Behav Ecol Sociobiol (2004) 56:34–41
DOI 10.1007/s00265-004-0760-2

ORIGINAL ARTICLE

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Male-male interference competition decreases spawning rate in the European bitterling (*Rhodeus sericeus*)

Received: 11 July 2003 / Revised: 13 January 2004 / Accepted: 15 January 2004 / Published online: 10 February 2004

Abstract We investigated the consequences of male-male interference competition associated with alternative male mating tactics in a freshwater fish, the European bitterling (*Rhodeus sericeus*). Male bitterling defend territories around living mussels and attract females to lay their eggs in the gill cavities of mussels. We experimentally manipulated spawning-site abundance and male density at two spatial scales. We showed that the total number of eggs spawned by females was constrained by the number of mussels available for oviposition. The effect was mediated by behavioral interactions among competing males because of variation in the Operational Sex Ratio (OSR) in close proximity to a mussel and not by a direct limitation in mussel capacity to accommodate the eggs. Both total and local male densities affected spawning behavior, and interacted in their effect on female spawning rate. Territorial male aggression caused courtship interruptions that prolonged the time until successful spawning and increased with male density. However, territoriality broke down at the highest male density, with a consequent stabilizing effect on spawning rate.

Keywords Alternative mating tactics · Density dependence · Operational sex ratio · Sperm competition · Reproduction

Communicated by J. Krause

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Introduction

Intra-sexual variation in reproductive behavior is common in many taxa (Taborsky 1994; Gross 1996; Birkhead and Møller 1998), and usually arises because of either frequency- or condition-dependent selection on males (Gross 1996). Such variation in mating behavior can lead to the emergence of alternative mating tactics, which are often associated with sperm competition (Parker 1970). In many species, males have evolved counter tactics to avoid sperm competition with rival males. These include mate guarding (Parker 1970; Alonzo and Warner 2000), sperm loading (Parker et al. 1996; Candolin and Reynolds 2002; Smith et al. 2002) and aggression towards rivals (Le Boef and Peterson 1969; Warner and Hoffman 1980). This complex of behavioral adaptations and counter-adaptations has clear direct consequences for individual fitness but may also affect population parameters (Mysterud et al. 2002).

Although population consequences of individual behavior have attracted considerable theoretical interest (e.g. Sutherland 1996; Anholt 1997; McNamara 2001), attempts to predict them for specific natural systems are scarce. Rose (1959) demonstrated that in artificial populations of guppies (*Poecilia reticulata*), cannibalism had direct benefits for cannibals, while regulating population size. In European bitterling (*Rhodeus sericeus*), Smith et al. (2000a) showed that females discriminated among spawning sites in relation to the probable survival of embryos. Using game-theory models, Smith et al. (2000b) demonstrated that female oviposition decisions had significant consequences for bitterling population size. Similarly, Spencer et al. (2002) showed that females of the mosquito *Culiseta longiareolata* avoided ovipositing in pools containing a predatory backswimmer *Notonecta maculata*. These decisions increased individual female fitness, and had the effect of increasing the equilibrium population size relative to populations of individuals unable to discriminate between oviposition sites.

However, in establishing whether behavior can affect population processes, it is also important to identify at what scale the effects of behavior are manifested (Ray and Hastings 1996; Mysterud et al. 2002). For example, Addicot et al. (1987) found that in the aphid-fireweed system, population parameters were affected by processes that occurred at a variety of scales; from behavioral interaction at the individual scale, competition for resources at the ramet scale, to competition for the services of ants at the fireweed clone scale. In the bluehead wrasse (*Thalassoma bifasciatum*), Warner and Hoffman (1980) showed that territorial male mating success and mating pattern varied with population density, sex ratio and the proportion of territorial and non-territorial males in the population. These factors interacted, with contrasting effects on small and large reefs (Warner and Hoffman 1980). These studies suggest that a hierarchical density scaling might better explain observed variability in the impacts of density dependence. Indeed, identification of density-dependent processes may vary according to the density scale at which they are tested (e.g. Poizat and Ponton 1996; Ray and Hastings 1996), with local density being essential for detecting behavioral interactions (Wiens et al. 1987).

The operational sex ratio (OSR) is the ratio of males to females that are ready to mate in a population. The OSR has been recognized as a central concept in understanding variation in mating competition (Emlen 1976; Kvarnemo and Ahnesjö 2002). A biased OSR usually increases intrasexual competition for matings, which can affect mating tactics, reproductive success and mate choice (Kvarnemo and Ahnesjö 2002).

Here, we investigate the consequences of male-male interference competition in the European bitterling (*R. sericeus*), a small cyprinid fish that lays its eggs on the gills of freshwater mussels. Competition among male bitterling over fertilizations frequently arises because of alternative reproductive behaviors between and within mating tactics (Smith et al. 2002). During the spawning season, female bitterling develop a long ovipositor to place their eggs onto the gills of a mussel through the mussel's exhalant siphon. Males defend small territories around mussels and attract females to spawn. Females forage in large shoals and visit male territories when they are ready to spawn. If a female with an extended ovipositor (i.e. ready to spawn) enters a male territory, he performs characteristic courtship behavior and leads her to a mussel. Once over the mussel, the male releases sperm into its inhalant siphon, so that water filtered by the mussel carries his sperm to the gills. The female inspects the exhalant siphon of the mussel, whilst the male continues to court her, aggressively attacking any intruders to his territory. If the female chooses to spawn in the mussel, she quickly inserts her ovipositor into the exhalant siphon and deposits one to six eggs. Once the female spawns, the male repeatedly releases sperm into the inhalant siphon and aggressively chases away the female and any intruders (Wiepkema 1961; Smith et al. 2004).

Alternative mating behavior is common in bitterling (Smith et al. 2004). While some males defend territories and attract females, other males adopt sneaking behavior. Both males that do not defend territories and males in adjacent territories often attempt to release their sperm over the inhalant siphon of mussels guarded by territorial males, before and after a female has spawned (termed pre- and post-oviposition ejaculation, respectively). On occasions, up to 60 males may release sperm into a mussel during a single spawning (Kanoh 1996; Smith et al. 2002). Territorial males respond to the presence of competing males by varying sperm expenditure and aggression directed towards competitors (Nagata 1985; Candolin and Reynolds 2002; Mills and Reynolds 2003; Smith et al. 2003).

The spawning season of *R. sericeus* is determined by photoperiod and water temperature; in our study area, spawning lasts from late April to late June. However, a peak of spawning usually occurs within a 3-week period, typically during May. Female bitterling spawn in bouts lasting 1–3 days (separated by a resting interval of 5–7 days) and are capable of spawning on up to five occasions on the same day at least (Smith et al. 2004). The typical breeding-season fecundity in the study population is between 80 and 250 eggs per female (Smith et al. 2000a). For the population of bitterling used in the present study, up to 257 bitterling embryos have been found in a single mussel (Smith et al. 2004), with up to 151 deposited in a mussel over 24 h (M. Reichard and C. Smith, unpublished data). Bitterling lay their eggs in all four species of unionid mussel occurring in the study area, but show preferences for *Unio tumidus*, *U. pictorum* and *Anodonta anatina* over *A. cygnea* (Smith et al. 2000b).

In the present study, we examine the mechanisms by which competition for fertilizations affect spawning rate and the spatial scale at which these effects manifest themselves. Because bitterling use freshwater mussels for oviposition, their spawning sites can be easily quantified and manipulated, making them ideal for tackling these questions. The study comprised two experiments. First, we experimentally tested whether the number of eggs spawned by females was dependent on the number of available spawning sites, and if so, whether fish behavior, local male density and local Operational Sex Ratio had any effect on spawning rate. In the second experiment, we partitioned the effects of total male density and local male density (manipulated by a proximity between adjacent territories) while holding the number of spawning sites constant.

Methods

Experimental set-up

Experiments were conducted in a large outdoor concrete pool at the Institute of Vertebrate Biology, Brno, Czech Republic in May 2002. The pool measured 12.4×6.0 m and water depth was 0.6 m. Water temperature varied naturally between 17.0 and 23.6°C. During experiments, mussels were presented to bitterling in sand-filled flowerpots positioned around three sides of the pool. One end of the pool was left free of mussels to enable divers to enter and leave the pool without disturbing experimental fish. Large plastic plants (0.7 m length) were placed around the margins of the pool as refuges for fish.

Fish used for all experiments originated from the River Kyjovka in the southeast of the Czech Republic, where European bitterling are abundant (Reichard et al. 2002). Fish were caught on 6 and 10 May along a 2-km stretch of the river using a DC electroshocker modified to catch small fish with minimal stress and injury. Captured fish were transported to the Institute of Vertebrate Biology aquarium facilities where they were separated by sex and housed in seven aquaria measuring 75 (length)×40 (width)×40 (depth) cm. All aquaria were equipped with an external filter to maintain water quality and aerated continuously. Fish were held under a natural light cycle and fed ad libitum with a mixture of live bloodworm (*Tubifex* spp.), live and frozen chironomid larvae, frozen *Daphnia* spp. and commercial flake food. Water temperature in aquaria matched natural variations and varied between 18 and 21°C.

Thirty *U. pictorum* and 150 *U. tumidus* were collected from an oxbow lake adjacent to the River Kyjovka (where both species are abundant and where bitterling occur) in mid-April (before the start of the bitterling spawning season) and transported to the Institute of Vertebrate Biology. They were stored in aerated, sand-filled containers in a shaded garden pond where phytoplankton food was abundant.

Experiment 1—the effect of mussel availability on spawning rate

The purpose of this experiment was to investigate the relationship between mussel abundance and the number of eggs spawned by bitterling. On 6 May (6 days after filling the experimental pool), 32 male and 70 female bitterling were stocked in the experimental pool. Two size classes of males were released: 16 large males [mean±SE Standard Length (SL, from tip of the snout to the end of caudal peduncle)=47.1± 0.66 mm] and 16 small males (SL=34.2±0.38 mm). Two distinct size classes are typically seen in bitterling populations and represent age cohorts (Smith et al. 2000a). The SLs of a random subsample of 45 females were measured as 45±0.6 mm (range 35–53 mm). Sixteen *U. pictorum* in sand-filled flowerpots were placed at 1 m intervals around three sides of the pool to enable stocked males to establish territories. Only mussels within the range 72–110 mm (mean 92±3.1 mm) were used, to avoid any effect of mussel size on the number of bitterling eggs spawned. There was no correlation between mussel size and number of eggs recovered from mussels for a subset of mussels measured (Pearson correlation, r=−0.06, n=14, P=0.840). The general lack of a strong mussel-size effect on embryo-carrying capacity (reviewed in Smith et al. 2004) may arise because embryos tend to be aggregated in the region of the gill nearest the exhalant siphon, in both large and small mussels. This effect appears to arise because the positioning of eggs on the gills is constrained by the length of the females' ovipositor (Smith et al. 2004).

Within 1 day of stocking, males had established territories around mussels and females started spawning. The experiment began 5 days after bitterling were stocked in the experimental pool. The original 16 mussels were removed from the flowerpots and, on each day of the experiment, a different number of *U. tumidus* mussels (4, 6, 8, 12 or 16 in a randomly predetermined order), in which no previous spawnings had occurred, were placed in the pool for 24 h. Each mussel was placed in 1 of 16 sand-filled flowerpots sited at a random, predetermined position. After the end of each trial (at 1700 hours), all mussels were collected and another group of mussels was placed in the pool according to the next treatment. After removal from the pool, mussels were dissected immediately and the number of bitterling eggs in the gills of each mussel was recorded.

During experiment 1, bitterling behavior and abundance around two randomly selected mussels were recorded by a snorkeller once each day (between 1200 and 1600 hours). The duration of each observation was 8 min. The number of rival males in proximity to a mussel (i.e. those interacting with the territorial male guarding the mussel) and the number of females with extended ovipositors visiting the mussel were recorded and used to calculate the operational sex ratio according to Kvarnemo and Ahnesjö (2002) as: OSR=males/(males+females). Male behavior (territorial male aggression, courting and leading behavior, ejaculations by territorial and rival males into the mussel, spawning attempts and courtship interruptions) was also recorded. For a full description of recorded behaviors, see Table 1. The presence of a snorkeller has not been observed to have any effect on bitterling spawning behavior during 9 years of bitterling behavior research (Smith et al. 2004).

Throughout the experiment, fish in the pool were fed daily, after completion of experimental work, on frozen bloodworm. Also, a carpet of algae, the principal component of the diet of bitterling (Przybylski 1996), quickly established on the walls and floor of the pool, on which fish grazed.

Table 1 Behavioral variables recorded during experiments 1 and 2. Modified from Wiepkema (1961) and Smith et al. (2004)

Behavior	Description
Aggression by territorial male towards rival	Aggression directed at other males, including fin-spreading, parallel swimming, jerking, and head-butting. Each individual aggressive event was scored once and no distinction was made between them.
Courting by territorial male and rivals	The male swam quickly towards the female, quivered his body at a high frequency and exposed his lateral side to her while extending dorsal and ventral fins.
Leading of females by territorial male and rivals	The male guided a female towards a mussel while courting. If a female followed the male, the behavior was scored as a lead.
Ejaculation by territorial male and rival males	Sperm release in a typical body posture—male sweeps forward and down quickly over the inhalant siphon of mussel. Sperm release was sometimes visible as a grayish cloud. Each ejaculation was scored as a separate event.
Spawning interruption	A territorial male stopped courting a female and engaged in aggression directed at a rival with the result that the female departed the territory.
Number of rival males	Number of males, excluding the territory holder, that released sperm over the inhalant siphon of a mussel before or after a spawning occurred.

Experiment 2—the effect of total and local male densities on spawning behavior

A second experiment was conducted to investigate male spawning behavior in relation to competitor density at two spatial scales. We defined total male density as the total number of males in the study pool, whereas local male density was manipulated by varying the distance between adjacent territories. Ten mussels in total, distributed in pairs among five arenas around the pool perimeter, were always present in the pool throughout the experiment. All mussels were placed individually in sand-filled flowerpots. A 1.2-m cylindrical net was set around each flowerpot to enable the capture of all fish that participated in a spawning. The net had a buoyant upper ring that was secured with weights during observations and released after a spawning occurred. Arenas were at least 6 m apart and separated by a clump of plastic vegetation, such that territory holders from different arenas did not interact.

Two levels of each treatment were tested. At the low total male density, 32 males were stocked in the pool. For the high total male density treatment, 64 males were used. Throughout experiment 2, 170 females were always present in the pool. Thus, there was also a change in the sex ratio between male density treatments, from 1:5.3 (low total male density) to 1:2.7 (high total male density). We did not manipulate female density to hold sex ratio constant since our aim was to look at the effect of male behavior on spawning rate, and a twofold increase in female density could be expected to increase spawning rate per se (see Discussion for more details). We investigated male density treatments sequentially, with all replicates within the low total male density completed between 16 May (1 day after fish were stocked) and 19 May. All replicates within the high total male density treatment were completed between 21 and 25 May. Total male density treatments were imposed sequentially for logistical reasons. However, there were no marked changes in water temperature during the experiment and female spawning rate was constant between the treatments (see Results). In addition, all territories were always occupied by a male, and the entire experiment was completed in 10 days during the peak spawning period. Consequently, we believe that total male density, rather than a seasonal change in spawning patterns, was likely to have the greater effect on male reproductive behavior.

Local male density was manipulated by varying the distance between adjacent mussels within a particular arena. A pilot study of territory stability and male intrusion rates showed that intrusion by adjacent territory holders was significantly affected by distance between mussels (logistic regression, $X^2=5.20$, $n=25$, $df=1$, $P=0.023$). We tested intrusion rates in adjacent male territories for five distances. If the distance between territories was 50 cm, one male usually controlled both mussels. For a distance of 75 cm, two males were able to defend territories, while their intrusion rates into adjacent territories was relatively high. Intrusion rates between adjacent territory holders declined with distance between mussels, with relatively low intrusion rates at 150 and 200 cm. Consequently, we defined high local male density where mussels were positioned 75 cm apart in an arena, and low local male density for mussels at a distance of 150 cm.

Throughout experiment 2, when observations were not taking place, two *U. pictorum* were placed 150 cm apart in sand-filled flowerpots in each arena to ensure territorial males were always present. For each replicate, a *U. tumidus* mussel in which no previous spawning had occurred was used to replace the resident *U. pictorum* in a randomly selected arena. A different mussel species was used to prevent any mix-up between resident and experimental mussels during the experiment. Within male density treatments, each mussel was assigned to either a high (75 cm) or low (150 cm) local male density treatment in a random predetermined order. Behavioral recording began once the newly placed mussel started to filter at a steady rate and the territorial male began defense of the mussel; typically within 2 min. During each test, a diver recorded fish behavior from a distance of approximately 1 m. The frequency of aggression by the territorial male, courting and leading females, ejaculations by territorial male and rivals, courtship interruptions and number of rival males interacting with the territory holder were recorded during each test (Table 1).

Once a successful spawning occurred, a note of the time was made and the cylindrical net was gently released. After release of the net, males continued to ejaculate into mussels and observations were continued until this behavior ceased. All the fish retained in the net were then captured using a hand net, measured to the nearest 1 mm and fin-clipped. The mussel in which spawning had occurred was isolated for 7 days and developing embryos were removed for a separate genetic study. Non-territorial males were immediately returned to the pool, while territorial males and females were placed in an aquarium pending release to the pool and were not used again in the study. All territorial males were captured following spawning, and replaced with another male of equivalent size from a stock of males held in a 2-m-length keep net in the pool. Females that had been caught and fin-clipped after spawning were similarly not used again. Some females that spawned were not caught in the cylindrical net (<20%) and may have participated in another replicate, though always with a different male and mussel. Some replicates may have had the same non-territorial males involved. Non-territorial males that had been fin-clipped and released were occasionally seen to participate in later spawnings, though this was not a recurring problem. Also, since territorial males and females were the main subject of our analyses and every territorial male was observed only once (one spawning) in a territorial role, we believe that our replicates (spawnings) were independent observations. Newly released males were seen to participate in spawnings and defend territories within 1 h following their release. Two divers observed spawning in five arenas, with a maximum of one observation in 5 h at an arena and a maximum of eight observations each day in total. A total of 52 spawnings was observed during experiment 2.

Data analyses

Courting and leading behavior, ejaculations and aggression by territorial male, ejaculations by rival males and courtship interruptions are presented as a rate per 1 min, and were transformed before analysis to meet assumptions of normality (Kolmogorov-Smirnov test, $\alpha=0.20$). A model 1 two-factor ANOVA with equal replication was used to test the effect of male densities on male behavior and spawning rate in experiment 2. Rival male leading behavior strongly deviated from normality and had a binomial distribution. Therefore, we separated cases based on whether rivals were able to lead females to mussels or not, and analyzed data using a chi-squared contingency test. When significant interaction effects in ANOVA were detected, each density scale was tested separately using an unpaired *t*-test. We calculated a post-hoc power analysis for tests where the *P* value was slightly larger then our alpha level for significance, as recommended by Jennions and Møller (2003) (but see Hoenig and Heisey 2001). Courting and leading behaviors were strongly positively correlated (Pearson correlation, $r=0.957$, $n=52$, $P<0.001$ and $r=0.911$, $n=52$, $P<0.001$ for territorial and rival males, respectively) and gave concordant results in all analyses. Therefore, only data for leading behavior are presented. Non-parametric tests were used in cases where data did not respond to transformation. Statistical analyses were conducted using Statistica 6.0.

Results

Experiment 1—Effect of mussel availability on spawning rate

The total number of eggs spawned each day was positively correlated with the number of mussels available (Pearson correlation, $r=0.941$, $n=5$, $P=0.017$; Fig. 1). The number of eggs laid in individual mussels ranged from 0

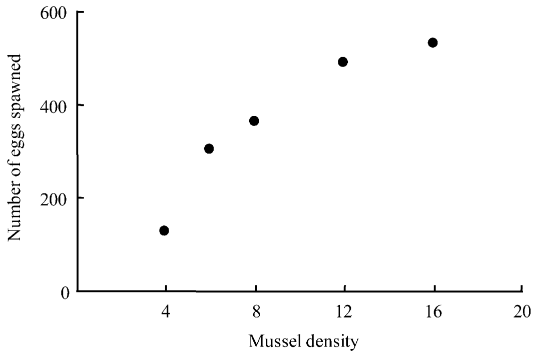

Fig. 1 The relationship between the number of bitterling eggs spawned over 24 h and the number of mussels available for oviposition

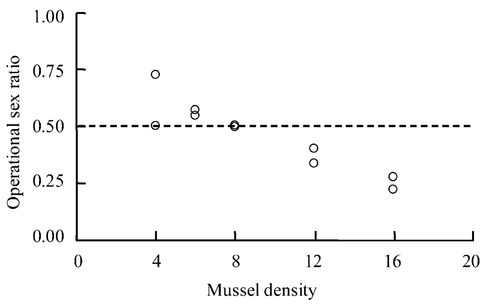

Fig. 2 Local male to female operational sex ratio (*OSR*) across different mussel densities. OSR was calculated according to Kvarnemo and Ahnesjö (2002) as OSR=males/(males+females). The *dashed line* indicates equal OSR; values from 0 to 0.49 females biased and from 0.51 to 1 male biased OSR

(1 case) to 132 (mean±SE: 40.5±3.86 eggs per mussel) and was not significantly correlated with the number of mussels available for oviposition (Pearson correlation, r=–0.088, n=46, P=0.562).

The number of courtship interruptions increased with number of males in proximity to a mussel (Pearson correlation, r=0.744, n=10, P=0.014) and declined with increasing mussel abundance (Pearson correlation, r=–0.791, n=10, P=0.006). Local OSR correlated with the number of mussels available for oviposition (Pearson correlation, r=–0.925, n=10, P<0.001), with local OSR male biased at low mussel densities and female biased at high mussel densities (Fig. 2). Territorial male aggression correlated positively with the number of rival male ejaculations (Pearson correlation, r=0.686, n=10, P= 0.028), but not with the number of spawning interruptions (Pearson correlation, r=0.603, n=10, P=0.065). The number of territorial male ejaculations also positively correlated with rival male ejaculations (r=0.934, n=10, P<0.001).

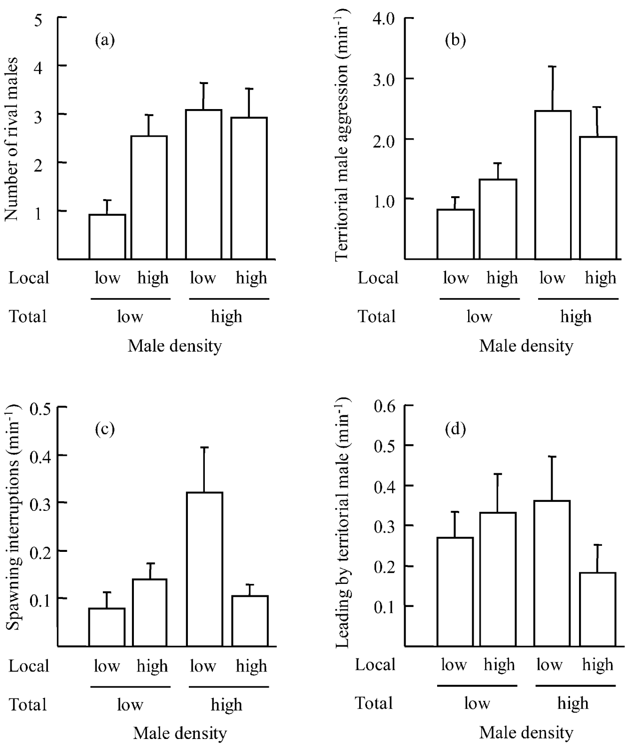

Fig. 3a–d Male behavior: **a** number of rival males that released sperm over the mussel; **b** rate of aggressive attacks by territorial male toward rival males; **c** rate of spawning interruptions; **d** leading rate by territorial male at different local and global male densities. *Error bars* represent 1 Standard Error

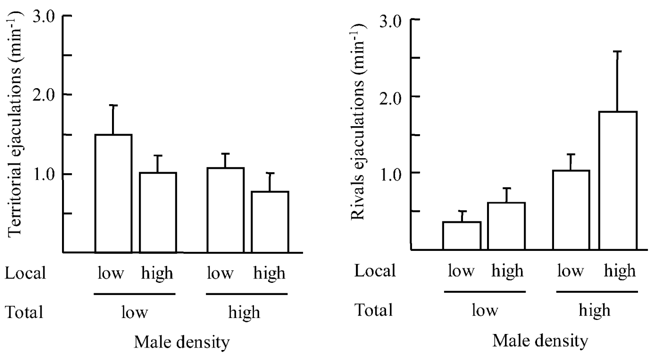

Fig. 4 Ejaculation rate of (*left*) territorial male and (*right*) rival males (pooled for all rival males) at different local and global male densities. *Error bars* represent 1 Standard Error

Experiment 2—effects of total male density

As anticipated, we detected a significant effect of total male density on the number of rival males involved in spawnings (ANOVA, $F_{1,48}$=6.79, P=0.012) and on rival ejaculation rate (ANOVA, $F_{1,48}$=6.08, P=0.017), with the highest numbers of rival males and rival ejaculations at high total male density (Figs. 3, 4). These effects were especially pronounced at low local male density (unpaired t-test, t_{24}=3.42, P=0.002 and t_{24}=2.81, P=0.010 for

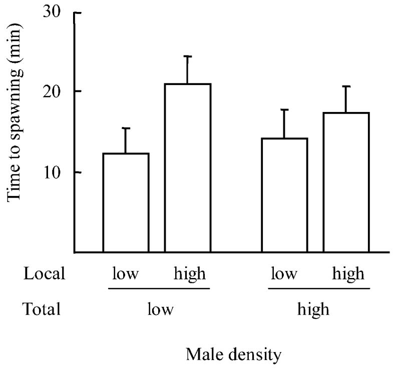

Fig. 5 Bitterling spawning rate (measured as time between mussel presentation and a successful spawning) at different male densities. *Error bars* represent 1 Standard Error

number of rivals and rival ejaculation rate, respectively). Consequently, at the high total male density, territorial males increased their aggression toward competing males (ANOVA, $F_{1,48}$=5.32, P=0.025; Fig. 3b), but this had no significant effect on courtship interruptions (ANOVA, $F_{1,48}$=3.50, P=0.067, power=0.45; Fig. 3c). There was no significant effect of total male density on leading rate by territorial males (ANOVA, $F_{1,48}$=0.25, P=0.620, Fig. 3d). However, we did detect a significantly higher incidence of rival leading behavior at high (39%) than at low (19%) total male density (contingency test, χ^2_1=4.06, P=0.044). Time from the start of the observation to a successful spawning was unaffected by total male density (ANOVA, $F_{1,48}$=0.01, P=0.976; Fig. 5).

Experiment 2—effects of local male density

At a high local male density, time to spawning increased significantly (ANOVA, $F_{1,48}$=4.42, P=0.041; Fig. 5), though the leading rate of territorial males (ANOVA, $F_{1,48}$=0.54, P=0.465; Fig. 3d) and rivals was unaffected (contingency test, P>0.50). Aggression by territorial males was not directly affected by local male density (ANOVA, $F_{1,48}$=0.19, P=0.663), though territorial aggression was highest at high total and low local male densities (Fig. 3b). Similarly, there was no direct effect of local male density on spawning interruptions (ANOVA, $F_{1,48}$=1.50, P=0.227), though there was a significant interaction with total male density ($F_{1,48}$=6.40, P=0.015), with the highest interruption rate at high total male density and low local male density (Fig. 3c). Courtship interruption rate increased significantly with local male density at the low total male density treatment (unpaired t-test, t_{24}=2.22, P=0.036), but not at the high total male density treatment (unpaired t-test, t_{24}=1.44, P=0.136). Surprisingly, the abundance of rival males in foreign territories was not affected by the local male density treatment in general (ANOVA, $F_{1,48}$=2.25, P=0.140) and the interaction with total male density was not significant ($F_{1,48}$=3.30, P=0.076, power=0.44). However, at the high local male density, rival males increased their presence in a foreign territory when the total male density was low (Mann-Whitney, U=33, n=26, P=0.007; Fig. 3a). At the high total male density, no effect was detected (Mann-Whitney, U=80, n=26, P=0.815). The number of rival ejaculations did not differ between local male density treatments (ANOVA, $F_{1,48}$=0.69, P=0.410; Fig. 4).

Discussion

We experimentally manipulated spawning site and male densities to investigate their effects on spawning rate and male reproductive behavior, and the potential to affect population parameters. Results showed the number of available spawning sites strongly affected spawning rate of the experimental population (Fig. 1). Restricted access to breeding sites frequently reduces population reproductive rates (Oliveira et al. 1999; Ekman et al. 2001) and the effect is typically mediated by the limit on breeding site availability per se (e.g. Village 1983; Nilsson 1987; Butchart et al. 1999). Thus, the number of eggs spawned by bitterling might be limited by mussel capacity to accommodate eggs. Indeed, we did not detect any difference in the average number of eggs in each mussel among mussel density treatments. However, the average number (41 eggs) recorded in our study was far below the carrying capacity of the mussels and we found no evidence of mussel saturation. Further, we found up to 132 eggs in a single mussel during the experiment and have frequently encountered much higher egg densities in field studies over the past 9 years of research on bitterling reproductive ecology (Smith et al. 2004).

Local Operational Sex Ratio changed significantly with mussel density during experiment 1 and OSR is known to affect male behavior (Kvarnemo and Ahnesjö 2002), with increased male-male interference competition for matings under male-biased OSRs (Emlen 1976; Enders 1993; Clutton-Brock et al. 1997; Jirotkul 1999). In experiment 1, territorial male bitterling adjusted their aggression across mussel densities according to the change in local OSR (Fig. 2). Although the effect of male aggression had no significant effect on the success of spawning attempts, this may have been due to a low number of replicates in our design. Based on our results in experiment 1, we proposed that male behavior related to male-male competition might have affected the total oviposition rate of the experimental population. We conducted experiment 2 to unambiguously test our correlative results from experiment 1.

Both total and local male densities were shown to affect bitterling spawning behavior in experiment 2 when the number of spawning sites was held constant. As more males participated in spawnings at the high total male densities (Fig. 3a), territorial male aggression increased (Fig. 3b). However, total male density had no direct significant effect on the rate of courtship interruptions (Fig. 3c). Nevertheless, total and local male densities significantly interacted in their effect on male spawning behavior. When total male density was low, territorial males aggressively defended their territories against rivals, and aggressive defense was amplified when local male density increased. Consequently, the number of unsuccessful spawning attempts significantly increased with higher local male density, with females abandoning spawning attempts as males interrupted courtship to engage in aggressive attacks against rivals. In contrast, at high total male density, territoriality appeared to break down with an increase in local male density. Territorial male aggression decreased (Fig. 3b), with the effect that courtship interruptions were considerably reduced (Fig. 3c) and females were able to spawn with fewer interruptions. At high total male density and high local male density, territorial males decreased their rate of leading females to mussels (Fig. 3d), and sometimes abandoned defense of their territories. The same response was observed by Smith et al. (2002) under natural conditions, with territorial males unable to defend a mussel against large groups of rival males that attempted to participate in spawnings. Results from a separate genetic study showed that rival males are often successful in fertilizing eggs and that the reproductive success of territorial males declines with the number of rivals (Reichard et al. 2004). Thus, the highest male density in the present study, which resulted in territoriality being relaxed, may be at the point at which territoriality is no longer adaptive.

In experiment 2, male density was manipulated at two spatial scales. However, by manipulating total male density (32 or 64 males in the pool) and holding female density constant (to control the effect of female abundance on spawning rate), we also manipulated overall OSR of the experimental population. Thus, a change in the population OSR may be responsible for effects we report rather than changes in total male density. However, we believe that total male density probably played a greater role than population OSR because: (1) female-mussel ratio (17:1), in contrast to male-mussel ratio (3.2:1 and 6.4:1), was constant between treatments, and (2) population OSR remained male biased in both treatments (1:5.3 and 1:2.7, respectively). In a comparable study, Alonso-Pimentel and Papaj (1996) studied the effect of both OSR and gender density on the copulatory behavior of the walnut fly (*Rhagoletis juglandis*). They showed that absolute male density, as well as density relative to female abundance, was important in determining male mating tactics. Moreover, the effect of one gender density did not depend on the density of the other gender, and gender densities were additive in their effects (Alonso-Pimentel and Papaj 1996).

Nagata (1985) and Mills and Reynolds (2003) have previously used aquarium studies to show that male bitterling adjust their mating behavior in relation to male density and male:mussel ratio, with a possible trade-off between territorial aggression and courtship behavior. Mills and Reynolds (2003) showed that unsuccessful spawning attempts increased twofold at a 18:3 male-biased OSR (male density of 28 ind. m^{-2}) relative to an equal (3:3, 5 males m^{-2}) OSR. They also observed a decrease in aggressive and courtship behavior and a change in mating tactics from pair to group spawning (Mills and Reynolds 2003). Interestingly, they detected no decrease in spawning rate, though this may have been because relatively few spawnings occurred in their experiment, even with an equal OSR (Mills and Reynolds 2003). In the related rose bitterling (*Rhodeus ocellatus*), Nagata (1985) observed that spawning rate was suppressed by territorial aggression and increased when territoriality broke down at a high local male density.

In the present study, male-male interference competition was found to significantly reduce bitterling spawning rates, and this effect was detectable at the experimental population level. However, it does not necessarily follow that this effect has the potential to alter recruitment patterns. The failure of female bitterling to spawn may be temporary, and females with eggs in their ovaries might be able to spawn eventually, albeit with the result that the spawning season is more protracted (Nagata 1985). However, female fish that fail to spawn tend to reabsorb their eggs through the process of ovarian atresia (Tyler and Sumpter 1996; Wootton 1998). For example, female seabream (*Sparus aurata*) showed high levels of atresia within 1 week of isolation from males (Meiri et al. 2002), and in rainbow trout (*Oncorhynchus mykiss*), up to 50% of the eggs in the ovaries of some females may undergo atresia (Tyler et al. 1990). Thus, the repeated failure to spawn as a result of male-male competition could reduce total population egg production for a spawning season. However, because bitterling embryo mortality inside mussels is density dependent (Smith et al. 2000a), a decrease in spawning rate may result in an improved survival of spawned eggs in mussels, with an attenuating effect on population size. These possible population consequences of male-male competition will be explored in a model of bitterling population dynamics.

Acknowledgements We are grateful to Tamsin Burland, Ulrika Candolin, and Lars Chittka for their comments. This research was supported by a Royal Society (NATO/Royal Society fellowship awarded to M.R. and Royal Society Joint Project Grant awarded to C.S. and P.J.), and Czech Academy of Sciences Internal Grant awarded to M.R. M.R. holds a license for conducting experimental work on vertebrates in accordance with Czech legal requirements, and experiments complied with current Czech laws.

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