FERTILIZATION, HATCHABILITY, SURVIVAL AND LARVAL BIOMETRY IN INTERSPECIFIC AND INTERGENERIC HYBRIDS OF CLARIID CATFISHES

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ABSTRACT

Interspecific and intergeneric hybridization studies were carried out in *H. longifilis*, *C. gariepinus* and *C. anguillaris* under controlled hatchery conditions to estimate their aquaculture potential in terms of fertilizability, hatchability and survival. Fertilization rate in all the nine genetic crosses ranges from 60-87.5%, the fertilization rates of the parentals being significantly higher($P<0.05$) with highest value of 87.5% obtained in *C gariepinus*. The intergeneric hybrids had the lowest rate of fertilization. Hatchability ranges between 75- 88.1%, with the parental *C anguillaris* being slightly significantly higher than the other genetic combinations. *C a x H l* had the lowest hatchability and there was no significant difference ($P<0.05$) in percent hatching among the the interspecific hybrids. The survival of all the nine genetic crosses from hatching up to the end of the two weeks indoor rearing period ranges between 78-89%, which wasn't significantly different among the interspecific and intergeneric hybrids. *C anguillaris* and *C gariepinus* had the highest percent survival which was significantly different from all the other mating combinations. The intergeneric hybrid larval had significantly greater ($P<0.05$) length in comparison to the interspecific hybrids. *H longifilis* and the hybrids produced from its eggs had greater body weight than that of *C gariepinus* and *C anguillaris*.

INTRODUCTION

The Clariid catfishes from wild and farmed sources are economically important food fish and have gained much prominence and species of *Clarias* and *Heterobranchus* are widely cultured (Huisman and Richter, 1987). *Clarias gariepinus is* closely related to *Clarias anguillaris*, both belong to the same genus (Teugels 1982, 1986). The two species are sympatric in Nigeria with very little external difference between them (Benech *et al.* 1993; Rognon *et al.* 1998; and Teugels 1998) and are only distinguished morphologically by the number of gill rakers on the first branchial arch. In *C. gariepinus*, the number is high (up to 110) while in *C. anguillaris* it is lower(less than 50). At the genetic level, the two species are also very close with a Nei's genetic distance of 0.16 (Rognon *et al.* 1998) and P distance 0.04 (Nwafili and Gao 2007). Genetic techniques have been applied to other animals to improve their quality but that of fish is not yet fully exploited. Globally, efforts aimed at genetic improvement have generated a lot of interest on the promising potentials of genetic principles to raise aquaculture productivity (Aluko, 1999). The most current advances in aquaculture production have been achieved through the application of genetic principles which includes selective breeding, hybridization chromosome manipulation, sex reversal and gene transfer (Aluko and Olufeagba, 1999). The application of these genetic tools is known to have increased fish production from a mere 0.6 t to a maximum of 10-12 t/ha/ year in India (Roderick, 1988). Hybridization has been found to be a breeding programme that tries to find mating combinations between different populations of fish which produce superior offspring for grow-out, which exhibit hybrid vigour (Tave, 1993).

Hybridization has been used to increase growth rate, manipulate sex ratios, produce sterile animals, improve flesh quality, increase resistance, improve tolerance to environmental extremes and improve a variety of other traits that make aquatic animals production more profitable (Dunham *et al.*, 2001). Hybridization between species can also result in offspring that are sterile or have diminished reproductive capacity. The more distantly related the two species, the greater likelihood of their hybrid being sub-viable or sterile (Chevassus, 1983). Interspecific hybridization has been carried out in *C. batrachus* x *C gariepinus* (Rahman *et al*,1995, Sahoo *et al* ;2000) and *C gariepinus x C macrocephalus*. (Yalkoob and Ali,1994) but with varying level of success. About eleven intergeneric hybrids have been produced among members of the three genera; viz ; *Catla, Labeo* and *Cirrhinus* involving five species of these hybrids. Those between Rohu *x Catla, Catla x* Rohu and *Catla x* Mrigal, showed improved growth performance and more flesh content than the parent species (Reddy *et al.*, 1997). There are limited studies on experimental hybridization in *C gariepinus x C anguillaris* . The hybrid cross between *Heterobranchus* and *Clarias species* is receiving considerable attention in Africa particularly Nigeria. These hybrids have been reported to show heterosis (Madu, *et al.*, 1992, Salami *et al.*, 1993). The study reported here with *C. anguillaris, H longifilis* and *C.*

gariepinus was undertaken to assess the viability and survival of their hybrid progenies during indoor rearing operation.

MATERIALS AND METHODS

The project was carried out using the facilities of the Hatchery Unit of Federal College of Freshwater Fisheries Technology, (FCFFT), New Bussa. The parental broodstocks used in this study; *C. gariepinus, C. anguillaris* and *H. longifilis* were obtained from the concrete tanks of the Fish Genetic Improvement laboratory of the National Institute for Freshwater Fisheries Research, New Bussa. Gravid females were weighed individually and injected intramuscularly with ovaprim at a dosage of 0.5 ml/kg of body weight (Leu and Chou, 1996). The injected breeders were kept in well aerated holding concrete circular tanks (2.200cm^3). Stripping of females was carried out after a latency period of 12 hours for *Clarias* (Madu, 1989) and 15 hours for *H. longifilis* (Olufeagba, 1999), by gentle hand stripping into clean bowls. In order to strip both *Clarias spp* and *H. longifilis* at the same time; the *H. longifilis* were injected 3 hours before the *Clarias spp.* The males were sacrified, testes is removed into clean petri dishes and milt were collected by maceration of the testes and mixed with 0.9% saline (NaCl solution) (Woynarovich and Horvath, 1980). The eggs and milt of the parents were mixed together to generate nine different mating combinations (genetic crosses) as follow

Parentals (Pure Line).
Clarias gariepinus (♂) x Clarias gariepinus (♀)
Clarias anguillaris (♂) x Clarias anguillaris (♀)
Heterobranchus longifilis (♂) x Heterobranchus longifilis (♀)
B Interspecific hybrids
Clarias gariepinus (♂) x Clarias gariepinus (♀)
Clarias anguillaris ♂) x Clarias anguillaris (♀)
C Intergeneric hybrids
. *Clarias gariepinus (♂) x Heterobranchus longifilis (♀)*
. *Heterobranchus longifilis (♂) x Clarias gariepinus (♀)*
Clarias anguillaris (♂) x Heterobranchus longifilis (♀)
Heterobranchus longifilis (♂) x Clarias anguillaris (♀)

Hybrids and control groups were produced on the same day in each experiment. One thousand (1,000) eggs were used for fertilization for each mating combination. The number of eggs were estimated using gravimetric method (i.e. no of eggs/g).The translucent eggs containing embryonic eyes at the time of polar cap formation (about 20minutes after fertilization) were considered fertilized and counted to calculate percentage fertilization. Opaque eggs were considered unfertilized. Percentage fertilization (%) was calculated as number of fertilized eggs/ total number of eggs fertilized x 100. Percentage hatchability was estimated using both numerical and volumetric methods. The number of hatchlings in each mating combination were obtained by (1) direct counting of unhatched eggs as well as the number of hatchlings in the incubating troughs (numerical method) and (2) volumetric method which involves random collection of fry using glass beaker of known volume in each hatching trough on third day of hatching using glass beaker of known volume ,when the swim- up fry were already evenly distributed. The mean number of fry was estimated used to extrapolate for the number of fry in the calculated volume of water in each trough, for each mating combination. Percentage hatchability (%) was calculated as; number of hatchlings/ total number of eggs fertilized x 100. When more than 60% of the eggs hatched, the length of the larvae, along with the size of the yolk sac was measured to below the nearest half mm using an ocular micrometer under a compound microscope at a magnification of 10x. Wet weight of the larvae was recorded to an accuracy of 0.01 mg using a digital electronic balance. Two hundred, three days old fry from each genetic cross were stocked in duplicate batches in fibre plastic tanks (60 x40x 10cm^3) with continuous flow through system (plate 2). Pooled weight of fry were taken, fry were fed ad-libitum with *Artemia nauplia* (shell free). Siphoning of uneaten food was done every morning and evening. The daily survival was monitored and the study lasted for 14 days.

Variations in the data generated from parameters in the various mating combination were evaluated using Analysis of variance (ANOVA) at 95% probability level, while Duncan New multiple Range Test (DNMRT) was employed to ascertain the difference between means of parameter with significant difference using SPSS version 15.0.

RESULTS AND DISCUSSION

The percentage fertilization, hatchability and survival of the various offspring produced by all the mating combinations are shown in Table 1.0. Percentage fertilization were highest in the parental combination with *C gariepinus* having the highest value of 87.5% which was significantly different ($p<0.05$). There were however no significant difference ($p>0.05$) between *C anguillaris* and *H longifilis*. Lowest percentage fertilization were recorded in the offsprings of the intergeneric combination with least significant value of 60% recorded in the *C anguillaris* (♂) *x* H *longifilis* (♀). The least significant difference showed significant difference ($P<0.05$) in the percentage fertilization in all the mating combinations. Hatchability ranges between 75-88.19% with the highest values recorded in the parental combination and least in the offspring of the intergeneric combinations. There was slight significant difference ($P<0.05$) in percentage hatchability in the various mating combination. *C. anguillaris* recorded the highest value of 88.19% while the least value of 75. % was recorded in *C. anguillaris* (♂) x *H. longifilis* (♀) (Table 1).

Table 1 Fertilization, hatchability and survival of fry in the various mating combinations.

Mating combination	No. of eggs fertilized	% fertilization	% hatchability	% survival at 2 weeks (indoor)
Parental				
Cg x Cg	500	87.5^{d}	86.5^{ab}	86^{b}
Ca x Ca	500	85^{cd}	88.1^{ab}	89^{b}
Hl X Hl	500	85^{cd}	80^{ab}	80^{a}
Interspecific				
Cg x Ca	500	80^{bcd}	79.1^{ab}	80^{a}
Ca x Cg	500	77.5^{bc}	83.3^{ab}	81^{a}
Intergeneric				
HL x Cg	500	80^{bcd}	77.2^{a}	80.5^{a}
Cg x HL	500	75^{b}	78.5^{ab}	80^{a}
HL x Ca	500	77.5^{bc}	76.8^{a}	82^{a}
Ca x HL	500	60^{a}	75^{a}	78^{a}

a,b,c,d values with different superscripts in each column are significantly different ($P<0.05$).
lg of *C gariepinus* egg was estimated to have 700 eggs;
lg of *C gariepinus* egg was estimated to have 692 eggs;
1g of *H longifilis* egg was estimated to have 520 eggs .

The length of the yolk sacs did not indicate any difference in the parental *H. longifilis* and hybrids of; *C g x H l* and *C a x H l (using maternal H longifilis)* as shown in Table 2.0. Their yolk sac length were significantly greater compared to all the other mating combinations. The average length of the intergeneric hybrid larvae were significantly greater ($P<0.05$) than that of the interspecific hybrids and the parentals. *C. anguillaris* larvae had the least length. *H. longifilis* larvae had the highest weight in all the mating combinations. A significant greater average larval weight were observed in the intergeneric hybrid produced from the *H. longifilis* eggs in comparison to the parental *C. gariepinus* and *C. anguillaris*. The larval weight did not vary between the parental *C gariepinus* and the hybrids produced from *C. gariepinus* eggs but were significantly greater than *C. anguillaris* larvae or its maternal hybrids.

The percentage survival of the offspring of the various mating combinations at the end of the two weeks in indoor rearing period is shown in Table 1.0. Considering the three parental mating combinations, the end of 2weeks indoor rearing, the offspring of *C anguillaris* had the highest percentage survival of 89%, closely followed by those of *C gariepinus* (86%) and least value of 80% were recorded in the offsprings of *H* longifilis. The interspecific hybrids of *C gariepinus* (♂) x *C* anguillaris (♀) had 80% while the reciprocal cross *C anguillaris* (♂) x *C gariepinus* (♀) produced offspring's with 81% and were not significantly different ($P>0.005$).The intergeneric hybrids had the least survival rate with highest value of 82% in *H l* (♂) x *C* a (♀) but not significantly different. Among the intergeneric hybrids, *H l* (♂) x *C* a (♀) had the highest survival of 74%, followed by those of C a (♂) x *H l* (♀) and *H l* (♂) x *C g* (♀) with values of 72% and 70%, respectively.

Table 2 shows the result of the phyto-chemical analysis of the various wood types used. Result showed the presence of tannins and Phenolic compounds in Parkia, tannins in Neem tree while terminalia had neither of these compounds. This might possibly explain the high percentage of protein in the experimental fish sample smoked-dried with Terminalia wood. Andrezey *et al*, (2005) reported the presence of polycyclic aromatic hydrocarbons deposited from wood smoke on fish while Rosa et al., (2007) reported the presence of volatile compounds in smoke –dried salmon. It appear from these findings that tannins affect the protein content in the smoked fish sample (Tables 1 and 2).

Table 2: Phytochemical Analysis of wood types.

Wood type	Tannin	Phenolic compounds
Neem	+++	-
Parkia	+	++
Terminalia	-	-

The organoleptic evaluation in Table 3 showed significant difference on the general acceptability of fish sample smoked dried using terminalia wood. Eyo (1985) reported significance difference in flavour and firmness of muscle from smoked-dried *Orechromis niloticus* using terminalia wood. He reported that *Afzelia africana* gave objectable colour and flavours to the fish. Balogun and Sumbella (2001) in their report indicated that wood type affect the colour and flavour of smoked dried fish samples. The findings from this study showed that wood type has effect on the nutrient composition of smoked dried fish. For the health of consumers, it may be necessary for fish processors to take into consideration the wood types used in fish smoking process.

Table 3: Organoleptic evaluation of fish samples using various wood types.

Wood type	SENSORY PARAMETERS				
	Flavour	Colour	Firmness	Aroma	General acceptability
Neem	4.19	4.52	4.57	4.23	3.66^{b}
Parkia	4.38	4.38	4.14	4.42	3.71^{b}
Terminalia	4.66	4.47	4.57	4.52	4.56^{a}

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