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Semi-traditional Production of Fry of a Fish Species [*Clarias gariepinus* (Burchell, 1822)] with a High Market Value in a Fish Farming Cooperative, COPAPE- Brazzaville, Congo

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This work aims to solve the problems of aquaculture in the Republic of Congo. Indeed, Congolese aquaculture is characterised by a lack of fry quality and in sufficient quantities. *Clarias gariepinus* (Burchell, 1822) has proved to be the most appropriate specy for Congolese fish farming. Two methods of producing fish rearing of this species were tested: the artificial method with hormonal treatment with Ovaprim and the semi-natural method. For both methods, 1027 and 1893 fry were respectively obtained ; the compliance test applied to these values shows a highly significant difference at the 95% probability threshold. The semi-natural method seems to be the best mastered by the cooperative. In order to reduce the acquisition cost of the ovaprim used, a dilution with a 25% normal saline solution can be made when inducing gametogenesis in *Clarias gariepinus*. All in all, that is by far the most productive in terms of fingerling production in the copape context.

Keywords: Pisciculture; Clarias gariepinus; ovaprim; artificial; semi-natural.

1. INTRODUCTION

The Republic of Congo is a Central African country on the right bank of the Congo River, covering an area of 342,000 km² and with a population of 5.61 million (Alagoz et al., 2023). Its economy is dominated by the oil sector, which currently accounts for 60% of GDP, 90% of exports and 80% of government revenue; the other sectors, including fishing, contribute less than 20% to the country's economic growth (Micha & Ruwet, 1974). Fish consumption in the Congo is estimated at 24.4 kg/inhabitant/year (Koumba, 2012) and national demand, which is around 100,000 tonnes/year, is met mainly by imports (Micha & Ruwet, 1974). Numerous pressures are therefore being exerted on Congolese fishery resources, reducing the reproductive potential of stocks. Given this situation, fish farming appears to be an alternative to the limitations of fishing. However, this is limited to continental waters and is practised in ponds, on a family and small-scale scale (Imorou Toko et al., 2007). The lack of quality fry in sufficient quantities (Koumba, 2012) is an obstacle to overcoming the problems of developing this sector in Congo. The aim of this work is to master the techniques for producing fry of a fish prized by the Congolese, with a view to making them available to traditional fish farmers. Our approach is to identify the obstacles to the abundant production of fry using the techniques adopted by COPAPE, for optimum fish production by traditional farmers.

2. MATERIALS AND METHODS

2.1 Presentation of the Experimental Site

The structure used to carry out this work is the *Coopérative de Production d'Aliments et de Poissons d'Elevage (COPAPE)*, located in the southern part of the city of Brazzaville, about 12 km from the central town hall at Mafouta in the eighth arrondissement (Madibou). The study was carried out between June 2022 and September 2022. COPAPE was recently set up (on 25 May 2019) with a remit to produce fry of known quantity and age, formulate feed rations for fish at different stages of physiological development, manufacture fish feed using local ingredients, promote fish farming in urban and peri-urban areas, enhance the value of local fish species, conduct research and development, set up above-ground experimental ponds, etc....

2.2 Biological Material

The biological material used consists of male and female *Clarias gariepinus* individuals from which offspring are obtained. The broodstock are fed with a granulated artificial feed (3 mm) imported from BioMar EFICO ALPHA 838F3. The larvae are also fed with an imported artificial crumb feed from Le GOUESSANT SUPRA S-mietAL0. The experimental work was carried out inside the hatchery and in open-air concrete ponds. The hatchery consists of 18 plastic tanks and 2 concrete ponds.

2.3 Production Methods

Two production techniques are being tested namely, the artificial method and the semi-natural method, according to the protocol inspired by the method described and tested by PROVAC in Benin in 2014. The artificial method involves the use or non-use of natural or synthetic hormones to promote final maturity (Seka, 1984). In order to reduce the acquisition cost of the ovaprim used, a dilution with 25% normal saline was made during the induction of gametogenesis in *Clarias gariepinus* (Buwono et al., 2023)].

Thus, the artificial method is used according to a modified protocol of the artificial method described and tested by PROVAC in Benin in 2014. It covers the following stages : cleaning and disinfection of ponds and experimental equipment, selection of broodstock, hormonal treatment of broodstock, egg collection, testicle collection, sperm extraction, gamete mixing, egg incubation, egg hatching and transfer of larvae to outdoor ponds.

The semi-natural method consists of obtaining fry with isolated pairs in large volume ponds, after treatment (Gilles et al., 2001). The semi-natural method is used according to a modified protocol of the semi-natural method described and tested by PROVAC in Benin in 2014. It covers the following stages : cleaning and disinfection of ponds and experimental equipment, selection of broodstock, hormonal treatment of broodstock, pairing of broodstock, natural fertilisation, removal of broodstock, hatching of eggs, transfer of larvae to hatchery and transfer of larvae to outdoor ponds (Buwono et al., 2023).

The temperature and pH of the water in the hatchery are measured every morning using a multi-parameter pH meter. The temperature of the water in the hatchery is kept as constant as possible (27°C) using a HOPAR immersion heater (220-240v 50Hz/60Hz). Using colorimetric strips, the alkalinity of the water expressed in mg/L (calcium carbonate equivalent ($CaCO_3$)) is regularly taken, in accordance with the colorimetric interpretation scale. During the first few days of their development, the larvae do not require exogenous food, but this is compensated for by vitelline reserves. Exogenous food is only provided after total or partial resorption of the yolk, by throwing 3 pinches of an imported artificial food from Le GOUESSANT SUPRA S-mietAL0, sieved beforehand to make it as fine as possible. They are fed three times a day (9am, 2pm and 7pm).

2.4 Larvae Monitoring

- **Measurement of water physico-chemical parameters**

For both production methods, the physico-chemical parameters of the water are measured throughout the growth of the larvae. These include water temperature, pH and alkalinity.

- **Calibration by sorting and classifying juveniles**

As the juveniles grow, calibration operations are carried out. Calibration is a method of sorting and classifying juveniles in order of size in order to separate those with rapid growth (jumpers) from those with slower growth (Smith & Reay, 1991).

To this end, a column of sieves of different mesh sizes is used and placed above a bucket filled with water. The size of the fry is matched to each class of juvenile retained by the strainer (Ndimele & Owodeinde, 2012).

For artificial reproduction, three calibrations are carried out : on the 21st day, the 34th day and the 50th day after hatching. For semi-natural reproduction, two calibrations are carried out : on the 35th day and on the 45th day after hatching (Uttej et al., 2023).

2.5 Data Analysis and Processing

The information collected was summarised and then analysed and processed using various programmes and software, namely : Microsoft Excel 2019 and SPSS IBM 22. Qualitative variables were expressed as means and standard deviations. Comparisons of means were made using statistical tests: Shapiro Wilk test (verification of normality), Kruskall-Wallis test (distribution comparison), $\chi 2$ homogeneity test and Wilcoxon test (comparison of means) at the 95% statistical significance level.

3. RESULTS

A total of eight (8) broodstock (4 males and 4 females) were handled for both methods:

3.1 Results of the Artificial Method

- Selection and hormonal treatment of broodstock

Four broodstock were selected, i.e. two males and two females. Ovules are released from female A by pressure on the individual's abdomen. When selecting the females, it is important to check whether or not the release of oocytes is accompanied by the release of blood. This shows whether the female has ever been artificially bred. Female A has never been handled, whereas female B has been handled many times for reproduction. Table 1 shows the weights of the selected broodstock and the doses of ovaprim administered.

Hormonal treatment is carried out at 18 hours 10 min. The doses of Ovaprim to be injected into the broodstock are proportional to the body mass of the broodstock. The average temperature of the water in which the pairs were placed was 26.6°C, resulting in a latency period of 16 hours 50 minutes, allowing females with mature oocytes to be produced the following morning (10 hours).

- **Collection of female gametes and sperm extraction**

Mature oocytes are collected from the females the day after the injection. The oocytes were weighed at 67g for female A and 76g for female B. Pairs of testes were taken from each male. The testicles of male A were pale pink and those of male B were blackish. After incision of the testicles, the testicles of male B contain almost no milt.

- **Fertilisation and egg incubation**

After adding the sperm-saline mixture and water to the tank containing the mature oocytes, the tank becomes creamy and a considerable number of white eggs are produced: these are unfertilised eggs. The immersion heater placed inside the pond was set to 27°C in order to obtain hatching 24 hours after incubation, but the temperature could not be stabilised because the heater was faulty.

- **Egg hatching**

Nearly 27 hours after fertilisation, the start of hatching was observed. The larvae leave the incubation supports for the bottom of the pond. The unfertilised eggs remain on the incubation supports, decomposing on the surface of the water. This decomposition is accompanied by a nauseating odour.

- **Transfer of larvae to nursery ponds**

On the tenth day after hatching, the larvae are moved from the hatchery to a concrete pond located outside. Mortality due to the transfer was observed.

3.2 Results of the Semi-Natural Method

- **Selection and hormonal treatment of broodstock**

Four broodstock were selected, i.e. two males and two females. These broodstock were divided into two pairs. Table 2 shows the weight measurements of the pairs of selected broodstock and the doses of Ovaprim administered to them.

The doses of Ovaprim to be injected into the broodstock were calculated on the basis of the broodstock weights. Hormonal treatment was carried out at 6pm.

- **Pairing and removal of broodstock**

Each pair was placed in an outdoor pond. The average temperature of the water in which the broodstock were placed was 23.4°C, leading to a lag time of 18 hours 30 minutes. When the lag time was reached the next day (12 hours 30 min), the spawners were removed from the ponds.

- **Egg hatching and transfer of larvae to the hatchery**

Not all the eggs hatch at the same time. The fertilised eggs gather in colonies in the corners of the pond. The first larvae are collected and transferred to the hatchery. The last eggs to hatch are left in their respective ponds, where they are also monitored.

- **Transfer of larvae from the hatchery to the growth ponds**

Six days later, the larvae transferred to the hatchery were moved to the concrete ponds located outside.

After incubation, the total number of eggs hatched was determined, as well as the number of unfertilised eggs that had turned whitish. The percentage of eggs that hatched was 65% for the first method and 44% for the second, a difference that was not significant ($P > 0.05$). Fry survival rates were 49.6% for the semi-articular method and 34% for the other method. Mortality due to the transfer was observed at 12%.

3.3 Larval Monitoring

Measurement of water physico-chemical parameters: Table 3 shows the average values of the physico-chemical parameters of the water during the experiment.

The mean values of the physico-chemical parameters of the water for the artificial method did not vary significantly ($p\text{-value} > 0.05$) from one pond to another. The limits of variation noted are 22.5 to 25.8 °C for temperature, 6.38 to 10.22 for pH and 40 to 240 mg/L for alkalinity.

Table 1. Weights of selected broodstock and doses of Ovaprim administered

	Males		Females	
Breeders	**A**	**B**	**A**	**B**
Weight (g)	1000	1500	1100	700
Ovaprim dose (ml)	0,2	0,3	0,5	0,3

Table 2. Weight of selected broodstock and doses of Ovaprim injected

	Pair 1		Pair 2	
Breeders	**Male**	**Female**	**Male**	**Female**
Weight (g)	1500	1300	2000	1400
Ovaprim dose (ml)	0,3	0,6	0,5	0,7

Table 3. Average values for physico-chemical water parameters

Méthode	Paramètres	T (°C)	pH	CaCo3 (mg/L)
Artificielle	Minimum	22,5	6,38	40
	Maximum	25,8	10,22	240
	Moyenne	23,88	7,97	100,87
	Ecart-type	0,95	0,98	46,51
Semi-naturelle	Minimum	21,9	6,82	40
	Maximum	25,6	10,41	240
	Moyenne	23,67	7,94	93,75
	Ecart-type	0,88	0,82	42,63

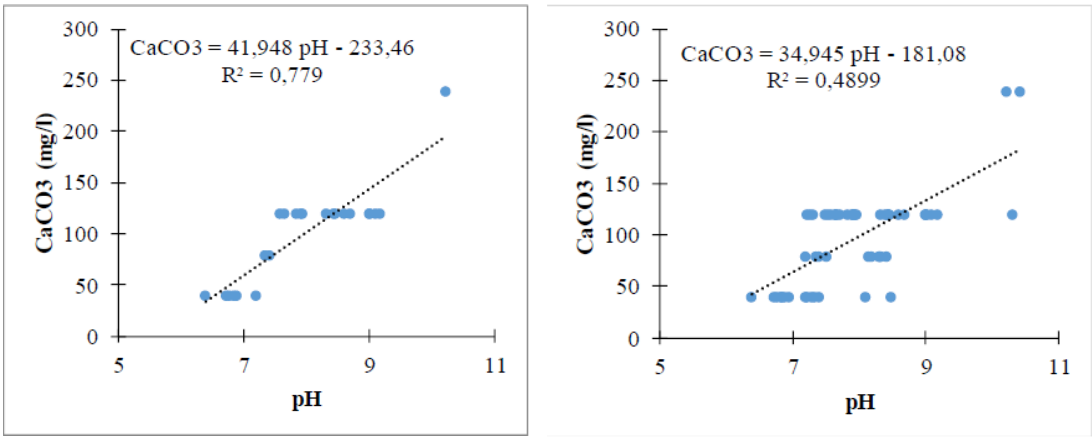

Fig. 1. pH-alkalinity relationship for (a) the artificial method and (b) the semi-natural method

The linear regression analysis shows that there is a significant linear relationship ($p < 0.05$) pH-CaCo3 in the hatchery pond (E1) with a p-value= 0.01917.

3.4 Comparison of Physico-Chemical Water Parameters for the Two Methods

The non-parametric Kruskal-Wallis test was used for both production methods. The mean values for alkalinity and temperature did not vary significantly in the semi-natural method (p-value > 0.05). The limits of variation noted were 21.9 to 25.6 °C for temperature, 6.82 to 10.41 for pH and 40 to 240 mg/L for alkalinity. There was a significant difference (p-value < 0.05) between the mean pH values. With E3 the pond within the hatchery, E1 E2 and E4 the ponds outside, the statistical analysis of linear regression is used to determine the relationship between pH and alkalinity during the experiment. Figs. 1a and 1b show the analyses of the relationship between pH and alkalinity for the artificial method and the semi-natural method respectively.

In the outdoor pond (E2), the linear pH-CaCo3 relationship was highly significant (p-value < 0.001) with a p-value=0.0003762. The coefficient of determination R^2= 77.9% indicates that variations in pH explain 77.79% of the variations in CaCO3. In fact, when the pH increases by an average of one unit, alkalinity increases by 41.948 mg/L. The linear regression analysis shows that there is a non-significant linear relationship (p-value > 0.05) for pH-CaCO3 in the different ponds. In fact, the coefficient of determination R^2= 28.55% indicates that variations in pH explain only 28.55% of the variations in CaCo3; when pH increases by an average of one unit, alkalinity increases by 27.751 mg/L.

3.5 First Feeding and Cleaning

In the first few days after hatching, the larvae consume the reserves contained in the yolk sac. Exogenous feeding did not begin until the yolk sac had resorbed. The exact moment of vitelline resorption for all the larvae could not be determined exactly, as the larvae did not hatch at the same time and waiting for the vitelline sac to resorb for all of them would have meant starving those that had hatched earlier. Yolk resorption for the greatest number was observed on the fourth day after hatching in both production techniques.

The first feeding included the first cleanings with the aim of expelling larval excrement and food remains which, by decomposing, would have polluted the water and consequently impacted larval growth.

3.6 Calibration of Fry

With regard to artificial reproduction, three calibrations were carried out. The first calibration was carried out on day 21. Four size classes were obtained from this calibration. The second calibration was carried out on the 34th day. At the end of the 2nd grading, fry not reaching a total length of 2 cm were discarded from the batch because they were considered slow-growing and/or non-performing. Finally, the third grading was carried out at day 50. At day 50, 1027 live fry were counted (including non-performing fry).

Two calibrations were carried out in the case of semi-natural reproduction : the first on the 35th day, from which three size classes were obtained, and the second on the 45th day. On the 45th day, 1893 live fry were counted.

Table 4. Calibration of live fry in artificial reproduction

		Class 1 (cm)	Class 2)	Class 3	Class 4
1st calibration	middle	4.2	2.8	2.1	1.2
	Standard deviation	0.57	0.17	0.26	0.22
2nd calibration	middle	4.8	3.3	2.4	1.4
	Standard deviation	0.14	0.28	0.22	0.22
3e calibration	middle	6.8	4.9	3.5	1.5
	Standard deviation	0.22	0.22	0.22	0.20

Fry at 21 days and 50 days post-hatching are shown in figures.

Fig. 2. (a) 21-day-old fry; (b) 50-day-old fry
(*source, author*)

Table 5. Calibration of live fry from semi-natural reproduction

		Class 1 (cm)	Class 2 (cm)	Class 3 (cm)
1st calibration	middle	5.9	3.9	3.1
	Standard deviation	0.28	0.2	0.28
2^{nd} calibration	middle	6.5	5	3.9
	Standard deviation	0.17	0.26	0.17

Variations in mean values of total fry lengths for artificial and semi-natural reproduction are shown respectively were 1.2 to 4.2 cm at day 21 and 1.5 to 6.8 cm at day 50 for artificial reproduction. However, they ranged from 3.1 to 5.9 cm on day 35 and from 3.9 to 6.5 cm on day 45 for semi-artificial reproduction.

4. DISCUSSION

Females are selected on the basis of ovule size and diameter (Chikhaoui, 2015). In this study, selection was based on weight and abdominal swelling. (Micha, 1976; Sheheli et al., 2021) shows that prior knowledge of the stage of maturity of the females to be induced is the most important factor to consider when standardising hormone dosage. However, weight-based doses of Ovaprim administered to females successfully induced oviposition (Malik et al., 2014).

In order to reduce the cost of the Ovaprim used, a dilution with 25% normal saline solution was made during the induction of gametogenesis in Clarias gariepinus (Buwono et al., 2023). During artificial reproduction, after the addition of the saline-sperm mixture and fresh water to the tank containing the mature oocytes, a considerable number of unfertilised and therefore dead eggs were observed. Non-fertilisation may be due to poor sperm quality; this could have been verified by a spermogram (Buwono et al., 2016).

Fresh water is added to the gamete mixture to activate the sperm. However, non-fertilisation can be attributed to the use of too much water during fertilisation. This means that many sperm will miss the micropyle of the egg. On the other hand, if there is not enough water, the micropyle may be recognised by another ovum or by the mucus of the ovary, which will have the same effect (Badiane, 2023).

The fertilised eggs gather in colonies in the corners of the pond, during semi-natural reproduction. Since catfish lay their eggs on a substrate (sandy-loam), it is important to provide adequate support to ensure maximum spawning, and if possible to provide a variety of backgrounds so that they can choose where to lay their eggs. Calibration of the fry proved advantageous in that it reduced the loss of fry through cannibalism (Smith & Reay, 1991; Clay, 1981).

The average water temperatures were 23.88°C for artificial reproduction and 23.67°C for semi-natural reproduction. The study was carried out during the dry season. From June to August, temperatures in the Republic of Congo are relatively low (Teugels, 1986). Although tolerant of temperatures ranging from 8 to 35°C, the optimum growth temperature for *Clarias gariepinus* varies from 28 to 30°C (Boyd, 1990). Average temperatures are below the recommended values, and could be a limiting factor in the growth and survival of *Clarias gariepinus* larvae. Temperature is very influential in the process of fish growth. As the water temperature increases, the fish appetite also increases. During the study, values obtained were within the normal range for the life of catfish fry. The results of other studies are good for supporting catfish survival, which ranges from 25-30ºC (Paul et al., 2020). It is also worth noting that The conservation, stability and storage of Ovaprim require room temperature (20 - 30°C) and protection from the sun (Buwono et al., 2016), but in the equatorial zone, water temperatures are very high (25°C on average). In addition, the pig droppings used to fertilise the ponds are a breeding ground for parasites (to be determined) that can be pathogenic for the larvae, thus reducing production yields. The average pH values are 7.94 for the artificial method and 7.97 for semi-natural reproduction. The best pH values in aquaculture are between 6.5 and 9 (Anusuya Devi et al., 2017; Arrigon, 1976) so the pH is conducive to larval development. However, the pH reached aberrant values (10.22 and 10.44) that could negatively influence the results. In fact, a pH above 9 is the lethal threshold for many species (Badiane, 2015; Buwono et al., 2016). The results showed that the average pH for each treatment was between 6.9 and 7.3. The optimum pH for growth and survival of catfish (Clarias sp.) is between 6.5 and 8 (Badiane, 2023). The level of acidity achieved is therefore considered good for the growth of Clarias gariepinus. Along with temperature and acidity, alkalinity is an equally important water quality parameter for growing fish.

The average alkalinity values were 93.75 mg/L and 100.87 mg/L for artificial and semi-natural reproduction respectively. These are within the range of optimal alkalinity values recommended by (Atse et al., 2012), i.e. 50 to 200 mg/L. Alkalinity is not a limiting factor for larval growth and survival. Nevertheless, alkalinity sometimes reached aberrant values (240 ml), which could have a negative impact on the results. The results of these measurements indicate that the quality of the water used during the study is not suitable for rearing catfish, although no recommended standards exist for the Congo as opposed to Indonesia and Benin (Paul et al., 2020).

5. CONCLUSION

Several studies on *Clarias gariepinus* fry production have been carried out around the world. For the Republic of Congo, no documentation is available to date. The study focused on the production of fry of a fish species with a high market value, *Clarias gariepinus,* by a cooperative, COPAPE. The objectives were to identify the obstacles to the abundant production of fry using the techniques adopted by COPAPE. The production techniques adopted by COPAPE are promising but hampered by the difficulty of maintaining the physico-chemical parameters of the water within the optimum value ranges for larval growth. The techniques can be applied on a larger scale, partly solving the problem of the very high demand for fish in the Republic of Congo through a permanent supply of fry and partly eliminating the need to import fish. This permanent supply of fry would also reduce the pressure on the natural stock, thereby helping to conserve biodiversity and maintain the ecological balance.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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