Preliminary report on the novel hybrid from crosses of *Clarias gariepinus* and *Hemibagrus nemurus*

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Abstract. This study was aimed at producing novel hybrids from reciprocal crosses of African catfish *Clarias gariepinus* (CG) and Asian redtail catfish *Hemibagrus nemurus* (HN). Spawning of the pure (♀CG×♂CG and ♀HN×♂HN) and reciprocal hybrids (♀CG×♂HN i.e. Clariomurus and ♀HN×♂CG i.e. Hemipinus) was done in two trials by hormonal injection, artificial fertilization, and incubation in aquarium tanks (0.5×0.5×0.5 m). The result obtained showed that fertilization was highest for the pure *C. gariepinus*, intermediate for the cross Hemipinus as well as the pure *H. nemurus* and least in the cross Clariomurus. No viable larvae were obtained from the cross Hemipinus. Hatchability in the Clariomurus, on the other hand, was significantly low (9.47%) compared to the pure *C. gariepinus* (42.15%) and *H. nemurus* (37%). The larvae of all the crosses were only reared for two weeks due to water quality problems. During this time, growth performance and morphology of the hybrid were intermediate of both parents. This study has been the first successful attempt to hybridize both catfishes, therefore, laid the foundation for further studies on the novel hybrid produced.
Key Words: African catfish, Asian redtail catfish, interspecific crosses, heterosis, hybridization.

Introduction. The role of research in solving aquaculture problems can never be over-emphasized. The future of aquaculture likely depends on the ability of researchers to develop strategies for solving various problems facing the industry. These include, disease and epizootics infections, poor breed and broodstock, increased stuntedness of fish, low feed conversion efficiency, reduction in environmental tolerance, increased mortality and high economic losses (Subasinghe et al 2003). Many of these problems are directly linked with genetic erosion caused by inbreeding of brood fish (Okomoda et al 2017). Hence, hybridization between different species or between strains of the same species may be an effective means of solving some of these aquaculture problems (Bartley et al 1997; FAO 1997; Rahman et al 2013).

Hybridization between different fish species also produces novel progenies which could lead to diversification of cultured species. A number of such hybrids have been reported to be more popular and commercially propagated than their pure sib. An example of this is the hybrid grouper whose market value is higher than either of its pure parents namely *Epinephelus fuscoguttatus* (Tiger grouper) and *E. lanceolatus* (Giant grouper) (Ch'ng & Senoo 2008). Similarly, the catfish hybrid is popular in Thailand (Pongthana 2001), and so is the striped bass hybrid in the US (Bartley et al 2001). Also, the tilapia hybrids dominate the fish production and sales in Israel (Hulata 1995). As the world, population continues to plunge and food crisis problems worsen, the production of more hybrids from novel crosses of different fish species may be the key to tackling the problem of food security as it can help diversify aquaculture production.

Hemibagrus nemurus (Valenciennes, 1840) is an economically important freshwater catfish widely distributed in many Asian countries (Rainboth 1996). It is nutritionally valued for its high protein and omega-3 poly unsaturated fatty acid besides it low cholesterol levels (Mesomya et al 2002). However, commercial propagation of this fish is largely limited by the problems associated with induced spawning (Muchlisin et al

2004). *Clarias gariepinus* (Burchell, 1822) on the other hand is native to West Africa and has been successfully introduced to many parts of the world largely because of its ease of spawning (Solomon et al 2015). Aside hardiness and the ability to grow faster, the *C. gariepinus* is also highly cannibalistic, hence leading to significant losses during culture (Almaza´n Rueda 2004; Olufeagba & Okomoda 2016; Okomoda et al 2017).

To our knowledge, successfully hybridization between these two species has not been scientifically reported till date. Hybrid progenies from these crosses could provide a new aquaculture candidate for research, production, and development. This study, therefore, describes the breeding performance, survival and early growth performance of hybrids from reciprocal crosses of *C. gariepinus* and *H. nemurus*. It is hypothesized that the novel hybrid produced in this study could be a commercial commodity holding to the popularity of the pure species in Asia, Africa and many parts of the world. However, this is based on the assumption that hybrid would perform better than the pure parents.

Material and Method. Sexually mature brood fish of *H. nemurus* were obtained from cages maintained by fish farmers at the Kenyir Lake in Terengganu, Malaysia. Broodstocks of *C. gariepinus*, on the other hand, were obtained from the School of Fisheries and Aquaculture Science hatchery of the Malaysia Terengganu University, Malaysia. They were maintained in different rectangular fiberglass tanks and fed commercially sold diet (35% crude protein) for two weeks. Two successful hybridization trials were performed to obtain progenies for this study using pairs of brood fish (per sexes and per species). Both, males and females of *H. nemurus* were injected twice commercial hormone containing OvaRH (sGnRHa) and a dopamine inhibitor. The first injection was given (at a rate of 0.2 mL) 8 h before the second (at a rate of 0.3 mL). The female *C. gariepinus* was injected (a one-time dosage of 0.5 mL hormone per kg) at the same time the second injection was administered to both sexes of the *H. nemurus*. This was to synchronize the timing of ovulation and stripping which was about 8 h after the second injection of *H. nemurus* or the one-time injection of *C. gariepinus.*

Eggs from the female of both species were stripped into two different bowls, after which they were divided into two to obtain the four batches of eggs for the various directional crosses in this study. The males of both species, however, were tranquilized with 150 mg/1 solutions of tricaine methane sulphonate (MS222) (Wagner et al 1997) and euthanized. The abdominal region was dissected with the aid of a scissors and the testes were removed. Milt content of both testes from a single male was mix in a clean small bowl separately for both species, half of which was used for the pure crosses and the hybrid crosses based on the direction shown below.

♀*C. gariepinus* × ♂*C. gariepinus*, (♀CG × ♂CG)
♀*C. gariepinus* × ♂*H. nemurus* (♀CG×♂HN)
♀*H. nemurus* × ♂*C. gariepinus,* (♀HN×♂CG)
♀*P. hypophthalmus* × ♂*P. hypophthalmus*, (♀PH × ♂PH)

The informal nomenclature system proposed by Rahman et al (2013) and adopted by Okomoda et al (2017) was used to name the reciprocal crosses in this study. This method simply adopts the first part of the generic taxonomy of the female broodstock and the end part of the specific taxonomy of the male broodstock to name hybrid crosses. Therefore, the cross between ♀CG×♂HN was regarded as "Clariomurus" while the reciprocal cross ♀HN×♂CG was called "Hemipinus". The eggs and milt content were mixed uniformly and sperm was activated by the addition of water. Triplicate batches of the different crosses were spawned on twelve nylon mesh substrate already prepared in twelve aquariums (0.5×0.5×0.5 m) with continuous aeration. The aquariums were tagged appropriately in accordance to the crosses they represent.

Fertilization and hatchability rate in this study was determined using the equation below;

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

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

Post-yolk absorption survival was estimated after which 100 larvae from each cross were stocked in 0.5×0.5×0.5 m aquarium tanks using a static system with continuous aeration. The fish were then fed live Artemia. Unfortunately, due to poor water quality, all progenies were lost on the 18th day post-hatching (dph). Hence, growth parameters were determined for the first two weeks of hatching of the fishes. The fish were bulk weighed using a sensitive weighing balance (accuracy 0.00 mg). A total length of ten randomly selected hatchlings was also taken (accuracy 0.00 cm) at the start of the exogenous feeding and at 14 dph using a micrometer gauge. The growth parameters recorded in this study include:

a. Mean length gained (cm) = $L_2 - L_1$
b. Mean weight gained (mg) = $W_2 - W_1$
c. Growth rate (mg/day) = $\frac{W_2 - W_1}{t_2 - t_1}$
d. 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)
L_1= initial length (cm)
L_2= final length (cm)
t_2-t_1= duration between W_2 and W_1(d)

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

Where, F1, P_1, and P_2 are the averages of the performance of the first generation of hybrids, Parent 1 and Parent 2, respectively.

At the 14th day of life, the gross morphology of the hybrid was compared to the pure species. Morphological descriptions were based on the fins configuration and characteristics. These seem to be the most obvious points of describable differences since fishes were too small to be morpho-meristically characterized in detail. Water quality parameters such as temperature, dissolved oxygen, and pH were monitored using a YSI professional plus multi-parameter water quality meter (Model 13M10065, Made in the USA).

Descriptive statistics in this study were analyzed using mini tab 14 computer software. Data were tested for normality and homogeneity of variance before Analysis of Variance (ANOVA) was done. Where significant differences occurred, means were separated using Fisher's least significant difference at a significance level of $p \leq 0.05$. However, when the assumptions of normality and homogeneity did not hold, data were analyzed using Kruskal-Wallis non-parametric test.

Results. Spawning performance of pure and reciprocal crosses of *C. gariepinus* and *H. nemurus* are summarized in Table 1. The pure *C. gariepinus* had the highest fertilization rate of 73.56 while the cross Clariomurus had the least value recorded (57.58%). However, Hemipinus and pure *H. nemurus* had similar intermediate values. Viable larvae were only obtained from the Clariomurus cross with hatchability about four times less (9.47%) compared to the values obtained in *C. gariepinus* (42.15%) and *H. nemurus* (37%). Negative heterosis was also observed for survival in the novel hybrid produced when compared to the performance of the mid parents (-67%).

Since viable larvae were not obtained in the Hemipinus, only the Clariomurus hybrid and the pure crosses were used to evaluate growth performance (Table 2). Feeding Artemia for two weeks led to a significant difference in the weight gained and the growth rate. The trend as observed in these parameters suggests intermediate early growth of the hybrid when compared to the pure crosses. Also, the early morphology of the Clariomurus hybrid showed that the progenies had combined features of both parents (Table 3). Water qualities during the study period were within recommended ranges for culture of the pure species ($t = 26.40\text{-}27.50^0$C; DO = 8.13-8.43 mg L^{-1}; pH = 7.00±0.26) (Table 4) as reported by Boyd (1982).

Table 1

Breeding parameters and heterosis (H) of pure and reciprocal crosses of *Clarias gariepinus* and *Hemibagrus nemurus*

Parameter	*♀CG × ♂CG*	*♀CG × ♂HN*	*♀HN × ♂CG*	*♀HN × ♂HN*	*P-Value*
Fertilization (%)	73.56±7.44[a]	57.58±13.00[c]	59.87±8.64[b]	60.57±7.30[b]	0.005
Hatchability (%)	42.15±15.26[a]	9.47±4.23[c]	0.00±1.10[d]	37.00±14.00[b]	0.001
H for hatchability (%)	-	-76.07	-100.00	-	-
Survival at first feeding (%)	25.65±11.73[a]	7.51±6.39[c]	-	20.48±11.73[b]	0.001
H for survival (at first feeding in %)	-	-67.44	-	-	-

Means in the same row with different superscript differ significantly (P≤0.05).

Table 2

Growth parameters and heterosis (H) of pure and reciprocal crosses of *Clarias gariepinus* and *Hemibagrus nemurus* reared for 2weeks

Parameter	*♀CG × ♂CG*	*♀CG × ♂HN*	*♀HN × ♂HN*	*P-Value*
Length 3 dph (cm)	0.030±0.001	0.025±0.002	0.020±0.001	0.531
Final length 35 dph (cm)	0.74±0.05	0.75±0.42	0.65±0.190	0.254
Length gain (cm)	0.71±0.10	0.73±0.29	0.63±0.08	0.093
Weight 3 dph (mg)	2.50±0.85	2.00±0.11	2.00±0.03	0.442
Weight 35 dph (mg)	60.1±2.13[a]	50.0±5.23[b]	40.2±1.55[c]	0.005
Weight gain (mg)	57.6±3.10[a]	48.0±1.99[b]	38.2±1.33[c]	0.005
H for growth (%)	-	-0.30	-	-
Growth rate (mg day^{-1})	1.65±0.03[a]	1.37±0.02[b]	1.09±0.19[c]	0.015
Specific growth rate	22.71±0.58[a]	22.99±0.39[a]	21.43±1.03[b]	0.015

Means in the same row with different superscript differ significantly (P≤0.05).

Table 3

Basic morphological differences between progenies of pure and hybrids of *Clarias gariepinus* and *Hemibagrus nemurus* larvae at two weeks of age

Parameter	*♀CG × ♂CG*	*♀CG × ♂HN*	*♀HN × ♂HN*
Body configuration	Dorsoventrally compressed	Dorsoventrally compressed	Laterally compressed
Dorsal fin configuration	Rectangular in shape and stretches to the tip of the caudal fin. Fin height is short	Fin divided into two: the first anterior end is with rays and stretches about two-third of the fish's trunk with relatively long height. The second posterior end is adipose in nature and short	Fin is divided into two parts: An anterior short end (one-fourth the trunk) with rays (long height) and a posterior short adipose fin.
Caudal fin shape	Rounded shape	Slightly furcated shape	Strongly furcated shape

Table 4

Mean water quality of the experimental unit during the study period

Parameter	*♀CG × ♂CG*	*♀CG × ♂HN*	*♀HN × ♂CG*	*♀HN × ♂HN*	*P- Value*
Temperature ^{0}C	27.50±0.56	26.40±1.32	27.50±0.57	26.80±0.87	0.410
DO (mg L^{-1})	8.33±0.09	8.33±0.27	8.13±0.24	8.43±0.22	0.230
pH	7.06±0.79	7.03±0.29	7.12±0.45	7.03±0.06	0.433

Mean in the same row with different superscript differs significantly (P≤0.05).

Discussion. Generally, the process of fertilization is not 100% successful even in pure crosses (Okomoda et al 2018b). Hence, the significantly lower fertilization percentages reported for the reciprocal crosses may be because of pre-zygotic isolation mechanism associated with inter-specific hybridization (Carrillo et al 2000). However, the extent of this effect on fertilization may differ from species to species and for different cross combinations. This study shows that successful hybridization between *C. gariepinus* and *H. nemurus* is achievable in one direction as viable progenies were only obtained in the Clariomurus. Similar conclusions have been made for the reciprocal crosses of *C. batrachus* and *P. hypophthalmus* (Boonbrahm et al 1977; Tarnchalanukit 1986) and the crosses between *C. macrocephalus* and *P. hypophthalmus* (Tarnchalanukit 1985; Na-Nakorn et al 1993).

Although a recent study between *C. gariepinus* and *P. hypophthalmus* produced viable progenies from both reciprocal crosses, progeny analysis confirmed that true hybrids were only gotten in one direction (Okomoda et al 2018a). This phenomenon has been attributed to asymmetric gamete incompatibility or asymmetric zygotic isolation in fishes (Zhang et al 2016). Generally, fertilizing a fish egg with a heterospecific sperm could lead to the production of viable progenies or death of all the fertilized eggs for the different reciprocal cross combination (Chevassus 1983). This is a common phenomenon in many previously reported intergeneric crosses of other fin fishes (Yang et al 1992; Goddard et al 1998), shell fishes (Strathmann 1981; Lessios & Cunningham 1990; Levitan 2002; Zigler et al 2003; Zhang et al 2016) and in insects (Kaneshiro 1976, 1980; Watanabe & Kawanishi 1979). Even though a number of models have been advanced to explain this pattern of reproductive outcome in some shell fishes (Levitan 2002), the underlying principles of its occurrence in fin fishes has not been unraveled, hence could be the focus of future researches.

However, it is important to note that hatchability in this study for the pure and hybrid crosses was generally low and could be linked to the quality of broodstocks used in the two trials. Hence, future studies using better brood fish may record better spawning performance for the novel hybrids reported in this study. Interspecific crosses of different species have been thought to be less successful due to the high rate of mortalities during the early life stages (Bartley et al 2001). The observation of low survival in this study may be linked to the abnormality of fry at hatching. Although not quantified, the level and kind of abnormality in the current study were thought to have affected the chances of survival of the hybrids in a similar manner to the opinions of Olufeagba & Okomoda (2016). A similar observation had been previously reported for the crosses between *C. gariepinus* and *C. batrachus* (Sahoo et al 2003) and the hybrids of Heteroclarias (Ataguba et al 2009). The levels of deformity observed for the different studies could be linked to gene compatibility of the species involved in the crosses reported. Importantly, this study shows that hybrids could live up to the fingerling stage (and possibly beyond). However, the mortality observed in the pure crosses many be due to different reason among which is cannibalism. Early mortality in *C. gariepinus* had been linked to cannibalism as a result of size differentiation within the progenies of the fish (Ataguba et al 2009; Olufeagba & Okomoda 2016).

Chevassus (1983) had earlier opined 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. This is different from the findings of other researchers such as Madu & Ita (1991), Jantrarotai (1993), Tober et al (1995), Ataguba et al (2010), Solomon et al (2013) Olufeagba et al (2016), Olufeagba & Okomoda (2016) who reported better performance in hybrids compared to their pure sibs. It may be too early to make a concrete assertion on the growth characteristics of the hybrid gotten in this study. However, our finding on the early growth pattern of this novel fish suggests intermediate performance. Similarly, morphological observations showed that all hybrid progenies are of the same morphotype which is significantly distinct from the pure parents. This observation suggests that there is no ploidy polymorphism in the progeny pool of the hybrid. Previous studies of crosses between different fish families had resulted in the observation of at least two or more different phenotypic offsprings with distinct chromosome numbers within the same hybrid pool (Varadaraj & Pandian 1989; Deng et al 1992; Na-Nakorn et al 1993; Cherfas et al 1994; Liu et al 2007; Liu & De-Mitcheson 2009).

The phenomenon of ploidy polymorphism with distinct morphotype is usually common in cases where the chromosome numbers or genetic distances of the crossed fishes are large (Gomelsky 2015). Hence, the complexity of the hybrid progenies gotten also tend to increase as the distance between the species becomes farther apart (Hu et al 2012). Therefore, with a chromosome number of 2n = 56 for the *C. gariepinus* (Okomoda et al 2018a) and 2n = 58 for *H. nemurus* (Supiwong et al 2014), it could be inferred that the genetic distance between the crossed species were not large enough to cause polyploid induction in the hybrids. Hence, theoretically, the produce hybrid progenies should all possess a 2n = 57 chromosomes if half the chromosome of both species is inherited. This is similar to the observations in the reciprocal crosses of *C. gariepinus* and *H. longifilis* (Teugels et al 1992), Kutum, *Rutilus frisii kutum,* and Bream, *Abramis brama orientalis* (Amini et al 2007), *C. gariepinus* and *P. hypophthalmus* (Okomoda et al 2018a). Close morphological observations of the hybrids in this study also showed evidence of shared features of the parent species however with significant fin modifications. Following the assertions of Chevassus (1983) and Wilkins et al (1994) who had earlier opined that true hybrids of interspecific hybridization would display an intermediate morphological character of both parents, our observations in this study suggest hybridization between the two species was successful.

Conclusions. Intergeneric cross of *C. gariepinus* and *H. nemurus* successfully produced viable progenies only in one direction (♀CG×♂HN). Early growth of the hybrid also showed intermediate performance when compared to the pure crosses. Building on this background report would involve detailed characterization and examination of the aquaculture potentials of the viable hybrids in future studies.

Acknowledgements. The authors are indebted to the management and staff of the School of Fisheries and Aquaculture Science, Malaysia Terengganu University, Malaysia in whose facility this research was conducted. We also acknowledge the help of students and staffs during the breeding trials and data collection of this study.

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Received: 14 September 2018. Accepted: 05 August 2019. Published online: 26 August 2019.
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How to cite this article:
Hassan A., Okomoda V. T., Austin J. G., 2019 Preliminary report on the novel hybrid from crosses of *Clarias gariepinus* and *Hemibagrus nemurus*. AACL Bioflux 12(4):1250-1259.