Effect of sex ratio variation on mating behaviour in a parasite, the salmon louse (*Lepeophtheirus salmonis*)

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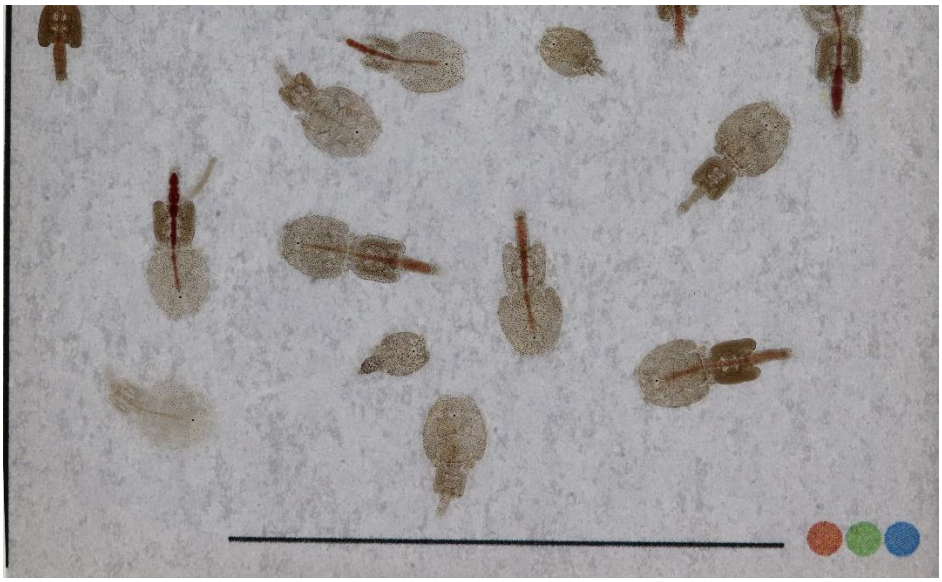

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Contents

Abstract

In sexual organisms, females produce large gametes and their reproductive success is generally more limited by resources compared to males that produce many small gametes and are more limited by the number of mates. Because of this difference, females tend to be choosy when selecting a mate, and there is a lot of competition among males for females.

To secure a mate, males may guard the female to prevent other males from mating with her. This ensures a higher chance of paternity, but also require time and energy that could otherwise be spent on finding additional mates. Because of this trade-off, one would expect that higher availability of unguarded females would decrease mate guarding duration. As the availability of unguarded females are based on the local sex ratio of males and females this means that one could expect shorter periods of mate guarding when the sex ratio is female biased (more females then males) and that the mate guarding duration is longer when the sex ratio is male biased (more males than females).

Here we study sexual selection and mate guarding behaviour in a parasite that has a major economic impact, the salmon louse (*Lepeophtheirus salmonis*). Sexual selection in parasites are often ignored with studies more often focusing on how to exterminate them, how they have adapted to their host and on evolution of virulence. We hypothesized that more male biased sex ratio would lead to longer mate guarding duration in salmon louse while more female biased sex ratio would lead to shorter mate guarding. Another hypothesis was that smaller males would be less competitive and as such the males that fell off during the experiment would on average be smaller than those that remained until the end.

The salmon louse is an ectoparasite which makes it easy to observe its behaviour on the host. We set up an experiment using 30 salmon and 100 salmon louse, 50 of each sex. We varied the initial sex ration in three experimental groups MMF, MMFF and MFF with M corresponding to male and F corresponding to female. The salmon louses were monitored every day with mate guarding behaviour being recorded as well as salmon louse that ended up in filters being taken pictures of for measurement.

We found that the adult males that were lost from the host during the experiment tended to be smaller than those that remained until the end while for females there was no noticeable size difference. When the initial sex ratio was male biased, males guarded their mates for longer. We also found that the start of mate guarding was impacting the duration of mate guarding and that sex ratio seemed to not impact when guarding behaviour ended. This pattern of longer mate guarding when there is a male biased sex ratio has also been observed in some non-parasitic Crustaceans like *Gammarus duebeni celticus, Alpheus angulatus* and *Caprella penatis.*

Introduction

Fitness is defined as an organism's contribution to the gene pool of the next generations. It results from the combination of survival until maturity and the production of viable and fertile offspring (Herron & Freeman, 2015). In sexual organisms a necessary element of reproduction is being able to find a mate, as such an important type of selection for these organisms is sexual selection, which determines their ability to successfully mate and produce offspring with individuals of the other sex. (Herron & Freeman, 2015).

Organisms that reproduce sexually are usually divided into males and females. Males are organisms that produce small gametes while females produce larger gametes. Due to the small size of the gametes males produce many gametes and tend not to spend much energy on their production, females on the other hand produce fewer, bigger gametes but invest more resources in each of them. These differences in the amount of resources invested into each gamete, causes there to be a difference in the optimal reproductive strategy for males and females (Trivers, 1972).

Since there is a higher cost for females to produce gametes (Hayward & Gillooly, 2011), they can only produce a limited amount of them. This means that their fitness will depend on the level of resources that they invest into each offspring. Male gametes on the other hand require lower amounts of nutrients and are produced in larger quantities. Because of this the fitness of males strongly depends on how many female gametes they are capable of fertilising.

This difference between males and females causes selection on females for expressing partner choice, *i.e.* for being "picky" regarding the male individuals that will fertilise the few gametes that they are investing valuable resources in (Queller, 1997). As for males, because of the general overproduction of relatively costless gametes, there is a more intense competition for access to female gametes, which leads to high variability in reproductive success. A few successful males can end up siring a high amount of offspring and thus have a high fitness, while a large majority of male gametes are lost.

(Nakatsuru & Kramer, 1982). Because of this there is generally strong selection on male traits that ensure them access to females.

For males the outcome of competition over females can be determined by two types of mechanisms. The first one is female choice, where the female chooses from a pool of potential partners the ones displaying certain traits deemed attractive (ornaments) (Pryke & Andersson, 2005). This represents a form of indirect competition among males. The second one is direct male-male competition, selecting for traits in males that enable them to exclude male competitors. Male-male competition can take many forms, but often comes down to competition for physical access to females or access to a resource that is attractive for the female (e.g., a large territory) (Wikelski & Baurle, 1996). A very direct display of this competition is aggression and combat among males, however it can also take other forms.

One of the forms that direct male-male competition can take is mate guarding. Mate guarding is when a male prevents a female the male already has mated with from remating with additional males (Evstigneeva, 1993). This ensures a high certainty of paternity. The negative consequence of mate guarding, however, is that the time spent preventing the female from remating with other males cannot be spent fertilising more females. This means that there is a fitness trade-off when it comes to mate guarding, where the male sacrifices potential future mating opportunities for a higher degree of paternity with its current mate (Komdeur, 2001).

As mate guarding has a trade-off between current and potential future reproductive success the optimal strategy should depend on a variety of factors, one of which is the ratio between males and females (Begon et al., 2006). With an abundance of females compared to males, the males would have a better chance of finding new females to mate with and as such it could be more beneficial to invest less in guarding the current female and more on finding new females to mate with. This would mean that there would be less competition among males as the males are occupied with finding females to mate with rather than compete against each other (Tania & Michelle, 2006).

Another possibility is that there are few females compared to males. In this case males would struggle finding females to mate with and as such one would expect it to be beneficial for them to spend more resources on the female they mate with to ensure paternity. This would then mean that males have a higher degree of competition over females when there are few females compared to males (Tania & Michelle, 2006).

When it comes to sex ratio there usually is a 1:1 ratio of males and females in a population if the cost to produce sons and daughters is the same (Metcalf, 1980). However even in the case where the population as a whole has a balanced sex ratio there could be fragmented parts of the larger population where there is a non-balanced sex ratio. In these fragmented smaller parts of the population one could expect different sexual selection pressures based on the local sex ratio (Aars et al., 1995). Here it could be expected that in parts of the population where there is an abundance of females compared to males the competition among males is weaker while in parts where there are fewer females than males the competition among males is stronger.

One type of organisms that often live in patchy areas are parasites, since they live inside or on a host they are often isolated from other parasites of the same species. As these populations within or on the surface of the host can be small there could be lots of variation in the sex ratio of populations of sexually reproducing parasites. This in turn means that high variation on sexual selection pressure could be found when examining different populations of a parasite species where the populations are defined by their individual host. With varying degrees of sexual selection, one would expect there to be a difference in the intensity of competition among males, which should be observable by examining certain behaviours related to sexual selection.

While the study of sexual selection is quite common in general as it has a high impact on fitness and thus is a large part of understanding evolution, there has been a lack of focus on this aspect when it comes to parasites. In general it seems like there primarily is a focus on finding out how to exterminate the parasites, the way in which parasites adapt to their host (including the evolution of virulence), and the way in which they

affect the evolution of the host. An example of this is the book Evolutionary Parasitology by Paul Schmid-Hempel which is used in the parasitology course at UiB. Using the preface of the book the only thing I was able to find related to sexual selection regarding parasites was the way in which the parasite affects its host and not anything about the sexual selection of the parasite itself. Taking into account that many parasites have sexual reproduction and that they are bound to the same evolutionary principles as other living organisms where sexual selection is important in sexual organisms, it seems negligent to ignore this aspect of parasites given their widespread impact.

As parasites are heavily dependent on their host, they are likely to have a lot of trade-offs differing from those of other similar, but non parasitic organisms. This is something that could impact the sexual selection of parasites and as such knowledge gained from research on non-parasitic organisms that are similar might not apply to the parasite. With regards to studies regarding parasites it is generally easier to observe ectoparasites, which are parasites living on the outside of the host rather than observing endoparasites that live inside the host, as monitoring what happens inside the host can be difficult.

Most ectoparasites are arthropods and one of these ectoparasitic arthropods that has a widespread economic impact is the Salmon louse (*Lepeophtheirus salmonis*). This parasite lives off the skin, blood and mucus of the salmon and has become a large problem for the salmon farming industries. In Norway for instance the mariculture stock of the Norwegian Atlantic salmon (*Salmo salar*) has increased from 160 thousand to 720 thousand metric tons from 1994-2015 (Norwegian Directorate of Fisheries, 2015). With this increase in amount of salmon compared to when there were only wild salmon and the fact that these farmed salmon are confined to small spaces has created an ideal situation for salmon louse to increase in numbers. More generally, intensive farming conditions are expected to select for increased virulence in parasites and pathogens (Mennerat et al. 2010) and the salmon louse seems to be no exception (Mennerat et al. 2017).

Since salmon louse are economically important there is a lot of research done on them and as such there are good facilities for them at UiB which makes it easy to maintain them in the lab. As an ectoparasite it is easily observable by looking at the skin of the fish which makes monitoring their behaviour easy. Salmon louse have sexual reproduction and males guard the females against remating. These factors make salmon louse a good species for researching mate guarding in parasites.

With salmon louse living in patchy areas (the fish they live on) and with mate guarding being an important factor in the fitness of male salmon louse the relationship between the duration of mate guarding and the sex ratio in the population is an interesting subject to study. One would expect in this situation for mate guarding to last longer the more males there are compared to females as there is higher competition for the females. Another thing to look into is how the size of male and female impact whether or not they manage to stay on the salmon. If a male salmon louse is small and as such not able to compete with other males this could lead it to abandon its current host in an attempt to find other females on another host, this would then mean that smaller males would be lost more frequent than larger males. To do this we set up an experiment with three different groups having different sex ratios and collected data from this experiment to look into whether or not there is a relationship between sex ratio and the start, end and duration of mate guarding as well as whether size matters with regards to whether the salmon lice were lost during the experiment.

Material and Methods

Study species

Salmon louse have a development that takes 40-52 days at 10 °C from fertilization to adulthood (Hamre et al., 2009). Their life cycle is divided into two free swimming non feeding larval stages followed by a non-feeding infective stage and then five parasitic stages on the fish which is divided into two chalimus stages where they are attached to the host by a frontal filament, two motile preadult stages and one adult motile stage where reproduction happens (Hamre et al., 2013). When they reach the adult stage

female louse are far larger than the males with the females having a length of roughly 10 mm while the males have a length of roughly 5 mm. The males reach the adult stage earlier than the female. At the time the males reach the adult stage the female is generally in her second pre adult stage. When the males reach adulthood, they start pre-copular mate guarding the pre adult females. Shortly after a female moult into an adult she is then impregnated by the male that guarded her during her pre adult stage. The female then produces eggs which are gathered in a pair of strings each having 107-1220 eggs. Salmon louse have an iteoparus life cycle meaning the female dies after she is done with reproduction. Under laboratory conditions female salmon louse have been reported to live for 191 days at 7.2 °C and there has also been a report from (Mustafa et al., 2000) that reported females living for up to 210 days. During this period where it lives as an adult after fertilization it continually produce new egg strings and up to 11 successive pairs of eggs trings have been reported (Hauch et al., 2000; Mennerat et al, 2012).

Sampling and preliminary rearing of *L. salmonis*

A total of 50 gravid, female salmon lice were collected the 15th of July 2019 at the field station of the Institute of Marine Research located at Austevoll, and brought back to the lab in refrigerated seawater. Egg strings were hatched and incubated in the lab for 14 days, *i.e.* until the larvae became infectious (copepodite stage).

The copepodites were used to infest 15 salmon maintained in a 500L tank with normal seawater (sea temperature). The infestation procedure is as described in Glover et al. (2001). The fish hosts received a dose of 30 copepodites / fish. After 30 days the fish were anesthetised and all lice collected and counted (172 lice in total, including 50 adult males and 50 preadult females).

Experimental setup

The following day (hereafter day 0 of the monitoring period) adult males and preadult females were transferred onto 30 new salmon hosts maintained in individual tanks. The individual fish hosts were split in three experimental groups with different sex ratios: (1) two males and one female (hereafter “MMF group”, n = 10), (2) one male and two

females (hereafter “MFF group”, n = 10) and (3) two males and two females (hereafter “MMFF group”, n = 10). Fish were lifted from their tanks using nets and sedated with a mix of benzocaine and metomidate, until they were not moving. Salmon lice were photographed for later measurements before being applied on each host fish. After making sure that the lice were attached to the fish the salmon was returned to its original tank.

Each fish was kept in their own tank with there being three rows of tanks each having 10 fish tanks. These were connected to a water system which runs from the top of the tanks at the highest shelf to the one at the bottom shelf. At the outlet of each tank there is a filter that catches anything that is lose in the tank which is used to collect the salmon louse that fell off the fish as well as to prevent them from entering another tank (Appendix, Figure A1).

Daily monitoring

From day 1 until day 35, salmon lice were observed on their fish hosts. The following information was recorded daily: sex (from day 1 to day 9 sex was not recorded as female lice were preadults and thus difficult to distinguish from males), female reproductive status (i.e. whether females were carrying egg strings), whether males were guarding females, and whether lice sat in pairs or away from each other (regardless of sex). Mate guarding was recorded whenever a female and male salmon louse were together, with the male sitting right behind the female, as opposed to both sexes sitting separately on the fish.

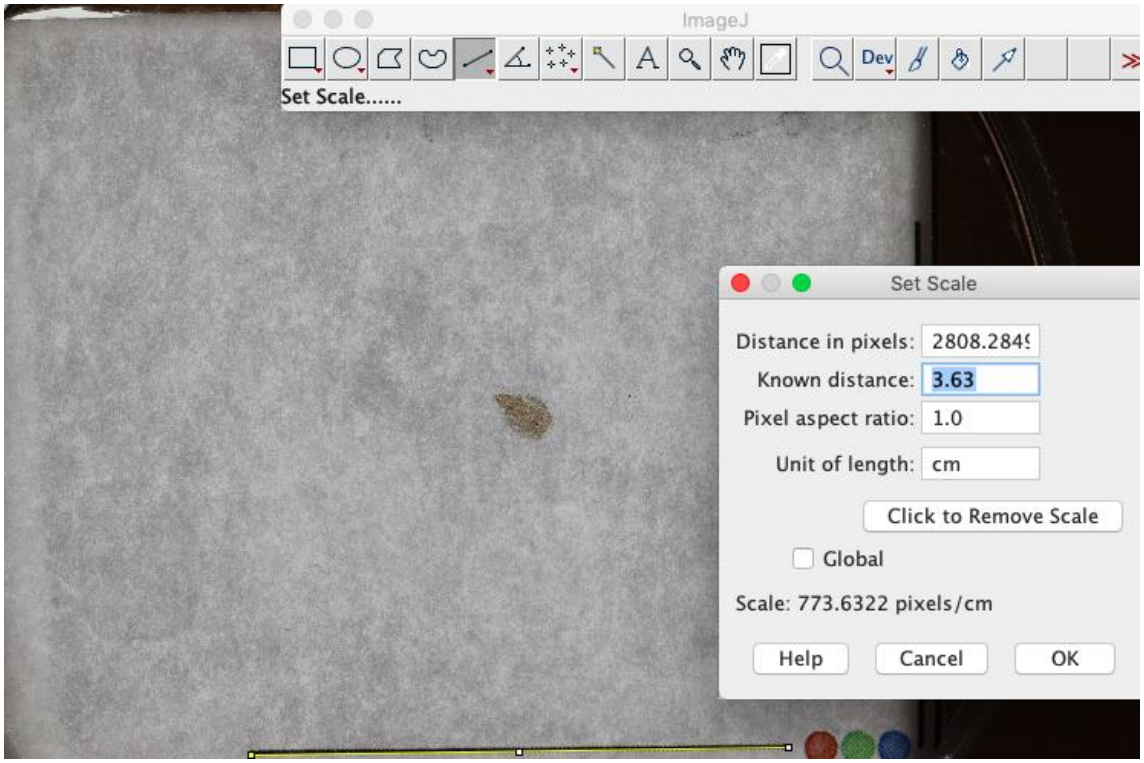
The filter on each individual tank was inspected once a day. Each filter was removed, its content flushed with seawater into a plastic box and the filter placed back on each tank. Each individual salmon louse found in the filter was photographed on a piece of wet paper with a scale, for later size measurement.

On day 36 the fish hosts were sedated again and all lice removed from the fish and photographed. All egg strings were also photographed separately. Pictures of spermatophores were also taken at a higher magnification for all females that were

removed from the fish the last day of the experiment. All females with egg strings were put into labelled egg hatcheries, and the day of hatching was recorded for each pair of egg strings.

Size measurements

When the salmon louse was taken pictures of for measurement, they were lying on a paper with a line of known length. The length of the cephalothorax was used as a proxy for body size and this was measured by using ImageJ-2 (Figure 1).



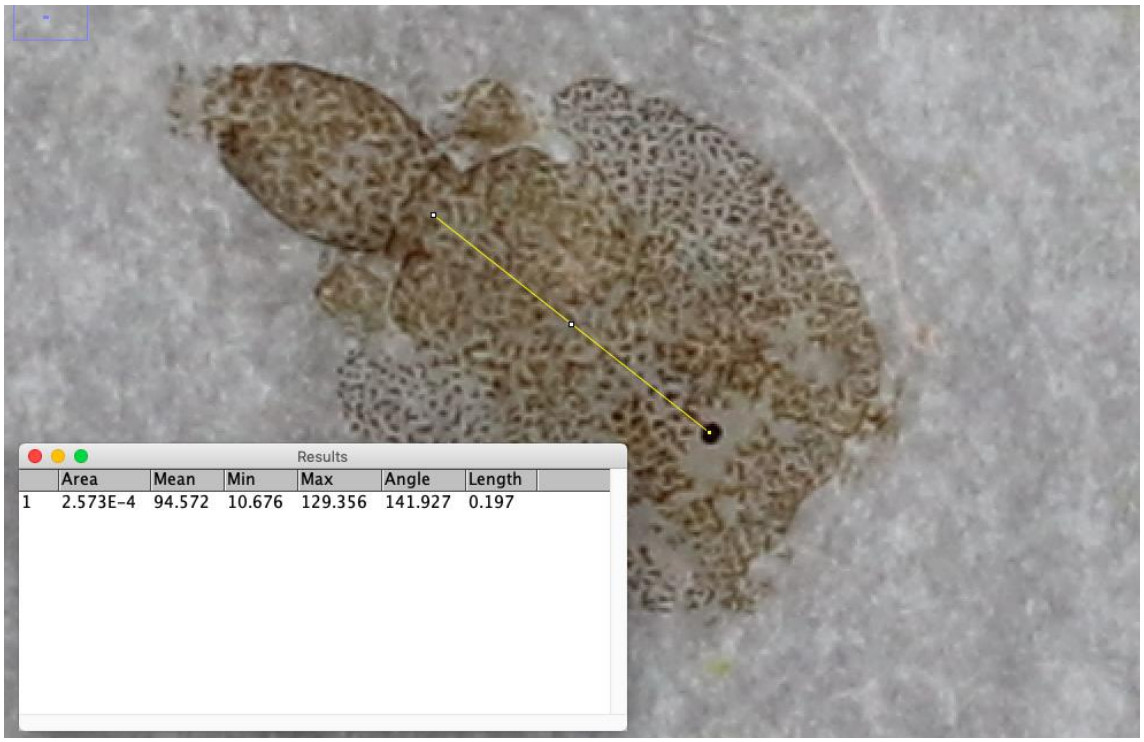


Figure 1. Measurement of body size of salmon lice from pictures. After setting the scale from the line visible on the picture (upper panel), the length of a line going from the eye spot down to the posterior end of the shell covering the cephalothorax (lower panel) was used as a proxy for body size.

Statistical analysis

The data from the daily recordings (see Appendix) was imported in R for statistical analysis. To figure out whether body size was related to whether the salmon louse fell off during the experiment, we compared the length of the cephalothorax between those individuals that were found in the outlet filters and those that remained on the fish. Welch Two Sample t-test was used on females and males separately. Welch two sample t-test was also used to compare the date at which the lice fell off the fish (centered around the day of extrusion of the first egg string) between males and females. We tested for differences across groups in start date, end date and the duration (time between first and last observation of mate guarding) of observed mate guarding using Anova. To further confirm the differences found across groups, we used linear regression to test whether the duration of mate guarding, and the dates at which males started guarding females (respectively, stopped), were correlated with the sex ratio at the start (respectively, end) of mate guarding.

Results

Body size *versus* the propensity to fall off the fish

For adult females there is no noticeable difference in the length of cephalothorax between lost and remained (t = -0.25, df = 5.0, P = 0.8). The mean size of the lost females was 3.00 mm while the remaining was 3.01 mm with 7 lost and 32 remaining adult females. Adult males have a more noticeable difference (t = -1.7, df = 32, P = 0.10), even though this difference is only marginally significant. Mean size of lost adult males is 2.09 mm while for remaining males it is 2.13 mm and the results are based on 31 lost and 20 remaining (Figure 2).

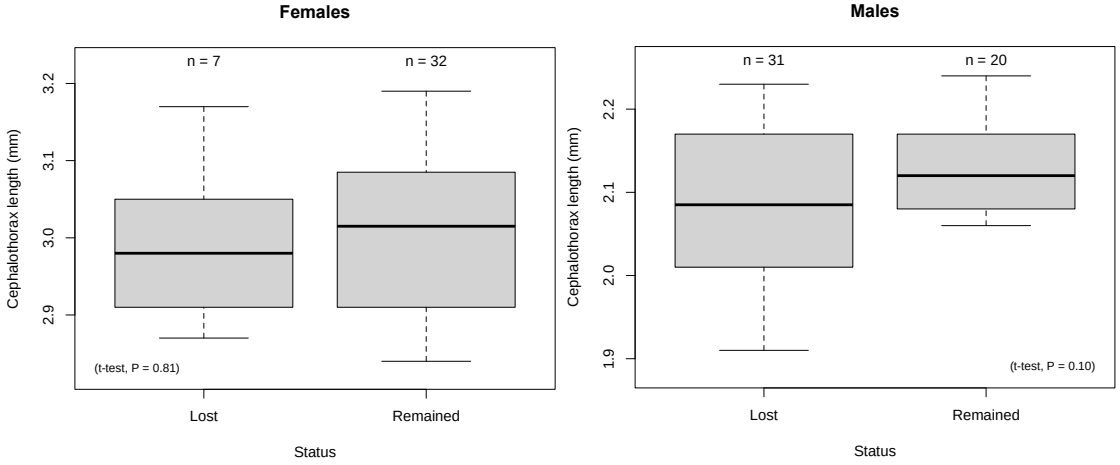


Figure 2: Length of cephalothorax in adult salmon louse and success of staying on the fish throughout the experiment. The Y axis shows the length of the cephalothorax in mm while the X axis divides the adult salmon louse into lost and remained where lost are louse that disappeared throughout the experiment while remained are those that still were on the fish at the end of the experiment.

Sex difference in the time at which lice fall off the fish

Adult males adult females fell off the fish at different times (t = -3.0, df = 14, P = 0.009). The mean for when adult males were lost is 1.4 days before the first clutch while the mean for adult females is 5.8. There is also a large difference in the amount that fell off with adult males being lost at a much higher rate (31 adult males *versus* 7 adult females, Figure 3).

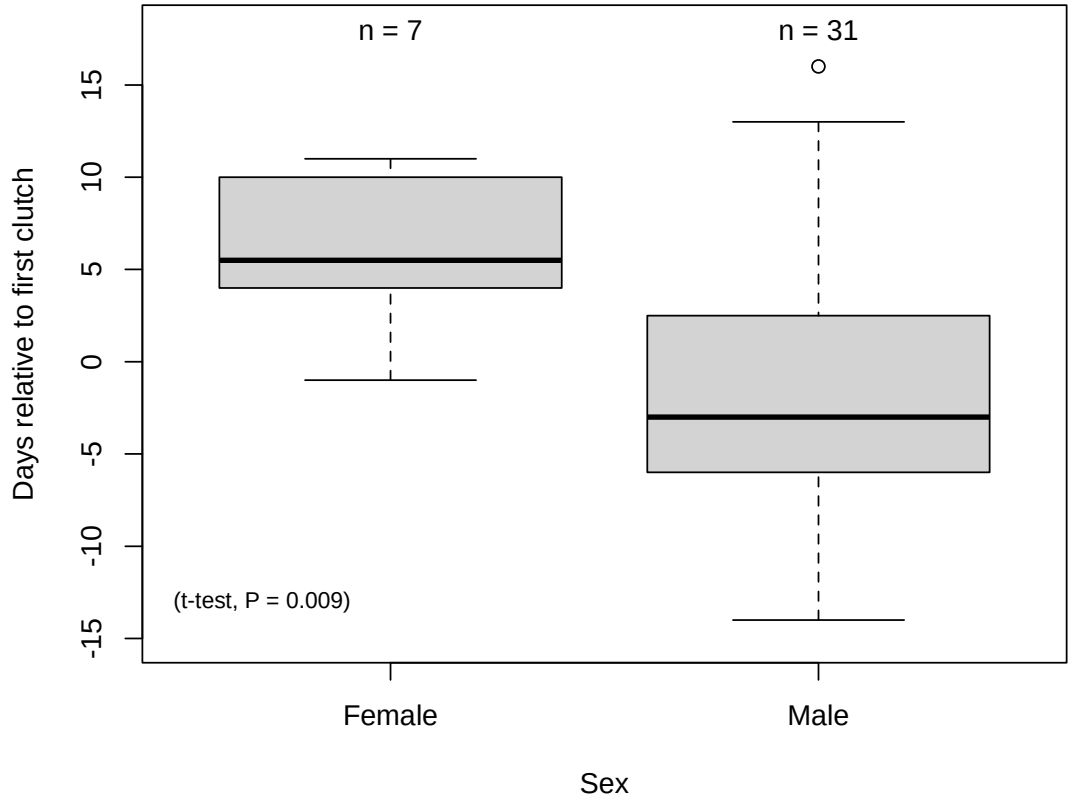


Figure 3: The time that adult females and males were lost from the host fish. The vertical axis gives days relative to first clutch which is when the first egg strings appeared.

Effect of sex ratio on timing and duration of mate guarding

Mate guarding tends to start earlier in the experimental group with the highest sex ratio, but the overall difference among groups is marginally significant (Anova, $F_{2, 25} = 3.03$; P = 0.068; Figure 4a). The end of mate guarding does not seem to differ significantly among the three experimental groups (Anova, $F_{2, 25} = 1.19$; P = 0.32; Figure 4b).

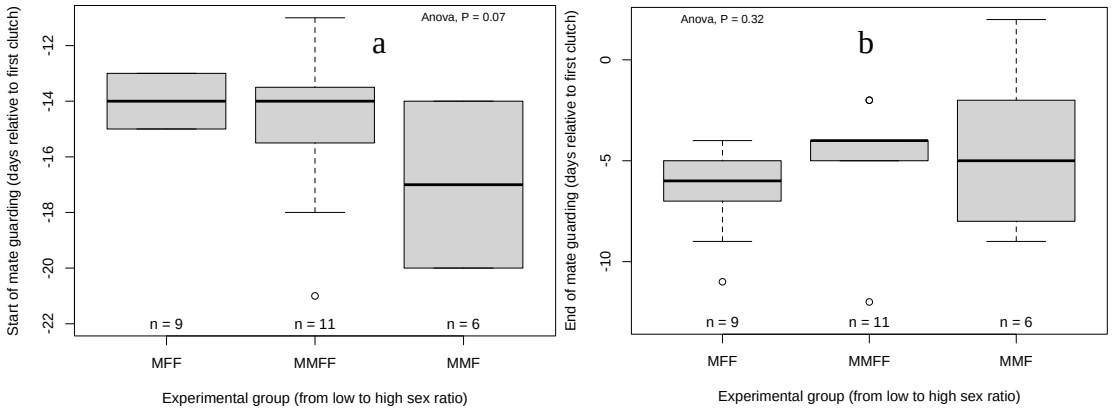


Figure 4: Time at which mate guarding started and stopped for each experimental group. The vertical axis gives start of mate guarding on figure a and end of mate guarding on figure b both relative to the first

clutch in days. The X axis has the experimental groups MFF, MMFF and MMF going from low to high sex ratio with low ratio meaning the least males compared to females. In the experimental groups M stand for male and F stand for female and they consist of the amount of males and females the letters indicate.

The total duration of mate guarding differed significantly among groups: it lasts longer when the sex ratio is higher and this difference being statistically significant (Anova, $F_{2, 25} = 4.36$; P = 0.02, Figure 5).

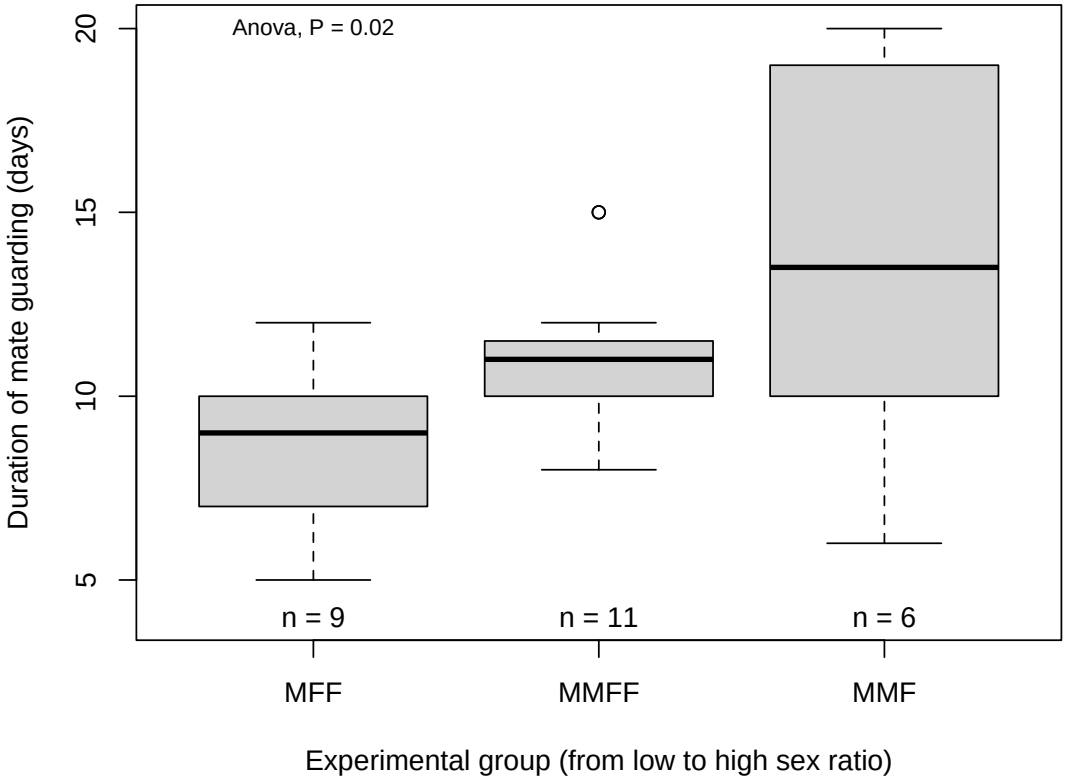


Figure 5: Duration of mate guarding in the different groups. The Y axes shows the duration of mate guarding in days while the X axes shows the experimental groups MFF, MMFF and MMF going from low to high sex ratio.

The trends found when comparing groups are confirmed by linear regression using dates and sex ratio as continuous variables. The higher the sex ratio, the earlier mate guarding starts (lm, $F_{1, 25} = 8.41$; P = 0.008; Figure 6a). The sex ratio at the end of mate guarding does not seem corrrelated to the end date of mate guarding (Anova, F1, 25 = 0.72; P = 0.40; Figure 6b).

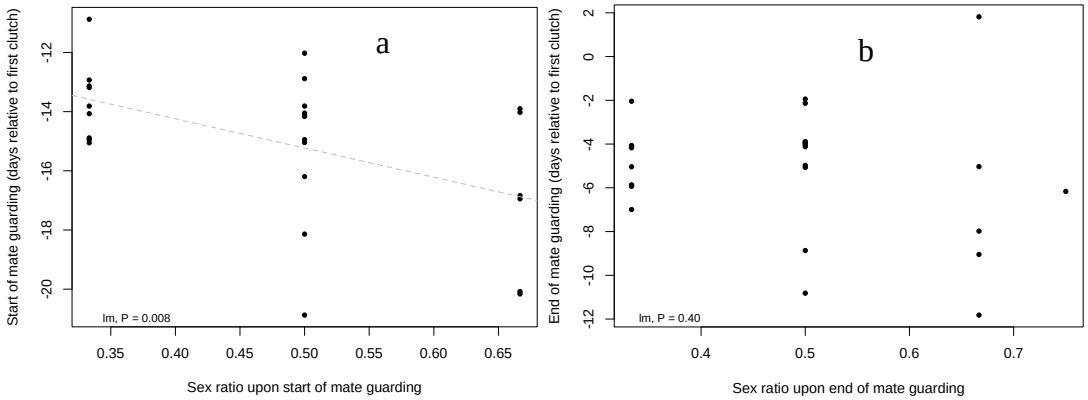


Figure 6: Sex ratio at start and end of mate guarding and when mate guarding started and ended. Figure a shows the relationship between start of mate guarding in days relative to first clutch which is the Y axis a nd sex ratio at the start of mate guarding which is the X axis with the sex ratio being the % of the louse on the salmon being male. Each black dot represents one case of mate guarding. Figure b shows the relation ship between end of mate guarding in days relative to first clutch which is the Y axis and sex ratio at the e nd of mate guarding which is the X axis.

In addition, the higher the sex ratio at the start of mate guarding, the longer the duration of mate guarding (Anova, F1, 25 = 7.23; P = 0.013; Figure 7a). Sex ratio at the end of mate guarding is not significantly correlated with duration of mate guarding (Anova, F1, 25 = 1.58; P = 0.22; Figure 7b).

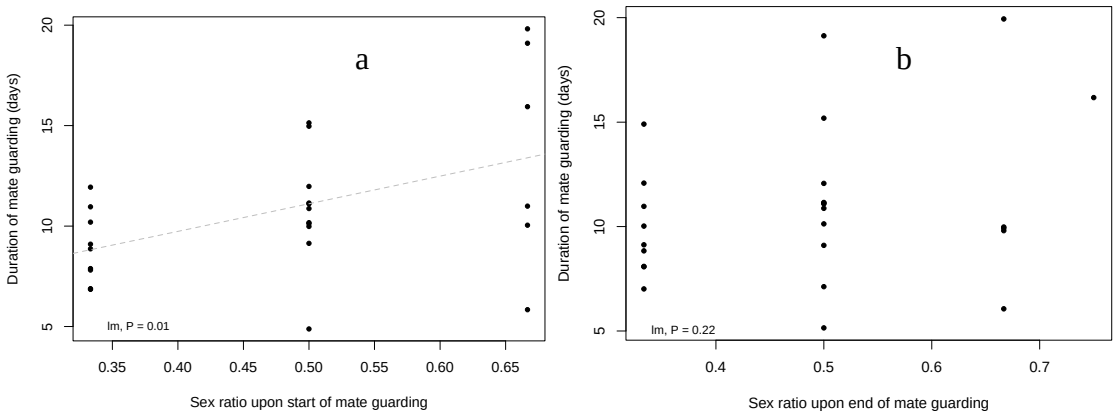


Figure 7: Sex ratio at start and end of mate guarding and the duration of mate guarding. The Y axis is the duration of mate guarding in days while the X axis on the left figure is sex ratio at start of mate guarding and on the right one it is the sex ratio at end of mate guarding.

Discussion

There was a noticeable difference in the size of males that fell off though this difference is not statistically significant while for females there is less of a difference in size of those that fell off and those that remained until the end. The sex of the adult salmon louse impacted the time in which they fell off their host with males falling off at a much earlier point on average and males falling off at a much higher rate than females. The sex ratio was found to not impact when mate guarding ended but to impact when it started as well as the duration of the mate guarding.

From the t-test there were no significant difference between cephalothorax length which is used here to indicate size and whether the salmon louse fell off during the experiment or not in either females or males. Among females the P value is 0.81 meaning there is 81% chance of the differences in size between those that fell off during the experiment can be explained by random chance. As for males the P value is 0.10 which is not considered statistically significant, with the threshold of statistical significance being 0.05 or lower. While not statistically significant the P value for males is still somewhat low with the trend being that males that fell off on average were smaller than those that remained until the end. Other experiments with higher sample sizes could possibly reveal a statistical significance in size of males and whether they fall off or not.

Looking at figure 2 the difference between the males that were lost and those that remained until the end of the experiment is that the ones that were lost includes the smallest individuals and on average are smaller. This difference could possibly be explained by smaller males being outcompeted by larger males and abandoning their current host to look for other potential mates at other hosts and thus ending up in the filter. Of course, the difference is only marginally significant and stronger evidence would be needed before one could conclude that body size may play a role in male-male competition in salmon lice.

When it comes to the difference between males and females and when they were lost the t-test shows a significant difference between the sexes in the timing of loss for adult salmon louse with the P value being 0.009. Adult males were on average lost earlier than adult females. Looking at the boxplot in figure 3 most of the males were lost between six days before the first clutch and three days after first clutch. As the time they were lost is around the time at which the females start producing eggs, i.e. posterior to mating, this suggests that males may not "fall off" accidentally, but that these events might reflect either male-male competition during the mating period or adaptive post-mating dispersal when no more unmated females are available on the host.

As for the adult females that were lost during the experiment this might have just been them randomly falling off. Adult males were lost at a much higher rate than females with there having been 31 lost adult males (xx %) during the experiment while only 7 adult females (xx %) were lost. These adult females were lost primarily after the production of the first clutch. It is unclear why adult females would actively leave their host once they have been fertilised and started egg production. Overall during the entire experiment females were mostly lost during the transition between the pre-adult and the adult stage (xx freshly moulted females lost). This may be caused by higher mortality during the moulting process

Mate guarding as mentioned in the introduction is an important behavioural trait under selection in salmon louse. Given that it is likely costly, one could expect it to vary according to sex ratio (i.e., expressed more when male-male competition is more intense). Consistent with this prediction we find that early mate guarding is most common in group MMF which is the group where there are the most males compared to females. Here there is only one female and ensuring the ability to mate with this one female and ensure paternity is then very important for the male to succeed evolutionary. Because of this it makes sense that this group is the one which has the earliest mate guarding. The MFF and MMFF group however seem to be quite close to each other with regard to when mate guarding started, one would expect in this case that mate guarding was earlier in the MMFF group than in the MFF group as there are more males here, however the difference here is much lesser than that between MMF and the two

other groups. Using an Anova test the P value is 0.07, which is close to significant. This trend for differences across experimental groups was confirmed by linear regression with continuous variables. The sex ratio at the start of mate guarding is negatively correlated with the date at which mate guarding starts, with a P value of 0.01 which is significant. This then means that males are capable of detecting the other males and females on the fish and change the time at which they start mate guarding accordingly to the sex ratio. A way in which they possibly could detect other males and females would be through chemical cues (Stephenson, 2012)

Due to some salmon louse having been lost before the mate guarding period started the experimental group is a less accurate indicator of sex ratio at the start of the mate guarding period. This, in addition to the fact that linear regression has higher power than comparisons across groups when sample sizes are small, can explain lower statistical significance for the group comparisons than for the linear regression approach.

The date at which mate guarding stops does not differ significantly across groups, which suggests that it is not influenced by the sex ratio at the start of the mate guarding period. It is nor significantly correlated to the sex ratio at the end of the mate guarding period either. This could possibly be because by the time mate guarding stops the female is already fertilized and further mate guarding does not provide any fitness advantage.

The significant of the difference between the sex ratio at the end of mate guarding and when mate guarding stopped however does not improve compared to the experimental group´s correlation to the end of mate guarding. In this case the P value is 0.40 while the one between experimental group and end of mate guarding was 0.32. From this it seems even less likely that the time at which mate guarding ends has to do with the sex ratio of the salmon louse.

The sex ratio at the end of mate guarding is not significantly correlated with the duration of the mate guarding (P value of 0.22). Since the end of the mate guarding

seems unaffected by sex ratio and the start of mate guarding seemingly being the deciding factor it makes sense for the sex ratio at the end of mate guarding not to affect the duration of the mate guarding.

When it comes to the correlation between the experimental group and the duration of mate guarding it actually is significant with the P value being 0.02. As this is the is the time between the start and the end of mate guarding it this should mean that there either is a correlation between one of those, likely start of mate guarding as it has a lower P value thus less likely to be caused by random chance or it could be both of them. If more data is gathered one could likely find this correlation to start of mate guarding or both. The correlation found here is again that the groups with the lowest sex ratio (least number of males compared to females) has the shortest mate guarding period. The MMF group has a much longer average mate guard duration than that of the other groups and mate guarding in the MMFF on average being somewhat longer than in the MFF group (figure 5). These results are what is to be expected as longer mate guarding indicates higher competition among males which should happen when there is scarcity of females compared to males.
Looking into the effect the sex ratio at the beginning has on the duration of the mate guarding the Anova test gives a P value of 0.01 showing a significant relationship. Considering how the sex ratio at the beginning effected the time at which mate guarding started this is quite expected as earlier mate guarding seem to result in mate guarding lasting longer as the end of the mate guarding seem to not be affected by sex ratio.

Summary

The body size of adult males or females does not differ according to whether the salmon louse was lost during the experiment, however while the results were not significant there is a noticeable pattern of smaller males being in the group that was lost and further research could show this to be significant. As for the difference in when males and females were lost there is a significant difference where males are lost earlier than females. As males are done mating with the females on its current host it might benefit them to move away from the current host which could explain this. As few adult

females were lost compared to adult males the pattern of females being lost here might just be random. As for mate guarding the results seem to indicate that the sex ratio at the start of mate guarding affects the date at which mate guarding starts, but that the end of mate guarding is unaffected by the sex ratio. As the start of mate guarding is affected and the end is unaffected this also means that the duration of the mate guarding depends on when it starts which means the duration of mate guarding also depends on the sex ratio at the start of mate guarding.

How this relates to other studies

This relationship between the duration of mate guarding and the sex ratio of a population is also something that has been found in other crustaceans. A study (Dick & Elwood, 1996) focusing on the Crustacean Amphipod *Gammarus duebeni celticus* found that the duration of mate guarding increased when a higher percentage of the population were male and that the density of the population had no effect on mate guarding duration. In this study the sex ratio varied between males accounting for 6-48% of the population which means they only tested situations where the majority of the *Gammarus duebeni celticus* were females.

Another study (Mathews, 2002) focused on mate guarding in snapping shrimp *Alpheus angulatus*. In natural populations the snapping shrimp has a sex ratio of 1:1 and they are socially monogamous. In the experiment they did they had experimental groups with 1 male for 5 females and 3 males for 3 females and they found that in when there was 1 male for 5 females there was a significantly higher chance of the male abandoning its mate.

A study of *Caprella penatis* the skeleton shrimp (Fumio & Yasuhisa, 2010) found that the mean duration of mate guarding was significantly longer when there was a male biased sex ratio. The male biased sex ratio in this experiment was 4 males and 2 females while the female biased sex ratio was 2 males and 4 females. In this they also found that larger males guarded for a longer period and that larger females were guarded for a longer period of time.

From this it seems like the result from this experiment is supported by other findings in other Crustaceans. With similarities being found in *Gammarus duebeni celticus, Alpheus angulatus* and *Caprella penatis.*

Future research

While the results from this experiment showed a significant correlation between start of mate guarding and sex ratio it is very limited with regards to how many situations it tests. As there are only three different groups and two of them are limited to having one individual of a sex and two of the other this might not represent how mate guarding is impacted by sex ratio when there are a higher number of males and females but a similar sex ratio. As such larger experiments using more groups should be used to find out if this difference in sex ratio effects mate guarding duration in general or if this is just limited to when there are very few salmon louse on the fish and if so to what degree the duration of mate guarding is impacted by different sex ratio.

Measurements of egg string length as well as of number of copepodites produced by the eggs could also be done in future research to look into how sex ratio effects a female's reproductive success. This was initially planned for this experiment, but the egg strings were not measured systematically, and we did not know which egg string we measured.

Future research could also focus on better understanding the dynamics of mate guarding in salmon louse. This could be done by tracking which salmon louse is mate guarding which female to see whether or not the same male stays on the same female for the entire duration or if the male switches. This could be done by adding tracking devices or markers or by using a camera which takes pictures at certain intervals and use that to track the movement of the salmon louse.

Since this situation where the salmon are isolated from other salmon is not something that is found in the wild or in the salmon farms experiments should also be done to look

into the movement of salmon louse between hosts. This is especially important as salmons live at a very high density in salmon farms which should make movement from one salmon to the other less challenging than in the wild where they are much more spread out. Properly understanding the dynamics of how the salmon louse moves between hosts is important for figuring out the trade-offs related to mate guarding as it gives insight into the cost of moving from one host to another.

To better understand the movement of male salmon louse effort could also be put into figuring out whether they have an ability to find out where female salmon louses are and move towards them. This could be done through an experiment where the male enters a tube with two exits where water is slowly running from both the exits. On one end there could be one or multiple female salmon louse which are unable to move but in contact with water so pheromones can be transferred while on the other it could be nothing. This can then be run multiple times using multiple females and if the male shows an ability to be able to locate and move towards the female further situations could be used. For instance, differing numbers of females could be used check the ability of the male to track where there are more females with few females on one side and a lot on the other. Males can also be introduced together with females having one group of males and females and another of only females to see whether they are able to locate males and go for the one without males as there is less competition there.

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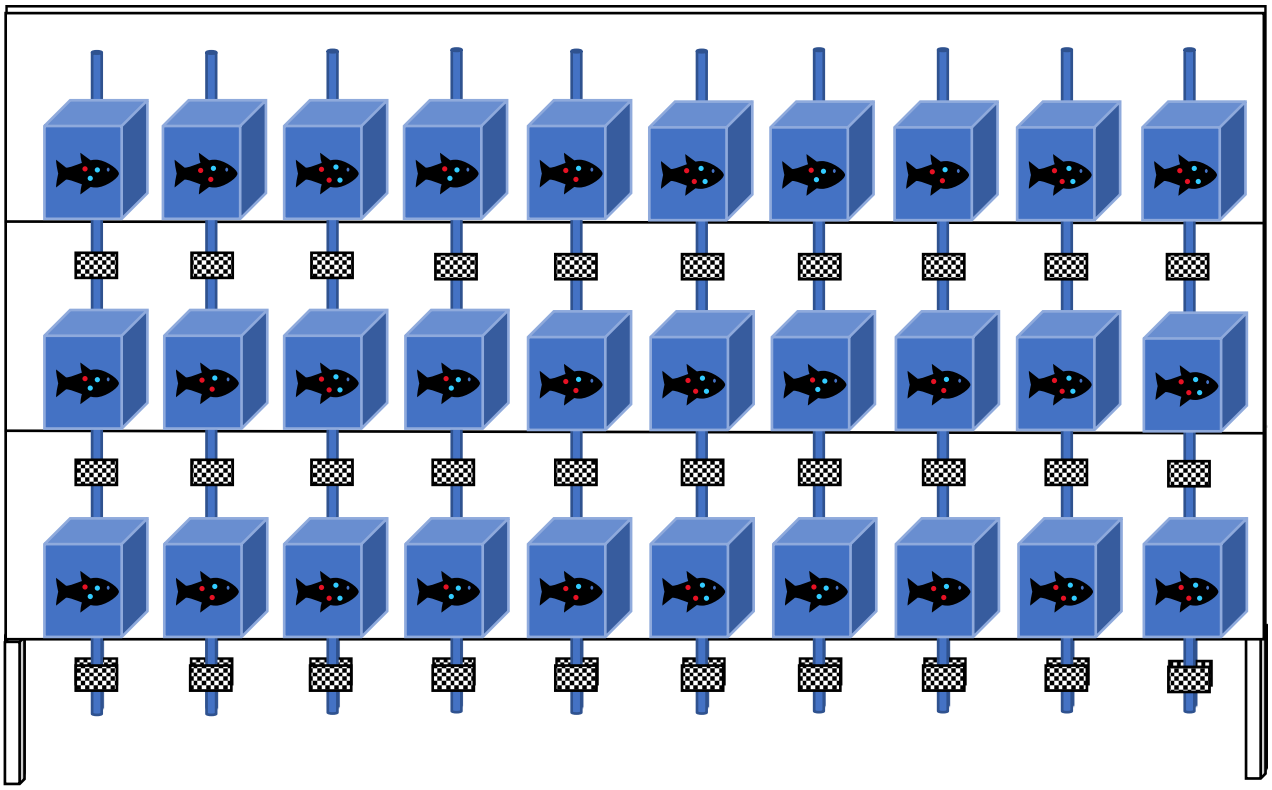
Appendix

A.1 Figure for experimental setup



Above is a fishtank with a fish, dots represent salmon louse. Male salmon louse are blue while females are red.

Filter between fish tanks.

A.2 R-syntax

```
data.df<-read.table('lost.txt', header=T, na.string=".")
attach(data.df)
various useful subsets
data.df<-read.table('lost.txt', header=T, na.string=".")
attach(data.df)
adult.df<-subset(data.df, stage == "Adult")
adultF.df<-subset(adult.df, sex == "Female")
adultM.df<-subset(adult.df, sex == "Male")
lostadult.df<-subset(adult.df, status == "Lost")
lostall.df<-subset(data.df, status == "Lost")
lostM.df<-subset(lostadult.df, sex == "Male")
lostF.df<-subset(lostadult.df, sex == "Female")

testing for differences in body size between lost and remained, for adults male only

t.test(bodysize ~ status, data=adultM.df)

        Welch Two Sample t-test

data:  bodysize by status
t = -1.7037, df = 31.87, p-value = 0.09817
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -0.091125353  0.008125353
sample estimates:
   mean in group Lost mean in group Remained
            2.0890              2.1305

Plotting differences in body size between lost and remained, for adults male only

boxplot(bodysize ~ status, data=adultM.df, xlab="Status", ylab="Cephalothorax length (mm)", ylim=c(1.
88,2.26), main="Males")
text(2.3, 1.89, labels="(t-test, P = 0.10)", cex=0.8)
text(1,2.26, labels="n = 31")
text(2,2.26, labels="n = 20")
```

testing for differences in body size between lost and remained, for adults female only

```
t.test(bodysize ~ status, data=adultF.df)

        Welch Two Sample t-test

data:  bodysize by status
t = -0.25363, df = 5.0336, p-value = 0.8098
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -0.1590827  0.1304577
sample estimates:
   mean in group Lost mean in group Remained
             2.996000              3.010312
```

Plotting differences in body size between lost and remained, for adults female only

```
boxplot(bodysize ~ status, data=adultF.df, xlab="Status", ylab="Cephalothorax length (mm)",
main="Females", ylim=c(2.82,3.23))
text(0.7, 2.83, labels="(t-test, P = 0.81)", cex=0.8)
text(1,3.23, labels="n = 7")
text(2,3.23, labels="n = 32")
```

testing for differences between sexes in timing of loss for adults

```
t.test(relative_date_lost ~ sex, data=lostadult.df)

        Welch Two Sample t-test

data:  relative_date_lost by sex
t = 3.023, df = 14.206, p-value = 0.009002
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
  2.101063 12.315604
sample estimates:
mean in group Female   mean in group Male
           5.833333            -1.375000
```

Plotting for differences between sexes in timing of loss for adults

```
boxplot(relative_date_lost ~ sex, data=lostadult.df, ylim=c(-15,18), xlab="Sex", ylab="Days relative to fi
rst clutch")
text(2,18, labels="n = 31")
text(1,18, labels="n = 7")
text(0.7, -13, labels="(t-test, P = 0.009)", cex=0.8)
```

importing data

```
pairtank.df<-read.table('pair_duration_tank.txt', header=T)
attach(pairtank.df)
```

testing if there are differences across groups in start date, end date, and duration of obse rved mate guarding

```
anova(lm(pair_start ~ group))
```

Analysis of Variance Table

```
Response: pair_start
          Df  Sum Sq Mean Sq F value  Pr(>F)
group      2  31.891 15.9454  3.0292 0.06796 .
Residuals 23 121.071  5.2639
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
pairtank.df$group<-factor(pairtank.df$group, levels=c("MFF", "MMFF", "MMF"))
boxplot(pairtank.df$pair_start ~ pairtank.df$group, ylab="Start of mate guarding (days relative to first clu
tch)", xlab="Experimental group (from low to high sex ratio)", ylim=c(-22,-11))
text(3,-11, labels="Anova, P = 0.07", cex=0.8)
text(1,-22, labels="n = 9")
text(2,-22, labels="n = 11")
text(3,-22, labels="n = 6")
```

Guard end group

```
boxplot(pairtank.df$pair_stop ~ pairtank.df$group, ylab="End of mate guarding (days relative to first clut
ch)", xlab="Experimental group (from low to high sex ratio)", ylim=c(-13,2))
```

```
text(1,2, labels="Anova, P = 0.32", cex=0.8)
text(1,-13, labels="n = 9")
text(2,-13, labels="n = 11")
text(3,-13, labels="n = 6")
```

Guard duration Group

```
anova(lm(pair_duration ~ group))

Analysis of Variance Table

Response: pair_duration
          Df  Sum Sq Mean Sq F value  Pr(>F)
group      2  91.142  45.571  4.3628 0.02475 *
Residuals 23 240.242  10.445
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
boxplot(pairtank.df$pair_duration ~ pairtank.df$group, ylab="Duration of mate guarding (days)", xlab="
Experimental group (from low to high sex ratio)", ylim=c(4,20))
text(1,20, labels="Anova, P = 0.02", cex=0.8)
text(1,4, labels="n = 9")
text(2,4, labels="n = 11")
```

Guard start correlation

```
anova(lm(pair_start ~ SR_start))

Analysis of Variance Table

Response: pair_start
          Df  Sum Sq Mean Sq F value   Pr(>F)
SR_start   1  39.686  39.686  8.4084 0.007865 **
Residuals 24 113.276   4.720
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

plot(jitter(pair_start) ~ SR_start, pch=20, xlab="Sex ratio upon start of mate guarding", ylab="Start of ma
te guarding (days relative to first clutch)")
```

```
abline(lm(pair_start ~ SR_start), col="grey", lty=2)
text(0.37,-21, labels="lm, P = 0.008", cex=0.8)
```

Guard end correlation

```
anova(lm(pair_stop ~ SR_stop))

Analysis of Variance Table

Response: pair_stop
          Df  Sum Sq Mean Sq F value Pr(>F)
SR_stop    1   6.343  6.3427  0.7239 0.4033
Residuals 24 210.273  8.7614

plot(jitter(pair_stop) ~ SR_stop, pch=20, xlab="Sex ratio upon end of mate guarding", ylab="End of mate
 guarding (days relative to first clutch)")
text(0.37,-12, labels="lm, P = 0.40", cex=0.8)
```

Guard duration correlation

```
anova(lm(pair_duration ~ SR_start))

Analysis of Variance Table

Response: pair_duration
          Df Sum Sq Mean Sq F value Pr(>F)
SR_start   1  76.76  76.760  7.2351 0.0128 *
Residuals 24 254.62  10.609
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
plot(jitter(pair_duration) ~ SR_start, pch=20, xlab="Sex ratio upon start of mate guarding", ylab="Durati
on of mate guarding (days)")
abline(lm(pair_duration ~ SR_start), col="grey", lty=2)
text(0.37,5, labels="lm, P = 0.01", cex=0.8)
```

Guard duration correlation end

```
anova(lm(pair_duration ~ SR_stop))
Analysis of Variance Table
```

```
Response: pair_duration
          Df  Sum Sq Mean Sq F value Pr(>F)
SR_stop    1  20.498  20.498  1.5824 0.2205
Residuals 24 310.886  12.954
plot(jitter(pair_duration) ~ SR_stop, pch=20, xlab="Sex ratio upon end of mate guarding", ylab="Duratio
n of mate guarding (days)")
text(0.37,5, labels="lm, P = 0.22", cex=0.8)
```

A.3 Raw Data

tank	group	sex	stage	date_lost	rank_lost	relative_date_lost			
	SR_date		othersex_when_lost		status	bodysize			
21A	MMF	Male	Adult	8	1	.	0.66	1	Lost	2.08
21A	MMF	Female	Preadult	8	1	.	0.66	2	Lost	.
21A	MMF	Male	Adult	27	2	.	1	0	Lost	2.17
21B	MMF	Female	Preadult	2	1	.	0.66	2	Lost	.
21B	MMF	Male	Adult	9	1	.	1	0	Lost	2.01
21B	MMF	Male	Adult	29	2	.	1	0	Lost	2.1
21C	MMF	Female	Preadult	6	1	.	0.666666667	2	Lost	2.04
21C	MMF	Male	Adult	12	1	.	1	0	Lost	2.17
21C	MMF	Male	Adult	31	2	.	1	0	Lost	.
22A	MFF	Male	Adult	13	1	-3	0.333333333	2	Lost	2.21
22A	MFF	Female	Adult	35	.	19	0	0	Remained	3.02
22A	MFF	Female	Adult	35	.	19	0	0	Remained	3.05
22B	MFF	Female	Moulting	6	1	.	0.333333333	1	Lost	2.54
22B	MFF	Male	Adult	8	1	.	0.5	1	Lost	.
22B	MFF	Female	Adult	15	2	.	0	0	Lost	2.91
22C	MFF	Male	Adult	35	.	20	0.333333333	1	Remained	2.06
22C	MFF	Female	Adult	35	.	20	0.333333333	2	Remained	3.13

22C	MFF	Female	Adult	35	.	20	0.333333333	2	Remained	2.99	
23A	MFF	Male	Adult	12	1	-4	0.5	2	Lost	2.23	
23A	MFF	Male	Adult	35	.	19	0.333333333	2	Remained	2.19	
23A	MFF	Female	Adult	35	.	19	0.333333333	1	Remained	3.07	
23A	MFF	Female	Adult	35	.	19	0.333333333	1	Remained	3	
23B	MMFF	Male	Adult	9	1	-7	0.5	2	Lost	2.08	
23B	MMFF	Male	Adult	27	2	11	0.33	2	Lost	.	
23B	MMFF	Female	Adult	35	.	19	0	0	Remained	3.08	
23B	MMFF	Female	Adult	35	.	19	0	0	Remained	2.96	
23C	MMFF	Female	Preadult	5	1	-9	0.5	2	Lost	.	
23C	MMFF	Male	Adult	12	1	-2	0.66	1	Lost	.	
23C	MMFF	Male	Adult	35	.	21	0.5	1	Remained	2.24	
23C	MMFF	Female	Adult	35	.	21	0.5	1	Remained	3.13	
24A	MMF	Male	Adult	26	1	4	0.75	1	Lost	2.13	
24A	MMF	Female	Adult	33	1	11	0.666666667	2	Lost	2.98	
24A	MMF	Male	Adult	35	.	13	1	0	Remained	2.08	
24A	MMF	Male	Adult	35	.	13	1	0	Remained	2.1	
24B	MMF	Male	Adult	13	1	-9	0.666666667	1	Lost	2.09	
24B	MMF	Male	Adult	30	2	8	0.5	1	Lost	2.04	
24B	MMF	Female	Adult	35	.	13	0	0	Remained	2.85	
24C	MMF	Male	Adult	3	1	-13	0.666666667	1	Lost	1.99	
24C	MMF	Male	Adult	36	.	20	0.5	1	Remained	2.16	
24C	MMF	Female	Adult	36	.	20	0.5	1	Remained	3.01	
25A	MFF	Male	Adult	16	1	-1	0.333333333	2	Lost	1.91	
25A	MFF	Female	Adult	36	.	19	0	0	Remained	2.99	
25A	MFF	Female	Adult	36	.	19	0	0	Remained	2.94	
25B	MFF	Female	Adult	23	1	6	0.333333333	1	Lost	2.87	
25B	MFF	Male	Adult	36	.	19	0.5	1	Remained	2.16	

25B	MFF	Female	Adult	36	.	19	0.5	1	Remained	3	
25C	MFF	Male	Adult	35	.	19	0.333333333	2	Remained	2.12	
25C	MFF	Female	Adult	35	.	19	0.333333333	1	Remained	3.19	
25C	MFF	Female	Adult	36	.	20	0.333333333	1	Remained	3.17	
26A	MMFF	Female	Preadult	3	1	-14	0.5	2	Lost	2.05	
26A	MMFF	Male	Adult	12	1	-5	0.66	1	Lost	.	
26A	MMFF	Male	Adult	36	.	19	0.5	1	Remained	2.08	
26A	MMFF	Female	Adult	36	.	19	0.5	1	Remained	2.84	
26B	MMFF	Female	Moulting	6	1	-17	0.5	2	Lost	2.53	
26B	MMFF	Male	Adult	15	1	-8	0.66	1	Lost	.	
26B	MMFF	Female	Adult	27	2	4	0.5	1	Lost	3.05	
26B	MMFF	Male	Adult	36	.	13	1	0	Remained	2.18	
26C	MMFF	Male	Adult	3	1	-14	0.5	2	Lost	1.99	
26C	MMFF	Male	Adult	18	2	1	0.333333333	2	Lost	2.22	
26C	MMFF	Female	Adult	35	.	18	0	0	Remained	3.16	
26C	MMFF	Female	Adult	36	.	19	0	0	Remained	2.96	
27A	MMF	Male	Adult	14	1	-5	0.666666667	1	Lost	2.03	
27A	MMF	Male	Adult	35	.	16	0.5	1	Remained	2.23	
27A	MMF	Female	Adult	35	.	16	0.5	1	Remained	3.06	
27B	MMF	Male	Adult	32	1	13	0.66	1	Lost	.	
27B	MMF	Male	Adult	35	.	16	0.5	1	Remained	2.14	
27B	MMF	Female	Adult	35	.	16	0.5	1	Remained	3.09	
27C	MMF	Male	Adult	36	.	20	0.666666667	1	Remained	2.08	
27C	MMF	Male	Adult	36	.	20	0.666666667	1	Remained	2.22	
27C	MMF	Female	Adult	36	.	20	0.666666667	2	Remained	3.14	
28A	MFF	Female	Preadult	3	1	-16	0.333333333	1	Lost		

	2.02									
28A	MFF	Male	Adult	18	1	-1	0.5	1	Lost	2.21
28A	MFF	Female	Adult	36	.	17	0	0	Remained	2.88
28B	MFF	Male	Adult	32	1	16	0.33	2	Lost	.
28B	MFF	Female	Adult	36	.	20	0	0	Remained	2.88
28B	MFF	Female	Adult	36	.	20	0	0	Remained	2.87
28C	MFF	Female	Preadult	3	1	-14	0.333333333	1	Lost	
	1.95									
28C	MFF	Male	Adult	24	1	7	0.5	1	Lost	.
28C	MFF	Female	Adult	36	.	19	0	0	Remained	3.06
29A	MMFF	Female	Preadult	6	1	-10	0.5	2	Lost	.
29A	MMFF	Male	Adult	12	1	-4	0.666666667	1	Lost	2.12
29A	MMFF	Male	Adult	36	.	20	0.5	1	Remained	2.12
29A	MMFF	Female	Adult	36	.	20	0.5	1	Remained	2.85
29B	MMFF	Female	Adult	15	1	-1	0.5	2	Lost	.
29B	MMFF	Male	Adult	36	.	20	0.666666667	1	Remained	
	2.06									
29B	MMFF	Male	Adult	36	.	20	0.666666667	1	Remained	
	2.08									
29B	MMFF	Female	Adult	36	.	20	0.666666667	2	Remained	
	2.86									
29C	MMFF	Female	Preadult	6	1	-10	0.5	2	Lost	2.09
29C	MMFF	Male	Adult	12	1	-4	0.666666667	1	Lost	1.99
29C	MMFF	Female	Adult	26	2	10	0.5	1	Lost	3.17
29C	MMFF	Male	Adult	36	.	20	1	0	Remained	2.09
30A	MMFF	Male	Adult	17	1	-3	0.5	2	Lost	.
30A	MMFF	Male	Adult	35	.	15	0.333333333	2	Remained	
	2.14									
30A	MMFF	Female	Adult	35	.	15	0.333333333	1	Remained	
	3.06									
30A	MMFF	Female	Adult	36	.	16	0.333333333	1	Remained	
	2.88									

30B	MMFF	Male	Adult	9	1	-9	0.5	2	Lost	2.01	
30B	MMFF	Female	Adult	23	1	5	0.33	1	Lost	.	
30B	MMFF	Male	Adult	35	.	17	0.5	1	Remained	2.08	
30B	MMFF	Female	Adult	35	.	17	0.5	1	Remained	3.05	
30C	MFF	Female	Preadult	9	1	-7	0.33	1	Lost	.	
30C	MFF	Male	Adult	15	1	-1	0.5	1	Lost	.	
30C	MFF	Female	Adult	35	.	19	0	0	Remained	3.11	

tank	group	pair_start	pair_stop	pair_duration	male_start	female_start	SR_start	male_stop	female_stop	SR_stop	av_male	av_female	av_SR
22A	MFF	-14	-6	9	1	2	0.333333333	1	2	0.333333333			
22C	MFF	-13	-7	7	1	2	0.333333333	1	2	0.333333333			
23A	MMFF	-13	-5	9	2	2	0.5	2	2	0.5	2	2	0.5
23B	MMFF	-14	-4	11	2	2	0.5	1	2	0.333333333	1.5	2	0.428571429
23C	MMFF	-12	-2	11	2	2	0.5	1	1	0.5	1.7	1.3	0.566666667
24A	MMF	-20	-6	16	2	1	0.666666667	3	1	0.75	2	1	0.666666667
24B	MMF	-20	-2	19	2	1	0.666666667	1	1	0.5	1.6	1	0.615384615
24C	MMF	-14	-4	11	2	1	0.666666667	1	1	0.5	1.1	1	0.523809524
25A	MFF	-15	-4	12	1	2	0.333333333	1	2	0.333333333			
25B	MFF	-15	-6	10	1	2	0.333333333	1	2	0.333333333			
25C	MFF	-13	-5	9	1	2	0.333333333	1	2	0.333333333			
26A	MMFF	-15	-4	12	2	2	0.5	1	1	0.5	1.8	1.1	0.620689655
26B	MMFF	-21	-12	10	2	2	0.5	2	1	0.666666667	2	1.4	0.588235294
26C	MMFF	-11	-4	8	1	2	0.333333333	1	2	0.333333333			
27A	MMF	-17	-8	10	2	1	0.666666667	2	1	0.666666667			
27B	MMF	-17	2	20	2	1	0.666666667	2	1				

	0.666666667	2	1	0.666666667							
27C	MMF	-14	-9	6	2	1	0.666666667	2	1		
	0.666666667	2	1	0.666666667							
28A	MFF	-15	-11	5	1	1	0.5	1	1	0.5	1
	1	0.5									
28B	MFF	-13	-6	8	1	2	0.333333333	1	2		
	0.333333333	1	2	0.333333333							
28C	MFF	-15	-9	7	1	2	0.333333333	1	1	0.5	
	1	1.1	0.476190476								
29A	MMFF	-14	-5	10	2	2	0.5	2	1	0.666666667	
	2	1.4	0.588235294								
29B	MMFF	-14	-5	10	2	2	0.5	2	2	0.5	2
	2	0.5									
29C	MMFF	-14	-4	11	2	2	0.5	1	1	0.5	1.9
	1.4	0.575757576									
30A	MMFF	-18	-4	15	2	2	0.5	2	2	0.5	2
	2	0.5									
30B	MMFF	-16	-2	15	2	2	0.5	1	2	0.333333333	
	1.5	2	0.428571429								
30C	MFF	-14	-4	11	1	2	0.333333333	1	1	0.5	
	1	1.6	0.384615385								