IS THERE EVIDENCE OF INBREEDING IN AFRICAN CATFISH (*Clarias gariepinus*) CULTURE IN NIGERIA?

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ABSTRACT

Anecdotal reports from fish farmers and government officials in Nigeria suggest that inbreeding may be a problem harming the availability of quality seed for the culture of African catfish although there is a paucity of studies and clear statistical evidence for this. African catfish broodstocks were collected from 10 different farms (Abuja, Benue, Kaduna, Kano and Adamawa and 5 farms in Oyo) and major rivers in Nigeria. The performance of catfish progeny produced from pure farm bred strains (10 tanks), hybrids formed by breeding together fish from different farms (5 tanks) and the progeny of wild fish (1 tank) was compared. Water quality, survival rate, specific growth rates and weight gain were monitored. The SGR of the wild fish was highest, lowest in the pure farm strain and intermediate in the hybrids which is accounted for by differences initial body mass. This suggests that the growth performance of the aquaculture fish has neither improved nor deteriorated compared with wild fish. The survival rate of the pure aquaculture strains was better than that of hybrids or wild fish. These results show no evidence of inbreeding depression of the aquaculture African catfish in Nigeria. This suggests that the perceived problems with production performance of African catfish in Nigeria could be attributed to other causes such as variation in the quality of the fish originating from different fish farms. These differences may relate to genetic differences between farm strains or differences in management practices of different hatcheries. It is important to note that adequate management practices still need to be put in place to maximize the potential of African catfish stocks in Nigeria. The growth performance of the different strains may be used as a guideline to form a base population for genetic selection to improve performance of *C. gariepinus* in Nigeria.

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1 INTRODUCTION

1.1 Aquaculture Production in Nigeria

The beginning of aquaculture in Nigeria can be traced back to be a government-driven venture in Panyam fish farm, Jos in 1951. Since then, aquaculture has expanded as a private industry (Akinrotimi *et al.*, 2011). Although production is mainly small-scale, there is a wide consensus that aquaculture has the potential to meet the growing demand for fish protein, contribute to growth of the economy and support the sustainable livelihoods of many communities (FAO, 2006, Omitoyin, 2007). From 2003 to 2014, aquaculture production grew rapidly in Nigeria, but in recent years, the production has not increased (Fig.1) and even declined slightly from 2015 to 2017. The reasons for this stall in aquaculture growth in Nigeria are not clear. However, further growth of aquaculture production in Nigeria can contribute significantly to increasing domestic fish supply (Olaoye & Ojebiyi, 2018).

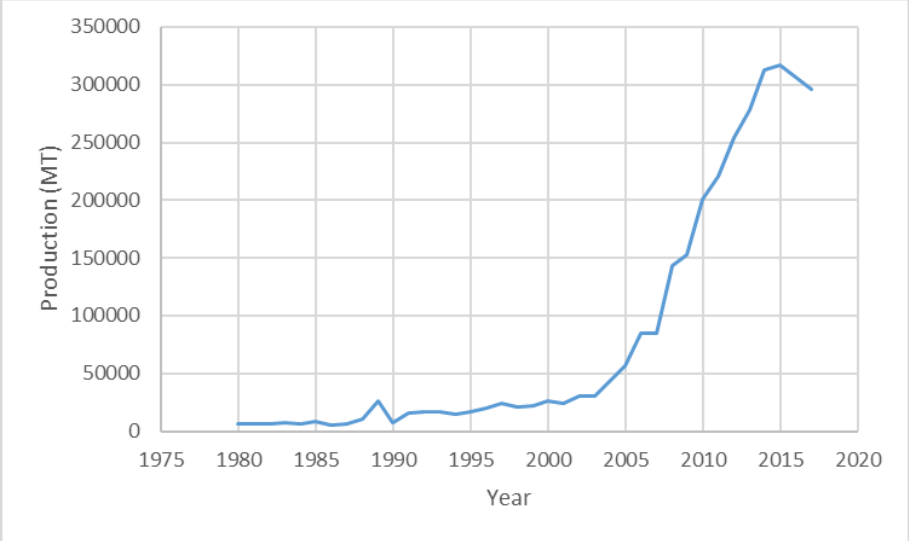

Figure 1. Aquaculture production in Nigeria from 1980 to 2017 (FAO, 2017)

Nigeria is the second largest aquaculture producer in Africa second only to Egypt (FAO, 2017). More than 80% of the total production in Nigeria are African catfishes, primarily *Clarias gariepinus and Heterobranchus spp* (Fig.2) and tilapia, with about 14% of total Nigerian aquaculture production. The country has large natural resources to support aquaculture development, including inland freshwater systems of 14 million hectares and available land area of 1.7 million hectares for aquaculture development (Adewumi, 2015; Omitoyin, 2018; Ajani, 2019).

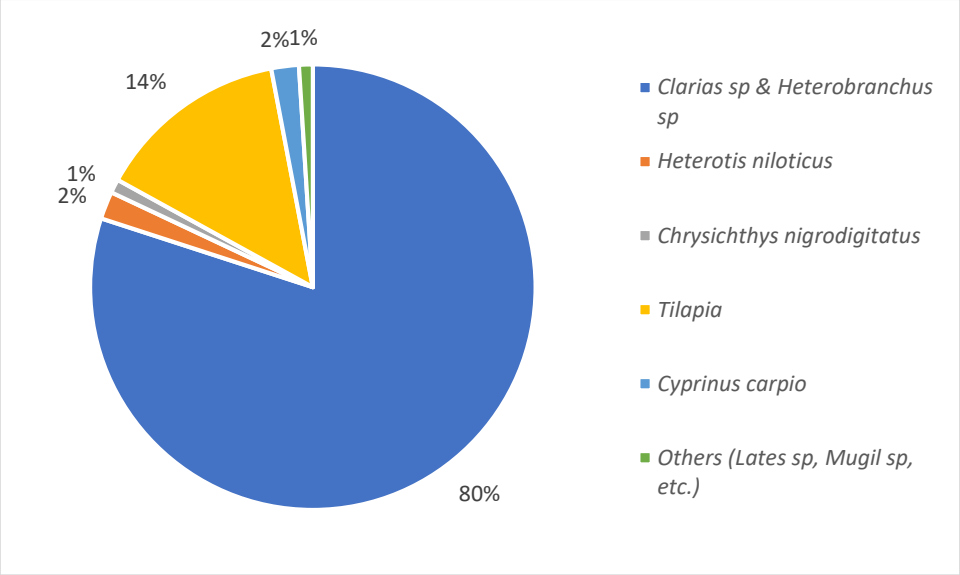

Figure 2. Proportions of common culture species in Nigeria (Adewumi & Olaleye, 2011)

According to the Nigeria Fisheries Statistics report (2016), the annual national demand for fish is estimated to be over 3 million tons. The demand for fish is likely to increase even further in Nigeria due to increasing population, rising awareness of the health benefits from consuming fish, and the fact that fish remains the preferred and affordable animal protein for most of the population. This creates fertile environment for further aquaculture growth in Nigeria. However, the current fisheries and aquaculture production is far from meeting the demand, at only 1.2 million MT, with 0.3 million MT coming from aquaculture (Nigeria Fisheries Statistics, 2016). This suggests that the gap between fish supply and demand is at least 2 million metric tonnes. Today, fish imports of 0.7 million MT supply part of the deficit and, in fact, Nigeria is the fourth largest importer of fish in the world, following China, Japan and the United States (ICIR, 2018). Nigeria spends about $1 billion annually on the importation of fish (Nworie, 2019). By importing such large amount of fish annually, Nigeria loses valuable foreign currency. The importation also stunts the growth and development of trade in local fish industries. One of the measures that the Federal Government of Nigeria has taken to counteract this is to limit the importation of food, including fish.

There are several bottlenecks to further growth of aquaculture in Nigeria. One of these is the availability of quality fish seed. The Federal Government of Nigeria has taken several measures to improve the quality and quantity of fish seed in Nigeria and one of these is the Growth Enhancement Support Scheme which distributes inputs such as catfish juveniles, fish feed, nets etc. at a subsidized price to fish farmers across the country. Furthermore, a broodstock production and seed multiplication project was initiated in 2016 (WAAPP, 2017). It is possible that poor seed quality is in part caused by inadequate broodstock management and inbreeding of broodfish. Anecdotal reports from fish farmers and government officials in Nigeria suggest that inbreeding may be a problem, although there is a paucity of studies and clear statistical evidence for this. Few studies exist on the performance of African catfish in Nigeria. The results of Iwalewa *et al.*, (2017) suggested that a domesticated strain of African catfish had lower fertilization rate, hatchability and survival than fish sourced from wild populations which indicates that there may be problems with genetic management of broodstock, rearing conditions of the brood fish or hatchery management. In most hatcheries, between 70 and 90% of fingerlings (2-5grams) are lost before reaching juvenile (5-52 grams) stage (Bondad-Reantaso, 2007). However, it is clearly necessary to establish a breeding program for African catfish in Nigeria to improve the quality of seed for the growing aquaculture.

1.2 Justification

Many fish producers in Nigeria claim that they have problems with poor egg quality resulting in low hatchability and poor growth rate. A few factors could contribute to this, including inadequate genetic management of broodstock and inadequate hatchery practices. Many fish hatcheries in Nigeria use catfish of the same parentage which could result in inbreeding depression over several generations, leading to a reduction in overall production (Olaleye, 2005). Ibiwoye (2017) found the number of broodfish and the effective breeding number to be low in several hatcheries. This could cause inbreeding depression and genetic drift. The most common practice in Nigeria is to purchase large fish from farmers growing fish for food and keep the fish for some time to use as broodstock. Unfortunately, the farmers cannot provide information on the pedigree of the fish. However, fish farmers in Nigeria appear to be aware of the problem with inbreeding and some take measures such as keeping records of broodstock sources and age to avoid it (Ibiwoye, 2017).

Although fish farmers suspect inbreeding to be a problem in catfish culture, this has not been confirmed with formal research. Adequate studies are still required to ascertain that inbreeding is a problem for the aquaculture industry. The present study will address this issue and provide useful information for the government and the aquaculture industry in Nigeria.

1.3 Objectives

The specific objectives of the study are:

- To evaluate the potential effects of inbreeding on growth and feed intake of African catfish, *Clarias gariepinus*.
- To evaluate the potential effects of inbreeding on the carcass quality of cultured and wild African catfish, *Clarias gariepinus.*

To this end, the experiment was performed to compare the performance of:
(1) catfish progeny produced at farms with local brood-fish
(2) hybrids formed by breeding together fish from different areas and
(3) the progeny of wild fish

1.4 Hypothesis

H01= If there is no significant difference in the growth of African catfish in the three groups of fish tested, then there is no evidence of inbreeding depression

H02 = If the hybrid groups grow better than the pure breed, then that could indicate inbreeding depression.

H03 = If wild fish grows better than the hatchery broodstock, there could be genetic issues with the broodstock, problems with rearing of broodstock or poor hatchery management

2 LITERATURE REVIEW

2.1 Broodstock Management

Broodstocks consists of mature individuals used for producing seed. Broodstock management involves the care and physical handling of the fish before and during reproduction and the maintenance of the genetic integrity of the broodfish by avoiding inbreeding which is important

for producing high quality seed (Azharul *et al.*, 2015). Poor broodstock management can inhibit gonadal development meanwhile good broodstock management and conditioning aid the survival and performance of the offspring (Ude *et al.*, 2005; Phelps, 2010, Migaud *et al.*, 2013). Suitable nutrition of broodstock is important for good reproductive performance of cultured fish, especially dietary protein levels (Coward & Bromage, 2000; El-Sayed *et al.*, 2003; Chong *et al.*, 2004; Muchlisin *et al.*, 2006). The type, nature, quantity and quality of feeds provided to the brood fish will influence and affect the quantity and quality of eggs produced which consequently have a bearing in the fertilization rates, hatchability and survival rates (Phelps, 2010; Ondhoro, 2013).

Another important part of broodstock management is good record keeping of pedigree and attention to the effective breeding number in the broodstock. To produce high-quality seed, the ideal situation is that hatcheries regularly receive broodstock from national breeding centres, produce seed from it, and replace it when its reproductive efficiency declines or ceases. In this way, hatcheries would be multiplying and distributing to farmers seed from the latest generation of the nucleus in the breeding centre.

2.2 Inbreeding

Inbreeding is the mating of relatives. Inbreeding can fix valuable traits in a stock to establish new breeds and varieties and improve the results of crossbreeding programmes (FAO, 1999). Diploid individuals have two complementary sets of chromosomes and, as a result, there are two genes coding for the same trait, one at each locus on the chromosomes. The two genes or alleles can be identical and then the individual is homozygous for the trait. Conversely, if the alleles are different, the individual is heterozygous. The genetic diversity of a population depends on the number of different alleles found in the population. Genetically, inbreeding increases homozygosity in the offspring and decreases heterozygosity (Howard *et al.*, 2017) and relatives are more alike genetically than non-relatives. Inbreeding could undermine genetic gains and genetic variability in fish stock if not monitored and controlled in a breeding program or production systems (Falconer & Mackay, 1996).

Securing a large enough effective breeding number is one effective way to guard against too high levels of inbreeding that can stunt growth and reduce survival, growth, reduce disease resistance, and cause physical deformities. The effective breeding number (N_e) is determined by the total number of potential broodfish (male and female) that make up the hatchery broodfish population and produce viable offspring (Tave, 1999), the sex ratio of the broodfish that spawned, the variance of family size, and the mating system that is used. If all males and females spawn, N_e is equal to the total number of males and females. However, hatchery managers often use unequal numbers of males and females, or in certain cases, not all the males or females spawn, reducing N_e. N_e can therefore be maintained by increasing the number of fish that are spawned and produce viable offspring.

The effective breeding number can be calculated as:

$$N_e = \frac{4N_mN_f}{N_m+N_f}$$

N_m= Number of males

N_f= Number of females

Inbreeding in a population is measured by the inbreeding coefficient (F) and is expressed as the amount of inbreeding that has accumulated starting from a specific point in the ancestry of the population. The inbreeding coefficient (F) is the probability that two alleles at any locus in an individual are identical by descent (i.e. both alleles can be traced back to a single common origin). It is possible to calculate the rate of accumulation of inbreeding with each generation, ΔF.

$$\Delta F = \frac{1}{2\mathrm{Ne}}$$

where N_e is the effective breeding number

Inbreeding (%) = $\frac{1}{2\mathrm{Ne}} \times 100$

2.3 Inbreeding Depression

As inbreeding increases, it often causes a decrease in productivity which is termed 'inbreeding depression'. Every individual carries some deleterious recessive alleles that are not expressed but are masked in the heterozygous state. In situations where deleterious alleles are on both loci (the individual is homozygous for the trait), they can have negative effects on the fitness of the carrier by decreasing larval viability, survival, growth rate, fecundity and reproductive ability of the fish (De Donato *et al.,* 2005). Studies in fish have shown that inbred fish have these clinical signs of inbreeding depression which usually do not occur immediately, but are expressed several generations after inbreeding has begun. The effects of inbreeding on juvenile viability have been well studied in both laboratory and captive populations from a range of taxa (Gjerde *et al.,*1983; Brewer *et al.,* 1990, Evans *et al.,* 2004; Fessehaye *et al.,* 2007). How quickly inbreeding depression occurs depends on the amount of inbreeding that has been produced and the trait. The severity of inbreeding depression also depends on the phenotype in question, and the population.

Inbreeding has a significant negative effect on the production traits of *Oreochromis mossambicus*, especially growth (Akinoshun, 2015). Inbreeding depression has been described for an array of fish species (Waldman & McKinnon, 1993). Inbred rainbow trout, *Oncorhynchus mykiss* had more body deformations and a reduced fry survival (Waldman & McKinnon, 1993), while the specific growth rate of inbred coho salmon (*Oncorhynchus kisutch*) was reduced (Gallardo & Neira, 2005). In other salmonid species, significant inbreeding depression has been reported for growth in Atlantic salmon (Rye and Mao, 1998) and rainbow trout (Pante *et al.,* 2001) for each 10% increment of inbreeding. Inbreeding in zebrafish reduced fertilization success, but not to a reduction in hatching rate. The inbred zebrafish also had a reduced survival, a lower growth rate and a higher number of fry suffering body deformations (Mrakovˇci´c & Haley, 1979). Inbreeding in guppies (*Poecilia reticulata*) reduced males' sexual activity in several populations (Mariette *et al.,* 2006).

2.4 Crossbreeding

In contrast to inbreeding, which increases the frequency of homozygotes, crossbreeding increases heterozygosity. Crossbreeding together with pedigree-based selection can produce improvements in aquaculture species (Vandeputte, 2009). An example of crossbreeding in the industry is the use of *Oreochromis niloticus* × *Oreochromis aureus* hybrids displaying beneficial all-male offspring and improved cold tolerance (Hulata *et al.,* 1993). Also, the *Morone chrysops* × *Morone saxatilis* hybrids, referred to as sunshine bass, grows faster and has better overall culture characteristics than either parental species under commercial culture conditions (Smith, 1988). However, despite the large numbers of reported hybrids, few have

been successfully cultured for extended periods because of the added complexity of production and the introgression of the hybrids back to the parental species (Penman, 1999) including loss of beneficial characteristics due to inbreeding.

2.5 Hatchery practices that may result in inbreeding problems in aquaculture

Often the potential occurrences of inbreeding in salmonids come from management and operational practices of aquaculture where inbreeding may arise at every operational step of broodstock programs (Campton, 1995; Hard & Hershberger, 1995). Factors that can give rise to inbreeding include breeding of relatives maintained as captive broods, insufficient number of breeders and inappropriate spawning practices such as pooling of gametes (Withler & Beacham, 1994). African catfish breeders often use low numbers of broodstock to produce each generation simply because the high reproductive rate of captive breeders increases operational efficiency and can easily exceed facility rearing constraints. This practice can lead to increased inbreeding in ensuing generations compared with wild populations because of the elevated chance that close relatives breed (Verspoor, 1988). The spawning procedures used in hatcheries also affect the inbreeding levels of the ensuing generations through their impact on Effective Breeding Number (N_e).

2.6 Is inbreeding depression a problem in Nigerian aquaculture?

The problem of inbreeding and potential inbreeding depression has not been evaluated in Nigeria before. However, hatchery owners appear to be aware of the problem and take precautionary measures to avoid inbreeding (Ibiwoye, 2017). Fish farmers and fisheries officials claim that inbreeding is a problem in the Nigerian aquaculture. This could be proven in a few ways:

1. Hatchery operators ignorantly select broodstocks from the same cohort. This singular act can lead to inbreeding (Anetekhai *et al.,* 2004). Salami *et al.,* (1993) and Nlewadim *et al.,* (2004) reported that selection of broodstock in the African catfish has largely been through a disjointed, isolated and occasional effort unlike in the case of the American catfish (*Ictalurus punctatus*).

2. The use of less effective breeding number (N_e) (due to high fecundity in fish species) over time can lead to inbreeding. Ibiwoye (2017) discovered that in some of the hatcheries the N_e could even be 20 or lower which could cause problems with inbreeding and genetic drift.

3. Most of the farms do not keep a female to male sex ratio 1:1 which further reduces the effective breeding number. Small effective breeding number increases the probability of genetic drift or inbreeding depression (Tave, 1999).

3 METHODS

3.1 Study population

Broodstocks were obtained from five fish farms in the Oyo zone and from fish farms in five other zones (Abuja, Adamawa, Benue, Kaduna and Kano) (Fig. 3). The driving distance between the farms in Oyo and other zones is 600-1200 km and therefore, there is likely little to no genetic mixing of broodfish between the regions. The broodstock from each farm was mated together to produce Purebred Farm Strains (PFS) (Table 1). To look for evidence of inbreeding depression, broodstock from each of the farms in Oyo was crossed with broodstock

from one of the farms in the other zones (Table 1). Finally, wild fish from two major river systems in Nigeria were crossed to compare the aquaculture strains to wild fish.

The broodstock used in this study were on average 800 grams and 1000 grams, for female and male respectively. The sex ratio of the broodfish was 1 male to 3 females.

Figure 3. Broodstock collection sites (5 farms from Oyo, 5 farms from Abuja, Adamawa, Benue, Kaduna, Kano and 2 major rivers)

3.2 Production of eggs

All broodstock were transported to the fish farm of University of Ibadan, where the growth experiment was conducted. The broodfish were acclimated to conditions at the University of Ibadan fish farm for two weeks before breeding. To induce ovulation, the females were injected with Ovulin (0.5 ml/kg). The hormone was injected intramuscularly without anaesthesia. After a latency period of 12 hours, eggs were collected from each female by gently pressing the abdomen. Milt was obtained by sacrificing the males, collecting testes and squeezing out the milt for fertilization. The stripped eggs were mixed with the milt in appropriate proportions using plastic spoon. Thereafter, 5 ml of isotonic saline solution was added. After thorough mixing, the eggs were incubated in a single layer in incubation troughs. On day 42 after fertilization, the fish were transferred to the grow-out tanks. Eggs, larvae and juveniles from each farm and from each crossing were kept in separate rearing units in the nursery.

Table 1. A list of progenies produced for the experiment

Tank	Origin of broodfish
1	Farm A in Oyo and Farm A in Oyo
2	Farm B in Oyo and Farm B in Oyo
3	Farm F in Benue and Farm F in Benue
4	Farm G in Adamawa and Farm G in Adamawa
5	Farm H in Abuja and Farm H in Abuja
6	Farm I in Kano and Farm I in Kano
7	Farm C in Oyo and Farm C in Oyo
8	Farm D in Oyo and Farm I in Kano
9	Farm J in Kaduna and Farm J in Kaduna
10	Farm D in Oyo and Farm in Oyo
11	Farm A in Oyo and Farm J in Kaduna
12	Farm B in Oyo and Farm in F in Benue
13	Wild fish and Wild fish
14	Farm E in Oyo and Farm E in Oyo
15	Farm C in Oyo and Farm H in Abuja
16	Farm E in Oyo and Farm G in Adamawa

3.3 Grow out phase

The fish from each group were stocked in 16 concrete tanks (width, length depth: 7m×7m×1.2m) from each origin of broodfish. Three hapas (2m×2m×1m) were placed in each tank for replication. The hapas were stocked with 15 fish per m^3, 60 fish in each hapa. The fish were fed to satiation with a 2mm commercial floating feed twice daily (at 9am and 4pm).

3.4 Sampling

Every two weeks, all fish in the hapa were netted out, weighed collectively, and counted to calculate average weight and survival.

3.4.1 Proximate Composition

Fish samples for the proximate composition were collected once during the 3rd week of the experiment. The proximate composition of the fish from the different groups were determined on dry matter basis using the method of AOAC (1990) during the growth trial in triplicates. Moisture content was determined by drying the fresh samples in hot air oven at 70°C to a constant weight. Protein was analysed with micro-Kjeldahl method (Kjeldahl digestion chamber) using 6.25 as the conversion factor for total nitrogen to protein. The crude lipid content was determined by the Soxhlet extraction using petroleum spirit. Ash content was determined by burning off organic material at 600°C for three hours and weighing the samples before and after.

3.4.2 Water Quality

The following water quality variables were measured weekly: dissolved oxygen, ammonia and pH using Hach's Aquaculture Test Kit (Model FF-2) following instructions by the manufacturer. Temperature was measured using mercury-in-glass thermometer by dipping the thermometer into the pond for two minutes with the mercury bulb fully immersed before taking readings.

3.5 Calculations of growth and diet utilizations

The following growth and feed intake indices were collected:

$$Feed\ intake\ (FI) = the\ amount\ of\ feed\ fed\ during\ the\ experiment$$

$$Weight\ gain = final\ average\ weight - initial\ average\ weight$$

The specific growth rate (SGR) was calculated by fitting exponential growth curves to mean weight over time.

$$w_2 = w_1 \times e^{(\Delta t \times \frac{SGR}{100})}$$

Where w_1 and w_2 are the initial and final body mass at time t_1 and t_2 and Δt is the number of days between t_1 and t_2. For each group, a linear curve was fitted to the natural logarithm of mean weights at different time:

$$\ln w = a + b \times days$$

The SGR was calculated from the slope (b) of the curve as:

$$SGR = 100 \times b$$

This approach gives better statistical power than when growth is compared as mean sizes at different times (Thorarensen, *et. al.,* 2015).

$\%Survival = 100 \times \frac{Number\ of\ fish\ harvested}{\text{Total number of fish stocked}}$ (Alatise & Otubusin, 2006)

$Feed\ Conversion\ Ratio = \frac{Feed\ fed\ (g)}{\text{Weight gain}}$ (Utne, 1979)

3.6 Statistical Analysis

The data collected was entered into Microsoft Excel and analysed using the R software (R Core Team, 2013). The growth data was analysed by comparing the mean body mass of Purebred Farm Strain (PFS) to that of the crosses and wild fish. In these analyses, the size was compared with a mixed model Anova with farms were nested within groups (PFS, Crosses and Wild). The mean size of fish from different PFS was also compared using one-way ANOVA. Tukey HSD tests were used to compare the mean values for groups. The SGR of the three groups was compared by comparing the slopes of the growth curves.

4 RESULTS

4.1 Water quality

There were no significant differences in the mean water quality values between the PFS, Cross and Wild groups, however, there were differences among tanks. There were significant ($p<0.0001$) differences in mean dissolved oxygen concentration among different tanks, ranging by 7% (highest to lowest) around the grand mean (5.05; Table 2). There were also significant ($p<0.0001$) differences in total ammonia nitrogen in different tanks where the lowest ammonia concentration (T 9 and T 14) was 33% of the highest ammonia concentration (T1). There were significant ($p<0.01$) differences in pH between different tanks, however, the range from highest to lowest value was less than 1% of the grand mean (7.09; Table 2). There were also significant ($p<0.02$) differences in mean temperature in different tanks, which ranged by 3% of the grand mean.

Table 2. Mean dissolved oxygen, ammonia, pH and temperature based on the individual tanks

Tanks	Dissolved oxygen (mg/l)	Ammonia (mg/l)	pH	Temperature (°C)
T1	5.18±0.10^{b}	0.305±0.04^{c}	7.00±0.12^{ab}	26.85±0.11^{a}
T2	5.29±0.10^{b}	0.180±0.04^{abc}	7.00±0.12^{ab}	27.15±0.11^{b}
T3	4.84±0.10^{ab}	0.300±0.04^{bc}	7.10±0.12^{ab}	27.35±0.11^{b}
T4	4.81±0.10^{ab}	0.220±0.04^{abc}	7.20±0.12^{ab}	27.05±0.11^{b}
T5	5.25±0.10^{b}	0.110±0.04^{ab}	6.90±0.12^{ab}	27.25±0.11^{b}
T6	5.11±0.10^{b}	0.210±0.04^{abc}	7.05±0.12^{ab}	27.50±0.11^{b}
T7	4.58±0.10^{a}	0.240±0.04^{abc}	6.75±0.12^{a}	27.00±0.11^{b}
T8	5.03±0.10^{ab}	0.140±0.04^{abc}	7.15±0.12^{ab}	27.55±0.11^{b}
T9	5.16±0.10^{b}	0.100±0.04^{a}	7.05±0.12^{ab}	27.45±0.11^{b}
T10	5.29±0.10^{b}	0.220±0.04^{abc}	7.00±0.12^{ab}	27.30±0.11^{b}
T11	4.88±0.10^{ab}	0.290±0.04^{abc}	7.20±0.12^{ab}	27.35±0.11^{b}
T12	5.13±0.10^{b}	0.260±0.04^{abc}	6.90±0.12^{ab}	27.10±0.11^{b}
T13	4.95±0.10^{ab}	0.155±0.04^{abc}	7.15±0.12^{ab}	27.50±0.11^{b}
T14	4.96±0.10^{ab}	0.100±0.04^{a}	7.10±0.12^{ab}	27.55±0.11^{b}
T15	5.17±0.10^{b}	0.290±0.04^{abc}	7.50±0.12^{b}	27.20±0.11^{b}
T16	5.09±0.10^{ab}	0.110±0.04^{ab}	7.35±0.12^{ab}	27.05±0.11^{b}

Means in the same column superscripted by different letters were significantly different ($P < 0.01$)

The water quality (dissolved oxygen, ammonia and temperature) varied over time and there were significant differences during different weeks (Table 3). The mean oxygen concentration during week 12 was significantly lower (Table 3). The total ammonia concentration rose by 158% from week 2 to week 14 when it was significantly higher than during all other weeks (Table 3). The temperature varied by 5% of the grand mean. There was no significant ($p=0.7$) change in pH over time.

Table 3. Mean dissolved oxygen, ammonia, pH and temperature based on culture period

Week	Dissolved oxygen (mg/l)	Ammonia (mg/l)	pH	Temperature (°C)
2	4.87±0.008^{a}	0.131±0.03^{a}	7.09±0.097^{a}	27.9±0.12^{d}
4	5.24±0.008^{bc}	0.181±0.03^{a}	7.06±0.097^{a}	27.8±0.12 ^{cd}
6	5.52±0.008^{c}	0.225±0.03^{a}	7.03±0.097^{a}	26.6±0.12^{a}
8	5.13±0.008^{b}	0.138±0.03^{a}	7.12±0.097^{a}	27.9±0.12^{cd}
10	5.07±0.008^{b}	0.175±0.03^{a}	7.09±0.097^{a}	28.1±0.12^{d}
12	4.49±0.008^{a}	0.169±0.03^{a}	7.09±0.097^{a}	27.9±0.12^{d}
14	4.94±0.008^{b}	0.388±0.03^{b}	7.03±0.097^{a}	27.3±0.12^{bc}
16	5.10±0.008^{b}	0.219±0.03^{a}	7.09±0.097^{a}	26.8±0.12^{ab}
18	5.04±0.008^{b}	0.212±0.03^{a}	7.06±0.097^{a}	26.7±0.12^{ab}
20	5.06±0.008^{b}	0.181±0.03^{a}	6.97±0.097^{a}	26.8±0.12^{ab}

Means in the same column superscripted by different letters were significant different ($P< 0.01$).

There was significant correlation among water quality and growth variables. Most notably, there was a significant negative correlation between both mean total dissolved ammonia concentration ($p=0.02$) and survival, and minimum pH and survival ($p=0.2$) (Table 4). In some cases, the results appear to reflect rather the effect of water quality on fish such as the negative correlation between mean oxygen concentration and final body mass ($p=0.02$) as well as increase in body mass ($p=0.02$) (Table 4). Similarly, there was a positive correlation between minimum total dissolved ammonia and initial ($p=0.006$), final body mass ($p=0.02$) and increase in body mass ($p=0.04$). However, other correlations may reflect negative effect of water quality on growth. There was a negative correlation between maximum temperature and increase in body mass ($p=0.02$) as well as final body mass ($p=0.02$) (Table 4). There was a significant

positive correlation between maximum pH and initial body mass (p=0.03), final body mass (p=0.004) the increase in body mass (p=0.007) and between minimum pH and final body mass (p=0.05).

Table 4. Correlation coefficients between water quality variables and growth indices. Values indicated by * are significantly different

	Initial body mass	Final body mass	Increase in body mass	Final number	Final biomass	Feed intake	FCR
Initial body mass		0.15	0.05	0.1	0.11	0.40*	0.01
Final body mass	0.15		0.99*	-0.19	0.7	-0.14	-0.52*
Increase in body mass	0.05	0.99*		-0.2	0.69*	-0.18	-0.52*
Final number	0.1	-0.19	-0.2		0.55	0.34*	-0.68*
Final biomass	0.11	0.70*	0.69*	0.55*		0.06	-0.93*
Feed intake	0.40*	-0.14	-0.18	0.34*	0.06		-0.03
FCR	0.01	-0.52*	-0.52*	-0.68*	-0.93*	-0.03	
Mean temperature	-0.2	-0.2	-0.18	0.03	-0.1	-0.18	0.06
Mean DO	0.14	-0.3*	-0.32*	0.18	-0.12	0.36*	0.11
Mean NH_3	0.17	0.23	0.21	-0.32*	-0.08	-0.01	0.16
Mean pH	0.22	0.06	0.04	-0.18	-0.07	-0.01	0.11
Maximum Temperature	-0.13	-0.35*	-0.34*	0.05	-0.22	-0.11	0.22
Maximum DO	-0.08	-0.22	-0.21	0.04	-0.17	0.09	0.15
Maximum NH_3	0.4*	0.22	0.18	-0.23	-0.04	0.2	0.14
Maximum pH	0.31*	0.41*	0.38*	-0.13	0.24	0.1	-0.1
Minimum temperature	-0.13	0	0.01	0.09	0.08	-0.19	-0.15
Minimum DO	0.27†	0.02	-0.01	0.14	0.11	0.25†	-0.06
Minimum NH_3	0.39*	0.34*	0.30*	-0.21	0.08	0.14	0.04
Minimum pH	0.22	0.29*	0.27†	-0.33*	0.01	-0.1	0.08

4.2 Survival Rate

The mortality rate was highest during the first six weeks for all the groups (Fig. 4). After that, the mortality rate appeared to be constant in all groups at 1.5-2% per week. The number of fish in all groups decreased significantly with time ($p<0.0001$). The survival rate in the PFS was 73% and significantly higher than in the Crosses (62%) and Wild fish (56%).

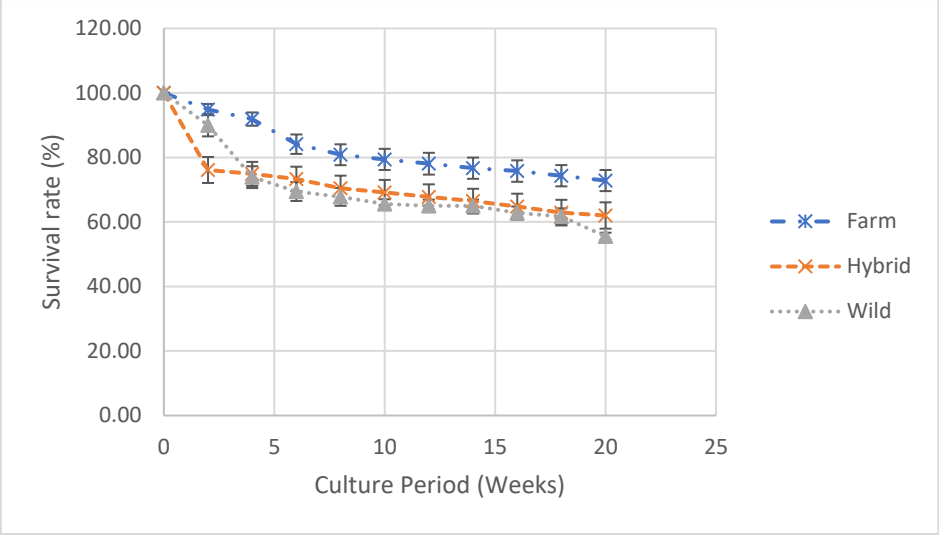

Figure 4. Survival over time of fish pure bred farm fish, crosses and wild fish

There was a significant difference in survival rate between tanks which ranged from 44% to 94% (Fig. 5).

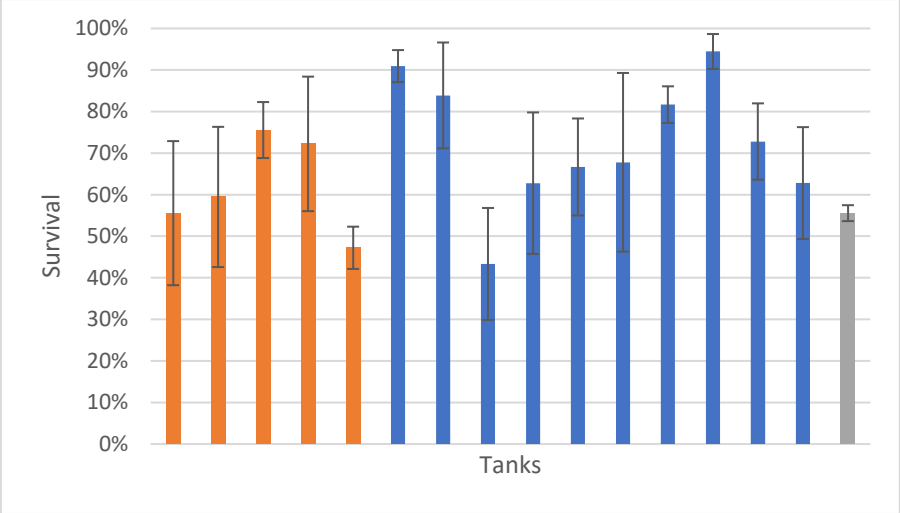

Figure 5. Survival in different tanks. Each of the bars represent individual tank, however, the orange bars are the crosses, the blue bars are the pure-bred farm strains and the grey bar is the wild fish

4.3 Body mass

The initial body mass of the wild fish was the smallest, although, there was no significant ($p=0.09$) difference in the initial body mass of fish from the three groups (Fig. 6). However, there was a significant ($p=0.003$) difference in the initial body mass between tanks (Fig. 6). Similarly, there was no significant ($p=0.2$) difference in the final mean body mass for different groups (Fig. 6) while there was a significant ($p=0.003$) difference in the final body mass of fish in different tanks.

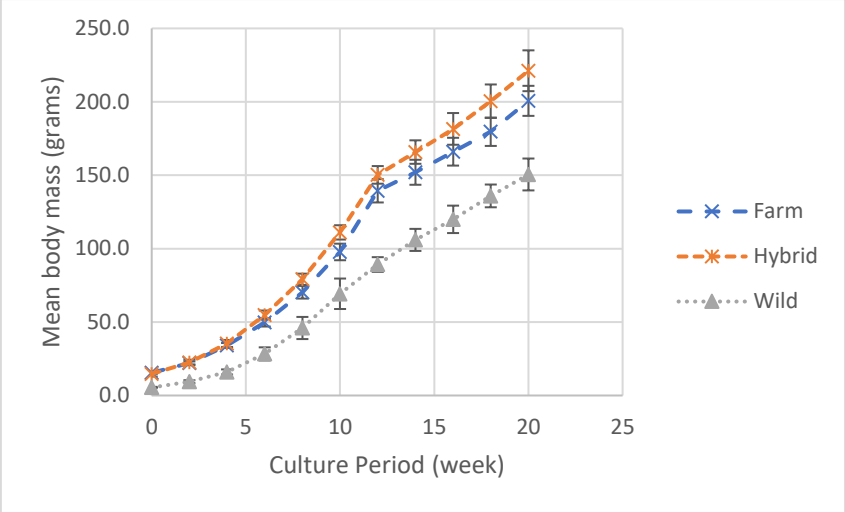

Figure 6. Growth of African catfish of different origin.

The Farm fish are pure farm bred strain; Hybrids are hybrid aquaculture strains and the Wild fish are descended from wild strain, growth on logarithmic scale is shown in Fig. 7.

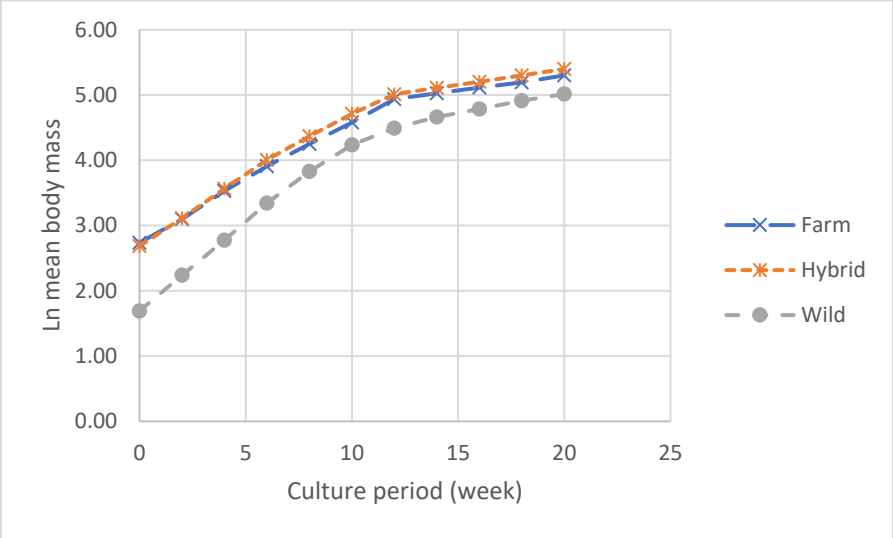

Figure 7. Body mass of African catfish stocks across the three groups (logarithmic scale)

Interestingly, there was not a significant correlation between initial and final body mass (Fig. 8 and 9).

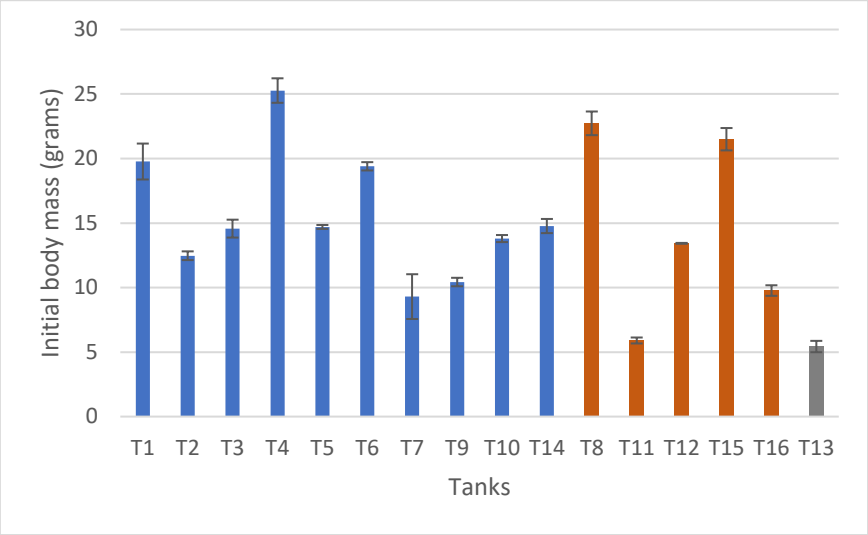

Figure 8. Initial body mass of different tanks. Each of the bars represent each tank, however, the blue bars are the pure-bred farm strains, the orange bars are the crosses and the grey bar is the wild fish

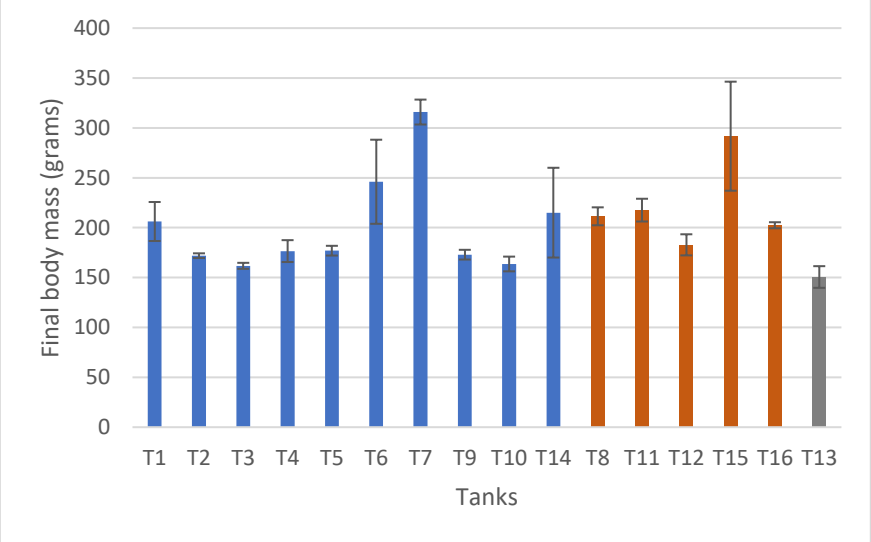

Figure 9. Final body mass of different tanks. Each of the bars represent each tank, however, the blue bars are the pure-bred farm strains, the orange bars are the crosses and the grey bar is the wild fish

There was a significant ($p<0.0001$) difference in the increase in body mass in different tanks (Table 5) both for the first 10 weeks and during the entire experiment. The difference in growth was more than two-fold, the lowest in tanks 1, 4, and 10, which were all pure farm strains, and highest in tanks 6 and 14, which were also pure farm strains.

Table 5. Increase in body mass in the different origin of broodfish

	Until week 20	**Until week 10**
Tanks	Mean Values	Mean Values
T1	149.8±22.2[a]	59.6±12.4[a]
T2	211.7±53.5[b]	103.9±30.0[b]
T3	168.3±53.5[b]	89.7±30.0[ab]
T4	145.1±53.5[b]	63.8±30.0[ab]
T5	200.3±53.5[b]	80.8±30.0[ab]
T6	270.2±53.5[c]	110.1±30.0[c]
T7	192.6±53.5[b]	92.5±30.0[ab]
T8	186.4±53.5[b]	82.6±30.0[ab]
T9	159.4±53.5[b]	51.8±30.0[ab]
T10	147.2±53.5[b]	74.1±30.0[ab]
T11	151.2±53.5[b]	77.9±30.0[ab]
T12	162.2±53.5[b]	70.6±30.0[ab]
T13	226.6±53.5[d]	106.7±30.0[d]
T14	306.6±53.5[e]	138.2±30.0[e]
T15	188.6±53.5[b]	85.7±30.0[ab]
T16	162.4±53.5[b]	80.4±30.0[ab]

Means in the same column superscripted by different letters were significant different (P< 0.01).

4.4 Specific Growth Rate (SGR)

Fish in all groups grew well until week 12, after which, growth rate decreased. It is not clear why growth rate decreased rapidly after week 12, but this issue will be addressed in the discussion.

Since the growth progressed in two stanzas, the SGR was analysed separately for the two periods. From week 0 to week 10 the SGR was significantly (p=0.0003) different in the three groups (Table 6). It was highest for the wild fish, lowest in the PFS group and, intermediate in the Hybrid group (Table 6). However, these differences appear to be primarily related to differences in initial body mass. From week 12 to the end of the experiment there was not a significant (0.0678) difference in SGR (Table 7).

Table 6. Specific Growth Rate (±SE) from week 0 to week 10 for different groups. The SGR was estimated by fitting the exponential curve $w = a \times e(day \times SGR/100)$. The table shows the intercept (a) and growth rate as SGR

	Farms	Hybrid	Wild
Intercept (g)	15.07±1.11[ab]	13.87±1.09[a]	5.60±1.24[b]
SGR (% day^{-1})	2.67±0.11[a]	3.06±0.09[b]	3.66±0.21[c]

Means in the same row superscripted by different letters were significant different (P< 0.01).

Table 7. Specific Growth Rate from week 12 to week 20

	Farms	Hybrid	Wild
Intercept (g)	77.37±1.16[a]	84.77±1.13[b]	41.86±0.39[b]
SGR (% day^{-1})	0.65±0.10[a]	0.67±0.08[a]	0.92±0.20[a]

Means in the same row superscripted by different letters were significant different (P< 0.01).

The log transformed body mass increased linearly with time in all groups from week 0 to week 10 and from week 12 to week 20 (Fig. 10).

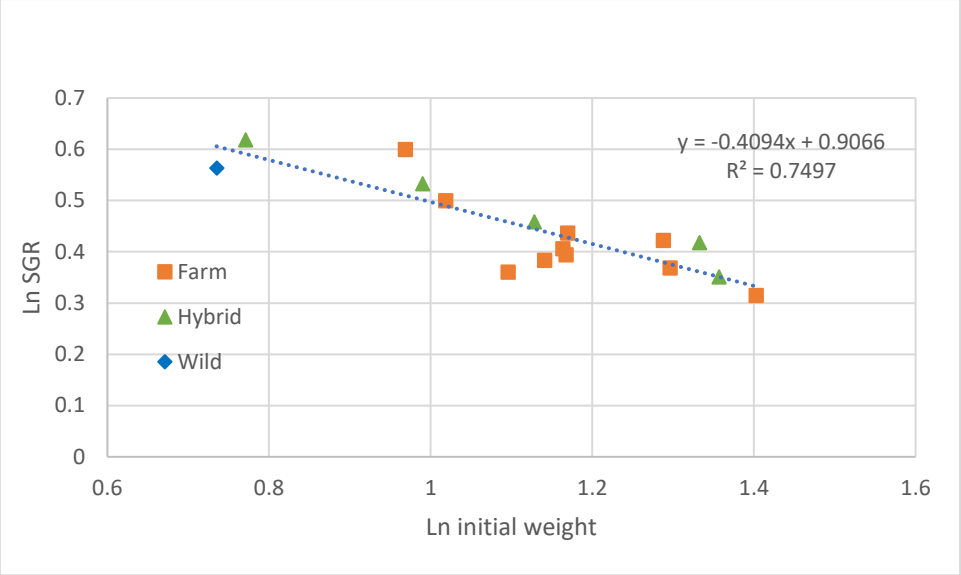

Figure 10. The relationship between the natural logarithm of initial weight and logarithm of specific growth rate

There were significant ($p<0.0001$) differences in SGR between fish from different origin (Table 8) from week 0 to week 10. The highest SGR was in tank 7, but that tank also had the lowest initial body mass. There were also significant differences between the intercepts, reflecting differences in initial weight. Similarly, there were also significant differences in SGR and the intercepts from week 12 to the end of the experiment.

Table 8. Specific Growth rate of only farm stocks until week 10

Tank	Intercept (g)	SGR (% day^{-1})
T1	3.03 ± 0.07^{a}	2.34 ± 0.17^{a}
T2	2.52 ± 0.18^{b}	2.37 ± 0.41^{b}
T3	2.77 ± 0.18^{c}	2.55 ± 0.41^{b}
T4	3.15 ± 0.18^{d}	2.07 ± 0.41^{b}
T5	2.79 ± 0.18^{d}	2.45 ± 0.41^{b}
T6	2.96 ± 0.18^{d}	2.64 ± 0.41^{b}
T7	2.28 ± 0.18^{e}	3.98 ± 0.41^{c}
T9	2.37 ± 0.18^{f}	3.16 ± 0.41^{d}
T10	2.58 ± 0.18^{g}	2.42 ± 0.41^{b}
T14	2.70 ± 0.18^{h}	2.73 ± 0.41^{b}

Means in the same row superscripted by different letters were significant different ($P< 0.01$).

4.5 Feed Intake

There was no significant difference in the increase in biomass, feed intake and FCR of the fish from the three different groups. The quantity of feed consumed reduced precipitously between weeks 12 and 14 resulting in reduced growth (Fig. 11).

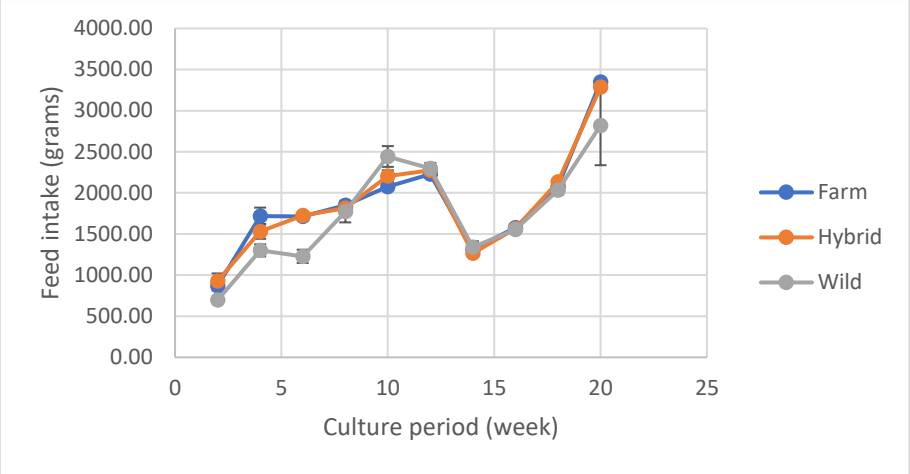

Figure 11. Total feed intake of African catfish of three groups

There was a significant (p=0.016) difference between tanks in increase in biomass (Table 9). The total biomass (harvest) from tank 7 was more than three-fold that of tank 1. There was also a significant (p=0.016) difference in FCR. The FCR in tank 7 (1.74) was 32% of the FCR in tank 1 (5.74).

Table 9. Mean increase in biomass, total feed intake and FCR in three groups of African catfish during the entire experiment

Group	Increase in biomass	Feed intake	FCR
Cross	6808.9±899.6	18725.2±261.2	3.2±0.4
PFS	6987.4±1101.7	18765.6±319.9	3.1±0.5
Wild	4523.1±2203.5	17480.5±639.8	3.9±1.1

4.6 Proximate Composition of the fish flesh of the different tanks

There is no significant difference in the proximate composition of the different strains between farms (Table 10).

Table 10. Proximate Composition of the fish flesh of the different tanks

Tanks	Crude Protein (%)	Ash (%)	Crude lipid (%)	Moisture content (%)	Dry matter (%)
T1	48.5	8.0	8.0	75.3	24.7
T2	45.8	7.3	7.8	75.2	25.2
T3	49.4	6.8	8.6	74.7	25.3
T4	48.9	4.3	7.8	72.2	27.8
T5	51.0	8.0	7.5	76.7	23.3
T6	41.9	6.9	6.8	71.7	28.3
T7	51.1	6.7	7.5	77.0	23.0
T8	32.6	6.7	6.8	74.3	25.7
T9	43.7	6.8	7.8	76.3	23.7
T10	50.4	8.3	8.1	75.1	24.9
T11	45.3	9.7	7.2	75.5	24.5
T12	38.9	7.8	8.0	75.9	24.2
T13	44.7	8.6	8.4	76.1	23.9
T14	49.2	6.7	9.0	75.2	24.8
T15	47.5	7.8	8.8	73.5	26.5
T16	56.6	6.5	9.2	77.1	22.9

5 DISCUSSION

This project addressed an important question for aquaculture of African catfish in Nigeria: Is inbreeding a problem that causes reduced growth and increased mortalities of aquaculture fish? The primary objective of the experiment was to answer this question by comparing the growth and survival of fish descended from broodstock in each of 10 fish farms to hybrids made by crossing fish from farms located more than 1000 km apart. The assumption was that the broodstock in distant farms were less related than the broodfish in nearby farms. If inbreeding is a problem, then the hybrids (assumed to be less inbred), would grow better, with reduced mortality compared with the pure farm strains. The growth and survival of these two groups was also compared to that of fish descended from broodfish sourced from the wild. This is an important benchmark to indicate if the production performance of the aquaculture strains is reduced or improved compared with their wild ancestors.

The results of the experiment suggest that there is no difference in growth performance between the PFS and the hybrids and the survival of the former was better. Therefore, although broodfish may be inbred, there is no evidence that it is affecting the production performance of the fish. However, there is a large difference in the growth and survival of fish from different fish farms, which may suggest that broodstock management and seed quality are problem.

It was important in the experiment that the rearing conditions are the same in all groups. Water quality in tanks was not significantly different among the three groups (PFS, hybrids and wild). However, there was a significant difference in the mean values of some water quality variables among different tanks. However, in most cases, these differences were relatively small. The mean temperature in different tanks ranged by less than 1 °C 26.9 to 27.6 °C and was near optimum temperature conditions (24-29 °C) for African catfish (Henken *et al.*, 1986). Some water quality variables appear to have been affected by increased biomass. Thus, oxygen concentration was negatively correlated with final body mass and the increase in body mass, suggesting that bigger fish consume more oxygen than smaller fish. However, there was a positive correlation between mean oxygen concentration and feed intake, suggesting that increased oxygen levels stimulate feed intake and growth of African catfish as it does for other species (Buentello *et al.*, 2000; Thorarensen & Farrell, 2011, Thorarensen *et al.*, 2010, 2017). However, in the present experiment, oxygen levels were within the recommended levels (2.8mg/l to 6.6mg/l) for African catfish (Keremah *et. al.*, 2014).

The results suggest that water quality may have affected the survival and growth of the fish in some cases. There was a negative correlation between survival and mean ammonia concentration, suggesting that increased mean ammonia levels may cause mortalities. The mean total ammonia levels (0.3 mg/l) and the maximum total ammonia levels (0.5 mg/l) were within the recommended levels for African catfish (<2 mg/l) (Schram *et al.*, 2010). Moreover, the growth rate of the fish in all groups was reduced from week 12, when the ammonia concentration increased to a peak. This suggests that high ammonia levels may have affected the growth of the fish during the experiment. There was also a negative correlation between survival and pH levels. The pH levels were within the recommended range (6.5-9) for African catfish (Pedapoli & Ramudu, 2014; Ajiboye *et. al.*, 2015). However, reduced pH indicates increasing CO_2 levels which in turn may lead to reduced growth or even increased mortality.

The measurements of water quality were performed only once each week and, therefore, it is possible that the measurements may have missed extreme values that could have affected survival and growth. However, this suggests that more focus should be placed on water quality to ensure maximum growth. In summary, these results suggest that different water quality may account for some of the differences in growth between tanks. However, there were no significant differences in water quality between the experimental groups (PFS, Hybrids, Wild).

The survival varied between the three groups with the best survival in the PFS group while survival in hybrids and wild fish was lower. There were also significant differences in the survival from different farms. The mortality rate during on-growing was highest in all groups just after the fish were stocked in the tanks and could be due to handling stress. Several factors can affect the survival rate of the fish including broodstock conditioning, hygiene, water quality management and handling (De Graaf & Janssen, 1996). Therefore, it is possible that differences in broodstock management may account for some of the differences in survival between fish from different farms. However, it is not clear why the hybrids had lower survival than the PFS fish. Some of the mortalities may have been caused by cannibalism, especially if there are shooters growing faster than other fish (Hecht & Appelbaum, 1988; Baras & Almeida, 2001). However, the fish were sorted within a narrow size range before they were stocked in the tanks and, therefore, size heterogeneity should have been minimal at the start of the experiment when the mortalities were highest. It is not clear if size heterogeneity was greater in the Hybrid and Wild fish than in the PFS fish.

Several approaches were used to measure the growth of the fish, including final body mass, increase in body mass and SGR. There were no significant differences in initial and final body mass or increase in body mass among the three different groups. However, there were significant differences in all these variables between fish originating from different farms. The analysis of the SGR has the highest statistical power of all the growth indices (Thorarensen *et al.*, 2015). There were significant differences in the SGR of the three groups until week 10 with the highest growth rate in the Wild fish and lowest in the PFS while the Hybrids were intermediate. However, in general, SGR decreases with increasing body mass such that the logarithm of SGR decreases linearly with increasing body mass (log transformed) with a slope between -0.3-0.45 (Jobling, 1994; Ali *et al.*, 2003). A similar linear relationship (slope -0.4) was obtained when the SGR of the fish in the present study were plotted as a function of their initial body mass. The initial body mass of the Wild fish was the smallest and, therefore, the SGR in this group was high. The SGR of other fish falls around the line suggesting that the observed differences in SGR in the Wild, Hybrid and PFS fish do not reflect differences in growth performance but merely differences in initial body mass. Therefore, there are no significant differences in the growth performance between the three groups. There are, however, very clear differences in the SGR from fish originating from different farms. For example, fish from tank 7 grow much better than would be predicted by their initial size. After week 10 there were no significant differences in growth rate between the three groups, but there were still differences in SGR between different farms. The findings that the growth of fish from different fish farms varies concurs with findings of other studies that there can be significant differences in growth performance in different populations of Africa catfish (Nguenga *et al.*, 2000; Giddelo *et al.*, 2002; Ibrahim *et al.*, 2013). This may suggest that it is important to compare the growth capacity of different populations of African catfish before establishing a breeding program.

Taken together, these findings show that there is no difference in growth rate among wild fish populations tested, the PFS and hybrids. This suggests that inbreeding depression does not

account for inadequate growth performance of the African catfish in Nigeria. Moreover, the survival of the PFS is slightly better than that of the hybrids and the wild fish. Crossbreeding is used to improve the performance of aquaculture fish (heterosis) (Mires, 1982; Bartley *et al.*, 1997; Dong & Yuan, 2002; Anita, 2004; Jothilakshmanan & Marx, 2013). However, we find no evidence for heterosis in the hybrids. Perceived problems with growth performance of African catfish are more likely due to variations in the quality of the fish originating from the different fish farms. Several factors could contribute to this, both genetic but also different management practices for broodfish such as feed quality or other environmental conditions in the farms. Having said that, it is still important to ensure the genetic integrity of aquaculture fish in Nigeria by establishing a centralised breeding program. The fact that the wild fish performed equally well as the aquaculture fish suggest that there has been no improvement in the quality of fish used for aquaculture and that also underlines the importance of establishing a breeding program.

The fact that the wild fish performed equally well as the aquaculture fish suggest that the domestication of the fish has not involved improvement in any production related traits. The aquaculture populations have not deteriorated compared with the wild fish, but this could happen if the broodstock are not properly managed genetically. This also emphasises the importance of establishing a centralised breeding program for African catfish in Nigeria to ensure access to good quality broodstock and to improve production related traits of the fish. Since genetic improvement through breeding progresses gradually over time, a breeding program should be established immediately.

Mean values of crude protein, dry matter, ether extract, moisture contents and ash content in the tissues of all the different farms of *C. gariepinus* exhibited no significant differences ($p>0.05$). The proximate composition seems parallel across all the various groups of fish stocks. This agrees with the fact that the various groups were fed with the same feed from the beginning of the experiment to the end of it. And so, variation is not expected in the fish flesh composition since the flesh composition is basically dependent on the quality of the nutrition given to the fish. The moisture content was within previously reported range in other fishes (Osibona *et al.,* 2006). Percentage moisture in the muscle was within the acceptable levels (30% - 80%) (Eyo, 2001). The crude protein contents were within the range previously reported for *C. gariepinus* and other fishes (Murray & Burt, 1991; Afolabi *et al.,* 1984; Eyo, 2001; Osibona *et al.,* 2006, Onyia *et al.,* 2007). This is within the range reported for fish (Mendez *et al.,* 1996).

6 CONCLUSION

The SGR of the wild fish was highest, lowest in the pure farm strain and intermediate in the hybrids which is accounted for by differences initial body mass. The growth of the fish descended from wild broodstock was comparable to that of the aquaculture fish. This suggests that the growth performance of the aquaculture fish has neither improved nor deteriorated compared with wild fish. These results show no evidence of inbreeding depression of aquaculture African catfish in Nigeria. This suggests that the perceived problems with production performance of African catfish in Nigeria are more likely due to variation in the quality of the fish originating from different fish farms. These differences may relate to genetic differences between farm strains or differences in management practices of different hatcheries.

7 RECOMMENDATION

Adequate management practices such as feed quality, water quality, etc still need to be put in place to maximize the potentials of African catfish stocks in Nigeria.

A selective breeding program should be set up by the Federal Government of Nigeria in order to make quality broodstocks available to the farmers across the country. The growth performance of the different strains may be used as a guideline to form a base population for genetic selection to improve performance of *C. gariepinus* in Nigeria. If the genetic improvement is targeted at the development of a fast-growing fish, then the best performing strain is appropriate to be included in the population for selective breeding program.

Again, further studies need to be carried out to detect an evidence of inbreeding depression in the aquaculture African catfish in Nigeria.

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