

Plasticity and selection of male sexual traits across operational sex ratios in Japanese

medaka (*Oryzias latipes*)

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ABSTRACT

In many species, the mating advantage associated with sexually-selected traits may vary with operational sex ratio (OSR; the number of sexually active males to fertilizable females in a population), and this metric is often used as a proxy for the intensity of competition for mates. The goal of this research was to assess whether OSR influenced male mating behaviour and morphology of sexually-selected traits. I examined these effects both intra- and inter-generationally by housing Japanese medaka (*Oryzias latipes*) in four different OSRs ranging from female-biased to highly male-biased. I found that: (1) courtship behaviour decreased and aggression tended to increase with increasing OSR, and male body size was the best predictor of aggression; (2) there is plasticity in mating behaviour, particularly courtship; (3) within males, larger primary and secondary sexual structures occurred in female-biased OSRs; and (4) overall levels of courtship and aggression were higher after one generation within OSRs.

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GENERAL INTRODUCTION

Sexual selection acts by favouring traits that give certain individuals an advantage over competitors of the same sex during competition for mates (Andersson, 1994). In general, this type of selection acts more commonly and strongly on males than females because of higher variation in reproductive success among males (Bateman, 1948). The positive relationship between reproductive success and the number of mates acquired is also generally stronger for males than for females, because females are often limited by the number of eggs they can produce, while males are generally limited by the number of mates they can obtain due to the relatively lower cost of their gametes (Bateman, 1948). Because their reproductive success is positively correlated with the number of mating events, in the absence of a requirement for parental care males should mate as often as possible, and selection is expected to act on traits that increase a male's abilities to acquire mates (Arnqvist & Rowe, 2005). These traits can be associated with intra- and/or intersexual selection during mate competition.

In many animal species, males compete with one another for access to females and have morphological or behavioural traits that can increase their chance of mating success pre- and post-copulation (Andersson, 1994; Arak, 1983). During competitive contests, males may possess specialized traits that lead to success during direct encounters (Andersson, 1994), including large body size (Bisazza & Marconato, 1988; Dickerson, Quinn, & Willson, 2002; Haley, Deutsch, & Le Boeuf, 1994), or weapons such as horns (Geist, 1966; Preston, Stevenson, Pemberton, Coltman, & Wilson, 2003). Competition among males can also occur during or after copulation via sperm

competition if females mate with multiple males, wherein sperm from multiple males compete to fertilize an egg in the female reproductive tract (Schulte-Hostedde & Millar, 2004). Sperm competition can lead to morphological changes in sperm characteristics (Gomendio & Roldan, 1991; Schulte-Hostedde & Millar, 2004; Stockley, Gage, Parker, & Møller, 1997) and increase testis size (Harcourt, Harvey, Larson, & Short, 1981; Schulte-Hostedde & Millar, 2004; Stockley et al., 1997) over evolutionary time.

Indirect competition among males exists due to female choice. Under this mechanism, ornaments, courtship displays and nuptial gifts can advertise male fitness (Williams, 1966), and these characteristics are useful criteria for mate selection (e.g. Grant & Green, 1996; Hill, 1990) because they provide females with information about male quality (Hill, 1990; Weir & Grant, 2010). Male traits that are favoured in competitive contests may also be favoured by females (Bateman, Gilson, & Ferguson, 2001; Howard, Martens, Innis, Drnevich, & Hale, 1998). However, the mating advantages associated with specific sexually-selected traits can change in association with the strength and/or direction of sexual selection (Emlen, 1976; Emlen & Oring, 1977).

Variation in mating scenarios, such as the operational sex ratio (OSR, defined as the number of males to females that can reproduce at any given time; Emlen, 1976), can influence the intensity of competition for mates and relative importance of different sexually-selected traits for mating success (Emlen & Oring, 1977). An increase in intrasexual competition for mates of the limiting sex can be observed in skewed OSRs, and the greater the shortage of females in a population, the stronger the intrasexual selection in males (Emlen & Oring, 1977). However, as OSR becomes increasingly skewed toward one sex, the cost of defense or production of exaggerated traits may

outweigh the benefits due to the required energetic investment and potential for lost mating opportunities because of time spent in direct fights (Brown, 1964). Both mating-related morphological (Fitze & Le Galliard, 2011; Jones, Arguello, & Arnold, 2004; Wacker et al., 2013a, 2013b) and behavioural traits (Clark & Grant, 2010; Grant & Foam, 2002; Grant, Gaboury, & Levitt, 2000; Weir, Grant, & Hutchings, 2011) can change with variation in OSR. Overall, the intensity of selection on male traits favoured in both male-male competition and female choice may be stronger in either male-biased (Jones et al., 2004; Wacker et al., 2013a) or female-biased OSRs (Fitze & Le Galliard, 2011; Klemme, Ylönen, & Eccard, 2007), depending on the level of competition, opportunity for mating events, and the mating/social system of the species.

Social environment, and specifically the OSR of a population, can change with variation in biotic (Trivers, 1972), abiotic (Fujimoto, Miyake, & Yamahira, 2015) and anthropogenic influences (Grü Ebler et al., 2008; Rowe & Hutchings, 2003). For example, differences in reproductive longevity among sexes, biased adult sex ratios, migration, differences in age at maturity among sexes, sex-biased mortality, and mortality during breeding season can all influence the OSR of a population (Kvarnemo & Ahnesjö, 1996). Therefore, it is important for animals to exhibit at least some behavioural plasticity in the context of mating systems so that their success is not limited to a specific set of conditions (Pfaus, Kippin, & Centeno, 2001). Plasticity in a mating context is also important in maintaining a high degree of phenotypic diversity of sexually-selected traits (Pfaus et al., 2001). If the mating advantage associated with a particular behaviour depends on an individual's social environment, it is considered a context-dependent behaviour (Cornwallis & Uller, 2010), and behavioural plasticity should be favoured for

mating behaviours that are context-dependent (Montiglio, Wey, Chang, Fogarty, & Sih, 2017; Patricelli, Uy, Walsh, & Borgia, 2002). Correlational selection should happen among traits if behaviours are dependent upon some other fixed characteristics of individuals (Brodie, 1992), and occurs when there is a mating advantage associated with some specific combination of traits (Montiglio et al., 2017). To maximize their reproductive success, males must adequately respond to both the presence and number of mating competitors in their environment (Bretman, Gage, & Chapman, 2011; Bretman, Westmancoat, Gage, & Chapman, 2011). Plasticity may be associated with a higher mating rate, and males may have the ability to adjust their behaviour with rapid changes (i.e., hour-to-hour) in their social environment (Montiglio et al., 2017). Despite this benefit, there are also limits and costs associated with such plasticity, such as the inability to produce more extreme phenotypes in individuals with plastic development compared to fixed development (i.e., plastic generalists versus specialists, respectively; Dewitt et al., 1998), reduced mating efficiency due to inaccurate or unreliable information acquisition about an individual's environment (Magurran & Nowak, 1991), and reduced fitness in plastic individuals relative to the population if two genotypes produce the same phenotype (Murren et al., 2015).

This study aimed to investigate variation in sexually-selected traits in male fish with respect to OSR. The first chapter focuses on the effects of OSR on behaviour and morphology within and across generations, and the relationships between behaviour and morphology with variation in OSR. The second chapter focuses on the ability of males to adjust their behaviour in novel OSR environments. This research will contribute to our

understanding of how populations may react to changes in operational sex ratio over a short temporal scale.

CHAPTER 1: Operational sex ratio influences sexually-selected behaviour and morphology

In many species, males possess specialized behavioural and/or morphological characteristics directly related to the competition for mates that provide an advantage over competitors in the acquisition of mates and the production of offspring; these are generally referred to as sexually-selected traits (Andersson, 1994). Sexually-selected traits can contribute to an individual's mating success through both intra- and intersexual selection (Andersson, 1994). During direct competition among males for access to females, structures used in combat, such as large horns in many ungulate species (e.g., Geist, 1966; Preston et al., 2003), are positively associated with mate acquisition. In addition to these specialized structures, large male body size (e.g., Bisazza & Marconato, 1988; Dickerson et al., 2002; Haley et al., 1994) and/or social ranking (Le Boeuf, 1974) is/are often associated with an advantage during aggressive contests. Competition among males can also occur during or after copulation via sperm competition if females mate with multiple males. Sperm competition can lead to morphological changes in both sperm characteristics and testis size. Testis size is positively correlated with sperm count (Ginsberg & Huck, 1989; Stockley & Purvis, 1993), and high sperm counts are usually positively correlated with fertilization success (Cohen, 1971, 1975; Miller et al., 2002; Stockley et al., 1997), so males who experience sperm competition may have a larger testes to body weight ratio than males who do not experience sperm competition (Harcourt et al., 1981; Schulte-Hostedde & Millar, 2004; Stockley et al., 1997).

In intersexual interactions, ornaments, courtship displays, and nuptial gifts can advertise male fitness (Williams, 1966). For example, large or colorful feather plumes of

many bird species function in mate choice by attracting females (Møller & Pomiankowski, 1993), where the most attractive males most often secure mates. These characteristics are good predictors of female choice (e.g., Hill, 1990), and females tend to choose males with more exaggerated colouring as this can be an indicator of male health and survival ability (Hill, 1990).

In some instances, both intra- and inter-sexual selection occur on the same traits. For example, large body size can confer an advantage during competitive contests, and also be preferred by females, (Bateman et al., 2001; Howard et al., 1998). Larger males may provide better parental care (Lindström & Hellström, 1993) and/or occupy more attractive territories (Candolin & Voigt, 2001). Furthermore, larger males may also be more successful after successive mating events than smaller males (Howard et al., 1998), as fertilization ability can be affected by successive mating events due to decreased sperm counts (Halliday, 1976; Jones, 1973).

The expression of sexually-selected traits is often correlated with the strength of sexual selection (Emlen & Oring, 1977). In populations where there is high variance in reproductive success among males, sexually-selected traits tend to be more exaggerated than in populations where mates and offspring are more evenly distributed among individuals (Andersson, 1994). The operational sex ratio (OSR), defined as the ratio of males to females in a population that are sexually receptive at any given time, is often used as a metric to estimate the intensity of competition for mates in a population; furthermore, the OSR can also affect the energetic cost of mate monopolization (Emlen, 1976). Variation in OSR can influence the degree to which secondary sexual characteristics contribute to an individual's success, and thus over time can affect the

evolution of these traits within and among populations by selecting for specific sexual traits in certain situations. An increase in intrasexual competition for mates of the limiting sex can be observed in skewed OSRs, and the greater the shortage of females in a population, the stronger the intrasexual selection in males (Emlen & Oring, 1977; Klemme et al., 2007). However, as OSR becomes increasingly skewed toward one sex, the cost of defense or production of exaggerated traits may outweigh the benefits due to the required energetic investment and potential for lost mating opportunities. Similarly, the frequency of alternative mating tactics tends to increase when OSRs become skewed (Grant, Casey, Bryant, & Shahsavarani, 1995; Jirotkul, 1999; Mills & Reynolds, 2003), further reducing the ability of one or a few individuals to monopolize mates. Furthermore, Ims (1988) observed a decrease in the variance of mating success among males at male-biased OSRs, and suggested that at highly male-biased OSRs, males who are typically “winners” cannot monopolize mates as easily, leaving subordinate males with a greater chance at securing females (Ims, 1988). Therefore, intrasexual selection in males may be more intense under female-biased OSRs (Ims, 1988).

From a behavioural perspective, changes in male aggression with respect to OSR have been examined in many studies (Clark & Grant, 2010; Grant & Foam, 2002; Grant et al., 2000; Weir et al., 2011), with relatively high male-male aggression in male-biased OSR situations (i.e., up to 2:1) that declines beyond this point, likely because the cost of aggressive defense of mates becomes too high (e.g., Brown, 1964; Emlen & Oring, 1977; Weir et al., 2011). In male-biased OSRs, courtship rates may also decrease (Weir et al., 2011) as the mode of competition switches from contest to scramble, and the use of alternative mating tactics increases (Grant, Casey, et al., 1995; Weir, 2013).

Similarly, selection on male morphological sexual traits may change with variation in OSR (Fitze & Le Galliard, 2011; Jones et al., 2004; Klemme et al., 2007; Wacker et al., 2013). The intensity of selection on male traits favoured in both male-male competition and female choice may be stronger in male-biased OSRs (Jones et al., 2004; Wacker et al., 2013), possibly due to an increase in the level of competition with respect to OSR (Wacker et al., 2013). Alternatively, selection on these type of traits may be highest in female-biased OSRs (Fitze & Le Galliard, 2011; Klemme et al., 2007) as the level of direct intrasexual competition decreases and male opportunity for multiple matings increases (Klemme et al., 2007).

Sexual selection on one trait may also influence the evolution of a correlated trait if specific behaviours are dependent upon some other fixed characteristics of an individual (Brodie, 1992). For example, dominance rank is associated with male body size in elephant seals, where larger males possess higher rank and obtain a mating advantage over smaller males (Haley et al., 1994). In some mating systems, body size is associated with participation in direct contests, such that larger males hold a ‘fighter’ role and participate fights, whereas smaller males tend to use alternative reproductive tactics to achieve some mating success (Fleming & Gross, 1994; Gross, 1985).

In understanding the association between OSR and sexually-selected traits, much of the literature to date focuses on measuring either intrasexual and/or intersexual behaviours, while manipulating both the OSR and density of individuals. This study aims to provide a more integrative understanding of the importance of sexually-selected traits by considering the relative importance of sexually-selected traits on an individual level with respect to OSR environments while controlling for density effects, and the inclusion

of both behavioural and morphological traits that may contribute to an individual's reproductive success, as well as the interaction between behaviour and morphology.

A good study species to explore the relationships between OSR and sexually-selected traits are Japanese medaka (*Oryzias latipes*), a small species of freshwater fish. They become sexually mature at approximately 3 months of age. Males actively compete for females using displays of aggression and courtship. They also closely follow females, which may be used by males as a courtship (Clark & Grant, 2010) and/or mate guarding tactic (Yokoi et al., 2016, 2015). During mating the male grasps onto the female with his anal fin and eggs released are externally fertilized if the female is receptive (Ono & Uematsu, 1957). Importantly, males exhibit easily identifiable behavioural and morphological sexually-selected traits.

Previous research using choice experiments has indicated that female medaka prefer larger males over smaller males (Howard, DeWoody, & Muir, 2004; Howard et al., 1998), perhaps because they have higher fertility after a number of mating events over smaller males (Howard et al., 1998). Males rely on their ability to compete using aggressive behaviour to win access to females (Hamilton, Walter, Daniel, & Mestler, 1969; Walter & Hamilton, 1970), and larger males generally tend to be better competitors compared to smaller males (Bisazza, Marconato, & Marin, 1989; Hoelzer, 1990). Females may also prefer males who court more, as this behaviour is an indicator of fertility in Japanese medaka (Weir & Grant, 2010).

Sexual dimorphism in the species allows for identification, as males have a larger anal fin than females (Briggs & Egami, 1959; Yamamoto & Suzuki, 1955; Yamamoto, 1967). This dimorphism exists because the male anal fin functions in mating (Fujimoto et

al., 2014) and fertilization (Koseki, Takata, & Maekawa, 2000) success, likely by situating females into position for egg release (Uematsu, 1990; Egami & Nambu 1961). Koseki et al. (2000) found that males with half-sized anal fins were still able to secure mates, but that larger anal fins are associated with higher fertility rates because they act as a barrier to contain released sperm. From a female choice perspective, males with larger anal fins may also be rejected less by females (Fujimoto et al., 2014). Anal fin size, mating behaviour, and OSR vary naturally in *Oryzias latipes* (Fujimoto et al., 2015), and selection experiments for more and less aggressive individuals have demonstrated observable changes in levels of aggression over a period of two to three generations (Ruzzante & Doyle, 1991, 1993), making them a good model species to study changes in sexually-selected behaviour in response to variation in OSR.

To better understand the relative selection on both mating-related behavioural and morphological traits in males in association with OSR, I examined how: i) OSR can affect morphology and behaviour within and across generations, and ii) the relationships between behaviour and morphology with variation in OSR. First, because direct competition is expected to be more intense in male-biased OSRs due to a relatively higher number of competitors (Emlen & Oring, 1977; Weir et al., 2011), I predicted that the frequency of aggression will increase with an increasing OSR. Similarly, because indirect competition is more intense in female-biased OSRs due to a relatively higher number of potential mates (Emlen & Oring, 1977), I predicted that the frequency of courtship behaviour will decrease with increasingly male-biased OSRs. Because males should mate with as many females as possible to maximize their reproductive success (Arnqvist & Rowe, 2005), I predict that primary and secondary sexual structures (i.e., relative testis

and anal fin size, respectively) will be larger in female-biased than male-biased OSRs due to a relatively higher number of potential mates present. Furthermore, because aggression and courtship are advantageous mating behaviours that are heritable (Fujimoto et al., 2015; Ruzzante & Doyle, 1991; Sasaki & Yamahira, 2016), I expect the frequency of these behaviours to change among generations within OSRs, such that generation 1 males will be more aggressive and court more than generation 0 males due to more offspring in the population with these phenotypes. Because body size is positively correlated with aggression in this species (Fujimoto et al., 2015; Howard et al., 1998), and smaller males generally tend to use sneaking tactics rather than aggression and courtship behaviours (Fleming & Gross, 1994; Gross, 1985), I expected larger males to be more aggressive and generally display higher frequencies of mating related behaviours (i.e. aggression, courtship and following) than smaller males.

Methods

Experimental Animals: Marking & Housing Conditions

Three hundred juvenile Japanese Medaka (*Oryzias latipes*) were obtained from Aquatic Research Organisms in New Hampshire USA. All procedures described hereon were repeated for two generations of fish and were approved under the SMU Animal Care Committee Protocol 17-04. At sexual maturity, fish were chosen at random from the stock population and used as generation 0, and their offspring were also selected at random to make generation 1. Fish used for generation 1 could not be chosen until they could be sexed, as specific numbers of males and females were needed for each OSR treatment. Fish were anaesthetized with 0.15g/L MS222 (Tricaine Methanesulfonate) and 0.3g/L sodium bicarbonate and tagged for individual identification using Visual Implant Elastomer tags (NorthWest Marine Technology) at least one week prior to the start of egg collection and behavioural experiments. Each fish was tagged in two locations out of a possible four (on the back, either anterior or posterior to the dorsal fin, on either the right or left side), using one or two of eight possible colours (pink, green, orange, red, yellow, brown, black or blue). Standard length was measured to the nearest 0.01mm using digital calipers. Caudal fin clips were cut from each fish using scissors and placed in 90% ethanol for later DNA analysis. Fish were housed in ten-gallon tanks containing fresh water (salinity 0.25ppt), an undergravel filter, kept at a temperature of 26°C-28°C, and a photoperiod of 14hr light:10hr dark with lights on at 0745h. Visual barriers were placed between each tank to eliminate any potential interactions. Fish were fed twice daily using a combination of commercial flakes, *Artemia* nauplii, and adult *Artemia*.

Experimental Design

Two hundred and eighty-eight fish (168 males and 120 females) were placed into 24 ten-gallon tanks (measuring 50.8cm x 27.9cm x 33.0cm) and raised under different operational sex ratios (male:female OSRs of 0.5, 1, 2 and 5). There were six replicates of each treatment. Tank density was kept constant throughout the experiment, with twelve fish per tank. Eggs were collected over a period of three weeks, reared to the zygotic stage and saved for paternity analyses to determine the distribution of mating success among males in each tank. Once this was complete, eggs continued to be collected each day during the morning and afternoon to avoid selecting for early versus late spawning, and were reared to be the adults of generation 1. Because all males experienced the same amount of time in their experimental treatment, I do not expect variation in age to influence the results.

Behavioural Observations

Behavioural traits were measured in both generations. Behaviour was quantified by observing males in the different competitive environments (i.e., OSRs) and was categorized as aggression, courtship or following. Aggression is defined as a chase, which occurs when one fish quickly charges at another individual, causing the other to either flee or be hit by the charging fish (Grant et al. 1995). Courtship is expressed as quick circles, which occur when the male swims in a fast arc in front of the female (Grant et al. 1995). Following occurs when a male pursues a female by swimming closely behind her, and is another male mating behaviour (Ono & Uematsu, 1957) which may be a form of

mate-guarding (Yokoi et al., 2016, 2015) or courtship behaviour (Clark & Grant, 2010). Fish were observed in the morning at 'lights on'. Each male was observed for two minutes, and a total of six observations of each male were conducted. All observations were taken before 1015h (as in Weir, 2013), as Japanese medaka use artificial dawn as a mating cue in a laboratory setting and spawn in the early morning. Therefore, a two-minute observation time was chosen so that behavioural data could be collected prior to the end of the spawning on a particular day, and so that data for all males could be obtained within one week for one of the six observation periods. Data were collected such that the time of each tank observation, and therefore each OSR treatment, were random. Furthermore, individual fish per tank were observed in the same order during each observation period to avoid sampling bias, resulting in observations of individual males occurring at various random times throughout the morning observation period on different days. Frequencies of aggression, courtship and following were determined for each male. An average behavioural frequency per two-minute observation was calculated for each behaviour type (i.e. aggression, courtship and following) for each male. The averages of each behaviour type (i.e., aggression, courtship and following) were summed to determine the total behaviour for each male. For males who died before all six observations were complete (nine males in generation 0, five males in generation 1), the number of observations on each male was taken into account when calculating behavioural frequency per two-minute observation to ensure accuracy of behavioural data used for analyses.

Morphological Data Collection

Sexually-selected morphological traits were examined for each generation; specifically, standard length, growth rate (mm/day), the area of the anal fin (mm²) and the mass of the testes (mg). The sample size of these measurements was 168 males per generation. To examine plasticity in anal fin size, photographs of each fish were taken under a dissecting microscope prior to placement in experimental treatments, twice throughout the experiment (at two and four months into the experiment) and immediately after euthanasia (at 6 months into the experiment), for a total of four measurements each taken two months apart. During these sampling times, standard length was measured to the nearest 0.01mm using digital calipers. To detect any effects of OSR on male growth, growth rate (mm/day) for each male was calculated using the first and last standard length measurements obtained, as social environment can select for different growth rates in Japanese medaka (Ruzzante & Doyle, 1991). ImageJ software was used for analysis of anal fin area. After euthanasia, fish were dissected and pictures of testis were taken prior to removal using methodology from Kinoshita et al. (2009), after which they were dried and weighed to the nearest 0.001mg to determine testis size.

Statistical Analyses

All analyses were carried out using R version 3.5.2 (R Core Team, 2018). Relationships between behaviour, morphology, OSR and generation were determined by generalized linear models (GLMs), and “tank” was used as an experimental replicate in these models whereby data on an individual level was used to calculate mean behaviour

and morphology for each tank. In the analyses of the interactive effects of OSR and generation on morphology, measurements taken directly after euthanasia were used to explore the potential of selection on morphological traits. Because anal fin and testes size are expected to scale with body size, linear regressions were used to determine the relationship between: i) anal fin area and body size, and ii) testis mass and body size for all males in each generation. From these models, anal fin and testis residuals were calculated for each male to account for body size differences among individuals, and these residuals were used in the analyses of anal fin size and testis size. Because residual analyses can be biased (Darlington & Smulders, 2001; Freckleton, 2001), I also used GLMs including body size to analyze anal fin and testis size data without correcting for body size (Figure S1; Tables S1a and S1b).

I compared the interactive effects of OSR and male morphology on male behaviour by linear mixed-effects models using the “lme4” package for mixed-model analyses (Bates, Maechler, Bolker, & Walker, 2015), with “tank” included as a random effect in these models to account for random differences among tanks. Measurements used in these models were those taken closest to the behavioural observations. Relationships between behaviour, OSR and generation (Figure S2; Table S2), as well as behaviour, morphology and OSR (Figure S3; Tables S3a and S3b) were analyzed using the proportions of each behaviour (i.e., aggression, courtship and following) that contributed to the total activity of each male. In all models, Akaike Information Criterion (AICc) criteria was used for model selection. Models that differed from others by a value of 2 or greater were considered the best explanations for the data.

Results

Morphology and OSR

I compared three male morphological traits (growth rate, residual anal fin size and residual testis size) across OSR treatment and between generations. Male growth rate was best explained by the effect of OSR treatment, generation, and their interaction (Table 1). Growth rate tended to increase with OSR in males from generation 0; however, the opposite pattern was observed in generation 1 from OSR treatments 0.5 to 2 (Table 1; Figure 1A). The model that best explained anal fin size included the effects of OSR treatment (Table 1), and anal fin residual values tended to decrease with increasing OSR (Figure 1B). There was no effect of generation, but a weak effect of OSR on testis size (Table 1). Male body size was best explained by the effect of generation (Table 1), and overall males from generation 0 were larger than males from generation 1 (Figure 1). This difference in body size between generations is most likely due to the effects of initial housing of juveniles, as generation 1 juveniles were reared to adults in smaller tanks than generation 0 fish. Generation 1 fish were moved into large tanks at sexual maturity, at which time the first standard length measurement was taken. Therefore, I do not expect growth rate data to be affected by a difference in juvenile rearing conditions.

Behaviour and OSR

Three behaviours were used to assess the influence of OSR on male mating behaviour (aggression, courtship and following, and the total amount of activity,

calculated as the sum of the three behaviours). The model that best explained the mean total activity of males included the effect of OSR and a weak additive effect of generation and OSR (Table 2), whereby male total activity decreased with increasing OSR (Figure 2A). Aggression was best explained by the effect of generation, and males from generation 1 had higher average aggression at all OSRs compared to males from generation 0 (Figure 2B). Courtship decreased with increasing OSR, and males from generation 1 tended to court more than males from generation 0 (Table 2; Figure 2C). The model that best explained male following behaviour includes the effects of OSR, generation, and their interaction (Table 2). Male following behaviour tended to decrease with increasing OSR, and males in generation 0 tended to follow more than males in generation 1 (Figure 2D).

Behaviour and morphology

I compared the interactive effects of OSR and male body size on behaviour. As male body sizes were not comparable between generations, models for each generation were created separately to avoid any effects of this difference on observed patterns.

Generation 0

Two models effectively explained variation in total activity of generation 0 males; these included only body size, and an additive effect of body size and OSR (Table 3a). My ability to discern patterns among OSR, body size and total activity was affected

by variation in the number of males across OSR treatments. However, total activity tended to increase with male body size in male-biased OSR treatments (i.e., OSR of 2 and 5; Figure 3A). Aggression tended to increase with body size in all OSR treatments (Table 3a; Figure 3A). There was no effect of body size on mean courtship, and the model which best explained mean male courtship included only the effect of OSR treatment (Table 3a; Figure 3A). Similarly, the frequency of following behaviour was best explained by the effect of OSR treatment, as well as a weak additive effect of body size and OSR (Table 3a; Figure 3A).

Generation 1

The model that best explained total activity of generation 1 males included body size, OSR treatment and their interaction (Table 3b). Total activity tended to increase with increasing body size in all OSR treatments, and this relationship is strongest at OSR = 5 (Figure 3b). Male aggression was best explained by body size, OSR treatment and their interaction, as well as weak additive effects of body size, and body size and OSR treatment (Table 3b). Mean aggression tended to increase with body size in all OSR treatments, and especially in OSR 5 (Figure 3b). The model that best explained mean male courtship included the effects of body size and OSR treatment; however, my ability to discern patterns among OSR, body size and courtship was affected by variation in the number of males among OSR treatments (Table 3b; Figure 3b). Two models effectively explained variation in male following behaviour; body size, OSR treatment and their interaction, and an additive effect of body size and OSR (Table 3b). In OSR treatments of 0.5, 1 and 5, following tended to increase with increasing body size, but this pattern did not occur for males in OSR = 2 (Figure 3d).

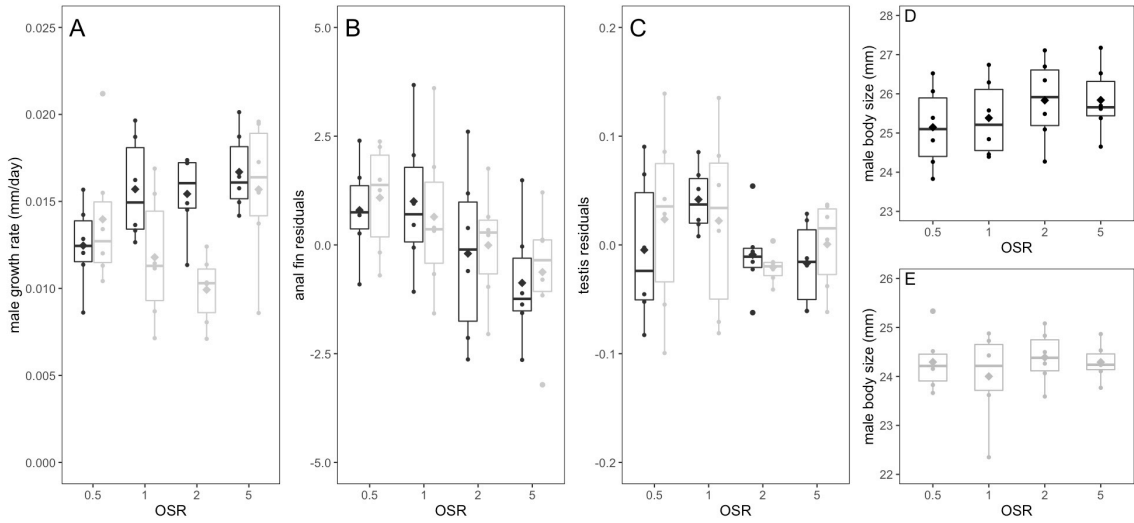


Figure 1. The relationships between OSR treatment and A) male growth rate (mm/day), B) male anal fin size using residuals, C) male testis size using residuals, D) body size of generation 0 males, and E) body size of generation 1 males. Generation 0 males are denoted by black fill and lines, generation 1 males by grey fill and lines. Boxes represent 25th and 75th quartiles, whiskers represent 1.5 x the inter-quartile range and horizontal bars indicate the median. Means are denoted by diamonds and statistical results are in Table 1.

Table 1. Generalized linear models indicating the effects of OSR treatment and generation on candidate male sexually-selected traits (growth rate, anal fin size and testis size). Degrees of freedom (*df*), Akaike Information Criterion (AIC_C), the differences between the model with the lowest AIC_C and all other models (ΔAIC_C), and model weights (ω_{AIC_C}) are included. Akaike Information Criterion (AIC_C) criteria was used for model selection. The best models are highlighted in bold.

Model	<i>df</i>	AIC _C	ΔAIC_C	ω_{AIC_C}
male growth rate				
OSR + generation + OSR x generation	9	-404.5	0.00	0.452
OSR + generation	6	-403.4	1.14	0.255
generation	3	-402.7	1.77	0.187
OSR	5	-400.3	4.22	0.055
(intercept only)	2	-400.2	4.35	0.051
male anal fin size				
OSR	5	181.2	0.00	0.643
OSR + generation	6	183.8	2.57	0.178
(intercept only)	2	184.3	3.15	0.133
generation	3	186.6	5.39	0.043
OSR + generation + OSR x generation	9	192.0	10.85	0.003
male testis size				
(intercept only)	2	-136.7	0.00	0.537
OSR	5	-134.9	1.75	0.224
generation	3	-134.4	2.24	0.175
OSR + generation	6	-132.3	4.33	0.062
OSR + generation + OSR x generation	9	-125.4	11.27	0.002
male body size				
generation	3	121.5	0.00	0.909
OSR + generation	6	126.2	4.67	0.088
OSR + generation + OSR x generation	9	133.4	11.90	0.002
(intercept only)	2	143.7	22.21	0.000
OSR	5	149.2	27.69	0.000

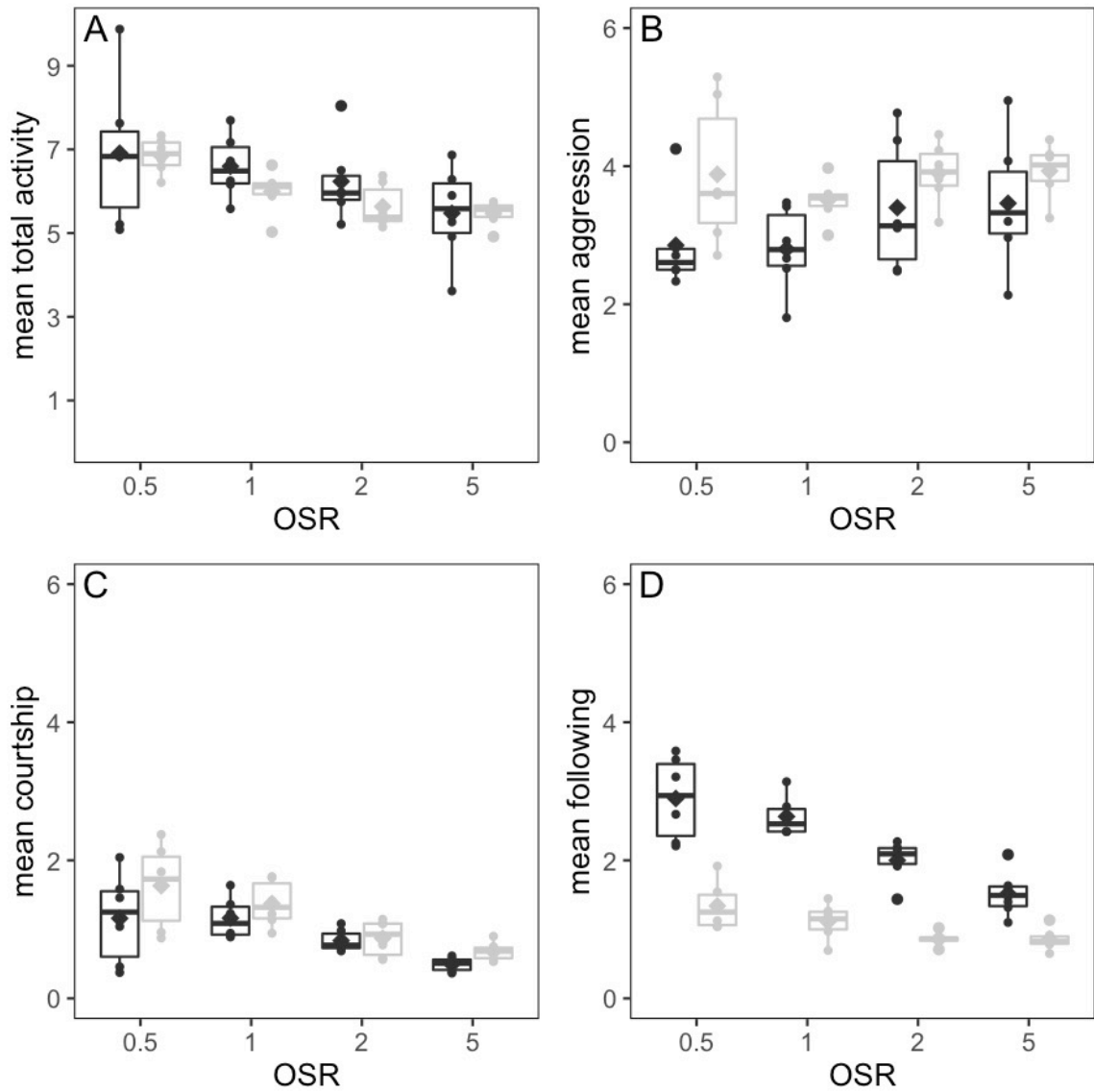


Figure 2. The relationships between OSR treatment and A) male mean total activity, B) mean male aggression, C) mean male courtship, and D) mean male following. Generation 0 males are denoted by black fill and lines, generation 1 males by grey fill and lines. Boxes represent 25th and 75th quartiles, whiskers represent 1.5 x the inter-quartile range and horizontal bars indicate the median. Means are denoted by diamonds and statistical analyses are summarized in Table 2.

Table 2. Generalized linear models indicating the effects of OSR treatment and generation on mean male mating behaviours. Degrees of freedom (*df*), Akaike Information Criterion (AIC_C), the differences between the model with the lowest AIC_C and all other models (Δ AIC_C), and model weights (ω_{AIC_C}) are included. Akaike Information Criterion (AIC_C) criteria was used for model selection. The best models are highlighted in bold.

Model	<i>df</i>	AIC _C	Δ AIC _C	ω_{AIC_C}
total activity				
OSR	5	134.3	0.00	0.615
OSR + generation	6	135.4	1.13	0.350
(intercept only)	2	141.5	7.20	0.017
generation	3	142.6	8.37	0.009
OSR + generation + OSR x generation	9	142.8	8.56	0.008
male aggression				
generation	3	110.8	0.00	0.764
OSR + generation	6	113.4	2.60	0.209
(intercept only)	2	118.2	7.47	0.018
OSR + generation + OSR x generation	9	120.7	9.97	0.005
OSR	5	121.4	10.67	0.004
male courtship				
OSR + generation	6	50.1	0.00	0.728
OSR	5	52.3	2.22	0.240
OSR + generation + OSR x generation	9	56.3	6.25	0.032
generation	3	71.7	21.61	0.000
(intercept only)	2	72.1	22.03	0.000
male following				
OSR + generation + OSR x generation	9	44.9	0.00	0.936
OSR + generation	6	50.3	5.35	0.064
generation	3	77.4	32.43	0.000
OSR	5	113.7	68.75	0.000
(intercept only)	2	117.9	72.94	0.000

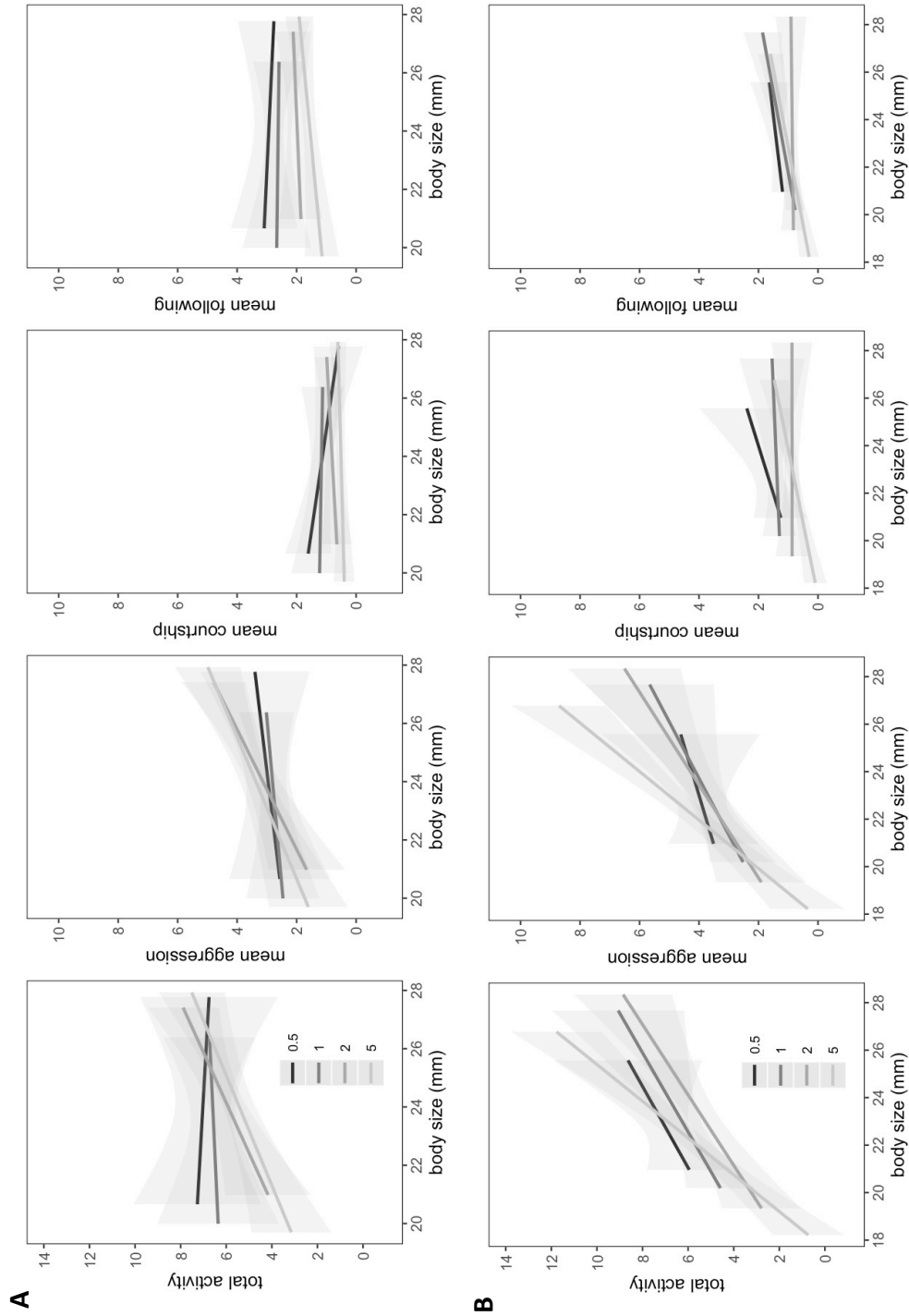


Figure 3. The relationships between male body size and mean male behaviours for A) generation 0 males, and B) generation 1 males, for each OSR treatment. Black lines denote OSR 0.5, and get lighter with each OSR treatment respectively (1, 2 and 5), and standard error with 95% confidence intervals are included for each OSR treatment. Statistical analyses are summarized in Tables 3a and 3b.

Table 3a. Linear mixed effects models indicating the effects of OSR treatment and male body size on mean male mating behaviour in males from generation 0, with tank included as a random effect. Degrees of freedom (*df*), Akaike Information Criterion (AIC_C), the differences between the model with the lowest AIC_C and all other models (Δ AIC_C), and model weights (ω_{AICc}) are included. Akaike Information Criterion (AIC_C) criteria was used for model selection. The best models are highlighted in bold.

Model	<i>df</i>	AIC _C	Δ AIC _C	ω_{AICc}
total activity				
body size	4	805.2	0.00	0.490
body size + OSR	7	805.8	0.55	0.371
body size + OSR + body size x OSR	10	808.2	2.97	0.111
(intercept only)	3	811.6	6.37	0.020
OSR	6	813.5	8.21	0.008
male aggression				
body size	4	657.6	0.00	0.899
body size + OSR	7	662.4	4.78	0.082
body size + OSR + body size x OSR	10	665.4	7.84	0.018
(intercept only)	3	673.9	16.36	0.000
OSR	6	677.4	19.86	0.000
male courtship				
OSR	6	383.2	0.00	0.710
body size + OSR	7	385.4	2.17	0.240
body size + OSR + body size x OSR	10	388.8	5.59	0.043
(intercept only)	3	393.1	9.85	0.005
body size	4	395.2	11.95	0.002
male following				
OSR	6	490.6	0.00	0.644
body size + OSR	7	491.9	1.32	0.334
body size + OSR + body size x OSR	10	497.4	6.75	0.022
(intercept only)	3	511.4	20.75	0.000
body size	4	513.0	22.34	0.000

GENERATION 0

Table 3b. Linear mixed effects models indicating the effects of OSR treatment and male body size on mean male mating behaviour in males from generation 1, with tank included as a random effect. Degrees of freedom (*df*), Akaike Information Criterion (AICc), the differences between the model with the lowest AICc and all other models ($\Delta AICc$), and model weights (ω_{AICc}) are included. Akaike Information Criterion (AICc) criteria was used for model selection. The best models are highlighted in bold.

Model	<i>df</i>	AIC _c	$\Delta AICc$	ω_{AICc}
total activity				
body size + OSR + body size x OSR	10	808.4	0.00	0.694
body size + OSR	7	810.7	2.28	0.221
body size	4	812.6	4.21	0.085
(intercept only)	3	848.2	39.85	0.000
OSR	6	850.5	42.11	0.000
male aggression				
body size + OSR + body size x OSR	10	724.8	0.00	0.477
body size	4	725.7	0.96	0.276
body size + OSR	7	725.7	0.97	0.276
(intercept only)	3	761.0	36.26	0.000
OSR	6	766.5	41.74	0.000
male courtship				
body size + OSR	7	423.0	0.00	0.616
body size + OSR + body size x OSR	10	425.0	2.08	0.218
OSR	6	425.6	2.63	0.165
body size	4	439.9	16.93	0.000
(intercept only)	3	442.5	19.51	0.000
male following				
body size + OSR + body size x OSR	10	285.8	0.00	0.506
body size + OSR	7	285.9	0.07	0.489
body size	4	296.5	10.74	0.002
OSR	6	296.9	11.13	0.002
(intercept only)	3	306.0	20.26	0.000

GENERATION 1

Discussion

In this study, male mating behaviour and morphology varied across OSRs, both within and between generations. Interestingly, OSR had the strongest influence on courtship behaviour, while male body size was the best predictor of aggression. The intergenerational difference detected in overall levels of both courtship and aggression behaviour may provide evidence that selection is acting on these behaviours in this species if males use these traits to gain a mating advantage, resulting in more offspring in the population that exhibit these phenotypes.

Male courtship decreased as OSR became more male-biased in both generation 0 and generation 1 males. These shifts in the frequency of courtship behaviour could be due to the difference in the number of competitors and potential mates in each OSR treatment, which may alter the number of intra- (Clark & Grant, 2010) and intersexual (Clark & Grant, 2010; Grant et al., 2000; Weir et al., 2011) encounters. Alternatively, this pattern could suggest that the costs (i.e., energetic expenditure during courtship displays and/or intrasexual fights to gain access to females to court) and benefits (i.e., mating advantage) of courtship behaviour may be context-dependent, and therefore sexual selection should favour behavioural plasticity in this trait (Montiglio et al., 2017; Patricelli et al., 2002), as observed in wild-caught *O. latipes* (Fujimoto et al., 2015). Because aggression is best explained by variation in male body size, it is possible that positive correlational selection occurs on aggression and body size (Brodie, 1992) if aggression is beneficial for mating success, or that larger males using fighting tactics rather than alternative reproductive tactics more than smaller males, as body size is positively correlated with aggression in Japanese medaka (Fujimoto et al., 2015). There is a notable trend of increasing aggression

and decreasing courtship with increasingly male-biased OSR (as in Clark & Grant, 2010; Kvarnemo & Ahnesjo, 1996), suggesting that there may be a trade-off between aggression and courtship behaviours as OSR increases.

If winning access to females using direct fights results in the opportunity to court females (Baxter, Mentlik, Shams, & Dukas, 2018) and potentially mate, male aggression may be beneficial in highly competitive environments (i.e., high OSRs). Because males in many species use tactics to repress reproductive success of competitors to enhance their own, especially in social environments where females are not defendable (Clutton-Brock & Huchard, 2013), males may also use aggressive behaviours not only to compete for access to females, but also to interrupt interactions between competitors and potential mates (Baxter et al., 2018; Wong, 2004; Yokoi et al., 2016). Interruption of mating events by competitor males has been documented in Japanese medaka (Weir, 2013), so it is possible that males in male-biased OSRs are using aggression to interrupt male-female interactions in this study. In fishes, the effects of social environment on the use of alternative reproductive tactics has been well documented (Grant, Bryant, et al., 1995; Jirotkul, 1999; Mills & Reynolds, 2003; Taborsky, 1994; Weir, 2013), and males use sneak mating and mating disruptions more frequently in situations where mating competition is more intense (i.e., male-biased OSRs, Grant, Bryant, et al., 1995; Mills & Reynolds, 2003; Weir, 2013). As larger males tend to be better competitors, and aggression was best explained by male body size, it is possible that courtship behaviour was limited to dominant males (Wong, 2004).

In Japanese medaka, it has remained unclear whether males use following as a form of courtship (Clark & Grant, 2010) or mate-guarding (Yokoi et al., 2015, 2016),

making it difficult to discern how this behaviour is affected by changes in OSR. However, it is unlikely that males used following behaviour as a form of mate-guarding in this experiment, as it is an energetically-costly activity that may only provide a mating advantage in situations where the presence of competitors is higher (Parker, 1974). Furthermore, males in this species may use following as a form of courting orientation, which is necessary when performing successful quick circles (Ono & Uematsu, 1957). Therefore, following behaviour was most likely used as a type of courtship in this study and resulted in the same pattern as male quick circles, which decrease with an increase in OSR. Interestingly, males from generation 1 followed significantly less overall than males from generation 0, which may be the result of ‘followers’ siring less offspring. This may suggest that following behaviour is not essential in acquiring mates and may not be beneficial, especially when the behaviour is not paired with quick circles.

There was a notable effect of generation on male body size; overall, males from generation 0 were larger than males from generation 1. Because body growth is density-dependent in fishes, particularly at the late juvenile life stage (Hazlerigg, Lorenzen, Thorbek, Wheeler, & Tyler, 2012), this can most likely be attributed to a difference in juvenile housing conditions between generation 0 and generation 1 males due to space restrictions in the aquatic facility. I did not detect a difference in male body size between OSR treatments, perhaps because of sizeable within-tank variance in body size or variation in sample size between OSR treatments. Male growth rate varied with OSR, and these patterns were different between generations. In fish, growth rate can vary as a consequence of behavioural interactions experienced by an individual (Ruzzante & Doyle, 1991). In generation 0, male growth rate increased with OSR, such that there was

a trend of increasing body size with an increase in OSR. This could be a result of the variation in total mating activity of individuals, as males in more male-biased OSRs expend less energy on mating activity, and less expenditure on courtship, than males in female-biased OSRs. Therefore, these energetic savings by participating less in social interactions could be used for growth. As both intra- and intersexual interactions were included in calculating total mating activity, it is unlikely that this decrease is a result of differences in the frequency of individual male encounters with males and/or females.

Relative anal fin and testis size also varied with respect to OSR. The anal fin functions in mating (Fujimoto et al., 2014) and fertilization success by acting as a barrier to contain released sperm (Koseki et al., 2000). For this reason, larger anal fins may be selected for in environments with higher sperm competition to prevent successful sneak mating by rival males. Similarly, larger testes are predicted to be selected for in environments with high sperm competition (Harcourt et al., 1981; Schulte-Hostedde & Millar, 2004; Stockley et al., 1997). However, in this study, relative anal fin size and testis size both decreased with an increase in OSR from 0.5-5. Because male reproductive success is positively correlated with the number of mating events, males should mate as often as possible (Arnqvist & Rowe, 2005). Males who have an opportunity to mate with multiple females may invest more in both mating-related behaviours, as well as testes and anal fins rather than overall body size to increase their reproductive success, provided that these traits are not directly linked to body size. Montiglio et al. (2017) found that in stream water striders, (*Aquarius remigis*), males with higher mating-related activity behaviour experienced a mating advantage when few rivals were present, but this advantage decreased as the number of rival males increased. Males in my study may

invest more time and energy into overall mating-related activity and structures to maximize their reproductive success in an environment where they can mate multiply compared to males in highly male-biased OSRs where fewer females are available.

Of more general interest is the higher frequencies of both aggression and courtship observed in generation 1 males compared to generation 0 males in the same OSRs. Fujimoto et al. (2015) found variation in male mating behaviours between wild populations of *O.latipes*; frequencies of courtship and aggression differed between Northern and Southern populations, and these differences were maintained in a laboratory setting during mating experiments which suggested a genetic basis of these behavioural traits. These differences in behaviour were attributed to a difference in OSR among the populations, and males from a population with an even OSR (i.e., OSR ~1) were less aggressive and courted less than males from a population with a male-biased OSR (i.e., OSR ~2, Fujimoto et al., 2015). As both aggression (Fujimoto et al., 2015; Ruzzante & Doyle, 1991; Sasaki & Yamahira, 2016) and courtship (Fujimoto et al., 2015; Sasaki & Yamahira, 2016) behaviours are presumed to have genetic basis in this species, perhaps males with higher aggression and courtship rates obtained a mating advantage in generation 0. Previous research using choice experiments has indicated that females prefer males who court more (Fujimoto et al., 2015; Grant & Green, 1996), as courtship may be an honest signal of fertility in this species (Weir & Grant, 2010). Certain mating behaviours may be restricted to dominant individuals (Wong, 2004) in male-biased environments, as larger males in this study exhibited higher total mating activity than smaller males. However, paternity analyses on males from this study are not yet completed.

In summary, male aggressive behaviour was positively associated with OSR, while male courtship behaviour and relative size of sexual structures were negatively associated with OSR. While courtship behaviour was best explained by social environment and tended to decrease as OSR became more male-biased, aggressive behaviour was best explained by male body size and tended to increase with OSR. Generation 1 males showed higher overall frequencies of both courtship and aggression when compared to generation 0 males. Structures used in reproduction were relatively larger and total mating activity higher in female-biased compared to male-biased OSRs. These results suggest that there may be trade-offs in the investment of time in behavioural traits with respect to an individual's social environment.

CHAPTER 2: Males rapidly adjust mating behaviours in response to a novel OSR

Behavioural plasticity is an adjustment of an individual's behaviour associated with changes in their environment (Dingemanse, Kazem, Réale, & Wright, 2010). In social settings, an animal's internal state, external stimuli (Stamps, 2016) and past social experiences (Pfaus et al., 2001) all interact to affect how an individual behaves in a given situation at a particular time. Furthermore, the payoff of specific behaviours in an individuals' repertoire can be changed by the presence of conspecifics (Laskowski & Bell, 2013). In a mating context, the relative number of sexually-receptive males and females, or the operational sex ratio (OSR; Emlen, 1976), of a population can influence the degree to which particular behaviour contributes to an individual's mating success, and may result in plasticity of mating behaviours with shifts in the intensity of competition for mates (Weir et al., 2011). It is important for animals to be flexible in their immediate responses to changing social environments to maximize their reproductive success (Pfaus et al., 2001).

Previous work has suggested that males are relatively consistent in the frequency their of mating behaviours, but are able to respond to changes in OSR by adjusting the levels of their behaviour (Magellan & Magurran, 2007; Montiglio et al., 2017). For example, Magellan & Magurran (2007) found high activity levels associated with specific mating behaviours in male guppies (*Poecilia reticulata*) were maintained in OSRs ranging from female-biased to male-biased (0.4, 1, 2.3, respectively). However, individual males may respond differently to changes in social environment (Montiglio et al., 2017) and differ in sexual activity from other males across all social environments (Polverino, Palmas, Evans, & Gasparini, 2019). Individual differences between males

may be consistent over time (Polverino et al., 2019), and this could be explained by an individual's preferred mating tactic, as some males may participate in intrasexual competition, while others may use alternative reproductive tactics (Montiglio et al., 2017).

When individual behaviour that confers a mating advantage is dependent upon that individual's current situation, sexual selection should favour behavioural plasticity (Montiglio et al., 2017; Patricelli et al., 2002). If behaviours are dependent upon some other fixed traits of an individual, sexual selection may be influenced by correlations among those traits (Brodie, 1992). It is important for animals to exhibit at least some behavioural plasticity in the context of mating systems so that their success is not limited to a specific set of conditions, and it is also important in maintaining a high degree of diversity of sexually-selected phenotypic traits (Pfaus et al., 2001). However, there are limits associated with plasticity, as well as phenotypic costs due to trade-offs between trait benefits and resource allocation (Murren et al., 2015). Costs in the form of reduced mating efficiency could be associated with inaccurate or unreliable information acquisition about an individual's environment (Magurran & Nowak, 1991). Because plasticity is limited by an individual's ability to acquire accurate information about its environment, inaccurate acquisition or interpretation of cues can lead to the expression of non-optimal phenotypes for a specific environment (Dewitt, Sih, & Wilson, 1998). For these reasons, plasticity is expected to persist only if the benefits equal or outweigh the costs (Dewitt et al., 1998), or the cost of plasticity is negligible.

A suitable study species to explore behavioural plasticity in response to variation in OSR are Japanese medaka (*Oryzias latipes*), and their relevance in studying mating

systems has been outlined in Chapter 1. Importantly, male mating behaviour in *O. latipes* may vary with respect to OSR; sneaking and mating interference (Grant, Bryant, et al., 1995) may increase with OSR, while courtship behaviour may decrease (Clark & Grant, 2010). Male aggression also increases with OSR up until a point (i.e., 2:1) where the energetic costs of fighting become unfavourable (Clark & Grant, 2010). However, results from Chapter 1 indicate that courtship behaviour decreases while aggression increases as the OSR becomes more male-biased, and body size is the best predictor of male aggression. These differences in behaviour in response to variation in OSR, as well as aggression perhaps being less context-dependent than courtship, provide an appropriate basis for examining the plasticity of male mating behaviour.

I examined the ability of individuals to adjust their mating behaviour in novel OSR environments, and the degree to which their previous environment may influence male behaviour when in a novel OSR environment. I also tested how quickly males may respond to changes in OSR, by examining male behaviour one day and one week following introduction to a novel OSR environment. This timeline was chosen to detect any rapid changes in behaviour due to changes in social environment (Montiglio et al., 2017). Because some males have the ability to alter their behaviour in response to changes in social environment (Magellan & Magurran, 2007; Montiglio et al., 2017), I predicted that males will alter their behaviour with respect to the presence of both rivals and potential mates in their environment by either increasing or decreasing their levels of aggression and courtship behaviour to match levels exhibited by males in their 'home' environments. Previous social environment can have an effect on future behaviour (Whitehouse, 1997), which led to the prediction that previous social environment will

have an effect on male behaviour in a novel social environment. Because Japanese medaka experience changes in social environment daily due to changes in female receptivity, I also predicted that males will be able to immediately recognize their new environment, and therefore changes in behaviour will be observed one day after males are introduced to a novel OSR treatment.

Methods

Experimental Animals: Marking & Housing Conditions

Generation 0 males from Chapter 1 were used to examine plasticity in mating behaviours of males with changes in OSR. Thus, tagging procedures and housing conditions are the same as outlined in Chapter 1.

Experimental Design

The experimental design for baseline data collection is outlined in Chapter 1. After spending eight months in their baseline OSR treatment, each male was moved into a novel OSR treatment to examine potential changes in mating behaviours (Figure 4). Females remained in the same tank (and therefore, same baseline OSR treatment), and the males were assigned to specific novel OSR treatment tanks to ensure they were not familiar with all other males or females in their given tank. Within new OSR treatments, each replicate tank was comprised of the same number of males from each original OSR treatment so that results from each replicate could be compared and combined during analyses. Different proportions of males from each original OSR were used among new OSR treatments due to the variation in sample size of males among original OSR treatments.

Behavioural Observations

Behavioural observations of each male were recorded for the baseline and novel

OSR environments. Behavioural data from Chapter 1 was used as baseline data in this experiment (see Chapter 1 – Methods). Immediately following the last baseline observation, individual fish were moved to a different OSR (i.e., ‘novel’ OSR; Figure 4) for one week. During this time, each male was observed for two minutes at ‘lights on’ for a total of two observation per male; one day following to the change in OSR, and one week following the change. Behaviour was quantified by observing males in the different competitive environments (i.e., OSRs) to determine frequencies of aggression, courtship and following (these behaviours are defined as in Chapter 1).

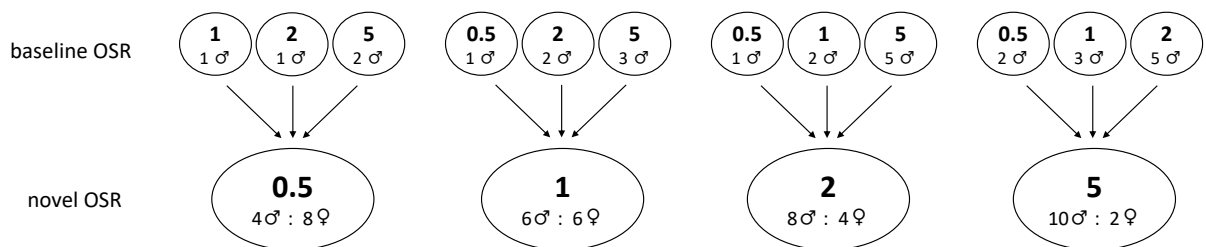


Figure 4. Experimental design where fish are moved from their baseline OSR treatment into a novel OSR treatment. The density was kept at 12 fish per tank in the novel OSR treatment.

Statistical Analyses

All analyses were carried out using R Version 3.5.2 (R Core Team, 2018).

Relationships between behaviour, OSR and treatment (i.e. baseline, day and week after change to novel OSR) were determined by Generalized Linear Models (GLMs).

Relationships between behaviour, OSR and treatment were also analyzed using the proportions of each behaviour (i.e., aggression, courtship and following) that contributed

to the total activity of each male (Figure S4; Table S4). Akaike Information Criterion (AICc) criteria was used for model selection as in Chapter 1.

I used intraclass-correlation coefficients (ICC) and random effect variances to examine behavioural change for individual males when placed in different treatments, including “individual” as a random effect in these models. Variance components were then transformed into percentages to determine the proportion of the total variation observed that can be attributed to among- or within-individual variation.

Results

Male behaviour in baseline and novel OSR treatments

I compared the differences in male behaviour among OSRs between baseline data and males in novel OSR environments. I examined the effects of OSR, observation time (i.e., baseline, one day after or one week after a change to a novel OSR) and their interaction on male behaviour. Total activity did not differ across the three time periods and was only weakly influenced by OSR (Table 4). Male aggression tended to increase with increasing OSR and did not differ across time periods among OSR treatments (Table 4; Figure 5). The best model to explain male courtship behaviour included the additive effects of OSR and treatment, which decreased as OSR increased, and was higher overall the day after the swap occurred (Table 4; Figure 5). Similarly, the additive effects of OSR and treatment explained following behaviour, which tended to decrease with increasing OSR (Table 4; Figure 5).

Change in male behaviour in novel OSR treatment

I used the baseline data to examine how introduction to a novel OSR treatment affected male behaviour by calculating differences in behavioural frequencies of each male between their baseline and novel OSR treatment. The model that best explained the difference in male aggression one day after introduction to a novel OSR treatment included the effect of the novel OSR treatment (Table 5a; Figure 6). The effect of males' baseline OSR and novel OSR treatment best explained the difference in both courtship

and following behaviour one day after introduction to the new OSR treatment (Table 5a; Figure 6).

The difference in aggression when comparing baseline behaviour to males after one week in their new OSR treatment was explained by the effect of baseline OSR treatment, but this did not differ markedly from a model containing only the intercept (Table 5b; Figure 6). The model that best explained a difference in courtship one week after introduction into a new social environment included the effects of baseline OSR and novel OSR, as well as a weak effect of their interaction (Table 5b; Figure 6). Baseline OSR and novel OSR, as well as a weak effect of only baseline OSR, explained the difference in following behaviour of males after one week in a new OSR treatment (Table 5b; Figure 6).

Among- and within-individual variation in behaviour

I examined the variance in baseline behaviour among and within individuals across 8 observations per male. Within-individual variation explained over half of the total variance in baseline observations in all three behaviours (Figure 7). Within-individual variation also explained over half of the total variance between baseline behaviour and one day after individuals were subject to a novel OSR environment, and between baseline behaviour and one week in a novel OSR treatment (Table 6; Figure 7). When comparing baseline to novel OSR treatments, within-individual variation in courtship and following explained more of the total variance in mating behaviour than it did for aggressive behaviour, and was also higher when comparing baseline and day after

behaviour levels than baseline and week after behaviour levels (Table 6). Over half of the total variance observed between one day and one week in a novel OSR treatment was explained by within-individual variance (Table 6; Figure 7).

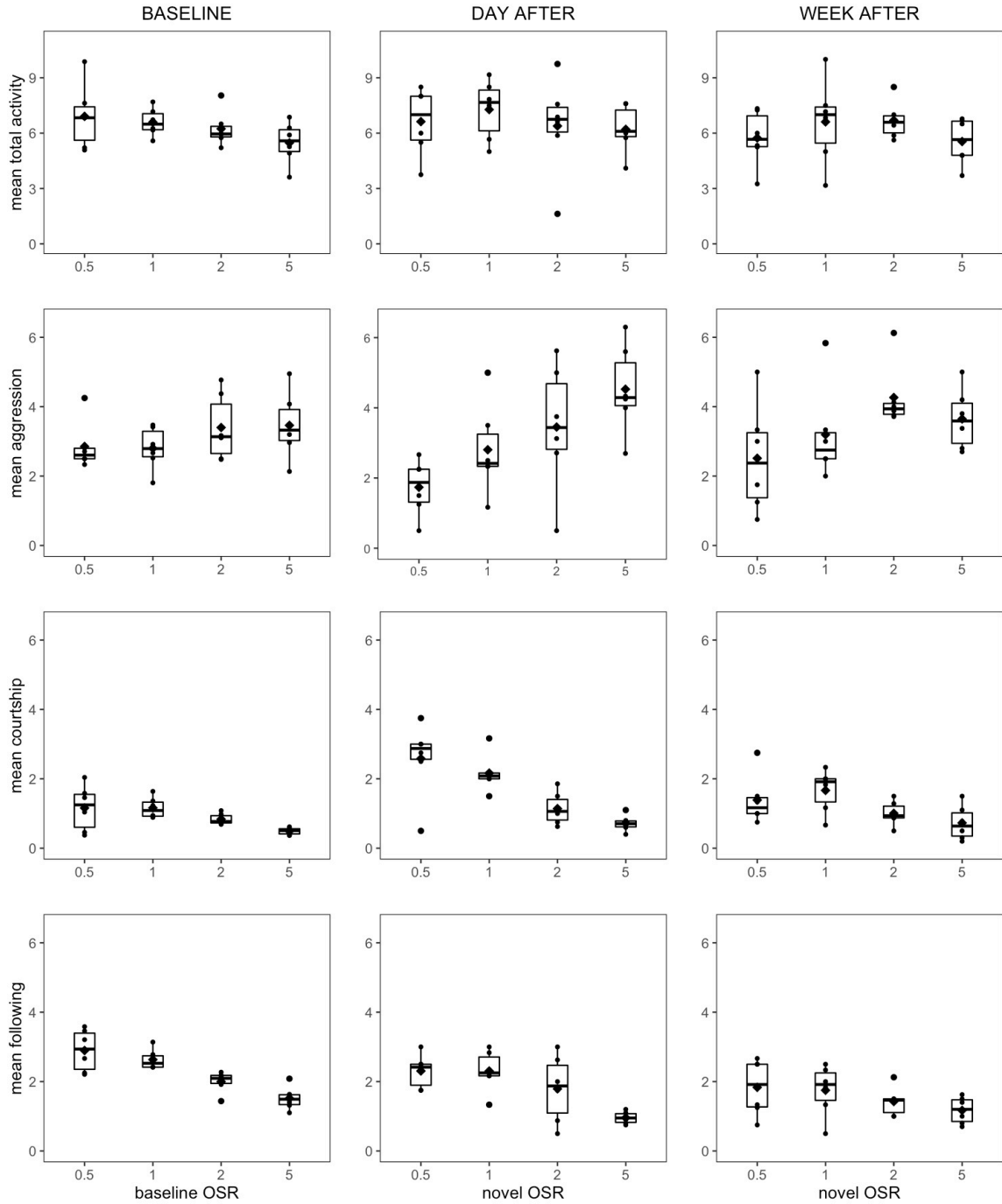


Figure 5. The relationships between OSR treatment and mating behaviours of males in their baseline OSR treatment, one day and one week after introduction into a novel OSR treatment. Boxes represent 25th and 75th quartiles, whiskers represent 1.5 x the interquartile range and horizontal bars indicate the median. Means are denoted by diamonds and statistical analyses are summarized in Table 4.

Table 4. Generalized linear models indicating the effects of OSR and treatment (i.e. baseline, one day after or one week after change to novel OSR) on male behaviour. Males from all OSR treatments were included in these models. Degrees of freedom (*df*), Akaike Information Criterion (AIC_C), the differences between the model with the lowest AIC_C and all other models (Δ AIC_C), and model weights (ω_{AICc}) are included. Akaike Information Criterion (AIC_C) criteria was used for model selection. The best models are highlighted in bold.

Model	<i>df</i>	AIC _C	Δ AIC _C	ω_{AICc}
total activity				
(intercept only)	2	274.4	0.00	0.570
OSR	5	275.9	1.45	0.276
treatment	4	277.7	3.30	0.109
OSR + treatment	7	279.5	5.08	0.045
OSR + treatment + OSR x treatment	13	292.3	17.88	0.000
aggression				
OSR	5	235.7	0.00	0.880
OSR + treatment	7	239.8	4.15	0.110
(intercept only)	2	245.7	9.98	0.006
OSR + treatment + OSR x treatment	13	246.9	11.23	0.003
treatment	4	249.5	13.85	0.001
courtship				
OSR + treatment	7	134.8	0.00	0.829
OSR + treatment + OSR x treatment	13	138.0	3.17	0.170
OSR	5	148.2	13.35	0.001
treatment	4	165.1	30.24	0.000
(intercept only)	2	172.0	37.18	0.000
following				
OSR + treatment	7	126.4	0.00	0.991
OSR + treatment + OSR x treatment	13	135.8	9.37	0.009
OSR	5	143.1	16.65	0.000
treatment	4	161.1	34.69	0.000
(intercept only)	2	169.5	43.06	0.000

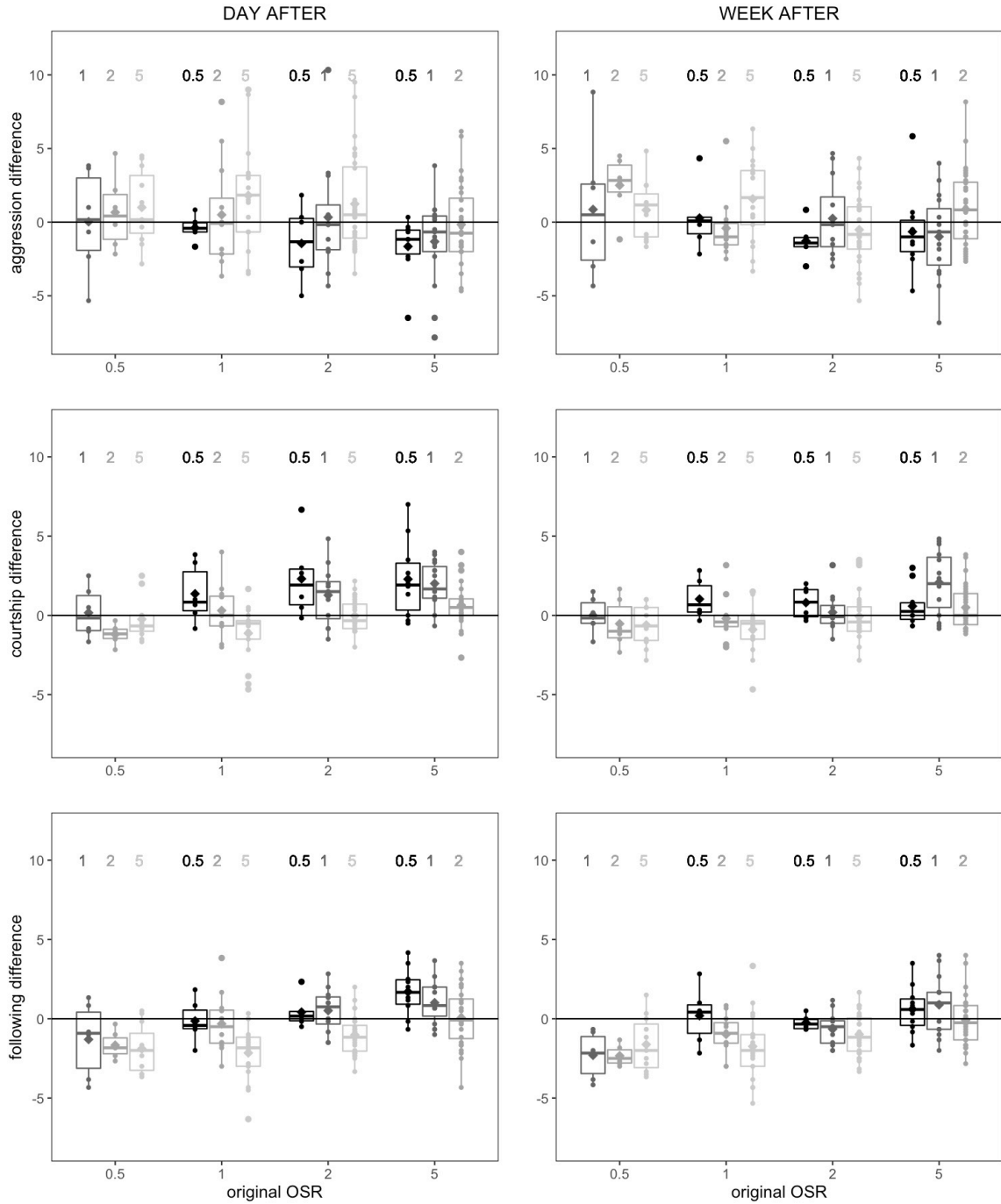


Figure 6. The relationships between original OSR treatment and the difference in male mating behaviour between baseline and novel OSR treatments. Individual boxes and labels represent the males' novel OSR treatments. Horizontal line represents baseline data collected on each male in their original OSR. Boxes represent 25th and 75th quartiles, whiskers represent 1.5 x the inter-quartile range and horizontal bars indicate the median. Means are denoted by diamonds and statistical analyses are summarized in Tables 5a and 5b.

Table 5a. Generalized linear models indicating the effects of baseline and novel OSR treatment on the difference in behaviour of males between their baseline and novel OSR treatment one day after introduction. Degrees of freedom (*df*), Akaike Information Criterion (AIC_C), the differences between the model with the lowest AIC_C and all other models (Δ AIC_C), and model weights (ω_{AICc}) are included. Akaike Information Criterion (AIC_C) criteria was used for model selection. The best models are highlighted in bold.

Model	<i>df</i>	AIC _C	Δ AIC _C	ω_{AICc}
aggression difference				
novel OSR	5	797.9	0.00	0.800
original OSR + novel OSR	8	801.4	3.44	0.143
original OSR	5	803.7	5.74	0.045
(intercept only)	2	806.4	8.52	0.011
original OSR + novel OSR x original OSR + novel OSR	13	811.8	13.84	0.001
courtship difference				
original OSR + novel OSR	8	599.0	0.00	0.836
original OSR + novel OSR x original OSR + novel OSR	13	603.0	3.93	0.117
novel OSR	5	604.8	5.79	0.046
original OSR	5	624.2	25.13	0.000
(intercept only)	2	641.2	42.14	0.000
following difference				
original OSR + novel OSR	8	595.3	0.00	0.925
original OSR + novel OSR x original OSR + novel OSR	13	600.3	5.06	0.074
novel OSR	5	609.9	14.60	0.001
original OSR	5	614.5	19.29	0.000
(intercept only)	2	645.7	50.45	0.000

Table 5b. Generalized linear models indicating the effects of baseline and novel OSR treatment on the difference in behaviour of males between their baseline and novel OSR treatment one week after introduction. Degrees of freedom (*df*), Akaike Information Criterion (AIC_C), the differences between the model with the lowest AIC_C and all other models (Δ AIC_C), and model weights (ω_{AICc}) are included. Akaike Information Criterion (AIC_C) criteria was used for model selection. The best models are highlighted in bold.

Model	<i>df</i>	AIC _C	Δ AIC _C	ω_{AICc}
aggression difference				
original OSR	5	758.8	0.00	0.468
(intercept only)	2	759.9	1.09	0.272
novel OSR	5	761.5	2.66	0.124
original OSR + novel OSR	8	762.3	3.49	0.082
original OSR + novel OSR x original OSR + novel OSR	13	763.1	4.28	0.055
courtship difference				
original OSR + novel OSR	8	576.0	0.00	0.504
original OSR + novel OSR x original OSR + novel OSR	13	576.4	0.35	0.423
novel OSR	5	581.1	5.06	0.040
original OSR	5	581.5	5.46	0.033
(intercept only)	2	596.1	20.02	0.000
following difference				
original OSR + novel OSR	8	594.0	0.00	0.540
original OSR	5	594.6	0.59	0.402
original OSR + novel OSR x original OSR + novel OSR	13	598.5	4.47	0.058
novel OSR	5	614.3	20.25	0.000
(intercept only)	2	625.1	31.11	0.000

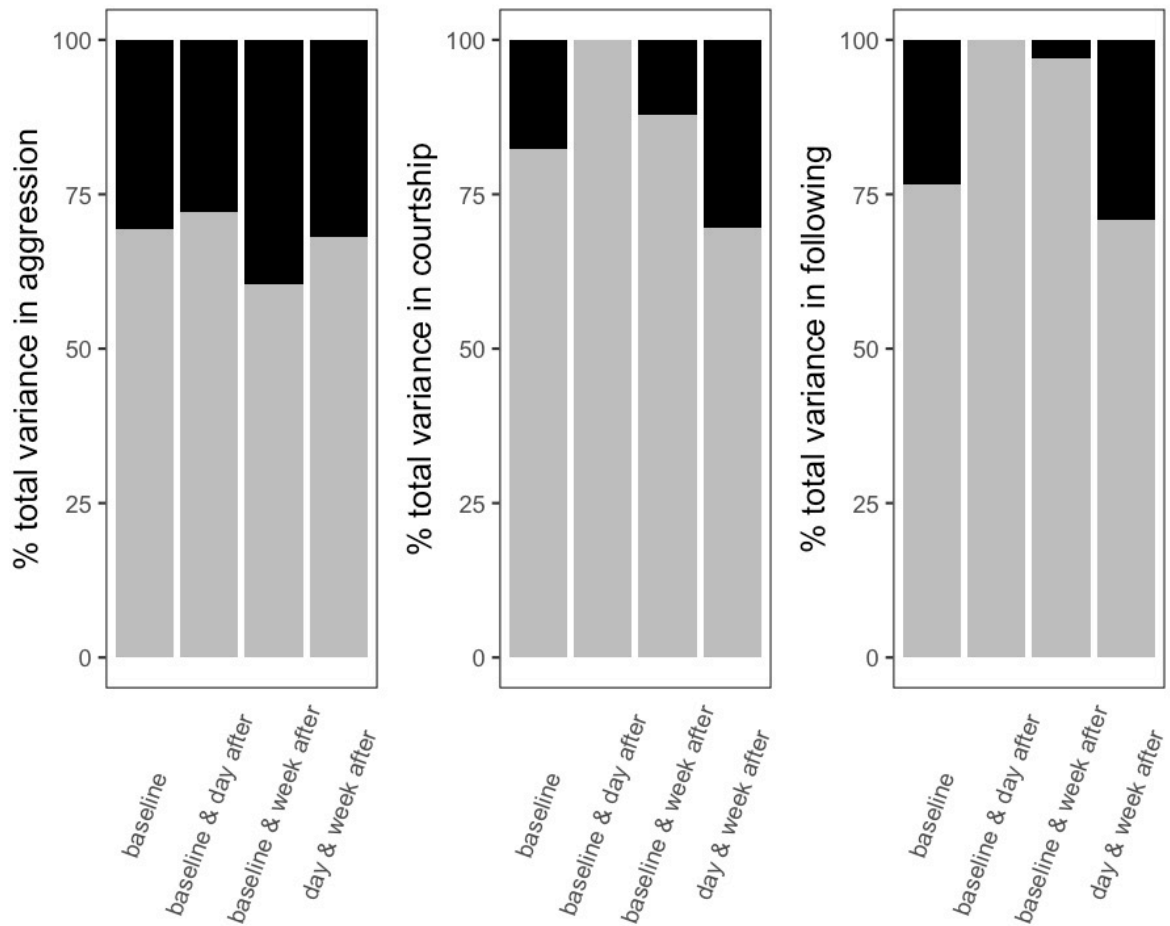


Figure 7. The percent of total variance observed in each behaviour between different treatments that is attributed to within-individual variance (grey) and among-individual variance (black). Statistical analyses are summarized in Table 6.

Table 6. Analysis of random effect variances and intraclass-correlation coefficients (ICC) to examine the effect of individual males on mating behaviours between different treatments. Variance components were then transformed into percentage of the total variance observed.

Model	variance	ICC	% of total variance
aggression			
baseline			
among individuals	2.639	0.3073	30.7%
within individuals	5.948		69.3%
baseline & day after			
among individuals	1.856	0.2790	27.9%
within individuals	4.796		72.1%
baseline & week after			
among individuals	2.341	0.3950	39.5%
within individuals	3.585		60.5%
day after & week after			
among individuals	2.859	0.3197	32.0%
within individuals	6.084		68.0%
courtship			
baseline			
among individuals	0.345	0.1770	17.7%
within individuals	1.602		82.3%
baseline & day after			
among individuals	0.000	0.000	0.0%
within individuals	1.723		100.0%
baseline & week after			
among individuals	0.185	0.1226	12.2%
within individuals	1.327		87.8%
day after & week after			
among individuals	0.758	0.3053	30.5%
within individuals	1.725		69.5%
following			
baseline			
among individuals	0.868	0.2334	23.3%
within individuals	2.851		76.7%
baseline & day after			
among individuals	0.000	0.000	0.0%
within individuals	1.732		100.0%
baseline & week after			
among individuals	0.046	0.0287	2.9%
within individuals	1.550		97.1%
day after & week after			
among individuals	0.578	0.2910	29.1%
within individuals	1.408		70.9%

Discussion

In this study, males rapidly changed the frequencies of mating behaviours when subjected to novel OSR environments, as over half of the total variation in mating behaviours was attributed to within-individual differences both one day and one week after introduction to a novel environment. Interestingly, within-individual variation explained more of the total variation in mating behaviour for courtship and following than aggression. Also, overall patterns in behaviour with respect to OSR in baseline treatments were maintained in novel OSR treatments after only one day in a new environment, but less so after one week in a new environment. Although different proportions of males from each original OSR were used among new OSR treatments, males from all original OSR treatments changed their behaviour in novel environments. From these findings, I suggest that males are able to quickly alter their behaviour with respect to OSR, and that courtship behaviour may be more plastic than aggressive behaviour in this species. I propose that male Japanese medaka may be able to adjust mating behaviour because they experience temporal variation in sex ratio each day due to changes in female receptivity, as behavioural plasticity is expected to increase with environmental variation (Carroll & Corneli, 1995; Karlsson, Eroukhmanoff, & Svensson, 2010; Komers, 1997).

Chapter 1 indicated that male courtship decreased as OSR became more male-biased. This pattern could suggest that the costs and benefits of courtship behaviour may be context-dependent, such that males in male-biased OSRs must first compete with rivals for access to females before they can perform courtship displays, whereas males in female-biased OSRs have the ability to court females mostly uncontested and obtain a mating advantage from this behaviour. Therefore, sexual selection should favour

behavioural plasticity in this trait (Montiglio et al., 2017; Patricelli et al., 2002), as observed in wild-caught *O. latipes* (Fujimoto et al., 2015). By contrast, male aggression did not vary as much as courtship. This may be because male body size and aggression are positively correlated in this species (Fujimoto et al., 2015), and males are maintaining their levels of participation in direct contests across OSRs. However, there is a notable trend of increasing aggression with OSR (as in Clark & Grant, 2010; Kvarnemo & Ahnesjö, 1996), suggesting that there may be a trade-off between the value of aggression and courtship behaviours as OSR increases. Following behaviour was probably used as a form of courtship in this study and resulted in the same trend as male quick circles, which decrease with an increase in OSR.

In fast-changing social environments, males must adequately respond to both the presence and number of mating competitors to maximize their success (Bretman, Gage, et al., 2011; Bretman, Westmancoat, et al., 2011). Males in a population may respond differently to changes in the level of competition (Montiglio et al., 2017; Natarajan, de Vries, Saaltink, de Boer, & Koolhaas, 2009). For example, more aggressive males may not alter their level of aggression in response to social environment, while less aggressive males do (Natarajan et al., 2009). Because the advantages of plasticity depend on a trade-off between current and future mating events, males who are less likely to mate in the future should invest only in their current mating opportunity, rather than in plasticity (Bretman, Gage, et al., 2011). In a mating context, an individual's experience in past social environments may affect behavioural plasticity in a current situation (Carroll & Corneli, 1995; Karlsson et al., 2010; Komers, 1997). For example, male soapberry bugs (*Jadera haemaiolom*) from a population with variable sex ratio reduced their level of

mate-guarding in a situation with high female availability, whereas males from a population with a static sex ratio did not show flexibility in this behaviour (Carroll & Corneli, 1995).

Overall patterns in courtship and aggression with respect to OSR were maintained in novel OSR environments one day after introduction, but less so after one week in a novel OSR. Because most of the total variation in behaviour was explained by individual differences in behaviour across OSRs, male *O. latipes* may be using cues from their social environment to adjust their levels of mating behaviours. All behaviours examined in this study appear to show flexibility; however, both courtship and following behaviours may be more context-dependent than aggression. Overall, within-individual differences contributed less to the total variation in aggressive behaviour than courtship and following when comparing the baseline OSR treatments to novel OSR treatments. Furthermore, male aggression measured one day after introduction to a novel OSR environment was best explained by the novel OSR treatment, but when measured one week after introduction it was best explained by individuals' original OSR treatments. This, along with an observed positive relationship between body size and aggression (Chapter 1), may provide evidence that aggressive behaviour is a more 'fixed' behaviour than courtship and following. It is important to consider that a change in sex ratio not only alters the level of male competition, but also the signals that males may receive about potential mating opportunities (Bretman, Gage, et al., 2011) and female choosiness (Fujimoto et al., 2015; Jirotkul, 1999). However, the degree to which males prioritize these signals from competitors and potential mates is unknown.

It is important for animals to be flexible in their immediate responses to changing social environments to maximize their reproductive success (Pfaus et al., 2001). My findings suggest that males are able to alter their mating behaviour when introduced to novel social environments that vary in mate availability and intrasexual competition. Moreover, I provide evidence that courtship behaviour is more plastic than aggressive behaviour in males both one day and one week after introduction to a new environment. Investigation into how males prioritize information they receive about their social environment in a mating context may provide further understanding for both the variation in behaviour with respect to OSR and the degree of variation that occurs on an individual level.

GENERAL DISCUSSION

I found that both male behaviour and morphology vary with OSR, and that behaviours vary inter-generationally within OSR treatments. Furthermore, males were able to rapidly adjust their behaviour when introduced to a novel OSR environment, such that their behaviour one day and one week after matched those individuals who were housed in specific OSR treatments for many months. My results show that OSR is an important determinant of mating system structure and male morphology. From an evolutionary perspective, OSR may lead to the evolution of male behaviour and morphology, if these traits are heritable.

The differences in behaviour observed in generation 0 males were a result of behavioural plasticity, as these males were randomly selected from a stock population and housed in specific OSR treatments. Results from Chapter 2 further support that male Japanese medaka adjust their mating behaviour in response to OSR. The evolution of adaptive plasticity relies on environmental variability for which different phenotypes are selected, and that no phenotype is favourable in all environments (Ghalambor et al., 2007). Furthermore, mating behaviours that are context-dependent should be plastic (Patricelli et al., 2002) so that individuals are able to maximize their success by adjusting behaviours with respect to social environment. This species may exhibit plastic behaviour in response to changes in OSR because they experience temporal variation in OSR over short (Leaf et al., 2011) and long (Fujimoto et al., 2015) timescales.

Sexual selection on courtship and aggression may be directly or indirectly influenced by female choice. Previous research has indicated that female medaka prefer larger males over smaller males; this was true in association tests, as well as mate choice

tests where larger males obtained a mating advantage over smaller males (Howard et al., 1998). Because males rely on their ability to compete using aggressive behaviour to win access to females (Hamilton et al., 1969; Walter & Hamilton, 1970), and larger males were more aggressive than smaller males in my study, more aggressive males may be selected. This may happen both indirectly and/or directly; females may be choosing to mate with larger males, and larger males may be better at gaining access to females through direct fights. Female medaka also prefer males who court more (Fujimoto et al., 2015; Grant & Green, 1996), and dominant males may have more opportunities for courtship (Wong, 2004). Because these behaviours may have a genetic basis (Fujimoto et al., 2015; Ruzzante & Doyle, 1991; Sasaki & Yamahira, 2016), perhaps males with higher aggression and courtship rates obtained a mating advantage in generation 0, leading to overall higher frequencies of both aggression and courtship in generation 1 males.

While male courtship rate was negatively associated with increasing OSR, aggression was positively associated with body size. Fujimoto et al. (2015) found a significant correlation between standard length and both quick circle and combat behaviours in wild *O.latipes*, and that courtship behaviour was plastic. I propose that courtship behaviour is more context-dependent than aggression because the total variation in behaviour explained by within-individual differences was less for aggressive behaviour than courtship, and aggression was positively correlated with body size. Understanding these relationships may help in explaining the contrasting pattern of decreasing courtship and increasing aggression as OSR becomes more male-biased; in social environments where the level of male-male competition is higher, dominant males may benefit from a

courtship advantage (Wong, 2004). Alternatively, if dominant males spend more time in direct fights with other males, subordinate males may have the chance to court females.

Relative anal fin and testis size, characteristics both involved in mating and fertilization success (Fujimoto et al., 2014; Koseki et al., 2000), also varied with respect to OSR. Previous work on wild *O. latipes* identified differences in relative anal fin sizes between two populations that differ in OSR; males from the male-biased OSR population (i.e. 2) had larger anal fins compared to males from the even OSR population (Fujimoto et al., 2015). Rather than seeing an increase in the size of male sexual structures often associated with an increase in sperm competition and the number of rivals males, males in my study showed an opposite trend. Because males can fertilize the eggs of up to 25 females per day, I propose that males invested in primary and secondary reproductive structures when more females were present (i.e., female-biased and equal OSR, rather than male-biased OSRs) to mate multiply to increase their reproductive success. In male-biased OSRs, males may rely on displays of aggression that are primarily fast charges and chases towards rivals. Having a smaller anal fin in these highly competitive environments may aid in an individual's ability to perform these displays effectively by reducing drag in the water, however the degree to which anal size affects this behaviour is unknown.

In summary, both mating-related behaviour and morphology varied with respect to OSR. These patterns suggest that males alter their behaviour based on social environment, and that these adults are also plastic in morphological traits related to mating and fertilization success. Furthermore, differences in courtship and aggressive behaviours between generations provide further evidence for a genetic basis in these traits. However, paternity analyses are needed to aid in the understanding of both the

behavioural patterns observed in this study with respect to OSR to explain which specific behaviours are 'optimal' and provide a mating advantage in each OSR treatment by determining which males had the most reproductive success, and to understand the inter-generational difference in behaviour. Additionally, investigation into behaviour and the degree of choosiness in females would provide a more detailed understanding of mating-related interactions and selection on sexual traits with variation in social environment. Overall, this study contributes to existing literature on the effects of OSR on male mating behaviour and plasticity in these behaviours over short temporal scales, as well as highlights intragenerational plasticity in male sexual structures associated with social environment.

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SUPPLEMENTARY MATERIAL

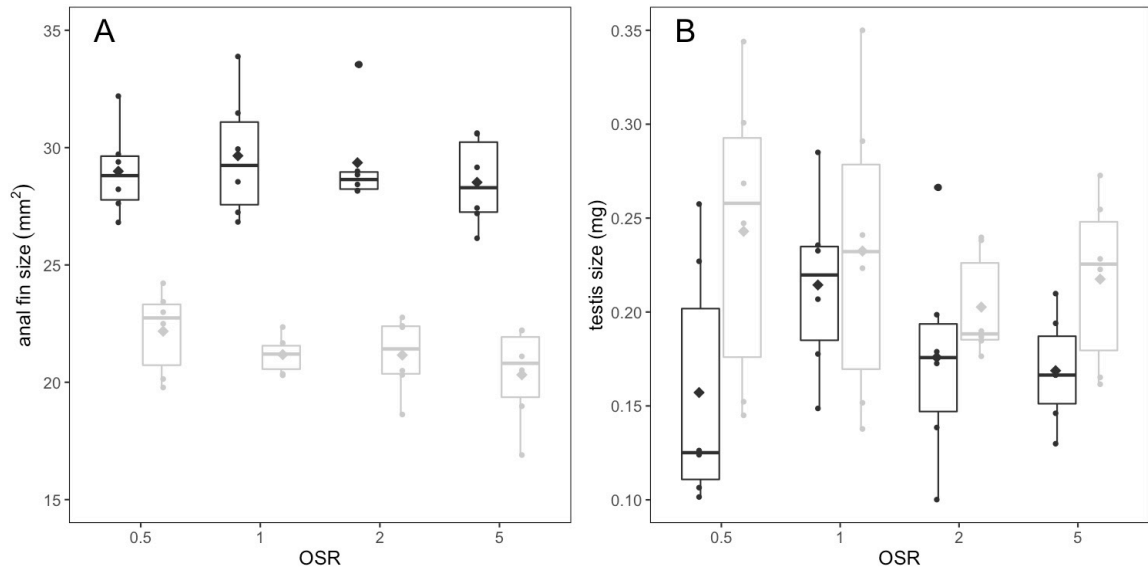


Figure S1. The relationships between OSR treatment and A) male anal fin size (mm²), and B) testis size (mg). Generation 0 males are denoted by black fill and lines, generation 1 males by grey fill and lines. Boxes represent 25th and 75th quartiles, whiskers represent 1.5 x the inter-quartile range and horizontal bars indicate the median. Means are denoted by diamonds and statistical results are in Table S1a (anal fin size) S1b (testis size).

Table S1a. Generalized linear models indicating the effects of body size, OSR and generation on male anal fin size (mm²). Degrees of freedom (*df*), Akaike Information Criterion (AICc), the differences between the model with the lowest AICc and all other models ($\Delta AICc$), and model weights (ω_{AICc}) are included. Akaike Information Criterion (AICc) criteria was

Model	<i>df</i>	AICc	$\Delta AICc$	ω_{AICc}
body size + OSR + generation	5	1541.7	0.00	0.358
body size + OSR + generation + OSR x generation	6	1543.3	1.57	0.164
body size + OSR + generation + body size x OSR	6	1543.6	1.90	0.139
body size + OSR + generation + body size x generation	6	1543.8	2.08	0.127
body size + OSR + generation + OSR x generation + body size x OSR	7	1545.4	3.64	0.058
body size + OSR + generation + body size x generation + OSR x generation	7	1545.4	3.66	0.058
body size + OSR + generation + body size x generation + body size x OSR	7	1545.7	3.99	0.049
body size + OSR + generation + body size x generation + OSR x generation + body size x OSR	8	1547.5	5.74	0.020
body size + OSR + generation + body size x generation + OSR x generation + body size x OSR + body size x OSR x generation	9	1548.2	6.50	0.014
body size + generation	4	1548.8	7.07	0.010
body size + generation + body size x generation	5	1550.8	9.12	0.004
body size + OSR	4	1690.0	148.26	0.000
body size + OSR + body size x OSR	5	1692.0	150.23	0.000
body size	3	1696.5	154.78	0.000
OSR + generation	4	1711.1	169.37	0.000
generation	3	1711.7	169.94	0.000
OSR + generation + OSR x generation	5	1713.0	171.29	0.000
OSR	3	1901.5	359.77	0.000
(intercept only)	2	1901.9	360.18	0.000

Table S1b. Generalized linear models indicating the effects of body size, OSR and generation on testis size (mg). Degrees of freedom (*df*), Akaike Information Criterion (AICc), the differences between the model with the lowest AICc and all other models ($\Delta AICc$), and model weights (ω_{AICc}) are included. Akaike Information Criterion (AICc) criteria was used for model selection. The best models are highlighted in bold.

Model	<i>df</i>	AICc	$\Delta AICc$	ω_{AICc}
body size + OSR + generation	5	-495.5	0.00	0.358
body size + generation	4	-495.4	0.12	0.164
body size + OSR + generation + body size x OSR	6	-495.0	0.50	0.139
body size + OSR + generation + OSR x generation	6	-494.2	1.28	0.127
body size + OSR + generation + body size x generation	6	-493.4	2.07	0.058
body size + generation + body size x generation	5	-493.3	2.19	0.058
body size + OSR + generation + OSR x generation + body size x OSR	7	-493.1	2.41	0.049
body size + OSR + generation + body size x generation + body size x OSR	7	-492.9	2.60	0.020
body size + OSR + generation + body size x generation + OSR x generation + body size x OSR + body size x OSR x generation	9	-492.2	3.31	0.014
body size + generation + body size x generation + OSR x generation	7	-492.2	3.36	0.010
body size + OSR + generation + body size x generation + OSR x generation + body size x OSR	8	-491.0	4.52	0.004
body size	3	-460.9	34.63	0.000
body size + OSR	4	-460.0	35.53	0.000
body size + OSR + body size x OSR	5	-459.3	36.17	0.000
generation	3	-439.7	55.85	0.000
OSR + generation	4	-438.5	57.07	0.000
OSR + generation + OSR x generation	5	-436.4	59.09	0.000
(intercept only)	2	-432.7	62.79	0.000
OSR	3	-431.3	64.18	0.000

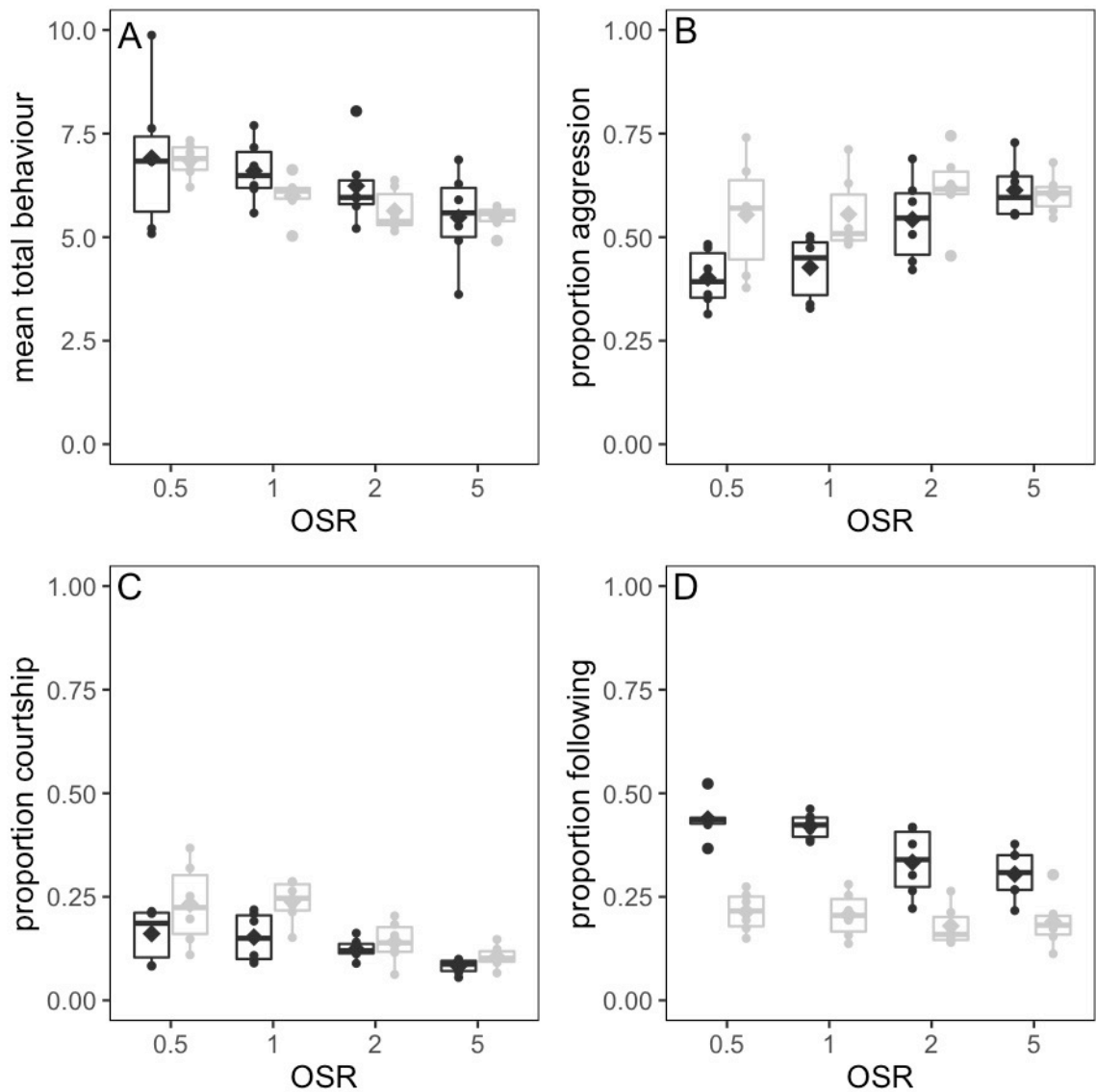


Figure S2. The relationships between OSR treatment and A) male mean total behaviour, B) proportion male aggression, C) proportion male courtship, and D) proportion male following. Generation 0 males are denoted by black fill and lines, generation 1 males by grey fill and lines. Boxes represent 25th and 75th quartiles, whiskers represent 1.5 x the inter-quartile range and horizontal bars indicate the median. Means are denoted by diamonds.

Table S2. Generalized linear models indicating the effects of OSR treatment and generation on proportions of male mating behaviours. Degrees of freedom (*df*), Akaike Information Criterion (AIC_C), the differences between the model with the lowest AIC_C and all other models (Δ AIC_C), and model weights (ω_{AICc}) are included. Akaike Information Criterion (AIC_C) criteria was used for model selection. The best models are highlighted in bold.

Model	<i>df</i>	AIC _C	Δ AIC _C	ω_{AICc}
total behaviours				
OSR	5	134.3	0.00	0.615
OSR + generation	6	135.4	1.13	0.350
(intercept only)	2	141.5	7.20	0.017
generation	3	142.6	8.37	0.009
OSR + generation + OSR x generation	9	142.8	8.56	0.008
male aggression				
OSR + generation	6	-82.1	0.00	0.755
OSR + generation + OSR x generation	9	-79.6	2.49	0.218
OSR	5	-74.4	7.64	0.017
generation	3	-73.4	8.66	0.010
(intercept only)	2	-68.2	13.92	0.001
male courtship				
OSR + generation	6	-132.1	0.00	0.885
OSR + generation + OSR x generation	9	-127.5	4.67	0.086
OSR	5	-125.3	6.80	0.029
generation	3	-114.6	17.58	0.000
(intercept only)	2	-111.0	21.11	0.000
male following				
OSR + generation	6	-124.8	0.00	0.567
OSR + generation + OSR x generation	9	-124.2	0.60	0.419
generation	3	-117.4	7.35	0.014
(intercept only)	2	-70.9	53.83	0.000
OSR	5	-68.6	56.13	0.000

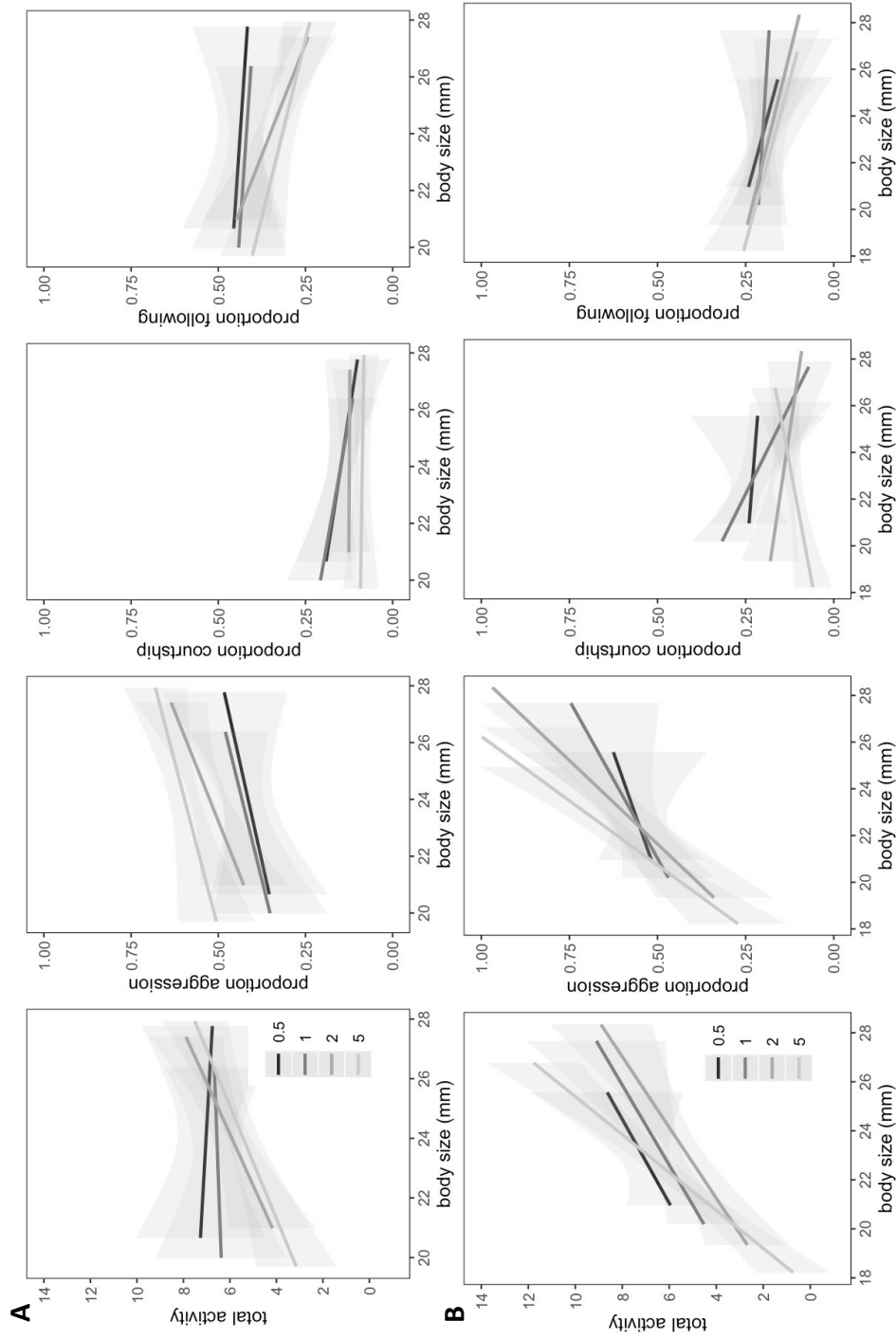


Figure S3. The relationships between male body size and proportions of male behaviours for A) generation 0 males, and B) generation 1 males, for each OSR treatment. Black lines denote OSR 0.5, and get lighter with each OSR treatment respectively (1, 2 and 5), and standard error with 95% confidence intervals are included for each OSR treatment.

Table S3a. Linear mixed effects models indicating the effects of OSR treatment and male body size on proportions of male mating behaviour for generation 0, with tank included as a random effect. Degrees of freedom (*df*), Akaike Information Criterion (AIC_C), the differences between the model with the lowest AIC_C and all other models (Δ AIC_C), and model weights (ω_{AICc}) are included. Akaike Information Criterion (AIC_C) criteria was used for model selection. The best models are highlighted in bold.

Model	<i>df</i>	AIC _C	Δ AIC _C	ω_{AICc}
total behaviour				
body size	4	805.2	0.00	0.490
body size + OSR	7	805.8	0.55	0.371
body size + OSR + body size x OSR	10	808.2	2.97	0.111
(intercept only)	3	811.6	6.37	0.020
OSR	6	813.5	8.21	0.008
male aggression				
body size + OSR	7	-103.6	0.00	0.929
body size + OSR + body size x OSR	10	-97.1	6.46	0.037
OSR	6	-96.7	6.82	0.031
body size	4	-92.3	11.26	0.003
(intercept only)	3	-85.3	18.28	0.000
male courtship				
OSR	6	-321.8	0.00	0.515
body size + OSR	7	-321.0	0.79	0.346
(intercept only)	3	-317.6	4.21	0.063
body size	4	-316.6	5.21	0.038
body size + OSR + body size x OSR	10	-316.6	5.23	0.038
male following				
body size + OSR	7	-165.0	0.00	0.850
body size + OSR + body size x OSR	10	-160.5	4.44	0.092
OSR	6	-158.2	6.77	0.029
body size	4	-158.1	6.83	0.028
(intercept only)	3	-150.0	15.00	0.000

Table S3b. Linear mixed effects models indicating the effects of OSR treatment and male body size on proportions of male mating behaviour for generation 1, with tank included as a random effect. Degrees of freedom (*df*), Akaike Information Criterion (AIC_C), the differences between the model with the lowest AIC_C and all other models (Δ AIC_C), and model weights (ω_{AIC_C}) are included. Akaike Information Criterion (AIC_C) criteria was used for model selection. The best models are highlighted in bold.

Model	<i>df</i>	AIC _C	Δ AIC _C	ω_{AIC_C}
total behaviour				
body size + OSR + body size x OSR	10	800.7	0.00	0.656
body size + OSR	7	802.6	1.88	0.256
body size	4	804.7	4.01	0.088
(intercept only)	3	840.2	39.49	0.000
OSR	6	842.5	41.81	0.000
male aggression				
body size	4	-21.1	0.00	0.569
body size + OSR	7	-20.0	1.13	0.324
body size + OSR + body size x OSR	10	-17.8	3.34	0.107
(intercept only)	3	13.1	34.22	0.000
OSR	6	17.7	38.78	0.000
male courtship				
body size + OSR + body size x OSR	10	-240.9	0.00	0.723
OSR	6	-238.2	2.72	0.186
body size + OSR	7	-236.8	4.16	0.091
(intercept only)	3	-224.8	16.19	0.000
body size	4	-223.5	17.44	0.000
male following				
body size	4	-143.3	0.00	0.680
(intercept only)	3	-141.3	1.95	0.257
body size + OSR	7	-137.8	5.44	0.045
OSR	6	-135.9	7.38	0.017
body size + OSR + body size x OSR	10	-131.6	11.69	0.002

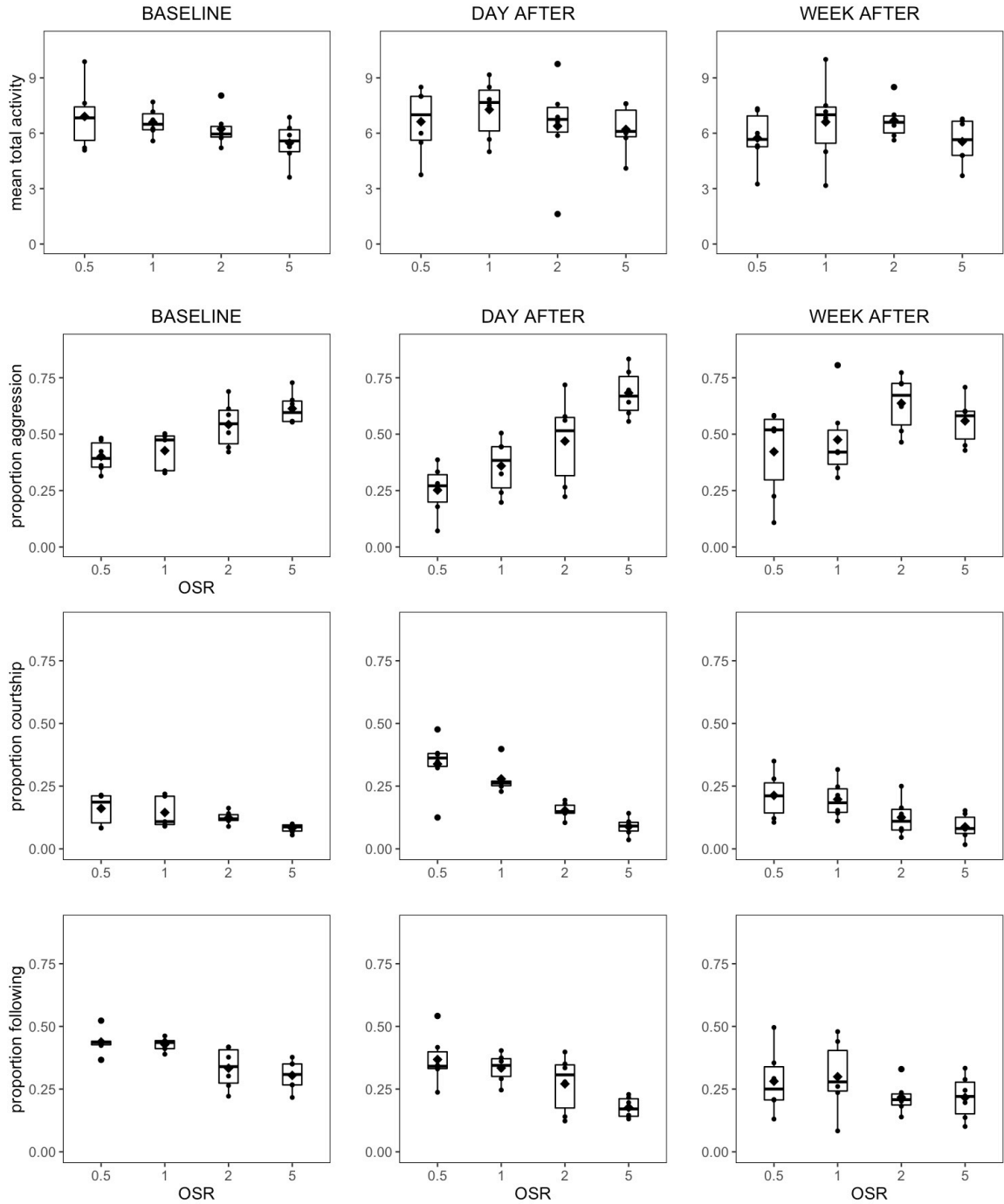


Figure S4. The relationships between OSR treatment and proportions of mating behaviours of males in their baseline OSR treatment, one day and one week after introduction into a novel OSR treatment. Boxes represent 25th and 75th quartiles, whiskers represent 1.5 x the inter-quartile range and horizontal bars indicate the median. Means are denoted by diamonds.

Table S4. Generalized linear models indicating the effects of OSR and treatment (i.e. baseline, one day after or one week after change to novel OSR) on proportions of male behaviour. Males from all OSR treatments were included in these models. Degrees of freedom (*df*), Akaike Information Criterion (AIC_C), the differences between the model with the lowest AIC_C and all other models (Δ AIC_C), and model weights (ω_{AICc}) are included. Akaike Information Criterion (AIC_C) criteria was used for model selection. The best models are highlighted in bold.

Model	<i>df</i>	AIC _C	Δ AIC _C	ω_{AICc}
total activity				
(intercept only)	2	274.4	0.00	0.570
OSR	5	275.9	1.45	0.276
treatment	4	277.7	3.30	0.109
OSR + treatment	7	279.5	5.08	0.045
OSR + treatment + OSR x treatment	13	292.3	17.88	0.000
aggression				
OSR + treatment	7	-73.3	0.00	0.499
OSR	5	-73.0	0.28	0.435
OSR + treatment + OSR x treatment	13	-69.2	4.02	0.067
(intercept only)	2	-48.0	25.21	0.000
treatment	4	-46.9	26.38	0.000
courtship				
OSR + treatment + OSR x treatment	13	-170.0	0.00	0.611
OSR + treatment	7	-170.0	0.91	0.388
OSR	5	-156.1	14.76	0.000
treatment	4	-136.1	34.82	0.000
(intercept only)	2	-129.3	41.55	0.000
following				
OSR + treatment	7	-137.7	0.00	0.997
OSR + treatment + OSR x treatment	13	-125.5	12.20	0.002
OSR	5	-122.1	15.51	0.000
treatment	4	-120.2	17.45	0.000
(intercept only)	2	-109.6	28.06	0.000