aminot A., Keroul R., 2004 [Hydrology of marine ecosystems. Parameters and analyses]. Edition IFREMER, 336 pp. [in French].
Arnal I. J., Pato C. F., 1978 [The growth of the clam (*Venerupis decussata L*.) in the Bay of Santander under natural conditions]. CIEM: Comité des coquillages et des crustacés. C.M. 1978/K: 28. 5p. Rapport [in French].
Besançon P. M., Cassard M., Chanut M., Colbalchini F., 2013 [The exploitation of invertebrates in the Thau Lagoon: bibliographical study and surveys concerning the areas and quantities fished. Reflection on a clam reseeding (*Ruditapes decussatus*)]. Cepralmar. Project report [in French].
Boglino A., Casanova T., Lourdou E., Ortega G., 2009 [Prospecting study for the diversification of sea shellfish farming in Languedoc-Roussillon]. Rapport bibliographique CREUFOP, Montpellier. 142p. [in French].
Bordeyne F., Robert S., Le Moine O., Léauté J. P., 2009 [Estimation of *Ruditapes decussatus* and *Ruditapes philippinarum* clam stocks on the Oleronian foreshore of the Marennes-Oléron basin] - June 2009 - IFREMER [in French].
Castaings J., 2008 [State of the art knowledge of the phenomenon of filling in lagoon environments]. Cepralmar 2008, lagoon-monitoring network [in French].
Cépralmar, 1987 [Assessment of the experimental clam farming carried out in the Port la Nouvelle lagoon]. 25 p. [in French].
Cépralmar, 2016 [Manila clam-Methodological test for monitoring a population of adult Manila clams in the Thau Lagoon] Project report [in French].
CNEXO, 1983 [The "clam" aquaculture biotech sheets.] Centre National pour l'Exploitation des Océans et institut scientifique et technique des pêches maritimes [in French].
Damayanti S., Kusdarwati R., Suprapto H., 2019 Bacterial resistance of *Escherichia coli* against antibiotics in *Clarias batrachus* digestion. AACL Bioflux 12(6):2195-2201.
Damsiri Z., Natij L., Khalil K., Loudiki M., Rabouille C., Ettahiri O., Bougadir B., Elkalay K., 2014 Spatio-temporal nutrients variability in the Oualidia lagoon (Atlantic Moroccan coast) International Journal of Advanced Research (2014), Volume 2, Issue 8, 609-618.
Derrien A., 2004 [Enumeration of Escherichia *coli* in sediments of Aiguillon Bay] Direction de l'environnement et de l'aménagement littoral, Laboratoire de La Rochelle. IFREMER [in French].
Devic E., 2010 [Diversification of shellfish farming in Languedoc-Roussillon: evaluation of the growth potential of the European clam (*Ruditapes decussatus*) and the black scallop (*Chlamys varia*) and trials on the capture of species of maricultural interest in the Thau lagoon and on the open sea longlines off Thau]. Thesis for the diploma of Advanced Agronomy (DAA), specialization in Fisheries, option Aquaculture, Agrocampus Ouest, Rennes, France, 35p. [in French].
Elmanama A. A., Afifi S., Bahr S., 2006 Seasonal and spatial variation in the monitoring parameters of Gaza Beach during 2002–2003. Environ. Res., 101, 25-33.
EPA, 2007 Mercury in solids and solutions by thermal decomposition, amalgamation, and atomic absorption spectrophotometry. Environmental Protection Agency, EPA method 7473, Rev 0.
FAO, 2006 Cultured aquaculture species fact sheets: *Ruditapes decussatus* (Linnaeus, 1758). http://www.fao.org/fishery/culturedspecies/*Ruditapes_decussatus*/fr.
Fleury E., D'Amico F., Annezo J. P., Barret J., Blin J. L., Bouget J. F., Claude S., Guilpain P., Grizon J., Hitier B., Langlade A., Souchu L., Martin A. G., Mary C., Normand J., Parrad S., Penot J., Pien S., Piriou J.Y., Pouvreau S., Robert S., Repecaud M., Bédier E., 2011 National Shellfish Farming Observatory Campaign 201 110.
Gallois D., 1973 [Study of two Veneridae from the Thau Lagoon: *Venerupis decussata* and *Venerupis aurea*]. Thesis to obtain the degree of Doctor of Specialty (Applied Biology - Option Ecology), Faculty of Science, Orléans, Orléans, 121p. [in French].
Gervasoni E., 2009 [Prospecting study for the diversification of sea shellfish farming in Languedoc-Roussillon]. CEPRALMAR – EG – 2009 [in French].

Gras M. P., Gras P., Guichard C., Peyre R., 1981 [Aquaculture of bivalves in claires in the Marennes-Oléron basin]. Science et Pêche, n°314, p.1-30 [in French].
Hartanto, Tjahjono A., 2020 Plankton indexes and heavy metal pollution in Kendal coastal waters, Indonesia. AACL Bioflux 13(1):46-63.
Harvey H. W., 1955 The chemistry and fertility of sea waters. Cambridge University Press, London, 244p.
Hervio-Heath D., Gourmelon M., Martial C., 2012 [Contamination of shellfish by bacteria pathogenic to humans. Western Mediterranean Marine Sub-Region]. Initial assessment DCSMM. MEDDE, AAMP, Ifremer, Ref. DCSMM/EI/PI/MO/28/2012, 7 p. [in French].
INRH, 2015 [Oualidia Lagoon: ecological and environmental health status]. Internal report, National Fisheries Research Institute [in French].
Joly J. P., 1982 [Contribution to the biology of the clam *Ruditapes decussatus* L. 189]. State Thesis, Univ, Paris 6, 1: ISTPM, France. [in French].
Kamara A., Rharbi N., Ramdani M., Berraho A., 2005 [Comparative study of the sexual cycle of the clam *Ruditapes decussatus* (L.) from three paralic environments of the Moroccan coasts]. Mar Life 15(1–2) :43–50. [in French]
Kamara A., Rharbi N., Ramdani M., 2008 [Preliminary research for the development of the culture of the European clam (*Ruditapes decussatus L*.) on the Moroccan coasts and the repopulation of overexploited sites]. Bulletin de la Société zoologique de France 10 [in French].
Karim H., Omar E., Mohammed I., Jamila L., Benyounes A., Zineb E., Abdellatif O., Ahmed M., 2017 [Hydrodynamic functioning of the lagoon of Oualidia (Morocco) before the tailing]. International Journal of Advanced Research 5, 2015–2027 [in French].
Klassen R. A., Douma S., Rencz A. N., 2010 *Environmental and human health risk assessment for essential trace elements: Considering the role for geoscience.* Part A: Current Issues: Journal of Toxicology and Environmental Health. 73: p. 242-252.
Labbardi H., 2006 [The *Ruditapes Decussatus* clam (Linnee, 1758) from the Moulay Bousselham lagoon, Morocco: Biometry, Reproduction, Food Activitiy and Interaction with the Ambient Environment]. PhD thesis. Univ, Hassan II, Mohammedia, Morocco, 107p. [in French].
Lakhlalki H., Jayed M., Benbrahim S., Rharbi N., Benhra A., Moutaki B., Maanan M., 2020 Assessment of contamination by organochlorine pesticides and polychlorinated biphenyl's from Oualidia lagoon water (Morocco). Arabian Journal of Geosciences.
Lavaud S., 2014 [Technical and economic feasibility study of support for the clam (*Ruditapes decussatus L*.) fishing industry in Languedoc-Roussillon by réensemencement] : updating of data on the industry and proposals for reseeding protocols 69 [in French].
Maitre-Allain T., 1983 [Follow-up of an attempt to repopulate clams in the Etang de Thau (Herault): Crescent, Mortality, Reproduction]. Thesis for the Diploma of Doctorate in Biological Oceanology, Pierre and Marie Curie University Paris 6, Paris, 136p [in French].
Makaoui A., Idrissi M., Agouzouk A., Larissi J., Baibai T., Ouehabi Z.E., Laamel M. A., Bessa I., Ettahiri O., Hilmi K., 2018 [Oceanographic State of The Oualidia Lagoon, Morocco (2011-2012)]. European Scientific Journal, ESJ 14, 93 [in French].
Mazouni, 1999 [Management of an exploited natural resource: Case of the clam (*Ruditapes decussatus*) in the Thau lagoon]. Sète (FR): Programme PALOURDE, 1999. 103p. Rapport final [in French].
Mejjad N., Laissaoui A., El-Hammoumi O., 2018 Geochemical, radiometic, and environmental approaches for the assessment of the intensity and chronology of metal contamination in the sediment cores from Oualidia lagoon (Morocco). Environ Sci Pollut Res25, 22872-22888(2018).
NM EN 13804, 2015 [Food products - Determination of elements and their chemical species - General considerations and specific requirements- Determination of elements and their chemical species] [in French].

NM EN 13805, 2015 [Food products - Determination of trace elements - Digestion under pressure] [in French].
NM EN 15763, 2012 [Food products - Determination of trace elements, Determination of arsenic, cadmium, mercury and lead by emission spectrometer with inductively coupled plasma and mass spectrometer (ICP-MS) after digestion under pressure] [in French].
NM ISO 16649-3, 2017 [Horizontal method for the enumeration of *Escherichia coli* β-glucuronidase-positive. Part 3: Most probable number technique using bromo-5-chloro-4-indolyl-3 β-D-glucoronate] [in French].
Oliveira J., Cunha A., Castilho F., Romalde J. L., Pereira M. J., A mini-review. Food Control, 22 (2011) 805-816.
Papp R., 2011 [The Environmental Properties of Trace Elements: Particularities and Consequences for Risk Analysis]. Rev 1. National College of Environmental Experts of the Chemical Industry [in French].
Parache A., 1982 [The clam]. La pêche Maritime 1254, 496-507 [in French].
Paulet Y. M., Lucas A., Gérard A., 1988 Reproduction and larval development in two *Pecten maximus* (L.) population from Brittany. J. expl mar. Biol. Ecol., 119 :145-156.
Plusquellec A., Beucher M., Legal Y., 1986 [Indicators of microbial pollution of coastal waters]. Ifremer*,* 3:541-548 [in French].
Puspitasari R., Suratno, Purbonegoro T., 2020 Health risk assessment of metal accumulated in marine bivalves from Semarang, Indonesia. AACL Bioflux 13(2):993-1002.
Ramsar, 2016 The List of Wetlands of International Importance published 25 November 2016 page 30.
Rharbi N., 2000 [Importance of hydrological and phytoplankton parameters on the growth of the oyster Crassostrea gigas in the lagoon of Oualidia]. State Thesis, Ben Msik Faculty of Sciences, Casablanca, 217 p. [in French].
Rharbi N., Ramdani M., Berraho A., 2003 [Elaboration of a rearing strategy for the *Crassostrea gigas* oyster in the lagoon of Oualidia (Morocco) based on the study of trophic relations]. Bull. Soc. zool. Fr., 128 (1-2):53-70 [in French].
Sarf F., 1999 [Sedimentary dynamics and state of pollution of the lagoon of Oualidia]. Postgraduate thesis, Faculty of Sciences. Rabat, 121p. [in French].
Sigwald R., Ledoux S., Spencer K., 2012 [Methodological guide on water injection dredging]. GEODE, Groupe d'études et d'observation sur les Dragages et l'environnement [in French].
Valentin A., Tremblay T., Gagnon F., Cartier J. F., 2000 [North Shore Regional Health and Social Services Board]. Direction de la santé publique*,* 90 p. [in French].
Waln P. R., Mann R., 1975 Growth and biochemical composition in *Ostrea edulis* and *Crassostrea gigas*. Proc. 9th Europ. Mar. Biol. Symp. 1975:587-607.
Wang W., Lu G., 2017 Heavy metals in bivalve mollusks. In: Chemical contaminants and residues in food. Dieter S., Cartus A. (eds), pp. 553–594, second edition, Elsevier.
Yap C. K., Wan H. C., Karami A., Ismail A., 2016 Health risk assessments of heavy metal exposure via consumption of marine mussels collected from anthropogenic sites. Science of the Total Environment 553:285–296.
Zbiry M., 2018 [Contribution to the study of the effects of contamination by organochlorine pesticides and polychlorinated biphenyls in different bivalve species of the Moroccan Atlantic coastal axis from Moulay Bousselham to Safi]. Faculty of Sciences Ain Chock, Hssan II University, Casablanaca, PhD Thesis [in French].

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