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Effects of Various Latency Periods on the Fertilization, Hatchability and Survival of *Clarias gariepinus*

O.T. Agbebi, O. Faseluka and A.A. Idowu
Department of Aquaculture and Fisheries Management, University of Agriculture, PMB 2240 Abeokuta, Ogun State, Nigeria

Corresponding Author: O.T. Agbebi, Department of Aquaculture and Fisheries Management, University of Agriculture, PMB 2240 Abeokuta, Ogun State, Nigeria Tel: +2348037045878, +2348072723153

ABSTRACT

The latency period of post-ovulation of *Clarias gariepinus* has been demonstrated to affect the viability of its eggs and embryos. This study has examined the effects of various latency periods on the viability of eggs, fertilization, hatchability and survival of the African catfish *Clarias gariepinus*. Progenies were produced using eggs successively stripped from the African catfish at 10, 12, 14, 16, 18, 20, 17, 14, 21 and 22 h of post-ovulation. Some eggs and sperm were delayed while others were used fresh. Low survival, hatchability and fertilization rates were observed in treatments 1 and 3 while an average percentage of fertilization, hatchability and survival rates were obtained in treatments 2 and 4. High rates of fertilization and hatchability as well as a considerable rate of survival of progeny after 21 days of indoor rearing was obtained in 14 h latency period at a temperature of 29.5°C and was significantly different ($p<0.05$). Therefore, any attempt to improve fingerlings production in *Clarias gariepinus* must consider the effects of latency period in relation to water temperature and the best latency period achieved in this study was 14 h.

Key words: Latency period, fertilization, progenies, hatchability, survival

INTRODUCTION

Fisheries have been recognized as one of the fastest growing sectors in the world. Fish is the most heavily traded food commodity in the market; with the continuous declining of natural fish production, it is crucial to improve fish production from aquaculture as it is one sector that can significantly contribute to World Fish Production (Gupta and Acosta, 2001). The production of marketable fish begins with the stocking of fry or juvenile into a rearing environment. These fish can come from wild capture, however the fish cannot be guaranteed that adequate numbers can be captured and stocked in the time corresponding to optimum production conditions; the fish farmer then naturally turns to other means of obtaining his stock which is invariably an artificial method (Oyelese, 2006). African catfish was one of the most suitable species in aquaculture it has been considered to hold a great promise for fish farming; the African catfish having a high growth rate, resistant to handling and stress, being very well appreciated and having a high market value. One key constraint to its culture is the limited availability of quality fingerlings as seed material (Sahoo *et al*., 2007). Induced breeding may be a dependable alternative for obtaining high quality seed material. The species of induced breeding of *Clarias gariepinus* depends largely on the Latency period (Hogendoorn and Vismans, 1980; Zonneveld *et al*., 1988). Latency period is being described as the time interval between injection of the female fish and stripping of eggs.

There are some other factors to be considered simultaneously with latency period, they include; the water in which the female brood-stock is kept after being injected has to be of the right temperature, as this will affect the latency period (Crandell *et al*., 1995). The higher the temperature, the lower the Latency period. The optimum temperature to keep the fish is 25°C and the fish will be ready in about 11-13 h (FAO, 1996).

The ripening of the ovary after injection depends on the type of hormone used to introduce the female fish (Crandell *et al*., 1995). For example, a fish injected with HCG will be ready to be stripped after 14-17 h of injection (Sahoo *et al*., 2007) whereas it is not so for other types of hormone.

The amount of hormone used to inject fish is also important. The higher the dosage, the faster the time of stripping. The fish breeder must properly monitor the exact latency period of this specie to avoid over-ripeness and under-ripeness of their eggs in order to achieve maximum fertilization, hatchability and survival of the hatched ones (Hogendoorn and Vismans, 1980; Zonneveld *et al*., 1988).

The effect of latency period on the spawning performance of the females have not been carefully studied, hence the study aims at achieving the best performance (fertilization, hatchability and survival), the viability of fresh and/or delayed gonad products in relation to the latency period of the fish species.

MATERIALS AND METHODS

The experiment was carried out towards the end of the wet season between September to October. A flow-through water system of twelve hatching troughs was adopted for this experiment. Twelve 14-months-old brood-stocks-Four gravid females weighed 900 g each and eight matured males weighed 1 kg each of *Clarias gariepinus* were acclimatized for one week.

Hormone injection: Each female received a single dosage of 0.45 mL per kilogram fish of ovaprim hormone to induce ovulation. The hormone was administered with a 2 mL syringe and inserted into the fish at the anterior parts of the dorsal fin. Each of the brood-stock injected were stripped according to their stipulated latency period, males were sacrificed to obtain their sperms. The gametes were mixed and fertilized accordingly and incubated in the flow through hatchery system, 20 g of eggs were stripped for each replicate according to their time frame:

1 g of eggs = 600 eggs, therefore, 20 g of eggs = 12,000 eggs

For all the treatments in Table 1, wet fertilization method was used, The testes from the male fish were removed and milt pressed into a sterile dry petri dish and diluted with physiological solution prepared by diluting 9 g of common salt in 1 L of water. The milt suspension was drawn to fertilized the stripped eggs. After 2 min of gentle stirring with plastic spoon, the fertilized eggs washed several times with fresh water to remove excess milt. The fertilized eggs were spread on a nylon mesh hatching substrate called kakaban in an incubation tank with a regulated flow of water until hatching occurs.

Incubation procedures: Immediately after fertilization, each of the replicates was incubated in a well aerated 7×49×49 cm troughs containing 20 L of water. Water was flowing in and out of the troughs to obtain a well oxygenated medium.

Table 1: Time of stripping of eggs, sperm removal and fertilization for each treatment

Treatments and replicates	Latency period (h)	Time of egg stripping	Delayed gonadal product	Time of sperm removal	Delayed for h	Fertilized at
T1R1	16	2 am	Both	2 am	2	4 am
T1R2	18	2 am	Both	2 am	4	6 am
T1R3	20	2 am	Both	2 am	6	8 am
T2R1	18	4 am	Eggs	6 am	2	6 am
T2R2	20	4 am	Eggs	8 am	4	8 am
T3R3	22	4 am	Eggs	10 am	6	10 am
T3R1	17	5 am	Sperm	3 am	2	5 am
T3R2	19	7 am	Sperm	3 am	4	7 am
T3R3	21	9 am	Sperm	3 am	6	9 am
T4R1	10	11 pm	None	11 pm	-	11 pm
T4R2	12	1 am	None	1 am	-	1 am
T4R3	14	3 am	None	3 am	-	3 am

Ten hours, after incubation, the observed white and opaque eggs were removed from each trough. This was done by siphoning out the dead/unfertilized eggs which appeared whitish. The percentage fertilization was estimated as:

$$\text{Fertilization (\%)} = \frac{\text{No. of fertilized eggs}}{\text{Total No. of eggs incubated}} \times 100$$

Counting of hatched larvae: Several hours after the incubation processes, hatching began. The time interval for hatching of eggs varied with replicates. The hatched larvae were counted while the un-hatched eggs were discarded; the percentage hatchability was estimated thus:

$$\text{Hatchability (\%)} = \frac{\text{No. of hatchlings}}{\text{Total No. of fertilized eggs}} \times 100$$

Physicochemical parameters: The following physicochemical parameters was monitored in the hatchery in which this study was carried out, temperature, Total Dissolved Solids (TDS), Dissolved Oxygen (DO), Electrical Conductivity (EC). The eggs and the milt that were delayed were kept secured at a temperature of 19°C.

Feeding rate and method: Each of the fry hatched in all the treatments were weighted. Feeding commenced 72 h after hatching. The fry were fed with *Artemia* for 14 days and were later fed with 0.2 mm of commercial fish feed. Each of the replicates was feed at 10% of the body weight thrice daily.

Daily survival values: The duration of this indoor experiment was 21 days. Daily survivals were obtained through the visual counting of the fry(s) on a daily basis. At the end of 21 days, the remaining fry(s) were counted and their percentages survival were estimated as:

$$\text{Survival (\%)} = \frac{\text{No. of fry at the end of study}}{\text{No. of fry at the beginning of study}} \times 100$$

Analysis of data: Data were analyzed using one-way analysis of variance in a completely randomized design. Significant means were separated using Duncan Multiple range test.

RESULTS AND DISCUSSION

The results from this study revealed that eggs and milt delayed for 4 h (Table 2) yielded 50% fertilization, 46.5% hatchability and 71.97% survival. The least percentages were obtained in eggs and milt kept for 6 h at 23°C before fertilization, the result obtained here was 45% fertilization. 23% hatchability and a survival rate of 49.03% after 21 days of indoor rearing (treatment 1). This implies that both eggs and sperm deteriorates rapidly when they stripped or pulled out of their sac.

Table 3 (treatment 2), involves fertilization carried out between 18 and 22 h after hormonal injection, where eggs were delayed for 2, 4 and 6 h and fertilized with fresh sperm at a temperature of 23°C recorded the lowest percentages. It gave 28% fertilization, 41.9% hatchability and 64.77% survival indoors. The highest percentages were obtained when the eggs were delayed for 2 h, 44.9% fertilization, 51.4% hatchability and 59.44% survival.

This is a clear indication that viability is inversely proportional to post-ovulation time. The decrease in the viability of Eggs might be due to over ripening as obtained in high latency periods (17-23 h) which resulted in poor fertilization and hatching rates (Ohata *et al*., 1996; Oyelese, 2006).

Table 4 shows (treatment 3) fertilization carried out between 17 h and 21 h after hormonal injection. Milt that has been delayed for 2, 4 and 6 h at 19°C, were used to fertilize freshly stripped eggs. Highest percentage were obtained when the sperm was delayed for 2 h 69.6% fertilization was achieved as well as 70% hatchability and 68.49% survival after 21 days of indoor rearing. When the sperm was delayed for 4 h, it gave 52.5% fertilization, 61.1% hatchability and 62.02% survival. The lowest percentages were obtained when the sperm was kept for 6 h before being used.

Table 2: Delayed eggs and delayed sperm

Replicates (h)	Weight of sperm sac (g)	No. of eggs incubated	No. of fertilized eggs	Fertilization (%)	Hatchability (%)	Unhatched eggs	Period of hatching (h)	No. of hatchlings	Feeding rate (at 10%) per day (g)	Number survived	Survival (%)
1: 16	2	12,000	7,200	60	59.2	1500	12	4263	1.80	3241	76.02
2: 18	2	12,000	6,000	50	46.5	32.1	14	2790	0.90	2008	71.97
3: 20	2	12,000	5,400	45	23.0	4155	14	1242	0.36	609	49.03

Table 3: Delayed eggs and fresh sperm

Replicates (h)	Weight of sperm sac (g)	No. of eggs incubated	No. of fertilized eggs	Fertilization (%)	Hatchability (%)	Unhatched eggs	Period of hatching (h)	No. of hatchlings	Feeding rate (at 10%) per day (g)	Number survived	Survival (%)
1: 18	2	12,000	5,388	44.9	51.4	2619	16	2769	0.83	1646	59.44
2: 20	2	12,000	4,320	36	50	2160	16	2160	0.65	1215	56.25
3: 22	2	12,000	3,360	28	41.9	1950	18	1408	0.42	912	64.77

Table 4: Fresh eggs and delayed sperm

Replicates (h)	Weight of sperm sac (g)	No. of eggs incubated	No. of fertilized eggs	Fertilization (%)	Hatchability (%)	Unhatched eggs	Period of hatching (h)	No. of hatchlings	Feeding rate (at 10%) per day (g)	Number survived	Survival (%)
1: 16	2	12,000	8,350	69.58	70.0	1670	14	5845	2.004	4003	68.49
2: 18	2	12,000	6,300	52.5	61.1	2.45	14	3849	1.17	2387	62.02
3: 20	2	12,000	5,760	48.0	36.5	3,660	16	2100	0.63	1940	53.89

Table 5: Fresh egg and fresh sperm

Replicates (h)	Weight of sperm sac (g)	No. of eggs incubated	No. of fertilized eggs	Fertilization (%)	Hatchability (%)	Unhatched eggs	Period of hatching (h)	No. of hatchlings	Feeding rate (at 10%) per day (g)	Number survived	Survival (%)
1: 10	2	12,000	5,400	45	76.0	1620	16	4104	1.14	3203	78.05
2: 12	2	12,000	4,800	40	80.8	920	16	3880	1.17	3377	87.08
3: 14	2	12,000	8400	70	83.9	1350	18	7050	2.12	6115	86.76

Table 6: Physicochemical parameters

Parameters	Measurement
Temperature (°C)	29.5
pH	7.2
Ammonium (mg L^{-1})	0.05
Dissolved oxygen (mg L^{-1})	4.8
Carbondioxide (mg L^{-1})	10

The result obtained shows that spermatozoa kept intact at 19°C can be potent for up to 4 h or even more if not exposed, because results obtained when the sperm was delayed was highest compared to treatment 2 when the eggs were delayed. Thus, it can be said that eggs deteriorates faster than sperm when gametes are outside their sac.

Table 5 (treatment 4) consists of fertilization carried out between 10 h and 14 h after hormonal injection. In this category, freshly collected milt, eggs stripped at 10 h yields 45% fertilization, 76% hatchability and a survival rate of 78.05%. Fourty percent fertilization was recorded for stripped at 12 h, the percentage hatchability and survival rate were 80.8% and 87.08, respectively. At 14 h of latency, it had a percentage fertilization of 70%, hatchability of 83.9% and an indoor survival of 86.76%.

The latency periods of 10 h are unarguably the best latency period for *Clarias gariepinus* at 29.5°C (Table 6). At period lower than that, there will be insufficient action of the hormonal treatment leading to failed ovulation (Tan-Fermin *et al*., 1997).

The study is in agreement with Viveen *et al*. (1986) in the latency period of 7 h and 18 to 20 h incubation time at the water temperature of 27 to 30°C in *C. gariepinus*; Rao *et al*. (1994) also agree with this on the same species.

There was no statistical difference ($p<0.05$) in the treatments except the treatment of 14 h latency period which was significantly different ($p<0.05$) in fertilization and hatchability.

The values were within the required levels recommended for a successful fish reproduction and in agreement with FAO (1992) except the fall in dissolved oxygen 4.8 mg L^{-1} compared to 6 mg L^{-1} recommended by FAO but this can be attributed to the securely locked indoor hatchery used for the study.

CONCLUSION

The success of artificial propagation of *Clarias gariepinus* through induced breeding under controlled environmental conditions is mostly dependent on the latency period and humidity/temperature.

This study has indicated that if eggs are stripped before the optimum time, there will be low fertilization, hatchability and survival of the hatchlings in both indoor and outdoor hatcheries. Also, if the time of stripping (i.e., latency period) is too high, there would still be loss of quality in the gonadal products.

The present study has demonstrated that *Clarias gariepinus* could be successfully induced in captivity 10-14 h latency period. Therefore, to obtain a high percentage of fertilization, hatchability and survival of *Clarias gariepinus,* the study recommends a latency period of 14 h at temperature of 29.5°C using fresh eggs and fresh sperm.

RECOMMENDATION

We recommend further studies to be carried out in fish breeding on the various delayed techniques of fish gametes with varying temperatures of between 35-37°C temperatures which is the actual African /tropical room temperature in the dry season.

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