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The Effect of Cold Storage of *Clarias gariepinus* Sperm on Hatchability and Survival

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ABSTRACT

This study examined the effect of cold storage of *Clarias gariepinus* sperm on hatchability and survival. Twelve (12) broodstocks were used for this experiment, testes were collected from the male and stored at $4^{o}C$ for the treatment of 12hrs, 36hrs and 48hrs respectively, while fresh sperms were also obtained for the controls. The stored testes were activated to room temperature with stored clean water before being used for fertilization all the fertilized eggs from the four trials were observed to hatch as well as the control. Percentage hatchability was observed to be very low for all the treatment. The percentage hatchability was (10% (12hrs), 20% (36hrs) and 10% (48hrs) respectively. Hatched eggs were under observation for seven days to estimate percentage survival, which was observed to be normal for all treatment, but having a significant differences ($P<0.05$), with the control having $5168 + 1014^{b}$, and the treatment $71.7 + 2.9^{a}$, $104 + 3.5^{a}$ and $110.3 + 20.5^{a}$ respectively. The treatment as compared with their control was observed to be relatively low in the percentage hatchability of eggs which may be attributed to sperm motility, milt quality and temperature of the activating medium. Therefore, because of this result it may be concluded that cold storage of sperm of *Clarias gariepinus* is possible and successfully but in very low capacity and except other methods of cold storage which utilizes extenders such as liquid nitrogen is adopted, it may therefore be recommended based on the poor hatchability observe in this work, that sperm storage may not be economical for commercial purpose except for experimental programs such as genetic studies, thus fresh sperm for fertilization should be considered the best, and encourage.

Keywords: *Clarias gariepinus*, Sperm, Cold Storage and Fertilization.

INTRODUCTION

Tobor (1985) stated that protein of animal origin is in short supply in Nigeria as increase in livestock population is being limited by several causes including virus diseases, scarcity, drought and high cost of feeds and low genetic potential of

indigenous livestock breeds. This situation has given rise to considerable increase in the demand for fish to supplement animal protein. Also, FAO (1996) revealed that West Africa countries of which Nigeria is one, obtain at least 50 percent of their animal protein needs from fish and fish products. According to Aromolaran and Akintunde (1998), the residents of Warri, in Nigeria preferred fish and actually consumed it more frequently than any other animal protein source. The average household in the area consumed fish at least once a day for 16days in one month. The number of species in aquaculture is growing and several important species rely on the collection of broodstock or seed from natural population.

With these present facts, it is very important that the aquaculture sector should be able to meet the challenges posed on the demand for fish to supplement animal protein. One way of achieving this, is by improving genetically on our broodstock (i.e. sperm management). The collection of fish sperm for storage (sperm management) is a good technique that may perhaps also improve the aquaculture sector. Spermatozoa motility (time of their motion after activation) is very important for successful fertilization of the eggs (Jezierska and Witeska, 1999). Thus together with morphologic characteristic, is considered an indicator of milt quality. Sperm motility and their ability to fertilize eggs are highest just after stripping (Jezierska and Witeska, 1999). In case of artificial spawning, storage of milt is sometimes necessary.

Reduction of spermatozoa quality (their ability to fertilized eggs) with time is well known, but there are little detailed data on motility time reduction. [Goodall *et al* (1989); Gluchoska and Jezieka (1994), and Jezierka *et al* (1995)] showed the effect of storage on motility time, while Ravinder *et al* (1997) on the percentage of spermatozoa motile after activation. According to Sarnowski *et al* (1997), however storage of sperm for 5hours in the refrigerator of about O^0C did not adversely affect fertilization rate. Possibility of milt storage at about O^0C was proved by [Babiak and Glogowski (1996); Hulat and Rothbard (1979); and Malczewski (1988)]. The authors observed that sperm stored at that temperature was able to fertilize eggs. However, storage affects sperm motility time. It seems that temperature of activating medium may affect activity of spermatozoa. Goodall *et al* (1989), and Babiak and Glogowski (1996) showed that at lower temperature time of motility is longer. Techniques of sperm management have been established in some freshwater fish species such as cyprinids (Billard, Cosson, Crim & Suquet, 1995) or Siluroids (Legendre, Linhart & Billard, 1996) and in Salmonids (Scott & Baynes 1980, Billard, 1992). Among

these techniques, sperm storage and cryopreservation are of special interest. At O^0C conditions, spermatozoa can be stored for a few hours up to several days, depending on the species while cryopreserved gametes can be theoretically stored between 200 and 32,000 years without deleterious effect (Ashwood-Smith, 1980). The use of cryopreserved spermatozoa can be delayed from the data of ova processing. The aim of this present study was to evaluate the effect of the hours of cold storage of *Clarias gariepinus* sperm on hatchability and survival.

MATERIALS AND METHODS

Collection of Broodstock

This experiment was carried out in Bernandez Fish Farm Hatchery in Oluwo, Abeokuta. For this study, a total of 12 broodstocks; four males and eight females of African Catfish *Clarias gariepinus* were collected for both control and treatment.

Experimental Unit

In this study, a mini hatchery was constructed using a flow through system. PVC pipes of 2" and ¾" were connected to supply adequate water to the hatchery from an overhead tank of 4.000litres. 12 plastic bowls of inner diameter 44cm and depth of 16cm was provided and fixed in the hatchery. Plastic mosquito nets were used as kakabans to provide attachment for the eggs after they have been fertilized by both the control and treatment sperm. Also, stones were provided and properly washed and sterilized with chlorine and were used as anchor for the kakabans to remain immersed in the plastic bowls of water.

Lastly, tarpaulins were obtained and used to cover exposed areas of the hatchery in order to control fluctuation temperatures.

Collection of Testes/Sperm

Four males of the broodstock were sacrificed and testes/sperm were obtained. Traces of blood and other tissues attached to the testes were wiped off using tissue paper. The testes were kept inside separate nylon bags which were tagged 12hrs. 24hrs. 36hrs and 48hrs respectively as the treatments and were placed inside a plastic container which was stored in the refrigerator at 4^0C until when used.

Collection of Testes/Sperm

Each female was injected with appropriate dosage of ovaprim hormone according to their body weight by injecting intramuscularly through the dorsal muscle. The females were injected at intervals of 12hours to be ready for stripping 12hours later.

Experimental Design

Four trials were conducted in the experiment with each trial having a control and treatment of three replicates each. In trial one, the first female was injected and was ready for stripping 12hours later, this was made possible by increasing the temperature of the room where the female was kept in a holding tank, knowing the latency time is affected by temperature. The eggs were stripped and the weight taken. The weighed eggs were then divided into two portions, one portion to be used for control and the other for the treatment i.e. a male broodstock was decapitated immediately after stripping and the sperm removed and used to fertilized along with the sperm stored in the refrigerator for 12hours. The same procedure was repeated for the remaining three trials of 24hrs, 36hrs and 48hrs each with a control for comparing. Experimental procedure is shown in Table 1.

Table 1: Experimental pattern

Trial	Day/Time	Activities
1 (12 hours)	Wednesday – 7:30pm	Four males were sacrifices and their sperm obtained and store in refrigerator in separate containers tagged 12hrs, 24hrs, 36hrs and 48hrs.
		The first female was injected and kept in a holding tank.
	Thursday – 7:30am	Female was stripped and the eggs divided into two portions and were fertilized with the control and 12hrs stored sperm.
2 (24 hours)	Thursday 7:30am	Second female was injected and kept in isolation.
	Thursday – 7:30pm	Eggs were stripped and also divided into two portions, one portion to be fertilized with the control sperm and the other portion with the sperm store for 24hrs.
3 (36 hours)	Thursday – 7:30pm	Third female injected
	Friday – 7:30am	Eggs were stripped and divided into two portions and were fertilized with control and sperm of 36hrs.
4 (48 hours)	Friday – 7:30am	The fourth and last female was injected.
	Friday – 7;30pm	Eggs were stripped and fertilized with both the control and stored sperm of 48hrs.

Incubation of Fertilized Eggs

Fertilized eggs as described in table 1 above were spread over the surface of kakabans (mosquito nets), in the three separate bowls, for the control and for the treatments giving a total of 6 bowls used as replicates

Observation and Data Collection

The eggs were under observation through incubation and hatching period. The number of eggs that hatched and survived in each trial were estimated and recorded. Data collected were used to calculate the percentage hatchability and survival.

Method of Estimating Percentage Hatchability and Survival

Hatchability was estimated by the percentage of normally hatched fry for all fertilized embryos i.e. they were compared with that of the control. For survival, a method was improvised by using plastic bowl to estimate the percentage survival after one week. The weight of the bowl was taken and recorded, then the weight of bowl plus water, and lastly the weight of bowl plus water and fry. The total number of fry in the bowl containing water was counted; and then number of bowls scooped from replicates of both the control and treatment was counted too in order to determine the overall total of fry

for each replicates. The weight of the fry and total numbers of fry were estimated as expressed below:

Wt. of fry = (wt. of bowl + H_20 + fry) – (wt. of bowl +H_20)
Total no. of fry = (no. of fry in bowl of water) x (the no. of scooped bowls)

Data Analysis

For comparison of treatments and control, statistical analysis was carried out using one way ANOVA.

RESULTS

Water Parameters

The results are presented in relation to the stored testes (treatment) and fresh testes (control). The mean temperature, dissolved oxygen and pH of the water in the plastic bowls were measured and recorded as 28.5^{0}C, 4.27 mg/l and 7.39 mg/l respectively. From this experiment, it was observed in each of the trials (12, 36 and 48 hours), that between 22 – 26hrs later after fertilization, most of the eggs hatched normally as that of the control, turning from dark green to brownish green colour before hatching and It was also observed that for the treatment, there was problem with the control and was therefore discarded. Table 2, shows the percentage hatchability and the approximate number of fry that survived seven days after hatching for both the treatment and control.

Table 2: Effect of Cold Storage (4^{0}C) of *Clarias gariepinus* Sperm on Mean Percentage Hatchability and Survival After 7 Days of Hatching

Trial	Hatchability	Weight of scooping bowl (g)	Weight of scooping bowl & water (g)	No of scooped bowls	Total no of fry in hatching bowl of water	Total no of fry that survived	Survivorship
12hrs	10	1.7	360	-	-	215*	3.3
Control	90	"	"	12	525	6.300	96.7
Total						6.515	100.0
36hrs	20	"	"	-	-	312*	9.0
Control	80	"	"	8	393	3,144	91.0
Total						3.144	100.0
48 hrs	10	"	"	-	-	331	5.2
Control	90	"	"	12	505	6,060	95.0
Total						6.391	100.0

*** Indicate that the fry were counted directly because of the relative small number for the treatment.**

Table 3: Statistical Analysis of the Number of Fry that Survived after the Treatment (ANOVA)

Control	12 hours	36 hours	48 hours
5168 ± 1014.4^{b}	71.7 ± 2.9^{a}	104 ± 3.5^{a}	110.3 ± 20.5^{a}

The Table 3 shows that there was a significant difference ($P < 0.05$) in the survival of fry between the controls and treatments. But within the treatments there is no significant difference. This shows that the duration of storage does not have any effect on the sperm but the difference is as a result storage of the sperm.

Table 4: Mean Percentage Hatchability for both Treatment and Controls

Parameter	Control	Treatment
Average mean %	86.67	13.33

Table 5: Mean Values of Survival of Hatchlings for both Treatment and Control

Parameter	12 Hours	36 hours	48 hours
Control	2100	1048	2020
Treatment	71.67	104.00	110.33

DISCUSSION AND CONCLUSION

From table 3, differences in survival of fry for the three treatment compared to that of the control was significant ($P < 0.05$), with control having 5168 ± 1014.4^{b}, and the treatments 71.7 ± 2.9^{a}, 104 ± 3.5^{a}, 110.3 ± 20.5^{a} respectively. This means that the duration of the storage does not have any effect on the sperm but the difference is as a result of storage of the sperm.

It was observed that for all the treatment, the eggs were able to hatch normally as the control, which was similar to what have been reported by various authors: Babiak and Glojowski (1996), Blaxter (1995), Hulata and Rothbard (1999), and Makzewski (1988) who proved the possibility of milt storage at 0^{0}C and also reported that sperm stored at that temperature was able to fertilized eggs, and that at lower temperature, time of sperm motility is longer. This may have contributed to the poor performance of the four treatments stored at a higher temperature (4^{0}C.). It was also observed that for all treatments, hatchability was very low. The percentage hatchability was (10% (12hrs), 20%

(36hrs) and 10% (48%) respectively. This low hatchability is not surprising because Jezierska and Witeska (1999) have reported that spermatozoa motility (time of motion after activation) is very important for successful fertilization of eggs. In their report, they said that sperm motility, and their ability to fertilized eggs is highest just after stripping. Therefore, it may be concluded that poor hatchability of the eggs is a result of spermatozoa motility and milt quality.

Jezierska and Witeska (1999) also observed that the spermatozoa of common carp were active for the longest time at 20^0C and Goodall *et al* (1989) in their report stated that temperature of activating medium may seem to affect activity of spermatozoa. It may therefore be concluded that the temperature of storage and the temperature of the activating medium could be responsible for the poor hatchability of the eggs. Jezierska and Witeska (1999), reported in their study of the effect of time and temperature of motility of spermatozoa of common carp and grass carps, that time of motility was considerably reduced in 24hrs post stripping did not exceed 10secs in series 1 while in series 2, time of sperm motility was reduced already in 8hrs and 24hrs, it almost ceased. If that be the case in common carp, then in catfish, *Clarias gariepinus* fertilization was successful even up to 48hrs. This may suggest that spermatozoa of *Clarias gariepinus* may be motile for up to or more than 48hrs, if the treatment may had proceeded further. Therefore, it may be concluded that cold storage of sperm of *Clarias gariepinus* is possible and successful but in very low capacity and except that other methods of cold storage which utilizes extenders such as liquid nitrogen is adopted. Based on the poor hatchability observed in this work, sperm storage may not be economical for commercial purposes except for experimental programs such as genetic studies. Finally, freshly stripped sperm for fertilization should be considered the best and be encouraged.

REFERENCES

Aromolaran A.B. and O.A. Akintunde (1988). "Socio Economic Analysis of Consumption Pattern and Consumer Preferences for Selected Animal Products in Delta State, Nigeria. In the Nigeria Livestock Industry in the 21st Century. Eds Ologhobo A.D. and Iyayi, E.A. pp. 272 – 275.

Ashwood-Smith M.J. (1980). Low Temperature Preservation of Cells, Tissues and Organs. In: Low Temperature Preservation in Medicine and Biology (ed. By M.J. Ashwood-Smith), pp. 19-44, *Pitman Medical Ltd., Turnbidge Wells*.

Babiak I., Glogowski J. (1996). Physiology of Fish Gametes, and the Factors Affecting Fertilization and Natural, and Controlled Conditions. Kom. Ryb. 3:14-17. (In Polish).

Billard R. (1992). Reproduction in Rainbow Trout: Sex Differentiation, Dynamics of Gametogenesis, Biology and Preservation of Gametes. Aquaculture 100, 263-298.

Billard R., Cosson J., Crim L.W. & Suquet M. (1995). Sperm Physiology and Quality. In: Broodstock Management and Egg and Larval Quality (ed. by N.R. Bromage & R.J. Roberts), pp. 53-76, *Cambridge University Press, Cambridge.*

Food and Agricultural Organisation (1996). "Agricultural Development in Nigeria" (1965 – 1980), FAO, Rome, Italy. Pp. 112.

Gluchowska E. Jezierska B. (1994). The Effect of Water pH on Common Carp Spermatozoa Kom. Ryb. 6, 21-22. (In Polish)

Goodall J.A., Blackshaw A.W., Capra M.F. (1989). Factors Affecting the Activation and Duration of Motility of the Spermatozoa of the Summer Whiting (Sillago ciliata). Aquaculture. 77; 243– 250.

Hulata, G. and Rothbard, S. (1979). Cold Storage of Carp Semen for Short Periods. Aquaculture 16:267-269.

Jezierska, B., Slominska, I. and Gluchowska, E. (1995). The Effect of Heavy Metal Pb, Cd and Cu on Activity of Common Carp Spermatozoa. Kom Ryb., 3:12-13. (In Polish).

Jezierska B. and Witeska M. (1999). The Effect of Time and Temperature on Motility of Spermatozoa of Common and Grass Carp. *In: Electronic Journal of Polish Agricultural Universities. Vol. 2. Issue 2*. P. 2-7.

Legendre M., Linhart O. and Billard R. (1996). Spawning and Management of Gametes. Fertilized Eggs and Embryos in Siluroidae. Aquatic Living Resources 9, 59-80.

Malczewski B. (1988). Modifications of Fish Breeding Technology Under Controlled Conditions. Gosp. Ryb. 3: 5-6. (In Polish).

Ravinder K., Nasaruddin K., Majumdar K.C and Shivaji S. (1997). Computerized Analysis of Motility, Motility Pattern and Motility Parameters of

Spermatozoa of Carp Following Short Term Storage of Semen. *J. Fish Biol.* 50: 1309 – 1328.

Sarnowski P., Sarnowska K. and Slominska I. (1997). The Effect of Lead and Copper on Embryonic Development of Grass Carp. 17 Zjazd Hydrob., Poznan, Sep. 8-11, 1997 (In Polish).

Scott A.P. and Baynes S.M. (1980). Relationship between Sperm ATP Content and Motility of Carp. Biochemical Cell Biology. 65, 474 -485.

Tobor, J.G. (1985). Fish Production and Processing in Nigeria. Nigerian Institute of Oceanography and Marine Research Technical Paper No. 22, pp 1-8.

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