

OBSERVATIONS ON COURTSHIP AND MATING BEHAVIOUR IN MALTESE POPULATIONS OF THE KILLIFISH *APHANIUS FASCIATUS* (PISCES: CYPRINODONTIDAE)

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ABSTRACT

Courtship and mating behaviour of a Maltese population of *Aphanius fasciatus* kept in laboratory culture is described. Males showed a brightening of their coloration, increased aggression towards other males, and paid more attention to females during the period April–October. Males took the lead in courtship. Courtship behaviour consisted of a number of acts. Some involved touching the female and these appeared to be designed to arouse sexual interest; others served to exhibit the male's body and to inhibit the female from swimming away. Mating commenced with the female pushing itself into floating algal mats; the male responded by wrapping its body tightly round that of the female, following which ejaculation of eggs and sperm occurred.

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INTRODUCTION

The killifish *Aphanius fasciatus* Nardo, 1827 occurs in coastal marine and brackish water habitats all round the Mediterranean except in the westernmost part (Tortonese, 1986). This species is very variable and numerous races and varieties have been described, most of which are of uncertain taxonomic status (Spillmann, 1961; Tortonese 1970a, b; 1973). *Aphanius fasciatus* is the only species of truly brackish water fish occurring in the Maltese Islands (Despott, 1919; Lanfranco, 1958). Maltese populations apparently show some differences from other Mediterranean populations and are sometimes regarded as a distinct race (Darmanin, 1979). Moreover, different sites in the Maltese Islands appear to support different ecotypes (Zammit & Van Es, 1980). Most Maltese populations are now declining and this species is listed as "vulnerable" in the Red Data Book for the Maltese Islands (Lanfranco & Schembri, 1989).

While there is a rich literature on the taxonomy, morphology and ecology of this species (for example: Steinitz, 1951; Cavicchioli, 1962; Torchio, 1967; Tigano, 1982; Penaz & Zaki, 1985), its behaviour has received much less attention. For local populations, some behavioural information has been given by Zammit (1975) and by Cilia (1986) but this is far from complete. The present work describes the courtship and mating behaviour in a Maltese population of *Aphanius fasciatus* kept in laboratory culture.

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## MATERIAL AND METHODS

Fish were collected using a hand net from the brackish water pool at the Ghadira Nature Reserve, northern Malta, where there is a thriving population, transported to the laboratory and housed in glass aquaria of dimensions 75 x 45 x 45cm kept outside out of direct sunlight. The water was aerated and salinity was allowed to vary between 24 ppt (winter) and 30 ppt (summer), while the water temperature was allowed to vary with the air temperature, to mimic the natural variations in salinity and water temperature in the fish's natural habitat (Hilli et al., 1990). The aquaria were provided with a 1cm layer of sand and with floating mats of green filamentous algae; sand and algae were obtained from the Ghadira pool. The fish were fed daily on commercial fish food (Tetra Fin and Sera-Raffy P) supplemented by live mosquito larvae.

Maltese *Aphanius fasciatus* are reported to breed between May and August (Zammit, 1975; Cilia, 1986). During this period, a close watch was kept on the culture tank for fish showing signs of sexual activity. Such fish were used for observations on courtship behaviour. For these observations, individual male fish were isolated in observation tanks identical to the communal culture tanks except that they were smaller (45 x 18 x 20cm) and the water was not aerated. After a settling period of 24 hours, a gravid female fish was introduced into the tank and observations made from behind a screen. Observations were made on ten pairs of fish during the period April - October 1988. Times were measured using a stopwatch. During the experimental period, water temperature varied between 27-31°C while salinity was a constant 33ppt.

## RESULTS

In the communal culture tanks, sexually active males were recognised by a brightening of their coloration, their aggression towards other males, and their attention to females. Based on observations of courting pairs kept in observation tanks, courtship behaviour was found to consist of a number of acts which are described below and illustrated in Fig. 1.

Watching The male hovers motionless watching the female from a distance of between 5 -15cm. Often the male's body is arched laterally, the caudal fin quivering vigorously.

Pursuing The male swims after the female at a speed just sufficient to maintain a position some 3cm behind.

Looping The male circles round the female in a horizontal plane at a distance of some 15cm. The female either remains stationary or moves forward slowly.

Zigzagging The male swims ahead of the female performing alternate sharp turns to the left and right.

Nudging The male prods the female on the dorsal part of her head with his snout. Occasionally the male nudges the female's head ventrally. Nudging behaviour is

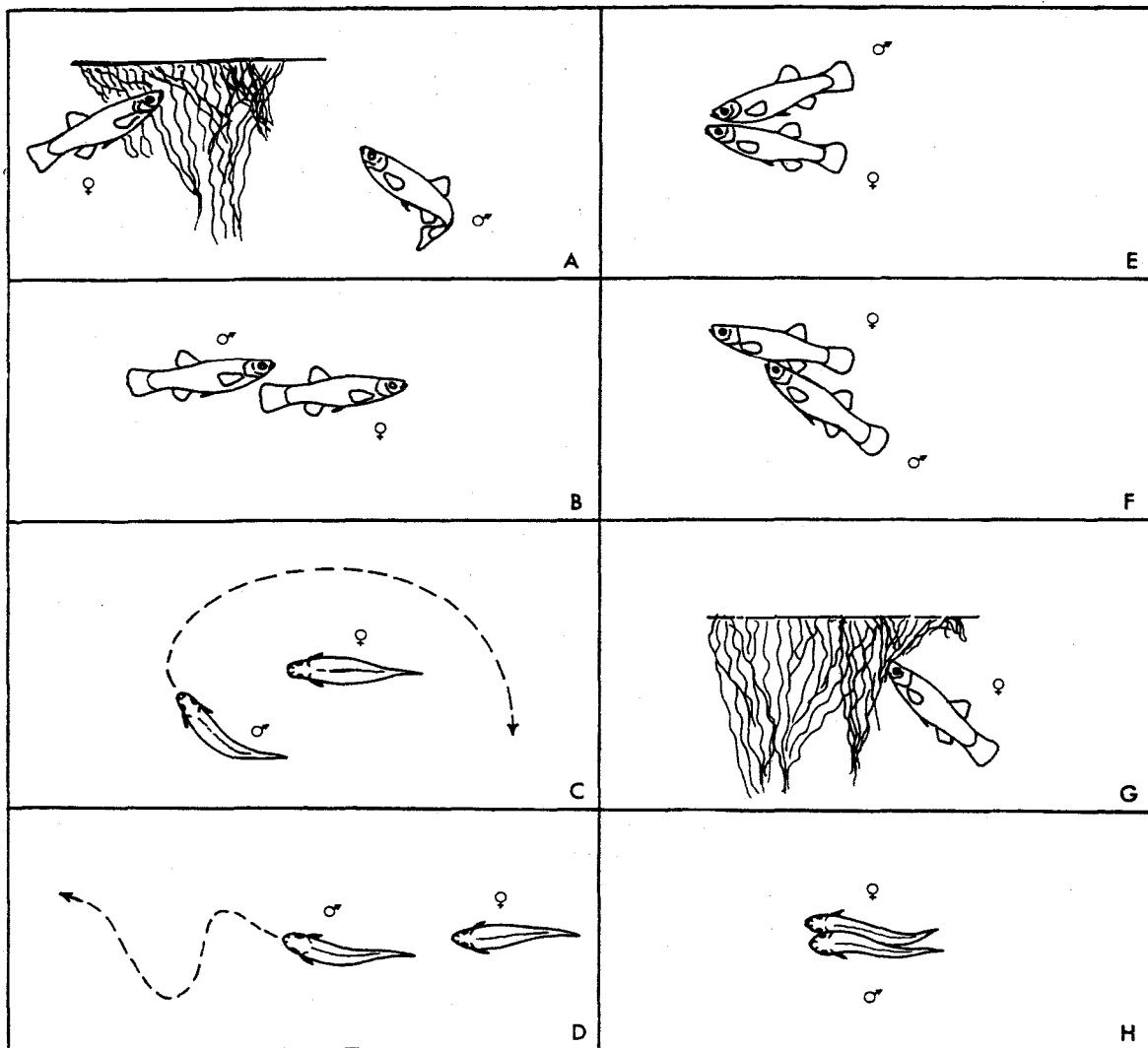


Fig. 1 Acts in the courtship behaviour of the killifish *Aphanius fasciatus* drawn from life. A, B, E, F and G show the fish in lateral view; Figs C, D and H show the fish in dorsal view.

followed by the pair swimming together with the male positioned just above the female and touching her on her head.

**Butting** The male prods the female's belly with his snout.

**Pushing** The female makes sudden attempts to push itself inside a floating algal mat.

**Clasping** This is the spawning posture and usually occurs after the female has pushed itself inside an algal mat. The male assumes an S-shape and wraps its body tightly round that of the female. Both fish quiver the posterior part of their body. During clasping the fish may assume a horizontal, vertical or oblique orientation and may even lie upside down with their ventral aspect facing the surface.

Jerking                      Claspings usually ends in a series of rapid jerks by the female. Following jerking, pair contact is often broken.

Courtship sequences were analysed by considering each of the nine acts defined above in turn and counting the number of times each of the other eight acts followed. Conditional probabilities for each two-act transition were calculated as described by Slater (1973). These data are presented in the form of an ethogram in Fig. 2.

On the basis of these data the typical courtship mating sequence seems to be as follows:

Following introduction of a gravid female into the observation tank, the male's initial reaction was 'watching' for a period of approximately 35 seconds, normally followed by furious 'pursuing' all over the available space. After approximately 2 minutes of this behaviour, the male started to 'nudge' the female. Females usually responded to this by attempting to move away from the male, whereupon the male showed 'looping' and 'zigzagging' displays. This whole sequence of 'nudging', 'looping' and 'zigzagging' lasted for periods of up to 14 minutes. The sequence either terminated abruptly with the male resuming 'watching' behaviour, or else the female started 'pushing' into an algal mat. The male most frequently responded to this by approaching her and either 'butting' or 'clasping'. 'Clasping' behaviour often occurred three or four times in succession with the female moving further into the algal mat each time. The sequence either terminated by 'jerking', if spawning was successful, or else reverted back to 'butting' and 'nudging'.

## DISCUSSION

In *Aphanius fasciatus*, the male plays the leading role and is more persistent, frequently spending long periods of time pursuing the female. Obviously this costs the male considerable expenditure of energy, and courting males appear to lose weight rapidly, as indicated by their receding abdomen. The different acts making up the courtship sequence seem to have different functions. Thus 'nudging' and 'butting', both of which involve touching the female's body, appear to serve in arousing mating interest on the part of the female. 'Nudging' behaviour is common in the courtship displays of several killifish species, for example, *Aphysemion occidentalis*, *Nothobranchius rachovi* and *Rivulus magdalenae* (Axelrod, 1987).

'Looping' and 'zigzagging' appear to serve a dual role. When the female is stationary, they serve to exhibit the male's body. However, if performed when the female is trying to swim away from the male, they may be strategies to inhibit the females from escaping; by moving slowly in front of the female in 'zigzagging', or by encircling her in 'looping', the male attempts to stop the female fleeing.

The role of 'clasping' appears to be that of ensuring maximum spawning success. By wrapping and pressing his body round the female's, the male may aid ejaculation of the eggs since in some fish, pressure on the ovary seems to facilitate expulsion of the ova from the ovarian lumen (Foster et al., 1969). Additionally such close proximity of the partners is likely to ensure fertilization of the majority of eggs. However, 'clasping' does not always lead to spawning. Careful examination of the algal filaments following 'clasping'

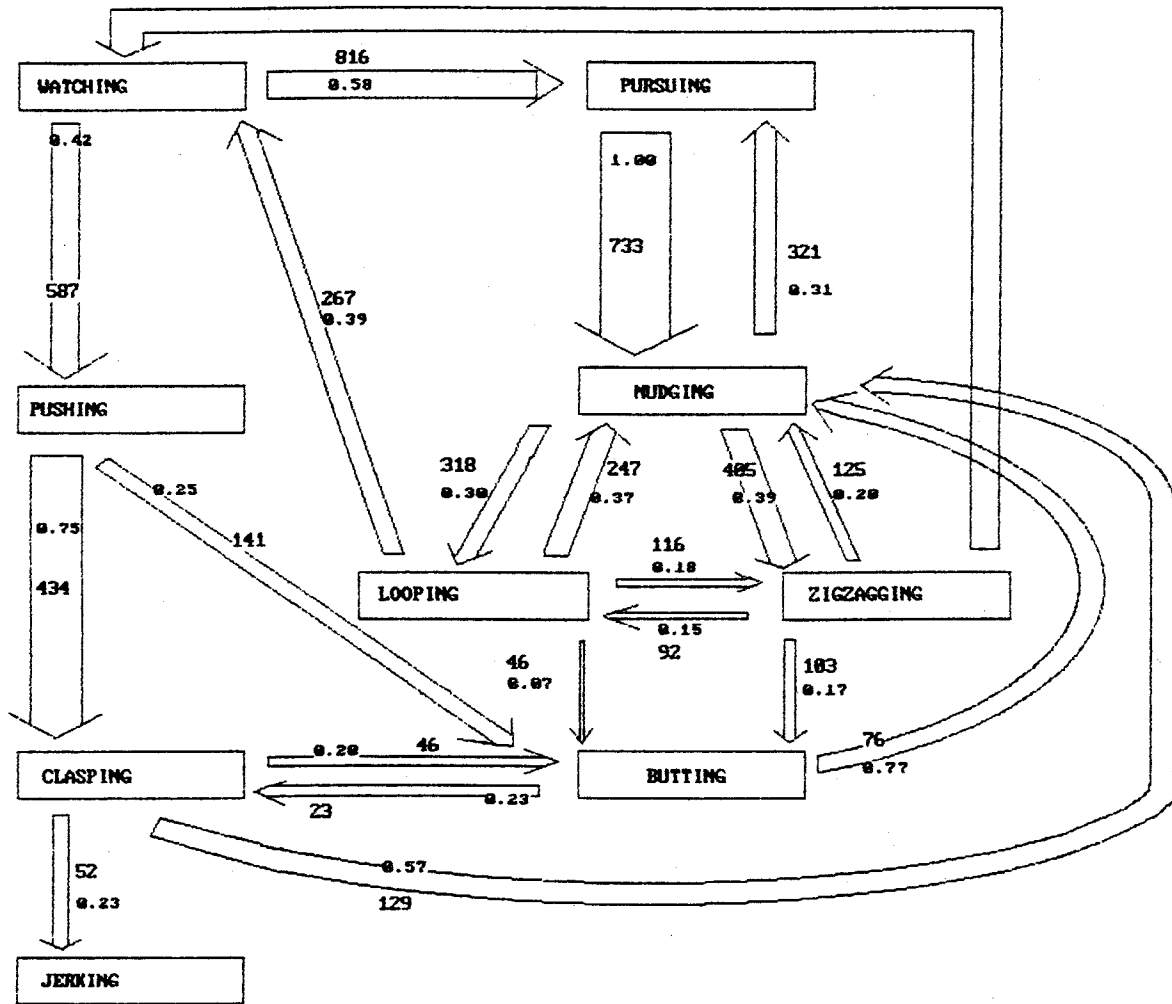


Fig. 2 Ethogram of courtship behaviour in the killifish *Aphantus fasciatus*. Arrow thickness is proportional to the conditional probability for the transition; conditional probabilities are also given as small figures; large figures are number of times the transition was observed.

behaviour by a pair of fish did not always reveal laid eggs. Thus, while courtship is always initiated by the male, it is likely that spawning success is affected mainly by female receptivity. Females often show resistance to courting males by fleeing from the site, or else, they may be induced to participate in the mating preliminaries, but then refuse to spawn. As in many other animal species, courtship behaviour in *Aphantus fasciatus* serves

to synchronize the activities of both partners, ensuring release of ova and sperm within a short interval of time, as well as providing an opportunity for mate choice, thereby reducing wastage of gametes (Halliday, 1983; Wootton, 1984).

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#### ACKNOWLEDGEMENTS

We are grateful to Charles Gauci, officer in charge of the Ghadira Nature Reserve, for supplying fish; to Mark Borg and Carmel Hill for information about the ecology of the Ghadira pool; to Joseph Cilia for his suggestions regarding culturing; and to Sandro Lanfranco for drawing Fig. 2.

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Received 28th November 1991