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# Embryonic and larvae development of reciprocal crosses between *Pangasianodon hypophthalmus* (Sauvage, 1878) and *Clarias gariepinus* (Burchell, 1822)

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ABSTRACT

The egg and larval development of reciprocal crosses of *Pangasianodon hypophthalmus* (Sauvage, 1878) and *Clarias gariepinus* (Burchell, 1822) were studied under laboratory conditions. Crosses between ♀*C. gariepinus* × ♂*P. hypophthalmus* (Clariothalmus) and ♀*P. hypophthalmus* × ♂*C. gariepinus* (Pangapinus) had embryonic stages similar to those of the pure sib, however, unequal cell cleavages were notable in the early development of both crosses, hence, leading to different forms of deformities. The critical stage where highest mortality occurred during the embryogenesis was the somite (21.68%) and hatching (48.1%) stages (respectively for the Clariothalmus and Pangapinus). However, both crosses produced viable larvae (60.21 vs 13.20% hatchability respectively), which survived (30.3 vs 2.1% respectively) until the end of the study (2 weeks). The external morphological features of the larvae were completely formed by the 14th day after hatching. The body forms of the crosses at this time were either phenotypic intermediary of the parent species (92% of Clariothalmus) or indistinguishable from the female parent (all Pangapinus and 8% of the Clariothalmus). This study thus laid the groundwork for further comparative studies on hybrid performance and characterization.



## Introduction

Hybridization is often used by aquaculturists in order to take advantage of potential desirable culture traits in offspring (Kiriyakit et al., 2011). This includes but not limited to increased growth rate, manipulation of sex ratios, increased disease resistance, improved tolerance to environmental extremes and improvement of other traits that make production more profitable (Dunham et al., 2000). However, despite the possibility of these numerous advantages, many previously reported hybrids have not gained aquaculture relevance. This among other reasons is due to the lack of adequate knowledge on several important aspects of the biology of the hybrids (Olufeagba et al., 2016).

Studies on the embryonic and larval development of fish are of great importance in understanding the ontogenetic processes. They are important in understanding the biology, functional trends and environmental preferences of the different developmental stages of any species (Borcato et al., 2004). It is also important in obtaining notable information on the developing abilities of the fish, comparing normal and altered developmental patterns (Morrinson et al., 2001). An understanding of the embryological and larval development is the basic knowledge required to improve the artificial propagation of any cultured species (Olufeagba et al., 2015). This is because of its aquaculture applicability. For instance, knowledge of the onset of exogenous feeding and yolk-sac absorption is important to know when to begin supplemental feeding. Hence, it is an integral step toward developing management and rearing technology for new species targeted toward commercial production (Ferosekhan et al., 2015).

The Pangasidae and the Clariidae are two important freshwater fish families with many of its species popularly cultured in Asia and Africa respectively. Their contribution to aquaculture production is exemplary (De Silva and Phuong, 2011; Solomon et al.,

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2013). However, the increasing need to diversify commercial aquaculture candidates of the world has inspired intergeneric hybridization attempts between the two families. Successful intergeneric hybridization has been reported between Bighead catfish *Clarias macrocephalus* Gunther, 1864, Philippine catfish *Clarias batrachus* (Linnaeus, 1758) and *Pangasianodon hypophthalmus* (Sauvage, 1878) (Tarnchalanukit, 1986; Na-Nakorn et al., 1993). In this study, we successfully hybridized *Clarias gariepinus* (Burchell, 1822) and *P. hypophthalmus* to produce viable offspring. In view of the aquaculture prospect of the novel hybrids produce, we investigated the embryogenic and larvae development under laboratory conditions to improve understanding about the structural changes of the hybrids during these developmental stages. This information will give insights to improving the rearing technique of the fish for better propagation.

## Materials and methods

### *Production of reciprocal hybrids*

Sexually mature broodstocks of *P. hypophthalmus* and *C. gariepinus* (between 1 and 2.5 kg) were obtained from the School of Fisheries and Aquaculture Sciences hatchery of the Universiti Malaysia Terengganu, in Malaysia. Embryogenetic and larvae development were studied in three trials using two pairs of male and female brood fish per species for each trial. Breeding procedure to obtain pure and reciprocal crosses were as described by Okomoda et al. (2017a). The informal nomenclature system proposed by Rahman et al. (2013) was used to name the reciprocal crosses by simply adding the first part of the generic name of the female broodstock with the end part of the specific name of the male broodstock. Therefore, the cross between the female of *C. gariepinus* and the male of *P. hypophthalmus* was regarded as "Clariothalmus" while the reciprocal cross was called "Pangapinus". The crosses were incubated in triplicate aquariums ($45 \times 30 \times 10\ cm^3$) with continuous aeration for each trial. Fertilization rate was determined according to the novel method proposed by Okomoda et al. (2017b). The principle involves discriminating "fertilized" and "hydrated" eggs in representative egg samples collected in a petri dish using the actively dividing animal pole and estimation was made using the equation shown below;

$$\%\,\text{Fertilization} = \frac{\textit{Fertilized eggs in the petri dish}}{\textit{Total number of eggs in the petri dish}} \times 100$$

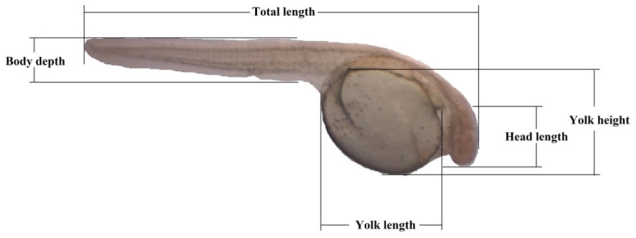


**Fig. 1.** Biometric parameters of the hatchling.

Hatchability rates were determined using the equation below

$$\%\,\text{Hatchability} = \frac{\textit{no. of hatched larvae}}{\textit{total no. of spawned eggs}} \times 100$$

The egg size (n = 20) before and after fertilization were obtained using a Nikon profile projector (Model number V-12BD/JA) attached with a Nikon digital counter (Model number SC-212).

### *Monitoring of embryonic and larval development*

Approximately 50 fertilized eggs were collected at regular intervals from each treatment and monitored under a Nikon dissecting microscope (Model number C-DSLS) following the method described by Ferosekhan et al. (2015) and Olufeagba et al. (2016). Pictorial evidence of the different developmental stages and observable abnormalities were captured using a Sony camera (Cyber-shot 16.2MP Model number: DSC-TX10 50i) fitted to the microscope. Upon hatching, biometric characteristics of the larvae (Fig. 1) were recorded using the Nikon profile projector. The yolk volume was calculated using the relation provided by Blaxter and Hampel (1963) ($V = (\pi/6)\ LH^2$, where V is the yolk size volume, L is the yolk size length and H is the yolk size height). Abnormality percentage was determined.

At the observation of mouth opening, larvae were fed freshly hatched Artemia *adlibitum* (three times a day at 08:00 h, 15:00 h and 21:00 h) through the time of larval observation. Morphological (phenotypic) and behavioural changes (feeding and swimming) of the reciprocal crosses were daily documented (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 daily. Observable differences in morphology within the same cross were documented and referred to as different morphotypes. These were described based on their resemblance to the parent stock (*Pangasius*-like and *Clarias*-like) and the direction of the reciprocal crosses (Clariothalmus and Pangapinus). Water quality was monitored (using VSI professional plus multi-parameter water quality meter Model 13M10065) and kept optimum by regular water change/continuous aeration (Temperature = 26.5 ± 0.7 °C; pH = 7.00 ± 0.26; Conductivity = 570 ± 2.90 $\mu Scm^{-1}$; 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 Minitab 14® computer software followed by one-way analysis of Variance (ANOVA). Where significant ($P < .05$) differences were observed, data separation was done using Fisher's least significant difference.

## Results

### *Egg characteristics, fertilization, and hatchability*

Egg and breeding characteristics of the different crosses are summarized in Table 1. Results reveal that eggs originating from *C. gariepinus* were significantly larger than those of *P. hypophthalmus* (0.92 vs 0.82 mm) before fertilization. However, after fertilization, egg size was largest for the pure *C. gariepinus* cross (1.14 mm),

**Table 1**
Egg and breeding characteristics of reciprocal crosses of *C. gariepinus* and *P. hypophthalmus* (n = 20 for egg size and 9 for fertilization/hatchability). Numbers are means ± standard errors.

| | Pure Clarias | Clariothalmus | Pangapinus | Pure Pangasius | P-Value |
|---|---|---|---|---|---|
| Egg size pre-fertilization (mm) | 0.92 ± 0.02[a] | 0.90 ± 0.06[a] | 0.82 ± 0.04[b] | 0.81 ± 0.04[b] | 0.001 |
| Egg size post fertilization (mm) | 1.14 ± 0.06[a] | 1.08 ± 0.04[b] | 0.96 ± 0.05[c] | 0.90 ± 0.04[d] | 0.001 |
| %Fertilization | 85.42 ± 3.21[ab] | 79.50 ± 2.01[b] | 70.33 ± 0.91[b] | 90.01 ± 4.21[a] | 0.001 |
| %Hatchability | 80.50 ± 0.44[a] | 60.31 ± 5.00[b] | 13.20 ± 4.20[c] | 63.13 ± 0.80[b] | 0.001 |

Mean in the same row with different superscripts differ significantly (ANOVA, $P \leq 0.05$).