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Seasonal change in the opportunity for sexual selection

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Abstract

Environmental and population parameters that influence the strength of sexual selection may vary considerably over the course of the reproductive season. However, the potential for sexual selection frequently fails to translate into variation in reproductive success among individuals. We investigated seasonal changes in variation in reproductive success, measured as the opportunity for sexual selection, using parentage analysis in 20 experimental populations of the European bitterling (*Rhodeus amarus*, Cyprinidae), a small freshwater fish with a promiscuous, resource-based mating system. We showed that although the largest males sired most offspring over the entire reproductive season, variation in reproductive success and hence the opportunity for sexual selection was low at the start of the season but increased significantly at its end. This seasonal difference probably arose from the superior competitive endurance of large males and from a higher temporal clustering of reproductively active females at the start of the breeding season than later in the season. The spatial distribution of oviposition sites had a negligible effect on the variation in reproductive success. We discuss the potential implications of our results for the importance and strength of sexual selection in natural populations.

Keywords: bitterling, body size, intensity of sexual selection, mating success, reproductive skew, *Rhodeus amarus*

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Introduction

The intensity of sexual selection depends on the variation in reproductive success within and between the sexes. Most mating systems are characterized by relatively large variation in reproductive success among males, with male fitness limited by the number of matings, and a smaller variation in females constrained by egg production (Bateman 1948; Clutton-Brock & Vincent 1991; Andersson 1994). Therefore, males can maximize their fitness by controlling access to females, either directly or through monopolizing resources critical to reproduction. The level to which fertilizations can be controlled by individual males is affected by the distribution of fertilizations in space and time. A relative scarcity and high patchiness of mating sites (high spatial clustering) and low reproductive synchrony of females (low temporal clustering) make monopolization of fertilizations by dominant males easier, leading to increased variance in reproductive success and a higher opportunity for sexual selection. In contrast, a high abundance and regular distribution of mating sites (low spatial clustering) and high synchrony of female reproduction (high temporal clustering) make monopolization of fertilizations difficult and are predicted to decrease the intensity of sexual selection (Emlen & Oring 1977; Ims 1988; Shuster & Wade 2003).

The role of the spatial and temporal clustering of fertilizations in underpinning the opportunity for sexual selection across species is one of the cornerstones of mating system theory (Emlen & Oring 1977; Ims 1988; Isvaran & Clutton-Brock 2007). However, there is profound disagreement about their role within species (Jones *et al*. 2001a; Griffith *et al*. 2002; Westneat & Stewart 2003; Singer *et al*. 2006; Lindstedt *et al*. 2007). While there is convincing support for differences in reproductive behaviour among populations with contrasting density and spatial components to the opportunity for fertilizations (e.g. Baird & Sloan 1997; Borg *et al*. 2002), parentage data have shown, surprisingly, that these opportunities do not necessarily translate into variation in reproductive success (Jones *et al*. 2001b; Griffith *et al*. 2002; Singer *et al*. 2006; Lindstedt *et al*. 2007). On a temporal scale, the reproductive synchrony of females has been related

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to paternity control by females in birds (Marthinsen *et al.* 2005), fish (Lindström & Seppä 2001) and mammals (Alberts *et al.* 2006), although the relationship appears to be complex and remains controversial (for discussions see Jones *et al.* 2001a; Griffith *et al.* 2002; Albrecht *et al.* 2007). Although there is good evidence for interannual differences in variation in reproductive success in relation to the temporal (Say *et al.* 2001) and spatial (Twiss *et al.* 2007) distribution of fertilizations in given years, striking changes in mating behaviour related to sexual selection regimes may occur over the course of a single reproductive season (Forsgren *et al.* 2004) and the seasonal pattern of the opportunity for sexual selection using genetic parentage data remains to be tested rigorously (Becher & Magurran 2004).

Reproductive seasonality is often linked to climatic factors, such as ambient temperature, patterns of rainfall, or photoperiod, and the onset of breeding can be rapid once a specific environmental threshold is exceeded. As a result, female receptivity is often highly synchronous (i.e. temporally clustered) at the start of the reproductive season, becoming less synchronized and relatively less frequent as females vary in the time they take to replenish energy reserves for subsequent bouts of breeding (Wootton 1998). Therefore, in accordance with mating system theory, mate monopolization by males is predicted to be more difficult at the start of the reproductive season. Another important aspect of seasonality is a general decline in offspring fitness value over the reproductive season (birth date effect). This effect arises from a competitive advantage to early offspring through a longer growing period and higher juvenile survival (Cargnelli & Gross 1996; Forchhammer *et al.* 2001; Lummaa & Tremblay 2003). Therefore, individual males might allocate disproportionately more energy to their reproductive effort early in the season; even at the expense of losing their high mating status later in the season (Andersson 1994). This strategy may still be adaptive if the birth date effect on offspring fitness outweighs the decline in overall number of progeny sired.

Body size is the most important determinant of dominance hierarchies (Andersson 1994). Large males enjoy higher success in male–male competition for access to females or resources and females also often prefer larger males. Female preference may arise irrespective of male dominance rank and the relative contribution of male size may be context-specific (Andersson 1994; Reichard *et al.* 2005). A further advantage of large body size to males stems from endurance rivalry through the greater energy reserves of larger-bodied individuals that can be allocated to the defence of resources and mate guarding (Judge & Brooks 2001; Lidgard *et al.* 2005). Although females may benefit from larger body size due to increased fecundity, their increase in reproductive success as a function of body size is assumed to be relatively lower than in males (Andersson 1994; Murphy 1998). The large variation in reproductive success among males can also lead to the evolution of alternative mating behaviour, when some males circumvent dominance hierarchies and gain access to fertilizations through forced copulations (Evans *et al.* 2003), sneaking (Jones *et al.* 2001a), deception (Fu *et al.* 2001) or female preference for subordinate males (Ophir & Galef 2003; Watters 2005).

Here, we investigated seasonal changes in variation in reproductive success using 20 experimental populations of the European bitterling (*Rhodeus amarus*, Cyprinidae), a small freshwater fish with a promiscuous, resource-based mating system. We tested the prediction that a high temporal clustering of reproductively active females, characteristic of the onset of the breeding season, would lead to a decrease in variation in reproductive success relative to the end of the season when the temporal clustering of reproductively active females is typically low. We further tested the prediction that variation in reproductive success would be greater when critical resources were clustered and therefore easier to monopolize by dominant males.

Materials and methods

Study species

During the reproductive season, male bitterling establish territories around living freshwater mussels and attract females to deposit their eggs in their territory. Females use long ovipositors to place their eggs into the mussel gills. Larger males establish dominance and control access to mussels, court females and lead them to mussels in their territory. Smaller males are often not able to establish their own territories, especially when mussels are scarce. Males often engage in sneaking behaviour by releasing sperm into a mussel guarded by a territorial male. Male mating behaviour is opportunistic and both large and small males may be territorial and sneak (Smith *et al.* 2004). Territoriality confers higher fitness, but the success of a territorial male is compromised in competition with two or more rivals (Reichard *et al.* 2004a). Experimental manipulation has revealed that female bitterling frequently prefer subordinate males, although a female's control of paternity is severely constrained by resource monopolization by dominant males (Reichard *et al.* 2005). The onset of reproduction in bitterling is initiated by elevated water temperature and increased photoperiod; in Central Europe, reproduction usually begins in late April. A typical breeding season fecundity for female bitterling is 80–250 eggs, which are spawned in several bouts during the approximately 2-month long reproductive season. A spawning bout lasts 1 day and the eggs are laid in 5–12 separate clutches consisting of one to six eggs. Most spawnings occur within a 3-week period at the start of the reproductive season when resting periods between female spawning bouts are shorter than later in the season (Asahina *et al.* 1980; Smith *et al.* 2004).

Bitterling do not provide parental care. Males mate repeatedly each day throughout the breeding season, resulting in a male-biased operational sex ratio (OSR, the number of sexually active males divided by the total number of sexually active adults; Kvarnemo & Ahnesjö 2002) throughout the season (Smith *et al*. 2002, 2004). The male bias in OSR increases as the reproductive season progresses as a consequence of the decrease in the overlap of female spawning bouts (Shimizu & Hanyu 1981; Asahina & Hanyu 1983; Nagata 1985; Smith *et al*. 2004), which is reflected in a significant reduction in spawning rate across the spawning season (see Fig. S1, Supplementary material).

Experiment

Experimental fish were collected from the River Kyjovka (Czech Republic) on 1 March 2005, before the start of the bitterling reproductive season. After a 4-day period of acclimatization in a large fibreglass tank (1700 L) in the garden of the Institute of Vertebrate Biology in Brno (IVB), fish were assigned to experimental treatments. Every fish was measured for standard length (SL, body size excluding the caudal fin), fin-clipped (a small part of lower or upper lobe of the caudal fin was taken and stored in 96% ethanol) and photographed. Individuals of each sex were assigned to three size categories (large, medium, small). The mean ± SE (95% confidence interval) SL was 42.2 ± 0.6 (40.9–43.5) mm for large males, 37.3 ± 0.6 (36.1–38.5) mm for medium males, 31.2 ± 0.6 (29.9–32.5) mm for small males, 41.4 ± 1.0 (39.2–43.5) mm for large females, 36.4 ± 0.7 (35.0–37.8) mm for medium females and 30.9 ± 0.6 mm (29.6–32.2) for small females. Sixty freshwater mussels *Unio pictorum* were collected by hand from an oxbow lake adjacent to the River Kyjovka before the onset of bitterling spawning.

Twenty experimental fibreglass tanks (130 cm × 130 cm) were arranged in the IVB garden in groups of four and filled to a depth of 60 cm with tap water before fish and mussel collection. Each tank was furnished with a thin layer of sand, six artificial plants at fixed positions and three sand-filled plastic pots. A single mussel was placed into each plastic pot. The position of plastic pots was assigned to two treatments according to a predetermined random pattern, with 10 populations per treatment. The spatial distribution of mussels was either regular (the distance between mussels was 120 cm, enabling the potential to establishment of three territories) or clumped (the three mussels positioned 5 cm apart in one corner, allowing formation of only a single territory) (Reichard *et al*. 2004b; M. Konečná, C. Smith, M. Reichard, unpublished observation and pilot study).

Three male and three female bitterling (one of each size class) were released into each tank on 5 April 2005 and allowed to spawn throughout the reproductive season until late July. Fish were fed daily with frozen chironomid larvae and frozen copepods and were also able to forage on algae and small invertebrates that established in tanks within 2 weeks. Water temperature (ranged from 6.5 to 26.2 °C, with a gradual increase from 22 April to 29 May followed by moderate fluctuations thereafter) and photoperiod (a maximum of 17 h of light on 22 June) varied naturally. Every tank was monitored daily (09:00–13:00) and any juvenile bitterling that emerged from mussels were captured using a fine-meshed dip net. Bitterling emerge from mussels at night and shoal near the water surface (Reichard 2002); therefore their collection did not disrupt adult fish. At emergence from mussels, juvenile bitterling are too large to be cannibalized by adults. Juvenile fish were transferred immediately after capture to a plastic box, killed with an overdose of anaesthetic and fixed in ethanol for genotyping. All juveniles (mean 208, range 71–391 per population) were released from mussels between 27 May and 26 August (median date 25 June), which matches natural patterns of release (Smith *et al*. 2000b).

Data analysis

Twenty-five individuals were randomly chosen from the first and the last 20% temporal quantiles of collected offspring in each population and subjected to parental assignment. In three populations, less than 125 offspring were produced, that is yielding less than 25 juveniles in each 20% quantile. In two populations (103 and 114 juveniles), we relaxed the quantiles to include the first and last 25% of offspring. In the third population (71 offspring in total), the interval was relaxed to 35%. All analyses were performed with the relaxed intervals (25 juveniles per replicate) and with strict 20% intervals (with 24, 21 and 14 juveniles per treatment for the three populations and 25 juveniles for the remaining 17 populations). The numerical differences in estimated parameters of variation in reproductive success were negligible and both analyses provided concordant results. We report results for the relaxed intervals throughout the study.

The opportunity for selection on each sex (I_{males}, I_{females}) was used as a measure of variation in reproductive success (Spence *et al*. 2006; Mills *et al*. 2007), calculated as variance in reproductive success of the given sex divided by the square of mean reproductive success of that sex (Shuster & Wade 2003). Equal reproductive success among individuals of a given sex results in a value of zero, with an increase in score demonstrating increased variation in reproductive success. Opportunity for sexual selection (I_{mates}) was calculated as the sum of the differences between the opportunity for selection on each sex ($I_{\mathrm{males}} - I_{\mathrm{females}}$), with a positive score indicating a greater intensity of sexual selection on males and negative on females (Shuster & Wade 2003).

The effects of temporal (start and end of the reproductive season) and spatial (distribution of oviposition sites) treatments on the opportunity for selection on each sex (I_{males}, I_{females}), opportunity for sexual selection (I_{mates}) and differences in reproductive success among size categories of each sex were tested using a general linear model ANCOVA with repeated measures (fixed factor: spatial effect, repeated measure: temporal effect, covariate: paired difference in temporal clustering of reproducing females within populations). One to three females may have been reproductively active on the same day resulting in varying temporal clustering of fertilizations (reproductive synchrony). We were unable to track reproductive episodes for individual females and their overlap because of logistical constraints. Therefore, we calculated a measure of temporal clustering of fertilizations retrospectively by comparing the number of days over which the first and the last 20% of offspring were collected. A paired *t*-test was used to compare the estimates between the start and end of the reproductive season directly. We further correlated variation in reproductive success with estimates of temporal clustering of fertilizations across and within the temporal treatments. Data were tested for normality (Shapiro–Wilk test) and for equality of variance (Bartlett test). All means are given with one standard error. For each statistical analysis, we estimated effect size in addition to conventional significance probabilities. The effect size measures the magnitude of a treatment effect independent of sample size. We used effect size correlation (*r*) (correlation between the independent variable classification and the individual scores on the dependent variable) for comparisons between two groups. The square of the *r*-value can be considered as the percentage of variance explained by the treatment effect. For designs with two or more factors, we used partial eta squared (η^2), which is the proportion of the total variance that is attributed to the treatment effect and is calculated as a ratio of the effect variance to the total variance (Cohen 1988). Data analyses were performed in STATISTICA 6.0 and SPSS 11.5.

Parentage analysis

DNA for 120 parental fish and 1000 offspring was isolated from fin tissue by boiling the tissue at 95 °C in a polymerase chain reaction (PCR) cycler in the extraction solution (25 mM NaOH, 0.2 mM EDTA, pH 12) for 45 min (Truett *et al*. 2000). The solution was then neutralized by adding the equal volume of 40 mM Tris-Cl (pH 5) and 1 µL of the solution was directly used for PCR. In one embryo (population L), DNA extraction was repeatedly unsuccessful and only 49 embryos were analysed in this population. All adult samples were initially genotyped for 12 variable microsatellite loci Rser01–06, Rser08–Rser12 (Dawson *et al*. 2003), and Rser13 for which the primers (forward: 5′–GCATACTGTTAA GCCACCCGC and reverse: 5′–AGCATTGGCAAGGTG GGAGAG) were designed from GenBank sequence no. AJ312848. Based on their informative value and compatibility, five loci were combined in a single multiplex PCR (Rser03, 04, 08, 11, 13), with a mean of 30 (range 4–64) alleles per locus. Mean observed heterozygosity in parental fish was 0.74 (range 0.31–0.93). The mean combined nonexclusion probability estimated in CERVUS 3.0 (Kalinowski *et al*. 2007) was 0.088 for the first parent and 0.019 for the second parent.

Forward primers were labelled with a fluorescent dye (FAM, HEX, NED) and the final concentration of primers in the reaction mixture was 0.15 µM for Rser03; 0.25 µM for Rser04; 0.10 µM for Rser08; 0.20 µM for Rser11; and 0.10 µM for Rser13. The 20-µL reaction volume contained primers for five loci, 1 µL of genomic DNA solution, 1 U of *Taq* polymerase (Fermentas), 1× Mg-free reaction buffer (Fermentas), 0.2 mM dNTPs, and 3 mM $MgCl_2$. The thermal profile of reactions consisted of an initial 3-min denaturation at 94 °C, 30 cycles of 94 °C for 40 s, 61 °C for 30 s, and 72 °C for 60 s, and a final 7-min extension at 72 °C. The PCR products (1 µL) were added to a denaturing mixture of size standard (GeneScan ROX 500, Applied Biosystems) and formamide. After 5-min denaturation in 96 °C and 2-min cooling on ice, the mix was run on the ABI PRISM 3130 Genetic Analyser (Applied Biosystems). The length of the DNA fragments was analysed using GENEMAPPER software (Applied Biosystems).

The observed heterozygosity enabled parental assignment by an exclusion of incompatible paternal and maternal genotypes for all but 13 embryos. For each of the remaining 13 embryos, one parent could be assigned by exclusion. The second parent was assigned using CERVUS 3.0. This procedure was highly effective; logarithm of odds (LOD) scores (log-likelihood that the putative parent is a true parent relative to other candidates) of the second most probable parent in 11 cases were considerably lower than the first (difference in LOD scores: mean ± SE = 1.4 ± 0.3, $n = 11$, range 0.7–3.7), making their assignment as true parents unlikely (Kalinowski *et al*. 2007). However, in two cases (population T) two females had similar LOD scores (difference < 0.5) for the same offspring and maternity was conservatively assigned to a female with the higher absolute LOD score. No mismatches between parents and offspring (genotyping errors or mutations) were observed. A detailed table with the number of offspring from all possible male–female pairings is included as Supplementary material (Table S1).

Results

Variation in reproductive success

The opportunity for selection on males was significantly lower at the beginning of the reproductive season than at the end (GLM ANCOVA with repeated measures, $F_{1,17} = 22.93$, $P < 0.001$, $\eta^2 = 0.574$; Fig. 1a). There was no significant difference in the opportunity for selection on males with regard to the spatial distribution of mussels ($F_{1,17} = 2.33$,

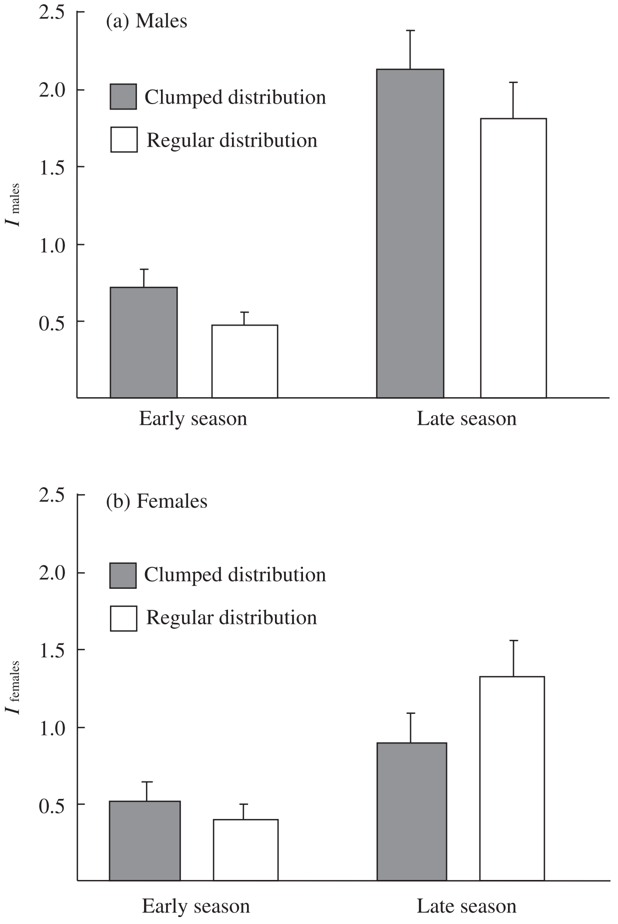

Fig. 1 Mean (+ 1 SE) opportunity for selection in (a) male and (b) female bitterling with regard to the spatial (clumped distribution of oviposition sites, dark columns; regular distribution, open columns) and temporal (start and end of reproductive season) distribution of fertilizations.

$P = 0.145$, $\eta^2 = 0.120$), although numerically higher values of I_{males} were obtained from populations where mussels were clumped (Fig. 1a). The difference in the temporal clustering of fertilizations was not a significant covariate ($F_{1,17} = 0.46$, $P = 0.505$, $\eta^2 = 0.027$). The interaction between temporal and spatial treatments was nonsignificant ($F_{1,17} = 0.04$, $P = 0.849$, $\eta^2 = 0.002$).

Like that for males, the opportunity for selection on females was significantly lower at the start than at the end of the reproductive season (GLM ANCOVA with repeated measures, $F_{1,17} = 9.03$, $P = 0.008$, $\eta^2 = 0.347$; Fig. 1b). The spatial distribution of mussels had no effect on estimates of I_{females} ($F_{1,17} = 0.23$, $P = 0.639$, $\eta^2 = 0.013$), although there was a significant interaction between factors ($F_{1,17} = 4.49$, $P = 0.049$, $\eta^2 = 0.209$; Fig. 1b); I_{females} was significantly higher at the end of the reproductive season with a regular mussel distribution than at the start, although not if mussels were clumped. Difference in temporal clustering of fertilizations was not a significant covariate ($F_{1,17} = 2.55$, $P = 0.128$, $\eta^2 = 0.131$).

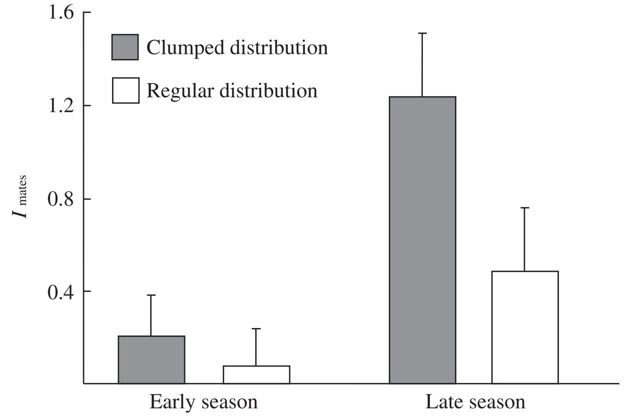

Fig. 2 Mean (+ 1 SE) opportunity for sexual selection in bitterling with regard to the spatial (clumped distribution of oviposition sites, dark columns; regular distribution, open columns) and temporal (start and end of reproductive season) distribution of fertilizations.

The opportunity for sexual selection (I_{mates}) significantly increased at the end of the reproductive season (GLM ANCOVA with repeated measures, $F_{1,17} = 7.27$, $P = 0.015$, $\eta^2 = 0.299$; Fig. 2). Estimates of I_{mates} were numerically higher when mussels were clumped, although this was not statistically significant ($F_{1,17} = 2.49$, $P = 0.133$, $\eta^2 = 0.128$). There was no significant interaction between temporal and spatial scales ($F_{1,17} = 2.06$, $P = 0.169$, $\eta^2 = 0.108$; Fig. 2) and difference in temporal clustering of fertilizations was not a significant covariate ($F_{1,17} = 0.58$, $P = 0.456$, $\eta^2 = 0.033$).

The effect of body size

Large males sired a significantly higher proportion of offspring compared to medium and small males (GLM ANCOVA with repeated measures, $F_{2,53} = 87.57$, $P < 0.001$, $\eta^2 = 0.768$; Tukey's honestly significant difference (HSD) post-hoc tests, large vs. medium: $P < 0.001$, $r = 0.809$, large vs. small: $P < 0.001$, $r = 0.781$, small vs. medium: $P = 0.165$, $r = 0.245$). There was a significant effect of season on the reproductive success of males from the three size classes ($F_{2,53} = 8.99$, $P < 0.001$, $\eta^2 = 0.253$), with the success of large males higher at the end of the season at the expense of medium and small males (Fig. 3a). There was no significant effect of the spatial distribution of mussels ($F_{2,53} = 0.67$, $P = 0.515$, $\eta^2 = 0.025$) nor an interaction between spatial distribution and size ($F_{2,53} = 0.09$, $P = 0.911$, $\eta^2 = 0.003$).

Large females produced significantly more offspring than small females (GLM ANCOVA with repeated measures, $F_{2,53} = 3.52$, $P = 0.037$, $\eta^2 = 0.117$; Tukey's HSD post-hoc tests, large vs. small: $P = 0.031$, $r = 0.296$, large vs. medium: $P = 0.120$, $r = 0.218$, small vs. medium: $P = 0.695$, $r = 0.108$). The effect of season on the reproductive success of the three

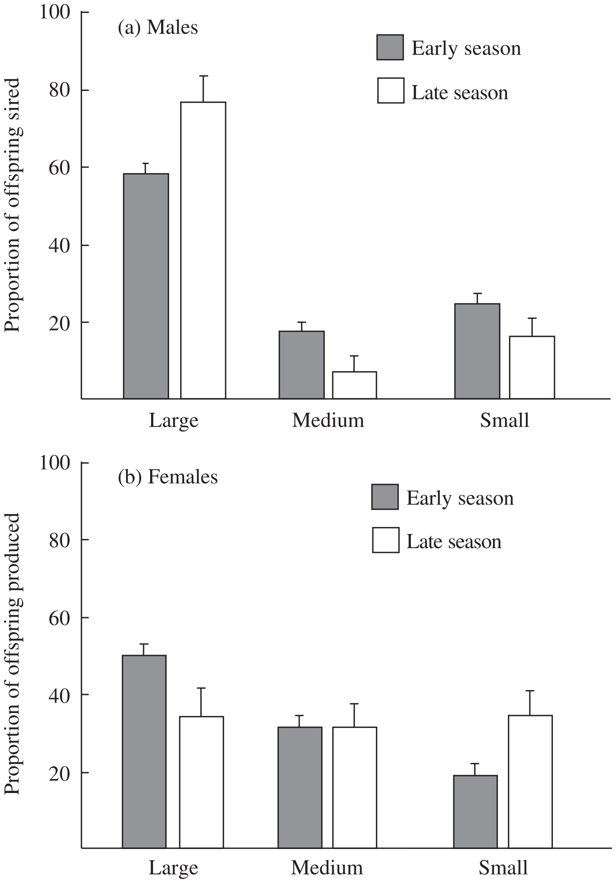

Fig. 3 Mean (+ 1 SE) proportion (in percent) of offspring sired by individual (a) male and (b) female bitterling in relation to the body size categories at the start (dark columns) and end (open columns) of the reproductive season.

female size classes was also significant ($F_{2,53} = 6.09$, $P = 0.004$, $\eta^2 = 0.187$), with a difference among size classes at the start of the season but no difference at the end of the reproductive season (Fig. 3b). The spatial distribution of mussels had no effect on differences in reproductive success among female size classes ($F_{2,53} = 0.40$, $P = 0.673$, $\eta^2 = 0.015$) and there was no interaction between spatial distribution and size ($F_{2,53} = 0.50$, $P = 0.609$, $\eta^2 = 0.025$).

Temporal clustering of fertilizations

The first 20% of offspring were released from mussels over a significantly shorter period than the last 20% (paired *t*-test, $t_{19} = 5.04$, $P < 0.001$, $r = 0.756$). The mean number of days was 11 ± 1.2 (95% CL: 8.6–14.3 days) for the start of the season and 24 ± 1.9 (95% CL: 20.3–28.0 days) for the end of the season. Across the entire reproductive season, the standardized number of days for the production of the first or last 20% of offspring positively correlated with estimates of the opportunity for selection on each sex (Pearson correlation, I_{males}: $r = 0.565$, $N = 40$, $P < 0.001$; I_{females}: $r = 0.456$, $N = 40$, $P = 0.003$), but not with the opportunity for sexual selection ($r = 0.272$, $N = 40$, $P = 0.089$). This result indicates that the variation in reproductive success within a given sex was high when the temporal clustering of reproductive females was low. There was no relationship between temporal clustering of fertilizations and I_{males}, I_{females} or I_{mates} during early and late phases of the breeding season (all $N = 20$, all $P > 0.219$).

Discussion

While it has been well established that factors responsible for the strength of sexual selection may vary considerably over the course of a reproductive season (Forsgren *et al.* 2004), the potential for sexual selection need not necessarily translate into variation in reproductive success (Jones *et al.* 2001b; Griffith *et al.* 2002; Singer *et al.* 2006; Lindstedt *et al.* 2007). Here, we used parentage analysis in 20 experimental populations of bitterling to show that the opportunity for sexual selection was relatively low at the start of the reproductive season, but increased significantly by the end of reproductive season. This has important consequences for the overall strength of sexual selection in natural populations, because many species reproduce over an extended breeding season (Hendry & Day 2005) although offspring survival (and hence their fitness value to parents) is often considerably higher early in the season (Cargnelli & Gross 1996).

Seasonal variability in the intensity of sexual selection may depend on changes in the OSR (Forsgren *et al.* 2004) and the underlying economic defendability of mates or resources critical to reproduction (Warner & Hoffman 1980; Quinn *et al.* 1996; Reichard *et al.* 2004a), changes in the strength or direction of mate choice (Kokko & Mappes 2005; Borg *et al.* 2006), sperm depletion (Gage *et al.* 1995; Preston *et al.* 2001) or endurance rivalry (Judge & Brooks 2001; Prenter *et al.* 2003; Lidgard *et al.* 2005). Likewise in other fishes (e.g. Quinn *et al.* 1996), in the bitterling, there is a male-biased OSR throughout the breeding season (Smith *et al.* 2004), despite an equal population sex ratio (Smith *et al.* 2000a). All males are always ready to mate while females go through stages of receptivity (1 day) followed by periods of reproductive quiescence to replenish their reserves for subsequent ovulations (Shimizu & Hanyu 1981; Smith *et al.* 2004). The peak of reproductive activity occurs early in the season (Asahina *et al.* 1980; Nagata 1985; Smith *et al.* 2004; Kitamura 2007), with an increase in the duration and variation in the intervals between female spawning bouts as the reproductive season progresses. This effect is widely recognized in fishes (Wootton 1998) and other taxa (Banks & Thompson 1987; Verboven *et al.* 2001), and may arise from complex effects of ambient temperature and photoperiod on ovulation (Asahina & Hanyu 1983) and the physiological costs associated with reproduction (Hirschfield 1980; Townshend & Wootton 1984). This increase in the interspawning interval results in progressive increase in male bias in the OSR later in the breeding season, which may potentially affect the strength of sexual selection in both directions (Kokko & Monaghan 2001).

We observed increased opportunity for sexual selection at the end of the season because of the higher reproductive success of large, presumably dominant, males. The alternative outcome was a reduction in the opportunity for sexual selection, resulting from a decrease in the economic defendability of resources (Quinn *et al*. 1996; Reichard *et al*. 2004a, b) or sperm depletion of dominant males (Preston *et al*. 2001), although this was not observed. The contribution of seasonality in female choosiness (Borg *et al*. 2006) to the overall strength of sexual selection in bitterling is likely to be negligible, because of constraints imposed by male dominance (Reichard *et al*. 2005). Female bitterling base their choice on the quality of host mussels (Smith *et al*. 2000b; Candolin & Reynolds 2001) and although dominant bitterling males are not always preferred by females, they enjoy high reproductive success by monopolizing high quality resources (Reichard *et al*. 2005). Superior endurance rivalry of large males, leading to their increased success at the end of the season, likely significantly contributed to the observed seasonal pattern of variation in reproductive success (Judge & Brooks 2001; Prenter *et al*. 2003). Hence, we hypothesize that the observed higher opportunity for sexual selection at the end of the reproductive season was a consequence of a superior endurance of large males and their increased ability to control fertilizations at lower temporal clustering of matings (Ims 1988; Lidgard *et al*. 2005). The relative contribution of the two factors cannot be untangled using the present experimental design, but is a subject of our ongoing research.

We also predicted that the spatial clustering of oviposition sites might affect variation in reproductive success, although this effect was negligible. At our low spatial clustering treatment, mussels were separated by a distance of 120 cm which is enough for independent territories to be formed and guarded by individual males (Reichard *et al*. 2004b). The lack of a significant effect may have arisen from: (i) a female preferences for mating with large males, (ii) a female preference for mating in the territory with the highest mussel quality that the largest male may have usurped, (iii) successful sneaking by large males in rival territories, or (iv) failure of small and medium males to establish territories in some populations with a regular mussel distribution. The four explanations are not mutually exclusive and all may have contributed to the observed pattern. Female bitterling generally mate more often with larger males (Smith *et al*. 2002), but the relationship between male size and female preference is complex. Experimental studies have shown that courtship vigour, irrespective of male body size, is the target of female choice, although larger males generally tend to court more vigorously (Reichard *et al*. 2005). Large males are also less likely to suffer disruptions to mating and occupy the best territories (Taborsky 1998; Smith *et al*. 2002; Reichard *et al*. 2007). Because the quality of their spawning sites declines with an increasing number of eggs deposited there (Smith *et al*. 2000b, 2001; Mills & Reynolds 2002), large males may change the location of their territories at the expense of smaller males, thereby severely reducing the ability of smaller males to attract a female. Sneaking is traditionally considered as 'making the best of the bad situation' and males resorting to sneaking are often viewed as inferior and unable to attract female themselves (Taborsky 1998; Jones *et al*. 2001a). However, large bitterling acting as territory holders often attempt to steal fertilizations in neighbouring territories (Smith *et al*. 2004). If large males are more successful in sneaking fertilizations in rival territories as well in preventing other males from sneaking in their own territory (Griffith *et al*. 2002; Le Comber 2004), it may further decrease the effect of the spatial arrangement of resources on variation in reproductive success, while increasing the overall opportunity for sexual selection (Albrecht *et al*. 2007). In this study, we could not systematically collect data on individual male spawning behaviour and territoriality, and we were also unable to compare offspring production from different territories or track territory ownership over the season. Consequently, we cannot directly link territory ownership and sneaking to the absence of an effect of mussel spatial distribution on variation in male reproductive success. Nevertheless, some males never become territorial, even if the number of vacant territories highly exceeds the number of males (Smith *et al*. 2004), making such a scenario plausible.

Large males sired most (67%) offspring over the entire reproductive season. Large females produced most (50%) offspring at the beginning of the season, but there was an equal share of offspring among female size classes at the end of reproductive season. While body size is one of the main traits under sexual selection in males (Andersson 1994), the higher proportion of offspring of large females early in the season may not be related to sexual selection (Becher & Magurran 2004). Larger female fish are generally more fecund than small females (Wootton 1998). In bitterling, mean estimated fecundity of females was 222, 168, and 118 eggs for large, medium, and small females respectively (calculated from the size-fecundity relationship of Smith *et al*. (2000a), using mean standard length for each size class). The higher fecundity of larger females is manifested as larger individual clutches, more eggs laid during spawning bouts and more clutches laid per season (M. Reichard, unpublished data). Consequently, larger females produced more eggs irrespective of the sexual selection regime and the higher proportion of offspring produced early in the season may simply be related to physiological constraints on oogenesis. Alternatively, males may preferentially court large females early in the season (Pélabon *et al*. 2003) and become less choosy as the OSR becomes more male biased later in the season.

Our parentage data, used for estimates of variation in reproductive success, are based on juvenile fish that successfully departed their mussel hosts. After fertilization, bitterling embryos suffer mortalities inside the mussel gill chamber from suffocation and ejection by mussels (Smith *et al*. 2004; Kitamura 2005). The mortality rates within the mussel host are density dependent, and typically range from 20% to 80% (Smith *et al*. 2000a; Mills & Reynolds 2002), which agree with estimates for our experimental populations (mean 64%, range 41–88%; M. Reichard, unpublished data). Other important sources of juvenile mortality in bitterling are predation by perch *Perca fluviatilis* L. and over-winter mortality (Smith *et al*. 2000a). Mortality at the juvenile stage is strongly affected by the birth date, with a disadvantage to later born offspring, which suffer interference competition from older bitterling and are forced to forage outside the safety of vegetated habitat where they are susceptible to predators (Smith *et al*. 2006). Hence, offspring produced at the start of the reproductive season (when the opportunity for sexual selection was weaker) contribute most to recruitment. This effect may have significant implications for estimates of the overall importance of sexual selection, effective population size, the opportunity for selection on male size and microevolutionary consequences (Hendry & Day 2005; Kokko & Rankin 2006; Mobley & Jones 2007; Ritchie *et al*. 2007; Seamons *et al*. 2007). It is likely that similar patterns of seasonal change in variation in reproductive success are widespread in other mating systems without parental care, which are common in fish, anurans, reptiles and invertebrates.

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Supplementary material

The following supplementary material is available for this article:

Fig. S1 Mean (+1 SE) number of bitterling eggs ejected per mussel in nine independent populations in the Czech Republic over four months in 1996.

Table S1 Experimental data from 20 populations (A to X) that were arranged in two treatments according to spatial distribution of spawning sites (clumped, regular).

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