NOTE

Effect of operational sex ratio on female–female versus male–male competitive aggression

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Abstract: We compared the patterns of female–female and male–male competition in Japanese medaka (*Oryzias latipes*) in response to changes in the operational sex ratio (OSR), the ratio of sexually active members of one sex to sexually active members of the opposite sex. As expected, courtship behaviour and intrasexual aggression were more frequent in males than in females. However, the overall patterns of female–female and male–male aggression were similar: intrasexual aggression increased with the OSR (female/male OSR for females and male/female OSR for males), consistent with predictions of mating-systems theory. A comparative analysis of our data and those from three other studies indicated that the rate of intrasexual aggression was greater in males than in females, but the patterns of intrasexual aggression were similar: the rate increased linearly and with a common slope with increasing OSR over a range of 0.4–3.

Résumé : Nous avons comparé les patterns de compétition entre femelles et entre mâles chez le médaka japonais (*Oryzias latipes*) en réaction aux changements du rapport des sexes opérationnel (OSR), soit le nombre de poissons d'un sexe qui ont une activité sexuelle sur le nombre de poissons sexuellement actifs de l'autre sexe. Comme prévu, le comportement de cour et l'agressivité intrasexuelle sont plus fréquents chez les mâles que chez les femelles. Cependant, les patterns généraux d'agression entre mâles et entre femelles sont semblables : l'agression sexuelle augmente chez les deux sexes à mesure qu'OSR (soit femelle/mâle pour les femelles, soit mâle/femelle pour les mâles) grandit, ce qui correspond aux prédictions de la théorie des systèmes d'accouplement. Une comparaison de nos données à celles de trois autres études montre que le taux d'agression intrasexuelle est plus élevé chez les mâles que chez les femelles, mais que les patterns d'agression intrasexuelle sont les mêmes chez les deux sexes: le taux croît de façon linéaire selon une pente commune à mesure qu'OSR augmente entre 0,4–3.

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Introduction

The operational sex ratio (OSR), first defined as the ratio of sexually active males to fertilizable females in a population at a given time (Emlen 1976), is a major organizing principle of mating-systems theory (Emlen and Oring 1977; Kvarnemo and Ahnesjö 1996; Reynolds 1996). It is predicted that as the OSR becomes increasingly male-biased, the intensity of male–male competition and sexual selection will increase (Emlen and Oring 1977; Kvarnemo and Ahnesjö

1996), and many studies have now confirmed this prediction (Kvarnemo et al. 1995; Jirotkul 1999; Grant et al. 2000).

The recognition that OSR is influenced by sexual differences in reproductive rate has facilitated quantitative predictions of which sex should be limiting, and hence the object of competition, in a variety of ecological situations (Clutton-Brock and Parker 1992; Kvarnemo 1994; Ahnesjö et al. 2001). There has recently been considerable interest in quantifying female–female competition in order to test these predictions (Gwynne and Simmons 1990; Debuse et al. 1999). However, this emphasis on determining the more competitive sex has perhaps deflected interest from the equally interesting question of whether the patterns of intrasexual competition with respect to OSR are similar in the two sexes, regardless of which sex is the more aggressive. We are aware of only three studies (Kvarnemo et al. 1995; Leggett 1995[2]; Debuse et al. 1999) that have simultaneously documented competition for mates by both sexes in response to changes in OSR; in general, the competitive behaviour of both sexes was affected by OSR.

The purpose of our study was first to describe the patterns of female–female and male–male competition with respect to OSR in a species where males are the more aggressive sex. To facilitate such a comparison, we defined OSR as the ratio of sexually active members of the sex of interest (i.e.,

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whose aggressive behaviour is being monitored) to sexually active members of the opposite sex. Hence, at any time we can calculate two OSRs: the male/female ratio and the female/male ratio. We used the Japanese medaka (*Oryzias latipes*) as our model species because males compete aggressively for females in the laboratory in a manner that can be predicted by the OSR (Grant et al. 1995*a*, 2000). To date, however, we have ignored the competitive behaviour of females, who have been reported to compete aggressively for access to males (Leggett 1995[2]). Our specific objectives were to verify that aggression was more frequent between males than between females, to confirm Leggett's (1995[2]) observation that females compete for males and to test whether competitive aggression increases with OSR in both females and males. Our fourth objective was to use data available in the literature to test quantitatively the hypothesis that the relationship between intrasexual aggression and OSR is similar in the two sexes.

Methods

Experimental subjects

While little is known about the behaviour of the Japanese medaka in the wild, it is an ideal species for manipulative studies of OSR. Individuals shoal and mate synchronously at dawn in a heavily vegetated environment (Grant et al. 1995*a*; Howard et al. 1998). Hence, OSRs likely vary widely in nature depending on the sex ratio of a shoal at a particular time and place.

Fifty Japanese medaka were purchased from a biological supply company and transferred to a stock tank measuring $60 \times 30 \times 30$ cm (l × w × h). The tank contained an undergravel filter, a heater, two airstones, gravel to a depth of 3 cm, and aged tap water. The sides and back of the tank were covered with black construction paper to minimize disturbance of the fish. The fish were exposed to a water temperature of 27–28°C and a 16 h light : 8 h dark cycle with lights on at 08:00.

The 30 females (1.40 ± 0.63 g and 32.6 ± 2.2 mm (mean ± SD)) and 20 males (0.72 ± 0.24 g and 33.0 ± 1.3 mm) were fed frozen brine shrimp daily to satiation. Experiments began when most females were producing eggs daily.

Experimental procedures

Groups of 5 females were exposed to three different female/male OSRs (1, 2.5, and 5) in random order in a repeated-measures design over a 3-day period. On the afternoon prior to day 1, 5 females were transferred to an experimental tank that was identical with the stock tank. At 08:20 the next day, 1, 2, or 5 males were added to the experimental tank. Because Japanese medaka typically spawn at dawn, the females were usually eager to spawn by 08:20. Each trial was videotaped from directly in front of the tank for 30 min. The males were removed after the trial and placed into a tank for "used" fish, whereas the females remained in the experimental tank for 2 additional days. On days 2 and 3, new males were added to the experimental tank at 08:20 to complete the three OSR treatments. These three trials also produced two male/female OSRs (0.4 and 1) at which male competition could be compared. After the trial on day 3, the males and females were transferred to the tank for used fish. We

used 10 groups of females in the study, so 10 females were used once and 20 were used twice, whereas all males were used 4 times. When all the fish of a particular sex were removed from the stock tank, it became the used-fish tank and vice versa. We reused fish to minimize the number required as per our animal-care permit. Nevertheless, it is unlikely that we reused the same group of females, so we treated each group as an independent datum in our study. The fish were cared for in accordance with the guidelines of the Canadian Council on Animal Care.

We scored male and female behaviour from the videotapes. Male–male and female–female aggression primarily took the form of chases, i.e., accelerated swimming toward another male. The most noticeable male courtship display was the quick circle (Hamilton et al. 1969; Grant et al. 1995*a*), in which a male swims in an arc above and in front of the female. Interference occurred when a female bumped or attempted to insert herself between a spawning pair. Following, i.e., a female approaching and following a male, appeared to be a subtle form of female courtship behaviour.

Because OSR within a trial changed with each spawning, we analyzed only the data collected before the first spawning of each trial. On average, the first spawning occurred after 4.29 min (range 0.57–19.35 min). In the two trials where no spawning occurred, we used the behaviour for the first 10 min of the trial.

We used a repeated-measures analysis of variance (ANOVAR) to test for treatment differences. The Huynh–Feldt correction was used for all tests of within-subjects effects because compound symmetry could not always be assumed (Potvin et al. 1990). Because the behaviour of males was not independent of that of females within a trial, we set α to 0.025 via the Bonferroni method (Sokal and Rohlf 1995).

Results

On average, 3.4 of the 5 females spawned in each trial; the numbers that spawned did not vary significantly among treatments (3.3, 3.8, and 3.0 for the treatments with OSRs of 1, 2.5, and 5, respectively; ANOVAR, $F_{[2,18]} = 1.02$, $P = 0.36$). Hence, the actual OSRs in our treatments were 0.66, 1.9, and 3.0. We used these actual female/male OSRs when making quantitative comparisons with other studies. We did not adjust the male/female OSRs because all the males seemed ready to mate, and males continue to court and compete for females that are unwilling to mate or have already mated (see Grant and Green 1996).

As predicted, male–male aggression increased marginally with OSR (Fig. 1; ANOVAR, $F_{[1,9]} = 6.50$, $P = 0.031$). The frequency of male courtship also differed significantly among treatments (ANOVAR, $F_{[2,18]} = 6.91$, $P = 0.0085$) and decreased significantly (paired *t* test, $P = 0.0024$) as the OSR increased from 0.4 to 1 (Fig. 1).

The frequency of female–female aggression, in the form of chasing, varied among treatments (Fig. 2; ANOVAR, $F_{[2,18]} = 5.34$, $P = 0.017$). Chasing was infrequent at a OSR of 0.66, increased at a OSR of 1.9 (paired *t* test, $P = 0.026$), and did not differ significantly between OSRs of 1.9 and 3 (paired *t* test, $P = 0.32$). The frequency at which females interfered with spawning pairs showed a similar pattern to chasing with respect to OSR (Fig. 2), but the differences were not

Fig. 1. Per-capita rates (mean ± SE; $n = 10$) of male–male aggression (●) and male courtship (▲) in Japanese medaka (*Oryzias latipes*) in relation to operational sex ratio (OSR).
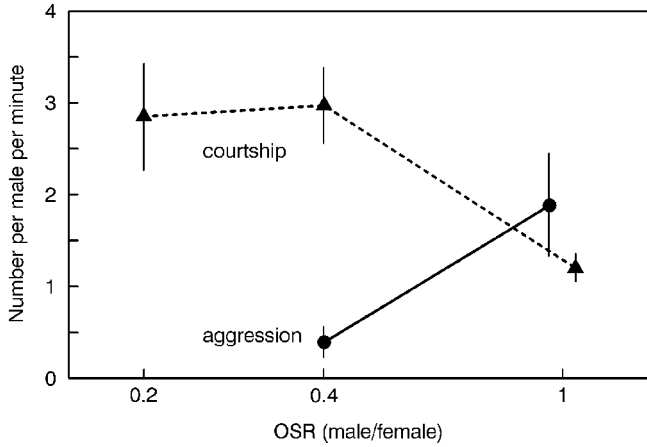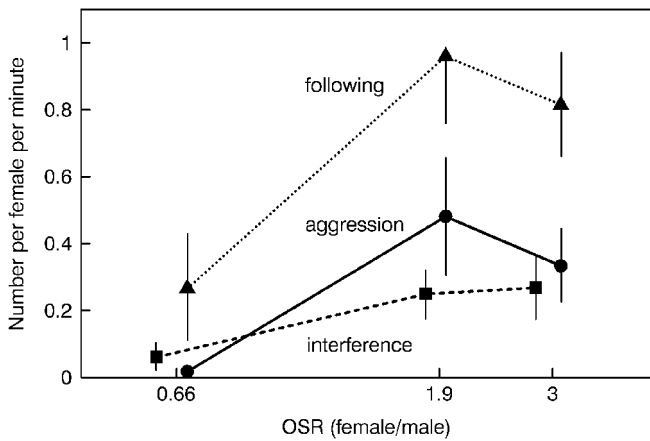

Fig. 2. Per-capita rates (mean ± SE; $n = 10$) of female–female aggression (●), interference with spawning pairs by females (■), and following, a form of female courtship (▲), in relation to OSR.

Fig. 3. Per-capita rates (mean ± SE; $n = 10$) of male–male (●) and female–female (▲) aggression in relation to OSR (male/female and female/male, respectively). For comparison, the thick line depicts the mean male–male aggression rate in relation to OSR for Japanese medaka from Grant et al. (2000).
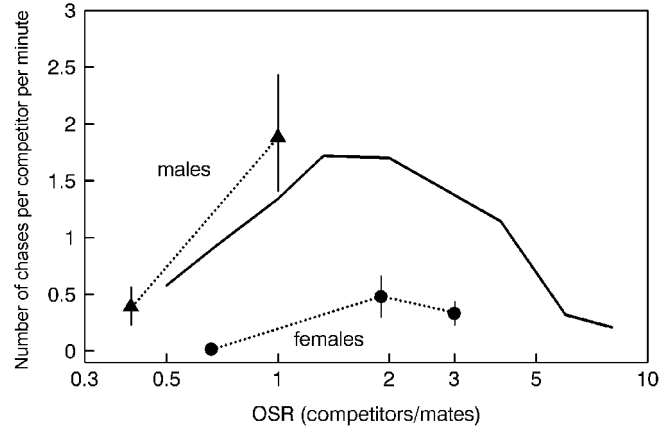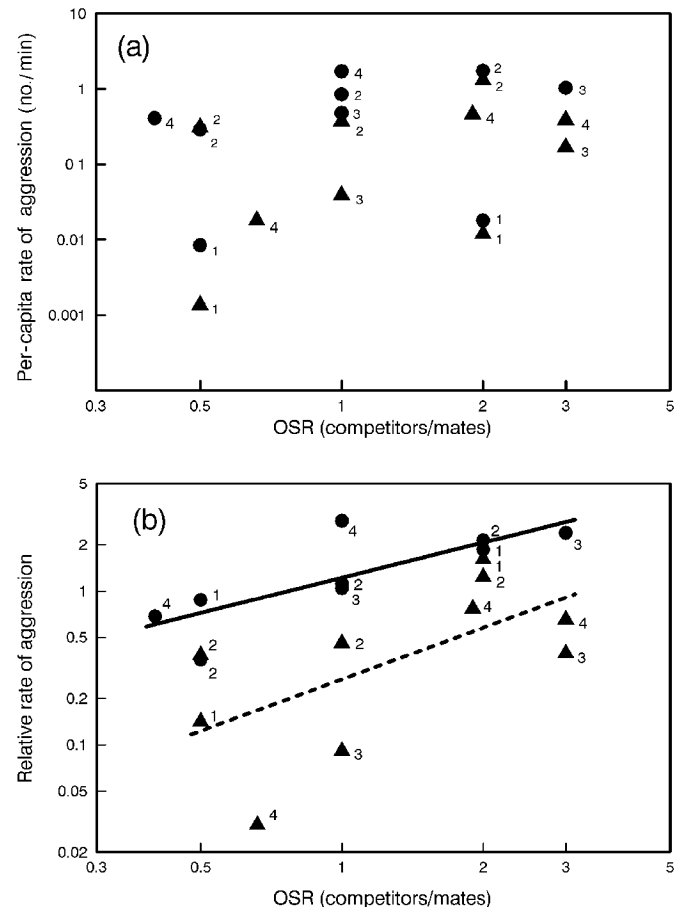

Fig. 4. (*a*) Per-capita rates of male–male (●) and female–female (▲) aggression in relation to OSR (male/female and female/male, respectively). 1, European lobsters, *Homarus gammarus* (Debuse et al. 1999); 2, sand gobies, *Pomatoschistus minutus* (Kvarnemo et al. 1995); 3, Japanese medaka (Leggett 1995[2]); 4, Japanese medaka (this study). (*b*) Relative rates of intrasexual aggression (data shown in *a* divided by the average rate of aggression for each study) by males (●) and females (▲) in relation to OSR. The solid and broken lines are the lines of best fit for males and females, respectively.

significant (ANOVAR, $F_{[2,18]} = 3.45$, $P = 0.064$). The rate at which females followed males also varied significantly among OSR treatments (ANOVAR, $F_{[2,18]} = 8.75$, $P = 0.0022$), primarily because of an increase between OSRs of 0.66 and 1.9 (paired t test, $P = 0.0042$).

As expected, the rate of male–male aggression was higher than the rate of female–female aggression (Fig. 3). In addition, male courtship behaviour was 4.5 times more frequent than female courtship behaviour (i.e., following) at a OSR of 1 (paired t test, $P = 0.0056$).

Data on the rates of male–male and female–female aggression in relation to OSR were extracted from three other studies in addition to our own (Fig. 4a). On average, male–male interactions were twice as frequent as female–female interactions at any given OSR (paired t test, $t = 2.52$, $n = 7$, $P = 0.045$). Only in sand gobies, *Pomatoschistus minutus*, at a OSR of 0.5 was intrasexual aggression more frequent in females than in males. Large differences in rates of aggression among species or studies were also evident that tended to obscure any patterns with respect to OSR. For example, at a OSR of 2, sand gobies interacted aggressively approximately 100 times more frequently than did European lobsters, *Homarus gammarus*.

To remove some of this variability in rates of aggression, we calculated the average per-capita rate of aggression for each study (e.g., mean of 4 OSR × gender combinations for lobsters = 0.0097 chases/min for each individual). These means were set to an arbitrary value of 1 to remove the variability related to average differences in aggression among species or studies. The effects of gender and OSR were retained by dividing the individual treatment means (i.e., each gender × OSR combination) by the average for the study, i.e., each datum in Fig. 4a is expressed as a fraction or a multiple of the average for the study to produce the data shown in Fig. 4b.

When the among-species differences in aggression were removed, the effects of gender and OSR were clear (Fig. 4b). Aggressive interactions were more frequent between males than between females (ANCOVA, $F_{[1,16]} = 16.33$, $P = 0.00095$), but the rate of intrasexual aggression increased linearly with OSR for both sexes (ANCOVA, $F_{[1,16]} = 12.12$, $P = 0.003$). However, the slopes of the relationships did not differ significantly between the sexes (ANCOVA, $F_{[1,15]} = 0.40$, $P = 0.53$).

Discussion

Male Japanese medaka were more aggressive and courted more frequently than females, presumably because of their higher potential rate of reproduction (Clutton-Brock and Parker 1992; Ahnesjö et al. 2001). Female Japanese medaka can produce at most one clutch of approximately 25 eggs per day (Howard et al. 1998), whereas males can fertilize the eggs of 4–30 females per day (Casey 1993[3]; Howard et al. 1998). Hence, females are the limiting sex and males compete actively for their attention.

The increase in male–male aggression with increasing male/female OSR was similar to the pattern observed previously (Grant et al. 2000; Fig. 3). The male chase rate at a OSR of 1 did not differ significantly between the two studies (i.e., their mean was within our 95% confidence interval). While our OSR of 0.4 was outside of the range of the previous study, our observed male chase rate was accurately predicted by the trends in their data (Fig. 3).

Female–female aggression also increased as the actual female/male OSR increased from 0.66 to 1.9, supporting the findings of three earlier studies (Kvarnemo et al. 1995; Leggett 1995[2]; Debuse et al. 1999). However, the frequency of female–female aggression in our study decreased slightly between actual OSRs of 1.9 and 3. A decline in aggression at high OSRs is predicted by resource-defence theory because competition among many individuals for each available mate may make aggression uneconomical (Grant et al. 2000). Such a dome-shaped relationship was observed in a previous study of competition between male Japanese medaka (Grant et al. 2000), but has not been documented for females because of the narrow range of OSRs used in this and previous studies.

The potential advantages of intrasexual competition are perhaps less obvious for female than for male Japanese medaka. Females spawn at dawn, presumably leaving the rest of the day free for foraging to produce the next day's clutch. Hence, spawning earlier in the morning means that more time is available for foraging. Second, male fertility declines with each spawning (Casey 1993[3]; Howard et al. 1998), so it is advantageous to spawn first with any male. While these advantages seem modest, they are apparently sufficient to induce female aggression and courtship at a female/male OSR of 1.9.

At male/female OSRs of less than 1, males competed primarily by scramble competition; each male typically courted a separate female and chased other males infrequently (Fig. 3; Grant et al. 2000). As OSR increased, male courtship declined (Grant et al. 2000), whereas male aggression peaked at a OSR of 2 and then declined significantly (Fig. 3). In contrast, courtship by females increased between female/male OSRs of 0.66 and 1.9. Presumably, males were sufficiently available at a OSR of 0.66, so the modest benefits of mating first with a preferred male may not have warranted a heavy investment in courtship or aggression by females. However, male courtship and aggression are presumably worthwhile at a OSR of 1 because males compete for access to large, fecund females (Grant et al. 1995b), and spawn with several females.

Our analysis of the data from four studies indicate that patterns of intrasexual aggression with respect to OSR are similar in males and females, despite the differences between the sexes in frequency of aggression. Male–male and female–female aggression increased linearly over a range of OSRs from 0.4 to 3. To determine if the rate of female–female aggression decreases at high OSRs, as it does in male Japanese medaka (Grant et al. 2000) and in males in general (J.W.A. Grant, unpublished data), will require further studies of female behaviour over a broader range of OSRs.

Our analysis may have broad implications for the study of mating systems, even though it is based on only four studies. While male–male competition will typically be more frequent and obvious than female–female competition, both sexes will usually be competing simultaneously for mating opportunities. Just as the mate-choice literature has evolved to embrace male choice behaviour, the mating-competition literature needs to embrace competition by females. The important and fundamental differences between the sexes that form the core of mating-systems theory should not obscure general principles that apply to both sexes. Because comparisons of male and female behaviour enrich our understanding of the dynamics of mating systems, further studies of the patterns of female competitive behaviour are needed, regardless of whether females are the more or less competitive sex.

Acknowledgements

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