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## **Reproductive Performances of African Catfish *Clarias gariepinus* according to the Type of Hormones and Substrates in Recycled Water in Southern Cameroon**

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### **Authors' contributions**

*This work was carried out in collaboration between all authors. Author CTT designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors DAEA and PN managed the analyses of the study. Author METT managed the literature searches. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aims:** Improve production of *Clarias gariepinus* fry through the use of local materials.  
**Place and Duration of the Study:** From 1<sup>st</sup> April to 09<sup>th</sup> May 2016 at the Akak Essatolo Fish Farm in Ebolowa, South Region Cameroon.  
**Study Design:** 10 broodstock of *C. gariepinus*, were used for the artificial reproduction. Pituitary extract and Ovaprim hormones were used to induce oocytes maturation on females. Eggs were obtained by the abdominal pressure of the female. Wicks (or Local raffia fibbers (*Raphia regalis*)) and mesh frame were used as incubators of fertilised eggs. 12 experimental batches each consisting of 50 g of fertilised eggs were spread in triplicates on both types of incubators previously arranged in closed-circuit tanks.  
**Methodology:** Fertilized eggs were enumerated by direct observation. At the end of the hatching (D0) and of vitelline resorption (D3), larvae of each experimental lot were counted.  
**Results:** Similar ( $P = .05$ ) absolute and relative fecundities used were recorded in all treatments. Female eggs induced with pituitary extracts and incubated on raffia fibbers recorded lower ( $P < .05$ ) fertilisation and hatching rates. All other treatments were comparable ( $P = .05$ ) for these parameters. Deformed larvae rates were comparable ( $P = .05$ ) for all treatments. Survival rates at the end of yolk sac resorption (J3) were higher ( $> 70\%$ ) in all treatment. However, treatment with Ovaprim and wick showed a survival rate (71.1%) significantly ( $P < .05$ ) lower than the other treatments ( $> 80$ ); which have otherwise remained comparable ( $P = .05$ ).  
**Conclusion:** The superiority of ovaprim at the beginning of reproduction is offset by the poor survival rate, which is better with the pituitary gland. It is therefore concluded that the use of the synthetic hormone is not economical for optimal production of *C. gariepinus* fry. In the same way a mastery of the use of the raffia fibres will improve the cost-effectiveness and consequently will decrease the production costs.

**Keywords:** Hormone; substrate; reproduction; fry; *Clarias gariepinus*; Cameroon.

## 1. INTRODUCTION

Africa has a great geo-climatic diversity resulting in the existence of a great potential for endogenous fish resources, of which only a small proportion is currently known and valued [1]. It is the case of African catfish *C. gariepinus* which is an endemic species in Africa, remains one of the most suitable species for African aquaculture [2]. *C. gariepinus* is a highly-valued species in aquaculture for its hardiness, its omnivorous diet, its fast growth, and the quality of its flesh [3]. Although this catfish has been domesticated for more than 40 years, its production has stagnated in Africa [4]. It was around the 1970s to 1980s that the basic techniques of artificial reproduction and feeding *C. gariepinus* began, allowing the development of semi-intensive and intensive of this species [2] through the recycled water. Recirculated fish farming systems have been developing for about 20 years, and their area of use is gradually expanding from the hatchery (breeders, eggs, larvae and fry) to the growing phase [5]. The production of catfish species faces a number of difficulties including poor control of larval rearing, which is a determining phase in cat-fish production. In some fish farming stations where *C. gariepinus* farming is a common activity, larval rearing does not

always achieve the expected results due to deficiencies in the control of environmental conditions (temperature, light, shelters, oxygenation, pH, nitrites, and ammonium) and artificial reproduction techniques [3]. Indeed, artificial reproduction as practised in fish farms in Cameroon still presents many challenges, including types and doses of hormones, as well as the incubation substrates. In Cameroon, existing synthetic hormones such as acetylated deoxycorticosterone (DOCA), Ovaprim (LHRHa or GnRH $\alpha$ ) and human Chorionic Gonadotropin (hCG) are commonly used to induction spawn in fish. Although they have been effective in improving results of artificial reproduction, their main disadvantages have been the high costs relative to the value of fish and can cause severe ulcers in injected females [6]. Pituitary extracts from animals such as fishes (carp, tilapia, perch, and catfish) and frogs are also used [7,8,6 and 9]. Nowadays, the hCG hormone is rarely used because of the long latency and its numerous failures [10 and 11]. Even though the pituitary extraction method is cheap, it is less used because of the lack of brood fishes to sacrifice [12].

However, the racks (or mesh frames) made using local materials available in Cameroon is

mostly common for egg incubation and give very good results. Moreover, other free local incubators such as plastic recovery bowls [9,10], the roots of water yacinths *Eichhornia crassipes* [9,13,4 and 14] or water lettuce *Pistia stratiotus*; brushes fibres or « kakaban » floating on the water [6,2] also give better results. The objective of this study was to improve production of *C. gariepinus* fry through the use of local materials at the Akak Essatolo Fish Farm in Ebolowa. Specifically, it was a question of determining, according to the type of hormones and the type of incubation substrates, the fecundity, fertilisation, hatching, deformed larvae and larval survival rates at vitelline resorption.

## 2. MATERIALS AND METHODS

### 2.1 Study Area

This study was conducted from 1st April to 09th May 2016 at Akak Essatolo, located in Ebolowa 1<sup>st</sup> District, Department of Mvila, South Cameroon: 02°48'993" N, 10°56'078" E. The relief consisted mainly of hills, and marshy lowlands dominated by *Raphias regalis* belonging to the Arecaceae family [15]. The climate was warm and humid equatorial type (Guinean), characterised by a short rainy season that extended from mid-March to June; a short dry season that runed from July to August; a long rainy season from September to mid-November and a long dry season from mid-November to mid-March. The average temperature was 25°C. Annual rainfall ranged from 1200 to 2000 mm [16].

### 2.2 Livestock Infrastructure

The assay was performed in a hatchery consisting of a surface building: 5 \* 3 m<sup>2</sup>. It includes 06 closed-circuit containers with a volume of 0.4 m<sup>3</sup> each, in which the reproduction, incubation and larval rearing operations were carried out. This device is fed by gravity by means of a fountain supplied by a pump, having a flow rate of 4 m<sup>3</sup> / h, itself fed by a well of 5 m deep. For this assay, each container was divided into 2 equal parts using a mesh screen of 0.5 mm mesh allowing easy circulation of water.

### 2.3 Assay Conduct and Data Collection

#### 2.3.1 Animal material

Ten broodstock of *C. gariepinus*: 5 females (mean weight = 2. 24 ± 200 kg) and 5 males (mean weight = 1. 2 ± 156 kg) (Table 1) from artificial propagation on the farm and raised in a

circular tray were selected according to the criteria used by Tiogué et al. [10]. Thus, the selected females presented: an inflated and soft belly, a protruding, reddish or pinkish urogenital papilla and whose yellowish or greenish oocytes, were easily obtained by slight pressure of the abdomen, with an oocyte diameter of at least 1.2 mm. The males chosen were the most vigorous and weighed > 200 g. The selected broodstock was weighed using a 0.5 kg precision kitchen scale and was then housed individually in basins at an average temperature of 26°C for 24 hours.

#### 2.3.2 Hormonal induction

Only females were induced. Each female received a single dose of hormone. After weighing each female and recording her weight, the hormone Ovaprim was administered to each with a 1 ml syringe at a rate of 0.5 ml / kg body weight (Table 1) in the dorsal muscle of the animal, as suggested by Tabaro [13] and Tabaro et al. [4].

All 5 selected male broodstock were sacrificed and their pituitary glands were extracted [17]. Each pituitary gland was crushed in a mortar, and diluted in 1 l of NaCl solution at 9 ‰ [17]. The pituitary extract which came from males of *C. gariepinus* having a weight greater than or equal to that of the female to be injected (Table 1) was administered according to the method used by Tiogué et al. [10]. Each female thus treated was stored individually in a basin, numbered and covered with sheets of aluminium sheet.

#### 2.3.3 Harvest gametes

The gonads of previously sacrificed males were removed entirely (total gonadectomy). All 10 testes were cut with blade, and pressed into a mortar containing 5 ml of physiological solution (NaCl at 9 ‰) for extracting milt. The milt thus obtained was stored in a freezer at 7°C until oocyte maturation [13,4]. Oocyte maturation occurred after 12 h 30 min and 14 h following the hormonal injection, respectively for pituitary extract and Ovaprim.

Oocytes of each female were collected by abdominal pressure from top to bottom, depending on the stripping method used by Tabaro [13] and Tabaro et al. [4], using plastic basins. These oocytes were then weighed using an electronic balance (d = 0.1 g). Oocytes from females induced by the same type of hormone have been mixed in a single bowl.

**Table 1. Characteristics of female broodstock and hormone dose received by each**

N°	Female	Oocytes		Hormone dose to inject
	TW (Kg)	Colour	D (mm)	
<b>Ovaprim</b>				
1	3.5	Yellowish	1.4	0.5 ml/kg
2	2.5	Yellowish	1.3	0.5, 0.5 ml/kg
<b>Pituitary extracts</b>				
3	3.4	Dark green	1.3	3 males, TW 3.5 kg (1.5+1+1)
4	1	Yellowish	1.3	1 male of 1 kg
5	0.8	Yellowish	1.3	1 male of 900 g

N°= Number, TW=Total weight, D = Diameter

### 2.3.4 Fertilization and incubation of eggs

The milt previously preserved has been divided into 2 parts. Each part was mixed with oocytes from females induced by the same type of hormone (Ovaprim or pituitary extract) until a homogeneous mixture is obtained. Then a rinsing solution (4 g of salt in 1 liter of water) was used to rinse the eggs for 60 seconds. The addition of this solution to eggs was noted as the time of fertilisation. 1 bowl containing 0.2 g of non-inseminated eggs and mixed with water was prepared for each type of hormone and served as a control. The time taken by the eggs of the control bowl to become white was noted after which all the other eggs were considered fertilised. Fertilised eggs were enumerated by direct observation.

Then, 12 experimental batches each consisting of 50 g of fertilized eggs (i.e  $33.825 \pm 2085.96$  eggs from the females induced by the two hormones) were spread in triplicates on both types of incubators previously arranged (with similar amount of surface area for egg attachment and set horizontally), in closed-circuit tanks (water height = 0.2 m and water flow rate = 6.4 ml / s). These incubators are:

- 3 incubation screen (mesh frame or screen designed with a synthetic mesh of rectangular shape bordered by a wooden frame): (50 x 30 cm<sup>2</sup>, 1 mm mesh), each carrying 50 g of eggs of females induced with the pituitary extract.
- 3 incubation screen (mesh frame or screen designed with synthetic mesh of rectangular shape bordered by a wooden frame): (50 x 30 cm<sup>2</sup>, 1 mm mesh), each carrying 50 g of eggs of females induced with the Ovaprim hormone.
- 3 wicks (local raffia fibbers (*Raphia regalis*)) each carrying 50 g of eggs of females induced with the pituitary extract.

- 3 wicks (local raffia fibbers (*Raphia regalis*)) each carrying 50 g of eggs of females induced with Ovaprim.

Each closed-circuit containers received two incubators.

The water temperature defined the incubation time after which the hatching rate of each lot was evaluated: the hatched larvae of each experimental lot were estimated according to the method proposed by Cacot [2]. Larvae with 2 heads, two tails, alive and immobile or dead were counted with the naked eye and were considered as deformed larvae. At the end of the hatching (D0), 50 normal larvae were taken from each batch using a net of 0.5 mm mesh, then placed quickly on the millimetric paper for the measurement of the total length.

### 2.3.5 Larval rearing up to the vitelline resorption (D3)

Following hatching (D1), 12 lots of  $10.000 \pm 69.22$  larvae each were distributed in triplicates in six closed-loop trays of 0.4 m<sup>3</sup> (that is 0.02 larvae / l). Water in the system was renewed at 50% / 24 h and the recirculation flow rate was 8.6 ml / s. The experimental device was equipped at the outlet with an overflow, the detachable part of which was covered with a net of 0.5 mm mesh. The moment at which the larvae showed an exogenous desire for nutrition, at the end of vitelline resorption (D3), was considered as the time of first nutrition. To evaluate the survival rate on D3, the total number of larvae was estimated according to the method proposed by Cacot [2].

## 2.4 Studied Characteristics

- Absolute fecundity (aF)

It is calculated according to the formula: aF = number of oocytes / g of oocytes laid.

- Relative fecundity (rF) or number of oocytes / kg of body weight of the female, is calculated according to the formula:
- $rF = (Pe \times Fa) / Pa$  where Pe is the weight of oocytes laid in g = Pa - Pf; with Pa (g) = weight of the female before laying and Pf (g) = weight of the female after laying.
- Fertilization rate (%) =  $(Nof / Noi) \times 100$  with Nof: number of fertilized oocytes and Noi: total number of incubated eggs.
- Hatching rate (%) =  $(Ne / Noi) \times 100$  where Noi is the total number of eggs incubated and Ne = the total number of hatching larvae.
- Survival rate of larvae at 3 days of age (%) =  $(NL3 / Ne) \times 100$  where Ne is the number of larvae at hatching and NL3 is the number of larvae after 3 days rearing.

## 2.5 Statistical Analysis

Data collected were collated and analysed using descriptive statistics (mean, standard deviation and percentage). Statistical comparison of fecundity between hormones was carried out using t-student test. Fertilisation, hatching, deformed larvae and survival rates were subjected to two-way analysis of variance (ANOVA II) between hormones and substrates. The analysis model was as follows:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \epsilon_{ijk}$$

Where  $\mu$ : Mean of the parameter considered;  $\alpha_i$ : Effect of type of hormone,  $\beta_j$ : effect of substrates and  $\epsilon_{ijk}$ : residual error.

Duncan's multiple test was then used to separate the averages when the differences were significant at the 5% probability level. All statistical analyses were performed using statistical software SPSS 20.0. The Excel software 2013 spreadsheet was used to draw the graphs.

## 3. RESULTS

### 3.1 Effect of the Type of Hormone on the Fecundity of *C. gariepinus*

Table 2 shows the fecundity according to the type of hormone used during the test. Females

induced by Ovaprim recorded similar ( $P = .05$ ) absolute and relative fecundities than those females treated with pituitary extracts of *Clarias*.

### 3.2 Fertilization and Hatching Rates according to the Type of Hormones and Substrates

Fertilization and hatching rates of *C. gariepinus* presented in Fig. 1 show that these parameters were significantly low ( $P < .05$ ) in females induced by pituitary extracts and whose eggs were incubated on wicks. In addition, female eggs induced by pituitary extracts and incubated on a rack have registered similar fertilization and hatching rates ( $P = .05$ ) to those of females induced by ovaprim regardless of the incubation substrate. The interaction between the hormone type and the substrate type have a small effect on these parameters.

### 3.3 Deformed Larvae Rates at Hatching by Type of Hormones and Substrates

Fig. 2 illustrates the deformed larvae rate at hatch obtained in each treatment. The results were relatively comparable ( $P = .05$ ) in all treatments. In addition, regardless of the hormone used, incubation on a rack showed similar ( $P = .05$ ) performance to those on the wick. The interaction between hormone type and substrate type do not bring significant information to explain the variability ( $P = .05$ ).

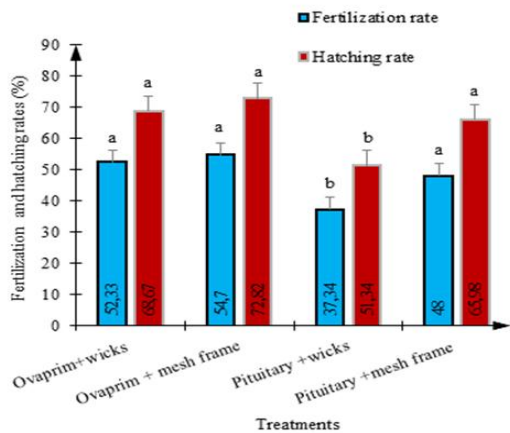
### 3.4 Survival Rate for Vitelline Resorption (D3) according to the Type of Hormones and Substrates

Survival rates reported of the resorption of the yolk sac (J3) are illustrated in Fig. 3. They were significantly ( $P < .05$ ) higher (> 70%) regardless of the treatment considered. However, treatment with Ovaprim with wick recorded a survival rate significantly ( $P < .05$ ) lower than other treatments, which are otherwise comparable ( $P = .05$ ). Moreover, whatever the hormone considered, incubation on rack showed the best survival rate ( $P < .05$ ) compared to that of wick. The Ovaprim hormone have a negative effect on survival rate, the substrate type and interaction between hormone type and substrate type have a small effect on the survival rate.

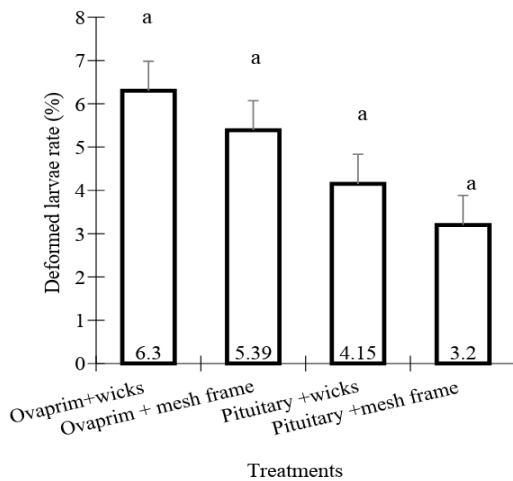
**Table 2. Effect of the type of hormone on the fecundity of *C. gariepinus***

Fecundity	Hormones		P value
	Ovaprim	Pituitary extracts	
aF (nog of oocytes laid)	676.5 ± 41.7 <sup>a</sup>	640.66 ± 9.07 <sup>a</sup>	P = .05
rF (nokg of female weight)	457 500 ± 94 045.2 <sup>a</sup>	189 333 ± 236 256.9 <sup>a</sup>	P = .05

a: values in the same line with the same alphabetical letter are not significantly different (P = .05). aF = absolute fecundity; rF = relative fecundity, nog = number of oocytes / g; nokg = number of oocytes / Kg

**Fig. 1. Effect of the type of hormone and substrate on fertilization and hatching rates of *C. gariepinus***

a, b : vertical bars with the same alphabetical letter are not significantly different (P = .05)

**Fig. 2. Effect of the type of hormone and substrate on deformed larvae rate at hatching**

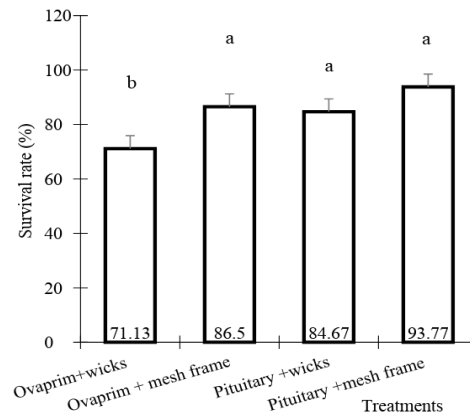
a, b : vertical bars with the same alphabetical letter are not significantly different (P = .05)

#### 4. DISCUSSION

The observed absolute fecundity values are similar to those of 631 and 642 eggs / g obtained

by Tabaro et al. [13], of 453.3 and 781.77 eggs / g reported by Okere et al. [18] in the same species under controlled conditions for pituitary extract and Ovaprim respectively. Relative fecundities are different from that (99.897 and 105.541 / kg) reported by Tabaro et al. [13] and those of (20.978.4 and 36.086 / kg) Okere et al. [18] for the pituitary gland and Ovaprim, respectively. The high values obtained for relative fecundity are related to the very high live weight (mean weight = 2.24 kg) of females, since Bromage et al. [19] already showed the significant linear variation in the number of eggs with the unit weight of females. The incubation success (< 60%) obtained would be due to the decrease in oxygen level (pump shutdown) caused by repeated power cuts. These results are lower than those recorded by Ndimele and Owodeinde [20]: 72 and 88% respectively for the pituitary and Ovaprim. However, they remain close to values obtained by Tiogue et al. [10] with domestic and wild strains of *C. gariepinus* (30.81 and 45.4%). The significantly lower fertilization and hatching rates of female eggs induced by pituitary extracts and incubated on raffia fibers compared to female ovaprim-inducing females and incubated on the same incubator would be related to an environmental problem (such as the water circuit which was interrupted for some time because of the power cut) in the incubation basin. However the high hatching rate (> 65%) recorded in the other treatments were comparable to those commonly observed in *C. gariepinus*: 67.42 and 71.76% [19], respectively for the pituitary gland and Ovaprim. In addition, the work of Tabaro et al. [13], report similar results to the average values of fertilisation and hatching rates on wicker and wick, by incubating *C. gariepinus* eggs on a wire frame (fertilisation rate: 65.67%, hatching rate: 44%). In the other hand, these results were very low compared to those reported by Natea et al. [21] from the same species exposed to different piscine pituitary and synthetic hormone (74.9 - 84.3 % and 51.5 - 73.3% respectively for fertilisation and hatching rates). The difference between this report and the present study could be due to differences in the condition at which

eggs incubated and hatchery facilities. The rate of deformed larvae was higher, with the hormone Ovaprim (wicking: 6.3%, tick: 5.39%), then with wicking incubation. Tiogu  et al. [10] reported similar results for domestic and wild strains of *C. gariepinus* (6.44 and 3.47%) using pituitary extracts and hCG hormones. However, Okere et al. [18] reported lower values: 0.1 and 0.9% for Ovaprim and pituitary extract respectively, in plastic recovery bowls. These high levels of deformed larvae are thought to be due to poor initial gamete quality [22], or to the young age of broodstock that was only in their first reproductive cycle at the beginning of this test. In addition, literature suggested that using synthetic or non- synthetic hormones ensure availability of matured quality eggs and ensuring good and viable milt production for commercial fish farming [23]. Survival rates of larvae at resorption of yolk sac are similar (> 70%) to those commonly found in *C. gariepinus*, at this stage of development: 81.9 and 77.7% by Okere et al. [18] for Ovaprim and pituitary gland respectively; 84 to 96.66% by Tiogu  et al. [10], for wild and domestic strains for pituitary extracts and hCG hormone. The results obtained with the incubation on the rack are greater than those recorded on wick; this would be because the wicking incubation system involves several risks that appeared during the test (detachment of the tongs, readjustment with respect to the height of the water, etc.). However, the use of wick is in all cases less expensive because raffia fibers are free and are permanent in the structure, unlike the mesh frames that are made on the local market. Nevertheless, good precautions in their use could make them better. In general, there is a predominance of Ovaprim on the pituitary, in terms of: Fecundity and hatching. According to Nwokoye et al. [24], this is explained by the fact that the GnRH analogues have better potential than the natural hormones. Similarly, Zohar [25] reports that GnRH analogues are more advantageous than native GnRH, because of their resistance to enzymatic degradation, after prolonged hormonal stimulation in mature females. However, results regarding deformed larval and survival rates after vitelline resorption show the superiority of the pituitary gland. According to Ajah [26], larval survival depends not only on factors such as: diet, pH, temperature, dissolved oxygen, and ammonium, nitrite and nitrate levels; but also on the type of hormone. Moreover, Okere et al. [18] indicate that the high mortality observed in larvae is due to the presence of toxic substances in Ovaprim.



**Fig. 3. Effect of the type of hormone and substrate on the survival rate of vitelline resorption**

a: vertical bars with the same alphabetical letter are not significantly different ( $P = .05$ )

## 5. CONCLUSION

The main results of this study are:

- Absolute and relative fecundities were comparable regardless of the hormone considered. So, due to its relatively cheaper cost, pituitary extract should be recommended to fish farmers.
- Eggs of females induced with pituitary extracts and incubated on wick recorded significantly low fertilization and hatching rates.
- Rates of deformed larvae were high and comparable between hormones or incubation substrates. Survival rates were significantly higher for female eggs induced by pituitary extracts regardless of substrate type.

In view of these results, the hormone Ovaprim expresses the best performances at the beginning of reproduction. But, this advantage is offset by poor survival rate, which is better with the pituitary gland. Therefore, it is clear that the use of the synthetic hormone is not economical for optimal production of *C. gariepinus* fry. In the same way a mastery of the use of the raffia fibres will improve the results and consequently will decrease the production costs.

An experiment on the effects of the physical and chemical characteristics of local incubators (raffia fibres and synthetic mesh) on fertilisation and hatching rates in this species would complete this study.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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## APPENDIX

### Physico-chemical parameters recorded during the test

Hormones	Temperature	pH	NO <sub>2</sub> <sup>-</sup>	NH <sub>4</sub> <sup>+</sup>
Ovaprim	27.28 ± 0.82 <sup>a</sup>	7.46 ± 0.05 <sup>a</sup>	0.22 ± 0.005 <sup>a</sup>	0.03 ± 0.009 <sup>a</sup>
Hypophyse	27.23 ± 0.66 <sup>a</sup>	7.42 ± 0.08 <sup>a</sup>	0.23 ± 0.008 <sup>a</sup>	0.04 ± 0.007 <sup>a</sup>

*a: values in the same line with the same alphabetical letter are not significantly different (P=.05)*

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