



**EFFICACY OF DIFFERENT CONCENTRATION OF PHYSIOLOGICAL SALINE
SOLUTION ON THE REPRODUCTIVE PERFORMANCE OF
Clarias gariepinus (BURCHELL, 1822)**

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ABSTRACT

The effect of different dilution factor of physiological saline solution in milt and its influence on reproductive performance of *Clarias gariepinus* were investigated. 2500g each of male and female *C. gariepinus* were collected from the teaching and research farm of the department fisheries and aquaculture, Federal University Dutse Jigawa. The experiment was conducted using a Completely Randomized Design (CRD) with four treatments, each replicated three times. The physiological saline concentration of each treatment was $T_1=0.3\%$, $T_2=0.5\%$, $T_3=0.7\%$ and $T_4=0.9\%$. Data on egg fertilization, hatching, and survival rates were collected. The result shows that fertilization rates were higher (82.08 ± 9.37) in T_2 (0.5g/1000ml), while hatching rates were better in T_2 and T_1 at 82.97 ± 16.71 and 75.04 ± 5.26 respectively, the values were not significantly different ($p > 0.05$). There was a higher significant difference ($p < 0.05$) in survival rate for T_1 (85.48 ± 19.53) than other treatments. The saline concentration dilution for optimum fertilization, hatchability and survival rate ranged from 0.3% to 0.5%. Higher saline concentrations negatively impact fertilization and survival rates in African catfish breeding. Optimal breeding outcomes may also depend on other water parameters and brooder fish health.

Key words: Concentration, Dilution, Fertilization, Reproduction, Induced Breeding

INTRODUCTION

Fish breeding constitutes a critical dimension of aquaculture that has undergone extensive research and innovative advancements aimed at augmenting fish production. In Nigeria, there exists a pronounced demand for high-quality fish seeds, a phenomenon attributed to the escalation of fish production facilitated by the heightened demand for fish protein (Katampe, 2023). Consequently, numerous studies have been undertaken to enhance the hatchability and survival rates of catfish larvae in controlled environments. Notwithstanding these extensive investigations, a significant number of hatcheries continue to provide substandard fish seeds to aquaculturist, which adversely affects both the growth rates and weight gain of the fish, as well as the overall profitability of the farmers (Samuel, 2021). Nevertheless, the inadequacy in supply and the comparatively elevated cost of *C. gariepinus* fingerlings, which arises from a low yield per breeding cycle (Suleiman, 2019), underscores the necessity to broaden the array of factors contributing to the diminished output. The collection of seeds from natural habitats is, however, inconsistent and restricted solely to the rainy season. Nonetheless, under specific cultural conditions, the ovulation of wild *C. gariepinus* can be facilitated through either environmental alterations or hormonal interventions (Abdulkarim and Yusuf, 2015). The failure of fertilization and hatching during induced spawning may not invariably stem from the maturity level of the parent stock fish, but could also be attributed to environmental conditions and various physico-chemical parameters, such as salinity. Saline solutions have been employed for the storage and preservation of isolated animal cells (Rours and Turner, 1916). In the context of induced spawning of African catfish, saline solutions serve a dual purpose, functioning both as a medium for pituitary homogenates and as a preservative for the milt (Olaoti *et al.*, 2021). Normal saline, characterized by



the presence of 9g of sodium chloride (NaCl) dissolved in one litre of water, is conventionally regarded as the optimal saline concentration for the process of fertilization; nevertheless, both excessive and insufficient saline concentrations may result in detrimental effects on cellular viability. Okomoda *et al.* (2024) observed elevated saline concentrations exert lethal effects on cellular integrity, which may subsequently impede fertilization, hatchability, and the survival rates of *Clarias gariepinus*. Furthermore, Okunsebor *et al.* (2014) and Orji, *et al.* (1997) documented that the percentage of fertilization exhibited an increase with a rise in the volume of normal saline, yet demonstrated a decline following the attainment of a specific saline volume. The objective of this study was to examine the impact of various concentrations of physiological saline solution on several facets of the reproductive performance of *Clarias gariepinus*.

MATERIALS AND METHODS

Study Area

The study was conducted in the Fish hatchery unit of the Department of Fisheries and Aquaculture, Faculty of Agriculture, Federal University Dutse, Jigawa state, Nigeria.

Experimental Fish

Gravid males and females *Clarias gariepinus* were collected from the departmental teaching and research farm, Department of Fisheries and Aquaculture, Faculty of Agriculture, Federal University Dutse. The fish were transported to the hatchery unit of the Department in 25 Litres Jerrycan cut one quarter horizontally. The fish were acclimatized in small separate holding 1 x 1 x 1m fish pond for 2 days prior to the breeding exercise sex wise.

Experimental Design

Induced breeding

Four females and males broodstock (1100 ± 132.29 - 944.00 ± 48.00 g) were used for the experiment. The female broodstock were induced with Ovaprim hormone at 0.5ml/kg of the fish via intramuscular injection. After eleven hours of the latency period, milt was collected from males, according to the method described by Diyaware *et al.* (2010). The induced female fish were stripped into receptacles and fertilized based on the following treatments of (0.3%, 0.5%, 0.7% and 0.9% g/100ml) of physiological saline dilution concentration as treatments 1, 2, 3 and 4, respectively. The treatments were replicated three times in a Complete Randomized Design (CRD) manner.

Preparation of Physiological Saline Solution

Sodium chloride were weighed 3, 5, 7 and 9 g using a sensitive weighing balance and dissolved in one thousand liter of distilled water to give final concentrations of 0.3%, 0.5%, 0.7% and 0.9% respectively. The solutions were stirred vigorously to obtain a homogenous mixture.

Milt Collection and Preparation

The male broodstocks were sacrificed to remove the testes. Small incisions were made on the testes and a slight pressure was applied for milt to be extracted, 0.5ml was used for each treatment (0.3%, 0.5%, 0.7% and 0.9% g/100ml) of physiological saline.

Stripping of Eggs and Fertilization

Pressure was applied gently on the abdomen of the female brooder induced with the Ovaprim suspensions and the Ovulated eggs was collected into plastic bowls with the aid of a 2ml graduated syringe. 2 grams each of the stripped eggs were used to fertilized with diluted Spermatozoa (milt) of (0.3%, 0.5%, 0.7% and 0.9% g/100ml) of physiological saline solution. Fertilization was done by pouring the milt on the ovulated eggs and stirring the mixture gently.

Incubation and Hatching of Fertilized Eggs

The fertilized eggs were incubated in an indoor incubating concrete tanks measuring 1 x 1 x 1 m³ with a substrate or egg collectors (cotton netting material). Hatching occurred between 24-30 hours of incubation at water temperature ranges of 26°C – 32°C with pH of 6.5 - 7.5. After hatching, the dead eggs and the egg collectors were removed. The hatched larvae fed on their yolk sac until it was completely absorbed within 2-3days.



Determination of Reproductive Performance Parameters

After fertilization, dead and unviable eggs which have turned whitish were counted and percentage fertilization was estimated. Percentage hatchability and survival were monitored and calculated at 24 to 48hrs and the fifth day after hatching respectively using the following formulae adopted from Haruna *et al.*, (2023).

$$\begin{aligned} (1) \text{ Fertilization rate (\%)} &= \frac{\text{Number of fertilized eggs}}{\text{Number of eggs}} \times 100 \\ (2) \text{ Hatching rate (\%)} &= \frac{\text{Number of hatched eggs}}{\text{Number of fertilized eggs}} \times 100 \\ (3) \text{ Survival (\%)} &= \frac{\text{Number of fingerlings stocked}}{\text{Number of fingerlings at the end of culture}} \times 100 \end{aligned} \quad \text{Onyia et al., (2017)}$$

Water Quality Parameters Monitored

Water temperature, dissolved oxygen and pH were monitored daily to maintain the quality of Water. Temperature was measured using mercury in glass thermometer in °C, pH was measured with digital EC/TDS/pH meter (Model: EC500 meter SANXIN-China) while dissolved oxygen was recorded using DO meter (Model: JPB-607A: 6V Shanghai REX) (DO).

Statistical Data Analysis

The study's data were subjected to One-Way Analysis of Variance (ANOVA), and SAS 10.0 was used to calculate differences between the means using the New Duncan's Multiple Range Test (1955) at a 95% confidence level.

RESULT AND DISCUSSION

Reproductive Performance of *C. gariepinus* Larvae at Different Concentration of Physiological Saline Solutions

Table 1 shows the mean Reproductive performance of *C. gariepinus* larvae at different concentration of physiological saline solutions.

Table 1: Mean Reproductive Performance of *C. gariepinus* Larvae at Different Concentration of Physiological Saline Solutions.

Parameter	T ₁ (0.3%)	T ₂ (0.5%)	T ₃ (0.7%)	T ₄ (0.9%)	p-value
Fertilization rate (%)	75.14±2.04 ^a	82.08±9.37 ^a	67.68±7.75 ^b	69.93±10.84 ^b	0.2211
Hatchability rate (%)	75.04±5.26 ^a	82.97±16.71 ^a	67.50±16.02 ^b	70.82±9.25 ^a	0.5140
Survival rate (%)	85.48±19.53 ^a	63.57±2.00 ^b	47.75±7.17 ^c	45.97±2.16 ^c	0.0060

All values on the row with different superscript are significantly different (P<0.05)

Higher mean fertilization (82.08±9.37) was recorded in 0.5% g/l physiological saline solution followed by that of 0.3% g/l with 75.14±2.04 fertilization. 0.7% g/l had the least 67.68±7.75 fertilization rates. There were significant differences (p<0.05) between the fertilization values among the treatments. The higher mean percentage fertilization (82.08±9.37) and hatching (82.97±16.71) rates obtained in this study is incongruent with the findings of Orji *et al.* (1997) obtained in their result as the highest fertilization was recorded at 0.4% salt concentration. This conforms with the report of Okunsebor *et al.* (2014), Ukwuani *et al.* (2016), Amachree *et al.* (2018) and Olaoti *et al.* (2021) who reported that high concentration of saline has a lethal effect on fertilized egg which could affect fertilization, hatchability and survival rate of *Clarias gariepinus*. However, the highest mean fertilization was quite lower in this study at 82.08±9.37 for T₂ and 75.14±2.04 for T₁ than that of Olaoti *et al.* (2021) at 93.67% and 93.00%. This might have occurred due to variations in physico-chemical parameters of the water used during the experimental process. This finding implies that fertilization is best carried out with milt mixed with saline solution within the range of 3.0g and 5.0g of salt to 1000ml of water.

The lowest mean hatchability was recorded in T₃ (67.50±16.02) while the highest was recorded in T₂ (82.97±16.71). However, this was found not to be statistically different from values obtained from T₁ (75.04±5.26). The high significant (p<0.05) hatchability rate observed in T₂ showed that the relative low



salt concentration in the treatment was optimum for the high hatchability recorded, this was earlier documented by Fashina-Bombata and Busari, (2003) for *Heterobranchus longifilis*, Fridman *et al.* (2012) for Nile tilapia *Oreochromis niloticus* and Olaoti *et al.* (2021) for *Clarias gariepinus*. This could also be attributed to fluctuations in physico-chemical parameters of the water which changes with metabolic processes of embryonic development and egg-hatching as well as the physiological state of the broodstock fish among many other factors which affect reproduction in catfishes. This finding implies that physiological saline solution of 5.0g salt can be used to substitute normal saline solution (9.0g). However, increase in saline concentration beyond this level could decrease the hatchability levels of the eggs.

The result of survival rate of fry's in the study showed the highest mean survival in T₁ (85.48±19.53) while the least was recorded in T₄ (45.97±2.16). However, mean survival values obtained in all the treatments were statistically different (p<0.05) in their survival rate. The values of T₁ and T₂ is indicative that the ideal salt concentrations for fry survival is within the range of 3.0 to 5.0 g of sodium chloride while values outside the range could lead to mortality and subsequent low survival rate as observed in T₃ and T₄ which were recorded in the two extremes. The high survival rate recorded in treatment T₁ was possibly due to better hatching rate and low population of unhatched eggs and reduced pollution and possible microbial infection. 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 hatching rate is directly proportional to the survival of fish larvae. Olaoti *et al.* (2021) also reported that highest survival was recorded at 0.4% saline concentration.

Physicochemical Parameters

The physico-chemical parameters of the water in the experimental tanks are shown in Table 2 below and 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 28.89±87 to 29.21±48 °C; mg/l; 7.22±15 to 7.32±10 and 6.49±11 to 6.79±07 mg/l for temperature, pH and DO, respectively. The water quality parameters recorded during the study were within the range for fish culture as suggested by (Boyd, 1979; Viveen *et al.*, 1985).

Table 2: Mean Physico-chemical Parameters of water in experimental tank

Water parameters	T ₁	T ₂	T ₃	T ₄	p-value
Temperature (°C)	29.09±62 ^a	28.99±71 ^a	28.89±87 ^a	29.21±48 ^a	0.7970
pH	7.28±03 ^a	7.27±07 ^a	7.22±15 ^a	7.32±10 ^a	0.2140
D O (mg/lit)	6.79±07 ^a	6.72±01 ^a	6.49±11 ^b	6.63±09 ^a	0.0000

All values on the row with different superscript are significantly different (P<0.05)

CONCLUSION AND RECOMMENDATION

The results obtained from this study revealed that 5 g/l physiological saline solution is an ideal concentration for optimal fertilization of eggs, hatching and subsequent survival of the larvae. Hence, the ranged of 4 to 5 g of salt in 1000ml of water concentration is suggested as the recommended concentration to fish farmers for optimum results during induced breeding while higher salt concentration leads to mortality. However, the current study should further be investigated to ascertain the relationships of different physiological saline concentrations and on the milt cells motility and morphology of *Clarias gariepinus*.

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