Research Journal of Biological Sciences 1 (1-4): 88-92, 2006

Fry Survival Rate under Different Anoxic Conditions in *Clarias gariepinus*

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Abstract: This study was conducted to determine the best dissolved oxygen level and culture medium for the survival of the African catfish Clarias gariepinus fry.The experiment was carried out for a period of 8 weeks, out of which the brood stock used to produce the fry were maintained for 4 weeks on a diet of 40% crude protein. The study was carried out in four plastic bowls subjected to different treatments in the departmental laboratory. The plastic bowls were designated I, II, 8.III, IV respectively each representing a treatment with bowl I containing deionised spring water unaerated kept in the laboratory, bowl II contain sprung water without aeration kept in the laboratory, bowl III contain constantly aerated spring water also kept in the laboratory and bowl IV contain spring water without aeration kept outside the laboratory under ambient conditions to serve as the control. The acclimatized Clarias broodstock were semi-induced under hatchery management conditions to produce Clarias gariepinus fry on a weekly basis for 4 weeks (as when required for experiment). Each experimental treatment bowl contain 100 Clarias gariepinus fry. The experimental treatment replicates was carried out for four weeks. The mean dissolved oxygen levels and survival rates of swim up fry values obtained for the 4 weeks are:- Oxygen levels 6.57, 7.04, 7.29 and 7.06 mg L^{-1} for treatments I, II, III and IV, respectively, survival rates were 84.80, 95.30, 100.00 and 96.80%, respectively for each experimental treatment. Analysis of variance shows that there is significant difference in the mean survival rate values of $p<0.05$ level of significance. Despite the anoxic conditions r was shown to be positively correlated ($r = 0.99$) for survival rate implying generally that the survival rate for Clarias gariepinus fry increases with increase in oxygen level in the water. Treatment III gave the highest percentage survival rate of 100% which coincided with a 7.29 mg L^{-1} oxygen level and ranking second to it is the survival rate/oxygen level of treatment IV with the values 96.80%/7.06 mg L^{-1}.

Key words: Anoxic condition, oxygen level, survival rate, clarias gariepinus fry

INTRODUCTION

Anoxic condition in this study strictly refers to oxygen tension as a major determinant of factors influencing survival, hatchability and successful breeding programme for Clarias gariepinus.

The unpredictable time of spawning of desirable indigenous fish species has led to various attempts by Aquaculurist to induce many species of fish to spawn including Clarias gariepinus which has been reported to be abundant in Nigeria and of importance in African aquaculture.

Success has been achieved in its spawning[1-3]. However, due to high mortality of eggs, larvae and fry/fingerlings; African catfish are difficult to find. The percentage survival of hatchlings of any fish depends to a large extent on the efficiency of management operated in the hatchery unit.

Huet[4] reported that Clarias is a popular cultured fish among the fish farmers in countries like India, Pakistan, Vietnam and with a high proportion in Thailand, Clarias gariepinus is widely distributed in African lakes, streams, rivers and rain-fed pools all the year round. This sp. is generally very hardy, tolerant to low dissolved oxygen more so with their accessory respiratory organ for breathing air and resistance to adverse weather conditions.

Clarias spawns are restricted to the edges of lakes, streams and pools where the water current is not strong enough to drift the egg substrates away.

After hatching the larva remains under the cover of the submerged vegetation at the water edges which would later serve as the nursery ground for the fry after the yolk sac or larvae stage. The advance stage subsists on the available plankton within this littorial zone before migrating as juveniles to the deeper and larger water bodies where they can prey on forage fish and other food items[5].

Adult Clarias are omnivores and are therefore not restricted to any trophic level in the water. During the dry season they seek for larger bodies of water by migrating either down or upstream. They sometimes travel over land to reach these bodies of water especially when the pond or water bodies where they live are drying up.

According to FAO[6] spawning in Nigeria occurs in August/ September but the presence of eggs in the female

ones are reported in May to October, at the peak of the rainy season[7] although some mature eggs are still found at all times in gravid Clarias.

Artificial propagation involves human intervention in the process of natural propagation and according to Woynarovich and Horuath[8] has the advantages of better rates of fertilization and hatching, protection against enemies and unfavourable environmental conditions; and better conditions for growth and survival.

Artificial propagation may start with the collection and further rearing of naturally produced eggs, spawn or fry, or with the production of the egg itself through artificial inducement, followed by controlled fertilization, hatching and rearing of larvae and fry.

Davy and Chouinard[9] reported that most problems in the breeding of species desired for aquaculture arises from the failure of capture fish most often the females to complete the second phase of the gonadal development (completion of maturation of gametes) and one solution to the problem is the injection of pituitary hormones either intramuscularly or intraperitoneally to induce the final maturation of gametes. Preliminary experiments using the above method were performed by Houssay in 1931 and has since undergone considerable refinement over the years[10].

FAO[11] stated that for hormone induced reproduction (semi artificial) for Clarias gariepinus the following hormones are generally used

- DOCA (Deoxycorticosteroid acetate) 2.5-5.0 mg per 100 gm of female. A disadvantage of using this hormone is that it is mostly suspended in oil which causes severe ulcers in the injected female.
- HCG (Human Chorionic Gonado trophin), 25 I.U per 100 gm of female. This hormone works well but it is expensive.
- Common Carp (Cyprinus carpio) Pituitary grand material 3-4 mg kg^{-1} of female or 1-2 whole pitiuataries per female. In general the common carp pituitary gland material has to be imported from abroad which means that it is not easily accessible to small scale fish farmers.
- Pituitaries of the African Catfish (Clarias gariepinus*)*. A female catfish will respond once it is infected with a pituitary of a catfish (male or female) of equal size.
- Pituitaries of the Nile tilapia (Oreochromus niloticus), 3-4 pituitaries of a Nile tilapia (100-150 gm) per female catfish will induce ovulation.
- Pituitaries of Nile perch (Lates niloticus*)*1-2 pituitaries female catfish will induce ovulation

The latter hormone:-

- OVAPRIM- which was not reported in the FAO[11] publication is a 6.synthetic hormone which has been found to be effective with a dosage 0.2-7.0.5 mg kg^{-1} weight of Clarias gariepinus at least as recorded about 90-100% success for semi-artificially induced spawning.

Mortality rates of hatchlings of Clarias gariepinus has been estimated to be between 90-100% after yolk absorption. This was attributed to lack of efficient management techniques which include the lack of adequate supply of good quality water that is rich in oxygen, high quality acceptable food and the maintenance of good cultural environmental conditions suitable for the hatchling at the early stages of development of the swim up fry.

The objectives therefore are:-

- To determine the best culture medium for the hatching and survival of Clarias gariepinus fry.
- To determine the best dissolved oxygen level for the fry of Clarias gariepinusunder hatchery management conditions.
- To study the effect of natural diets on the survival rate of the fry of Clarias gariepinus reared in the hatchery.

MATERIALS AMD METHODS

Experimental fish: Fifteen females and ten male broodstock of Clarias gariepinus of size ranges 350-375gm were used for this experiment. The broodstock were kept inside hapas in one of the experimental nursery ponds for 4 weeks. The experiment was carried out using the Departmental experimental fish farm and laboratory.

Three major stages were involved in this experiment.

- Conditioning of the broodstock and artificial propagation-hormonal inducement, stripping, dry fertilization, incubation and larval rearing.
- Fry rearing using natural diet consisting of zooplankton, cultured in a concrete outdoor tank.Monitoring survival rate of the swum-up fry with dissolved oxygen levels.

Conditioning of the broodstock: The brooders were conditioned inside hapas for 4 weeks. During this period the male and female broodstock were separately kept in 4 big hapas rigged in one of the experimental ponds. The broodstock were fed on a 40% crude protein diet throughout the conditioning period.

Preparation of pituitary gland solution: The donor fish (gravid females) were killed by cutting off the heads with a 21kitchen knife. The brain case cut round with the aid of a sharp knife and the whitish pituitary gland was picked with a pair of sterile scissors (after clearing the hypothalamus area of flesh) just below the brain. A physiological solution of 0.9% saline solution was used to macerate the pituitary gland in a porcelain mortar. The mixture was allowed to settle and the clear solution was taken into a 5ml hypodermic syringe.

Injection of breeders: 1.5 mL supernatant solution of the pituitary gland was slowly injected intramuscularly into the dorsal muscle of each of the selected female breeders above the lateral line just below the anterior part of the dorsal fin. The selected males were injected 0.5 mL of the supernatant solution each. The injected supernatant help the fish to complete their ovulation and sperm formation to the ripe stage where fertilization can take place. Prior to injection the head of the fish was covered with a wet towel.

The spawners were injected between the hours of 8.00-9.00 p.m. Injected breeders (male and females) were kept in separate bowls overnight in the laboratory. The water temperature was in the range of 27°C.

Collection and fertilization of ripe eggs: After about 10-12 h of injection ripe eggs from the spawners were stripped into a plastic plate by gentle pressure along the ovaries towards the genital aperture on the abdomen until some blood comes out with the freely running eggs in thick jets as a result of the hormonal treatment. The males were also sacrificed for their testis, which were split open with the aid of a sharp blade to release the milt. The milt was then washed down on the eggs with 0.9% saline solution mixing carefully with a white plastic spoon to effect fertilization.

Incubation and hatching: Fertilized eggs were incubated in circular plastic bowls filled to $^{2}/3^{rd}$ levelwith freshwater from the spring water close to the laboratory. The fertilized eggs were homogenously spread in a single layer on small meshed sized mosquito nets spread inside suspended perforated plastic trays filled with water.

Aeration was provided on a continuous basis by connecting each bowl to a powerful aerator. The water inside the incubation plastic were being changed every 30 min to prevent fowling. Unfertilized eggs, eggshells and crippled larvae were constantly removed by siphoning to avoid the development of fungi while the swim-up fry hatched out between 12 to 14 h after fertilization at the water temperature of about 27°C.

The containers were generally disinfected with mild dilute formaldehyde solution (10%). Adding a few drops to a litre of distilled water to wash the bowls.

Fertilization, hatchability and survival of swim-up fry
Determination of the number of eggs spawned: Total number of egg = 700 x difference in body weight after spawning (gm) N.B - 1 gm of egg mass contains about 700 eggs[5].

Number of fertilized eggs:

$$\%\,\text{Fertilization} = \frac{\text{Number of fertilized eggs} \times 100}{\text{Total number of eggs incubated.}}$$

N.B- Total number of fertilized eggs were determined by direct counting of unfertilized eggs (white eggs) and subtracting it from the total number of eggs incubated.

Hatchability:

$$\%\text{Hatchability} = \frac{\text{Number of hatchling(dayold)} \times 100}{\text{Total number of fertilized eggs}}$$

Survival rate of swim-up fry:

$$\%\,\text{Survival Rate} = \frac{\text{Number of swim up fry at } 5^{th} \text{ day} \times 100}{\text{Initial number of Hatchling at day1}}$$

Natural food: Two outdoor concrete tanks were used for plankton culture following the method of Woynarovich and Horvath[8]. The tanks were manured with chicken dung and exposed to sunlight every day. A rich culture of plankton developed over time. Using a plankton net (64 micro net) a mixed culture of zooplankton were collected from the tanks and fed to the fry immediately every 6 hours ad libitum for each treatment.

Determination of dissolved oxygen (D.O) level for the experimental treatments: Dissolved oxygen levels for the experimental treatments were determined using the Winkler method (Winklers solutions (A) and (B).

Statistical analysis: Regression analysis were computed to test correlation (r) between oxygen levels and survival rates.

Table 1: Number of eggs spawned and fertilized/percentage fertilization per female clarias gariepinus per week

Week	No. of spawned female/week	Weight of female before stripping (gm)	Weight of female after stripping (gm)	Difference in weights (gm)	Total No. of eggs spawned female per week	Total No. of eggs fertilized per female/week	Percentage of eggs fertilized from a female per week
1	1	350	330	20	20x700 = 14.000	11.200	80
2	1	375	350	25	25x70 = 17.500	15.050	86
3	1	362	332	30	30x700 = 21.000	17.010	81
1	1	375	350	23	23x700 = 16.100	13.524	84
Total				98	68.600	Average	82.75%

* Using Viveen *et al*[5] method

Table 2:Estimated number of fertlized eggs hatched into day old hatchlings and percentage hatchability per week

Week	No. of spawned female per week	Total No. of egges fertilized from a female per week	Total No. of day old hatchlings from a female per week	Percentage hatchability (%)
1	1	11.200	10.975	98
2	1	15.050	14.300	95
3	1	17.010	16.500	97
4	1	13.524	12.848	95
			Average	96.25%

Table 3: The four plastic experimental bowls used with the different oxygen tension and environmental conditions per week

Experimental bowls	Different Oxygen tensions and conditions per week
1	Spring de-ionized water kept in the laboratory.
2	Spring water without aeration kept in the laboratory
3	Aerated-spring water kept in the laboratory
4	De-ionized spring water kept outside the laboratory under ambient conditions

Table 4: Weekly/average estimated survival rate of *clarias garieoinus* under dufferebt oxygen tension and conditions using 100 swim-up fry per experimental bowl for 5 days weekly

		Weekly/Average oxygen tension (mg L^{-1}) per bowl/swim up survival rate at day 5 weekly									
		Week 1		Week 2		Week 3		Week 4		Week 5	
Experimental bowl	No. of swim up fry stocked bowl per week	0_2T	Surv	0_2T	Surv	0_2T	Surv	0_2T	Surv	0_2T	Surv
1	100	6.65	85	6.60	85	6.49	86	6.52	83	6.57	84.8
2	100	7.09	95	7.06	95	7.00	96	7.01	95	7.04	95.3
3	100	7.27	100	7.29	100	7.29	100	7.30	100	7.29	100.0
4	100	7.09	98	7.08	96	7.05	96	7.03	97	7.06	96.8

N.B -100 swim up fry were used per week (i.e.. for the 4 week experimental period) and their survival rates were estimated at day 5 weekly

Analysis of variance (ANOVA) was also used to test level of significance ($p<0.05$) or ($p>0.05$) in the survival rate values observed for the experimental treatments replicates while the LSD (least significant difference) was used to select the means which produced the highest survival rate.

RESULTS

The results of the experimental are presented in Table 1-4.

DISCUSSION

The oxygen levels of the 4 experimental treatments were positively correlated r = 0.99 with the survival rates. This implies as the oxygen levels in the treatment bowls increased the survival rates of the swim up fry increases. This is clearly shown in Table 4 with treatment 3 (swim-up fry in aerated spring water kept in the laboratory) recording 100% survival at highest oxygen level of 7.29 mg L^{-1}, this is followed by treatment 4 (De-ionized spring water kept outside the laboratory under ambient condition) recording 96.80% survival rate at an oxygen level of 7.06 mg L^{-1}, next to it is treatment 2 with a survival rate 95.30% at an oxygen level of 7.04 mg L^{-1}, while treatment 1 (spring de-ionized water kept in the laboratory) recording the least performance with a survival rate of 84.80% at dissolved oxygen level of 6.57 mg L^{-1}.

From the ongoing it could be seen that slight changes in the oxygen levels of the culture medium (i.e., the treatments) had marked significant ($p<0.05$) effects on the survival rates of the swim up fry.

Also since the Clarias broodstocks used for the production of the swim-up fry were fed 40% crude protein diet and also maintained in hapas under the same standard conditions an average percentage (%) fertilization of eggs of 82.75% and percentage (%) hatchability of eggs of 96.25% were recorded during the 4 week oxygen/swim up fry survival rate experimental study as shown in Table 1 and 2.

Artificial propagation involves human intervention in the process of natural propagation and according to Woynavorich and Horvath[8] has the advantages of better rates of fertilization and hatchling, protection against enemies and unfavourable environmental conditions and better conditions for growth and survival. This findings is in line with the results obtained for this study.

Also the injection of pituitary hormones intrasmuscularly especially to the female broodstock must have completed the second phase of the gonadal development (completion of maturation of females) which according to Davy and Chouinard[9] has been the major problem in the breeding of fish species desired for aquaculture.

Also the high average percentage (%) fertilization of eggs of 82.75% and average percentage hatchability of eggs of 96.25% was achieved because the dissolved oxygen levels in all the 4 treatments on the average (6.57 mg L^{-1}, (Trt 1), 7.04 mg L^{-1} (Trt 2), 7.29 mg L^{-1} (Trt 3) and 7.06 mg L^{-1} (Trt 4)) were all above the minimum desirable limit of 5.0 mg L^{-1} (Dissolved Oxygen) recommended by Boyd[12] for culture purposes. Hence since it appears there is a strong correlation between fertilization/Hatchability fry survival rate and dissolved oxygen concentrations in culture medium, the highest fry survival rate of 100% recorded for treatment 3 (Aerated spring water in the laboratory) maintained at 7.29 mg L^{-1} of dissolved oxygen cannot be overemphasized.

CONCLUSION

The success of artificial propagation of fish through induced breeding under controlled environmental condition especially as in fish hatchery situations 4.is mostly dependent on the dissolved oxygen levels available for the fry coupled with adequate feeding especially after the yolk absorption stage.. Therefore a high dissolved oxygen level in the culture medium (above a minimum of 5.0 mg L^{-1}) will result in high fertilization/hatchability and a high fry survival rate.

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