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BRIEF COMMUNICATIONS

Deceptive female oviposition behaviour elicits male ejaculation in the European bitterling

C. Smith*†, Y. Zhu‡§, H. Liu§ and M. Reichard*¶

**Department of Biology, University of Leicester, University Road, Leicester, LE1 7RH, U.K., ‡Graduate School, Chinese Academy of Sciences, Beijing 100039, China, §Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China and ¶Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Květná 8, 603 65 Brno, Czech Republic*

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In European bitterling *Rhodeus amarus*, fish that lay their eggs in the gill chambers of living freshwater mussels, females perform conspicuous behaviours associated with spawning that increases the probability of males performing ejaculatory behaviour and participating in a spawning. A significant positive association was detected between behaviour in which a female performs a spawning action but without releasing eggs, here termed 'deceptive female oviposition', and ejaculatory behaviour by courting males.

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Key words: mating tactics; pre-oviposition ejaculation; signal evolution; sneaking sperm competition.

In many taxa, females mate with several males, and this behaviour can confer indirect benefits to females by increasing offspring fitness (Jennions & Petrie, 2000). A further benefit to mating with multiple males may be to ensure the fertilization of ova, and several empirical studies have shown that female infertility rates in nature can be high (Eberhard, 1996). Explanations for infertility include genetic incompatibility between sperm and ova or sperm depletion in males (Nakatsuru & Kramer, 1982; Wedell *et al.*, 2002). In some species, there may be an intersexual conflict in optimum ejaculate size and ejaculation frequency. The optimal tactic for males may be to distribute ejaculates among several females, while for females the optimal tactic might be for a high level of sperm expenditure that ensures fertilization (Wedell *et al.*, 2002).

European bitterling *Rhodeus amarus* (Bloch) have a promiscuous, resource-based mating system (Smith *et al.*, 2004). Males either guard territories around living freshwater mussels and attract females to deposit their eggs in mussels in

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†Author to whom correspondence should be addressed. Tel.: +44 (0) 116 252 3342; fax: +44 (0) 116 252 3330; email: cs152@le.ac.uk

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their territory, or act as 'sneakers', parasitizing the courtship of territorial males. Large males usually adopt a territorial role while smaller individuals act as 'sneakers'. 'Territorials', however, also sneak in the territories of neighbours and 'sneakers' will sometimes adopt a territorial role if the opportunity arises. 'Territorials' have higher reproductive success than 'sneakers', at least at low male densities (Reichard *et al.*, 2004*a*). Females use long ovipositors to place their eggs onto the gills of a mussel through the mussel's exhalant siphon; males fertilize the eggs by releasing sperm into the inhalant siphon. Female European bitterling base their mate choice on mussel quality as a site for oviposition (mussel species and fullness with other European bitterling eggs and embryos) and male size and colour, with consistent preferences for certain mussel characteristics and for larger and more colourful males (Candolin & Reynolds, 2001; Smith *et al.*, 2000, 2002, 2004). Under natural conditions, most European bitterling spawnings involve 'sneaking' (Smith *et al.*, 2002, 2003) and sperm expenditure per spawning is high (Candolin & Reynolds, 2002; Reichard *et al.*, 2004*a*, *b*). Males mate repeatedly each day throughout the breeding season (Smith *et al.*, 2004), raising the possibility of sperm depletion (Reichard *et al.*, 2004*b*). Notably, pre-oviposition, rather than post-oviposition, ejaculation correlates most closely with male reproductive success in European bitterling (Reichard *et al.*, 2004*a*). Pre-oviposition sperm release occurs in other fish species (Sevenster-Bol, 1962; Pilastro *et al.*, 2002; Le Comber *et al.*, 2004) and may be a widespread, but overlooked, feature of reproduction in fishes.

Female European bitterling suffer reduced fertility in pair spawnings compared with group spawnings, possibly through sperm limitation (Reichard *et al.*, 2004*a*; Smith & Reichard, 2005). Female European bitterling perform behaviours associated with spawning more frequently over mussels when potential sneaking males are in close proximity. One such behaviour is 'deceptive female oviposition', in which a female performs a spawning action but without releasing eggs (Smith *et al.*, 2004). This behaviour was termed 'touching' by Wiepkema (1961), 'skimming' by Smith *et al.* (2001) and as a 'failed spawning attempt' by Candolin & Reynolds (2002). 'Deceptive female oviposition' is a conspicuous behaviour and entails the female making contact or almost making contact with the mussel siphon with the base of her ovipositor, but without inserting the ovipositor into the mussel siphon or releasing eggs. 'Deceptive female oviposition' behaviour is distinct from 'missed' ovipositions, in which the female attempts to spawn but misses the mussel siphon and deposits her eggs on the substratum (Smith *et al.*, 2004). Smith *et al.* (2001) speculated that 'deceptive female oviposition' might have a role in enabling a female to assess mussel quality, though this function has never been demonstrated. Notably, 'deceptive female oviposition' closely mimics spawning behaviour; it attracts the attention of males, and is displayed at a significantly higher frequency by females exposed to more than a single male (Smith & Reichard, 2005; C. R. Pateman-Jones, unpubl. data). Consequently, this behaviour may function to signal to males a female's readiness to spawn, though it is not a prelude to spawning; Smith *et al.* (2001) detected a highly significant negative association between the frequency of 'deceptive female oviposition' behaviour and spawning rate. The aim of the present study was to investigate the pre-oviposition ejaculatory behaviour of male European bitterling in response to 'deceptive female oviposition'. Given a hypothesis that 'deceptive female oviposition' behaviour is used by male European bitterling as a cue for sperm release, two predictions were made. It was first predicted that the frequency of 'deceptive female oviposition' behaviour would correlate positively with male ejaculation frequency. A further prediction was of a significant positive association in the temporal sequence of 'deceptive female oviposition' and male ejaculatory behaviours, with the transition from one behaviour to the other significantly more frequent than expected by chance.

Fish and mussels were collected in April 2003 from the River Kyjovka (fish) and adjacent oxbow (mussels *Unio pictorum* Philipsson) in the Czech Republic and transported to the Institute of Vertebrate Biology, Brno, where experiments were performed during May 2003. Tests were conducted inside mesh observation cages placed in a large outdoor concrete pool (12·4 × 6·0 m, depth of 0·6 m) stocked with 250 adult European bitterling. Observation cages (1·5 × 1·3 × ·7 m) were constructed from rigid netting (mesh-size 5 × 5 mm). They contained two pairs of sand-filled flowerpots in the opposite back corners of the cage (1·1 m apart); each with a single, size matched *U. pictorum* of 81 ± 1·4 mm length (mean ± S.E.). A male European bitterling was haphazardly captured from the pond and released in the cage. Once the male established a territory around both mussels (1–2 h), a female with an extended ovipositor was added to the cage in a square-sided glass box placed in the cage centre, and after 5 min gently released. In addition, a second male fish was confined in a glass box as a stimulus to the territorial male and female. The position of the confined male (left or right) was randomly determined. Other aspects of this study, to investigate female responses to territorial males and rivals, are presented in Smith & Reichard (2005), though no results are repeated in this study. The fish were videoed by a diver until the female spawned, and for at least a further 2 min. Thus, a territorial male, a female and a potential rival of the territorial male were always present in the observation cage. The mean ± S.E. standard length (L_S) of males was 46 ± 1 mm and females 47 ± 1 mm. If the female failed to spawn after 40 min, the experiment was halted. After a spawning, the mussels and fish were removed and replaced. The dimensions of the observation cages enabled males and females to perform all the normal behaviours associated with courtship, territorial defence and spawning that can be observed under natural conditions (Smith *et al.*, 2000, 2001, 2002, 2003, 2004). After completion of the study, fish and mussels were returned to sites from which they were collected.

Only video recordings for trials that resulted in spawnings were scored for behaviour. Behaviour was recorded using a palmtop computer with the FIT-System behaviour recording software; this software provides data on both the frequency of individual behaviours as well as the precise timing (to the nearest second) of each behaviour performed by a subject from the start of behaviour recording, thereby permitting the temporal pattern of behaviour to be analysed.

A total of 58 trials were conducted, of which 36 concluded in a spawning. Of those trials ending in a spawning, only 29 involved either male pre-oviposition ejaculations or 'deceptive female oviposition' behaviour; data from these trials were used to test a correlation between the frequency of ejaculations and 'deceptive female oviposition' behaviour. Including trials in which neither pre-oviposition ejaculation nor 'deceptive female oviposition' behaviour occurred made no difference to the outcome of the subsequent statistical test. In only 19 of the 29 trials, both ejaculatory and 'deceptive female oviposition' behaviour took place over one or both test mussels sequentially. An analysis of the temporal relationship in behavioural sequences between pre-oviposition ejaculation and 'deceptive female oviposition' behaviour in one of two mussels from these 19 trials, using a sociometric transition matrix (Lehner, 1996), was used to test whether 'deceptive female oviposition' and sperm release occurred in sequence at a frequency higher than expected from a null model.

There was a significant positive correlation between the frequency of 'deceptive female oviposition' and male sperm release (Pearson's correlation, data square root transformed, d.f. = 1, 27, $P < 0{\cdot}001$; Fig. 1). In addition, there was a significant positive association in the sequence of 'deceptive female oviposition' and male ejaculatory behaviours, with the transition from 'deceptive female oviposition' to male sperm release significantly more frequent than expected by chance (χ^2 contingency test, d.f. = 1, $P < 0{\cdot}001$).

In pair spawnings, the fertility of female European bitterling is limited by either sperm availability or sperm and egg compatibility (Smith & Reichard, 2005). Females perform behaviours, particularly 'deceptive female oviposition', that increases their conspicuousness to males, and may be adaptive in the context of ensuring fertilization of ova (Smith & Reichard, 2005). In this study, an association was demonstrated between 'deceptive female oviposition' behaviour and ejaculatory behaviour by courting males, with 'deceptive female oviposition' behaviour appearing to serve as a cue for sperm release by males. Though a significant positive correlation between 'deceptive female oviposition' behaviour and ejaculatory behaviour by courting males was detected, and an association between the temporal sequence of these behaviours, other cues may also influence male ejaculation rates. Male European bitterling also use the proximity of rival males to a mussel as a cue for the intensity of sperm competition,

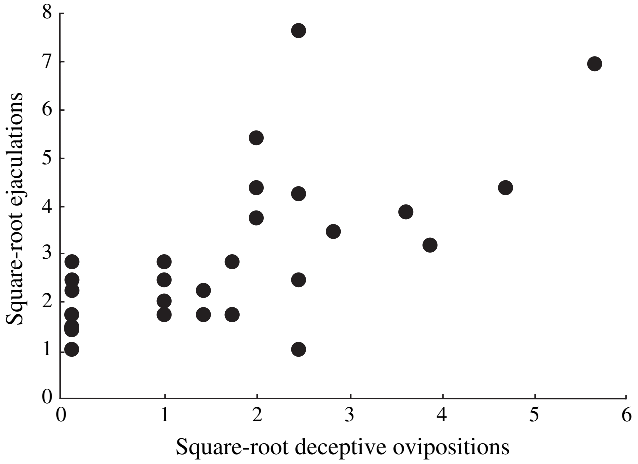

FIG. 1. Plot of the square root of 'deceptive female oviposition' frequency and square root of male pre-oviposition ejaculation frequency.

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responding by either attempting to lead females to other mussels if alternatives are available (Smith *et al.*, 2003) or by sperm loading; increasing their rate of ejaculation into mussels into which a rival has already released sperm (Smith *et al.*, 2002). 'Deceptive female oviposition' appears to be a further cue used by males to adjust rates of ejaculation. 'Deceptive female oviposition' behaviour may enhance the reproductive success of females by ensuring the fertilization of ova, either by increasing the volume of sperm released by a courting male and thereby increasing the chance that sperm and egg meet inside the mussel gill chamber. Thus, 'deceptive female oviposition' behaviour appears to represent a deceptive female signal (Searcy & Nowicki, 2005), since it is not always a prelude to spawning. Further research should explore the evolution of male responses to 'deceptive female oviposition' behaviour.

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