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Original Article

Strategic sperm allocation and a Coolidge effect in an externally fertilizing species

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Because sperm is costly and limiting, males are predicted to allocate sperm differentially across matings, according to the level of sperm competition, female reproductive quality, and female novelty. We investigated sperm allocation in the European bitterling (*Rhodeus amarus*), an externally fertilizing species of fish that spawns and incubates its eggs in the gills of freshwater mussels. We predicted that males would allocate sperm differentially according to the quality and novelty of mussels. Dominant males responded to rivals by increasing both sperm investment and aggression, whereas subordinates responded chiefly through sperm investment. Dominant males invested more sperm in novel mussels in accordance with predictions for a Coolidge effect, the mussel representing a new fertilization opportunity. However, subordinate males were not influenced by mussel novelty but were responsive to order of mating. Males did not allocate sperm according to mussel quality, suggesting that certainty of paternity is a more important variable than offspring survival in shaping bitterling sperm allocation strategies. This study demonstrates strategic ejaculate expenditure in an externally fertilizing species, analogous to that in internal fertilizers, but with sperm investment operating on the level of the site of fertilization rather than the female. *Key words*: ejaculate, mate choice, mating system, mussel, oviposition, sexual selection, sperm depletion. *[Behav Ecol]*

INTRODUCTION

Sperm competition arises when females mate with more than 1 male so that the ejaculates of different males compete simultaneously to fertilize the same set of ova (Parker 1990, 1998; Andersson 1994; Simmons 2001). It represents a form of postcopulatory male–male competition that functions in both internal and external fertilizers (Pitnick and Hosken 2010). Sperm characteristics, as well as those of seminal fluid, can experience strong sexual selection and contribute to the outcome of sexual conflict. A substantial body of empirical and theoretical work over the past 40 years has placed sperm competition at the forefront of efforts to understand mating system evolution (Pizzari and Parker 2009).

Although maximizing ejaculate size may ensure fertilization success, sperm is physiologically expensive to produce and sperm depletion can limit male reproductive success (Dewsbury 1982; Nakatsuru and Kramer 1982; Møller 1991; Van Voorhies 1992; Olsson et al. 1997; Preston et al. 2001; Wedell et al. 2002). Thus, males are predicted to allocate sperm differentially according to the circumstances of each mating in which they participate, that is, the risk and intensity of sperm competition and the quality of the female (Wedell et al. 2002; Pizzari et al. 2003). It also pays males to invest more in females with higher fecundity (Reinhold et al. 2002). However, the fitness gain from any particular female decreases with increasing investment, so it additionally pays to invest in novel females, a phenomenon known as the "Coolidge effect" (Dewsbury 1981; Wedell et al. 2002). Sperm allocation strategies are also mediated by male social status; dominant and subordinate males differ in their ability to attract and monopolize females and experience different risks of sperm competition (Leach and Montgomerie 2000; Pilastro et al. 2002; Cornwallis and Birkhead 2006; Rudolfsen et al. 2006; Parker and Pizzari 2010). Thus, subordinates may experience more intense sperm competition than dominants because dominant individuals are more likely to aggressively exclude rivals from a mating and will thereby experience relatively lower levels of sperm competition (Petersen and Warner 1998). In contrast, subordinates will tend only to have the opportunity to mate in competition with dominants and other subordinates. A prediction of sperm competition theory is that subordinates will experience stronger selection to invest more in sperm production (Parker 1990).

Males from a broad range of taxa that show internal fertilization appear sensitive to both sperm competition risk and intensity, and female status, with males tailoring sperm investment to maximize their long-term reproductive success (Pitnick and Markow 1994; Pound and Gage 2004; Byrne and Rice 2006; Bretman et al. 2009; Parker et al. 1999; Pizzari et al. 2003; Jordan and Brooks 2010; Kelly and Jennions 2011). Pizzari et al. (2003) demonstrated sophisticated status-dependent sperm allocation in the domestic fowl, *Gallus gallus*, with males increasing sperm investment in response to female promiscuity, novelty, and reproductive quality. In the present study, we investigated sperm allocation in a species with external fertilization, the European bitterling, *Rhodeus amarus*, a small freshwater fish that spawns and incubates its eggs in the gills of freshwater mussels, fertilization taking place within the mussel gills. In the context of experimental studies of reproduction, the mussel can, thus, be considered as an extension of the female reproductive tract. Consequently, following Pizzari et al. (2003), we tested the hypothesis that males would allocate sperm differentially among mussels according to the risk

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of sperm competition within the constraints of their social status, and that males would invest sperm in relation to the novelty and quality of mussels, rather than the female. Mussel quality appears to have a greater impact on reproductive fitness than female quality in the bitterling mating system (Smith et al. 2004). Clutch size in bitterling is not correlated with female size (Casalini et al. 2009), but bitterling egg and embryo mortality is strongly density dependent, and mussel species vary in their quality as hosts (Smith et al. 2004).

The bitterling mating system is promiscuous; both males and females spawn repeatedly with multiple partners and in multiple mussels. Dominant males aggressively defend territories to monopolize mussels and lead females to a mussel for spawning (Kanoh 2000; Smith et al. 2004). Females use long ovipositors to place their eggs into the gills of a mussel through the mussel's exhalant siphon. Females inspect mussels before spawning, basing their spawning site choices on mussel and male quality (Kitamura 2005; Casalini et al. 2009; Agbali et al. 2010), with consistent preferences for certain mussel characteristics that relate to enhanced embryo survival (Smith et al. 2004). Males fertilize the eggs by releasing sperm over the inhalant siphon of the mussel, repeatedly in the course of a single mating. Water filtered by the mussel carries the sperm to the eggs where they are fertilized and complete development in 3–4 weeks. Preoviposition sperm releases, whereby males ejaculate into the siphon of a mussel before a female spawns, are a common feature of male courtship and mating tactics. Bitterling sperm remains viable within the mussel gills for a prolonged period, being rich in mucins, and appears capable of fertilizing eggs at least 14 minutes after ejaculation (Reichard, Smith et al. 2004; Pateman-Jones et al. 2011). Those males that control access to mussels enjoy high reproductive success (Reichard, Jurajda et al. 2004; Reichard, Smith et al. 2004; Reichard et al. 2005). Male dominance is determined by size (Casalini et al. 2009), with smaller males adopting alternative mating tactics, although these roles are not fixed and male mating behavior is opportunistic (Candolin and Reynolds 2001; Smith et al. 2002). Males respond to the resulting sperm competition in accordance with theoretical predictions, elevating their ejaculation rate and ejaculate size when competing with a rival (Candolin and Reynolds 2002; Smith et al. 2003, 2004, 2009). For further details on bitterling reproductive biology, see Smith et al. (2004).

We conducted 3 experiments to test the following specific predictions: 1) Males would show status-dependent investment in mussels according to the risk of sperm competition, 2) they would preferentially allocate sperm to novel mussels, and 3) they would preferentially allocate sperm to mussels of superior quality in which offspring survival was greatest. Male sperm investment was not measured directly in experimental trials, and the number of ejaculations was instead used as a proxy. Ejaculation rate, along with other proxy measurements, including sperm volume, copulation duration, number of ejaculations during a mating bout, and number of sperm remaining in a male after ejaculation, appears to be reliable measures of sperm investment in a female or mating (Kelly and Jennions 2011).

MATERIALS AND METHODS

Experimental conditions

Experiments were performed in the aquarium facility at the Institute of Vertebrate Biology, Brno, Czech Republic, during May 2011. Fish for experimental work were collected by electrofishing from the River Kyjovka in the southeast of the Czech Republic and transported to the Institute of Vertebrate Biology. A stock tank of females and males with a range of sizes was set up with a group of mussels in order to encourage competition for territories and the establishment of dominance relationships. Fish were held under natural light conditions and fed twice each day with a mixture of frozen chironomid larvae and commercial flake food. Water temperature matched natural conditions and varied between 18 and 21 °C. Each aquarium contained a sand substrate and artificial plants as refuges. Mussels were collected from Lake Hvězda, a site with abundant mussels but few bitterling, which limited the risk that mussels already contained bitterling eggs. All mussels were checked for the presence of eggs using a mussel-opening device (Kitamura 2005) prior to their use in experiments. Mussels were stored in large outdoor fiberglass tanks (1700 l) with abundant phytoplankton food. All experiments were conducted in aquaria measuring 75 × 40 × 40 cm isolated by opaque barriers.

Risk of sperm competition

A pair of males was placed in each aquarium, together with a female with an extended ovipositor (indicating her readiness to spawn) and a single mussel (*Unio pictorum*) in a sand-filled pot. Each pair of males consisted of 1 large 2-year old and 1 smaller 1-year old male. They were left for a minimum of 1 h to settle and enable dominance to be established, which in all cases resulted in the larger male being dominant. Depending on the experimental treatment (assigned randomly), either the dominant or the subordinate male was captured and placed in a glass box in the corner of the aquarium, or removed entirely. There were, thus, 2 factors each with 2 levels, dominance status of focal fish (dominant/subordinate) and sperm competition risk (rival present/absent). In the no-rival treatment, a nonreproductive female was placed in the glass box as a control; this control enabled us to differentiate between the effect of sperm competition risk and the effect of a third fish in the aquarium. The mussel was replaced to ensure that the behavior of the remaining male was not influenced by sperm released into the mussel prior to the start of trials.

Observations commenced once the focal male had approached and inspected the mussel. The following behaviors were recorded over a period of 10 min: sperm release (male inspects mussel siphon and then sweeps forward and down over it), courtship (male swims toward female and then approaches a mussel while "quivering"), aggression (male attempts to chase the fish in the glass box), male inspection of mussel (the fish positions its snout close to the exhalant siphon of the mussel), female inspection of mussel, and skimming (a common behavior where the female performs a spawning action but does not insert her ovipositor into the mussel siphon or release eggs) (Smith et al. 2007). If a female spawned during the observation period, observations were suspended until the male recommenced courting the female; male behavior changes radically immediately after spawning, the rate of sperm release increasing significantly and he becomes aggressive toward the female, chasing her away from the vicinity of the mussel. Normal courtship behavior resumes after approximately 2-5 min. If the male did not approach the mussel, no behavior was recorded, and the pair was not included in the analysis. Six replicates of each treatment were completed, a total of 24 independent trials.

At the end of the observation period, males and nonspawning females were measured for standard length (tip of the snout to base of the caudal fin) and mussels for total length (maximum length of the shell) and placed in an outdoor stock tank and were not used again in the experiment. There was a limited number of females in spawning condition at any

one time, so these females remained in experimental aquaria for an entire day and thus may have been used in more than 1 observation session. As females only stay in spawning condition for a day at a time, females were measured and replaced each morning. The purpose of the experiment was to observe male behavior, and female identity was treated as a random factor in the analysis. A total of 24 focal males and 14 females were used.

Mussel novelty: dominant males

A 2-year old male and a female in spawning condition were placed in an aquarium together with 2 mussels (*U. pictorum*) adjacent to each other, each in separate sand-filled pot. One mussel was covered with a transparent plastic pot with holes pierced in it, so the fish could not inspect it closely or use it for spawning, although they could see and smell it. The pairs were left for a minimum of 2 h to enable the male to inspect the mussel, release sperm into it, and court the female. Depending on treatment, the covered mussel was uncovered and the original mussel covered (novel mussel), or the already uncovered mussel was disturbed but otherwise left uncovered (familiar mussel).

Observations commenced once the male had approached and inspected the mussel; in each instance, this happened within 10 min of adjusting the mussels. The same behaviors as in the previous experiment (with the exception of aggression as there was no rival) were recorded for a period of 10 min. At the end of this time, the cover was placed over the mussel that had been uncovered during behavior recording. Once the male inspected the uncovered mussel, the behavior of the pair was recorded for a further 10 min. Thus, observations were paired, the behavior of each male being recorded in response to both the original mussel and the new mussel, the order of presentation being determined randomly. A total of 19 paired replicates were completed. Males and mussels were used only once, whereas females were reused while they remained in spawning condition (6 females were used twice).

Mussel novelty: subordinate males

Testing the effect of mussel novelty in subordinates followed the same protocol, with the addition of the following preliminary procedure. Two males (a 1- and a 2-year-old) were placed in the aquarium on the evening prior to experimental observation. In the morning, a female in spawning condition was added to each aquarium in a glass jar next to the mussels. The fish were left for an hour during which time the larger 2-year-old male established dominance. The female was released and allowed to interact (including spawning) with both males. After 2–3 h, the dominant male was captured and removed and the subordinate male allowed to interact with the female for 15–30 min. This procedure ensured that the subordinate male was able to inspect an uncovered mussel and interact with the female but was within the typical time span, based on field observations, that a dominant male may be absent from a mussel in his territory. Experimental observations then commenced as previously described. Mussels and fish, including females, were used only once, and a total of 15 paired replicates were completed.

Mussel quality

Two experiments were conducted using different procedures for manipulating mussel quality, fullness with eggs and embryos, and species identity. Bitterling egg mortality in mussel gills is strongly density dependent, and female bitterling avoid spawning in mussels that already contain high densities of developing eggs and embryos (Smith et al. 2001). To manipulate fullness with eggs, we presented males with mussels of the same species (*U. pictorum*) but differing in the number of eggs already present in the gill chamber. High-quality mussels were stored in an outdoor tank without fish, whereas low-quality mussels were exposed to bitterling spawning for 1 week prior to the experiment.

The experimental protocol was similar to that used for mussel novelty; a male and a female in spawning condition were placed in each aquarium together with 2 mussels (*U. pictorum*), both covered with a transparent plastic pot with holes pierced in it. The fish were left for 90 min during which time they had no access to mussels. At the end of this period, both mussels were removed and replaced with a high- and a low-quality mussel, one of which was covered and one exposed, the order randomly determined.

Once the male began mussel inspections, the same behaviors were scored as previously described for a period of 10 min After this time, the cover was transferred from the covered mussel to the exposed one, and once the male began inspection of the uncovered mussel, behavior was recorded for a further 10 min. A total of 18 paired replicates were completed. Males and mussels were used only once, whereas 4 females were used twice. All fish and mussels were measured to the nearest 1 mm. The gill chambers of low-quality mussels were dissected and bitterling eggs and embryos inside the gills counted. Eggs deposited during the trial, which were obvious from their early stage of development, were not included in the count.

Mussel species vary in their quality as hosts for bitterling eggs, and female bitterling avoid those in which egg mortalities are elevated (Smith et al. 2004). To manipulate mussel quality on the basis of species identity, the focal mussels were *U. pictorum* (preferred, high-quality host) and *Anodonta anatina* (nonpreferred, low-quality host). The experimental protocol was otherwise identical to that used for testing an effect of mussel fullness. A total of 17 paired replicates were completed.

Data analysis

The relationship between male behavior (response variables: male mussel inspection, courtship, ejaculation, and, where applicable, aggression) and treatment was analyzed using linear mixed models (LMM, normally distributed data) or generalized linear mixed models (GLMM, Poisson error structure, log-link function), implemented with the *nlme* (LMM) and *lme4* (GLMM) packages for the R environment (R Development Core Team 2009). Male and female identities were included as random factors where applicable (paired design for males and repeated use of females), and potentially informative variables (female behavior, fish and mussel size) were included as covariates.

For each male behavior, we evaluated the fit of all candidate models using Akaike information criterion corrected for a small sample size (AICc)–based approach in the *MuMIn* package (Burnham and Anderson 2002; Barton 2012). First, we built a set of all candidate models (*dredge* function in *MuMIn* for the global model including all variables). The global model included treatment level as a fixed factor and female behavior (inspection rate and skimming rate) and length of male, female, and mussels as covariates. Where applicable (Experiments 2 and 3), the order of mussel presentation (1st or 2nd) was included as an additional fixed factor. Interactions between fixed factors were included only when biologically meaningful. This included an interaction between dominance and rival presence in Experiment 1 (sperm competition), and interactions between treatments and order of presentation in Experiments 2 and 3. All potential models were ranked

according to their AICc, and ΔAIC (the difference between the AIC of the best model and the compared model) was calculated. A subset of the best models (ΔAIC < 2) was entered in the model averaging procedure (*model.avg* (*model, subset= ΔAIC < 2*)) and used to calculate model-averaged coefficients (estimate, standard error [SE], *z*-value and *P*). For the effect of sperm competition risk on ejaculation rate, there was a single model with ΔAIC < 2, and estimates were calculated solely from this best model. For several male behaviors (courtship rate in the risk of sperm competition and mussel novelty experiments, male inspection in experiments on the risk of sperm competition, and mussel quality in terms of fullness), there was no significant association with explanatory variables; those behaviors were concluded not to be affected by our experimental treatments or covariates. We provide model estimates with their standard errors in the text. Error bars in figures denote 95% confidence intervals. A subset of the best models (ΔAIC < 2) for each analysis is provided as Supplementary Material.

RESULTS

Risk of sperm competition

Males significantly increased their sperm investment when a rival was present (LMM: 0.77±0.27, $z = 2.87$, $P = 0.004$, Figure 1A). There was no effect of social status (−0.41±0.38, $z = 1.07$, $P = 0.284$), but a significant interaction between social status and presence of a rival (1.19±0.43, $z = 2.73$, $P = 0.006$), with subordinate males responding to rival presence with a greater increase in ejaculation rate, was detected (Figure 1B). There was a significant effect of female inspection of a mussel on the rate of male sperm release (0.10±0.02, $z = 5.20$, $P < 0.001$). Males increased their rates of aggression in response to the risk of sperm competition (GLMM: 2.47±0.74, $z = 3.35$, $P = 0.001$), and dominant males were more aggressive than subordinates (2.91±0.69, $z = 4.24$, $P < 0.001$, Figure 1B). There was a significant effect of rival male size on sperm investment (0.30±0.07, $z = 4.06$, $P < 0.001$), with larger rivals eliciting more frequent sperm releases ($r = 0.546$, $P = 0.006$, $n = 24$). The risk of sperm competition did not affect the courtship rate or mussel inspection rate of either dominant or subordinate males (LMM: $P > 0.05$).

Mussel novelty

Dominant males increased their sperm investment when presented with a novel mussel compared with a familiar one (GLMM: 1.46±0.36, $z = 4.11$, $P < 0.001$, Figure 2A). There was a positive effect of female mussel inspection (0.14±0.03, $z = 4.32$, $P < 0.001$) and a negative effect of male length (−0.09±0.04, $z = -2.37$, $P = 0.018$) on sperm release. Subordinates did not significantly increase sperm investment with a novel mussel (LMM: $P > 0.05$) but maintained a high rate of sperm release across both treatments (Figure 2B). The best model included female skimming behavior (1.81±0.44, $z = 4.03$, $P < 0.001$), and there was a significant effect of order of mussel presentation (2.62±0.83, $z = 2.84$, $P = 0.005$), more sperm being invested in the first mussel presented, irrespective of novelty. Both dominant (GLMM: 9.79±1.55, $z = 6.34$, $P < 0.001$) and subordinate (LMM: 5.86±2.51, $z = 2.33$, $P = 0.020$) males spent more time inspecting novel than familiar mussels (Figure 3A,B). Courtship rate did not vary with mussel novelty, social status, or any other covariate (LMM: $P > 0.05$). The mean courtship rate of dominant males (17.8±2.4 SE) was twice that of subordinates (8.3±1.9), although this cannot be formally tested as the data derive from separate experiments.

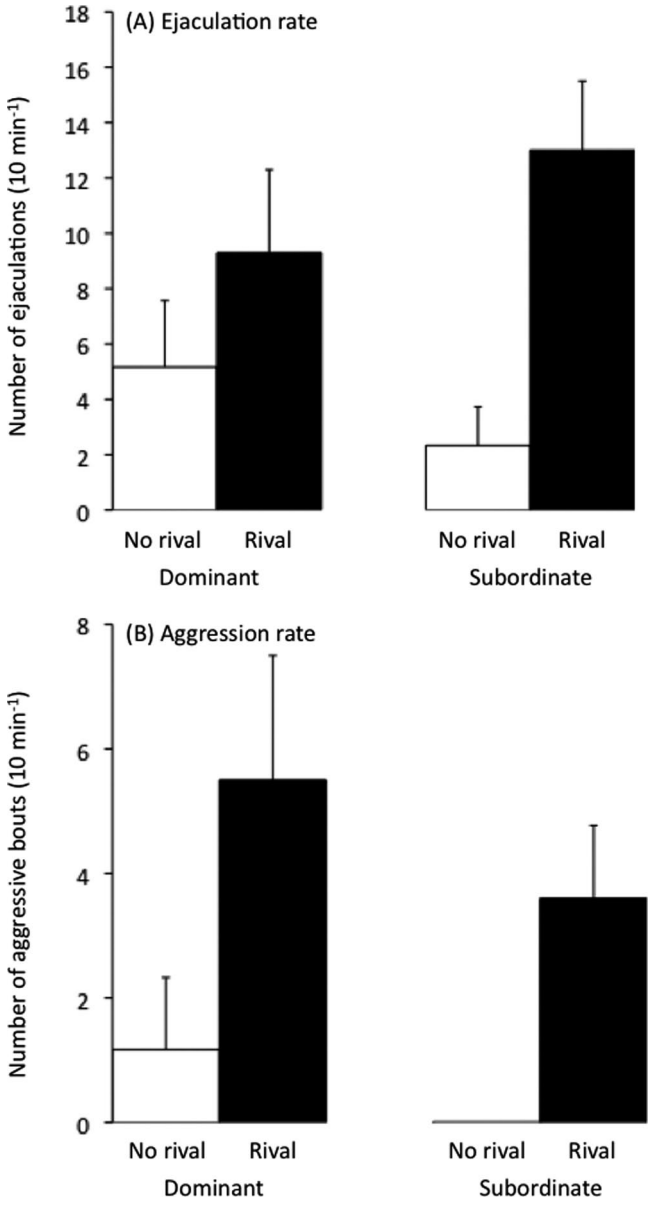

Figure 1
Male response to the risk of sperm competition. Mean number of ejaculations (A) and aggressive bouts (B) during a 10-min observation period of dominant and subordinate males when a rival was absent (white bars) or present (black bar). Error bars denote 95% confidence intervals.

Mussel quality

Females spawned significantly more frequently in high-quality mussels (measured in terms of fullness with eggs and mussel species), preferring those that contained no eggs and *U. pictorum*. However, there was no effect of either measure of mussel quality (fullness with eggs and embryos and mussel species) on any male behavior (GLMM for sperm release, LMM for courtship and male inspection: all $P > 0.05$). Rather, female inspection elicited higher rates of sperm release in both treatments (GLMM: 0.13±0.05, $z = 2.36$, $P = 0.018$ for mussel fullness; LMM: 0.59±0.09, $z = 6.55$, $P < 0.001$ for mussel species), although in the mussel species treatment, order of presentation was also significantly associated with rates of sperm release (−4.60±1.40, $z = 3.34$, $P = 0.001$), and lower rates were observed during the second trial. There were also the effects of

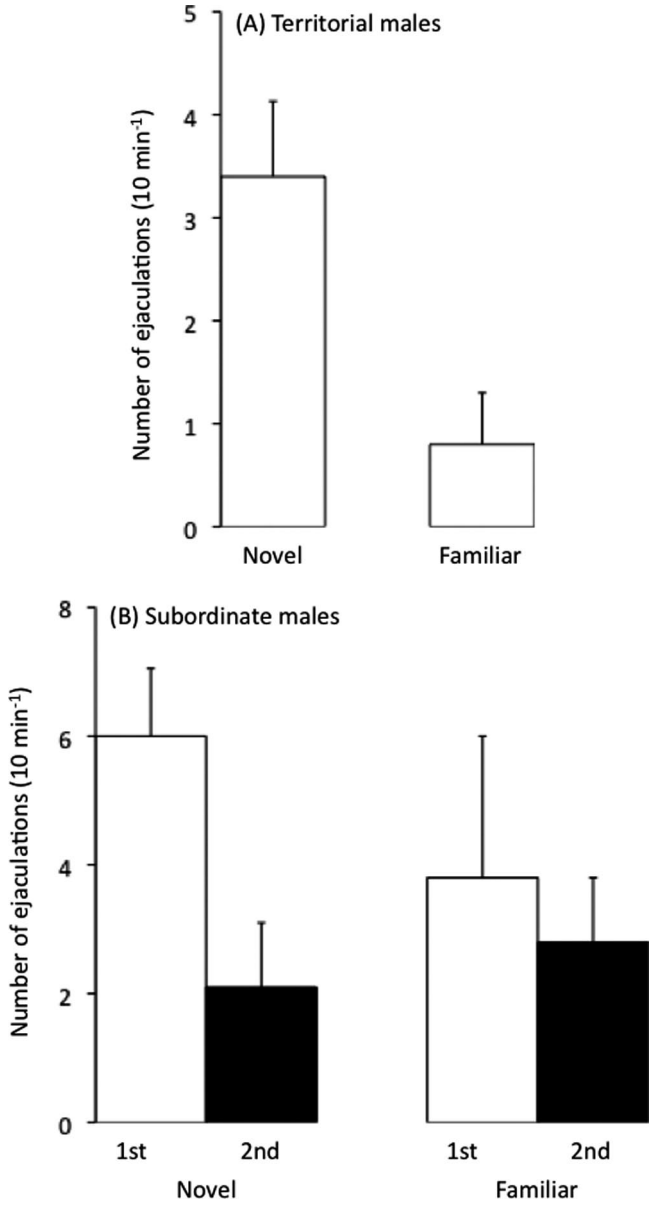

Figure 2
Sperm allocation in response to novelty. Mean number of ejaculations of territorial (A) and subordinate (B) males during a 10-min observation period. For subordinate males, mean values are additionally shown by order of presentation. Error bars denote 95% confidence intervals.

female inspection (LMM: 0.33±0.16, z = 1.98, P = 0.048) and female body size (0.81±0.36, z = 2.25, P = 0.024) on courtship rate in the mussel quality experiment, and male body size (LMM: −1.76±0.71, z = 2.47, P = 0.013) in the mussel fullness experiment. In the species treatment, female inspection rate (LMM: 0.42±0.17, z = 2.46, P = 0.014) and order of presentation (−5.38±2.03, z = 2.65, P = 0.008) also affected male inspection.

DISCUSSION

Our results point toward highly flexible male sperm allocation in European bitterling, sensitive to changes in sperm competition risk, mussel novelty, male status, and female behavior. Dominant males allocated sperm differentially among mussels according to the risk of sperm competition and mussel novelty. Subordinate males also elevated sperm investment when presented with a rival, but, although they showed a behavioral response to mussel novelty, we did not detect a significant difference in their investment in novel and familiar mussels. Males, irrespective of social status, did not differentiate between mussels on the basis of quality.

Both dominant and subordinate males invested more sperm in response to an elevated risk of sperm competition (Figure 1) in accordance with theoretical models (Parker 1998) that is consistent among taxa (Kelly and Jennions 2011). Dominant males were more aggressive than subordinates (Figure 1B), reflecting the fact that dominant and subordinate males consistently experience different levels of sperm competition. Dominant males also showed preferential sperm investment when presented with a new mussel, the stimulus female remaining unchanged (Figure 2A). Thus, the Coolidge effect, whereby males show a decline in sexual interest with one female, which is revived by a new female, appears to operate at the level of the site of fertilization in bitterling.

The Coolidge effect mediates differential sperm allocation, enabling males to keep sperm in reserve in order to exploit new fertilization opportunities (Wedell et al. 2002), and has been demonstrated in several species with internal fertilization, including mammals (Dewsbury 1981), birds (Pizzari et al. 2003), and mollusks (Koene and Ter Maat 2007). In the context of bitterling reproduction, it is the mussel, not the female, that represents a new fertilization opportunity. The response to a new mussel by dominant males, of loading them with multiple ejaculates, appears to be directly analogous to the same behavior observed in internally fertilizing species (Dewsbury 1981; Wedell et al. 2002; Pizzari et al. 2003) in response to new females as a means of distributing sperm adaptively across multiple females. Notably, a Coolidge effect has hitherto never been demonstrated in a species with external fertilization, though the analogy of females with sites of oviposition suggests that species with comparable mating systems to bitterling, including those with discrete nest sites, will also show this response. The cues used by males to recognize mussel novelty are unclear but require a mechanism for recognizing individual mussels. In the case of internal fertilization, female recognition is achieved through visual (Pizzari et al. 2003) or olfactory (Koene and Ter Maat 2007) cues. In the case of bitterling, cues may involve recognition of site-specific odors or the male's own ejaculate.

Subordinate ejaculation rates were high with both novel and familiar mussels (Figure 2B). Although we detected no significant increase in their ejaculation rates in response to novel mussels, subordinate males did spend more time inspecting novel mussels, which suggested that they recognized mussel novelty. Our experimental design demonstrated that subordinates allocated most sperm to the first mussel presented, possibly an opportunistic response reflecting the fact that subordinates typically have fewer mating opportunities than dominant males. This finding accords with that of Cornwallis and Birkhead (2007) who showed that subordinate male fowl were similarly more influenced by copulation order than female reproductive value. These findings lend support to the prediction that dominant and subordinate males have different optima in response to sperm competition. Dominant males modulated ejaculation rates in response to sperm competition risk and mussel novelty, which presumably maximized fertilization success while limiting the chance of sperm depletion (Pateman-Jones et al. 2011; Smith et al. 2009). Subordinates allocated sperm more consistently, thereby maximizing their likelihood of fertilization success during the more limited mating opportunities with which they were presented, and where they faced a relatively higher sperm competition risk (Parker and Pizzari 2010).

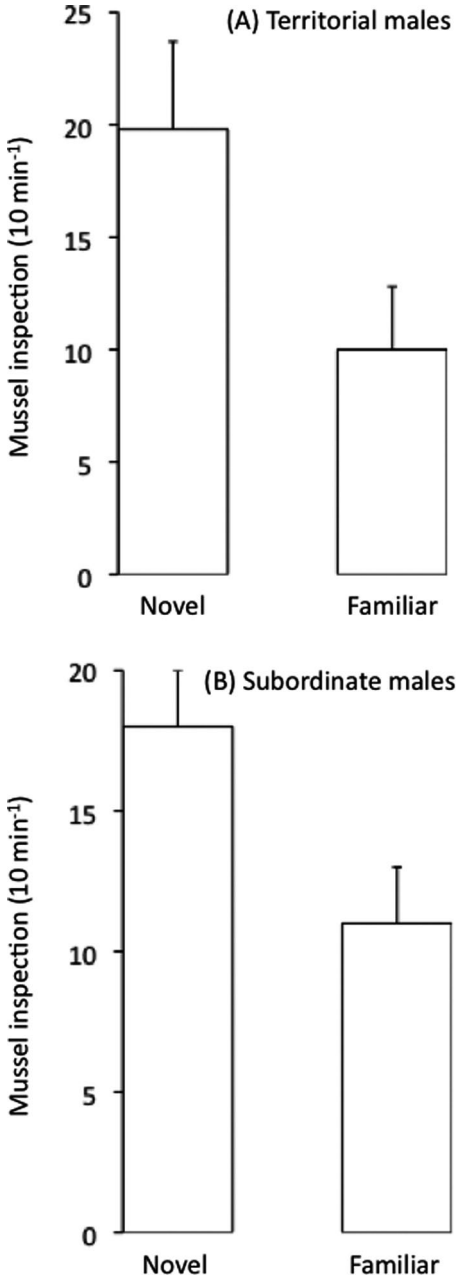

Figure 3
Mussel inspection in response to novelty. Mean number of male inspections of mussels by territorial (A) and subordinate (B) males of novel and familiar mussels. Error bars denote 95% confidence intervals.

Mussel quality appears to have a greater impact on reproductive fitness than female quality in the bitterling mating system (Smith et al. 2001, 2004; Casalini et al. 2009). Female spawning clearly followed the predicted pattern, only occurring in *U. pictorum* containing few or no eggs and embryos. However, male bitterling did not differentiate in sperm allocation among mussels on the basis of their quality as hosts for incubating offspring. The main driver for male responses in this experiment was female inspection rate, with no significant treatment effect. Thus, it is clear that mussel novelty is distinct from mussel quality and the cues for measuring each also appear to be different. The response to novelty relates to the mussel as a new fertilization opportunity rather than its quality as a host. This result suggests that while mussel host quality is an important determinant of female reproductive fitness, because of the prevalence of sperm competition in the bitterling mating system, male fitness may depend more on maximizing fertilization opportunities. This result contrasts with that of Pizzari et al. (2003) in fowl (*G. gallus*), in which males responded to both female novelty and quality. Results from the present study demonstrate that these 2 components of male decision making in sperm allocation are discrete.

In summary, in promiscuous mating systems, males are able to achieve a high reproductive success through strategic ejaculate expenditure. Responses are mediated by male social status, dominant males being more strategic and subordinates more opportunistic. In species with external fertilization, allocation strategies are likely to operate on the level of the site of fertilization rather than the female. The Coolidge effect appears to be a response to a new fertilization opportunity that is independent of variation in reproductive quality.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at http://www.beheco.oxfordjournals.org/

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