Assisted reproduction of *Clarias gariepinus* Burchell, 1822

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Abstract

The objective of this work was to set up an experimental protocol concerning artificial reproduction in the catfish *Clarias gariepinus* with the induction of spawning using GnRH. The experiment was carried out at an aquaculture farm in Khemis Meliana (Ain Defla), Algeria. In the study, five African catfish broodstock (3 females and 2 males) were used. Hormonal injection was made into the back muscle below the fin, and doses of GnRH were determined according to the weight of each individual. Fertilisation was performed artificially using the dry method. After incubating the eggs, a binocular magnifying glass was used to check egg condition and embryonic development over time. The results obtained show that artificial insemination of this species, and survival and growth of larvae, are possible. After injection with GnRH, *C. gariepinus* females displayed successful ovulation, fertilisation, larval hatching, and larval monitoring. For this species, a latency period of 22 hours was recorded, and approximately 35,700 larvae were obtained. The fertilization rate was 48%. At the end of this experiment, we can conclude that it is possible to improve reproduction through the proper use of hormonal stimulation techniques and by improving the diet and abiotic factors that are dominant in fish farming.

Key words: *Clarias gariepinus; GnRH; artificial insemination*

Introduction

Aquaculture is a rapidly growing food production sector that currently provides nearly 50% of the fish consumed worldwide, and it is considered to have the greatest potential to meet the growing demand for aquatic food (Laffoley et al., 2019). There are several methods of breeding farmed fish, selected on the basis of the reproductive biology of the species of interest, local environmental conditions and available facilities. These methods can be classified into

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three categories: natural, semi-natural, and artificial reproduction (FAO, 2012; Tkacheva et al., 2020).

The African catfish *Clarias gariepinus* Burchell, 1822 is a major warmwater aquaculture species distributed in Africa and Asia (Khan and Abidi, 2011; Zidan et al., 2020). It is an excellent intensive culture species due to its tolerance to poor water quality, its ability to maintain strong growth at high density, resilience to disease, and the ability to accept cheap food (Rouabah et al., 2016; Shourbela et al., 2017). Likewise, it is an excellent farmed fish, due to its economic nature compared to other production species with market value. It can constitute a piscicultural community in reservoirs and is wide used in breeding thanks to its characteristic adaptation to a wide range of temperature, dissolved oxygen concentrations, and its short trophic chain (Bakos and Gorda, 2001; Géoffroy et al., 2019).

According to Legendre et al. (1996), the seasonality of fish reproduction is one of the main features that hamper supply to the international market, and mastering artificial reproduction has thus become a mandatory step in optimising aquaculture production. To this purpose, we performed this study at an aquaculture farm in Khemis Meliana (Ain Defla, Algeria) to achieve artificial reproduction in catfish *Clarias gariepinus* with spawning induction using GnRH.

Material and methods

Study area and period

This study was carried out at the Laribi Sadek aquaculture farm, in Ain Sultane (Khemis Miliana, Ain Defla, Algeria), over a five-month period (spring season). The farm contains about ten large volume tanks (each with a diameter of 15 m), 8 medium tanks, 5 small tanks, 2 raceways and 2 small tanks for aquaponics, with a hatchery with 12 indoor basins each of 15 m^3 volume. The farm is supplied with borehole water with a temperature of 21°C. This farm applies the principles of fish farming integrated with agriculture, where water is reused to irrigate orange and mandarin plantations (Figure 1). The breeder also uses a boiler at the entrance to the hatchery to control temperatures. There are several species raised on this farm such as: catfish, king carp, tilapia, and pike-perch.

Figure 1. Aquaculture farm

Experimental design

In this study, we used five parents of African catfish (*Clarias gareipinus*), 3 females and 2 males, reared on the Laribi farm.

Choice of broodstock

The capture of adult fish consists of fishing the broodstock during their natural spawning period, or during their migration to their spawning grounds. Broodstock must be kept in ponds at a maximum density of one fish per m^2 of water:

Mature females were selected on the basis of belly roundness, indicating the proper development of ovaries at a temperature above 23°C. Artificial reproduction may be practiced all year round and the same female can lay eggs every 7 weeks (Figure 2). For males, the larger ones were selected since their testes are well developed and full of semen. Males and females can easily be distinguished due to pronounced sexual dimorphism (Figure 3).

Figure 2. Clarias female

Figure 3. Clarias male

Figure 4. Sedative based on *Eugenia Caryophyllata*

Figure 5. Intramuscular injection of GnRH under the dorsal fin

Anaesthesia

The parents were anaesthetized so as not to stress them during handling to avoid any disturbance to gonad maturation. A sedative based on *Eugenia Caryophyllata* (clove) was used as follows: 1 drop per 10 litres of water (Figure 4).

Hormonal induction

The hormonal induction techniques of oocyte maturation and ovulation followed by artificial fertilisation are often favoured as they allow for better control of all phases of reproduction and larval rearing. There are mainly two hormonal induction techniques: an invasive method that consists of a hormone injection to the back muscle under the fin, and a non-invasive method that is based on inserting a probe into the genital papilla to release the hormone (Figure 5).

Dose calculations

The doses of GnRH injected were determined as a function of the weight of each parent, with a total dose of 3 mg/

kg of live weight of broodstock, i.e., 3 mg hormone is applied per 1 kg bodyweight in females and per 2 kg in males.

Latency

The latency time (interval between injection and ovulation) is the maturation time of the oocytes; this is a function of the average temperature to which the females are subjected. Indeed, there is an inversely proportionate relationship between latency time and temperature, where latency time decreases as temperature rises.

Fertilisation

Sampling of testes and semen

Both males were sedated with clove oil before the experiment. An incision was made in the belly of the fish using a scalpel from top to bottom until reaching the anus. A finger is inserted under the incised skin to avoid damaging the internal organs. The testes are located on either side of the spine and mature testicles can be recognized by their white and opaque outer fringe. The testes were then detached from the dorsal part of the abdominal cavity (Figure 6). After the testes are removed, the males are sutured and a dose of antibiotics is given by IM.

Semen collection

The testes are held over a glass container such as a beaker that has been previously dried to prevent sperm activation upon contact with water. Multiple transverse incisions of the testes were made using a scalpel and the sperm was collected.

Figure 6. Observation of testes in situ

Egg harvest

The eggs were collected by abdominal massage of the female; this is called "stripping" (Gilles et al., 2001) (Figure 7). First, the genital papilla and its periphery were cleaned and dried; then, firm pressure was exerted on the abdomen with manual pressure applied to the area of the ovaries to cause the eggs to be released into a small basin or a dry basin. Ripe eggs are more or less transparent, small, with approximately 600 eggs per gram.

Figure 7. Stripping

Fertilisation

Artificial insemination generally consists of mixing the eggs with dry milt, then adding a volume of water equivalent to that of the eggs in order to mobilise the spermatozoa to penetrate the eggs and thus fertilize them. Here is the approach we took for fertilisation of the eggs: a sufficient amount of milt was poured onto the eggs. It should be noted that 1 mL milt can fertilise 20 million eggs.

The container containing the milt and eggs was gently shaken for two minutes to mix the sperm with the eggs before adding water. The time available for the ripe egg to be fertilised is very short since the eggs begins to swell immediately in contact with water, causing closure of the micropyle and thus preventing entry of the spermatozoa and the fertilisation of the eggs. We the added a volume of water equivalent to that of the eggs, i.e., 100 mL per 100 gram eggs, with a volume of fertilizing solution (40 g NaCl per 10 L water) to activate and mobilise the sperm. We rinsed with clean water 3 to 4 times while agitating between rinses to remove all dead and decaying cells.

The fertilized eggs were mixed with whole milk at a rate of 250 mL per 100 gram eggs or with powdered milk to prevent clumping. We can also use the clay which also has a colloidal effect; it will surround the eggs to prevent their agglutination. The eggs were rinsed with clean water to remove the milk and sperm using a sieve. Eggs were then incubated under the conditions most favourable to normal development and capable of ensuring their survival.

Egg incubation

Immediately upon fertilisation, the egg begins to develop, following a process of complex events. The technique consists of spreading the eggs in a single layer on frames of mosquito net or fine mesh at 1 mm. Grass-based spawning grounds can also be used as an incubation medium. The time it takes for the fertilized egg to turn into a larva depends mainly on the temperature and the dissolved oxygen content of the water.

Hatching

The movements of the fish embryo intensify until the eggshell is ruptured and larvae weighing about 1 mg are produced (Figure 8).

Figure 8. 1-day old larvae

All experiments were carried out according to the guidelines of the Institutional Animal Care Committee of the Algerian Higher Education and Scientific Research (Agreement Number 45/DGLPAG/DVA.SDA.14).

Results

Clarias garipeinus was artificially induced. Eggs were easily collected by light abdominal massage and were already ripe with a greenish coloration (Table 1).

Table 1. Result of egg retrieval and latency time

Broodstock	Average temperature	Latency	Result	Egg weight (g)
Female 1	26 °C	22 h	Ovulation	133
Female 2		22 h	Ovulation	109
Female 3		22 h	Ovulation	121

Fertilisation

Fertilization was performed *in vitro*. This consisted of sprinkling the milt of males on the eggs. We sprinkled the milt evenly on the surface of the eggs, and added water in the same proportion as the quantity of eggs to speed up fertilisation. The fertilisation took place in a minute. The fertilised eggs showed a marked increase in size. Egg adhesion is possible if the operation is not done with proper handling.

The quantity of eggs produced was determined using two methods:

- Theoretical method: According to (Ducarme and Misha, 2003), the females of *Clarias gariepinus* produce around 30,000 ova/kg live weight.
- Volumetric method: The volume of eggs and their number are recorded (number of eggs in 1 mL reduced to the total volume of each laying) (Table 2).

Incubation

The eggs were placed in tanks on laying pens. Unfertilised eggs were easily distinguished by their whitish colouring. Incubation lasted about 36 hours, until the appearance of larvae in the tanks.

Hatching

After hatching, the larvae carried yolk sac vesicles which constitute their nutritional reserve. The larvae were very small and needle-shaped, very active and seeking to hide in dark corners. The hatching rate corresponded to the number of living larvae divided by the number of incubated eggs x100, as reported by Ducarme and Misha (2003). In this study, the hatching rate was 85%.

Survival of fry

The larvae of *Clarias gariepinus* began to take food three days after hatching.

Figure 9. Fry of *Clarias gariepinus*

Figure 10. 25-day-old *Clarias gariepinus*

Table 2. Number of eggs for each female

Broodstock	Female weight (kg)	Egg weight (g)	Number of eggs /mL	Total number of eggs
F1	1.4	133	217	28,861
F2	1.3	109	192	20,928
F3	1.1	121	208	25,168

At this stage, they are called alevins. The first food should be diluted egg yolk, then zooplankton (Figures 9 and 10).

Discussion

Artificial reproduction in catfish includes the choice of hormone and dose to be injected, stripping, *in vitro* fertilisation, and incubation of eggs with the use of artificial reproduction techniques that may or may involve the use of natural or synthetic hormones promoting final maturation (Gilles et al., 2001; Ashley-Dejo et al., 2020).

Different hormones include fish pituitary extracts, hCG hormone, gonadotropin hormone (GTH), luteinising hormone-releasing hormone (LHRH) and LHRH agonists (LHRHa), gonadotropin-releasing hormone (GnRH) and GnRHa, Ovatide, Dagin, Ovaryprim, Ovaprim, Ovopel, Ovupin-L, Ovulin and Aquaspawn, which are commonly used to induce final maturation or ovulation in *Clarias* females (Zidan et al., 2020). Otémé et al. (1996) obtained an ovulation rate of 100% after a single intramuscular injection of HCG at the optimal dose of 1.5 IU/g. According to Munsur Ali et al. (2016), hormone injection into the muscular basal region of the pectoral fin gives the best result of induction. Although induction by hormone therapy appears to be most effective method in artificial reproduction (Zidan et al., 2020), this widely used method must be performed with great caution, respecting the concentrations prescribed for each fish species (Zakes and Demska-Zakes, 2009; Géoffroy et al., 2019). In the same context, the main constraint linked to this form of reproduction for small producers is the high cost of commercial hormones (Géoffroy et al., 2019).

The injection of GnRH replaces the natural discharge of hormone which is secreted by the pituitary gland into the bloodstream under the control of the hypothalamus. It thus induces the final maturity of dormant eggs in the selected females. Water temperature is essential for the final maturation of the eggs. In our experiment, it was stable for 12 hours at approximately 25°C. Temperature has a direct effect, since gametogenesis only takes place in a given species within a determined temperature range (Munsur Ali et al., 2016). They also reported that most catfishes breed from June to August when water temperatures are from 29 to 33°C in Bangladesh.

According to several reports, the selection of females to be induced is made on the basis of the size homogeneity of the fish and of the ova and their diameter, generally between 1.4 and 1.6 mm (Viveen et al., 1985; De Graaf and Janssen, 1996; Gilles et al., 2001). The broodstock used for this experiment was selected from the tanks, on the basis of body weight, degree of abdominal bloating in females and the development of urogenital papilla in males. According to Viveen et al. (1985), specimens of *Clarias gariepinus* are sexually mature after seven to ten months at a weight of 200 to 500 grams. All selected broodstock weighed more than 1 kg.

Eggs were collected by abdominal massage of the female in a procedure called "stripping" (Gilles et al., 2001). Concerning the harvest of milt and fertilisation, the males are always sacrificed in order to remove their testes. According to the literature, taking larger males is enough to ensure that their testes are well developed. Females of *C. gariepinus* produce from approximately 30,000 (Ducarme and Misha, 2003) to approximately 99,897 eggs/kg bodyweight (Rukera et al., 2005). For males, as reported by Janssen (1985), 1 mL milt is sufficient to fertilise 15 million eggs (knowing that 1 mL milt contains between 10 and 20 billion sperm).

In the current study, fertilisation was performed artificially according to

the dry method described by Janssen (1985), consisting of first mixing the eggs and sperm dry before adding water and then activating the sperm that were near the eggs and fertilised them. Egg fertilisation depends on sperm quality (Ronyai, 2007; Kristan et al., 2013; Blecha et al., 2016). Other papers have reported a new method to deliver sperm to eggs in common carp and revealed that sperm injection into the ovary can also result in successful fertilisation (Müller et al., 2018; Müller et al., 2020).

Indeed, Gilles et al. (2001) reported that white eggs should be checked quickly in incubators, as their rotting can cause significant water pollution. Hatching takes place after 22 hours of incubation at a temperature of 26°C. A similar result was given by Tkacheva et al. (2020) who found that hatching occurred after 20 hours at a temperature of 27.2°C. In a hatchery, egg-laying can be induced throughout the year in *C. gariepinus* when the temperature is kept constant at 25°C; however, a higher temperature (30°C) leads to an increase in the proportion of atretic oocytes in the ovary and to regression of the testes (Ducarme and Micha, 2003).

In this study, the average hatching rate was 85%. Other papers report mentioned different hatching rates in fish: 73% in Pangus and 68% in Shing (Munsur Ali et al., 2016); 85.1 and 83.7% in C. *gariepinus* receiving 4000 IU hCG/kg fish, 4000 plus 10 mg dopamine antagonist, respectively (El-Hawarry et al., 2016).

Larval rearing is the most delicate phase in the life of a fish. The larvae consume their yolk reserves and they do not require any food (Gilles et al., 2001). Ducarme and Micha (2003) started feeding *C. gariepinus* larvae on the second day after hatching. At this stage, they are called alevins. The first food should be diluted egg yolk, then zooplankton. Vandecan et al. (2011) reported that the complete replacement of live food by artificial food leads to poor growth and poor survival power. According to Ashley-Dejo et al. (2020), after the first three days of yolk absorption, the swim-up larvae need an exogenous source of food to live and grow. Thus, suitable food must be provided in sufficient quantities to avoid mortality. Food deficiencies in eggs can result in the inhibition or cessation of embryogenesis that can cause deaths in the new organisms before hatching or the occurrence of abnormal growth of the larvae produced (Rawung et al., 2020).

Rukera et al. (2005) revealed that the sex ratio of *Clarias gariepinus* is the tanks is approximately equal, with an average weight of about 1 kg at a density of 2 or 3 individuals / m^2. After spawning, males prefer dark places, while females show a curious and ambient dynamism when taking water quality parameters, and show a preference for currents when draining and filling raceways. Munsur Ali et al. (2016) reported that fishes become calm and quiet after spawning. After releasing eggs and milt, they were found to stay on the bottom of the tank.

Temperature and dissolved oxygen content are factors that limit growth performance. Temperature acts on the nitrogen retention coefficient, and therefore, on the weight growth of fish. Water temperature is very important for embryonic development and the total duration of the incubation. It has been shown that hatching in *Clarias gariepinus* is temperature dependent, requiring from 20 to 57 hours for the eggs to hatch. Temperature is a determining ecological factor for the survival of species in running waters, and which has significant ecological repercussions (Makhoukh et al., 2011). Hence, water temperature directly affects aquatic life and the chemical conditions in water, in particular the absorption capacity of gases (Munsur Ali et al., 2016).

In order to maintain high productivity during artificial reproduction, it is

necessary to control the reproductive cycle of the species, especially the period of reproduction, the size at sexual maturity, and environmental factors, particularly appropriate oxygen concentrations, water temperature and pH, all of which can be species specific (Wang et al., 2010; Munsur Ali et al., 2016).

Conclusion

The study carried out at the Laribi Sadek farm in Khemis Miliana aimed to set up an experimental protocol for the artificial reproduction of catfish (*Clarias gariepinus*). This species is prized due to its flesh quality, their dietary requirements in breeding, and their resilience to disease. Based on these strengths, we carried out artificial reproduction trials of these species using the hormone-assisted spawning induction method (GnRH) on broodstock from reservoirs. These results indicate that it is possible to improve reproduction through the proper use of hormone stimulation techniques and by improving the diet and abiotic factors that are dominant in fish farming. Controlling artificial reproduction is the solution to increasing production, and can reduce the imports of fish as a commodity that is very expensive for the national economy.

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Potpomognuta oplodnja *Clarias gariepinus* Burchell, 1822.

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Cilj je ovog rada bio uspostaviti eksperimentalni protokol u svezi potpomognute oplodnje soma *Clarias gariepinus* s induciranjem mriještenja pomoću GnRH. U tu smo svrhu proveli studiju na akvakulturnoj farmi u Khemis Meliana (Ain Defla), u Alžiru. U radu smo koristili pet jedinki afričkog soma iz matičnog jata, 3 ženke i 2 mužjaka. Hormonske injekcije ubrizgane su u leđni mišić, ispod peraje. GnRH doze određene su ovisno o težini svakog roditelja. Oplodnja je obavljena umjetno, suhom metodom. Tijekom vremena, za provjeru stanja jajašaca i razvoja embrija nakon inkubiranja jajašaca, rabljeno je binokularno povećalo. Dobiveni rezultati pokazali su da je potpomognta oplodnja ove vrste moguća, kao i preživljavanje i rast ličinki. Naime, nakon ubrizgavanja GnRH, ženke *C. gariepinus* pokazale su uspješnu ovulaciju, oplodnju, rast ličinki, kao i njihovo praćenje. Za ovu vrstu, zabilježeno je vrijeme latencije od 22 sata uz dobivanje oko 35700 ličinki vrste *Clarias*. Stopa oplodnje iznosila je 48 %. Na kraju ovog eksperimenta možemo zaključiti da je moguće poboljšati reprodukciju ispravnom primjenom tehnika hormonalne stimulacije i poboljšanjem prehrane te abiotičkih čimbenika koji prevladavaju u uzgoju ribe.

Ključne riječi: *Clarias gariepinus*, *GnRH*, *umjetno osjemenjivanje*