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Embryonic and Larval Development of Reciprocal Crosses between *Clarias gariepinus* (Burchell, 1822) and *Clarias jaensis* (Boulenger, 1909) in West Cameroon

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Abstract

Establishing intraspecific breeding and hybridization programs and determining genetic variability are two important issues for aquaculture. However, interspecific hybridization to improve growth and feeding efficiency is limited. For this purpose, the embryonic and larval development of reciprocal crosses of *Clarias gariepinus* (Burchell, 1822) and *Clarias jaensis* (Boulenger, 1909) were studied under laboratory conditions. The fertilization rate varied from 63.33% to 92%, while the hatching rate ranged from 55.68% to 76% with the highest value in hybrids ♀Cg × ♂Cj. Crosses between ♀Cj × ♂Cj, ♀Cg × ♂Cj and ♀Cj × ♂Cg had embryonic stages similar to those of the pure sib ♀Cg x ♂Cg. All crosses, however, had different timing for the various embryological stages. Hatching occurred at 32 h 15 min and 38 h for ♀Cj × ♂Cj and ♀Cj × ♂Cg, and 23 h and 23 h 30 min, respectively, for ♀Cg × ♂Cg and ♀Cg × ♂Cj. However, both crosses produced viable larvae until the first external feeding. The external morphological features of the larvae were completely formed by the 10th day after hatching. The body forms of the crosses at this time were indistinguishable from the pure sib. This study thus laid the groundwork for further comparative studies on hybrid performance and characterization.

Keywords

Embryonic Development, Larval Development, Interspecific Hybridization,

Clarias gariepinus, *Clarias jaensis*

1. Introduction

The main objective of hybridization in aquaculture is to combine favorable characteristics of one species with another in order to generate offspring that exhibit superior quality compared to the parent species [1] [2]. Hybridization has since been used as a biotechnological tool to enhance aquaculture production [1]. Aquaculturists utilize this breeding method to create aquatic organisms with a combination of valuable traits from two species while minimizing unwanted reproduction through the production of sterile hybrids [3]-[6]. These desirable traits may include enhanced growth rate, increased productivity, superior flesh quality, heightened disease resistance, improved environmental adaptability, and more efficient food conversion [7]. Nevertheless, despite the potential benefits, a significant number of previously documented hybridizations have not gained significance in aquaculture. This can be attributed, in part, to the insufficient understanding of crucial biological aspects concerning the hybrids, as highlighted by [8].

Research on the embryonic and larval development of fish plays a crucial role in understanding ontogenetic processes in aquaculture. These studies provide valuable insights into the biology, functional trends, and environmental preferences of different developmental stages of fish species [9] [10]. Additionally, they offer significant information on the developmental capabilities of fish, enabling comparisons between normal and altered developmental patterns [11] [12]. A thorough understanding of embryological and larval development is essential for enhancing the artificial propagation of cultured species, given its relevance in aquaculture [13]. This knowledge is particularly crucial for aquaculture applications. According to [14] and [15] the study of fish embryology involves the examination of egg development and their survival rate before and after hatching and up to the time of swimming. It also includes the study of the rate of yolk absorption until the beginning of exogenous feeding of the fry [16]. Consequently, it represents a fundamental step towards developing management and rearing techniques for newly targeted species aimed at commercial production.

African catfish *Clarias gariepinus*, a non-native species in Cameroon, has been successfully introduced into various river systems for aquaculture purposes since the 1970s [17] [18]. As mentioned by [19] and [20], *C. gariepinus* is recognized for its robustness, omnivorous feeding habits, rapid growth rate and high-quality flesh, and has been regarded as a promising candidate for aquaculture development in the region. *C. gariepinus* is widely cultured and distributed in various aquatic environments throughout the country, including the Mbô Floodplain, where it cohabits with the indigenous species *C. jaensis*. However, due to increased fishing pressure, *C. jaensis* is facing a decline and may be at risk

of disappearing [18]. Moreover, various research has indicated a low survival and growth rates in captivity for *C. jaensis* with many failures documented in the artificial propagation of *C. jaensis* [21] [22]. The growing demand for diversification of commercial aquaculture species worldwide has led to interest in interspecific hybridization between these species. The hybridization between the African catfish *Clarias gariepinus* (Burchell, 1822) and the indigenous *Clarias jaensis* (Boulenger, 1909) has been reported as successful in aquaculture literature [23]. However, there is limited data available regarding the embryonic and larval development of reciprocal crosses.

In this study, we conducted a study on the embryonic and larval development under controlled hatchery conditions to enhance our understanding of the morphological changes during these developmental stages. This knowledge will provide valuable insights for optimizing fish rearing techniques to facilitate better propagation strategies.

2. Materials and Methods

Broodstock Selection and Breeding

The study was conducted at the technical facilities of the Group of Common Initiatives for Integrated Western Aquaculture (GIC IAO) in Batié, located in the West Region of Cameroon. The climatic conditions in Batié are characterized as Guinean type, with a dry season from mid-November to mid-March, featuring temperatures ranging from 19 ˚C to 27˚C, and a rainy season from mid-March to mid-November with temperatures between 20˚C and 24˚C and significant diurnal variations. The average annual precipitation in Batié ranges from 1621 to 1800 mm. The topography of the area consists of mountainous terrain, with an average altitude of 1700 meters above sea level [24].

Two species of African catfish from the *Clarias* genus were used in the study. Twenty (20) *C. jaensis* (10 males and 10 females) with a mean weight of 413.73 ± 108.17 g and mean total length of 35.36 ± 4.98 cm, and twenty (20) *C. gariepinus* (ten male and 10 female) with a mean total weight of 614.20 ± 222.45 g and mean total length of 45.90 ± 4.67 cm were captured by fishermen from the Menoua River at the Mbô Floodplain. The broodstock were transported in a 10-liter container to the fish culture station in Batié. Upon arrival at the farm hatchery, same-sex broodstock of each species were acclimatized in a concrete tank measuring ($2 \times 0.86 \times 0.53$ m^3) for one month. During this acclimatization period, the broodstock were fed twice daily with an imported fishmeal containing 35% crude protein.

Eight broodstock individuals were selected for this study comprising one pair each of male (♂) and female (♀) of *C. jaensis* and *C. gariepinus*. The final oocyte maturation was induced using the Ovulin® hormone at a dose of 0.5 ml of stock solution per kg of body weight. Each female received two injections at 6h intervals, one as a preparatory injection (1/3) and a second (2/3) as the effective dose.

The administered dose was proportional to the weight of the fish. Males were not injected. After the injection, the broodstock were kept separately till the time of the stripping. Eggs from the different females were stripped into two separate bowls according to the species and gently mixed for 10 s using a rinsed soft spatula (Figure 1).

(a) (b) (c)

Figure 1. Broodstock of Clariidae used during the study: (a) Female *C. jaensis*; (b) Female *C. gariepinus*; and (c) Genital papilla of male of *C. jaensis* (to the left) and *C. gariepinus* (to the right).

Half of the eggs in each bowl were then transferred into another bowl using the spatula to provide four set of eggs (two from each species) for the four directional crosses (comprising two pure strain and two hybrid crosses). The male fish were tranquilized through immersion in an anesthetic solution containing clove powder as described by [25] before the milt collection using the technique of partial gonadectomy described by [26]. The milt from each testis was homogenized and subsequently diluted in a physiological solution of 0.9% NaCl in separate bowl based on species to combine the sperm from two males of the same species. The resultant mixture was used for fertilizing the eggs following specific crossing directions, as follows:

- ♀ *C. gariepinus* x♂ *C. gariepinus* (♀Cg × ♂Cg) (crossing A)
- ♀*C. jaensis* × ♂ *C. jaensis* (♀Cj × ♂Cj) (crossing B)
- ♀*C. gariepinus* × ♂ *C. jaensis* (♀Cg × ♂Cj) (crossing C)
- ♀*C. jaensis* × ♂ *C. gariepinus* (♀Cj × ♂Cg) (crossing D)

After adding the milt into each bowl of eggs, the eggs and milt were mixed gently using clean and dry spatula for 1 min to ensure uniform mixing, after which 100 ml of a fertilization solution (NaCl: 3 g/l and urea: 6 g/l in 1 l of water) was added and the contents mixed again for another minute. The excess of the fertilization solution was then decanted from each bowl leaving behind the fertilized eggs. Triplicate bowls of equal egg masses (10 g approximating about 7,000 eggs using the relationship of 700 eggs/g reported by [27] for *C. gariepinus* and 3,500 eggs using the relationship of 350 eggs/g for *C. jaensis* according to our observations) were evenly spread on 12 racks and incubated inside the hatchery in four concrete tank (2 m × 0.86 m × 0.53 m), equipped with a water pump and an immersion heater (water height = 0.37 m and water flow = 5.18 ml/s) at an average temperature of 26˚C.

Three replicate bowls of each cross line, each containing 100 eggs from specific cross directions, were placed in their respective incubators. After a designated period (1 - 2 hrs), the eggs in the bowls were observed to determine the fertilization rate. Fertilized eggs were distinguished from unfertilized eggs based on the presence of a transparent shell with a distinct gray or black spot, while unfertilized eggs exhibited an opaque appearance.

The egg size (n = 20) before and after fertilization were obtained using a stereo zoom photomicroscope bms 143 with usb camera 3MP, led (Model number MIK749597).

$$\%\text{Fertilization} = \frac{\text{Fertilized eggs in the bowl}}{\text{total number of eggs in the bowl}} \times 100$$

$$\%\text{Hatchability} = \frac{\text{number of hatched larvae}}{\text{total number of spawned eggs}} \times 100$$

$$H(\%) = \frac{F_1 - 1/2(P_1 + P_2)}{1/2(P_1 + P_2)} \times 100$$

where F_1, P_1, and P_2 are the average values for the first generation of hybrids, parent 1, and parent 2, respectively, and H is the percentage of heterosis in the F_1 hybrids.

Approximately 50 fertilized eggs were sampled at regular intervals from each cross line and examined under a stereo photomicroscope BMS 143 with USB camera 3MP, led (Model number MIK749597) following the protocols outlined by [8] and [14]. Pictorial evidence of the various developmental stages and any visible anomalies was captured. After hatching, the morphological features of the larvae (**Figure 2**) were measured using the BMS PhotoLib Software of the stereo photomicroscope. The yolk volume was calculated using the formula provided by [28]. The percentage of abnormalities was determined.

$$\text{V} = \left(\frac{\pi}{6}\right) lh^2$$

V represents the yolk volume; L is the yolk length; H is the yolk height.

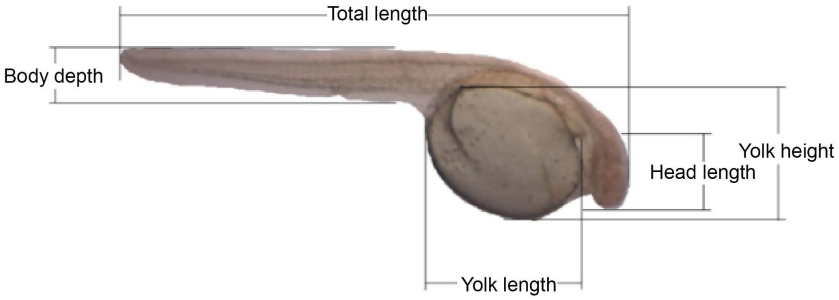

Figure 2. Biometric parameters of the hatchling.

Morphological(phenotypic) and behavioral changes (feeding and swimming) of the reciprocal crosses were documented every two days (n = 15). The feeding

and swimming pattern of the larvae were also observed before, during and after feeding. Measurement of the total length was also done every two days. Water quality was carefully monitored using a RCYAGO Dissolved Oxygen Meter with Electrode Filling Fluid and 5-in-1 Pentype Multi-Paramater and kept optimum by regular water change/continuous aeration (Temperature = 26.5 ± 0.7˚C; pH = 7.00 ± 0.26; Conductivity = 570 ± 2.90 μS·cm^{-1}; Total dissolved solid = 245.0 ± 0.80 mg·l^{-1}; Dissolved oxygen = 4.59 ± 0.50 mg·l^{-1}). Descriptive statistics for breeding and hatchling characteristics were performed using the AgroR package of R software followed by one-way analysis of Variance (ANOVA). Where significant ($p < 0.05$) differences were observed, data separation was done using Fisher's least significant difference.

3. Results

3.1. Egg Characteristics, Fertilization, and Hatchability

Table 1 summarizes the egg and breeding characteristics of the various crosses. According to the results, before fertilization, the eggs of *C. jaensis* were substantially bigger than those of *C. gariepinus* (1.06 vs. 0.70 mm). The ovulated or fertilized eggs were round, adhesive in nature and looked transparent. After fertilization, the size of the eggs varied and was highest (1.18 mm) for the pure *C. jaensis* cross, intermediate (1.16 and 0.96 mm respectively) for the reciprocal ♀Cj × ♂Cg crosses and pure *C. gariepinus*, and lowest (0.89 mm) for the reciprocal ♀Cg × ♂Cj crosses. Fertilization rate and hatchability were relatively greater in all crosses of female *C. gariepinus* (♀Cg × ♂Cj = 92%; ♀Cg x ♂Cg = 81% for fertilization rate and ♀Cg × ♂Cj = 76%; ♀Cg × ♂Cg = 70.66% for hatchability) compared to the other crosses of female *C. jaensis* (♀Cj × ♂Cj = 67% and ♀Cj × ♂Cg = 63.33% for fertilization rate and ♀Cj × ♂Cj = 65.93%; ♀Cj × ♂Cg = 55.68% for hatchability).Hence, heterosis for hatchability was positive for hybrids ♀Cg × ♂Cj and negative for ♀Cj × ♂Cg.

3.2. Embryogenesis

The stages of embryonic development, their characteristics, and times of occurrence after fertilization are detailed in Table 2 and illustrated in Figure 3. The

Table 1. Breeding and egg characteristics of reciprocal crosses between *C. gariepinus* and *C. jaensis* (n = 20 for egg size). Data are expressed as means ± standard errors.

Characteristics	♀Cg × ♂Cg	♀Cj × ♂Cj	♀Cg × ♂Cj	♀Cj × ♂Cg	p-value
Egg size pre-fertilization (mm)	0.70 ± 0.01^{b}	1.06 ± 0.03^{a}	0.70 ± 0.01^{b}	1.08 ± 0.04^{a}	$p < 0.001$
Egg size post fertilization (mm)	0.96 ± 0.02^{b}	1.18 ± 0.02^{a}	0.89 ± 0.05^{c}	1.16 ± 0.01^{a}	$p < 0.001$
%Fertilization	81 ± 6.55^{a}	67 ± 2.24^{b}	92 ± 3.00^{a}	63.33 ± 4.51^{b}	0.001
%Hatchability	70.66 ± 2.08^{a}	65.93 ± 2.96^{ab}	76 ± 4.58^{a}	55.68 ± 9.57^{b}	0.012
H for hatchability (%)			11.27	−18.48	

Mean in the same row with different superscripts differ significantly (ANOVA, $p < 0.05$).

Table 2. Stages of embryonic development in *C. gariepinus* and *C. jaensis* pure and reciprocal hybrids under laboratory conditions. Mean time was calculated by determining the average time using data from all crosses.

Stage	Mean time				Description
	♀Cg × ♂Cg	♀Cj × ♂Cj	♀Cg × ♂Cj	♀Cj × ♂Cg	
Mature oocytes/eggs	0 min	0 min	0 min	0 min	The unfertilized egg of *C. jaensis* was golden brown and oval, and *C. gariepinus* was light golden brown. Fresh eggs were adhesive and surrounded by uniform transparent layer of cytoplasm.
Fertilized egg	0 min	0 min	0 min	0 min	The fertilized egg expands a few seconds after fertilization and become hardened. The fertilized egg of *C. gariepinus* was greenish while the one of *C. jaensis* was yellowish.
Animal and vegetal pole	25 min	50 min	25 min	50 min	Expansion of the yolk away from the membrane and accumulation of cytoplasm at the anterior pole to form the animal pole and vegetal pole.
2-cell stage	45 min	3 h	45 min	3 h 15 min	This was observed as a vertical division of the animal pole producing two cells of equal size.
4-cell stage	50 min	7 h	50 min	7 h 30 min	Second line division producing four cells of equal size.6. eight-cellstage1 h, 10 min Third cell division, irregular cell heaps seen on top of the round lower part of the yolk.
8-cell stage	1 h 20 min	9 h	1 h 20 min	9 h 30	Third cell division, irregular cell heaps seen on top of the round lower part of the yolk.
16-cell stage	1 h 25 min	10 h	1 h 25 min	11 h	Irregular cells seen but difficult to count.
32-cell stage	1 h 40 min	11 h	1 h 40 min	12 h	Further division of cells, some cells tended to lie on another cell, irregular in size.
64-cell stage	4 h 15 min	12 h 30 min	4 h 15 min	15 h	Further division of cells producing many more cells but small blastomeres, the morula decreased.
Blastula stage	5 h 10 min	13 h	5 h 10 min	20 h	Further division producing mass of cells elevated over the general outline of the yolk mass (like a dome-shaped head).
Gastrula stage	9 h 15 min	17 h 30 min	9 h 15 min	24 h 15 min	Embryo develops germ rings. Cephalic and caudal edges evident, which formed at the advanced stage of the blastula.
First wriggling movement	18 h	26 h	18 h	28 h	Initiation of wriggling; the long somite starts to move both sides within the chorion wall, but this rate gradually increases with time.
Hatching	23 h	32 h 15 min	23 h 30 min	38 h	Vigorous movement at the tail to either side against the chorion wall, the chorion wall then ruptures and hatching occurs.

eggs were protected by a chorion wall containing cytoplasm and a substantial yolk surrounded by the vitelline membrane. Initial cleavage, resulting in the formation of two blastomeres, occurred within 25 min post fertilization (mpf) in ♀Cg × ♂Cg and ♀Cg × ♂Cj and within 3 h and 3 h 15 min respectively for cross ♀Cj × ♂Cj and ♀Cj × ♂Cg, forming the animal and vegetal poles; the yellow cy-

toplasm has moved toward the vegetal pole, leaving an optically clear animal pole. The chorion expanded away from the egg, creating the perivitelline space. Subsequent cleavages in *C. gariepinus* and *C. jaensis* and their hybrids led to the multiplication of blastomere and the reduction in their size. The gastrula stage was reached in 9 hours and 15 minutes in female *C. gariepinus* hybrids, occurring 8 h and 15 h later respectively in female *Clarias jaensis* hybrids (**Figure 3**).

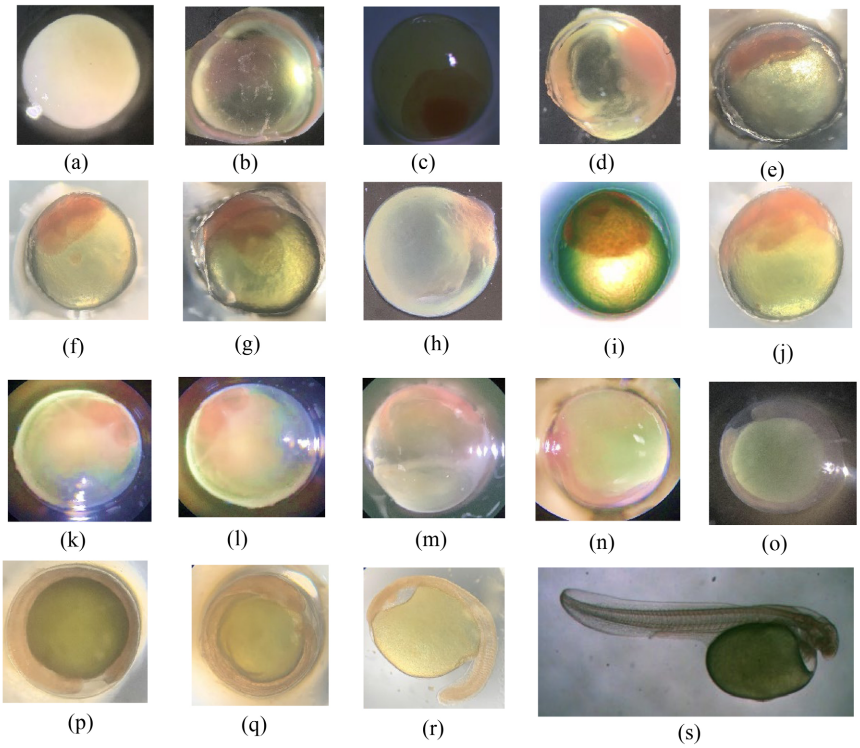

Figure 3. Normal embryological development of hybrid crosses between *C. gariepinus* and *C. jaensis*. (a) Unfertilized egg; (b) Fertilized egg; (c) Animal and vegetal pole; (d) Two-cell stage at first cleavage; (e) Four-cell stage; (f) Eight-cell stage; (g) 16-cell stage; (h) 32-cell stage; (i) 62-cell stage; (j) Morula stage; (k) Advanced morula stage; (l) Blastula stage; (m) Gastrula stage; (n) 75% epiboly; (o) Somite begins; (p) Somite cell; (q) Prime; (r) Freshly hatched; (s) Hatchling.

Subsequent to that, pigmentation was observed in the somites and the lens was fully formed. In this study, the somite stage was reached after 18 hours for ♀Cg × ♂Cg and ♀Cg × ♂Cj compare to 26 and 28 hours respectively for cross ♀Cj × ♂Cj and ♀Cj × ♂Cg. Further embryonic development led to a yolk sac enveloped by a girdle. By the 17th hour in ♀Cg × ♂Cg and ♀Cg × ♂Cj and the 25th and 27th hour there was an increase in the embryo's wriggling within the chorion sac. Prior to hatching, frequent embryonic movements were observed as the embryo attempted to breach the perivitelline membrane. Subsequently, the chorion wall ruptured from the caudal region, allowing the hatchlings to emerge. The incubation period at an average temperature of 26.7 ± 0.3˚C was 23 hours in pure *C. gariepinus*, 23 hours and 30 min in ♀Cg × ♂Cj cross, compare to 32 h 15

min and 38 h respectively in ♀Cj × ♂Cjand ♀Cj × ♂Cg hybrids.

3.3. Larvae Development

The hatching process began with the larvae making powerful movements against the wall in rapid succession using the tail area. The majority of larvae of had an elongated trunk tail that was approximately equal to or longer than the circumference of the yolk. Nevertheless, a significant number of larvae were trapped in the sac due to the limited length of the trunk, resulting in sluggish periodic movement within the eggs. Normal larvae were transparent with a straight body, approximately twice the length of the yolk. After hatching, larvae from ♀Cg × ♂Cg and ♀Cg × ♂Cj crosses were transparent and slightly brown, while larvae from ♀Cj × ♂Cj and ♀Cj × ♂Cg crosses were yellowish with laterally compressed bodies. Hatchlings had unpigmented eyes and no defined mouths or fins.

In general larvae from reciprocal crosses showed larger early biometric traits compared to those from pure parent crosses. Larvae from *C. gariepinus* eggs had a small head that was not clearly separated from the yolk sac, while larvae from *C. jaensis* eggs had a distinct separation between the head and yolk sac. The yolk sac was oval and pale greenish in color for *C. gariepinus* eggs crosses, and yellowish for *C. jaensis* eggs crosses. When viewed from above, the head and yolk sac resembled a bulb-like structure (Table 2). The hybrid larvae from ♀Cj × ♂Cg had significantly greater total length (9.62 mm) and yolk related characteristics (yolk volume = 22.69 mm; yolk length = 3.92 mm; yolk height = 3.32 mm). On the other hand, larvae from ♀Cg × ♂Cj had the highest head length (2.24 mm) and body depth (0.96 mm). The pure *C. gariepinus* larvae had the lowest biometric characters. A dark pigmented and prominent eye spot emerged on each side of the head within 24 hours after hatching. Barbels in the form of small knobs were visible in one-day-old larvae from all crosses. The mouth was visible but not yet open, and the anal opening remained closed. The yolk size of the offspring decreased gradually until it was fully absorbed, with an average absorption time of 10 days for ♀Cj × ♂Cj, 8 days for ♀Cj × ♂Cg, 7 days for ♀Cg × ♂Cj, and 3 days for ♀Cg × ♂Cg (Table 3).

Table 3. Larvae characteristic of pure and reciprocal crosses of C. *gariepinus* and *C. jaensis* (n = 15) Numbers are means ± standard errors.

Characteristics	♀Cg × ♂Cg	♀Cj × ♂Cj	♀Cg × ♂Cj	♀Cj × ♂Cg	p-value
Yolk Sac duration	3.2 ± 0.40^{d}	10.00 ± 1.78^{a}	7.03 ± 0.66^{c}	8.56 ± 0.67^{b}	$p < 0.001$
Total length	6.64 ± 0.4^{d}	7.69 ± 0.5^{c}	8.34 ± 0.37^{b}	9.62 ± 0.35^{a}	$p < 0.001$
Head Length	1.03 ± 0.12^{b}	1.05 ± 0.03^{b}	2.24 ± 0.16^{a}	0.85 ± 0.15^{c}	$p < 0.001$
Yolk height	1.78 ± 0.18^{d}	2.10 ± 0.06^{c}	3.1 ± 0.08^{b}	3.32 ± 0.10^{a}	$p < 0.001$
Yolk length	2.62 ± 0.29^{d}	3.16 ± 0.18^{c}	3.5 ± 0.16^{b}	3.92 ± 0.31^{a}	$p < 0.001$
Yolk volume	4.47 ± 1.22^{d}	7.35 ± 0.52^{c}	17.73 ± 1.8^{b}	22.69 ± 2.49^{a}	$p < 0.001$
Body depth	0.87 ± 0.06^{b}	0.88 ± 0.05^{b}	0.96 ± 0.06^{a}	0.95 ± 0.01^{a}	$p < 0.001$

Mean in the same row with different superscripts differ significantly (ANOVA, $p < 0.05$).

In Figure 4, the morphological variations amongst hatchlings from the different crosses are illustrated. The mouth was not visible until approximately 12 hours after hatching. Both the excretory system and the anus were primitive. Above the mouth of the larvae, two olfactory pits were seen, and the eye vesicles had dark patches on them. A very low rate of deformity was observed among all the crosses with the highest rate in Cg♀ × Cj♂ hybrids (16%). The low level of abnormality observed in all the crosses hybrids included curvature of the body, bent tail, truncated tails, bent or wavy trunk, detached yolk or reduced yolk size. Conversely, the percentage of larvae that survived until the total yolk sac resorption was 56.33%for ♀Cg × ♂Cj, 62.33% for ♀Cj × ♂Cg, 52% for ♀Cj × ♂Cj and 96.66% for ♀Cg × ♂Cg (Figure 5). This indicates that the period following hatching is the most crucial for larvae development.

By the time the larvae reached the 10th dph, their fin configuration had fully developed, giving them an adult appearance. On the other hand, the whole hybrid pool possessed a fin configuration that implies a shared inheritance from both parents, making them visually identical to their respective parents. Three distinct colors were noted in the reciprocal hybrid pool: a uniform yellow-brown, a dark brown, and a grey shade. The hybrids from ♀Cj × ♂Cg, however, had the longest total length (16.80 ± 0.79 mm) at 10 dph, with ♀Cg × ♂Cj hybrids coming in second (14.19 ± 0.99 mm).

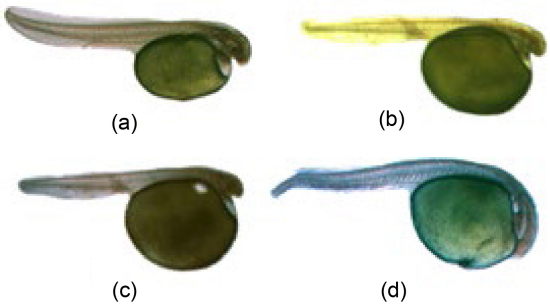

Figure 4. hatchlings from the different crosses. (a) ♀Cg × ♂Cg; (b) ♀Cj × ♂Cj; (c) ♀Cg × ♂Cj; (d) ♀Cj × ♂Cg.

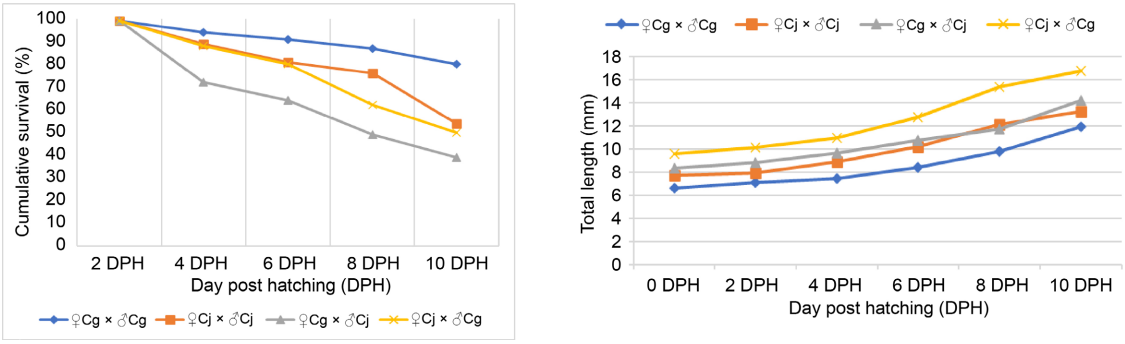

Figure 5. (a) Cumulative survival of larvae for the different crosses from hatching to 10 DPH; (b) Evolution of total length of larvae for the different crosses from hatching to 10 DPH.

4. Discussion

In this study the ovulated eggs of all the crosses increased in size after incubation of fertilized eggs in hatchery, which might be due to hydration of the eggs. Swelling of egg has also been documented in progenies of pure *Rita rita* [29] and pure *H. fossilis* [30]. However, the differences observed before and after fertilization between the egg sizes of the different species in this study are clearly due to the egg characteristics of the species used as the parent. In pure and reciprocal crosses between *Pangasianodon hypophthalmus* and *C. gariepinus* [31] and between *C. gariepinus* and *H. longifilis* [8], there was an increase in egg size before and after fertilization that was comparable to the findings of this study.

In this study the mean fertilization rate for crosses derived from the same female specie eggs did not significantly differ from one another. It was within the range that had previously been reported for *C. jaensis* (68.33% to 87.57%) by [22] and [23] and for *C. gariepinus* (80 to 95%) by [8] [31] [32]. However, the hybrid crossing ♀Cj × ♂Cg had the lowest fertility rate (63.33%). Given the high number of reported failures in artificial reproduction of *C. jaensis*, the low rate of fertilization of the crosses derived from the female *C. jaensis* eggs can be related to the species [21] [22].

Research on early life is crucial for novel fish crosses; this cannot be overstated. Nevertheless, prior reports of hybridization trials rarely address this.The pattern of egg cleavage observed in pure *C. gariepinus* was similar to those previously reported for the parent [8] [31]. These were discoidal meroblastic mitotic divisions resulting in relatively equal blastomeres [33] in normally developing cells leading to somite development. Although in this study the stages of embryonic development were the same in all crossbred lines, there was a notable difference in the timing of these stages principally due to the fact that embryonic development is influenced by a reciprocal interaction between genes and the organism's fitness. The biological features of distinct fish species and the dominant environmental factors, particularly temperature, can thus account for variations in the duration of the incubation period between crosses made from female *C. gariepinus* and *C. jaensis* eggs, respectively [8] [34].

Moreover, the hatching rates were relatively higher in all genetic combinations (55.68% to 76%), suggesting a possible close genetic compatibility between these species as explained by [35]. These results were higher compared to those obtained from the same hybridization trial in the same station (42.77% to 67.77%) by [23] and could be due to difference in maturity reflected in egg and milt qualities. Therefore, the successful reciprocal hybridizations between *C. gariepinus* and *C. jaensis* at relatively high fertilization and hatchability rates reported in this study are an indication of the high quality of broodfish used.

In this study, larvae from different crosses ranged in length from an average of 6.64 to 9.62 mm at hatching, with the ♀Cj × ♂Cg hybrids having the largest average length. The length of freshly hatched larvae of the same crossing combination was measured by [23] to be between 3.5 and 5.5 mm, whereas hybrids from

the hybridization of *C. gariepinus* and *P. hypophthalmus* were measured by [31] to vary from 3.44 to 4.54 mm. The size of the eggs at the time of laying, which is dependent on the spawners' age and size, may be the main cause of these variations. In general, older females lay larger eggs, which hatch into larger larvae; however, this could also be because the genetic types of the parents have adapted to the environment [8]. According to [36] and [37], there is a positive correlation between egg size and a variety of larval traits, including hatching success and length at hatching. Similarly, [38] and [39] had reported that larger eggs provide more energy for larvae development which is explained by the presence of a larger yolk sac. The hatching and survival rate of larvae continue to be critical to the sustainable and profitable production of catfish [40].

The results of this study are in contrast with those of [41] and [42], who reported high larval mortality from crosses between ♀ *C. gariepinus* and ♂ *C. batrachus*. The reason behind the findings, according to those researchers, could be either chromosome incompatibility or the hybridization effect. In the reciprocal crosses of *C. gariepinus* × *C. batrachus*, [41] [43] also reported total mortality.

Larvae development was completed within 10 dph for all the crosses in this study. The full larval development in 14 days that was documented in earlier studies [14] [31] was not consistent with this observation. In other families of catfish, like *Heterobranchus spp*. according to [44], larvae may continue the morphological development after the third week of hatching. Despite the fact that this study demonstrates that hybrid larvae of pure and reciprocal crosses between *C. gariepinus* and *C. jaensis* attain their final morphological development in less than two weeks, variations between 10 and 14 days can be attributed to differences in rearing conditions and experimental protocols. However, the large yolk sac, which supplies the required energy during this crucial phase, continues to be the key factor in this rapid development [45]. The greater total length achieved in reciprocal hybrids could be due to the increase in genetic diversity of reciprocal hybrids, which may lead to better adaptation to environmental conditions and overexpression of growth-promoting genes [46] [47].

5. Conclusion

The present study summarized the embryonic and larval development of hybrids from pure and reciprocal crosses between *C. gariepinus* and *C. jaensis*. The development of the embryo and larvae in reciprocal crosses between *C. jaensis* and *C. gariepinus* shows that there is some degree of reproductive compatibility between these two fish species. Both species are capable of successfully fertilizing and developing embryos when crossed with each other. The findings suggest that the reproductive barriers between the two species are not insurmountable, indicating potential genetic similarities and shared evolutionary history. This conclusion highlights the importance of investigating the reproductive compatibility between closely related species in understanding their evolutionary relationships and potential for hybridization. Further studies are needed to explore

the extent and implications of such hybridization events in the wild and their long-term consequences for these fish populations.

Conflicts of Interest

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

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