‘Fasting male-feeding female’ behaviour in *Bombina orientalis* during amplexus

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ABSTRACT – The feeding and reproductive behaviours of *Bombina orientalis* pairs were observed in captivity for approximately one month. Pairs performed amplexus irrespective of their reproductive states. In reproducing pairs, the males rejected food, but the females ate. This particular sexual dimorphic behaviour is termed ‘fasting male-feeding female’ (FM-FF), and it contrasts strikingly with the behaviour of non-reproducing amplectic pairs, in which both the male and female continued to feed.

THE term amplexus literally describes a sort of coupling posture by a male and a female, and in amphibians such behaviour is particularly associated with reproduction. The amplexus of amphibians has been extensively studied since the early observations by Spallanzani in 1786 (Aronson & Noble, 1945), chiefly to elucidate triggering factors and underpinning neurological mechanisms. Very few studies have examined amplexus in the context of its association with feeding behaviour.

Savage (1932) first noticed females’ active feeding during amplexus in the discoglossid frog *Bombina variegata*, although he did not mention instances of feeding by amplectant males. Later, Birkenmeier (1954) clarified the presence of two kinds of amplexi in *B. variegata* and *B. bombina*: the real (echt) amplexus, and the false (scheinbar) amplexus. The real amplexus is restricted to the reproductive season (spring) and the amplectic female actively swims around without leaving the water, or trying to free herself from the male. The amplextant male remains on the female´s back without attempting to feed (Birkenmeier, 1954). The false amplexus takes place any time during the remaining seasons (from summer to winter). The amplectic female swims sluggishly, trying to get out of the water and free herself from the male. Eating and male-avoidance are reported to be common in females involved in this type of amplexus (Birkenmeier, 1954).

In this and other studies, however, no systematic descriptions were made on the occurrence of feeding behaviour in both sexes and for the two kinds of amplexi. We here describe these issues on the basis of captive observations.

In November and December (spring in the Southern Hemisphere) five pairs of adult males and females of *B. orientalis* were housed in individual aquaria. Each of these frogs had been kept under room conditions, and fed a standard diet of insects. Each aquarium was a glass cube of 0.4 m-side with an open top, and was filled with 0.1 m depth of tap water; its bottom was covered with a layer of gravel. A piece of tree bark and a few aquatic plants were placed on the gravel.

During the study period (28–29 days) water temperature was 22°C during the day and 19°C at night. Food items consisted of live insects, *Tenebrio, Zophobas*, or *Acheta*, offered with tweezers daily to each individual frog at ca. 15:00 hr during 28 or 29 consecutive days, except on weekends and holidays. On every occasion, the frogs were persistently stimulated to eat, until they rejected the offered insects.

We recorded the following data: (a) the number of feeding occurrences in each individual; (b) the number of individual insects consumed by each frog at each feeding; and (c) the number of eggs laid by the female of each pair during the observational period. Statistical significances in observed differences were tested using Mann-Whitney U-test. Type I error probability was set at 0.05.

After about 5 to 10 minutes of being housed with a female in an aquarium, each male clasped the female by its groin with his forelimbs, thus initiating amplexus (inguinal). This lasted almost throughout the entire observation period. Occasionally, however, the female freed herself from the male’s grip for relatively brief periods. On such occasions, both the male and the female either alternately approached or withdrew from

each other; otherwise the male clamped the female between his forelegs. This free-state of a pair ended when the male was in close proximity to the female and carried out two sequentially linked actions: (a) rejection of food (b) resumption of amplexus with the female.

The females of four pairs (CII-CV) laid 171–423 eggs during the observation period (Table 1); we refer to these as 'fertile pairs'. During amplexus in these fertile pairs, males invariably firmly pressed their jaws against the females' backs. Bodies of the males were curved in such a way as to locate their cloacae close to the female's cloacae. Males of these fertile pairs consistently rejected the insects offered during the amplexus. In contrast, females readily accepted and ate insects ad libitum every time they were offered to her.

When the fertile pairs (except pair III) were not in amplexus – between 1 to 3 days during the 29 days of observation – both males and females ate the offered insects, and no statistically significant difference due to sex was found in terms of the number of insects eaten (Table 1). The number of insects consumed per feeding event by non-amplectant females was not statistically different from the number of insects consumed per feeding event by amplectant females.

The female of pair 1 did not lay eggs. At variance with the other pairs, the male's body was in a rather horizontal position, being away from the female's body. The male's head did not seem to be pressed firmly against the dorsum of the female.

A week after the beginning of observations, the clasping male of this pair showed an unusual form of behaviour when offered with food: although the male initially avoided insects by pushing them away (using its hindlimbs and/or swimming away), it started to accept them after persistent stimulation (as performed with all males) to accept them. When the male accepted an insect, the female could easily free herself from him; however when the male clasped the female again, he started to reject any food offered to him. From that day on, the male in amplexus ate about one insect every second day until the end of the observational period. The day when the male was out of amplexus he ate one insect, whilst the female ate two.

Although amplectic clasping is necessary for reproduction in most anurans it is not essential, because the false amplexus illustrates the case of the physical embracing of male and female without reproduction.

Table 1. Frequency of feeding occurrences, number of insects eaten, and number of eggs laid in *Bombina orientalis* couples either out of or in amplexus. CI: infertile pair; CII-CV: fertile pair. * = number of times insects were accepted by a frog.

Pair	**Length of observation period (days)**	**Pair behaviour**						**Total number of laid eggs**
		Out of amplexus			*In amplexus*			
		Feeding occurrences*	Number of insects eaten		Feeding occurrences*	Number of insects eaten		
			male	female		male	female	
				Fertile pairs				
C II	29	3	7	13	15	0	67	423
C III	29	-	-	-	16	0	67	384
C IV	29	2	5	4	14	0	41	148
C V	29	1	5	6	11	0	59	171
X		1.5	4.3	5.8	14.0	0.0	58.5	281.5
SE		0.65	1.49	2.72	1.1	0.00	6.1	71.0
				Infertile pairs				
C I	28	1	1	2	10	17	57	0

Despite the low number of frog pairs observed, they show the two kinds of amplexus previously described by Birkenmeier (1954) for *B. bombina*. One is the real amplexus, in which the male firmly grasps the female whatever the kind of stimuli is given to him to free her, and results in ovulation and fertilization. The other is the false amplexus, in which the male releases his grasp on the female when he is stimulated to eat, and no eggs are produced by the female.

Real amplexus in *B. orientalis* is characterised by a reproducing pair in which the male fasts and the female eats. This fasting behaviour of the male seems to be constitutive of the phenomenon because males reject food during the amplexus stage, even when they had been fasting for one whole month (observed in pair III). We propose to call this behaviour 'fasting male-feeding female' (FM-FF), which, when demonstrated by an amplectant pair, is indicative of their reproductive state.

One may wonder what the proximal and ultimate factors are compelling the amplectic male to abstaining from taking food. There are two factors among many possible ones that may explain such behaviour: (a) stress provoked by retaining a firm grasp on the female in order to defend her from other males which presumably would take his position if given the opportunity to do so; (b) Mechanical hindrance to mouth opening due to the firm pressing of his head against the female's back during ovulation.

The fact that the female does not discontinue feeding during the reproductive period could possibly be to offset the expenditure of energy for gametogenesis, which in females is much greater than in males, as found in many reptile species (Bonnet *et al*., 2001, Aubret & Bonnet, 2002, Schneider *et al.*, 2002, Lagarde *et al*., 2003). In natural conditions egg-laying in *B. orientalis* takes place many times during a single reproductive season (spring and summer: Kawamura *et al*., 1972). For this reason, the false amplexus during the reproductive period in the couple CI would be an exceptional event, that deserves to be studied with particular detail.

There are no other published reports of FM-FF in Amphibia. However, one of us (ITM) also observed this behaviour in the leptodactylid frog *Ceratophrys ornata* in captivity. Behaviour analogous to the FM-FF was reported for the rat. In this mammal the presence of a receptive female inhibited food intake in males, but not their sexual behaviour. The latter was neither affected by the offer of food (Saito *et al*., 1999).

In the light of the similarity between proteins and hormones on the one hand, and of the general mechanisms of organic functions in vertebrates on the other hand, the FM-FF behaviour in *Bombina orientalis* seems to be a suitable experimental model for the investigation of the complex linkages between feeding behavior and the liberation of sexual hormones in higher vertebrates.

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