Available online at www.elixirpublishers.com (Elixir International Journal)

Aquaculture

ISSN: 2229-712X

Elixir Aquaculture 43 (2012) 6696-6700

Fertilization, hatchability, survival and larval biometry in interspecific and intergeneric hybridization in *heterobranchus longifilis,clarias gariepinus* and *clarias anguillaris* under controlled hatchery conditions

A. A Akinwande[1], O.A Fagbenro[2] and O.T Adebayo[2]

[1]Department of Aquaculture, Federal College of Freshwater Fisheries Technology, New-Bussa,Niger State.

[2]Department of Fisheries and Aquaculture Technology, Federal university of Technology, Akure, Ondo State.

ARTICLE INFO

Article history:

Received: 29 December 2011;
Received in revised form:
19 January 2012;
Accepted: 4 February 2012;

Keywords

Interspecific,
Intergeneric,
Hybridization,
Hatchability,
Fertilization.

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 their was no significant difference ($P<0.05$) in percent hatching among the interspecific hybrids. The survival rate in 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 had significantly greater ($P<0.05$) larval length in comparison to the interspecific hybrids. *H .longifilis* and the hybrids produced from its eggs had significantly 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 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 vigor (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 rate of survival of their hybrid progenies during indoor rearing operation.

Tele:
E-mail addresses: akinwande_fish@yahoo.com

Materials and method

Hypophysation and artificial hybridization

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 (Madu, 1989). 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 sacrificed, 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

A. Parentals

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 (♀)
* (♂) Male; (♀) Female

Hybrids and control groups were produced on the same day in each experiment.

Fertilization and Hatchability

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 from:

$$\frac{\text{Number of fertilized eggs}}{\text{Total number of eggs fertilized}} \times 100$$

The mean number of hatchlings in each mating combination was obtained by direct counting of unhatched eggs as well as the number of hatchlings in the incubating troughs(numerical method).

Percentage hatchability (%) was calculated from:

$$\frac{\text{Number of hatchlings}}{\text{Total number of eggs fertilized}} \times 100$$

Larval biometry

Yolk sac length, larvae length and weight of each mating combination were taken when more than 60% of the eggs were observed to have hatched. Length measurements were taken under the binocular Olympus microscope (CX 60) with the aid of calibrated micrometer where fifty divisions equal 0.5mm using x10 objective lens. Weight wet of larvae was recorded to an accuracy of 0.01mg using a digital Acculab 300-electronic balance. Pooled means of forty hatchlings (20 from each replicate) were taken.

Survival rate

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. Pooled weight of fry were taken, fry were fed ad-liditum with *Artemia nauplia*(shell free). Siphoning of uneaten food was done every morning and evening. The daily survival were monitored and the study lasted for two weeks(14 days).

$$\text{Survival rate (\%)} = \frac{N_i - N_f}{N_i} \times 100$$

Where N_f = final number of fish at end of experiment

N_i= initial number of fish at beginning of experiment.

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 multiple Range Test 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 is 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 offspring of the intergeneric combination with least significant value of 60% recorded in the *C .anguillaris (♂)x* H. *longifilis(♀)*. There was 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.* a*nguillaris* recorded the highest value of 88.19% while the least value of 75. % was recorded in *C .anguillaris* (♂) x *H . longifilis* (♀) (Table 1.0 and Fig 2.0).

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 was 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 offspring 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 percentage 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..

Several works have indicated that catfish species can be hybridized to produce offspring with varying characters (Tarnachalanukit, 1986; Mukhopadathy and Dehedrai, 1987; Legendre *et al.*, 1992). Richter *et al;* (1995) in an earlier finding reported that all the hybrid of the African catfish, female *C. gariepinus* and the Asian Catfish, male C. *batrachus* died within 20 hours after fertilization. Laywornyawut *et al.,* (1992) also could not get any viable fry from hybridization between *C. macrocephalus*(♂) × *C.gariepinus*(♀) .

The hatchability percentage reported in this present study indicates the viability of interspecific hybrids of *C. gariepinus* and *C. anguillaris.*

The yolk sac length of *H longifilis* larvae and its maternal hybrids were similar to each other and were greater significantly greater than that of pure *C. gariepinus*, *C. anguillaris* or their hybrids. This could be due to the bigger sized *H. longifilis* eggs. The higher mean wet weight of the *H. longifilis* hybrid larvae than that of all other genetic crosses could also be attributed to the greater length of the intergeneric hybrid larvae.

The parental crosses except *H. longifilis* had the highest survival in this study. Legendre *et al.*, (1992) however had earlier recorded that after 15 days of larval rearing in rec irculatory tap water, the survival rate was higher for *H. l* (♂) x *C. g* (♀) and *H. longifilis* when compared with other combinations. This variation may be due to difference in method employed in the rearing operation in this study. Survival was very high in all the hybrid crosses. Both hybrids of *C. gariepinus* and *C. anguillaris* exhibited very high survival rates compared to that reported in *C. gariepinus* (♂) x *C. batrachus*(♀) with over 30% mortality resulting from abnormal and deformed larvae(Rahman *et al.*, 1995). Survival rates of the interspecific hybrids were however not comparable to the parental .*C gariepinus* and *C. anguillaris*. The result slightly differs from that obtained by Nwadukwe (1995) who noted that F_1 hybrids from *H. longifilis* maternal parent had the best survival rates at the end of 18 days. This study shows the hybridization potentials of various catfish hybrids and their survival in indoor rearing system with continuous flow-through system . It is suggested that further studies be carried out to evaluate their growth performance and survival at fingerlings and grow-out stages.

References

1. Aluko PO, and Olufeagba SO (1999) Genetics and food security in Nigeria in the 21st century: Genetically modified fishes; risk and prospects. . *Genetic Society of Nigeria Annual Conf.* 205-206

2. Aluko PO (1999) Genetic approaches in catfish hybridization: Effort of NIFFR, Nigeria. *NIFFR Newsletter* 16, 2 : 3-8.

3. Benech AA, Abenech V, Teugels GG and Gourene GA. (1993) Useful character to distinguish two African catfishes, *Clarias anguillaris* and *C.gariepinus* (Siluriformes; *Clariidae*). *Cybium* 17, (1): 83-85.

4. Chevassus B (1983) Hybridization in fishes. *Aquaculture 33,* 245-262.

5. Dunham RA, Majumdar K, Hallerman E and Main G(2001) Review of the status of aquaculture genetics. In K.R.P. Subasinghe, P. Buemo, M.J. Philipa; C. Haugh; S.E. and J.R. Arhur (eds). *Aquaculture in the third millennium*. *Proceedings of the conference on Aquaculture in the third millennium*, Bangkok, Thailand, 20-25 February, 137-186. NACA, Bangkok and FAO, Rome.

6. Huisman EA, and Richter CJ (1987). Reproduction, growth, heat control and aquacultural potential of the African catfish, *Clarias gariepinus* (Burchell, 1822*). Aquaculture 63*, 1-14.

7. Laywonyawut K, Penman D, Mc Andrew BJ and Roongratri N (1992) Preliminary study on Karyotyping of the hybrid catfish Pladuk Big-oui (male *C. gariepinus* × female *C. macrocephalus*). In: *Genetic in Aquaculture and Fisheries Management* (ed.D.J. Penman, N. Roongratri and B.J. Mc Andrew), 141-142.

8. Legendre M., Teugels GG, Cauty C and Jalabert B (1992) A comparative study on 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.

9. Madu CT (1989) Hatchery management of the mudcatfish, *Clarias anguillaris*. *Ph.D. Thesis.* University of Jos, 221pp.

10. Madu CT, Mohammed S , Mezie A, Isa J and Ita EO (1992) Comparative growth, survival and morphometric characteristics of *Clarias anguillaris*, *Heterobranchus bidorsalis* and their hybrid fingerlings. *Annual Report of the National Institute for Freshwater Fisheries Research, New Bussa, Nigeria*. 7pp..

11. Mukhopadathy SM, and Dehadrai PV (1987) Survival of hybrids between air breathing catfishes ; *Heteropneustes fossilis* (Bloch) and *Clarias batrachus* (Linn.). *Matsya* 12- 13, 162-164.

12. Nwafili SA and Gao T (2007) Is the Dutch domesticated strain of *Clarias gariepinus* (Burchell 1822) a hybrid? *African J. Biotech* . 6, 1072-1076.

13. Olufeagba SO, (1999) Induced triploid *Heterobranchus longifilis*, and its aquacultural potentials. *Ph.D. Thesis*, University of Ilorin. 140pp.

14. Rahman MA, Bhadra A, Begum N, Islam MS and Hussain MG (1995) Production of hybrid vigour through crossbreeding between *Clarias batrachus* and *Clarias gariepinus Aquaculture* 138, 125-130.

15. Richter C J, Eding EH, Ver Reith JJ and Fleure, WG (1995) African catfish (*Clarias gariepinus): In broodstock management and Egg and Larval Quality*. Eds Bromage and Roberts Published by Blackwell Science Ltd, London .242-276pp.

16. Roderick E (1998) Latest Research benefits on versatile tilapia. *Fish* F*armer* 12, (43) 23.

17. Rognon X, Teugels GG, Guyomard R, Galbusera P, Andriamanga M Volckaert F and Agnese JF (1998) Morphometric and allozyme variation in the African catfishes *Clarias gariepinus* and *C. anguillaris*. *J. Fish Biol* 53, 192-207.

18. Sahoo SK, Giri SS, Sahu AK, and Ayyappan S (2003) Experimental Hybridization between Catfish *Clarias batrachus* (Linn.) x *Clarias gariepinus* (Bur.) and Performance of the Offspring in Rearing Operations. *Asian Fisheries Science* 16, 157-166.

19. Salami AA, Fagbenro OA, and Sydenham DHJ (1993) The production and growth of Clariid catifish hybrids in concrete tanks. *The Israeli Journal of Aquaculture-Bamidgeh 45(1),* 18-25.
20. Tarnchalanukit W (1986) Experimental hybridization between catfishes of the family Clariidae and Pangasiidae in Thailand. *Environmental Biology of Fishes 16,* 317-320.
21. Tave D (1993) Genetic for fish hatchery managers second edition, van Nostrand Reinhold, New York, 415pp.
22. Teugels GG (1982) Preliminary results of a morphological study of five African species of the subgenus *Clarias* (*Clarias*) (Pisces: Clariidae). *Journal of Natural History 16,* 439-464.
23. Teugels GG, (1986) A systematic Revision of the African species of the genus, Clarias (Pisces, Clariidae). *Ann. Mus. Roy. Afr. Centr., Zool*. 247, 199p.
24 . Teugels GG (1998) Intra- and inter-specific morphometric variation in *Clarias gariepinus* and *C. anguillaris* (Siluroidei, Clariidae), 241- 247pp. In J.F. Agnese (ed.) *Genetics and aquaculture in Africa*. Office de la Recherche Scientific et Technique Outre-Mer (ORSTOM), Paris.
25. Yaalkob WAA and Ali AB (1994) Portable canvas tanks for culture of hybrid catfish(*Clarias gariepinus x C. macrocephalus*) by small scale farmers in malaysia. NAGA, *ICLARM Quarterly* 17(1), 25-28

Table 1.0 Fertilization, hatchability and survival of fry in the various mating combinations

Mating combination	No of eggs fertilized	% fertilization	% hatchability	% survival at 2weeks (indoor)
Parentals				
C. gariepinus (♂) x *C. gariepinus* (♀)	500	87.5[d]	86.5[b]	86[b]
C. anguillaris (♂) x *C. anguillaris* (♀)	500	85[cd]	88.1[b]	89[b]
H. longifilis (♂) x *H. longifilis* (♀)	500	85[cd]	80[ab]	80[a]
Interspecific hybrids				
C. gariepinus (♂) x *C. anguillaris* (♀)	500	80[c]	79.1[ab]	80[a]
C. anguillaris (♂) x *C. gariepinus* (♀)	500	77.5[bc]	83.3[b]	81[a]
Intergeneric hybrids				
H. longifilis (♂) x *C. gariepinus* (♀)	500	80[c]	77.2[a]	80.5[a]
C. gariepinus (♂) x *H. longifilis* (♀)	500	75[b]	78.5[ab]	80[a]
H. longifilis (♂) x *C. anguillaris* (♀)	500	77.5[bc]	76.8[a]	82[a]
C. anguillaris (♂) x *H. longifilis* (♀)	500	60[a]	75[a]	78[a]

Values with different superscripts in each column are significantly different from each other ($P < 0.05$).
lg of *C. gariepinus* egg was estimated to have 700 eggs
lg of *C. anguillaris* egg was estimated to have 692 eggs
lg of *H. longifilis* egg was estimated to have 520 eggs

Table 2. Yolk sac and larval biometry of the various mating combinations at more than 60% hatching

Combination	Yolk sac length (mm)	Larval length (mg)	Larval weight (mg)
Parentals			
C. gariepinus (♂) x *C. gariepinus* (♀)	1.5[ab]	4.5[a]	2.3[ab]
C. anguillaris (♂) x *C. anguillaris* (♀)	1.2[a]	4.4[a]	2.0[a]
H. longifilis (♂) x *H. longifilis* (♀)	1.8[b]	4.5[a]	3.0[c]
Interspecific hybrids			
C. gariepinus (♂) x *C. anguillaris* (♀)	1.3[a]	4.6[a]	2.1[a]
C. anguillaris (♂) x *C. gariepinus* (♀)	1.4[a]	4.5[a]	2.3[ab]
Intergeneric hybrids			
H. longifilis (♂) x *C. gariepinus* (♀)	1.4[a]	4.8[b]	2.4[b]
C. gariepinus (♂) x *H. longifilis* (♀)	1.7[b]	4.9[b]	2.7[bc]
H. longifilis (♂) x *C. anguillaris* (♀)	1.5[ab]	5.0[b]	2.4[b]
C. anguillaris (♂) x *H. longifilis* (♀)	1.8[b]	4.8[b]	2.6[bc]