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Optimization of Hormonal Induction in African Catfish Using 70% Ethanol-Preserved Pituitary Glands in Environments without Cold Chain

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Abstract

This study, which focused on the artificial reproduction of *Clarias gariepinus*, examined the effects of different preservation methods for pituitary induction hormones (fresh or in 70% ethanol) on larval vigor. The results demonstrate that the method of pituitary preservation directly influences the number of eggs obtained. The use of fresh pituitaries produced significantly higher egg mass (112.91 g) and absolute fecundity (75,944 eggs) compared to pituitaries preserved in 70% ethanol (77.97 g and 52,070 eggs). This difference reflects the full potency of hormones in fresh glands, whereas storage results in a slight loss of potency. However, the biological quality of the gametes remains intact: the fertilization rates (82.33%) and larval survival rates (86.67%) obtained with pituitary glands preserved in 70% ethanol for 7 days are statistically identical to those of the fresh batch. This simplified preservation method therefore proves to be an effective and low-cost alternative, particularly suited to fish farmers in rural areas lacking a cold chain, in settings where hatcheries have no access to 97% - 100% ethanol.

Keywords

Clarias gariepinus, Hypophysation, 70% Ethanol, Growth, Survival

1. Introduction

The expansion of catfish aquaculture is often hampered by a major bottleneck: the

chronic and structural shortage of high-quality fry. However, self-sufficiency in fry is recognized in the international scientific literature as a *prerequisite* for ensuring the predictability of production cycles and the economic profitability of intensive fish farms [1] [2]. As a result of this biotechnical dependence, producers remain subject to constant biological uncertainty, where access to fry depends on uncontrollable external factors, thereby limiting any inclination toward long-term investment or scaling up to a significant commercial level in the sector [3] [4].

In the absence of functional local hatcheries capable of synchronized production, fish farmers often rely on wild fry, a practice with severe zootechnical and health drawbacks. In fact, the growth rate of these wild fry is very slow due to environmental changes and inbreeding. Furthermore, their quantities are insufficient and unpredictable. These wild-caught fingerlings systematically introduce opportunistic pathogens into aquaculture systems and exhibit growth heterogeneity that promotes cannibalism, leading to extremely high mortality rates that deter investment [5] [6]. This harmful dependence on the unpredictability of wild biological factors not only undermines the resilience of farms, but it also constitutes a major obstacle to the emergence of regional food sovereignty capable of breaking free from costly, often irregular, and risky cross-border imports due to transport-related mortality and exposure to diseases resulting from poor quarantine compliance [7].

Despite the established importance of artificial reproduction, there remains a significant gap in the optimization of specific protocols for African catfish, particularly regarding the long-term viability, efficiency, and ethical considerations of reproductive material management. The current literature lacks a comprehensive comparative analysis of different methods for preserving pituitary glands—such as preservation in alcohol at concentrations < 96%, which is unavailable in our region, versus the use of fresh glands—and their subsequent impact on reproductive performance. This is especially true given that imported hormones are also unavailable here and may pose a risk to human health [8] [9]. The effects of pituitary gland preservation on vital aquaculture outcomes, such as growth and survival rates of fry, are not sufficiently documented. The development of species-specific assisted reproduction technologies requires dedicated research to characterize unique reproductive mechanisms and develop effective protocols, which are often lacking for many species [10].

Addressing these identified gaps is essential for improving the efficiency, sustainability, and ethical standards of aquaculture and the conservation of African catfish biodiversity. Optimizing pituitary gland preservation methods will enable better planning, reduce the immediate need to sacrifice donor fish, and could improve the consistency and reliability of hormonal induction, thereby enhancing hatchery operations. Understanding the direct impact of these reproductive techniques on the growth and survival of fry is essential for maximizing production yields, improving the economic viability of fish farmers, and ensuring the successful establishment of new populations. Ultimately, this research will contribute to

morebetter, responsible, and economically viable aquaculture practices for the African catfish, supporting both food security and biodiversity conservation.

This study stands out for its pragmatic approach aimed at securing local production of *Clarias gariepinus* fry in South Kivu through artificial reproduction. It validates the use of 70% ethanol as an accessible preservation solution for pituitary glands and reaffirms the use of fresh gonadotropins, thereby addressing the lack of costly synthetic hormones.

The overall objective of this study was to contribute to food security through the promotion of aquaculture. Specifically, it aimed to evaluate the effectiveness of methods for preserving the pituitary glands of *Clarias gariepinus* (70% ethanol and use of fresh pituitary glands) without access to a cold chain for ovulation induction. The survival and growth rates of *Clarias gariepinus* larvae were compared based on the pituitary gland preservation method used.

2. Study Area, Materials, and Methods

2.1. Site of Broodstock Procurement and Experimental Station E

Broodstock were captured in the coastal zones of the Bukavu basin on Lake Kivu, one of the region's main artisanal fishing sites. These sites have a natural abundance of adult catfish and offer favorable conditions for their capture and live transfer to the hatchery [11]. This study showed that these coastal habitats, consisting of grassy areas, are particularly conducive to the presence of *Clarias gariepinus*, thereby confirming their strategic role in supplying broodstock for artificial reproduction.

The experimental work was conducted at the hatchery of the Kivu Fish Corporation (KFC), located in the Kadutu commune within the grounds of the Lycée Wima school, at the following geographic coordinates: latitude 20˚30'17.73"S, longitude 28˚50'52.23048", and an elevation of 1583 asl. This site was chosen due to its proximity to the fishing areas of Lake Kivu, which ensures the availability of broodstock, and the presence of established infrastructure for artificial reproduction and fish farming.

The experimental trials took place over a three-month period, from January to April 2026, corresponding to part of the rainy season in South Kivu, which is favorable for the natural reproduction of *Clarias* in tropical environments and for the availability of facilities to serve as a hatchery.

2.2. Materials

Biological Material and Products

The Clarias gariepinus fish (males and females) were sourced from Lake Kivu. A 0.9% w/v NaCl physiological saline solution (500 ml) from Shijiazhuang No. 4 Pharmaceutical was used. Cloves, used as an anesthetic prior to surgery, were purchased at the local market. The feed used for the fish (broodstock and larvae) was the Koudijs brand (44% crude protein) with a particle size of 4.0 mm (floating extrudates) for optimal ingestion (Koudijs Animal Nutrition 2022) [12] and Skret-

ting Perla Larva Pro (62% protein), with the particle size (0.2 mm then 0.3 mm) adapted to the mouth opening of the larvae [13]. Incubation of the fertilized eggs was carried out in circular polyvinyl chloride (PVC) tanks with a capacity of 50 liters. These incubators were equipped with a continuous water exchange system (closed system with physical and biological filtration) to maintain an optimal level of dissolved oxygen. The bottoms of the tanks were fitted with drainage pipes and overflow control valves. A UV lamp was installed on these incubators to ensure that the water was free of microorganisms. The plastic tanks held 200 liters of water for rearing broodstock. The polyvinyl chloride (PVC) rearing tanks for *Clarias gariepinus* larvae had a capacity of 20 liters. The equipment included a mini oxygenator for aeration pumps, a siphon hose to remove impurities and dead larvae, nets for collecting broodstock and larvae, microtest tubes for measuring ammonia and nitrite levels, and an OHAUS electronic scale, model: Pioneer PX4201 with an accuracy of 0.1 g for weighing *Clarias gariepinus* broodstock, feed, and larvae. The incubation rack was used to arrange the eggs inside the incubator. A clean towel was used to secure the *Clarias gariepinus* females before stripping to prevent the mucous membrane from slipping and to avoid handling accidents. Small basins were available for collecting eggs prior to fertilization.

2.3. Methods

Preparation of male pituitary inducers and experimental design

Six males were captured from Lake Kivu in the evening of February 27, 2026, and euthanized in the morning of February 28, 2026. Afterwards, they were stored for seven days prior to female induction. To evaluate the effect of the type of hormonal inducer on the reproduction and the development of *Clarias gariepinus* larvae, a completely randomized design was implemented, encompassing six 20 liters plastic basins as replicates of each of the 2 treatments (**Figure 1**). This design included two treatments corresponding to the two types of hormonal inducers: 70% ethanol conservation, and fresh extracts. Twelve breeding females, among which 6 were induced by fresh male pituitary gland extracts, and 6 other by 70% ethanol-preserved male pituitary gland extracts were used in the experiment.

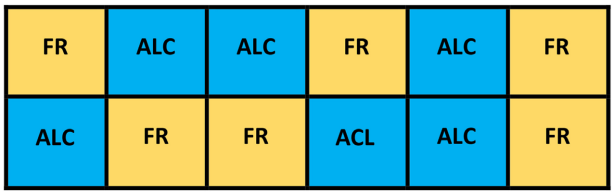

Figure 1. Sketch of the experimental setup; ALC = preserved in 70% alcohol; FR = fresh pituitary gland. Each square represents a 20-liter plastic basin containing one female per batch.

Hormonal Induction protocol

The pituitaries were stored for seven days at the hatchery's ambient temperature in sterile, opaque vials protected from light. Each day during the seven-day

storage period, The average daily ambient temperature was 19.65˚C. Prior to injection, the pituitary extracts were rinsed with a sterile buffered saline solution (NaCl), except for the samples that were used fresh. Hormonal induction was performed by injecting the pituitary extracts into mature females. On the one hand, 6 fresh pituitaries were mixed with NaCl and crushed. On the other hand, six 70% ethanol-preserved male pituitaries were mixed with NaCl and crushed. The pituitary mass used was ca. 0.5 ml per female, at the rate of 1 ml per kg. extraction/preparation steps, the injection route was intramuscular below the dorsal fin and the exact latency time before stripping was equivalent to 9.30 hours.

Fertilization Procedure

At the end of the required thermal latency period, mature oocytes were manually extracted by gentle abdominal pressure (*stripping*). The expelled ovarian fluid was directly collected in a clean, dry container previously tared on a precision electronic balance OHAUS, Pioneer PX4201, 0.1 g. This step allowed the recording of the total mass of the spawn (Wf) [14]. The conventional method of sperm collection in *Clarias gariepinus* relies on the sacrifice of male breeders, followed by the removal of the testes and their maceration to extract the milt [15].

After removal of the testes using surgical forceps, they were placed in a previously dried dish to avoid any premature activation of the spermatozoa. A fine incision was then made with sterile scissors, followed by gentle manual pressure allowing the release of the milt into a dry beaker. The collected sperm was then aspirated using a sterile 5 ml syringe in order to precisely measure the volume obtained for each breeder, in accordance with the methodology described by Sayah (2016) [16]. Sperm was assigned per female, and only one male contributed to each fertilization batch containing one female. Male effects were not controlled. The low variability in female weight after spawning (CV = 9.15%) highlighted the good homogeneity of the biological sample.

Morphometric Parameters

The morphometric parameters of males (n = 6) were measured before pituitary gland collection. Total weight (W) was determined using a precision electronic scale. Linear measurements (LT, LS, LH, H) were performed according to the standardized protocol of Viveen *et al.* (1985) [15]. The body condition of the fish was assessed using Fulton's condition index (K), calculated using the formula $K = \left(W/L^3 \times 100\right)$, in accordance with the recommendations of Froese (2006) [17]. The weight-length relationship was modeled using the allometric equation $W = a \times L^b$ after logarithmic transformation to determine the growth type [18].

Reproductive parameters

The parameters evaluated included ovulation latency over a 9.5-hour period, the weight of oocytes produced (g), and the number of oocytes produced, or absolute fertility (F), calculated using the formula:

$F = \dfrac{\text{Gonad weight} \times \text{number of eggs in the sub} - \text{sample}}{\text{Weight of the sub} - \text{sample}}$, relative fertility (FR) cal-

culated using the formula $FR = \frac{\text{Total number of eggs}}{\text{Weight of the female}}$, gonadosomatic index (GSI) calculated using the formula $IGS = \left(\frac{\text{Weight of gonads}}{\text{Weight of the female}}\right) \times 100$, [19], the fertilization rate (%), the hatching rate (%), and post-yolk sac resorption survival rate. This protocol is based on the work of [20], who compared the efficacy of pituitary extract and synthetic hormones for the reproduction of *Clarias gariepinus*. Their study showed that pituitary extracts can induce effective ovulation, with fertilization and hatching rates comparable to those obtained with commercial hormones.

Larval rearing conditions, Growth and Survival

The Perla Skretting brand was applied to 50 larvae per 20-liter tank according to the protocol of El-Sayed (2006) [21], characterized by six daily meals during the first fifteen days, then three meals until weaning at 30 days post-hatching. Water monitoring, based on the standards of Boyd (1990) [22] and APHA (2023) [23], was carried out twice daily (07:00 and 18:00) during the 30 days following yolk sac resorption in order to capture thermal and oxygen extremes without stressing the stock. Electronic parameters such as temperature (27.3 ± 0.45˚C), pH (6.9 ± 0.18), conductivity (312.4 ± 85.20 µS·cm^{-1}), and dissolved oxygen (6.4 ± 0.55 mg·l^{-1}) were measured using probes at mid-depth, while chemical analyses of nitrogen compounds were performed by colorimetry on 5 ml of water using the indophenol method in two minutes for ammonia (<0.2 mg·l^{-1}) and the Griess method in eight minutes for nitrites (0.02 ± 0.01 mg·l^{-1}).

The parameters measured included the survival rate (%) at D10, D20, and D30 as younger larvae (<day 10 were too small, and larve are fragile and sensitive to stress in case of more frequent mesurements; and 10 individual were pooled, making it impossible for repeated measures), as well as growth in length (cm) and weight (g), based on sampling 10 larvae per tank every 10 days up to 30 days of rearing, using a digital caliper and a digital scale. The condition factor (K) was calculated according to Fulton, $K = \left(\frac{\text{Weight}}{\text{Length}^3 \text{ in cm}}\right) \times 100$.

Similarly, Santi *et al.* (2022) [24] used Ovaprim at a dose of 0.5 ml/kg to induce reproduction in *Clarias gariepinus*, emphasizing the importance of the injection protocol and storage conditions for optimizing reproductive outcomes.

Animal Ethics

This study was conducted in strict compliance with universal principles of animal welfare and international guidelines on the use of fish in research. The research team and hatchery technicians implemented all required skills to minimize suffering, stress, and discomfort of the fish at each invasive stage of the protocol. To this end, all invasive manipulations were performed under deep anesthesia by immersing the broodstock in a clove oil (*Syzygium aromaticum*) solution, ensuring total loss of motility and pain sensitivity.

Statistical Analyses

Statistical analyses were performed using R software version 4.5.0. In addition to descriptive statistics, a one-way ANOVA was used to compare survival and growth between the two storage conditions. Means were compared using Tukey's HSD test ($p < 0.05$). When data did not follow a normal distribution, the nonparametric Kruskal-Wallis test was used. The relationship between survival and growth was explored using linear regression models.

3. Results

Egg-laying and Fertility Performance

The reproductive performance data obtained during the experiment, specifically egg weight and absolute fertility as a function of pituitary preservation method, are summarized in Table 1.

Table 1. Egg weight and fertility by treatment (n = 6).

Parameters	Groups	Mean ± Standard Deviation	CV (%)	*p-value*
	Storage			
Egg weight (g)	Ethanol	77.97 ± 9.87	12.66	0.001
	Fresh	112.91 ± 16.32	14.46	
	Storage			
Absolute Fertility	Ethanol	52,069.50 ± 4726.38	9.08	0.001
	Fresh	75,943.83 ± 11,987.41	15.78	
Female Weight (g)	**Total**	616.67 ± 56.44	9.15	-

Table 1 shows that the method of pituitary preservation has a significant influence on reproductive parameters. Fresh pituitaries had a significant advantage ($p = 0.001$), resulting in a significantly higher egg-laying weight (112.91 g) and absolute fecundity (75,944 eggs) compared to pituitaries preserved in ethanol (77.97 g and 52,070 eggs). The low variability in female weight after spawning (CV = 9.15%) highlights the good homogeneity of the biological sample. It was consistently observed that the use of fresh pituitaries resulted in a significantly higher egg-laying weight than that obtained with pituitaries preserved in 70% ethanol.

Hormonal Induction, Biometric Characteristics, and Sexual Maturity Status of Broodstock

The influence of pituitary preservation method on the biometric characteristics and sexual maturity status of *Clarias gariepinus* broodstock was evaluated. Table 2 presents a comparison between individuals induced with a pituitary preserved in alcohol for seven days and those induced with a fresh pituitary.

Table 2. Homogeneity by induction methods (n = 6).

Pituitary preservation method	Body weight (g)	Maturity (IGS in %)
Alcohol (7 days)	571.33 ± 31.21	13.67 ± 1.97
Fresh	662 ± 31.02	17.17 ± 2.64

Table 2 shows that pituitary glands preserved in alcohol perform less well. However, a closer look at the figures reveals that while the group treated with fresh glands shows the highest values—with a weight of 662 ± 31.02 and a GSI of 17.17 ± 2.64—the true efficiency of these results lies in the response of the group preserved in alcohol. Indeed, although these fish are significantly lighter, with an average weight of 571.33 ± 31.21, they still reached a hight maturity index of 13.67 ± 1.97. This demonstrates that alcohol effectively preserves the hormones' inductive capacity, even in broodstock of more modest weight. Thus, the observed statistical difference does not diminish the practical value of this method. This demonstrates that preservation in alcohol is a reliable alternative, capable of ensuring high-quality maturation under conditions where access to synthetic hormones is problematic. This ability to induce a strong hormonal response in smaller individuals confirms the technical viability of this process for the day-to-day management of a hatchery.

Comparison of the Performance of Fresh versus Preserved Pituitaries on Key Reproductive Parameters

The efficacy of pituitaries preserved in 70% alcohol compared to fresh pituitaries was evaluated based on key reproductive parameters. Table 3 presents the average fertilization, hatching, and post-vitelline rates obtained for each preparation method.

Table 3. Reproductive performance according to pituitary preservation method (n = 6).

Parameter Evaluated	Ethanol Group	Fresh Group	*p*-value	Significance (5% threshold)
Fertilization rate (%)	82.33 ± 6.83	76.67 ± 5.57	0.1465	Not significant ($p > 0.05$)
Hatching rate (%)	48.17 ± 6.55	53.67 ± 10.84	0.3125	Not significant ($p > 0.05$)
Post-yolk survival (%)	71.67 ± 9.07	70.50 ± 6.75	0.8055	Not significant ($p > 0.05$)
IGS (%)	13.67 ± 1.97	17.17 ± 2.64	0.0263	Significant ($p < 0.05$)

The results in Table 3 indicate that the preservation method significantly influences the IGS ($p < 0.04$), clutch weight, absolute fertility, relative fertility, and final body weight of females ($p < 0.006$). The GSI was significantly higher with fresh storage compared to ethanol storage (17.17 ± 2.64% versus 13.67 ± 1.97%). Similarly, egg-laying weight, fertility indicators, and the body weight of females after egg-laying were consistently higher when stored fresh. Nevertheless, an analysis of zootechnical performance shows that this difference in gross ovarian weight does not impair the biological efficacy of 70% ethanol. On the contrary, the ethanol group tended to show a slightly higher average fertilization rate than the fresh group (82.33% versus 76.67%), while the hatching rate (approximately 50%) and post-vitelline survival rate (71%) remained statistically equivalent ($p > 0.05$) between the two treatments.

Relationship Between Gonadosomatic Index (GSI) and Hatching Rate

The influence of the females' stage of maturity on incubation yield was analyzed

using a correlation study. Figure 2 illustrates the relationship between the Gonadosomatic Index (GSI) and the hatching rate of eggs obtained after 14 days of conditioning.

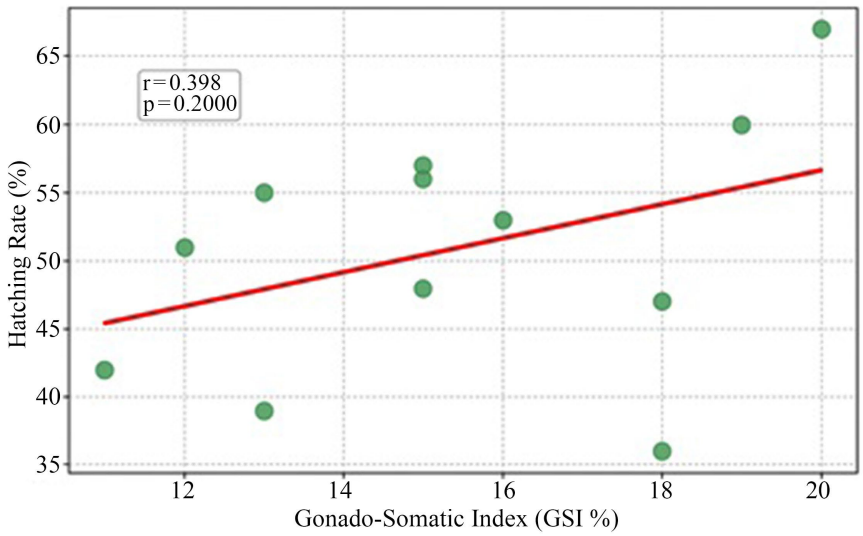

Figure 2. Relationship between the gonadosomatic index (GSI) of females and the hatching rate of eggs in *Clarias gariepinus*.

Figure 2 shows a weak positive correlation (r = 0.398) between ovarian mass (GSI) and hatching success. The upward slope suggests that a high GSI is a favorable indicator for hatchery performance. This result highlights that the 14-day conditioning protocol enabled the attainment of an ovarian maturity stage suitable for fry production, while noting that the intrinsic quality of the oocytes remains a determining factor in embryonic success.

Reproduction of Females

The biometric characteristics and maturity levels of the broodstock, categorized by the pituitary preservation method used for induction, are presented in Table 4. These data allow for an assessment of the initial condition of the individuals prior to analyzing their spawning performance.

Table 4. Average reproductive performance of females.

Parameters	Mean ± SD (n = 12)
Egg weight (g)	95.44 ± 22.18
Absolute fertility (oocytes)	64,007 ± 15,219
Relative fertility (oocytes/g)	103.25 ± 19.8
IGS (%)	15.42 ± 2.87

The zootechnical characteristics in Table 4 for the twelve breeding females illustrate a strong reproductive potential and well-controlled physiological readiness. The average weight of the egg mass, which stands at 95.44 ± 22.18 g, indicates

a massive release of oocytes during abdominal stripping. This productivity is confirmed by an average absolute fertility of 64,007 ± 15,219 oocytes, reflecting intense vitellogenesis supported by the 14-day conditioning regimen. Relative fertility stands at 103.25 ± 19.8 oocytes per gram of female body weight, underscoring the metabolic efficiency of the group. The average gonadosomatic index of 15.42 ± 2.87% further attests to complete and uniform ovarian maturation.

Correlation Between Weight and Absolute Fertility

To validate the use of body weight as an indicator of hatchery performance, a fertility model was developed. The results in Figure 3 reveal a statistically significant relationship, allowing for the estimation of oocyte production even before hormonal induction.

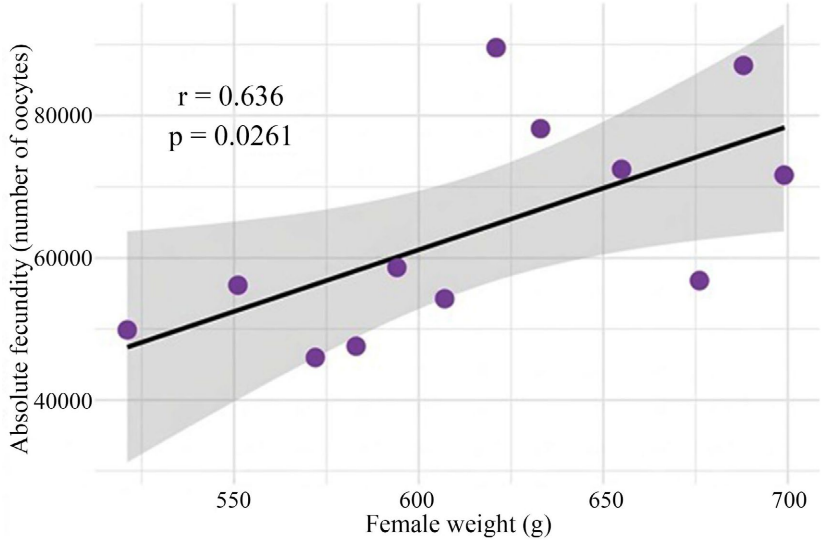

Figure 3. Analysis of the correlation between weight and absolute fertility.

Figure 3 reveals a positive correlation (r = 0.636) that is statistically significant ($p = 0.026$) between the weight of broodstock and their absolute fertility. This result demonstrates that egg-laying capacity increases predictably with fish size. This proves that the females underwent normal biological development. This linear relationship is an indicator of technical proficiency: it shows that fry production can be predicted simply by weighing the broodstock before induction, even in a local hatchery.

Survival and Growth in *Clarias gariepinus* Larvae

The results of the samples' evolution according to the two preservation methods over the 30-day monitoring period are summarized in Table 5. The analysis highlights a gradual but equivalent decrease in values in both experimental batches.

Changes over time in Table 5 show a gradual and continuous decrease from baseline values over the 30-day follow-up period for both treatment protocols tested. At the end of the experiment (Day 30), the ethanol-treated group retained an average of 86.67% ± 4.84% of its original value, compared to 84.00% ± 5.06%

for the group kept fresh. Although preservation in ethanol showed slightly higher averages throughout the follow-up period (notably on Day 10, with 96.33% ± 2.94% versus 93.00% ± 7.13%), statistical analysis reveals that no significant difference exists between the two methods, as the *p-values* consistently remained above the critical threshold of 5% ($p > 0.05$). It is nevertheless interesting to note that the ethanol batch exhibits significantly lower and better-controlled internal variability than the fresh batch, whose standard deviations begin to rise as early as the tenth day. Thus, 70% ethanol proves to be statistically as effective as the fresh batch over the course of a month, while providing greater stability and homogeneity in the structure of the experimental data.

Table 5. Degradation kinetics and analysis of the relative stability of samples preserved in 70% ethanol and kept fresh.

Time	Ethanol Group (Mean ± SD)	Fresh Group (Mean ± SD)	*p*-value	Significance (at the 5% level)
Initial J	100.00% ± 0.00%	100.00% ± 0.00%	1.0000	identical
Day 10	96.33% ± 2.94%	93.00% ± 7.13%	0.3266	Not significant ($p > 0.05$)
Day 20	91.67% ± 2.94%	90.00% ± 7.38%	0.6241	Not significant ($p > 0.05$)
Day 30	86.67% ± 4.84%	84.00% ± 5.06%	0.3731	Not significant ($p > 0.05$)

4. Discussion

Hormonal Induction and Pituitary Gland Preservation

The effectiveness of hormonal induction is the key factor in the success of *Clarias gariepinus* hatchery operations. Our results highlight a significant influence of the pituitary gland preservation method on spawning parameters, while validating the viability of ethanol preservation. We observed that fresh pituitary glands resulted in a higher spawning weight (112.91 ± 16.32 g) and absolute fecundity (75,944 eggs) compared to glands preserved in ethanol (77.97 ± 9.87 g and 52,070 eggs; $p = 0.001$). The superiority of fresh material reflects the intact integrity of gonadotropic hormones (GtH), which have not undergone any protein denaturation process. As Nagahama and Yamashita (2008) [25] point out, the final maturation of oocytes depends on a specific hormonal peak that is at its maximum when the gland is harvested and injected immediately.

However, our study demonstrates that, despite a reduced egg-laying volume, the biological quality of the gametes is preserved by storage in 70% ethanol. The fertilization rates (82.33 ± 6.83%) and larval survival rates (86.67 ± 4.97%) obtained with pituitary glands stored in 70% ethanol are statistically similar to those of the fresh group. This finding corroborates the work of Okomoda *et al.* (2015) [26], who demonstrated that 95% ethanol acts as an effective fixative, stabilizing hormonal glycoproteins and preventing their enzymatic degradation. Our experimental results show that the use of 70% ethanol allows for a Gonadosomatic Index (GSI) of 13.67 ± 1.97 to be achieved in induced females. This value exceeds

the minimum maturity threshold of 10% - 12% generally accepted in the scientific literature for successful ovulation in *Clarias gariepinus* [27].

According to Ataguba *et al.* (2013) [28], the use of fish pituitary gland extract (FPGE) as an inducer remains a more comprehensive method than synthetic hormones, as it provides a spectrum of complementary hormones that facilitate overall ovulation. While our results confirm the superiority of the fresh extract in terms of volume, they primarily validate the effectiveness of our preservation protocol: the use of 70% ethanol for a period of 7 days. Our observations align with the conclusions of Musa *et al.* (2019) [29], who note that even though induction potency decreases slightly with storage, it remains more than sufficient for profitable commercial production. The major benefit of this simplified preservation method is emphasized by Adebayo and Popoola (2008) [30]: it offers fish farmers in rural areas, who lack a cold chain, a self-sufficient and low-cost solution for building up stocks of effective hormonal inducers.

Finally, we established a positive, albeit weak, correlation ($r = 0.398$) between the IGS and hatching success. Although this relationship is moderate, it underscores that the 14-day conditioning protocol enabled the achievement of optimal ovarian maturity. This dynamic is consistent with the principles of neuroendocrinology described by Zohar *et al.* (2010) [31], in which the gonadal response to induction is determined by the parent's prior physiological state. The overall success of the induction in our two experimental batches, coupled with high larval survival, demonstrates that ethanol is a preferred alternative for biosecurity and sustainability in small-scale hatcheries in Central Africa, as recommended by Fermon (2011) [32] and Adewumi (2015) [33].

Limitations of the study

The study focused on evaluating the early stages of larval development, specifically the growth and survival of fry, as influenced by the aforementioned reproductive techniques and methods for preserving pituitary glands. The study did not examine the long-term ecological impacts of these practices beyond the immediate environment of Lake Kivu for broodstock collection and the Kivu Fish Corporation (KFC) hatchery for artificial reproduction. Furthermore, the rearing period did not extend to four months—specifically from January to April 2026—and we did not explore advanced genetic manipulation techniques or comparative studies with other fish species, except as general context.

5. Conclusions

From a hormonal perspective, our study provides a concrete solution to supply challenges. By validating the use of 70% ethanol for 7 days to preserve pituitary glands, we have demonstrated that a simple and inexpensive solution can replace the cold chain and address the unavailability of 97% - 100% ethanol. Admittedly, fresh pituitary glands remain the most effective in terms of egg-laying volume (112.91 g versus 77.97 g for ethanol), but the biological quality of the larvae remains similar. This means that a local fish farmer can now plan production

using their own stocks of spawning inducers, thereby eliminating the need for imported synthetic hormones, which are costly and, above all, unavailable in the region.

The regional focus of this work is its greatest significance. By choosing to use wild broodstock sourced directly from Lake Kivu, we have promoted the use of our local strains in this pioneering study. Our results show that this local strain is every bit as good as domesticated strains: it is resilient, adapts perfectly to tank farming, and responds exceptionally well to precision nutrition such as that provided by Perla feed. The growth dynamics we recorded—with a specific growth rate of 21.2% per day—prove that the genetic potential of our local fish is a treasure that remains underutilized for the country's food security.

This study stands out for its pragmatic approach aimed at securing local production of *Clarias gariepinus* fry in South Kivu through artificial reproduction. It validates the use of 70% ethanol as an accessible preservation solution for pituitary glands and re-establishes the extraction of fresh gonadotropins, thereby addressing the lack of costly synthetic hormones.

In light of the results obtained in this study, we make the following recommendations: 1) Widespread adoption of alcohol preservation: We recommend adopting 70% ethanol as the standard method for storing pituitary glands, particularly in areas without electricity to provide refrigeration. This practice increases the resilience and self-sufficiency of local hatcheries in the face of logistical constraints; 2) Protection of Lake Kivu's genetic heritage: There is an urgent need to establish a genetic conservatory dedicated to local strains of *Clarias gariepinus* from Lake Kivu. Such a facility is essential to protect this unique natural heritage from uncontrolled hybridization resulting from imports; 3) Optimization of production centers: We strongly encourage fry production centers to adopt 70% alcohol preservation to safeguard their strains of high genetic value and stabilize their hormonal production.

Author Contributions

Conceptualization, Elie Namegabe BACIRHEBA and Jean-Berckmans Bahananga MUHIGWA; methodology, Désiré Akonkwa BALAGIZI; software, Elie Namegabe BACIRHEBA and Jean-Berckmans Bahananga MUHIGWA; validation, X.X., Y.Y., and Z.Z.; formal analysis, X.X.; investigation, Guillain Andanga Machumu and Elie Namegabe BACIRHEBA; resources, Désiré Akonkwa BALAGIZI; data curation, Jean-Berckmans Bahananga MUHIGWA; writing—original draft preparation, Elie Namegabe BACIRHEBA; writing—review and editing, Elie Namegabe BACIRHEBA and Gabriel Mukabo OKITO; visualization, Jean-Berckmans Bahananga MUHIGWA; supervision, Désiré Akonkwa BALAGIZI; project administration, Elie Namegabe BACIRHEBA and Guillain Andanga Machumu; funding acquisition, Elie Namegabe BACIRHEBA and Guillain Andanga Machumu. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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