Further Observations on the Induced Spawning of the Sharptooth Catfish, *Clarias gariepinus* (Clariidae: Pisces)

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Abstract

Combinations of human chorionic gonadotrophin (HCG), pituitary gland extracts (PGE) of common carp and catfish, follicle stimulating hormone (FSH), luteolytic hormone and oxytocin were used in induced spawning trials with the catfish, *Clarias gariepinus*. Based on increases in the Body Girth Index (BGI), as an indicator for gonadal hydration, it was concluded that catfish PGE alone is the most suitable hormone for the induced spawning of the catfish. The results, however, also show that ovulation in the catfish can be effected without the use of PGE. It was unsuccessfully attempted to cold store catfish semen prior to stripping. Refined fertilization, incubation and egg washing techniques are described and illustrated.

Introduction

Growing interest has in recent years been shown in culture techniques of numerous clariid species in Africa, India and the Far East (Ramaswami and Sunderaraj, 1957; Tongsanga *et al.*, 1963; Micha, 1972, 1973 & 1975; Van der Waal, 1972 & 1974; Carreon *et al.*, 1973; Carreon *et al.*, 1976; De Kimpe and Micha, 1974; Hogendoorn, 1979; Hogendoorn and Vismans, 1980; Hogendoorn, 1980 and Schoonbee *et al.*, 1980). *Clarias lazera* has since 1970 been used in fish culture in central Africa (Hogendoorn, 1979). The interest shown in *Clarias* species is a result of the growing realization that these fishes may be ideally suited for aquaculture purposes (Van der Waal, 1972). Bruton (1979) also emphasized the necessity for catfish culture.

The propagation of the wels catfish *Silurus glanis* has developed into a successful large scale industry in eastern European countries (Horvath, 1977) and it is felt that the same success can be achieved with the culture of *Clarias gariepinus* in southern Africa.

In the majority of the papers mentioned above it is stressed that the culture techniques, especially those to induce spawning under controlled hatchery conditions and to effect the commercial rearing of the larvae have to be optimized, as the large-scale propagation of catfish in the hatchery is critical to the development of catfish farming as a whole. It has been found that semi-natural or hormone induced reproduction in ponds does not prove to be a reliable method for fingerling production (Van der Waal, 1972; Micha, 1975; and Hogendoorn, 1979 & 1980).

During the summer season of 1979 we successfully induced the sharptooth catfish, *C. gariepinus* to spawn in our laboratories (Schoonbee *et al.*, 1980). During these trials a combination of human chorionic gondadotrophin (HCG) and an extract from the pituitary gland (PGE) of the common carp, *Cyprinus carpio* was used to induce spawning. The successful use of a full cream milk powder solution to rid the eggs of their adhesiveness, in order to incubate them in breeding funnels, was also described. Although the spawning results were satisfactory it was decided to investigate further combinations of mammalian gonadotrophic hormones (either in combination with common carp PGE or without) as well as the use of catfish PGE alone, to ascertain whether even better gonadal hydration and subsequent release of eggs could be achieved.

Apart from testing the various other hormone combinations it was also endeavoured to refine some of the techniques such as egg washing and incubation procedures. It was also attempted to collect and cold store catfish semen prior to stripping the female in order to facilitate the smooth running of the entire operation.

Experimental Procedures

Collection of spawners

Fish were caught on two occasions in November 1980, with gill nets in Turfloop Dam (approx. 2 km east of the University of the North campus). Twenty-one females and 27 males were selected on site for the first trial and 20 females and 25 males were selected for the second spawning trial two weeks later. The males were selected as spawners if the genital papilla was prominently swollen. It was found that the testes of males with a swollen papilla were significantly more developed than in males of which the papillae were normal. The females were selected on the basis of distention of the abdomen and the appearance of the genital papilla, which, if found to be well vascularized, was indicative of gonadal maturity.

The fish were transported to the hatchery and the females transferred into four 1 000 ℓ plastic bins (1 150 mm x 1 000 mm x 680 mm). All the males were transferred into two such containers. A water exchange rate of 500 ℓ/h was maintained in these bins, which were also covered with 27 mm netting material to prevent the fish from jumping out.

The physical and chemical conditions of the water during both spawning trials did not differ significantly and are shown in Table 1.

TABLE 1
THE PHYSICAL AND CHEMICAL CONDITIONS OF THE HATCHERY WATER ($\bar{x}$ ± SD)

Temperature °C	23 ± 0,85
O_2 (% saturation)	97 ± 1,5
pH	8,8
Conductivity (mS/m)	89,0 ± 1,4

Hormonal injection programmes

In contrast to our previous induced spawning trials (Schoonbee *et al.*, 1980) during which only HCG and common carp PGE combinations were used, combinations of the following hormones were tested during the present trials:

1. HCG sold under the tradename of Pregnyl (HCG consists primarily of luteinizing hormone (LH) but showing some follicle stimulating hormone (FSH) characteristics — Pickford and Atz, 1957).
2. Extract of the common carp pituitary gland (PGE).
3. Sharptooth catfish PGE — (Hoar, 1969, mentions that teleost pituitary glands show strong LH characteristics and traces of FSH activity).
4. A mammalian prostaglandin luteolytic preparation sold

TABLE 2
RESPONSES OF CLARIAS GARIEPINUS TO COMBINATIONS OF TREATMENT WITH HCG, PGE, FSH, LUTALYSE AND OXYTOCIN (gl = gland)

TRIAL 1

Treatment	No. of Fish	Sex	$\bar{x}$ Mass (kg)	PGE Donor	TIME AND DOSAGE DAY 1	DAY 2	RESULT
1	4	F	1,2	Carp	15h30 1 gℓ/kg + 350 IU HCG/kg 22h30 1,5 gℓ/kg		Total spawn, all fish at 21h30 on Day 2
2	3	F	1,28	Catfish	15h35 1 gℓ/kg + 350 IU HCG/kg 22h35 1,5 gℓ/kg		Total spawn, all fish at 21h40 on Day 2
3	3	F	1,3	Catfish	15h40 1,5 gℓ/kg 22h40 1,5 gℓ/kg		Total spawn, all fish at 21h00 on Day 2
4	3	F	1,1	Carp	15h45 1,5 gℓ/kg 22h45 1,5 gℓ/kg		Total spawn, 2 fish at 21h15 on Day 2
5	4	F	1,4	Carp	15h50 1 500 IU HCG/kg 22h50 1,5 gℓ/kg		Total spawn, all fish at 19h15 on Day 2
6	2	F	1,36	—	15h55 100 IU Foligon/kg 22h55 0,3 mℓ Lutalyse/kg	06h30 5 IU Oxytocin/kg	Total spawn, 1 fish at 00h15 on Day 3
7	2	F	1,5	Carp	16h00 1 000 IU HCG/kg + 0,5 gℓ/kg 23h00 0,3 mℓ Lutalyse/kg	06h35 5 IU Oxytocin/kg	No spawn by 04h00 on Day 3 Returned to holding pond
8	25	M	2,5	Catfish	16h00 1 gℓ irrespective of weight		Distinct thinning of sperm in comparison to Treatment 9
9	2	M	2,5	—	NO TREATMENT		Sperm active, viable but viscous

TRIAL 2

1	4	F	1,2	Catfish	06h00 1 000 IU HCG/kg 14h00 1 gℓ/kg		Total spawn, 3 fish at 09h00 on Day 2
2	4	F	1,4	Catfish	06h15 500 IU HCG/kg 14h15 1,5 gℓ/kg		Total spawn, all fish at 09h15 on Day 2
3	4	F	1,4	—	06h30 0,5 mℓ Lutalyse/kg 14h30 5 IU Oxytocin/kg		Total spawn, 2 fish at 09h30 on Day 2
4	4	F	1,5	—	06h45 1 500 IU HCG/kg 14h45 5 IU Oxytocin/kg		Total spawn, 2 fish at 10h00 on Day 2
5	4	F	1,4	Catfish	07h00 1 gℓ/kg 15h00 1 gℓ/kg		Total spawn, all fish at 10h30 on Day 2
6	25	M	2,2	—	08h00 1 000 IU HCG irrespective of weight		Distinct thinning of sperm

under the tradename of Lutalyse (7- (3 alpha-dihydroxy-2 beta-(3S)-3 hydroxy trans-1-octenyl)-1 alpha-cyclopentyl-'cis-5-heptenoic acid with 2-amino-2-(hydroxymethyl)-1,3-propanediol).

5. An FSH preparation sold under the tradename of Foligon.

6. Oxytocin (synthetic).

The combinations and dosages administered during the two injection programmes (Trial 1 and 2) as well as the time of injection are shown in Table 2.

It was found unnecessary to administer a "starter" dosage as suggested for the Chinese carps *Ctenopharyngodon idella* and *Hypophthalmichthys molitrix* by Schoonbee *et al.* (1978) and Brandt and Schoonbee (1980). Two main fractional doses were given approximately 7 h apart in Trial 1 and approximately 8 h apart in Trial 2. In treatments 6 and 7 of Trial 1 the fish were given a third injection of 5 IU Oxytocin per kg approximately 8 h after the second fractional dose.

Oxytocin was injected to determine whether induced ovarian contractions could be achieved so as to ease the release of eggs, a practice which is generally carried out during the induced spawning of the sparid fish *Sparus aurata* in Israel (Gordin, 1980).

During the induced spawning trials conducted in the summer of 1979 (Schoonbee *et al.*, 1980) one female was injected with the PGE of catfish only. This female spawned freely in the aquarium 10 h after the first injection. It was therefore decided to repeat this treatment on a more controlled basis during these trials in order to test the efficacy of this treatment.

The males used during Trial 1 were all, except two, injected with catfish PGE. The PGE was prepared by homogenizing the gland in a 0,9% physiological saline solution, after it had been carefully dried on tissue paper. The dosage consisted of one pituitary gland per fish irrespective of mass and the time of injection coincided approximately with the time the females received the first dosage. During Trial 2 all males received 1 000 IU HCG irrespective of their mass. Similarly to Schoonbee *et al.* (1978) it was assumed that a pituitary gland from a 1 kg donor had the same potency as 1 000 IU HCG. These males also received their hormonal dosage at approximately the same time as the females received their first injection.

This procedure of injecting the males, which is widely followed for Chinese carp (Schoonbee *et al.*, 1978), facilitates the thinning and release of the semen from the testis.

Care was taken in all cases that the dosage volume did not exceed 2 mℓ and the fish were injected intramuscularly in the nape region. Carp pituitaries preserved in absolute ethyl alcohol were obtained from the Israeli Fish Breeders Association, Haifa and were collected from fish weighing 1 kg each. Catfish pituitaries were collected a week before commencing with the spawning trials, from both males and females with a mean mass of 1,3 kg and similarly preserved in absolute alcohol. According to Chiu Liao (1981) and Schoonbee (1979) the Taiwanese fishery biologists find no difference in the potency of male and female catfish pituitaries, provided the glands were collected from fish that are mature and have not spawned during that season.

Hormone effectiveness

During Trial 2 one of the aims was to determine the potency (or effectiveness) of the various hormones administered in combination with catfish PGE and also the potency of catfish PGE when used alone. For this purpose the body circumference of marked females was measured before hormonal treatment and at intervals after hormonal injections up to the time of spawning and afterwards.

Body circumference was measured in the region just anterior to the dorsal fin. Antalfi and Tölg (1971), Rothbard *et al.* (see Brandt and Schoonbee, 1980) and Brandt and Schoonbee (1980) found that increases in body circumference, expressed as a factor of total length (Body circumference/Total length = Body Girth Index — BGI), of Chinese carp species can serve as a measure of gonadal hydration. Consequently the index can serve as an indicator of the effectiveness or potency of the administered hormone (Table 3).

Longevity of *C. gariepinus* sperm

Catfish sperm cannot be released over the eggs by applying pressure to the abdomen as is the case for carp species. The males have therefore to be anaesthetized, sacrificed, the testes removed, given an incision and the sperm squeezed out over the eggs. Hulata and Rothbard (1979) reported that carp semen could be stored for up to 45 h at temperatures between 0°C and 5°C and used with no loss of potency. Such a practice would greatly facilitate the work in the hatchery by separating the time of semen collection and fertilization. Moreover, Hogendoorn (1979) reported that unfertilized eggs of *Clarias lazera* develop "normally" until the 8 or 16 cell stage. Should such a similar

TABLE 3
BODY GIRTH INDEX (BGI) INCREASES OF *CLARIAS GARIEPINUS* UNDER DIFFERENT HORMONAL TREATMENTS

$$BGI = \frac{\text{Pre-dorsal body circumference}}{\text{Total body length}}$$

Treatment No. as in Trial 2	No. of fish	Initial BGI	BGI after 8,5 h	% BGI increase	BGI after 25 h	% BGI increase	BGI after spawn	% BGI decrease
1	4	0,443	0,457	2,99	0,478	7,27	0,399	20,15
2	4	0,438	0,472	6,98	0,482	11,05	0,408	19,47
3	4	0,437	0,452	3,34	0,474	7,54	0,398	19,53
4	4	0,441	0,461	5,78	0,475	9,39	0,400	10,59
5	4	0,438	0,442	4,14	0,486	9,78	0,390	24,63

development occur in *C. gariepinus* eggs, then it is imperative that the eggs be fertilized as quickly as possible after stripping.

Six mℓ of catfish semen were collected to test whether it could be stored until the females had been stripped. The semen was divided into two equal portions.

Similar to the procedures followed by Hulata and Rothbard (1979) one portion was mixed with a 0,3% urea and 0,4% NaCl solution at a ratio of 5 parts semen to 1 part solution. The urea and NaCl solution is generally used to rid carp eggs of their stickiness (Woynarovich, 1962). The second portion was not diluted.

Both portions were stored at 3°C in a refrigerator. Subsamples of these portions were microscopically examined at short intervals and their viability evaluated on the basis of their motility. Similarly to Lindroth (1946) we assumed that non-motile spermatozoa are unlikely to be capable of fertilization, although motility according to Lindroth (1946) is not necessarily synonymous with fertilizing capability. Before each examination the sperm was activated with distilled water.

Breeding funnels

Clarias gariepinus eggs are non-pelagic and the larvae have an extremely large yolk sac in comparison to *Cyprinus carpio* larvae. In previous spawning trials (Schoonbee *et al.*, 1980) it was found that large (32,25 ℓ) perspex breeding funnels (Züger Gläser), which are commonly used for incubating carp eggs, are unsuitable for catfish eggs. Therefore inverted 1 ℓ cooldrink bottles from which the bottoms had been removed, were used.

During the present trials a battery of 16 funnels using 1,25 ℓ rain guages was constructed (Fig. 1). Each funnel was fitted with a short drain pipe, inserted just below the lip of the funnel. From here the water flowed into a halved 55 mm plastic pipe.

The water supply to the funnels was from a filtration system and entered the battery of funnels by one of two supply manifolds. The two manifolds (one for each row) were connected by means of a hosepipe of the same diameter. The second supply manifold was fitted with a stopper at its end. The water flow in the funnels was regulated by screw-type laboratory clamps and this system was found to be ideal for catfish eggs. Moreover, once the larvae had hatched they could easily reach the overflow drain pipe, whereafter they were transported via the run-off pipe into one of the 1 000 ℓ plastic bins.

Figure 1
The adapted rain gauge funnels for the hatching of C. gariepinus *eggs. Note overflow drain pipes inserted below the funnel lip*

Results and Discussion

The response to the various hormonal treatments during the two trials are shown in Table 2. In all cases where Oxytocin was administered (treatments 6 and 7 in Trial 1 and treatments 3 and 4 in Trial 2), the spawning results were neither as successful as expected nor as good as with the other hormonal treatments. We concluded that catfish do not have the same desired response to this hormone as *Sparus aurata*.

The results of treatment 6 in Trial 1 and treatments 3 and 4 in Trial 2 show that *C. gariepinus* can be induced to spawn without PGE. This is of great consequence when no pituitary material of common carp can be obtained and/or if one has a limited initial stock of catfish.

An analysis of the BGI values, determined during Trial 2 (Table 3), reveals that treatment 2 appears to be a most suitable hormone combination in terms of gonadal hydration. The results of treatment 5 (two consecutive doses of catfish PGE), however, also showed good hydration of the gonads. In this case the high percentage decrease in the BGI, after spawning was completed, would suggest that catfish PGE is an extremely suitable gonadotrophin for the induced spawning of this species. It is therefore assumed that catfish PGE contains traces of FSH and is high in LH, as is also found in most other teleosts (Hoar, 1969). From the high percentage decrease of the BGI it was concluded that catfish PGE provides for better hydration of the gonads prior to the release of the eggs than any of the other combinations which were injected. Carreon *et al.* (1976) also found more successful results using pituitary homogenates from donors of the same species (*Clarias macrocephalus*) than using HCG to induce spawning.

On average the females could be stripped of their eggs 21 h after the second injection (expressed in hour degrees this is 483 h°C). Stripping was accomplished by gently stroking the abdomen in the direction of the genital papilla. The eggs of most females were stripped in the form of a paste into a completely dry plastic bowl (Fig. 2). The eggs of one of the females (treatment 5, Trial 1) were found to be watery when stripped (probably an indication of over-hydration). This group of eggs was fertilized and incubated separately and ultimately showed an extremely poor percentage of hatching success (10% in comparison to an average of 68% in all other groups). These eggs were also reddish in colour whilst those obtained in paste form were brown-green.

The hormonal treatment of the males had a distinct thinning response on the milt, which facilitated obtaining semen from the testis. There was no difference in the degree of thinning of the semen when either HCG or PGE was injected. There was also no noticeable difference in fertilization success when either semen from injected or uninjected males was used, although as mentioned above, the hormonal treatment made it easier to squeeze the milt from the testis.

The results of the observation on sperm motility (Table 4) clearly suggest that catfish eggs should be fertilized with freshly collected semen, as sperm motility was reduced by 50% within 60 min. In a recent paper by Hogendoorn (1980) in which he investigated the viability of stored sperm from *C. lazera*, he found that the semen of this species could be stored for 24 h at 5°C with only a 4% reduced fertilizing capability in comparison to fresh sperm.

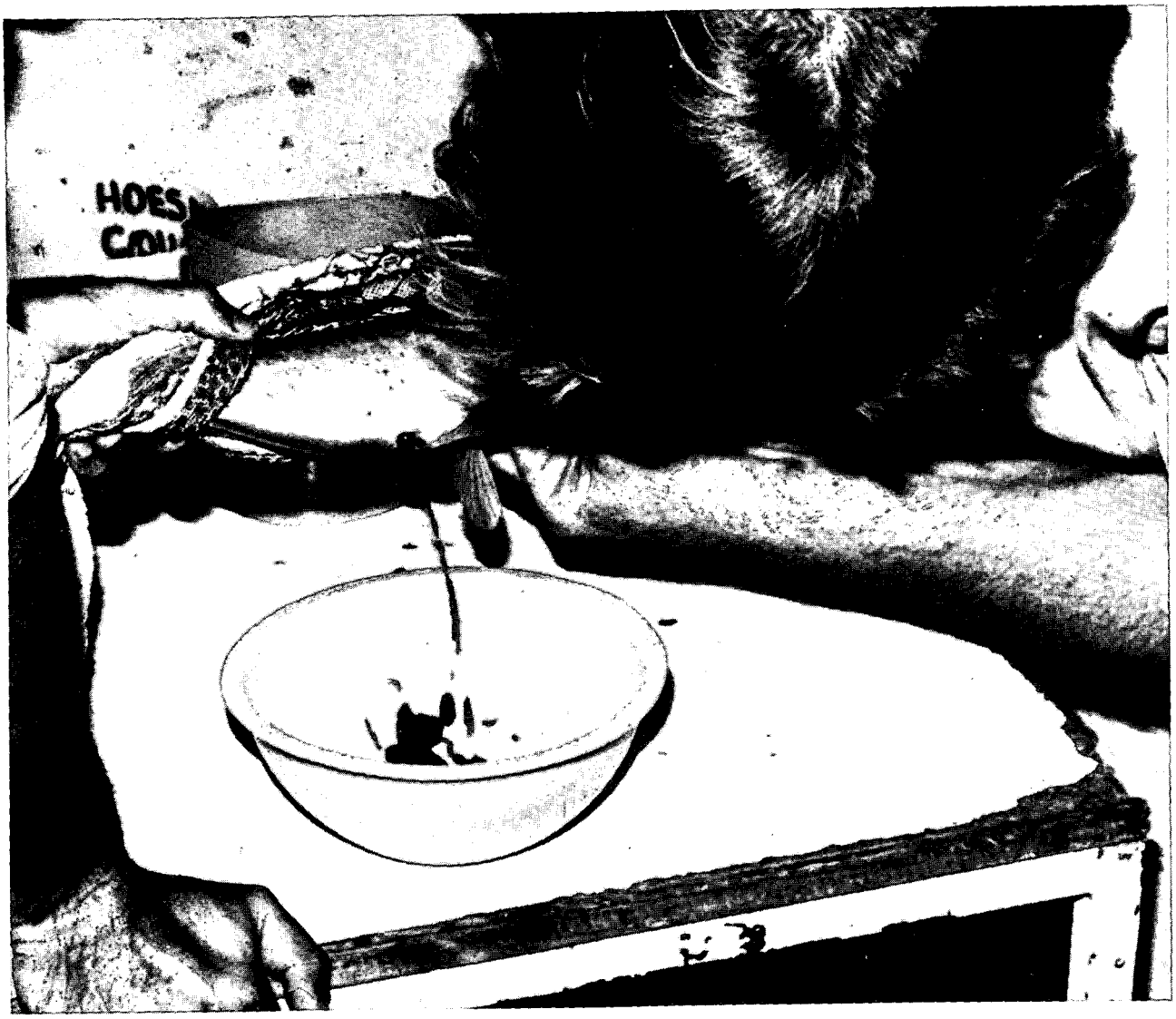

Figure 2
The process of stripping eggs from a female C. gariepinus. *Note sticky nature of eggs adhering to the sides of the plastic bowl*

TABLE 4
THE PERCENTAGE MOTILITY OF *CLARIAS GARIEPINUS* SPERM KEPT AT 3°C FOR TWO HOURS

Elapsed Time (minutes)	Undiluted	Diluted at 5:1
0	All motile	All motile
15	All motile	All motile
30	± 30% non motile	± 20% non motile
45	± 40% non motile	± 30% non motile
60	± 60% non motile	± 50% non motile
75	± 90% non motile	± 60% non motile
90	No motile spermatozoa	± 90% non motile
105	No motile spermatozoa	Isolated motile spermatozoa
120	No motile spermatozoa	Isolated motile spermatozoa

As a result of our findings we recommend that male *C. gariepinus* be anaesthetized before stripping the females. On having obtained sufficient eggs the males should be sacrificed and their testes removed to fertilize the eggs. This is to be done quickly in order to lose as little time as possible between stripping and fertilization to prevent spontaneous cleavage of the eggs, should this phenomenon occur in *C. gariepinus* as it does in *C. lazera*.

A similar experiment, investigating the motility of *C. gariepinus* sperm was carried out at ambient room temperature (23°C). During this experiment sperm motility decreased at an even faster rate than when the sperm was kept at 3°C. Kuchnov and Foster (1976) found that exposure of spermatozoa to air resulted in their activation and consequently interfered with their storability.

The semen obtained from 1 male of approximately 2 kg was found to be sufficient to fertilize eggs from 3 females (i.e. approximately 467 mℓ eggs). On average 1 female was found to release 155,7 ± 30,7 mℓ (n = 32 females) of eggs i.e. an average

of 44 900 eggs per female ($\bar{x}$ mass of females = 1,34 ± 0,12 kg). These calculations of fecundity for females of this weight group correspond closely with those of Bruton (1979).

During the spawning trials in the summer of 1979 (Schoonbee *et al.*, 1980) the semen was expressed directly over the eggs and the combination mixed for 3–4 min with a soft plastic spoon to promote fertilization. The results obtained with this method were satisfactory although a number of eggs were mechanically damaged by the stirring process, as the combination of sperm and eggs turned into a rather stiff paste after fertilization. To overcome this during these (summer 1980) spawning trials either a solution of 3 g urea and 4 g NaCl/ℓ water (Woynarovich A solution – Woynarovich, 1962) or a solution of 20 g full cream milk powder per ℓ water in a ratio of 1:7 was added to the eggs (1 volume solution : 7 volumes eggs) prior to expressing semen over the eggs. This procedure was followed for 2 samples of eggs from 6 females. After stirring the combination for 4 min 5 subsamples of each sample were examined microscopically.

Fertilization success in both solutions was approximately 98% and there were no mechanically damaged eggs. Investigation of subsamples of eggs not diluted prior to fertilization showed that 2% of the eggs were damaged. Had these eggs not been damaged, fertilization would also have been approximately 98% successful.

To rid the eggs of their stickiness they were initially washed in the Woynarovich A solution for 20 min, whereafter they were rinsed in a solution of 20 g full cream milk powder per ℓ water for 50 min. This rinsing process effectively coats the eggs with fat globules (Klotsch *et al.*, 1977 and Schoonbee *et al.*, 1980). During the entire washing time (70 min) the solutions were decanted and replaced every 5 min. At all times the washing solutions were added to completely cover the eggs. The results of this washing recipe were more satisfactory than the results obtained in the previous trials in 1979.

After the eggs had been treated in this manner they were transferred to the breeding funnels. The funnels (Fig. 1) were filled with eggs up to approximately the 700 mℓ mark and maintained at a flow rate of 1,8 ℓ/min. This flow rate was found to be the maximum permissible rate beyond which eggs would be washed out of the funnels. At this rate the eggs moved vigorously throughout the funnel and this flow was maintained until all eggs were loose from each other, whereafter the flow was reduced to 1,3 ℓ/min, at which the eggs rolled gently in the funnels. This reduced flow rate was maintained until the larvae started to hatch, whereafter it was again reduced to 0,75 ℓ/min to facilitate the separation of the larvae and the dead eggs as discussed below. The reduction in the flow rate in the funnels from 1,8 – 0,75 ℓ/min did not affect the percentage oxygen saturation during the incubation period, which remained constant at 97 ± 1,5%.

The eggs obtained during Trial 1 started to hatch 38–45 h after fertilization (39,7 Day °C). Bruton (1979) generally found *C. gariepinus* eggs to hatch 24–25 h at 19–24°C after fertilization, although he found some eggs to hatch after 36–48 h. Hogendoorn (1979), however, found the eggs of *C. lazera* to hatch 48 h after fertilization. Our previous results (Schoonbee *et al.*, 1980) and those of Holl (1968) are in virtual agreement with the present hatching times.

The mean hatching success in all the funnels was determined to be 68% ranging from 45–89%, which is a considerable improvement over the previous trial (Schoonbee *et al.*, 1980) during which hatching success ranged from 14–80%. During the second trial, two weeks after Trial 1, heavy rains occurred in the catchment area of Turfloop Dam from which the hatchery obtains its water.

This resulted in a heavy silt load of the water. Due to a mechanical failure in the filter system at this time the silt in the water killed all the eggs 25 h after fertilization.

Prophylactic treatment of the eggs during incubation consisted of injecting a solution of Malachite Green into the funnel supply hose (Fig. 3) which was then immediately diluted and moved through all the funnels. This treatment proved to be very effective in preventing the fouling of dead eggs.

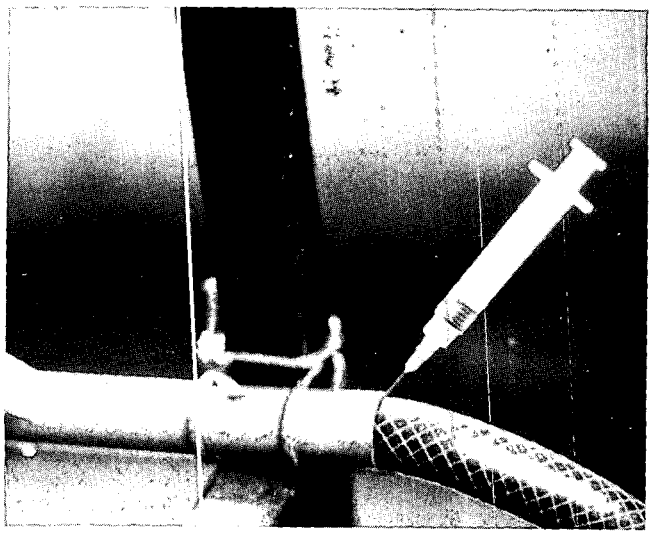

Figure 3
Application of Malachite Green by syringe into the funnel supply hose

During the 1979 breeding trials (Schoonbee *et al.*, 1980) the larvae were decanted from the funnels 8–10 h after all the larvae had hatched, as they showed no sign of "swarming" or "up-swim" to the surface. During the present trials the larvae were left in the funnels to see whether "swarming" would occur and how long after hatching. The first larvae started to swim to the surface approximately 18 h after the majority had hatched. This phenomenon greatly facilitates the separation of the larvae from the dead eggs, the majority of which remained behind in the funnels. As mentioned previously, in order to achieve an even better separation of the larvae and the dead eggs the flow rate through the funnels was reduced to 0,75 ℓ/min.

The larvae were reared more successfully on various feeds (Hecht, 1982) and on a larger scale than previously (Hecht, 1981) at a density of 300/ℓ. As a result of these refined induced spawning techniques as well as the successful rearing of the larvae, it is now possible to commence with the farming of *C. gariepinus* on a pilot scale.

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