

International Journal of Biosciences | IJB | ISSN: 2220-6655 (Print) 2222-5234 (Online) http://www.innspub.net Vol. 22, No. 6, p. 171-179, 2023

OPEN ACCESS

Intraspecific hybridization of native and exotic strains of *Clarias gariepinus* and growth performance of hybrid fry in South Western, Cameroon

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Key words: Exotic-stain, Intraspecific hybridization, Native-strain

http://dx.doi.org/10.12692/ijb/22.6.171-179

Article published on June 13, 2023

Abstract

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Introduction

Global population expansion has resulted in a persistent and drastic rise in demand of aqua-foods hence increased aquaculture production is clearly needed to meet this demand (Mair et al., 2018). Capture fisheries are at capacity or showing precipitous declines due to over fishing (Pauly and Zeller, 2017). The estimated minimum daily crude protein requirements of an adult ranging from about 65g and 85g to be obtained from animal sources (Scholtens, 2019). Encouraging fish production through aquaculture and improvement of culture species by hybridization and selective propagation will take advantage of the prolificacy, development and fast growing hybrids produced thereby shortening culture time (Oloyede, 2005; Oben et al., 2015). Hybridization is the mating of genetically differentiated individuals or groups of individuals and may involve crossbreeding within a species (strain crossing) or crosses between separate species [Rahman et al., 2013]. The process is precipitated by a rapid demand for high quality protein especially from aquatic sources (Adene et al., 2017).

The scarcity of genetically improved fish seed is one of the major constraints to the rapid development of the aquaculture industry and stock management (Yisa et al., 2018) in Cameroon. The production of improved and environmentally stable fish seed will in no doubt increase aquaculture productivity and hence increase accessibility to aqua-protein to meet the minimum daily crude protein intake requirements (Oloyede, 2005). A good supply of high quality fish seed is essential for successful aquaculture production and this can be produced through the union of gametes from two different species strains to produce a new organism of desirable character traits with the aim of improving the genetic quality of the offspring compared to the parents (Adene et al., 2017). This involves mating combinations between genetically isolated populations.

To combat the problem of the introduction of nonnative species, selective breeding programs in which researchers chooses the next generations broodstock based on predetermined criteria ranging from weight, resistance to disease, environmental variability, survival, increase fecundity, fry survival and ability to withstand adverse environmental conditions from domesticated native stocks (Palmer *et al.*, 2007; Abualreesh, 2020), are ongoing. This work therefore investigated the hatchability and survival rate of Native and Exotic hybrid *Clarias gariepinus* strains and their reciprocal crosses and compared their growth performance with two pure strains of *Clarias gariepinus*.

Materials and methods

Study site

This research was carried out at an experimental site setup the Limbe Nautical Arts and Fisheries Institute (LINAFI), the OKF fish hatchery complex, Limbe and the University of Buea CORAF/WECARD Fisheries laboratory in the Department of Fisheries and Aquatic Resources Management University of Buea mount Cameroon Region. Broodstock selection, and hormone injection and all laboratory procedures were done as described by Slembrouck *et al.* (2003), Sutton, (2006) and Ogunsina, (2014).

Experimental Crosses

The following genetic crosses of the two *Clarias gariepinus* strains (exotic hybrid *C. gariepinus* strain and native strain) were carried out.

	Males
Х	Exotic hybrid (EH)
Х	Native strain (NS)
Х	Exotic hybrid (EH)
X	Native strain (NS)
	X X X X

Each of the four crosses was then incubated in separate 1000L capacity breeding recirculatory aquaculture system where hatching took place. Three 1L capacity flow through translucent hatching bowls were cleaned and disinfected mosquito netting material (for egg collection) of appropriate size were placed in them and also in the recircurlatory tanks. Each cross had three replicates making a total of 12 bowls. The fertilized eggs were spread on the netting material and the temperature maintained at 28°C with thermostatic heaters in each hatching bowl. The egg diameter of 100 eggs from each female was measured to the nearest 0.01mm.

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After hatching, all the dead eggs, dead and live hatchlings were counted in each of the triplicate flowthrough bowls. Percentage hatchability calculated using the formula:

% Hatchability = $\frac{\text{total N}^{\circ} \text{ hatched of eggs}}{\text{total N}^{\circ} \text{ferilized of eggs}} x100$

The fertilization rate was check at 10 hours from the time of incubation and the percentage hatchability at 10 hours was calculated as

 $Fertization \ rate = \frac{total \ N^{\circ} \ fertized \ of \ eggs}{total \ N^{\circ} \ incubated \ of \ eggs} x100$

Setting of indoor Experiment and daily survival of hatchlings

Each treatment was in triplicates. Two hundred and fifty (250) hatchlings after taking the pool weight were place in a 50x30x26 cm3 plastic container filled with 30L of water complete with aeration each. The survival of fry in each container was taken daily for 14 days while the length and weight of the fry was taken every 72 hour. Weights of 20 hatchlings and individual lengths were taken with a sensitive hooded electronic weighing balance and a Vernier Caliper to the nearest 0.01g and 0.01mm respectively.

Feeding of Larvae and Water quality monitoring

Three days post hatching, the larvae were transferred into 12 indoor tanks. After all the yolk were thought to have been completely absorbed, the fry were feed shell free artemia nauplii for 14 days at 3 times a day. During this period, measurements such as lengths and weight were taken on a 72 hours basis and on the 14th day.

The growth parameters record during this study include

- Mean length gained (cm) = (L2 L1)
- Mean weight gained (mg) = (W2 W1)
- Growth rate DGR (mg/day)

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DGR = (W2-W1)/(t2-t1)
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Specific growth rate SGR (%/day)
SGR = (lnW2-lnW1)/(t2-t1)*100
Where W1 is initial weight (mg)
W2 is final weight (mg)

L1 is initial length (cm) L2 is final length (cm) t2-t1 is duration between W2 and W1 (days)

Heterosis (H)

$$H = \frac{F1 - 0.5(P1 + P2)}{0.5(P1 + P2)}$$

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

The essential water quality parameters such as dissolved oxygen, pH and temperature were monitored thrice daily while ammonia content was measured in the hatchery tanks and once weekly in the indoor rearing tanks using a HANNA Ammonia Medium Range meter (portable spectrophotometer HI96715) with reagents HI93715-01A and HI93715-1B.

Statistical Analysis

The data collected during the experiment were subjected to analysis of variance and the mean separated with Duncan multiple range tests for testing treatment means using the statistical package for social sciences (SPSS) version-21. Polynomial regression was performed on increase in fish lengths with time for each cross to predict trends and reveal hidden dynamics in fish growth.

Results

A comparison of the reproductive characters of female spawners from the native strain and the exotic strain of *C. gariepinus* showed that the female of the exotic hybrid (EH) strain were significantly (P<0.05) larger and produced more eggs than the native Cameroon strain (NS), but NS produced significantly larger and heavier eggs than EH (P=0.05). Both NS and EH were however in comparable (not statistical significance) physical condition as shown in Table 1.

Table 2 shows the breeding characteristics: fertilization rate, hatchability, survival and heterosis of NS, EH and their reciprocal hybrids. The fertilization rate of the pure NS (28.5%) and EH (28.1%) cross were significantly lower (P<0.05) compared to their reciprocal hybrids (32.9% and 45.5%), while the hatchability of the two pure cross NSxNS (36.82%) and EHxEH (41.14%) was significantly higher at P<0.05, compared to the hybrid crosses (20.6% and 8.73%). However, the hatchlings of the pure NS (75.87%) cross showed a significantly higher survival at P<0.05, while the pure EH (50.93%) cross showed a significantly lower survival at P<0.05 relative to the reciprocal hybrids (64.27% and 65.33%).

The pure EH and NS recorded comparable length gain (48mm and 56mm respectively) to their hybrids (48mm and 55mm) at P<0.05 the growth parameters of initial weight, final weight and weight gain. Results of all the mating combinations showed that NS had a significantly better performance for final weight

(28.33mg) and weight gained (22.85mg). The intraspecific mating combination were comparable with EH for both parameters as shown in Table 4.

Table 1. Reproductive characteristic of femalespawners of Native Cameroon *Clarias gariepinus*strain (NS) and Exotic hybrid strain (EH).

Parameter	NS	EH	P-value
K-factor	2.25	2.30	
Fecundity	15152±330 ^a	82408±4604 ^b	0.000
Egg diameter (mm)	1.64 ± 0.22^{a}	1.56 ± 0.19^{b}	0.002
Egg weight (mg)	2.92 ± 0.23^{a}	2.44 ± 0.29^{b}	0.000

Means in the same row with the same superscript are no significantly different ($P \le 0.05$).

Table 2. Breeding parameters and heterosis (H) of pure and reciprocal crosses of native Cameroon strain (NS) and exotic hybrid strain (EH) of *Clarias gariepinus*.

Parameter	♀NS × ♂NS	$PEH \times ONS$	$PNS \times CEH$	$Q EH \times \mathcal{F}EH$	P-Value
Fertilization (%)	28.10±0.36ª	32.90 ± 0.33^{a}	45.50±0.69 ^b	28.50 ± 0.49^{a}	0.001
Hatchability (%)	41.14 ± 1.22^{a}	20.60 ± 1.23^{b}	08.73±0.50 ^c	36.82±1.53ª	0.000
H for Hatchability (%)	-	-0.472	-0.152	-	-
Survival (%)	50.93 ± 1.22^{b}	64.27±1.22 ^a	65.33±1.22ª	75.87±1.22 ^c	0.000
H for Survival (%)	-	0.013722	1.032986	-	-
	1 7 71 66		•		

Means in the same row with different superscript differ significantly ($P \le 0.05$).

Table 3. Growth parameters and heterosis (H) of pure and reciprocal crosses of *Clarias gariepinus* strains reared for 14-days under indoor hatchery conditions.

Parameter	$QNS \times \Im NS$	$Q EH \times \Im NS$	$PNS \times CEH$	$\mathcal{P}\mathbf{EH} \times \mathcal{F}\mathbf{EH}$	P-Value
Length (cm)	0.0739±0.017	0.0729 ± 0.02	0.0729±0.017	0.0739±0.017	0.25
Final length (cm)	0.121±0.17	0.117 ± 0.23	0.123±0.20	0.127 ± 0.20	0.11
Length gain (cm)	0.048 ± 0.10	0.048±0.29	0.055 ± 0.08	0.056 ± 0.08	0.093
Weight (mg)	5.48±0.117	5.47±0.115	5.53 ± 0.113	5.49±0.116	0.370
Final Weight (mg)	28.33 ± 0.33	15.00±0.63ª	16.00 ± 0.27^{ab}	17.00 ± 0.52^{b}	0.000
Weight gain (mg)	22.85±3.10	9.53 ± 1.99^{a}	10.47 ± 1.33^{a}	11.51 ± 1.33^{a}	0.005
H for growth (%)	-	0.014	1.033	-	-
Growth rate (mg day-1)	1.6 ± 0.03	0.68 ± 0.02	0.75±0.19	0.82±0.19	0.150
Specific growth rate	10.20 ± 0.58	3.97 ± 0.39^{a}	4.56 ± 1.03^{a}	5.29 ± 1.03^{a}	0.000

Means in the same row with different superscript differ significantly ($P \le 0.05$)

In the indoor experimental units, water temperature ranged from 28.99 ± 0.16 to 29.19 ± 0.15 °C, pH (8.33 ± 0.02 to 8.40 ± 0.02), and dissolved oxygen (6.44 ± 0.11 to 6.73 ± 0.097 mg/l), (Table 4). The spatial variations

in temperatures in the indoor rearing space ranged from 23.99° C in the morning 6-9am to -31.5 °C afternoon during the study period November–December in Limbe, Cameroon.

Table 4. Mean water quality parameter	recorded in the experimental unit	t during the 14-day study period.
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Parameter	Q NS × \Im NS	$Q EH \times \Im NS$	$PNS \times CEH$	♀EH × ♂EH	P-Value				
Temperature °C	29.04±0.15	29.01±0.15	28.99±0.16	29.01±0.15	0.995				
DO (mgL ⁻¹)	6.73±0.097	6.54±0.11	6.49±0.11	6.44±0.11	0.098				
pH 8.40±0.02 ^a 8.37±0.02 ^{ab} 8.33±0.02 ^b 8.33±0.02 ^b 0.033									
Mean in the same row with different superscript differs significantly (P≤0.05)									

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There was a general increase in fry length and weight throughout the experimental period and through all the individual crosses as shown in table 5 and 6 for lengths and weights respectively. The length increase from an initial length of 0.0173cm to a final length ranging from 0.1174±0.023 for $\Im EH \times \Im NS$ to 0.127±0.020 for $\Im EH \times \Im EH$ (Table 5). Similarly, the fry also gained weight from an initial of about 5.48mg to a final weight ranging from 15.0±0.63mg for $\Im EH \times \Im NS$ to 28.33±0.26mg for $\Im NS \times \Im NS$.

Table 5. Growth Performance (length changes in cm) of the fry/fingerlings of pure Native Cameroon strain (NS), pure Exotic hybrid strain (EH) of *Clarias gariepinus*, and their reciprocal crosses, at the aquaculture experimental unit of LINAFI Limbe, Cameroon.

		$\mathcal{P}\mathbf{EH} \times \mathcal{O}\mathbf{NS}$	$\operatorname{PNS} \times \operatorname{CH}$	$Q EH \times C EH$	P-Value
Initial	0.074±0.017	0.073±0.017	0.073±0.017	0.074±0.017	0.025
Day 3	0.073±0.017	0.0712 ± 0.015	0.068±0.015	0.071±0.018	0.01448
Day 6	0.075±0.014	0.0697±0.019	0.082±0.016	0.078±0.018	0.24
Day9	0.0941±0.014	0.0933±0.014	0.102 ± 0.015	0.105 ± 0.008	0.31
Day12	0.103±0.014	0.102 ± 0.017	0.1102 ± 0.013	0.113 ± 0.018	0.28
Day14	0.121±0.017	0.1174±0.023	0.123 ± 0.020	0.127 ± 0.020	0.11

Mean in the same row with different superscript differs significantly ($P \le 0.05$)

Table 6.	Growth	Performance	(weight	changes	in mg)	of the	fry/	fingerlir	igs of	fpure	Native	Cameroon	strain.
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	♀NS × ♂NS	$PEH \times \Im NS$	$PNS \times \mathcal{O}EH$	♀EH × ♂EH	P-Value
Initial	5.48±0.117	5.47±0.115	5.53 ± 0.113	5.49±0.116	0.125
Day 3	8.17±0.61	8.17±0.61	8.17±0.61	8.17±0.61	0.370
Day 6	7.50 ± 0.35^{a}	6.00±0.22	7.17 ± 0.73^{a}	7.23±0.15 ^a	0.002
Day9	8.50 ± 0.43^{a}	7.67±0.15 ^a	9.67 ± 0.25	13.00 ± 0.22	0.000
Day12	11.17±0.34	9.0±0.28	13.67±0.21 ^a	13.24 ± 0.35^{a}	0.000
Day14	28.33±0.26	15.0±0.63ª	16.0 ± 0.27^{ab}	17.0 ± 0.52^{b}	0.000

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

Predictive polynomial functions of lenght increase with time in the various crosses

The relationship between length and time in the various crosses along with their respective polynomial growth models (of degree 2) are shown in Fig.s 1, 2, 3 and 4. The goodness-of-fit representation of the data was good in all the crosses, ranging from 95.47% ($\text{QEH} \times \text{CNS}$) to 97.66% ($\text{QNS} \times \text{CNS}$).



Fig. 1. Growth model for increase in fish length with time for $QNS \times CNS$.





Fig. 2. Growth model for increase in fish length with time for $\text{QEH} \times \text{CNS}$.



Fig. 3. Growth model for increase in fish length with time for $QNS \times CEH$.





Fig. 4. Growth model for increase in fish length with time for $\Im EH \times \Im EH$.



Fig. 5. Growth model for increase in fish length and weight with time showing a general increase within the exprimental period.



Fig. 6. Growth model for increase in fish weight with time showing changes in weight of Fish for the different experimental crosses.

Discussion

The intraspecific crosses of NS and EH showed that the highest percentage hatchability of 41.10% was recorded by $QNS \times \ImNS$ which is contrary to the results reported by Adene *et al.* (2017) who hybridized a native African catfish strain in Nigeria with improved "hollandaise" strain, but is similar to the result obtained in an interspecific hybridization

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by Tiogue et al. (2020). This was followed by the cross between ♂NS x ♀EH with 36.82% and the least was 8.73% for $QNS \times CEH$. There was a significant difference in percentage hatchability between the pure strains and their reciprocal cross. Although there has been limited research work on this area, but the high percentage hatchability recorded in EH might be due to generic improvement through selective breeding. Tine et al. (2021), reported in the average of 4.76% and 95% percentage hatchability and mortality respectively wish is significantly lower that obtained in this work at 33.75% Hatchability and 35.95% mortality respectively Clarias gariepinus crosses with the highest indoor percentage survival of 75.87% recorded for the parental exotic hybrid and the least of 50.93% recorded for parental native strain of Clarias gariepinus which could be due to the lack of adaptation of the native strain to indoor culture conditions. The highest survival recorded for the parental Exotic hybrid cross might be due to its hardness and adaptation to the indoor rearing environment results from selective breeding for resistance while the native strain which are less adapted to indoor rearing conditions recorded the least survival. Olufeagba et al. (2000) recorded a high indoor percentage survival of (72.5%) for Clarias angularis as an introduced species in aquaculture.

The indoor growth performance showed that there were no significant differences (P<0.05) in the crosses but the exotic hybrid *Clarias gariepinus* performed better in length gain (1.21cm) while the native strain performed better in weight gain at 28.33mg. This might also be attributed to genetic improvement, on the exotic strain and the hardiness and adaptability of the native strain to its environment. The cross $PNS \times SEH$ performed significantly better (P<0.05) for all parameters when compared to the cross $PEH \times SNS$ with 10.47mg in weight and 0.73cm, length increase and weight increase respectively.

All through the experimental period, the growth curve of experimental fish was in the lag and log phase as show by the growth models in Figs 2-6 of the growth models. Body weights and body length increased among the experimental crosses with a significant difference (p>0.05) though the curves for length increase showed minimal variation in shape from one experimental cross to the other. Similarly, the change in weight was also in the increasing phase of growth and had a significant difference from one cross to the other (p>0.05) among treatments as from the 4th day of the trial till the end of the trial period.

Conclusion

The combination with the best hatchability, survival rate and growth performance was $QNS \times CEH$. This hybrid had a comparablely high survival rate, percentage hatchability and increase in length close to the pure parental breed and the percentage growth rate was also close to that of the exotic parental cross. All four crosses however showed promising performance under indoor experimental conditions. All the fry produced for the experiments were eliminated to avoid contamination of the local water bodies which is a concern as expressed by Vitule et al. (2006) on the introduction of a potential invasive species (the African catfish) into Brazilian freshwater aquaculture which caused detrimental effects on the local aquatic biodiversity.

Acknowledgments

The authors are grateful to The Director and all the Staff of the Limbe Nautical Arts and Fisheries Institute (LINAFI) for having allowed the realization of this work on their Campus, their infrastructural and moral support contributed greatly to the success of this work., to the proprietor Dr. Oumarou N. of OKF Fish hatchery complex for making available his hatchery, and to Professor Pius Mbu Oben, the principal investigator and research team of the UB-CORAF/WECARD project for providing the parent stock used in the experiment. A big thank you to all the members of the Limbe screw for endless hours of work to ensure the successful completion of the research.

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