*Environmental Biology of Fishes* **42**: 1–6, 1995.
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# Sperm drinking by female catfishes: a novel mode of insemination

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Received 30.6.1994 Accepted 20.7.1994

*Key words:* Reproductive behaviour, *Corydoras aeneus,* T-position, Egg fertilization, Ventral pouch, Callichthyidae, New mode of sperm delivery

**Synopsis**

We report on unique reproductive behaviour and a new mode of egg insemination in a small catfish *Corydoras aeneus* (Callichthyidae). A male courts a female by presenting his abdomen to her. Before releasing eggs, the female attaches her mouth to the male's genital opening and directly drinks his sperm. The sperm pass through her intestine and are discharged together with eggs into the 'pouch' formed by her pelvic fins. Thus, eggs are mixed with fresh non-dispersed sperm in an enclosed space, ensuring effective insemination. This mode of insemination is novel to fishes, but is likely not restricted to catfishes of the genus *Corydoras.*

## Introduction

A variety of reproductive behaviours is exhibited by catfishes (Axelrod & Burgess 1986, Sato 1986, Burgess 1989, 1992) which include species of various sizes and food habits (Burgess 1989, 1992). Small catfishes of the genus *Corydoras* (Callichthyidae), consist of more than 120 species occurring in South America (Matsuzaka 1993). These are popular among aquarists, and their reproductive behaviours have been observed in aquaria by many authors (Sakurai et al. 1985, Burgess 1987, 1992, Matsuzaka 1993).

Most species of *Corydoras,* observed hitherto, exhibit a unique reproductive behaviour, so called 'T-position' where the female attaches her mouth to the anal region of the male (Burgess 1987, 1992, Sakurai et al. 1985, Matsuzaka 1993). The female releases eggs into a pouch formed by her pelvic fins located near the anus, and carries them to egg deposition sites. Despite numerous observations of spawning behaviour, how eggs are inseminated is still unknown. *Corydoras aeneus* is one of the most popular catfish in aquaria. In this paper, we report on the reproductive behaviour of *C. aeneus,* and examine how its eggs are inseminated.

## Reproductive behaviour of *C. aeneus*

### *Methods*

*Corydoras aeneus* is a small catfish (6 cm in maximum total length). We obtained 150 fish from a commercial breeder. Males and females were kept in different tanks (70 l) and were fed artificial food (Tetramin). Water temperature was kept between 23–24° C. One female with a large abdomen and two males were put in a 40 l aquarium constructed of one-way mirrors. So we could observe reproductive behaviours. After one female had deposited eggs, she was replaced by another female with an enlarged abdomen.

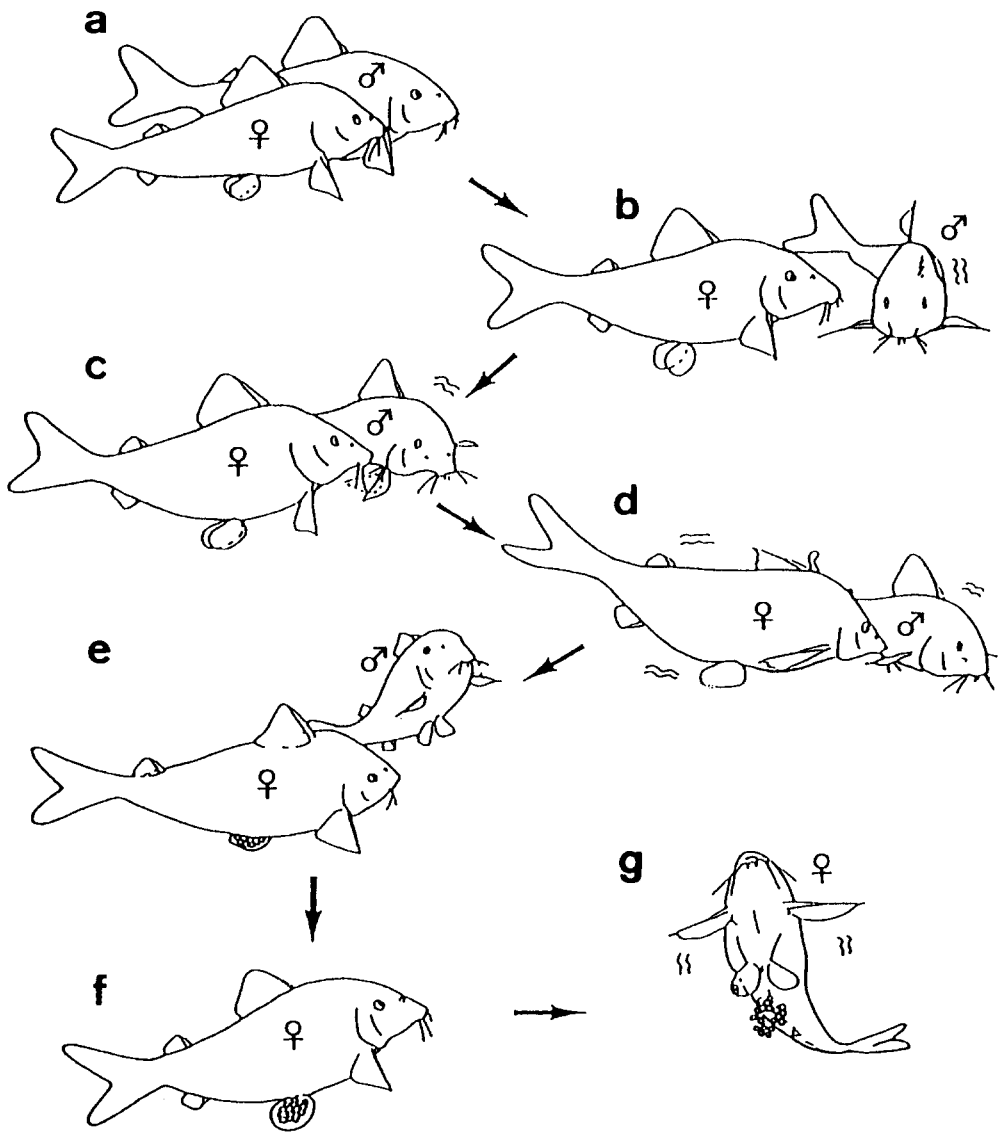


*Fig. 1.* An ethogram of reproductive behaviour of *Corydoras aeneus:* a and b – A male presents his abdominal side to a female. c and d – The female attaches her mouth to the males anal region in T-position. e – She deposits eggs into her pelvic pouch, just after the mouth attachment. Then she is stationary on the bottom. f – She swims and looks for an egg deposition site. g – She deposits eggs alone (the figure shows a female depositing eggs on a glass wall).

*Results*

During courtship behaviours, males usually followed the female and frequently presented their abdominal sides to her (Fig. 1a, b). She usually ignored the presentations, but occasionally attached her mouth to the male's anal region (Fig. 1c). This mouth attachment in the 'T-position' continued for 5.9 sec (± 1.4 SD, n = 51) (Fig. 1d), while the male would ejaculate. From the beginning of the attachment, the female closed her gill covers tightly for 7.1 sec (± 1.6 SD, n = 51). The duration of closed gill covers was the same (18 cases of 51) or longer (33 of 51) than that of the mouth attachment in each event. Concurrently with the mouth attachment, the female made a 'shell-shaped' pouch with her ventral fins. Soon after releasing her mouth attachment (0.6 sec later, n = 37), she deposited one clutch of 30.2 (± 11.1, n = 41) eggs of 1.5 mm in diameter into the pouch. Then she usually remained stationary at

the bottom for 94.6 sec (± 24.8 SD, n = 55), during which the eggs were retained in the pouch (Fig. 1e). Then she swam and carried eggs inside her pouch and deposited them by herself (Fig. 1f, g). After about 5.5 min, she repeated this sequence during 2–3 h, 17.2 times (± 5.2 SD, n = 17). Eggs were attached in a single layer usually on a small area (ca. 3 cm in diameter) of the glass wall. Deposited eggs were not guarded.

## Experiments on the egg insemination

Several hypotheses have been proposed as to how eggs are inseminated in *Corydoras* (Sakurai et al. 1985, Burgess 1987, Matsuzaka 1993). These fall into three categories: insemination occurs (1) by sperm spread in the aquarium at various times after spawning; (2) on the egg attachment site by sperm there discharged from the mouth of the female held from the time of T-position, and (3) inside her pouch by sperm coming from a male in the T-position. To confirm how eggs are inseminated, 3 experiments were conducted in a 40 l aquarium.

### *Experiment 1: Whose sperm inseminates eggs*

#### *Methods*

We examined whether or not eggs of one clutch are inseminated by sperm obtained during a single anal site attachment. An albino female (albinism is caused by a recessive allele at a single locus) was mated with two males (an albino and a normal type) in an aquarium, and the phenotype of the young one week after hatching was examined. If hypothesis 1 is correct, young of both phenotypes will appear within a clutch.

*Table 1.* Coloration of young of an albino female mating with albino (A) and normal (N) males.

| Sequence of mating | Colour of males | Colour of young | |
|---|---|---|---|
| | | Albino | Normal |
| 1 | A | 12 | 0 |
| 2 | N | 1 | 14 |
| 3 | A | 12 | 0 |
| 4 | N | 0 | 15 |
| 5 | N | 0 | 2 |
| 6 | A | 5 | 0 |
| 7 | N | 0 | 10 |
| 8 | A | 25 | 0 |
| 9 | A | 2 | 0 |

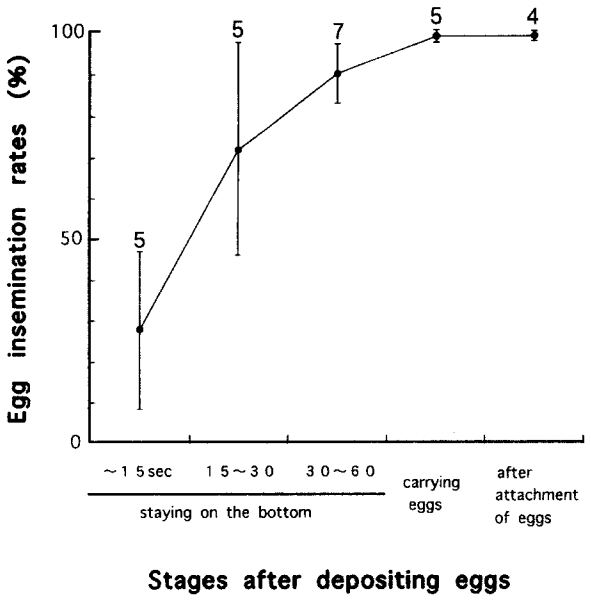


*Fig. 2.* Insemination rates after egg release into fin pouch. Vertical lines are SD and figures are sample sizes.

#### *Results*

The female mated with both albino and normal males (Table 1). When the albino female attached to the anal area of the albino male, all 56 young from 5 clutches demonstrated the albino phenotype. In contrast, when the female attached to the normal coloured male, 41 of 42 young of 4 clutches were all normally coloured. This indicates that all eggs of one clutch were inseminated by sperm of the mate at that time, and rejects hypothesis (1).

### *Experiment 2: Timing of egg insemination*

#### *Methods*

To examine the timing of the insemination, eggs were collected at various times after release. The collected eggs were held in another aquarium with appropriate conditions. On the next day, it could be distinguished whether embryos were developing

normally. Dead eggs were considered as unfertilized.

*Results*

About 25% of the eggs were inseminated within 15 sec after release into the pouch (Fig. 2). After that, the insemination rates increased. When the female brought eggs to the deposition site in her pouch, as many were inseminated as those attached on the deposition site. This clearly indicates that eggs are inseminated inside the pouch, while the female is stationary on the bottom, and rejects hypothesis (2).

*Experiment 3: How sperm arrive inside the pouch*

*Methods*

The results of the two experiments support hypothesis (3) that eggs of one clutch are inseminated inside the female's pouch with sperm of the respective mate. Possible means of passage of the sperm from the genital pore of a male to the female's pouch are: (1) sperm flow or swim along the outside of the body of the female and arrive inside the pouch, or (2) sperm are swallowed by the female and pass through her intestine and are discharged into her pouch (an incredible scenario but conceivable), or both (1) and (2).

To examine the passage of sperm, in still water aquaria, 0.2–0.4 ml of diluted methylene-blue solution was deposited by a pipette at the mouth of a female which was just in the 'T-position' (Fig. 3a), and the flow of the blue water was observed.

*Results*

After 4.2 sec (± 0.8 SD, n = 22) of the release of the solution, blue water appeared from her anus and was funnelled into her ventral pouch (Fig. 3b)! During the time that the gill covers were closed (about 7 sec), the blue solution did not flow from her gills. The blue solution remaining around the mouth of the female did not reach the abdominal area (Fig. 3b). Eggs were released 1–2 sec after the appearance of the blue water from the anus, and were inseminated at high rates (87.6% ± 12.7 SD in 16 clutches). The flow of the blue water clearly indicates that the female drinks sperm together with some water from around the anal region of the male and discharges this into her pouch.

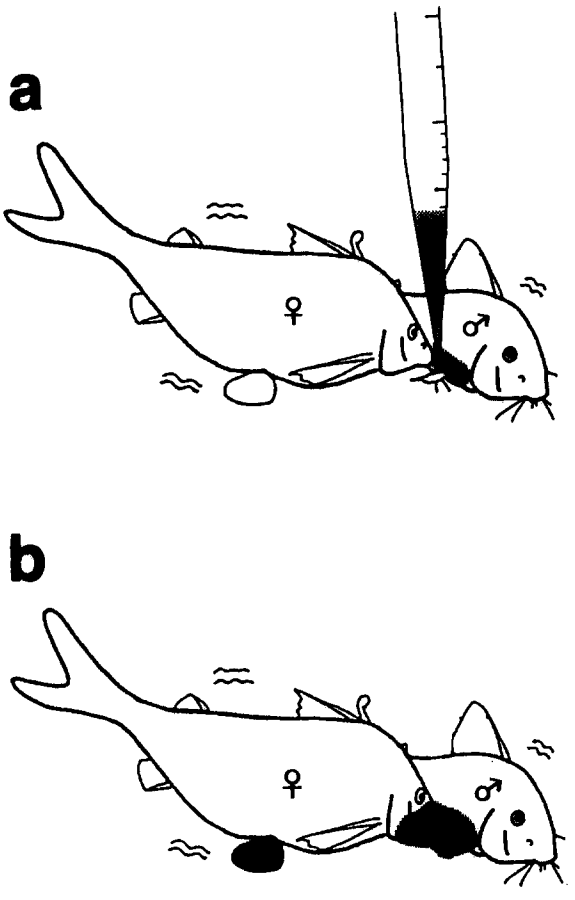


*Fig. 3.* A sketch of releasing methylene-blue solution at the mouth of a female in T-position (a) and the flow of bluish water 5–7 sec after the release, when the female's gill covers were still closed (b).

In some cases, after gill ventilation of females started again, the blue solution streamed from the gill posterior openings of the females, but moved backward ca. 30° along the body and the colour was much fainter than the blue in her pouch. The blue water did not flow along the female's body and did not arrive in her pouch.

## Discussion

The reproductive behaviour of *Corydoras aeneus* includes a female's mouth attachment to a male's

anal region in the T-position and releasing eggs into her pouch formed by ventral fins, as in congeners (Sakurai et al. 1985, Burgess 1987, Matsuzaka 1993). The flow of the blue water strongly indicates that while the gill covers of the female of *C. aeneus* are closed during the T-position, she directly drinks the sperm together with some water, which is transported quickly through her intestine and discharged into her pouch. Eggs are released just after the discharge of sperm and will be inseminated inside her pouch, while she remains still on the bottom. Although fishes exhibit a variety of reproductive behaviours (Breder & Rosen 1966, Balon 1975, 1981, 1990, Baylis 1981, Gross & Shine 1981), this mode of egg insemination in *C. aeneus* is novel among fishes, and perhaps in the animal kingdom.

Let us consider the timing of some reproductive behaviours of the *C. aeneus* female. A female usually attaches her mouth to a male's anal region for 6 sec, and the duration of drinking sperm and water while closing her gill cover is ca. 7 sec (Fig. 1). If a female drinks water for a longer period, the sperm in her pouch will be diluted by additional water from her anal region. On the other hand, shorter periods of drinking would not be long enough to transport sperm into her pouch. An interval of 7 sec of drinking sperm would probably result in an optimal density of sperm in the pouch. The female was stationary on the bottom for 70–120 sec after egg deposition. During this time eggs in the pouch were inseminated. The duration of this stationary period is probably an optimal length of time required for maximal insemination of her eggs.

The water containing sperm swallowed by the female passes through the intestine very quickly. How can females accomplish such a unique performance? *Corydoras* use intestinal breathing in which they gulp air and pass it through the stomach to the end of the intestine (Burgess 1987). *Corydoras aeneus* also frequently do intestinal breathing (Kohda & Tanimura personal observation). This ability of swallowing and passing air would facilitate the evolution of the behaviour of the fast drinking and passing water through the intestine.

The reproductive behaviours of more than 20 species of *Corydoras* reported hitherto include the T-position and releasing eggs into the pouch (Sakurai et al. 1985, Burgess 1987, Matsuzaka 1993). This similarity of behavioural patterns suggests that insemination by sperm after passage through a female's intestine could be common in fishes of this genus.

Three characteristics of *Corydoras* may be preconditions for the evolution of this unique mode of insemination: (1) a short gut of benthivores (in *C. aeneus* gut length is 5.4 cm (± 0.4 SD, n = 21)); (2) the location of their pelvic fins beside the anus; and (3) their frequent swallowing of air for intestinal breathing. The three preconditions are widespread among other small catfishes (Burgess 1989), suggesting that this mode of insemination may not be restricted to the genus *Corydoras*. Indeed, the T-position is observed in some small catfishes other than *Corydoras* and other than Callichthyidae (Burgess 1989, Kanda & Minoguchi 1990).

Unfortunately, we have little information on the reproductive ecology of *Corydoras* in natural habitats. We predict that eggs mixed with active sperm in the narrow space of the pelvic pouch would ensure a high insemination rate even in a turbulent stream which is one of the common habitats of *Corydoras* (Burgess 1987, 1992, Matsuzaka 1993).

## Acknowledgements

We thank the members of Laboratory of Animal Sociology of Osaka City University and Y. Nagata of Osaka Kyoiku University for valuable discussion during the study, and A.P. Møller, T. Sato, T. Kuwamura, E.K. Balon and D. Noakes for reviewing the manuscript. This work was partly supported by the Grant-in-Aid for Scientific Research on Priority Areas (# 319) from the Japan Ministry of Education, Science and Culture.

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