

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

Abstract

Studying the effects of multiple batches of stripped eggs contributes to the sustainability and viability of aquaculture practices by improving our understanding of reproductive performance and informing best practices in breeding programs, hence, the need to investigate on the effects of multiple batches of stripped egg on reproductive performances of *Heterobranchus longifilis* became necessary. The aim was to provide valuable information for decision-making processes in aquaculture operations. Nine brood stocks of *H. longifilis* (6 male & 3 female) with average body weight of 2.3 kg and average length of 64.2 cm where 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 where 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 brood stock where in four batches, each batch measured 50g and labeled A, B, C & D. 3g of eggs containing approximately 2000 oocytes (eggs) where measured out from each batch and mixed with the diluted milt and incubated in a 2 x 1 x 10cm³ 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% 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%. Sequel to the findings of the present study, the least batch of stripped eggs should be avoided for use in fertilization since the unhatched or dead eggs has some detrimental 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

Different batches of eggs of eggs can exhibit variability in quality and reproductive success, which can impact breeding programs and aquaculture practices. This study will help identify factors influencing this variability. By understanding how different of eggs batches of eggs perform, researchers and aquaculturists can optimize breeding practices to improved overall efficiency and productivity.

The reproductive performance of fish species particularly in controlled environments like concrete tanks, is of significance interest to aquaculturists and researchers. *Heterobranchus longifilis* commonly known as African catfish, is a species of commercial importance in aquaculture due to its fast growth rate, hardiness and adaptability to various rearing conditions (Oyeleye *et. al.*, 2016; Otoh and Udoh 2018 a, b; Otoh and Udoh 2019). They are a popular food fish in many parts of Africa and are also exported to other regions, additionally they are sometimes kept as pet in aquariums (Ekanem, *et. al.*, (2000; Nlewadim, *et. al.*, 2011; Nya, *et. al.*, 2017; Udoh and Otoh, 2017; Otoh, 2020 a, b; Otoh, *et. al.*, 2022; Otoh, *et. al.*, 2023 a, b).Its uniqueness to grow to table size within a short period of time makes it acceptable as an aquaculture candidate (Nlewadim, *et. al.*, 2011; Otoh and Udo, 2018 b) compare to other species. *H. longifilis* is ubiquitous in nearly all the freshwater ecosystems ranging from lakes, rivers and streams (Adewunmi and Olaleye, 2011).

Understanding the reproductive performance of different batches of stripped eggs of *Heterobranchus longifilis* in concrete tanks is crucial for optimizing breeding practices and improving production efficiency. In this study we aim to investigate the reproductive performance of multiple batches of stripped of *H. longifilis* in concrete tanks by analyzing parameters such as eggs viability, hatchability, survival rate of larvae and growth performance. Additionally, comparing the reproductive performance of different batches of eggs can provide valuable information on variations in eggs quality and the effectiveness of breeding management practices (Paul *et al.*, 2023).

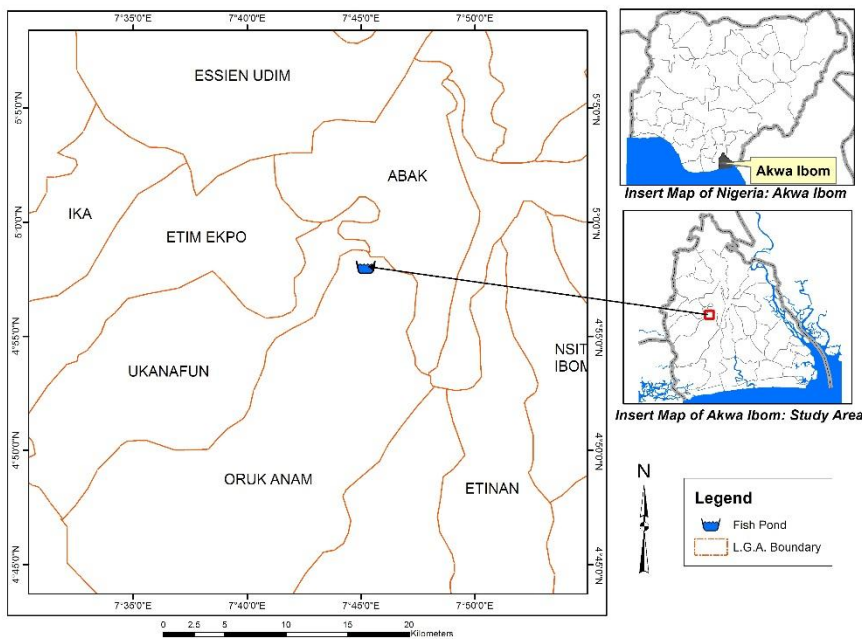
This research will not only contribute to the advancement of knowledge regarding the reproductive biology of *H. longifilis* but also offers practical implication for the sustainable production of this economically importance species in aquaculture settings. Ultimately, a better understanding of the reproductive performance of *H. longifilis* in concrete tanks can lead to improved breeding strategies, increased productivity and enhanced profitability for fish farmers. Finally, identifying factors that contribute to variability in reproductive performance can help mitigate risks

associated with poor hatch rate or low-quality offspring, which can have significance economic implications on aquaculture.

2.0 Materials and Methods

2.1 Study Area

The present study was carried out in Akwalbom State University (AKSU) fish farm complex, ObioAkpa Campus, AkwaIbom State, which is located between latitude 51017'N and 70 27'N, Longitude 7⁰27⁰E and 7⁰58' E. The study area has an annual rainfall ranging from 3500mm to 500mm and average monthly temperature of 25°C. AkwaIbom State is a coastal state lying between latitude 4028'N and 5'3'N and between longitude 7⁰27⁰E and 8-degree 20'E with a relative humidity between 60 to 70%. It is in the tropical rainforest zone of Nigeria. (Otoh *et. al.*, 2019).



Map1: A Map showing the location of the Akwa Ibom State University fish farm complex.

2.2 Acquisition and Care of Brood Stocks

20 matured brood stocks (10 males and 10 females) were randomly selected and stocked in a concrete pond at the rate of 2 fish / m² and fed at 5% body weight twice daily for three months using Coppens commercial feed. six (6) sexually mature males and three (3) females of uniform size with average body weight of 2.3kg and length of 64.7cm were carefully selected according to Otoh, *et. al.*, (2020). The rationale behind the stocking of 20 brood stock was to feed them for a while and select those ones that are of uniform size. Twelve (12) indoor breeding tanks of equal dimension 1x1x1cm³ were used for the study. Water levels and Temperature in each breeding tank was maintained at 30cm³ and 26⁰C respectively.

2.3 Hormone Induced Spawning

Six (6) matured male brood stock 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 (triplicate) 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 approximately 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 $2 \times 1 \times 10\text{cm}^3$ at temperature of $26\text{ }^\circ\text{C}$ and replicated three times. During incubation, water levels were maintained at 30cm^3 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).

$$F_s (\%) = K_f.K_h.K_s/10,000$$

Where;

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

K_f = Fertilization rate (%) of eggs

K_h = Hatching rate (%) of fry

K_s = 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 (K_s) 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 ± 0.20 - 5.66 ± 0.20 , (mg/l) 26.25 ± 0.05 - 26.82 ± 0.04 ($^\circ\text{C}$) and 6.90 ± 0.02 - 6.95 ± 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 ($^\circ\text{C}$)		26.25 ± 0.05	26.50 ± 0.01	26.80 ± 0.02	26.82 ± 0.04
pH		6.90 ± 0.02	6.93 ± 0.01	6.95 ± 0.25	6.95 ± 0.01
Dissolved oxygen (mg/L)		5.21 ± 0.20	5.61 ± 0.40	5.65 ± 0.150	5.66 ± 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 brood stock on reproductive performances of *Heterobranchus longifilis* for (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 ± 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 brood stock 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 ± 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.

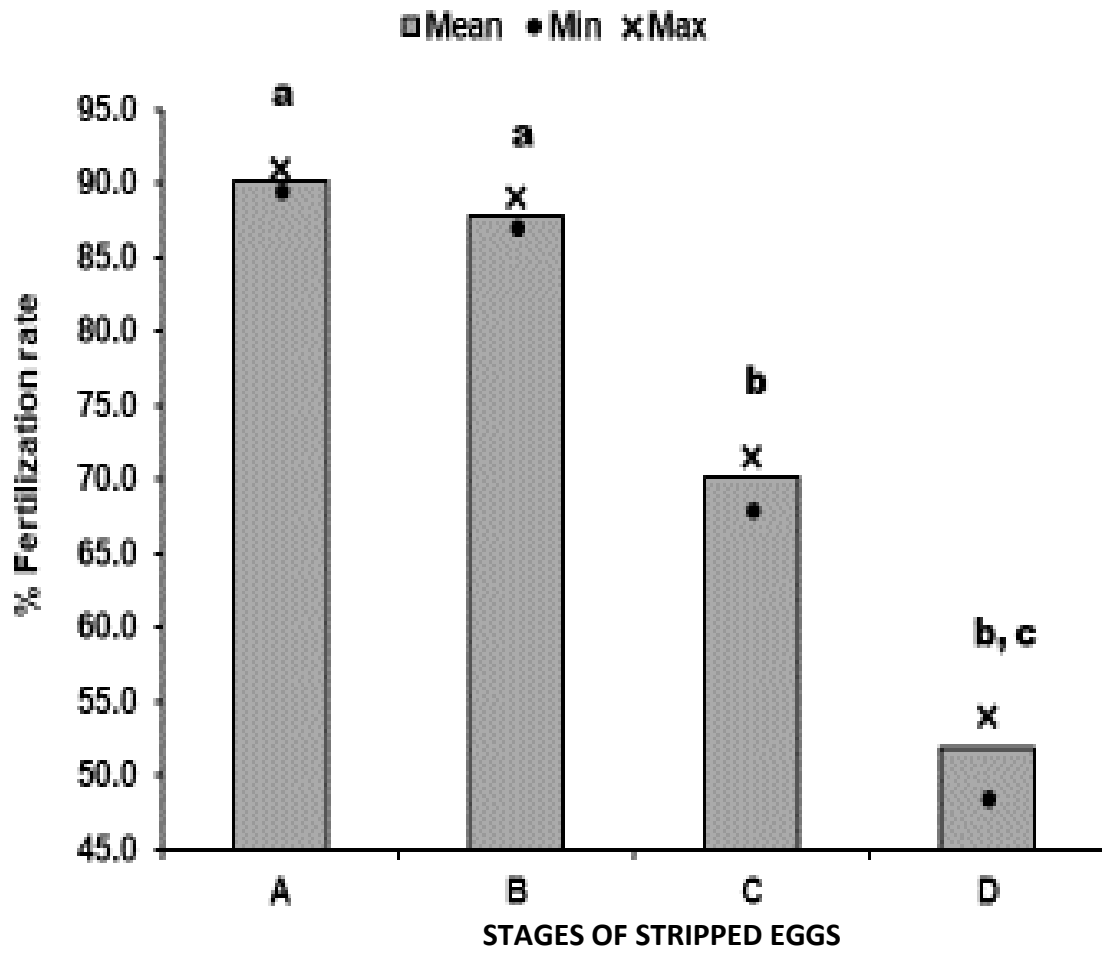


Figure 1: Percentage fertilization of Different Batches of Stripped Egg of *H. longifilis* Brood Stock

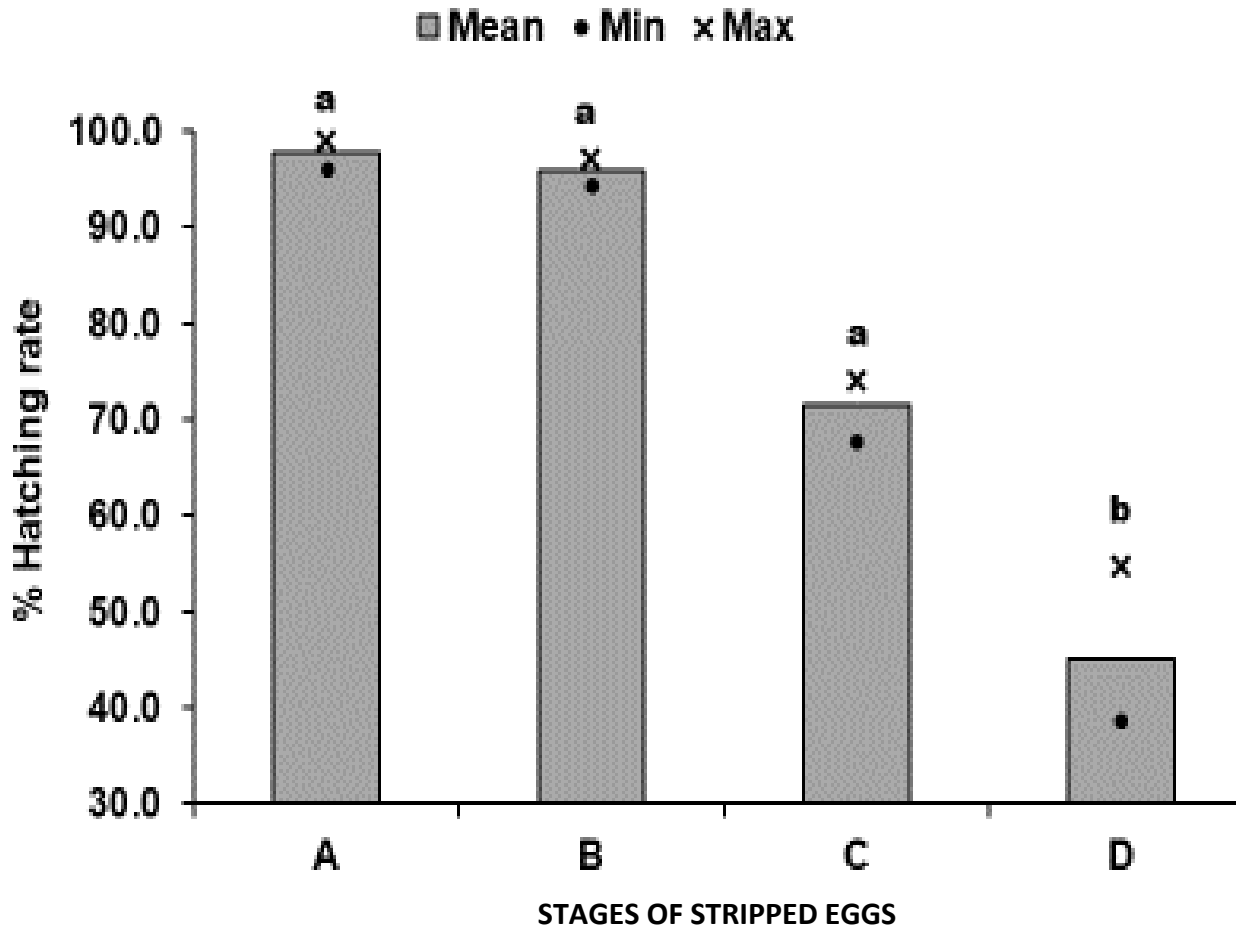


Figure 2: The percentage hatchability of different batches of stripped eggs of *H. longifilis* Brood Stock

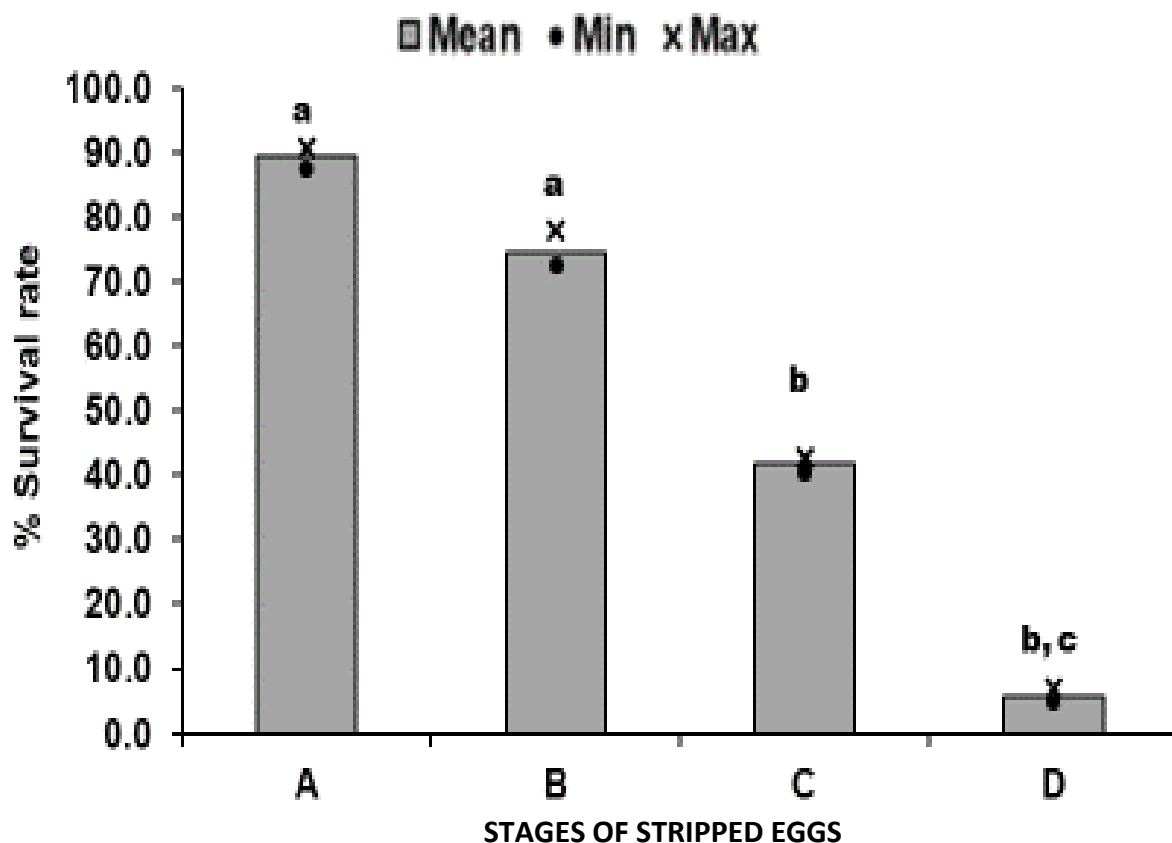


Figure 3: The percentage survival of different batches of stripped eggs of *H. longifilis* Brood Stocks

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).

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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 (Matured gravid females) in the wild (natural habitat) do not release immature egg whereas under forceful maturity of eggs (artificial insemination), achievement of 100% fertilization and hatchability is uncertain.

This study reveals that first batch of stripped eggs show percentage fertilization of 90.17 ± 0.44 significantly ($p < 0.05$) higher than 87.69 ± 0.42 obtained from the second batch of stripped eggs and 70.17 ± 1.09 obtained from batch 3, the least batch of eggs had percentage fertilization of 51.83 ± 1.69 . This result reveal 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 inducement of hormones via artificial insemination, the level of egg maturity varies depending on the developmental stages of the embryo. 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* is primarily a function of viable eggs. The results of % fertilization, % hatchability and % survival rate was 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 brood stock should be used during artificial spawning while the last 25% should be ignored for the security of the entire hatching process.**

5.1 Acknowledgements

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