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Effectiveness of Partial Orchiectomy in African Catfish (*Clarias gariepinus*) as a Sustainable Non-Lethal Alternative to Elite Male Culling

Elie Namegabe Bacirheba[1,2,3], Jean-Berckmans Bahananga Muhigwa[2*], Gabriel Mukabo Okito[4], Désiré Akonkwa Balagizi[2], Guillain Andanga Machumu[1]

[1]Kivu Fish Corporation (KFC), Bukavu, Democratic Republic of the Congo
[2]Université Officielle de Bukavu (UOB), Bukavu, Democratic Republic of the Congo
[3]Action Sociale Kesho Congo (ASKC), Bukavu, Democratic Republic of the Congo
[4]Centre de Recherche et d'enseignement en Hydrobiologie, Pêche-Aquaculture et Toxicologie Environnementale (CREHYPATE) de l'institut Supérieur de Pêche de Goma (ISPÊ-GOMA), Goma, Democratic Republic of the Congo
Email: elienamegabe1996@gmail.com, *jeanbahananga61@gmail.com, mgokito@gmail.com, akonkwadesire@gmail.com, guillainmachumu1987@gmail.com

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Abstract

This study compares partial orchiectomy with the conventional sacrifice of males for sperm collection in African catfish. It evaluates fertilization, hatching, larval survival, and growth. Two treatment groups of males were used, and offspring performance was monitored during 30 days of rearing. The main result shows that sperm collected surgically produced reproductive outcomes similar to those obtained by sacrifice. Moreover, operated males survived the postoperative period, and progeny survival was higher in the surgery group. In quantitative terms, the technical feasibility of this surgical method is confirmed by the collection of a semen volume (2.6 mL) that is statistically equivalent to that obtained using the conventional euthanasia method (3.12 mL). At 30 days of age, larvae from operated males exhibited a high survival rate of 88.67%, a result significantly higher than that of the control group derived from sacrificed males (82.00%); with a larval Specific Growth Rate (SGR) exceeding 21% per day. Partial orchiectomy preserves elite broodstock and optimizes the overall productivity of hatcheries by maximizing larval survival and growth rates.

Keywords

Clarias gariepinus, Orchiectomy, Survival, Growth

1. Introduction

In developing countries, food security remains a major structural challenge char-

acterized by persistent socioeconomic instability and rapid population growth. Protein-energy malnutrition affects a large portion of the population due to unsustainable access to locally produced animal protein sources [1]. In this context, African catfish (*Clarias gariepinus*) aquaculture is recognized by international organizations and researchers as a major strategic lever due to the species' rapid growth, physiological hardiness, and high nutritional value [2].

The expansion of catfish aquaculture is often hampered by a major bottleneck: the chronic and structural unavailability of high-quality fingerlings. Self-sufficiency in fingerlings is, however, recognized by the international scientific literature as a *prerequisite* for ensuring the predictability of production cycles and the economic profitability of intensive fish farms [3] [4]. 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 [5].

In the absence of functional local hatcheries capable of synchronized production, fish farmers are forced to obtain fry from the wild with great difficulty—a practice with disastrous zootechnical and health consequences. 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 [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 protocols specific to African catfish, particularly regarding the long-term viability, efficiency, and ethical considerations of reproductive material management. Although sperm extraction is a fundamental step, there has been insufficient comparative research on less invasive methods—such as testicular surgery—versus lethal methods (slaughter of males), regarding their effects on sperm quality, the longevity of male broodstock, and the overall sustainability of breeding programs. The ethical implications of reproductive technologies, including gamete collection, are a recognized area of concern, particularly when it comes to postmortem collection or invasive procedures [8] [9]. Crucially, the downstream consequences of these specific sperm extraction techniques 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 is often lacking for

many species [10].

A thorough comparative analysis of sperm extraction techniques—particularly an evaluation of testicular surgery versus terminal methods—is crucial for developing more humane and sustainable breeding practices. This could enable the repeated use of genetically superior males, preserving valuable genetic diversity and reducing the overall impact on broodstock populations, in line with broader conservation goals for endangered species where postmortem gamete collection is being explored to maintain genetic diversity [8]. 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 more robust, responsible, and economically viable aquaculture practices for the African catfish, supporting both food security and biodiversity conservation.

This study adopts a pragmatic approach aimed at securing local production of *Clarias gariepinus* fry through artificial reproduction. Furthermore, the use of testicular microsurgery preserves elite broodstock.

The overall objective of this study is to contribute to food security through the promotion of aquaculture. Specifically, it aims to:

1) Compare different methods of sperm collection (sacrificing the fish and testicular surgery—orchiectomy) for the fertilization and hatching of *Clarias gariepinus* eggs.

2) Determine the survival and growth rates of *Clarias gariepinus* larvae based on the sperm collection method.

2. Materials and Methods

Location

Lake Kivu served as the source of broodstock. It is a mountain lake located in a region of tectonic depressions formed by the East African Rift Valley. From east to west, the lake receives water from several mountains and rivers. To the south, it is connected to the Mitumba mountain range and Lake Tanganyika, thus forming part of a complex network of mountainous terrain and ecosystems [11].

During the experimental period, sampling of broodstock was conducted in the coastal areas of the Bukavu basin, which is among the region's primary sites for artisanal fishing. These sites have a natural abundance of adult catfish and offer favorable conditions for their capture and live transfer to the hatchery [12]. The riparian habitats, consisting of grassy areas, are particularly conducive to the presence of *Clarias gariepinus*.

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 (latitude 20˚30'17.73684"S, longitude 28˚50'52.23048", and elevation 1583 m).

2.1. Materials

Biological Materials 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)) [13] and Skretting Perla Larva Pro (62% protein), with the particle size (0.2 mm then 0.3 mm) adapted to the mouth opening of the Skretting larvae, (2023) [14]. Following the partial orchiectomy and suturing of the abdominal wall, a methylene blue solution was applied topically to the incision site. This local antiseptic treatment was administered to prevent opportunistic fungal infections, primarily caused by *Saprolegnia* sp., and to promote skin healing in male broodstock in an aquatic environment [15] [16].

The fertilized eggs were incubated in circular polyvinyl chloride (PVC) tanks with a capacity of 50 liters. These incubators were equipped with a continuous water-renewal system (a closed system with physical and biological filters) 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 rearing tanks for *Clarias gariepinus* larvae were made of polyvinyl chloride (PVC) and had a capacity of 20 liters. Equipment included a mini oxygenator for aeration pumps, a siphon hose for removing 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 broodstock, feed, and *Clarias gariepinus* larvae; *a* 5-ml syringe for collecting sperm; latex gloves to ensure hygiene when handling fish; a workbench to ensure comfort during handling; and a surgical kit (scissors, forceps, disposable pipettes, and a usable scalpel) for performing orchiectomy on male broodstock. The incubation rack was used to arrange the eggs in the incubator. A clean towel was used to restrain the female *Clarias gariepinus* prior to stripping to prevent the mucous membrane from slipping and to avoid handling accidents. Small basins were available for collecting eggs prior to fertilization.

2.2. Methods

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

Experimental Design and Broodstock Distribution

A total of 30 mature *Clarias gariepinus* broodstock were mobilized for this

study. This experimental stock included 12 reproductive females and 18 mature males. Distribution and use of these 18 males were as follows: 12 males were directly integrated into the factorial comparison plan of sperm collection methods, with 6 males subjected to partial orchiectomy (surgery group) and 6 males subjected to conventional sacrifice for direct milt collection. To optimize biological material use, pituitary glands (hypophyses) of the 6 sacrificed males were immediately collected and preserved for 7 days to evaluate their hormonal induction capacity after storage in 70% ethanol for 6 females. The remaining 6 males (surplus broodstock) were sacrificed on day 8 of the trial for fresh pituitary collection, allowing preparation of crude hormonal extract necessary and sufficient for ovulatory induction of the other 6 reproductive females.

Surgical Sperm Collection

To ensure a continuous supply of sperm without having to slaughter the males, the researchers tested surgical techniques that allow for sperm extraction while ensuring the fish's survival. According to Diyaware *et al.* (2010) [17], non-lethal sperm collection in *C. gariepinus* can be performed via a minor abdominal incision under anesthesia, followed by partial extraction of the testicular lobes. This approach preserves individuals for future use and reduces broodstock stress and mortality [18].

The surgical procedure was performed in three successive steps on six males. The first step involved anesthetizing the fish by immersing them in a clove solution at a concentration of 0.5 g of powder per liter of water**.** The cloves, purchased at the local market, were finely ground before being completely dissolved in water. The active ingredient in this solution is eugenol, a natural anesthetic widely used in fish farming. The immersion lasted five minutes [18].

The second step consisted of the surgical procedure and removal of the testes. After anesthesia, the male was removed from the solution and placed in a supine position on a damp cloth laid out on the operating table. The abdomen was disinfected with 70% technical ethanol, then incised with a scalpel using a fine longitudinal incision along the peritoneal cavity [18]. The incision, ranging from 3 to 8 cm in length depending on the fish's size, extended from the posterior to the anterior part of the urogenital papilla and was made carefully to avoid any damage to the internal organs. The organs within the peritoneal cavity were then gently retracted using forceps to gain access to the testes. One of the testes was carefully removed using forceps and scissors. After removal, the peritoneal cavity was closed and sutured using absorbable suture material (2/0 chromic gut) with a simple discontinuous suture consisting of 5 to 9 stitches. Postoperative disinfection was performed with methylene blue [18].

The third step is post-surgical recovery. After suturing, the six fish were placed in recovery tanks for the convalescence period. The first feeding took place 48 hours after suturing to prevent complications related to food ingestion and to minimize stress on the sutures. Wound healing was monitored once a week, with each fish briefly removed from its tank using a net for examination [18].

Postoperative monitoring involved daily checks of the physicochemical parameters of the rearing water during the ten days required for the broodstock to heal completely. This monitoring focused on temperature, dissolved oxygen, pH, and electrical conductivity. At the same time, ammonia and nitrite concentrations were assessed by visual colorimetric analysis, using a color chart for comparison. These analyses were conducted in accordance with standard protocols for monitoring water quality in aquaculture [19]. At the same time, the post-surgery survival rate of the fish was assessed throughout the observation period [18]. With regard to the physicochemical parameters, changes in the values were monitored over a ten-day period for the entire experimental group.

Sperm Collection via Euthanasia

Sperm collection is a crucial step in the artificial reproduction process for *Clarias gariepinus*, as it determines the quality of fertilization and the long-term availability of male broodstock. Traditionally, sperm collection in this species involves the sacrifice of the breeding male, followed by testicular removal and maceration to obtain usable sperm [20]. Although this method is commonly used in Africa, it has a major drawback: the systematic loss of the male broodstock, which limits the sustainability of broodstock populations in hatcheries [21] [22].

Sperm collection and recovery were performed with particular care to avoid any premature activation of the spermatozoa. After removal using forceps, the testes were placed on a pre-dried dish. Extraction then involved making an incision in the testes with fine scissors, followed by gentle manual pressure to release the seminal fluid into a beaker. Finally, the seminal fluid was aspirated using a 5-ml syringe, allowing for precise measurement of the total volume collected from each male, in accordance with the procedure described by Sayah (2016) [23].

The volume of semen (ml) collected from each *Clarias gariepinus* male, both surgically and by euthanasia, was recorded individually.

Hormonal Induction and Female Reproduction Protocol

Hormonal induction in African catfish *Clarias gariepinus* relies on the use of pituitary extracts, a technique widely employed in aquaculture to stimulate ovulation in mature females. The induction product was prepared from homologous pituitary glands collected from mature males. The crude hormonal extract was prepared at a concentration of 1 g/mL in 0.9% NaCl physiological saline, produced by Shijiazhuang No. 4 Pharmaceutical. This pituitary solution was then administered by a single intramuscular injection at a dose of 1 g/mL of female body weight, according to the protocol described by Okomoda *et al.* (2015) [24]. Following injection, females were kept in individual holding tanks under controlled hatchery thermal conditions, respecting a strict ovulatory latency time of 9 h 30. This experimental protocol was inspired by the work of Otoh *et al.* (2025) [25], which demonstrated that pituitary extracts yield fertilization and hatching rates comparable to those obtained with commercial hormones.

Egg Collection by Stripping and Fertilization Steps

Artificial reproduction was carried out using a non-invasive protocol to pre-

serve the integrity and survival of aquaculture broodstock females, following the approaches of Esa *et al.* (2023) [26]. At the end of the required thermal latency period, mature oocytes were manually extracted by gentle abdominal pressing (stripping). The expelled ovarian fluid was directly collected in small clean, dry collection basins pre-weighed on a precision electronic balance (OHAUS Pioneer PX4201, accuracy 0.1 g), allowing recording of the total spawning mass (Wf).

Immediately after stripping, artificial fertilization was performed dry according to the conventional method described for Siluriformes by Viveen *et al.* (1985) [20]. Milt (sperm), precisely measured using a sterile 5 ml syringe, was added directly to the oocytes in the dry container. The oocyte-sperm mixture was gently homogenized dry for a few seconds. Sperm activation and fertilization were triggered by adding a small volume of clean hatchery water, followed by gentle continuous stirring for about 1-2 minutes. Fertilized eggs were then immediately rinsed with clean water to remove excess seminal fluid, then spread evenly on incubation frames. These frames were placed in circular PVC incubators with a capacity of 50 liters, equipped with a closed continuous water renewal system to maintain optimal oxygenation conditions, in accordance with hatchery standards for Siluriformes by Biegniewska *et al.* (2010) [27].

Environmental Conditions and Larval Rearing Parameters

Intensive larval rearing was conducted for 30 days in experimental units consisting of small PVC tanks with a useful capacity of 20 liters. Stocking density per tank was uniformly set at 50 larvae per unit (*i.e.*, an initial density of 2.5 larvae·L^{-1}). Water renewal and removal of organic waste (feces and food residues) were ensured daily using appropriate siphon tubes, in accordance with water quality management recommendations in inland aquaculture [19] [28].

To rigorously interpret survival and growth dynamics, physicochemical variables of the environment were strictly monitored twice daily (07:00 and 18:00). During the entire fry rearing phase, from Day 3 to Day 30 post-yolk sac resorption, average water physicochemical conditions were maintained stable and homogeneous among experimental tanks (ANOVA, $p > 0.90$) within the biological comfort ranges described for *Clarias gariepinus* by Haylor (1991): temperature increased progressively and controlled from 26.58˚C ± 0.14˚C to 28.2˚C ± 0.09˚C at the end of rearing; average dissolved oxygen saturation was maintained between 6.79 ± 0.1 mg/L and 6.47 ± 0.09 mg·L^{-1} thanks to a continuous mini-pump aeration system; and pH stabilized between 7.44 ± 0.04 and 6.92 ± 0.03. Toxic nitrogenous compounds were rigorously controlled: total ammonia (NH3) remained null or below detection threshold (<0.2 mg/L), while nitrite concentration (NO2) evolved from 0 ± 0 mg/L at the start of incubation to very low stable traces of 0.03 ± 0.01 mg/L at the end of larval rearing.

Feeding

In this study, a single diet was used to feed all larvae from the different treatment groups. This diet is Perla, produced by Skretting; it is a high-performance starter diet specifically formulated for fish larvae. It comes in the form of extruded

micro-pellets with an extremely fine particle size (0.2 mm from 0 to 10 days, then 0.3 mm from 11 to 30 days), which is suited to the small size of the larvae's mouth openings [14]. The diet was tested in 20-liter tanks containing 50 larvae per rearing tank. The larvae were fed 6 times a day for the first 15 days, then 3 times a day until weaning at 30 days post-hatching [29].

Comparison of Fertilization Rates, Egg Hatching Rates, Survival, and Larval Growth according to Sperm Collection Methods

The reproductive performance of *Clarias gariepinus* was evaluated by the fertilization rate—based on the count of transparent eggs—and the hatching rate—calculated as the ratio of viable larvae to fertilized eggs—according to the methodology of Tilahun *et al.* (2016) [30]. These parameters allow for the quantification of the effectiveness of artificial reproduction, with the fertilization rate calculated from an initial sample and the hatching rate determined at the end of incubation.

- the fertilization rate (%), calculated according to Tilahun *et al.* (2016) [30].
- Weight in g
- Length in cm
- $\mathrm{TF} = \dfrac{\text{Number of transluscent fertilized eggs}}{\text{Total number of eggs (incubation)}} \times 100$

  the hatching rate (%), calculated according to Tilahun *et al.* (2016) [30].

$$\mathrm{TE} = \frac{\text{Number of hatched live larvae}}{\text{Total number of fertilized eggs}}$$

- The measured parameters included: Survival rate (%): proportion of surviving larvae on Day 10, Day 20, and Day 30; survival rate (%) on Day 10, Day 20, and Day 30, according to the formula by Tilahun *et al.* (2016) [30];

$$\mathrm{TS} = \frac{\text{Final number of living individuals at stage d10, d20, or d30}}{\text{Total number of individuals initially counted at stage d4}} \times 100$$

- Total number of individuals initially counted at stage d4

Growth in length (cm) and weight (g): measured by sampling 10 larvae per tank every 10 days up to 30 days of rearing, using a digital caliper and a digital scale; Condition factor (K): calculated according to Fulton $\mathrm{K} = \left(\dfrac{\text{Weight in g}}{\text{Length}^3 \text{ in cm}} \right) \times 100$.

Experimental Design and Statistical Analyses

The experimental unit was strictly defined for each level of protocol analysis. For evaluation of morpho-ponderal characteristics, growth, and broodstock condition, the experimental unit was the individual fish (n = 12 females and n = 6 males per treatment group). For monitoring water physicochemical parameters (temperature, pH, dissolved oxygen, electrical conductivity, nitrites, and ammonia), the experimental unit was the individual rearing tank (n = 12 tanks in total). For zootechnical evaluation of fertilization, hatching, and overall larval survival at 30 days, the experimental unit was also the tank, with (n = 3) replicate tanks per treatment combination (12 tanks in total).

For monitoring the offspring weight and length growth, the 10 larvae randomly sampled from each tank at D10, D20, and D30 constituted subsampling units (technical replicates). To avoid pseudo-replication bias, individual measurements of these 10 larvae were averaged per tank. It is the mean value per experimental unit (tank, n = 3 per combination) that was subjected to two-factor ANOVA, Student's t-test, and Principal Component Analysis (PCA), thus ensuring strict independence of observations. Data normality was verified beforehand by the Shapiro-Wilk test. The sketch of the experimental design is shown in Figure 1.

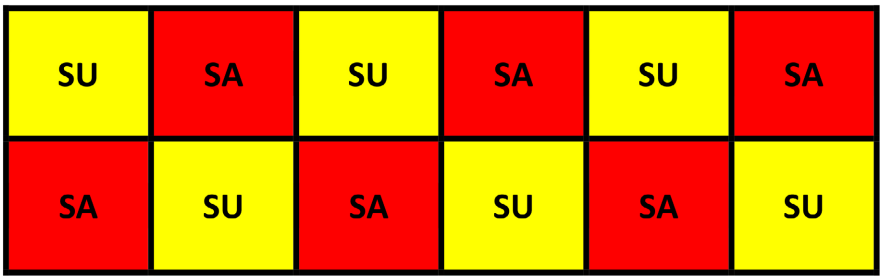

Figure 1. Sketch of the experimental design. SU = Surgery; SA = Sacrifice.

3. Results

3.1. Male Orchiectomy and Euthanasia

The effectiveness of surgical semen collection was evaluated by direct comparison with the conventional method involving sacrifice. The results in Table 1 detail the maturity parameters (IGS) and quantitative semen yields obtained for each treatment.

Table 1. *Comparison of surgical and sacrificial methods* based on sperm volume and IGS.

Method	Sample Size	Sperm volume (mL)	Relative volume (mL)	IGS (%)
Surgery	6	2.6 ± 0.71	4.56 ± 1.22	0.93 ± 0.26
Sacrifice	6	3.12 ± 0.88	4.71 ± 1.35	0.96 ± 0.29
p:		0.2895 (ns)	0.723 (ns)	0.8212 (ns)

Table 1 shows encouraging results for the management of male broodstock of *Clarias gariepinus*.

First, the sexual maturity of the two groups was equivalent, with a nearly identical Gonado-Somatic Index (GSI) of approximately 0.93 ± 0.26 for the operated individuals and 0.96 ± 0.29 for the sacrificed individuals. This similarity in GSI validates the rigor of the sampling and allows for a fair comparison of collection performance.

Quantitatively, the average volume of semen collected per sacrifice is similar to that obtained surgically ($p = 0.29$). This demonstrates that the surgical method

allows for the extraction of a semen volume that is similar to that of the conventional lethal method.

3.1.1. Relationship between Sperm Volume Produced, Male Body Size, and Testicular Weight

To determine whether male stature is an effective selection criterion, the relationship between body weight and the volume of semen collected was analyzed in Figure 2 and Table 2.

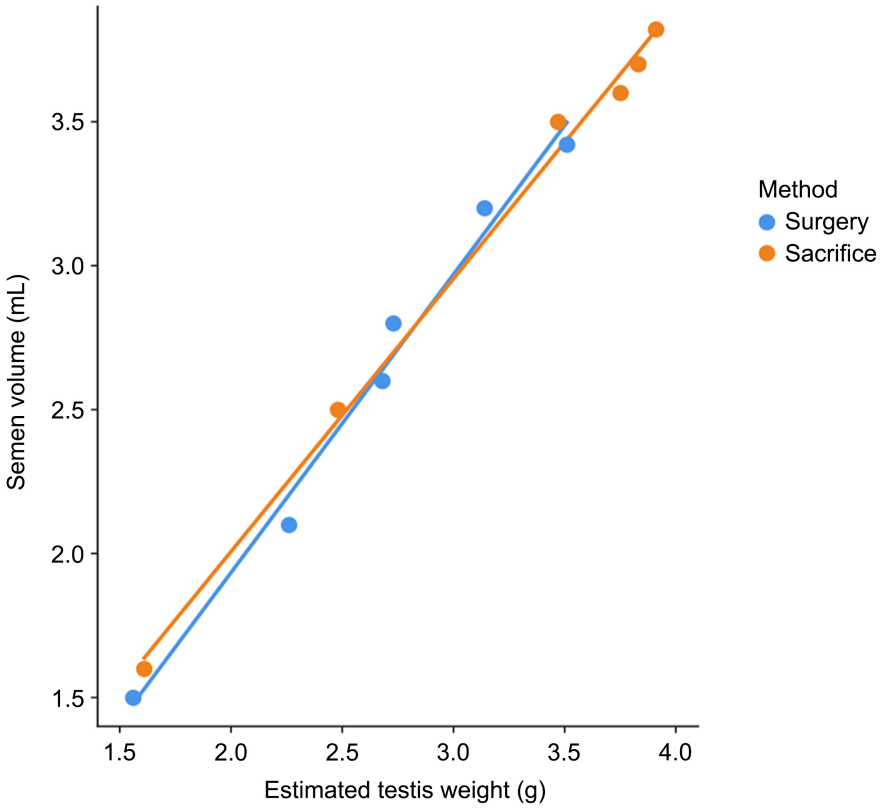

Figure 2. Relationship between sperm volume produced and testicular weight.

Table 2. Correlation between body size, testicle weight, and sperm production.

Correlated parameters	Pearson's correlation coefficient (r)	*p*	Decision
Body weight vs. semen volume	0.410	0.186	Not significant (ns)
Body weight vs. testicular weight	0.277	0.383	Not significant (ns)
Testicular weight vs. semen volume	**0.985**	<0.0001	Highly significant

Figure 2 and Table 2 show a strong positive linear relationship between testicle weight and collected semen volume ($r = 0.985$; $p < 0.0001$), proving that semen production is strictly determined by gonadal mass. There is a near-perfect align-

ment of the data points where the trend lines for "Surgery" and "Sacrifice" completely overlap. This overlap provides clear biological evidence that partial removal from a live fish yields exactly the same volume of semen as traditional sacrifice for the same gonadal mass. The non-lethal method guarantees maximum collection yield while sparing the lives of the broodstock.

Conversely, the fish's total body weight proves to be a misleading indicator, showing no significant correlation with testicular weight ($p = 0.383$) or sperm volume ($p = 0.186$). This finding shows that a heavy broodstock is not necessarily more fertile and scientifically validates the exclusive use of the Gonadosomatic Index (GSI), rather than visual gross weighing, to select high-performing males in hatcheries.

3.1.2. Survival of Male Breeding Stock after Orchiectomy

Assessing the survival of broodstock following surgery is the primary indicator of the protocol's success. Table 3 summarizes the survival rate observed over a ten-day period (D10) following orchiectomy.

Table 3. Summary of post-surgical survival of males.

Indicator	Total number (n)	Living individuals	Survival rate
Survival rate at Day 10 after orchiectomy	6	6	100%

Table 3 illustrates the daily monitoring of the six male broodstock during the ten days following the surgical procedure and reveals a survival rate of 100%. No individual died as a result of the surgery or postoperative handling. This success, combined with the optimal water quality maintained during the stabilization phase ($O_2 > 7$ mg/L), confirms the safety of the orchiectomy-based semen collection protocol for *Clarias gariepinus*. This result confirms the technical feasibility of this non-lethal method, which preserves the entire broodstock for future breeding cycles, thereby optimizing the hatchery's profitability.

3.1.3. Impact of the Sperm Collection Technique on Male Gamete Viability

To evaluate the impact of the sperm collection technique on the viability of male gametes, a comparison of fertilization, hatching, and larval survival rates was conducted between the two groups. Table 4 and Figure 3 present the reproductive performance data and the results of the associated Student's t-test, which allows assessment of the biological efficacy of partial orchiectomy compared to traditional sacrifice.

Table 4 and Figure 3 illustrate the reproductive performance and provide biological evidence of the protocol's success. The results show that semen quality is preserved, regardless of whether the male undergoes surgery or is euthanized using the traditional method. In fact, the fertilization rate obtained through surgery

(80% ± 8.05%) is virtually identical to that obtained through sacrifice (79% ± 5.62%), a similarity confirmed by a very high p (0.8086). This trend stabilizes in subsequent stages, particularly for hatching and larval survival, where statistical tests reveal no significant differences ($p > 0.05$). These rates are consistent with the biological standards observed in *Clarias gariepinus* and demonstrate that the fry produced is equally vigorous, regardless of the sperm origin.

Table 4. Reproductive performance (surgery and culling).

Sperm source	n	Fertilization %	Hatching %	Larval Survival %
Surgery	6	80 ± 8.05	52.5 ± 7.31	88.67 ± 4.32
Sacrifice	6	79 ± 5.62	49.33 ± 10.91	82.00 ± 2.83
Student's t-tests:	p(fec) = 0.8086	p(hatch) = 0.5698	p(surv) = 0.011	

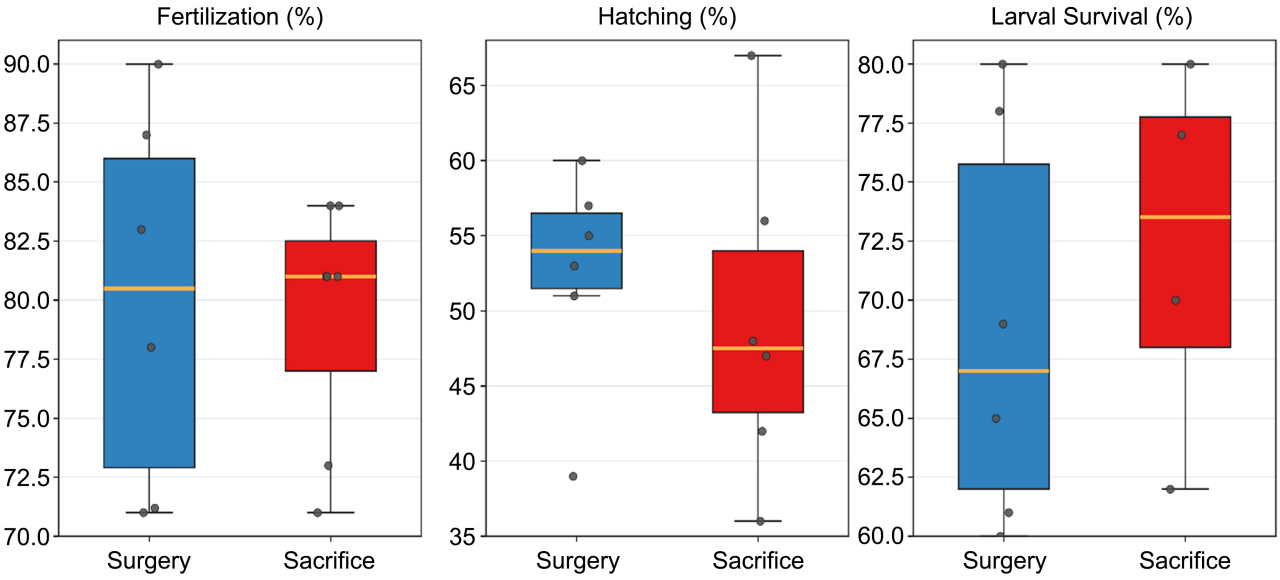

Figure 3. Comparison of reproductive performance by sperm source (surgery or sacrifice).

The distribution of the reproductive performance for each collection method is illustrated by the box plots in Figure 3. This graphical representation allows for visualization of the data dispersion and the overlap of results between surgical collection and sacrifice. Visual inspection reveals near-complete overlap of the boxes for the three indicators (fertilization, hatching, and survival). This closeness of the medians confirms the absence of any significant difference between the two methods.

Although there is slightly greater variability in the hatching rate in the "Sacrifice" group, the overall performance of the surgical method remains highly stable. This graph provides definitive visual evidence that non-lethal collection guarantees fry production identical to that of the traditional method, thereby validating its adoption in hatcheries.

3.1.4. Egg-Laying and Fertility Performance by Sperm Collection Method

The reproductive performance data obtained during the experiment—specifically, clutch weight and absolute fertility as a function of the type of male parent—are summarized in Table 5.

Table 5. Egg-laying and fertility performance.

Parameters	Groups	N	Mean ± standard deviation	CV (%)	*p*
	Collection method				
Egg weight (g)	Surgery	6	94.47 ± 23.28	24.64	0.927
	Sacrifice	6	95.74 ± 23.86	24.92	
	Collection method				
Absolute fertility	Surgery	6	62650.00 ± 14260.11	22.76	0.772
	Sacrifice	6	65362.67 ± 17560.94	26.87	
Female weight (g)	**Total**	12	616.67 ± 56.44	9.15	-

Table 5 shows that semen collection method (surgery vs. euthanasia) does not significantly affect clutch weight and absolute fertility ($p > 0.05$). Regarding egg weight, the mean values recorded were 94.47 ± 23.28 g for the Surgery group and 95.74 ± 23.86 g for the Sacrifice group. Statistical analysis confirms the absence of a significant difference between these two methods ($p = 0.927$). The coefficients of variation, at 24.64% and 24.92% respectively, indicate similar dispersion and homogeneity of the data between the two groups.

Similarly, absolute fecundity does not vary significantly depending on the treatment applied to the males ($p = 0.772$). Females in the Surgery group had an average fecundity of 62650.00 ± 14260.11 eggs, while those in the Sacrifice group had an average of 65362.67 ± 17560.94 eggs.

The associated coefficients of variation (22.76% for surgery and 26.87% for sacrifice) reflect broadly comparable intra-group variability with high stability in both cases.

Partial orchiectomy does not affect the females' physiological response to induction or the final oocyte yield.

3.2. Survival and Growth in *Clarias gariepinus* Larvae

Larval survival rates over time shows disparities during the 30-day rearing period, a critical phase of development, as shown in Figures 4(a)-(c).

Figure 4 presents the results of the longitudinal study of larval viability conducted during the first thirty days of rearing. These results illustrate larval survival and allow for a comparison of the robustness of the offspring produced using the two semen collection methods. Survival rates on the 20th and 30th days varied significantly depending on the surgical method (p20d = 0.07; p30d = 0.03), unlike

on the 10th day, when no notable difference was observed (p10d = 0.41). It thus appears that the euthanasia method resulted in lower larval survival compared to that achieved through surgery.

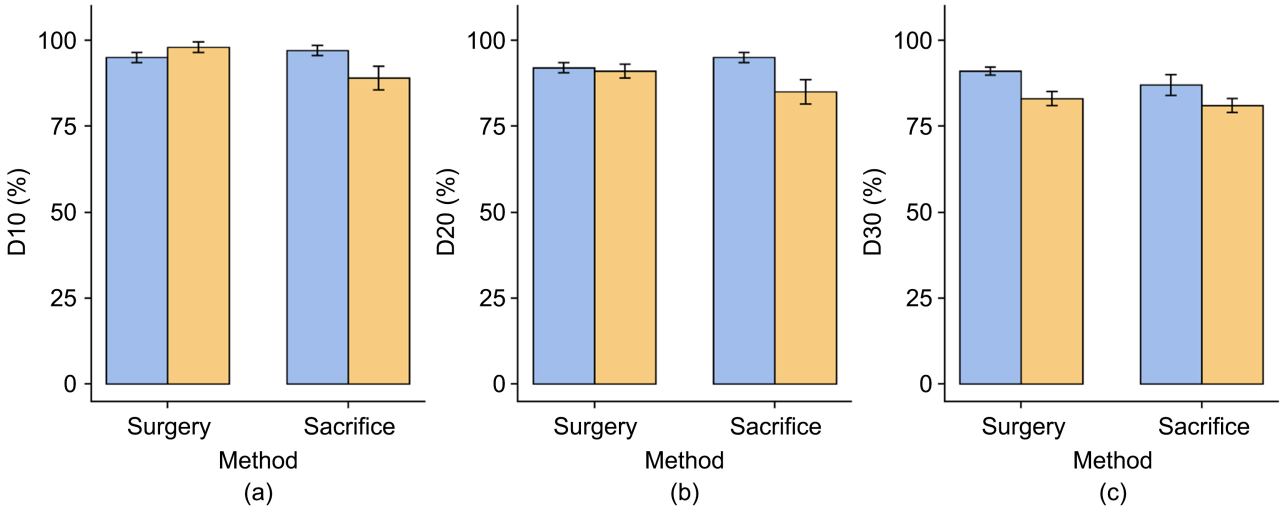

Figure 4. Larval survival after 10 days (a), 20 days (c), and 30 days of rearing (c).

Figure 5, which shows larval survival over 30 days, reveals a gradual decline in the population, with no peaks of mass mortality. It can be observed that larvae from the surgical group (blue curve blue curve) consistently maintained a higher survival rate than those from the sacrifice group (red curve), ending with approximately 88.6% survivors versus 82%.

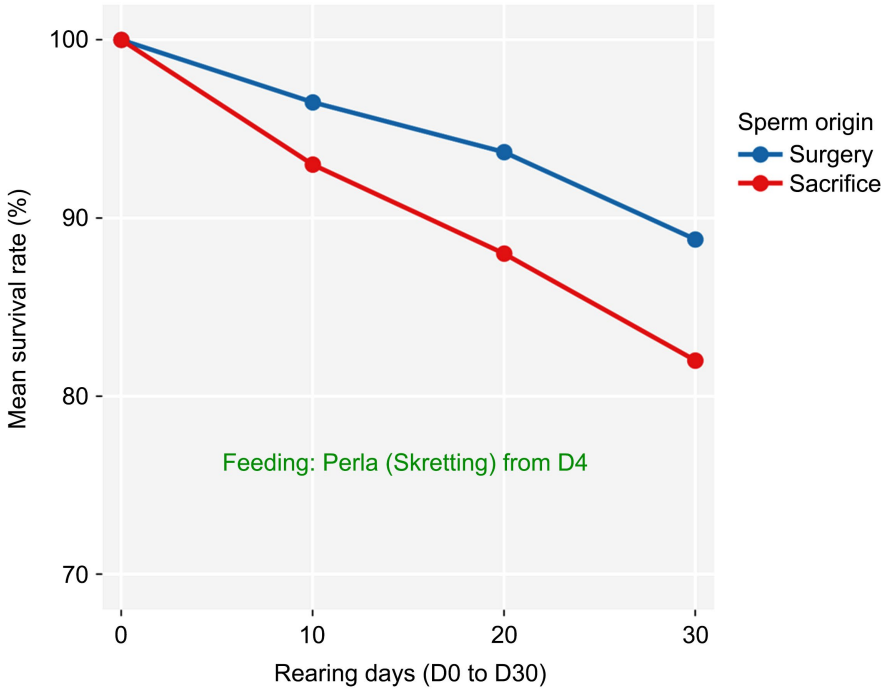

Figure 5. Survival rate dynamics of larvae over 30 days.

This result is crucial because it proves that semen collection via partial orchiectomy in no way impairs the vigor of the offspring. Finally, the stability of the slopes starting on the fourth day confirms that the use of Perla feed (Skretting) successfully secured the critical phase of dietary transition, thereby ensuring the production of vigorous and high-performing fry for the hatchery. Furthermore, the surgical method and the K index on the ^{10th}day are negatively correlated. This indicates that the sacrifice resulted in larvae with a lower K index on the ^{10th}day.

The growth dynamics of the larvae, monitored from the 10th to the 30th day of rearing, are illustrated in Figure 4. This figure allows us to visualize the evolution of average weight and to compare the metabolic efficiency of the offspring produced using the two sperm collection methods.

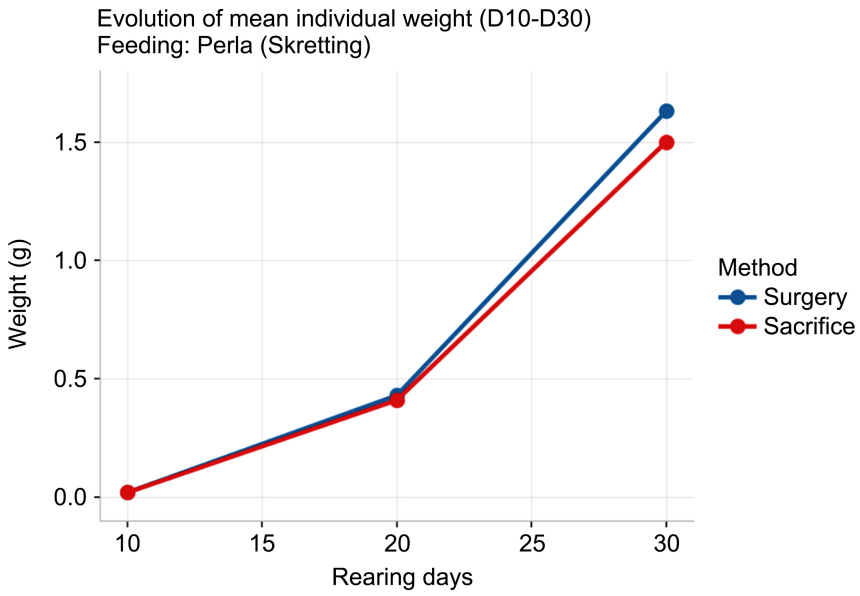

Figure 6. Weight growth kinetics of larvae fed Perla (Skretting) according to the method of male semen collection.

Figure 6 illustrates the trend in average individual larval weight between the 10th and 30th days of rearing. It highlights a quasi-exponential growth, which is particularly marked after the Day-20. At the start of the study on Day 10, weights are very low and identical for both groups, then increase gradually until Day 20, when the two curves converge at around 0.4 g. The final phase between D20 and D30 shows a sharp acceleration in weight gain, confirming the complete assimilation of Skretting's Perla feed during the transition to exogenous feeding. In comparison, the blue curve representing the larvae from the surgery group stands out and remains above the red curve of the sacrifice group at the end of the experiment. By Day 30, the surgery group clearly exceeded the 1.5 g threshold, while the control group barely reached this value.

3.2.1. Body Condition of Fry at the End of Rearing

The health and vigor of the fry at the end of the 30-day rearing period were as-

sessed by calculating the Fulton condition index (K). Table 6 presents a comparison of this index among the offspring fed Perla (Skretting).

Table 6. Body condition of fry at 30 days.

Male origin	Number of tanks	Condition index K (Mean ± SD)
Surgery	6	0.6 ± 0.05
Euthanasia	6	0.57 ± 0.06

Student's t-test (homogeneity): $p = 0.363$.

Table 6 summarizes the results of the K Condition Index analysis on Day-30 and confirms the good health and body condition of the fry. It can be observed that the index remains stable and consistent around an average value of 0.60, with no significant difference between larvae from the surgical group and those from the traditional sacrifice group ($p = 0.363$) and it indicates that the larvae did not suffer from nutritional deficiencies and that the Perla feed (Skretting) was fully assimilated in all batches.

3.2.2. Growth Performance

The efficiency of feed assimilation and the rate of larval development were evaluated by calculating the Specific Growth Rate (SGR). Table 7 summarizes the daily growth performance for the two experimental groups during the critical period from the 10th to the 30th day.

Table 7. The specific growth rate from the first to the 30th day.

Male origin	Specific growth rate (SGR)
Surgery	22.42 ± 1.13%/day
Euthanasia	21.97 ± 1.3%/day

Student's t-test (Surgery vs. Sacrifice): $p = 0.537$.

Table 7 summarizes the results of the Specific Growth Rate (SGR) analysis and provides definitive evidence of the success of the rearing protocol. With average values of 22.42%/day for the surgery group and 21.97%/day for the sacrifice group, the larvae exhibited exceptional growth between the 10th and 30th days. Mathematically, this means that the individual biomass of the fry increased by nearly one-quarter of their own weight every day, confirming the exponential growth phase and the perfect assimilation of the Perla feed (Skretting). In fact, there is no significant difference between the two sperm collection methods.

4. Discussion

Neuroendocrine Correlations and Hatching Kinetics

A moderate positive correlation (r = 0.398) was found between the females' in-

itial IGS and the final hatching success of the eggs following hormonal treatment.

This interaction is consistent with the neuroendocrine models described by Zohar *et al.* (2010) [31] as well as with the reviews by Schulz *et al.* (2010) [32] on fish reproductive physiology, which state that the sensitivity of the gonads to an exogenous signal depends on the prior state of receptivity of the target tissues.

This dynamic indicates that the 14-day conditioning protocol enabled the females to reach an advanced stage of vitellogenesis. The more the oocyte is loaded with vitelline reserves (as indicated by a high IGS), the more functional the membrane receptors for gonadotropins are and the more ready they are to trigger the cellular mechanisms of oocyte maturation following activation by EGPP, whether fresh or stored.

Postoperative Survival and Management of Recovery Conditions

The assessment of postoperative survival revealed a 100% success rate among all six individuals that underwent partial orchiectomy, with no pathological complications during the ten-day follow-up period. This success was accompanied by strict stabilization of water quality, characterized by an average temperature of 25.75˚C ± 0.40˚C, a neutral pH of 7.41 ± 0.39, a saturation oxygen level of 7.10 ± 0.60 mg/L, and extremely low nitrite levels of 0.04 ± 0.06 mg/L.

This 100% survival rate with no loss of stock is consistent with the observations of Legendre and Billard (1996) [33], who confirmed that the lobular structure and anatomical compartmentalization of the testes in *Clarias gariepinus* allow for surgical removal without impairing its basic vital functions. It also corroborates the clinical guidelines by Wildgoose (2000) [16] and Santi *et al.* (2022) [18] on the importance of maintaining physiological conditions during convalescence, as well as the follow-up studies by Rouabah *et al.* (2016) [34] regarding the preventive effect of chemically pure water on postoperative osmotic stress.

From a physiological standpoint, maintaining a high concentration of dissolved oxygen (7.10 mg/L) stimulated the respiratory metabolism of the operated animals, facilitating post-anesthetic recovery and accelerating the healing process of sutured skin and muscle tissues. Furthermore, the neutral pH (7.41) and the absence of toxicity from nitrogen compounds (nitrites at 0.04 mg/L and ammonia < 0.2 mg/L) eliminated the risk of chemical irritation of the wounds, thereby preventing the development of secondary bacterial infections.

Volumetric Yields and Characteristics of the Collected Semen

The average volume of semen extracted surgically was 2.6 ± 0.71 mL (relative volume of 4.56 ± 1.22 mL), a result that was statistically equivalent ($p = 0.2895$) to the raw value of 3.12 ± 0.88 mL obtained using the conventional euthanasia technique. The mean Gonado-Somatic Index (GSI) of the operated donors was 0.93 ± 0.26%, showing a moderate but non-significant positive correlation ($r = 0.410$; $p = 0.186$) between body weight and semen production.

This consistency in yield is consistent with the observations of Mansour *et al.* (2004) [35] and Tınkır *et al.* (2023) [36], demonstrating that the seminal structure and fluid ejection mechanisms in Siluriforms allow for the expulsion of consistent

volumes regardless of whether the collection is lethal. Similarly, the obtained IGS value (0.93%) is consistent with the maturity criteria described by Okomoda *et al.* (2015) [24], while the relative independence between weight and seminal volume is in line with the conclusions of Viveiros and Godinho (2009) [37].

The lack of statistical significance in the volumetric difference ($p = 0.2895$) indicates that partial ablation does not cause any mechanical or physiological obstruction of excretion. Semen isometry can be explained by the gonad's full maturity, with available volume being linked to the degree of internal spermatogenesis rather than solely to the animal's body size, as evidenced by the weak influence of gross weight ($p = 0.186$).

Reproductive Performance and Offspring Viability

Reproductive performance confirmed absolute parity for the average fertilization rate (80% ± 8.05% for the surgery group versus 79% ± 5.62% for the sacrifice group; $p = 0.8086$) and for the hatching rate (52.5% ± 7.31% vs. 49.33% ± 10.91%; $p = 0.5698$). Conversely, the survival rate of larvae from the surgery group showed a highly significant statistical advantage (88.67% ± 4.32% vs. 82.00% ± 2.83%; $p = 0.011$) compared to the control group.

The overlap of the medians for fertilization and hatching confirms the biological efficacy of the non-lethal technique, consistent with the work of Viveiros and Godinho (2009) [37]. Furthermore, the increase in larval vigor (88.67%) supports Vandeputte's (2003) hypotheses regarding the added value of preserving high-performing strains to stabilize offspring in controlled aquaculture. [38]

The equivalence of fertilization and hatching rates indicate that the fertilizing capacity of the gametes is not impaired by the surgical procedure. The significant increase in larval survival ($p = 0.011$) suggests that the surgical collection s avoids the massive release of catecholamines and stress hormones associated with the agony of the sacrificed fish, thereby preserving the seminal fluid from any early biochemical alterations and conferring greater physiological vigor to the fry.

Sustainability and Zootechnical Management of the Breeding Stock

The implementation of this non-lethal surgical protocol has made it possible to extract high-quality semen volumes while keeping the entire male broodstock alive (100% survival rate at Day 10).

This finding is supported by Santi *et al.* (2022) [18] in Burkina Faso, who view non-lethal collection as a solution to difficulties in securing broodstock. In this regard, Diyaware *et al.* (2010) [17] demonstrated that the economic time required to reuse a male Clarias after collection is 90 days, with IGS and fertility parameters not differing significantly from those observed at 120 days. This opportunity for sustainable exploitation meets the economic imperatives outlined by Aisyah *et al.* (2018) [39] in intensive aquaculture, as well as the zootechnical analyses by Santi *et al.* (2022) [18] in Burkina Faso, which advocate the use of non-lethal collection methods to overcome constraints on the supply of high-quality broodstock in sub-Saharan Africa.

In *Clarias gariepinus*, the ability to heal rapidly and metabolic resilience allow

the male to survive unilateral orchiectomy. By preserving the fish, the hatchery eliminates the need to continuously replenish its donor stocks, allowing it to capitalize on elite individuals selected for their growth performance, thereby optimizing genetic progress over production cycles.

Feeding Performance and Weight Growth Kinetics of Larvae

The evaluation of growth kinetics reveals exponential development of the larvae starting on the twentieth day of rearing (D20) on the Perla diet (Skretting). The Specific Growth Rate (SGR) reached exceptional values of 22.42 ± 1.13%/day for the surgery group and 21.97 ± 1.30%/day for the sacrifice group. At the end of 30 days (D30), individual weight exceeded the 1.50 g threshold for the surgery group. Furthermore, the final Fulton Condition Index (K) remained consistent and stable between the two groups (0.60 ± 0.05 vs. 0.57 ± 0.06; $p = 0.363$).

These TCS values exceeding 21.0% per day align with the high productivity standards of de Graaf and Janssen (1996) published by the FAO.[40] The weight gain kinetics obtained using the high-energy Perla feed also validate the performance documented by Chepkirui-Boit *et al.* (2011) [41] regarding the importance of an optimized nutritional transition protocol using a dry micro-starter diet to maximize linear and somatic growth in young Clariidae larvae. Furthermore, the morphological balance achieved ($K \approx 0.60$) in these 30-day-old fry reflects growth primarily focused on body elongation, which is typical of this early developmental stage. This result is consistent with the nutritional assimilation dynamics described by Ng *et al.* (2003) for the African catfish, with the condition index expected to increase gradually as the juveniles accumulate muscle mass reserves. [42]

An increase in individual biomass of nearly one-quarter of its own weight each day implies the effectiveness of the transition to exogenous feeding on the fourth day (D4). The isometric relationship of the K index ($p = 0.363$) between the two batches demonstrates that the origin of the male broodstock does not alter the fry's intestinal absorption capacity or digestive anatomy. The feed's amino acid and phospholipid content thus simultaneously supported both the fish's maintenance metabolism and rapid musculoskeletal development.

5. Conclusions

One of the findings of this study is the complete success of partial orchiectomy. In a field where the sacrifice of male fish has been the norm for decades, seeing that the broodstock from Lake Kivu survived at a 100% rate after surgery is promising. The rapid recovery, and the production of a sperm whose quality is equivalent to that of the sacrificed males (80% fertilization rate). By achieving a 30-day larval survival rate of 91% among the offspring of these operated males—a rate higher than that of the control group—it is shown that protecting the broodstock's life is not a luxury but a profitable biological investment.

There is also a regional focus. By choosing the wild broodstock sourced directly from Lake Kivu, the use of local strains is promoted. The results show that this

local strain is comparable to domesticated strains: it is resilient, adapts to tank-based aquaculture, and responds 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.

The use of testicular microsurgery preserves elite broodstock.

In light of the results, here are the recommendations:

- Promotion of partial orchiectomy: It is imperative to promote this technique among fish hatcheries to put an end to the systematic and unnecessary culling of high-performing males. This approach allows elite individuals to be preserved for multiple breeding cycles.
- Protection of Lake Kivu's genetic heritage: There is an urgent need to establish a genetic conservatory dedicated to the 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.

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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, Jean-Berckmans Bahananga MUHIGWA, Désiré Akonkwa BALAGIZI; formal analysis Jean-Berckmans Bahananga MUHIGWA; 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.

Conflicts of Interest

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

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