UNIVERSITI PUTRA MALAYSIA

COMPARATIVE ASSESSMENT OF REPRODUCTIVE PERFORMANCE IN PUREBRED CLARIAS GARIEPINUS AND HYBRID (CLARIAS NIEUHOFII X CLARIAS GARIEPINUS)

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COMPARATIVE ASSESSMENT OF REPRODUCTIVE PERFORMANCE IN PUREBRED *CLARIAS GARIEPINUS* AND HYBRID (*CLARIAS NIEUHOFII X CLARIAS GARIEPINUS)*

By

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A Project Report Submitted in Partially Fulfilment of the Requirement for the Degree of Bachelor of Science in Aquaculture with Honours in the Faculty of Agricultural and Forestry Sciences University Putra Malaysia Bintulu Sarawak Campus

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ABSTRACT

The African catfish, *Clarias gariepinus,* is a widely adopted culture species globally due to its hardy nature and adaptation to a wide range of climatic conditions. Crossbreeding can increase hybrid vigour through heterosis in *Clarias* species with an overall increase in their average performance characteristics. Therefore, this study investigated the embryonic development of pure breed *Clarias gariepinus* and hybrid *C. nieuhofii* with *C. gariepinus* under similar laboratory conditions**.** Observations on the embryonic development of *Clarias gariepinus* and hybridized form with *C. nieuhofii* for a 24 hour period was microscopically monitored and recorded. The pure cross of *C. gariepinus* successfully fertilized and hatched within 24 hours. However, there was fertilization failure and lack of progress in embryonic development for the hybrid form of *Clarias nieuhofii* with *Clarias gariepinus* under similar conditions. This study on embryonic development of *C. gariepinus* has bridged a gap in the knowledge of developing eggs and larvae morphology of the species in Malaysia. Although the attempted hybridization of *C. gariepinus* with *C. nieuhofii*, the two common species of catfish in Malaysia has failed, further experimentation should be conducted to address the intrinsic and extrinsic variables that are known to impede embryogenesis in hybridization experiments to optimise the process.

Keywords: catfish; *C. nieuhofii*, *C. gariepinus*; hybrid; Malaysia

ABSTRAK

Ikan keli Afrika, *Clarias gariepinus*, adalah spesies ternakan yang paling banyak diternakdi seluruh dunia kerana sifatnya yang tahan lasak dan menyesuaikan diri dengan pelbagai keadaan iklim. Pembiakan silang boleh meningkatkan prestasi pembiakan hibrid melalui heterosis dalam spesies *Clarias* dengan peningkatan keseluruhan dalam ciri prestasi purata mereka. Oleh itu, kajian ini mengkaji perkembangan embrio baka tulen *Clarias gariepinus* dan hibrid *C. nieuhofii* dengan *C. gariepinus* di bawah keadaan yang sama. Pemerhatian terhadap perkembangan embrio *Clarias gariepinus* dan bentuk hibrid dengan *C. nieuhofii* untuk tempoh 24 jam dipantau dan direkodkan secara mikroskopik. Persilangan di antara *C. gariepinus* tulenberjaya disenyawakan dan menetas dalam masa 24 jam. Walau bagaimanapun, terdapat kegagalan persenyawaan dan kekurangan kemajuan dalam perkembangan embrio untuk hibrid *Clarias nieuhofii* dengan *Clarias gariepinus* dalam keadaan yang sama. Kajian tentang perkembangan embrio *C. gariepinus* ini telah merapatkan jurang dalam pengetahuan membangunkan telur dan morfologi larva spesies di Malaysia. Walaupun percubaan penghibridan *C. gariepinus* dengan *C. nieuhofii*, kedua-dua spesies ikan keli yang biasa di Malaysia telah gagal, percubaan selanjutnya perlu dijalankan untuk menangani pembolehubah intrinsik dan ekstrinsik yang diketahui menghalang embriogenesis dalam eksperimen hibridisasi untuk mengoptimumkan proses tersebut.

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APPROVAL SHEET

I clarify that this research project report entitled "Comparative Assessment of Reproductive Performance in Purebred *Clarias Gariepinus* and Hybrid (*Clarias Nieuhofii X Clarias Gariepinus)*" has been examined and approved as a partial fulfilment of the requirement for the degree of Bachelor of Science in Aquaculture with Honors in the Faculty of Agricultural and Forestry Sciences University Putra Malaysia Bintulu Sarawak Campus.

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CHAPTER 1

1.0. INTRODUCTION

According to the United Nations, the world's population is expected to increase from 7 billion in 2011 to 9 billion in 2023, with a concurrent increased demand in food consumption (United Nations Statistics, 2023). Most of the world's population depends on fish as an affordable food and global aquaculture provides approximately 47% of the world's fish production, outpacing other major food sources (FAO, 2023). Annual fisheries and aquaculture production is forecast at 185 million tons in 2023, 0.6 percent more than 2022 levels, principally driven by expansion and intensification of aquaculture in the North Atlantic and small pelagic in the Eastern Pacific (FAO, 2023). In Malaysia, fish and other products from the aquaculture industry make a substantial contribution to food supplies (Jumatli and Ismail, 2021) and generate revenue through the exportation of freshwater fish, shrimps, marine finfish, molluscs, and seaweed (Roslina and Amir, 2014).

Catfishes include a wide variety of ray-finned fish with prominent barbells that resemble cat whiskers, hence their name (Ouma & Barasa, 2022). The catfishes in the genus *Clarias* have an anguilliform body, long dorsal and anal fins, flattened head bone, and a broad mouth with 4 pairs of barbels. Catfishes also possess an accessory air-breathing organ that allows them to survive for a limited time outside water (Hee, 1999). *Clarias* species generally inhabit swamps, paddy fields, small water pools, and various kinds of rivers. They are distinguishable from the related species by their large dorsal fin and the short and rounded occipital process (Othman et al., 2011).

The *Clariidae* family occurs in Asia, Syria, and Africa, where they live in the muddy bottoms of freshwater habitats and graze on a variety of food materials, exhibiting a typical

omnivorous feeding behaviour (Ouma & Barasa, 2022). In Southeast Asia, several *Clarias* species have been described, identified, and validated (Lim & Ng, 1999). In Malaysia, indigenous *Clarias* species such as *Clarias anfractus* and *Clarias planiceps* were morphologically identified in Borneo (Hee, 1999). *Clarias batu*, a species of clariid catfish, was also identified on Tioman Island off the eastern coast of Peninsular Malaysia (Lim & Ng, 1999). Four species of walking catfish, including *Clarias batrachus, Clarias macrocephalus, Clarias meladerma,* and *Clarias nieuhofii*, were identified based on the cytochrome B gene in peat swamp forest, Selangor (Othman et al., 2011). *Clarias nieuhofii* (also known as the slender walking catfish) commands a high market value compared to other *Clarias* species because of its good taste and appealing appearance. *C. nieuhofii* has beautifully patterned skin, which is reddish brown with 13-14 vertical rows of white spots.

The *Clarias* species has a great economic importance in both fisheries and aquaculture fields (Othman et al., 2011). Catfishes are important biodiversity resources with significant ecological roles in the aquatic food chains. They form important food fish for local communities and generate income to support livelihoods in tropical South America, Africa, and Asia (Ouma & Barasa, 2022). According to the Food and Agriculture Organization (FAO 2023), a total of 27 catfish species were farmed in 86 countries worldwide in 2017, with a total production of 5.5 million tonnes, worth US$10.5 billion of farm gate value. In Malaysia, catfish is an economically important food that has gained popularity among consumers due to its high nutritional value and good taste (Chong et al., 2000).

Thailand, Indonesia, and Malaysia are the top three catfish breeders in Southeast Asia. Catfish breeding in Malaysia is gaining popularity due to the ease of cultivation, high market demand, and the short breeding period which takes approximately 3 months to reach market size, makes it an attractive enterprise for small, medium, and large-scale producers. The culture of catfish in Malaysia is currently limited to freshwater species such as the walking

catfish or *C. batrachus*, *C. macrocephalus*, and *C. gariepinus*, which is an exotic fast-growing and resilient species of catfish that is readily acceptable by the local people (Adan, 2000). Most of the *Clarias* currently cultured in Malaysia are hybrids between *C. macrocephalus* and *C. gariepinus*, which resemble the indigenous species, are readily accepted by consumers, and have a high demand and market value (Adan, 2000). Despite several years of significant production gains in the fisheries subsector, there are still challenges in maintaining and enhancing the value and productivity of fish breeding in Malaysia. The present challenges of fish breeding in Malaysia include environmental threats such as pollution, fish stock depletion, and climate-related concerns, reflecting broader issues in the aquaculture sector (Fathi et al., 2018).

1.1. Statement of the problem

The wild population of indigenous *Clarias* species is fast depleting because of human exploitation due to indiscriminate capture for food or economic benefits. *Clarias nieuhofii* population density in Asia has experienced a severe decline during the last 2 decades due to overexploitation and habitat losses because of the rapid ongoing reclamation of peat swamp forests and the indiscriminate application of insecticides in paddy fields (Pechsiri & Vanichanon, 2016). The African catfish, *Clarias gariepinus,* is the most widely adopted culture species of catfish globally due to its hardy nature and adaptation to a wide range of climatic conditions. However, its propagation is constrained by insufficient seed supply due to the poor survival rate of the fingerlings (Ouma & Barasa, 2022). The vulnerability of the species requires conservation, recovery, and management strategies. Moreover, previous studies have shown that crossbreeding can increase hybrid vigour through heterosis in *Clarias* species with an overall increase in their average performance characteristics (Rahman et al., 1995). Therefore, this study was designed to investigate the embryonic development of

the hybrid progeny following induced hybridization of *Clarias nieuhofii* with *Clarias gariepinus* under laboratory conditions**.**

1.2. Objectives of the study

The objectives of this study are to:

i. To compare the morphological characteristics of parental bloodstock of both purebred *Clarias gariepinus* and the hybrid *Clarias nieuhofii* x *Clarias gariepinus*.

ii. To evaluate the complete egg development stages in purebred *Clarias gariepinus* and the hybrid *Clarias nieuhofii* x *Clarias gariepinus* under laboratory conditions.

1.3. Research questions

i. What are the morphological characteristics of parental bloodstock of both purebred *Clarias gariepinus* and the hybrid *Clarias nieuhofii* x *Clarias gariepinus*?

ii. What is the egg development stages in purebred *Clarias gariepinus* and the hybrid *Clarias nieuhofii* x *Clarias gariepinus* under laboratory conditions?

CHAPTER 2

2.0. LITERATURE REVIEW

2.1. Overview of the aquaculture industry in Malaysia

Aquaculture, which involves the farming of finfish, molluscs, crustaceans, and seaweeds is one of the fastest growing food production enterprises in the world (FAO, 2023). Globally, fish provide about 3.2 billion people with 20% of their animal protein intake, and per capita fish consumption has increased from 9 kg in 1961 to 20.3 kg in 2016 (FAO, 2023). The rapid development of aquaculture has been considered the blue revolution, which is an approach to increasing global fish production to contribute to human nutrition and food security (Ahmed & Thompson, 2019).

The Malaysian aquaculture industry has seen a steady rise in production which translates into employment generation and revenue that makes significant contributions to the gross domestic products (GDP). For instance, in 2019, the industry accounted for 1.1 percent of the global total, with 0.4 percent coming from aquaculture causing 8.9 percent increase in the national agricultural GDP. In the same year, the Malaysian aquaculture sector produced approximately 411,781 tons of total aquaculture production with an estimated economic value of USD 700 million (Tan et al., 2023). The production value of the fisheries industry increased by 7.5% from RM13.84 billion (in 2020) to RM14.88 billion (in 2021) (Department of Fisheries Malaysia, 2023). Despite the huge economic potentials of the industry, there are many challenges that hinder future developments in aquaculture in Malaysia. Prominent among these problems is sustainability including environmental concerns. Understanding and addressing these issues are crucial for the long-term viability of fish breeding practices. To achieve the desired success, the industry is currently exploring challenges and opportunities

in sustaining aquaculture, emphasizing the importance of adopting sustainable practices for the continued success of fish breeding in Malaysia.

2.2. Catfishes

Catfishes belong to a diverse group of ray-finned fish belonging to the Phylum Chordata, class Actinopterygii, and of Order siluriform (Ouma & Barasa, 2022). The catfishes, so named because of their prominent barbells which resemble cat whiskers, consist of 40 families distributed worldwide. Catfishes of the genus *Clarias* (Clariidae) are characterised by their slender bodies: dorsal and anal fins with long bases, flat bony head. a broad terminal mouth with four pairs of long barbels, and an accessory air-breathing organ (Lim & Ng, 1999). The catfish species are very important to the sustainability of the aquaculture industry (Owodeinde and Ndimele, 2011). In Southeast Asia, several *Clarias* species have been described, identified, and validated (Lim & Ng, 1999). In Malaysia, indigenous *Clarias* species such as *Clarias anfractus* and *Clarias planiceps* were morphologically identified in Borneo (Hee, 1999). *Clarias batu*, a species of clariid catfish, was also identified on Tioman Island off the eastern coast of Peninsular Malaysia (Lim & Ng, 1999). Four species of walking catfish, including *Clarias batrachus, Clarias macrocephalus, Clarias meladerma*, and *Clarias nieuhofii*, were identified based on the cytochrome B gene in peat swamp forest, Selangor (Othman et al., 2011). *Clarias nieuhofii* (also known as the slender walking catfish) commands a high market value compared to other *Clarias* species because of its good taste and appealing appearance. *C. nieuhofii* has beautifully patterned skin, which is reddish brown with 13-14 vertical rows of white spots.

Figure 1. *Clarias nieuhofii* broodstock

The African catfishes include fish of the family *Clariidae* which is widely distributed tropical South America, Africa, and Asia, where it is regarded as an important culturable fish species for fish farming (Adah et al., 2014). Catfishes are commonly exploited by fishermen in natural waters and cultured by farmers as an important source of animal proteins which has gained great economic significance (Legendre et al., 1999). *Clarias gariepinus* has gained wide acceptance as a very suitable species for aquaculture because of its high growth rate, high fecundity rate, resistance to diseases, and the ability to tolerate a wide range of environmental parameters under culture conditions (Temitope, 2017). *Clarias* species also accepts a wide range of natural and artificial food and adapts to a variety of feeding modes in different environments, good meat quality and smoking characteristics and can be cultured all year-round (Adah et al., 2014).

Figure 2. *Clarias gariepinus* broodstock

2.3. Fish breeding

The increasing popularity of catfish among fish consumers has created an overwhelmingly increased demand for *Clarias* and conventional breeding can no longer meet up with these high demands (Temitope, 2017). To increase fish production for a growing global population, aquaculture must grow sustainably while significantly reducing its environmental impacts (Ahmed & Thompson, 2019). Increased production of fry and fingerlings with higher food conversion ratios, faster growth rates, and better environmental tolerance is almost necessary to provide fish food security towards the increased global demand (Adah et al., 2014). One of the sustainable practices for growing aquaculture is through hybridization. To produce fish that have a higher development rate, a higher feed conversion capacity, a shorter production cycle, and a higher tolerance for unfavourable water conditions, genetic approaches are required (Temitope, 2017). Hybridization is the process of combining genes from two closely related species or subspecies of organisms to create a new hybrid progeny which bear the

characteristics of the parents either naturally or by human mediation (Adah et al., 2014). It is recognised as an essential tool for the genetic improvements in the aquaculture industry which has been used extensively for stock improvement and management purposes (Owodeinde et al., 2012). Hybridization is a widespread and more commonly observed phenomenon in fish than any other groups of vertebrate animals under natural conditions (Scribner et al., 2001). According to Campton (1987), the high incidence of hybridization among closely related fish families, genera and species is favoured by external fertilization, weak behavioural isolating mechanisms, unequal distribution of parental species, limited spawning environment, decreasing habitat complexity, and susceptibility to secondary contact between recently evolved forms. Previous studies focusing on stock manipulations and growth performances at different dietary compositions have shown that *Clarias gariepinus* × *Heterobranchus bidorsalis* hybrids had greater growth, superior survival, and resistance characteristics than the actual breed of either *Clarias gariepinus* or *Heterobranchus bidorsalis* alone (Owodeinde et al., 2012).

In fish, hybridization may involve two different species, genera, or families to produce hybrid first filial generation that may be backcrossed, outcrossed, or crossed again to maintain desirable traits on the progeny (Adah et al., 2014). Intraspecific hybridization may involve crossbreeding between members of the same species (strains) with different characteristics (Adah et al., 2014). Interspecific hybridization occurs widely across a taxonomically diverse array of fish species naturally. Multiple interacting factors affect the outcome of hybridization events with human influences contributing to nearly 50% of hybridization. Aquacultural activities, species introductions, and habitat losses also influence hybridization events under natural conditions (Legendre et al., 1999). Interspecific cross breeding in fish may lead to hybrid vigour due to heterosis for disease resistance or growth rate. For instance, a study by Onyia et al. (2010) reported that cross breeding of *Clarias anguillaris* strains produced a

progeny with better performance in terms of hatching success and survival rates under cultural conditions. Cross breeding between *C. batrachus* and *C. gariepinus* is known to increase average performance traits such as hatching rate, viability of larvae, growth and survival rates of the F1 individuals than their reciprocal and control sibs (Rahman et al., 1995). Interspecific hybridization involving genetic variants provides an alternative to conventional selective breeding of fishes for qualitative or quantitative improvements in commercial traits (Adah et al., 2014). Although certain interspecific hybrids fail to show heterosis for any trait, they are still important for aquaculture application due to a good blend of beneficial traits from both parent species (Hulata, 2001). Moreover, catfish hybrids were reported in *Clarias gariepinus*, *Heterobranchus longifilis* (Hecht and Lublinkhof, 1985), *H. fossilis* and *C. batrachus* (Padhi et al., 1995) and *C. batrachus* x *C. gariepinus* (Sahoo et al., 2003). Legendre et al. (1999) has shown that the reciprocal intergeneric hybrid catfish between *C. gariepinus* and *H. longifilis* can be produced. Sogbesan et al. (2005) working with an interspecific hybrid, 'hetero*clarias*' reported a positive net gain and cost benefit ratio in all the diets.

CHAPTER 3

3.0. MATERIALS AND METHODS

3.1. Experimental site

The study was performed at the Department of Animal Science and Fisheries hatchery unit at Universiti Putra Malaysia Campus Bintulu Sarawak, which was located between latitude 3.211475 and longitude 113.092918. The study period corresponds to the Northeast Monsoon, which is the peak of the rainy season in Sarawak.

3.2. Broodstock selection

The gravid cultured type of *Clarias gariepinus* male (n=3, weighing 960 ± 50g) and females (n=3, weighing 1000 ± 100g) were purchased from Pasar Tamu Baru Bintulu (3.2672° N, 113.1012° E) while the wild type of *C. nieuhofii* Males (n=3, weighing 850 ± 20g) and females (n=3, weighing 920 ± 30g) were purchased from Pasar Tamu Bintulu (3.1706° N, 113.0405° E). The maturity of the genital organs were considered in selecting the brood stocks. Thus, gravid females were chosen based on swollen, reddish genital openings, and the gravid males were chosen based on the presence of reddish and pointed genital papillae. The broodstock were transported by car in a flat-button, translucent, breathable plastic container 43cm x 20cm x 27cm in dimension to the Department of Animal Science and Fisheries hatchery unit for acclimatization before breeding.

3.3. Broodstock rearing in the hatchery.

During the 14-day acclimatization period, the broodstock was housed in 4 separate 100-liter tanks according to their gender and species. The tanks were half filled with water and maintained at a pH of 7.0, ammonia level of 0.0 ppm, 3 mg/l of dissolved oxygen, 0.1 mg/L

of nitrite, 0-4ppm of nitrate, and a temperature of 25°C throughout the period. Fed with pellets twice a day (5% of body weight). Feeding was done twice daily using a floating commercial adult fish pellet (manufacturer) at a rate of 5% of body weight. The water was partially replaced every week, and chlorine was removed from the water by adding Aquadine® (10mls per tank) anti-chlorine solution. The water quality parameters are shown in Table 1.

Table 1. Water quality used for brood stock and breeding.

Parameter	**Level**
pH	6.5-8.0
Ammonia	0.0 ppm
Dissolved oxygen	3 mg/l
Temperature	22-28 °C
Nitrite	0.1mg/l
Nitrate	0-40 ppm

3.4. Induced breeding in *Clarias* species.

Clarias species were identified by observing their body colour, body pattern and shape of the body, size of the eye, occipital process, barbells, and fins (Othman et al., 2011). Both males and females of both species were induced with an intramuscular injection containing a combination of salmon gonadotropin-releasing hormone (GnRH) analogue 20 µg/ml + Domperidone 10 (Ovaprim®) at a dose rate of 0.5ml/kg for females and 0.25ml/kg for males given at 12 hours prior the ova and milt collection (Esa et al., 2023). The females of both *Clarias gariepinus* and *C. nieuhofii* with a swollen and soft belly, protruding reddish genital papilla, showing greenish coloured matured oocytes (1.1 and 1.6 mm) after oocyte maturation and ovulation following a latency period of 12 hours after induction were processed for milt and egg collection. Matured eggs were collected from the females into separate clean, dry containers by the application of manual gentle pressure on the abdomen, forcing the eggs to exit the genital opening.

Figure 3. Eggs of *Clarias* species collected by hand stripping.

The males of both species were chosen based on their well-developed genital papillae and sacrificed to collect milt, which was diluted with 0.9% NaCl.

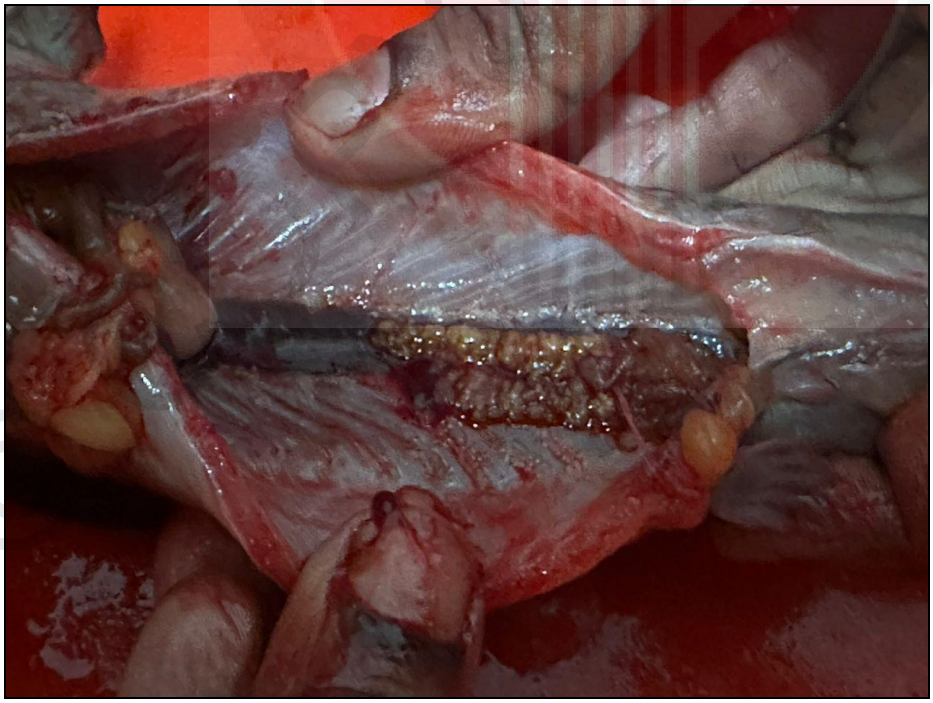


Figure 4. Collection of milt from a sacrificed male *Clarias* species after dissection.

For each cross, 10 g of oocytes (1000 to 1830 oocytes) were taken from each species and introduced into twelve separate labeled plastic bowls (three bowls per cross). The milt was collected with a syringe and mixed with oocyte in bowls for fertilization. A fertilization solution (NaCl: 2 $g \cdot l^{-1}$ and urea: 4 $g \cdot l^{-1}$ water) was used to rinse the mixture for 1 minute. One bowl containing unfertilized oocytes of each cross was used as a control to evaluate the fertilization rate. The oocytes in each bowl were spread with a chicken's feather. The fertilized eggs were observed under the compound and dissecting microscope for 24 hours until hatching. Every stage of embryonic development will be recorded by taking a serial photograph.

3.5. Determination of Fecundity

The total number of eggs produced per kilogram of female body weight was determined by the number of quality eggs produced by each species after the acclimatization period. The female fish was induced with Ovaprim® and weighed using a sensitive scale to the nearest gram, stripped into a dry plastic bowl, and weighed the eggs to calculate the number of eggs from each egg mass of the female. A one-gram sample was taken from each egg mass and fixed in 10% buffered for 12 hrs and placed in 70% ethanol for storage before counting in a calibrated Petri dish using a tally counter under a dissecting microscope at ×20 magnifications (Esa et al., 2023).

The number of eggs spawned = weight of the egg mass (per female) x the number of eggs per g of the female's egg mass.

3.6. Total egg number release

The total number of eggs released by each female was calculated by subtracting the weight of the broodstock after stripping (W_b) from the total weight of the broodstock before stripping (W_a) multiplied by the number of egg counts per gram (N) as follows (Esa et al., 2023):

Number of eggs released = ($W_b - W_a$) g × N

3.7. Determination of gonadosomatic Index

The male and female gonads of each species were collected after dissection and weighed. The testes and egg lobes were removed and weighed to the nearest gram using a sensitive balance. The male brood stock was carefully dissected using standard procedures to remove both testes, weigh, and collect semen. Both the gonadal weight (g) and volume of sperm (mL) in each testis was determined. After obtaining the testicular weights, a longitudinal incision was made on each testicular lobe to collect the milt in calibrated 2mL falcon tubes to determine the semen volume. The gonadosomatic index (GSI) was calculated based on the following formula (Esa et al., 2023):

Gonadosomatic Index (GSI) = weight of gonad (g) eviscerated weight (g) × 100

The condition factor K = (W/L3) × 102

where W = weight (g) and L = length (mm).

3.8. Estimation of fertilization and hatching rate

The fertilization and the hatchability rates were determined using 1 g of eggs (approximately 183–200 oocytes) from each cross. The egg number was estimated using the gravimetric method (eggs per gram) and 1 g of eggs from each species was used to determine the fertilization rate. Each bowl was placed below a UV light source with a water height of 0.35 m and a flow rate of 5.18 mL−1 during at an average temperature of 27 °C in the hatchery. The time taken for the eggs in the control bowl to turn white was monitored and recorded. The eggs containing embryonic eyes 45–60 min after fertilization will be considered fertilized and counted to estimate the fertilization rate. The number of hatchlings per container were recorded by direct counting of the hatchlings and unhatched eggs for each

cross combination. At the end of hatching, deformed or dead larvae was counted and siphoned off to distinguish them from normal larvae. The larvae was counted directly with the naked eye during the day. A thermometer was used to record the water temperature in the rearing tanks every day in the morning, noon, and evening. The following formulas was used to calculate the rates of fertilization, hatching, and larval survival (Esa et al., 2023):

(i) Fertilization rate (%) = Number of fertilized eggs/ numbers of estimated eggs × 100

(ii) Hatchability (%) = Total number of hatched eggs/ total number of fertilized eggs × 100

(iii) Survival (%) = Total number of larvae − number of dead larvae total number of larvae × 100%

3.9. Data analysis

Photomicrographs of different stages of embryonic development were captured using a Smartphone digital camera (iPhone 14 Promax 2022) and presented descriptively. Comparisons between pure *C. gariepinus* and the hybrid cross of *C. gariepinus* and *C. nieuhofii* embryos was done by using morphological features of the different stages of embryonic development.

CHAPTER 4

4.0 RESULTS

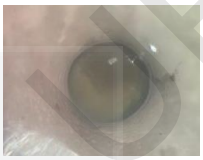
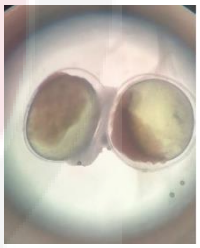
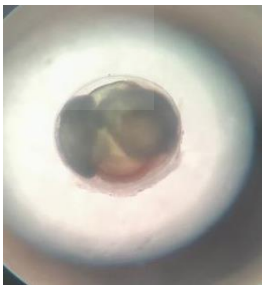
4.1 Observations on embryonic development in *Clarias gariepinus*

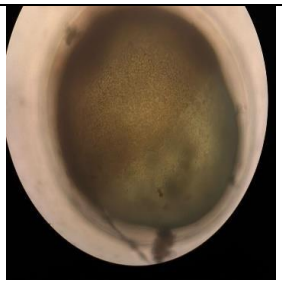
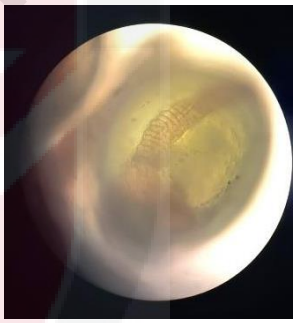
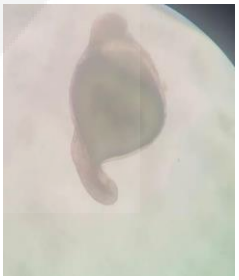
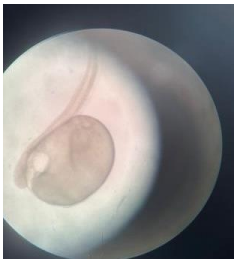
Table 1 summarizes the observations on embryogenetic development of *Clarias gariepinus* immediately after fertilization up to the hatching stage of larva 24 hours post fertilization in the laboratory culture.

The fertilized egg (Figure 1) appears brownish in colour spherical and sticky. The animal and vegetal pole (blastodisc) was observed at 45 minutes post fertilization (Figure 2). The blastodisc was formed by the expansion of the yolk further from the membrane and accumulation of cytoplasm at the anterior portion. The 2 - cells stage at first cleavage was observed as a vertical division of the animal pole producing two cells of equal sizes observed 40 minutes post fertilization (Figure 3). The 4 – cell stage was observed 55 minutes post fertilization marked by a second line of division perpendicular to the first line producing 4 equal cells (Figure 4). The 16-32- cell stage/morula was observed at 2 hours after fertilization. The morula stage is formed by a mass of dividing cells which were seen to be irregular in size, overlapping, and difficult to count (Figure 5). The blastula stage was observed at about 3 hours after fertilization. The structure was formed by further division of the morula cells producing a mass of cells elevated over the general outline of the yolk mass like a dome-shaped structure (Figure 6). The Gastrula was observed at 7 hours after fertilization. At this stage, the embryo was seen to have developed several germ rings from the cephalic and caudal edges which were formed at advanced stages of the blastula formation in figure 6 (Figure 7). The First wiggling movement of the larva was observed at 21 hours post fertilization. The long somite was seen to have originated from both sides within the chorion

wall. It started with 4-5 movements per minute and gradually increased over time (Figure 8). The hatching of larvae was observed at 22 hours post fertilization. The hatching was initiated by a marked violent movement of the tail to either side against the chorion wall, followed by contraction to break the chorion wall breaks (Figure 9).

Table 1. Stages of embryonic development in *Clarias gariepinus*

Time/ Period after fertilization	**Formation of embryo**
Fertilized egg. Appears brownish in colour spherical and sticky.	 **Figure 1**
Formation of the animal and vegetal pole (blastodisc). Expansion of the yolk away from the membrane and accumulation of cytoplasm at the anterior to form animal and vegetal pole observed 45 minutes post fertilization.	 **Figure 2**
2 - cells stage at first cleavage was observed as a vertical division of the animal pole producing two cells of equal sizes observed 40 minutes post fertilization	 **Figure 3**
4 – cell stage was observed 55 minutes after fertilization with a second line of division perpendicular to the first line producing 4 cells which of equal sizes	 **Figure 4**
16-32- cell stage (morula) was observed at 2 hours after fertilization, the morula stage is formed by a mass of cells. The cells were seen to be irregular in size, overlapping, and could be difficult to count.	

	 **Figure 5**
Blastula This stage develops 3 hours after fertilization. Further division producing a mass of cell elevated over the general outline of the yolk mass (like a dome-shaped)	 **Figure 6**
Gastrula This stage was seen 7 hours after fertilization. The embryo develops germ rings. Cephalic and caudal edges which were formed at advanced stages of the blastula.	 **Figure 7**
First wiggling movement was observed at 21 hours after fertilization. At this stage, the long somite started from both sides within the chorion wall. It started with 1 movement in 25 seconds, but this rate gradually increased with time.	 **Figure 8**
Hatching 22 hours after fertilization, hatching occurs, marked by a violent movement of tail to either side against the chorion wall, followed by contraction. As a result of which the chorion wall breaks and hatching occurred	 **Figure 9**

4.2 Observations on embryonic development following the hybridization of *Clarias nieuhofii* and *Clarias gariepinus* hybrid.

Figure 4.1 & 4.2 summarizes the observations on embryogenetic development following the hybridization of *Clarias nieuhofii* and *Clarias gariepinus* hybrid up to 22 hours post fertilization in the laboratory culture. Serial photographs captured every two hours after mixing the sperms and oocytes together showed varying degrees of fertilization and failure of embryonic development during the first, second, third, fourth, and fifth experimental attempts.

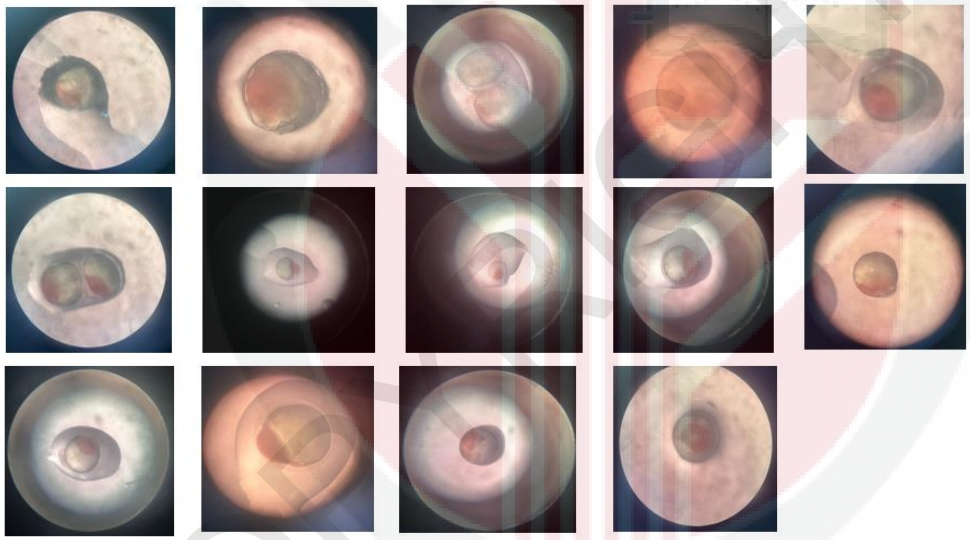


Figure 4.1. Failure of embryonic development in *Clarias nieuhofii* with *Clarias gariepinus* hybrid (1st attempt)

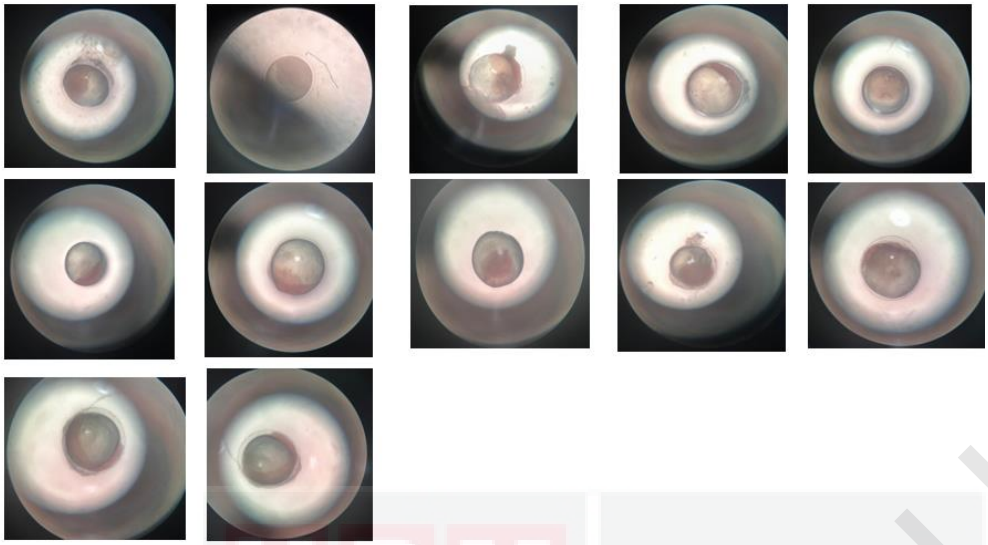


Figure 4.2. Failure of embryonic development in *Clarias nieuhofii* with *Clarias gariepinus* hybrid (2nd attempt)

CHAPTER 5

5.0 Discussion

5.1 Observations on embryonic development in *Clarias gariepinus*

The production of catfishes through hybridization to ensure faster growth rate, high food conversion ratio and environmental tolerance is an age long practice in Asia and African countries (Adah et al., 2014). Several hybridization studies either for mere scientific interest or commercial purposes in fishes have been reported (Colombo et al., 1998). The contributions of hybrid fishes to global aquaculture production are grossly underestimated (Rahman et al., 2018). In this study, a comparative assessment of the morphological characteristics and complete egg development stages of parental bloodstock of both purebred *Clarias gariepinus* and the hybrid *Clarias nieuhofii* x *Clarias gariepinus* in the aquaculture system were monitored and recorded.

The results of this study have shown that the pure cross of *C. gariepinus* successfully passed through various stages of embryonic development and hatching during the 24-hour period of observation. The developmental stages observed for *C gariepinus* were like a previous study (Dirisu & Kwu, 2004). The embryology of *Clarias gariepinus* is like other Clarias species (Mollah and Tan, 1983; Kamler et al., 1994). The completion of cleavage and hatching within 22 hours in this study agrees with the reports of Kamler et al. (1994) and Dirisu & Kwu (2004) for *Clarias gariepinus*.

5.2 Observations on embryonic development following the hybridization of *Clarias nieuhofii* and *Clarias gariepinus* hybrid.

This study also attempted to hybridize *Clarias gariepinus* with *C. nieuhofii* to enhance hybrid vigour. After several attempts at hybridization experiments, the results showed that there was

fertilization failure and lack of progress in embryonic development. Although there is lack of information on crossbreeding involving *Clarias gariepinus* x *C. nieuhofii*, the crossbreeding involving *Clarias nieuhofii* has been performed to determine the weight ratio for crossbreeding between male brood of *Clarias nieuhofii* and female brood of *Clarias batrachus*. The study showed 83.335% survival rate of crossbred seeds and proves that this crossbreeding is possible between the species (Restu & Nataleo, 2016). Additionally, there is information on interpopulation crossbreeding involving the closely related African catfish, *Clarias gariepinus*, in Indonesia. The study evaluated the growth and survival performances of reciprocal interpopulation crossbreeding, comparing farmed and wild African catfish at the nursing stage (Sunarma et al., 2016).

Fertilization failure and lack of embryonic development in fish hybridization experiments can be attributed to either intrinsic factors related to species involved or the experimental conditions. Genetic incompatibility, mismatched spawning conditions, hormonal imbalances and poor gamete quality are the common intrinsic factors that may influence fertilization and embryonic development in hybridization experiments (Liu et al., 2020). Genetic deterioration due to hybridization has been reported as a common source of degradation resulting from interspecies hybridization due to poor selection of brood stock (Rahman et al., 2018). The most important extrinsic factors that are known to affect fertilization and embryogenesis in aquaculture experiments are water quality, water temperature, and handling stress (Njiiri et al., 2015). Other factors that could impede embryonic development include poor acclimatization conditions, and nutrition. Nutrition is an important factor affecting the reproductive performance of all vertebrates, including fish. In many species, reducing feeding rates of mature fish during the reproductive cycle decreases growth, and can reduce gonado-somatic index (GSI), gonadal maturation, final oocyte maturation and egg quality, as well as spawning in females, which are more affected than the males (Volkoff & London, 2018).

Therefore, overall, that quantity and quality of food supplied to the broodstock during grown, acclimatization, and breeding is crucial for a successful reproductive cycle. Thus, the observed failure of fertilization in attempted hybridization of *Clarias gariepinus* x *C. nieuhofii* could be related to either intrinsic or extrinsic factor or both. It is noteworthy that although we observed the ideal 2 weeks acclimatization period in this study, there were several challenges during the period that could compromise the oocyst quality and cause the fertilization failure observed in the hybridization experiment. One of the major challenges was inability to maintain the ideal water quality due to production of large amounts of waste by *C. nieuhofii*, feed pollution, and build-up of ammonia which required constant water change that makes it cumbersome. Moreover, differences in quality of the various types of commercial feed used for feeding the broodstock could compromise the nutritional needs, affect hormonal balance, impede gonadal development, and cause poor oocyte quality, which may cause the fertilization failure observed in the hybridization experiment.

5.3 Conclusion

This study on embryonic development of *C. gariepinus* has bridged a gap in the knowledge of developing eggs and larvae morphology. Although the attempted hybridization of *C. gariepinus* with *C. nieuhofii*, the two common species of catfish in Malaysia has failed to achieve the desired objective, further experimentation should be conducted to address the intrinsic and extrinsic variables that are known to impede embryogenesis in hybridization experiments to optimize the process. Hybridization is expected to increase the average performance traits such as hatching rate, viability of larvae, growth, and survival rate of hybridized *Clarias* specie derived from crosses between *C. nieuhofii* and *C. gariepinus*. Furthermore, useful information is provided for routine fish hatchery operators.

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