The Zoologist Vol. 6: 39-44 (2008)

EFFECTS OF SHORT TERM STORAGE ON THE VIABILITY OF MUDFISH *CLARIAS GARIEPINUS* (BURCHELL 1844) TESTICULAR HOMOGENATE

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Abstract
Freshly prepared testicular homogenate of sexually mature mudfish *Clarias gariepinus* stored undiluted, diluted with distilled water or with acetone (1: 1 by volume) at -0.5 to – 2^0C was tested for its viability in fertilizing the eggs of the female brood fish of the same species. Results show that the homogenate retained its initial fertility up to 24 hours of storage in all media used. After 24 hours of storage at – 0.5 t0 – 2^0C, its fertility decreased significantly (P> 0.5) such that at 30 hours it was no longer fertile. The ability of the testicular homogenate to retain its initial fertility for 24 hours is of immense benefit to artificial fertilization in the culture of this species.

Key words: Testicular homogenate, *Clarias gariepinus,* viability, short-term storage.

Introduction
In the artificial fertilization of *Clarias gariepinus* eggs, it is necessary to sacrifice the males to obtain the required milt since milt could not be procured by manual stripping. Since the male pituitary hormone could be used to induce spawning in *C. gariepinus* in 13-16 hours (Ufodike and Madu 1998), it was reasoned that if the milt from the ripe testes could be preserved in a viable condition on a short term basis, then the milt of the same fish sacrificed for their pituitaries could also be used to artificially fertilize the ovulated and stripped eggs, thus minimizing the number of male fish that would have to be killed. The carp pituitary extract to date appears to be the most appealing, popular and effective inducing agent in a majority of fish species (Woynarovich and Hovath (1980) and this has led to the carp being referred to as a "universal donor". However, it becomes economical in the present situation to practice homoplastic hypophysation. In other to make use of the milt, several methods have been employed to improve the short- term storage of fish sperm. Withler and Humphreys (1967) Withler and Morley (1968) and Mollah (1985) have reported that chilling appears to be the most common method employed. In their study on Sockeye Salmon (*Oncorhynchus nerka)* Withler and Morley (1968) showed that cooler temperatures prolonged the period over which stored sperm would remain viable. *Sockeye salmon* milt stored at 2.9^0C fertilized over 90% of the fresh ova up to 94 hours where as the milt stored at 9.9^0C showed similar percentage fertility up to 46 hours. Mollah (1985) reported that the addition of a fluid to stored milt may increase its fertilization rate. Hogendoorn and Vismans (1980) reported an average increase of 9% hatchability of *C. lazera* eggs fertilized with stored milt or fresh milt diluted with 0.9% Nacl solution in a proportion of 10^{-1} 10^{-2} or 10^{-3} . Plosila *et al* (1972) noted that the addition of an equal volume of "dilution water" to brook trout. *(Salvelinus fontinalis)* milt in the fresh condition or after 24 hours storage at – 2^0C also increased the fertilization rate. These fertilization enhancers are advantages for those species which produce limited quantity of milt and where sacrificing the males are necessary for milt collection.

This experiment was designed to determine if milt or testicular extract of *C. gariepinus* could be preserved on a short term basis and for what duration it can be stored without significant loss in its fertility. Dilution of testicular homogenate with equal volume of distilled water or acetone was tested to determine if the dilution decreased the fertility rate significantly in comparison to the undiluted.

Materials and Methods

A total of 100 male *C. gariepinus* broodstock weighing 1000g +1.5 were procured from the Magnifical fish farm Nunya, Abia State. The fish were transported to the Fisheries Laboratory of the Abia State University Uturu. Testes of 50 sexually mature males were homogenized in a 10ml glass homogenizer. The homogenate was divided into 3 equal portions and kept in glass vials. One portion was undiluted with an equal volume of distilled water or acetone (1:1). The testicular homogenates were placed on ice in a polystyrene box in a fridge which maintained an air temperature ranging from -0. 5 to - 2^0C. Each of the vial was stoppered with a cotton plug to permit exchange of gases.

Six batches of ovulating fish, each comprising of 12 fish in three replicates (216 female fish in all) were used over the 48 hours experimental period. The fish ovulated 12-13 hours after receiving intra muscular injection of 5mg dry Carp Pituitary Extract (CPE) per kg body weight of the female as a “knock out” dose. Fish in batch II were similarly injected 8 hours after those in bath 1 so that they started ovulating at 8 hours post ovulation period of the Batch 1 fish. Batch III fish were treated 8 hours after those of batch 1 for the same reason. The same schedule of CPE administration was maintained for all the six batches of fish. Ovulation was assessed according to the methods described by Hogendoorn and Vismans (1980). In each batch, egg samples from the individual fish were used for fertilization purposes up to 8 to 9 hours post ovulation because the ovulated eggs in *Clarias species* declines in viability 10 hours post ovulation (Mollah and Tan, 1983, Ufodike and Madu, 1998).

Two samples each of about 200-300 eggs, were stripped from each of the 12 ovulated fish at 5 min, 20 min, 2 hrs, 4 hrs and 8 hrs post ovulation. One egg sample from each fish was fertilized using 15µl of normal testicular homogenate while the other using 30µl of diluted testicular homogenate (in distilled H_20 or acetone). The vials containing the testicular homogenate was well mixed using an electric mixer prior to use. Stripping and fertilization of the egg for the 8 hour sample of each batch except batch IV overlapped with those of 0 hours of the neat batch. This was done to minimize double variations in the quality of the eggs used for this experiment.

The three types of testicular homogenate were assessed for their fertility by the hatching rate of the eggs spread in a mono layer in a water flow- through incubator maintained at a temperature of 28^0C$\pm$ 0.5C. Each of the hourly run was triplicated. After fertilization, a random sample of 300 eggs were siphoned out at various portions of each incubation tray for estimation of fertility and hatching. Fertilized eggs which were light brown coloured and transclucent were readily distinguished from those unfertilized which were white and opaque.

Statistical Analysis

The results presented in percentages were transformed to Arcsine (Zar 1974) before a one way analysis of variance was performed. The reason for this was because percentages or proportion form a binomial, rather than a normal distribution. If the square of percentage or population is transformed to it arcsine, the angle whose sine is *x*, then the resulted data will have an underlying distribution that is nearly normal

Duncan's Multiple Range test at P= 0.05 was then employed for further analysis of the result (Vann 1972).

Results

The results of the hatching rate of eggs fertilized with testicular homogenate following storage from 5 minutes up to 28 hours at -0.5 to – 2.0^{0}C is presented in Table 1. There was no hatching in the eggs treated with the testicular homogenate stored after 32 hours or more. Hence, data presented in Table 1 are only for testicular homogenate following storage for up to 28 hours. Generally, the result showed that with each series there was no significant difference (P 0 > 0.05) between the fertility of both normal and diluted testicular homogenate (distilled water or acetone) to up to 20 hours of storage. However within both series, the fertility of both diluted and undiluted testicular homogenate decreased significant (P< 0.05) at 24 hours although the percentages at that time were not significantly (P>0.05) less than for batch 1 fish at 8 hours.

The present result did not show any apparent variation in fertility of testicular homogenate stored without the addition of water or upon 1:1 dilution with distilled H_20 or acetone(-0.5 to -2^{0}C)

Discussion

In this study *Clarias gariepinus* testicular homogenate appeared capable of retaining initial fertility up to 20 hours when stored at a temperature of – 05 to -2^{0}C. This duration is comparable to those obtained for *Clarias macrocephalus* (Mollah 1985) Carp *Cyprinus carpio* (Keke 1992) Labeo rohits *Clenopharyngodon idella* and catfish *Pangassuis sutchi* milt which remained potent for up to 24 hours when chill stored in refrigerator or on ice after extrusion (Withler, 1980). The work of Barrett (1951) indicated that sperm from salmon *Oncorhynchus keta* could be held at 2.5 – 5^{0}C for periods of as much as 8 days, though the fertilization rate was highly variable and dropped below 50% after 4 days. Withler and Humphreys (1967) noted that pink Salmon (*Oncorhynchus gorbuscha)* milt retained initial fertility up to 33 hours when stored undiluted at a temperature of 8-9^{0}C.

Results from this study indicate no apparent variations in fertility of testicular homogenate stored undiluted or at a 1:1 dilution with acetone or distilled water at -0.5 to – 2^{0}C. This finding is inconsistent with the earlier works of other workers such as Gray (1919) who reported that Chinook salmon spermatozoa lost motility and became inactive within 2 minutes of introduction into an aqueous solution. The reasons for the inconsistency may not be far fetched. Apart from possible species difference, it is possible that collection and storage techniques may have varied between these studies. Additionally Withler (1980) had cautioned that collected milt should be kept free of contamination from blood, mucus, faeces or moisture. Mollah (1985) has posited that addition of water in the presence of such contaminants could cause furious activity which soon uses up the sperms energy so that they become inactive within less than a minute.

A positive correlation exists between the fertility of the fish spermatozoa and their motility. The fertility of the fish spermatozoa is also dependent on the temperature. Turdakou (1971) reported that in *Leuciscus bergi* as the temperature was raised to 25^{0}C above the normal range of 5-15^{0}C, the fertility rate of the spermatozoa reduced sharply. This could be explained in several ways. Electromicrographic studies of teleosts eggs has according to Amanze and Iyengar (1990) revealed the detailed structure and physiology of the micropyle which is open for only a limited

Table 1 Fertility of *Clarias gariepinus* (Burchell 1844) testicular Homogenate following storage at -0.5 to – 2^0C for various time intervals as assessed by the hatching rate of ovulated mudfish eggs (%). Storage period of testicular homogenate

Normal testicular homogenate						8 hour *			16 hour *		24 hour *			
	Replication	5min	30m	2h	4h	Batch I	Batch II	12 h	Batch II	Batch II	20 h	Batch III	Batch Iv	28h
	1	54	50	56	60	55	56	74	67	75	62	59	58	3
	2	60	69	62	63	60	67	79	72	68	74	36	45	0
	3	71	65	70	72	58	70	68	78	83	75	42	30	10
	Mean ±S.D	61.6+8.2ab	61.3+3.3ab	62.7ab±0.6	65.0ab±2.3	57.6bc±3.2	64.3ab±2.4	73.7ab±1.6	72.3ab±2.2	75.3ab±1.6	70.3ab±1.8	45.6c±10.6	44.3c±8.6	4.3d+3.3
Distilled water diluted testicular homogenate	1	56	54	62	74	54	62	64	73	79	78	60	34	0
	2	62	60	71	62	60	70	78	57	74	69	43	59	10
	3	73	70	65	68	68	68	66	68	70	65	38	36	0
	Mean ±S.D.	63.7ab±3.6	61.3ab±2.6	66.0ab±3.4	68.0ab±7.5	60.7bc±7.0	66.3ab±2.4	69.7ab±6.6	66.0ab±7.2	74.3ab±0.4	70.6ab±7.1	47.0c±2.3	43c±3.6	3.3±3.3d
Acetone diluted testicular homogenate	1	58	55	71	75	52	64	65	74	76	75	58	36	8
	2	60	61	66	61	54	68	76	60	75	74	40	42	0
	3	71	70	61	69	69	72	68	64	70	63	42	50	0
	Mean ± S.D	63.0ab ±0.3	62.0ab ±3.4	66.0ab±2.4	68.3ab ±1.4	58.3bc±3.3	68ab±1.8	69.7ab±7.0	66.0ab±1.4	73.7ab±2.3	70.7ab±7.0	46.6c±3.3	42.7c±0.4	2.7d ±7.0

* Times where sampling and fertilization of eggs from the preceding batch and the succeeding batch overlapped. Means not followed by the same letter differ significantly when the comparison was made within normal or diluted a testicular homogenate using Duncan's Multiple range lest (P=0.05). 100-200 ovulated eggs were mixed with 15µl normal testicular homogenate and 30µl of diluted testicular homogenate and incubated at 28^0C for 35 hours

time for the passage of the sperm during fertilization. The sperms accordingly become infertile once they cannot penetrate the egg micropyle. The loss of activity may either be due to loss of motility and therefore unable to find or penetrate the micropyle or due to a general deterioration in which the sperm cell capacity to fertilize is lost (Truscott *et al* 1968; Ufodike and Madu 1998).

From this work, testicular homogenate retention of initial fertility under conditions described in this work is very important for achieving success in controlled breeding of this species. It is documented (Vivien *et al* 1986; Ufodike and Madu 1998) that *C. gariepinus* responds by ovulation within 13-16 hours of pituitary injection which is presumably within the 20 hour range of stored testicular homogenate viability. It follows that both the testicular homogenate and the pituitary of a male can be used to induce the female to ovulate and to fertilize the pituitary induced ovulated eggs.

By so doing, the cost of fry production can be reduced. It is noted that the quantity of milt produced by *C. gariepinus* was not affected by dilution with equal volume of distilled water or acetone and did not affect the fertility rate.

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