Improvements in the large scale artificial propagation of the sharptooth catfish, *Clarias gariepinus* (Burchell), in South Africa

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Induced spawning procedures developed under local conditions in the Transvaal for the sharptooth catfish, *Clarias gariepinus*, using a combination of the chorionic gonadotrophin Chorulon with pituitary gland homogenate from the European common carp, *Cyprinus carpio*, or from *C. gariepinus* donors, are described. Egg-hatching procedures in which the glutinosity of the fertilized eggs of *C. gariepinus* are first neutralized using a full-cream powdered milk solution and then hatched in funnels, are compared with a method in which the eggs are directly transferred to nylon screens in trout-hatching trays after fertilization. The hatching trays proved superior and the advantages of this method are discussed.

Kunsmatige teelttegnieke ontwikkel onder plaaslike toestande in Transvaal vir die skerptandbaber, *Clarias gariepinus*, word beskryf waarin gebruik gemaak word van die chorioniese gonadotropien Chorulon in kombinasie met pituïtêre klierekstrakte van die baber, *C. gariepinus*, of van die gewone karp, *Cyprinus carpio*. Metodes waarvolgens die eiers van die baber na bevrugting eers geskei word van mekaar voordat dit uitgebroei word in tregters, word vergelyk met dié waar die eiers direk na bevrugting oorgedra word na foreleierbroeirakke. Laasgenoemde metode blyk die voordeligste te wees en voordele hiervan word bespreek.

Keywords: Induced spawning of fish, *Clarias gariepinus*, trout-hatching trays, chorionic gonadotropin, pituitary hormones

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Introduction

Of the indigenous freshwater fish species in southern Africa suitable for production purposes, only the cichlid *Oreochromis mossambicus* (Peters) has been widely used as a pond fish in aquaculture (Schuster, 1949, 1952; Vaas & Hofstede, 1952; Atz, 1954; Chimits, 1955, 1957; Chen, 1976). In recent years two other African fish, namely the catfishes *Clarias gariepinus* (Burchell) and *C. lazera* (C. + V.), [now regarded as a junior synonym of *C. gariepinus* (Teugels, 1982a and b)], have received increasing attention from fish culturists (de Kimpe & Micha, 1974; Richter, 1976; Hoogendoorn, 1979, 1983; van der Waal, 1978, 1985; Schoonbee, Hecht, Polling & Saayman, 1980; Hecht, 1981, 1982; Hecht, Saayman & Polling, 1982; Steyn, van Vuren, Schoonbee & Chao, 1985).

Despite the progress made in South Africa in domesticating the sharptooth catfish *C. gariepinus* for aquaculture, some problems are still being encountered concerning its large-scale artificial propagation. One of these, which forms the subject of this paper, includes the development of stickiness and clumping of the eggs once they are released by the fish and come into contact with water, a factor which impedes the mass incubation of fertilized eggs in breeding jars (Schoonbee, *et al.*, 1980). Another problem is the variable success rate obtained in the induced breeding of spawners collected from the wild as compared with fish raised to maturity in captivity. It was felt that an account of these two problems, and the way in which they were successfully dealt with, should be published so that other fish culturists who are working on the domestication of this fish species, could benefit from our experience.

Materials and Methods

Spawners used in breeding trials

Wild male and female spawners of *C. gariepinus* used during the present breeding trials were all approximately 1 kg in size. Fish of such a relatively small size are not only easy to handle during the induced spawning procedures but also yield adequate quantities of viable eggs which may vary between 60 000 and 70 000 per female. The fish were obtained from impoundments within a radius of about 100 km from the hatchery. Fish were mainly collected using 110 mm mesh gill nets. To avoid unnecessary handling stress, fish selected for spawning were removed from the nets immediately after capture and transferred to containers with well-aerated water.

Selection of ripe, gravid females was made at the sampling sites. The choice of the correct gravid spawners is extremely important for the eventual success in egg fertilization and egg hatching rates. Females were selected on the basis of distended, well-developed gonads. Evidence of swollen and slightly reddened genital papillae, in both male and female spawners, is another good indication that fish are ready to spawn (Schoonbee, *et al.*, 1980). Females already in the process

of releasing eggs spontaneously during handling, are known to be overripe, and usually do not produce viable eggs during spawning, and these were rejected. The condition just described may occur more frequently amongst fish collected from the wild late in the breeding season.

Even though the sharptooth catfish has the ability to utilize oxygen from gulped air in its special suprabranchial organ (Jubb, 1967), and is known to survive low oxygen levels in water, the numbers of spawners transported in containers are always kept at comparatively low densities, usually not more than ten fish per 75 litres of water, i.e. at a mean mass of 1 kg per 7,5 litres.

In addition to the wild spawners, laboratory-reared male and female fish which grew to maturity in captivity within a period of 9 months after hatching were also used in the breeding trials. These fish were raised in outside earthen ponds during summer, but transferred to the hatchery during winter, where they were kept at temperatures around 26°C. To avoid damage to the breeders in the tanks, from their naturally aggressive spawning behaviour, males and females were separated.

A high protein (38%) water-stable commercial trout pellet was used as feed.

Prophylactic treatment of spawners against parasites

As occasional attacks on larvae of *C. gariepinus* by ectoparasites, including the gill fluke *Quadricanthus clariadis clariadis* Paperna, were experienced, all spawners, as a standard procedure, were treated prophylactically against ectoparasites according to Leteux & Meyer (1972), using a mixture of 0,05 mg per litre zinc-free malachite green and 25–40 mg per litre 40% formalin for a period of 4–6 hours prior to the commencement of the spawning program.

Holding facilities

Holding tanks in the hatchery were all supplied with well-aerated, fresh, aged, chlorine-free recirculated water. Fish transported to the laboratory were first conditioned to the water used in the hatchery by gradual replacement of the transportation water over a period of 1–2 hours. To minimize stress, spawners in the hatchery were prevented as far as possible from being disturbed. Particular care was taken in this respect during the induced spawning program. Depending on holding tank availability, the numbers of female spawners were kept in densities not exceeding one fish (*ca.* 1 kg) per 15 litres of water. Males were kept at higher densities.

Spawning procedures

Clarias gariepinus is one of the easiest fish to spawn artificially and a number of procedures are presently being followed with success to induce it to spawn in captivity. Even so, it is important in any large-scale spawning program to obtain consistently good results. The following procedures, developed over a number of years in our hatchery, were found to produce the best results under local environmental conditions and were also used in the present project:

1. Prophylactic treatment of spawners against ectoparasites using the method described by Leteux & Meyer (1972).
2. Transfer of male and female fish to separate tanks with clean, aerated, aged water for 4–6 hours before commencement of artificial spawning.
3. First intramuscular injection of all female spawners, lateral to dorsal fin, using 200 IU of Chorulon, a veterinary chorionic gonadotropin produced by Intervet, Netherlands.
4. Replacement of holding water of spawners (which may accumulate faeces) with fresh water after 18 hours.
5. Second injection of female spawners, 24 hours after first injection. Dosage consists of 400 IU Chorulon per female plus an extract of homogenized pituitary gland (PGE) dissolved in a 0,9% NaCl solution, from a *C. gariepinus* (male or female) or *Cyprinus carpio* (male or female). The donor must be of approximate equivalent mass to that of the recipient female. A specially designed glass homogeniser is used to prepare PGE for injections. Care is taken to restrict the volume of each dosage, as far as possible to below 1 ml.
6. The first injection of male spawners with one pituitary gland homogenate, is given to facilitate the thinning of seminal fluid, and the easier release of sperm during the fertilization process of the eggs. This coincides with the second injection of the females. The mass of the donor fish is not as important as in females, but if a high thinning response of sperm is required the donor selected can be of a larger size than that of the recipient male. Pituitary glands of *C. gariepinus* used can be from live fish, can be alcohol preserved, or even freeze dried.
7. Third injection of female spawners 30 hours after the first injection with Chorulon. Dosage consists of PGE homogenate from one male or female donor of equivalent mass.
8. Second injection of male spawners with one *C. gariepinus* or *Cyprinus carpio* PGE homogenate irrespective of the body mass of the recipient male, approximately 6 hours after first injection.
9. Inspection is made of female spawners for any sign of spontaneous release of eggs approximately 40–42 hours after first hormonal injection. If this is observed, spawners are gently dried with towels and then stripped of their eggs by holding the fish firmly over the mouth with one hand, and applying pressure on the gonads in the direction of its genital opening. To avoid damage to the gonads of the females, no excessive pressure is exercised during the stripping of eggs. Unripe and developing eggs are thus retained in the gonads. This procedure facilitates the possibility of more than one spawning effort with the same female, within one summer season.

Fertilization of eggs

A dry fertilization technique is followed. Semen

obtained from testes removed from freshly sacrificed males (Schoonbee *et al.*, 1980; Hecht *et al.*, 1982), is released on the eggs and then gently mixed with a rubber-tipped cake-spatula. Small quantities of a 0,9% NaCl solution are added during mixing, which not only facilitates the mixing process of eggs and sperm, but may promote sperm motility and the incidence of fertilization. Recently it has been shown that male spawners need not be sacrificed, as a successful technique has been developed by van der Waal (1985) to hand-strip adequate quantities of semen needed for fertilization from live *C. gariepinus* males.

Hatchery procedures

Variable hatching results were obtained following the technique of egg separation with full-cream powdered milk as recommended by Schoonbee *et al.*, (1980). The large scale hatching of *C. gariepinus* in breeding funnels usually showed high egg mortalities, probably due to the prolonged stirring and mechanical handling of the eggs during the milk treatment.

In the present breeding trials, investigations were made of hatching rates obtained on subdivided batches of fertilized eggs, from ten different females artificially spawned according to the procedures described (Table 1). One batch from each of the ten females was treated with full-cream powdered milk, according to procedures described by Schoonbee *et al.*, (1980), and then transferred to breeding funnels connected to a water recirculating system at temperatures fluctuating between 23° and 26°C.

The other batches of non-treated fertilized eggs were transferred to 'Heath Techna' trout-hatching trays fitted with bottom screens of 1,0 mm mesh (Figure 1), through which water flowed during recirculation. This mesh size prevented even the smallest eggs of *C. gariepinus* of an average size of 1 kg, from falling through during deposition in the trays. Eggs were spread onto screens, 1–2 layers deep. The tray system, which comes in batteries of eight trays, stacked one upon the other, was

Table 1 Comparison of hatching success (%) of two sets of fertilized eggs of *Clarias gariepinus*, one treated with full-cream milk powder solution and hatched in funnels and the other transferred directly to trout hatching trays

	Hatching %	
Female no.	Treated eggs in funnels	Non-treated eggs in trays
1	58	60
2	61	80
3	27	80
4	56	70
5	47	85
6	42	95
7	59	62
8	22	44
9	36	81
10	44	41
	$\bar{x}$: 45,2	$\bar{x}$: 69,8
	SD: 13,70	*SD*: 17,80
	SE: 4,33	*SE*: 5,63

Pooled variance estimate			Separate variance estimate		
T value	Degrees of freedom	2-Tail prob.	*T* value	Degrees of freedom	2-Tail prob.
-3,46	18	0,003	-3,46	16,89	0,003

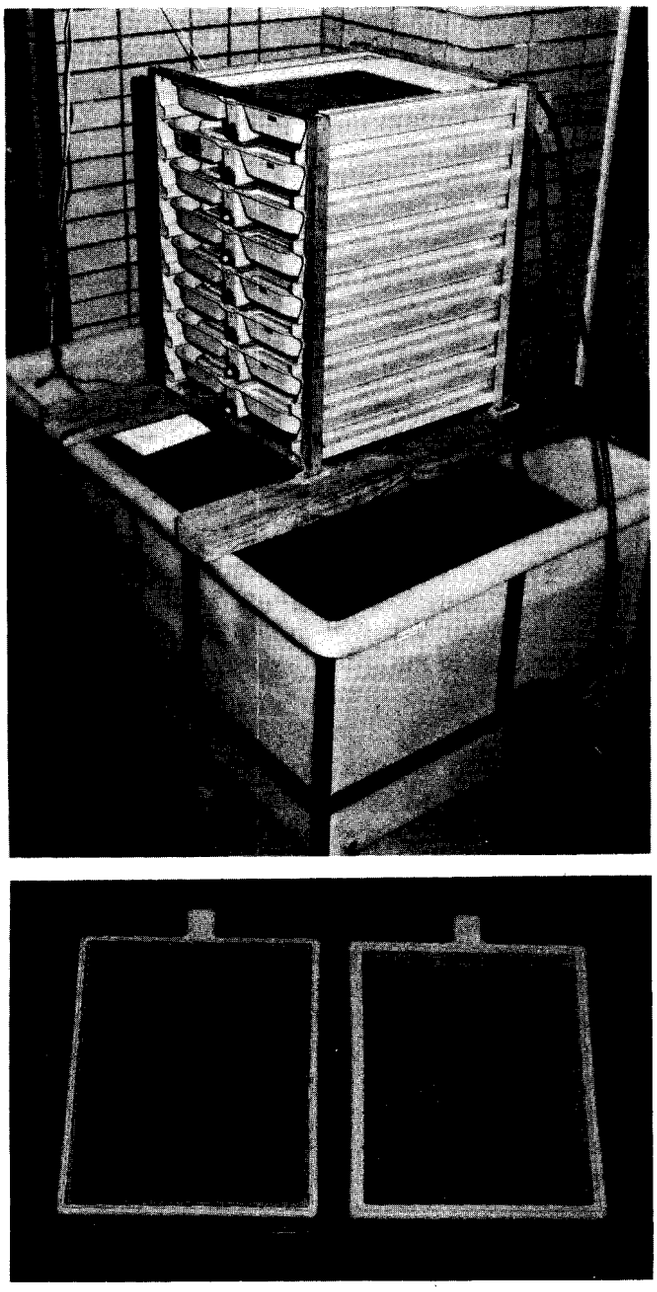

Figure 1 Battery of trout hatching trays (top) connected to recirculating water system. Each tray is provided with a fine mesh screen (below left) onto which the fertilized eggs are deposited. The tray may be covered with a coarse mesh screen through which the larvae can escape during the hatching process (below right)

then connected with a recirculating water through-flow system, flowing at a rate of 6–10 litres per minute. Water temperatures were maintained at 23–26°C. Once the eggs came into contact with the water they became adhesive and remained firmly attached to the screens until the larvae hatched.

Statistical evaluation of data

Paired *T* tests were made to compare the difference in hatching success between eggs treated with full-cream powdered milk solution hatched in funnels with that of eggs deposited on the screens in the hatching trays.

Results and Discussion

Prevailing drought conditions in the Transvaal, and the general absence of rain during early summer which appears to be one of the trigger factors in the gonadal maturation and subsequent natural spawning of *Clarias* species (Holl, 1966, 1968; Bear & Thomas, 1964; van der Waal, 1978), played an important role in the difficulties experienced in the induced spawning of *C. gariepinus* during the present breeding trials.

The use of Chorulon, a chorionic gonadotropin, clearly facilitated the oocyte maturation during the induced spawning programmes with this fish species. Results obtained also verified the value of human chorionic gonadotropin in induced spawning programmes with *Clarias* (Ramaswami & Sunderaraj, 1957; Schoonbee *et al.*, 1980) and carp species (Bailey & Boyd, 1970; Lin, 1974; Schoonbee, Brandt & Bekker, 1978). One disadvantage associated with the use of chorionic gonadotrophins is, however, that proportions of the eggs released tend to become overripe and only develop partially, causing a substantial percentage of the eggs to die off during the first 6–12 hours after commencement of embryonic development. A similar situation probably occurred during the present breeding trials where a high incidence of fertilization was followed by a generally low and variable hatching success of 41–95% (Table 1). Another factor which also contributed towards the reduced hatching rates in most of the spawners was the mechanical handling of the eggs during their separation. This assumption is substantiated by a paired *T* test which was applied to the two sets of hatching results achieved, following separation of the eggs (funnel breeding procedure) and direct hatching in trays (Table 1). A significantly higher survival of embryos were recorded in the latter case.

The use of trout hatching-trays for the breeding of eggs of *C. gariepinus* in the present investigation showed definite advantages over methods in which the eggs are first separated from each other prior to transfer to breeding funnels. The simplicity of the hatching-tray system, and the speed with which large batches of eggs can be handled, together with the fact that the large-scale spawning of *C. gariepinus* is possible within a confined laboratory space using stacks of trays with recirculating water-systems, makes this procedure in the mass propagation of this and probably other fish species with sticky eggs, the preferable method.

This method also adopts artificial spawning procedures already in use for snakehead fish *Channa maculata* and *Clarias* in the Republic of China, where eggs are stripped, and either deposited in plastic trays or on nylon screens, in well-oxygenated water, where they then hatch (Chen, 1976; Schoonbee, personal observations).

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