

# Effects of Different Batches of Stripped Eggs on The Reproductive Performances of *Heterobranchus Longifilis*

## Abstract

Owing to the fact that about 50% of the total stripped egg of catfish do not survive till fingerling stage during artificial propagation, the need to investigate on the effects of different batches of stripped egg on reproductive performances of *Clarias gariepinus* became necessary. Nine brood stocks of *Clarias gariepinus* (6 male & 3 female) with average body weight of 2.3kg and average length of 64.2 cm were carefully selected for the study. Six matured males were sacrificed for the removal of testis without hormonal inducements. The milt collected from the six males were pulled together and divided into twelve portions each diluted with 2ml of normal saline solution. Three female brood stocks were separately induced at single dosage of 0.5ml/kg body weight and allowed for a period of 10 hours before stripping. Stripping of eggs from each broodstock was in four batches, each batch measured 50g and labeled A, B, C & D. 3g of eggs containing approximately 2000 oocytes (eggs) were measured out from each batch and mixed with the diluted milt and incubated in a 2 x 1 x 10cm<sup>3</sup> of water in a concrete pond at temperature of 26°C in three replicates. The result revealed that the reproductive performances considered in this study decreased as the batches of stripped eggs increased from 1-4 batches. The first batch of stripped eggs produced 90.17 ± 0.44, 97.78±0.86, 89.48 ±1.08 percentage fertilization, hatchability and survival respectively, while the least batch of stripped eggs (batch 4) produced the least value of fertilization (5.83 ± 1.69); 45.00 ±4.90 hatchability and survival value of 5.59 ± 0.61. Therefore, from the above result, the least batch of stripped eggs should not be used for fertilization since the unhatched or dead eggs has negative effects on the fertilized eggs including fry at the hatchery level.

**Keywords:** Batches of stripped Eggs, Reproductive performances, Fertilization and *Heterobranchus longifilis*.

## 1.0 Introduction

Fish remained a universal protein source acceptable at all ages of humanity, easily to come by at affordable cost compared to other sources of protein. Globally, the demand for fish is daily increased as the world population continues to expand whereas the supply of fish and fish products from the wild is drastically reduced due to environmental challenges and degradations leaving behind aquaculture as the only alternative achieving maximum production of fish to meet the global needs.

African catfish such as *Heterobranchus* and *Clarias* species remained the most culturable species of significant in Nigeria and beyond (Otoh and Udoh, 2018 a, b; Oyeleye *et. al.*, 2016). This is due to the unique characteristic of the 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; Udoh and Otoh, 2017; Otoh, 2020 a, b, Otoh, *et. al.*, 2023 a, b; Otoh, *et. al.*, 2022). Although the growth of fish depends on availability of good feed of which a single feed stuff component cannot achieve, according to Ekanem *et. al.*, 2000, *Heterobranchus* and *Clarias*, readily accept any supplementary feed and their growth rate is unique within a short period of culture (Nlewadim, *et. al.*, 2011; Asanaung, *et. al.*, 2020) compared to other species.

This species dominates fresh water settings such as streams, lakes and rivers (Adewunmi and Olaleye, 2011). It has high commercial value (Oyeleye *et. al.*, 2016; Shourbela *et. al.*, 2019). Problems associated with fish seed in the natural environment necessitated artificial propagation techniques (induced spawning) under more controlled conditions for a reliable source of fingerlings (Akande and Dieiouadi, 2010). To obtain larvae of acceptable quality during artificial propagation depends mostly on quality gametes which is at times difficult to achieve (Cejko, *et. al.*, 2013; Szabo, *et. al.*, 2015 and Kristan *et. al.*, 2018). This situation called for intervention of biotechnologists for the collection of high-quality gametes, short term gamete storage (Kucharczyk *et. al.*, 2018) and fertilization (Muller *et. al.*, 2018).

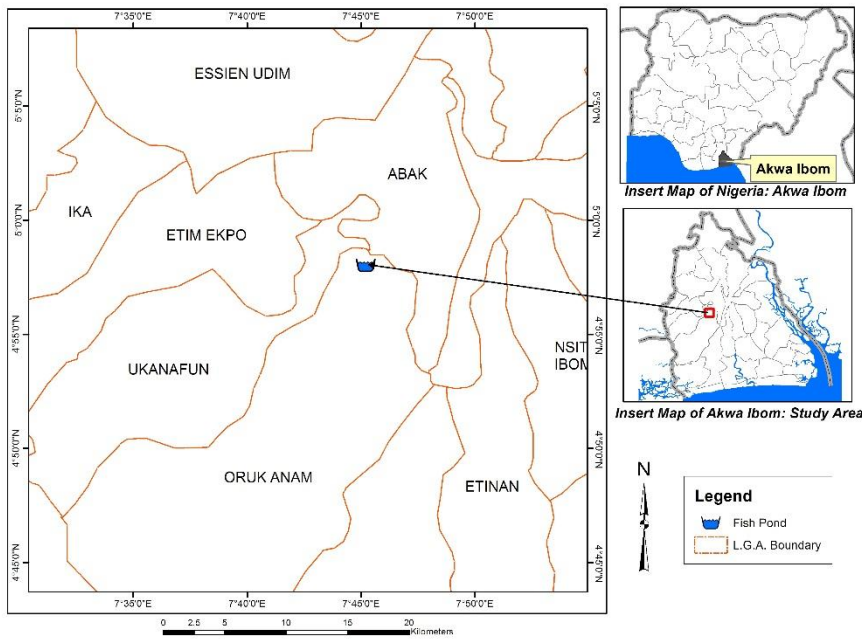
Catfish has gained huge recognition in our society hence the need for intensification of breeding techniques for mass production of fish seed needed for farm stocks. Low survival of fry at the hatcheries level could be attributed to unidentified factors of which differences in the batches of stripped egg from the brood stocks might not be exceptional (Otoh and Udoh 2019), hence the focus of this study. Practically, it has been observed that all eggs in the gonad do not mature at the same time.

However, through human intervention with the use of artificial hormones, maturity of entire eggs occurs at the same time and the need to consider if all the mature eggs through human intervention do fertilize, hatched and survived as expected remained very crucial. This is to overcome the danger of mass mortality since the unhealthy eggs cause the mortality of fry at the early stage of development.

## 2.0 MATERIALS AND METHODS

### 2.1 Study Area

This research study was carried out in Akwa Ibom State University (AKSU) fish farm complex, Obio Akpa Campus, Akwa Ibom State, which is located between latitude 5°17'N and 7°27'N, Longitude 7°27'E and 7°58'E. The study area has an annual rainfall ranging from 3500mm to 5000mm and average monthly temperature of 25°C. Akwa Ibom State is a coastal state lying between latitude 4°28'N and 5°3'N and between longitude 7°27'E and 8°20'E with a relative humidity between 60 to 70%. It is in the tropical rainforest zone of Nigeria. (Otoh and Udoh, 2019)



**Fig. 1: A Map showing the location of the Akwa Ibom State University fish farm complex.**

### 2.2 Acquisition and Care of Brood Stocks

20 matured broodstocks (10 males and 10 females) were separately stocked in a concrete pond at the rate of 2 fish / m<sup>2</sup> and fed at 5% body weight twicedaily for three months using Coppens commercial feed. six (6) sexually mature males and three (3) females with average body weight of 2.3kg and length of 64.7cm were carefully selected according to Otoh, *et. al.*,(2020). Twelves (12) indoor breeding tanks of equal dimension 1x1x1cm<sup>3</sup> were used for the study. Water levels and Temperature in each breeding tank was maintained at 30cm<sup>3</sup> and 26°C respectively.

### 2.3 Hormone Induced Spawning

Six (6) matured male broodstock were sacrificed for sperm removal without hormonal inducement. milt collected from the six sample were pooled together in a plastic container and divided into twelve (12) portions each diluted with 2ml of normal saline solution and preserved separately. Three female breeders were separately transferred to hatcheries for inducement with ovaprim hormone at single dosage of 0.5ml/kg body weight and allowed for a period of 10 hours under the same temperature before stripping manually to obtain eggs (Otoh,*et. al.*, 2020 and otoh, *et. al.*, 2023)

### 2.4 Eggs Stripping and Fertilization

Four batches of stripped eggs (50g each) were separately obtained from each of the 3 breeders through gentle pressing of the abdominal region ventrally and labeled A, B, C and D respectively. 3g of eggs containing about 2000 oocytes were measured out of each of the A, B, C and D each mixed with a portion of the diluted milt for artificial fertilization and activated with 100ml of normal saline solution. After 3 minutes, the saline solution was decanted while the fertilized eggs were uniformly spread on the Kakaban (shredded nylon sack) and incubated in aerated indoor concrete breeding 2 x 1 x 10cm<sup>3</sup> at temperature of 26<sup>0</sup>C and replicated three times. During incubation, water levels were maintained at 30cm<sup>3</sup> depth.

## 2.5 Fertilization

The colour variations between the eggs were observed, clear and transparent eggs were considered fertilized while dead /white and opaque one was regarded as unfertilized (Udoh 2000 and otoh, *et. al.*, 2023). Based on the counts, the reproductive performances of different stripped eggs were observed such as; percentages fertilization, hatchability, survival and fry production success. Efficiency of these productions was evaluated following the method of Rana (1995).

$$Fs (\%) = \frac{Kf.Kh.Ks}{10,000}$$

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

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 100 (Hanjavanit, *et. al.*, 2008)

Survival rate (Ks) were calculated during initial feeding according to the following formula

Survival rate = (number of live larvae/ total number of larvae hatched) x 100 (Hanjavanit, *et. al.*, 2008)

## 2.6 Monitoring of Water quality

Dissolved oxygen and pH of the water were monitored daily using pH meter (VIVOSUN pH Meter) and dissolve oxygen meter (Extech 407510 Dissolved Oxygen Meter) while mercury in glass thermometer was used to take temperature readings.

## 2.7 Statistical Analysis

Data were processed using Microsoft Excel 2010 for their mean values and presented in graphs. The Data was analyzed using one-way ANOVA at 0.05 significant levels to check the significant difference in fertilization, hatchability and survival rates.

## 3.0 Results

### 3.1 Mean Water Quality of the Incubating Tanks

The physiochemical parameters of each of the treatment showed no significance ( $P > 0.05$ ) difference. Dissolved oxygen, temperature and PH measurement ranged between  $5.21 \pm 0.20$ - $5.66 \pm 0.20$ , (mg/l)  $26.25 \pm 0.05$  -  $26.82 \pm 0.04$  (<sup>0</sup>C) and  $6.90 \pm 0.02$  -  $6.95 \pm 0.01$  respectively.

**Table 1: Mean water Quality Parameters of the Incubating Tanks**

		Stripped egg stages			
		Stage 1	Stage 2	Stage 3	Stage 4
Temperature (°C)		$26.25 \pm 0.05$	$26.50 \pm 0.01$	$26.80 \pm 0.02$	$26.82 \pm 0.04$
pH		$6.90 \pm 0.02$	$6.93 \pm 0.01$	$6.95 \pm 0.25$	$6.95 \pm 0.01$
Dissolved oxygen	(mg/L)	$5.21 \pm 0.20$	$5.61 \pm 0.40$	$5.65 \pm 0.150$	$5.66 \pm 0.20$

### 3.2 Effect of Different Batches of Stripped Eggs on the Reproductive Performances of *Heterobranchus longifilis*

The results on the effect of different batches of stripped eggs from the broodstock on reproductive performances of *Heterobranchus longifilis* (fertilization rate, hatching rate and survival rate) is shown in figure 1-3. Results revealed that's the percentage fertilization of the first batch of stripped eggs was  $90.17 \pm 0.44$  significantly ( $P < 0.05$ ) higher than the  $87.83 \pm 0.60$  observed in the second batch of stripped eggs, which significantly ( $P < 0.05$ ) increased more than the third and last batch of stripped eggs with the least value of percentage fertilization of  $51.83 \pm 1.69$  recorded for batch 4 (Fig. 1). The percentage fertilization of different batches of stripped eggs showed an interesting pattern in the order of  $A > B > C > D$ .

The result on the percentage hatchability of different batches of stripped eggs showed a similar trend decreasing significantly ( $P < 0.05$ ) from the first to the least in the order of  $97.78 \pm 0.86$ ,  $95.83 \pm 0.80$ ,  $71.52 \pm 1.88$  and  $45.00 \pm 4.90$  respectively (Fig. 2).

The result of different batches of stripped eggs from the broodstock on the percentage survival of the fry is presented in figure 3. The percentage survival of fry obtained from the first batch of stripped eggs was  $89.48 \pm 1.05$  significantly ( $P < 0.05$ ) higher than  $74.52 \pm 1.76$  percentage survival obtained from the second batch of stripped eggs. The percentage survival of eggs  $41.62 \pm 0.67$  obtained from the third batch of stripped eggs was significantly ( $P < 0.05$ ) higher than the least value of percentage survival from the last batch of stripped eggs  $5.59 \pm 0.61$ .

The result of this study revealed that the entire reproductive parameters considered in this study significantly ( $P < 0.05$ ) decrease from the first batch of stripped eggs to the least as shown in figure 1-3.

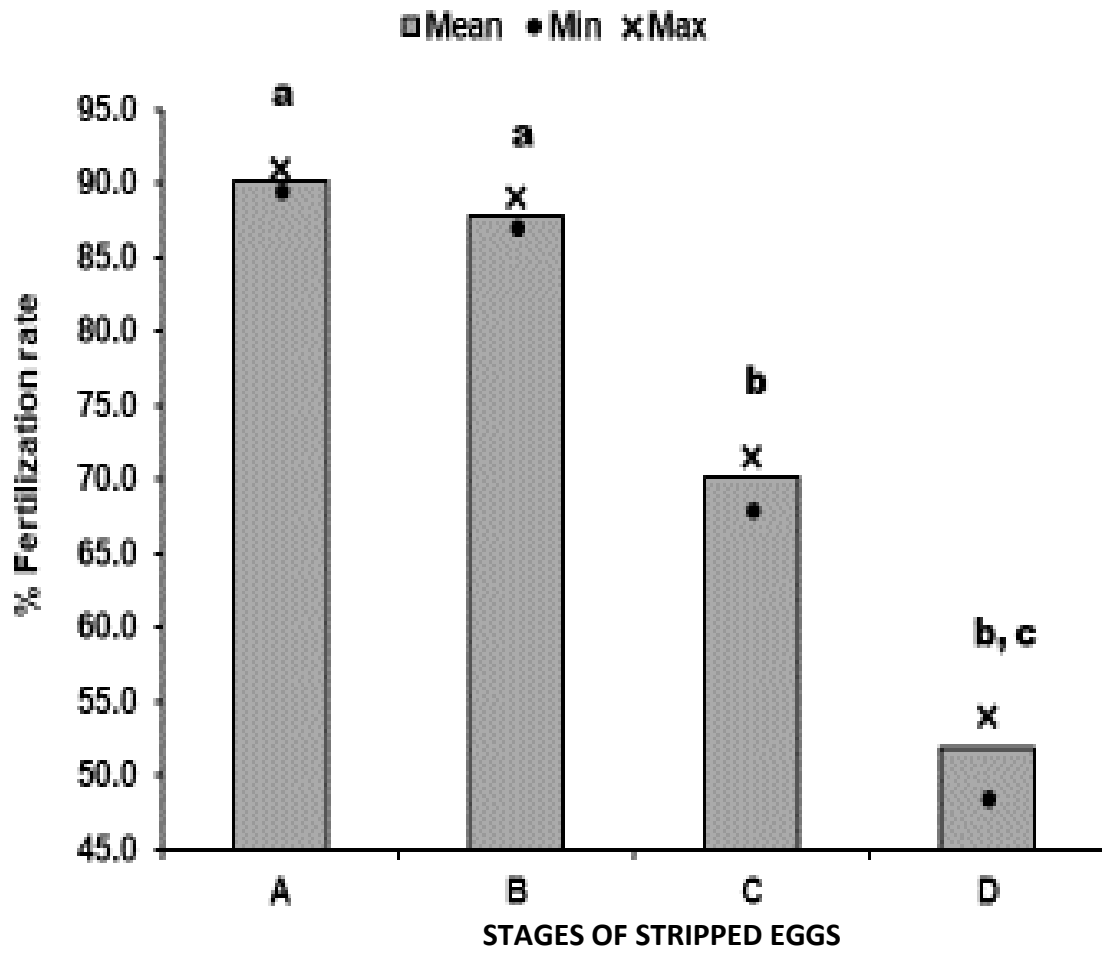


Figure 1:Percentage fertilization of Different Batches of Stripped Egg of *H. longifilis* Brood Stock

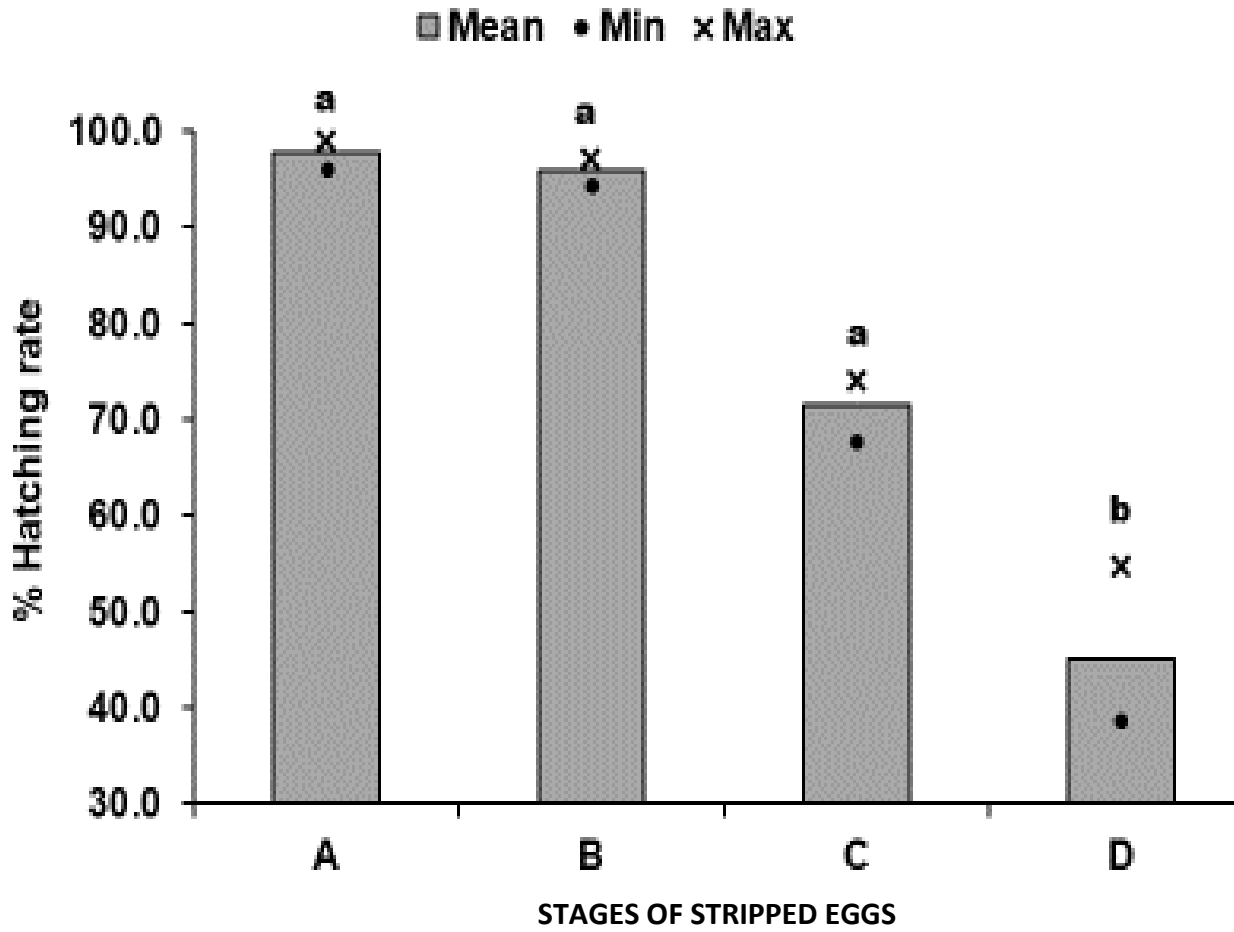


Figure 2: The percentage hatchability of different batches of stripped eggs of *H. longifilis* Brood Stock

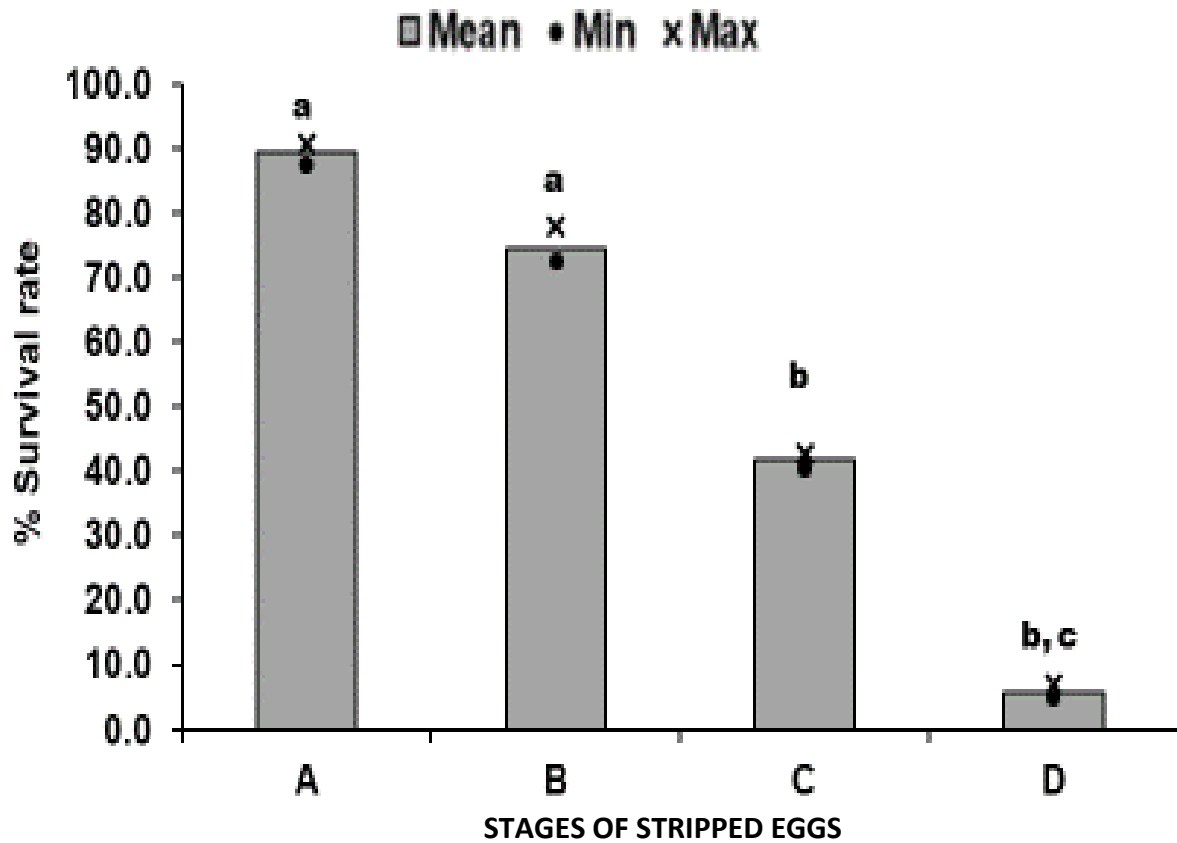


Figure 3: The percentage survival of different batches of stripped eggs of *H. longifilis* Brood Stocks

#### 4.0 Discussion

Water parameters considered in this study were not significantly different among all the batches and were within the recommended range for catfish breeding (Eyo *et al.*, 2003; George and Atakpa, 2015; Jonah *et al.*, 2020).

It has been observed that all eggs in the gonad do not mature at the same time. But in artificial environment under aquaculture system entire eggs in the gonad mature at the same time through human intervention using artificial hormone. In a natural environmental condition, entire eggs are not released at the same time rather in batches based on the stages or levels of maturity. 100% fertilization and hatchability are certain in a natural environment based on the viability of the released eggs, sufficient sperm for fertilization and conducive environmental parameters. This is because breeders in the wild do not release immature egg whereas under forceful maturity of eggs, achievement of 100% fertilization and hatchability is doubtful.

This study reveals that first batch of stripped eggs show percentage fertilization of  $90.17 \pm 0.44$  significantly ( $p < 0.05$ ) higher than  $87.69 \pm 0.42$  obtained from the second batch of stripped eggs and  $70.17 \pm 1.09$  obtained from batch 3, the least batch of eggs had percentage fertilization of  $51.83 \pm 1.69$ . This result reveals that the percentage fertilization of stripped eggs decreased as the batches of stripped eggs shifted from the first to the least. This result could be as a result of maturity stage and viability of the eggs. The percentage hatchability observed from the different batch of stripped eggs showed a similar trend to that of percentage fertilization with a decreasing pattern observed from the first batch to the least. This result could be attributed to different levels or stages of eggs maturity (Otoh and Nlewadim, 2019). It is observed that although all eggs matured through artificial induction of hormones, the level of egg maturity differs. The percentage survival of the fry obtained from the stripped eggs reduced significantly as the batches changed from batch 1 to 4 in the order of  $A > B > C > D$ .

This study revealed that the first batch of stripped eggs during artificial spawning produced excellent reproductive performances followed by the second batch while the least and the poor reproductive performance was observed in the least batch of stripped eggs.

## 5.0 Conclusion

Based on the result of this study, it is observed that the reproductive performance of *Heterobranchus longifilis* primarily a function of viable eggs. The results of % fertilization, % hatchability and % survival rate were excellent for the first and second batch of stripped eggs when compared to the third and fourth batch. Upon the findings in this study, it is recommended that only 75% of stripped eggs from catfish broodstock should be used during artificial spawning while the last 25% should be ignored for the security of the entire hatching process.

## 6.0 Reference

- Adewunmi, A. A. and Olaleye, V. F. (2011). Catfish culture in Nigeria: Progress, Prospects and Problems. *African Journal of Agricultural Research*, 6(6): 1281 – 1285.
- Akande, G., and Y. Diei-Ouadi. (2010). Post-harvest losses in small-scale fisheries: Case studies in five sub-Saharan African countries. FAO Fisheries and Aquaculture Technical Paper. No. 550, FAO, Rome.
- Asangusung, P. S., Uka, A., & Otoh, A. J. (2020). Economic evaluation of three hormonal preparations in artificial propagation of *Heterobranchus longifilis* (Valenciennes, 1840). *Journal of Aquatic Sciences* 35(2):173-179.
- Cejko, B. I., Źarski, D., Krejszef, S., Kucharczyk, D. and Kowalski, R. K. (2013). Effect of Hormonal Stimulation on Milt Volume, Number of Sperm and Sperm Motility in the Crucian carp, *Carassius carassius* (L.). *Isr. J. Aquacult.*, 65: 912-919.
- Ekanem, S. B., Otoh, A. J., Enyehihi, U. K., Taeye, M. (2000), The Response of Juvenile *Chrysichthys nigrodigitatus* (lacepede) To different components. *African Journal of Fisheries and Aquaculture*, 2(2000): 59 -67.
- George, U. U. & Atakpa, E. O. (2015). Seasonal Variation in Physico-chemical Characteristic of Cross River Estuary, South Eastern Nigeria. *Nature and Science*; 13(12):86-93.
- Jonah, U. E. George, U. U. & Avoaja, D. A. (2020). Impacts of Agrochemicals on Water Quality and Macro-invertebrates Abundance and Distribution in Ikpe Ikot Nkon River, South-South Nigeria. *Researcher*; 13(12):36-43.

- Hanjavanit, C., Kitancharoen, N. and Rakmanee, C. (2008). Experimental Infection of Aquatic Fungi on eggs of African Catfish, *Clarias gariepinus*, (Burchell 1882), Kwame Kruma University of Science. 36: 36-43.
- Kristan, J., Źarski, D., Blecha, M., Policar, T., Malinovskyi, O., Samarin, A.M., Palinska-Zarska, K., Nowosad, J., Krejszeff, S. and Kucharczyk, D. (2018). Fertilizing ability of Gametes at Different Post-activation times and the Sperm–oocyte ratio in the artificial Reproduction of pikeperch Sander *luciooperca*. *Aquacult. Res*; 49 (4):1383–1388.
- Kucharczyk, D., Nowosad, J., Kujawa, R., Dietrich, G., Biegaj, M., Sikora, M. and Luczynski, M.J. (2018). Comparison of spontaneous and hormone-induced reproduction of burbot (*Lota lota* L.) under hatchery conditions. *Aquaculture*; 485: 25–29.
- Müller, T., Kucska, B., Horvath, L., Ittzes, A., Urbanyi, B., Blake, C., Guti, C., Csorbai, B., Kovacs, B. and Szabo, T. (2018). Successful, induced propagation of African catfish (*Clarias gariepinus*) by ovarian lavage with sperm and hormone mixture. *Aquaculture*; 485 (2): 197–200.
- Nlewadim, A. A., Udoh, J. P. and Otoh, A. J. (2011), Growth response and survival of *Heterobranchus Longifilis* cultured at different water levels in outdoor concrete tanks. *Aquaculture, Aquarium, Conservative & Legislation*; 10(1): 113-122.
- Nya E., Udosen I. and Otoh A. (2017). Effect of Herbal based immunostimulant diets for disease control in African catfish *Clarias gariepinus* against *Aeromonas hydrophilia* Infections. *Journal of Biology, Agriculture and Healthcare*; 7(16):49-54.
- Otoh A. J., M. T. Udo and U. U. George. (2022). Comparative Effect of Inducing Broodstock with Natural and Artificial Hormones on Reproductive Performances. *Tropical Freshwater Biology*, 31: 95-102.
- Otoh A. J., M. T. Udo and U. U. George. (2023a). Comparative Growth Performance and Sex Ratio of *Heterobranchus longifilis* and its Offspring Induced with Synthetic Hormone and Pituitary Gland of *Heterobranchus longifilis*. *Journal of Wetlands and Waste Management*; 5(1): 106- 111.
- Otoh, A. J., Udoh, J. P., Nya, E. and Asuquo, I. E. (2023b). Effect of Incremental Dilution of Catfish Sperm with Normal Saline Solution on Reproductive performance of *Clarias gariepinus*. *Journal of Wetlands and Waste Management*; 5(1):74-78.
- Otoh, A. J. and Udoh, J. P. (2018a). Semen quality of adult male offspring of *Heterobranchus longifilis* of different parental age groups fed two different iso-nitrogenous feeds. *Tropical fresh water biology*, 27: 1-22.
- Otoh, A. J. and Udoh, J. P. (2018b). Age Related Sperm Quality of Male *Heterobranchus longifilis* Brood Stock Fed two different Isonitrogenous feeds. *Tropical fresh water biology*, 27: 31-42.
- Otoh, A. J and Udoh, J.P (2019), Intergenerational consequences of maternal feed type and age on the egg quality of F1 offspring of *Heterobranchus longifilis*. *Journal of wetlands and waste management*, 3(1):53-58.
- Otoh, A. J., Umanah S. I. and Udo, M. T. (2020a): Comparative study of the effects of feed types and ages of Brood Stock on Reproductive Performances of *Heterobranchus longifilis* in concrete ponds. *Nigerian Journal of Agriculture, Food and Environment*. 16(4): 42-49.
- Otoh, A.J., Umanah, S.I., Udo, M.T. and Umoh, M. (2020b), Seminal study on the morphometric, meristic and sexual dimorphism of the calabar snake fish, *Erpetoichthys clabaricus* [polyteridae] [smith, 1866] in a rain forest stream, Nigeria. *Nigerian Journal of Agriculture, Food and Environment*, 16(4): 76-81.
- Oyeleye, O. O., S. I. Ola, and O. G. Omitogun (2016). Ovulation induced in African catfish (*Clarias gariepinus*, Burchell 1822) by hormones produced in the primary culture of pituitary cells. *International Journal of Fisheries and Aquaculture*, 8:67–73.
- Rana, K. (1995). Preservation of Gametes. In: Bromage, N. R. & Roberts, R. J., ed., *Broodstock Management and Egg and Larval Quality*, Cambridge University Press, Cambridge, 53-76.

- Shourbela, R. M., H. G. Tohamy, and N. Waleed (2019). Induced spawning of African catfish (*Clarias gariepinus* Burchell 1822) after pre-spawning prophylactic disinfection; the breeding performance and tissue histopathological alterations are under scope. *Iranian Journal of Fisheries Sciences*, 18 (2): 309–324
- Szabo, T., Radics, F., Borsos, Á. And Urbányi, B. (2015). Comparison of the results from induced breeding of European catfish (*Silurus glanis* L.) broodstock reared in an intensive system or in pond conditions. *Turk. J. Fish. Aquat. Sci*; 15: 385–390.
- Udoh, J. P. and Otoh, A. J. (2017). Growth Performance of Silver Catfish, *Chrysichthys nigrodigitatus* fingerlings Fed salt-rich diet in Fresh Water System. *Aquaculture, Aquarium, Conservation and Legislation*; 10(1): 113 – 122.
- Udoh, J. P. (2000). Survival, development and hatching of *Clarias gariepinus*: (Burchell) eggs in responses to fish extender composition. *African Journal of Fisheries and Aquaculture*, 2, 49-58.

UNDER PEER REVIEW