# Successful handstripping of male *Clarias gariepinus*

**B.C.W. VAN DER WAAL AND L. POLLING**
*Department of Zoology, University of the North, Private Bag X1106, Sovenga 0727, South Africa*

## Abstract

In the past when *Clarias gariepinus* was artificially spawned, males had to be sacrificed to obtain semen for fertilization. It has now been found that one day after administration of hypophysis extract, males can be successfully stripped and ova fertilized, resulting in hatching success of up to 98 per cent. It was also found that testes stored for 24 h at 4°C were effective for fertilization of eggs. This method will enable long-term genetic selection for aquaculture.

## Introduction

*Clarias gariepinus* is regarded as a suitable candidate for aquaculture as it shows some of the most favourable characteristics required for fish farming viz. hardiness, fast growth rate, large size, air breathing ability and an omnivorous feeding habit (Van der Waal, 1974, 1978; Clay 1977, 1981; Bruton, 1979 and Schoonbee, Hecht, Polling and Saayman, 1980).

One provision for successful aquaculture is the availability of fish fingerlings and in this regard the basic work for large-scale production of *C. gariepinus* larvae has been done (Van der Waal, 1972, 1974, 1978; Schoonbee *et al.*, 1980; Hecht, 1981, 1982; Hecht, Saayman and Polling, 1982), but there are still a number of problems including egg adhesiveness, larval mortalities and the fact that males have to be sacrificed to obtain milt with which to fertilize eggs (Schoonbee *et al.*, 1980; Hecht *et al.*, 1982).

The procedure of sacrificing the males has also been in use for *C. lazera* (Hogendoorn, 1977, 1979; Hogendoorn and Vismans, 1980; Richter and Van den Hurk, 1982). *C. lazera* is probably synonymous to *C. gariepinus* (Bruton, 1983).

This technique whereby valuable brood fish have to be sacrificed is not only wasteful but is also a major obstacle in future selection and genetic improvement attempts.

During artificial spawning trials with *C. gariepinus*, a successful attempt was also made to strip semen from the males.

## Material and methods

Spawners were collected during the early summer of 1982-83 from Turfloop Dam, 2 km east of the University of the North, Pietersburg. Fish used in November 1982 were collected from the Nyl River Pans near Potgietersrus.

The following basic gonadal stimulation procedure was followed:

Day one: (11h00 – 16h00) 200 IU Chorulon (Chorionic gonadotrophic hormone (Intervet, Netherlands)) per female (~1,5 kg).
Day two: (08h00 – 11h00) 400 IU Chorulon plus one homogenized hypophysis from a *Clarias* of similar weight.
Day two: (16h00) One homogenized hypophysis per female all administered intramuscularly, with 0,9 per cent saline as solvent.

Females were ready for stripping early on day three. Males were also hypophysized, early on day two. This procedure was

*Figure 1*
*Handstripping of Clarias gariepinus* male. Note droplet of viscous semen on tip of cake spoon and position of thumb.

TABLE 1
RESULTS OF TRIALS WHERE *CLARIAS GARIEPINUS* EGGS WERE FERTILIZED BY HANDSTRIPPING MALES OVER THEM OR WHERE MACERATED TESTES WERE USED

| Date | Time | Female | Male | | Egg survival after 24h % | Notes |
|---|---|---|---|---|---|---|
| | | | Handstripped | Testes macerated | | |
| 11-12-82 | 08h00 | No 1 | | 1 male | 95 | |
| | | No 1 | 1 male | | 80 | |
| | | No 2 | | same male | 80 | |
| 16-02-83 | 08h00 | No 2 | | 1 male | 25 | |
| | | No 2 | 1 male | | 21 | |
| 17-03-83 | 00h45 | No 1 + 2 | | 3 males | 80 | |
| | | No 3 + 4 | 1 male | | 10 | few eggs, difficult to strip |
| | | No 5 | | 3 males | 90 | |
| | | No 6 | 1 male | | 5 | few eggs, difficult to strip |
| 20-04-83 | 21h00 | No 1 | Male No 1 | | 0,1 | |
| | | No 2 | No 2 | | 50 | male 3,5 kg |
| | | No 3 | No 3 | | 0 | |
| | 22h00 | No 4 | No 2 | | 97 | |
| | | No 5 | No 4 | | 60 | |
| | | No 6 | No 5 | | 98 | |
| | | No 7 | | 1 male | 70 | |
| | | No 8 | | 1 male | 98 | |
| | 23h00 | No 9 | No 2 | | 95 | |
| 19-10-83 | 08h00 | No 1 | No 1 | | 81 | Egg batches split in two |
| | | No 1 | | No 1 | 87 | |
| | | No 2 | No 2 | | 72 | ,, |
| | | No 2 | | No 2 | 87 | |
| | | No 3 | No 3 | | 57 | ,, |
| | | No 3 | | No 3 | 66 | |
| | | No 4 | No 4 | | 55 | ,, |
| | | No 4 | | No 4 | 82 | |
| | | No 5 | No 5 | | 68 | ,, |
| | | No 5 | | No 5 | 57 | ,, |
| | | No 6 | | No 6 | 85 | ,, |
| | | No 7 | No 7 | | 7 | Male ***not*** hypophysized, batch split in two |
| | | No 7 | | No 7 | 84 | |
| | 09h00 | No 8 | | No 8 | 89 | ,, |

followed where males were to be stripped.

### Procedure to handstrip males

A male fish was dried in a towel, one hand of the operator holding the jaws of the male, the other hand being used to exert moderate pressure with the thumb ventrally on the abdomen starting at a point half-way between the pectoral and pelvic fins, hindwards towards the genital papilla, repeating at least ten times (Figure 1). An assistant steadies the fish by the tail with a towel, as is customary with females. In a few instances, testes of males killed 24h earlier were stored in a refrigerator at 4°C before fertilization was attempted.

When fertilizing eggs with macerated testes, males were sacrificed, the testes removed, slit open with a razor and macerated with the fingers above the eggs so that semen dripped onto the eggs.

Immediately after stripping or squeezing sperm over the eggs, a few millilitres of saline (0,9% NaCl) was added. The eggs and sperm were well mixed, allowed to stand for one minute and washed once with clean water after which the fertilized eggs were deposited directly on loose sheets of fine synthetic cloth in the stacked trays. Recirculating water was used for incubation. After 24h a subsample of >200 eggs was collected from each tray and the percentage survival calculated by microscopic inspection.

## Results and discussion

Results of trials are summarized in Table 1. A somewhat lower survival (and hatching) success was realized (53,1%) when males were stripped over eggs as compared to fertilization by the macerated testes method (78,3%). The maximum ranges however, compare well, i.e. 98% survival rate, indicating that the former technique is inherently as good as using macerated testes.

Stripping of males was unsuccesful only in one instance when a later autopsy showed very underdeveloped testes. When performing the stripping of a male fish, an assistant held a plastic cake mixer under the genital papilla to direct the semen onto the eggs. It was noted that with the first two or three strokes very little fluid was produced, followed by steady squirts of an often slightly yellow fluid, very watery and containing only a relatively low density of actively swimming spermatozoa upon microscopic inspection. It is assumed that this fluid is mainly urine, pressed from the well developed urinary bladder. After this, a smaller

quantity of more viscous, clear to somewhat cloudy fluid was expelled upon each stroke. Microscopic inspection showed high densities of actively swimming spermatozoa. In most cases three to five drops of this fluid could be obtained whereafter the flow of semen virtually stopped. This volume of fluid was used for batches of eggs of 10 to 100 g (5 000 to 50 000 eggs). In one trial (20-4-83) a large male (No 2) was, however, stripped on three consecutive occasions over a period of one and a half hours to fertilize eggs from three females (with a total mass of 29g) with no indications of diminishing semen flow. The lower hatching success in the case of Female No 2 (50 per cent) compared with 97 and 95 per cent of females No's 4 and 9 shows how sensitive *Clarias gariepinus* eggs are for correct timing of fertilization after the artificial inducement programme. Females No 1 and No 3 were definitely stripped too early.

**TABLE 2**
**RESULTS OF TRIALS USING TESTES STORED FOR 24H AT 4°C TO FERTILIZE *CLARIAS GARIEPINUS* EGGS.**

| Date | Female | Testes 24h old | Fresh testes | Percentage survival after 24h |
|---|---|---|---|---|
| 09-11-82 | No 1 | Male No 1 | | 10 |
| | No 2 | Male No 1 | | 50 |
| 13-01-83 | No 4 | One male | | 59 |
| | No 4 | | One male | 67 |

In the trial conducted on 19-10-83, male No 7 was not hypophysized, and only seven per cent live eggs were obtained compared to an average of 67 per cent for all other batches of that trial where males had been stripped.

In an attempt to economize on fish that had to be sacrificed to obtain hypophyses and semen, a few batches of eggs were fertilized with semen obtained from testes that had been kept at 4°C for 24h. Results are presented in Table 2. Results are similar for both treatments. This is in contrast to the results of Hecht *et al.*, (1982) who found that motility of sperm is reduced by 50 per cent within one hour at 3°C. However Hecht *et al.*, (1982) used semen obtained from testes whereas in the present study whole testes were stored. Hogendoorn and Vismans (1980) report that semen of *C. lazera* was kept for 24h at 5°C with a reduction in hatching rate of only four per cent compared to fresh semen. Hulata and Rothbard (1979), successfully used refrigerated *Cyprinus carpio* semen, stored for 45h to fertilize egg batches.

Whilst inspecting *Clarias* semen microscopically, it was noted that spermatozoa are not active in the undiluted form but that after contact with water or saline, vigorous activity ensued which stopped abruptly after 30 s with only a few spermatozoa still moving after two minutes. Storing in a diluted form, or storing semen extruded from testes (as practiced by Hecht *et al.*, 1982), may thus not be a suitable method to preserve viable spermatozoa of *C. gariepinus* over a short period.

## Conclusions

Male *C. gariepinus* can be successfully stripped of semen one day after hypophysation. An egg hatching success of up to 98 per cent may be achieved, although the mean value is lower than where macerated testes of sacrificed males are used.

There are indications that males can be used more than once in a season. This technique should find application especially where brood fish material is at a premium or is being used for selection and genetic improvement. Spermatozoa of *C. gariepinus* have a short period of activity of only 30 s. Testes kept refrigerated for 24h yield similar results as fresh testes, adding an additional technique to economize on parent material.

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