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Research Article

Effects of Different Physiological Saline Concentrations on the Reproductive Performance of *Clarias gariepinus* (Burchell, 1822)

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Abstract: This study aimed to investigate the effect of different concentrations of physiological saline (0, 3, 6 and 9 g/l as control) on the reproductive performance of *Clarias gariepinus* within 7 days of induced breeding. At the end of the experiment, there were significant increases (ANOVA, $p>0.05$) in fertilization, hatching and survival rate with an increase in physiological saline concentration up to 6 g/l. Values were (mean ± standard deviation, SD, %) 94.3 ± 1.1, 88.8 ± 3.0 and 78.7 ± 2.3 for fertilization, hatching and survival rate respectively. The physico-chemical parameters were measured and values were within the ideal range for *C. gariepinus* production. Overall, the results indicated that milt prepared in 6 g/l physiological saline concentration is the most ideal for fertilization, hatching and survival of *C. gariepinus* larvae

Keywords: Physiological saline concentration; Survival; Reproductive performance; Hatching; Clarias gariepinus

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Introduction

The African catfish is known as a vital tropical aquaculture fish in Africa, Asia and Europe (Hocutt, 1989). The wide choice of the African catfish in aquaculture is ascribed to its various culture qualities such as good growth performance; resistance to disease; high fecundity; ability to survive in captivity (Borode *et al.,* 2002). However, the constraint faced in commercial aquaculture in Nigeria is the inadequate production of good quality and quantity of seeds (Nwadukwe *et al.,* 1993). African catfish fingerlings are produced commercially by inducing breeding techniques either with the use of synthetic hormone or crude pituitary extracts. However, reports have shown high egg production rates accompanied by low hatchability and survival rates (Haylor, 1993). The reason for this is unclear. According to Orji *et al.* (1997), failure in fertilization and hatching rates may be due to environmental and physico-chemical factors e.g., salinity other than the maturity stage of the fish since that had already been established during the selection of the brood stocks for breeding.

The use of normal physiological saline solution (Sodium Chloride, 0.9% w/v) for the osmotic protection of cells has been established (Wolf, 1963). Physiological saline has been used as preservative for Channel catfish sperm (Guest, 1976). During induced breeding of African catfish, physiological saline has been used as a carrier for pituitary homogenates, preservatives for milt and diluent of artificial hormones (Ayinla, 1994). However, there are concerns on the concentration of physiological saline used. Complaints from farmers indicated low fertilization, hatching and survival of the larvae. More so, only a few documentation has been made (Orji, 1997). This study aimed to investigate the effect of different concentrations of physiological salineon some aspects of the reproductive performance of *C. gariepinus.*

Materials and Methods

Study Area, test organisms and spawning agent

The study was carried out in the Fisheries laboratory of the Rivers State University, Nkpolu-Oroworukwo, Port Harcourt. *C. gariepinus was* used as the test organism for this study. The spawning agent, Ovaprim, manufactured by Syndel International Inc., Canada, is a liquid preparation and contains 20 µg of salmon gonadotropin releasing hormone (D-Arg6, Pro9, Net-sGnRH) and 10 mg of domperidone, a dopamine antagonist. The recommended dose from the manufacturer is 0.5 ml/kg body weight of the fish. However, the dose varies from species to species and depends on the location and physical condition of fish

Procurement, transportation and acclimation of brood stocks

A total of eight (8) brood stocks of *C. gariepinus* were collected from Momoh farm, Kpite, Tai Local Government Area (4 female) and Ansa Farm, ARAC, Port Harcourt (4 male), both in Rivers State, Nigeria. All the brood stocks were of similar weight of ~1 kg. Selection of brood stocks were based on the readiness of the genitals (Ayinla, 1994). The gravid female brood stocks of *C. gariepinus* were selected based on the swollen, reddish genital opening while male were based on reddish and pointed genital papillae. Brood stocks were transported in plastic troughs from the farms to the Fisheries laboratory of the Rivers State University. Brood stocks were acclimated and conditioned in separate tanks for one week and were fed with 40% crude protein commercial pelleted feed at 3% body weight twice daily at 9.00 and 18.00 hours. Feeding was suspended 24 hours prior to inducement.

Administration of hormone

Female brood stock of *C. gariepinus* was injected intramuscularly into the dorsal muscle above the lateral line with ovaprim at 0.5 ml/kg fish and kept in a trough with minimal water for conditioning (Achionye-Nzeh, 2012).

Preparation of physiological saline solution

Sodium chloride were weighed 0, 3, 6 and 9 g using a sensitive weighing balance and dissolved in one liter of distilled water to give final concentrations of 0, 3, 6 and 9 g/l respectively. The solutions were stirred vigorously for homogenous mixture.

Collection and preparation of milt

All the male *C. gariepinus* brood stock was sacrificed and testes removed. Milt were collected from individual fish by cutting the testes into smaller pieces. Milt were squeezed and washed into already prepared concentrations (0, 3, 6 and 9 g/l) of physiological saline to keep the sperm dormant but alive. Thereafter, milt was sieved to remove dead tissues (Achionye-Nzeh, 2012).

Experimental design

The complete randomized design was used for this experiment. Female brood fish were stripped of their eggs into four individual plastic basins. Thereafter, the milt already prepared in the different concentrations (0, 3, 6 and 9 g/l) of physiological saline concentration was added for fertilization. Fertilized eggs for each treatment were randomly distributed in triplicate design (i.e., 3 troughs/ treatment) into a total of 12 plastic troughs for incubation and subsequent rising of hatchling for 1 week (7 days).

Feeding

The larvae in each of the experimental troughs were fed on commercial diet (Coppens) at 3% of their body weight (Viveen, 1985). Feeding was done twice daily to enhance optimum growth (Davies, 2006). The daily ration was divided into two equal parts. One part was fed at 9 hours and the other part of 18 hours. Feed was dispensed evenly on the water surface in each tank to allow equal opportunity for feeding.

Measurement of reproductive performance

Calculation of number of eggs released: The number of eggs released in each experimental trough was determined (Viveen *et al.,* 1985; Davies *et al.,* 2006; Ndimele *et al.,* 2011; Owodeinde, 1822). Briefly, the weight of the brood stock after stripping (W_b, grams) was subtracted from the weight of the brood stock before

stripping (W_a) and the difference multiplied by 700 (1 g=700 eggs).

The formula is shown as: No of eggs released=(W_b-W_a) g × 700

Where W_a is the weight of brood stock before stripping; W_b is the weight of brood stock after stripping; g is gram; 700 is the constant.

Percentage fertility rate: Prior to hatching, the number of fertilized and unfertilized eggs was counted physically for each treatment. The fertilized eggs were green, transparent and flattened whereas the unfertilized ones were whitish in color and thick.

Percentage fertility rate was determined (Florence, 2012) using the formula:

$$\% \text{ Fertility rate} = \frac{\text{Number of fertilized eggs}}{\text{Total number of eggs counted}} \times 100$$

Percentage hatchability rate: After hatching, the number of larvae in each experimental trough was carefully counted and the percentage hatchability rate was determined FAO (1996) using the formula:

$$\% \text{ Hatchability rate} = \frac{\text{Number of eggs hatched}}{\text{Number of eggs incubated}} \times 100$$

Percentage survival rate: Percentage survival rate was determined by the end of the experiment according to FAO (1996) using the formula:

$$\% \text{ survival rate} = \frac{\text{Final number of fry harvested}}{\text{Initial number of fry stocked}} \times 100$$

Determination of water quality

Temperature, pH and Dissolved oxygen (DO) of the water in the experimental troughs were measured daily using a mercury with a digital meter (Multi 3410 Digital Multiparameter Meters, Global Water Instrumentation, College Station, Texas, USA).

Table 1: Number of Fertilized Egg, Hatchings and Survivals of *C. gariepinus* Larvae at Different Concentration of Physiological Saline Solutions.

Concentrations Of Physiological saline Solutions (g/l)	No. of Fertilized Egg	No. of Hatchlings	No. of Survivals
0	128.7 ± 12.0^a	95.7 ± 5.6^a	9.0 ± 1. 0^a
3	320. 3 ± 23.6^b	249.8 ± 29.7^b	30.3 ± 4.0^b
6	616.3 ± 5.7^c	577.7 ± 19.4^c	39.3 ± 1.1^c
9	570.6 ± 20.4^d	404.7 ± 39.1^d	31.0 ± 4.5^b

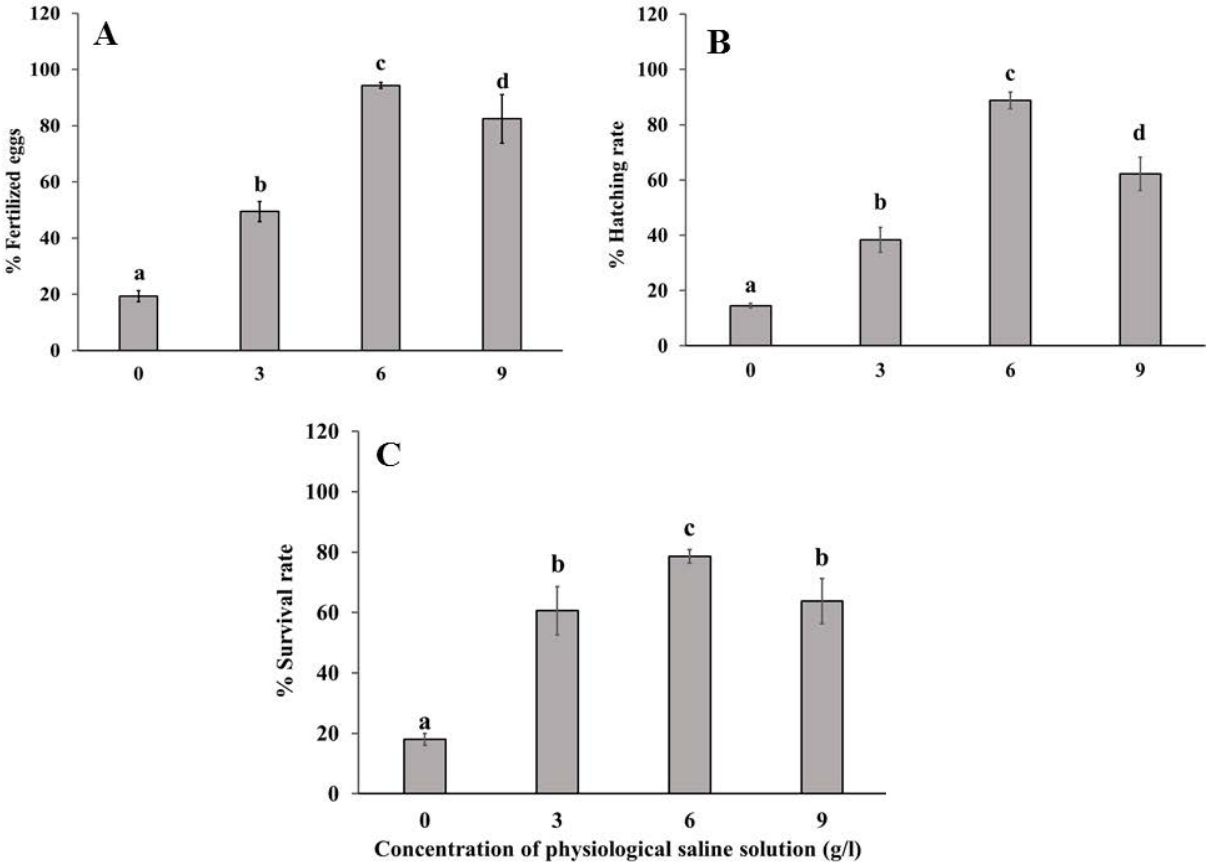

Figure 1: Percentage fertilized eggs (A), Percentage hatching rate (B), Percentage survival rate (C) of *C. gariepinus* at different concentration of physiological saline solutions. Data are means ± SD, *n*=3 replicates/ treatment. Different letters indicates statistically significant difference (ANOVA, $p>0.05$).

Statistical analysis of data

Statistical analysis was carried out on all data using ANOVA with SSPS. The Turkey post hoc test was used to 95% confidence level to provide specific information on which means are significantly different from each other.

Results

Physico-chemical parameters of water in experimental troughs

The physico-chemical parameters of the water in the experimental troughs were measured daily during the experimental period. There were no significant differences in the experimental water in all treatments (ANOVA, $p>0.05$). Values (means ± SD) ranged from 5.5 ± 0.2 to 5.6 ± 0.9 mg/l; 6.4 ± 0.1 to 6.5 ± 0.5 and 26.1 ± 0.1 to 26.9 ± 1.1°C for DO, pH and temperature respectively.

Reproductive performance

Number of fertilized egg, hatchings and survivals of *C. gariepinus* larvae at different concentration of physiological saline solutions: The number of fertilized eggs, hatchings and survival of *C. gariepinus* at different concentrations of physiological saline solution were monitored **(Table 1)**. Overall, the results showed significant differences (ANOVA, $p<0.05$) with an increasing physiological saline solution. However, highest numbers of fertilized eggs (616.3 ± 5.7), hatching (577.6 ± 20.4) and survival (39.3 ± 1.1) were observed at 6 g/l physiological saline solution **(Table 1)**. Significant decreases were seen at 3 g/l and 9 g/l compared to the 6 g/l physiological saline solution for fertilized eggs, hatching and survival of larvae **(Table 1**; ANOVA, $p<0.05$). There was no significant difference (ANOVA, $p<0.05$) for the number of survivals at 3 and 9 g/l physiological saline solutions.

Percentage fertilized eggs, hatching and survival rate in *C. gariepinus* at different concentrations of physiological saline solution: The percentage of fertilized eggs in *C. gariepinus* at different concentration of physiological saline solution **(Figure 1)**. The percentage fertilized eggs increased with increased concentrations of physiological saline solution and peaked (94.3 ± 1.1%) at 6 g/l of the solution. However, it declined slightly (82.4 ± 8.6%) at 9 g/l of physiological saline solution. Likewise, the percentage hatching rate in C. gariepinus at different concentration of physiological saline solutions **(Figure 1)**, showed a significant increase (ANOVA, $p>0.05$) and peaked (88.8 ± 3.0 %) at 6 g/l concentration of the solution. A decline (62.2 ± 6%) was observed at 9 g/l. The highest percentage survival rate (78.6 ± 2.3%) was recorded at 6 g/l. However, no significant difference (ANOVA, $p<0.05$) was observed at 3 and 9 g/l of the physiological saline solution for percentage survival rate **(Figure 1)**.

Discussion

Physico-chemical Parameters

The physico-chemical parameters of water are vital in the biology and physiology of aquatic organisms. The values of dissolved oxygen (DO) obtained in this study (5.5 ± 0.2-5.6 ± 0.9) were within the required levels recommended for a successful fish reproduction FAO (1996). Ufodike and Garba (1992), reported DO concentration of 4.0 mg/l as the minimum adequate for different stages of most species of aquatic life. Temperature (°C) values were within 26.1 ± 0.1°C and 26.9 ± 1.1°C. The value agrees with that reported by Madu *et al.* (1988). They reported best temperature range for optimum production of *Clarias* species as 25-31°C [18]. The pH values obtained in this study ranged from 6.4 ± 0.1 to 6.5 ± 0.5. This was similar to the report of Yang *et al*. (2009) in a similar experiment with the catfish*, Silurus asotus*.

Reproductive Performance

Egg Fertilization rate: The egg fertilization in *C. gariepinus* increased with increasing concentration of physiological saline solution with the optimum fertilization at 6 g/1. The result showed that fertilization could take place without the use of physiological saline solution, but performance was better when a physiological saline solution was used **(Table 1 and Figure 1)**. Our results are in line with those reported by other authors (Nwadukwe, 1993; Haylor, 1993; Orji *et al.,* 1997). For example, Orji *et al*. (1997) reported egg fertilization in physiological saline solutions from 0 to 1%, with 0.4% being the most ideal. This may be because the time taken for the eggs to fertilize is dependent on the activating solution (Zarski, 2012). It has been demonstrated during artificial fertilization, that physiological saline solution opens the micropyle of eggs for longer periods when compare to fresh water (Leifritz, 1976).

Hatching rate: The hatching rate increased significantly as the concentration of the salinity increases. This agrees with the findings of Rasowo *et al.* (2007) who reported that physiological saline solution provides a conducive environment for egg hatching. In addition, it has been reported that both freshwater and brackish water fish species possess hatching enzymes with salt-dependent characteristics (Kawaguchi, 2013).

Survival of larvae: The optimum survival of fish larvae was recorded at 6 g/l of physiological saline solution, and while the lowest was observed at 0 g/l. This finding shows the predominant effect of egg fertilization and hatching rate on larva development and survival in *C. gariepinus* at different concentration of physiological saline solution. Borode (1998), reported that survival of fish larvae in Clariids during hatchery operations could be dependent on the level of egg fertilization and hatching rate. They further asserted that good egg fertilization and latching rate is directly proportional to the survival of fish larvae.

Conclusion

The results obtained from this study showed that 6 g/l physiological saline solution is an ideal concentration for optimal fertilization of eggs, hatching and subsequent survival of the larvae. Hence, the 6 g/l concentration is suggested as the

recommended concentration to fish farmers for optimum results during induced breeding.

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