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**Operational sex ratio affects female Japanese medaka (*Oryzias latipes*) behaviours**

By  
Amanda L. Gove

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

May 1st, 2020, Halifax, Nova Scotia

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Date: May 1st, 2020

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## Abstract

Operational sex ratio (OSR) is the ratio of sexually active males to fertilizable females in a population. The OSR can be used to predict variation in mating behaviours across different social contexts and is used to predict how selection and conflict will vary within species. There exist two characteristics that influence the way species behave in regard to reproduction: sexual conflict and sexual selection. Within the literature about sexual behaviour, most focus on males rather than females. The goal of this research is to determine how OSR influences the behaviour of female Japanese medaka (*Oryzias latipes*). During the experiment, behavioural observations of individual female medaka were recorded across four OSRs (male: female): of 0.5, 1, 2, and 5. Rates of behaviour associated with conflict and competition were recorded for two minutes per female over three discrete observation periods. Conflict behaviours, female refusal and male-female aggression increased with increasing OSR, while female-male aggression decreased. Competition behaviours, females receiving aggression and females initiating aggression, decreased with increasing OSR. Body size was another factor analyzed to see the influence on both conflict and competition. Two of the conflict behaviours, female refusal and male-female aggression were influenced by the body size of the female, but none of the competition behaviours were affected by body size. Determining which behaviours are influenced by OSR and body size can be used to predict the outcomes of sexual conflict and sexual selection in different mating systems.

May 1<sup>st</sup>, 2020

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# **1. Introduction**

## ***1.1 Sexual Selection and Sexual Conflict***

Sexual selection occurs when some individuals possess characteristics that give them an advantage over others of the same sex and species solely with respect to reproduction (Darwin, 1871; Hosken & House, 1998). Sexual selection is often characterized by two mechanisms: mate choice and mate competition (Andersson, 1994; Hosken & House, 1998). Although male choice and female-female competition are prominent in some mating systems, females are typically the choosy sex and males compete with one another for access to females or mating-related resources (Harris & Moore, 2005; Hosken & House, 1998). This trend is based on males' reproductive success often being positively correlated with the number of matings they obtain, such that they compete for access to as many females as possible (Hosken & House, 1998). Females may be choosy because they invest more energy in the production of fewer, larger gametes, whereas males produce large quantities of smaller gametes at a lesser cost (Dewsbury, 1982; Hosken & House, 1998; Weir & Grant, 2010). Females should be able to discriminate among males, either choosing mates that provide some benefit or avoiding males that impose a high cost to mating (Harris & Moore, 2005). Female mate choice depends on the interpretation of cues or signals that are indicated by the overall information of mate quality (Harris & Moore, 2005). Males and females can differ in their investment in different components of reproduction, and may therefore approach mating with differing strategies (Krasnec, Cook, & Breed, 2012).



Beyond sexual competition, another evolutionary phenomenon that contributes to the framework of sexual interactions is sexual conflict. Conflict occurs between individuals of the opposite sex, when there is increased investment by one partner that permits decreased investment by the other (Arnqvist & Locke, 2006; Parker, 1970, 2006). For example, male and female water striders (*Gerris lacustris*) exhibit genital morphology that is indicative of an ‘arms race’; males have structures that enhance their ability to copulate with females, while females evolve structures to prevent copulation (Han & Jablonski, 2010). When a male water strider mounts a female, she uses her “genital shield” to avoid the mating attempt (Han & Jablonski, 2010). However, if the female refuses, the male will tap on the surface of the water to attract predators from below (Han & Jablonski, 2010). This action puts the female at risk of an attack (as the female is the closest to the water surface), so females open their genital shield to reproduce to decrease the probability of such an attack (Han & Jablonski, 2010). Therefore, what is good for the male is not good for the female and this creates conflict. Throughout a female's lifetime, reproductive fitness costs arise through interactions with potential mates and will be influenced by the frequency of such interactions (Arnqvist & Locke, 2006; Parker, 1970, 2006). In addition, conflict may arise between males and females because of mating interference or sperm limitation (Dewsbury, 1982; Nakatsuru & Kramer, 1982); females may refuse to mate with particular males if other females signal that these males have decreased fertility (Harris & Moore, 2005; Nakatsuru & Kramer, 1982; Weir & Grant, 2010). Because it is difficult to assess how females could directly assess sperm stores in males, females may be using indirect signals such as social interactions as indicators of male fertilization ability (Harris & Moore, 2005). Based on

work by Darwin (1871), Bateman (1948), and Trivers (1972), it is generally agreed that the sex that invests more into the offspring evolves to be the more selective one. In the majority of species, it is the females that invests strongly in eggs, as compared to the very small investment of males in sperm (Schlupp, 2018; Trivers, 1972). Gross and Sargent (1985) state that parental care has only one benefit: the increase in survival of zygotes with care relative to those without care. In externally fertilizing species, the general rule will be for no care to give rise to uniparental male care (Gross & Sargent, 1985). Because females invest more than males, they can be choosy when picking their mate, which can lead to more competition among males.

Males and females can show preferences in mating, such as for large body size, but likely for very different reasons. Female preferences for large males are thought to be due not only to direct benefits such as protection that larger males provide, but indirect genetic benefits for their offspring (Reynolds & Gross, 1992; Schlupp, 2018). Male preferences are related to a direct benefit, via increased fecundity and therefore a greater offspring output by larger females (Dosen & Montgomerie, 2004). Grant et al. (1995) conducted an experiment to see if males preferred larger or smaller bodied females. They found that males spent more time with the large rather than the small female in 22 of 23 trials from their experiment, indicating that males preferred the larger of two females when courting (Grant, Casey, Bryant, & Shahsavarani, 1995). This finding shows that body size of an individual can impact the way they behave in a population and can influence both mechanisms of sexual selection, specifically male choice or male-male competition. The effect of male-male competition was shown by Lehtonen & Lindström (2004), wherein they found the sand gobies (*Pomatoschistus minutus*) larger body size

had an effect on mating success, relating to the intensity of male-male competition: the number of eggs in a male's nest correlated with his body size only in habitats where scarcity of nest sites promoted competition among males.

### ***1.2 Operational Sex Ratio (OSR)***

Both sexual selection and conflict are influenced by the frequency of inter- and intra-sexual encounters. Population density and operational sex ratio (OSR; the ratio of sexually active males to fertilizable females in a population; Emlen, 1976) influence an individual's social environment by determining the number of potential competitors and mates encountered (Clutton-Brock & Parker, 1992; Grant & Foam, 2002). Sexual conflict and sexual selection can influence mating behaviour in species, especially when taking into account various OSRs, as the number of males to females in a population will influence what interactions are going to occur (e.g., more/less aggression, refusals, and mating). OSR has been a cornerstone of sexual selection theory since Emlen and Oring's (1977) review of the evolution of mating systems. It is used to predict the intensity of intrasexual competition for mates and mating system structure by predicting which sex is non-limiting and therefore, competes for access to the limiting sex (Emlen & Oring, 1997). Thus, in populations with male-biased sex ratios, male-male competition should be more intense than in even sex ratios, and females are expected to be choosier.

Males typically have a higher potential rate of reproduction than females; therefore, the ratio of males to females in a population is often biased in favour of males (Clark & Grant, 2010; Clutton-Brock & Parker, 1992; Vincent et al., 1992). Because of this bias, it leads to not all individuals being available for mating at any given time as the

operational sex ratio is rarely equal to one, even if the adult sex ratio in the population approaches unity (Kokko & Jennions, 2008; Kokko & Johnstone, 2002). For example, the age at sexual maturity often differs between the sexes, leading to an imbalance in the OSR (Emlen & Oring, 1997). Conventionally, males are the more competitive sex, which often leads males to have more intense intra-sexual competition and leads to greater choosiness in females than in males (Clutton-Brock & Parker, 1992). However, when the OSR is female-biased, typical sex roles may be reversed, leading to female-female competition and male choosiness (Clark & Grant, 2010; Forsgren, Amundsen, Borg, & Bjelvenmark, 2004). The OSR can be used to predict mate choice and competition behaviour between sexes. For example, in syngnathid fishes, such as pipefishes (*Syngnathus typhle* and *Nerophis ophidion*), females compete more intensely for access to mates and males exhibit pregnancy. This produces more intense female competition for mates and imposing greater sexual selection pressures on females (Vincent et al., 1992).

Biased sex ratios can also influence the degree to which sexual conflict occurs. In many species, males have alternative mating tactics that tend to occur more frequently when a larger number of males are present and their mating behaviours/strategies may change to gain access to females (Grant, Bryant, & Soos, 1995; Weir, 2013). For example, male water striders use three main tactics to obtain and guard mates: they may guard females until oviposition, not guard at all, and/or intimidate the female into mating (Han & Jablonski, 2010; Vepsalainen & Savolainen, 1995). The use of alternative male tactics is dependent on the OSR; guarding usually occurs at a high OSR ratio and no guarding typically occurs at a low OSR ratio (Vepsalainen & Savolainen, 1995). Overall,

this causes conflict towards females as they now have to try to mate with these new strategies (Gross, 1996).

### ***1.3 Japanese Medaka as Model Species***

Japanese medaka (*Oryzias latipes*) are small freshwater beloniform fish (Shima & Mitani, 2004) that are frequently used as a model species to test predictions about sexual selection and sexual conflict. They have courtship rituals and spawn daily under laboratory conditions, meaning they are ideal to use for creating multiple OSR scenarios based on their various observable behaviours (Grant & Foam, 2002; Shima & Mitani, 2004).

Medaka are sexually dimorphic (distinguished by differences in anal fin morphology and abdomen shape; Howard et al., 1998; Kamito, 1928) and do not exceed 40 mm in length. They sexually mature at 10-12 weeks, and once mature, they are able to spawn daily (Howard et al., 1998; Yamamoto, 1975). Their mating is described as ‘pseudocopulation’ because males and females are in close physical contact during mating (Ono & Uematsu, 1957). During pseudocopulation, the male grasps the female with his anal and dorsal fins and quivers, followed by sperm and egg release if the female is receptive (Howard et al., 1998; Ono & Uematsu, 1957). Throughout the mating season, female medaka can spawn daily, producing 10-50 eggs that are fertilized externally (Howard et al., 1998). These fish are oviparous, with eggs fertilized and embryogenesis occurring externally (Shima & Mitani, 2004). The sticky egg masses remain attached to the female for as little as a few hours until they become attached to vegetation as the female rubs her abdomen against the surfaces (Howard et al., 1998). Medaka are not monogamous, and they do not show any parental care towards their offspring. Males are able to reproduce with up to 4-30

females per day (Howard et al., 1998), whereas females are only able to mate once per day and have a short reproductive cycle of 24h (Ono & Uematsu, 1957; Yokoi et al., 2016). Therefore, female medaka are generally the limiting sex and males compete intrasexually for mating attempts (Nakatsuru & Kramer, 1982).

Japanese medaka have several easily observable behaviours. Male courtship behaviours include manoeuvring underneath females and swimming in a rapid circle (or 'quick circle' (Howard et al., 1998; Ono & Uematsu, 1957)). Females signal rejection by assuming a more vertical position in the water column (Ono & Uematsu, 1957). Both males and females partake in following and aggressive behaviours which can be used to measure the intensity of competition and conflict within various OSR ratios that are either female-biased, male-biased or similar. The behaviours such as following and aggression are not only used between mates, but also between members of the same sex for competition. For example, females were aggressive or nudged other females that were being courted by males to try and compete for courtship (Clark & Grant, 2010).

#### ***1.4 Research Goals***

The current research aims to determine the effects of OSR and body size on female mating behaviours which include both intrasexual and intersexual competition in Japanese medaka. Females are studied to address the following questions: (1) How does the operational sex ratio affect sexual conflict and female intrasexual competition? and (2) Does body size of the female influence her mating behaviour? To answer these questions, I observed several behaviours in female medakas that are associated with competition and conflict and analyzed the body size of each female with the associated

behaviour. Allen (2019) found the mean courtships rates of two generations of male Japanese medaka decreased with the increasing sex ratios. Theoretically, the decreased rate could be from the females rejecting the males, thus female rejecting rates should increase with the increasing OSR. Therefore, I predict that female conflict behaviours will increase with increasingly male-biased OSRs. Another factor for this prediction is that males will still try and court the females even when they have a clutch of eggs, considering females can only mate once per day, they will have to reject the males. Accordingly, this also causes higher conflict rates. For female competition behaviours, I predict that as the OSR changes from female- to male-biased, female intrasexual competition will decrease. In previous studies, male medaka have shown a preference towards larger bodied females over smaller bodied females. If the larger bodied females are being courted more often than the smaller, I predict the larger body size will have a higher rate of conflict behaviours. This prediction is made under the speculation that if larger bodied females are being preferred, they will be courted more. Seeing that females are able to have their choice, I also predict that larger females will cause more competitive behaviours to establish dominance over the small females, so they are able to pick the best mate.

## 2. Materials and Methods

### 2.1 Experimental Animals

Two hundred and eighty-eight Japanese medaka (*Oryzias latipes*) were used in this experiment; these animals are the second generation of lab-bred fish at Saint Mary's University, Halifax, Nova Scotia. Prior to the experiment, all fish were anaesthetized with 0.15g/L MS222 (Tricaine S) and 0.3g/L sodium bicarbonate and tagged using visual implant elastomer (North West Marine Technology) for individual identification. The fish were tagged using one or two of eight possible colours on either the anterior or posterior section of the dorsal fin, on either the left or right side. The fish were housed in ten-gallon tanks, 20 x 10 x 12 inches, containing fresh water with a salinity of 0.57ppt (parts-per-thousand), measured with a Hanna probe HI 98192.

Within the laboratory, there were a total of 24 tanks. Fish were housed at four male: female sex ratios in groups of 12 animals, 0.5, 1, 2, and 5. For example, the sex ratio of 0.5 had 4 males and 6 females, 1 had 6 males and 6 females, 2 had 8 males and 4 females, and 5 had 10 males and 2 females. The physical location of tanks containing each OSR was randomized on each of the six shelves to minimize any bias associated with proximity to light or the entry way of the room. Each tank contained an undergravel filter and were kept at a temperature of 21°C - 28°C with aquarium heaters that are monitored by HOBOMobile phone application, and a photoperiod of 14hr light:10hr dark with lights turning on at 07h45. Fish were fed twice per day from Monday – Friday and once on weekends. During the morning feeding, between 0800 -1000h, fish were fed frozen brine shrimp (Hik Ari Bio-Pure), while the afternoon feeding, 1400 – 1600h, fish were fed brine shrimp nauplii and flakes (TetraMin Tropical Flakes). Water quality tests



such as pH, ammonia, nitrite, and nitrate were conducted weekly to maintain the health of the fish. Similarly, water changes occurred weekly, or as needed, given the outcome of water testing. Tank water consisted of RODI (reverse osmosis deionized water) that includes 1.6 mL/gal of Red Sea salt, 0.145 mL/gal of alkaline buffer, and 0.5 mL/gal of stress coat. The fish were monitored during daily feeding and water quality/changes to ensure they were healthy. All procedures were in accordance with the Animal Care Committee at Saint Mary's University protocol 17-04.

## ***2.2 Experimental Design***

During the experiment, I observed individual female medaka behaviours in all 24 tanks across three different observation periods. On a particular day, females within each of the four OSR treatments were observed in random order. Within each tank, I randomized the order in which I would observe females. Observations were completed in this manner for all six replicate tanks. Thus, over a six-day period, all females were observed. This process was repeated three times. Each observation was conducted between 0800h and 1000h, before morning feeding. During the observations, I recorded the frequency of the behaviours outlined in section 2.3. This was recorded on a scoresheet with each behaviour listed, I would observe a female for a two-minute time interval and tally any behaviours that were occurring in the timeframe, then I would move on to the next female on the list and continue each day until one replicate block was completed. The sample size of the females was 119, it was supposed to be 120 but a male was wrongly tagged as a female prior to the experiment. Additionally, the body size of the female was considered for analysis. The body sizes of the females ranged from 18.4mm to 26mm. These were analyzed to see how she reacted to aggression against her and being the aggressor.

### ***2.3 Behavioural Observations***

Japanese medaka have several behavioural characteristics that correspond with mating and traits that occur between individuals of the same sex and opposite sex. For sexual conflict behaviours, these were categorized by female to male interactions. The behaviours for sexual conflict included: female refusal of male courtship or mating attempts, female aggression toward males, and male aggression toward females. Refusal is identified by a characteristic ‘head-up’ display, whereby a female raises the anterior end of her body, creating a 30°–60° angle between her body and the male (Ono & Uematsu, 1957). Resistance by the female also includes the ‘swim-up’ behavior, whereby females swam to the surface of the water with their heads raised (Ono & Uematsu, 1957; Weir, 2013). This occurs after a male is trying to court the females with their quick-circling behaviour, where a male maneuvers his body underneath and around the female in rapid circles (Ono & Uematsu, 1957). Male-female aggression is when the female is experiencing aggression from the male, this occurs when the male is behind the female and will dart at her to get attention (Clark & Grant, 2010; Ono & Uematsu, 1957). Another behaviour that is categorized as conflict is female-male aggression, which is shown when the female is the aggressor, as she will target the observed male in the tank by swimming towards him rapidly (Clark & Grant, 2010; Ono & Uematsu, 1957).

Female competitive behaviours are categorized as female-female interactions. There are three main behaviours displayed during female-female interactions. Firstly, if a female is experiencing aggression from another female, this is recorded as “females receiving aggression” and characterized when the female is fleeing from an individual swimming rapidly behind (Clark & Grant, 2010; Ono & Uematsu, 1957). Secondly,

“females initiating aggression” is shown when the observed female is the aggressor, as she will target another female in the tank by swimming towards her rapidly (Clark & Grant, 2010; Howard et al., 1998; Ono & Uematsu, 1957). Finally, interruption occurs when a female physically disrupts an ongoing mating event by inserting her body between two members of a mating pair.

#### ***2.4 Data Analysis***

Prior to analysis, the recording of refusals, initiating aggression towards females/males, receiving aggression from females, male-female aggression and interruptions were tallied per round to quantify the occurrence of each behaviour for each female. These values were averaged across the three observation periods, so there was only one data point per female. Female body size was recorded prior to observations and incorporated into data. The package “lme4” was used for generalized linear mixed models (glmer) to determine the influence of OSR and body size on female mating behaviours. For these analyses, OSR and female body size were fixed effects, and tank was included as a random effect to avoid pseudoreplication. The package “MunMIn” was used to compare all possible models for each behaviour. All analyses were conducted using R version 3.6.1 (R Core Team, 2019).

## 3. Results

### 3.1 Female Conflict Behaviours

The three female medaka conflict behaviour frequencies were compared across increasingly male-biased OSRs of 0.5, 1, 2, and 5. Conflict behaviour consisted of: female refusal, male-female aggression and female-male aggression. The frequency of male-female aggression (Figure 3.1A) and female refusal (Figure 3.1B) increased with increasing OSR (Table 3.1). By contrast, when OSR increased, female-male aggression decreased, however when analyzed, there was no significance of the OSR on this behaviour (Figure 3.1C, Table 3.1). The predictor that best explained female's refusal was the OSR (Table 3.1). For male-female aggression, the predictor that held the most weight for the behaviour throughout the observations was the effect of OSR (Table 3.1).

Additionally, female body size was assessed as a potential predictor of conflict behaviour; larger females tended to exhibit higher refusal rates and were more aggressive toward males than were smaller females (Table 3.1, Table 3.2). To assess how body size was being influenced on these two behaviours, male-female aggression and female refusal, the OSR was divided per ratio in the experiment (Figure 3.2, Figure 3.3). For male-female aggression, the predictor of body size best explained the OSR 0.5, 1, and 5 (Table 3.2). Also, this behaviour shows an increasing trend for OSR 0.5 and 1, and a decreasing trend for OSR 2 and 5 (Figure 3.2). For female refusal, the predictor of body size best explained the OSR of 0.5 (Table 3.2). There is an increasing trend for this OSR in regard to the increasing body size (Figure 3.3A).

Table 3.1. Model selection of the effects of OSR, body size, and intercepts on the conflict behaviour frequencies: male-female aggression, female refusal, and female-male aggression. Female refusal and male-female aggression were assessed using linear mixed effects models, while female-male aggression was assessed using a glm without the effect of body size due to singularity in the data. Below are the degrees of freedom (df) for the predictors, Akaike Information Criterion corrected for small sample sizes (AICc), the variance between the lowest AICc value predictor compared to all other predictors ( $\Delta$ AICc), and models weight. The best models are shown in bold and differ from other models by a  $\Delta$ AICc value of 2 or more.

Behaviour	Predictor	df	AICc	$\Delta$ AICc	Weight
Male-female aggression	<b>OSR</b>	<b>6</b>	<b>378.3</b>	<b>0</b>	<b>0.46</b>
	<b>Intercept</b>	<b>3</b>	<b>379.9</b>	<b>1.66</b>	<b>0.2</b>
	<b>OSR + Body size</b>	<b>7</b>	<b>380.1</b>	<b>1.84</b>	<b>0.183</b>
	Body size	4	380.8	2.55	0.128
	OSR + Body size x OSR + Body size	10	383.9	5.63	0.028
Female refusal	<b>OSR</b>	<b>6</b>	<b>341.5</b>	<b>0</b>	<b>0.688</b>
	<b>OSR + Body size</b>	<b>7</b>	<b>343.1</b>	<b>1.69</b>	<b>0.295</b>
	OSR + Body size x OSR + Body size	10	348.9	7.44	0.017
	Body size	4	368.5	27.06	0
	Intercept	3	368.6	27.18	0
Female-male aggression	<b>Intercept</b>	<b>2</b>	<b>-47.6</b>	<b>0</b>	<b>0.943</b>
	OSR	5	-42	5.6	0.057

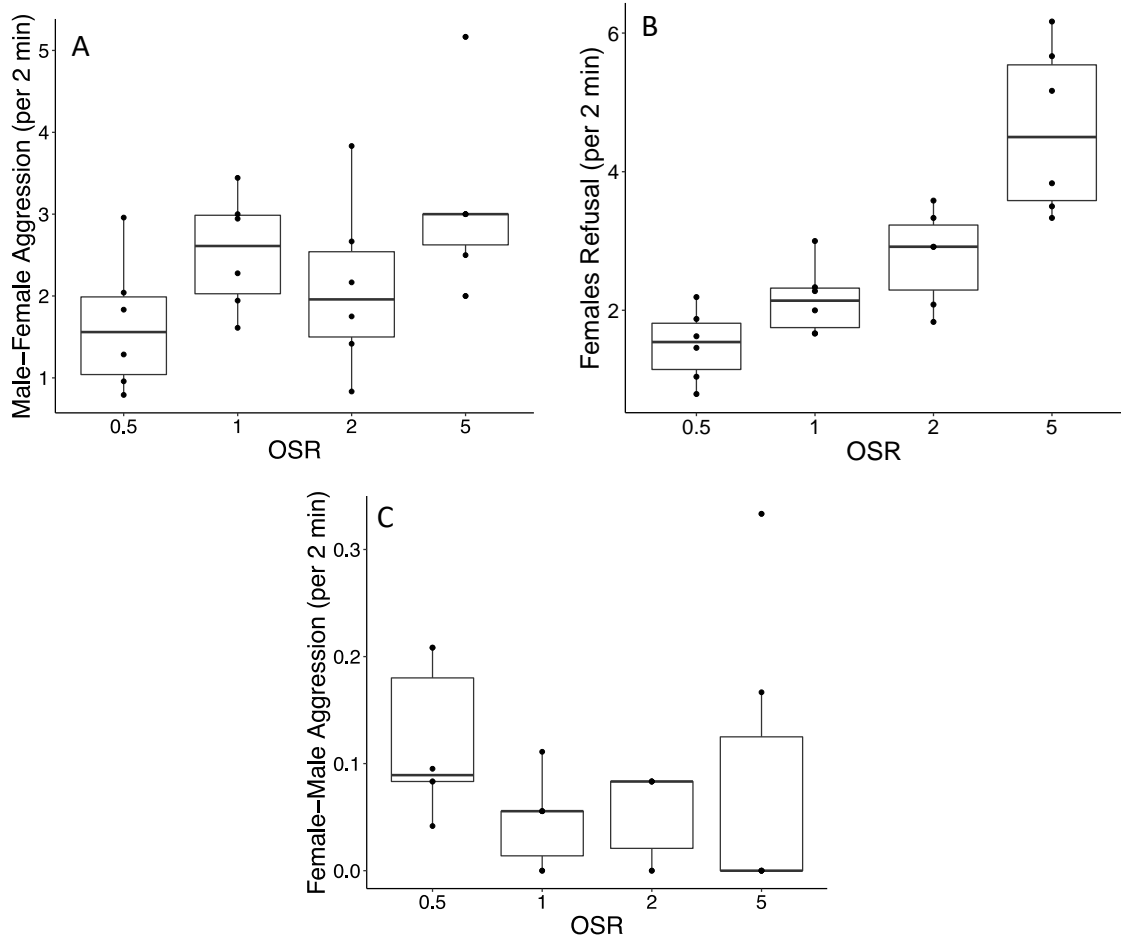


Figure 3.1. Conflict behaviours across operational sex ratio. (A) the average rate of male aggression towards females. (B) the average rate of females' refusal. (C) the average rate of females' aggression towards males.

Table 3.2. Model selection of the effects of body size, and intercepts on the conflict behaviour frequencies: male-female aggression and female refusal. These behaviours were assessed using linear mixed effects models. Below are the degrees of freedom (df) for the predictors, Akaike Information Criterion corrected for small sample sizes (AICc), the variance between the lowest AICc value predictor compared to all other predictors ( $\Delta$ AICc), and models weight. The best models are shown in bold and differ from other models by a  $\Delta$ AICc value of 2 or more.

Behaviour	OSR	Predictor	df	AICc	$\Delta$ AICc	Weight	
Male-Female Aggression	0.5	<b>Intercept</b>	<b>2</b>	<b>153.1</b>	<b>0</b>	<b>0.702</b>	
		<b>Body Size</b>	<b>3</b>	<b>154.8</b>	<b>1.71</b>	<b>0.298</b>	
	1	<b>Intercept</b>	<b>2</b>	<b>121.8</b>	<b>0</b>	<b>0.708</b>	
		<b>Body Size</b>	<b>3</b>	<b>123.5</b>	<b>1.77</b>	<b>0.292</b>	
	2	<b>Intercept</b>	<b>2</b>	<b>83.1</b>	<b>0</b>	<b>0.734</b>	
		Body Size	3	85.1	2.03	0.266	
	5	<b>Intercept</b>	<b>2</b>	<b>40.7</b>	<b>0</b>	<b>0.669</b>	
		<b>Body Size</b>	<b>3</b>	<b>42.1</b>	<b>1.41</b>	<b>0.331</b>	
	Female Refusal	0.5	<b>Intercept</b>	<b>2</b>	<b>127.9</b>	<b>0</b>	<b>0.615</b>
			<b>Body Size</b>	<b>3</b>	<b>128.8</b>	<b>0.94</b>	<b>0.385</b>
1		<b>Intercept</b>	<b>2</b>	<b>100.9</b>	<b>0</b>	<b>0.767</b>	
		Body Size	3	103.3	2.38	0.233	
2		<b>Intercept</b>	<b>2</b>	<b>76.9</b>	<b>0</b>	<b>0.753</b>	
		Body Size	3	79.1	2.23	0.247	
5		<b>Intercept</b>	<b>2</b>	<b>44.1</b>	<b>0</b>	<b>0.861</b>	
		Body Size	3	47.7	3.64	0.139	

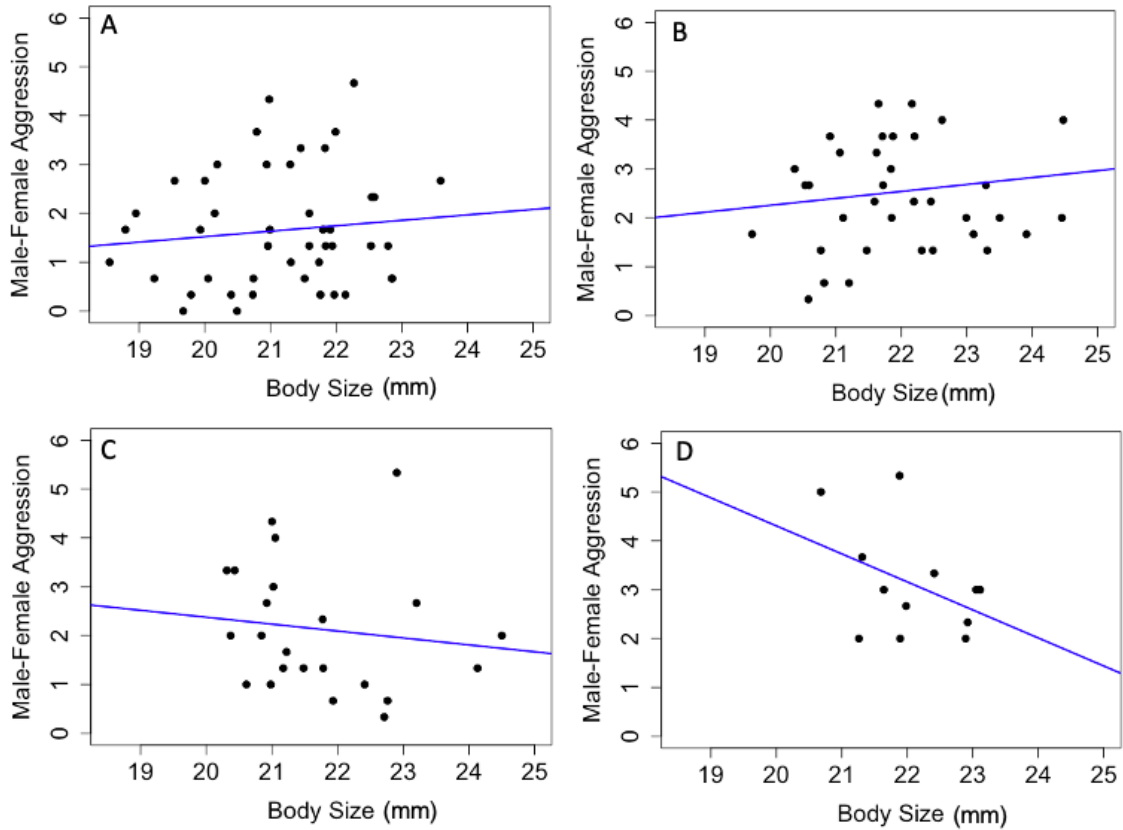


Figure 3.2. Female body size versus the average rate of male-female aggressive behaviour for the subset data. The line of best fit is indicated in blue. (A) the average rate of OSR 0.5 with an increasing trend. (B) the average rate of OSR 1 with an increasing trend. (C) average rate of OSR 2 with a decreasing trend. (D) average rate of OSR 5 with a decreasing trend.



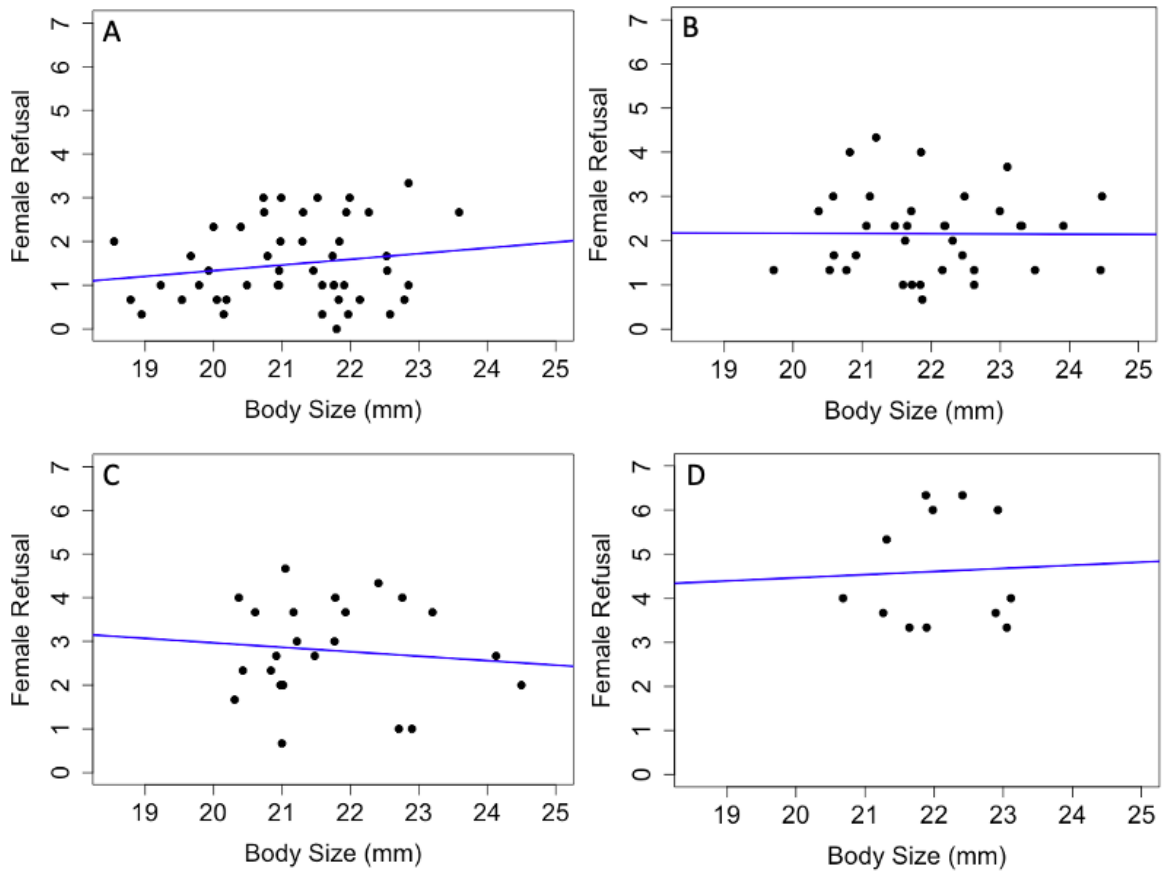


Figure 3.3. Female body size versus the average rate of female refusal behaviour for the subset data. The line of best fit is represented in blue. (A) the average rate of OSR 0.5 with an increasing trend. (B) the average rate of OSR 1 with no trend. (C) average rate of OSR 2 with a slight decrease. (D) average rate of OSR 5 with a slight increase

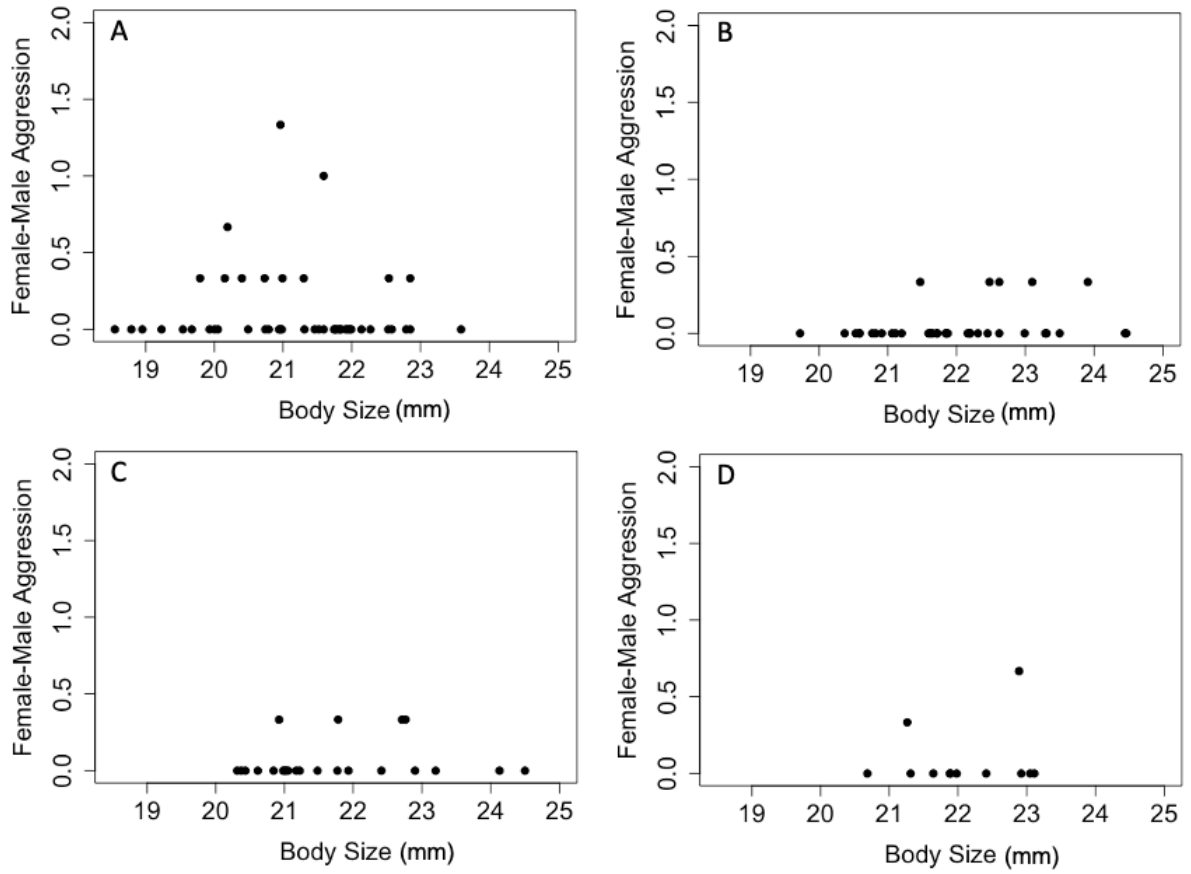


Figure 3.4. Female body size versus the average rate of female-male aggressive behaviour for the subset data. (A) the average rate of OSR 0.5. (B) the average rate of OSR 1. (C) average rate of OSR 2. (D) average rate of OSR 5.

### ***3.2 Female Competition Behaviour***

The three female medaka competition behaviour frequencies were compared across increasingly male-biased OSRs of 0.5, 1, 2, and 5. Competition behaviours consisted of: female receiving aggression and female initiating, and interruptions. The frequency of females receiving aggression (Figure 3.5A) and females initiating aggression (Figure 3.5B) decreased with increasing OSR (Table 3.2). Interruption was only recorded once throughout the experiment, so analysis wasn't considered for this behaviour. The predictor that best explained females initiating aggression was the OSR, as this behaviour was influenced by the increasing OSR (Table 3.2). For females receiving aggression, the predictor was also best explained by the effects of the increasing OSR and there was a slight effect by the intercept as well (Table 3.2).

Additionally, female body size was assessed as a potential predictor of competition behaviour. Neither the female initiating aggression or female receiving aggression behaviours were affected by the predictor body size (Table 3.2, Figure 3.6, Figure 3.7).

Table 3.3. Model selection of the effects of OSR, body size, and intercepts on the competition behaviour frequencies: female initiating aggression and female receiving aggression. These competition behaviours were assessed using linear mixed effects models. Below are the degrees of freedom (df) for the predictors, Akaike Information Criterion corrected for small sample sizes (AICc), the variance between the lowest AICc value predictor compared to all other predictors ( $\Delta$ AICc), and models weight. The best models are shown in bold and differ from other models by a  $\Delta$ AICc value of 2 or more.

Behaviour	Predictor	df	AICc	$\Delta$ AICc	Weight
Female initiating aggression	<b>OSR</b>	<b>6</b>	<b>112.5</b>	<b>0</b>	<b>0.399</b>
	<b>Intercept</b>	<b>3</b>	<b>112.8</b>	<b>0.32</b>	<b>0.34</b>
	OSR + Body size	7	114.7	2.18	0.134
	Body size	4	114.9	2.37	0.122
	OSR + Body size x OSR + Body size	10	121.4	8.92	0.005
Female receiving aggression	<b>OSR</b>	<b>6</b>	<b>22.3</b>	<b>0</b>	<b>0.453</b>
	<b>Intercept</b>	<b>3</b>	<b>23.3</b>	<b>0.98</b>	<b>0.278</b>
	OSR + Body size	7	24.4	2.12	0.157
	Body size	4	25.4	3.11	0.096
	OSR + Body size x OSR + Body size	10	29	6.68	0.016

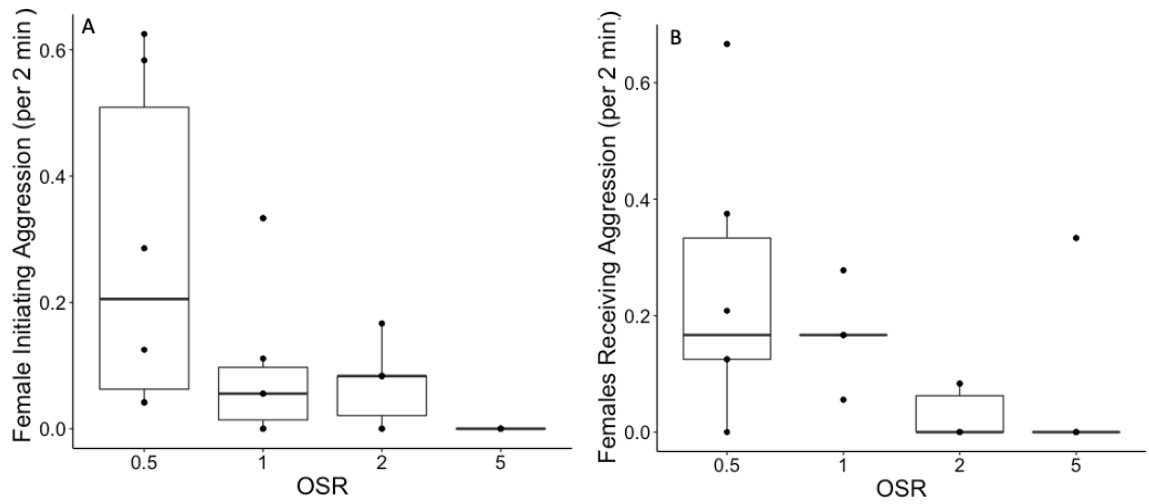


Figure 3.5. Competition behaviours across operational sex ratio. (A) the average rate of female initiating aggression towards females. (B) the average rate of female receiving aggression.

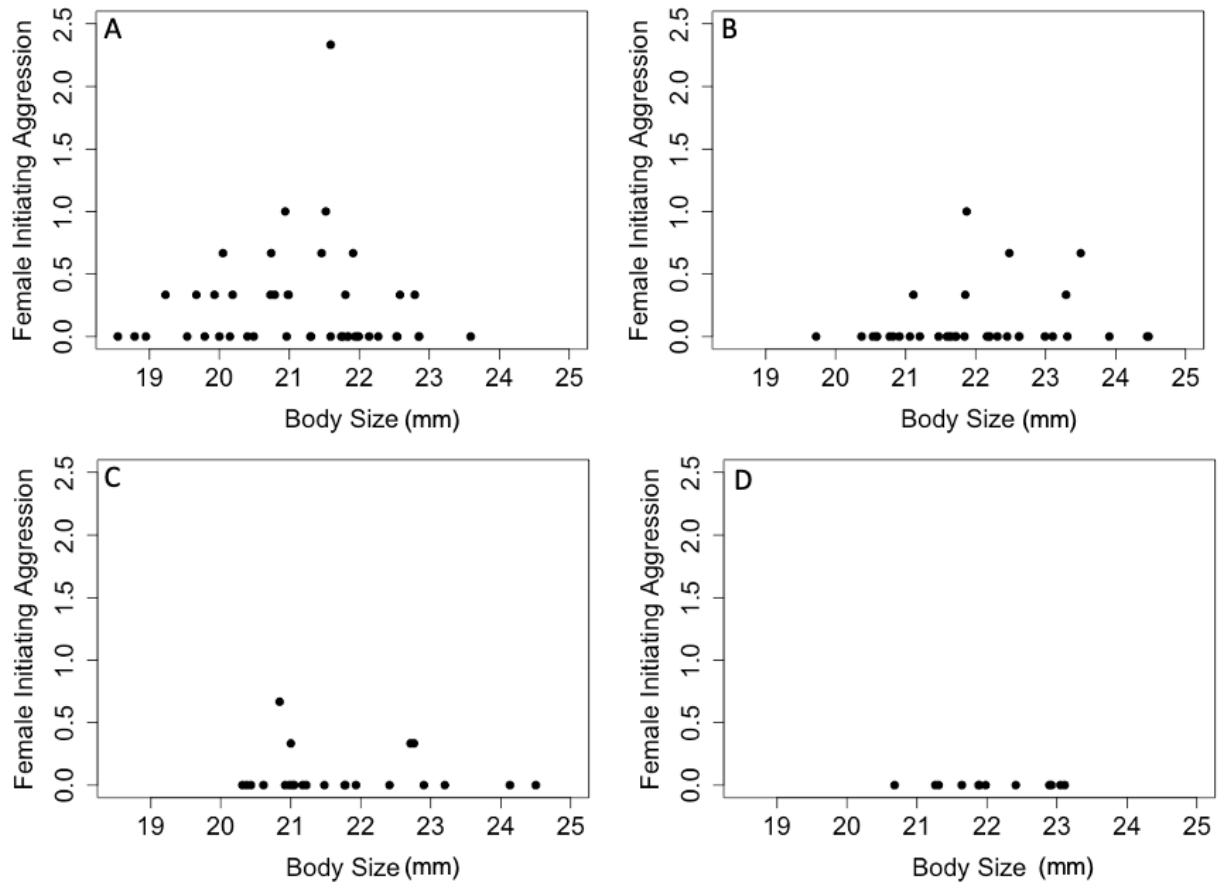


Figure 3.6. Female body size versus the average rate of female initiating aggressive behaviour for the subset data. (A) the average rate of OSR 0.5. (B) the average rate of OSR 1. (C) average rate of OSR 2. (D) average rate of OSR 5.

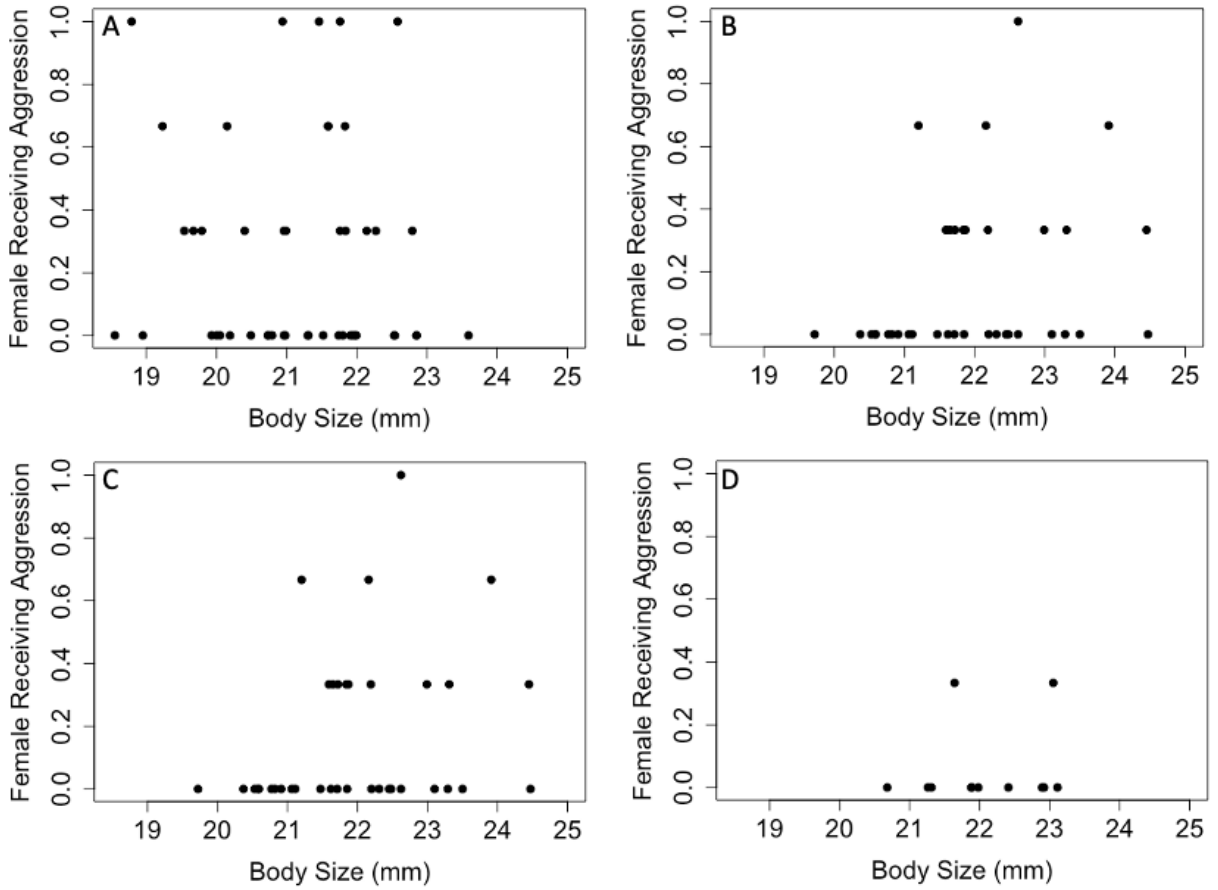


Figure 3.7. Female body size versus the average rate of females receiving aggression behaviour for the subset data. (A) the average rate of OSR 0.5. (B) the average rate of OSR 1. (C) average rate of OSR 2. (D) average rate of OSR 5.

## **4. Discussion**

The main objective of this study was to quantify the relationship between OSR and female Japanese medaka mating behaviour. The frequency of conflict and competition behaviours can potentially be due to the difference in the number of mates and competitors within the specific OSR treatments, which can alter the number and intensity of inter- (Clark & Grant, 2010; Grant et al., 2000; Weir, Grant, & Hutchings, 2011) and intrasexual (Clark & Grant, 2010) encounters. I predicted that conflict behaviours would increase with increasingly male-biased OSR due to an increased number of interactions with males. In support of my prediction, I found that both female refusal and male-female aggression increased with increasingly male-biased OSR. In both of these cases, body size also influenced the rate of behaviour. By contrast, I predicted that competition behaviours would decrease when OSR changed from female- to male-biased, as intrasexual competition among females would decline. Two of the competition behaviours (female receiving aggression from females and female initiating aggression to females), decreased with increasing OSR. There was no effect of body size on either of these competition behaviours.

### ***4.1 Conflict***

Female refusal and male-female aggression increased with increasing OSR. However, female-male aggression was not influenced by the OSR. An OSR of 0.5 can mean that females are not being constantly courted, but instead have to try and be courted so they would not be showing as much aggressive behaviour to the males. These findings are consistent with those of Clark and Grant (2010), who found that the changes in reproductive behaviour within sexes can be explained by encounter rates with members of



the same and opposite sex. As the OSR increased, the density of opposite-sex individuals increased the rate of courtship behaviour by both males and females. Therefore, for male-biased OSRs, a male's propensity to court females (i.e., proportion of encountered females that are courted (Forsgren et al., 2004)) will likely increase (Weir et al., 2011). Allen (2019), reported that successful male courtship rates for two generations of male Japanese medakas decreased with an increasing OSR. Based on this finding, when the males are unsuccessful in their courting attempts, females would be showing more refusals when the OSR is male-biased (Clark & Grant, 2010; Kvarnemo & Ahnesjö, 1996). In addition, rejection by female can occur if she is unable to mate with any male due to her spawning event being complete for that day or because the female is reluctant to mate with that particular male (Grant & Green, 1996).

Along with the OSR, female body size had an effect on two of the conflict behaviours: females refusal and male-female aggression. I predicted that for each of the conflict behaviours, larger body size would be associated with higher conflict rates due to the males preferring larger body size of females. Because there was an interaction between the overall OSRs and body size (Table 3.1), the OSRs were separated to determine which individual sex ratio was being influenced by body size. For three of the OSRs, 0.5, 1, and 5, body size significantly influenced male-female aggression (Table 3.2). For these three OSRs, there is an increasing trend for the ratios 0.5 and 1, but a decreasing trend for the ratio of 5 (Figure 3.2). The decreasing trend could be due to the lower sample size in the OSR 5 as there are only two females in the tank, compared to the higher number of females in the other ratios. For female refusal, refusal increased with body size only at OSR 0.5 (Table 3.2, Figure 3.3). The highest sample size is present in

OSR 0.5 as it contains the highest ratio of females to males, this higher sample size may have more power to detect patterns within that OSR.

Female fecundity is tightly linked to size in most fishes, because larger females typically can carry more eggs (Helfman, Collette, Facey, & Bowen, 2009). Almost all studies that looked at male preferences for size did find a preference for larger females (e.g. Dosen & Montgomerie, 2004; Grant, Bryant, et al., 1995). Grant et al. (1995) had an experimental set-up that showed males spending more time in the zone near the large female than in that near the small female in 19 of 25 trials. When the male was allowed to interact with both females, the males spent more time with the large rather than the small female in 22 of 23 trials (Grant, Casey, et al., 1995). In other species, preference of larger body size was also shown: Dosen & Montgomerie (2004) studied male preferences for female guppies (*Poecilia reticulata*), they found that when males were simultaneously presented with two females of unequal size, they spent significantly more time associating with the larger female. Also, male three-spined sticklebacks, *Gasterosteus aculeatus* preferred larger females (Rowland, 1982). Because males have a preference for larger body size, they will continuously court these females more often, thus causing higher conflict behaviour from the females.

#### **4.2 Competition**

When increasing the OSR, both females receiving and initiating aggression decreased. The interruption behaviour was only recorded once throughout the whole experiment, therefore there was insufficient data and could not be addressed in analysis. Interruption can potentially be explained as a rare behaviour for females as Jirotkul (1999) found that

male guppies (*Poecilia reticulata*), engaged in more interruptive behaviours than females, indicating that this behaviour occurs more from males than it does from females and that is why it was not observed. For the other two competition behaviours, my findings are congruent with those of Kvarnemo & Ahesjö (1996), Grant et al. (2000), Grant & Foam (2002), and Grant and Clark (2010) as they found the proportion of female-female competitions increased with the degree of female-biased OSR. However, when switching from a female-biased OSR to male-biased, female intrasexual interactions decreased. In populations with a biased OSR, the mate-limited sex is expected to compete more for access to mates and to affect sexual selection, mainly via effects on same-sex competitive interactions (Kokko & Rankin, 2006). Overall, the availability of mates influences female–female competitive interactions in species with standard and reversed sex roles (Darwin, 1871; Rosvall, 2011; Trivers, 1972). When males are limited in number, the frequency and intensity of female–female competitive interactions are expected to increase. In addition, aggressive behaviors also increase when females compete for high-quality mates that provide either direct benefits or indirect benefits (Rosvall, 2011).

The two competition behaviours, females receiving aggression and females initiating aggression, were not affected by body size. I predicted that the larger females would be more competitive as they would be dominant over smaller females. However, females rarely showed aggression; males participated in aggression more frequently than females. As the OSR shifted from female-biased to male-biased, the two competition behaviours decreased. This decrease could be explained by the lower number of females in the tank, as a result the females would not have to compete against one another. Another reason why body size was not a factor could be due to the biased OSRs. There

was only one OSR that was female biased, one that had even number of males and females, and 2 that were male-biased. If there were more sex ratios that showed female biased, body size could have potentially been a factor, but having only one female-biased OSR led to females not having to compete against one another.

#### ***4.4 Conclusion***

The operational sex ratio can influence both conflict and competition behaviours of Japanese medaka. A biased OSR can predict which sex will compete for access to mates and how intense the competition will be. As the OSR becomes unequal, more-intense mating competition is predicted, and the sex that is in excess, predicted to become the predominant competitor for access to mating partners, will be under stronger sexual selection (Kvarnemo & Ahnesjö, 1996). Not only can changes in the OSR alter the intensity of competition for mates, but the body size of the female can as well. The body size of females can make her more appealing to males as conflict behaviours were influenced by body size. Multiple studies, along with mine, found males courting larger females more often than smaller. This led to more conflict behaviour by larger females as they were rejecting the constant courtship attempts. My results suggest that the conflict and competition behaviour of females are just as interesting as those of males. Further studies need to include not only males, but females. These studies should specifically look at how the presence and absence of eggs can affect sexual conflict and selection. The ability to compare male and female based studies allow researchers to expand on the topic of mating systems to further research on developmental evolutionary studies.

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## Appendix A



Table A. Raw data that was collected during the observational period. Data collection includes the three rounds, the tank number and shelf number, the female identification, the six-behaviour observed, the presence of eggs, the operational sex ratio the fish belong too, and the body size of the female. The following behaviour are extended based on their abbreviations: FFA is female initiating aggression, FMA is female-male aggression. CBM is male-female aggression, and CBF females receiving aggression.

Round	Tank/ Shelf	Female	Refusal	Interrupt	FFA	FMA	CBM	CBF	Eggs	OSR	Body
1	5B	123	1	0	1	1	1	1	Y	0.5	20.99
1	5B	122	3	0	0	0	1	0	Y	0.5	20.96
1	5B	132	4	0	0	0	0	0	Y	0.5	20
1	5B	121	1	0	1	0	1	0	Y	0.5	19.23
1	5B	125	0	0	1	0	0	1	Y	0.5	19.67
1	5B	126	2	0	0	0	4	0	Y	0.5	20.94
1	5B	128	2	0	0	0	2	0	Y	0.5	20.98
1	5B	124	0	0	0	0	1	0	Y	0.5	19.54
1	6B	221	0	0	0	0	0	0	Y	1	20.77
1	6B	218	3	0	0	0	5	0	Y	1	21.62
1	6B	219	2	0	0	0	2	0	Y	1	20.37
1	6B	222	0	0	0	0	2	0	Y	1	21.72
1	6B	217	3	0	0	0	5	0	Y	1	21.11
1	6B	220	0	0	0	0	0	0	Y	1	22.31
1	7B	254	1	0	0	0	1	0	Y	2	24.13
1	7B	256	4	0	0	0	0	0	Y	2	22.76
1	7B	253	1	0	0	0	2	0	Y	2	21.77
1	7B	255	3	0	0	0	1	0	Y	2	21.17
1	8B	265	7	0	0	0	0	0	Y	5	22.41
1	8B	266	4	0	0	0	1	0	Y	5	21.98
1	17E	181	8	0	0	0	1	0	Y	5	21.88
1	17E	182	5	0	0	0	6	0	Y	5	20.68
1	18E	242	0	0	0	0	5	0	Y	1	22.16
1	18E	241	3	0	0	0	3	0	Y	1	22.45
1	18E	243	3	0	0	0	1	0	Y	1	23.29
1	18E	245	2	0	2	1	0	0	N	1	22.48
1	18E	246	2	0	0	0	7	0	Y	1	22.62
1	18E	244	0	0	3	0	2	0	Y	1	21.87
1	19E	39	3	0	2	0	0	0	Y	2	20.84
1	19E	37	0	0	0	0	0	0	Y	2	25.69
Round	Tank/ Shelf	Female	Refusal	Interrupt	FFA	FMA	CBM	CBF	Eggs	OSR	Body
1	19E	40	3	0	0	0	1	0	Y	2	20.98

1	20E	158	3	0	0	0	0	0	N	0.5	22.53
1	20E	161	1	0	0	0	1	0	Y	0.5	20.05
1	20E	164	1	0	0	0	1	1	Y	0.5	22.14
1	20E	157	1	0	1	0	0	0	Y	0.5	22.79
1	20E	159	1	0	0	0	0	0	Y	0.5	21.97
1	20E	160	3	0	0	0	0	0	Y	0.5	20.49
1	20E	163	1	0	0	0	1	1	Y	0.5	19.79
1	20E	162	0	0	0	0	1	0	Y	0.5	18.95
1	13D	97	5	0	0	0	3	0	N	2	23.2
1	13D	100	2	0	0	0	4	1	N	2	21.02
1	13D	98	1	0	0	0	3	0	N	2	22.9
1	13D	99	1	0	0	0	4	0	N	2	21
1	14D	62	1	1	0	0	3	1	N	5	23.05
1	14D	61	3	0	0	0	2	1	N	5	21.64
1	15D	54	3	0	0	0	0	0	N	0.5	20.74
1	15D	56	2	0	0	0	0	0	N	0.5	18.55
1	15D	52	2	0	0	0	3	0	N	0.5	21.3
1	15D	50	1	0	1	0	2	0	N	0.5	19.93
1	15D	49	1	0	0	0	0	0	N	0.5	22.85
1	15D	55	1	0	2	0	0	0	Y	0.5	21.91
1	15D	51	2	0	0	0	0	0	N	0.5	20.73
1	16D	28	5	0	0	0	2	1	N	1	22.99
1	16D	25	4	0	0	0	2	0	N	1	24.45
1	16D	26	1	0	0	0	2	1	Y	1	23.31
1	16D	30	6	0	0	0	1	0	N	1	20.82
1	16D	27	2	0	2	0	3	0	N	1	23.5
1	16D	29	4	0	0	1	2	0	Y	1	23.91
1	1A	113	2	0	0	0	2	0	Y	1	19.72
1	1A	112	4	0	0	0	2	2	N	1	21.2
1	1A	114	1	0	0	0	2	0	Y	1	21.47
1	1A	110	2	0	0	0	5	1	Y	1	21.65
1	1A	109	6	0	0	0	2	0	Y	1	21.85
1	1A	111	6	0	0	0	0	0	Y	1	23.1
1	2A	134	3	0	0	0	0	0	Y	5	21.26
1	2A	133	3	0	0	0	0	0	Y	5	21.89
1	3A	200	3	0	0	0	2	0	N	0.5	21.94
1	3A	193	1	0	0	0	3	1	Y	0.5	21.83
<b>Round</b>	<b>Tank/ Shelf</b>	<b>Female</b>	<b>Refusal</b>	<b>Interrupt</b>	<b>FFA</b>	<b>FMA</b>	<b>CBM</b>	<b>CBF</b>	<b>Eggs</b>	<b>OSR</b>	<b>Body</b>
1	3A	199	0	0	0	1	0	2	Y	0.5	20.15

1	3A	194	3	0	0	0	3	0	Y	0.5	23.59
1	3A	195	6	0	0	0	6	0	Y	0.5	22.27
1	3A	198	0	0	0	0	5	0	Y	0.5	20.79
1	3A	197	4	0	0	0	2	0	N	0.5	21.99
1	4A	172	4	0	0	0	1	0	Y	2	21.78
1	4A	169	4	0	0	0	0	0	Y	2	20.31
1	4A	170	8	0	0	0	7	0	N	2	21.05
1	4A	171	4	0	0	0	1	0	Y	2	20.37
1	21F	229	2	0	0	0	1	0	N	2	22.71
1	21F	232	2	0	0	0	3	0	N	2	22.41
1	21F	231	2	0	0	0	4	0	N	2	21.48
1	21F	230	3	0	0	0	0	0	N	2	21.93
1	22F	146	0	0	0	0	1	1	N	1	22.19
1	22F	149	2	0	0	0	2	0	N	1	22.2
1	22F	148	1	0	0	0	0	0	N	1	20.59
1	22F	150	0	0	0	0	2	0	N	1	21.59
1	22F	147	1	0	0	0	2	0	N	1	20.91
1	22F	145	1	0	0	0	1	1	N	1	21.84
1	23F	20	2	0	0	0	2	0	N	0.5	20.96
1	23F	13	2	0	0	1	1	0	N	0.5	22.85
1	23F	14	0	0	0	0	0	1	N	0.5	21.76
1	23F	17	3	0	0	0	1	1	N	0.5	21.84
1	23F	19	0	0	0	0	3	0	N	0.5	21.8
1	23F	15	4	0	0	0	1	0	N	0.5	21.31
1	23F	18	0	0	0	0	0	0	N	0.5	21.74
1	23F	16	3	0	0	0	0	0	N	0.5	20.4
1	24F	206	5	0	0	0	4	0	Y	5	23.11
1	24F	205	6	0	0	1	3	0	Y	5	22.89
1	9C	2	7	0	0	0	3	0	N	5	22.92
1	9C	1	4	0	0	0	7	0	N	5	21.31
1	10C	76	3	0	0	0	0	0	N	2	20.61
1	10C	73	3	0	0	0	2	0	N	2	21.22
1	10C	75	2	0	0	1	4	0	Y	2	20.92
1	10C	74	4	0	0	0	3	0	N	2	20.43
1	11C	86	6	0	0	0	1	0	N	1	24.47
1	11C	88	0	0	0	1	4	3	N	1	22.62
1	11C	89	2	0	0	0	4	0	Y	1	21.06
<b>Round</b>	<b>Tank/ Shelf</b>	<b>Female</b>	<b>Refusal</b>	<b>Interrupt</b>	<b>FFA</b>	<b>FMA</b>	<b>CBM</b>	<b>CBF</b>	<b>Eggs</b>	<b>OSR</b>	<b>Body</b>
1	11C	85	2	0	0	0	0	0	N	1	20.58

1	11C	87	4	0	0	0	3	0	N	1	21.71
1	12C	281	0	0	0	0	1	1	Y	0.5	21.59
1	12C	278	0	0	0	0	2	0	Y	0.5	22.58
1	12C	279	1	0	0	0	2	0	N	0.5	20.19
1	12C	284	4	0	2	0	0	0	N	0.5	21.52
1	12C	280	2	0	2	0	3	0	Y	0.5	21.46
1	12C	277	0	0	6	2	2	0	Y	0.5	21.59
1	12C	283	0	0	0	0	2	2	Y	0.5	18.79
1	12C	282	2	0	0	0	1	2	Y	0.5	21.76
2	21F	231	3	0	0	0	0	0	Y	2	21.48
2	21F	229	1	0	0	0	0	0	N	2	22.71
2	21F	232	7	0	0	0	0	0	Y	2	22.41
2	21F	230	4	0	0	0	1	0	Y	2	21.93
2	22F	147	2	0	0	0	4	0	Y	1	20.91
2	22F	146	5	0	0	0	6	0	Y	1	22.19
2	22F	150	0	0	0	0	2	0	N	1	21.59
2	22F	148	3	0	0	0	2	0	Y	1	20.59
2	22F	145	1	0	0	0	2	0	Y	1	21.84
2	22F	149	2	0	0	0	7	0	Y	1	22.2
2	23F	14	2	0	0	0	0	0	Y	0.5	21.76
2	23F	19	0	0	1	0	0	0	Y	0.5	21.8
2	23F	18	4	0	0	0	2	0	N	0.5	21.74
2	23F	17	2	0	0	0	2	0	Y	0.5	21.84
2	23F	16	4	0	0	0	1	1	Y	0.5	20.4
2	23F	13	1	0	0	0	1	0	Y	0.5	22.85
2	23F	15	2	0	0	0	2	0	N	0.5	21.31
2	23F	20	1	0	0	0	0	0	Y	0.5	20.96
2	24F	206	3	0	0	0	4	0	N	5	23.11
2	24F	205	1	0	0	1	0	0	N	5	22.89
2	9C	2	5	0	0	0	2	0	N	5	22.92
2	9C	1	8	0	0	0	1	0	N	5	21.31
2	10C	74	2	0	0	0	3	0	Y	2	20.43
2	10C	75	5	0	0	0	2	0	Y	2	20.92
2	10C	76	4	0	0	0	2	0	N	2	20.61
2	10C	73	3	0	0	0	1	0	Y	2	21.22
2	11C	90	1	0	0	0	5	0	N	1	20.53
2	11C	85	4	0	0	0	0	0	N	1	20.58
<b>Round</b>	<b>Tank/ Shelf</b>	<b>Female</b>	<b>Refusal</b>	<b>Interrupt</b>	<b>FFA</b>	<b>FMA</b>	<b>CBM</b>	<b>CBF</b>	<b>Eggs</b>	<b>OSR</b>	<b>Body</b>
2	11C	88	0	0	0	0	5	0	N	1	22.62

2	11C	87	2	0	0	0	4	0	N	1	21.71
2	11C	86	2	0	0	0	4	0	Y	1	24.47
2	12C	280	0	0	0	0	2	3	Y	0.5	21.46
2	12C	281	0	0	0	0	2	1	Y	0.5	21.59
2	12C	277	0	0	0	0	0	0	Y	0.5	21.59
2	12C	282	1	0	0	0	0	1	Y	0.5	21.76
2	12C	279	1	0	1	0	1	0	N	0.5	20.19
2	12C	284	4	0	1	0	2	0	N	0.5	21.52
2	12C	278	0	0	0	0	0	3	Y	0.5	22.58
2	12C	283	1	0	0	0	1	1	Y	0.5	18.79
2	13D	100	2	0	0	0	3	0	Y	2	21.02
2	13D	98	0	0	0	0	6	0	Y	2	22.9
2	13D	99	0	0	0	0	3	0	Y	2	21
2	13D	97	5	0	0	0	1	0	N	2	23.2
2	14D	62	4	0	0	0	2	0	Y	5	23.05
2	14D	61	1	0	0	0	1	0	Y	5	21.64
2	15D	51	1	0	1	1	1	0	Y	0.5	20.73
2	15D	52	2	0	0	1	4	0	Y	0.5	21.3
2	15D	56	1	0	0	0	0	0	N	0.5	18.55
2	15D	50	2	0	0	0	1	0	Y	0.5	19.93
2	15D	54	2	0	1	0	1	0	N	0.5	20.74
2	15D	49	4	0	0	0	1	0	N	0.5	22.85
2	15D	55	1	0	0	0	3	0	Y	0.5	21.91
2	16D	30	3	0	0	0	1	0	Y	1	20.82
2	16D	25	0	0	0	0	0	1	Y	1	24.45
2	16D	26	3	0	0	0	0	0	Y	1	23.31
2	16D	29	2	0	0	0	1	2	N	1	23.91
2	16D	28	0	0	0	0	3	0	Y	1	22.99
2	16D	27	1	0	0	0	0	0	Y	1	23.5
2	1A	113	2	0	0	0	3	0	Y	1	19.72
2	1A	109	1	0	1	0	2	0	Y	1	21.85
2	1A	111	0	0	0	0	5	0	Y	1	23.1
2	1A	110	2	0	0	0	2	0	N	1	21.65
2	1A	112	7	0	0	0	0	0	N	1	21.2
2	1A	114	4	0	0	1	1	0	Y	1	21.47
2	2A	133	3	0	0	0	4	0	Y	5	21.89
2	2A	134	4	0	0	1	0	0	N	5	21.26
<b>Round</b>	<b>Tank/ Shelf</b>	<b>Female</b>	<b>Refusal</b>	<b>Interrupt</b>	<b>FFA</b>	<b>FMA</b>	<b>CBM</b>	<b>CBF</b>	<b>Eggs</b>	<b>OSR</b>	<b>Body</b>
2	3A	200	3	0	0	0	2	0	N	0.5	21.94

2	3A	197	2	0	0	0	7	0	N	0.5	21.99
2	3A	193	0	0	0	0	0	0	Y	0.5	21.83
2	3A	198	0	0	1	0	4	0	Y	0.5	20.79
2	3A	196	1	0	0	0	3	0	Y	0.5	22.54
2	3A	194	0	0	0	0	5	0	Y	0.5	23.59
2	4A	195	0	0	0	0	5	0	Y	0.5	22.27
2	4A	171	0	0	0	0	2	0	N	2	20.37
2	4A	172	2	0	0	0	2	0	Y	2	21.78
2	4A	170	2	0	0	0	4	1	Y	2	21.05
2	4A	169	0	0	0	0	4	0	Y	2	20.31
2	5B	124	0	0	0	0	3	0	Y	0.5	19.54
2	5B	125	5	0	0	0	0	0	N	0.5	19.67
2	5B	126	1	0	1	0	3	0	Y	0.5	20.94
2	5B	123	5	0	0	0	2	0	Y	0.5	20.99
2	5B	121	2	0	0	0	0	0	Y	0.5	19.23
2	5B	122	1	0	4	4	1	0	N	0.5	20.96
2	5B	128	4	0	1	0	4	0	Y	0.5	20.98
2	5B	132	2	0	0	0	3	0	Y	0.5	20
2	6B	220	4	0	0	0	4	0	N	1	22.31
2	6B	217	5	0	0	0	0	0	N	1	21.11
2	6B	218	2	0	0	0	2	0	N	1	21.62
2	6B	219	3	0	0	0	5	0	Y	1	20.37
2	6B	221	1	0	0	0	2	0	Y	1	20.77
2	6B	222	2	0	0	0	4	0	N	1	21.72
2	7B	253	3	0	0	0	0	0	N	2	21.77
2	7B	254	5	0	0	0	1	0	N	2	24.13
2	7B	255	4	0	0	0	3	0	N	2	21.17
2	7B	256	4	0	0	0	1	0	N	2	22.76
2	8B	266	7	0	0	0	1	0	Y	5	21.98
2	8B	265	4	0	0	0	2	0	Y	5	22.41
2	17E	182	5	0	0	0	2	0	Y	5	20.68
2	17E	181	6	0	0	0	7	0	Y	5	21.88
2	18E	244	0	0	0	0	5	0	Y	1	21.87
2	18E	242	3	0	0	0	3	2	N	1	22.16
2	18E	245	3	0	0	0	2	0	N	1	22.48
2	18E	241	2	0	0	0	4	0	Y	1	22.45
2	18E	246	1	0	0	0	8	0	Y	1	22.62
<b>Round</b>	<b>Tank/ Shelf</b>	<b>Female</b>	<b>Refusal</b>	<b>Interrupt</b>	<b>FFA</b>	<b>FMA</b>	<b>CBM</b>	<b>CBF</b>	<b>Eggs</b>	<b>OSR</b>	<b>Body</b>
2	19E	38	3	0	0	0	1	0	Y	2	24.5

2	19E	40	0	0	0	0	0	0	N	2	20.98
2	19E	37	3	0	0	0	5	0	N	2	25.69
2	19E	39	1	0	0	0	3	0	N	2	20.84
2	20E	158	1	0	0	0	2	0	Y	0.5	22.53
2	20E	161	1	0	0	0	1	0	Y	0.5	20.05
2	20E	157	0	0	0	0	3	0	Y	0.5	22.79
2	20E	162	0	0	0	0	4	0	Y	0.5	18.95
2	20E	163	1	0	0	1	0	0	Y	0.5	19.79
2	20E	160	0	0	0	0	0	0	N	0.5	20.49
2	20E	164	1	0	0	0	0	0	Y	0.5	22.14
2	20E	159	0	0	0	0	1	0	Y	0.5	21.97
3	17E	181	5	0	0	0	8	0	Y	5	21.88
3	17E	182	2	0	0	0	7	0	Y	5	20.68
3	18E	245	4	0	0	0	2	0	N	1	22.48
3	18E	243	2	0	0	0	3	0	N	1	23.29
3	18E	241	0	0	0	0	0	0	N	1	22.45
3	18E	244	2	0	0	0	4	1	N	1	21.87
3	18E	242	1	0	0	0	5	0	Y	1	22.16
3	18E	246	0	0	0	0	4	0	Y	1	22.62
3	19E	40	3	0	0	0	2	0	N	2	20.98
3	19E	39	3	0	0	0	3	0	Y	2	20.84
3	19E	37	3	0	0	0	1	0	N	2	25.69
3	19E	38	3	0	0	0	3	0	Y	2	24.5
3	20E	159	0	0	0	0	0	0	N	0.5	21.97
3	20E	157	1	0	0	0	1	1	Y	0.5	22.79
3	20E	163	1	0	0	0	0	0	Y	0.5	19.79
3	20E	164	0	0	0	0	0	0	Y	0.5	22.14
3	20E	161	0	0	2	0	0	0	Y	0.5	20.05
3	20E	158	1	0	0	0	2	0	Y	0.5	22.53
3	20E	160	0	0	0	0	0	0	N	0.5	20.49
3	20E	162	1	0	0	0	1	0	Y	0.5	18.95
3	9C	1	4	0	0	0	3	0	N	5	21.31
3	9C	2	6	0	0	0	2	0	N	5	22.92
3	10C	75	1	0	0	0	2	0	Y	2	20.92
3	10C	73	3	0	0	0	2	0	Y	2	21.22
3	10C	76	4	0	0	0	1	0	Y	2	20.61
3	10C	74	1	0	0	0	4	0	Y	2	20.43
<b>Round</b>	<b>Tank/ Shelf</b>	<b>Female</b>	<b>Refusal</b>	<b>Interrupt</b>	<b>FFA</b>	<b>FMA</b>	<b>CBM</b>	<b>CBF</b>	<b>Eggs</b>	<b>OSR</b>	<b>Body</b>
3	11C	88	4	0	0	0	3	0	N	1	22.62

3	11C	87	2	0	0	0	4	0	Y	1	21.71
3	11C	90	1	0	0	0	2	0	N	1	20.53
3	11C	85	3	0	0	0	1	0	N	1	20.58
3	11C	89	3	0	0	0	1	0	Y	1	21.06
3	12C	284	1	0	0	0	0	0	N	0.5	21.52
3	12C	282	0	0	0	0	0	0	Y	0.5	21.76
3	12C	281	1	0	0	0	3	0	Y	0.5	21.59
3	12C	277	3	0	1	1	2	2	N	0.5	21.59
3	12C	279	0	0	0	2	6	0	Y	0.5	20.19
3	12C	278	1	0	1	0	5	0	Y	0.5	22.58
3	12C	280	2	0	0	0	5	0	N	0.5	21.46
3	12C	283	1	0	0	0	2	0	Y	0.5	18.79
3	21F	230	4	0	0	0	1	0	N	2	21.93
3	21F	229	0	0	1	1	0	0	Y	2	22.71
3	21F	232	4	0	0	0	0	0	N	2	22.41
3	21F	231	3	0	0	0	0	0	Y	2	21.48
3	22F	150	3	0	0	0	3	1	N	1	21.59
3	22F	148	1	0	0	0	6	0	N	1	20.59
3	22F	146	2	0	0	0	0	0	N	1	22.19
3	22F	147	2	0	0	0	5	0	Y	1	20.91
3	22F	149	3	0	0	0	2	0	N	1	22.2
3	22F	145	1	0	0	0	6	0	N	1	21.84
3	23F	19	0	0	0	0	2	0	N	0.5	21.8
3	23F	17	1	0	0	0	1	0	N	0.5	21.84
3	23F	20	0	0	0	0	2	0	N	0.5	20.96
3	23F	18	1	0	0	0	1	0	N	0.5	21.74
3	23F	14	1	0	0	0	1	0	N	0.5	21.76
3	23F	13	0	0	0	0	0	0	N	0.5	22.85
3	23F	15	2	0	0	0	0	0	N	0.5	21.31
3	23F	16	0	0	0	1	0	0	N	0.5	20.4
3	24F	205	4	0	0	0	3	0	N	5	22.89
3	24F	206	4	0	0	0	1	0	N	5	23.11
3	5B	123	3	0	0	0	2	0	N	0.5	20.99
3	5B	125	0	0	0	0	0	0	N	0.5	19.67
3	5B	126	0	0	2	0	2	3	Y	0.5	20.94
3	5B	128	0	0	0	0	7	0	N	0.5	20.98
3	5B	122	0	0	4	0	2	1	N	0.5	20.96
<b>Round</b>	<b>Tank/ Shelf</b>	<b>Female</b>	<b>Refusal</b>	<b>Interrupt</b>	<b>FFA</b>	<b>FMA</b>	<b>CBM</b>	<b>CBF</b>	<b>Eggs</b>	<b>OSR</b>	<b>Body</b>
3	5B	121	0	0	0	0	1	2	N	0.5	19.23



3	5B	124	2	0	0	0	4	1	N	0.5	19.54
3	6B	222	1	0	0	0	2	1	N	1	21.72
3	6B	219	3	0	0	0	2	0	N	1	20.37
3	6B	218	1	0	0	0	3	0	N	1	21.62
3	6B	217	1	0	1	0	1	0	Y	1	21.11
3	6B	221	3	0	0	0	2	0	N	1	20.77
3	6B	220	2	0	0	0	0	0	N	1	22.31
3	7B	256	4	0	1	1	1	0	N	2	22.76
3	7B	254	2	0	0	0	2	0	N	2	24.13
3	7B	253	5	0	0	0	5	0	N	2	21.77
3	7B	255	4	0	0	0	0	0	N	2	21.17
3	8B	266	7	0	0	0	6	0	Y	5	21.98
3	8B	265	8	0	0	0	8	0	Y	5	22.41
3	13D	99	1	0	1	0	6	0	Y	2	21
3	13D	98	2	0	0	0	7	0	Y	2	22.9
3	13D	100	2	0	0	0	2	0	Y	2	21.02
3	13D	97	1	0	0	0	4	0	N	2	23.2
3	14D	62	5	0	0	0	4	0	Y	5	23.05
3	14D	61	6	0	0	0	6	0	N	5	21.64
3	15D	49	5	0	0	0	1	0	N	0.5	22.85
3	15D	56	3	0	0	0	3	0	Y	0.5	18.55
3	15D	51	6	0	0	0	0	0	N	0.5	20.73
3	15D	50	1	0	0	0	2	0	Y	0.5	19.93
3	15D	54	3	0	1	0	1	0	N	0.5	20.74
3	15D	52	2	0	0	0	2	0	N	0.5	21.3
3	15D	55	1	0	0	0	2	0	Y	0.5	21.91
3	16D	29	1	0	0	0	2	0	Y	1	23.91
3	16D	28	3	0	0	0	1	0	Y	1	22.99
3	16D	26	3	0	0	0	2	0	Y	1	23.31
3	16D	30	3	0	0	0	0	0	N	1	20.82
3	16D	27	1	0	0	0	3	0	N	1	23.5
3	16D	25	0	0	0	0	4	0	N	1	24.45
3	1A	113	0	0	0	0	0	0	N	1	19.72
3	1A	114	2	0	0	0	1	0	N	1	21.47
3	1A	111	5	0	0	1	0	0	N	1	23.1
3	1A	110	3	0	0	0	6	0	N	1	21.65
3	1A	109	5	0	0	0	2	0	N	1	21.85
<b>Round</b>	<b>Tank/ Shelf</b>	<b>Female</b>	<b>Refusal</b>	<b>Interrupt</b>	<b>FFA</b>	<b>FMA</b>	<b>CBM</b>	<b>CBF</b>	<b>Eggs</b>	<b>OSR</b>	<b>Body</b>
3	2A	134	4	0	0	0	6	0	Y	5	21.26

3	2A	133	4	0	0	0	2	0	Y	5	21.89
3	3A	197	3	0	0	0	2	0	Y	0.5	21.99
3	3A	200	2	0	0	0	0	0	N	0.5	21.94
3	3A	194	5	0	0	0	0	0	Y	0.5	23.59
3	3A	193	1	0	0	0	7	1	Y	0.5	21.83
3	3A	198	5	0	0	0	2	0	Y	0.5	20.79
3	3A	199	1	0	0	0	3	0	N	0.5	20.15
3	3A	196	0	0	0	0	0	0	N	0.5	22.54
3	3A	195	2	0	0	0	3	1	N	0.5	22.27
3	4A	172	6	0	0	1	1	0	N	2	21.78
3	4A	170	4	0	0	0	1	0	N	2	21.05
3	4A	169	1	0	0	0	6	0	Y	2	20.31
3	4A	171	8	0	0	0	3	0	N	2	20.37