Courtship rate signals fertility in an externally fertilizing fish

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Sperm limitation is widespread across many animal species. Several mechanisms of sperm allocation have been proposed, including optimal allocation according to clutch size and equal allocation across females. However, considerably less effort has been directed at investigating the behavioural signals associated with sperm limitation in males, which may include mating rate and the intensity of courtship. We investigated whether multiple successive spawnings affect individual male fertilization success, mating rates and courtship rates in Japanese medaka (*Oryzias latipes*). Across an average of 17 spawning events per male, fertilization success decreased from 83.7 per cent for the first spawning to 40 per cent for the last spawning while courtship rate decreased from 3.4 to 1.5 min⁻¹. Females appeared to respond to male sperm depletion by reducing clutch size. Our results suggest that male Japanese medaka are sperm-limited, and that courtship rate may be an honest indication of fertilization ability.

Keywords: sperm depletion; courtship; mating rate; fertilization success

1. INTRODUCTION

Traditional ideas about mating system evolution predict that males have the ability to generate a sufficient amount of sperm to fertilize an unlimited number of eggs, whereas female reproductive output is constrained by the cost of producing large gametes (Bateman 1948; Trivers 1972). However, seminal work by Dewsbury (1982) and Nakatsuru & Kramer (1982) resulted in considerable research into sperm limitation, much of which suggests that males are limited in their ability to successfully fertilize eggs over many successive matings. Consequently, males may use different tactics to maximize reproductive success, such as optimizing sperm allocation according to clutch size (Shapiro & Giraldeau 1996) or partitioning sperm equally across females (Warner *et al.* 1995).

Depletion of sperm reserves can result in a reproductive ‘time out’ during which males do not attempt to fertilize eggs (Clutton-Brock & Parker 1992). As such, reproductive rates may decrease as males replenish sperm supplies. ‘Time out’ periods may also be characterized by a decrease in courtship and other

energetically costly mating behaviour, such that males are providing an honest signal of fertilization capability (e.g. Markow *et al.* 1978). However, empirical evidence suggests that males of some species may not alter rates of reproductive behaviour, despite having depleted sperm reserves (e.g. Markow *et al.* 1978; Nakatsuru & Kramer 1982; Preston *et al.* 2001; Damiens & Boivin 2006; Lemaître *et al.* 2009). Thus, the frequency or duration of mating behaviour may not be an honest indicator of fertilization ability in some species (but see Hettyey *et al.* 2009).

Japanese medaka (*Oryzias latipes*) is an ideal species for testing predictions related to sperm depletion and associated behavioural changes. Males court mature females using round dances (Ono & Uematsu 1957) and there is some evidence of sperm limitation in male medaka indicating that small males become sperm depleted much faster than larger males (Howard *et al.* 1998). Herein, we test the predictions that: (i) male fertilization ability will decrease over subsequent spawning events; (ii) latency to spawning will increase over subsequent spawning events as males become sperm-depleted; (iii) courtship will decrease over subsequent mating events; (iv) changes in fertility and reproductive behaviour will be greater in smaller fish; and (v) clutch size will decrease over subsequent spawning events in response to male behaviour.

2. MATERIAL AND METHODS

(a) Experimental protocol

Japanese medaka were held in large stock tanks until experiments began. Two days before a trial, 30 ripe females and five males were removed from stock tanks and placed in sex-specific holding tanks. The experiment tank, measuring 60 × 30 × 33 cm (l × w × h), was separated into two unequal compartments (40 and 20 cm in length, respectively) by a black Plexiglas divider. Prior to a trial, mass (g) and standard length (mm) were recorded for an actively courting male. One male was used per day for a total of 10 males. At the start of a trial, a male was placed in the larger section of the experimental tank and 10 females were placed in the smaller section of the tank. Following a 10 min acclimation period, one female was released into the larger section of the tank through a sliding Plexiglas door and remained there for a maximum of 10 min, during which the number of round dances and the latency to spawning were recorded. After removing the female, this procedure was repeated until males had either spawned with 20 females or did not spawn with three consecutive females.

Following spawning, the female was captured in an open, clear Plexiglas box. Eggs were removed from the ventral surface following spawning by gently washing with an inverted pipette and were placed in a Petri dish with a methylene blue solution (3 mg l⁻¹) to prevent microbial infection. Fertilization was assessed 24 h after spawning.

(b) Statistical analyses

We used the angular transformation of the proportion of eggs fertilized and the logarithmic transformation of round dance rate and latency to spawn to meet the assumptions of linear analyses. We examined the effect of spawning order on the proportion of eggs fertilized, latency to spawn and courtship rate using linear mixed-effects models, with spawning order as a fixed effect, body size measures as covariates, and the intercepts and slopes for individual males as random variables (see the electronic supplementary material for details of model selection). Clutch size was included as a main factor in analyses of proportion of eggs fertilized. We also examined pairwise correlations between round dance rate, latency to spawning, fertilization success and clutch size.

3. RESULTS

(a) Spawning and fertilization success

Individual males spawned an average of 17 times (range 12–20; table 1). Clutch size ranged from 7 to

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Table 1. The number of females presented and the number of spawnings for each male in the experiment.

male	mass (g)	standard length (mm)	number of females presented	number of spawnings
1	—	—	26	20
2	0.506	30.1	27	20
3	0.335	26.2	24	16
4	0.433	28.8	28	16
5	0.444	29.2	26	20
6	0.409	27.8	27	18
7	0.390	28.2	19	12
8	0.540	31.4	27	19
9	0.401	28.6	27	15
10	0.425	28.6	28	18

51 eggs (mean = 22). Male size had no effect on the number of spawnings per male (linear regression, mass: $F_{1,7} = 4.69$, $p = 0.07$; standard length: $F_{1,7} = 2.26$, $p = 0.18$), although our power to detect an effect was low. However, none of the measures of body size was significant covariates in the mixed effects model for the proportion of eggs fertilized over successive spawnings. There was no interaction between spawning order and clutch size ($t = 1.61$, $p = 0.11$; see the electronic supplementary material, table S1), and clutch size alone had no effect on the proportion of eggs fertilized ($t = -0.69$, $p = 0.49$; see the electronic supplementary material, table S1). However, there was an overall decrease in fertilization success ($t = -7.79$, $p < 0.001$; see the electronic supplementary material, table S1; figure 1) from 83.7 ± 5.4 per cent (mean $\pm$ s.e.) for the first spawning to 40 ± 17 per cent for the last spawning (mean = -2.5% per spawning). Furthermore, there was a decrease in clutch size over successive spawnings ($t = -3.93$, $p < 0.001$; see the electronic supplementary material, table S1; figure 2) and females had larger clutches in the presence of longer males ($t = 2.59$, $p = 0.036$; see the electronic supplementary material, table S1).

(b) Pre-spawning behaviour

Overall, spawning occurred within 168 ± 10 s from the start of a trial and was not affected by spawning number or any measures of size (see the electronic supplementary material, table S1). However, the rate of round dances decreased with increasing spawning order from 3.38 ± 0.50 round dances per minute at the first spawning to 1.52 ± 0.13 round dances per minute at the last spawning ($t = -3.07$, $p = 0.003$). Larger males courted at a higher rate than smaller males, so mass ($t = 2.62$, $p = 0.034$) was retained in the model as a covariate (see the electronic supplementary material, tables S1 and S2). These same variables were significant when all trials were included (figure 1).

(c) Correlates of fertilization success

Pairwise correlations within males between the rate of round dances, latency to spawning, proportion of eggs fertilized and clutch size did not yield consistent

significant results (table 2). Nevertheless, courtship rate tended to decrease with fertilization success for 9 of 10 males (Binomial test, $p = 0.02$). Furthermore, model II regression slopes of the proportion of eggs fertilized versus courtship rate calculated for each male were significantly positive ($t = 3.83$, d.f. = 9, $p = 0.004$).

4. DISCUSSION

Our results suggest that male Japanese medaka are sperm-limited (e.g. Dewsbury 1982; Nakatsuru & Kramer 1982). However, males in our experiment did not experience a reproductive 'time out' and spawned with females at a similar rate over successive mating events. By contrast, courtship rate declined with spawning order, suggesting that a change in courtship rate may reflect the energetic state or fertility of males after repeated copulations.

We observed an overall decrease in fertilization success as the number of spawnings increased, although some males spawned with up to 20 females before ceasing to attempt copulation. These results are consistent with previous findings (e.g. Nakatsuru & Kramer 1982), and suggest that male Japanese medaka do not allocate sperm according to clutch size or across females. Intense intrasexual competition, coupled with daily synchronous spawning of females, may result in a male strategy that maximizes reproductive output by releasing as much sperm as possible during the first spawning. However, there was no decrease in mating rate, suggesting that males may also increase success by spawning quickly with many females.

Because courtship rate declined over successive spawnings and was positively related to fertilization success, it may signal male fertility and energetic state. Males with relatively high courtship rates are expected to have a mating advantage over others (Farr 1980; Reynolds 1993) and energy depletion in courting males has been observed in some species (e.g. Shine & Mason 2005). In Japanese medaka, females prefer males with higher courtship rates (Grant & Green 1995; Grant *et al.* 1995). While females of certain species avoid spawning with already mated males (e.g. Nakatsuru & Kramer 1982; Harris & Moore 2005), Japanese medaka females do not avoid mating with males that had previously spawned and tend to copy the mate choice of others (Grant & Green 1995). Despite this behaviour, females released fewer eggs at later spawning events, and may be assessing fertility based on male behaviour.

In contrast to the findings of Howard *et al.* (1998), we found no effect of body size on fertilization success or the number of spawnings per male, perhaps because our males were less variable in size. Consistent with Howard *et al.* (1998), heavier males tended to spawn with more females before experiencing exhaustion than lighter males in our study, and females released more eggs for larger males.

Overall, both fertilization success and courtship rate declined as the number of spawnings increased, indicating that courtship rate may be a reliable indicator of male quality. Sperm-depleted males continued to spawn at a cost to females, although females decreased

Figure 1. Proportion of eggs fertilized versus spawning order and male courtship rate (round dances per min) versus the order of female presentation for the 10 males in the experiment. Regression lines are predicted values back-transformed from the best fit model.

Figure 2. Clutch size versus spawning number for the 10 males in the experiment. Regression lines are predicted values back-transformed from the best fit model.

Table 2. Correlation coefficients within males across spawnings for clutch size, latency to spawn, courtship and proportion of eggs fertilized. Significant values are marked with an asterisk.

male	<i>n</i>	clutch– latency	clutch– courtships	clutch– fertilization	latency– courtship	latency– fertilization	courtship– fertilization
1	20	–0.229	0.513*	0.087	–0.439	–0.276	0.295
2	20	–0.141	–0.098	–0.184	–0.425	–0.431	0.529*
3	16	–0.508*	0.437	–0.121	–0.202	–0.116	0.163
4	16	–0.267	0.447	0.302	–0.443	–0.002	0.343
5	20	–0.735*	0.133	0.241	0.178	–0.176	0.250
6	18	–0.294	–0.284	0.550*	0.047	–0.085	–0.514*
7	12	–0.199	0.078	0.413	–0.067	0.144	0.556*
8	19	0.478*	–0.131	0.164	0.238	–0.014	0.171
9	15	–0.242	–0.198	0.328	–0.307	0.163	0.127
10	18	–0.364	–0.070	0.183	0.623*	0.352	0.246

clutch size and may avoid sperm-depleted males if other males are available (see Harris & Moore 2005; van Son & Thiel 2006). Furthermore, sperm-depleted males may decrease the number of spawning opportunities of their competitors, thereby influencing the fitness of other males in the population. The interaction between courtship rates and fertilization success warrants further investigation, particularly in the context of mate choice and intrasexual competition.

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