Signature page

# The Effects of Novel Mates and Competitors on the Courtship and Aggression Behaviour of Japanese Medaka (*Oryzias latipes)*

By

Maggie C. Kelly

A Thesis Submitted to
Saint Mary's University, Halifax, Nova Scotia
in Partial Fulfillment of the Requirements for
the Degree of Bachelor of Science with Honours.

April 2024, Halifax, Nova Scotia

| | |
|---|---|
| Approved: | Dr. Laura Weir<br>Supervisor |
| Approved: | Dr. Linda Campbell<br>Reader |
| Date: | April 30th, 2024 |

# The Effects of Novel Mates and Competitors on the Courtship and Aggression Behaviour of Japanese Medaka (*Oryzias latipes)*

By

Maggie C. Kelly

A Thesis Submitted to
Saint Mary's University, Halifax, Nova Scotia
in Partial Fulfillment of the Requirements for
the Degree of Bachelor of Science with Honours.

April 2024, Halifax, Nova Scotia

| | |
|---|---|
| Approved: | Dr. Laura Weir<br>Supervisor |
| Approved: | Dr. Linda Campbell<br>Reader |
| Date: | April 30th, 2024 |

# The Effects of Novel Mates and Competitors on the Courtship and Aggression Behaviour of Japanese Medaka (*Oryzias latipes)*

by Maggie Kelly

## Abstract

Mating with novel individuals is a beneficial strategy to cope with environmental instability and increases the probability of offspring fitness. Additionally, for males to mate successfully, they must appropriately allocate their limited mating energy towards courtship (intersexual) and aggression (intrasexual) behaviour. In male Japanese medaka (*Oryzias latipes*), previous research has shown that sex ratio influences both male and female behaviour. However, there remains a research gap in investigating how the identity, or the novelty, of the surrounding individuals affects this balance. The goal of this study is to measure the effects of mate and competitor novelty on the ratio of inter- and intrasexual behaviour of male Japanese medaka, as well as the effects of mate novelty on female reproductive output. Male behaviour, both aggression (chasing and hitting) and courtship (following and circling), was quantified by observing each male on two different days for two minutes each and the frequency of inter- and intrasexual behaviour was recorded. For males, these observations were collected for all four sex ratios in three scenarios: familiar mates and familiar competitors, novel mates and familiar competitors, and novel mates and novel competitors. I found that in response to both novel mates, and novel mates and competitors, males prioritized intersexual interactions by increasing their allocation to courtship and following behaviour. I suggest that males use mate novelty as bet hedging strategy to increase offspring fitness in an unstable environment. In addition, female reproductive output, measured by the presence of eggs, was recorded daily in the first two scenarios (first with familiar and then with novel mates). I found that females decrease reproductive output in response to novel mates. I suggest that females are more receptive to familiar males because they take less risk relative to males with their limited gametes, and instead bet hedge with post-fertilization mechanisms.

April 30th, 2024

## Acknowledgments

I would first like to thank my supervisor Dr. Laura Weir, both for her valuable guidance during my honours project, as well as for continuing to foster my curiosity in animal behaviour and ecology. Thank you to my reader Dr. Linda Campbell for her valuable feedback and for bringing this ‘fishy soap opera’ to life, as well as my Honours professors Dr. Anne Dalziel and Dr. Carla Crossman for their continued support and guidance during the year.

I also want to extend my gratitude to my lab mates at the SMU fish lab, as well as the members of my Honours class. Thank you to Sarah Young-Veenstra and Kenzie Melanson for being PowerPoint geniuses, and for your advice which was immensely helpful. Thank you to Taylor Adams for our many Starbucks runs, ‘water change debriefs’ and general antics. Thank you to Kelsey Mercer for our countless laughs and philosophical discussions and thank you to Julia MacIsaac and Isadora Schumann Munhoz for your support, both in the lab and on our elite trivia team.

Finally, I would like to thank my mother, Dawnise, and Father, Robin, for always pushing me to do my best. Thank you to my Grandparents, Don, and Anne, for their good luck calls before every big presentation. Thank you to my dog, Rudy, for being his adorable self, and thank you to my sister, Norah, for always being my biggest cheerleader.

## Table of Contents

## *1 Introduction*

### *1.1 Sexual selection*

Sexual selection is defined as mode of natural selection whereby an individual’s fitness is determined by their ability to compete for and secure mates (Andersson, 1994) . This ability is determined by traits associated with both inter- and intrasexual interactions (Fitze et al., 2008). Intersexual interactions are primarily courtship behaviours between members of the opposite sex, and intrasexual interactions are primarily aggression behaviours between members of the same sex (Fitze et al., 2008). Males are typically the more competitive sex, as male fertility is primarily influenced by their number of partners (Bateman, 1948). However, males have limited energy that they can spend on mating, and therefore must appropriately allocate their energy towards inter- and intrasexual behaviour (Fitze et al., 2008).

### *1.2 Effects of sex ratio and mate novelty on mating behaviour*

Various extrinsic factors have been shown to influence how males prioritize their behaviour. One of these factors is sex ratio, where when the number of available mates is limited, members of the unlimited sex will modify their behaviour to be competitive and increase mating opportunities (Emlen & Oring, 1977). For example, in Japanese medaka, males in male-biased sex ratios will allocate more effort toward intrasexual aggression, and as a trade-off, less effort for intersexual courtship (Grant et al., 2010). The opposite is true as sex ratio becomes more female-biased, and available mates outnumber competitors (Grant et al., 2010).

In addition to sex ratio, mate identity can influence the intensity of behaviour associated with competing for and obtaining mates. The effects of mate novelty, or the Coolidge effect (Beach & Jordan, 1956), on mating behaviour has also been studied in a variety of species. This phenomenon was first observed in rats refers to a male's heightened sexual interest in a novel female compared to a familiar female, in tandem with a declining interest in a familiar female with each repeated mating (Steiger et al., 2008).

The Coolidge effect may be a strategy to maximize reproductive investment (Kelley et al., 1999). Reproduction is an inherently costly process, where the investment in reproduction is associated with trade-offs such as engaging in mating activities that could expose individuals to increased risk of predation (Hoffman et al., 2008). Mating with a variety of partners can be beneficial for multiple reasons. Increased mate novelty can increase genetic variation among offspring and can increase fitness in variable environments (Steiger et al., 2008). Mate novelty allows for bet hedging, which is a reproductive strategy that evolves when offspring environmental conditions cannot be predicted (Shama, 2015). Bet hedging individuals will mate with a diverse range of phenotypes to produce a variety of different offspring (Lips, 2001). Under these conditions, increasing the genetic variance among offspring may increase the probability that a portion of offspring will inherit a suitable phenotype for the prevailing environment (Lips, 2001; Shama, 2015).

In addition to maximizing reproductive investment, female mate choice is an important factor in reproductive success. In general, females are more discriminating than males when choosing a mate (Janetos, 1980). This is attributed to the high costs of egg production relative to sperm, the female's limited capacity for breeding frequency and the responsibility of parental care which usually falls on the female (Janetos, 1980). Despite this, most research on the Coolidge effect only focuses on the male mate choice with little research exploring how mate novelty influences female sexual interest. Lisk & Baron (1982) observed that female golden hamsters decreased their courtship with each repeated exposure to a familiar male. Since then, attempts to replicate this type of study have been scarce and there remains a gap in the literature for research that thoroughly investigates the effect of mate novelty on female choice.

### *1.3 Japanese medaka (Oryzias latipes) as a study species*

Japanese medaka (*Oryzias latipes*) are a small freshwater fish native to Japan. Their natural habitat includes streams, marshes, and rice paddies (Hiramatsu & Shikasho, 2004). These habitats are shallow, close to land, and influenced by river discharge, meaning they are highly variable and subjected to rapid changes in temperature, oxygen level and pH (Franz et al., 2019). Medaka are polygamous and do not provide parental care to their offspring (Leaf et al., 2011). Males exhibit distinct and observable mating behaviours which include aggression towards competing males by chasing or hitting, and courtship towards available females by following and performing courtship circles in front of the female (Ono & Uematsu, 1957)Female reproductive output is also easily observed as their eggs are fertilized externally (Iwamatsu, 2004). These characteristics make Japanese medaka a good candidate to study the influence of mate novelty on of male-or female-directed mating behaviour and female reproductive success.

### *1.4 Research goals*

The main goal of this project is to investigate the effects of novel mates and competitors on the courtship and aggression behaviour of Japanese medaka. First, I investigate how the introduction of novel mates affects the courtship and aggression behaviour of male medakas. Next, to address the research gap in the effects of novel mates on female behaviour, I investigate how the introduction of novel mates affects the mating behaviour of female medaka. Finally, I investigate how the introduction of novel competitors influences the aggression behaviour of male medakas. We predict that both male and female medaka will exhibit the Coolidge effect, indicated by changes in mating behaviour by males, and egg production by females.

## 2 Methods

### *2.1 Experimental Animals*

This experiment used 288 lab-bred Adult Japanese medaka (*Oryzias latipes)* raised for six generations in the aquarium facility at Saint Mary's University, Halifax, Nova Scotia. The fish were originally obtained from Aquatic Research Organisms (New Hampshire, USA). Individual fish were assigned a number and tagged with Visual Implant Elastomer tags (NorthWest Marine Technology, Washington, USA) in two out of four possible locations on the dorsal side of the fish (left front, left back, right front, right back) in one or two of eight colour options (black, blue, brown, green, orange, pink, red, or yellow). To identify individuals during the experiment, tags were inserted while the fish were anesthetized using 0.15g/L MS-222 (Tricaine methane sulfonate) buffered with 0.3 g/L sodium bicarbonate ($NaHCO_3$).

The Japanese medaka were housed in 10-gallon tanks (20 in x 10 in x 12 in) equipped with a water aeration device, water heater and under gravel filter. Tanks contained fresh water between 23-28 degrees Celsius (verified with daily temperature tests) and pH levels of 7.4-8.4. pH and other water quality parameters such as nitrite, nitrate and ammonia levels were tested weekly. Partial water changes (25% volume) were also performed weekly on all tanks after water quality testing using water from a drum that contained 55 gallons of RODI (reverse osmosis deionized) water, 88mL of red sea salt (to maintain water conductivity between 800-1600 mS/cm), 8mL of alkaline buffer (to maintain pH between 7 and 8), and 27.5mL of stress coat. All tanks experienced a 14:10 hour light: dark photoperiod starting at 0700h to mimic the light cycle of their natural habitat.

All fish were fed twice daily, once in the morning (before 1000h) and once in the afternoon (after 1400h). In the morning, they were fed 15g of frozen adult brine shrimp (Hikari Bio-Pure) and flake food (TetraMin Tropical Flakes). In the afternoon, they were fed live 1.5g *Artemia nauplii* (brine shrimp larvae hatched from rehydrated cysts in the lab).

## ***2.2 Experimental design***

### *2.2.1 Tank set up*

Twenty-four experimental tanks were used for observations. Each tank contained 12 fish in one of four male: female sex ratios (0.5, 1, 2 and 5). Sex ratio (SR) 0.5 contained 4 males and 8 females, SR 1 contained 6 males and 6 females, SR 2 contained 8 males and 4 females, and SR 5 contained 10 males and 2 females. Each of the four sex ratios had 6 replicates. Tanks were located on 6 different shelves in the aquarium facility, each shelf housing one tank of each SR. To minimize bias based on shelf location and varying proximity from the entryway, the order of SRs on each shelf was randomized. All males from the 24 tanks were observed under three experimental conditions (familiar mates, novel mates, and novel competitors).

### *2.2.2 Familiar mate phase*

To familiarize the males with their mates and competitors, the Japanese medaka lived in their original tanks for one month before observation. After one month, I began the first two rounds of observations. I observed 6-8 tanks a day, such that one round of observations on all males took one week. Each male was observed for 2 minutes each round, and all observations took place between 0800h and 1200h. To avoid sampling bias, males were observed in the same random order for each round.

During each observation, I recorded instances of aggression, courtship and following by the focal male. Aggression is defined as a chase, where a male quickly charges at another male, causing the other to be hit or flee from the charging fish (Grant et al., 1995). Courtship is defined as a quick circle performed by a male around a female, where the male swims in a fast arc in front of the female (Grant et al., 1995). Following occurs when a male follows closely behind a female. (Ono & Uematsu, 1957).

Each female was also checked daily for the presence of eggs as a measure of their reproductive output. The eggs are located on the ventral surface, anterior to the anal fin.

### *2.2.3 Novel mate phase*

In this phase, males from each tank were moved as a group to a novel tank of the same SR as their original tank (Figure 1). To ensure that behavioural observations could occur within the same timeframe as the familiar phase, I randomly paired replicate SR tanks and swapped their males. One pair of replicates were swapped each day, meaning that it took three days to move all males to their new replicate tank. Observations of the male behaviour from each replicate pair occurred one day and one week after their switch occurred. Individual males were observed for two minutes and instances of aggression, following and courtship were recorded. Females were checked daily for eggs as was done previously in the familiar phase. After observations were completed, the fish were moved back into their original tanks.

### *2.2.4 Novel mate and novel competitor phase*

In this phase, males from each tank were moved individually to a novel tank of the same SR as their original tank (Figure 2) meaning males were exposed to as few familiar competitors as possible, while experiencing the same sex ratio. To ensure that the behavioural observations could occur within the same timeframe as the familiar phase, I mixed males together from one sex ratio per day. Moving all males to their novel competitor tanks took four days. Observations of the six sex ratio replicate tanks occurred one day and one week after their switch. Individual males were observed for two minutes and instances of aggression, following and courtship were recorded. Females were not checked for eggs during this phase.

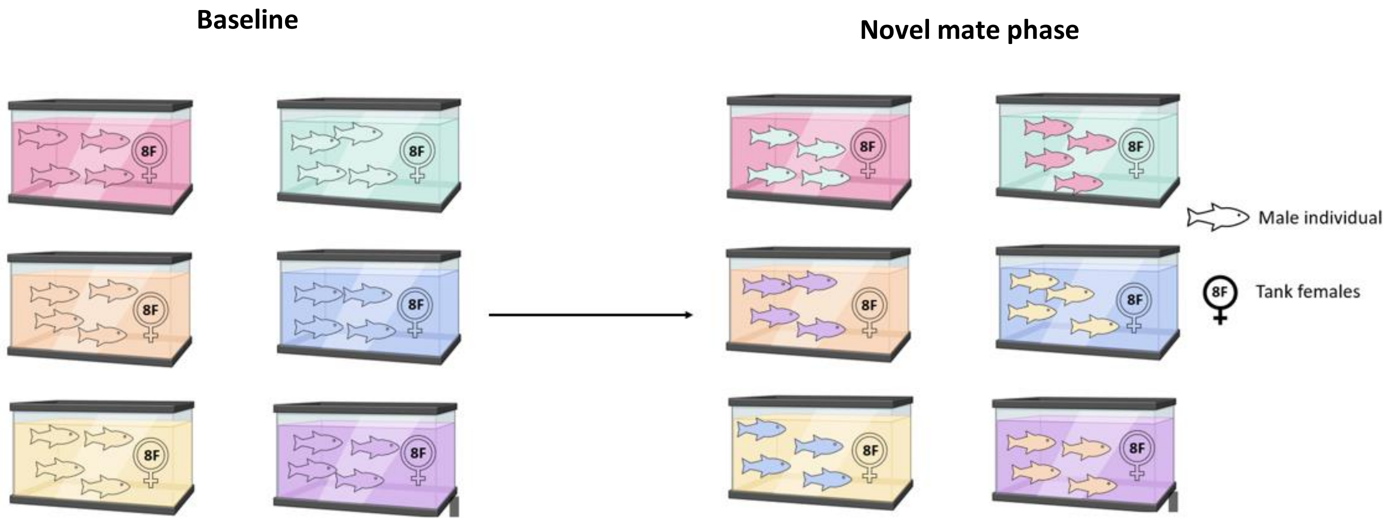

Figure 1. Visual representation of the novel mate phase showing how fish are switched among the six sex ratio 0.5 tanks. The fish symbols represent individual males, and the female symbol represents the females of the tank. Males from each SR tank are moved as a group to a novel tank of the same sex ratio. This exposes the males to novel mates with familiar male competitors.

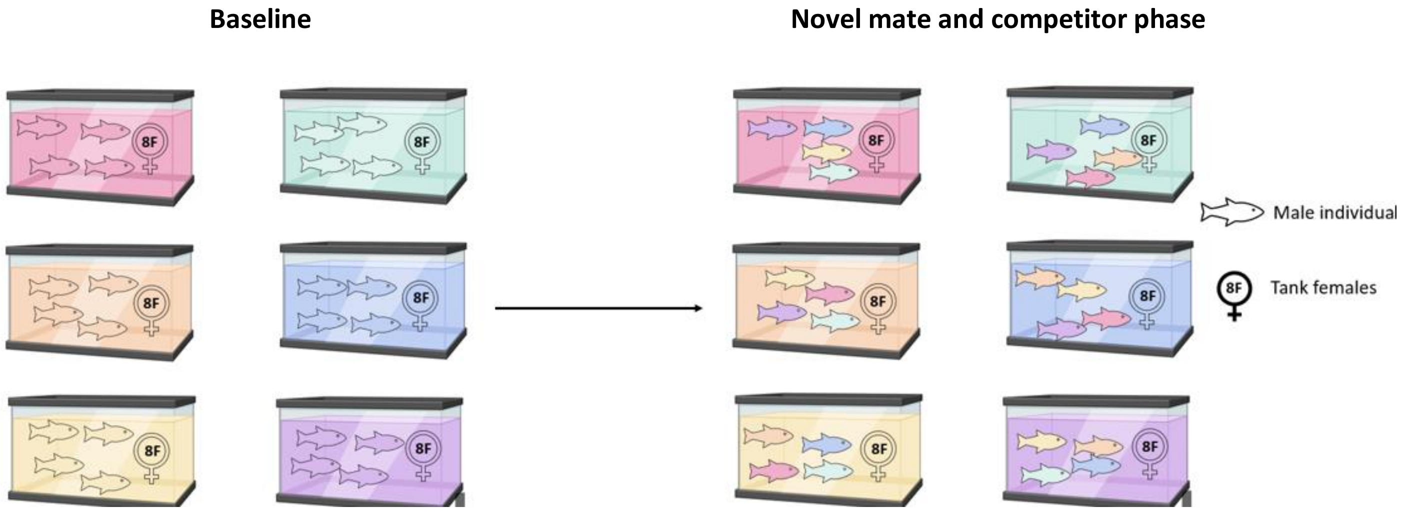

Figure 2. Visual representation of the novel mate and novel competitor phase showing how fish are switched among the six sex ratio 0.5 tanks. The fish symbols represent individual males, and the female symbol represents the females of the tank. Males from each sex ratio0.5 tank are moved as individuals to a novel tank of the same sex ratio. This exposes the males to novel mates and novel competitors.

### *2.3 Statistical analysis*

All analysis were performed using R version 4.3.0 (R Core Team, 2023). Relationships between behaviour, novelty and sex ratio were determined using generalized linear models (GLMs). The data collected on individuals was used to calculate mean behaviour for each individual tank. “Tank” was used as an experimental replicate in these models.

Linear mixed-effects models using “lme4” package for mixed model analysis (Bates et al., 2015) to compare the interactive effects of sex ratio, mate novelty, individual behaviour, and tank. Individual behaviour and tank are included as random effects in this model to account for difference among tanks and individual behaviour. Behavioural observations were averaged both by tank and individual fish. All models used Akaike Information Criterion (AICc) criteria for model selection. I selected the model with the lowest AICc value as the best fit for the data; models that were lower than all others by an AICc value of 2 or greater were considered the sole ‘best’ model.

Post-hoc analyses comparing differences between different combinations of sex ratio and novelty treatment were performed with estimated marginal means (EMMEANS) using the “emmeans” package (Length, 2023). EMMEANS values were used to assign compact letter displays (CLD) to each pair-wise comparison using the “multcomp” package (Hothorn et al., 2008).

## *3 Results*

### *3.1 Total behaviour frequency*

I compared the total frequency of male behaviours (aggression, following, and courtship) between males with familiar mates and males with novel mates, and novel mates and competitors. In both novelty treatment, total behaviour tended to decrease compared the familiar treatment (Figure 3). The model that best explained total behaviour frequency in both novelty treatments includes the interactive effects of sex ratio and novelty treatment (Table 1; Figure 3). In the novel mate treatment, the interaction was primarily driven by a distinct difference between familiar and novel treatments in sex ratio 5, whereas the differences in frequency between treatments among all other sex ratios were similar (Figure 3). In the novel mate and novel competitor treatment, the interaction was primarily driven by the distinct differences in frequency between familiar and novel treatments in sex ratio 0.5 and 5, whereas the differences in frequency between treatments among all other sex ratios were similar (Figure 3). To account for possible changes in activity level due to time passing between experiments, I used the proportion of total behaviour to standardize all treatments for further comparison.

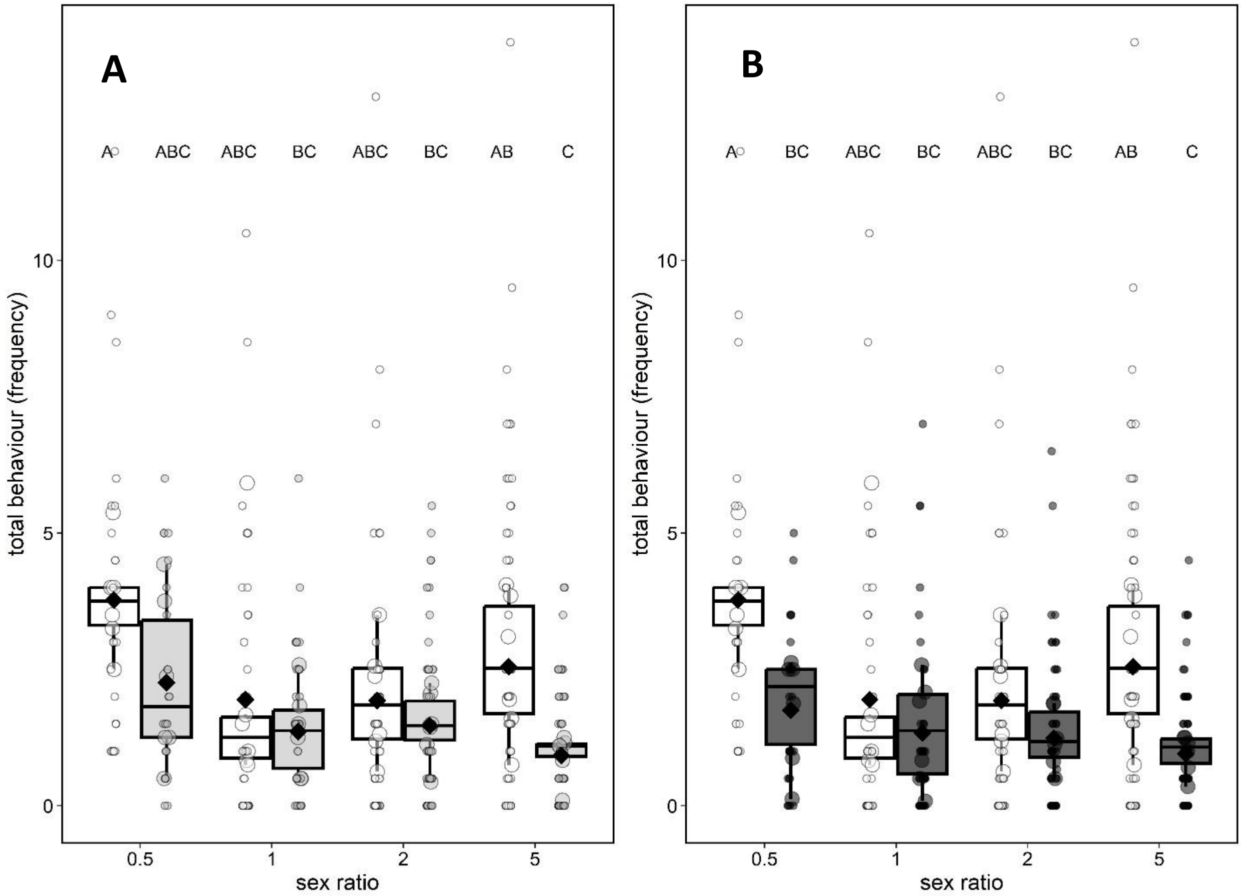

Figure 3. The relationship between sex ratio and frequency of total inter- and intrasexual behaviour of males exposed to base familiar mates (white boxes) and novel mates (A) (grey boxes), or males exposed to novel mates and competitors (B) (black boxes). Large dots represent tank average for each sex ratio replicate, small dots represent averages of individual males. Compact letter displays (CDLs) above each box denote pairwise comparisons between treatments, where sharing common letters represents similarity, and sharing no common letters represents difference. 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 1.

*Table 1*. Generalized linear models demonstrating the effects of familiar and novel mate treatments and sex ratio (SR) on the frequency of total inter- and intrasexual behaviours of male medaka. Individual males and tank are included as a random effect. Included are degrees of freedom (df), Akaike Information Criterion ($AIC_c$), the difference between the model with the lowest $AIC_c$ and all other models ($\Delta AIC_C$ ), and the model weight ($w_{AICc}$ ). Model selection is based on Akaike Information Criterion criteria and the best fit models are highlighted in bold.

| Model | *df* | $AIC_C$ | $\Delta AIC_C$ | $w_{AICc}$ |
|---|---|---|---|---|
| *Novel mates* | | | | |
| **Sex ratio x novelty** | **11** | **3222.3** | **0.00** | **0.617** |
| **Sex ratio + novelty** | **8** | **3224.0** | **1.70** | **0.264** |
| Novelty | 5 | 3225.6 | 3.29 | 0.119 |
| Sex ratio | 7 | 3249.6 | 27.29 | 0.000 |
| (Intercept only) | 4 | 3251.4 | 29.03 | 0.000 |
| *Novel mates and competitors* | | | | |
| **Sex ratio x novelty** | **11** | **3230.2** | **0.00** | **0.637** |
| **Sex ratio + novelty** | **8** | **3232.5** | **0.58** | **0.192** |
| Novelty | 5 | 3232.8 | 5.35 | 0.170 |
| Sex ratio | 7 | 3268.6 | 13.59 | 0.000 |
| (Intercept only) | 4 | 3269.1 | 13.76 | 0.000 |

### ***3.2 Novel mate phase***

#### *3.2.1 Proportion of male inter- and intrasexual behaviour*

I compared the proportion of intrasexual and intersexual behaviour between males exposed to familiar mates and males exposed to novel mates. The model that best explained the change in behaviour includes effects of mate novelty, as well as the additive effects of treatment and sex ratio (Table 2). When exposed to novel mates, males tended to spend more of their total behaviour on intersexual interactions (courtship and following) than intrasexual interactions (aggression) (Figure 4). Additionally, the proportion of aggression increases as sex ratio became more male biased, and courtship increases as sex ratio becomes more female biased (Figure 4).

Following contributed the most to the additive effects of sex ratio and novelty treatment in intersexual behaviour, where the best models to explain following proportion included novelty treatment, or the additive effects of sex ratio and novelty treatment (Table 2; Figure 2). Following increases in response to mate novelty and increases as sex ratio becomes more female biased (Figure 4). Changes in courtship are best explained by the model that included the novelty treatment and tends to increase in response to mate novelty but does not change significantly between sex ratios (Table 2; Figure 4).

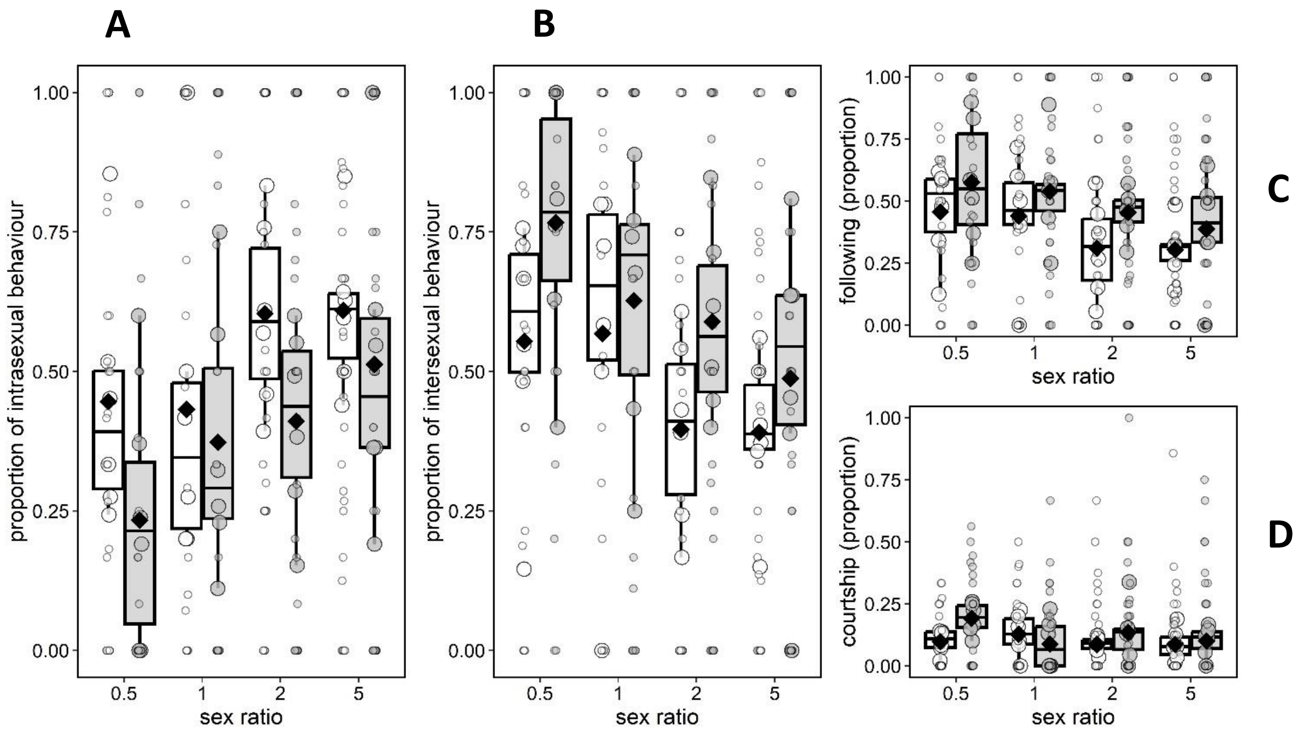

Figure 4. The relationship between sex ratio and proportion of inter- (A) and intrasexual (B) behaviour of males exposed to baseline familiar mates (white boxes) and to novel mates (grey boxes). The proportion of intrasexual behaviour is influenced only by aggression behaviour, the proportion of intersexual behaviour are composed of both following (C) and courtship (D) behaviours. Large dots represent tank average for each sex ratio replicate, small dots represent averages of individual females. 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 2.

Table 2. Generalized linear models demonstrating the effects of baseline familiar and novel mate treatments and sex ratio (SR) on the proportion of male inter- and intrasexual behaviour. Individual males and tank are included as a random effect. Included are degrees of freedom (df), Akaike Information Criterion ($AIC_c$), the difference between the model with the lowest $AIC_c$ and all other models ($\Delta AIC_C$ ), and the model weight ($w_{AICc}$ ). Model selection is based on Akaike Information Criterion criteria and the best fit models are highlighted in bold.

| Model | *df* | $AIC_C$ | $\Delta AIC_C$ | $w_{AICc}$ |
|---|---|---|---|---|
| *Intrasexual behaviour* | | | | |
| **Novelty** | **5** | **365.1** | **0.00** | **0.549** |
| **Sex ratio + novelty** | **8** | **365.7** | **0.58** | **0.411** |
| Sex ratio x novelty | +11 | 370.5 | 5.35 | 0.038 |
| Sex ratio | 7 | 378.7 | 13.59 | 0.001 |
| (Intercept only) | 4 | 378.9 | 13.76 | 0.001 |
| *Intersexual behaviour* | | | | |
| **Novelty** | **5** | **365.1** | **0.00** | **0.549** |
| **Sex ratio + novelty** | **8** | **365.7** | **0.58** | **0.411** |
| Sex ratio x novelty | +11 | 370.5 | 5.35 | 0.038 |
| Sex ratio | 7 | 378.7 | 13.59 | 0.001 |
| (Intercept only) | 4 | 378.9 | 13.76 | 0.001 |
| *Following* | | | | |
| **Novelty** | **5** | **289.5** | **0.00** | **0.568** |
| **Sex ratio + novelty** | **8** | **290.2** | **0.73** | **0.395** |
| Sex ratio x novelty | +11 | 296.2 | 6.75 | 0.019 |
| (Intercept only) | 4 | 297.9 | 8.39 | 0.009 |
| Sex ratio | 7 | 297.9 | 8.43 | 0.008 |
| *Courtship* | | | | |
| **Novelty** | **5** | **-159.2** | **0.00** | **0.677** |
| (intercept only) | 4 | -156.6 | 2.54 | 0.190 |
| Sex ratio + novelty | 8 | -154.8 | 4.40 | 0.075 |
| Sex ratio x novelty | +11 | 153.2 | 5.95 | 0.035 |
| Sex ratio | 7 | 152.4 | 6.77 | 0.023 |

### *3.2.2 Female reproductive output*

I compared the effect of mate novelty on female reproductive output between four sex ratios. Overall, females tended to have eggs less frequently when exposed to novel mates than when exposed to familiar mates (Figure 5). The model that best explained the variation in egg presence include the interactive effects of the novelty treatment and sex ratio (Table 3; Figure 5). This interaction was primarily driven by the distinct difference in egg presence between familiar and novel treatments in sex ratio 0.5, whereas the differences between treatments among all other sex ratios were similar (Figure 5).

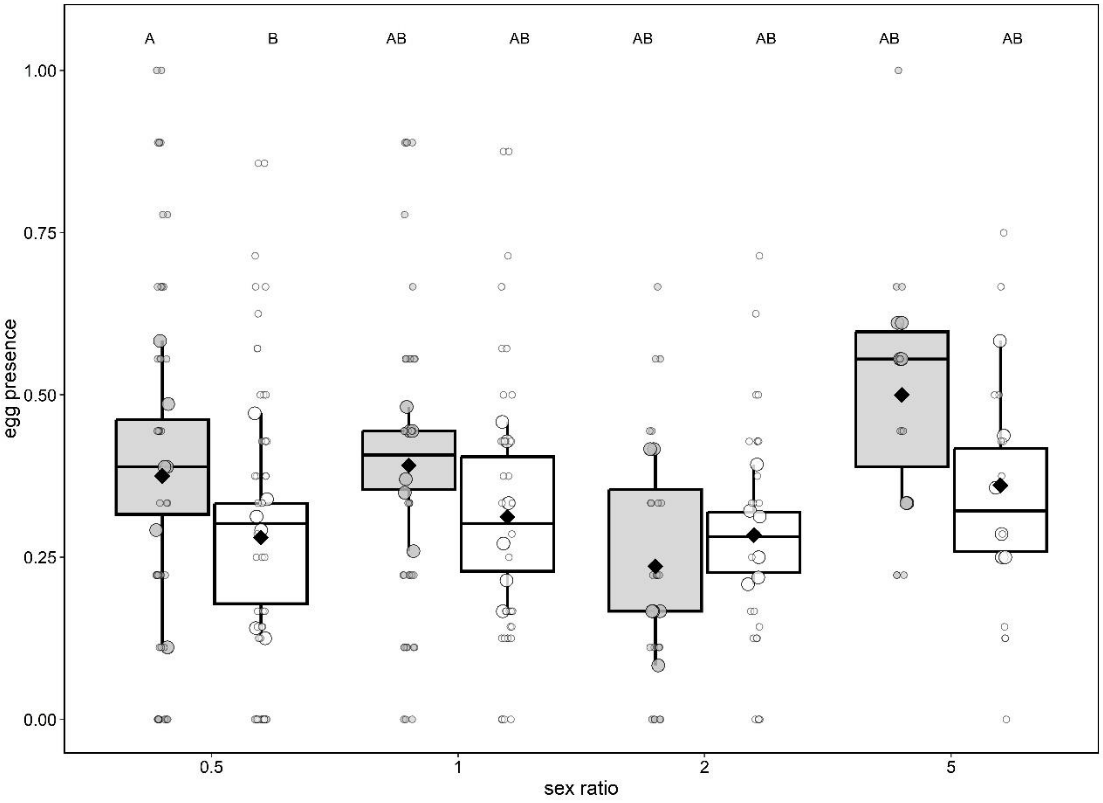

Figure 5. The relationship between sex ratio and egg presence of females exposed to familiar (white boxes) and novel mates (grey boxes). Females were observed daily for the presence of eggs posterior to the anal fin and were scored 1 for egg presence and 0 for egg absence. Large dots represent tank average for each sex ratio replicate, small dots represent averages of individual females. Compact letter displays (CDLs) above each box denote pairwise comparisons between treatments, where sharing common letters represents similarity, and sharing no common letters represents difference. 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 3.

*Table 3.* Generalized linear models with a binomial distribution demonstrating the effects of familiar and novel mate treatments and sex ratio (SR) on the proportion of female reproductive output. Individual females and tank are included as a random effect. Included are degrees of freedom (df), Akaike Information Criterion ($AIC_c$), the difference between the model with the lowest $AIC_c$ and all other models ($\Delta AIC_C$ ), and the model weight ($w_{AICc}$ ). Model selection is based on Akaike Information Criterion criteria and the best fit models are highlighted in bold.

| Model | *df* | $AIC_C$ | $\Delta AIC_C$ | $w_{AICc}$ |
|---|---|---|---|---|
| *Female reproductive output* | | | | |
| **Sex ratio x novelty** | **+11** | **2391.3** | **0.00** | **0.669** |
| Novelty | 5 | 2393.6 | 2.29 | 0.213 |
| Sex ratio + novelty | 8 | 2394.6 | 3.52 | 0.115 |
| (Intercept only) | 4 | 2403.7 | 12.41 | 0.001 |
| Sex ratio | 7 | 2404.9 | 13.59 | 0.001 |

## *3.3 Novel mates and competitor phase*

### *3.3.1 Proportion of male inter- and intrasexual behaviour*

I examined the effects of novel mates and competitors, and sex ratio on the proportion of male inter- and intrasexual behaviour. The models that best explained these changes included effects from sex ratio, and the additive effects of sex ratio and novelty treatment (Table 4). Overall, males did not significantly increase their proportion of intersexual behaviour when exposed to both novel mates and competitors (Table 4; Figure 6). However, they did increase intersexual behaviour in response to female-biased sex ratio (Table 4; Figure 6).

Following contributed the most the effects of sex ratio on the proportion of intersexual behaviour, where the proportion of following increased in response to female-biased sex ratio but is not significantly affected by novelty treatment (Table 4; Figure 6). The proportion of courtship was not significantly influenced by novelty or sex ratio (Table 4; Figure 6).

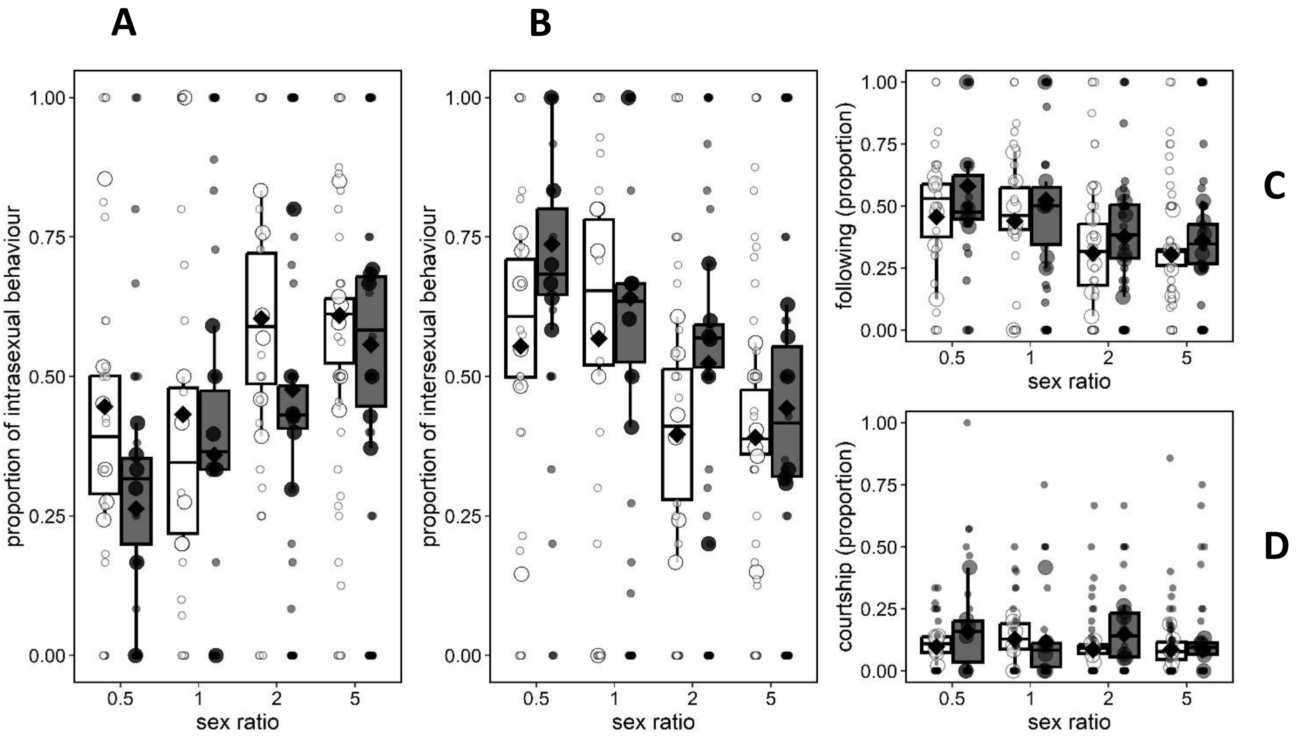

Figure 6. The relationship between sex ratio and proportion of inter- (A) and intrasexual (B) behaviour between males exposed to familiar (white boxes) and novel mate + competitors (black boxes). The proportion of intrasexual behaviour is influenced only by aggression behaviour, the proportion of intersexual behaviour are influenced both following (C) and courtship behaviours (D). Large dots represent tank average for each sex ratio replicate, small dots represent averages of individual females. 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 demonstrating the effects of baseline and novel mate + competitor novelty, and sex ratio on the proportion of male inter- and intrasexual behaviour. Individual males and tank are included as a random effect. Included are degrees of freedom (df), Akaike Information Criterion ($AIC_c$), the difference between the model with the lowest $AIC_c$ and all other models ($\Delta AIC_C$ ), and the model weight ($w_{AICc}$ ). Model selection is based on Akaike Information Criterion criteria and the best fit models are highlighted in bold.

| Model | *df* | $AIC_C$ | $\Delta AIC_C$ | $w_{AICc}$ |
|---|---|---|---|---|
| *Intrasexual behaviour* | | | | |
| **Sex ratio** | **7** | **365.2** | **0.00** | **0.395** |
| **Sex ratio + novelty** | **8** | **365.7** | **0.49** | **0.309** |
| (Intercept only) | 4 | 367.5 | 2.22 | 0.130 |
| Novelty | 5 | 367.7 | 2.43 | 0.117 |
| Sex ratio x novelty | +11 | 369.4 | 4.20 | 0.048 |
| *Intersexual behaviour* | | | | |
| **Sex ratio** | **7** | **365.2** | **0.00** | **0.395** |
| **Sex ratio + novelty** | **8** | **365.7** | **0.49** | **0.309** |
| (Intercept only) | 4 | 367.5 | 2.22 | 0.130 |
| Novelty | 5 | 367.7 | 2.43 | 0.117 |
| Sex ratio x novelty | +11 | 369.4 | 4.20 | 0.048 |
| *Following* | | | | |
| **Sex ratio** | **7** | **286.3** | **0.00** | **0.396** |
| **(Intercept only)** | **4** | **287.1** | **0.84** | **0.260** |
| Sex ratio + novelty | 5 | 287.7 | 1.47 | 0.190 |
| Novelty | 7 | 288.3 | 2.06 | 0.141 |
| Sex ratio x novelty | +11 | 293.2 | 6.95 | 0.012 |
| *Courtship* | | | | |
| **(Intercept only)** | **4** | **-122.1** | **0.00** | **0.466** |
| **Novelty** | **5** | **-121.6** | **0.52** | **0.360** |
| Sex ratio | 7 | -118.8 | 3.33 | 0.088 |
| Sex ratio + novelty | 8 | -118.2 | 3.93 | 0.065 |
| Sex ratio x novelty | +11 | -115.8 | 6.30 | 0.020 |

### *3.4 Comparing novelty phases*

I compared the behavioural difference between the two novelty phases (novel mates, and novel mates and competitors). The model that best explained this difference in both intersexual (following and courtship) and intrasexual behaviour did not include novelty or sex ratio (Table 5). There was no difference between the change in behaviour caused by both novelty phases and by sex ratio (Figure 7; Table 5). Additionally, there was no difference in following between novelty phase or sex ratio, or in courtship between novelty phase or sex ratio (Figure 7; Table 5).

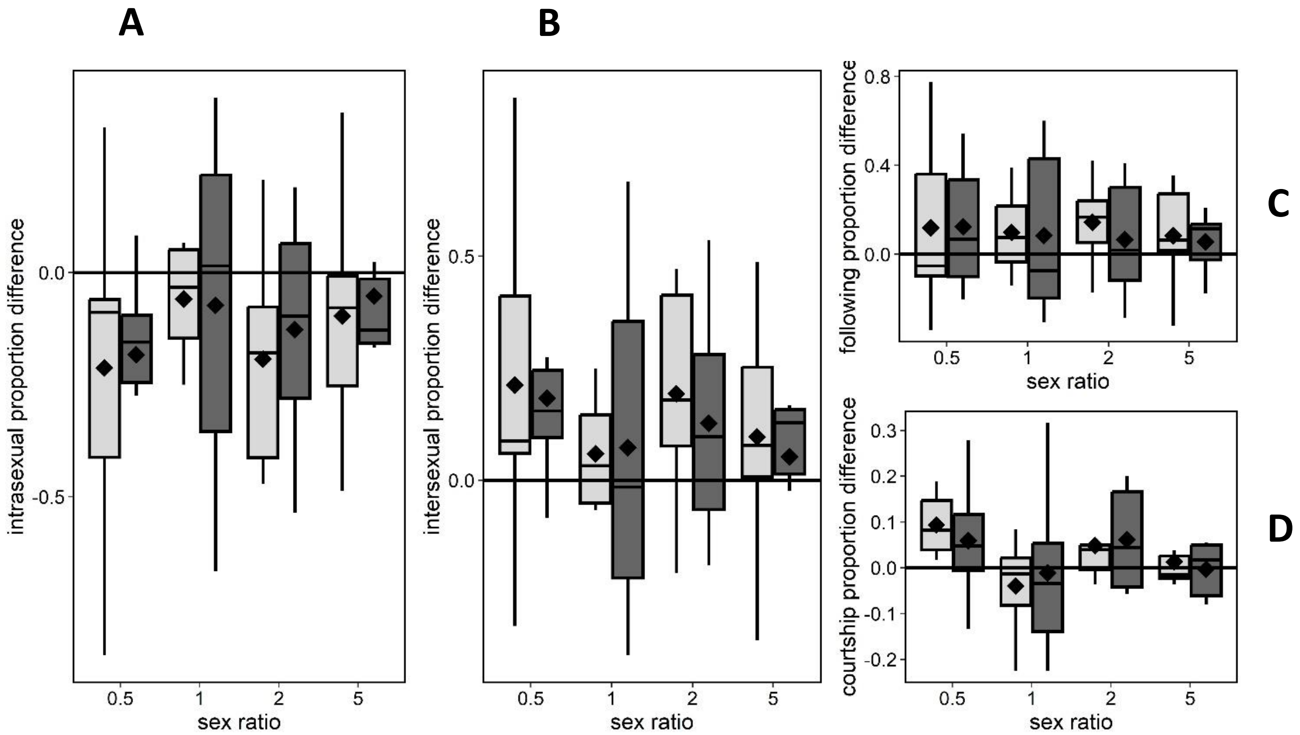

Figure 7. The relationship between difference in inter- and intrasexual behaviour from baseline behaviour between novel mate (grey boxes), and novel mate and competitor phases (black boxes) and sex ratio. Horizontal line represents baseline behaviour from observation of all males before novelty was introduced. 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 5.

Table 5. Generalized linear models demonstrating the effects of baseline and novel mate + competitor treatments, and sex ratio on the proportion of male inter- and intrasexual behaviour. Individual males and tank are included as a random effect. Included are degrees of freedom (df), Akaike Information Criterion ($AIC_c$), the difference between the model with the lowest $AIC_c$ and all other models ($\Delta AIC_C$ ), and the model weight ($w_{AICc}$ ). Model selection is based on Akaike Information Criterion criteria and the best fit models are highlighted in bold.

| Model | *df* | $AIC_C$ | $\Delta AIC_C$ | $w_{AICc}$ |
|---|---|---|---|---|
| *Intrasexual difference* | | | | |
| **(Intercept only)** | **3** | **12.7** | **0.00** | **0.708** |
| Novelty | 4 | 14.9 | 2.12 | 0.245 |
| Sex ratio | 6 | 18.6 | 5.91 | 0.037 |
| Sex ratio + novelty | 7 | 21.1 | 8.40 | 0.011 |
| Sex ratio x novelty | +10 | 30.1 | 17.32 | 0.000 |
| *Intersexual difference* | | | | |
| **(intercept only)** | **3** | **12.7** | **0.00** | **0.708** |
| Novelty | 4 | 14.9 | 2.12 | 0.245 |
| Sex ratio | 6 | 18.6 | 5.91 | 0.037 |
| Sex ratio + novelty | 7 | 21.1 | 8.40 | 0.011 |
| Sex ratio x novelty | +10 | 30.1 | 17.32 | 0.000 |
| *Following difference* | | | | |
| **(Intercept only)** | **3** | **9.5** | **0.00** | **0.724** |
| Novelty | 4 | 11.7 | 2.11 | 0.252 |
| Sex Ratio | 6 | 16.9 | 7.34 | 0.018 |
| Sex Ratio + novelty | 7 | 19.4 | 9.82 | 0.005 |
| Sex Ratio x novelty | +10 | 28.2 | 18.65 | 0.000 |
| *Courtship* difference | | | | |
| **(Intercept only)** | **3** | **-67.6** | **0.00** | **0.516** |
| **Novelty** | 4 | **-66.2** | **1.37** | **0.260** |
| Sex ratio | 6 | -65.2 | 2.38 | 0.157 |
| Sex ratio + novelty | 7 | -63.5 | 4.12 | 0.066 |
| Sex ratio x novelty | +10 | -55.0 | 12.59 | 0.001 |

## 4 Discussion

The objective of this study was to measure the effects of mate and competitor novelty on the courtship and aggression behaviour of Japanese medaka. To do this, I posed three main questions: 1) how does male courtship and aggression behaviour change in response to novel mates, 2) how does female reproductive output change in response to novel mates, and 3) how does male courtship and aggression behaviour change in response to novel mates and competitors?

### *4.1 Novel mate phase*

#### *4.1.1 Effects on male behaviour*

In this experiment, I predicted that exposure to novel females would increase male courtship behaviour, that males in female biased sex ratios would allocate more energy to intersexual courtship than intrasexual aggression behaviour, and that the change in behaviour would be greater in male biased sex ratios. This is because I anticipated that males in female biased sex ratios will be less familiar with the individual females in their tank. I predicted that this would illicit a greater change in their behaviour relative to males in males biased tanks.

In response to novel mates, males allocated more of their energy towards intersexual courtship behaviour. As a trade-off, less energy was allocated towards intrasexual aggression behaviour. Additionally, sex ratio influenced behavioural allocation, where males in male biased sex ratios prioritized intrasexual aggression behaviour, and as sex ratio becomes more female biased, intersexual behaviour was increasingly prioritized. Finally, there was no difference in behavioural change between male and female biased sex ratio.

The behavioural change in response to sex ratio variation is consistent with the expected dynamic of sex ratio and mate competition intensity, where mate competition increases as sex ratio becomes increasingly male biased (Kvarnemo & Ahnesjo, 1996). This mate competition increase results in increased intrasexual interactions, and as a trade-off, decreased intersexual interactions in male biased sex ratios (Kvarnemo & Ahnesjo, 1996).

The increase in courtship behaviour in response to novel mates supports the hypothesis that male medaka experience the Coolidge effect. I propose that this is the result of a male bet hedging strategy. It is well established that male fertility increases with mate frequency (Bateman, 1948). However, securing mates is a costly investment associated with trade-offs such as engaging in mating activities that could expose an individual to predation or that could take away from other survival efforts (Hoffman et al., 2008). Therefore, to maximize this investment in a variable environment, males will employ a bet hedging strategy, where individuals will mate with a diverse range of phenotypes to produce a variety of offspring, with the intent of siring a portion of offspring that inherit a suitable phenotype for the prevailing environment (Steiger et al., 2008). Males investing more time in courting unfamiliar females is consistent with this strategy, as mating with new females increases phenotypic variety in mates.

This phenomenon has been observed in other species of fish such as the *Poecilia reticulata,* the wild guppy (Kelley et al., 1999). They found that wild guppies housed temporarily in aquaria spent more time courting unfamiliar females; however, males in their wild environment did not discriminate between familiar and unfamiliar females (Kelley et al., 1999). This is explained by wild males maximizing their mating opportunity by moving amongst schools of females, and therefore do not need to discriminate between familiar and unfamiliar females (Kelley et al., 1999). For males in aquaria, where there is no flux of new individuals, identifying unfamiliar females proves as an advantage reproductive strategy (Kelley et al., 1999). This is because the chance of encountering a novel female is lower when new individuals are not able to enter or exit the tank (Kelley et al., 1999).

However, not every species can exhibit the Coolidge effect. For example, invertebrate hermaphrodite species such as the freshwater snail *Biomphalaria globrata* are not influenced by mate novelty (Häderer et al., 2009). This is because the cost of male mating is not high enough for strategic mating the be worth the energetic cost, due to the high rate of seminal fluid restoration in this species (Häderer et al., 2009). This reduces the benefits of prudent mate discrimination (Häderer et al., 2009). Additionally, in order for an organism to exhibit the Coolidge effect, it must have the physical ability to distinguish between individuals. It is hypothesized that *B. glabrata* are incapable of discriminating between novel and familiar mates because they lack the ability to remember visual and chemical familiarity cues (Häderer et al., 2009).

Conversely, it has been demonstrated that medaka are able to distinguish between one another by both visual and olfactory cues (Wang & Takeuchi, 2017). This is consistent with the idea that to exhibit the Coolidge effect, the organism must have the ability to distinguish between individuals. Additionally, the medaka used in this study were housed in aquaria, and there is currently no data on how mate novelty influences wild medaka behaviour. If the benefits of mate discrimination are outweighed by other opportunities in a wild environment for wild medaka, as was the case for guppies, then mate novelty could have no effect on wild medaka, and therefore may be ecologically insignificant. However, this claim would require more research.

### *4.1.2 Effects on female reproductive output*

I predicted that female medaka would exhibit the Coolidge effect, meaning that female reproductive output would increase in response to novel mates. Additionally, I predicted that females in male biased sex ratios would have a higher reproductive output than females in female biased sex ratios. Finally, I predicted that the difference in reproductive output would be greater in male biased sex ratios than in female biased sex ratios. This is because I anticipated that females in male biased sex ratios will be less familiar with the individual males in their tank. I predicted that this would illicit a greater change in behaviour relative to females in female biased tanks.

In this experiment, female reproductive output tended to decrease in response to mate novelty across most sex ratios. Reproductive output was highest in sex ratio 5, the most male biased sex ratio. The difference in reproductive output because of mate novelty was greatest in sex ratio 0.5, the most female biased sex ratio.

In terms of sex ratio, the result of higher overall reproductive output in male biased sex ratios is consistent with the observation that mate competition intensity increases as sex ratio becomes more male biased (Kvarnemo & Ahnesjo, 1996). I propose that this increase in mate competition intensity causes females to experience higher courtship efforts by males, which leads to more consistent reproductive output.

The decrease in reproductive output in response to novel mates is consistent with the observation that female medaka are more receptive to familiar males (Yokoi et al., 2016). Females may be less receptive to novel males because they invest greater energy in fewer gametes compared to males (Andersson, 1994). This may result in females being choosier when selecting a mating partner and therefore, females will prefer familiar males that they know are quality partners that can reproduce successfully (Andersson, 1994).

Additionally, unlike males, females' fertility does not primarily depend on mate frequency (Kvarnemo & Ahnesjo, 1996). Therefore, bet hedging with mate frequency is not necessarily a cost-effective option for females, creating less appeal for mate novelty (Kvarnemo & Ahnesjo, 1996). Alternatively, I propose that females likely bet hedge with post-fertilization mechanisms such as varying egg and clutch size, and hatch time to cope with varying environmental conditions (Crean & Marshall, 2009).

### ***4.2 Novel Mate and Competitor Phase***

In this experiment, I predicted that in response to novel mates and competitors, male medaka would allocate more energy to intrasexual aggression behaviour over intersexual courtship. I predicted that aggression would be higher overall in male biased sex ratios, and that the difference in behaviour caused by aggression novelty would be greater in female biased sex ratios. This is because I anticipated that males in female biased sex ratios will be less familiar with the individual females in their tank. I predicted that this would illicit a greater change in their behaviour relative to males in males biased tanks.

The results of the experiment demonstrated that male medaka allocate more of their total behaviour towards courtship in response to novel mates and competitors. Additionally, the change in behaviour allocation across both sex ratio and novelty treatment did not differ significantly between the novel mate, and the novel mate and competitor phase.

I propose that males did not increase their aggression compared the familiar baseline observations because they were already acting at their maximum aggression capacity. Increasing aggression past this maximum would take too much energy away from courting novel females, which as mentioned previously is a method that increases the likelihood of offspring fitness (Lips, 2001). While aggression behaviour is important for securing mates, competing with novel over familiar competitors does not have the same fitness advantages as courting novel over familiar females (Lips, 2001).

## 4.3 Conclusion

In response to novel mates, males allocated more of their total behaviour towards courtship over aggression behaviour. This response is likely a result of a male bet hedging strategy, where mating with novel females could increase offspring diversity (Steiger et al., 2008). Alternatively, females decreased reproductive output in response to novel mates. This could result from more conservative mate choice by females, as well as an alternative focus on bet hedging through post-fertilization mechanisms (Andersson, 1994; Crean & Marshall, 2009). Finally, males did not increase their aggression behaviour in response to novel mates and competitors. This could be because male medaka are already acting at their maximum aggression capacity, and increasing aggression does not have the same reproductive benefits as increasing courtship (Steiger et al., 2008).

## 5 References

Andersson, M. (1994). *Sexual Selection*. Princeton University Press.

Bateman, A. J. (1948). Intersexual Selection in Drosophila. *Heredity*, 349–368.

Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, *67*(1), 1–48.

Beach, F. A., & Jordan, L. (1956). Sexual Exhaustion and Recovery in the Male Rat. *Quarterly Journal of Experimental Psychology*, *8*(3), 121–133.

Crean, A. J., & Marshall, D. J. (2009). Coping with environmental uncertainty: Dynamic bet hedging as a maternal effect. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *364*(1520), 1087–1096. https://doi.org/10.1098/rstb.2008.0237

Emlen, S. T., & Oring, L. W. (1977). Ecology, Sexual Selection, and the Evolution of Mating Systems. In *New Series* (Vol. 197, Issue 4300).

Fitze, P. S., Cote, J., MartÍnez-Rica, J. P., & Clobert, J. (2008). Determinants of male fitness: Disentangling intra- and inter-sexual selection. *Journal of Evolutionary Biology*, *21*(1), 246–255. https://doi.org/10.1111/j.1420-9101.2007.01447.x

Franz, M., Lieberum, C., Bock, G., & Karez, R. (2019). Environmental parameters of shallow water habitats in the SW Baltic Sea. *Earth System Science Data*, *11*(3), 947–957. https://doi.org/10.5194/essd-11-947-2019

Grant, J. W. A., Bryant, M. J., & Soos, C. E. (1995). Operational sex ratio, mediated by synchrony of female arrival, alters the variance of male mating success in Japanese medaka. In *Anim. Behav* (Vol. 49).

Grant, J. W. A., Gaboury, C. L., & Levitt, H. L. (2010). Competitor-to-resource ratio, a general formulation of operational sex ratio, as a predictor of competitive aggression in Japanese medaka (Pisces: Oryziidae). In *Behavioral Ecology* (Vol. 11, Issue 6).

Häderer, I. K., Werminghausen, J., Michiels, N. K., Timmermeyer, N., & Anthes, N. (2009). No effect of mate novelty on sexual motivation in the freshwater snail Biomphalaria glabrata. *Frontiers in Zoology*, *6*(1). https://doi.org/10.1186/1742-9994-6-23

Hiramatsu, K., & Shikasho, S. (2004). GA-based model optimization for preference intensity of Japanese Medaka Fish (Oryzias latipes) to streamflow environments. *Paddy and Water Environment*, *2*(3), 135–143. https://doi.org/10.1007/s10333-004-0052-5

Hoffman, C. L., Ruiz-Lambides, A. V., Davila, E., Maldonado, E., Gerald, M. S., & Maestripieri, D. (2008). Sex differences in survival costs of reproduction in a promiscuous primate. *Behavioral Ecology and Sociobiology*, *62*(11), 1711–1718. https://doi.org/10.1007/s00265-008-0599-z

Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous Inference in General Parametric Models. *Biometrical Journal*, *50*(3), 346–363.

Iwamatsu, T. (2004). Stages of normal development in the medaka Oryzias latipes. In *Mechanisms of development* (Vol. 121, Issues 7–8, pp. 605–618). https://doi.org/10.1016/j.mod.2004.03.012

Janetos, A. C. (1980). Strategies of Female Mate Choice: A Theoretical Analysis. In *Behav. Ecol. Sociobiol* (Vol. 7, Issue 2).

Kelley, J. L., Graves, J. A., & Magurran A. E. (1999). Familiarity breeds contempt in guppies. *Nature*, *401*, 661–661. www.nature.com

Kvarnemo, C., & Ahnesjo, I. (1996). The dynamics of operational sex ratios. *Trends in Ecology and Evolution*, *11*(10), 404–408.

Leaf, R. T., Jiao, Y., Murphy, B. R., Kramer, J. I., Sorensen, K. M., & Wooten, V. G. (2011). Life-history characteristics of Japanese Medaka Oryzias latipes. *Copeia*, *4*, 559–565. https://doi.org/10.1643/CI-09-190

Length, R. V. (2023). *emmeans: Estimated Marginal Means, aka Least-Squares Means* (R package version 1.9.0).

Lips, K. R. (2001). Reproductive trade-offs and bet-hedging in Hyla calypsa, a neotropical treefrog. *Oecologia*, *128*(4), 509–518. https://doi.org/10.1007/s004420100687

Lisk, R. D., & Baron, G. (1982). Female Regulation of Mating Location and Acceptance of New Mating Partners following Mating to Sexual Satiety: The Coolidge Effect Demonstrated in the Female Golden Hamster. In *BEHAVIORAL AND NEURAL BIOLOGY* (Vol. 36).

Ono, Y., & Uematsu, T. (1957). Mating Ethogram in Oryzias latipes. *Zoology*, *13*, 1–4.

R Core Team. (2023). *R: A language and environment for statistical computing* (4.3.0). R Foundation for Statistical Computing.

Shama, L. N. S. (2015). Bet hedging in a warming ocean: Predictability of maternal environment shapes offspring size variation in marine sticklebacks. *Global Change Biology*, *21*(12), 4387–4400. https://doi.org/10.1111/gcb.13041

Steiger, S., Franz, R., Eggert, A. K., & Müller, J. K. (2008). The Coolidge effect, individual recognition and selection for distinctive cuticular signatures in a burying beetle. *Proceedings of the Royal Society B: Biological Sciences*, *275*(1645), 1831–1838. https://doi.org/10.1098/rspb.2008.0375

Wang, M.-Y., & Takeuchi, H. (2017). Individual recognition and the “face inversion effect” in medaka fish (Oryzias latipes). *ELIFE*. https://doi.org/10.7554/eLife.24728.001

Yokoi, S., Ansai, S., Kinoshita, M., Naruse, K., Kamei, Y., Young, L. J., Okuyama, T., & Takeuchi, H. (2016). Mate-guarding behavior enhances male reproductive success via familiarization with mating partners in medaka fish. *Frontiers in Zoology*, *13*(1). https://doi.org/10.1186/s12983-016-0152-2