



Intraspecific Hybridization, Growth Performance and Survival of Purebreds and Reciprocal Hybrids of two *Clarias Gariepinus* (Burchell, 1822) Strains in the Mount Cameroon Region

Fidelis Narika Ambeno^{1*}, Benedicta Oshuware Oben¹, Mbeng Ashu Arrey¹, Joseph Ebobenow², Geneva Ojong Nkongho^{1,3}, Minda Sandra Nfuah¹, Pius Mbu Oben¹

¹Department of Fisheries and Aquatic Resources Management, Faculty of Agriculture and Veterinary Medicine, University of Buea, P.O. Box 63 Buea, Cameroon.

²Department of Physics, Faculty of Science, University of Buea, P.O. Box 63, Buea Cameroon.

³Institute of Agricultural Research for Development - Limbe, P.O Box 77, Cameroon

***Corresponding Author:** Fidelis Narika Ambeno, Department of Fisheries and Aquatic Resources Management, Faculty of Agriculture and Veterinary Medicine, University of Buea, Buea, Cameroon

Abstract: Reduced fish growth and survival may result from artificial propagation when broodstock that are successive filial generations of a limited number of parents are used. To solve this problem, hybridization can be employed to obtain strains with improved performance, which can then be reared as broodstock. Purebreds and reciprocal hybrids from a cultured strain and a Cameroonian grassland strain of *Clarias gariepinus* (Burchell, 1822) were produced artificially and grown for 63 days to assess their growth performance and survival. Stripped fecundity was higher in the Cultured females ($P < 0.05$). Fertilization and hatching rates were highest in the Grassland purebred (79 ± 4.6 and 75.0 ± 6.2 %, respectively), intermediate in the reciprocal crosses, and lowest in the Cultured purebred ($P < 0.05$). Mean weight gain was highest in the Cultured female x Grassland male hybrid (1.2187 ± 0.0173 g) with heterosis value of 70.85%, and lowest in the Grassland purebred (0.2845 ± 0.0076 g) ($P < 0.05$). Predicted weight increase followed the same trend. Fry survival was higher in the reciprocal hybrids (3.25 ± 1.76 and 2.67 ± 1.84 %) than the purebreds ($P < 0.05$). While the Cultured female x Grassland male hybrid is recommended for use as broodstock, its further hybridization with the Grassland female could maximise the number of hatchlings, growth and survival in one strain.

Keywords: Hybridization, cultured strain, Cameroonian grassland strain, *Clarias gariepinus*, weight gain, heterosis, predicted weight, fry survival

1. INTRODUCTION

Although projections show that significant growth in global fish production will come from aquaculture [1], the serious lack of adequate supplies of good quality fish seed for local farmers and producers in developing countries has been recurrently identified as an important constraint to aquaculture development [2-6]. In these countries the obtention of fish seed by artificial propagation using parents that are successive filial generations of a limited number of broodstock has gained grounds [7- 9]. However, such seed may suffer from the undesirable effects of inbreeding, such as lower growth and survival [10], reduced number of offspring [11], reduced fitness [8], decreased fecundity [12] and structural deformities [7].

The hybridization of fish species with other but closely related species or strains has been employed as a means to address the above constraint and obtain individuals with improved performance [13]. Hence, this study involved the hybridization of a cultivable Cameroonian clariid species with the exotic aquaculture strain of *Clarias gariepinus* (Siluriformes: Clariidae), a popular aquaculture species in many African countries [3-5], to evaluate the growth performance and survival of their purebreds and reciprocal hybrids.

2. METHODS AND MATERIALS

2.1. Broodstock source and experimental sites

Mature broodstock of the aquaculture stock of *Clarias gariepinus* (Cultured) and a *C. gariepinus* strain (Grassland), collected from the Mezam River Basin in Lower Bafut, North West Region,

Cameroon, and characterized by [14], were obtained from the Fish, Animal Production and Agroforestry (FAPA) hatchery complex in Muea – Buea. Spawning experiments were carried out at the OKF AquaFish Center, Limbe. The facility integrates modern recirculation aquaculture (RAS) units, each with a 1m³ capacity and connected to a biofilter for detoxification, a UV radiation source (RESUN® 008 – 9 V) for killing of any eventual pathogens, and a RESUN® ACO-004 electromagnetic air pump for aeration. The water in each RAS unit was recycled by a 0.5hp electric pump with a maximum flow rate of 42L/min adjustable by fitted taps. Replicated growth experiments were carried out at the Limbe Nautical Arts and Fisheries Institute (LINAFI) in 60 L capacity plastic tanks. Buea and Limbe are both located in Fako Division of the South West Region of Cameroon (Figure 1).

Figure 1. Sites for broodstock holding, spawning and larviculture.



2.2. Gamete Collection, and Egg Fertilization and Incubation

Two gravid female broodstock specimens and two mature male fish of each *Clarias gariepinus* strain (Cultured and Grassland) were selected according to the descriptions of [15] and [16]. The weights of the female broodstock are presented on Table 1. The weights of the male broodstock were 2.0 and 1.4 kg (Cultured), and 1.2 and 1.2 kg (Grassland). Each broodstock specimen was kept separately in a labelled plastic basin containing a little water at 28°C. The females were injected intramuscularly just below the dorsal fin with the synthetic hormone Ovulin® at 0.5 mL/kg body weight, followed 3 minutes later by a 3ml suspension of acetone-dried *C. gariepinus* hypophysis/kg body weight, to induce final oocyte maturation and ovulation. The females were monitored periodically until the females began releasing eggs. The males were injected both hormones at half the dose given the females. The eggs were obtained by stripping and the milt by aspiration with a hypodermic syringe [4], [17], [18]. The eggs from each female were weighed to give the stripped ova weight. From the stripped ova of each female broodstock three subsamples of ~2 g each were randomly collected, weighed in petri dishes, mixed with normal saline to facilitate counting, and counted. The mean number of eggs per gram from the subsamples was multiplied by total weight of stripped eggs to obtain total number of stripped eggs ≈ stripped fecundity [19]. The diameter of each of 50 oocytes stripped from female broodstock was measured to the nearest 0.01 mm using digital callipers and the mean computed [20]. The stripped ova index (SOI) was calculated from the stripped ova weight for each female, using the formula:

$$SOI = (100)(\text{stripped ova weight})/(\text{body weight}) \text{ ----- (1) [19].}$$

The quantities of milt from the two males of each strain were pooled together, as were the eggs from the two females of each strain [18]. Fertilisation was completely randomised, with each of the following combinations constituting a treatment:

Treatment 1 = Cultured female x Cultured male;

Treatment 2 = Grassland female x Cultured male;

Treatment 3 = Cultured female x Grassland male;

Treatment 4 = Grassland female x Grassland male.

Half of the pooled Cultured eggs were fertilized with half the quantity Cultured milt and incubated by spreading in a single layer on the “kakaban” in the culture tank of a recirculation aquaculture system (RAS) unit at the OKF AquaFish Center. A sub-sample of ~2 g fertilized eggs was collected in a plastic vial for determination of fertilization rate, while a sub-sample of ~1 g of the fertilized eggs was incubated in separate triplicate 1 L bowls for determination of hatchability [21]. The procedure was repeated for the remaining quantities of eggs and milt to obtain the other treatments. The water in the RAS units was kept running with constant aeration. At the end of incubation, the “kakaban” was removed from each RAS tank and a flow-through run for 2 – 3 hours to remove any accumulated ammonia.

At 10 hours post-fertilization [22], the total number of eggs and the number of unfertilized (opaque) eggs in the 2 g sample of eggs from each treatment were determined. Fertilization rate (FR) was then calculated as follows:

$$FR = (100)(\text{Total number of eggs} - \text{Number of opaque eggs})/(\text{Total number of eggs}) - (2) [4].$$

At the end of hatching, the number of larvae in each small bowl was counted and hatching rate (HR) computed as follows:

$$HR = (100)(\text{Number of hatched larvae})/(\text{Number of incubated eggs}) \text{ ----- (3) [21].}$$

Table 1. Body weight, total egg weight, stripped ova index, stripped fecundity and egg diameter of the Cultured and Grassland strains of *Clarias gariepinus* female broodstock.

Parameter	+Mean±SD				*T-test / W-Anova
	Cultured female 1	Cultured female 2	Grassland female 1	Grassland female 2	
Female weight (kg)	1.80±0.35 ^a	2.70±0.45 ^b	1.70±0.32 ^a	2.20±0.40 ^c	P<0.05
Total egg weight (g)	350.30±16.61 ^a	525.40±17.00 ^b	206.40±4.78 ^c	347.60±5.36 ^a	P<0.05
Pooled egg weight (g)	875.70±12.75 ^a		554.00±19.51 ^b		P<0.05
Stripped ova index (%)	19.46±4.50 ^a	19.46±3.52 ^a	12.14±1.74 ^b	15.80±3.21 ^{a,b}	P<0.05
Number of eggs/g	899±66 ^a	1004±167 ^b	852±65 ^c	943±45 ^d	P<0.05
Egg diameter (mm)	1.22±0.18 ^{a,b}	1.15±0.17 ^a	1.16±0.27 ^a	1.32±0.39 ^b	P<0.05
Pooled egg diameter (mm)	1.1850±0.0086 ^a		1.2500±0.0089 ^b		P<0.00
Stripped fecundity	314,920±23,080 ^a	527,502±76,100 ^b	175,853±22,660 ^c	327,787±42,900 ^a	P<0.05
Pooled stripped fecundity	421,211 ^a		251,821 ^b		P<0.05

+SD = standard deviation. *T-test for two means, Welch’s ANOVA for more than two treatments.

^{a,b,c,d}Games-Howell: Values on same row with same superscript letters are not significantly different (P > 0.05).

2.3. Feeding of Larvae in Ras Units

The larvae were fed ad libitum from the 4th day right up to the 7th day in the RAS tanks with shell-free *Artemia* (protein 54%, lipid 9%, ash 4%, fibre 6%, moisture ≤5%) (INVE[®], USA).

2.4. Fry Transportation, Stocking and Grow-Out in Indoor Plastic Tanks

At one week of culture in the RAS tanks, the post-larvae of each treatment were transported in aerated water in a separate plastic container from the OKF AquaFish Center to the LINAFI premises about 8 km away, for replicated growth experiments. For each treatment, 400 randomly selected fry were stocked in a 60-litre plastic tank in triplicate, at the rate of 10 fry per litre [23]. Each tank was fitted with a pipe connected to a RESUN[®] ACO-004 electromagnetic air pump for aeration.

Feeding continued from Day 7 with a transition (Table 2) from shell-free *Artemia* (INVE[®]) to the commercial extruded feed Le Gouessant[®] (58% crude protein, 12% lipid, 0.2% crude fibre, 8.76% ash, 1.58% calcium, 1.27% phosphorus, 0.82% sodium), at 08:00h, 13:00h and 18:00h, at 5% body weight. Daily, before the first rations, any uneaten feed, faecal matter and dead fish were removed from each tank and the water changed [24, 25].

Once a week, 20 fry were randomly collected from each tank, placed on a petri dish containing a very little film of water, and the total length [26] of each fry measured by means of digital callipers (TOTAL[®]) to the nearest 0.01mm. The fry were then placed on paper filter to absorb water and weighed on an electronic balance (ATOM[®]) to the nearest 0.01mg. The fry were then carefully returned to their respective tanks [20, 24]. The number of dead fry from each tank was recorded to determine the percentage survival, and the ration per tank adjusted to the new fish biomass in the tank [27]. The experiment lasted 63 days.

Table 2. Feeding protocol for the larvae and fry during experimental period.

Day	% shell-free <i>Artemia</i>	% commercial extruded feed ^a	
		0.2-0.3mm ^b	0.3-0.5mm ^b
0 – 3	0	0	0
4 – 6	100	0	0
7	75	25	0
8	50	50	0
9	25	75	0
10 – 20	0	100	0
21 – 23	0	50	50
24 – 63	0	0	100

^aLe Gouessant[®], ^bFeed grain diameter.

The following growth parameters were determined for the fish in each tank:

Mean weight gain (MWG) = Final mean weight (Y) – Initial mean weight (X) ----- (4).

Average daily growth rate (ADGR):

ADGR = (Final weight of fish – initial weight of fish)/Number of days of experiment ---- (5).

Specific growth rate (SGR) = (100)(ln Y – ln X)/(Rearing period, in days) ----- (6),

where ln is the natural logarithm.

Condition factor (K) = W x 100/ L³ ----- (7),

where W is weight of a fish and L is its length.

Survival rate (SR) = (100)(Final number of fish)/Initial number of fish ----- (8). [28, 29].

The length–weight relationships of fishes of the various treatments were estimated using the equation:

W = aL^b ----- (9), where W is the body weight (g), L is the total length (cm), “a” is the y-intercept and “b” is the slope or regression

coefficient of the regression line. The length and weight data were log-transformed, so that the above Equation 9 became the linear expression:

$$\ln(W) = \ln(a) + b \ln(L) \text{-----} (10).$$

Daily, just before feeding, uneaten feed, faecal matter and any dead fish were removed the water changed [30].

2.5. Water Quality Parameters

In the RAS tanks and the indoor culture tanks at grow-out, water temperature, pH and total dissolved solids were monitored with electronic meters, and dissolved oxygen with the Lutron® DO-5509 probe, thrice daily. Ammonia-N content was monitored with the H96715-01 Hanna Instruments® medium range kit, generally weekly; following the manufacturer’s manual, readings were multiplied by a factor of 1.214 to obtain parts per million (ppm) of ammonia. [4], [30], [31].

2.6. Data Analysis

All data were analysed with IBM SPSS 21®. The data were screened for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests. Levene’s test was used to screen the data for homogeneity of variance, which is a strict requirement of the ANOVA test. Since this requirement was not met in several parameters under study, Welch’s ANOVA was used to compare the means of more than two sets of data, and the Games-Howell post-hoc test for pair comparisons. The independent samples t-test was used to compare the means of two independent sets of data. Polynomial regression was performed on increase in fish weight with time for each treatment to predict trends in fish growth. Confidence level was set at 95%.

3. RESULTS AND DISCUSSION

3.1. Physicochemical Water Quality Parameters in Recirculation Aquaculture System Tanks and Indoor Plastic Tanks

The physicochemical water quality parameters in both the RAS tanks and the grow-out tanks are presented on Table 3 and Table 4, respectively. The parameters recorded were generally within acceptable limits for tropical aquaculture [32], [33], [34].

Table 3. Mean, standard deviation (SD) and range of physicochemical water quality parameters in the RAS culture tanks from Day 1 to Day 7.

	Statistic	*Treatment 1	Treatment 2	Treatment 3	Treatment 4	*W-Anov a	Recommend d range
Temperature (°C)	Mean±SD	26.33±0.54 ^a	25.95±0.73 ^{ab}	25.52±0.60 ^b	25.24±0.70 ^b	P<0.05	20-32 [32]
	Range	24.40 – 27.10	23.80-27.50	23.90-26.50	23.90-26.60		
Dissolved oxygen (mg/l)	Mean±SD	7.86±0.42 ^a	7.83±0.35 ^a	8.18±0.30 ^a	7.98±0.49 ^a	P>0.05	5-8 [33]
	Range	7.10-8.50	7.31-8.35	7.73-8.51	6.86-8.47		
pH	Mean±SD	8.45±0.43 ^a	8.29±0.45 ^a	8.24±0.52 ^a	8.26±0.41 ^a	P>0.05	6.5-9.0 [34]
	Range	7.84-8.90	7.61-8.75	7.44-8.75	7.70-8.90		
Total dissolved solids (mg/l)	Mean±SD	114.85±1.13 ^a	113.98±0.96 ^{ab}	113.22±0.90 ^b	113.93±0.85 ^{ab}	P<0.05	≤500 [33]
	Range	113-116	112-115	111-114	112-115		
Ammonia (mg/l)	Mean±SD	0.24±0.08 ^a	0.00 ^b	0.00 ^b	0.00 ^b	P<0.05	≤0.05 [32]
	Range	0.00-0.29	0.00	0.00	0.00		

*Treatment 1 = Cultured female x Cultured male; Treatment 2 = Grassland female x Cultured male; Treatment 3 = Cultured female x Grassland male; Treatment 4 = Grassland female x Grassland male. *Welch’s Anova; ^{a,b,c} Games-Howell: Values on same row with same superscript letters not significantly different (P > 0.05).

Intraspecific Hybridization, Growth Performance and Survival of Purebreds and Reciprocal Hybrids Of Two *Clarias Gariepinus* (Burchell, 1822) Strains in The Mount Cameroon Region

Table 4. Physicochemical water quality parameters of indoor plastic rearing tanks at LINAFLI, Limbe, from Week 2 to Week 9. ^{a,b,c}Games-Howell: Values on same row with same superscript not significantly different.

	Statistic	*Treatment 1	Treatment 2	Treatment 3	Treatment 4	Welch's Anova	Recommended range
Temperature (°C)	Mean±SD	28.16±1.35 ^a	28.08±1.31 ^a	28.28±1.33 ^a	28.20±1.33 ^a	P>0.05	20-32 [32]
	Range	25.00-30.56	24.78-30.11	25.00-30.44	25.00-30.33		
Dissolved oxygen (mg/l)	Mean±SD	7.13±0.71 ^a	7.05±0.82 ^a	7.09±1.11 ^a	7.08±0.82 ^a	P>0.05	5-8 [33]
	Range	5.40-8.64	4.82-8.97	5.29-13.30	5.36-8.98		
pH	Mean±SD	8.49±0.32 ^a	8.37±1.23 ^a	8.47±0.25 ^a	8.51±0.25 ^a	P>0.05	6.5-9.0 [34]
	Range	7.28-8.94	7.60-8.06	7.90-8.87	80.3-9.02		
Total dissolved solids (mg/l)	Mean±SD	136±2 ^a	141±3 ^a	137±2 ^a	136±3 ^a	P>0.05	≤500 [33]
	Range	130-139	131-250	131-141	131-140		
Ammonia (mg/l)	Mean±SD	0.32±0.04 ^a	0.31±0.04 ^a	0.54±0.21 ^b	0.21±0.04 ^c	P<0.05	≤0.05 [32]
	Range	0.24-0.39	0.21-0.38	0.24-0.81	0.11-0.28		

3.2. Female Reproductive Indices

From the data observed in this study, the heavier female of each strain recorded higher fecundity and egg weight than the lighter female (P<0.05) (Table 1), in agreement with [35 – 37], who reported that smaller fish tended to yield fewer eggs than larger fish. This is probably because smaller fish invest more energy in other activities such as predation avoidance, especially in cannibalistic fish like *Clarias*, instead of for growth and reproductive activity [38]. The lowest (87,927, Grassland 1, 1.7kg) and the highest (263,751, Cultured 2, 2.7kg) fecundity observed in this study are generally within the range of values of fecundity for *Clarias gariepinus* reported elsewhere [38], [39]. A stripped fecundity value of 52,480 had been previously observed (female, 2.6kg) in the Grassland strain [20], much lower (P<0.05) than the values (Grassland female 1: 1.7kg 87,927 and Grassland female 2: 1.7kg 163,894) observed in this study.

In fish reproduction under controlled conditions attempts are made to obtain the largest eggs possible and of the best quality [18]. Egg diameter in this study ranged from 1.15 – 1.22 mm (Cultured strain) and 1.16 – 1.32 mm (Grassland strain). These fall within ranges of values observed in other studies (1.00 – 1.6 mm) [40], (1.41 – 1.66 mm) [39] and (1.06 – 1.78 mm) [41]. A higher mean egg diameter (1.63±0.25 mm) was previously recorded in the Grassland strain [20] than the value recorded in this study (1.2500±0.0089 mm). Differences in oocyte maturation stage may have contributed to a higher egg diameter in [20]. Substantial disparity in egg size would be expected in *Clarias gariepinus* between October (when this study was done, when most of the oocytes would still be immature and of a smaller size), and January [20] (when the oocytes would be in an advanced stage of maturation and of a larger size) [15]. Additionally, the larger egg size in [20] could be due to the lower mean temperature (26.5 °C) of the latency period compared to the 28 °C in this work [42].

Strain-wise, egg size was greater (P<0.05) in the Grassland than in the Cultured fish. This variation could be attributed to inherent genetic differences, since all the females were kept in the same conditions, right up to ovulation. Based on egg diameter, therefore, the Grassland females in this study would be more desirable for aquaculture than the Cultured females.

Both fertilization and hatching rates were highest (P<0.05) in the Grassland purebred, lowest in the Cultured purebred, but not significantly different between the reciprocal crosses (Table 5). Hatching rate in catfish has been related to egg quality [18], suggesting that the higher hatching rates in combinations with Grassland females could be attributed to the larger (P<0.05) eggs they produced. This suggests that the differences in fertilization and hatching between the treatments in this study

could be due to genetic variations between the two strains, a trend which has been hitherto observed [43].

Table 5. Fertilisation and hatching rates of the pure lines and reciprocal crosses of the Cultured and Grassland strains of *Clarias gariepinus*.

Parameter	Crosses				*Welch's Anova
	Treatment 1	Treatment 2	Treatment 3	Treatment 4	
Fertilization rate (%)	27±2.9 ^a	70±8.0 ^b	73±7.3 ^b	79±4.6 ^c	P<0.05
Hatchability (%)	30±5.2 ^a	34±4.0 ^a	31±4.0 ^a	75±6.2 ^b	P<0.05

^{a,b,c,d}Games-Howell: Values on same row with same superscript not significantly different (P > 0.05).

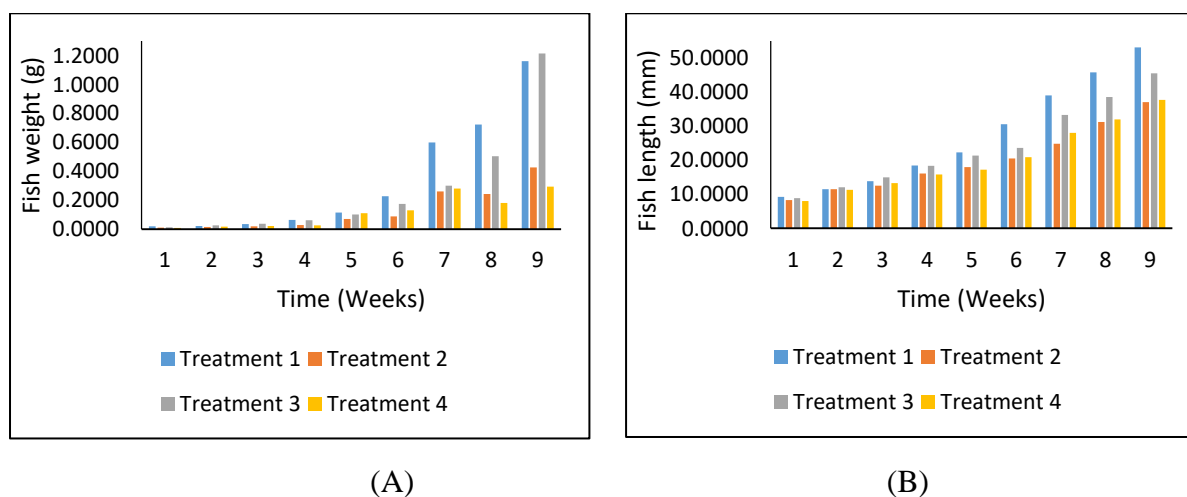
3.3. Larval and Fry Growth

Weekly progress in fish weight and total length are presented in Figure 2, while the mean values of all the growth variables and survival rate are presented on Table 6. Although the Cultured females produced smaller eggs (P<0.05) than the Grassland females, they yielded progeny (Treatments 1 and 3) which grew faster than those of the Grassland females, throughout the experiment. Smaller fish eggs have been reported to produce individuals that grew faster than those from larger eggs [44].

The fish in Treatment 1 (Cultured purebred) had the highest weight at Week 1 (P<0.05), and from Weeks 4 to 8. However, Treatment 3 (Cultured female x Grassland male) crossbreds recorded higher weight than Treatment 1 at Weeks 2, 3, and 9. Such a random progress in weight or length where sibling crosses overtake each other at various points in time has been observed in clariid fish propagation [45–46]. This could be attributed mainly to inherent genetic factors that determine the growth pattern of each treatment [47].

Although it was observed that Treatment 1 had a higher temperature than Treatment 3 (P<0.05) at Week 1 (Figure 2), which would relate to increased food intake [48] and faster growth in the former [49], both treatments had the two highest weights, which did not differ significantly from each other, suggesting that the high growth in Treatment 3 resulted from genetic causes.

Figure 2. Increase in fish weight (a) and total length (b) of the pure and reciprocal crosses from the Cultured and Grassland strains of *Clarias gariepinus* from Week 1 to Week 9.



Key: Treatment 1 = Cultured female x Cultured male; Treatment 2 = Grassland female x Cultured male; Treatment 3 = Cultured female x Grassland male; Treatment 4 = Grassland female x Grassland male.

At the end of the experiment, Treatment 3 (Cultured ♀ x Grassland ♂) performed the best (P<0.05) in weight gain (1.2187±0.0173 g), exhibiting heterosis in weight increase of 70.85% over the parental lines (Table 6). This reveals that Treatment 3 fish added more mass per unit increase in length than the other treatments, resulting in a higher condition factor, a desirable attribute in aquaculture.

Intraspecific Hybridization, Growth Performance and Survival of Purebreds and Reciprocal Hybrids Of Two *Clarias Gariepinus* (Burchell, 1822) Strains in The Mount Cameroon Region

Table 6. Mean values and standard deviation of growth variables and survival rate of the pure and reciprocal crosses of the Cultured and Grassland strains of *Clarias gariepinus*.

Parameter	*Treatment 1	Treatment 2	Treatment 3	Treatment 4	Levene	Welch's ANOVA/T - test
Initial total length (mm)	9.22±0.97a	8.27±0.45b	8.86±0.41a	8.01±0.75b	P=0.001	P<0.05
Final total length (mm)	53.13±7.38a	37.01±7.15b	45.52±8.32c	37.66±6.19b	P=0.146	P<0.05
Mean length gain (mm)	43.91±7.83a	31.01±8.31b	38.51±9.13a	29.65±5.77b	P=0.341	P<0.05
Initial body weight (g)	0.0190±0.0133a	0.0105±0.0009 b	0.0135±0.0018 a,b	0.0095±0.0011 b	P=0.000	P<0.05
Final body weight (g)	1.1610±0.0003a	0.4256±0.0014b	1.2135±0.0637c	0.2940±0.0076d	P=0.000	P<0.05
Mean weight gain (g)	1.1420±0.0133a	0.4149±0.0018b	1.2187±0.0173c	0.2845±0.0076d	P=0.000	P<0.05
% Heterosis for weight gain	–	-41.83	70.85	–		P<0.05
Average daily growth rate (%)	2.00±0.02a	0.73 ± 0.00b	2.14 ± 0.03c	0.50 ± 0.01d	P=0.000	P<0.05
Specific growth rate (%/day)	7.69±1.13a	6.62±0.16b	8.08±0.25a	6.14±0.21b	P=0.000	P<0.05
Feed conversion ratio	1.51a	3.23b	1.84c	3.22b	P=0.001	P<0.05
Final condition factor (K)	0.86±0.36a	0.88±0.58a	1.51±1.12b	0.64±0.30a	P=0.000	P<0.05
Survival rate (%)	1.75±0.54a	3.25±1.76b	2.67±1.84a,b	0.08±0.03c	P=0.000	P<0.05
% Heterosis for survival rate	–	255.19	191.80	–		P<0.05

a,b,c,d Games-Howell: Values on same row with same superscript are not significantly different.

3.4. Prediction Equations for Weight Increase

Predictive modelling of fish weight with time are shown on Figures 3, 4, 5 and 6 for Treatments 1, 2,

3 and 4, respectively [50]. Growth patterns based on the models show that if culture time were extended under the prevailing conditions, Treatment 3 would far outgrow the other treatments (Figure 7), rendering it more desirable than the other treatments for aquaculture. The goodness-of-fit representation of the data was good in all the treatments.

Figure3. Polynomial growth model for increase in fish weight with time for Treatment 1 (Cultured female x Cultured male).

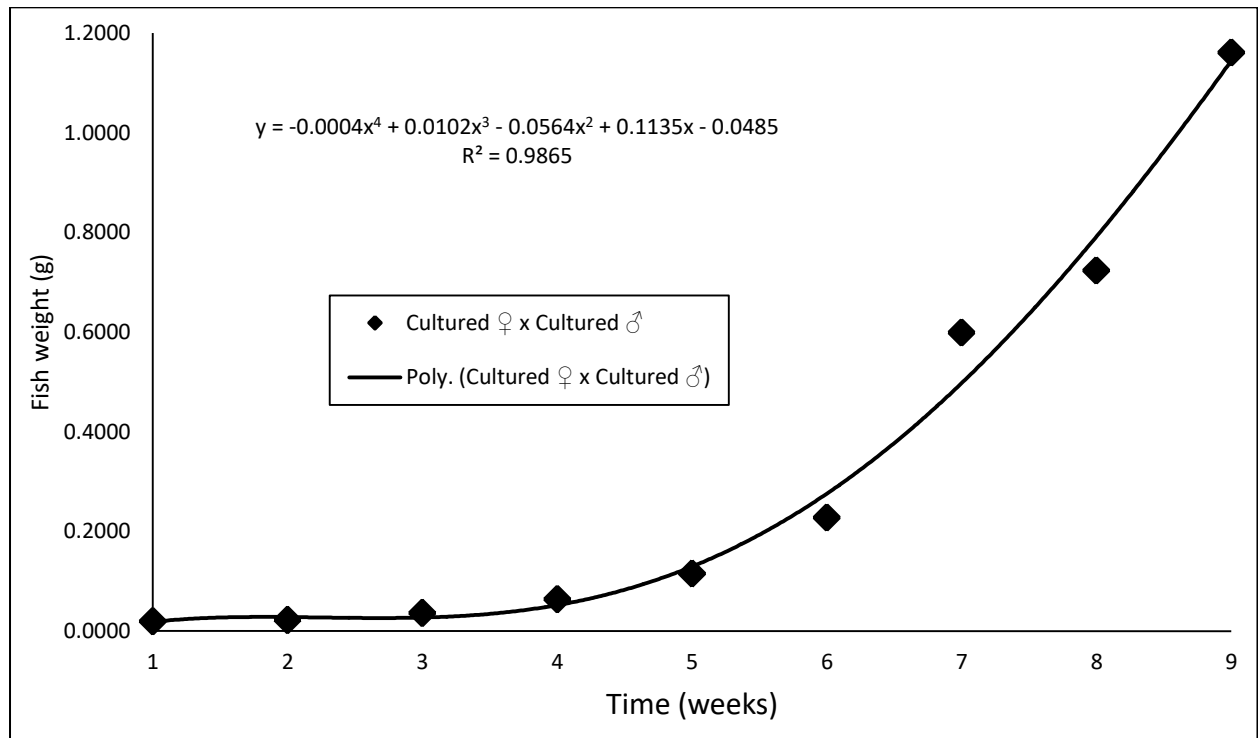


Figure4. Polynomial growth model for increase in fish weight with time for Treatment 2 (Grassland female x Cultured male).

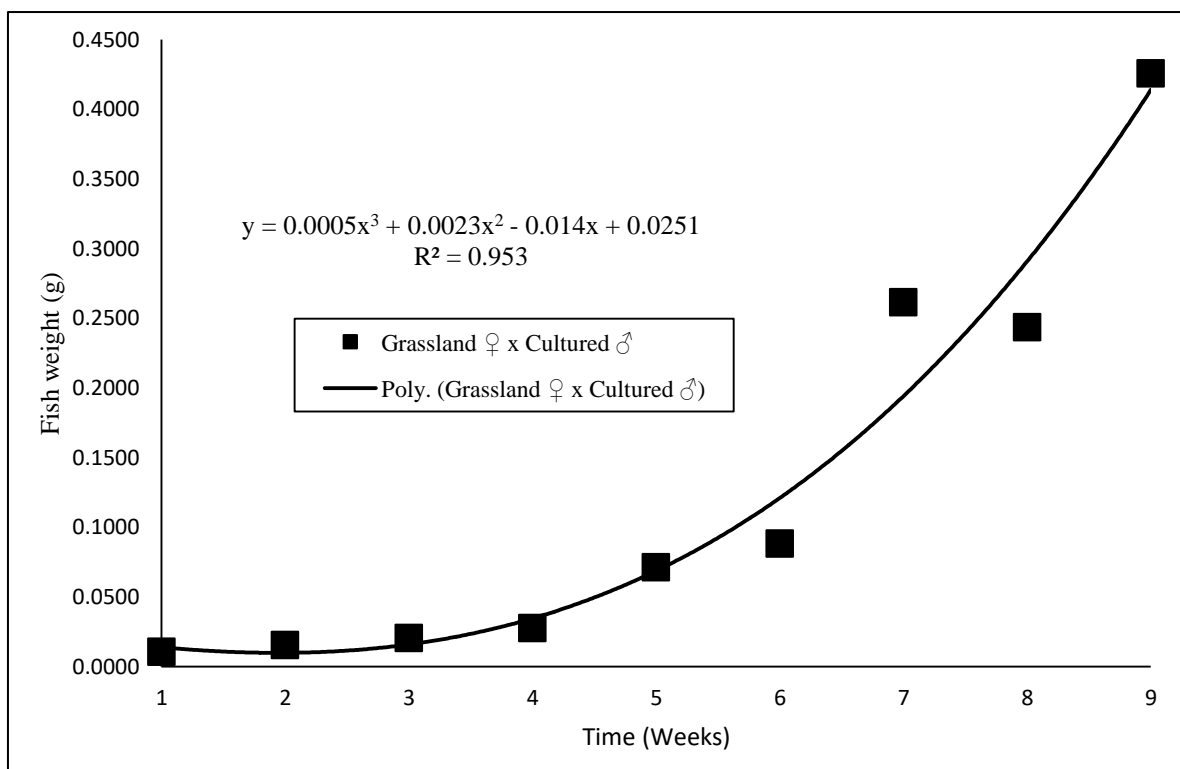


Figure5. Polynomial growth model for increase in fish weight with time for Treatment 3 (Cultured female x Grassland male)

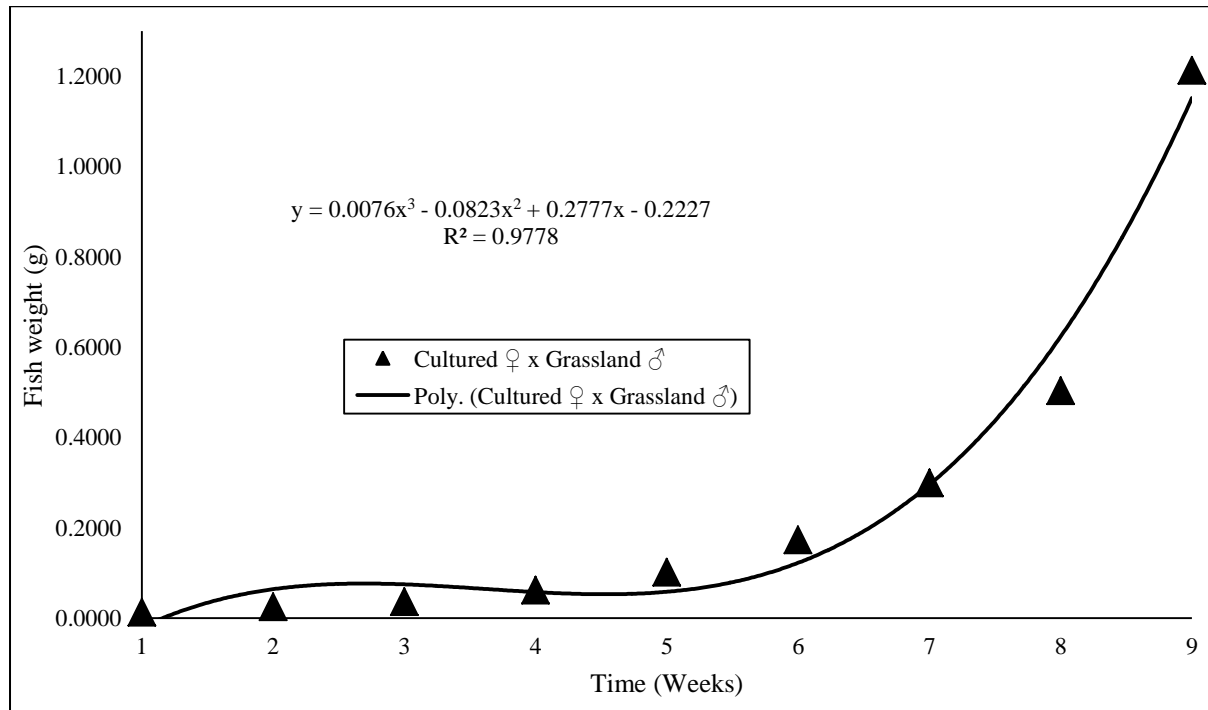


Figure6. Polynomial growth model for increase in fish weight with time for Treatment 4 (Grassland female x Grassland male).

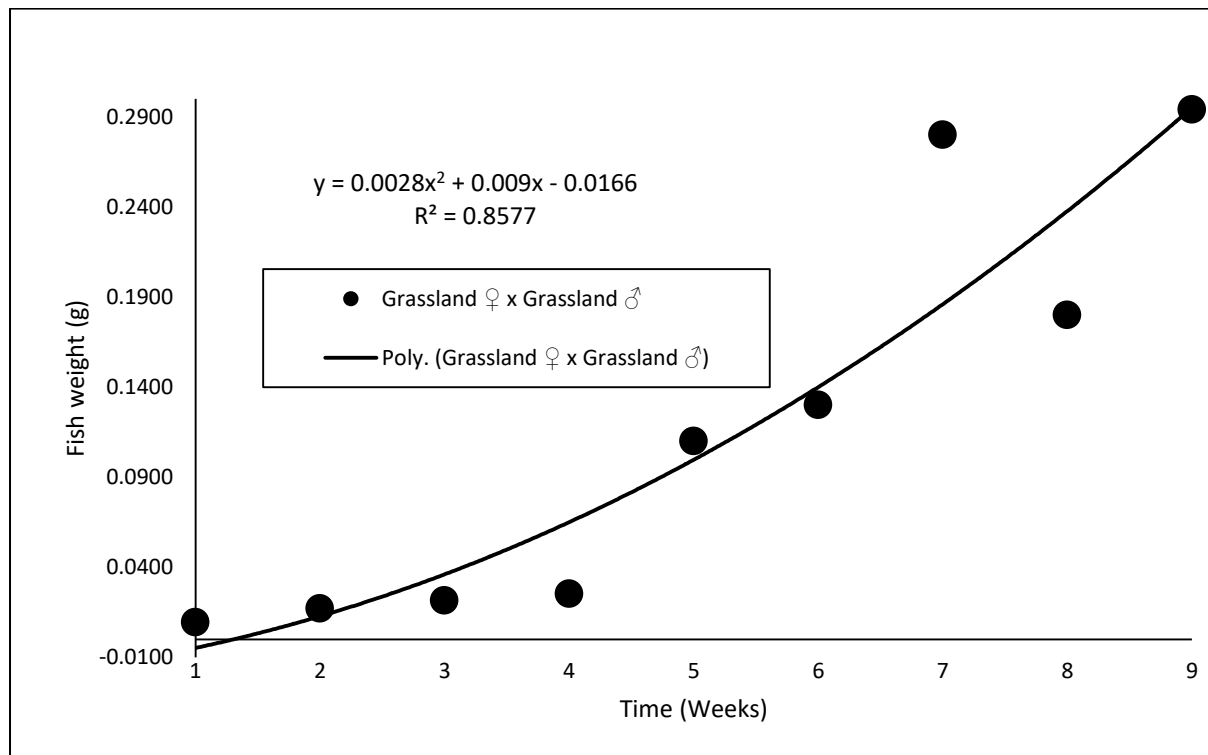
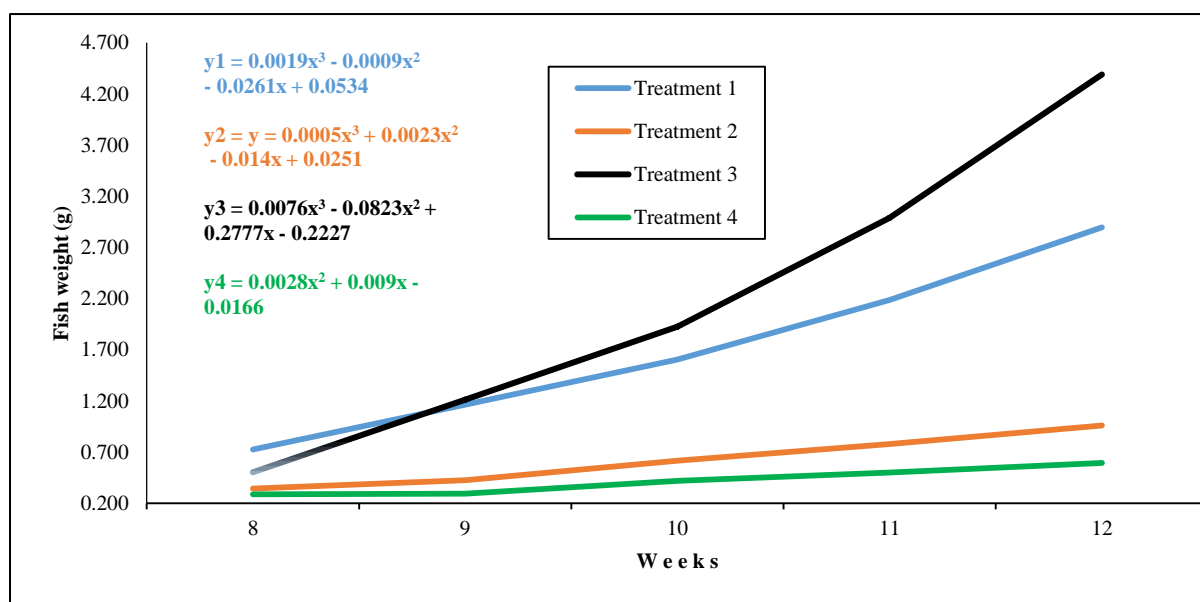


Figure7. Predicted weights of the pure lines and reciprocal crosses of the Cultured and Grassland strains of *Clarias gariepinus* used in this study, from Week 10 to Week 12, based on the regression functions obtained for each treatment.

Key: y_1 , y_2 , y_3 , y_4 = Regression functions of Treatments 1, 2, 3, 4, respectively.



3.5. Fry Survival

Highest survival rate was observed in Treatment 2, although not significantly higher than Treatment 3 ($P > 0.05$) (Table 6). The toxic ammonia range of 0.1495 – 0.1678 mg/l expected in this study, based on prevailing temperatures [37], was exceeded in all the treatments (Table 4). This may have led to the poor survival rates observed, compared to survival rates of 65.2 and 30.5% in clariid purebreds and 85.5% in their hybrids after 2 months obtained by [4] who worked with flow-through outdoor concrete tanks in the same geographical region where this study took place. These authors employed a flow-through system for continual ammonia removal from the culture tanks, which perceptibly proved effective for fish survival.

4. CONCLUSION

Based on the higher fecundity of the Cultured females, the higher observed and predicted growth of their progeny, as well as their comparatively high survival, Treatment 3 (Cultured female x Grassland male) presented the best prospects for use as broodstock. This hybrid would reduce dependence on a limited number of broodstock in *Clarias gariepinus* culture. However, the higher rates of fertilization, hatching and survival associated with the Grassland females could be incorporated genetically into the Treatment 3 fish by further hybridization, in order to maximise the number of hatchlings, growth and survival in one strain.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the University of Buea, the West and Central African Council for Agricultural Research and Development (CORAF/WECARD) SIAD—RP and SIARP—ESEA Projects in Cameroon, the Fish, Animal Production and Agrofarms (FAPA) hatchery complex in Buea, the OKF AquaFish Center, Limbe, and the Limbe Nautical Arts and Fisheries Institute (LINAIFI), for funding and logistical support.

REFERENCES

- [1] World Bank, Fish to 2030: Prospects for fisheries and aquaculture, Agriculture and environmental services discussion paper 03 (2013).
- [2] Tekwombuo J., Hatchery design and broodstock management policy as a tool for sustainable aquaculture: case of Cameroon, United Nations University, Fisheries (2014).
- [3] Kaktcham P. M., Zambou N. F., Fonteh A. F. and Pérez-Chabela M. L., Aquaculture in Cameroon and potential of lactic acid bacteria to be used as diseases controlling agents. A Review, Nacameh, 9(1): 1-18 (2015).
- [4] Oben P. M., Oben B. O., Akoachere R. and Ebobenow J., Induced spawning, survival and growth of an African catfish hybrid (female *Clarias gariepinus* and male *Clarias anguillaris*) fingerlings relative to their parental species in the Mount Cameroon region, Trop. Fresh. Biol, 24: 63 – 88 (2015).

- [5] Olanrewaju A. N., Kareem O. K. and Orisasona O., Cryopreservation: a viable tool for sustainable catfish aquaculture industry in Nigeria, *J. Fish. & Ltvsck Prod*, 3(4) ISSN: 2332-2608 (2015). DOI: 10.4172/2332-2608.1000149.
- [6] Adeleke, B., Robertson-Andersson D., Moodley G. and Taylor S., Aquaculture in Africa: a comparative review of Egypt, Nigeria, and Uganda vis-à-vis South Africa. *Rev. Fish. Sci. & Aquac.*, 29(2): 1 – 29 (2020).
- [7] Brummett R. E., “Freshwater fish seed resources in Cameroon,” In: M.G. Bondad-Reantaso (ed.), Assessment of freshwater fish seed resources for sustainable aquaculture. FAO Fisheries Technical Paper, No. 501, Rome, FAO, 628p. (2007).
- [8] Christie M. R., French R. A., Marine M. L. and Blouin M. S., How much does inbreeding contribute to the reduced fitness of hatchery-born steelhead (*Oncorhynchus mykiss*) in the wild? *J. Hered.*, 105(1): 111–119 (2014). DOI: 10.1093/jhered/est076.
- [9] Chattopadhyay N. R., “Current status of hatchery operations in some leading seed-producing states of India and its impact on aquaculture.” In: Induced fish breeding: A practical guide for hatcheries, 1st ed. London: Academic Press, 2017, ch. 7, pp. 143–206.
- [10] Wang J., Ge Q., Li J., and Li J., Effects of inbreeding on growth and survival rates, and immune responses of ridgetail white prawn *Exopalaemon carinicauda* against the infection of *Vibrio parahaemolyticus*. *Aquac.*, 519: 734755 (2020).
- [11] Vega-Trejo R., Head M. L. and Jennions M. D., Evidence for inbreeding depression in a species with limited opportunity for maternal effects, *Ecol. and Evol.*, 5(7): 1398–1404 (2015).
- [12] Fessehaye Y., Bovenhuis H., Rezk M. A., Crooijmans R., van Arendonk J. A. M. and Komen H., Effects of relatedness and inbreeding on reproductive success of Nile tilapia (*Oreochromis niloticus*). *Aquac.*, 294: 180–186 (2009).
- [13] Rahman M. A., Arshad A., Marimuthu K., Ara R. and Amin S. M. N., Inter-specific hybridization and its potential for aquaculture of fin fishes, *Asian J. An. & Vet. Adv.*, 8 (2): 139 – 153 (2013). DOI: 10.3923/ajava.2013.139.153.
- [14] Nkongho G. O., Oben B. O., Sanni M. T., Agbebi O. T., Obie A. E., Makombu J. G. and Oben P. M. Morphological and molecular characterization of some grassland and cultured *Clarias* (*Clariidae*, *Siluriformes*) fish species from Cameroon. *Int. J. Res. BioSciences.*, 7(3): 16-26 (2019).
- [15] de Graaf G. J. and Jansen J. A. L., Handbook on the artificial reproduction and pond rearing of the African catfish, *Clarias gariepinus*, in sub-Saharan Africa. FAO Fisheries Technical Paper. No 362. Rome, FAO. 1996. 73 p (1996).
- [16] Marimuthu K., Nirmell S., Rahman M. A., Arshad A., Raj M. G. and Arockiaraj J., *Journal of Environment and Biotechnology Research*, 1(1): 2-9 (2015).
- [17] Brown G. G. and Moore A. A., “Comparative storage methods and fertility of walleye semen,” In: R. C. Summerfelt (ed). Walleye culture manual. NCRAC Culture Series 101. North Central Regional Aquaculture Center Publications office, Iowa State University, Ames. 45 – 49 (1996).
- [18] Brzuska, E., Artificial propagation of European catfish (*Silurus glanis*): application of a single dose of pellets containing D-Ala6, Pro9NET-mGnRH and dopamine inhibitor metoclopramide to stimulate ovulation in females of different body weight. *Czech J. Anl. Sci.*, 48(4): 152–163 (2003).
- [19] Santos H. B., Sampaio E. V., Arantes F. P. and Sato Y., Induced spawning and reproductive variables of the catfish *Lophiosilurus alexandri*, Steindachner, 1876 (*Siluriformes: Pseudopimelodidae*). *Neot. Ichth.*, 11(3): 607-614 (2013).
- [20] Nkongho G. O., Morphological and molecular characterization, reproduction and fry nutrition of Grassland and domestic clariid catfish species from Cameroon, Ph.D. Thesis, University of Buea, Cameroon (2019).
- [21] Gadissa S. and Devi L. P., Evaluation of spawning induction of African Catfish (*Clarias gariepinus*) by heteroplastic hypophysation, *Int. J. of Fish. Aquat. Stud.*, 1(1): 22-25 (2013).
- [22] Ayoola S. O., Kuton M. P., and Chukwu S. C., Comparative study of piscine and non-piscine pituitary extract and Ovulin® for inducing spawning in catfish (*Clarias gariepinus*), *Afr. J. Food, Agric, Nut. & Dev.*, 12(6): 6809-6822 (2012).
- [23] Atse B. C., Konan K. J., Alla Y. L. and Pangini K., Effect of rearing density and feeding regimes on growth and survival of African Catfish, *Heterobranchus longifilis* (Valenceinnes, 1840) larvae in a closed recirculation aquaculture system, *J. Appl. Aquac.*, 21:3, 183-195 (2009).

- [24] Adewolu M. A., Akintola S. L. and Akinwunmi O. O., Growth performance and survival of hybrid African catfish larvae (*Clarias gariepinus* x *Heterobranchus bidorsalis*) fed on different diets, *The Zool.*, 7: 45-51 (2009).
- [25] Musefiu T. A., Growth Performance of the African catfish, *Clarias Gariepinus*, fed varying inclusion levels of *Talinum Triangulare* as feed additives, *Biomed. J. Sci. & Tech. Res.*, 9(3): 7185 – 7189 (2018). DOI: 10.26717/BJSTR.2018.09.001812.
- [26] Xue Y., Ren Y., Xu B., Mei C., Chen X. and Zan X. Length–weight relationships of fish species caught by bottom trawl in Jiaozhou Bay, China, *J. Appl. Ichth.*, 27: 949–954 (2011).
- [27] Nabulime M., Rutaisire J., Ssekaayi J., Smith D. and Kabasa J. D., Development of fry diets for African catfish (*Clarias gariepinus*) diets larvae in Uganda, *J. Fish. and Aquat. Sci.*, ISSN 1816-4927, 1 – 9 (2015). DOI: 10.3923/jfas2015.
- [28] Promya J. and Chitmanat C., The effects of *Spirulina platensis* and *Cladophora* algae on the growth performance, meat quality and immunity stimulating capacity of the African sharp tooth catfish (*Clarias gariepinus*), *Int. J. Agric. and Biol.*, 13: 77–82 (2011).
- [29] Olaniyi C. O and Akinbola D. O., Comparative studies on the hatchability, performance and survival rate of african catfish (*Clarias gariepinus*) larval produced: using Ovulin® and catfish pituitary extract hormones, *J. Biol. Agric. and Healthc.*, 3(9): 57-62 (2013).
- [30] Ndimele P. E. and Owodeinde F. G., Comparative reproductive and growth performance of *Clarias gariepinus* (Burchell, 1822) and its hybrid induced with synthetic hormone and pituitary gland of *Clarias gariepinus*, *Turk. J. Fish. and Aquat. Sci.*, 12: 619 – 626 (2012).
- [31] Dhara K. and Saha N. C., Controlled breeding of Asian catfish *Clarias batrachus* using pituitary gland extracts and Ovulin® at different temperatures, latency periods and their early development, *Aquac. Res. and Devt.*, 4: 4. (2013). DOI: 10.4172/2155-9546.1000186.
- [32] Viveen W. J. A. R., Richter C. J. J., van Oordrot P. G. W. J., Janssen J. A. L. and Huisman E. A., Practical manual for the culture of the African catfish, *Clarias gariepinus*. Directorate General International Cooperation of the Ministry of Foreign Affairs, The Hague, the Netherlands; Department of Fish Culture and Fisheries of the Agricultural University of Wageningen, the Netherlands; and Research Group for Comparative Endocrinology, Department of Zoology of the University of Utrecht, the Netherlands. Pp 1-128 (1985).
- [33] Boyd C. E. and Lichtkoppler F., Water quality management in pond fish culture. International Center for Aquaculture Agricultural Experiment Station and Auburn University, Alabama: Research and Development Series No. 22, Project: AID/DSAN-G 0039 (1979).
- [34] Wurts W. A. and Durborow R. M., Interactions of pH, carbon dioxide, alkalinity and hardness in fish ponds. Southern Regional Aquaculture Center, SRAC Publication No. 464 (1992).
- [35] Macgregor J. S., Ovarian development and fecundity of five species of California. *Current Fishes, California Co-operative Oceanic Fisheries Investigations Report*, 18: 181 - 187 (1975).
- [36] Eyo J. E. and Mgbenka B. O., Aspects of the Biology of *Clarias gariepinus* in Anambra River Basin. 1. Oocyte Diameter, Fecundity and Sex Ratio, *J. Agric. Sci. and Technol.*, 2(1): 47 – 51 (1992).
- [37] Absalom K. V., Anpe J. A., Igoche L. E. and Okunsebor S. A., Fecundity and egg size of *Clarias gariepinus* in Pandam Lake, Quan-Pan L.G.A. Plateau State, Nigeria, *J. Agric. and Vet. Sci.*, 10(2): 19 – 22 (2017). DOI: 10.9790/2380-1004021922.
- [38] Ataguba G. A., Solomon S. G. and Onwuka M. C., Broodstock size combination in artificial spawning of cultured *Clarias gariepinus*, *Livest. Res. Rur. Devt.*, 24(223) (2012).
- [39] Yalçın S., Solak K. and Akyurt I., Certain reproductive characteristics of the catfish (*Clarias gariepinus* Burchell, 1822) living in the River Asi, Turkey, *Turk. J. Zool.*, 25: 453 – 460 (2001).
- [40] FfigBichi A. H., Isyaku S., Danba E .P., Kurawa I. A., and Nayawo A. A., Effect of brood stock size on egg fertilization and hatchability, *Bayero J. Pure Appl. Sci.*, 7(2): 150 – 154 (2014).
- [41] Ogidan I. M., Chukwuka A. V., Omiwole R. A., Ibor O. R., and Adeogun A. O., Gamete quality and reproductive performance in *Clarias gariepinus* chronically exposed to industrial effluent mixtures, *Afr. J. Biomed. Res.*, 21: 201 – 210 (2018).
- [42] Robertson D. R. and Collin R., Inter- and intra-specific variation in egg size among reef fishes across the isthmus of Panama, *Front. Ecol. Evol.*, 2(84): 1 – 15 (2015).
- [43] Ndeham V. R., Ladu B. M. B. and Onyia L. U., Comparison of fertilization, hatchability, growth and survival of *Clarias gariepinus* from Adamawa and Katsina States, *Adv. Life Sci. Tech.*, 53:1 – 7 (2018).
- [44] Self K. E., Schreck C. B., Cogliati K. M., Billman E. J. and Noakes D. L. G., Egg size and growth in steelhead *Oncorhynchus mykiss*. *J. Fish Biol.*, 93(3): 465 – 468 (2018).

- [45] Tiogué C. T., Nyadjeu P., Mouokeu S. R., Tekou G. and Tchoupou H., Evaluation of hybridization in two African catfishes (Siluriformes, Clariidae): exotic (*Clarias gariepinus* Burchell, 1822) and native (*Clarias jaensis* Boulenger, 1909) species under controlled hatchery conditions in Cameroon. *Adv. Agric.*, 2020: 1 – 11 (2020).
- [46] Omeji S., Obande R. A. and Oyaje J., Intra-specific hybridization of local and exotic *Clarias gariepinus*, *Int. J. Modern Biolog. Res.*, 1: 35-41 (2013).
- [47] Martins C. I. M., Schrama J. W. and Verreth J. A. J., Inherent variation in growth efficiency of African catfish *Clarias gariepinus* (Burchell, 1822) juveniles, *Aquac. Res.*, 36: 868 – 875 (2005).
- [48] Handeland S. O., Imsland A. K. and Stefansson S. O., The effect of temperature and fish size on growth, feed intake, food conversion efficiency and stomach evacuation rate of Atlantic salmon post-smolts. *Aquac.*, 283: 36 – 42 (2008). DOI: 10.1016/j.aquaculture.2008.06.042.
- [49] Orina P. S., Rasowo J., Oyoo-Okoth E., Musa S., Munguti J. M. and Charo-Karisa H., Combined effects of photoperiod and temperature on growth and survival of African catfish (*Clarias gariepinus*, Burchell 1822) larvae under laboratory conditions. *J. Appl. Aquac.*, 28(1): 17 – 25 (2016). DOI: 10.1080/10454438.2015.1113763.
- [50] Hopkins, K. D., Reporting fish growth: A review of the basics, *J. World Aquac. Soc.*, 23(3): 173 – 179 (1992)

Citation: Ambeno FN, et al. *Intraspecific Hybridization, Growth Performance and Survival of Purebreds and Reciprocal Hybrids of two *Clarias Gariepinus* (Burchell, 1822) Strains in the Mount Cameroon Region. International Journal of Research Studies in Biosciences. 2021; 9(3): 01-14. DOI: <https://doi.org/10.20431/2349-0365.0903001>.*

Copyright: © 2021 Authors. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.