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Ponds culture of juveniles of freshwater oyster *Etheria elliptica* Lamarck, 1807 in Benin (West Africa)

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Abstract
In order to domesticate the freshwater oyster *Etheria elliptica*, a 42-day freshwater pond rearing trial was conducted in two ponds of equal size (12 m^2 x 1.10 m deep), one of which was fertilized with poultry droppings and the other one was a control (unfertilized). Three depths (30 cm, 60 cm, 90 cm from the bottom of the pond) were tested with one repetition. For each depth, 02 sieves (40 cm diameter and 15 cm depth) were used. A total of 180 oysters with an average size of 37.56±1.95 mm and an initial average weight of 30.35±0.72 g were used with a density of 15 oysters per colander. pH, temperature, transparency, and phytoplankton density were measured daily between 7:00 am and 8:00 am in June-July 2018. Each week, the colanders were checked. Oysters were counted, measured and weighed. The physico-chemical parameters measured in the ponds were consistent with the requirements of tropical water oysters. At the end of the experiments, the survival rate (50±35.36%) was identical for all depths in both ponds. The best growth in size was recorded in the fertilized pond. The sizes increased 2.06 mm and 3.8 mm at 90 cm and 30 cm depth respectively). In contrast, the smallest increases were obtained in the control pond, 0.93 mm and 1 mm at 90 cm and 30 cm depth respectively. In the fertilized pond, the average daily gain ranged from 0.017 g/d (at 90 cm depth) to 0.027 g/d (at 30 cm) while it was between 0.005 g/d (90 cm) and 0.017 g/d (30 cm) in the control pond. As for the specific growth rate, it varied from 0.014%.d^{-1} (90 cm) to 0.047%.d^{-1} (30 cm) in the control pond while it was between 0.047%.d^{-1} (90 cm) and 0.078%.d^{-1} (30 cm) in the fertilized pond. The best zootechnical performance was recorded between 30 cm and 60 cm in both ponds. Therefore, depths between 30 cm and 60 cm seem to be suitable for rearing *Etheria elliptica* in ponds. Also, fertilization of the rearing ponds with poultry droppings would improve the growth of the reared oysters.

Keywords: Fertilization, rearing, *Etheria elliptica*, survival, growth

Introduction
In developed countries, oyster culture is an industrialized economic activity while it continues to be a wild collection activity in developing countries [1, 2, 3]. Oysters are mainly marine and brackish water with a very limited number of freshwater species [4]. Marine and brackish species are the most studied and reared in the world [5,6]. *Etheria elliptica* (Mollusca: Bivalvia: Etheriidae) is a freshwater oyster widely distributed in tropical Africa and Madagascar [7, 8]. In West Africa, oysters are an important source of aquatic animal protein after fish [9, 10, 11]. Their exploitation generates significant income for riparian populations [10, 11]. However, consumers of oysters face a problem of food poisoning because of the way oysters are fed [12, 13, 14]. Indeed, oysters are filter feeders, and by doing so accumulate pollutants and heavy metals in their body [4, 13, 15]. Knowing that rivers and water bodies are increasingly polluted by human activities and given the growing interest of populations in eating oysters, it is necessary to consider the breeding of the species most appreciated by consumers in controlled environments. In Benin, two species of oysters currently inhabit continental waters. In brackish waters, the mangrove oyster *Crassostrea gasar* grows on mangrove roots while the freshwater oyster *Etheria elliptica* colonizes rivers and streams [2, 16]. Among these species, the mangrove oyster is traditionally farmed along the coastal lagoon in southern Benin [13, 17]. As for *Etheria elliptica*, attempts at its farming are limited to the farming trials conducted by [3] in the Pendjari River. As the species *Etheria elliptica* is subject to increasing harvesting by riparians of the rivers and water bodies where it occurs, the study of its population dynamics in the Pendjari River noted that it is overexploited [18]. Studies on the ecology [19, 20, 21], survival and growth performance [3], feeding [22] and reproduction [23, 24] of *Etheria elliptica* have revealed that the species is a potential candidate for aquaculture.

For the conservation of the species and for the safety of consumers, it is therefore of great necessity to reduce the pressure of harvesting the species through its introduction into oyster aquaculture in a controlled environment. As *Etheria elliptica* is freshwater oyster, its culture in ponds appears as an opportunity for the numerous promoters of freshwater fish farms in Benin. The specific goals of the present work are to evaluate 1) the survival and growth of juveniles of *Etheria elliptica* reared in freshwater ponds at different depths and 2) the effect of fertilization on their growth rate.

Materials and methods

Biological material

The oysters used in this study come from the Ouémé River in southern Benin. They were collected in the wild in the localities of Lokossa and Ananme (commune of Adjohoun) and Asrossa (commune of Bonou) (Fig 1). These localities have high densities of oysters. These oysters live isolated or in colonies adjacent to each other. Juvenile oysters living in isolation are the most suitable for growth studies. The take-off of young oysters living in colonies results in high mortality rates due to the fragility of the lower valves [24]. Fifty-liter drums were used to transport them from the wild harvest stations to the experimental farm.

Location of the rearing site and experimental setup

The trials were conducted on the Assiki farm located in the Vakon district in the commune of Akpro-Missérété (South Benin) (Fig 1). The experimental set-up is composed of sieves (40 cm in diameter and 15 cm deep) and 2 ponds of 12 m^2 with a depth of 1.10 m. Three depths (30 cm, 60 cm and 90 cm from the bottom of the pond) were tested for each of the two ponds. Per pond, two (02) sieves containing oysters were placed at each depth, *i.e*. 06 sieves per pond. The sieves were attached to supports (made of bamboo) with a rope (made of nylon). Thus, the sieves placed at 30 cm are closer to the bottom of the pond while the sieves located at 90 cm are closer to the surface of the ponds. One of the ponds was fertilized with poultry droppings (dose: 600 g/m^3) 4 days before the oysters were stocked while the second pond was not fertilized (control pond). The stocking density was 15 oysters per pond.

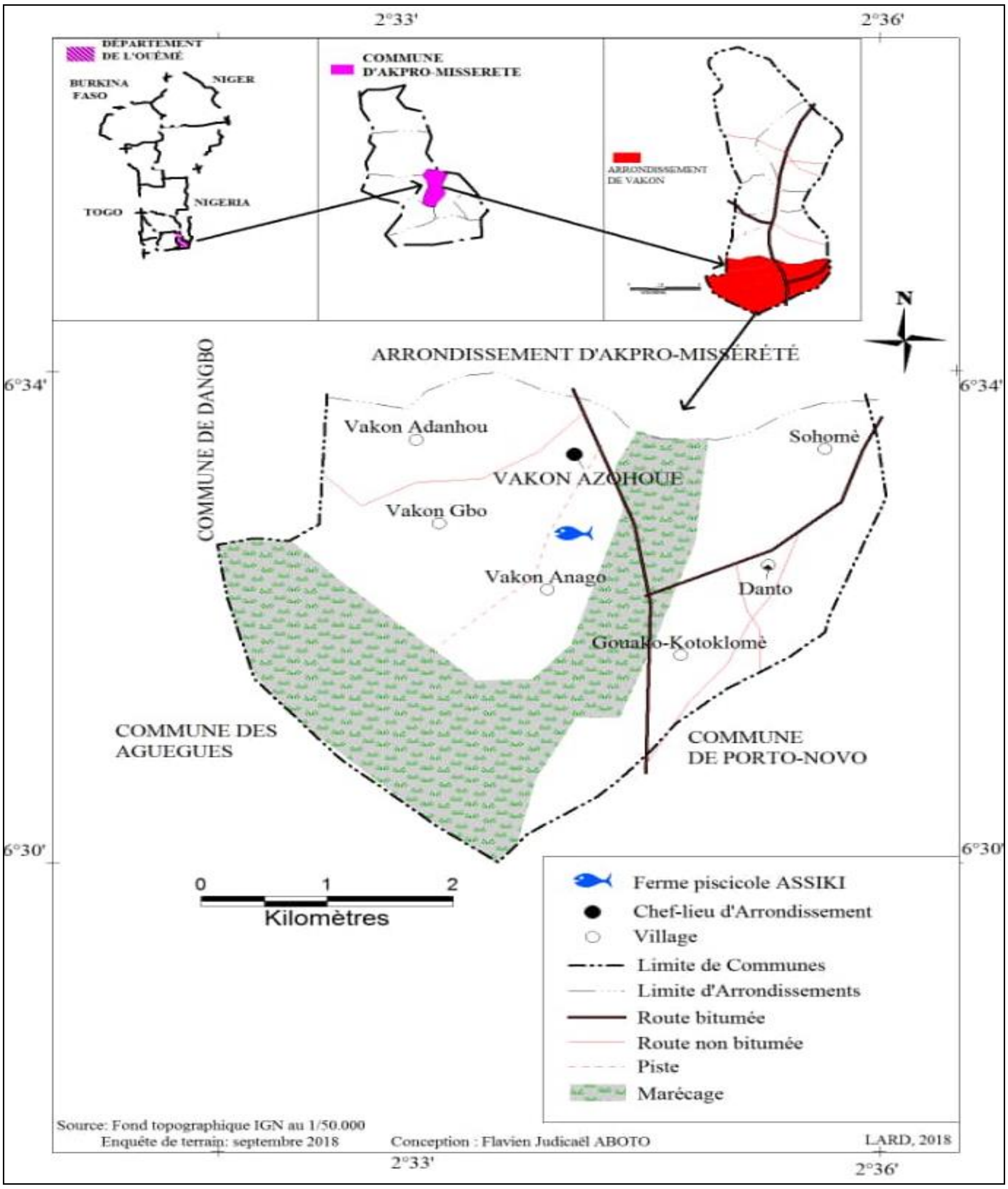

Fig 1: Location of the Assiki experimental farm and the collection sites for juvenile *Etheria elliptica* oysters along the Ouémé River.

Loading of oysters
Sexual maturity in the freshwater oyster *Etheria elliptica* is reached between 58 mm for males and 65 mm for females [24]. Juveniles of more or less homogeneous size were collected for this experiment. Oysters were measured with a caliper and weighed with an electronic scale model MH-999 before loading. A total of 180 juveniles of *Etheria elliptica* with average size 30.35±0.72 mm) and average weight = (37.56±1.95 g) were used for this study.

Methods of data collection
Data collection
The trials lasted 06 weeks and measurements were performed once per week. Physicochemical parameters such as pH, temperature and transparency were measured once a day between 7:00 and 8:00 am. The pH was measured with pH-papers, the water transparency with a Secchi disk and the temperature was taken with a thermometer. The phytoplankton density of the pond water was measured with a densimeter.
A control fishery was carried out every week to evaluate the survival and growth of the oysters. At each control fishing, oysters from each colander were counted. The number of dead individuals per colander was recorded. Size and weight were measured for each oyster specimen.

Data processing
Descriptive statistics (means, standard deviations) were calculated for the physicochemical parameters of the pond water. The average size and weight of oysters were calculated for each of the three depths of each pond.
The growth performance and survival of oysters were determined using the zootechnical parameters with the following formulas:

Weight gain (WG): WG (g) = Wf-Wi,

Average Daily Gain (ADG): ADG (g/d) = (Wf - Wi)/ Δt,
with Wi= Initial average weight; Wf = Final average weight and Δt = Experimental duration in days.

Specific growth rate (SGR): SGR (%.d-1) = 100 × (LnWf - LnWi)/Δt,

where LnWi = natural logarithm of initial weight; LnWf = natural logarithm of weight.

Change in mean shell length (ΔSL) : ΔSL = SLi- SLf,

with SLi = initial mean shell length and SLf = final mean shell length.

Survival rate (S): S (%) = (Nf / Ni) x 100,

where Ni = initial number of individuals and Nf= final number of individuals at the end of the experiment.

Statistical analysis
The non-parametric test of Kruskal-Wallis was used for the comparison between ponds of the values of each physicochemical parameter of the water. When this test shows a variation between ponds for a parameter, 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 morphometric measurements (height and weight), data were processed by pond and depth. Results are presented as mean±standard deviation between duplicates. The comparison of these parameters was performed 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
Water quality of the ponds
The descriptive statistics of the physico-chemical parameters of the ponds measured each day during this study, are summarized in Table 1.

Table 1: Descriptive statistics of physico-chemical parameters recorded in the breeding ponds

Control pond				
Parameters	**Temperature (°C)**	**pH**	**Transparency (cm)**	**Density (Ind /L)**
Average	26.76	7	38.86	751.58
SD	1.09	0	1.23	135.72
Maximum	28.6	7	40.2	1003
Minimum	25.5	7	36.9	701
Fertilized pond				
Parameters	**Température (°C)**	**pH**	**Transparence (cm)**	**Densité (Ind/L)**
Average	26.72	7	31.26	1690.72
SD	0.99	0	3.76	969.89
Maximum	28	7	39.4	3033
Minimum	25.2	7	25.3	695

The temperature ranged from 25.5 °C to 28.6 °C with an overall average of 26.76±1.09 °C in the unfertilized (control) pond while it ranged from 25.2 °C to 28 °C with an average of 26.72±0.99 °C in the fertilized pond (Table 1). pH remained neutral (pH=7) in the ponds throughout the experiment (Table 1). As for water transparency, it varied from 36.9 cm to 40.2 cm with an average of 38.86±1.23 cm in the control pond while it varied from 25.3 cm to 39.4 cm with an average of 31.26±3.76 cm in the fertilized pond (Table 1). For these three parameters (temperature, pH and transparency), the Kruskal-Wallis test revealed no significant difference ($p>0.05$).
Phytoplankton density in the pond water ranged from 701 individuals/L water to 1003 individuals/L water with an average of 751.58±135.72 individuals/L water in the control pond. In the fertilized pond, phytoplankton density ranged from 695 individuals/L water to 3033 individuals/L water with an average of 1690.72±969.89 individuals/L water in the fertilized pond (Table 1). There is a significant difference between the phytoplankton density of the water in the control pond and that in the fertilized pond ($p<0.05$).

Oyster survival and growth
Survival rate and growth parameters such as average shell length difference (ΔSL), Average Daily Gain (ADG) and Specific Growth Rate (SGR) of oysters after 42 days of rearing are summarized in Table 2. There were no significant differences for morphometric measurements, survival rate and growth parameters ($p>0.05$).

Table 2: Mean values of growth parameters of the freshwater oyster *Etheria elliptica* in the rearing ponds.

	Control pond			Fertilized pond		
Depths	30 cm	60 cm	90 cm	30 cm	60 cm	90 cm
SLi (mm)	29.93	29.93	29.73	31.06	31.46	30
SLf (mm)	30.93	30.86	30.66	34.86	34.46	32.06
ΔSL	1	0.93	0.93	3.8	3	2.06
Wi (g)	36.4	37.96	40.64	34.78	38.06	37.5
Wf (g)	37.12	38.64	40.88	35.94	39.14	38.24
WG (g)	0.72	0.68	0.24	1.16	1.08	0.74
ADG (g/d)	0.017	0.016	0.005	0.027	0.025	0.017
SGR (%.d^{-1})	0.047	0.042	0.014	0.078	0.067	0.047
Survival (%)	50±35.36	50±35.36	50±35.36	50±35.36	50±35.36	50±35.36

Oyster survival

The survival rate was the same in both ponds and for all depths. Survival was 50±35.36 after 42 days of rearing oysters in ponds (Table 2).

Average shell length: The difference in mean shell length (ΔSL) of oysters after 42 days of rearing was 1 mm for depth 30 cm and 0.93 mm for 60 cm and 90 cm in the control pond. In the fertilized pond, it was 3.8 mm, 3 mm, and 2.06 mm for depths 30 cm, 60 cm, and 90 cm, respectively (Fig 2; Table 2).

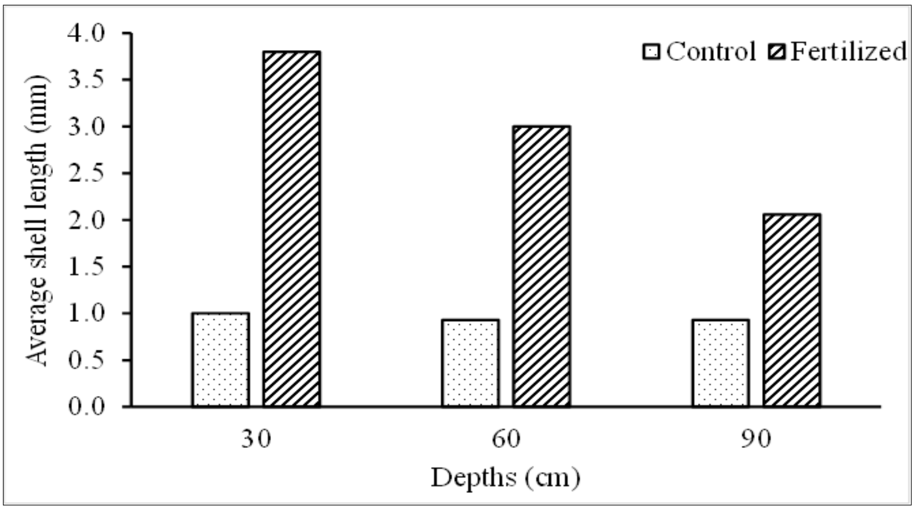

Fig 2: Shell length gain of oyster *Etheria elliptica* individuals reared in ponds at different depths for 42 days.

Average daily gain

The lowest values of daily average gain were recorded in the control pond compared to those recorded in the fertilized pond (Fig 3). For both ponds, the values of daily average gain decreased with depth (Table 2). In fact, the average daily gain varied between 0.005 g/d (90 cm depth) and 0.017g/d (30 cm depth) with a value of 0.016g/d for the 60 cm depth in the control pond (Table 2). In contrast, it ranged from 0.017 g/d (90 cm depth) to 0.027 g/d (30 cm depth) and was 0.025 g/d for 60 cm depth in the fertilized pond (Table 2).

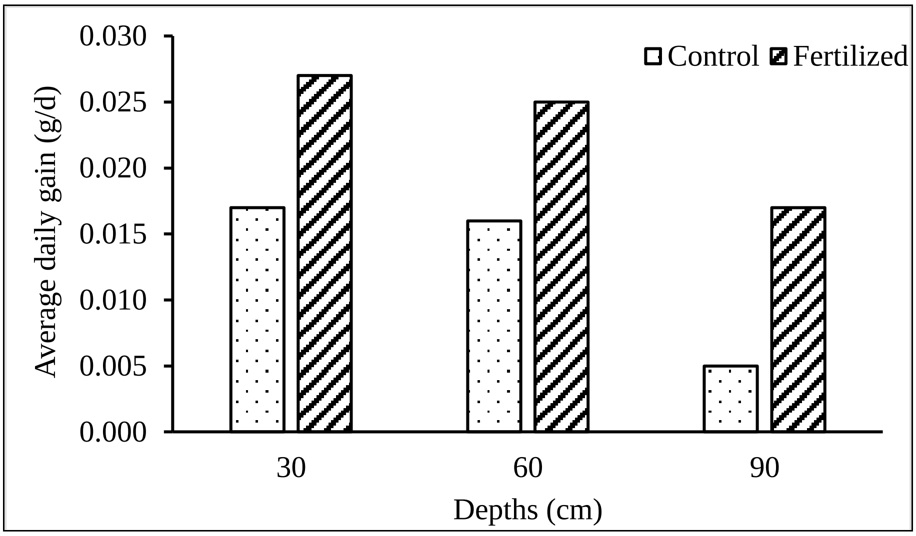

Fig 3: Average daily weight gain of *Etheria elliptica* oyster individuals reared in ponds at different depths for 42 days.

Specific Growth Rate
At the end of the experiments, the specific growth rate (SGR) values decreased with depth in both ponds (Fig 4). The highest values were recorded in the fertilized pond while the lowest were noted in the control pond (Table 2). In the control pond, SGR ranged from 0.014%.d^{-1} (90 cm depth) to 0.074%.d^{-1} (30 cm depth) with a value of 0.042%.d^{-1} for the 60 cm depth. In contrast, it has 0.078%.d^{-1}, 0.067%.d^{-1} and 0.047%.d^{-1} for depths 30 cm, 60 cm and 90 cm respectively in the fertilized pond (Table 2).

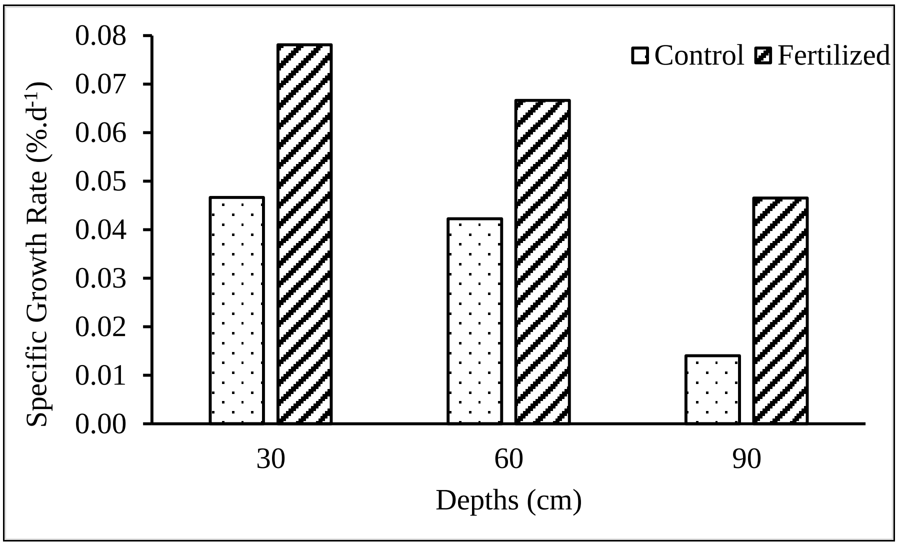

Fig 4: Specific growth rate of oyster specimens *Etheria elliptica* reared in ponds for 42 days at different depths.

Discussion
Physico-chemical parameters
The biology of oysters is conditioned by the physico-chemical parameters of the water. Parameters such as temperature, pH, water transparency and phytoplankton density were monitored during the 42 days of testing to assess their influences on oyster survival and growth. Throughout the experiment, the water temperature in the ponds varied between 25.2 °C and 28.6 °C and corresponded well to temperature values in tropical waters. The nature of the water in the ponds was neutral (pH = 7) throughout the experiment. These temperature and pH ranges recorded in the experimental ponds correspond to the ecological preferendum of tropical bivalves [2]. Consequently, oyster survival and growth were not influenced by these parameters during these trials. As for phytoplankton density of pond water, a mean value of 1690.72±969.89 individuals/L water was recorded in the fertilized pond compared to 751.58±135.72 individuals/L water in the control pond. Poultry droppings are known to be soluble in water and rich in phosphorus [25]. The use of poultry droppings in a pond would result in the release of large amounts of nutrients, particularly phosphorus, which would explain the high phytoplankton densities recorded in the fertilized pond [25, 26]. Correlatively, the low values of water transparency were recorded in the fertilized pond while the highest values were noted in the control pond.

Oyster survival and growth
After 42 days of pond rearing, the survival rate of *Etheria elliptica* specimens was 50±35.36% in both ponds regardless of depth. This survival rate is low compared to 79% obtained by [3] for a rearing trial in the Pendjari River in Benin and 97% obtained by [27] in the White Volta and Oti River in Ghana after 6 months of rearing. Indeed, among the oyster specimens used in the present study, there were free specimens (unglued shells) but also colonies of oysters (glued shells) that were detached in station. Mortalities were recorded in the first week and early in the second week and would be due to poor handling during shell peeling. Furthermore, the oyster *Etheria elliptica* is a whitewater species, the constant renewal of water in rivers contributes to a better oxygenation of juveniles and their survival in the natural environment [21, 28]. On the other hand, breeding ponds are closed environments.
Oysters showed growth in size (shell length) in the ponds regardless of depth (Fig 2). This growth was greater (ΔSL between 2.06 mm and 3.8 mm) in the fertilized pond compared to the unfertilized pond (ΔSL between 0.93 mm and 1 mm). The values of average daily gain (ADG) and specific growth rate (SGR) indicate that the growth of the cultured oysters decreases with increasing depth. Thus, the lowest values of ADG and SGR were recorded at 90 cm while the best performance was noted at 30 cm depth for both ponds (Fig 3 and 4). This trend is due to the fact that oysters are benthic species, living naturally at the bottom of the water [2, 10]. Indeed, oysters settled at 30 cm depth live near the bottom of the pond and would benefit from the best trophic and ecological conditions. However, growth is better in the fertilized pond compared to the control pond for all depths combined (Figs 2, 3 and 4). GMQ ranged from 0.005 g/d to 0.017 g/d in the control pond and from 0.017 g/d to 0.027 g/d in the fertilized pond. As for SGR, it was between 0.014%.d^{-1} and 0.047%.d^{-1} for the control pond while it varied between 0.047%.d^{-1} and 0.078%.d^{-1} in the fertilized pond. The better growths recorded in the fertilized pond could be attributed to the availability of food due to high primary production [25, 26]. Indeed, the oyster *Etheria elliptica* is planktonophagous with a diet based mainly on benthic diatoms (Eunotia bilunaris and Coscinodiscus rudolfii) (65.84% IP) as preferential foods [2]. Consequently, the availability of phytoplankton induces better linear and weight growth of the bivalve (Vaughn *et al*., 2008) [2].
Further studies are needed to determine if poultry droppings fertilization leads to the production of the oyster's preferred phytoplankton species. However, diet studies of *E. elliptica* in the Pendjari River revealed the consumption of 64 food types,

indicating opportunistic and plastic feeding behavior [29]. The plasticity of its diet constitutes an asset for the promotion of its rearing in controlled environment, in this case freshwater ponds.

Conclusion

This study showed that the culture of the freshwater oyster *Etheria elliptica* in freshwater ponds is a good opportunity for fish farm promoters in Benin, on the one hand, and in Africa, on the other. The best growth performances were obtained at depths of 30 cm and 60 cm. Fertilization of the ponds with poultry droppings improved the growth performance of the reared specimens through a better production of phytoplankton, the main food source of the oyster *Etheria elliptica*. Trials with free-living, non-housed oyster juveniles will result in higher survival rates.

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