Study on tThe Effects of Different Batches of Stripped Eggs on tThe Reproductive Performances of *Clarias gariepinus*

AbstractABSTRACT

This study was conducted at the hatchery complex of Akwa Ibom State Universityto investigate the effects of different batches of stripped egg on reproductive performances of *Clarias gariepinus*. Nine brood stocks of *Clarias gariepinus* (six6 malesand&three3 females), with average body weight of 2.3kg and 64.2cm, where carefully selected for the study. Six matured males weresacrificed euthanizedfor the removal of testis without hormonal inducements. The milt collected from the six males were poolled together and divided into 12twelve portions each diluted with 2mL1 of normal saline solution. Three female brood stocks where separately induced at single dosage of 0.5mL1/kg body weight and allowed for a period of 10 hours before stripping. Stripping of eggs from each broodstock where in four batches, each batch measured 50g and labeled A, B, C, and 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 2x1x10cm^3 of water in a concrete pond at temperature of 26^{o}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 rate 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 rate value of 5.59 ± 0.61. Therefore, from the above result, the least batch of stripped eggs should not be used for fertilization since becausethe unhatched or dead eggs has anegative effect on the fertilized eggs including fry at the hatchery level.

Keywords:Batches of stripped Eggs, Reproductive performances, Clariasgariepinus, *Hatchability, Fertilization.*

1.0INTRODUCTIONNTRODUCTION

The success of aquaculture at the hatchery level would have no comparism if the total no of stripped eggs duringartificial propagation of catfish survived till fry or fingerling stage.Fish remains auniversal protein source acceptable at all ages of humanity, easily to come by at affordable cost,comparecompared to other sources of protein. Globally, the demand for fish is at daily increase as the world population continue to expand,whereas the supply of fish and fish products from thewildisdrasticallyreduceddue toenvironmental challenges anddegradations leaving behind aquaculture as the only alternative means to meet the global needs.

African catfishes such as *Heterobranchus* and *Clarias*species remains the most culturable species of significance in Nigeria and beyond (Otoh*,et.et al.*, 2017; Otoh and Udoh, 2018 a, b; Oyeleye*,et.et al.*, 2016). This is due to the unique characteristic of the species suchas; fast growth rate, good taste, generally accepted for consumption, high market price and high resistance to disease, and ability to reproduce in captivity (Nlewadim*,et.et al.*, 2011; Nya*,et.et al.*, 2017; Otoh*,et.et al.*, 2020 a, b; Otoh*,et.et al.*, 2022; Otoh *et.etal.*, 2023 a, b). Although, the growth of fish depends on availability of good feed of which a single feed stuff component cannot achieve (Ekanem*,et.et al.*,2000).*Heterobranchus* and *Clarias* readily accept any supplementary feed and their growth rate is unique within a short period of culture according to (Nlewadim *et.etal.*, 2011) compared to other species (Otoh and Udoh, 2018;Asangusung *et al.*, 2020). These species dominate fresh water setting such as streams, lakes, and rivers (Adewunmi and Olaleye, (2011) and has high commercial value (Oyeleye*,et.et al.*, 2016; Shourbela*,et.et al.*, 2019).

Problems associated with fish seed at the natural environment necessitated artificial propagation technique (induced sSpawning) 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 gamete which is at times difficult to achieve (Cejko *et.et al.*, 2013; Szabo*,et.et al.*, 2015; Kristan*,et.et al.*, 2018). This situation called for intervention of bio technologist for the collection of high-quality gametes, short term gamete storage (Kucharczyk *et.et al.*, 2018) and fertilization (Muller*,et.et al.*, 2018).

Catfish had gained huge recognition in our society hence the need for intensificationof breeding technique for mass production of fish seed needed for farm stocks. Low survival of fry at the hatcheries level could be attributed to unidentified factors (Otoh and Udoh, 2019) ofwhich differences in the batches of stripped egg from the brood stocks might not be exceptional hence the focus of this study.

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2.0MaterialsandMethods

2.1 Study Area

This research study was carried out in AkwaIbom State University (AKSU) fish farm complex, ObioAkpa Campus, AkwaIbom 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. AkwaIbom 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 and to 70%. It is in the tropical rainforest zone of Nigeria. (Otoh and Udoh, 2019)

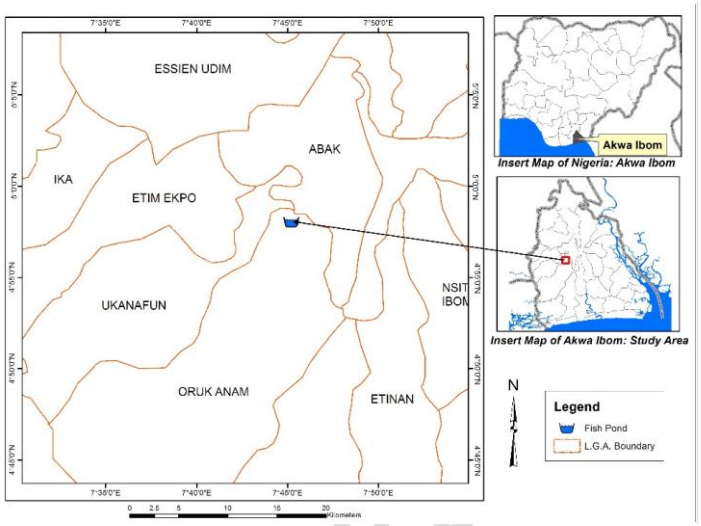

Fig. 1. A Map showing the location of the Akwa Ibom State University fish farm complex.

2.2 Acquisition and Care of Brood Stocks

Nine (9)matured broodstocks (six6 males and three3 females) were separately stocked in a concrete pond at the rate of 2twofishes/m^2 and fed at 5% body weight twicedaily for three months using Coppens commercial feed. Six sexually mature males and three females with average weight of 2.3kg and 64.2cm were carefully selected according to Otoh, *et. et al.*, (2020a).Twelve (12) indoor breeding tanks of equal dimension 1x1x1cm^3 wereused for the study. Water levels and t Temperature in each breeding tank were as maintainedat 30cm^3 and 26°C, respectively.

2.3 Hormone Induced Spawning.

Six(6) matured male broodstock were sacrificed euthanizedfor sperm removal without hormonal inducement. The milt collected from the three samples were pooled together in a plastic container and dividedinto 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. et al.*,2020a and;Ootoh, *et. et al.*, 2023).

2.4 Eggs Stripping and Fertilization.

Four batches of stripped eggs (50g each) were separately obtained from each of the three3breeders 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 three3minutes, the saline solution

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was decanted while the fertilized eggs were uniformly spread on the Kakaban(sShredded nylon sack) and incubated in aeratedindoor concretebreeding tanks 1x1x1m^3 at temperature of 26-°C and replicated three times. During incubation, water levels were maintained at 30cm^3 depth.

2.5 Fertilization.

The cColour variations between theineggs wasereobserved, with clear and transparent eggswere considered as fertilized whileanddead /white and opaque one was regarded as unfertilized (Udoh, 2000;. andOtoh,*et.etal*., 2023).Based on the counts, the reproductive performancesof different stripped eggs were observed, such as: percentages of fertilization, hatchability, survival, and fry production success. Efficiency of these productions was evaluated following 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

Percentage hatchability wasobtained 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 (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.et al*., 2008)

2.6 Determination Water quality Parameters

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.

2.7Statistical Mmethods

Data were processed using Microsoft Excel 2010analyzed for with their mean values, standard deviations, and ranges and presented shown 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.

3.0RESULTS

3.1 Physico-Chemical Parameters of Culture Media

The water parameters considered in this study, showed no significance (P>0.05) difference among the treatments.Dissolved oxygen, temperature, and PH measurements ranged between 5.21 ± 0.20–5.66 ± 0.20 (ML-1),26.25 ± 0.05–26.82 ± 0.04 (°C-C) and 6.90 ± 0.02–6.95 ± 0.01, respectively.

Table 1: Mean Water Quality Parameters of tThe Incubating Tanks

	Stripped egg stages			
	Stage 1	Stage 2	Stage 3	Stage 4
Temperature (˚C)	26.24 ± 0.05	26.40 ± 0.01	26.80 ± 0.02	26.81 ± 0.04
pH	6.90 ±0.01	6.92 ±0.01	6.94 ±0.25	6.94 ±0.01
Dissolved oxygen (mg/L)	5.21 ± 0.20	5.62 ± 0.40	5.64 ± 0.150	5.65 ± 0.20

3.2 Effect of Different Batches of Stripped Eggs on the Reproductive Performances of *Clarias gariepinus*

The results on the effect of different batches of stripped eggs from the broodstock on reproductive performances of *Clarias gariepinus* revealed that's the percentage in fertilization of the first batch of stripped eggs was 87.69±0.42significantly (P<0.05) higher than the 85.38 ± 0.60observed in the second batch of stripped eggs, which significantly (P<0.05) increased more than the third and last batches of stripped eggs, with the least value of percentage fertilization of50.33±1.67recorded for batch 4 (Table 2).The percentage fertilization of different batches of stripped eggs decreased in the order of A, B, C,and D.

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The result on the percentage hatchability of the stripped eggs equally decreases significantly (P<0.05) from the first to the least in the order of 97.06±0.84, 93.87±0.80, 68.85±1.89, and 31.93±4.94~~respectively~~ (Table 3).

The result of different batches of stripped eggs from the broodstock on the percentage survival of the fry~~'s~~ is presented in Table 4. The percentage survival of fry obtained from the first batch of stripped eggs was 84.29±1.05significantly (P<0.05) higher than 76.93±1.76 percentage survival obtained from the second batch of stripped eggs. The percentage survival of eggs 56.21±0.67 obtained from the third batch of stripped eggs was significantly (P<0.05) higher than the least value of percentage survival recorded for the last batch of stripped eggs with a percentage survival rate of 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 lasts batch as shown in table (2-4).

Table 2: The percentage fertilization of different batches of stripped eggs of *Clarias~~,~~ gariepinus* reared in concrete fish ponds.

	Batches of stripped eggs.			
	Batch 1	**Batch 2**	**Batch 3**	**Batch 4**
Total Weight (kg)	66 .00 ± 0.00[a]	66.00 ± 0.00[a]	66.00 ± 0.00[a]	66.00 ± 0.00[a]
Total Length (cm)	2.23 ± 0.00[a]	2.23 ± 0.00[a]	2.23 ± 0.00[a]	2.23 ± 0.00[a]
No. of eggs used	200.00 ± 0.00[a]	200.00 ± 0.00[a]	200.00 ± 0.00[a]	200.00 ± 0.00[a]
No. of whitish (dead) eggs	24.62 ± 0.84[a]	29.23 ± 1.20[b]	64.65 ± 2.19[c]	99.33 ± 3.34[d]
No. of brown (fertilized) eggs	175.38 ± 0.84[a]	170.77 ± 1.20[b]	134.35± 2.19[c]	100.67 ± 3.34[d]
Fertilization (%)	87.69 ± 0.42[a]	85.38 ± 0.60[b]	67.17 ± 1.09[c]	50.33 ± 1.67[d]

Table3: The percentage hatchability of different batches of stripped eggs of *Clarias~~,~~ gariepinus* reared in concrete fish ponds.

	Batches of stripped eggs.			
	1	**2**	**3**	**4**
Total Weight (kg)	66.00 ± 0.00[a]	66.00 ± 0.00[a]	66.00 ± 0.00[a]	66.00 ± 0.00[a]
Total Length (cm)	2.23 ± 0.00[a]	2.23 ± 0.00[a]	2.23 ± 0.00[a]	2.23 ± 0.00[a]
No. of eggs used	200.00 ±0.00[a]	200.00 ± 0.00[a]	200.00 ± 0.00[a]	200.00 ± 0.00[a]
No. of whitish (dead) eggs	24.62 ± 0.84[a]	29.23 ± 1.20[b]	64.65 ± 2.19[c]	99.33 ± 3.34[d]
No. of brown (fertilized) eggs	175.38 ± 0.84[a]	170.77 ± 1.20[b]	134.35 ± 2.19[c]	100.67 ± 3.34[d]
No. of eggs hatched	170.23 ± 2.16[a]	160.31 ± 0.68	92.51 ± 2.41	32.15 ± 3.52
Hatchability (%)	97.06 ± 0.84[a]	93.87 ± 0.80[b]	68.85 ± 1.89[c]	31.93 ± 4.94[d]

*Means with the same superscript within the same row are significantly different (p<0.05)

Table4: The percentage survivalRate of different batches of stripped eggs of *Clariasgariepinus* reared in concrete fish ponds.

Batches of stripped eggs

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	Batch 1	Batch 2	Batch 3	Batch 4
Total length (cm)	65.00 ± 0.00[a]	65.00 ± 0.00[a]	65.00 ± 0.00[a]	65.00 ± 0.00[a]
Total weight (kg)	2.3 ± 0.00[a]	2.3 ± 0.00[a]	2.3 ± 0.00[a]	2.s3 ± 0.00[a]
No. of eggs hatched	170.23 ± 2.16[a]	160.31 ± 0.68[b]	92.51 ± 2.41[c]	32.15 ± 3.51[d]
Survival after 10 days	152.00 ± 2.00[a]	123.33 ± 2.84[b]	52.00 ± 0.58[c]	13.32 ± 1.86[d]
Percentage Survival (%)	84.29 ± 1.05[a]	76.93 ± 1.76[b]	56.21 ± 0.67[c]	42.69± 0.61[d]

*Means with the same superscript within the same row are significantly different ($p<0.05$)

4.0DiscussionDISCUSSION

The experimental water quality parameters were in line with the recommended standard for aquaculture operation for catfish breeding (Eyo *et al.*,2003; George and Atakpa, 2015; Jonah *et.et al.*, 2020), as significant differencesin water quality parameters wereas not observed in all the batches throughout the duration of study.

This study stem from the fact that all eggs in the gonad do not mature at the same time. Though, through human interference with the use of artificial hormones maturity of entire eggs occur at the same time and the need to consider if all the mature eggs through human interference do fertilize, hatched, and survived as expected remained very crucial for a successful breeding programme. This is to overcome the danger of mass mortality since the unhealthy eggs are primarily responsible for the mortality observed at the early stage of fry development.

Considering the gonad development of catfish, like *Clarias gariepinus*species,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 arecertain in a natural environment based on the viability of the eggs released, sufficient sperm for fertilization and conducive environmental conditions. This is because breeders in the wild do not release immature eggs. Under artificial propagation (forceful maturity of eggs) achievement of 100% fertilization and hatchability is doubtful. This study reveals that first batch of stripped eggs show percentage fertilization that was significantly higher than the valueobtained for the second batch of stripped eggs, third batch and forth batch respectively.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 similar pattern of results with decreasing order from the first to the last batch. This result could be attributed to different levels or stages of eggs maturity. It is observed that although all eggs matured through artificial inducement of hormones, the level of egg maturity differs.

The percentage survival rate of the fry production success of stripped eggs reduced significantly as the batches increased from batch 1-4 in the order 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.

Overall, the observed differences in reproductive performance between the first and second batches compared to the third and fourth batches may be attributed to a combination of factors including the age and condition of the brood stock, timing of egg stripping, environmental conditions, and genetic variability.

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4.1 Conclusion

The research investigated the impacts of different batches of stripped eggs on the reproductive performance of *Clarias gariepinus*. Results showed that the first and second batches exhibited better reproductive performances compared to the third and fourth batches. This suggests that the timing or quality of egg stripping may influence the reproductive success of the species. The success recorded by the first and second batches of stripped eggs when compared to the third and fourth batches could be attributed to timing of stripping and

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environmental conditions during egg collection. Higher quality eggs and optimal timing of stripping may have led to better reproductive success, while eggs from the later batches may have been of lower quality or collected under less ~~favourable~~favorable conditions, impacting their viability and subsequent reproductive performance.

4.2 Recommendations

Further research is needed to fully understand the underlying mechanisms driving these differences and to optimize egg collection practices for enhanced reproductive success in *Clarias gariepinus* aquaculture.

6.0REFERENCE~~eference~~

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., Żarski, D., Krejszeff, 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., Taege, M. (2000), The Response of Juvenile Chrysichthys nigrodigitatus (lacepede) To different components. African Journal of Fisheries and Aquaculture, 2(2000): 59 -67.

Eyo, A. A. (2003). Fundamentals of fish nutrition and diet development –An overview, in Eye, A. A. (Ed). National Workshop on Fish Feed Development Feeding Practices in Aquaculture FISON, NIFFR *and FAO – NSPFS, New Bussa, Nigeria*, PP 1 –33.

George, U. U. & Atakpa, E. O. (2015). Seasonal Variation in Physico-chemoical Characteristic of Cross River Estuary, South Eastern Nigeria. Nature and Science; 13(12):86-93.

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.

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.

Kristan, J., Żarski, 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 lucioperca. *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 hydrophia* 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.

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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 (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. 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., 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 (*Silurusglanis* 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, *Chrysicthys nigrodigitatus* fingerlings Fed salt-rich diet in Fresh Water System. Aquaculture, Aquarium, Conservation ad 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.

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