SUCCESSFUL HANDSTRIPPING OF HATCHERY BRED AND REARED MALE AFRICAN CATFISH, *C. gariepinus* (*BURCHELL*, 1822)

S.L. LAMAI.

Department of Fisheries,
Federal University of Technology, Minna, Nigeria.

ABSTRACT

A total of 45 Male and 5 female *Clarias gariepinus* bred and reared in the hatchery in sonning for one year were obtained for this experiment. The fish were then housed separately according to their sexes and maintained on trout diet at 10% body weight for two days before they were subjected to induction.

These were then induced using both human chorionic gonadotropin (hCG - 500iu) and carp pituitary suspension (CPS -3mg kg^{-1} suspended in 0.9% saline) either as priming or resolving doses. The milt produced was used to fertilize eggs tripped from females. The results indicated high milt production,motility and fertility in most males.

INTRODUCTION.

From the experience of breeding and culture techniques, it became clear that one of the major constraints of rapidly building a stock of *C. gariepinus* was the inability to strip the males. Despite its enormous potential as an aquaculture candidate (Huisman and Richter, 1987), the problem of production of this fish has always been the inability to strip the male of milt like most fishes. The traditional method of obtaining milt has been to sacrifice the male, remove its testis and macerate over stripped eggs. A single successful attempt to strip the male was made by van de Waal & Polling (1984) in South Africa. They recruited male spawners from the wild, supposed to have received natural cues (Bruton, 1979) and by a single hypophysis for a period of 24 hours were then handstripped. Several advantages go with this technique including the fact that the spawners were naturally mature with naturally high endocrine levels. These implied that both milt thinning and release could be easily stimulated with favourable results (van de Waal & Polling, 1984).

A technique whereby feral stock has to be recruited can not be recommended for a successful handstripping of hatchery reared stock, since conditions in the hatchery are different from those in nature, especially for a fish which does not reproduce in captivity like *C. gariepinus*.

The absence of natural cues, crowding, artificial rearing methods, etc all contribute to suppress natural spawning in captivity (Bruton, 1979; Huisman and Richter, 1987).

The aim of this work was to develop a technique for successfully handstripping male fish which have been bred and reared wholly in the hatchery.

During an artificial spawning trial, thirteen hatchery reared nine-month old male C. gariepinus were used to attempt handstripping of the males. The results were favourable. The second experiment was to confirm the earlier work and to establish the procedure, hence, the number of fish and size range were increased to 32 one-year-old males weighing 195-671g.

MATERIALS AND METHODS

Induction

During the first attempt, thirteen male and two female spawners were selected while in the second experiment, thirty-two male and two female spawners were selected from a group of *Clarias* spawned and reared in the hatchery. They were separated from the others except for the females that were kept individually in aquaria in the recirculation system. They were fed trout pellets once daily at 5% body weight, but this was increased to 10% one week before the start of induction. They were observed for a period of two weeks to ensure that all fish were both healthy and feeding well.

24 hours before priming the fish were further regrouped into 4 groups of three for the first and four groups of four for the second experiment for treatment with the hormones, and 4 groups of four for the controls (without hormones) in the second experiment only, the females and the thirteenth male in experiment one remaining as they were. All fish were then removed, anaesthetized, weighed and measured for lengths (Table 1a & b), and all fish were returned to their respective aquaria in the recirculation system.

Table 1a. Weights and lengths of 13 male and 2 female *C. gariepinus* used during handstripping of males 1st batch.

Treatment	Group	Wt (g)	L (mm)	Sex
A1	1	470	430	m
	2	203	208	"
	3	191	208	"
A2	1	374	396	m
	2	217	205	"
	3	539	452	"
B1	1	369	400	"
	2	369	380	"
	3	224	318	"
B2	1	440	400	"
	2	337	380	"
	3	179	318	"
	Uninduced fish	320	370	"
	1	340	332	f
	2	260	280	"

Table 1b. Weights and lengths of 32 male and 2 female *C. gariepinus* used during handstripping of males 2nd batch.

Treat.	Group	Wt (g)	Len. (mm)	Sex
A1	1	338	370	male
	2	336	377	"
	3	389	398	"
	4	365	385	
A2	1	534	440	"
	2	441	403	"
	3	395	403	"
	4	339	400	"
Ctrl.1a	1	268	345	m
	2	471	405	"
	3	372	392	"
	4	434	434	"
Ctrl.1b	1	338	400	"
	2	401	409	"
	3	586	440	"
	4	466	406	"
B1	1	643	462	"
	2	501	445	"
	3	443	414	"
	4	671	480	"
B2	1	303	282	"
	2	348	402	"
	3	611	462	"
	4	487	436	"
Ctrl.2a	1	619	455	"
	2	378	394	"
	3	290	374	"
	4	233	380	"
Ctrl. 2b	1	438	420	"
	2	629	472	"
	3	509	443	"
	4	195	320	"
	1	315	352	female
	2	505	423	"

The male fish were primed (see Table 2) using 500iu (i.e 0.5ml) hCG (Sigman Co. Ltd), dissolved in distilled water. This was injected intramuscularly at two different times of the day. The first two groups of fish (treatment A in duplicate) were primed at 0800h, while the second two groups (treatment B in duplicate) were primed at 200h. The controls were all primed with 0.5 ml distilled water (the vehicle in which hCG was dissolved) at their respective times. The next day, at 0800h, the resolving dose of one gland, each an equivalent of 3mg of cPS (Inter-fish, German) suspended in 1.5ml of 0.9% saline, was injected intramuscularly to all fish treated earlier with hCG, irrespective of weight. The controls were all given 1.5ml of 0.9% saline as their own resolving dose. The females were then given their only induction of the same dose of CPS. Table 4.2 gives a summary of the gonadal stimulation procedure.

Table 2. Gonadal stimulation procedure for male handstripping experiments.

Experiment 1	n	T1	delay	T2	delay	T3
Treatment	3+3	0800	24th	0800	8h	1600
A1 +A2	3+3	2000	12th	0800	8h	1600
B1 +B2	2	-	-	0800	8h	1600
Females						
Experiment 2						
Treatment	n	T1	delay	T2	delay	T3
A1 +A2	4+4	0800	24th	0800	8h	1600
Cont.A1+2	4+4	0800	24th	0800	8h	1600
B1 +B2	4+4	2000	12h	0800	8h	1600
Cont.B1+2	4+4	2000	12h	0800	8h	1600
Females	2	-	-	0800	8h	1600

n =number of fish, T1 =time of priming injection of 500iu hCG in 0.5ml water (controls 0.5 ml water only), T2 =time of resolving injection of 3mg cPS in 1.5ml saline (controls 1.5ml saline only), T3 =time of stripping.

Stripping of males and females.

8 hours after the resolving does was administered to all the fish, they were removed from their tanks according to their groups, beginning with Al, and anaesthetized. Males were stripped by the application of strokes between the anal fins and genital papilla using the right thumb (van de Waal & Polling, 1984). When performing the stripping, an assistant held a 20ml analytical grade plastic cup, under the genital papilla to catch the milt that was coming in steady squirts in some cases or flowing gently in a few cases. Strokes were applied and continued until milt no longer flowed from the fish. The milt so collected was then stored in a refrigerator until all the males were stripped. The two females were then stripped of eggs. The stripped fish were then left in freshwater to recover from the effect of the anaesthesia. The thirteenth male in the first experiment was sacrificed and the testis squeezed to measure milt volume. This milt was then kept in the refrigerator at 4°C to assess durational viability.

Determination of milt volume produced.

When all the fish including the females were stripped, the milt from each fish that gave milt was drawn into a plastic syringe and the volume recorded in ml or ml (Table 3a & 3b).The milt was then returned to the fridge until required for the next stage.

Table 3a

Handstripping of male *C. gariepinus* carried out. The results show the volume of milt produced in ml per fish (v), in the two treatment groups A & B (Table 2), and the sperm motility (M), sperm density (D) and hatchability of eggs fertilized with the milt (H).

		V	M	D	H
A1	1	0.05	1	2	30
	2	0.05	1	2	45
	3	-	0	-	-
	Mean	0.05			37.5
A2	1	0.1	1	1	30
	2	0.05	1	2	26.7
	3	0.1	1	3	70.92
	Mean	0.083			42.54
B1	1	-		-	-
	2	-		-	-
	3	0.05	1	1	7.3
B2	1	0.05	1	2	37
	2	-	0	-	-
	3	0.2	1	3	42
	Mean	0.125		-	39.5
Squeezed		1.5ml	1	3	not tested

Table 3b.

The handstripping of male *C. gariepinus* carried out. The results show the quantity of milt produced in ml per fish (V), the two treatments A & B (Table 4.2), and the Sperm motility (M), sperm density (D), and % fertilizability (F) of eggs fertilized with the milt. No. milt was produced by the control fish. * =blood produced.

		V	M	D	F
A1	1	0.13	1	1	23.39
	2	0.00	0	0	-
	3	1.05	1	3	38.65
	4	0.20	1	1	27.55
	Mean	0.345	1	1.666	29.86
A2	1	0.95	1	2	20.80
	2	0.1	1	1	-
	3	0.0	1	1	11.60
	4	*	1	1	-
	Mean	0.35	0.75	1.333	10.8
B1	1	0.28	1	1	30.34
	2	0.05	1	1	25.40
	3	0.03	1	1	-
	4	0.29	1	1	7.94
	Mean	0.16	1	0.75	15.92
B2	1	1.95	1	3	19.78
	2	0.23*	1	1	9.76
	3	0.00	0	0	-
	4	0.02*	1	1	-
	Mean	0.537	0.666	1	9.846

Determination of fertilizability/hatchability

The eggs obtained from the females were weighed out in aliquots of one gram and placed in petri-dishes. Each petri dish then received two drops of milt from a different male, followed by distilled water to both activate the milt and to cover the eggs. The eggs and milt were then swirled gently (Hecht et al., 1982) and left for a few minutes at room temperature for fertilization to occur. In the first experiment, eggs were incubated and after 24 hours were examined and hatchability determined. In the second experiment, after 15h, the eggs were examined under a binocular microscope, fertilized eggs counted to determine percent fertilizability.

Determination of sperm motility and density.

The motility of milt determination was carried out 30-45 minutes after stripping by inspection of a drop of milt taken from each remaining sample from the fridge, under a light microscope. To a drop of the milt on a clean slide was added a drop of distilled water in order to activate it. This was then examined under the light microscope at a magnification of x400. The motile spermatozoa could easily be seen moving sometimes in high density and sometimes scantly. The presence or absence of motility of spermatozoa was expressed as present (1) or absent (0). The density was rated 1,2 & 3 (i.e. low, medium and high).

RESULT AND DISCUSSION

The results of the handstripping exercise are shown in Tables 3a & b. In the first experiment, eight out of 12 gave milt, and in the second experiment, 12 fish out of 16 gave milt. None of the control fish gave milt in response to stripping. The quantity of milt produced by individual fish in experiment 2 was more than that produced by individual fish in the earlier trial (Table 3a & b). The quantity of milt produced by B2 1 (1.95ml) in the second experiment is more than was obtained (1.5ml), when one squeezed testis dissected from one control fish. The results demonstrate the need and effectiveness of the hormones used. However, not all fish responded and milt volumes were very variable. There were no apparent differences between treatments A and B, indicating that the delay between the priming and resolving injection is not critical.

During stripping, it was noted in some fish that the first stroke was sufficient to produce a few drops of milt. However, in a few instances milt was only produced after several strokes. In some cases urine preceded the milt and hence, the first few drops had to be discarded, the drops that followed which were white and more viscous were collected. This observation agrees with that made by van de Waal & polling. (1984). In some cases blood was expelled with the milt.

The effect of weight individually on milt production in this work was not visible as R^2 = 0.355, while in the first trial, the weight mean effect was visible though not strong as the simple regression analysis was R^2 = 0.628. On the whole, more milt was produced in the first experiment than in the second as mean values of 0.054ml and 0.33ml were produced in the first and second experiments respectively.

The mean fertilizability % of the milt in experiment 2 was generally low in all cases, perhaps due to the fact that there was a long delay before the milt was added to the eggs. In contrast, the first trial showed a higher mean % hatchability than the mean % fertilizability in the second experiment. This was attributed to the number of the experimental fish. That is, while the first trial had only 15 fish (13 males and two females), the second had 34 (32 males and two females). This means it took longer to carry out the process of fertilization. Hogendoorn (1979a) reported that unfertilized eggs of *Clarias lazera* develop 'normally' until the 8 or 16 cell stage. This might occur in *C. gariepinus,* which is probably synonymous to . *C. lazera* (Bruton, 1983) and suggests that any delay would adversely affect fertilizability of the eggs. Hecht *et al.* (1982) recommended that *C. gariepinus* eggs should be fertilized as quickly as possible. The sperm are also active or motile for only 30 seconds after activation (van de Waal & Polling, 1984). *C. gariepinus* sperm motility was observed to last for a short time at room temperature by Hecht *et al.*(1982); they also observed a % motility decrease with time of diluted and undiluted sperm. For example, they found that at 45 minutes undiluted sperm motility was 40% while the diluted was 30%. However, I observed that the sperm squeezed from the testis of the control male in the first experiment and stored at 4¯C remained viable after 8 days. Low fertilizability from stripped milt was observed by Polling and his colleagues (1984) among some of their treatments, and they observed an overall 53.1% hatching success. The fertilizability in my second experiment showed highest in the duplicate Al which had a mean value of 29.86% and the least in duplicate B2 (9.84%). On the effects of weight on fertilizability or hatchability, it was observed that there was no relationship as the correlation calculated for the first and second experiments were R2 were 0.062 and 0.183 respectively.

Motility, seemed to be higher where more milt was obtained though this was not statistically significant. The calculated values of R^2 for the first and second experiments were 0.265 and 0.052 respectively. The presence of blood in the milt affected the observation of motility as sperms could not be seen clearly under the microscope during observation, this was true of B2 1.

There was a strong relationship between the motility of milt produced and the % hatching in the first trial (R^2 =0.984), but not in the second experiment (R^2 =0.413). A negative relationship was observed between weight and density. There was a positive relationship between the mean volume of milt produced and % hatching in the first experiment, corresponding to the motility as stated above.

The milt production and hatching success do suggest that the method of handstripping can provide an alternative to the traditional method for maceration of testis. Polling *et al.*(1984) reported the same observation, where they realised a mean hatching success of 53% when males were stripped, and 78.3% when testes were macerated over eggs. They observed that the maximum ranges however, compared well i.e 98% survival rate, indicating that this technique is inherently as good as using macerated testis. The fact that up to 1.95 ml (B2,1) was obtained in one individual compares well with 1.5ml of milt obtained by squeezing in one individual in the control of the first trial. This quantity can fertilized 10,000 eggs or more, indeed, Hecht *et al.* (1982) estimated that semen obtained from a 2kg male (5-6ml) was sufficient to fertilize eggs from 3 females (i.e approx. 200,000 eggs).

Handstripping the male is by far more useful than the maceration method, because it economises on males. It also has the advantage of allowing continuing genetic investigations as the male parent will not be lost through sacrifice.

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