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Performance and characteristics of the progenies from the reciprocal crosses of *Pangasianodon hypophthalmus* (Sauvage, 1878) and *Clarias gariepinus* (Burchell, 1822)

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ABSTRACT

This study was designed to evaluate the culture performance and characteristics of the offspring from the reciprocal crosses of *Pangasianodon hypophthalmus* and *Clarias gariepinus*. The hybrid ♀*C. gariepinus* × ♂*P. hypophthalmus* (Clariothalmus) gave better hatchability than ♀*P. hypophthalmus* × ♂*C. gariepinus* (Pangapinus), similar to *P. hypophthalmus* but lower than the value recorded in the *C. gariepinus*. Cytogenetic analysis of the Clariothalmus reveals the presence of diploid hybrid (2n = 58 representing 60% of the population) and gynogenetic diploid offspring (2n = 56 representing 40% of the population), while, Pangapinus offspring were all gynogenetic diploid (2n = 60). Hence, the diploid hybrid exhibited phenotypic divergence from both parents, while the gynogenetic diploids were phenotypically indistinguishable from the maternal parent and had similar meristic counts. After 112 days of culture, gynogenetic diploid of the Clariothalmus offspring had better growth than the other crosses, however, cannibalism and aggressive behaviour was higher in this group of fish compared to other. Hence, culturing the diploid hybrid Clariothalmus separately from its gynogenetic diploid may be a management practice of interest as demonstrated in this study. This study provides evidence to support the success of hybridization between African and Asian catfish in one direction and demonstrates their potential for culture.

1. Introduction

The feasibility of producing and commercially propagating new aquaculture candidate with fast-growing potential is pivotal to aquaculture diversification and achievement of food security. Aquaculture diversification can either be through successful domestication and culture of wild species or successful hybridization between genetically distinct groups (FAO, 1997). Hybridization, as a means of species modification, is based on the possibility of integrating genetic material from different groups into one single group (Seehausen, 2004; Mallet, 2007; Stelkens et al., 2009; Liu, 2010). Crossing between two different genus, subfamilies, families or order is often referred to as distant hybridization (Pandian and Koteeswaran, 1998). Progenies of distance hybridization often produce different artificial hybrid offspring groups with phenotypic, cytogenetic and genotypic alterations (Hu et al., 2012; Zhuo et al., 2015). These offsprings have been reported to exhibit heterosis in performance due to the combination of different beneficial traits from both parents (Gray et al., 1993). This includes but not limited to, increased disease resistance, better food conversion, higher growth rate, improved productivity, good flesh quality, increased environmental tolerance and sexual dimorphism (Deng et al., 1992; Cherfas et al., 1994; Zhong et al., 2012; Rahman et al., 2013).

However, performance evaluation has always been between progenies of the pure crosses and the hybrid pool despite the observation of ploidy polymorphism (e.g. studies of Varadaraj and Pandian, 1989; Deng et al., 1992; Cherfas et al., 1994; Liu et al., 2007). Aside from establishing some characteristics of the different ploidy group in the hybrid pool (i.e. morphological, cytogenetic and genetic), seldom has the growth performance of these individual groups been evaluated in comparison with one another and to the pure crosses. This is probably due to the low percentage occurrence of some ploidy group observed in many of the previously reported studies (e.g. Richter et al., 1995; Zou et al., 2007; Na-Nakorn et al., 1993). However, proper matching of the information on growth with morphological, genetic and cytogenetic

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characters could be useful in designing future studies which could be aimed at producing higher percentages of the advantageous ploidy group.

We recently successfully hybridize *Pangasianodon hypophthalmus* (Sauvage, 1878) and *Clarias gariepinus* (Burchell, 1822) in a quest to produce a novel aquaculture candidate and explore the possibility of combining desirable traits from both species (Okomoda et al., 2017a). The resultant progeny from our preliminary investigation consisted of two distinct morphotypes (Clarias-like and Panga-like) which suggested the presence of ploidy polymorphism in the cross between ♀*C. gariepinus* × ♂*P. hypophthalmus*. However, progeny from ♀*P. hypophthalmus* × ♂*C. gariepinus* was all Panga-like. This study is therefore designed to determine the breeding, growth, morphological (meristic) and cytogenetic characteristics of the different groups within the hybrid pool in comparison with the pure sib. The data generated in this study will provide guidance for future work on the breeding of the novel hybrid described herein.

2. Materials and methods

2.1. Hybrid production from reciprocal crosses of P. hypophthalmus and C. gariepinus

Sexually mature broodstocks of *P. hypophthalmus* and *C. gariepinus* (between 1 and 2.5 kg) were obtained from the School of Fisheries and Aquaculture Science hatchery of the Universiti Malaysia Terengganu, Malaysia. Four successful hybridization trials were done to obtain progenies for this study. The breeding method described by Okomoda et al. (2017a, 2017b) for the pure (♀*C. gariepinus* × ♂*C. gariepinus* and ♀*P. hypophthalmus* × ♂*P. hypophthalmus*) and reciprocal crosses (♀*C. gariepinus* × ♂*P. hypophthalmus* and ♀*P. hypophthalmus* × ♂*C. gariepinus*) was employed for each trial. In brief, four female/species were injected with Ovaprim® at a dosage of 0.5 ml/kg for each trial. Four males of *P. hypophthalmus* were also injected using the same dosage. The injection was done systematically as described by Okomoda et al. (2017a) to ensure simultaneous and synchronized stripping of eggs from both species at the same time. The eggs were stripped and pooled separately for the two species. Then the pooled eggs were divided into two portions; each portion was fertilized with either the pooled milt from four male *C. gariepinus* (obtained from testis of sacrificed fish) or milt from four male *P. hypophthalmus* (through striping). The informal nomenclature system for naming hybrids proposed by Rahman et al. (2013) and adopted by Okomoda et al. (2017b) for the same crosses were used in this study. Therefore, the cross between ♀*C. gariepinus* × ♂*P. hypophthalmus* was regarded as "Clariothalmus" while the reciprocal cross (♀*P. hypophthalmus* × ♂*C. gariepinus*) was called "Pangapinus". The fertilized eggs were spread on fine-mesh nylon net immersed in hatching tanks ($1 \times 2 \times 1\ m^3$) fitted with continuous aeration. Fifty minutes after fertilization, the percentage of actively dividing cell was determined as fertilization percentage according to the method recently described by Okomoda et al. (2018). Hatching commenced at about 23 h and prolonged to the 36th hour for the Pangapinus cross. Hence, hatching percentage was determined earlier in the other crosses than the latter. The data from the six trials for each group were pooled and analyzed by one-way analysis of variance (ANOVA) using Minitab 14.

2.2. Hybrid culture and growth performance evaluation

One hundred and fifty fry of each group were cultured in quadruplicate rearing tanks ($80 \times 60 \times 40\ cm^3$) using a re-circulatory aquaculture system (4 × 4 experimental design). The water quality was monitored using an YSI professional plus multi-parameter water quality meter (Model 13M10065, Made in the USA) and maintained at optimum level (temperature = 36.2 ± 1.0 °C; pH = 7.05 ± 0.22; conductivity = 219 ± 0.34 mg l^{-1}; total dissolved solids = 90.4 ± 2.11 mg l^{-1}; dissolved oxygen = 5.9 ± 0.52 mg l^{-1}). The larvae started exogenous feeding on the third-day post-hatching (dph). They were fed to satiation with freshly hatched Artemia thrice daily till the 14 dph. Subsequently, they were fed commercially sold fish pellet (45% Crude protein, 8.2% Moisture, 9.5% Ash, 12% Ether extract, 1.5% Crude fiber) of different sizes based on the size of the fish. Under this condition, larvae were monitored till the 56 dph (2 months). At two month of age, the stocking density was reduced to 50fish per tank. Also, the two morphotypes observed in the Clariothalmus offspring were separated and cultured as individual groups. Hence, the second phase of the growth study which lasted another 56 days had a 5 × 4 experimental design. The weight and fish numbers were recorded every two weeks using a sensitive weighing balance. Performance index was computed as shown in the equations below:

a. Mean Weight Gained (mg) = $W_2 - W_1$
b. Growth rate (mg/day) = $\frac{W_2 - W_1}{t_2 - t_1}$
c. Specific growth rate (%/day) = $\frac{\log_e(W_2) - \log_e(W_1)}{t_2 - t_1}$
where
W_1 = initial weight (mg)
W_2 = final weight (mg)
$t_2 - t_1$ = duration between W_2 and W_1(d)
d. Survival rate (%) = $\frac{\text{fish stocked} - \text{mortality}}{\text{fish stocked}} \times 100$
e. %fish bitten = $\frac{\text{Total number of fish bitten} \times 100}{\text{Total number of fish at the end of the experiment}}$
f. Cannibalism mortality (%) = $\frac{(\text{Dead fish with missing parts} + \text{Unobserved mortality})}{\text{Total number of mortality}.} \times 100$
g. Heterosis H (%) = $\frac{F1 - 1/2(P1 + P2)}{1/2(P1 + P2)} \times 100$

The data were analyzed by one-way analysis of variance (ANOVA) using Minitab 14.

2.3. Determination of chromosome number

To determine the ploidy level, karyotyping procedure by Liu et al. (2001, 2007) was optimised with some modifications. In brief, ten fish samples of the pure and hybrid progenies were injected with freshly prepared 0.05% colchicine (at the rate of 1 ml kg^{-1}) solution and allowed to swim for 2–3 h. Kidney or gill tissues were removed and chopped in a 1.5 ml tube containing 0.075 M KCL. The cell suspension was aspirated and transferred into a new 1.5 ml tube leaving behind the tissue suspension. After 1 h, the tissues suspension was centrifuged (2500 rcf for 10 min) and the supernatant discarded (leaving 1 ml solution above the cell pellet). The cell was re-suspended in freshly prepared cold methanol-acetic acid fixative (3:1) for three changes (20 mins interval for each change). Cells suspension was allowed to age for three days before dropping on ethanol cleaned slides and staining with 10% Giemsa stain (prepared 0.01 M phosphate buffer at pH 7) for 1 h. Slides were air dried and fixed with 2–3 drops of DPX. The slides were microphotography using a Nikon Eclipse 80i compound microscope, and the images processed using the NIS element Basic Research software (at 100× magnification). About 20 metaphase spreads of chromosomes from 10 individuals were counted and analyzed to determine the ploidy and chromosome number for each group. Good quality metaphase spreads were used for karyotype analysis.

2.4. Meristic comparisons of hybrid and pure crosses

Due to the size independent of meristic counts (Strauss, 1985; Murta, 2000), it was selected for the characterization of the morphology of the pure and hybrid crosses. The parameters recorded include the numbers of dorsal fin ray, pelvic fin ray, pectoral fin ray, anal fin ray, and caudal fin ray. To provide an objectively defined score that summarizes the major components of these variables, multiple group