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Relationships of Egg Size of Clarias Gariepinus on Fertilization, Hatching and Frys Survival Rates

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ABSTRACT

Eggs from matured female brood stocks were obtained and classified into 3 egg size classes, large, medium and small based on the size of eggs. 1.0-1.2mm, 0.5-0.9mm and 0.3-0.5mm respectively. They were fertilized with milt obtained from sacrificed males. The data obtained was used to assess the fertilization, hatching and frys survival rates of Clariasgariepinus. The result shows that the size of egg has a significant effect on the fertilization, hatching and fry survival rates of Clariasgariepinuus. That is the bigger the egg size the higher the fertilization, hatching and frys survival rates and vice versa. The physic-chemical parameter (ie the temperature PH and dissolved oxygen) values are within the expected range.

Keywords: Egg sizes, Fish, Fertilization, Hatching, Survival rate, Fecundity, Physico-chemical parameters.

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1. INTRODUCTION

Catfishes constitute a large group of primarily fresh water fishes which are widely distributed throughout the world. The *Clarias*species are omnivorous with a propensity for being carnivores. They live mostly on river bottom, swamps and muddy places (hence the nomenclature mud fish) where they feed on variety of food ranging from mud, planktons, weed to frogs, small fish insects and their larvae, crustaceans, worms, snails, etc [5]. *Clariasgariepinus*and *Clariasanguilaris*grow to a large size one (1) meter or more in length and more than (7) seven kilograms in weight. *Clariassubmarginatus*is much smaller, 186mm being the largest recorded in Nigeria. *Clarias*species exhibits many qualities which make it suitable for culture. These include hardiness, high disease resistance, high yield potential, consumer acceptability, high fecundity and more salinity tolerance. *Clarias*is able to withstand adverse environmental conditions and can easily be spawned under capture conditions. It has a wide tolerance of relatively poor water quality condition in which other freshwater fishes would find it difficult to survive. The ‘hardiness” of the beast makes it an ideal candidate for highly intensive culture without prerequisite pond aeration or high water exchange rates [18]. Reproduction could be natural or artificial.

1.1. Reproduction in *Clarias* Species

In its natural environment, *Clarias*breed generally during the rainy season but the presence of large quantities of eggs in females are reported from May to October although some matured eggs are still found at all times. The male has elongated conical urinogential papilla while in the female the vent (oviduct) is more rounded with a longitudinal grove/cleft [13].

There is often a massive aggregation before spawning during which the males fight themselves for the right court with the females in river massive migration of catfish may take place before spawning sometimes numbering thousands of fishes [35]. At full maturity, a gravid female show a deeper and more founded soft abdomen, a reddish vent, prominence of blood vessels in the belly region and the appearance of a few eggs upon slight manual pressure on the abdomen [2].

Ripe males are characterized slight vascularised genital papilla. Unlike the females, the males do not respond to the ejection of milt on slight manual pressure on the abdomen.

*C. gariepinus*shows a seasonal gonodal maturation which is usually associated with the rainy season. The maturation process of *C. gariepinus* are influenced by annual changes in water temperature and photoperiodicity and the final triggering of spawning is caused by a rise in water level due to rainfall. A complete breakdown of the natural annual reproductive cycle can be obtained after the broodstock have been kept for one year indoors and reproduction can then be carried out continuously throughout the year. However, this method is not recommended as it depends on the availability of high quality feed and often encounter diseases such as crank head and retarded growth in the breeders, and an oedemic disease in the developing larvae [9].

An example of maturation and spawning of *C. gariepinus*in Lake Victoria (Kenya) shows that the reproduction start in March just after the start of the first heavy rains as is indicated by the decrease in the Gonad Somatic Index (GSI). Natural reproduction is completed in July and the GSI remains low till November, therefore the gonads start maturing gradually and become ripe again in March [9].

$$GSI = \frac{\text{weight of ovary}}{\text{Total weight}} \times 100$$

Spawning usually takes places at night in the shallow inundated areas of the river lakes and streams. Courtship is preceded by highly aggressive encounters between males. Courtship and mating takes place in shallow waters between isolated pairs of males and females. The mating posture, a form of amplexus (the male lies in a V-shape curved around the Head of the female) is held for several second, a batch of milt and eggs is released followed by a vigorous swish of the female's tail to distribute the eggs over a wide area. The pair usually rest after mating (from seconds up to several minutes) and then resume mating. There is no parental care for ensuring the survival of catfish offspring except by the careful choice of a suitable site. Development of eggs and larvae is rapid and the larvae are capable of swimming within 48-72 hours after fertilization at 23-28^{0}0 C [9].

There is often massive aggregation before spawning during which the males fight among themselves for the right to Court the females. In river massive migration of catfish may take place before spawning, sometimes numbering thousands of fishes [9]. Fertilization of the eggs take place externally, catfish sperm in motile for 60 to 120 seconds which is a very short period compared with sperm mortality duration in *Tilapia* of 10 -12 minutes [8]. Field observation suggest that the male release its sperm before the female releases her eggs, she swishes her tail vigorously from inside to side using her snout as an anchor and mixes and distributes the eggs. Under natural conditions a pair may consequently mate 2 to 5 minutes before they are distributed and separated [8]. The development of the occytes of the African catfish is mostly related to temperature (as in common with large number of fish species). Within the development of the occyte, six chronological stages can be seen [28].

1.2. Effect of Egg Size on Larval Size

Understanding the evolution of life histories required an accurate knowledge of the trade-offs that govern reproductive strategies [21, 22]. Trade-offs occur because no simultaneous maximization of all life history traits influencing reproductive success is possible, so optimization therefore entails an allocation compromise between conflicting trait [29, 31]. Within this framework, there has been particular interest in examining the compromise between the size and number of descendants [11] since it affects both fecundity and offspring fitness which are the two crucial features of female fitness [25]. The concrete development of this balance is conditioned by the consequences on parental fitness of variation in both components. If offspring fitness is not size dependent, then natural selection will favour the production of a large number of offsprings, each approach the physiologically minimum viable size. Nevertheless, there is frequently a positive size-dependence of several offspring performance traits [20, 29]. Therefore, if juvenile size is influenced by egg size, there will be an increase of parental investment per descendant until the gain in juvenile survival offsets the loss of fecundity.

*Clariasgariepinus*exhibited considerable variability in egg size, both within and among populations, partially accounted for by female age and size [4, 14]. Whereas inter-population variability in egg size could be adaptive, variable environmental, inter-population patterns of variation almost certainly indicate that some advantages are associated with large egg size.

1.3. Aim

The aim is to carry out an investigation on the effect of egg size on fertilization, hatching and fry survival rate in *Clariasgariepinus.*

1.4. Specific Objectives

* To determine the effect of egg size on the fertilization and hatching rates in *Clariasgariepinus*species.
* To assess fry survival rates within a stipulated time with respect to different egg sizes.
* To determine the physic-chemical parameters of the hatching water of Bagauda Fish Seed Multiplication Centre.

2. MATERIALS AND METHODS

2.1. Research Site

The research involving the investigation of the effect of egg size on fertilization, hatching and fry survival rates in the African catfish *Clariasgariepinus* was carried out at the hatching complex of Bagauda Fish Seed Multiplicaton Centre, Kano State for a period of six months (March-September 2015).

2.2. Fish Brooders

Twelve brood stocks (six males and six females) were procured from a reputable fish farm in Kano State – FAGAM Integrated Farms. Both males and females were selected for qualities required for fingerlings production. Three different class sizes of the broodstock were procured from the FAGAM farm which were between 0.6kg and 2.0kg body weight. They were transported to Bagauda fish seeds multiplication centre in 50 litreJericans and starved for 36 hours prior to inducement of the broodstock.

2.3. Selection of Female Spawner

Six female broodstock whose belly became soft and containing ripe eggs were selected for induced ovulation [18]. The eggs were obtained by slightly pressing the abdomen towards the genital papilla. They were classified into three eggs class sizes based on the sizes of the eggs. Eggs that ranged between 0.3-0.5mm were classified as small, eggs that ranged between 0.6-0.9mm were classified as medium and those that ranged between 1.0-1.2mm were classified as large.

2.4. Selection of Male Broodstock

Six males broodstock of between 600 – 800gm body weight and 8 months old and above were selected. Presence of ripe sperms was indicated by white, opaque and milky colour extending from the distal margin into the body of the testes, while unripe testes are smaller and translucent in colour.

2.5. Induced Ovulation

Ovaprim which is a synothetic hormone was used to induced ovulation in the female African catfish *Clariasgariepinus*at 0.5ml/kg body weight after using a meter scale to determine the average weights of individual breeder [16, 35]. The metler scale was also used to determine the average weights of the ovulated eggs of each female fish under investigation. After 8 hours they started to ovulate and by 12 hours ovulation was complete.

2.6. Tests Extraction and Milt Harvest

The testes were located at the dorsal part of the abdominal cavity of the male. They show white opaque milky colour. Once ovulation is complete in the females and the eggs were ready for fertilization, the male breeders were sacrificed in order to collect the milt. A sharp knife was used to open up the stomach of the male broodstock where the gonad were located. The milt was then extracted and preserved in 0.9% saline in plastic containers.

2.7. Stripping

An ovulating female was recognized by a thickness and softness of the belly region. The female was then held by two persons. The abdominal region was gently cleaned with a dry towel to prevent water from coming in contact with eggs. The eggs were then collected into a clean dry plastic bowl by gently using the thumb to press the abdomen from the pectoral fin towards the genital area. The eggs were seen flowing easily from the genital vent. The eggs were then classified into three class sizes, larges, medium and small based on the size of the eggs. This is in accordance with the stripping technique of Hogendoorn, [19] and Viveen et al., [34] where ripe breeders were injected with pituitary hormone to induce egg ovulation. The males were sacrificed for milt since the milt cannot be procured by stripping.

2.8. Latency

The latency period was determined as the time at which the eggs collected gave high yield of normal fertilized larvae with minimum number of deformed larvae.

2.9. Fecundity

This is the amount of eggs found in a female prior to the next spawning season. It was calculated using the formula

Fecundity = wt of egg stripped x 750 (1g eggs = 750 eggs) [34].

2.10. Fertilization

The male was sacrificed and the milt was collected in 0.9% saline solution. The milt was then mixed with three different egg size classes, eggs of between 0.3-0.5mm (Small) eggs of between 0.7-08mm (medium) and eggs of between 1.0-1.2mm (large) and stirred very well with a feather. Fertilized eggs appear light brown and transparent while unfertilized eggs appears whitish and opaque.

Percentage fertilization was calculated using the formula

$$\% \text{ fertilization} = \frac{\text{Mean no. of eggs fertilized}}{\text{Mean no. of eggs milted}} \times 100$$

2.11. Determination of Egg Size

The female broodstock were classified into three class sizes based on the size of eggs. Large (1.0-1.2mm), medium (0.6-0.9mm) and small (0.3-0.5mm). eggs obtained from each class size was also classified into large, medium and small respectively by collecting a sample of ten unfertilized eggs from each female size class. Measurement of the diameter of the eggs were made with a dissecting microscope with an ocular micrometer.

2.12. Hatching

Six (6) concrete hatching tanks measuring (2m x 2m x 1m) at the Bagauda Fish Seeds Multiplication Centre indoor hatchery were used in conducting the investigation. A spawning mat for each hatching tank was constructed based on the size of tank using a mosquito net size polymopylene material, plastic pipes and ½” diameter, elbow pipes and T-pipes. This is to enable good spread of the fertilized eggs to facilitate high rate hatching of the fertilized eggs. The hatching mats were then placed in each of tanks, the fertilized eggs based on the three different class sizes was poured on each spawning mats and water allowed to pass through continuously. After about 24 hours the eggs start to hatch. Water was passed continuously at low pressure for good aeration and to prevent the egg shell from decomposition.

$$\% \text{ hatching} = \frac{\text{mean no. of frys hatching}}{\text{mean no. of eggs fertilized}} \times 100$$

2.13. Feeding of Larvae

From the first day to the second day after hatching the larvae still have egg yolk which serve as food. After this mouth become open, the egg yolk has been completely absorbed and after the third day they start taking food. Three experimental tanks were set up based on the larvae that emerged from the three egg sizes classes, large, medium and small. They were fed on *Artemia*(Shell free) for two weeks. Feeding was done three times per day at 8.00am, 2.0pm and 5.00pm at 2 times their body weight. After this they were fed on 0.5mm prepared coppens feed for one week three times per day at 8.00am, 2.00pm and 5.00pm. In the fourth week they were feed on 0.8mm prepared coppens feed. After which they were fed on 1.2mm and 2.0mm for two weeks.

2.14. Survival Rates

The survival rates of fingerlings was assessed using the formular.

$$\frac{\text{No. of hatched eggs}}{\text{No. dead}} \times 100$$

2.15. Phsico-Chemical Parameters of Experimental Water

2.15.1. pH

The hydrogen ion concentration of the experimental water was analyzed using a pocket sized pH meter (model-PHeP). The pH was analyzed bi-weekly.

2.15.2. Temperature

The degree of coldness or hotness of the experimental water was analyzed bi-weekly using a mercury in glass thermometer which is graduated in degree centigrade (^{o}C).

2.15.3. Dissolved Oxygen (DO)

A digital oxygen meter model H1-9146 was used to determine the dissolved oxygen concentration bi-weekly for a period of six weeks.

2.15.4. Statistical Analysis

The fertilization and hatching rates were analyze using the T-test (SPSS 11.5 version) and the survival rates were analyzed using the Time Series Analysis.

3. RESULT

Table-1. Percentage Fertilization of Experimental Eggs

Treatments **Egg Sizes**	**I** **(Large)**	**II** **(Medium)**	**III** **(Small)**
Average wt. of female before stripping (gm)	1650	1,100	700
Average wt. of female after stripping	1485	990	630
Weight of ovulated eggs	165	110	70
No. of eggs per gm	750	750	750
Estimated no of ovulated eggs	123,750	82,500	52,500
Estimated no of eggs milted	6,250	6,900	7,350

Continue

No. of eggs fertilized	6,028	6,451	4,698
No. of unfertilized eggs	222	449	2,652
% of fertilized eggs	**97**	**94**	**64**

Source: Fish seed multiplication centre Kano state, 2015

The above result shows that the percentage number of eggs fertilized for large sized eggs was 97, for medium sized eggs was 94 and 64 for small sized eggs.

Table-2. Percentage of Hatching of Experimental Eggs of *Clariasgariepinus*

Treatments **Egg Sizes**	**I** **(Large)**	**II** **(Medium)**	**III** **(Small)**
Average of female before stripping (gm)	1650	1,100	700
Average of female after stripping	1485	990	630
Weight of ovulated eggs	165	110	70
No. of eggs per gm	750	750	750
Estimated no of ovulated eggs	123,750	82,500	52,500
No of eggs milted	6,250	6,900	7,350
No. of eggs fertilized	6,028	6,451	4,698
No. of eggs hatched	5,484	5,806	3,289
No. unhatched	544	645	1,409
% of fertilized eggs	**91**	**90**	**70**

Source: Fish seed multiplication centre Kano state, 2015

The above result shows that the percentage hatching for large sized eggs (1.0-1.2mm) was 91% and 90% for medium sized eggs (.06 to 0.9mm) and 70% for the small sized eggs (0.3-0.5mm).

Table-3. Percentage Survival of Frys after six weeks

Treatments **Egg Sizes**	**I** **(Large)**	**II** **(Medium)**	**III** **(Small)**
Wt. of female before stripping (gm)	1650	1,100	700
Wt. of female after stripping	1485	990	630
Weight of ovulated eggs	165	110	70
No. of eggs per gm	750	750	750
Estimated no of ovulated eggs	123,750	82,500	52,500
No of eggs milted	6,250	6,900	7,350
No. of eggs fertilized	6,028	6,451	4,698
No. of eggs hatched	5,484	5,806	3,289
Mortality	1,979	2,241	2,137
No survived	3,505	3,565	1,152
% mortality	**36**	**39**	**65**
% Survival rate	**64**	**61**	**35**

Source: Fish seed multiplication centre Kano state, 2015

From the above result the percentage survival was 64% for large sized eggs, 61% for medium sized eggs and 35% for small sized eggs.

Table-4. Morphometric Characteristics of Female Brood Stock of *Clariasgariepinus*

Treatment	**I (Large)**		**II (Medium)**		**III (Small)**	
Replicates	A	B	A	B	A	B
Wt (gm)	1,800	1,500	1,200	1,000	800	600
TL (cm)	58	56	52.5	50	50	48
SL	50	49	45	44	44	42
AVTL (cm)	57.0		51.3		49	
AVSL (cm)	49.5		44.5		43	

Source: Hatching complex of Bagauda Kano, 2015

The above table show that the average total length for large sized eggs was 57.0cm, 51.3cm for medium sized eggs and 49cm for small sized eggs while the average standard length was 49.5cm for large sized eggs, 44.5cm for medium sized eggs and 43cm for small sized eggs.

Table-5. Weekly Standard Length and Total of Frys of *CLariasgariepinus*

Treatment	I (Large)		II (Medium)		III (Small)	
	SL	**TL**	**SL**	**TL**	**SL**	**TL**
Weeks						
1	2.8	2.9	1.9	2.0	0.9	1.1
2	3.3	3.4	2.0	2.1	1.0	1.2
3	3.5	3.7	2.1	2.2	1.2	1.3
4.	3.9	4.1	2.2	2.3	1.3	1.4
5.	4.4	4.6	2.3	2.4	1.4	1.5
6.	4.9	5.1	2.4	2.5	1.5	1.6
Total	22.8	23.8	12.9	13.5	7.3	8.1
AV	3.8	4.0	2.2	2.3	1.2	1.4

Source: Hatching complex of Bagauda Kano, 2015

The above table shows the average standard length and average total length was 3.8 and 4.0cm for large sized eggs, 2.2 and 2.3cm for medium sized eggs, 1.2 and 1.4cm for small sized eggs respectively.

Table-6. Percentage Survival of Frys after six weeks

Weeks/Treatments	I (Large)	II (Medium)	III (Small)
1	0.018	0.0017	0.0007
2	0.019	0.018	0.018
3	0.300	0.028	0.025
4	0.582	0.580	0.043
5	0.865	0.860	0.051
6	1.148	1.120	0.068
Wt. gain (gm)	1.13	1.118	0.067

Source: Hatching complex of Bagauda Kano, 2015

The table shows the total weight gain of frys of *Clariasgariepinus*was 1.13gm for frys obtained from large sized eggs, 1.118gm for frys obtained from medium sized eggs and 0.067 for frys obtained from small sized eggs for the period of six weeks.

Table-7. Physico-Chemical Parameters of Hatching Water of Bagauda Fish Seeds Multiplication Centre for six weeks

S/No	Parameters	Range (mean)
1	Temperature (^{0}C)	23.0-24.0(25.5)
2	pH	7.3-7.4(7.35)
3	Dissolved Oxygen (DO) (mg/L)	5.5-6.0 (5.75)

Source: Hatching complex of Bagauda Kano, 2015

From the table above the mean temperature of the hatching water of Bagauda fish seeds multiplication centre for the period of six weeks was 23.5^{0}C, the mean pH of the water was 7.35 and the mean Dissolved Oxygen (DO) was 5.75mg/L.

4. DISCUSSION

Female *Clariasgariepinus* of the same population produced noticeable variability in egg size. This can mainly be due to differences in female weight and size. The number of fertilized eggs and hatching rates was strongly dependent upon he initial egg size. This relation between offspring size and original egg size persisted throughout the early development of the frys. In fact egg size determined most of the variation in final fingerling size which is thought to be an important component of juvenile fitness [29].

From this study (Table 1) the eggs size range was found to be between 0.3-0.5mm for small females (Small sized eggs), 0.6-0.9mm for medium sized females (Medium sized eggs), and 1.0-1.2mm for large size female (large size eggs), which agreed with the findings of Douglas, [10] who encountered size range of 0.3-1.2mm diameter for *Clariasgariepinus*eggs in lake Kariba, south Africa. The findings of Bichi, [2006] who encountered size range of 0.2-1.5mm for *Heterobronchuslongifilis*was also in agreement with the present study. As larger eggs came from large females, percentage fertilization was higher for large sized eggs, followed by the medium sized eggs and lowest for the small sized eggs. This implied that the size of eggs have a significant effect on the rate of fertilization [6]. From this result egg size determined most variation in final fingerlings size. The hatching rate for the three egg size classes, large, medium and small was 91%, 90% and 70% respectively which appeared to be significant at P=0.05 (Appendix V). this implies that the size of the egg have a significant effect on the number of eggs hatched [11, 12, 15]. Size at hatching is positively correlated (P=0.5) with egg size. This is in agreement with the findings of [4, 30, 23, 20] and larger fry at hatching have advantage in survival and fitness is also in agreement with the findings of [24, 27, 20].

Since large sized eggs have a higher finger of reduction in weekly mortality rate, which implies a higher survival rate. That is large size eggs have the highest survival rates followed by medium size eggs and finally small sized eggs Therefore, the size of egg is directly related to the survival rates. Larvae produced from larger egg size have higher chances of survival [25, 17, 20, 29]. The latency period of 8-12 hours obtain in this study agrees with the latency time of 12 hours for *Clariasgarepinus* according to Hogendorn and Vismas, [19]. It also agrees with the findings of Sule, [32] that the latency time for stripping of *Clariasgariepinus*after hormone injection is 9-11 hours. The values for Dissolved Oxygen (DO), pH temperature obtained in this study compare with those obtained by other workers for optimum reproduction to take place in catfishes. Bichi, [6] stated that for reproduction to take place in catfishes there should be an optimum temperature of between 19.0-29.5^{0}C and optimum pH of 7.3-8.6 Umar, [33] stated that the optimum temperature for reproduction in *CLariasgariepinus*is between 23^{0}C and 29.5^{0}C. Boyd, [7] stated that for reproduction to take place in fishes there has to be an optimum temperature of between 25-31^{0}C, a pH of 6.0-8.5and Dissolved Oxygen of between 2.0mg/1-6.00mg/1. Adeniji and Ovie, [1] found in their study a temperature of 29-310C, pH value of 2.9mg/1-6.10mg/1 suitable for reproduction in fish [3].

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

Based on the data obtained from this work, it can be concluded that the bigger the size of the female broodstock the bigger the size of the eggs produced and the bigger the size of eggs, the higher the fertilization, hatching and frys survival rates in the African catfish (*CLariasgariepinus).*

5.2. Recommendation

Since reproduction is correlated to egg size, it is recommended that fish farmers should use large fish of at least 1.0-2.0kg since they can produce large eggs for reproduction purposes.

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