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First report on the successful hybridization of *Pangasianodon hypophthalmus* (Sauvage, 1878) and *Clarias gariepinus* (Burchell, 1822)

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Summary

Breeding and larval performance of novel hybrids from reciprocal crosses of Asian catfish *Pangasianodon hypophthalmus* (Sauvage, 1878) and African catfish *Clarias gariepinus* (Burchell, 1822) were investigated in this study. Spawning was by hormonal injection of brood fish, artificial fertilization, and incubation in triplicate aquarium tanks ($0.5 \times 0.5 \times 0.5$ m^3) with continuous aeration. Reciprocal crosses (♀*C. gariepinus* × ♂*P. hypophthalmus* and ♀*P. hypophthalmus* × ♂*C. gariepinus*) had lower hatchability (≤50%) than their pure siblings (≥75%). Fish from all crosses survived until the juvenile stage but survival at 35 days post hatching (dph) was higher for pure *C. gariepinus* sib. ♀*C. gariepinus* × ♂*P. hypophthalmus* was observed to be less resistant to degradation of water quality than the other crosses, however it had higher body weight compared with the other crosses that showed similar performance. Morphological comparison of surviving juvenile at 35 dph, showed that all ♀*P. hypophthalmus* × ♂*C. gariepinus* and 13% of the ♀*C. gariepinus* × ♂*P. hypophthalmus* exhibited the very same morphology as that of their maternal parent species, while the other portion of the ♀*C. gariepinus* × ♂*P. hypophthalmus* cross exhibited morphological traits that were intermediate between those of both parent species. This study been the first successful attempt to hybridize both species and therefore, laid the groundwork for further studies on the aquaculture potentials of the novel hybrids.

Keywords: African catfish, Asian catfish, Hatching success, Heterosis, Hybridization, Morphotype

Introduction

Hybridization has been used as one of the range of biotechnology tools in solving many aquaculture problems (Rahman *et al.*, 2013), the mating of genetically differentiated individuals or groups, (i.e. crosses within a species or between different species) (Bartley *et al.*, 2001). Hence, it is able to transfer desirable traits from one group/species to another (Rahman *et al.*, 2000, 2005). The desirable trait may include, but not be limited to, an increase in growth rate, improved productivity, better fillet quality, disease resistance, increase in environmental tolerance, better food conversion, sexual dimorphism and increased harvesting rate in culture systems (Rahman *et al.*, 2013).

Pangasianodon hypophthalmus (Sauvage, 1878) and *Clarias gariepinus* (Burchell, 1822) are among the most emblematic and important freshwater aquaculture species in south-east Asia, West Africa and many parts of the world (Hung *et al.*, 1999; Solomon *et al.*, 2013). *P. hypophthalmus* has a better fillet quality and can grow to a very large size (Hung *et al.*, 1999; Chattopadhyay *et al.*, 2002). The artificial breeding pattern involves stripping both male and female, however transient mortality during the first feeding days is a major challenge in the larva nursing of this catfish (Baras *et al.*, 2010). It also has a late maturity period which

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is between 2–3 years (Hung *et al.*, 1999). *C. gariepinus,* however, has a faster growth and matures early (9 months). The possession of a suprabranchial organ as opposed to the vascularized swim bladder found in *P. hypophthalmus* makes the African catfish more tolerant to anoxic water than its Asian counterpart (Ahmed *et al.*, 2008). However, the gonad morphology of the male *C. gariepinus* makes sperm collection by stripping impossible. Hence, artificial breeding procedure involves sacrificing the male to obtain the sperm. This breeding system limits the possibility of stock improvement by selective breeding (Legendre *et al.*, 1992). Furthermore, the high rate of cannibalism due to social interaction in the African catfish can significantly reduce the number of fish at harvest (Almaza´n Rueda, 2004; Olufeagba & Okomoda, 2016).

Intergeneric and interspecific hybridization of *P. hypophthalmus* and *C. gariepinus* with many close species has been successfully carried out, some of which showed positive heterosis for growth, survival and many other desirable traits (Tober *et al.*, 1995; Sahoo *et al.* 2003; Gustiano 2004; Hassan *et al.*, 2011; Olufeagba *et al.*, 2016). Earlier studies by Boonbrahm *et al.* (1977), Tarnchalanukit (1985, 1986) and Na-Nakorn *et al.* (1993) had also demonstrated successful hybridization between some species of the Clariid family [Philippine catfish *Clarias batrachus* (Linnaeus, 1758) and Bighead catfish *Clarias macrocephalus* Gunther, 1864] with the Asian catfish *P. hypophthalmus*. However, successfully hybridization between *P. hypophthalmus* and *C. gariepinus* has not been scientifically reported to date. Successful hybridization of these fish could offer solutions to some of the problems associated with the breeding of both pure species. For instance, the killing of male *C. gariepinus* to obtain testis is eliminated due to the ease of sperm stripping from the male *P. hypophthalmus*. Also, the early maturity and high fecundity of the female *C. gariepinus* complements the difficulty faced with the female *P. hypophthalmus* brood fish. The present study therefore analyses for the very first time the breeding performance, survival and growth of the hybrids and pure crosses of these two species during the embryonic and larval periods.

Materials and Methods

Brood fish procurement and breeding

Eight brood fish each of *P. hypophthalmus* (mean weight of 1.7 kg) and *C. gariepinus* (mean weight of 1 kg) of reproductive age (above 3 years and 1 year respectively) were obtained from the School of Fisheries and Aquaculture Sciences hatchery of the Universiti Malaysia Terengganu, in Malaysia (1:1 male to female). They were acclimatized for 2 weeks in rectangular fibreglass tanks and fed on a commercial diet (35% crude protein). In two breeding trials, hybridization was attempted between *P. hypophthalmus* and *C. gariepinus* using eight brood fish for each trial (i.e. two pairs of male and female for both species). Both sexes of *P. hypophthalmus* were first injected with Ovaprim® hormone at a rate of 0.2 ml and 0.5 ml of hormone per kg of the fish (for female and male respectively). Female *P. hypophthalmus* were given a second injection 8 h later at a dosage of 0.3 ml of Ovaprim® hormone per kg (to make up recommended dosage of 0.5 ml per kg) (Chaturvedi *et al.*, 2015). Female *C. gariepinus* were injected a one-time dosage of 0.5 ml Ovaprim® hormone per kg at the same time the second injection was administered to the female *P. hypophthalmus*. This procedure was aimed at synchronising the timing of ovulation and stripping for both species that had different latency period of 16 h and 8 h respectively for *P. hypophthalmus* and *C. gariepinus*. The fish were maintained in eight separate tanks according to their sex and species. Eggs from each female were stripped into two bowls according to their species. This was gently mixed and half of the eggs from each species transferred into another bowl to obtain four batches of eggs for the various directional crosses (comprising of two pure and two hybrid crosses). A small portion of the eggs (15–25 eggs) from both species was also isolated till they become opaque to determine fertilization rate. Milt from male *P. hypophthalmus* was obtained by stripping. The males *C. gariepinus,* however, were tranquilized with 150 mg/1 solutions of tricaine methane sulphonate (MS222) (Wagner *et al.*, 1997) before they were sacrificed. The testes were macerated into a small bowl to mix the sperm content of both males (of *C. gariepinus*). Half of the content was used for the pure crosses while the other half was used for the hybrid crosses based on the direction shown below:

- ♀C. gariepinus × ♂C. gariepinus, (♀CG × ♂CG)
- ♀C. gariepinus × ♂P. hypophthalmus (♀CG × ♂PH)
- ♀P. hypophthalmus × ♂C. gariepinus, (♀PH × ♂CG)
- ♀P. hypophthalmus × ♂P. hypophthalmus, (♀PH × ♂PH).

The eggs and sperm content were mixed uniformly for 1 min, after which a small quantity of water (100 ml) was added and the content mixed again for another minute. The excess water and sperm were decanted leaving behind the fertilized eggs. Triplicate batches of equal eggs mass (10 g) were spawned on 12 nylon mesh substrate suspended over continuously oxygenated water in 12 aquarium bowls (0.5 × 0.5 × 0.5 m3). The aquaria were tagged appropriately in accordance to the crosses they represent. The

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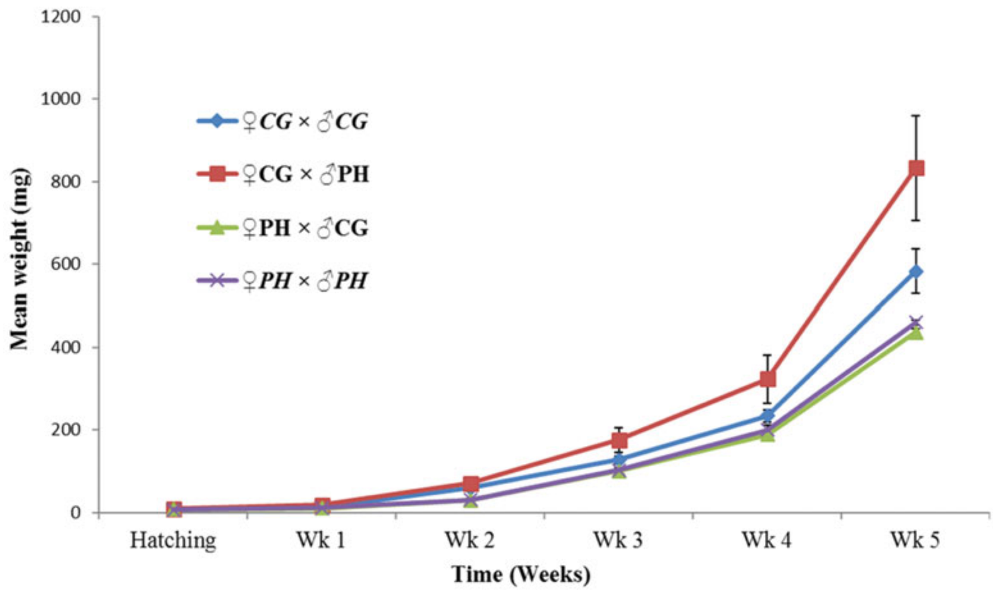

Figure 1 Weekly growth of pure and reciprocal hybrids of *Pangasianodon hypophthalmus* and *Clarias gariepinus*.

numbers of egg in 1 g of fertilized egg mass were also determined for each cross to estimate the number of eggs spawned and determine associated breeding parameters.

Determination of breeding and growth performance

The time taken for the small portion of the eggs initially separated to become opaque (dead eggs) was noted to estimate fertilization rate using the formulae specified by Ella (1987) as shown below:

$$\%\ \text{fertilization} = \frac{N - b}{N} \times 100$$

where (N) represents the total number of eggs spawned, (b) number of bad eggs and was obtained by counting.

The hatching rate of each cross was evaluated by expressing the value of hatch fry as a percentage of the total number of eggs incubated:

$$\%\ \text{hatching rate} : \frac{\textit{no. of hatched larvae}}{\textit{total no. of spawned eggs}} \times 100$$

The number of normal and deformed larvae was also determined by direct observation and counting. Generally, the criteria used to determine normality of hybrid hatchlings were the presence of a straight body and a distinct head distinguished from the yolk. Divergence from this form was considered abnormal. Post-yolk absorption survival was estimated (at first feeding), 100 larvae from each cross were then stocked in 0.5 × 0.5 × 0.5 m^3 aquarium tanks using a static system with continuous aeration (natural photoperiod of 12 h daylight and 12 h darkness). Each group was fed sequentially a dietary regime of live *Artemia*; fishmeal and commercial micro-pellets feed. *Artemia* was fed as the first diet between the 3rd to 21st days post hatching (dph). They were incubated and hatched in salt water 24 h prior to feeding. Each batch of hatched *Artemia* was fed continuously to the fish for a maximum of 3 days, after which newly hatched *Artemia* was used. *Artemia* was administered four times daily (between 8:00 h and 21:00 h). The fishmeal was only fed to the larvae on the 22nd dph (feeding was stopped due to increased mortality of the fish). The fishmeal was made into a dough and stocked to the side of the aquarium tanks for sequential release into the culture system, this was done three times a day. From the 23rd to the 35th dph commercial micro-pellets feed (45% crude protein) were distributed *ad libitum* by hand three times a day. Growth parameters of the hatchlings were observed under these feeding regimes. Fish were bulk weighed weekly using a sensitive weighing balance (nearest 0.00 mg) and mean weights obtained (Fig. 1). Total lengths of 15 randomly selected hatchlings were also taken (nearest 0.00 cm) at the start of the exogenous feeding and at the end of the 35 dph using a micrometre gauge.

All fish were returned to the appropriate rearing tank after measurements were taken. Mortality in the rearing tanks was noted daily and recorded appropriately. Also, dead fishes were observed for missing parts which are evidence of incomplete cannibalism (Almaza´n Rueda, 2004; Olufeagba & Okomoda, 2016). Upon weekly checks for the measurement of growth, survivors were counted and missing fish were assumed to have succumbed to complete cannibalism

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Table 1 Breeding parameters and heterosis (*H*) of performance for the reciprocal hybridization between *Pangasianodon hypophthalmus* and *Clarias gariepinus*

Parameter	♀CG × ♂CG	♀CG × ♂PH	♀PH × ♂CG	♀PH × ♂PH	*P*-value
Fertilization (%)	89.33 ± 1.33	86.67 ± 1.76	86.00 ±2.08	87.60 ± 2.73	0.678
Hatchability (%)	88.00 ± 2.53^{a}	49.04 ± 4.58^{b}	12.19 ± 3.51^{c}	76.25 ± 1.39^{c}	0.001
H for hatchability (%)		−40.29	−85.16		
Survival at first feeding (%)	75.33 ± 0.88^{a}	50.33 ± 6.39^{b}	45.00 ± 2.89^{b}	45.20 ± 7.21^{b}	0.001
H for survival (at first feeding in %)		−16.49	−25.33		
% Abnormality	5.00 ± 1.73^{c}	67.33 ± 5.61^{a}	28.00 ± 4.16^{b}	3.33 ± 0.88^{c}	0.001

[a,b,c]Means in the same row with different superscript letters differ significantly ($P \leq 0.05$).

(Solomon & Okomoda, 2012; Olufeagba & Okomoda, 2016). Growth parameter determined in the fishes includes:

mean length gained (cm) = L2 − L1
mean weight gained (mg) = W2 − W1
growth rate (mg/day) = $\frac{W_2 - W_1}{t_{2-t_1}}$
specific growth rate (%/day) = $\frac{log_e(W_2) - log_e(W_1)}{t_{2-t_1}}$
where W1 = initial weight (mg); W2 = final weight (mg); L1 = initial length (cm); L2 = final length (cm); t2 − t1= duration between W2 and W1(d).
survival rate (%) = $\frac{\text{fish stocked} - \text{mortality}}{\text{fish stocked}} \times 100$

Note: Survival was recorded both in cumulative terms (i.e. based on the number of larvae initially stocked in the aquarium at 3 dph) and in relative terms (i.e. based on the previous number of surviving fish in the culture tanks before the occurrence of the current mortality):

cannibalism mortality (%) = $\frac{\text{(Dead fish with missing parts + Unobserved mortality)}}{\text{Total number of mortality.}} \times 100$

heterosis H (%) = $\frac{F1 - {}^1\!/\!_2(P1 + P2)}{{}^1\!/\!_2(P1 + P2)} \times 100$

where, F1, P1, and P2 are the averages of the performance of the first generation of hybrids, parent 1 and parent 2, respectively.

At the end of the experiment, the gross morphologies of the surviving hybrids and pure species were compared. Morphological variation in the hybrids was described and assumed as different morphotypes. Their ratios based on the pooled surviving hybrids in all the rearing tanks were calculated and recorded morphotype were described based on the observable fin and body characteristic. Detailed morphological comparison could not be made at 35 dph due to the size of the fish.

Water quality parameter

Water quality parameters such as temperature, dissolved oxygen, total dissolved solids, pH, and ammonia concentration were monitored daily throughout this study using a YSI professional plus multi-parameter water quality meter (Model 13M10065, USA). Also, when mass mortality (≥50%) was noticed within 24 h in any of the group (between 3rd and 35th dph), water quality parameter during that period were separately and analysed to see if mortality could be linked to degradation of water quality. A 24 h challenge test was undertaken on the 36 dph to roughly evaluate the tolerance of the different crosses to low dissolved oxygen. During this challenge test, the surviving fishes from the previous study were raised under the same static condition with no aeration. Water quality parameters were recorded and survival determined.

Data analysis

Descriptive statistics were analysed using mini tab 14 computer software followed by one-way analysis of variance (ANOVA). When significant ($P < 0.05$) differences were observed, data were separated using Fisher's least significant difference.

Results

Breeding performance in this study is summarized in Table 1. Both pure and reciprocal hybrids had comparable ($P > 0.05$) fertilization rates (86–89%). Artificial propagation of the reciprocal hybrid was successful in producing viable larvae. However, hatchability was about four times as high for ♀CG × ♂PH than for ♀PH × ♂CG (49 vs 12%) but generally lower than values recorded in the pure sibling (88 and 76% for ♀CG × ♂CG and Panga respectively); 67.33 and 51.09% of ♀CG × ♂PH and ♀PH × ♂CG respectively were abnormal while pure siblings had abnormality less than 5%.

At the end of the endogenous feeding, close to half of the hatched larvae of *P. hypophthalmus* (45.20%), ♀CG × ♂PH (50.33%) and ♀PH × ♂CG (45%) survived. Survival was much higher in pure *Clarias* (75%) compared with pure Panga (45.2%). During the *Artemia* feeding period (Table 2), survival was significantly higher in fish originating from *Clarias*

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Table 2 Survival of pure and reciprocal hybrids of *Pangasianodon hypophthalmus* and *Clarias gariepinus* under different feeding regimes

Parameter	♀CG × ♂CG	♀CG × ♂PH	♀PH × ♂CG	♀PH × ♂PH	*P*-value
% Survival during *Artemia* feeding	82.00 ± 4.10^{a}	71.43 ± 3.60^{b}	27.00 ± 3.03^{d}	49.00 ± 6.80^{c}	0.007
H for survival at *Artemia*		33.33	−58.78		
% Survival during fishmeal feeding	96.50 ± 1.50^{a}	38.00 ± 7.00^{b}	89.50 ± 2.50^{a}	93.00 ± 3.00^{a}	0.001
H for survival at fishmeal feeding		−59.89	−5.54		
% Survival during micro-pellets feeding	81.00 ± 3.00^{b}	85.00 ± 3.02^{a}	91.50 ± 3.50^{a,b}	91.00 ± 2.00^{a,b}	0.008
H for survival at micro-pellets feeding		−1.16	6.40		
% Cannibalism mortality	96.50 ± 3.50^{a}	69.30 ± 3.00^{b}	15.00 ± 2.00^{c}	14.50 ± 4.50^{c}	0.001

[a,b,c]Means in the same row with different superscript letters differ significantly ($P \leq 0.05$).

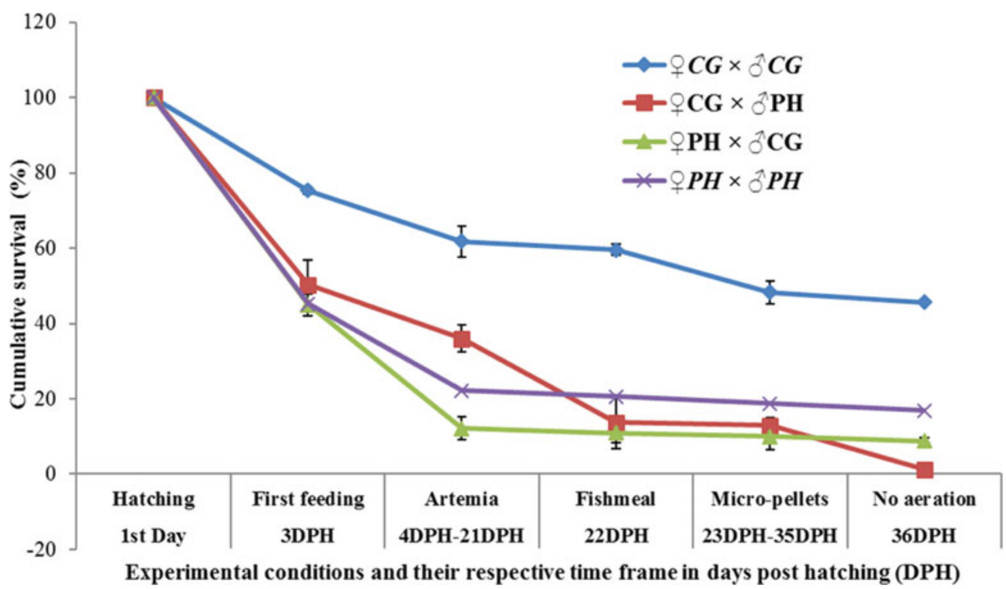

Figure 2 Cumulative survival of pure and reciprocal hybrids of *Pangasianodon hypophthalmus* and *Clarias gariepinus* under different experimental conditions.

eggs (82–87%) than in those originating from Panga eggs (49−56%) (Fig. 2). However, when fish were offered micro-pellets, survival was high in all groups, but proportionally lowers in pure *Clarias* and ♀CG × ♂PH (81–85 versus 91%). This was partly due to different rates of cannibalism, which accounted for over 95% of total mortality in pure *Clarias* and 69.3% in ♀CG × ♂PH as against ≤15% recorded in the other crosses.

The 24-h challenge test (i.e. tolerance to low dissolved oxygen) at the end of the rearing period (Table 3) revealed a good hardiness of pure *Clarias*, pure Panga and ♀PH × ♂CG. They all exhibited survival rates over 90% during this test. By contrast, less than 10% of ♀CG × ♂PH survived this test. The greater fragility of ♀CG × ♂PH to degradation of water quality is supported by the lower survival rate that had been observed at 22 dph, at the transition between the two types of food: only 38% of the ♀CG × ♂PH survived this transition, whereas survival in the other crosses ranged from 89.5 to 96.5% (Table 2).

The performance of larvae after a 35 dph feeding is shown in Table 4. The final mean body weight varied from 833 to 436 mg and specific growth rate from 11.64 to 12.85% day^{-1}. These parameters were significantly lower in *P. hypophthalmus* and ♀PH × ♂CG compared with the rest of the crosses. However, ♀CG × ♂PH hybrid displayed a 60.37% heterosis in mean body weight relative to the performance of the mid parents, while ♀PH × ♂CG had a negative heterosis (−16.49%).

Two morphotypes were observable in the ♀CG × ♂PH at 35 dph (Table 5). The first morphotype (87% of the hybrid pool) has combined features of both parents, while the other morphotype (13% of the hybrid pool) was hardly distinguished from the *C. gariepinus* parent. The ♀PH × ♂CG hybrid, however, has only one morphotype which looks closely and indistinguishable from the *P. hypophthalmus* parent.

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Table 3 Survival of pure and reciprocal hybrids of *Pangasianodon hypophthalmus* and *Clarias gariepinus* and the mean water quality of the experimental unit during the 24 h challenge test without aeration

Parameter	♀CG × ♂CG	♀CG × ♂PH	♀PH × ♂CG	♀PH × ♂PH	*P*-value
Survival (%)	94.59 ± 0.10[a]	9.57 ± 1.88[c]	89.17 ± 0.833[b]	90.11 ± 2.20[a,b]	0.001
H for survival (%)		−89.64	−3.44		
Temperature °C	29.10 ± 0.10	28.95 ± 0.09	28.50 ± 0.20	28.80 ± 0.89	0.346
DO (mg l^{-1})	0.22 ± 0.05[b]	1.42 ± 0.32[a]	1.47 ± 0.26[a]	0.65 ± 0.55[a,b]	0.008
TDS (mg l^{-1})	109.4 ± 20.2	105.70 ± 14.2	101.80 ± 15.9	102.00 ± 16.3	0.252
pH	6.82 ± 0.09	6.85 ± 0.12	6.97 ± 0.11	6.86 ± 0.15	0.684
NH_4 (mg l^{-1})	11.12 ± 0.86[b]	17.62 ± 1.61[a]	12.13 ± 1.19[a,b]	14.73 ± 2.93[a,b]	0.013

[a,b]Means in the same row with different superscript letters differ significantly ($P \leq 0.05$).

Table 4 Growth parameters and heterosis (*H*) of performance for the reciprocal hybridization between *Pangasianodon hypophthalmus* and *Clarias gariepinus*

Parameter	♀CG × ♂CG	♀CG × ♂PH	♀PH × ♂CG	♀PH × ♂PH	*P*-value
Length 3 dph (cm)	0.534 ± 0.015[b]	0.624 ± 0.035[a]	0.452 ± 0.024[c]	0.467 ± 0.033[c]	0.014
Final length 35 dph (cm)	3.60 ± 0.30[b]	4.69 ± 3.40[a]	3.80 ± 0.20[b]	3.60 ± 0.10[b]	0.050
Length gain (cm)	3.07 ± 0.32[b]	4.03 ± 0.15[a]	3.35 ± 0.22[a,b]	3.13 ± 0.07[b]	0.050
Weight 3 dph (mg)	2.98 ± 0.08[a]	2.67 ± 1.07[b]	0.98 ± 0.03[c]	0.88 ± 0.06[c]	0.364
Weight 35 dph (mg)	583.3 ± 23.3[b]	833.00 ± 67.0[a]	436.51 ± 7.94[b]	460.2 ± 16.2[b]	0.015
Weight gain (mg)	580.4 ± 83.3[b]	831.00 ± 167.0[a]	435.63 ± 8.00[b]	459.3 ± 5.2[b]	0.015
H for growth (%)		59.64	− 16.54		
Growth rate (mg day^{-1})	16.58 ± 2.38[b]	23.73 ± 4.76[a]	12.45 ± 0.23[c]	13.12 ± 2.43[c]	0.015

[a,b,c]Means in the same row with different superscript letters differ significantly ($P \leq 0.05$).

Discussion

This study demonstrates the possibility of producing viable hybrids from reciprocal crosses of *P. hypophthalmus* and *C. gariepinus*. Tarnchalanukit (1986), and Boonbrahm *et al.* (1977) had earlier reported successful hybridization between *C. batrachus* and *P. hypophthalmus*. Similarly, Na-Nakorn *et al.* (1993) and Tarnchalanukit (1985) also reported their findings between *P. hypophthalmus* and *C. macrocephalus*. Glamuzina *et al.*, (1999, 2001). Frisch & Hobbs, (2007) had stated that successful hybridization between different species could be pointers to close evolutionary relationship. However, the low hatchability observed in the hybrids and the percentages of deformed larvae suggest a possible genetic incompatibility between these species from different families. Similar findings have been reported in reciprocal crosses of *C. gariepinus* with *C. batrachus* (Sahoo *et al.*, 2003; Olufeagba & Okomoda, 2016) and in the hybrids between *C. gariepinus* and Sampa catfish *Heterobranchus longifilis* Valenciennes, 1840 (Ataguba *et al.*, 2009). However, Glamuzina *et al.* (1999, 2001) observed no difference in the deformity rates of hybrid and non-hybrid groupers, while Owodeinde & Ndimele (2011) reported higher hatchability rates of reciprocal crosses of *C. gariepinus* and African catfish *Heterobranchus bidorsalis* Geoffroy Saint-Hilaire, 1809 compared with the pure sibling. The differences observed between these studies could be linked to the differences in the gene compatibility of the species involved in the different crosses reported.

Interspecific hybridization has been thought to be less successful due to the high rate of mortalities during the early life stages (Bartley *et al.*, 2001). However, about half the percentage of hatched larvae survived at first feeding. The low survival of the *P. hypophthalmus* at post-yolk absorption has earlier been justified by the possession of oral teeth and spines, which facilitate grasping of large food items including siblings, hence, accounting for the mortality at this stage (Baras *et al.*, 2010). This is in contrast with the situation in pure *Clarias*, which never grows long oral teeth, and in which cannibalism essentially takes place at an older age and developmental stage, once sufficient size disparity has taken place (Mollah *et al.*, 1999). Hence, observations made in this study on cannibalism suggest a preponderant influence of maternal origin upon embryo. This is because mortality linked to cannibalism was more in ♀CG × ♂PH than ♀PH × ♂CG. Many dead fish were found on the bottom of the rearing facility with some missing body parts. This observation is similar to the kind of mortalities described by Solomon & Okomoda (2012); Appelbaum & McGeer (1998) and attributed to cannibalism. However, a recent study by Olufeagba & Okomoda (2016) suggested that

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Table 5 Morphological features of pure and reciprocal hybrids of *Pangasianodon hypophthalmus* and *Clarias gariepinus* larvae that survived up to 35 dph

Parameter	♀CG × ♂CG	♀CG × ♂PH	♀PH × ♂CG	♀PH × ♂PH	
Picture					
Morphotype percentage	100	87%	13%	100%	100%
Head shape	Broad	Broad	Broad	Not broad	Not broad
Eye	Small	Large	Small	Large	Large
No of barbels	8	Between 4 and 6	8	4	4
Body configuration	Dorsoventrally compressed	Dorsoventrally compressed	Dorsoventrally compressed	Laterally compressed	Laterally compressed
Caudal fin shape	Rounded	Slightly furcated	Rounded	Strongly furcated	Strongly furcated
First dorsal fin configuration	Rectangular short height fin which stretches as far as the tip of the caudal fin	Fin with long height but stretches two-thirds of the fish's trunk	Rectangular short height fin which stretches as far as the tip of the caudal fin	Long height fin but stretches one-fourth of the fish's trunk	Long height fin but stretches one-fourth of the fish's trunk
Second dorsal fin	Absent	Short and adipose in nature	Absent	Short and adipose in nature	Short and adipose in nature
Anal fin	Rectangular	Ellipse	Rectangular	Falcate	Falcate

Table 6 Mean water quality parameters of the experimental unit during the *Artemia*/micro-pellet diet regime and the fishmeal diet regime

Parameter		♀CG × ♂CG	♀CG × ♂PH	♀PH × ♂CG	♀PH × ♂PH	*P*-value
Artemia/micro-pellet feeding regime	Temperature °C	29.07 ± 0.96	28.77 ± 0.81	29.27 ± 0.98	29.00 ± 0.86	0.983
	DO (mg l^{-1})	5.33 ± 0.43	6.17 ± 0.63	5.29 ± 0.34	5.29 ± 0.17	0.431
	TDS (mg l^{-1})	82.70 ± 34.5	85.10 ± 25.5	80.70 ± 36.0	87.88 ± 6.66	0.722
	pH	7.45 ± 0.24	7.41 ± 0.24	7.59 ± 0.31	7.68 ± 0.14	0.849
	NH_4 (mg l^{-1})	7.59 ± 0.35	7.11 ± 0.66	7.37 ± 1.00	6.97 ± 0.65	0.925
Fishmeal feeding regime	Temperature °C	30.33 ± 0.12	30.30 ± 0.15	30.20 ± 0.10	30.23 ± 0.09	0.848
	DO (mg l^{-1})	4.30 ± 0.72	4.17 ± 0.41	4.67 ± 0.31	4.40 ± 0.55	0.914
	TDS (mg l^{-1})	198.13 ± 7.59	198.00 ± 11.1	190.40 ± 19.1	192.00 ± 3.61	0.948
	pH	6.69 ± 0.15	6.65 ± 0.16[b]	6.76 ± 0.22	6.73 ± 0.44	0.991
	NH_4 (mg l^{-1})	13.59 ± 1.19	15.45 ± 0.67	13.87 ± 0.56	15.02 ± 1.48	0.565

[b]Means in the same row with different superscript letters differ significantly ($P \leq 0.05$).

hybridization between *C. gariepinus* and *C. batrachus* led to significant reduction in cannibalism. Hence, they hypothesized that the genes responsible for cannibalism in *C. gariepinus* could be recessive to that in *C. batrachus*. This seems not to be the case in the present study. Nevertheless, in view of the results presented here, larval mortality and thus cannibalism among the hybrids and pure species can be maintained at an 'acceptably low rate', if sorting is routinely carried out.

This study shows that reciprocal hybrids can survive up to and reach juvenile stage (and possibly beyond). Nevertheless, it turned out that ♀CG × ♂PH hybrids were more sensitive than others to a degradation of water quality, for reasons that remain to be clearly elucidated. However, the reduced fitness and tolerance of the ♀CG × ♂PH may be linked to a hybridization effect which was earlier reflected in high abnormality percentages in the hatched larvae. Tarnchalanukit (1985) had earlier reported that the hybrid between ♂*P. hypophthalmus* and ♀*C. macrocephalus* survived up to 4½ months but was less tolerant to poor water quality. Hence, the issue of survival for the novel hybrid ♀CG × ♂PH might outweigh any other advantage over the parent species whenever water quality is not optimal. The poor water quality observed under the fishmeal-based feeding regime as compared with *Artemia* or commercial micro-pellets diet justifies the need to optimise feed types administered to larvae of fishes in their early life. The differences in the water stability of the feeds are the likely reasons for the discrepancies observed in the water quality of the system under these feeding regimes.

Many researchers have earlier proposed better performance of reciprocal hybrids over their pure breeds (Madu & Ita, 1991; Jantrarotai, 1993; Tober *et al.*, 1995; Ataguba *et al.*, 2010, Solomon et al 2013; Olufeagba *et al.*, 2016; Olufeagba & Okomoda, 2016). However, Chevassus (1983) stated that growth of hybrids mostly appears to be intermediate between that of parental species because of partial transmission of traits of the parent to the hybrids. The findings of this study, revealed a positive heterosis for growth in ♀CG × ♂PH and a negative heterosis for ♀PH × ♂CG. The pattern of inheritance as a result of the different crosses may have resulted in the differences in performance observed for the reciprocal crosses. Bartley *et al.* (1997) had earlier opined that the high preference of Thai fish farmers for hybrids catfishes (between Thai catfish *C. macrocephalus* and African catfish *C. gariepinus*) is due to the possession of desirable qualities from both parents. This could be a pointer to possible acceptability of the ♀CG × ♂PH as a large proportion of this hybrid possesses combined features from both parents.

Morphological observations of the hybrids showed two morphotype in the ♀CG × ♂PH hybrid and one in the ♀PH × ♂CG hybrid. The number of morphotypes observed in this study is less compared with the morphotypes reported by Tarnchalanukit (1986), Boonbrahm *et al.* (1977) and Na-Nakorn *et al.* (1993). However, it is possible that the reduced morphotypes number observed in this study was due to mortalities observed before 35 dph. In line with the morphotype naming format used in similar studies by Tarnchalanukit (1986), Boonbrahm *et al.* (1977) and Na-Nakorn *et al.* (1993), the *Clarias*-like hybrids (which is about 13% in the ♀CG × ♂PH and 100% of the ♀PH × ♂CG) were indistinguishable from the pure *C. gariepinus*. However, the Panga-like hybrid found in the ♀CG × ♂PH showed evidence of shared features (significant modification in fin configuration) from both parents but look like the *Pangasius* in many ways. Chevassus (1983) and Wilkins *et al.* (1994) had earlier opined that offspring of interspecific hybridization displayed intermediate morphological characteristics of maternal and paternal species. However, the observation of the two different

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morphotypes in the ♀CG × ♂PH suggests the possible presence of ploidy polymorphism in the hybrid pool**.** This is a usually phenomenon associated with many previously reported distant hybridizations trials (Kurita, *et al.*, 1995; Pandian & Koteeswaran 1998; Liu *et al.*, 2010). Furthermore the study by Na-Nakorn *et al.* (1993), on the chromosome characteristics of hybrids between ♂*P. hypophthalmus* × ♀*C. macrocephalus* confirmed the presence of diploid, triploid and aneuploidy hybrids corresponding respectively to two intermediate morphotypes (pangasiid-like and clariid-like) and one morphotype indistinguishable from its clariid parent.

Conclusion

The intergeneric cross of Asian catfish *P. hypophthalmus* and African catfish *C. gariepinus* in this study produced viable hybrids with potential for culture. However, some hybrids demonstrate lower tolerance to poor water quality when compared with the pure sibs. Future research could be focused on characterising the hybrids and determining the developmental and physiological traits in early and later life stage. Furthermore, the performance of hybrid based on the phenotypic morphotypes can be assessed and compared. However, it is important to state that hybridization between geographically distant species such as this should be conducted very carefully, because escapees from fish farms could just have dramatic and largely unexpected consequences upon wild populations.

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References

Ahmed, A.E., Mohamed, K., Ahmed, S.A. & Masoud, F. (2008). Anatomical, light and scanning electron microscopic studies on the air breathing dendretic organ of the sharp tooth catfish (*Clarias gariepinus*). *J. Vet. Anat* **1**, 29–37.

Almaza´n Rueda, P. (2004). Towards assessment of welfare in African catfish, *Clarias gariepinus*: the first step. PhD Dissertation, Wageningen University, Wageningen, The Netherlands.

Appelbaum, S. & McGeer, J.C. (1998). Effect of diet and light regime on growth and survival of African catfish (*Clarias gariepinus*) larvae and early juveniles. *Aquacult. Nutr.* **4**, 157–64.

Ataguba, G.A., Annune, P.A. & Ogbe, F.G. (2009). Induced breeding and early growth of progeny from crosses between two African clariid fishes, *Clarias gariepinus* (Burchell) and *Heterobranchus longifilis* under hatchery conditions. *J. Appl. Biosci*. **14**, 755–60.

Ataguba, G.A., Annune, P.A. & Ogbe, F.G. (2010). Growth performance of two African catfishes *Clarias gariepinus* and *Heterobranchus longifilis* and their hybrids in plastic aquaria. *Livestock Res. Rural Dev*. **22**, article 30.

Baras, E., Slembrouck, J., Cochet, C., Caruso, D. & Legendre, M. (2010). Morphological factors behind the early mortality of cultured larvae of the Asian catfish, *Pangasianodon hypophthalamus*. *Aquaculture* **298**, 211–9.

Bartley, D.M., Rana, K. & Immink, A.J. (1997). The use of inter-specific hybrids in aquaculture. FAO Aquaculture Newsletter No. 17, Rome, Italy, 7–13 December 1997. ISSN 1020–3443.

Bartley, D.M., Rena, K. & Immink, A.J. (2001). The use of inter-specific hybrids in aquaculture and fisheries. *Rev. Fish Biol. Fish.* **10**, 325–37.

Boonbrahm, M., Tarnchalanukit, W., Suraniranat, P. (1977). [Notes on the larvae of hybrids between Pla Duk Ui and Pla Sawai. Report for The Department of Aquaculture]. Faculty of Fisheries, Kasetsart University. 9 pp. [In Thai].

Chattopadhyay, N.R., Mazumder, B. & Mazumdar, B. (2002). Induced spawning of *Pangasius sutchi* with pituitary extract. *Aquacult. Asia* **7**, 43–4.

Chaturvedi, C.S., Ambulkar, R.S., Singh, R.K. & Pandey, A.K. (2015). Induced spawning in *Pangasianodon hypophthalmus* and hatching of eggs in three different types of hatching systems at Raipur (Chhattisgarh), India. *Natl J. Life Sci.* **12**, 215–21.

Chevassus, B. (1983). Hybridization in fish. *Aquaculture* **33**, 245–62.

Frisch, A.J. & Hobbs, J.P.A. (2007). In vitro hybridization of coral trouts, *Plectropomus leopardus* (Lacepede, 1802) and *Plectropomus maculatus* (Bloch, 1790): a preliminary investigation. *Aquacult. Res*. **38**, 215–8.

Glamuzina, B., Glavić, N., Skaramuca, B., Kozul, V. & Tutman, P. (2001). Early development of the hybrid *Epinephelus costae* ♀ × E. *marginatus* ♂. *Aquaculture* **198**, 55–61.

Glamuzina, B., Tutman, P. & Skaramuca, B. (1999). Hybridization of Mediterranean groupers: *Epinephelus marginatus*♀ × *E. aeneus*♂ and early development. *Aquacult. Res*. **30**, 625–8.

Gustiano, R. (2004). Morphometric analysis of the artificial hybridization between *Pangasius djambal* and *Pangasionodon hypophthalmus*. Proceedings of Symposium PERIPI, 5–7 August 2004. RIFA, Indonesia.

Hassan, A., Azmi, A.M. & Putra, A.A.S. (2011). Cross-breeding of *Pangasianodon hypophthalmus* (Sauvage, 1878) and *Pangasius nasutus* (Bleeker, 1863) and their larval development. *J. Sustainable Sci. Manage*. **6**, 28–35.

https://doi.org/10.1017/S0967199417000259 Published online by Cambridge University Press

Hung, L.T., Tam, B.M., Cacot, P. & Lazard, J. (1999). Larval Rearing of Mekong Catfish *Pangasius bocourti*. Substitution of *Artemia nauplii* with live and artificial feed. *Aquat. Living Resour*. **12**, 229–32.

Jantrarotai, W. (1993). Nutrients requirements of Thai walking catfish. NIFI Newsletter, **3**, 2–3.

Kurita, J., Oshiro, T., Takashima, F. & Sakaizumi, M. (1995). Cytogenetic studies on diploid and triploid oogenesis in interspecific hybrid fish between *Oryzias latipes* and *O. curvinotus*. *J. Exp. Zool.* **273**, 234–41.

Legendre, M., Teugels, G.G., Cauty, C. & Jalabert, B. (1992). A comparative study of morphology, growth rate and reproduction of *Clarias gariepinus* (Burchell, 1822). *Heterobranchus longifilis* Valenciennes, 1840 and their reciprocal hybrids (Pisces; Clariidae) *J. Fish Biol.* **40**, 59–79.

Liu, D., Liu, S., You, C., Chen, L., Liu, Z., Liu, L. & Liu, Y. (2010). Identification and expression analysis of genes involved in early ovary development in diploid gynogenetic hybrids of red crucian carp × common carp. *Mar. Biotechnol.* **12**, 186–94.

Madu, C.T. & Ita, E.O. (1991). Comparative growth and survival of hatchlings of *Clarias* sp., *Clarias*. hybrid and *Heterobranchus* sp. in the indoor hatchery. *Annals Rep. Natl Inst. Freshw. Fish. Res. (Nigeria)*, pp. 47–50.

Mollah, M.F.A., Islam, M.S., Faroque, M.A.A. & Khan, M.H.K. (1999). Minimization of cannibalism of African catfish (*Clarias gariepinus* Burchell) larvae in indoor culture system. *Bangladesh J. Fish. Res.* **3**, 181–6.

Na-Nakorn, U., Rangsin, W. & Witchsunkul. (1993). Suitable condition for induction of gynogenesis in the catfish, *Clarias macrocephalus*, using sperm of *Pangasius sutchi*. *Aquaculture* **118**, 53–62.

Olufeagba, S.O. & Okomoda, V.T. (2016). Cannibalism and performance evaluation of hybrids between *Clarias batrachus* and *Clarias gariepinus*. *Croatian J. Fish.* **74**, 124–9.

Olufeagba, S.O., Okomoda, V.T. & Shaibu, G. (2016). Embryogenesis and early growth of pure strains and hybrids between *Clarias gariepinus* (Burchell, 1822) and *Heterobranchus longifilis* Valenciennes, 1840. *North Am. J. Aquacult*. **78**, 346–55.

Owodeinde, F.G. & Ndimele, P.E. (2011). Survival, growth and feed utilization of two clariid catfish (*Clarias gariepinus*, Burchell 1822 and *Heterobranchus bidorsalis*, Geoffroy, 1809) and their reciprocal hybrids. *J. Appl. Ichthyol*. **27**, 1249–53.

Pandian, T.J. & Koteeswaran, R. (1998). Ploidy induction and sex control in fish. *Hydrobiologia* **384**, 167–243.

Rahman, M.A., Uehara, T. & Aslan, L.M. (2000). Comparative viability and growth of hybrids between two sympatric species of sea urchins (Genus *Echinometra*) in Okinawa. *Aquaculture* **183**, 45–56.

Rahman, M.A., Uehara, T. & Lawrence, J.M. (2005). Growth and heterosis of hybrids of two closely related species of Pacific sea urchins (Genus *Echinometra*) in Okinawa. *Aquaculture* **245**, 121–33.

Rahman, M.A., Arshad, A., Marimuthu, K., Ara, R. & Amin, S.M.N. (2013). Interspecific hybridization and its potential for aquaculture of fin fishes. *Asian J. Anim. Vet. Adv.* **8**, 139–53.

Sahoo, S.K., Giri, S.S., Sahu, A.K. & Ayyappan, S. (2003). Experimental hybridization between catfish *C. gariepinus*(Bur.) × and *C. batrachus* (Linn.) and performance of the offspring in rearing operations. *Asian Fish. Sci.* **16**, 157–66.

Solomon, S.G. & Okomoda, V.T. (2012). Growth response and aggressive behaviour of *Clarias gariepinus* fingerlings reared at different photoperiods in a water re-circulatory system. *Livestock Research for Rural Development* **24**, article 191.

Solomon, S.G., Okomoda, V.T. & Ochai, L. (2013). Growth responses of pure bred *Heterobranchus bidorsalis*, *Clarias gariepinus* and their intergeneric crosses fed commercial diet. *Banat's J. Biotechnol*. **8**, 71–6.

Tarnchalanukit, W. (1985). Experimental hybridization between catfish of the families Clariidae and Pangasiidae in Thailand. Kasetsart University. *Fish. Res Bull.* No. **16**, 8 pp.

Tarnchalanukit, W. (1986). Experimental hybridization between catfishes of the families Clariidae and Pangasiidae in Thailand. *Env. Biol. Fish.* **16**, 317–20.

Tober, B., Gabriele, H. & Hans-Jurgen, L. (1995). Species crosses in African catfish *Clarias gariepinus* × *Heterobranchus longifilis*. *Aquaculture* **137**, 325–32.

Wagner, E.J., Jeppsen, T., Arndt, R., Routledge, M.D. & Bradwisch, Q. (1997). Effects of rearing density upon cut throat trout haematology, hatchery performance, fin erosion and general health and condition. *Prog. Fish Cult.* **59**, 173–87.

Wilkins, N.P., Courtney, H.P., Gosling, E., Linnane, A., Jordan, C. & Curatolo, A. (1994). Morphometric and meristic characters in salmon, *Salmo salar* L., trout, *Salmo trutta* L., and their hybrids. *Aquacult. Res* **25**, 505–18.

https://doi.org/10.1017/S0967199417000259 Published online by Cambridge University Press