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FEMALE BROODSTOCK SIZE AND MATERNAL EFFECTS ON PROGENY OF *Clarias gariepinus* (BURCHELL 1822)

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ABSTRACT

Catfish farmers have been enduring having to spawn with very big females at high cost avoiding medium and small sizes. The relationship between female broodstock weight and the growth rate of progeny has been a major concern among catfish producers. To examine the impact of different female broodstock sizes on the reaction to growth of the fish seed, induced breeding of the study fish was done. The experiment was designed a completely randomized design (CRD) made of three treatments replicated three times. The treatment were three categories of weight of broodstocks as Treatment I (1000g (Treatment I or Small size), Treatment II (1500g or Medium size) and Treatment III (2500g or Large size). There was no significant fluctuation in the physico-chemical parameters of the water. There was no significant difference in the fry survival among Treatments (P>0.05). The maternal effect on growth of the study fish was 10.5 weeks of culture as Treatment 11 fingerlings then grew fastest. At the end of 40 weeks culture period there was significant difference (P<0.05) in the final weight of the table fish (1231.67 g, 1156.67g, and 1006.67g, for Treatment 11, Treatment 111, and Treatment 1, respectively). Overall, the results of these growth evaluations showed that the selected group had improved growth performance. Medium sized female broodstock can therefore deliver substantial genetic improvements in induced breeding of the study fish.

Keywords: *Broodstock size, Clarias gariepinus, Female, Maternal effect.*

INTRODUCTION

The continuous growth of aquaculture, reported by Galappaththi *et al*. (2020), greatly depends on the production of fish seeds with good survival and growth rates and better feed conversion efficiency, among other factors. The production output of the major species cultured in Nigeria are Tilapia 5.4%, Carp 14.6%, Clariid Catfishes, 73.2 %, Ornamental 5.4%, and Others 1.8% (Wally, 2016). Thus, aquaculture in Nigeria is essentially about Clariid catfish culture and our hope of increased sustainable fish supply therefore hangs on its development and improved. To achieve this improved state of aquaculture, the sustainable supply of best quality fish seed is essential. High quality seed can only be produced from proper breeding practices and utilization of biotechnology (Achegbulu *et al*., 2013).

Selection is the primary breeding programme targeted at the broodstock, and directs to a large degree, the quality of offspring, and subsequently fish production. Fish hatcheries have been in operation for many cultured fish species with inadequate corresponding programmes for genetic improvement leading to poor and deteriorating outputs in hatcheries. This deterioration was earlier attributed to the combined effect of selection in the wrong direction and to inbreeding by Eknath and Doyle (1990). This often leads to large variation in fish size, low survival, low growth rate, and poor feed conversion ratio. This eventually causes poor returns to fish farming. Efforts have been made by Ponzoni *et al*. (2012), to explain the genetic basis of the deterioration of stock performance and to prescribe methods to avoid it.

The maternal effect is a directional filtering of traits used in selective breeding. 'Maternal effects are the impacts made by the size, age and condition of the female on quality of the eggs, and growth and viability characteristics of the embryo after fertilization' according to Falconer and Mackay (1996). They defined maternal effect as the influence attributable from the mother to the phenotypes of her offspring. They added that variability of the larvae stages due to paternal effects is insignificant as males have much smaller gametes than females. Falconer and Mackay (1996), suggest that detailed knowledge of the maternal effect is necessary for any breeding selection programme to be successful. Such directional filtering of traits has been shown to be an important and indispensable step in genetic breeding programs (Xu *et al*., 2015). Xu *et al*. (2015) noted that 'the longevity of the maternal effect is smaller in fish that are grown in higher water temperatures and have more rapid developmental rates'. For instance, *Oreochromis niloticus* has rapid embryonic development and spawns at high temperature and its maternal effect on fry size lasts for only 20 days (Siraj *et al*., 1983), suggesting that maternal effects should not bias selection beyond this point.

Palada-de Vera and Eknath (1993) noted that *Oreochromis niloticus* fingerlings initial size did not affect growth; albeit, replication used in the experiment was minimal. No such study has been done for the African Catfish *Clarias gariepinus*. The closest of such studies are those of Megbowon *et al*. (2013), where the breeding performance of specie from different Nigerian waters was studied. The most relevant study so far in Nigeria on broodstock selection using size rage of *Clarias gariepinus* is that of Atagbuna *et al*. (2012), where not only the female but also the male broodstock weight was the selection criteria. The study did not particularly consider maternal effect of the selection, a tool for directional filtering of traits, which has been shown by Xu *et al*. (2015), to be an important and indispensable step in genetic breeding programs. This work thus, seeks to initiate a genetic breeding programme (the biological technology of selective breeding, specifically the traditional selective breeding) that will provide data for such directional filtering of traits, for the African Catfish *Clarias gariepinus*.

MATERIALS AND METHODS

This study was conducted over a period of 18 months at the fish hatchery facility of Marine Farms. Marine Farms has a reputable hatchery, and it is located at No.2 Orogun Street, Isiohor Village, Benin City

Preparation of fish hatchery and culture tanks

A concrete grow out tank (10m x 12.5m x 1.5m), three rectangular hatching tanks (2.3m x 1.0m x 1.0m) each partitioned into 3 compartments, nine 5 l plastic bowls, nine 60 l plastic aquaria, and nine grow-out ponds (2m x 8m x 1.5m), were used for this study. The tanks were washed using a detergent and a disinfectant and properly rinsed with clean water prior to impoundment with water. Grow-out tanks were fertilized using poultry droppings to raise plankton prior to stocking, while in the hatching tanks, the kakabans were laid overnight inside clean water of about 0.3m depth. Broodstock tank and grow-out tanks were filled with water to 0.8m depth from a nearby borehole via an overhead tank all within the farm. The nursery ponds were filled with water up to about 0.6-1m depth.

Selection of experimental fish

i. Selection of foundation stock

Five hundred (500) *Clarias gariepinus* fingerlings were selected from a reputable hatchery in Benin City based on uniformity of size, age, health status (only fish seeds that were healthy and active were selected), nutritional history (all fish seed raised on the same diet) and parental origin (all fish seed raised from the same parents). These fingerlings were raised for 12 months.

ii. Selection of broodstock from the foundation stock (selection within cohort)

From the foundation population (500 individuals), using individual selection method based on biological fitness and weight, one-year old *Clarias gariepinus* females with average weight category of 1kg, 1.5kg and 2.5kg were selected. The biological fitness was based on presence of a round and turgid papilla, softness of abdomen and uniform size of intra-ovarian oocytes as selection criteria (Sahoo *et al*., 2004). Oocytes were obtained by applying a slight pressure on the abdomen (Egwenomhe *et al*., 2019). Random selection and measurement, using a micrometer screw gauge, of thirty to forty oocytes followed. The selected females were grouped into three categories based on their weights e.g. 1,000g, 1,500g and 2,500g. Each of these weight categories represented a treatment.

iii. Selection of male broodstock

Individual selection method was used to also obtain the males. Progeny of a different set of parents, weighing about 2.5-3kg each, were sourced from a farm in Ibadan, South-West Nigeria and selected on the basis of elongated and turgid, reddish genital papilla (Billard *et al*., 1984). One male was used for each of the 3 trials thus a total of three male fishes were selected.

Eggs obtained, as recommended by Egwenomhe *et al*. (2022), from a total of 9 selected females and milt from one selected male, each trial were used for the study.

Experimental design

The experiment was designed as Completely Randomized Design (CRD) made of three treatments. The treatments were three broodstock weight categories namely; Treatment 1 (Small size females of 1000g average weight), Treatment 11(Medium size females of 1,500g average weight) and Treatment 111 (Large size females of 2,500g average weight). Each treatment was replicated three times

Experimental procedure

The experiment was conducted in four stages. The stages and activities in each stage is as follows;

Stage 1; Rearing of the selected five hundred (500) post fingerlings for 12 months thus providing broodstock recruitment.

The fish were stocked in a concrete grow out pond (10m x 12.5m x 1.5m) at four fish per meter square (Ozigbo *et al*., 2014). Feeding to satiation was done two times daily in the morning (10.00hrs) and evening (16.00hrs) with a 40% crude protein commercial feed. Pond water quality was maintained by regular flushing while raising the fish for the 12 months to ensure sexual maturity.

Stage 2; **Propagation of fish species (induced spawning with synthetic hormone) and rearing the fry till 10 weeks old.**

Nine broodstocks were selected based on the established criteria. A single intramuscular dose of Ovaprim hormone was administered to each of the selected females in each treatment with 2ml hypodermic syringe having 0.1ml graduation and No. 22 needle (Egwenomhe and Obi 2012). Female fish were injected at 20.00hr with the various quantity of hormone based on weight at 0.5ml/kg of fish as recommended by the manufacturers. The injected fish were returned to a holding tank with water while observing the latency period.

Eggs were striped into dry plastic bowl. The sperm sacs were collected from the selected males just before striping the eggs. Milt were extracted by dissecting the sperm sac using scalpel and diluted (Egwenomhe *et al*., 2019) using adequate normal saline to increase the volume of the solution. The eggs were quickly fertilized by mixing with adequate drops of sperm suspension using plastic spoon. Only physiological saline solution was used for this purpose as recommended by Egwenomhe *et al*. (2019). Fertilized eggs were quickly rinsed with more saline water. The eggs were spread in single layers on a suspended nylon mesh net (kakabans) in the shape of trays immersed below water of about 15cm depth in a flow -through system with 0.2 l / min flow rate for aeration and removal of metabolites during incubation (Olumuji and Mustapha 2012).

The newly hatched larvae were raised till 10 weeks old in the hatchery. Exogenous feeding began by evening of the third day of life of the hatchlings. Total flushing of ponds was done every four days.

Larvae were fed with dried decapsulated cysts of Artemia salina nauplii to satiation twice daily. The fish were slowly introduced to artificial dry diet of 200 – 300 µm catfish feed after one week of feeding. During the weaning period of 4 days, the diets were offered alternately to the larvae.

Culturing of 14 days old fry for another 35days was done using nine 60l plastic tanks out door. Fish larvae were feed 0.5mm commercial feed till fish were 25days old and subsequently fed ad lib with 43% crude protein 0.8-1.2mm feed till fish were 49 days old.

Stage 3. Rearing 10 weeks (fingerlings) old fish seed to table size for six months.

This was done using nine concrete grow-out ponds (i.e. 2m X 8m X 1.5m). The fish were stocked in the grow-out ponds at 200 fish per weight category (i.e. 12 fish per m^2). The sizes of the fingerlings were carefully selected for even match to avoid death via cannibalism. Also, each tank had fingerlings from each size category to nullify possible environmental influence in result. Feeding of fish to satiation with a 40% CP commercial feed was done throughout the culture period. The fish were fed in the morning (10.00hrs) and evening (16.00hrs). Water quality was maintained through occasional flushing and fish were cultured for 6 months to table size.

Measurement of water quality parameters

This was done at six days interval. DO was measured in situ with WTM, Oxical-SL portable electronic probe, pH and Temperature were monitored using AZ® pH meter Pen type (Model; 8685) and adequate oxygen level (above 5mg/l) was maintained with RESUN LP- 100 low noise air-pump. Ammonia and Nitrate was measured, treated with Nessler's reagent, with the aid of a visible spectrophotometer. Total Alkalinity was measured by titration method.

Data collection

Data were collected at the various stages following the recommendation of Egwenomhe and Obi (2012) as follows; Percentage survival from hatching to first feeding was estimated using 50 hatchlings from each treatment. Inception of first signs of exogenous feeding was closely monitored, particularly at 72 hours after hatching (swim up fry stage). Survival at this stage was evaluated by enumerating the remaining live fish in each bowl.

Growth rate of fish seed; for growth comparism of the study fish in each Treatment from the swim up fry stage up to 10th week post hatching, 150 fish seed/Treatment were stocked while 10 fish were randomly sampled per replicate every four days using a fine mesh scoop net, for weighing using Dura scale D2TM 300g x 0.01g capacity pocket scale (Precision 0.01g), while they are in a 10 ml plastic cup containing water with pre-determined weight. By the end of this larvae stage trial (10 weeks old), enumeration of surviving fry and their bulk weights was recorded.

Stage 4; Rearing 10 weeks old fish seed (fingerlings) to table size (6 months).

Growth rate of fingerlings; Weight was taken biweekly, as recommended by Sotolu (2010), throughout the culture period. Weighing of 10 randomly selected individuals was done using electronic scale of model KERN 572 for the first one month. Subsequently a table scale (Nops kitchen scale 5000g capacity, Grad 40g and Camry 50kg,Grad 200g) was used to weigh samples of 10 fish per Treatment.

All growth parameters were evaluated as follows;

$$\text{Weight gain} = W1 - W0\ (\text{mg}); \quad \ldots\ldots 1$$

$$\text{Growth rate} = \frac{W1 - W0}{T} \quad \ldots\ldots 2$$

and Specific Growth Rate (SGR%day) were calculated and converted into percentage thus:

$$\text{Specific growth rate (SGR)} = 100\ \frac{\text{LnW}_1 - \text{LnW}_0}{\text{T (days)}} \quad \ldots\ldots 3$$

Where: W0 = initial mean body weight, W1 = final mean body weight and T = time in Days (duration of culture or culture period) and ln= natural logarithm (Benedict *et al*., 2005).

Food Conversion Efficiency (FCE):

$$\text{FCE} = \frac{\text{final mean weight of fish}}{\text{Weight of feed given}} \text{x100 (TBoujard } et\ al., 2002) \ldots\ldots 4$$

Food Conversion Ratio (FCR) as determined as;

$$\text{FCR} = \frac{\text{Weight of dry feed fed (g)}}{\text{life weight gain (g)}} \ldots\ldots 5$$

Survival Rate (SR) was calculated according to Coulibaly *et al*. (2007) as;

$$\text{SR (\%)} = \frac{\text{Total number of fish harvested}}{\text{Total number of fish stocked}} \text{x100} \ldots\ldots 6$$

Performance Index (PI) was given as;

$$\text{PI} = \frac{\text{survival rate x mean final weight (g)} - \text{initial mean body weight (g)}}{\text{Rearing duration in days}} \text{ (Engle and Valderrama, 2001)} \ldots\ldots 7$$

Data Analysis

Data collected were subjected to analyses of variance (ANOVA) using Genstat statistical package Version 2005. Using Duncan Multiple Range Test (DMRT) at p<0.05, differences in means were compared.

RESULTS AND DISCUSSION

Water Quality Parameters

The values of the water quality parameters were within optimal range for fish growth and survival as reported by Albaster and Lloyd (1980). The dissolve oxygen, ammonia, total alkalinity concentrations varied from 5.8, 0.18, and 120 (minimum) to 6.7, 0.28, and 128 (maximum) mg/l, respectively. This suggests that the parameters could not negatively influence the test fish. Optimal water condition was maintained for the fish throughout the experiment to forestall such undue influence on fish growth.

Weight of female spawners

The weight of the female spawners was significantly different (p<0.05) among the various treatments. This was intended to generate possible maternal effects base on deliberate selection- 'a situation where the phenotype of an organism is determined not only by the environment it experiences and its genotype, but also by the environment and genotype of its mother' as noted by Falconer and Mackay (1996).

Survival and weight of larvae

There was no significant difference in the survival of the larvae among treatment (P<0.05). The survival rate by the fourth day of life suggests that the yolk was adequate to sustain the endogenous feeding stage in all treatments. This is owing to the fact that all the females used in this study were matured and thus produced fully matured eggs. Gunder and Fink (2004), reported that *Clarias* species matures at about 8 months old. Four days old fry of Treatment 111 (0.2165 g) were significantly ($p<0.05$) larger than those of Treatment 11 (0.1068g) which are in turn larger than the larvae of Treatment 1 (0.0850g). Similar size ranges where earlier reported in newly hatched larvae of *Clarias* spp. that weighed 0.50 – 0.6 mg (average 0.53 ± 0.06 mg), by Omer *et al*. (2016). They added that the fry, post yolk sac absorption, weighed 0.84 – 0.96 mg (average 0.90 ± 0.06 mg). The significant difference in weight of 4 days old fry observed in this work can only be traced to difference in maternal weight i.e. genetic variability of the broodstocks, with larger broodstocks producing larger larvae. Breidy *et al*. (2017) reported that egg quality and larvae development are affected by genetic variability of broodstocks of *Oreochromis niloticus*. Also, Phu *et al*. (2015) observed variability in quality of tilapia progeny when females from different sources were fed the same diet, suggesting that variability is partly attributed to differences in genetic characteristics of females.

Growth Rate, Specific Growth Rate, Survival, and Performance of Fish Seed from the Different Sized Female *Clarias gariepinus* by the 10th week of culture

Table 1. shows that there was significant difference ($p>0.05$) in the growth performance of the fish seed from the various female size categories. The survival rate of the fish seed was very high ranging from 61% in small broodstock (Treatment 1) to 73.33% in large broodstocks (Treatment 111). It was significantly different between with the Treatment 11 and 111 which were better than Treatment 1. Treatment 111(big size) with absolute growth rate of 0.053g/day had the highest growth rate which is significantly different ($p<0.05$) from other treatments. Treatment 11(medium size) which had 0.047g/day was significantly ($P<0.05$) higher than Treatment 1(small size) with value of 0.0378 g/day. The same trend was observed and recorded for specific growth rate as shown in table 1.

Survival of fish seed

Jamabo *et al*. (2015) reported lower survival rate range of 44 - 63% for this species. Omer *et al*. (2016), while working on the fecundity of three different size groups of *Clarias gariepinus*, females and the growth performance of larvae fed different starter food types, reported high survival rates for fish fed frozen Artemia (86.83 ± 8.76%) and frozen rotifers (81.06 ± 9.40%). The survival rate of hatchlings of *C. gariepinus* increased with increase in parent brood stock size. The survival rate of fry was high in large brood stock size while the lowest survival rate of fry was obtained for the small brood stock size (Table 1). This was supported by an earlier study by Miller *et al.* (1988) where larger fry at hatching were noted to have some advantages in survival and fitness. Bichi *et al*. (2014), while studying reproductive performance in the African catfish (*Clarias gariepinus*), similarly reported that large size eggs had the highest survival rates followed by medium size eggs and finally small sized eggs. Bichi *et al*. (2014) opined that fry obtained from large sized brood stock were bigger at hatching and thus have advantages in survival and fitness. Sule and Adikwu (2004) attributed this to large egg yolk reserve in large eggs. Therefore, the size of broodstock and thus eggs is directly related to the survival rates. Larvae produced from larger broodstock/egg size have higher chances of survival.

Growth rate and Specific growth rate of fish seed

The absolute growth rate and specific growth rate were highest in Treatment 111 (large size), with values of 0.053g/day and 6.71 %/day respectively and significantly different ($p< 0.05$) from Treatment 11(medium size) with values of 0.047g/day and 6.56% day respectively and Treatment 1 (small size) with values of 0.038 g/day and 6.29 %/day respectively. Uedeme-Naa and Nwafili (2017) also reported a superiority in growth performance found in the fingerlings produced by larger broodstock group (1.75 kg(♂)x1.75 kg (♀)) of *Clarias gariepinus*, and was also confirmed by the results of Ahmed *et al*. (2004) who found that growth performance was significantly ($P \leq 0.05$) affected by fish size.

Fish seed growth performance index

Treatment 111 (large size), performed expectedly significantly better than both Treatment 11 and Treatment 1. Treatment 1(small broodstock) had the least growth performance. Growth performance is a function of survival and increase in weight of the study fish. Although, the survival of the fish seed was not significantly different ($p>0.05$), Treatment 111 (large broodstock) had the highest significantly different ($p<0.05$) increase in weight, hence best growth performance while Treatment 1 (small broodstock) had the lowest increase in weight hence least growth performance. This is similar to the findings of several authors including Einum (2003), while studying the brown trout, Salmo trutta.

Bimonthly Weight of the Grow-Out Fish from the Different Sized Female *Clarias gariepinus* from the 12th to the 40th week of culture

There were significant differences ($p<0.05$) between and among the mean treatment weight gain for the three treatments. Treatment 11 (559.56g) had the highest mean weight gain, followed by Treatment 111 (528.30g). Treatment 1 (493.31g) had the least mean weight gain among the three treatments. Fig 1 shows increased growth rate in all treatment over time with sharp increase between the 14th and 16th week of age. Treatment 11 and 111 continued to increase in weight consistently throughout the study while Treatment 1 showed reduced growth rate by the 36th week of age.

Fig 1 shows Treatment 111 offspring only grow more than those from Treatment 11 up to approximately the 10 weeks and three and half days (10.5 weeks) and ≈5.50g offspring weight. There after Treatment 11 offspring grow more than those from Treatment 111 throughout the culture period of 40 weeks.

Elvingson and Johansson (1993) earlier reported that 'when fish are first reared separately, then mixed, thus with common environmental effects, the initial heterogeneity in performance between offspring of different dams progressively vanishes'. Similarly, *Oreochromis niloticus* has rapid embryonic development and spawns at high temperature and its maternal effect on fry size lasts for only 20 days as reported by Siraj *et al*. (1983), suggesting that maternal effects should not bias selection beyond this point.

Survival rate

There was no significant ($p<0.05$) difference in the survival of the fish seed from the different sized female *Clarias gariepinus* from the 12th to the 40th week of culture. This might have been due to high quality broodstock used and the supply of feed of the same nutritional quality, under the same feeding regime and good water quality management. Jamabo *et al*. (2015) reported lower survival rate range of 44 - 63% while studying the effect of feeding levels on growth performance, showing how poor quality of feed affects survival. Ajayi *et al*. (2016) also recorded (93.33%) survival for juvenile of this species raised for 13 weeks and this is within the range recorded in this study (91.2-97.1%).

Performance index of fish seed as grow out

There was no significant difference ($p<0.05$) in the performance of fish seed from Treatment II and III from the 12th to the 40th week of culture but they were significantly ($p>0.05$) different from treatment I. Jamabo *et al*. (2015) also reported performance index range from 3.07 ± 0.52 - 3.68 ± 0.27, while studying the effect of feeding levels on growth performance, feed conversion ratio of *Clarias gariepinus*. Jokthan (2013) attributed the performance of this catfish to both size and age of the female brooders. The higher performance index recorded in treatment II and III (in which there was no significant difference ($p<0.05$)) may be in connection with the size of the female broodstock used, indicating that medium and large size female broodstocks gives better growth performance in *Clarias gariepinus*.

CONCLUSION

Maternal effects induced differences in the growth performance of the individuals. This maternal effect on larvae size lasts for only 10.5 weeks in *Clarias gariepinus*. Larvae produced from larger broodstock/egg size have higher chances of survival. This work is also attesting to the fact that the growth of fish larvae is highly influenced by the size of parent stock and as such larger broodstock is recommended for use when faster growing fish seed are desired by fish breeders during fish seed multiplication in hatcheries. Finally, medium and large size female broodstocks gives better growth performance in *Clarias gariepinus* as such hatchery operators should use these size categories for spawning.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

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