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INDUCED BREEDING OF AFRICAN CATFISH (*Clarias galiepinus*) AS INFLUENCE BY MILT DILUTION THROUGH DIFFERENT LEVELS OF NORMAL SALINE INCLUSION

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ABSTRACT
An experiment was conducted at the Fish Hatchery Unit, Department of Fishery Technology, Enugu State Polytechnic, Iwollo to evaluate the induced breeding of African catfish (*Clarias gariepinus*) as influenced by milt dilution through different levels of normal saline inclusion. The treatments were; 0.4 millilitre of Normal Saline plus 2 millilitres of Milt (0.4 ml NS + 2 ml M), 0.8 millilitre of Normal Saline plus 2 millilitres of Milt (0.8 ml NS + 2 ml M), 1.2 millilitres of Normal Saline plus 2 millilitres of Milt (1.2 ml NS + 2 ml M), 1.6 millilitres of Normal Saline plus 2 millilitres of Milt (1.6 ml NS + 2 ml M) and 2 millilitres of Normal Saline plus 2 millilitres of Milt (2 ml NS + 2 ml M). The control treatment was left with no saline inclusion (0 ml NS + 2 ml M). The experiment was laid out in completely randomized design with three replications. Data were collected on fertilization rate of the eggs, hatching rate of the eggs and survival rate of hatchlings. The data collected were analysed using analysis of variance (ANOVA) for completely randomized design and the treatment means separated using least significant difference (LSD) at 5% probability level. The result showed that all the normal saline inclusion treatments performed better than the control .There were significant differences ($p<0.05$) among the treatments in fertilization rate, hatching rate and survival rate with 1.2 having superior values in hatching rate and survival rate. It can be concluded that milt dilution through normal saline inclusion improved induced breeding of Clarias gariepinus. 2 millilitres of milt diluted with 1.2 millilitres of normal saline for every 20 grams of egg is therefore recommended for improved induced breeding of Clarias gariepinus under normal environmental factors.

KEY WORDS: *Clarias gariepinus*, Fertilization, Hatching rate, Induced breeding, Milt, Normal saline, Survival rate.

INTRODUCTION

Fish farming is recognized as one of the most important aspects of Nigerian agricultural sector in the early eighties because of its lucrative nature and cheap supply of amino acid (protein). Most fishes reared from seeds (fingerlings) during this period were captured from the wild. Difficulties encountered and poor output obtained from the captured fingerlings resulted in scarcity of fish produce and encourages diseases infestation to the stock. The high increase rate in the demand for fingerlings in the phenomenal growing aquaculture production has stimulated the demand for artificial reproduction of cultured fishes (Nwokoye *et al*., 2007). Induced breeding on the African catfish became pertinent to strategically address these shortcomings. Nwokoye (2007) and Ngueku (2015) unraveled that synthetic and non- synthetic hormones have been used in the artificial propagation of *Clarias gariepinus*. They include Human Chorionic Gonadotrophin (HCG), Decorticosterone Acetate (DOCA), Pituitary (PT) extracts, Ovaprim, Ovatide, Ovaryprim in their researches and obtain positive responses. Similar researches were done on the efficacy of hormonal sources. Olubiyi *et al*. (2005) reported the use of varying doses of between 0.2 and 0.5ml of ovaprim for the induction of *Clarias gariepinus.* Adigun *et al*. (1983) performed on the induced breeding of African catfish, *Clarias lazera*, using acetone dried pituitary extract, DOCA and HCG. Various types of fishes have been induced to spawn, using various hormonal substances (non-synthetic) HCG (Brzuska, 2002) ovatide and ovaprim (Haniffa and Sridhar, 2002), pituitary extract (Haniffa *et al*., 2000; Nwadukwe *et al*., 1993). Ovaprim has been known to have better result because of its potency and quality as well as its relatively cheaper cost, ease handling and better survival of hatchlings (Nwokoye *et al*., 2007). However, Orji *et al*. (1997) stated its limitations on induced spawning thus farmers encounter shortage of fingerlings with which to stock their ponds irrespective of many hatcheries in different locations. Failure in fertilization and hatching during induced spawning may also be due to environmental and physico-chemical factor such as salinity. Salinity as defined by Wikipedia (2016) is the saltiness or dissolved salt content of a body of water. Anne (2016) defined saline solution or normal saline as a sterile mixture of salt and water. Normal saline is produced by dissolving 9g of NaCl in 1 litre of water. Orji *et al.* (1997) also pointed that saline solution has been used for storing and preserving isolated animal cells. Many

researchers have done great work on improving the output of fish seeds production. Saline solution has been used during induced spawning which serve a dual purpose. Orji *et al* (1997) reported that the success of induced spawning is as a result of the application of saline solution. Milt quality is a measure of the ability of sperm to successfully fertilize an egg and such ability mostly depends on qualitative parameters of milt composition of seminal fluid, milt volume, sperm density and sperm motility (Rurangwa *et al*. 2004). The quantity of eggs produced from a female broodstock of an average of one kilogram varies from 500g-700g depending on the season. Saline solution when included to the milt increases the volume of the spermatozoa and as well aids in spatial distribution of fertilized eggs since the quantity often extracted from male catfish is small, a maximum of 5ml per male broodstock. Irregularities in the level of the output during spawning were observed and the quantity of the saline solution applied became a suspect. Okunsebor *et al*. (2014) opined that the quantity of saline that is premixed with milt before its use to fertilize stripped egg is suspected to have an effect on fertilization rate, hatchability of eggs and survival of the hatchlings. The need to evaluate the quality of different milt dilutions became imperative in achieving high performance on fish seed yield. This research work therefore was to study the induced breeding of African catfish (*Clarias gariepinus*) as influenced by milt dilution through different levels of normal saline inclusion by determining fertilization rate of the eggs, hatching rate of the eggs and survival rate of the hatchlings.

MATERIALS & METHODS

Study Area

The study was carried out at the Fish Hatchery Unit, Department of Fishery Technology, Enugu State Polytechnic, Iwollo (ESPOLY) from November to December 2015.

Brood Stock Collection and Management

The male and female brood stocks of *Clarias gariepinus* were collected from Animal a reputable Fish Farm in Asaba, Delta State, Nigeria and acclimatized for 3 days. The brood stocks were kept in small separate outdoor concrete ponds with water level of ¾ of the ponds according to sexes. The weight of the stocks was determined using weighing balance.

Hormone Administration

Ovaprim (0.5ml per kg of fish) was administered to the female brood stocks in between the dorsal fin and the lateral line towards the abdominal region using syringe and needle as described by Oyelese (2006) to aid spawning.

Gonad Removal

The male brooders were sacrificed by dissecting to collect the testes. The sperm were collected by laceration of the milt with a clean razor blade.

Stripping

After 24 hours latency period, injected female brood stocks were removed from the ponds and stripped into bowl.

Experimental design and treatments

The experiment was laid in completely randomized design (CRD) with three replications. A total of 18 trials were carried out comprising five treatments replicated three times. The treatments were;

0.4 millilitre of Normal Saline plus 2 millilitres of Milt (0.4 ml NS + 2 ml M)
0.8 millilitre of Normal Saline plus 2 millilitres of Milt (0.8 ml NS + 2 ml M)
1.2 millilitres of Normal Saline plus 2 millilitres of Milt (1.2 ml NS + 2 ml M)
1.6 millilitres of Normal Saline plus 2 millilitres of Milt (1.6 ml NS + 2 ml M)
2 millilitres of Normal Saline plus 2 millilitres of Milt (2 ml NS + 2 ml M)
0 millilitre of Normal Saline plus 2 millilitres of Milt (0 ml NS + 2 ml M)

Fertilization

20 grams of egg was collected into bowl for each treatment. 2 millilitres of milt from the male bloodstocks was collected and diluted with different levels of normal saline as treatments. Fertilization was carried out by mixing milt with eggs aided by feather in order to ensure maximum fertilization. Fertilized eggs were poured directly on the spawning mop that served as egg collector.

Incubation

Each of the replicates was incubated in a well aerated trough containing 20 litres of water. Water was flowing in and out of the trough (flow-through system) to obtain a well oxygenated medium.

Data collection

Data were collected on fertilization rate, hatching rate and survival rate.

Fertilization rate

Fertilization rate (%) was calculated as stated by Adebayo (2006) as;

$$\frac{\text{Number of fertilized eggs}}{\text{Number of eggs counted}} \times \frac{100}{1}$$

Hatching rate

Hatching rate (%) was estimated at 24 hours after fertilization as;

$$\frac{\text{Number of hatched eggs}}{\text{Number of eggs fertilized}} \times \frac{100}{1}$$

Survival rate

Survival rate of hatchlings was determined 5 days after hatching as described by Adebayo and Popoola (2008).

$$\text{Survival rate (\%)} = \frac{\text{number of larvae alive}}{\text{Number of hatchlings}} \times \frac{100}{1}$$

Data analysis

All the data collected were analysed using analysis of variance for completely randomized design and significant means separated using least significant difference (lsd) at 0.05 probability level as described by Obi (2002).

RESULTS

The results of the analysis of variance as presented in Table 1 showed that there were significant differences ($p<0.05$) in fertilization rate, hatching rate and survival rate amongst the treatments. The highest fertilization rate (80%) was obtained in eggs fertilized with milt solution comprising 1.6 ml NS + 2 ml M while the lowest rate (46.33%) was obtained in 0 ml NS + 2ml M (control). The trend of the fertilization rate was 1.6 ml NS + 2 ml M > 1.2 ml NS + 2 ml M > 0.8 ml NS + 2 ml M > 0.4ml NS + 2 ml M > 2 ml NS + 2 ml M > 0 ml NS + 2 ml M (control).

Consequently, hatching rate increased with increased volume of normal saline; from 75.18% obtained in 0.4 ml NS + 2 ml M to 85.33% obtained in 1.2ml NS + 2 ml M, then decreased with further increase in volume normal saline; from 73.42% obtained in 1.6 ml NS + 2 ml M to 51.45% obtained in 2ml NS + 2 ml M and the lowest value of 38% was obtained in 0 ml NS + 2 ml M (control). The trend of the performance of hatching as influenced by normal saline inclusion was 1.2 ml NS + 2 ml M > 0.8 ml NS + 2 ml M > 0.4ml NS + 2 ml M > 1.6 ml NS + 2 ml M > 2 ml NS + 2 ml M > 0 ml NS + 2 ml M (control). All the normal saline inclusion treatments performed significantly ($p<0.05$) better than the non saline inclusion (control). The hatching rate obtained in 0.4ml NS + 2 ml M (75.18%) was statistically at par with 73.42% obtained in 1.6 ml NS + 2 ml M.

Similarly, the result showed that optimum survival rate (90%) was obtained in1.2 ml NS + 2 ml M. This differed significantly ($p<0.05$) with other treatments but was significantly at par with 89.06% obtained in 1.6 ml NS + 2 ml M. The minimal survival rate (49.4%) was obtained in 2ml NS + 2 ml M. The survival rate obtained in 0.8ml NS + 2 ml M (85.07%) was statistically at par with 84.08% obtained in 0.4ml NS + 2 ml M. All the normal saline treatments performed significantly ($p<0.05$) better than non saline treatment (control)

TABLE 1: Effect of different levels of normal saline inclusion on Fertilization, Hatching and Survival rate of *Clarias galiepinus*

Milt solutions	Fertilization rate (%)	Hatching rate (%)	Survival rate (%)
0.4ml NS + 2ml M	67.00^{d}	75.18^{c}	84.08^{b}
0.8ml NS + 2ml M	72.33^{c}	82.33^{b}	85.07^{b}
1.2ml NS + 2ml M	75.00^{b}	85.33^{a}	90.8^{a}
1.6ml NS + 2ml M	80.00^{a}	73.42^{c}	89.06^{a}
2ml NS + 2ml M	54.00^{e}	51.45^{d}	67.77^{c}
0 ml NS + 2ml M	46.33^{f}	38.00^{e}	49.4^{d}
$LSD_{0.05}$	*2.011*	*2.956*	*3.278*
S.E	*1.130*	*1.662*	*1.842*
CV (%)	*1.7*	*2.5*	*2.4*

NS = Normal Saline; M = Milt; LSD = Least significant difference; S. E. = Standard error; CV = Coefficient of variation. Mean value with the same letter are not significantly different (P>0.05).

DISCUSSION

Normal saline inclusion in milt in induced breeding of fish has been a practice adopted by farmers to improve fertilization, hatching and survival of African catfish (*Clarias gariepinus*). However, Okunsebor *et al*. (2014) reported that high concentration of saline has a lethal effect on the cell which could affect fertilization, hatchability and survival rate *Clarias gariepinus*. The ideal saline concentration for better result remains a problem for farmers. The result of this study showed that there were significant differences ($p<0.05$) in fertilization rate, hatching rate and survival rate as influenced by milt dilution though different levels of normal saline inclusion. All the normal saline treatments recorded above 50% in all the parameters evaluated and performed significantly better than the non saline inclusion treatment (control) which recorded below 50% in all the parameters showing that although fertilization could happen in milt without normal saline inclusion, improved fertilization was obtained when milt was diluted through normal saline inclusion. The result was in agreement with the findings of Okunsebor *et al* (2014) and Orji *et al.* (1997).

The percentage fertilization as influenced by the treatments increased with increase in volume of normal saline and decreased after a certain volume of normal saline as shown in Table 1. The decreased fertilization rate at 2ml of normal saline could be an indication of an exceeded tolerable range. Non normal saline inclusion (only milt) probably provided little volume when compared to the quantity of eggs available for fertilization and as a result the spermatozoa probably did not distribute widely over the surface area of the eggs to ensure high rate of egg fertilization resulting in many unfertilized eggs. At moderate normal saline inclusion, more fertilization happened probably because of tolerable salinity and wider spermatozoa distribution. At high normal saline inclusion, the milt viability probably was affected by high saline concentration. Orji *et al.* (1997) and Okunsebor *et al.* (2014) reported that too little or too much saline concentration has a lethal effect on the cells.

Consequently, the hatching rate increased as the volume of normal saline increased; from 75.18% in 0.4ml of normal saline to 85.33% in 1.2ml of normal saline then decreased with further increase in the volume of normal saline; from 73.42% in 1.6ml of normal saline to 51.45% in 2ml of normal saline and the lowest value (38%) was obtained in 0 ml of normal saline (only milt). All the normal saline treatments performed significantly ($p<0.05$) better than the non normal saline (0 ml NS + 2ml M) treatment. Hatching was an induction of fertilization under normal environmental factors like pH, temperature, water hardness and dissolved oxygen. The optimum survival rate (90%) obtained in 1.2ml NS + 2ml M was possibly due to

high hatching rate that minimized pollution in the incubation pond. The minimal survival rate (49.4%) obtained in 0ml NS + 2 ml M (non normal saline inclusion) was probably due to high pollution in the incubation pond as result of deposition of high quantity of unfertilized eggs and unhatched eggs. Orji *et al*. (1997) in a study on *Heterobranchus bidorsalis* reported that it was possible that lower salinity might have given a better result. They opiend that the lower salinity tolerance might be based on the fact that *Heterobranchus bidorsalis* was a freshwater fish and higher salinity would probably result in the shrinkage of the cells and result to death. *Clarias gariepinus* just like *Heterobranchus bidorsalis* is a freshwater fish and could be affected by high salinity. This could explain why low survival percentage was obtain in 2ml of normal saline inclusion compared to other lower saline concentration treatments. Nwokoye *et al.* (2007) in a study on Clariid catfish cited by Okunsebor *et al* (2014) reported that there was the possibility of carry over effects from fertilization to hatchability, and survival of Clariid catfish fry. Okunsebor *et al* (2014) suggested that when sperm cells of fish are not protected by the fish skin, an isotonic medium must be provided to extend the cell's life span. 1.2 ml NS + 2 ml M appeared to be the most favourable isotonic solution for induced breeding of *Clarias gariepinus.* This possibly explained why above average fertilization and best hatchability and survival rate was obtained in 1.2 ml NS + 2 ml M compared to other treatments.

CONCLUSION & RECOMMENDATION

Induced breeding of African catfish (*Clarias gariepinus*) ensures availability of fish seeds for commercial fish farming. One of the problems associated with fertilization, hatching and survival of fish seeds in induced breeding was resolved in this study by means of milt dilution through normal saline inclusion. The study revealed that although fertilization, hatching and survival were achieved when only milt was used to fertilize *Clarias gariepinus* eggs, improved results were obtained when milt was diluted through normal saline inclusion. The best result was obtained when 2 millilitres of milt diluted with 1.2 millilitres of normal saline was used to fertilize 20 grams of egg. 2 millilitres of milt diluted with 1.2 millilitres of normal saline for every 20 grams of egg is therefore recommended for improved induced breeding of *Clarias gariepinus* under normal environmental factors.

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