

Effect of Graded Level of Normal Saline Solution on Fertilization Rate, Hatchability and Survival of *Clarias Gariepinus* Fry

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ABSTRACT: The effect of graded level of normal saline solution on fertilization rate, hatchability and survival of *Clarias gariepinus* fry was conducted in Department of Aquaculture and Fisheries Management, Nasarawa State University Keffi. six (6) brood stocks of *C. gariepinus* consisting of 3 males and 3 females were used for the study. Sodium chloride was weighed at 0g/l, 2.25g/l, 4.50g/l, 6.75g/l and 9.00g/l using a sensitive weighing balance representing T1, T2, T3, T4, and T5 respectively. Milt was squeezed and washed into already prepared concentrations (0, 2.25, 4.50, 6.75 and 9.00 g/l) of physiological saline to keep the sperm dormant but alive. Statistical analysis was carried out on all data using ANOVA with STAR and means were separated at 95% confidence level. The study revealed that fish highest fertilization was recorded in 6.75g/l (82.41%) saline solution which is not significantly different ($P>0.05$) from the result obtained in 9.0g/l (82.22%) saline solution. However, the lowest fertilization rate was observed at 0g/l (37.56%) respectively. The hatchability rate at 6.75% (63.42%) saline solution was the highest and lowest at 0g/l (23.53%) respectively. The highest survival rate was at 6.75% (54.29%) and the lowest was obtained at 0g/l (15.00%) respectively. The study concluded that saline solution had positive effect on egg fertilization, hatching rate and survival of *Clarias gariepinus* at different concentrations of physiological saline solution. This study recommends 6g/l physiological saline solution as an ideal concentration for optimal fertilization of eggs, hatching and subsequent survival of the larvae.

KEYWORDS: Hatchability, Survival Rate, Fertilization rate, Fry.

INTRODUCTION

The high increase in the demand for fingerlings in the phenomenal growing aquaculture industry has stimulated the demand for artificial reproduction of cultured fishes (Okunsebor *et al.*, 2014). Artificial propagation of fish is a most promising and reliable way of ensuring availability of good quality fish seeds all year round and sustainability of the Aquaculture industry. It involves the use of natural (hypophysation) or synthetic hormones to induce ovulation and spawning in farmed fishes (Olumuji and Mustapha, 2012). It is a method used for fishes that do not normally breed in captivity.

Salinity therefore has been observed to influence growth, survival and production potentials of fish by its effect on osmoregulatory and metabolic activities of aquatic animals. At unfavourable concentrations, it can cause stress or even death of fish depending on the species. Most estuarine and marine fish species which are euryhaline have optimal salinity range within which the scope for growth and other biological activities are optimal (Okunsebor *et al.*, 2014). Salinity tolerance tests with fish have been performed using different saline solution. Salinity tolerance tests with fish have been performed using different saline solutions for example, sodium chloride, diluted seawater and synthetic seawater.

The quantity of saline mixed with milt before it is used to fertilized stripped eggs is suspected to have an effect on the fertilization rate, hatchability rate and survival rate of the eggs (Okunsebor *et al.*, 2014). Failure in fertilization and hatching rate may be due to environmental and physiological factors such as salinity. Many fish hatchery in Nigeria, the quantity of normal saline used as diluent for milt often constitute dilution ratios different from the 0-6-0.7% recommended by Dekuboba *et al.*, (2018) either to increase or reduce the concentration of milt during induced breeding of *Clarias gariepinus*. There is paucity of knowledge on the effect of dilution on the potency and viability of spermatozoa. Okunsebor *et al.* (2014) studied the effect of different Dilutions of saline on some sperm parameters and their influence on fertilization, hatchability and survival rate of hatchlings. While Ukwuani *et al.* (2014) conducted a study on the Induced breeding of catfish (*C. gariepinus*) as influenced by milt dilution through different levels of normal saline inclusion. The study determined the effect of graded level of normal saline solution on fertilization rate, hatchability and survival of *Clarias gariepinus* fry

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MATERIALS AND METHODS

Description of the Study Area

The study was carried out at the Department of Aquaculture and Fisheries Management, Faculty of Agriculture Shabu, Lafia Campus, Nasarawa State University Keffi. The study area falls within the southern guinea savanna zone of Nigeria and located between latitude 8°33'22"N and longitude 8°32'82"E. Rainfall usually starts from April-October and average monthly rainfall is from 40mm-350mm (NIMET, 2016).

Procurement, and Transportation of Brood Stocks

A total of six (6) brood stocks of *C. gariepinus* consisting of 3 males and 3 females was collected from Lafia Local Government Area, Nasarawa State. Brood stocks was transported in plastic troughs from the Departmental Fish Farms.

Administration of Hormone

Female brood stock of *C. gariepinus* were injected intramuscularly into the dorsal muscle above the lateral line with ovatide 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 was weighed at 0g/l, 2.25g/l, 4.50g/l, 6.75g/l and 9.00g/l using a sensitive weighing balance representing T1, T2, T3, T4, and T5 respectively. The solutions were stirred vigorously for homogenous mixture.

Collection and Preparation of Milt

All the male *C. gariepinus* brood stocks were sacrificed and testes removed. Milt sacs were collected from individual fish by cutting the testes into smaller pieces. Milt was squeezed into already prepared concentrations (0, 2.25, 4.50, 6.75 and 9.00 g/l) of physiological saline solution.

Experimental Design

The complete randomized design was used for this experiment. Female brood fish were stripped of their eggs into five individual plastic bowls. Thereafter, the milt already prepared at different concentrations (0, 2.25, 4.50, 6.75 and 9.00 g/l) of physiological saline concentration was added for fertilization. Fertilized eggs for each treatment were randomly distributed in replicate into a total of 15 plastic bowls of 45litre capacity for incubation and subsequent raising of hatchling for 1 week (7 days).

Calculation of number of eggs released

The number of eggs released in each experimental bowl was determined according to Davies *et al.* (2006); Ndimele *et al.* (2011). 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).

No of eggs released = (W_b - W_a) g × 700

Percentage fertility rate

Prior to hatching, the number of fertilized and unfertilized eggs were counted physically for each treatment. Percentage fertilization rate was determined (Florence, 2012) using the formula:

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

Total number of eggs counted

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

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

Number of eggs incubated

Determination of Water Quality

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

Statistical Analysis

Statistical analysis was carried out on all data using ANOVA with STAR statistical package and means were separated at 95% confidence level.

RESULTS

Fertilization Rate of *Clarias gariepinus* at Different Saline Solution

The result presented in Figure 1 below showed the percentage fertilization of *Clarias gariepinus* at different Saline Solution. Significant differences (P<0.05) was observed in the percentage fertilization of the fish at different saline solution. It was observed that fish highest fertilization was recorded in 6.75g/l (82.41%) saline solution which is not significantly different (P>0.05) from the

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result obtained in 9.0g/l (82.22%) saline solution. However, the lowest fertilization rate was observed at 0g/l (37.56%) and was not significantly different ($P>0.05$) from fertilization rate at 2.25g/l (40.39%) respectively.

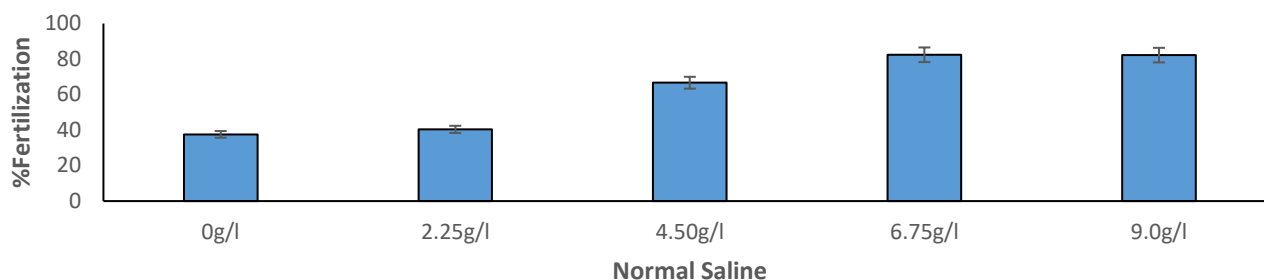


Figure 1: Percentage Fertilization of *Clarias gariepinus* at Different Saline Solution

Hatchability Rate of *Clarias gariepinus* at Different Saline Solution

The result in Figure 2 below showed the hatchability rate of *Clarias gariepinus* at different saline solution. The result obtained in Figure 2 showed significant differences ($P<0.05$) in the hatchability rate of fish. The hatchability rate recorded at 6.75% (63.42%) saline solution was the highest and then was slightly higher than 9.0g/l (62.87%) followed by 4.50g/l (45.86%), 2.25g/l (33.07%) while fish at 0g/l (23.53%) was the lowest respectively.

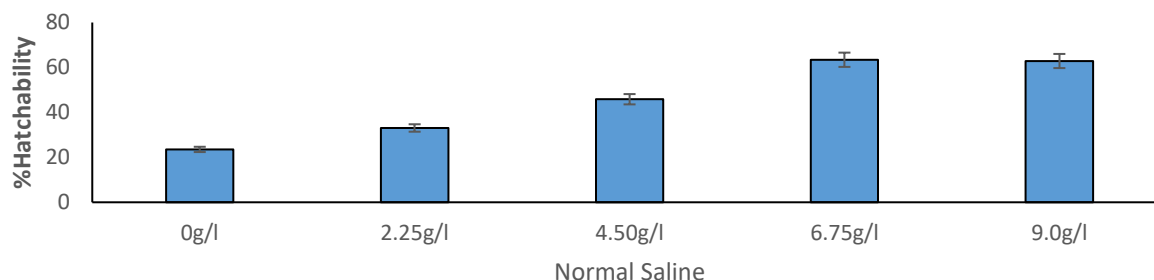


Figure 2: Hatchability Rate of *Clarias gariepinus* at Different Saline Solution

Survival Rate of *Clarias gariepinus* at Different Saline Solution

The survival rate of *Clarias gariepinus* at different saline solution is presented in Figure 3 below. The survival rate of the fish showed significant differences ($P<0.05$) across treatments. The highest survival rate was recorded at 6.75% (54.29%) followed by 9.0g/l (52.58%), 4.50g/h (25.47%), 2.25g/l (20.35%) while fish at 0g/l (15.00%) was the lowest. This result showed that saline solution was effect at 6.75g/l and 9.0g/l in survival rate of fish than other treatments respectively.

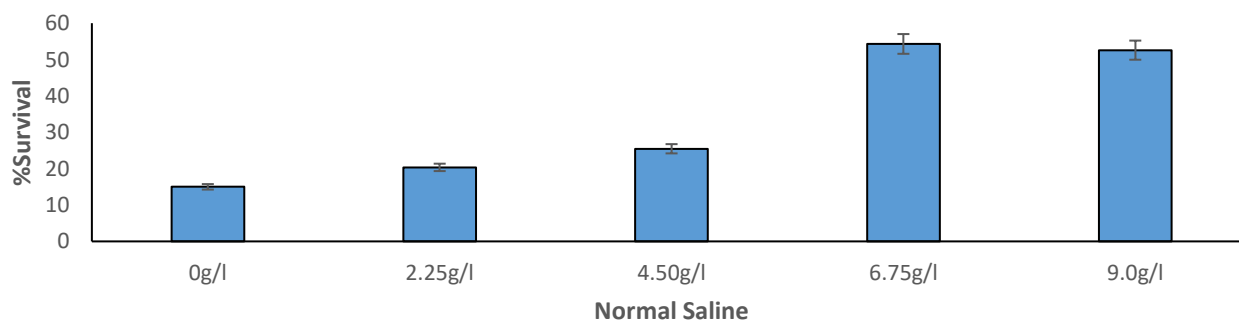


Figure 3: Survival Rate of *Clarias gariepinus* at Different Saline Solution

Water Quality Parameters

The water quality parameters of the experimental tanks used in the study is presented in Table 1 below. There is no significant differences ($P>0.05$) observed in the result presented for water quality parameters in the study. The temperature ranged between 26.89 – 27.45°C, pH (6.29 – 6.46), Dissolved Oxygen (5.53 – 5.83mg/l) and ammonia (0.63 – 0.64mg/l) respectively.

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Table 1: Water Quality Parameters

Parameters	(0g/l)	(2.25g/l)	(4.50g/l)	(6.25g/l)	(9.00g/l)	SEM	LOS
Temperature (°C)	27.34±0.12 ^a	26.89±0.17 ^a	27.45±0.14 ^a	26.89±0.17 ^a	27.45±0.14 ^a	0.01	0.735
pH	6.46±0.02 ^a	6.38±0.01 ^a	6.29±0.04 ^a	6.38±0.01 ^a	6.29±0.04 ^a	0.00	1.263
DO	5.53±0.02 ^a	5.83±0.01 ^a	5.64±0.05 ^a	5.83±0.01 ^a	5.64±0.05 ^a	0.02	0.000
Ammonia	0.63±0.00 ^a	0.64±0.00 ^a	0.63±0.01 ^a	0.64±0.00 ^a	0.63±0.01 ^a	0.00	0.000

SEM = Standard Error of Mean; LOS = Level of Significant; Means with the same superscripts are not significantly different (P>0.05) from each other

DISCUSSION

Fertilization Rate of *Clarias gariepinus* at Different Saline Solution

The result of the fertilization rate of fish in the study indicated that fish egg can be fertilized without normal saline solution however treatment at different normal saline solution showed significant results for fertilization and the performance was better in treatments containing saline solution. Treatment without normal solution had the lowest fertilization rate and this could be due to low sperm motility. Studies showed that using normal saline can initiate sperm activation and open egg's micropyle but without saline solution the sperm life span is limited to few minutes. It was observed in the study that fertilization rate increase with increase in saline solution even though the result obtained at 6.25g/l and 9.00g/l were not significantly different from each other. The result of this study is consistent with Dokuboba *et al.* (2018) reported that egg fertilization in *Clarias gariepinus* increased with increasing concentration of physiological saline solution at 6 g/l. The study showed that fertilization of *Clarias gariepinus* is better when saline solution is used. The result of the study is evident enough to prove that normal saline improve fish fertilization by providing a stable, physiological environment that maintains sperm integrity and enhance sperm motility for fertilization to take place effectively. Since significant variation was not observed in the fertilization of the fish eggs at 6.25g/l and 9.0g/l, it means that it could be economical for fish farmers to utilize saline solution at 6.25g/l concentration to obtain good result similar to the usual 9.0g/l used by farmers for decades.

Hatchability Rate of *Clarias gariepinus* at Different Saline Solution

The hatchability rate of the study significantly increasing with increase in saline solution. The highest hatchability was obtained at 6.25g/l of saline solution higher than other treatments. Slight variation was recorded between hatchability of fish at 6.25g/l and 9.00g/l but the differences was not significant. It can be concluded that, saline solution at these doses could be effective in *Clarias gariepinus*. This agrees with the findings of Dokuboba *et al.* (2018) who reported similar observation that physiological saline solution provides a conducive environment for egg hatching. In addition, it has been reported that *Clarias gariepinus* possess hatching enzymes with salt-dependent characteristics (Kawaguchi, 2013). It could be clearly seen from the study that lower saline solution leads to decline in hatchability rate. This study disagree with José *et al.* (2019) who reported that increase saline solution can impact negative effect to fish hatchability, however this claim can be true if the saline solution goes beyond the level used in the study. Egwenomhe and Oghenevwairhe (2025) reported 93.34% hatchability in *Clarias gariepinus* at 9g/l of saline solution.

Survival Rate of *Clarias gariepinus* at Different Saline Solution

The optimum survival of *Clarias gariepinus* fry was recorded at 6.25 g/l of physiological saline solution, and 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 *Clarias gariepinus* at different concentration of physiological saline solution. Adebisi and Ewuola (2019) opined 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. The better survival rate of fish recorded in the study could be linked to the rate of fertilization and hatchability obtained in the study because both parameters contributes to higher survival of fish (Fregene *et al.*, 2024). Survival is optimum in low levels of salinity considering the fact that *Clarias gariepinus* fry is a freshwater fish at a developing stage of growth, higher concentration of salinity can lead to stress and death at this stage due to osmotic effect (Torsabo *et al.*, 2024). However, the percentage survival was however relatively low compared to results obtained by Nwokoye *et al.* (2007), and this could be attributable to the size of the tanks in which the experiment was conducted which was relatively smaller and also more eggs were fertilized and the bowls were not placed on aerators and also inconsistent power supply could have hinder the ability to place the incubation tanks on a flow-through system.

Water Quality Parameters

The result of water quality parameters showed that all the values were within the range for African catfish. All the treatments tanks were having similar water quality parameters. The implication of water quality effect on the fish was evident in the result of the survival rate obtained in this study. The result of the water quality parameters recorded at the end of the study indicated that the water quality parameters were at recommended levels for rearing and culture of *Clarias gariepinus* for optimum growth, survival

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and reproduction (Oladipupo and Salami, 2020). This occurred as a result of continuous changing of the water during the study period

CONCLUSION

The study concluded that saline solution had positive effect on egg fertilization, hatching rate and survival of *Clarias gariepinus* at different concentration of physiological saline solution. The result of the fertilization rate of fish in the study indicated that fish egg can be fertilized without normal saline solution however treatment at different normal saline solution showed significant results for fertilization and the performance was better in treatments containing saline solution. The hatchability rate of the study significantly increasing with increase in saline solution. The highest hatchability was obtained at 6.25g/l of saline solution higher than other treatments. The optimum survival of *Clarias gariepinus* fry was recorded at 6.25 g/l of physiological saline solution, and the lowest was observed at 0 g/l. Fertilization, hatchability and survival rate of *Clarias gariepinus* increase with increase in saline solution and attain the highest performance at 6.25g/l of saline solution. The result of water quality parameters showed that all the values were within the range for African catfish. All the treatments tanks were having similar water quality parameters.

RECOMMENDATION

This study recommends 6g/l physiological saline solution as an ideal concentration for optimal fertilization of eggs, hatching and subsequent survival of the larvae.

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