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Controlled reproduction test of the African catfish *Clarias gariepinus* (Burchell.1822) in captivity, at the Saharan hatchery of Ouargla.

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Informations	Abstract
Keywords : Reproduction, Ovaprim, Fish, Ouargla, Captivity. *Correspondance : ibrahim.behme@univ-mosta.dz	The objective of the study is to master artificial reproductive techniques, in the simulation of ovulation and the response to spawning of the African catfish <i>Clarias gariepinus</i> at the Saharan hatchery of Ouargla for the selected species using the hormone «ovaprim» at different doses as an inducing agent. For this purpose, two mature females and two males weighing from 0.8kg to 2.3 kg were selected for two different doses of hormonal injection. Females (T1) and (T2) received a single dose of 0.4 and 0.5 mL Ovaprim /kg body weight, respectively. After 9.5 and 10.37 h of latency time, the females were individually (stripping) in dry plastic containers, and pre-weighed to record the weight of the recovered egg. The ovulation rate was observed at (100%) in both hormone injection doses. Reproductive performance is evaluated on the basis of total egg mass, pseudo-gonad somatic index (PGSI %), latency time, fertilization rate, and hatching time are examined on the basis of the two administered doses. The results indicate that the total weight of skinned eggs is higher in individuals who received 0.4 ml of Ovaprim/kg body weight, compared to those who received higher doses. The average weight of eggs collected is estimated at T1=152.2 g, and T2 = 97.4 g. The highest percentage of pseudo-gonad somatic index (PGSI %) (11%), the highest fertilization rate 85.56 ± 3.7 %, the shortest latency time 9:50 h and hatching time 18:30 h were recorded in subjects who received 0, 4 ml Ovaprim/kg body weight. As a result, our results indicate that Ovaprim at a dose of 0.4 ml/kg body weight may be used as the optimal reproductive dose appropriate for <i>C. gariepinus</i>.

1. Introduction

Aquaculture is a for-profit business. In addition to inputs, the quality and quantity of fish produced are determinants of the company's profitability.

The African catfish, *Clarias gariepinus*, is the most sought-after farmed fish species in Africa, commercially farmed using expensive synthetic hormones, one of which is Ovaprim.

The objective is to master the artificial reproduction technique, in the simulation of ovulation and the

response to spawning of the African catfish *C. gariepinus* using the hormone «ovaprim» at different doses as an inducing agent.

2. Materials and methods

2.1. Presentation of the study environment

The experiment took place in June 2018, at the Aquaculture Development Saharan Station, which is located 820 km south of the capital in the commune of Hassi ben Abdallah 25 km from the chief town of the willaya of Ouargla (Fig. 1).

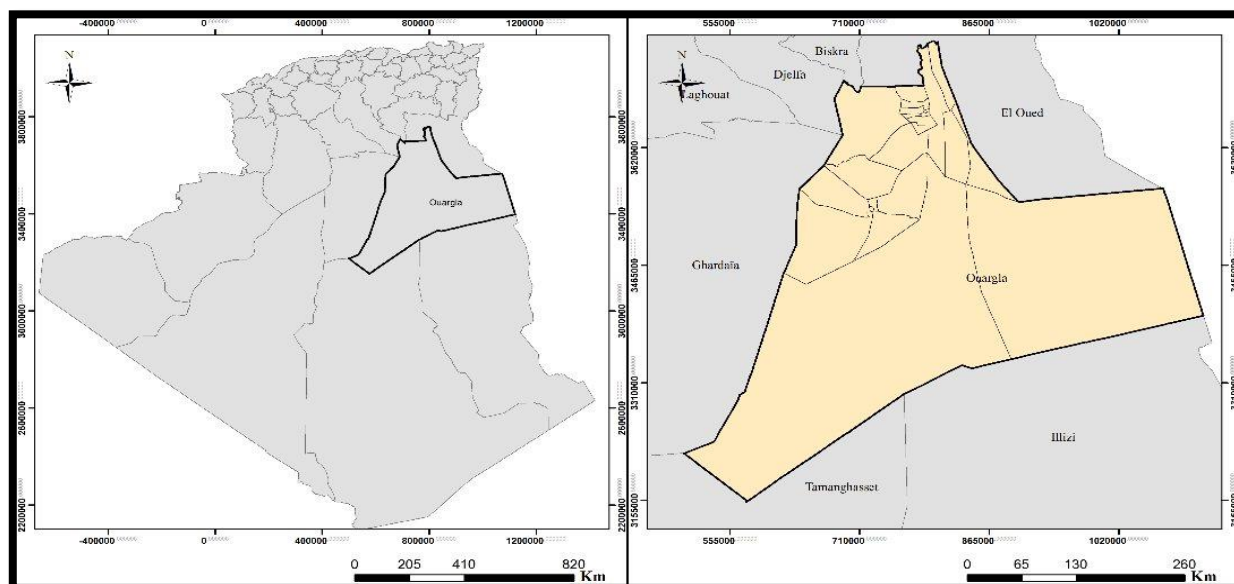


Fig. 1. Geographical location of Ouargla (BEHMENE ,2020)

Table 4: Hormonal treatment of broodstocks

N°	broodstocks	Number of individuals	The hormone used	Injections (ml / kg)	Body weight (g)	Total dose of hormone (ml)	Length (cm)
T1	female	1	Ovaprim	0.4	1294.5	0.52	59
T2	female	1	Ovaprim	0.5	1152	0.58	54
T3	male	1	Ovaprim	0.25	2225	0.56	/
T4	male	1	hormone-free	0	823.3	0	53

T*: Hormonal treatment of genitures.

2.2. Selection of broodstocks

African catfish *Clarias gariepinus* are used, live and healthy during the breeding season (May-June). They were identified on the basis of their external morphological characteristics (Fig. 2).

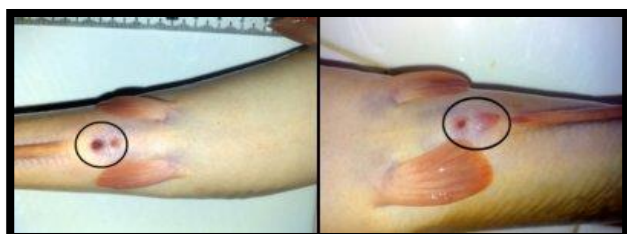


Fig. 2. A female (left) ; male (right) individual of *C. gariepinus* (Burchell, 1822) (BEHMENE ,2020)

2.3 Management of broodstocks

The selected broodstock were disinfected with 0.15 ml/10 L formaldehyde and potassium permanganate

for 6 hours, and fed into circular housing ponds for 48 hours without feed.

2.4 Synthetic hormones (Ovaprim)

Ovaprim is a commercial product that contains 10mg/ml of a gonadotropin releasing hormone from salmon (GnRHa).

2.5 Experimental design

For each dose of ovaprim hormone, (01) a single selection test was conducted to determine the response to spawning. A total of two females and two males were used in this study. Injection doses are shown in (Tab. 4):

To stimulate female ovulation, females were weighed individually. Ovaprim was injected at a dose of 0.4 ml for the female (T1) and 0.5 ml/kg for the female (T2) according to Legender , 1986; Madu, 1989). The Females of each treatment were kept

separately. To stimulate spermiation, the hormone Ovaprim was injected for a dose of (0.25 ml/kg) for the male progenitor (T3) but the male progenitor (T4) without hormonal injection.

All hormones were administered intramuscularly over the lateral line to the dorsal section and directed to the ventral surface. The dose of ovaprim depends on the weight of the broodstock. (Nwokoye et al., 2007).

2.6 Hormone injection for broodstock

The injection is made in the evening at 23:30 h and the fish are kept in the pond in aerated stall until the end of the experiment.

2.7 Housing

The injected female is kept alone in water with a temperature of 28°C. To determine latency using the degree hour formula, corresponding to 300 (Monsengo, 2017).

2.8 Harvest of Milking

The males were sacrificed and the testicles were removed, macerated and pressed in a saline physiological solution (NaCl 0.9%) at the rate of 1 g of testis per 5 ml of saline solution. Kept in refrigerator (5°C) until used for fertilization (Phelps et al. 2007).

2.9 Manual stripping of eggs

After the positive reaction of the female to the injection, the eggs are easily released from the genital papilla. To extract the eggs, the female was carefully removed using a dip net and carefully held by two people with wet wipes and then a soft press was made at the flanks until the first drops of blood appeared.

2.10 Fertilization of eggs

The eggs released by the female are fertilized by uniform sprinkling of drops from freshly dissected testicles. Total eggs were fertilized with 2.5 ml of sperm suspension per 100 g of eggs. A 0.9% saline solution (Na Cl) (Woynarovich and Horvath, 1981) was added, causing mild sperm movement, activating fertilization. After 45 seconds to a minute, no further fertilization will take place as the sperm are no longer mobile. To activate the sperm, a small amount of clean water was immediately added to the container equal in volume to the eggs.

2.11 Incubation of fertilized eggs

Immediately after fertilization, the eggs are spread in a single layer on the incubation frames (0.5 mm mesh) immersed in water (Fig. 3).



Fig. 3. 100 L incubation aquariums

The incubation is carried out in well aerated water in six (06) aquarium filled with 100 liters of water, the water level in the tank is adjusted with the position of the springboard (Table 5).

Table 5: Distribution of aquariums by injected dose

	Dose of 0.5 ml/l			Dose of 0.4 ml/l		
Aquariums	A1	A2	A3	A4	A5	A6
Number of eggs	50g	25g	20g	50g	50g	50g

A*: Aquarium

The water flows slowly, incubating in an open circuit system to obtain a well-oxygenated medium and not disturb the eggs. Incubated eggs are kept in six separate aquariums with a water temperature of 28°C. After 24 hours of incubation, the nylon fillet is removed. Egg incubation is carried out according to the method described by Brzuska and Adamek (1999), after hatching the embryos penetrate through the hatching dish nets and gather in the corner of the aquariums as to the shells of dead eggs, and deformed embryos, they stay on the surface.

2.13. Reproduction performance parameters

The analysis of reproductive performance induced *Clarias gariepinus* in our case concerns only four (04) parameters, namely: ovulation rate (%), latency time (heurs), pseudo-gonado-somatic index (PGSI %), and fertilization rate (%).

2.13.1. Ovulation Rate

$$\text{Ovulation rate (\%)} = \frac{\text{Number of ovulated females}}{\text{Number of injected females}} * 100$$

2.13.2. Latency Time (h)

Latency time (h): this is the period from injection to ovulation.

2.13.3. Pseudo-gonado-somatic index (PGSI%):

$$\text{pseudo - gonado - somatic index (PGSI\%)} = \frac{\text{total weight of eggs}}{\text{total weight before stripping}} * 100$$

The aim of this index is to evaluate the production of eggs induced by the hormone at different doses.

2.13.4. Fertility rate

The fertilization rate is calculated by random sampling by examining three samples from each incubation basin. Fertilized eggs counted to calculate the percentage of fecundity.

$$\text{Fertility rate} = \frac{\text{No. of fertilized eggs}}{\text{Egg Sample Number}} * 100$$

(Alam et al. 2006).

Fertilization rates from the two ovaprim doses used (0.4 and 0.5 ml/kg) are compared with the use of the reduced gap (t) test at the 0.05 significance rate using the following formula (Lamotte, 1971).

$$t = \frac{|q_1 - q_2|}{\sqrt{Qe(1-Qe)(\frac{1}{n_1} + \frac{1}{n_2})}} \text{ Avec } Qe = \frac{n_1 q_1 + n_2 q_2}{n_1 + n_2}$$

The calculations are performed by programming under Excel 2016.

3. Results and discussion

3.1. Selection of spawners

The successful breeding choice is confirmed by certain criteria in particular: a protruding urogenital papilla, a good dilatation of the abdomen emitting a few ovules under a slight pressure in the female and a good corpulence in the male.

3.2. Selection of broodstock

The diameter of each oocyte was measured with a binocular magnifier, where 90% of the oocytes were found to have a diameter greater than 1.05 mm. The mature ovaries are brown-green and generally uniform in size (Ayinla et al., 1994).

3.3. Collection of testicles and semen

After dissection of the sacrificed male, the identification of the testicles is done what allowed to recover a quantity of sperm of (1ml), although this sire received a hormonal injection (Table. 6).

Table 6: Amount of Semen by Dose

treatment	Hormone dose (ml/kg)	Weight (g)	Amount of semen (ml)
T3	0.25	2225	1
T4	hormone-free	823.3	4

T*: Hormonal treatment of genitures.

As a result of these results (Table. 3), another male (T4) reared at the experimental station was sacrificed, and the amount of semen collected was (4 ml) although its weight was less than the spawner (T3) and received no hormonal dose. Hogendoorn (in Janssen, 1985) estimated that one ml of milk is sufficient to fertilize 50 kg of eggs. The use of hormones can produce poor results if the

progenitors are not well conditioned. Under such conditions, partial or no egg-laying may occur and others may not respond to hormonal treatment even if they are in relatively good condition (Piper et al., 1982).

3.4. Ovulation rate

The percentage of ovulation is 100% in terms of ovulation quality; it is obvious that Ovaprim is more effective in ovulation induction in *C. gariepinus* (Table. 4).

3.5. Number of eggs

In our 1g sample of eggs, 650 to 680 eggs are present, which is consistent with the results reported by (Janssen, 1985). The total number of eggs for the 0.4 ml/kg dose is 98,930 eggs and for the 0.5 ml/kg dose is in the order of 63,310 eggs (Table. 7).

Table 7: Results of artificial reproduction as a function of hormonal doses

treatment	Hormone dose (ml/kg)	Quantity of eggs (g)	Ovulation rate (%)	Latency time (h).	No. of eggs
T(1)	0.4	152.2	100	9h et 50 min	98 930
T(2)	0.5	97,4	100	10h et 35min	63 310

3.6. Latency time:

The latency time (Fig. 4) is 9 h 50 minutes, which is lower in the subject who received a dose of 0.4ml/kg. The maturation of the eggs in the ovary requires water absorption and swelling of the female's belly during ovulation.

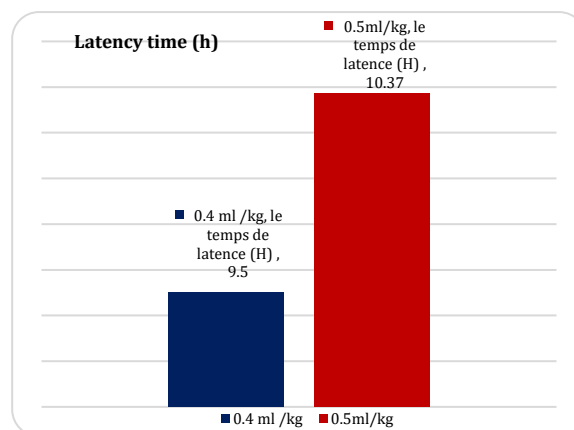


Fig.4. Latency time versus time

3.7. Pseudo-gonadosomatic index (%)

The purpose of this index is to assess hormone-induced egg production at different doses. In (Fig.5), the percentage of eggs obtained by manual extraction is 11% of the body weight of the female who received a hormone dose of 0.4 ml/kg and 8.45% for the hormone dose of 0.5 ml/kg. Janssen (1985) argues that the amount of eggs obtained

represents 10 to 15 per cent of the body weight of females (March October). From November to February a relative fertility rate of 5-10% is obtained. Indeed, the results indicate that the total weight of stripped eggs is higher in females with a hormonal dose of 0.4 ml, compared to those with higher doses. These results are similar to the work of (kasi, 2015) in Malaysia on the same doses of the hormone ovaprim and for the same species.

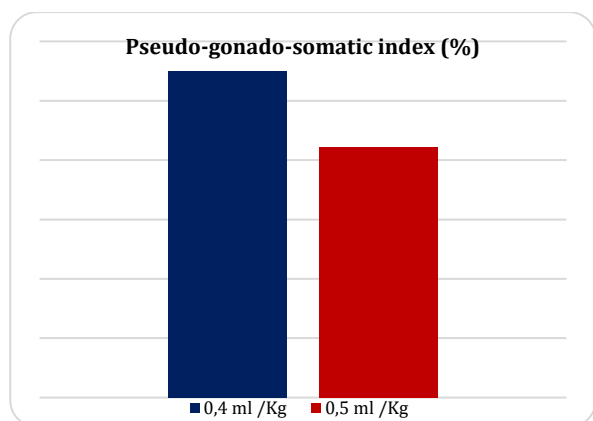


Fig. 5. Pseudo-gonado-somatic index results (%)

3.8. Fertility rate (%)

The rate of fertilization is of the order of 85.56 and 77.11% (Table .5) in injected females at the respective doses of 0.4 and 0.5 ml/kg of ovaprim. The comparison of the two rates, by the reduced gap test (t) (Table .8), shows a significant difference between the fertility rates from the two different ovaprim doses used at a safety rate of 95 %, indicating a higher fertilisation rate in females injected at 0.4 ml/kg compared to those injected at 0.5 ml/kg. This translates that the use of a dose of 0.4 ml/kg of ovaprim, provides an optimum and economical fertilization rate for an aquaculturist interested in the production of fry of *C.gariepinus* on a large scale that the use of a dose of 0.5 ml/kg. Because ovaprim is expensive.

Table 8: Comparison of the two fertility rates

Ovaprim dose (ml/kg)	Fertilization rate (%)	t observed	t theoretical
0.4	77,11 ± 0,039	3,25	1,96
0.5	85,56 ± 0,032		

KatherHaniffa and Sridhar (2002) reported (70%) fertilization rates in *Heteropneustes fossilis* (Clariidae) also treated with the synthetic hormone Ovaprim at a dose of 0.3 ml/kg body weight. Variation in the rate of fertilization can be attributed to the varied quality of eggs and semen, physiological difference in brood stock, seasonal variation and difference in hormonal dosage (Gheyas et al., 2002; KatherHaniffa and sridhar , 2002; Nwokoye et al., 2007). According to kasi et al., (2015) the highest percentages of fertilization rate see 97.88% were also recorded in females who received

0.4 ml of ovaprim compared to those who received higher doses.

3.9. Duration of hatching

The hatch time recorded in this study is 42h for induced aquariums with 0.5ml/kg and 44 hours for aquariums injected by 0.4 ml/kg (Table .9).

Table 9: Duration of Hatching as a Function of Hormone Doses

Aquariums A1 /A2/A3 (0.5 ml/kg)	Aquariums A3/A4/A5 (0.4ml/kg)
18.5 h	20h

The time of outbreaks recorded in our case differs from that of (Haniffa et al., 2000) which indicates an outbreak time of 39 to 43 hours for *C. gariepinus* treated with pituitary gland, 23 hours for Ovaprim-induced eggs and 36-38 hours for HCG-induced eggs at an average temperature of $25 \pm 0.3^{\circ}\text{C}$.

The hatching rate was not estimated due to massive egg mortalities in aquariums containing poor quality water that infiltrated the livestock system. The low incubation rate of eggs would be attributed to exposure and fungal infection of eggs, as the amount of dead eggs provides a nutritional basis for fungal growth (Tucker and Robinson, 1990). Other disturbances in hatching rates are due to fluctuations in water temperature and egg hatching times (Piper et al., 1982).

The relationship between incubation period duration and light intensity is well advanced by (Prokesova, 2016). Eggs incubated in the dark take about 25% more (6 h) to hatch compared to those with the highest light intensity. Lighting can increase the frequency of muscle contractions of the embryo, with more frequent movements leading to earlier rupture of the chorionic membrane. Therefore, it is recommended to the CNRDPA of Ouargla to use light intensities of 70 to 500 Lx rather than total darkness, because they can facilitate the work of operators and minimizing electricity costs.

4. Conclusion

Through this study and taking into account the pressing needs of Alevins for the development of aquaculture in the Saharan region, the results advanced in this study concerning in particular the use of an optimal dose of ovaprim of 0,4 ml/ kg, are promising and could serve as a baseline for future experiments conducted at the experimental station of Ouargla for the controlled reproduction of the catfish *C. gariepinus*. The analysis of the induced reproductive performance through four selected parameters, ovulation rate, latency time, pseudo-gonado-somatic index, and fertilization rate are encouraging. However, it is recommended to follow carefully the rearing circuit conducted in an open system, to adopt light intensities of 70 to 500 Lx in

the experimental phase only in total darkness, and to ensure water supply to aquariums to avoid fungal attacks on eggs that affect the evidence of successful induced reproduction.

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