Development of a new rearing technique for the clam *Ruditapes decussatus* (Linnaeus, 1758) and evaluation of its zootechnical performance in the Oualidia Lagoon, Morocco

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Abstract. The growth of the European clam, *Ruditapes decussatus* (Linnaeus, 1758), was studied in Oualidia Lagoon according to two rearing techniques, one traditional in traps on the ground and the other developed in suspended traps. This study aimed to evaluate the zootechnical performance of the two techniques, the cumulative mortality and the growth gain of the clam. The monitoring of the rearing was monthly from July 2017 to February 2019, as well as the monitoring of physicochemical parameters. The results showed that at the end of the study, the length was statistically identical for individuals from both techniques: 36.52 mm and 36.47 mm for suspended and on the ground techniques, respectively. The Mann Whitney test indicated that there was no significant difference between the total fresh weight of individuals from suspended technique (12.58 g) and those from the on the ground rearing (11.38 g). The cumulative mortality was statistically lower in suspended reared clams (26%), compared to the on the ground rearing (29%).

Key Words: European clam, Atlantic Ocean, shellfish farming, growth, mortality, suspended traps.

Introduction. The European clam, *Ruditapes decussatus*, is a burrowing bivalve mollusc naturally distributed in lagoons and estuaries environments in Mediterranean and Atlantic coasts of Morocco, namely Marchika Lagoon in Nador, mouth of Moulouya river, Merja Zerka of Moulay Bousselham, Oualidia-Sidi Moussa Lagoons, Khnifiss Lagoon and Dakhla Bay. This species highly valued by consumers, has long been exploited in untimely and poorly controlled manner. Indeed, its fishing represents a main activity for the local populations of its distribution sites, including Oualidia Lagoon. This subsistence exploitation, which has become commercial has affected the durability of this resource (Rharbi et al 1996; Kamara et al 2005; Belbachir et al 2014).

Thus, aquaculture sector plays an important role to display the growing demand for seafood products, including clams. *R. decussatus* is one of the most commercialized shellfish species both nationally and internationally due to its nutritional and gastronomic values (FAO 2020; ANDA 2020). The sector is promising to alleviate the problems of overexploitation. Usually, clam farming is done on the ground, after a pre-growth of the spat to a size of 12-13 mm. The young clams are then sown directly into the first centimeters of sediment with or without a protective net. In addition, many authors reported issues in on the ground reared clam's culture, from shell deformations to loss of the entire clam crop (Boglino et al 2009; Gervasoni 2009; Besançon et al 2013). In addition, Devic (2010) reported that rearing European clams on chains in Thau Lagoon showed encouraging results in terms of growth (to 22 mm) before showing shell deformations. The author then suggested that clams should be grown to commercial size on the ground in the sediment. Furthermore, in France, clams rearing in "suspended tanks containing sediment" was tested and gave satisfactory results (Cépralmar 2016). On the Moroccan Atlantic coast, despite its commercial importance, *R. decussatus* has so far been the subject of few studies on the improvement of its exploitation in Moulay Bousselham Lagoon (Rharbi et al 1996), its morphometric analysis and its genetic structuring, by microsatellites (Amane et al 2019, 2021). No study was focused on the influence of the rearing technique on the evaluation of spat's zootechnical performance.

Our study is part of a research and development program of the aquaculture department of the National Institute of Fisheries Research. It aims to develop spat clam rearing to attend authorized commercial size (30 mm) using the suspended technique in horizontal traps, compare its zootechnical performance with the traditional technique (on the ground) and to analyze the effect of physicochemical parameters on its zootechnical performance. Our study could serve as a basis for future clam farming trials in other lagoons in the country.

Material and Method

Description of the study sites. The study was carried out in Oualidia Lagoon (32°45'N and 9°03'W), located on the Moroccan Atlantic coast, at 76 km south of El Jadida and 62 km north of Safi (Figure 1). The major axis of the lagoon is oriented NE-SW, extends in parallel to the coast over an approximate distance of 7.5 km long and 0.5 km wide, with a total area of about 4 km^2 (Bidet & Carrusco 1982; Hilmi et al 2017). Oualidia Lagoon is among the most important paralic sites in Morocco. It is a site of biological and ecological interest (SIBE), characterized by an important faunistic and floristic biodiversity, and also by the regular or occasional reproduction of various bird species and aquatic species (El Hamoumi et al 2003; El Asri et al 2015, 2017, 2021a, 2021b). In addition, this lagoon is also a popular tourist center and has long been considered the capital of Moroccan oyster farming and clam fishing par excellence. It has been the subject of various studies, including spatio-temporal variations in phytoplankton assemblage (Natij et al 2021; Somoue et al 2020), spatial and temporal variation of physico-chemical parameters of the water and its impact on the algae distribution (Al Qoh et al 2022).

Figure 1. The location of Oualidia Lagoon, Morocco.

Experimental design and sampling. In this study, two rearing techniques were used to evaluate the zootechnical feasibility of *R. decussatus* rearing, namely the traditional rearing technique known as "on ground" and a second technique newly developed for environmental considerations. This is the rearing technique "on horizontal traps with double nets suspended on spinnerets". For this study, T8 spats (9±1.3 mm) from Dakhla shellfish hatchery (Morocco) were pre-grown in suspended bags from July 2017 to October 2017. Starting in October 2017, the 18.3±2.4 mm young clams were divided into two rearing (grow-out) batches, on 8 racks: 4 horizontal suspended racks and 4 racks placed on the ground. During 18 months of grow-out, monthly samples of the reared individuals were taken to monitor the evolution of linear and weight growth. Similarly, monthly mortalities were also monitored. The different size measurements were made with a caliper to the nearest 0.01 mm. Then, using a scalpel, each clam was opened and the meat was carefully separated from the shell. The meat and shell were weighed fresh (after draining) using an electronic scale to the nearest 0.001 g.

Environmental parameters. Physicochemical parameters such as temperature, salinity and chlorophyll a were analyzed throughout the study to determine variations in environmental conditions. They were monitored monthly and measured in situ with a multiparameter probe (Hanna HI 9829).

Growth gain. The instantaneous growth rate is determined by the coefficient (K) according to the equation proposed by (Malouf & Bricelj 1989):

$$K = (L2 - L1) / (t2 - t1) \quad K = (W2- W1) / (t2- t1)$$

Where:
L1 and L2 - the length of the shell at the beginning and end of the experiment;
W1 and W2 - the average weights of individuals at the beginning and end of the experiment.
t - the duration of the experiment (in months), it corresponds to the period between two samplings.

Mortality and deformation monitoring. Dead and live individuals were counted monthly, in horizontal suspended and ground traps. Dead individuals are removed to determine the mortality rate according to the equation proposed by (Fleury et al 2011):
Instantaneous mortality rate (IM) at time t:

$$IM = \text{Number of dead individuals}/(\text{Number of dead individuals} + \text{Number of living individuals})$$

The cumulative mortality rate (CM) at time t:

$$CM(t) = 1-[(1- CM(t-1)) *(1-IM(t))]$$

The deformation rate corresponds to a monthly count that was conducted to determine bulging (deformed) individuals.

Statistical analysis*.* The Kolmogorov-Smirnov test was used to check the distribution of the data. Spearman correlations were performed between biological parameters (length, thickness, height, total wet weight) and environmental parameters (temperature, salinity and chlorophyll *a*) to evaluate the influence of environmental variables on the growth of *R. decussatus* and on the performance indices of the two rearing techniques. The T-test or Mann Whitney test was applied to detect significant differences between the means of the different variables. Statistical tests were performed by SPSS 25.0 software.

Results

Environmental parameters. During the study period, the monthly temperature variation (Figure 2A) showed two phases: temperature increase phase from December to August and temperature decrease phase from August to December. The highest temperature was recorded in September 2018 (23.49°C) while the lowest was in December 2017 (12.02°C). The results recorded for salinity (Figure 2B) showed that it varied between (32.0 psu), recorded in August 2018 (summer season as the maximum value), and (25.0 psu), recorded in November 2017 (fall season as the minimum value). Chlorophyll *a* concentration (Figure 2C) varied with maximum values in August 2018 (2.43 μg L^{-1}) and minimum values in November 2018 (0.63 μg L^{-1}).

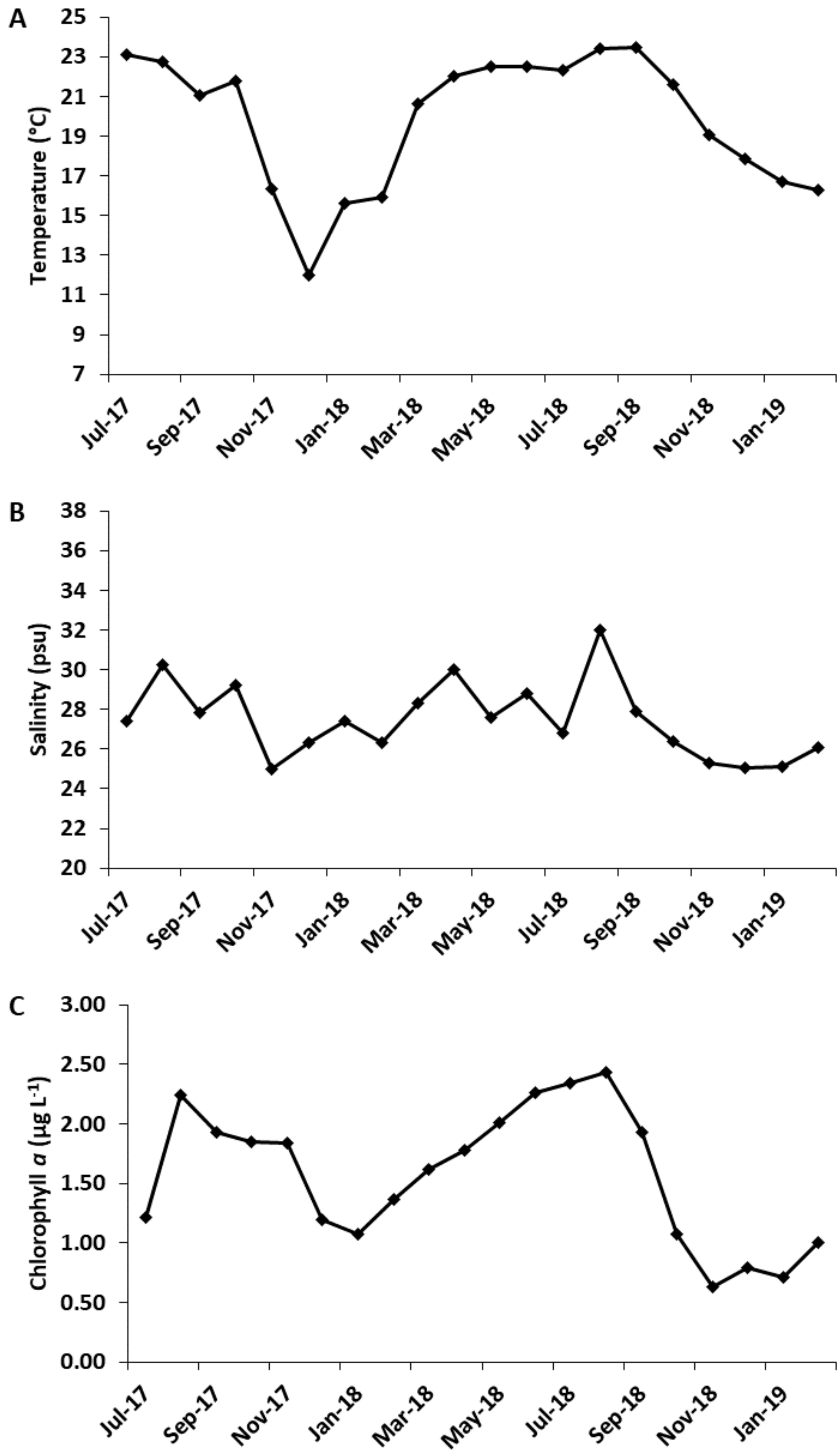

Figure 2. Monthly variation of temperature (◦C) (A), salinity (psu) (B) and chlorophyll *a* (C) (µg L^{-1}) of Oualidia Lagoon recorded during the study period.

Linear growth. The monthly evolution of linear growth is represented in Figures 3, 4 and 5 for length, height and thickness, respectively. The results showed that the monthly evolution of the three parameter profiles were statistically identical (Student's t-test, $p<0.05$) for the two rearing techniques. A slowdown in growth was observed during the winter period (from December to February). Concerning length growth, the individuals from the on the ground rearing reached the commercial size (30 mm) in August, after 13 months of culture. The average length reached by these individuals at the end of the study was 36.52±1.06 mm, while the individuals from the suspended rearing reached the commercial size after 14 months of rearing (in September). At the end of the study, their average size was 36.47±1.54 mm (Figure 3).

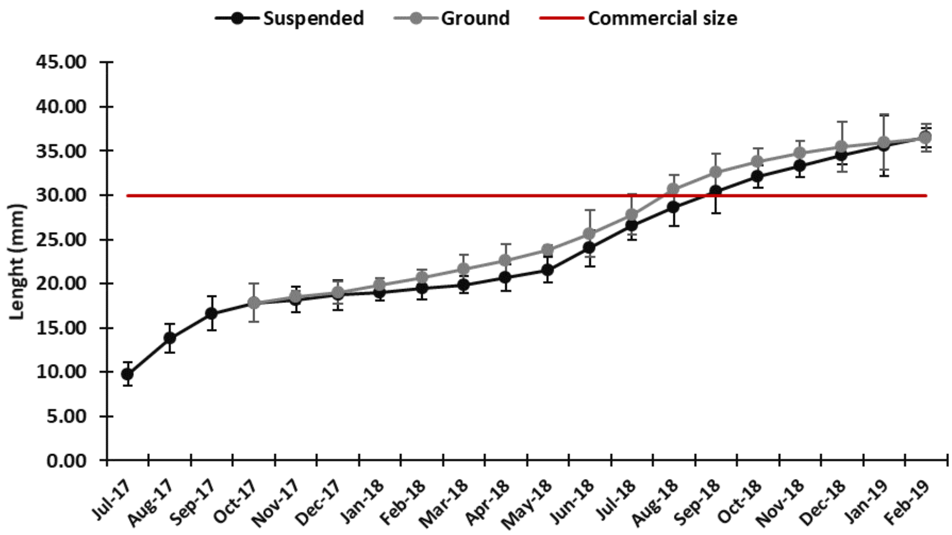

Figure 3. Evolution of length growth of clams from suspended and on the ground rearing techniques at Oualidia Lagoon from July 2017 to February 2019.

In addition, for height growth, the results showed that at the beginning of the rearing the spat measured (6.76±0.86 mm) in height. At the end of the study, the average height of the individuals reared on the ground was (26.10±0.56 mm), while for the suspended rearing individuals it was 27.04±0.99 mm (Figure 4). Student's test (pvalue<0.05) confirmed that no significant difference was noted between the two techniques.

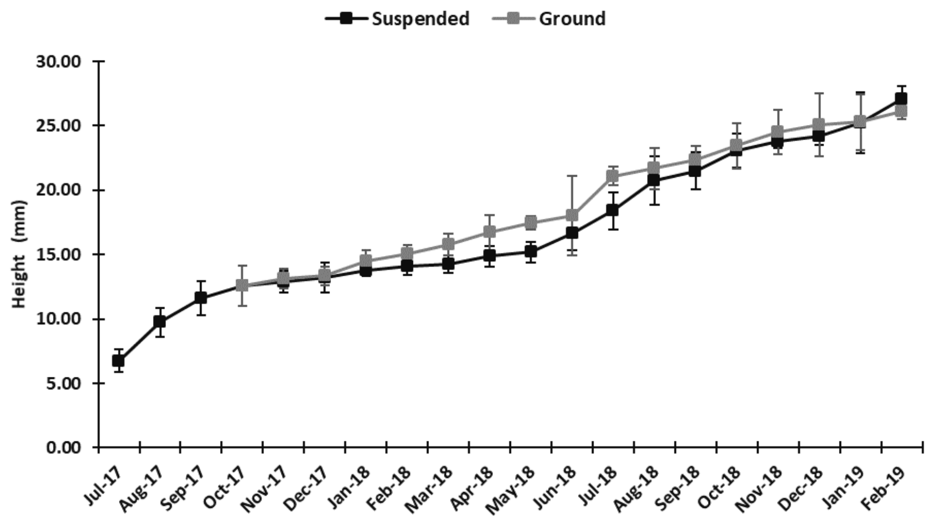

Figure 4. Evolution of height growth of clams from suspended and the ground rearing techniques at Oualidia Lagoon from July 2017 to February 2019.

Regarding thickness, at the beginning of the rearing, spats thickness was 3.52±0.45 mm in July. At the end of the study, the thickness average was similar for both techniques (Student's test, p<0.05). It was 17.68±1.12 mm and 18.89±1.21 mm for on the ground and suspended rearing, respectively (Figure 5).

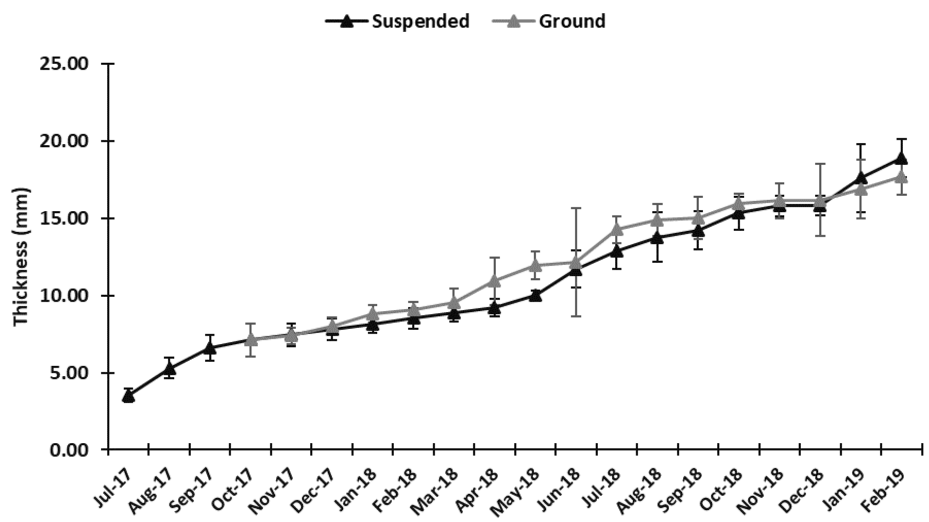

Figure 5. Evolution of thickness growth of clams from suspended and the ground rearing techniques at Oualidia Lagoon from July 2017 to February 2019.

Linear growth. The results showed that the total weight growth rate was slow during the first months of rearing from October 2017 to March 2018 (autumn-winter) for both techniques. However, in late spring and summer (April to September 2018), the weight gain was exponential and more important for individuals from on the ground rearing. In addition, from October growth rates were reversed and total weight growth was greater for the suspended rearing individuals. At the end of the study, reared clams reached an average total weight of 11.38±0.74 g and 12.58±1.02 g for on the ground and suspended techniques, respectively. On the other hand, it should be noted that the average total weight reached at the commercial size (30 mm) was about 6.5 g for the suspended reared individuals, and it was reached after 13 to 14 months of rearing. This weight almost doubled (i.e. approximately 12 g) after 18 months of rearing (at the end of the rearing).

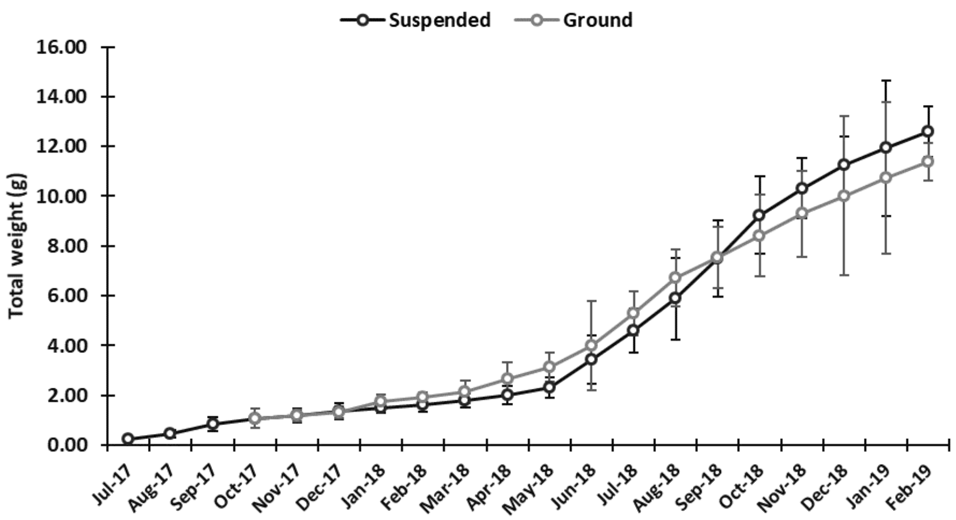

Figure 6. Evolution of total weight growth of clams from suspended and the on the ground rearing techniques at Oualidia Lagoon from July 2017 to February 2019.

Spearman correlations revealed a negative correlation between biometric parameters of suspended clams and physicochemical parameters, while they showed a positive correlation with the temperature, a negative correlation with the chlorophyll *a* for the clams reared on the ground (Table 1).

Table 1

Results of the Spearman's correlation between physicochemical parameters and biometric parameters of reared *Ruditapes decussatus* sampled at Oualidia Lagoon

Rearing techniques	*Parameter*	*Spearman correlation*	*Temperature*	*Salinity*	*Chlorophyll a*
Suspended	Length	Coefficient	- 0.03	- 0.28**	- 0.56**
		P value	0.48	0.00	0.00
	Height	Coefficient	- 0.04	- 0.28**	- 0.57**
		P value	0.36	0.00	0.00
	Thickness	Coefficient	- 0.03	- 0.25**	- 0.60**
		P value	0.57	0.00	0.00
	Total weight	Coefficient	- 0.03	- 0.24**	- 0.59**
		P value	0.60	0.00	0.00
On the ground	Length	Coefficient	0.48**	0.04	- 0.53**
		P value	0.00	0.58	0.00
	Height	Coefficient	0.47**	0.03	- 0.54**
		P value	0.00	0.65	0.00
	Thickness	Coefficient	0.46**	0.04	- 0.53**
		P value	0.00	0.56	0.00
	Total weight	Coefficient	0.47**	0.03	- 0.55**
		P value	0.00	0.63	0.00

***Growth gain**.* Figures 7 and 8 represent the monthly variations of growth gain for length and weight, respectively. The results showed that the growth gain for both parameters was higher in summer periods (from June to September) and lower during the rest of the year. The maximum values of length and weight gains for clams reared on the ground (2.87 mm and 1.43 g) were recorded in August (after 14 months of culture). For suspended clams, the maximum value of length gain (2.6 mm) was recorded in July and that of weight gain (1.74 g) in October, while the minimum values were observed during the cold periods, for both rearing techniques with (0.47 mm and 0.10 g) and (0.31 mm and 0.11 g), for clams reared on the ground and for the suspended clams, respectively. In addition, it should be noted that the Mann Whitney Test showed that there was no significant difference between the two rearing techniques ($p<0.05$). Similarly, the Student's T-test indicated that weight gain means was similar between the two techniques.

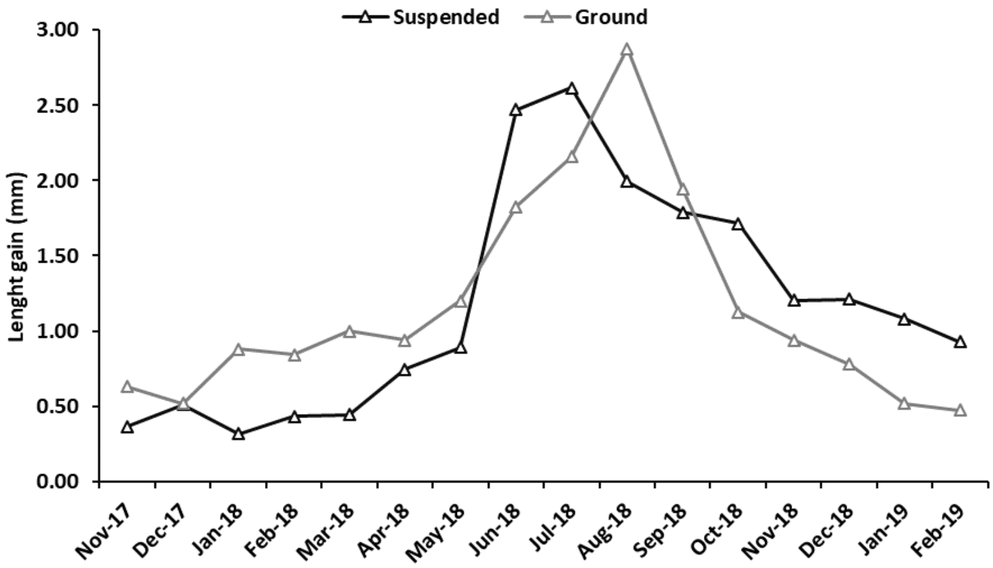

Figure 7. Monthly variations of length gain of suspended clams and reared clams on the ground at Oualidia Lagoon from July 2017 to February 2019.

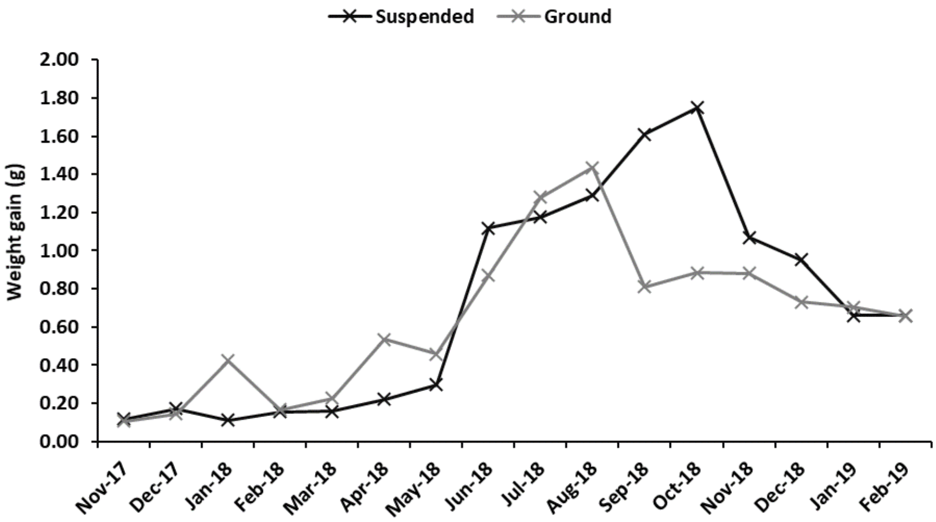

Figure 8. Monthly variations of weight gain of suspended clams and reared clams on the ground at Oualidia Lagoon from July 2017 to February 2019.

Figures 9 and 10 represent the evolution of growth gain of individuals according to the rearing technique. For on the ground rearing (Figure 9), the variations in length and weight gains were similar, with higher growth gains during the summer period and lower during the cold period. Similarly, the length gain was greater than weight gain, except during the last rearing months, when the trend was reversed.

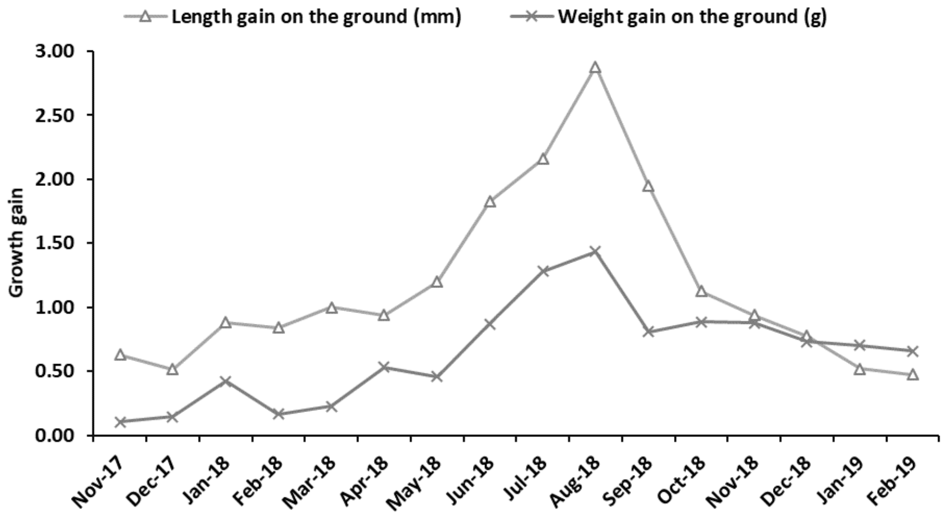

Figure 9. Monthly variations of growth gain of on the ground reared clams at Oualidia Lagoon from November 2017 to February 2019.

For suspended reared clams (Figure 10), the growth rates for weight and length were also similar. Indeed, the patterns variation of length and weight gain were similar with growth gain increasing from May to August for length and from May to October for weight. These growth gains were low at the beginning of rearing and decreased at the end of rearing. Similarly, as for individuals from on the ground rearing, the gain in length was also greater than that in weight.

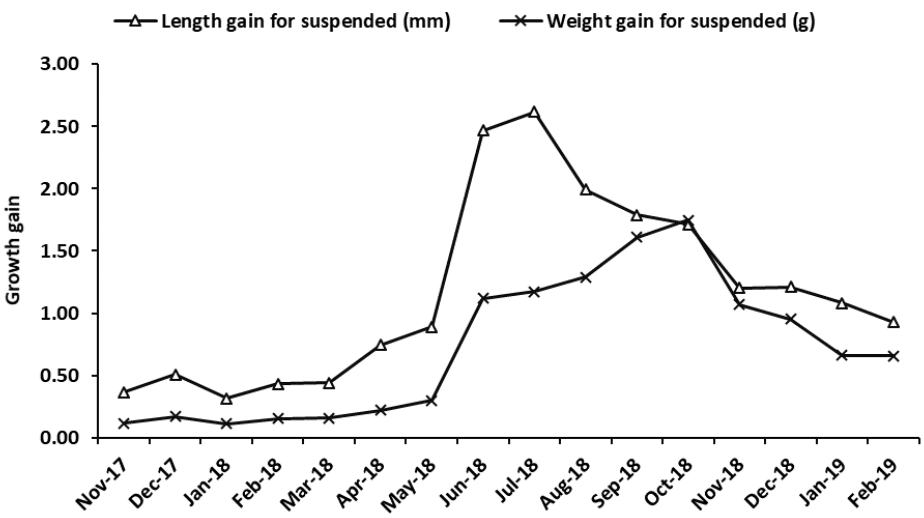

Figure 10. Monthly variations of growth gain of suspended reared clams at Oualidia Lagoon from November 2017 to February 2019.

Cumulative mortality. The results showed that the evolution of cumulative mortality was similar for both rearing techniques (Student's t-test, $p<0.05$). Similarly, the monthly cumulative mortality was higher and more important for suspended reared clams at the beginning of the study (from October 2017 to May 2018). Thereafter, it was almost similar for both rearing techniques from June to November 2018. However, at the end of the rearing, it was more important in individuals from the on the ground rearing. At the end of the study, the cumulative mortality rate was 29.13% and 25.89% for the on the ground reared and suspended reared clams, respectively. Furthermore, it should be noted that during the study period, no shell deformation was observed for all individuals from both rearing techniques.

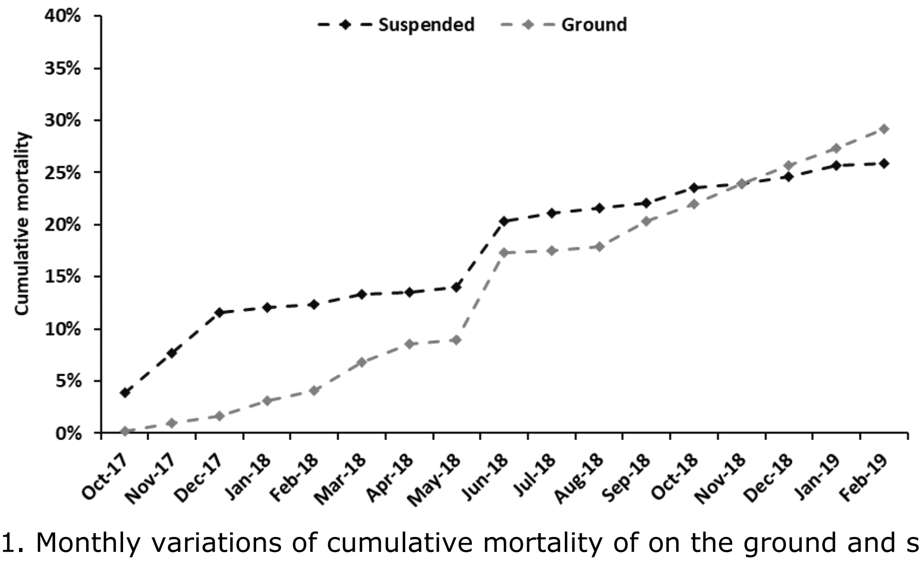

Figure 11. Monthly variations of cumulative mortality of on the ground and suspended reared clams at Oualidia Lagoon from July 2017 to February 2019.

Discussion. The study of physicochemical parameters during the study period showed that the temperature values were at their maximum in summer and at their minimum in autumn and winter. The results obtained are in agreement with other previous works at Oualidia Lagoon, where the authors report that the average temperature varied between 14.5°C and 27°C, and this during two distinct periods, winter with low temperatures and summer with high temperatures (Benbrahim et al 2015; Doukilo et al 2021). Fluctuations in salinity during the study period were due to rainfall and also to the presence of more than 50 freshwater sources identified in the lagoon (Hilmi et al 2009). Concerning chlorophyll-*a*, it increased during the summer and decreased during the cold period of the year. The results obtained during the study period varied between 0.63 and 2.43 μg L^{-1}, and are in agreement with a previous work on Oualidia Lagoon, where the authors reported that chlorophyll *a* value ranged from 0.2 to 4.6 μg L^{-1} (Makaoui et al 2018). A study suggests that variations in chlorophyll a level are related to the magnitude of nutrient inputs through the upwelling of marine water into the lagoon (Idhalla et al 2005).

From the rearing results we noticed that linear and weight growth followed almost the same trend in clams from both rearing techniques, with a faster growth for suspended reared clams than for those from on the ground reared clams. Also, we found that the seasonal evolution of the different biometric parameters was closely related to the variations of environmental parameters where the growth accelerates in spring and summer and decreases during the cold periods of the year. Our results are in line with other studies where the authors reported that rearing in clusters or in net bags gave better growth rates during spring (Gras & Gras 1981; Dhraief et al 2009). The results obtained showed that for both rearing techniques, the growth gain of *R. decussatus* is related to variations in environmental parameters and the type of rearing. We observed that during the summer the growth gain increased rapidly in contrast to the cold period of the year when it decreased. Our results are in agreement with the results of Ramos & Cendrero (1965) who indicated that clam growth was faster in August, in Santander Bay (Spain). Also, according to another study, clam growth in the wild is likely to increase by 5 to 7 mm $month^{-1}$ during this season (Gomez 1975). These fluctuations have been explained in the literature, where it has been confirmed that favorable environmental conditions, namely temperature and food abundance, are closely related to increased clam growth (Baud & Bacher 1990; Besançon et al 2013). Dreno et al (1979) found that the growth rate of clam spat in oyster beds on the southeast coast of Bourgneuf Bay was higher between May and July. From the month of August, a slowdown in the speed of growth was noted and from October it was accentuated and they explained this variation by the quality and (or) the quantity of food but most certainly also to the decrease of temperatures. It is known that its phytoplankton abundance in Oualidia Lagoon fluctuates seasonally and its abundance is highest in autumn and summer when temperatures are high (Somoue et al 2020). This favors the growth of clams during the summer period. Oualidia Lagoon presents favorable conditions (of salinity, temperature and primary production) that are suitable for bivalves rearing, this has been confirmed by several authors who have reported that the lagoon is characterized by environmental parameters for spat and adult oysters *Crassostrea gigas* rapid growth (Kaddioui et al 2018; Shafee & Sabatie 1986) The slowdown in clam growth noted during the study can be explained by the green algae *Ulva lactuca* invasion in the rearing structures. By accumulating, these algae could probably cause the total or partial closure of the meshes of the bags, which would reduce the exchange of water inside the traps and consequently the rate of nutrition of the clams would also be reduced (Pieterse et al 2012). Thus, water exchange is considered to be a key determinant of feeding, proper distribution of nutrients throughout the traps and pockets and growth gain (Walne 1972; Wilson-Ormond et al 1997). Other authors suggest that the progressive decrease in growth rates in different bivalve species with the age increasing is related to the energy use for reproduction rather than for growth (Bataller et al 1999; Mitchell et al 2000; Pouvreau et al 2000). The authors explain that the variations in growth gain values in terms of weight, in clams from suspension culture compared to those on land, are also related to the duration of immersion in water (Boscolo et al 2003). This indicates that the rate of filtration or food absorption is constant in suspended clams as opposed to those on land. In our study, the cumulative mortality rates of clams were significant for both culture structures (29.13% for the on the ground and 25.89% for the suspension culture). We observed that the intensity of cumulative mortality was greater during the summer period. Our results are in agreement with the observations of Goulletquer et al (1986), who reported a low mortality rate between April and August, in the Manila clam *R. philippinarum*. Lipovsky & Chew (1972) speculated that mortality is related to seawater temperature.

As mentioned, the invasion of *U. lactuca* around the suspended traps allowed us to suggest that the mortality observed in suspended individuals could probably be due to a respiratory deficit. At the end of the study, we found the absence of deformation in individuals from both techniques; several authors state that clams deform (bulging of the animals) as soon as they are reared above ground and explain this by the absence of pressure by the sand (Boglino et al 2009). This indicates that the technique used in our study is adequate for growing clams without any deformation, but further testing and measurements are needed to confirm the hypothesis that deformation is negligible in suspension.

Conclusions. Oualidia Lagoon offers favorable conditions for the growth of the European clam *R. decussatus*. The new suspension culture technique for European clam spat has shown better growth in size and weight than those reared on land. It promotes growth gain by avoiding clam emergence during tidal cycles. This type of farming ensures a better phenotypic quality of the clams than the one on land. Overall and despite the technical constraints encountered, such as the proliferation and accumulation of green algae, by the suspended rearing technique, that require labor and permanent cleaning efforts of the structure, the objectives of the study were achieved. This new culture technique seems promising and could be tested in other sites with appropriate environmental characteristics for clam growth, in order to enhance the development of their production from spat to commercial size.

Conflict of interest. The authors declare no conflict of interest.

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