

Comparative Study of Different Ages on The Reproductive Performances of *Clarias gariepinus*

Effect of different ages of African catfish (*Clarias gariepinus*) broodstock on reproductive performance and fries production

Abstracts.

This study was conducted at the hatchery complex of Akwa Ibom State University to investigate the effect of different ages of African catfish (*Clarias gariepinus*) on their reproductive performances. Eighteen (18) broodstock of *Clarias gariepinus* (3 males and 3 females) from each age group, six months, twelve months and eighteen months old with average body measurement of 2.5 kg and 65.7cm were selected from AKSU fish farm labeled treatment A, B and C for the study. 3 male broodstock from each treatment were sacrificed for milt extraction without hormonal inducement. The milt from each treatment were separately pooled into one volume and divide into three portions each diluted with 2ml of normal saline solution. Three female broodstock from each treatment were separately induced with ovaprim at a single dosage of 0.5ml/kg body weight of fish and allowed for a latency period of ten hours at water temperature of 26°C before stripping manually. 3g of egg from each broodstock was mixed with the diluted milt and activated with 100 mls of normal saline. The fertilized eggs were incubated in aerated indoor concrete tanks in 3 replicates. Six hours after incubation, the percentage fertilization, hatchability, survival and fry production success in each treatment was accessed. The results reveal that the reproductive parameters considered in this study significantly ($p < 0.05$) increased with age of broodstocks. The percentage fertilization of egg from the least to the oldest broodstock were 63.67 ± 0.88 , 69.50 ± 0.87 and 81.83 ± 5.05 respectively. The percentage hatchability of the oldest broodstock was 82.62 ± 1.6 significantly ($P < 0.05$) higher than 64.79 ± 2.74 (12 months) with the least value of 49.04 ± 5.76 recorded for six month old brood stock. The oldest broodstock had the highest percentage survival 83.14 ± 3.16 and fry production success of 56.05 ± 0.06 while the least broodstock (6 month old) had the least value of survival 54.59 ± 2.42 and fry production success of $17.17 \pm 2.59\%$.

1.0 Introduction

The need for more knowledge of artificial propagation of African catfish require urgent attention since captured fisheries is declining as result of climatic and environmental challenges (Otoh, et al., 2023). Fish remained the cheapest source of protein, readily available, affordable and acceptable at all ages of humanity. The declining of captured fisheries projected aquaculture as the only alternative source for achievement of mass production of fish to meet the increasing need of protein for human sustainability (Otoh, et al., 2022, Udoh and Otoh, 2017). Generally, importance of fish in human diet and economic progress of our society is already recognised (Otohet, et al., 2023 a, b) and this is achieved through aquacultural practice.

However, the success of aquaculture begins at the hatchery levels where the reproductive performances of the fish will be determined for seed productions. African cat fish such as *Heterobranchus* and *Clarias* species remained the most culturable species of significant in Nigeria and beyond (Otoh and Udoh, 2018a,b., Oyeleye et al., 2016). This is due to the unique characteristic of these species such as fast growth rate, good taste, generally accepted for consumption, high stocking density, high market price and high resistance to disease and ability to reproduce in captivity (Nlewadim et al., 2011; Nya, et al., 2017, Otoh, et al., 2020a; Otoh and Udoh, 2018a; Otoh and Udoh, 2019; Otoh, et al., 2022, Udo and Otoh, 2023; Otoh, et al., 2023; Otoh and Udoh, 2020).

Although the growth of fish depends on availability of good feed (Otoh and Udoh, 2018; Ekanem et al., 2000) of which a single feed stuff component cannot achieve. *Heterobranchus* and *clarias* readily accept any supplementary feed and their growth rate is unique within a short period of culture according to Nlewadim et al., (2011) compare to other species.

Effects of fish age on breeding success and gamete quality varies. Some studies (Madu, et al., 2016) have reported increase while others (Masoumeh, et al., 2012) reported decline in some traits. Some studies also suggest that increasing paternal age may be associated with degenerative and declining gamete quality. Otoh and Udoh, (2018a) demonstrated that males of 3½ and 2½-year old produced better fertilization ability based on parental age. Viable and profitable hatchery management require choosing the best-suited male and female broodstock from different ages in the farm. Age remains a natural parameter for assessment of lifetime of individual and a biological prerequisite for determination of sexual maturity of living organism of which fish is included (Otoh and Udo, 2019). Since the age of fish has influence on the quality of the gametes, which determine the success and reproductive performances of fish at the hatchery levels, the need for investigation of age of African catfish (*Clarias gariepinus*) on reproductive performances become necessary which is the focus of this study.

Comment [hh1]: The numbers of fish in each treatment are small. The number is not enough to judge age.

Comment [hh2]: Is the weight for all ages? What is the weight of a 6 month old? The weight of the fish must be clarified at each age separately (6, 12, 18 months). Weights should vary with age. If the weight is equal to the age, then the fish of large ages are not good.

Comment [hh3]: How did the eggs hatch after 6 hours of incubation? Catfish eggs hatch after 25-36 hours.

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Comment [hh5]: Standardizing the writing of the references are as follows for all (Otoh et al., 2022)

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2.0 Material and Methods

2.1 Location of Study

The research was conducted at the Fish Farm Complex (Fig. 1b) of Akwa Ibom State University, Obio Akpa campus (Fig. 1a), located between latitude 5°17'N and 7°27'N, Longitude 7°27'E and 7°58'E. The area has an annual rainfall ranging from 3500– 5000 mm and average monthly temperature of 25°C in the tropical rainforest zone of southeast Nigeria (Otoh and Udoh, 2018a).

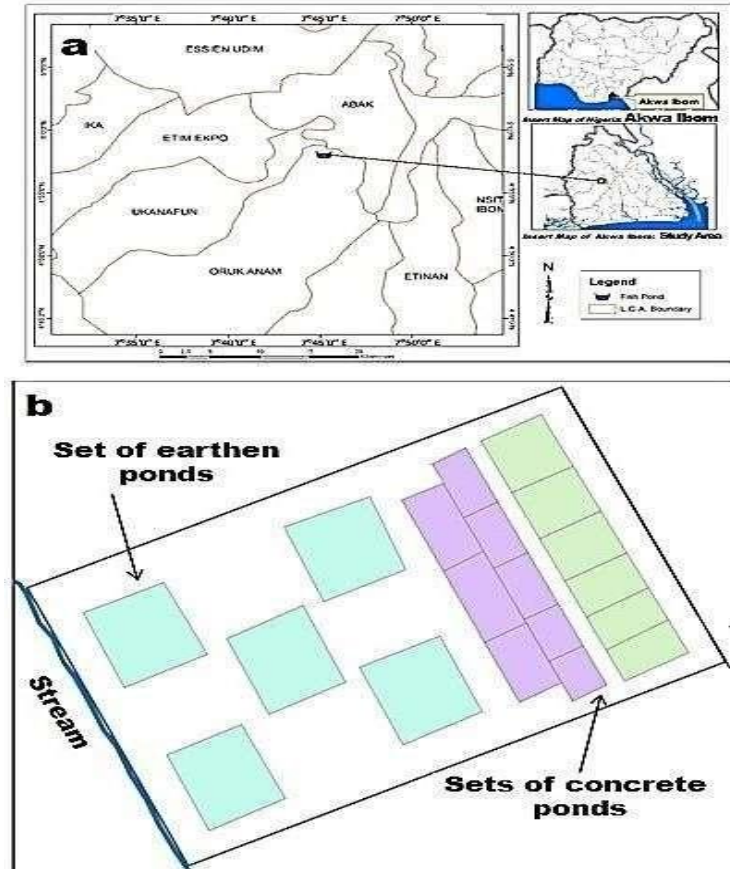


Fig. 1: A cross section of map of Nigeria showing Akwa Ibom with an inset showing the location (a) and layout (b) of the University Fish Farm Complex

2.2 Acquisition of Brood Stocks

A total of 18 broodstocks (3 male and 3 female) each belonging to 3 different age group such as 6 months old, 12 months old and 18 months old with average body measurement of 2.5 kg and 65.7cm were selected from AKSU fish farm for the study according to Otoh, *et. al.*, (2022).

2.3 Milt Extraction:

Three males from each of the three age group were sacrificed for milt extraction without hormonal inducement. The milt from each age group were pooled together into one volume and divided into 3 portions, each was diluted with 2ml of normal saline (9g salt) and preserved below the temperature of 7°C.

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2.4 Hormonal Inducement:

3 female broodstock from each age group were transferred to hatchery and induced with ovaprim hormones at a single dosage of 0.5 ml/kg body of fish (Asangusung, *et. al.*, 2020) and allowed at a latency period of ten hours before stripping.

2.5 Stripping of eggs:

Eggs from each sample was stripped into a separate plastic containers. 3g of eggs from each sample containing approximately 2000 oocytes was measured into 9 separate containers. Eggs in each container was mixed with the diluted milt for fertilizations.

2.6 Incubation:

Incubation of the fertilized eggs was carried out in nine breeding tanks, 3 tanks per treatment. The fertilized eggs were spread on a kakaban on a single layer in 30cm³ water level. Dead eggs were siphoned out of the spawning tanks after 35 hours (Otoh, *et. al.* 2023; otoh, *et. al.*, 2020 a, b) and activated with 100ml of 0.9 % saline solution, after the first 5 minutes, the saline solution was decanted and the fertilized eggs uniformly spread in a monolayer on a kakaban (Shredded Nylon Sacks) and incubated in aerated indoor concrete tanks (during incubation, water levels were maintained at 30cm³ depth). Six hours after fertilization, the colour variations between the eggs was observed clear and transparent, eggs were considered fertilized while dead/white and Opaque ones were regarded as unfertilized (Otoh and Udoh, 2019; Udoh, 2000; Otoh, *et. al.*, 2020a; Otoh, *et. al.*, 2022).

The percentage fertilization were calculated by five days determination of the fertilized eggs per 200 eggs siphoned based on colour. Five different locations of the breeding tanks were marked and 200 eggs siphoned from each location and the white eggs recorded (Udoh, 2000; Otoh, *et. al.*, 2020b). The total larvae survival was determined by 10 days post hatching. Egg fertilization, hatching and survival rate were determined as follows: Fertilization rate (%) of eggs = (No. of eggs fertilized / total number of eggs) x 100 Hatchability (%) = (number of hatchlings / total number of fertilized eggs) x 100. Percentage hatchability was obtained by direct counting of unhatched eggs as well as the numbers of eggs hatched in each incubating tank. Hatching rate = (No of healthy fertilized eggs / No of fertilized eggs used) x 10 (Udoh, 2000). Survival rate (Ks) was calculated during initial feeding according to the following formula:

Survival rate = (number of live larvae / total number of larvae hatched) x 100 (Udoh, 2000). Efficiency of hatching was evaluated by the method of Rana (1995): Fs (%) = Kf.Kh.Ks/10,000 where:

Fs = Success rate (%) of fry production at 10-day post hatching.

Kf = Fertilization rate (%) of eggs

Kh = Hatching rate (%) of fry

Ks= survival rate (%) of 10-day-old swim-up fry (Rana, 1995).

2.7 Statistical Analysis

Data collected were subjected to one-way analysis of variance (ANOVA) to test for significant difference with the aid of predictive analytical software version 18.0. Probability level of (P<0.05) was considered significant. Replicates were treated as random effect while age of the fish were treated as fixed effect.

3.0 Result

The research on the comparative effect of different ages of broodstock in the reproductive performances of *Clarias geriepinus* revealed that the physiochemical parameters of each treatment in this study shows no significant difference (p>0.05). Dissolved oxygen, temperature and pH measurement ranged between 5.21±0.01-5.26±0.01 (Mg/L) mg/L, 27.03±0.01-27.06±0.01(° C) and 6.92± 0.01-6.96± 0.01 (Table 1) respectively.

Comment [hh14]: The reference is a or b

Comment [hh15]: How were dead eggs activated????!!!!
Paragraph reformulation

Comment [hh16]: What is the importance of measuring the hatching rate and what is the difference between it and the percentage hatchability.

Comment [hh17]: (SR) not (Ks)

Comment [hh18]: The table shows the water quality of stripped egg stages, and does not display the water quality under the different ages.

Table 1: Mean water Quality Parameters of the Incubating Tanks

Stripped egg stages

	Stage 1	Stage 2	Stage 3	Stage 4
Temperature (°C)	27.05 ± 0.05	27.03 ± 0.01	27.04 ± 0.02	27.06 ± 0.04
pH	6.92 ± 0.02	6.93 ± 0.01	6.95 ± 0.25	6.96 ± 0.01
Dissolved oxygen (mg/L)	5.21 ± 0.20	5.23 ± 0.40	5.25 ± 0.150	5.26 ± 0.20

Effect of broodstock age (months) on percentage fertilization rates of *Clarias gariepinus* broodstock reared in concrete aquaculture ponds is presented in Fig. 2. The percentage fertilization of eggs obtained from six(6) months old broodstock was 63.67 ± 0.88 which shows significant ($p < 0.05$) difference with the percentage fertilization of eggs obtained from twelve (12) months old broodstock 69.50 ± 0.87 of *Clarias gariepinus*. The oldest broodstock of Eighteen (18) months old had the highest percentage fertilization value of 81.83 ± 5.05 which is significantly ($p < 0.05$) different from the percentage fertilization of eggs obtained from 6 and 12 months old broodstock. Therefore, fertilization Increase with age of broodstock.

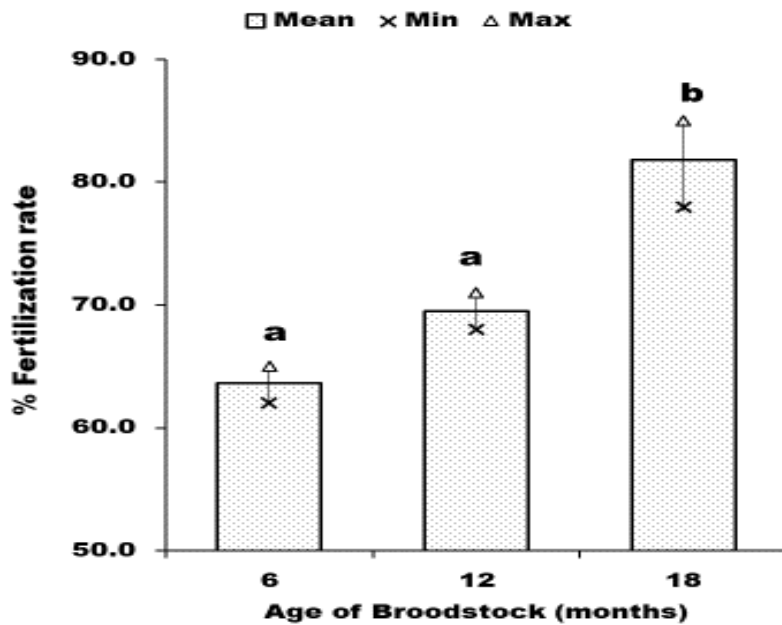


Fig. 2: Percentage Fertilization Rate of *Clarias gariepinus* Brood Stock at Different Ages

Effect of broodstock age (months) on percentage hatching rates of *Clarias gariepinus* broodstock reared in concrete aquaculture ponds is presented in Fig. 3. From the statistical analysis, six (6) months old broodstocks showed percentage hatchability value of 49.04 ± 5.79 while Twelve (12) months old broodstocks showed percentage hatchability value of 64.79 ± 2.74 . With the variation in the hatchability value among the two treatment, statistical analysis revealed that they were significantly ($p < 0.05$) different. Eighteen (18) months old broodstocks had percentage hatchability value of 82.62 ± 1.6 and showed significant ($p < 0.05$) difference with percentage hatchability value of eggs obtained from six and twelve months old broodstocks.

Comment [hh19]: What is displayed in the results is the hatching rate or percentage hatchability, as there is a different equation to calculate each of them in materials and methods.

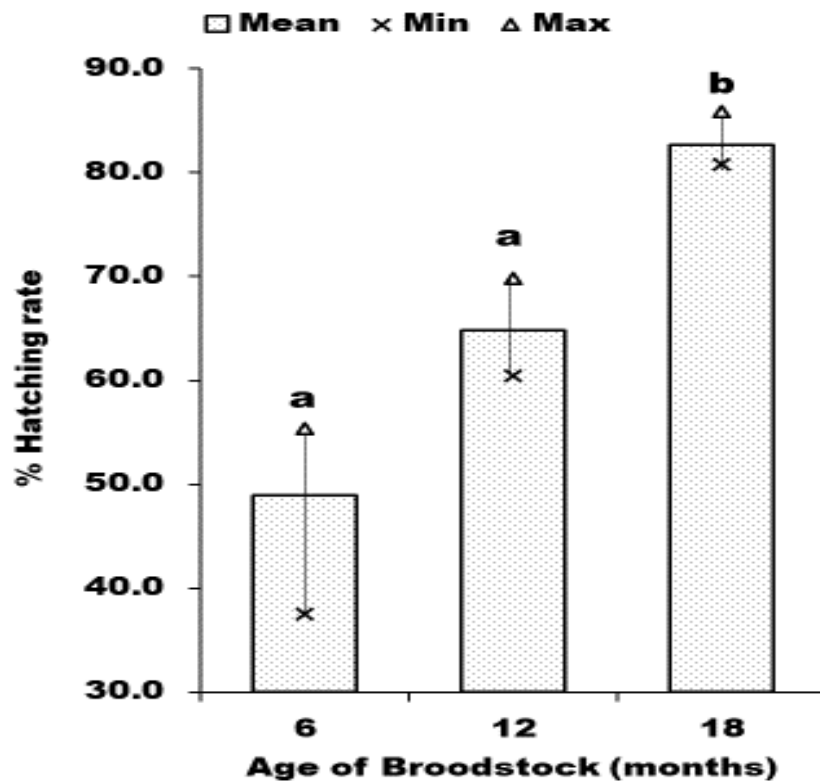


Fig. 3: Percentage Hatchability Rate of *Clarias gariepinus* Brood Stock at Different Ages

Effect of broodstock age (months) on percentage fry survival rates at 10-day-old of *Clarias gariepinus* broodstock reared in concrete aquaculture ponds is shown in Fig. 4. The percentage survival of **Frys fries** obtained from six (6) months old broodstock had percentage survival value of 54.59 ± 2.42 which is significantly ($p < 0.05$) higher than the percentage survival value of **Frys fries** obtained from Twelve (12) months old broodstock whereas the percentage survival value of **Frys fries** obtained from Eighteen (18) months old broodstock was 83.14 ± 3.61 and is significantly ($p < 0.05$) different from that of six and Twelve months old broodstock. The least survival value was observed in twelve (12) months old broodstock.

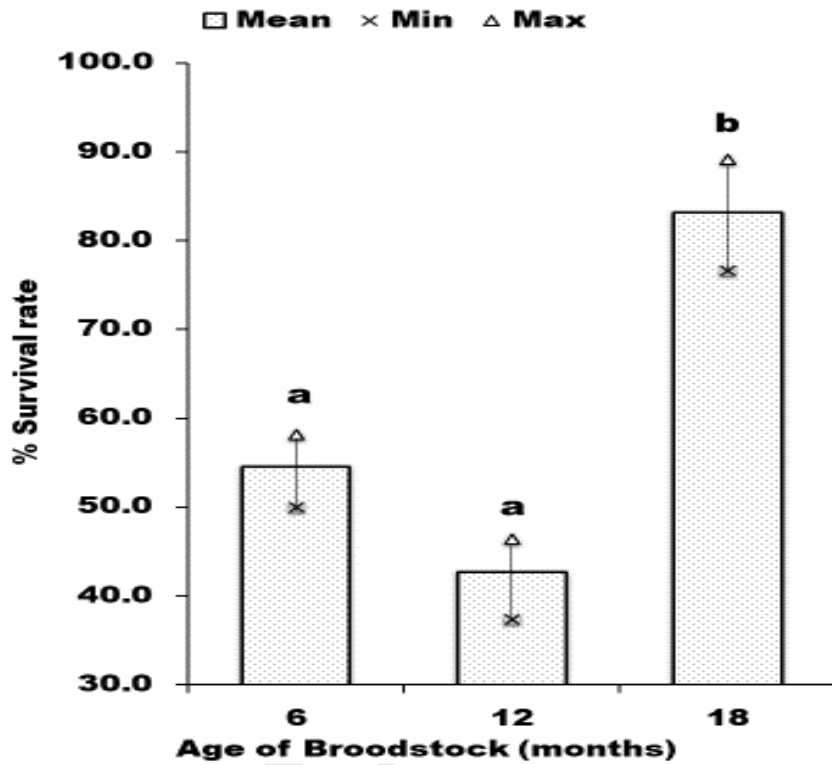


Fig. 4: Percentage Survival Rate of *Clarias gariepinus* Brood Stock at Different Ages

Effect of broodstock age (months) on percentage fry production success rates of *Clarias gariepinus* broodstock reared in concrete aquaculture ponds is presented in Fig. 5. According to the analysis, six(6) months old broodstock produced percentage fry production success value of 17.17 ± 2.59 while Twelve (12) months old broodstock produced fry production success value of 19.17 ± 1.17 but showed no significant difference ($p < 0.05$) among the two. However, Eighteen (18) months old broodstock of *Clarias gariepinus* produced the highest value of percentage fry production success of 56.05 ± 0.06 which significantly ($p < 0.05$) differ from that of six and twelve months old.

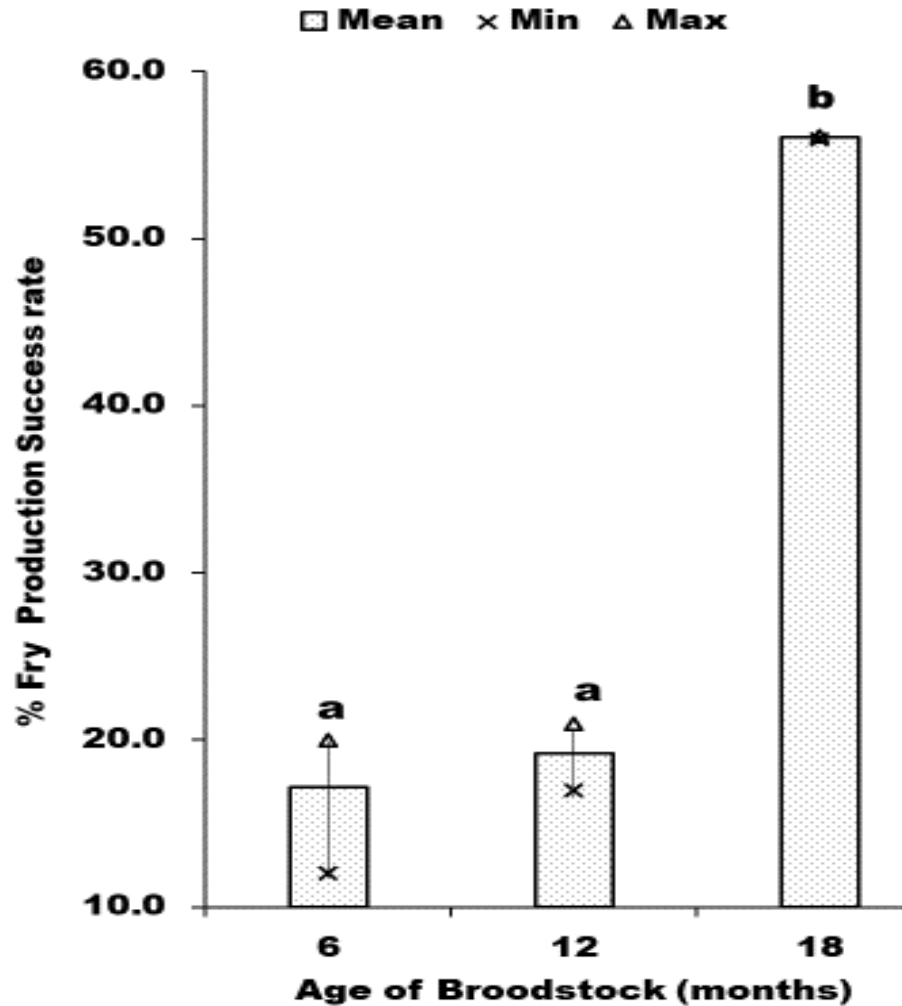


Fig. 5: Percentage Fry Production Success of *Clarias gariepinus* Brood Stock at Different Ages

4.0 Discussion

The knowledge of the fact that over 50% of the stripped eggs from African catfish do not survive till fingerling stage during artificial propagation, called for investigation of age effects on the reproductive performances of *Clarias gariepinus*. The study revealed that the physico-chemical parameters of water observed in this study showed no significant difference ($P < 0.05$) and were within the tolerable limit according to (Eyo, 2023, George and Atakpa, 2015; Jonah *et al.*, 2020).

Analysis revealed that reproductive parameters such as ; fertilization, hatchability, survival and fry production success observed in this study significantly ($p < 0.05$) increased with the age of the broodstocks. The oldest broodstock (18month old) had the highest percentage fertilization value of 81.83 ± 2.05 followed by 12 month old 69.50 ± 0.87 while 6 month old broodstock had the least fertilization value of 63.67 ± 0.88 [This result

could be possible since the oldest broodstock produced the biggest size of eggs which provided the larger surface area for sperm contact and fertilization. This result is in line with the observation of Otoh and Udoh, (2019) and Umana, (2020) but disagree with Bovand and Khara, (2017) whose report of the age effect on the reproductive performances was the reverse of this study.

A significant ($P < 0.05$) difference was also observed in the percentage hatchability with the age of broodstock. The oldest broodstock had the highest hatchability value of 82.62 ± 1.63 which is significantly ($P < 0.05$) higher than 69.50 ± 0.87 and 63.67 ± 0.88 hatchability obtained from 12 and 6 months old broodstock. Similar result was observed by Otoh, *et. al.*, (2020 a, b) who observed the hatchability increase with the age of broodstock and this could be attributed to the egg size from the broodstock and Yolk size which also provide potential energy for the hatchability as it increased with age of broodstock.

The percentage survival of the oldest broodstock 84.14 ± 3.61 was significantly ($P < 0.05$) higher than the percentage survival of the other two ages of survival, the least percentage survival of 42.67 ± 2.73 was observed in 12 month old broodstock while 54.59 ± 2.42 percentage survival was observed in 6 months old broodstock. This result could be attributed to the management system at the hatchery level. Broodstock age on fry production success also revealed significant ($p < 0.05$) difference with age. This could be as a result of fertilization and hatchability rate of eggs as well as the management system at the hatchery level. However, Otoh, *et. al.*, (2020a) in his study observed that the survival of the fry increases with the age of the broodstock.

5.0 Conclusion

Based on the result of findings in this study, it is observed that the reproductive performance of *Clarias gariepinus* is primarily dependent on the age of the brood stock. The results of % fertilization, % hatchability, % survival rate and fry production success were excellent for *C. gariepinus* brood stock of one year and six months (18 months). Therefore, *C. gariepinus* broodstock of a higher age group will give a better reproductive performance and is recommended for effective breeding programme in artificial or man-made environment.

6.0 References

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Comment [hh20]: There are no results in this manuscript that support this interpretation. Egg diameter was not measured in this manuscript.

Comment [hh21]: Also, there are no results in this manuscript that support this interpretation.

Comment [hh22]: Materials and methods do not include a method for rearing and feeding of larvae

Comment [hh23]: 2003 or 2023
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