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Survival and growth of mangrove oyster *Crassostrea gasar* (Dautzenberg, 1891) reared at different depths in ponds in Benin (West Africa)

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Abstract
The objective of this work was to study the survival and growth of juveniles of the oyster *Crassostrea gasar* reared in ponds at different depths. Rearing trials were carried out for 42 days (6 weeks) in two ponds (12 m^2 x 1.10 m deep), one of which was fertilized with poultry droppings and the other with no fertilization. For each pond, 03 depths (30 cm, 60 cm and 90 cm) were tested in duplicate. At each depth, two iron mesh bags (40 cm x 20 cm x 10 cm) were used. Oysters with an average size of 40.50 mm and average weight of 15.36 g were cultivated at a density of 30 individuals per bag. Phytoplankton density, pH, temperature, transparency and salinity of the water were daily measured between 7:00 and 8:00 am. Oysters were counted weekly. Individual length and weight were measured to evaluate their survival and growth. The values of physico-chemical parameters measured during this study met the requirements of tropical water oysters. After 6 weeks of rearing, the best survival rates were observed at 60 cm in the ponds (86.67% and 81.67% in the fertilized and unfertilized ponds, respectively) while the lowest survival (46.67% and 41.67 in the fertilized and unfertilized ponds, respectively) was recorded at 90 cm depth. The highest mean final sizes (40.584 mm and 40.568 mm in the fertilized and unfertilized ponds respectively) were recorded at 60 cm depth while the lowest values (40.557 mm and 40.553 mm in the same order) were recorded at 90 cm depth. The average final weight ranged from1 5.32 g (at 90 cm depth) to 15.38 g (at 60 cm) while it overlapped between 15.3 g (90 cm depth) to 15.35 g (30 cm depth) in the unfertilized pond. The average daily gain was 0.0004 g/day and 0.0005 g/day respectively for the 30 cm and 60 cm depths of the fertilized pond. The best zootechnical performance was recorded between 30 cm and 60 cm depth in the fertilized pond. Fertilized pond culture highlight future prospects for the promotion of *Crassostrea gasar* oyster culture in controlled environment.

Keywords: mangrove oyster, *crassostrea gasar*, phytoplankton, survival, growth, culture

Introduction
Oysters are bivalve molluscs of marine origin, some species of them are have colonized coastal and continental waters [1]. They play a very important ecological role in the management of pollution by removing pollutants from water through filtration [2]. They are also subject to industrial exploitation (oyster farming) in developed countries and artisanal exploitation (harvesting) in developing countries generating significant income for operators [3, 4, 5]. The genus *Crassostrea* groups hollow oysters and represents the most cultivated genus worldwide because of its zootechnical performance [6]. Mangrove oysters are consumed by coastal populations and in some large African cities such as Dakar (Senegal), Abidjan (Ivory Coast) and Cotonou (Benin) [6, 7, 8] because of their organoleptic qualities.The trade in mangrove oysters results in the abusive destruction of mangrove trees [6, 7, 8]. Mangroves (*Rhizophora racemosa* and *Avicennia germinans*) are characteristic of mangrove ecosystems and constitute the natural habitat of the mangrove oyster [9]. In Benin, the mangrove oyster is subject to artisanal exploitation at the coastal water levels especially in Lake Nokoué and the coastal lagoon [8, 10].Several scientific studies have been conducted on the reproduction, feeding and growth of the mangrove oyster [6, 10, 11, 12], but there is a lack of scientific data on rearing in controlled systems of the species. Oyster research is absent so that currently at the coastal lagoon, oyster farming remains traditional without any improvement.

It is therefore necessary to improve existing production techniques in order to optimize oyster production in Benin. Knowing that oysters feed mainly on phytoplankton and that the latter is not distributed in equal proportions in the water column of an aquatic ecosystem [13], the growth and survival of oysters can be influenced by depth. The present work aims to investigate the influence of depth and fertilization on the survival and growth of juvenile mangrove oysters *Crassostrea gasar* reared in ponds.

Materials and Methods
Study Environment
Oysters were reared in ponds at Tokpa-zoungo in the area of Abomey-Calavi in southern Benin (Fig 1). It is a marshy area close to the Nokoué lagoon. The breeding site is accessible both in the dry and flood seasons. The experimental site has ponds of 12 m^2 and 1.10 m in depth. These ponds are filled with lagoon water. The water is brackish and rich in phytoplankton.

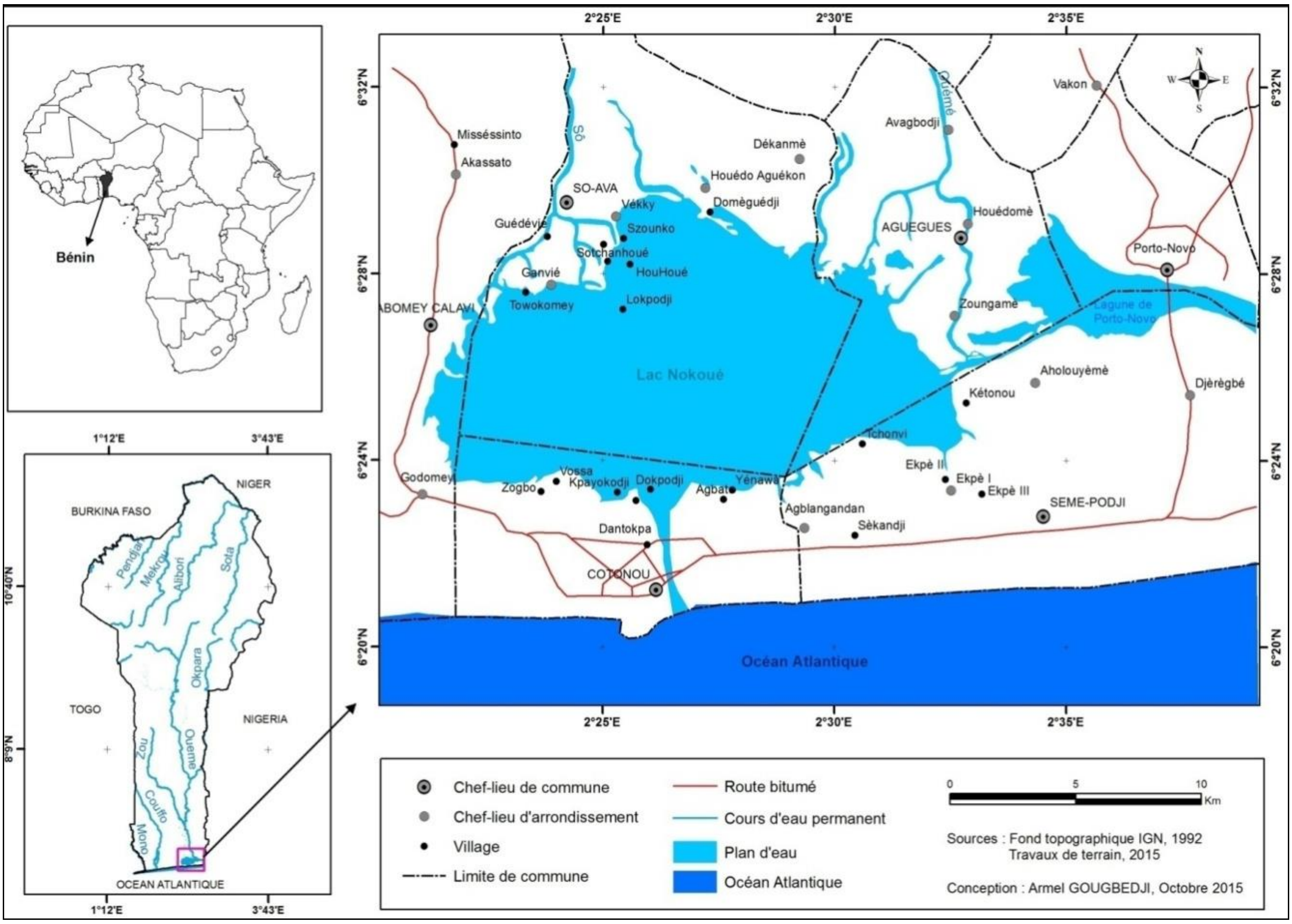

Fig 1: Location of Nokoué lake and experimentation site

Materials
Origin of the oyster juveniles
The juveniles of *C. gasar*, ranging in size from 40.5 mm to 41 mm, were collected at Agbato, south of the lagoon, from women oyster harvesters. The spat were then transported in jute bags between 7 and 10 a.m. to the rearing site located about 15 km away.

Experimental design
The experimental setup is composed of two ponds of 126 m^2 and 1.10 m in depth. Each of the ponds was fed with brackish water from the coastal lagoon using a motor pump. One pond was fertilized with dry poultry droppings at a density of 600 g/m^3 [14] four (04) days prior to oyster stocking while the control pond was not fertilized. Two chairs 100 cm apart from each other were placed in each pond. The chairs were designed with teak legs in the form of a scaffold with three (03) levels 30 cm apart. The three levels are located at 30 cm, 60 cm and 90 cm depth respectively (Fig 2). The chairs are used to hold the oyster bags at precise depths of 30 cm, 60 cm and 90 cm. On each chair, a pocket made of iron mesh (dimensions: 40 cm x 20 cm x 10 cm) is placed at each depth, i.e. 3 pockets per chair. A total of six (6) pockets containing the spat are installed in each pond (Fig 2). The water of the site being brackish, varnishing was carried out on the porches with antirust.

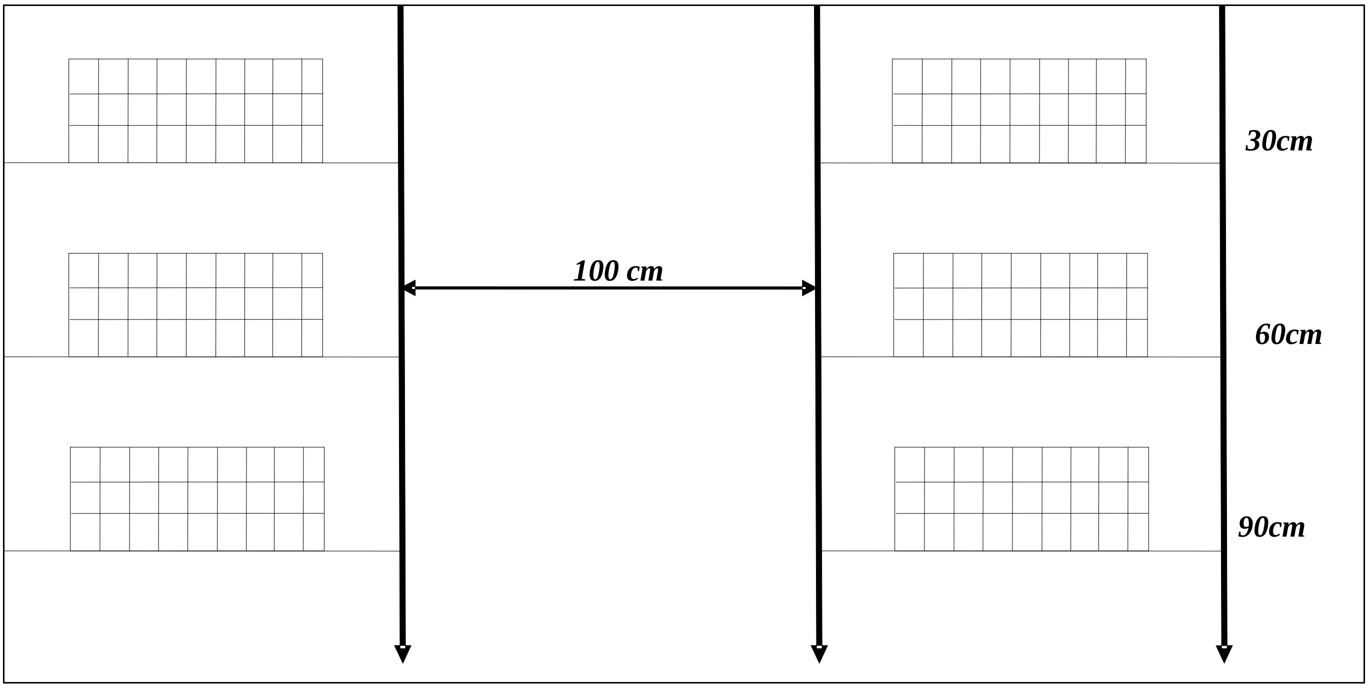

Fig 2: Experimental design for each oyster pond

Data Collection and Processing

Pouching of oyster juveniles

Oysters were acclimatized for 7 days before the start of the experiments. Before loading, the shell length (size) and weight of each oyster specimen were measured with a caliper and an electronic scale respectively. They were then distributed in the bags at a density of 30 individuals/pocket. The initial average size was 40.5 mm with an average weight of 15.36 g.

Data collection

The experiment lasted six (06) weeks. The physico-chemical parameters of the pond water were measured every day. Thus, the salinity, pH, temperature and transparency of the pond water were measured once per day between 7:00 and 8:00 am, respectively with a refractometer, a pH meter, a thermometer and a Secchi disk. The density of phytoplankton in the water was taken with a densimeter.

For the growth study, ten (10) oyster specimens were selected weekly at random by bag. They were measured and weighed. The biomass per pocket was estimated by extrapolation. The number of dead individuals per bag was recorded at each control.

Data Processing

Means and standard deviations of the physico-chemical parameters of the pond water were calculated. For the oysters sampled, mean sizes and weights were calculated for each depth and for each pond.

Average daily gain (ADG) and specific growth rate (SGR) were calculated:

$$ADG\ (g/d) = (Wf - Wi)/\ \Delta t;$$

With Wi= Initial mean weight; Wf = Final mean weight and Δt = duration of the experiment in days.

$$SGR\ (\%.d^{-1}) = 100 \times (LnWf - LnWi)/\Delta t;$$

With LnWi = neperian logarithm of the initial weight; LnWf = neperian logarithm of weight.

By pocket, survival rate (SR) was calculated at each growth check according to the following formula:

$$SR\ (\%) = 100 \times Nf / Ni;$$

Where,

Ni: Initial number of individuals and Nf: Final number. Averages were calculated between duplicates of each depth per pond to have average survival rates for each depth.

Statistical Analysis

For each physico-chemical parameter, the non-parametric Kruskal-Wallis test was used to highlight possible variation between ponds. When there was a difference, the Mann-Whitney U test was used to test the significance of the variation. Comparison of survival rates was performed using the χ^2 test. For morphological parameters (oyster size and weight), data were processed by pond and depth. Results are presented as mean±standard deviation between duplicates. The comparison of these parameters has been done by analysis of variance (ANOVA). When these tests revealed a significant difference, post hoc comparisons (Least Significant Difference: LSD) were performed. Differences were considered significant at the 5% level for all analyses.

Results

Physico-chemical parameters of the water

The extreme values and averages of the physicochemical parameters measured during the experiment are summarized in Table 1.

Table 1: Physico-chemical parameters measured in the fertilized and unfertilized ponds during the experiments (Mini, minimum; Max, maximum; Avg, average; SD, standard deviation).

	Unfertilized pond				**Fertilized pond**				**p**
	Mini	**Maxi**	**Mean**	**SD**	**Mini**	**Maxi**	**Mean**	**SD**	
Temperature (°C)	26.3	28.1	27.10 [a]	0.81	26.1	28.1	26.87 [a]	0.78	> 0.05
Salinity (‰)	14	17.7	16.13 [a]	1.59	15	18	16.49 [a]	1.16	> 0.05
Transparency (mm)	37.1	40.8	39.06 [b]	1.29	26.1	33.7	29.64 [a]	2.92	< 0.05
pH	6	7	6.14 [a]	0.38	6	7	6.71 [a]	0.49	> 0.05
Phytoplankton density (ind/litre)	603	902	751.86 [a]	111.15	1196	2363	1677.14 [b]	459.91	< 0.05

For each line, different letters next to the mean values indicate a significant difference $p < 0.05$ (Post Hoc: LSD).

Water temperature ranged from 26.3 °C to 28.1 °C with an overall average of 27.1±0.81 °C in the unfertilized pond while it ranged from 26.1 °C to 28.1 °C with an average of 26.87±0.78 °C in the fertilized pond (Table 1). As for water salinity, it ranged from 14‰ to 17.7‰ in the unfertilized pond with an average of 16.13±1.59‰. In the fertilized pond, it ranged from 15‰ to 18‰ with an average of 16.49±1.16‰ (Table 1). pH varied between 6 and 7 in both ponds with an average of 6.14±0.38 and 6.71±0.49 in the unfertilized and fertilized pond, respectively (Table1). For these three parameters (temperature, salinity, and pH), there was no significant difference in values between ponds ($p > 0.05$). Water transparency varied from 37.1 mm to 40.8 mm with an overall average of 39.06±1.29 mm in the unfertilized pond. In contrast, in the fertilized pond, it ranged from 26.1 mm to 33.7 mm with an average of 29.64±2.92 mm (Table 1). Phytoplankton density ranged from 603 individuals/L water to 902 individuals/L water with an average of 751.86±111.15 individuals/L water in the unfertilized pond. In the fertilized pond, it ranged from 1196 individuals/L water to 2363 individuals/L water with an average of 1677.14±459.91 individuals/L water (Table 1). For water transparency and phytoplankton density, there was a significant difference between ponds ($p < 0.05$). The fertilized pond had the highest phytoplankton densities versus the low densities recorded in the unfertilized pond. Correspondingly, the low transparencies were recorded in the fertilized pond while the highest values were noted in the unfertilized pond (Table 1).

Oyster survival and growth

The survival rate and growth parameters of oysters reared in ponds for 42 days are summarized in Table 2.

Table 2: Survival and growth parameters of juvenile *Crassostrea gasar* oysters reared in ponds (SLi, mean initial shell length; SLf, mean final shell length; Wi, mean initial weight; Wf, mean final weight; ADG, Average daily gain; SGR, specific growth rate).

	Unfertilized pond				**Fertilized pond**			
	30 cm	60 cm	90 cm	P	30 cm	60 cm	90 cm	p
SLi (mm)	40.500 [a]	40.500 [a]	40.500 [a]	> 0.05	40.500 [a]	40.500 [a]	40.500 [a]	> 0.05
SLf (mm)	40.567 [a]	40.568 [a]	40.553 [a]	> 0.05	40.582	40.584	40.557	> 0.05
Wi (g)	15.360 [a]	15.360 [a]	15.360 [a]	> 0.05	15.360 [a]	15.360 [a]	15.360 [a]	> 0.05
Wf (g)	15.350 [a]	15.340 [a]	15.300 [a]	> 0.05	15.375 [a]	15.382 [a]	15.320 [a]	> 0.05
ADG (g/d)	-0.0002 [a]	-0.0005 [a]	-0.0014 [a]	> 0.05	0.0004 [a]	0.0005 [a]	-0.0010 [a]	> 0.05
SGR ($\%.d^{-1}$)	-0.0016 [a]	-0.0031 [a]	-0.0093 [a]	> 0.05	0.0023 [a]	0.0034 [a]	-0.0062 [a]	> 0.05
Survival rate (%)	80.00 [a]	81.67 [a]	41.67 [b]	< 0.05	68.33 [ab]	86.67 [a]	46.67 [b]	< 0.05

For each pond, different letters next to values in the same row indicate a significant difference $p < 0.05$ (Post Hoc: LSD).

Variations in survival rates

After 6 weeks of rearing, the survival rate in the bags placed at 30 cm depth decreased to 80% and 68.33% in the unfertilized and fertilized ponds, respectively (Table 2 & Fig 3). The lowest survival was recorded in the fertilized pond. However, there was no significant difference in survival rates recorded at 30 cm between the two ponds ($p > 0.05$, Table 2). At 60 cm depth, survival rates were 81.67% and 86.67% in the unfertilized and fertilized ponds, respectively (Table 2 & Fig 4). There is no significant difference between the survival rates recorded at 60 cm from one pond to another ($p > 0.05$, Table 2). For the bags placed at 90 cm, the survival rates were the lowest and reached 41.67% in the unfertilized pond and 46.67% in the fertilized pond (Table 2 & Fig 5). There is no significant difference between the survival rates recorded at 90 cm from one pond to another ($p > 0.05$, Table 3).

A comparison of survival rates among depths shows that the lowest survival rates were recorded at 90 cm depth while the best survival rates were recorded at 60 cm depth in both ponds (Figs 3, 4 & 5). In each pond, the survival rates recorded at 90 cm depth are significantly lower than those recorded at 30 cm and 60 cm depth ($p < 0.05$).

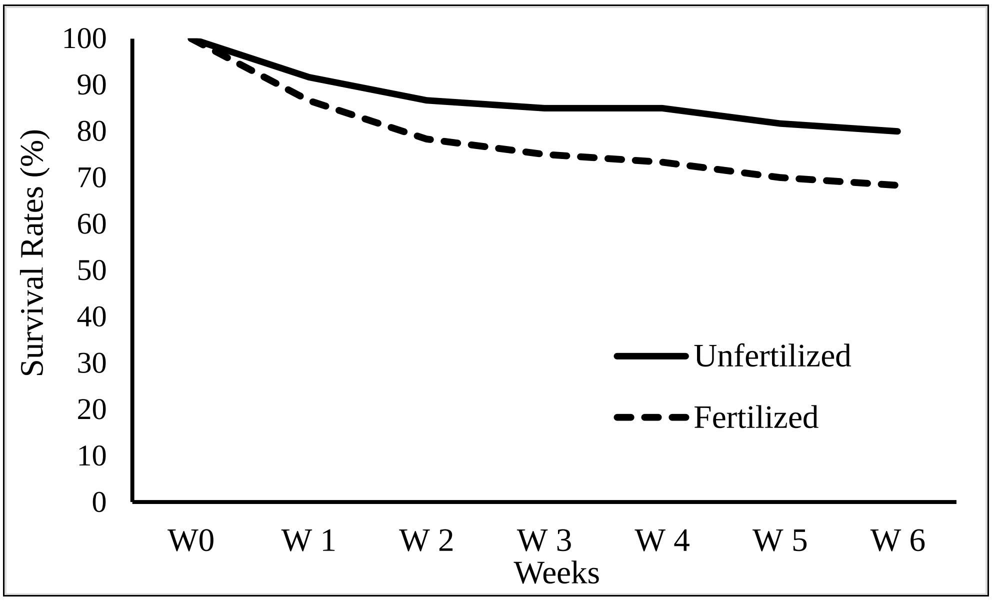

Fig 3: Evolution of the survival rate of juvenile oysters *Crassostrea gasar* reared in fertilized and unfertilized ponds at 30 cm depth.

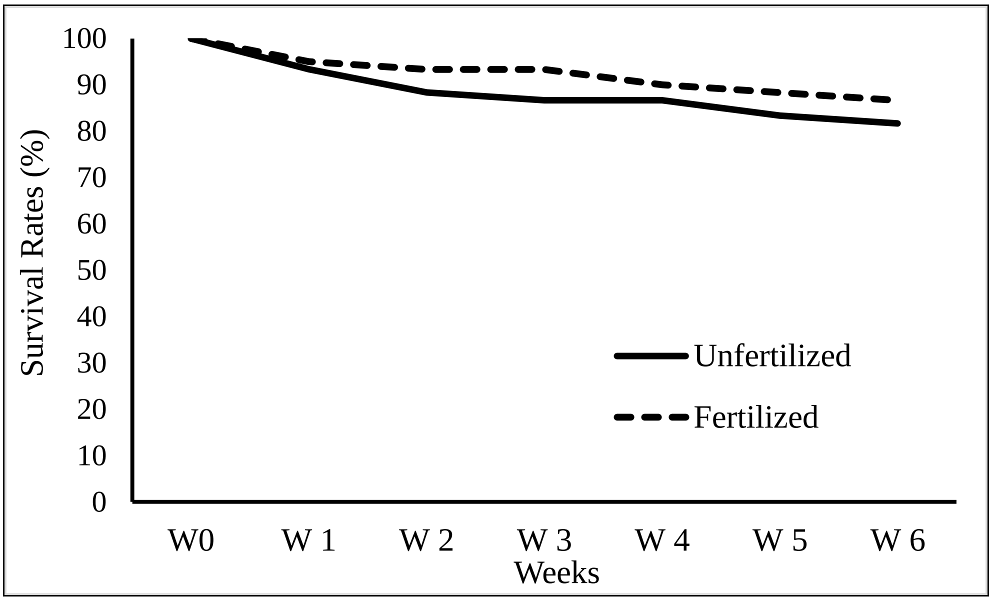

Fig 4: Evolution of the survival rate of juvenile oysters *Crassostrea gasar* reared in fertilized and unfertilized ponds at 60 cm depth.

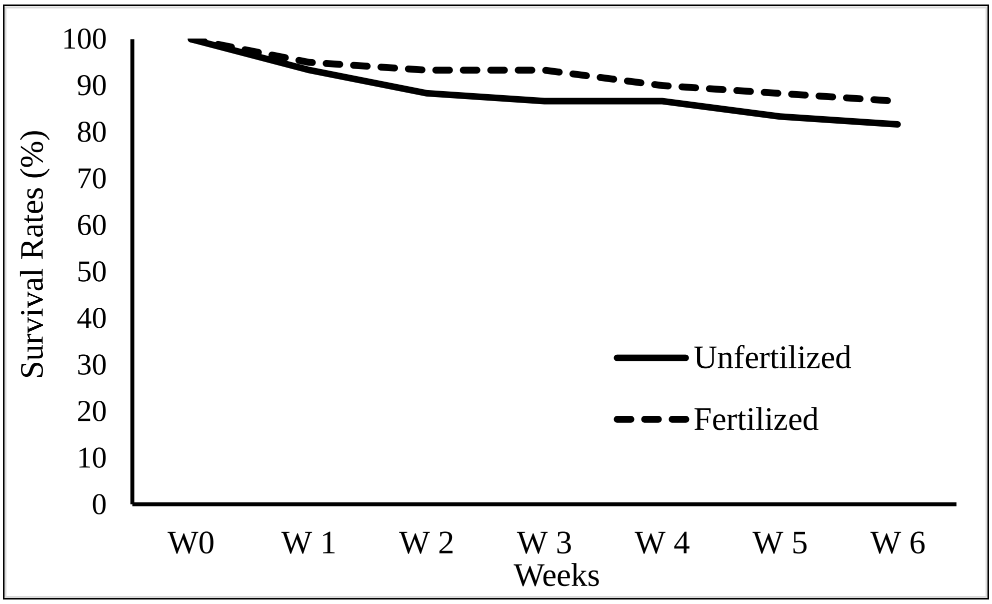

Fig 5: Evolution of the survival rate of juvenile oysters *Crassostrea gasar* reared in fertilized and unfertilized ponds at 90 cm depth.

Variations in mean weight

After the 6 weeks of experimentation, the highest final mean weights were recorded in the fertilized pond while the lowest values were recorded in the unfertilized pond regardless of depth (Figs 6, 7 and 8). In the unfertilized pond, the average weight of oysters decreased from 15.36 g at the beginning of the experiment to 15.35 g; 15.34 g and 15.30 g respectively at 30 cm, 60 cm and 90 cm depths after 6 weeks of rearing (Figs 5, 6 and 7). On the other hand, in the fertilized pond, a slight increase in the average weights of the oysters was observed. The average weight increased from 15.36 g to 15.375 g; 15.382 g and 15.32 g respectively at 30 cm, 60 cm and 90 cm depths after 6 weeks of rearing (Figs 6, 7 and 8). No significant difference was recorded between the values of the different depths of each pond, nor between the values of the same depth for both ponds ($p > 0.05$, Table 2).

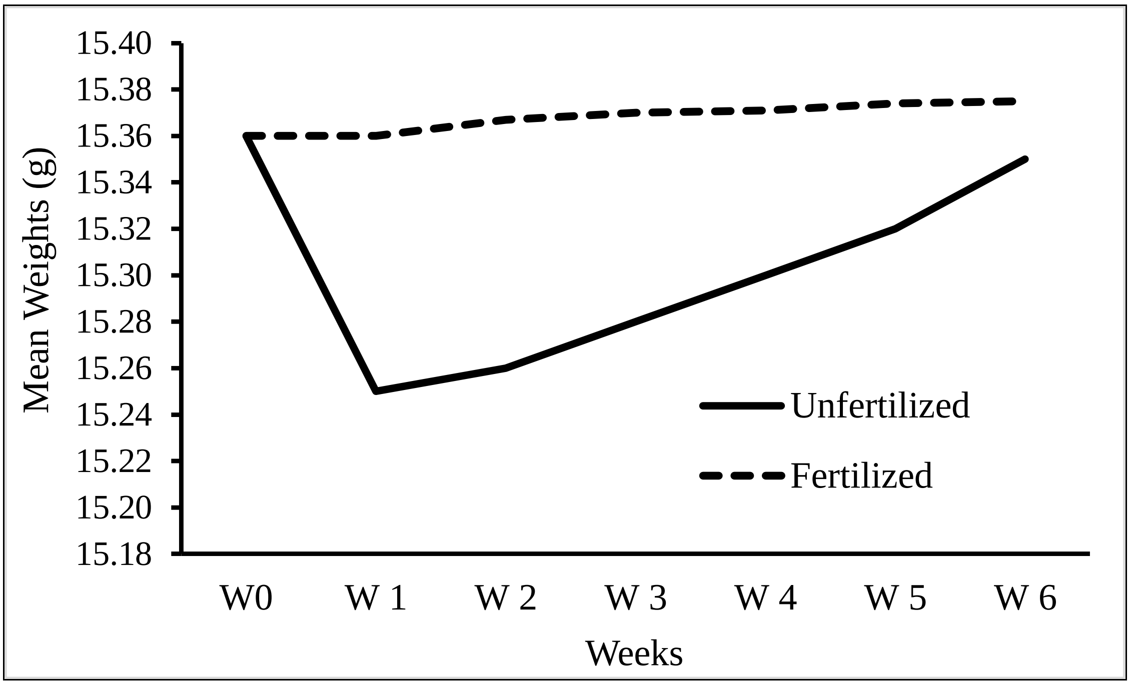

Fig 6: Variations in mean weights of oysters in unfertilized and fertilized ponds at 30 cm depth

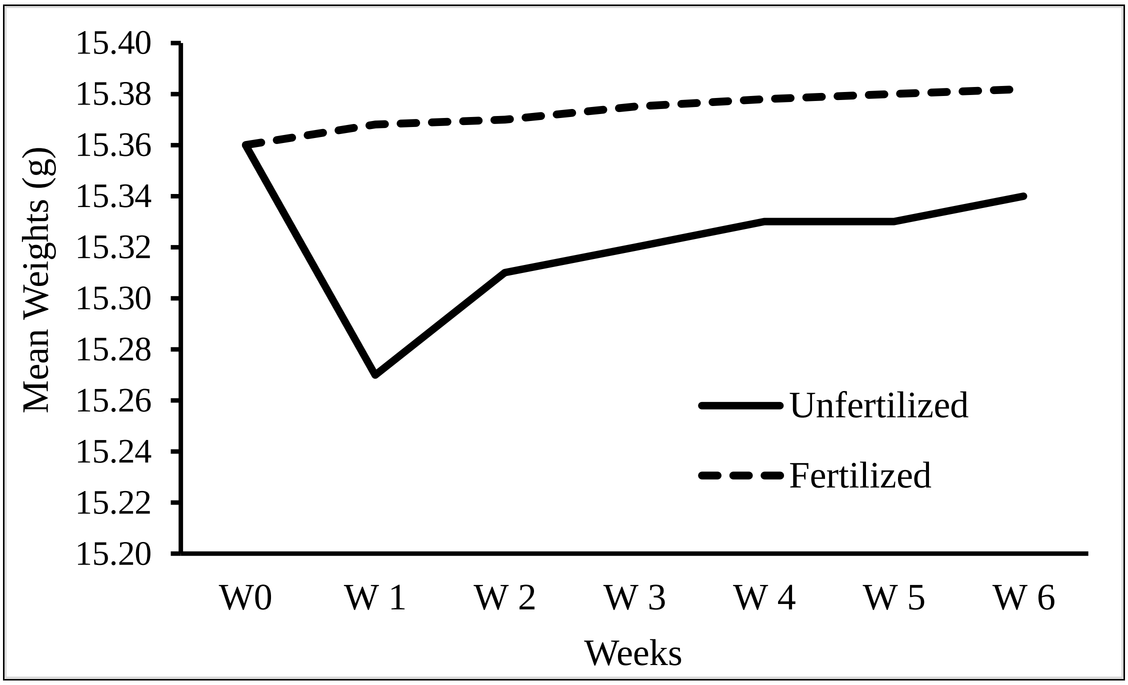

Fig 7: Variations in mean weights of oysters in unfertilized and fertilized ponds at 60 cm depth

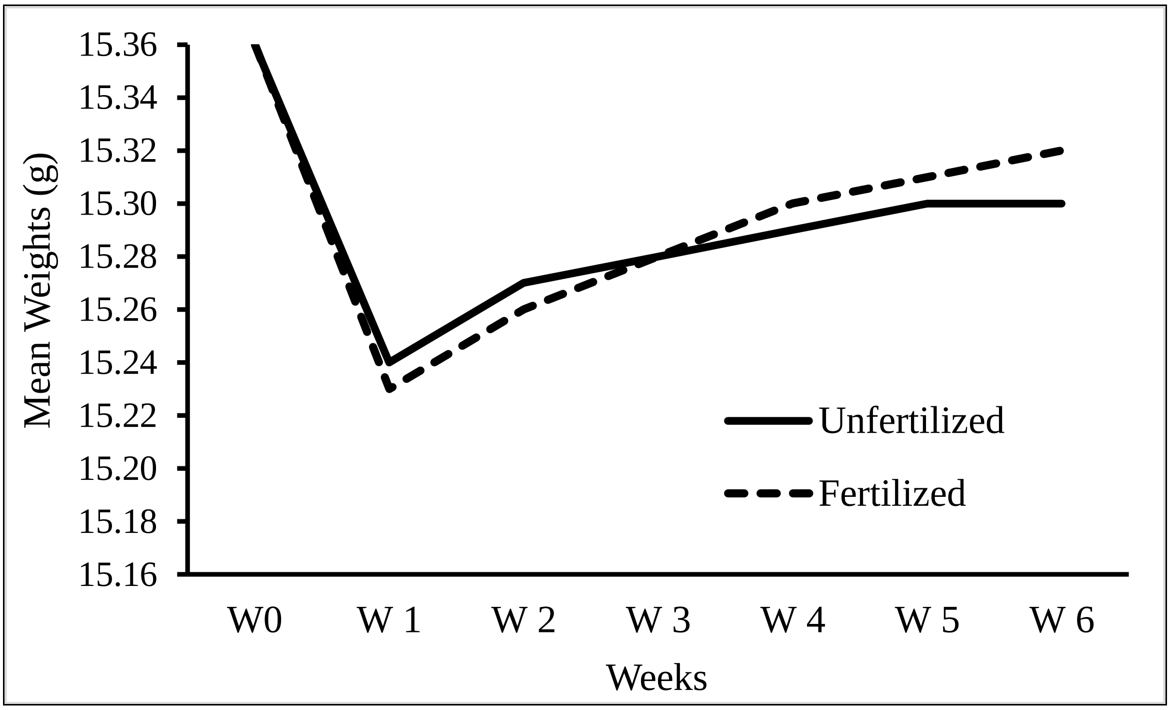

Fig 8: Variations in mean weights of oysters in unfertilized and fertilized ponds at 90 cm depth

Growth in size of reared oysters

For all depths combined, a slight increase in shell length was observed in both ponds. At the end of the experiment, the average shell length was higher in the fertilized pond than in the unfertilized pond regardless of depth. At 30 cm depth, the average shell length increased from 40.50 mm to 40.567 mm and 40.582 mm in the unfertilized and fertilized ponds, respectively (Fig 9). The initial average length (40.50 mm) increased to 40.568 mm and 40.584 mm at 60 cm depth (Fig 10) and to 40.553 mm and 40.557 mm at 90 cm depth (Fig 11) in the unfertilized and fertilized pond, respectively. However, there was no significant difference either between the values of the different depths of each pond or between the values of the same depth for both ponds ($p > 0.05$, Table 2).

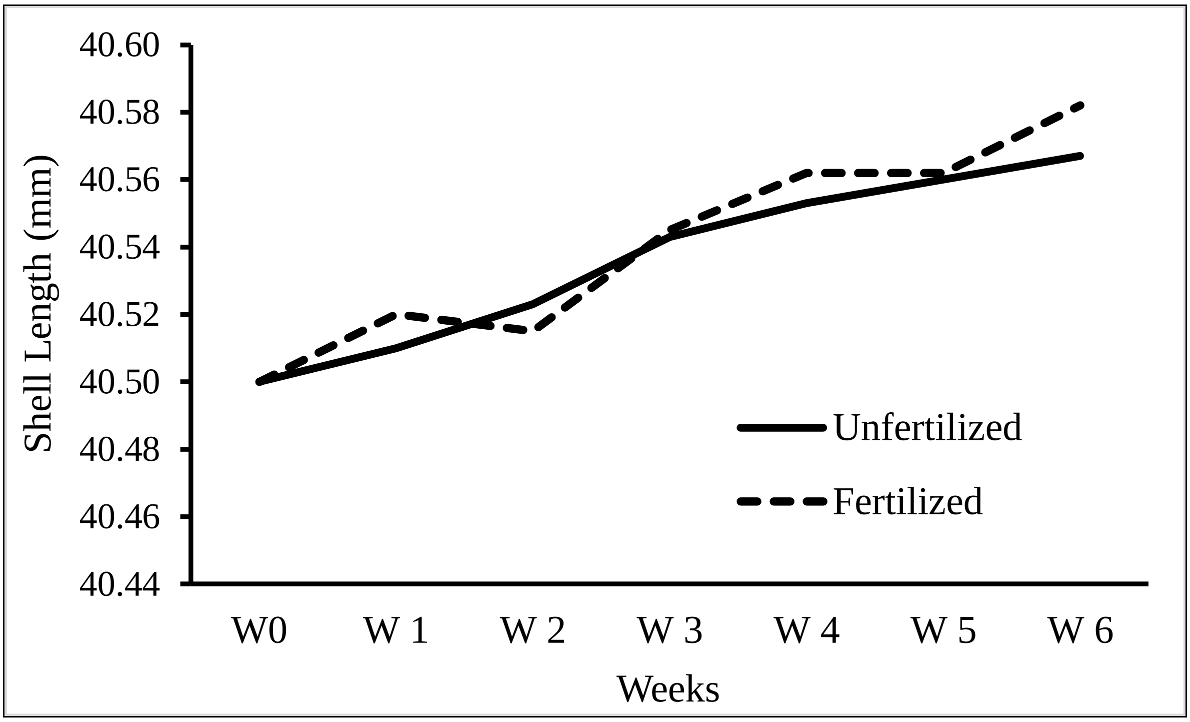

Fig 9: Variation in mean length of oysters in unfertilized and fertilized ponds at 30 cm depth

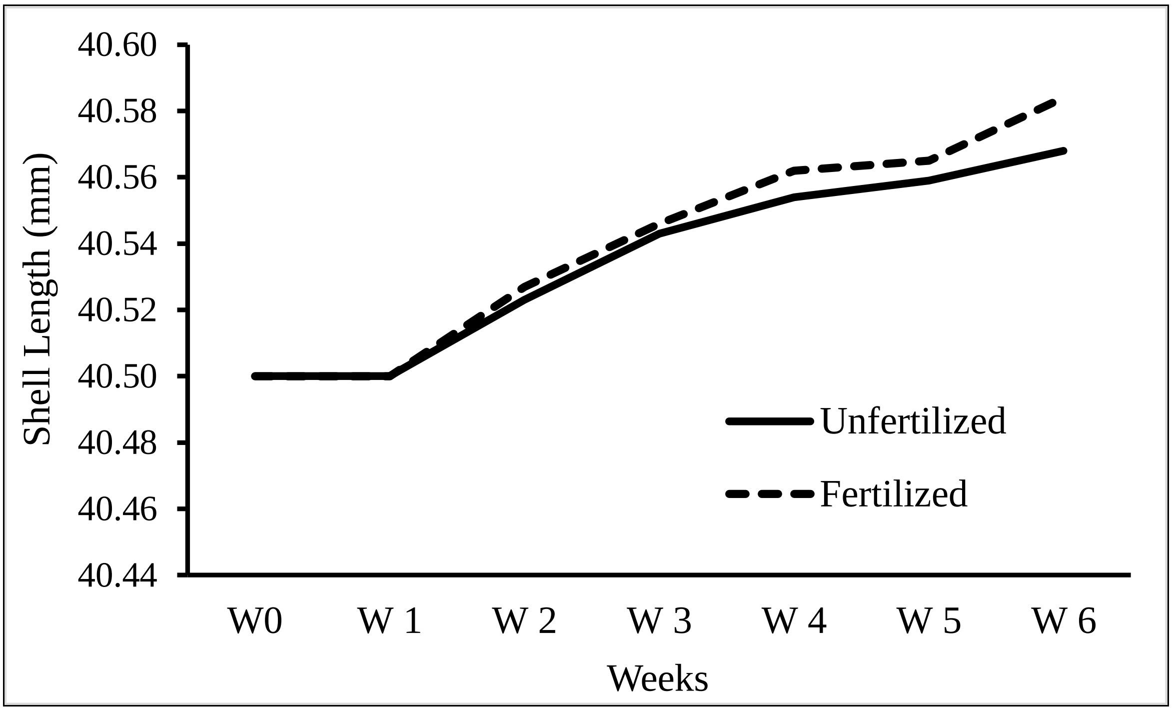

Fig 10: Variation in mean oyster length in unfertilized and fertilized ponds at 60 cm depth

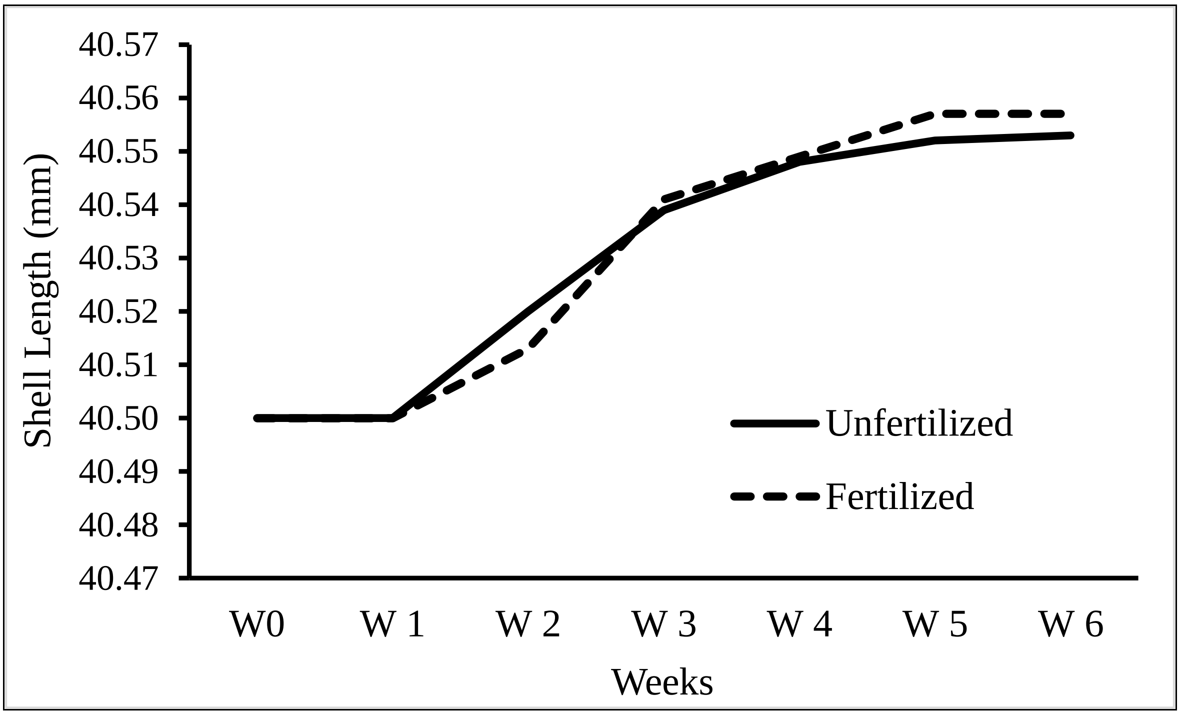

Fig 11: Variation in mean length of oysters in unfertilized and fertilized ponds at 90 cm

Average daily gain and specific growth rate

The average daily gain was 0.0004 g/d and 0.0005 g/d for the 30 cm and 60 cm depths of the fertilized pond, respectively (Table 2). The values of average daily gain and specific growth rate were negative for all depths of the unfertilized pond and for the 90 cm depth of the fertilized pond (Table 2). The specific growth rate was 0.0023%. d^{-1} and 0.0034%.d^{-1} respectively for the same depths of the fertilized pond (Table 3). There was no significant difference between the values of the different depths of each pond, nor between the values of the same depth for both ponds ($p > 0.05$, Table 2).

Discussion

The purpose of the present study was to rear juveniles of *Crassostrea gasar* in a controlled environment. *Crassostrea gasar* belongs to the group of brackish water oysters that live in mangroves. They grow and survive in a very wide range of salinity (6‰ to 60‰) [15]. Salinity values recorded in the present study ranged from 14‰ to 18‰. Thus, salinity was not a limiting factor for oyster survival and growth in this study. During the 6 weeks of rearing, water temperature varied between 26.1 °C to 28.1 °C while pH varied between 6 and 7. For all three parameters, no extreme or lethal values were recorded. The average phytoplankton density was 1677.14±459.91 individuals/L of water in the fertilized pond versus 751.86±111.15 individuals/L of water in the unfertilized pond. As for transparency, the average was 39.06±1.29 mm in the unfertilized pond while it was

29.64±2.92 mm in the fertilized pond. Thus, the fertilized pond had the highest phytoplankton concentrations and the lowest water transparency values compared to the unfertilized pond. These results could be explained by the faster solubility and high phosphorus content of poultry droppings [14]. Indeed, poultry droppings would have released a significant amount of nutrients, especially phosphorus, which resulted in a better primary production (high density of phytoplankton) and consequently a low transparency in the fertilized pond.

To study the survival of oysters, the survival rate was calculated per pond for each depth. Overall, the lowest survival rates were recorded at 90 cm while the highest survival values were recorded at 60 cm and 30 cm depth regardless of pond. Indeed, the soil of the study area being clayey, pond maintenance operations and any other manipulations lead to an excessive deposition of mud at the bottom of the ponds. At 90 cm, the oysters are close to the pond bottom and therefore may be influenced by the turbid nature of the bottom water during growth control or pond maintenance operations. Since oysters are known as filter-feeding organisms, they would die from gill plugging [10]. Poor handling during control fisheries is another cause of the mortalities recorded in the present study [16]. The study of oyster growth consisted of taking total weight and shell length measurements of the oysters at each control fishing. For both ponds, the highest values of average weight were recorded at 60 cm and 30 cm while the lowest were recorded at 90 cm. A comparison between the ponds showed that the best average weights were recorded in the fertilized pond compared to the unfertilized pond for the depths 30 cm and 60 cm. The average daily gain (ADG) and specific growth rate (SGR) showed that oysters reared in the unfertilized pond (all depths) and at depth 90 cm in the fertilized pond did not grow in biomass, but instead lost weight after 42 days of rearing. The values of these parameters are all negatives for all depths (30 cm, 60 cm and 90 cm) of the unfertilized pond and for the 90 cm depth of the fertilized pond. In contrast, oysters reared in the fertilized pond at 30 cm and 60 cm depth gained weight. ADG varied from 0.0004 to 0.0005 g/d while SGR varied from 0.0023%.d^{-1} to 0.0034%.d^{-1} respectively at 30 cm and 60 cm depth in the fertilized pond. This difference in growth can be explained by the high primary production (high phytoplankton density), thus the availability of food in the fertilized pond compared to the unfertilized pond. At 90 cm depth, not only are the final average weights of the oysters in both ponds lower than the initial average weights, but also the curve of the average weights of the oysters in the fertilized pond is almost merged with the unfertilized pond. This low growth of oysters is probably caused by a low primary production at 90 cm (even in the fertilized pond) due to the absence of sunlight for photosynthesis to occur [13]. Indeed, primary production in a pond depends not only on nutrients (mineral salts) but also on sunlight. The absence or presence in low proportion of one of these factors limits primary production [13].

With respect to shell length, the shape of the growth curve in shell size revealed an increasing trend for all depths and for both ponds.

Conclusion
For the three depths (30 cm, 60 cm and 90 cm) tested in the present study, the best survival and growth were recorded at 60 cm and 30 cm while the lowest values were recorded at 90 cm regardless of pond. In addition, growth was higher in the fertilized pond than in the unfertilized pond for all depths, due to high primary production (high phytoplankton density) as a result of fertilization with poultry droppings. For pocket culture of the oyster, *Crassostrea gasar*, depths between 30 cm and 60 cm and fertilization of the ponds with poultry droppings resulted in better oyster productivity.

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