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Water Temperature a Determinant of Fertilization and Hatchability Rates in Artificiallly Induced Breeding of *Clarias Gariepinus* (Teleostei: Clariidae)

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Abstract: This study is aimed at determining the effect of water temperatures on fertilization and hatchability rates of artificially induced *Clarias gariepinus*. Sixty six (665 broad-stock 132 females and 34 males) were purchased from Agodi fish farm, Ibadan of average weight 398 g and conditioned for two weeks after which some were sacrificed for fresh pituitary hormone and milt. Four trials were carried out. Injection of females was done at different periods of the day (8.00 am, 4.30, 8.00 and 12.00 pm) that is 0800, 1630, 2000 and 2400 h at an average temperature of 26.00, 24.30, 27.66 and 25.00°C, respectively and stripped after 10 h for eggs. Significant variations exist ($p<0.05$) between water temperature at the time of injection and hatchability/fertilization rates, this is further confirmed by the strong positive correlation of $r = 0.95$ in both cases. The highest hatchability rate of 77.84% and highest fertilization rate of 66.65% was recorded at 8.00 p.m with water temperature of 27.66°C this is followed by 75.51% at 26.00°C hatchability rate/51.23% fertilization rate for fish infected at 8.00 a.m and stripped 6.00 p.m (10 h later).

Key words: Water temperature, fertilization, hatchability rates, artificially induced, *Clarias gariepinus*

INTRODUCTION

Artificial propagation is the stimulation of some conditions necessary for the production of frys and fingerlings (fish seed) and this involves several methods.

- Natural spawning without hormonal treatment, by stimulating natural spawning conditions.
- Natural spawning with hormones final maturation of reproductive organs is brought about by infection of hormones, after which the fish spawn naturally in ponds or tanks.
- Artificial spawning with or without hormonal treatment, sexually mature broodstock are obtained through hormonal treatment, the eggs are stripped manually, fertilization is done manually and eggs are incubated.

Artificial propagation by induced breeding through hormone treatment, followed by artificial fertilization and incubation of fertilized eggs and subsequent rearing up to fingerling size has several advantages as listed below:

- Better rate of fertilization and hatching
- Protection against enemies and unfavourable environmental conditions.
- Better conditions for growth and survival

The artificial propagation of *Clarias gariepinus* is a chain of activities. These activities are:

- Selection of broodstock from the wild or fish ponds.
- Rearing of broodstock
- Inducing final maturation and ovulation with hormone treatment.
- Treatment of male to collect mitt.
- Stripping of the female broodstock to collect eggs
- Artificial fertilization
- Incubation and hatching of eggs
- Rearing of larvae and fry to fingerlings various hormone preparations have been used to induce spawning of *Clarias*. The commonly used ones are
- Pituitary extract or Hypophysis (as used in this study).
- Deoxy corticosterone Acetate (DOCA)
- Human Chorionic Gonadotropin (HCG)
- A combination of pituitary hormone and human Chorionic Gonadotropin (CG)

The inducement of spawning in *Clarias* has not been very successful with HCG[1-3]. However the use of DOCA (deoxy corticosterone acetate) was found to be successful and effective Dazie *et al.*[4] noticed that the hormones which gives the best result for induced spawning of *Clarias* are DOCA in oil suspension at a rate of 5 mg 100 gm^{-1} body weight of fish.

The use of pituitary hormone in induced spawning was described as traditional method of fish propagation Lam[5] and Huisman[6] described the use of pituitary hormone as the most reliable method. Pituitary extracts from the common carp (*Cyprinus carpio*) can also be used effectively and thus generally referred to as a "universal donor Woynarovich and Horvath[7]. Pituitary glands can be obtained fresh from fish or as preserved dried extracts in drug markets. Hogendoorn[8] and Adigun *et al.*[3] in their various inducement trials obtained good spawning results using acetone dried carp pituitary at 4 mg kg^{-1} of female body weight.

When fresh pituitary lands of the same fish are to be used, the weight of the donor (s) must balance the weight of the recipient. If dried pituitary are to be used 4mg dry material per kilogram of female body weight is recommended and usually administered in one or two doses using 0.6-0.9% saline solution or distilled water as carrier Huismann *et al*[6] Hogendoorn[9]. The males receive a dose equivalent to the introductory dose but at the time of the administration of the resolving dose to the female sometimes it might become necessary to repeat the hypophysation three or more rimes at regular intervals.

A study of the 'Latency period' for C*larias gariepinus* was carried out and reported by Hogendoorn[8] to be water temperature dependent, as shown in Table 1.

The knowledge of the latency period or hour grade which is shown to be temperature dependent as shown in Table 1 helps the farmer to know exactly when to expect ovulation, spawning or stripping.

Ayinla[10] observed that the time interval between the start of embryonic development (fertilization) and hatching, (incubation period) changes with the increase in temperature. At 25°C hatching takes place about 28-32 h after fertilization while it is about 23-24 h at 30°C.

One of the failure in breeding trials has been due to impropriate monitoring water temperature in breeding exercises, lack of basic information on the biology of the fish concerned in relation to season and other successful hatching and fertilization or raising of fingerlings. Hence, there is need to get the appropriate water temperature required in the artificial propagation of *Clarias* sp. For mass production of its eggs, milt, fertilization and hatching of eggs.

It is also the aim of this study to determine the best tune of the day for the artificial propagation of *Clarias*. Such a result could then be recommended to fish farmers for the artificial induced breeding of *Clarias gariepinus*.

It is therefore the objective of this study to

- Determine the effect of temperature on the hatchability of artificially induced *Clarias gariepinus*.

Table 1: Time between hypophysation and stripping of *clarias gariepinus* females at different temperatures (after Hogendoorn 1980)

Water temperature°C	Latency period in hour for various groups of fish				
	1	2	3	4	5
20	17.00	19.00	21.00	23.00	30.00
25	8.00	9.00	10.00	11.00	13.50
30	5.50	6.50	7.00	7.50	8.50

Source: (Hogendoorn, 1980)

- Establish the effect of temperature on the rate of fertilization of artificially induced *Clarias gariepinus*.
- To determine the most suitable temperature and time for injection and stripping of *Clarias gariepinus*.

MATERIALS AND METHODS

The effect of temperature on artificially induced breeding of *Clarias gariepinus* was monitored as follows:

Broodstock conditioning and artificial propagation: The conditioning of 66 broodfish was carried out in the nursery ponds at the University of Ibadan fish farm using $1m^3$ hapas for 6 broodfish (3 females and 3 males). They were fed to selection two times daily with 40% crude protein diet for 4 weeks to promote the maturation of the gonads.

The females which showed the most advanced maturity were chosen for artificial propagation using the following criteria.

- Well rounded and soft abdomen which extends anteriorly beyond the pectoral in to the urinogenital papilla.
- Freely running matured eggs which showed clearly the nucleus at the centre was obtained easily by gentle or slight pressure on the abdomen.
- The genital opening was swollen and reddish in colour.
- The males were chosen on the basis of having slightly dongated swollen reddish (richly supplied with blood) high vascularised urinogenital papilla.

Method of gland collection and preparation of pituitary gland solution: The head of the fish was separated from the body and the top of the brain box was removed by a sharp butchers knife. With oblique strokes a portion of the scalp was removed without much damaging of the head.

The grey matters and fatty substances over the brain were removed, the olfactory nerves were separated and also the optic nerves and the anterior portion of the brain were separated anteriorly by means of tweezers and the brain lifted up and laid back. The pituitary gland was invariably left behind in the cavity on the floor of the

brain box. This is encapsulated by a membrane called durameter, this membrane was then detached posteriorly by a force and slowly lifted up when the pituitary gland was exposed. It is then carefully removed by means of tweezers.

Preparation of pituitary gland solution, injection of female spawners, stripping of female for eggs: Two mililitre of 9% saline solution was used as solvent for the pituitary gland solution. The required fresh pituitary gland was extracted from the donor fish, defattened and dehydrated in acetone. The pituitary was macerated in the 9% saline solution and allowed to settle. The supernal with an solution was loaded with syringe gland solution was injected on a calculated dose of 1 mL 500 gm^{-1} body weight (1 mL 500 gm^{-1} body weight of fish).

Females of more or less equal size with male donor were selected by weighing hormone solution was slowly infected into the dorsal muscle of the broodstock above the lateral line just below the posterior part of the dorsal fin and between the 4th and 6th dorsal ray.

The infection of the female spawners was done at four different times (hours of the day)-at 08.00 a.m, 6.30, 20.00 and 24.00 pm). They were then gently replaced in their containers was then taken with the aid of thermometer. Four trials were carried out each occasion .The ovulated females were carefully taken from the plastic containers covered with net and held by using wet towel. The abdomen was gently pressed with thumb from the pectoral fin region to the genital papilla. The stripping was done for each spawner at constant latent period of hours. The eggs were collected into a dry plastic.

Procurement of milt fertilization,incubation and hatching, separation of larvae from egg shells: Sperm (milt) was obtained from a male spawner by sacrificing and dissecting the testis. Some small incisions were made into the cream coloured lobes of the testis. The milt was squeezed out and collected into a small-sterilized bottle. The collected milt was then diluted with the 9% physiological salt solution.

After stripping the females eggs in a dry bowl, few drops of milt solution were added on to the eggs and the sexual products were mixed by gently shaking the bowl and properly mixed with feather, also with addition of approximately the same volume of clean water to fertilized eggs were spread homogenously in one single layer in the incubation tray which is really supplied with clean well aerated water.

The incubation trays were removed as soon as hatching was completed and normal larvae had gathered under the incubation trays .eggs shells dead eggs, shells, dead eggs and deformed larvae were removed by siphoning.

Determination of the number of eggs spawned, percentage fertilization: The weights of the females were taken before the infection and after spawning. This was done to know the weight of the eggs. The drop in weight was taken as the weight of the eggs. The number of eggs spawned was calculated by volumetric method. The eggs were put in known volume of water. 10 mL was taken out of it and eggs in it were counted. This was used to calculate the spawned eggs. The weights of the eggs were also used to calculate the number of eggs spawned. 1gm weight of *Clarias gariepinus* eggs = 700eggs[11].

The number of unfertilization eggs were estimated by counting the number of unfertilization eggs 10 to 12 h after fertilization and subtracting from total number of eggs stripped. The unfertilization eggs were white and opaque while the fertilized eggs were transparent and light brown. The percentage fertilization was estimated from the number of unfertilized eggs by the equation % fertilization =

$$\frac{N-n}{N}\times\frac{100}{1}$$

Where N = Total number of eggs stripped
n = number of unfer

Estimation of the number of hatchlings and percentage hatchability: The numbers of hatchlings were estimated also using the volumetric method. The hatchlings were put in a known volume of water. Ten mililitre was taken out of it and eggs in it were counted. This was used to calculate the number of fry's.

The percentage hatchability was calculated from the number of fry counted i.e.

$$\frac{N-n}{N}\times\frac{100}{1}$$

where

Where N = number of hatchlings
N = number of fertilized eggs

Statistical analysis: All data in this project work were subjected to Analysis of Variance (ANOVA) (Steel and Torrie, 1960) regression and correlation analysis.

- *% FERTILIZATION =(No of fertilized eggs÷ No of eggs stripped)×100
- *% HATCHABILITY = (No of fry ÷ No of fertilized eggs)×100
- *% PRODUCTIVITY = (No of fry ÷No of eggs)×100

The highest hatchability rate of 77.84% and highest fertilization rate of 66.65% was recorded at 8.00 p.m (2000 h) as shown in Table 6 at an incubation period of 27 h.

Table 2: Effect of water temperature on the rate of fertilization and hatchability of *clarias gariepinus* injected at O800h

Weight of female spawner (G)	Weight of eggs stripped (G)	Number of fertilized eggs	No of eggs stripped 1g = 700 GGES	Water temperature (°C)	Incubation period (H)	No of fry hatched out	Latency period (H)
424	16.00	6.554	11.200	26.00	27	4.676	10
476	13.00	6.460	9.100	26.00	27	4.844	10
483	23.80	6.537	16.660	26.00	27	4.800	10
477	18.40	5.980	12.880	26.00	27	4.920	10
465	17.80	6.383	12.460	26.00	27	4.820	10

Table 2 shows 5 replicates of number of fry hatched out at 0800 h

Table 3: Effect of water temperature on the rate of fertilization and hatchability of *clarias gariepinus* injected at 1630 h

Weight of female spawner (G)	Weight of eggs stripped (G)	Number of fertilized eggs	No of eggs stripped 1g = 700 GGES	Water temperature (°C)	Incubation period (H)	No of fry hatched out	Latency period (H)
360	22	1.370	15.400	24.30	30	1.005	10
370	18	1.462	12.600	24.40	30	1.060	10
380	15	1.439	10.500	24.50	30	1.062	10
410	25	1.473	17.500	24.00	30	1.028	10
380	20	1.436	14.000	24.30	30	1.039	10

Table 2 shows 5 replicates of number of fry hatched out at 1630 h

Table 4: Effect of water temperature on the rate of fertilization and hatchability of *clarias gariepinus* injected at 1630 h

Weight of female spawner (G)	Weight of eggs stripped (G)	Number of fertilized eggs	No of eggs stripped 1g = 700 GGES	Water temperature (°C)	Incubation period (H)	No of fry hatched out	Latency period (H)
396	26	18.200	13.966	27.65	25.00	10.900	10
354	29	20.300	13.870	27.70	25.00	11.895	10
412	33	23.100	14.057	27.80	25.00	11.033	10
358	32	22.400	14.127	27.50	25.00	9.780	10
380	30	21.000	14.005	27.66	25.00	10.902	10

Table 4 shows 5 replicates of number of fry hatched out at 200 h

Table 5: Effect of water temperature on the rate of fertilization and hatchability of *clarias gariepinus* injected at 2400 h

Weight of female spawner (G)	Weight of eggs stripped (G)	Number of fertilized eggs	No of eggs stripped 1g = 700 GGES	Water temperature (°C)	Incubation period (H)	No of fry hatched out	Latency period (H)
354	17	11.900	1.975	25.00	29	1.378	10
377	23	16.100	1.987	24.90	29	1.464	10
361	20	14.000	2.086	25.10	29	1.063	10
388	24	16.800	2.080	25.00	29	1.555	10
370	21	14.700	2.032	25.00	29	1.500	10

Table 5 shows 5 replicates of number of fry hatched out at 2400 h

DISCUSSION

From the experiment as shown in (Table 6) there was no direct proportional relationship between average female body weight and the weight of the eggs. This observation did not correspond to Fagede and Adebisi's (1976) findings that fecundity of fish is directly proportional to its body weight. This unproportionality might be due to.

- During stripping, all the eggs could not be stripped because it was only the ovulated eggs that could come out.
- Some eggs might have been over-riped and reabsorbed or not well ovulated due to the variation in temperature an latency period.

Therefore, total weight of eggs stripped from a female is depended on the number of eggs ovulated at the time of stripping hence not a time representation of the total quantity of eggs obtained from a female spawner.

From all the breeding trials in this study (Table 6) the spawning temperature ranged between 24.30-27.66°C for *Clarias gariepinus*, although the highest hatchability of 77.84% was recorded at 27.66% for fish infected at 8.00 p.m. This is in line with Dupree and Humer, (1984) which reported that warm water fishes from best at temperatures between 25-32°C. A significant effect ($p<0.05$) exists between water temperature and average percentage hatchability. Also the strongly positive correlation of $r = 0.95$ suggests that hatching of eggs is favoured at higher temperatures.

Also the different percentage fertilization rates obtained at different injection temperature, with a constant latency period of 10 h further confirmed the significant effect ($p<0.05$) temperature has on positive correlation $r = 0.95$ between water temperature and average percentage fertilization implies that fertilization of eggs is favoured at higher temperature.

In Table 6, it was observed that as temperatures increased incubation period also decreased. This means that water temperature and incubation period

Table 6: Effect of average water tempetature on average fertilization and hatchability of *clarias gariepinus*

Mean latency period(h)	Average weight of female spawner (g)	Average weight of egg stripped (g)	Average no. of egg stripped	No of egg fertilized	*% Fertilization	Average of fry hatched out	*% Hatchability	*% Productivity	Average water temperature	Incubation period
10 4.30pm to 3.00am	380	20.00	14,000	1436	10.25	1039	72.35	7.42	24.30	30
10 12.00pm to 10.00am	370	21.00	14,700	2,032	13.65	1,500	73.82	10.20	25.00	29
10 8.00am to 6.00pm	465	17.80	12,460	6,383	51.23	4,820	75.51	38.68	26.00	27
10 8.00pm to 6.00am	380	30.00	21,000	14,005	66.65	10,902	77.84	51.88	27.66	25

The highest fertlization rate (66.65%) and hatchability rate (77.84%) was recorded for fishes injected at 8.00pm at an average temperature of 27.66%,also with the lowest incubation period of 25 h. This is closely followed by fish injected at 8.00am with fertilization rate of 51.23% and hatchability rate of 75.51% at an injection temperature of 26.00°C and incubation period of 27 h.While the least fertilization rate of 10.25%. Hatchability rate 72.35% was recorded for fish injected at 4.30pm at a temperature of 24.30°C and the highest incubation period of 30 h.

are inversely proportional. When water temperature increased from (24.30-27.66) incubation period decreased from (30-25 h).

CONCLUSION

The pituitary injection done on matured females at 8.00 p.m. And (stripped at 6.00 a.m) at a temperature of 27.66°C gave the highest percentage fertilization rate of 66.65%, highest percentage hatchability rate of 77.84% and highest percentage productivity of 51.88%.

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