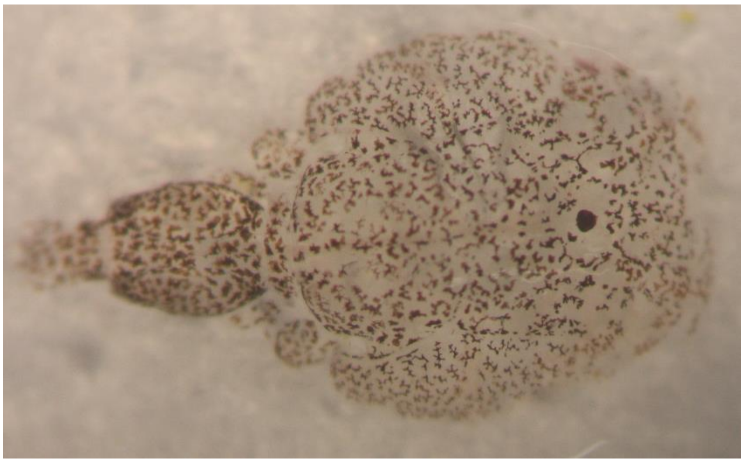
Should I stay or should I go?

The factors affecting male dispersal in an ectoparasite, the salmon louse (*Lepeoptheirus salmonis*)



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Cover photo: Salmon louse photo taken by Marie Hauso.

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For the reader, parasites are so much fun and I hope in reading this paper you can see why. I will at least never stop learning about this cool varied group of organisms.

Bergen, June 2022

Marie Hauso

Abstract

In sexual organisms where females invest more than males in each of their offspring the reproductive success of males is often limited by access to females. This selects for different reproductive strategies, where females are often choosy, and males often use more energy than females in finding, and competing for, the other sex.

Many parasitic species reproduce sexually in or on their host, which represent a very patchy habitat. As migration among hosts entails a risk of starvation, and given that females are more dependent than males on a constant food supply to produce propagules, males should be the dispersing sex in sexually reproducing parasites. The effects of the parasitic salmon louse (*Lepeoptheirus salmonis)* on salmon (*Salmo salar*) have been extensively studied as they pose a problem for the salmon farming industry and threaten wild salmon populations. Previous experimental studies have had little focus on the behavioural ecology of salmon lice, in part due to the difficulty in individually tracking individual parasites.

In the present project I have investigated male salmon lice through different experimental and correlational set ups to understand aspects of their dispersal and mating behaviour. The main hypothesis is that males should disperse among fish hosts based on female availability on their initial host, as well as perceived female availability on alternative hosts present nearby. I predicted that males should disperse more when the sex ratio on the fish is high, and that they should disperse based on pheromone scent in water. Moreover larger males should have more energy reserves and therefore be more successful at both dispersal and guarding their females (as they cannot eat while mate-guarding). The main part of this master's project is the pheromone experiment, combined with correlative approaches to explore the other predictions.

I found no significant relationship between sex-ratio on either the initial host or the host of destination and male dispersal. Pheromones alone might not be enough to promote dispersal in males, although there were signs that males might disperse towards pre-adult females. Neither male body size nor reproductive investment related to male dispersal. Lastly mate-guarding behaviour costs time and energy and some less well-nourished males do not stay attached to the female until she reaches adulthood.

Introduction

Behavioural ecology is the study of interactions between an organism an its environment from an evolutionary perspective (Seymoure et al., 2021). The evolutionary study of behaviour looks at the "why" questions by analysing the costs and benefits of an action in terms of *fitness*, or *selective value* (Davies et al., 2012). Tinbergen formulated a framework to address biological questions (Davies et al., 2012), distinguishing between proximate and ultimate factors. Proximate factors include how an individual comes to behave in a certain way over the course of their lives (Davies et al., 2012). Ultimate factors are the ones underlying the adaptive costs and benefits and therefore explaining why an individual has evolved a particular behaviour (Davies et al., 2012).

All organisms face challenges in surviving and reproducing, and species that reproduce sexually have the additional challenge of finding a mate. In species with separate sexes the reproductive success for females is often found to be limited by their access to food resources, while that of the males is often limited by the number of females they can mate with (Trivers, 1972). This may select for sex-biased dispersal[1], whereby one sex will tend to stay in the same area as long as it does not run low on resources, while the other sex will disperse and thus gain access to mates elsewhere. Dispersal can bear a cost in terms of energy or time expenditure, risk, and lost opportunities (Bonte et al., 2012), but on the other hand it can have benefits in terms of inbreeding avoidance and increased access to mates (Clobert et al., 2004).

Animals use a range of cues to signal their presence to potential mates or locate them, assisting in dispersal behaviour. Visual or olfactory cues, temperature, and vibration are all stimuli perceived by terrestrial (e.g. insects) and marine arthropods and that can change their behavioural output (Matthews, 1998). Copepods in particular use semiochemicals to interact with their environment and with conspecifics (EL-Shafie & Faleiro, 2017). Semiochemicals diffused in an aquatic environment have been shown to influence aquatic life immensely (Martin & Hine, 2008). They can modify the behaviour of the receiver, in contexts related to finding a mate, a host or avoid enemies (Sharma et al., 2019). Semiochemicals include pheromones (Martin & Hine, 2008), and in this text both wordings will be used.

[1] Dispersal encompasses all movements of individuals or propagules with potential consequences for gene flow across space (Ronce, 2007).

Parasitism[2] is a lifestyle that has evolved many times (Bush et al., 2002) and a large majority of all living species are parasites (Schmidt-Hempel, 2021). Parasites are an extremely diverse group and there are parasitic species in almost all classes of organisms. They possess highly adaptive features that enable them to infect and exploit their hosts, which in turn selects for immunological and behavioural defences in their hosts (Grabda, 1991).

Parasites live in typically patchy habitats (their hosts). Because of that their spatial distribution and mobility will depend on those of the hosts that carry them around. But this may not be the only way for them to disperse. In particular, a number of species called ectoparasites living at the surface of their hosts (as opposed to endoparasites) have kept at least in some life stages the capacity to move and even switch hosts, provided alternative hosts are nearby. This may prove beneficial in cases when the host's quality decreases, its immune response compromises the survival or reproduction of parasites, or when host survival itself is compromised.

Migrating from host to host may be particularly beneficial for parasites with separate sexes. The fitness gains of inter-host dispersal may be considerable, because at low parasite abundances not all hosts will bear members of both sexes, and even if they do, males may gain significantly higher fitness by fertilising females present on more than one host individual. The potential fitness costs of dispersing may also be particularly high for parasites, because most of them cannot survive without a host. Given the high gain / high cost potential of dispersal for parasites one can expect to find clear adaptations in parasites for dispersing based on cues that reliably indicate the presence of other fish or members of the opposite sex nearby. Furthermore, for parasites females are often highly fecund, and thus strongly depend on a constant supply of nutrients from the host. It can therefore be expected that males would be adapted to disperse more than females. Sex-biased dispersal has been documented in a broad range of species. Notably, male-biased dispersal is widespread in mammals while dispersal in birds is mostly female-biased (Zwahlen et al., 2021: Trochet et al., 2016). There is to date, however, no study documenting sex-biased dispersal in parasites. More generally, little is known about the behaviour of parasites, due to the notorious difficulty in marking and tracking them, and to the traditional way of seeing them as "one entity" when in fact they are populations made of individuals.

[2] Parasites utilizes another organism (host) as habitat and depends on it for resources, a type of symbiosis that benefits the fitness of the parasite and reduces that of its host (Raffel et al., 2008).

Here I will study, using correlative and experimental approaches, some of the mechanisms that may be involved in dispersal in an ectoparasitic copepod, the salmon louse *L. salmonis*, as part of a larger study where individual parasites are being identified and marked.

L. salmonis was previously thought to spend their entire life on the same host (Wootten et al., 1982). Recent behavioural studies of salmon lice carried out at University of Bergen (UiB) have, however, shown that males are the dispersing sex, while females tend to stay on the same host throughout their adult lives (Timothee Michaux, Master thesis in prep., University of Liège, Belgium). Males become adult about one week earlier than females and start guarding immature females by firmly hanging on to the female's abdomen with their mouth parts. This stage when the male is clasped on to the female is called "precopula" (Boxshall, 1990) when the female becomes an adult the male grasped on mates with her. The higher the sex ratio (proportion of males versus females), the earlier the onset of mate-guarding. Males seem to leave their fish host shortly after females have reached adulthood, *i.e.* once females on the fish are fertilised (Steinar Trengereid, Master thesis, 2020, UiB). By leaving their fish host, however, males take the risk of not finding another one, or of finding another fish without any female salmon lice on it. One should therefore expect that male salmon lice base their decision of jumping on cues indicating the nearby presence of other fish hosts bearing fertile females. This seems confirmed by observations made in the lab, where we see that males jump more often when there are more fish gathered in the same tank (Timothee Michaux, Master thesis in prep., University of Liège, Belgium).

The salmon louse (*Lepeoptheirus salmonis*) is a threat to both the salmon farming industry and to wild salmonid populations. In the past 40 years aquaculture has been developed massively worldwide, especially in Norway where the aquaculture industry is the second biggest industry in the country. The production of farmed Atlantic salmon (*Salmo salar*) has increased from 160 to 720 metric tonnes per year between 1996 and 2015. Salmon farming is done using floating cages in the fjords and along the coast (Pike & Wadsworth, 1999). This stationary cage setup containing fish hosts all year round represent ideal conditions for parasites to grow in large numbers, causing an estimated cost exceeding 300 million euros annually to the farming industry. In addition, marine cages are not isolated from the rest of the marine environment. The huge amounts of parasitic larvae constantly released from farms into the sea also represent a major threat to already declining populations of wild salmonids. Young salmon coming out at sea cannot survive high parasite loads acquired from having to swim through the farmed coastal areas (Hayward et al., 2011). The primary control measure so far had been the use of chemotherapeutants, but unsurprisingly triple drug resistance has now become a major issue. Therefore, basic research using salmon lice behaviour is encouraged in the hope to gather general knowledge of potential use for biological control. It is crucial to protect wild salmon as the population almost faced extinction due to salmon lice on smolts and escaped farmed salmon (Sægrov, 1997;Barlaup, 2013). Norwegian fish farms have obligations to report salmon lice found and if lice load exceeds criteria treatments against the parasite is needed (Heuch & Schram, 1999; Ministry of Trade Industry and Fisheries, 2012).

It has been suggested that finding a way to disrupt semiochemical signals in fish farms could solve the salmon lice problem in a more environment-friendly method as pheromones are not harmful to the environment (Sharma et al., 2019). Chemical camouflage and competitive attraction are two mechanisms that could be effective (El-Shafie & Faleiro, 2017). Competitive attraction is male responding to an artificial pheromone instead of a calling female, while camouflage is the process of hiding the natural chemicals from a female by saturating the environment by artificial pheromones rendering the male unable to determine female location (El-Shafie & Faleiro, 2017).

Aim of study

In this study I investigate experimentally whether chemical cues in the water affect the dispersal of salmon lice males through two different setups. I also tested for correlations between the dispersal and morphology of males and their mate-guarding behaviour. A core aim of this study is to identify some of the factors that may affect male reproductive decisions. To do so I addressed four types of questions.

1. Sex-ratio influence on dispersal patterns

The hypothesis here is that males should base their dispersal decisions on the relative availability of females on their host of origin compared to the host of destination. This will be done by examining whether the propensity of males to leave their host is related to the sex-ratio on the fish of origin, or the destination fish, or both. Male lice will be identified, and their movements among fish hosts in the same tank tracked from one week to the following one. The prediction is that they should tend to stay on their host if the sex ratio is low (i.e., low proportion of males to females), and when they jump this should be towards a fish with a higher number of females.

2. Effect of pheromones in water on dispersal

The hypothesis here is that male *L. salmonis* should base their decision of jumping on olfactory cues, namely pheromones present in the water, indicative of the presence of nearby fish carrying virgin females. To do so I performed an experiment where I recorded the propensity of unpaired male lice to leave their fish host when exposed to water corresponding to various conditions (fish / no fish, with / without female salmon lice, fertilised / virgin adult or pre-adult females). The prediction here is that males should jump more often when exposed to water coming from tanks with female sea lice, especially if these females are unfertilised

3. Relationship between male body size and dispersal

The hypothesis here is that males of different qualities (reflected by their body size) differ in their propensity to disperse. In a previous study a trend was found for males detaching from their fish being smaller than those remaining on the fish during the short period when mating occurred (Steinar Trengereid, Master thesis, 2020, UiB). This might indicate some degree of male-male competition on the fish, with the relatively smaller males being kicked out by the bigger ones. Male body size will be measured from a larger set of pictures, as well as their genital size. Taking genital size relative to body size as a proxy for reproductive investment, I postulate that males investing more in reproduction should guard their mate more than those with lower reproductive investment.

4. Male-male competition costs

Here I hypothesise that mate-guarding males pay a physiological cost because they are less nourished than those that do not guard a mate. Males are photographed on the female and until copulation and identified. The prediction is that males should keep guarding the same female until she is adult, at the expense of filling their guts with blood. Additionally, I estimated how long it takes after a blood meal for a male to have an empty gut.

Methods

Model species

The salmon louse (*Lepeophtheirus salmonis*) is the model species of this master thesis. The salmon louse is a species of copepod parasitic on marine salmonids and has a direct life cycle (i.e., needs passage through only one host species to complete its life cycle). Krøyer published a scientific description of the salmon louse in 1837, although its life stages were not fully described before 1993 (Scram, 1993) and were further updated by Hamre et al. In 2013 (figure 1). The infective larvae first develop on the fish while being attached to it by a filament. From the pre-adult stage they become mobile and are both able to move on the surface of their host, and to swim among hosts. This makes it possible to pick them up temporarily for measurements and place them back on their host without damaging them. In addition, like many crustaceans they carry chromatophores, whose unique positions allow individual recognition (Hansen et al. *in prep.*). All in all, this makes them an ideal species for the study of dispersal in parasites.

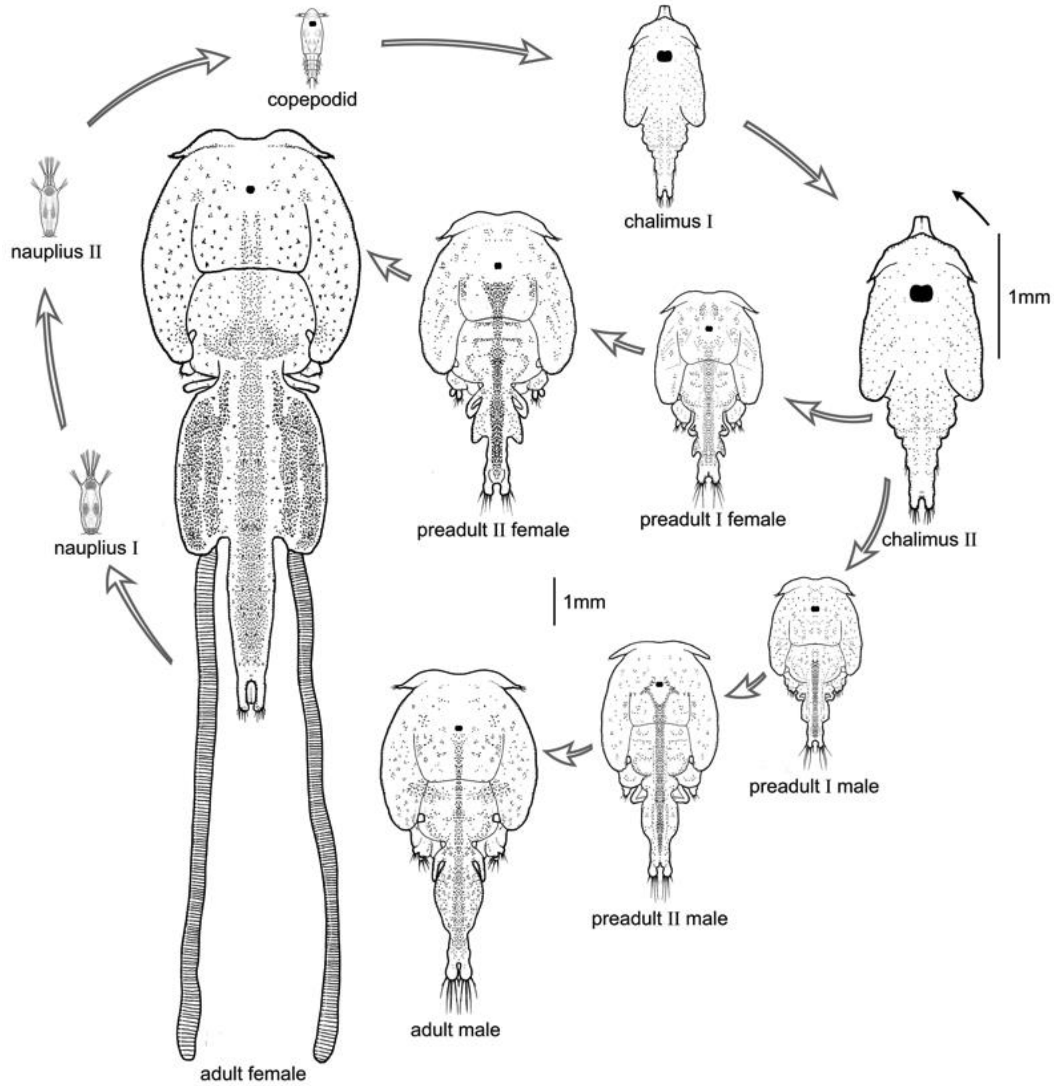


Figure 1. *Salmon louse (Lepeophtheirus salmonis) have eight stages after hatching from egg. The three first stages are living off host, the last five stages are on host. After chalimus 2 stages identifiers of sex are present. Illustration: Sea Lice Research Centre (2020).*

The salmon louse is a dioecious species with sexual dimorphism where females have a larger body size than males. Males also have a varied appendage modified to clasp onto female (Bush et al., 2002). Generally speaking, for most species females make a larger parental investment than their male counterparts (Herron & Freeman, 2014), and this is also the case for the salmon louse. The reproductive success of males is based on access to females, but we can also see their sexual investment based on their genital segment proportion to body size (Brennan & Prum, 2015). Male salmon lice may also have different reproductive strategies based on its habitat's other occupants.

Salmon lice have a tubular mouth, and their digestive tract is a straight tube from mouth part throughout body (Bush et al., 2002). Salmon lice feed from the mucus, the skin and the blood of their host. Females graze intensely, and continuously produce eggs during their adult life (lasting up to several months), and previous studies suggest that their fecundity is resource-limited (Ugelvik et al. 2017).

Type of data collected

During this project I collected data myself for effect of pheromones on dispersal in two parts. "Single host pheromone experiment" and "multiple host pheromone experiment", which was the planned experiment for this master project on behaviour. I also assembled data available from previous studies. For dispersal, size collection and mate guarding I assembled and completed a dataset partly collected by Timothee Michaux (guest student in 2021, size data) and exploited pictures taken for the larger ParAnthropE led by Dr Adele Mennerat (hereafter referred to as "ParAnthropE data collection"). Finally, upon own initiative I set up a small additional experiment to document how long it takes for male sea lice to digest a blood meal and thus infer a potential cost to mate-guarding named "digestion time experiment".

Anaesthesia and handling of the fish

To prevent damages to the fish (stress, escapes) and allow easier collection of lice, all fish that are to be handled or transported are to be under anaesthesia. Anaesthesia is done using a 20L bucket filled with 10L of water from the original fish tank and 1.3 grams of tricaine mesylate or MS-222 (Finquel vet.) mixed into the water. The fish is in the bucket for as little time as possible. Fully anesthetized takes approximately 2 minutes. After the lice are picked off the fish, the fish is placed in a recovery bucket containing plain and fresh seawater.

Photography method and processing

All lice have been photographed in the same method as stated here unless otherwise said in figure text. The camera used is a *Canon E05 800D* with a *60mm ultrasonic* lens. The camera is mounted on a foot and there is a light source (light table) from underneath the samples (Figure 2). The lice to be photographed are placed in a petri dish, on a sheet of wet paper showing scale bares of known length and that has been sprayed with seawater to prevent lice from dissecting. Size measurements were done using ImageJ.

Figure 2. *Camera set up for photographs of salmon lice picked off fish temporarily. Camera mounted on a stand at 45.5cm. A cut cardboard box with a hole for placing the Petri dish with salmon lice is placed under the camera and back lit from an externals source*.

Data collection from ParAnthropE

To address questions 1 and 3 I collected data from pictures taken in the larger ParAnthropE project. I gathered information on sex-ratio, male dispersal, male body size and mate-guarding behaviour.

I identified males from three tanks with five fish carrying salmon lice. Each of these tanks contained lice initially sampled at three different locations and brought back and reared in the lab. This allowed me to trace individual males from one week to the following one (n = 56). To identify male lice I looked at the patterns (location and relative sizes) of chromatophores located on the head, around the eye spots. These pigmentations have been shown to be individually unique (Hansen et al., *in prep*); even though the pigmented dots might slightly change form, they remain at the exact same position throughout adult life (Figure 3).

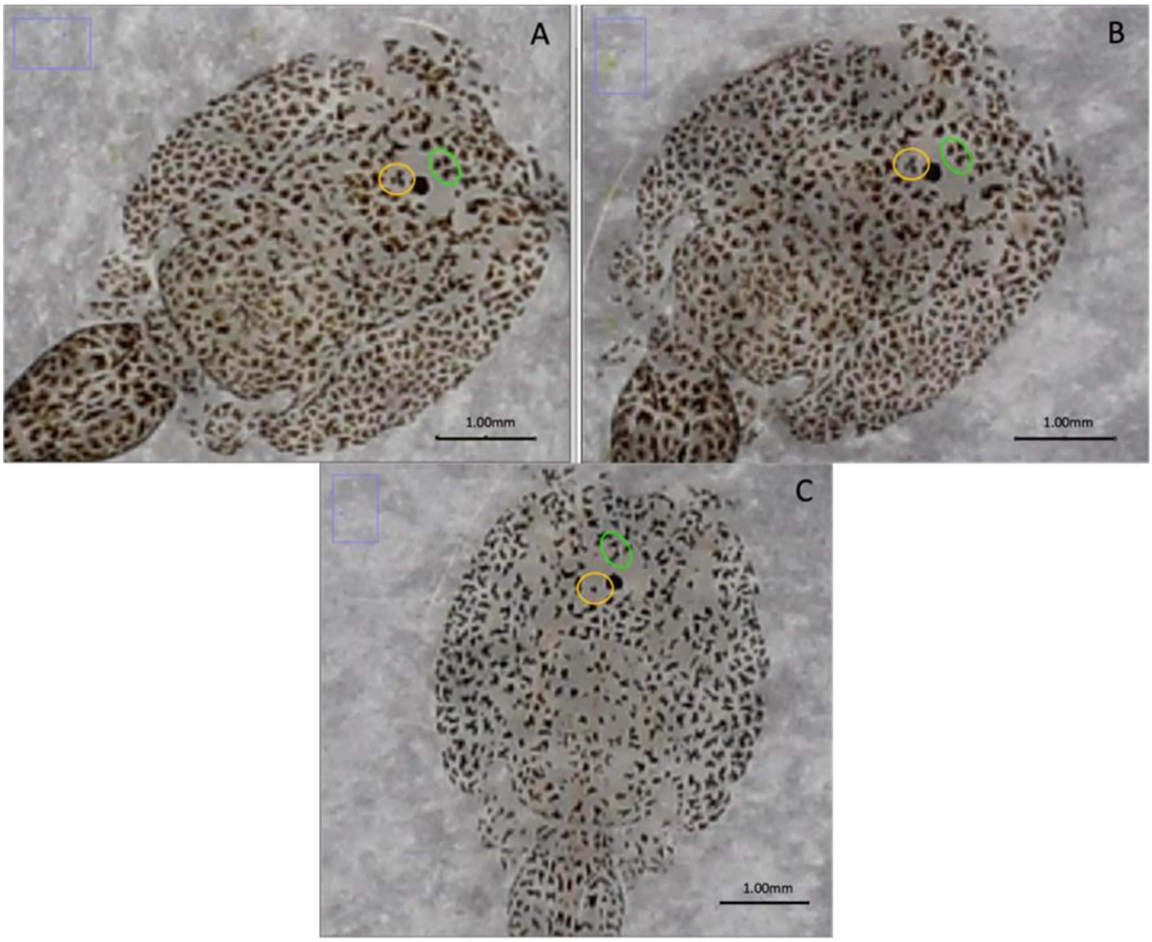


Figure 3. *Photographs of male sea lice spots to process identification. Picture A and B shows the same sea louse with one week between, picture C shows a different sea louse with one week in between from A. Identification is done by looking at pattern around eye spot. Marks in orange and green are done to highlight some of the patterns.*

In addition to identification of individual salmon lice I explored whether dispersal related to body size in male salmon lice I used photos and data from the ParAnthropE project ranging over several weeks. Measured salmon lice were also added to previous work done on dispersal of males, where (n=56) males that lived 6-8 weeks were recorded to be able to find size effect on dispersal patterns. Size data was also collected from all photos from the two week time period (n= 315) that looked at different origins from the pictures were mate guarding behaviour also was assessed, in this dataset, stages of salmon lice were varied, and only adult males was recorded unless the female was mate guarded. The scale of each set of pictures was set using the microchips glued on adult females, and that are 0.5mm wide Body size of male salmon lice is measured as the length of the cephalothorax segment, measured from the centre of the eye spots to the posterior end of the cephalothorax carapace. The length of the genitalia (abdomen) is measured as shown in Figure 4. This process was done to all photos of males in ParAntheropE data collection.

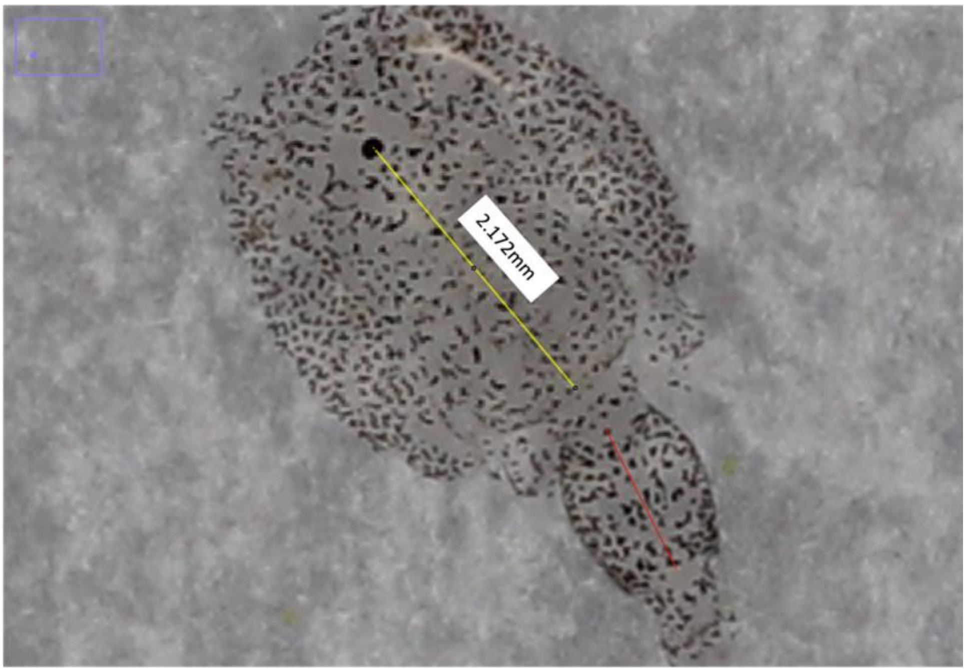
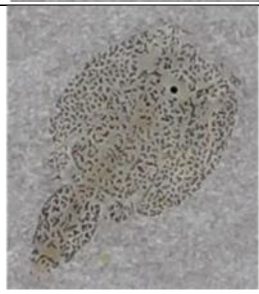
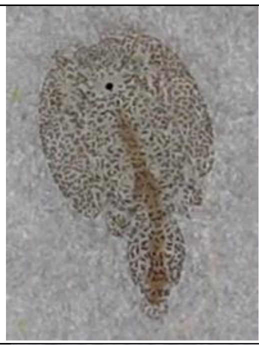


Figure 4. *Example of size measurement of a male sea louse. Body size is measured aa the length from the middle of the eye spot to the posterior end of the cephalothorax carapace (yellow line). The red line shows how the length of the genital segment was measured (red line).*

Analysis of mate-guarding males was done by identification and measurements, but in addition to the processed salmon lice non-mate guarding males received, males and females in precopula were also scored based on nourishment in digestive tract (males mate guarding, n = 156). A scoring system for digestion stages was created and can be read in table 1.

Table 1. *Descriptive digestive state sorted into categories (from 0 – 3) used to determine when nourishment intake has happened last. The table gives approximately where the salmon lice is in its digestive process since blood meal was eaten (category), where 3 is most recent.*

Category	Percent of blood colour present	Description	Example pictures
0	< 5	Gut line: No visible gut line Colouration: No visible colour pigment General: No traces of blood meal eaten, may have slight tint of colour pigmentation on genital segment.	
1	5 - 30	Gut line: Somewhat visible gut line, may be less clear near mouth Colouration: No red colour present General: May or may not be visible without zoom at first sight in lice photos	
2	31 - 70	Gut line: Visible gut line Colouration: brown to reddish General: complete gut line, always visible without zoom on photo	
3	71 - 100	Gut line: Visible gut line Colouration: Red General: Very clear red colour, complete line	

Experimental setup: Effect of pheromones in water on dispersal

Infection method

The copepodites (infective stages) used to infect the fish with were donated from the bigger ParAnthropE project. The larvae produced by five different females whose eggs hatched within the same 3-day window were pooled together. These larvae were 12-15 days old (post-hactching) upon infection.

A total of 20 salmon fish were maintained in two 500L tanks filled with seawater pumped from the harbour and filtered. Infection was carried out following standard procedure. The larvae were pooled in a small bucket and lightly stirred to ensure homogenous copepodite density. 10mL were collected and poured onto a small filter. The sample was then flushed with salt ethanol on to a counting grid to estimate the number of copepodites per mL. We used a dose of 30 copepodites per fish, which usually ensures an approximate number of around five adult salmon lice per fish. Additionally, we temporarily lowered the water level in the tanks for about one hour.

When the female lice were expected to be in the preadult stage (39 days post-infection) all salmon from both tanks was anesthetized and all the lice were removed from all fish hosts. The lice were then placed back on fish so as to separate male lice from female lice. In the one tank containing nine fish only female lice were placed (15♀, 7 pre-adult ♀). In the other tank containing 10 fish all males were placed, together with one female per fish to increase the chance that the males would stay on their fish (5♀, 2 pre-adult ♀, 24♂, 2 pre-adult ♂). Because some of the females had already reached the adult stage, we checked whether they had already been fertilized or not by checking for the presence of a spermatophore under a magnifier at the lower ventral part of the genital complex (figure 4).

Figure 4. *Photograph of two adult female sea lice that have been placed on their back. Difference between A and B shows unfertilized female (A) and fertilized female (B). At end of genital complex before abdomen the male place his sperm package, shown as white substance in female lice B (missing in A). Photo taken with no additional light source.*

This part corresponds to question 2, which was addressed using two slightly different setups, both based on the same principle: male lice were placed on a test fish in a so-called 'response' tank receiving water from various 'treatment' tanks, and their propensity to jump off their host was monitored. The first setup involves fish that are maintained individually in the response tanks, single host pheromone experiment. The second setup tests for similar effects but with two test fish per response tank, one of them initially free of lice, multiple host pheromone experiment. All fish used as 'test' fish in this experiment were taken from a separate tank where they had never been infected with salmon lice (naive fish).

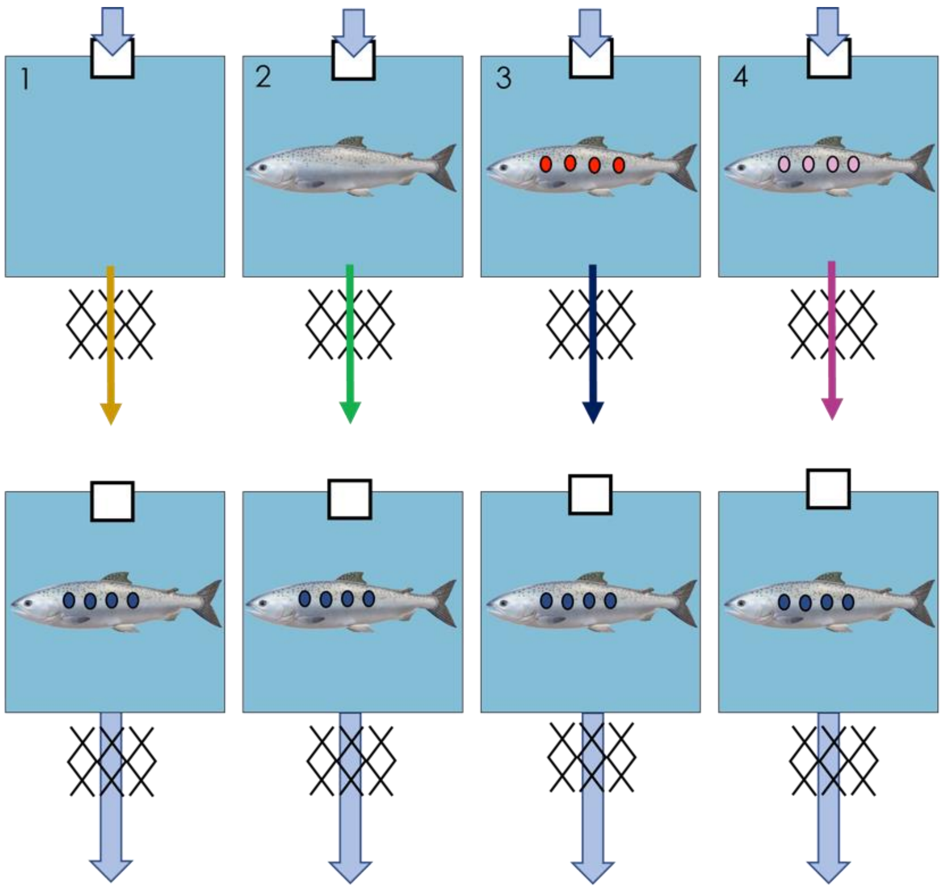
The tanks used for this experiment are flow-through, plastic tanks. The flow of water is continuous, and the water kept at a constant level. The volume of water (50L) is replaced in less than five minutes. Each trial were water flows from a treatment tank to a response tank lasts for one hour. At the end of the experiment, given that there was time left during the daytime was extended for the last trials, to see if longer trial time had affected the results.

Lice used in the experiment are limited to those reared originally, no new male lice were introduced. With first counting of males on fish grown to adult stage 32 lice were counted.

In total 23 males were identified as repeats during the trials (appendix 1) of 29 males in total. Some lice were not identified and was only found once, so they do not count for repeats. Ideally, they should be repeated as few times as possible as handling can stress and potentially damage them. On average each lice was used twice in the different setups at different days. One individual was used in each set up.

First setup, Single host pheromone experiment

To increase sample size in this experiment we monitored four response fish simultaneously. Each test fish in the response tanks initially carried four adult male salmon lice. The treatment tanks were kept on a rack above the response tanks, with a filter between them to prevent lice from the treatment fish from ending up in the response tanks. The fish in the response tanks were presented water from four different treatment tanks: (I) only seawater, (II) one uninfected, naive fish, (III) one fish with four fertilised female lice, and (IV) one fish with four virgin female lice. A coloured water hose system allowed to easily switch treatment and response tanks at the end of each 1-hour trial period (Figure 5A & 5B). Dispersal was counted as success when at the end of 1h, the male salmon lice were either in the filter or free swimming in the tank.



Entry point for water

Filter that catches salmon lice from entering another tank

Salmon lice represented. Pink is virgin females; red is fertilized females and blue is males

Water movement that stays the same thought the experiment

Water movement that is manipulated throughout the experiment

Figure 5A. *Experimental schematic set up for single host pheromone experiment. Top row have the control and treatment groups, bottom row is the response.*

Figure 5B. *Picture of the actual setup in the lab, during one trial. On the floor is the response lice group and treatment in the row above.*

Second setup, multiple host pheromone experiment

During each one-hour trial water from one of the treatment groups flows through one of the response tanks. Thirty minutes after the start of the trial, the placement of male lice on the test fish is noted. After one hour the trial is ended; male lice are counted on the fish, in the tank and in the outlet filter. Then water hoses are switched among response tanks and a new one-hour trial starts. This experiment lasted for two days, with four response fish carrying 16 male lice tested each day. Treatment fish remained the same for the two days, but all test fish were renewed the second day. 11 lice were reused on day two (appendix 1). In total the behaviour of 32 male lice was recorded over two days.

Part two used four different, 500L treatment tanks: (I) eight fish with no lice on them, (II) 15 fish carrying a large mix of adult male and female salmon lice, but also a large number (65-70 individuals) of preadult females, (III) nine fish with adult unfertilized female lice (<22 individuals), and (IV) eight fish with mostly males (n<32) and very few fertilised females (n<10). Two response tanks were used in this setup, with two fish per tank, one of them carrying four male lice at the start of the trial. Each trial lasted one hour and during that hour water from one treatment tank was flowing though the response tank. Tank treatment was chosen at random, but secured that as few patterns as possible was repeated. Each single tank received all four treatments. Lice were photographed at the start of each trial and the ones having left their fish hosts were photographed again at the end for identification. As in the first setup, the location of male lice on the fish was noted after 30 minutes. This experiment was repeated over three days. In total the behaviour of 25 salmon lice was recorded with this setup. Dispersal was counted as success in two ways here, first if salmon lice moved to the other potential host in tank, then additionally if male salmon lice were not on its original host at the end of the hour, this includes then new host, in filter and free swimming salmon lice (figure 6).

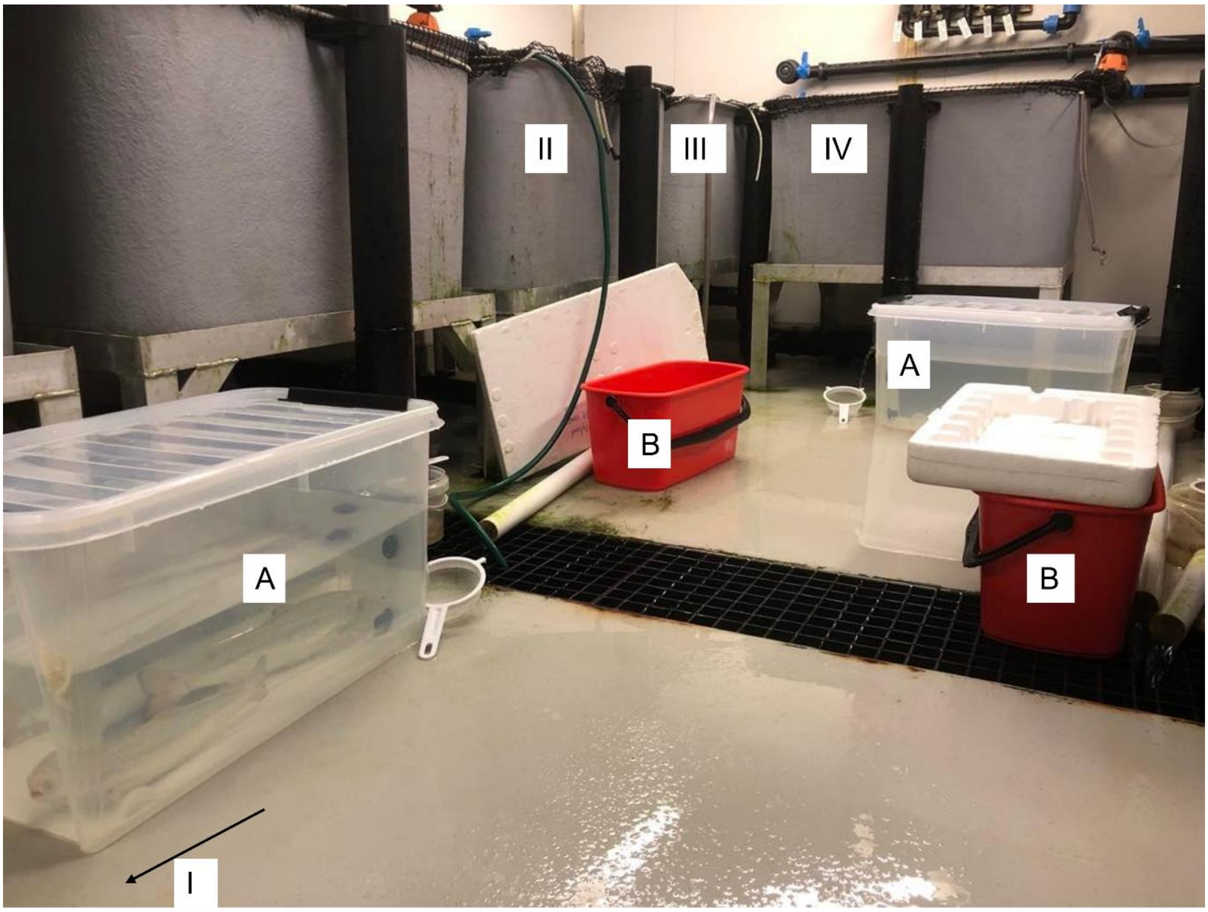


Figure 6. *Experimental set up for part two of the project. Labelled numbers are the tanks used to feed water to the response group. The different numbers represent the different predictors (I,II,III and IV). Tank number I is not photographed in this photo, but is behind the camera and is the same tanks as the three others. Label A is the single tanks with two fish in them that is the experimental group. Label B are anaesthesia buckets.*

Digestion time experiment

To address question 4 and better understand the potential cost of mate-guarding behaviour exhibited by male sea lice, a small addition to this master thesis was added. Salmon lice males (n=6) at 61 days post-infection were taken off their host and placed in incubators individually and photographed each day with 24 h interval (+/-1h). The status of their digestion was recorded from day 1 to day 14.

Statistical analyses

All analyses of data were conducted using R studio version 1.4.1717.

1. Sex-ratio influence on dispersal patterns

Differences in sex-ratio on hosts were measured from ParAnthropE data collection. I used a t-test to compare sex ratio between the first and second week, for those males that moved, with dispersal between two consecutive weeks as a binomial response variable, and numbers of adult males and females on the fish of origin and the fish of destination as covariates.

2. Effect of pheromones in water on dispersal

2A. Single host pheromone experiment

To test whether male dispersal differed among treatments I used a generalised linear model with the number of males having left the fish as a response variable (fitted assuming a Poisson distribution), treatment as a factor, and the number of lice having remained on the fish as a covariate.

2B. Multiple host pheromone experiment

To test whether male dispersal was affected by treatment when an alternative fish host was nearby I used a generalised, linear mixed-effects model with numbers of lice having left and remained on the initial fish as a binomial response variable (success = moved, failure = remained), and treatment as an explanatory variable. The identity of the fish where the lice were initially placed was included as random effect factor.

To assess a potential fish effect I also used a linear model with the proportion of males that moved during the trial as a response variable, and treatment, trial, and fish ID as factors.

3. Relationship between male body size and dispersal

To address this question I tested whether male body length was correlated to the proportion of weeks that individual males had moved among fish during an 6 to 8-week period. Similarly I tested whether investment in gonads relative to body size (a common measure of reproductive investment) correlates with male dispersal.

4. Male-Male competition costs

To explore the potential costs of mate-guarding behaviour in males I compared stomach content in males that were mate-guarding to that of males that were not. I used a generalised linear model with mate guarding status as a binomial response variable and gut content and fish as explanatory variables.

I also monitored daily the emptying of the gut on six male salmon lice after they had been prevented from feeding. The rate of blood digestion was obtained by fitting a linear mixed-effect model with digestion score as response variable, days since last meal as an explanatory variable, and male louse ID as a random effect factor.

Results

1. Sex-ratio influence in dispersal patterns

Out of 46 males that I identified and tracked from one week to the following one, 10 changed fish, 10 were washed out of the tank, and 26 remained on the same fish (Figure 7).

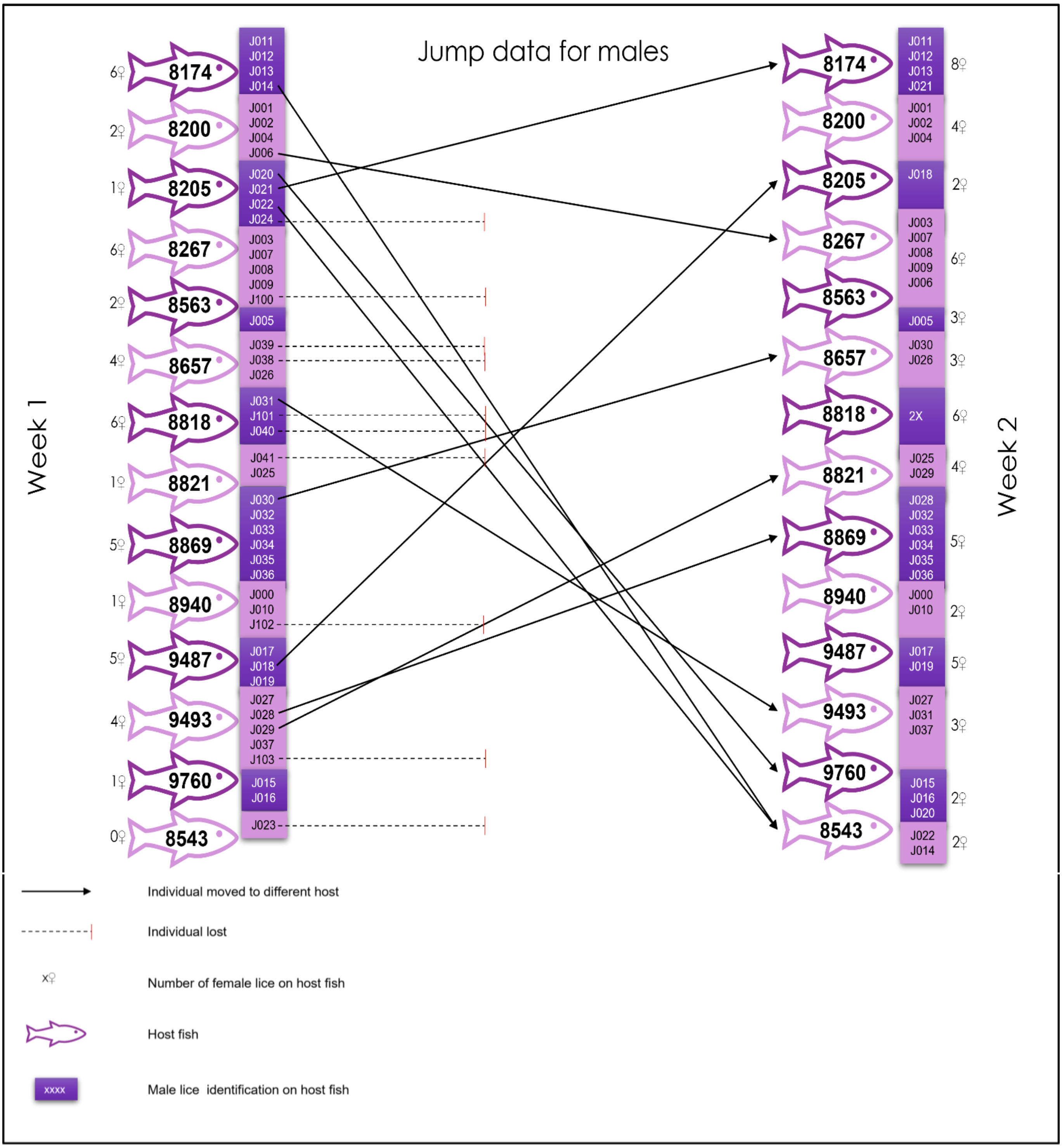


Figure 7. *Individual male sea louse tracking from one week to the following one. Movement is shown with lines between fish. Number of female lice are marked besides each fish for each week. For these two weeks there were no pre-adult males (all males had already become adult). Some females moulted from pre-adults II to adults during that period.*

In all 10 cases when males changed fish, they moved to a fish with a lower sex ratio (lower proportion of males) than their fish of origin (Figure 8; t-test: df = 17, t = 4.08, P = 0.0007).

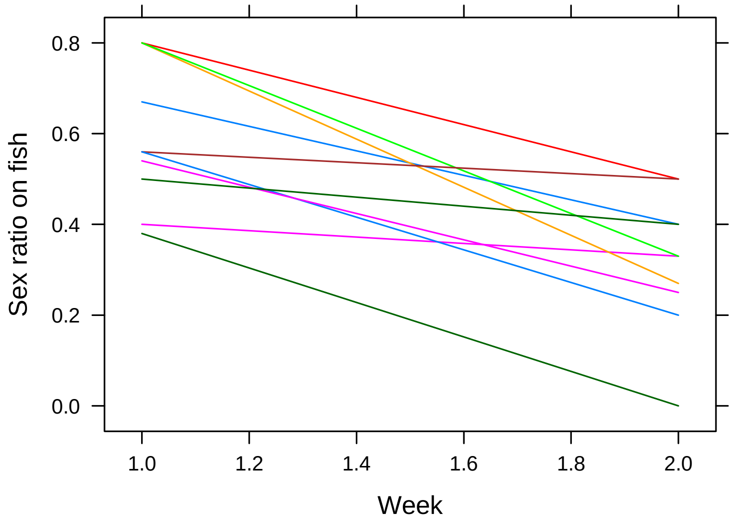


Figure 8. *Those 10 male salmon lice that changed fish went from a fish with a relatively higher ratio to a fish with a relatively lower sex ratio of lice. Each line represents the change in sex ratio for one individual male salmon louse.*

2. Effect of pheromones in water on male dispersal

Experimental setup 1

Almost no leaping was observed using the first setup (figure 9) and thus no significant difference in jumping frequency was found among treatments (ANOVA, P = 0.44). Out of 32 possible jumps that could be done during the two days, only two males jumped, on one occasion each. Direct observations revealed that males were very mobile on their host, even though they did not leave it.

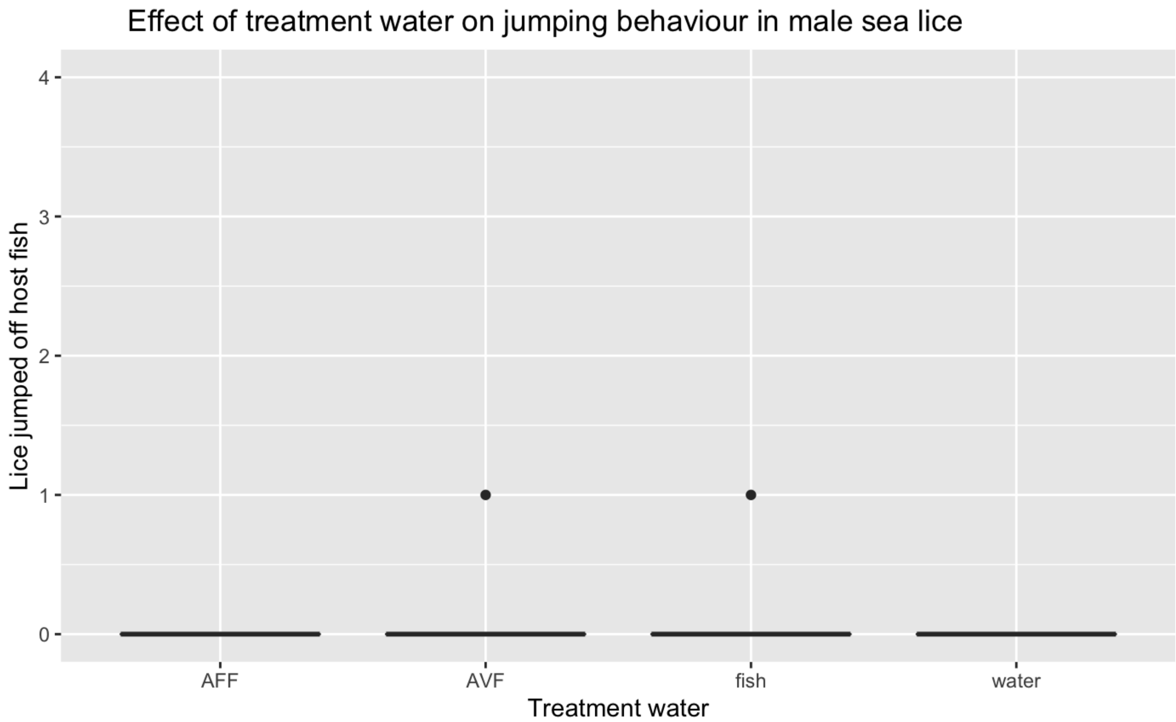


Figure 9. *Frequency of individual male lice that jumped off their host during the first setup (single tank: single fish experiment). The four treatments differed in the contents of the tank where the water came from (AFF = adult fertilised females on fish; AVF = adult virgin females on fish; fish = fish with no louse (control 1); water = .plain seawater with no fish and no louse (control 2).*

Experimental setup 2

Using the second experimental setup host-switching behaviour was occurring at a slightly higher frequency when males were given water from a tank containing preadult II females (Figure 10), although the overall treatment effect among all four groups was not significant (p = 0.852).

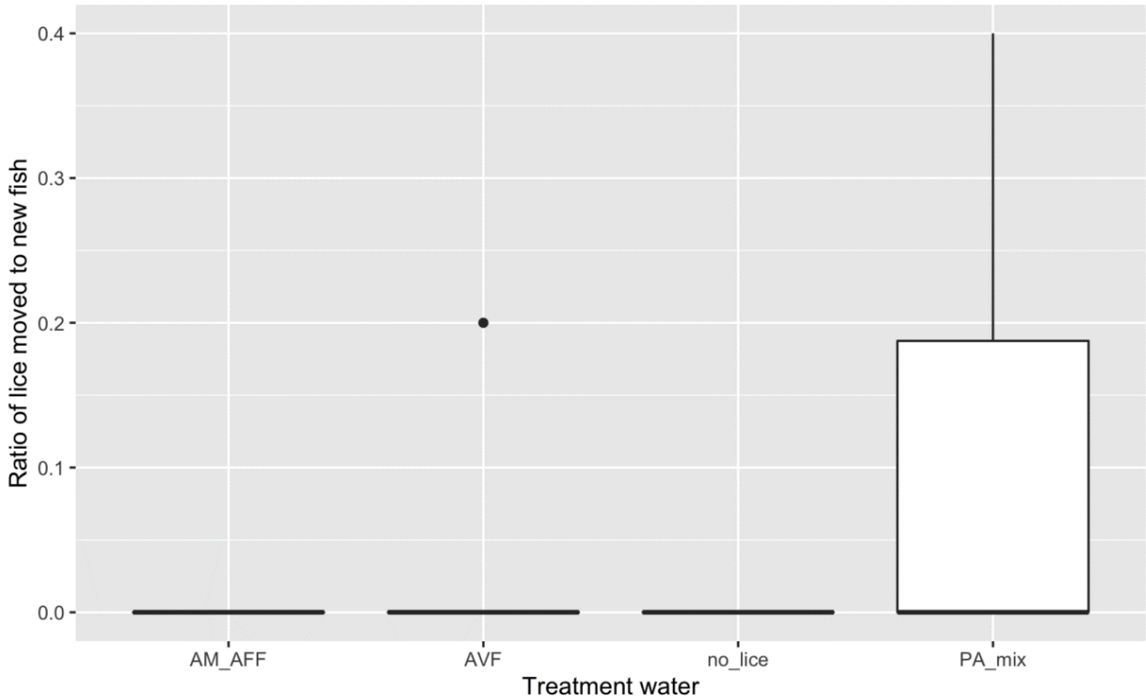


Figure 10. *Frequency of individual male lice that changed fish host during the second setup (single tank: two fish experiment). The four treatments differed in the contents of the tank where the water came from (AM_AFF = adult males and adult fertilised females on 10 fish; AVF = adult virgin females on 10 fish; no_lice = 15 fish with no lice on them (control); PA_mix = mix of adult males and females, and preadult females on 15 fish).*

The frequency of male jumping behaviour increased when defining jumping behaviour as leaving the initial fish, which includes all cases of male lice that fell into filter and those who were found swimming freely or attached to the tank wall. When these numbers are added together, it seemed that the distribution of male jumping was comparable among treatment groups, except for the control water coming from a tank with only fish and no salmon lice (Figure 11). The overall effect of treatment was however not significant.

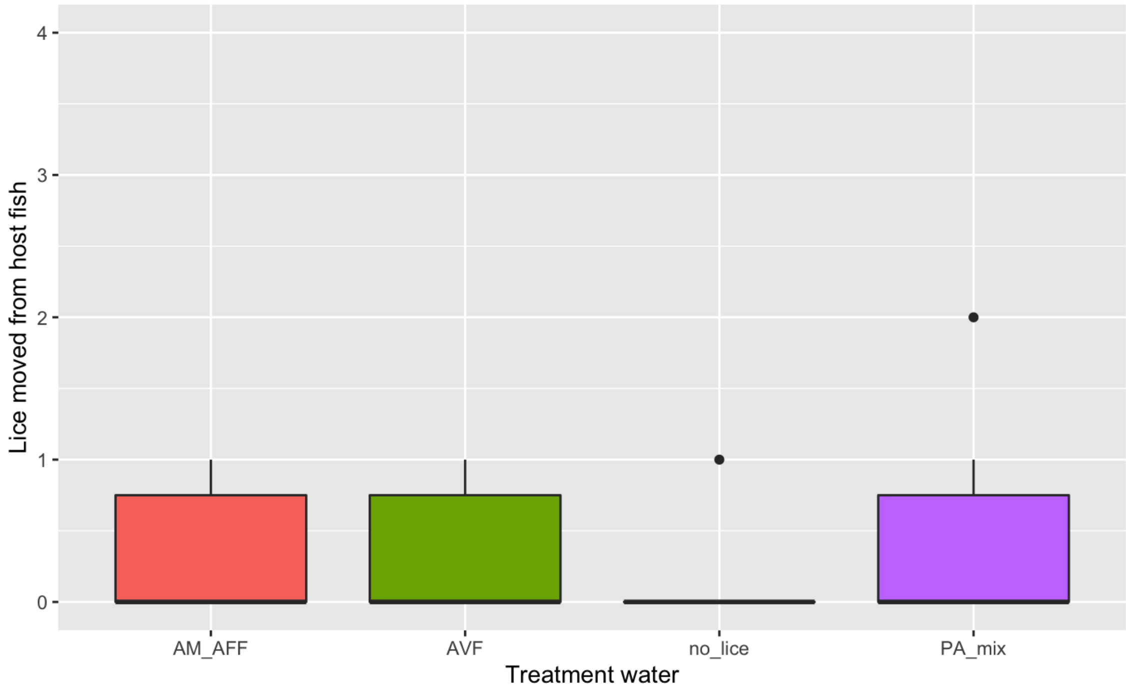


Figure 11. *Frequency of individual male lice that left their initial fish host during the second setup (single tank: two fish experiment). The four treatments differed in the contents of the tank where the water came from (AM_AFF = adult males and adult fertilised females on 10 fish; AVF = adult virgin females on 10 fish; no_lice = 15 fish with no lice on them (control); PA_mix = mix of adult males and females, and preadult females on 15 fish).*

3. Relationship between male body size and dispersal

The size of cephalothorax for males (n=92) ranges from 1.78 to 2.34cm, but is not significantly correlated (p=0.83) to their propensity to move among host fish, here taken as the proportion of males that moved over a period of six to eight weeks (Figure 12).

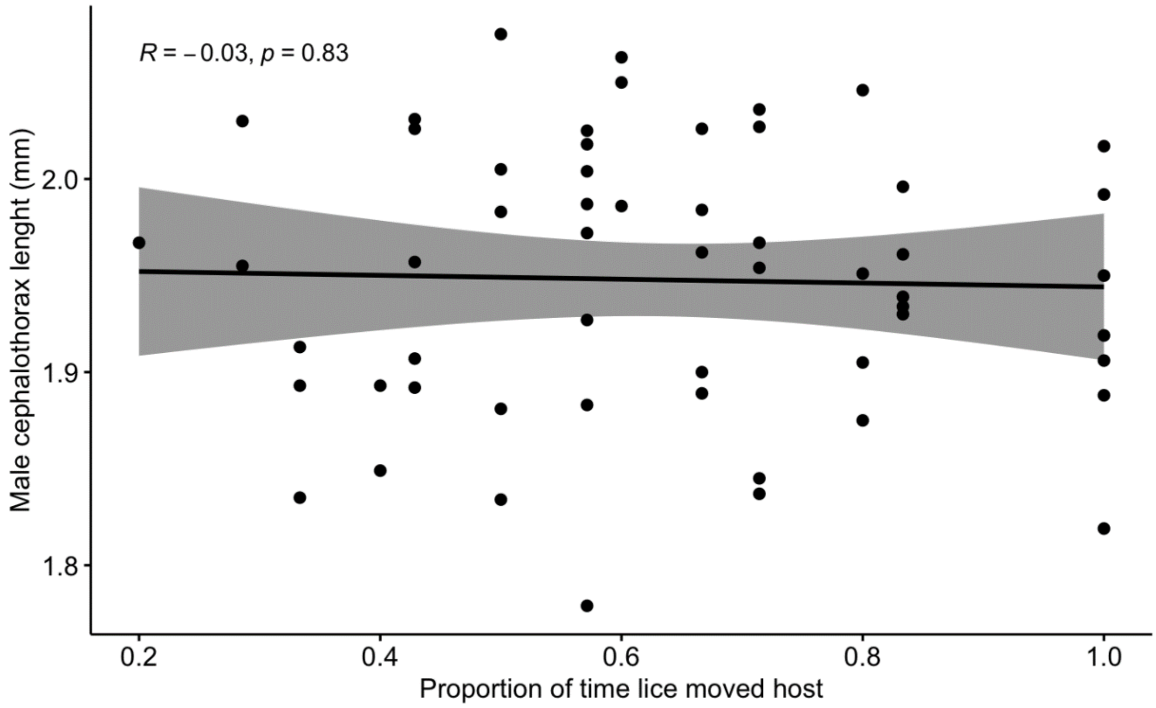


Figure 12. *Proportion of times that male lice moved among fish between two consecutive weeks, during a 6-8 week period (n=92), in relation to their body size (measured as cephalothorax length). The regression line is shown in black and the confidence interval is represented by the grey area.*

Reproductive investment, measured as the size of genitalia divided by body size, tends to relate negatively to male dispersal, but the relationship is not significant (P = 0.33) and the data quite spread out (Figure 13).

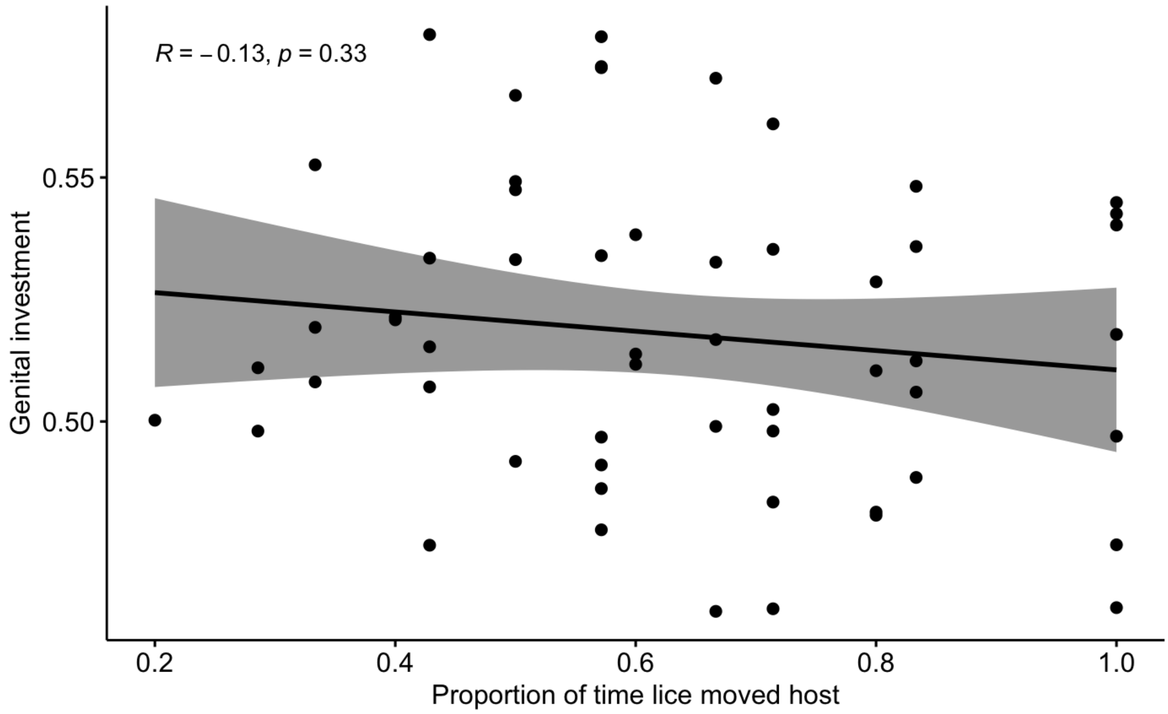


Figure 13. *Proportion of times that male lice moved among fish between two consecutive weeks, during a 6-8 week period (n=92), in relation to their relative genital size (taken as a proxy for reproductive investment). The regression line is shown in black and the confidence interval is represented by the grey area*.

4. Male-male competition costs

Surprisingly most males did not guard their mate consistently from one week to the following one. In my sample few males stayed with the same female more than one week (Figure 14).

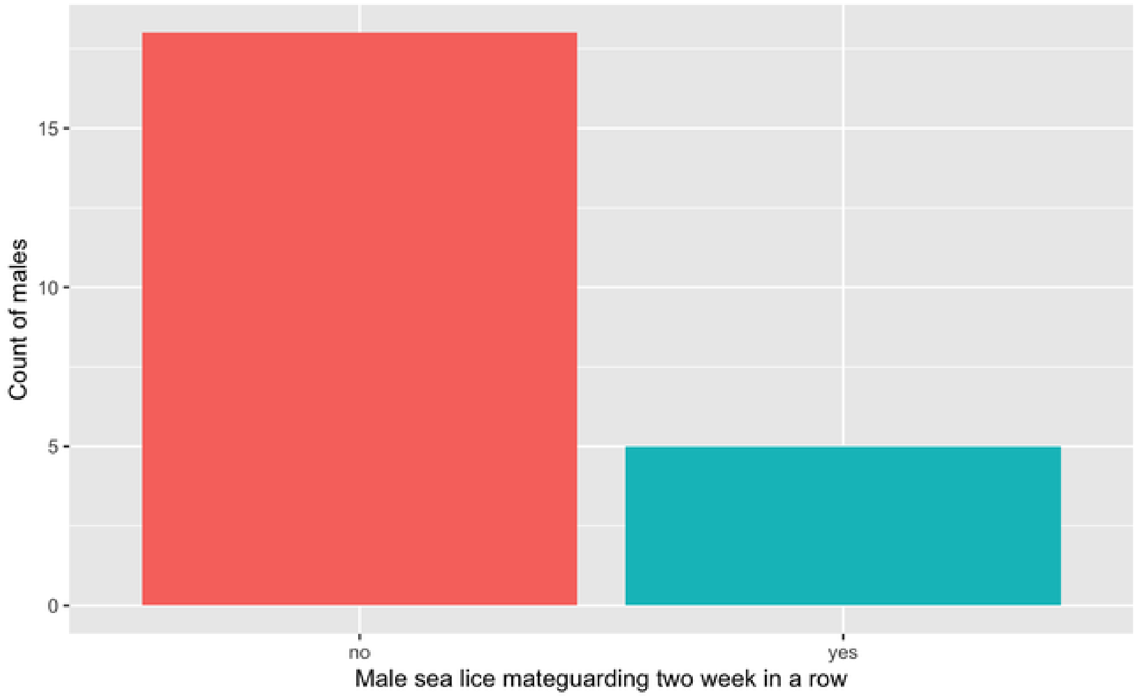


Figure 14. *Distribution of males that either guarded the same mate over two weeks or that did not*.

For each week those males that guarded a mate had a significantly lower gut content score than males that are not (Figure 15, glm, $P < 10^{-4}$).

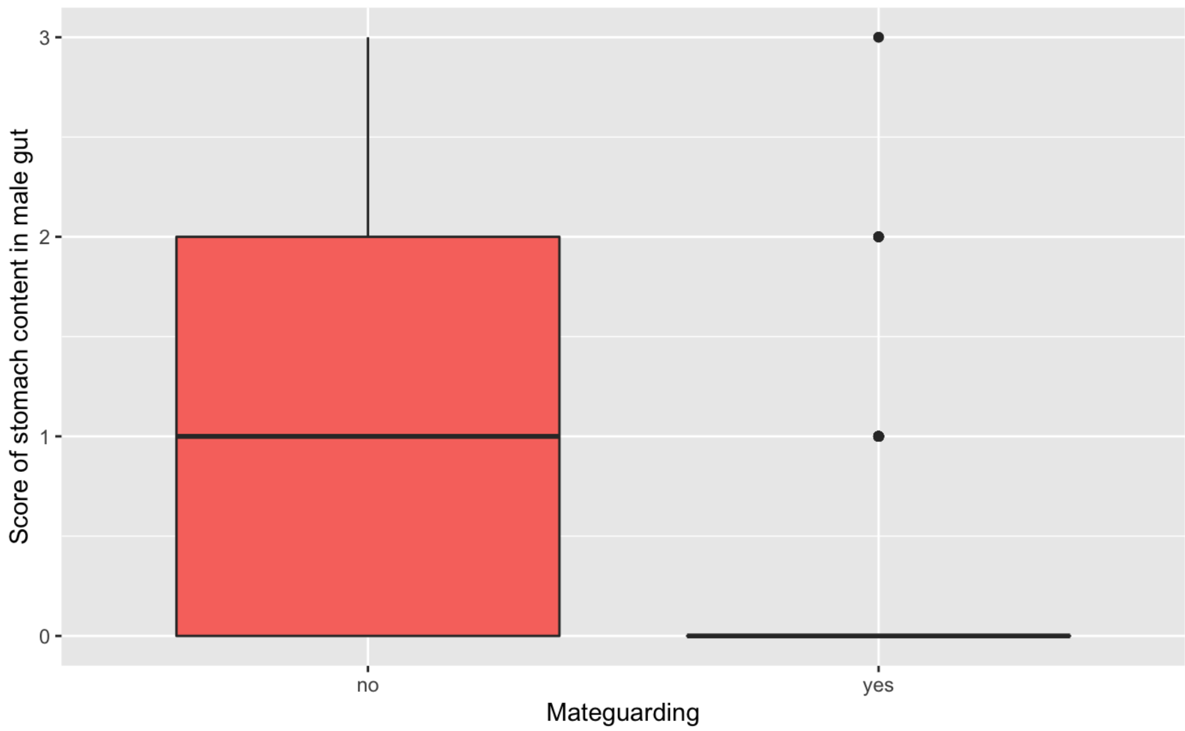


Figure 15. *Gut content score (ranging from 0 to 3) in males that are mate guarding and males that are free swimming.*

There was no significant difference in cephalothorax length between those males that guarded a mate and those that did not (Figure 16, glm: P = 0.51).

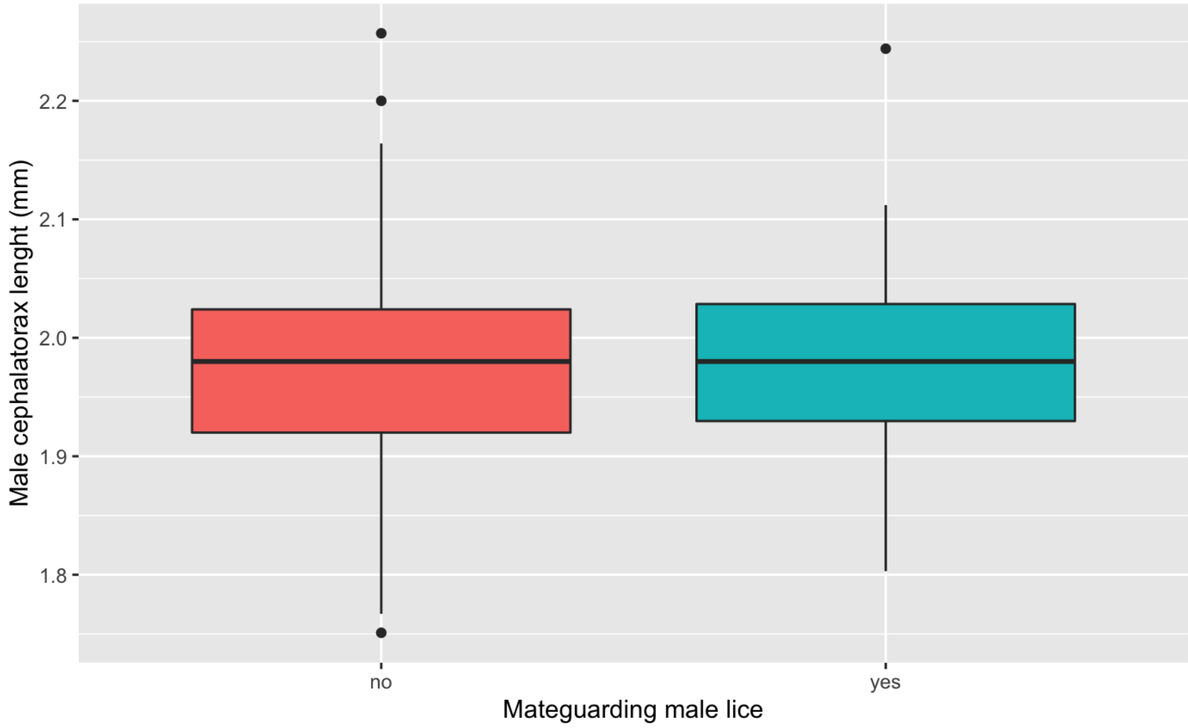


Figure 16. *Cephalothorax length (in mm) of male salmon lice in relation to whether they guard a mate or not.*

The relative size of the genital segment compared to body size was slightly, but not significantly higher, for those males that guarded a female compared to those that did not (Figure 17).

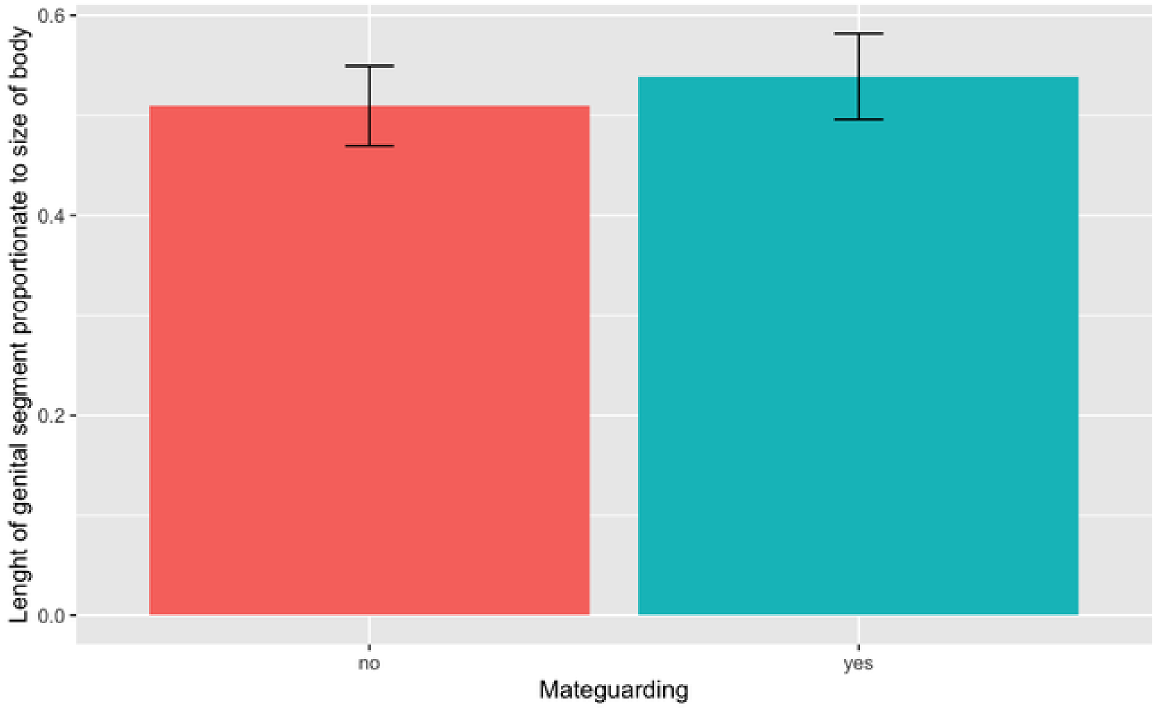


Figure 17. *Relative genital size of male salmon lice in relation to whether they guard a mate or not.*

During the monitoring of gut contents all lice (n=6) either had a score of 0 or had already died by day 12. Two lice survived for 12 days after having been taken off their hosts, and these started their fasting period with a blood meal score of 3. All other lice had an initial score lower than 3 and lasted for fewer days (Figure 18). There is a strong correlation between blood meal score and days since lice were removed of host ($R = -0.66$, $P < 10^{-4}$).

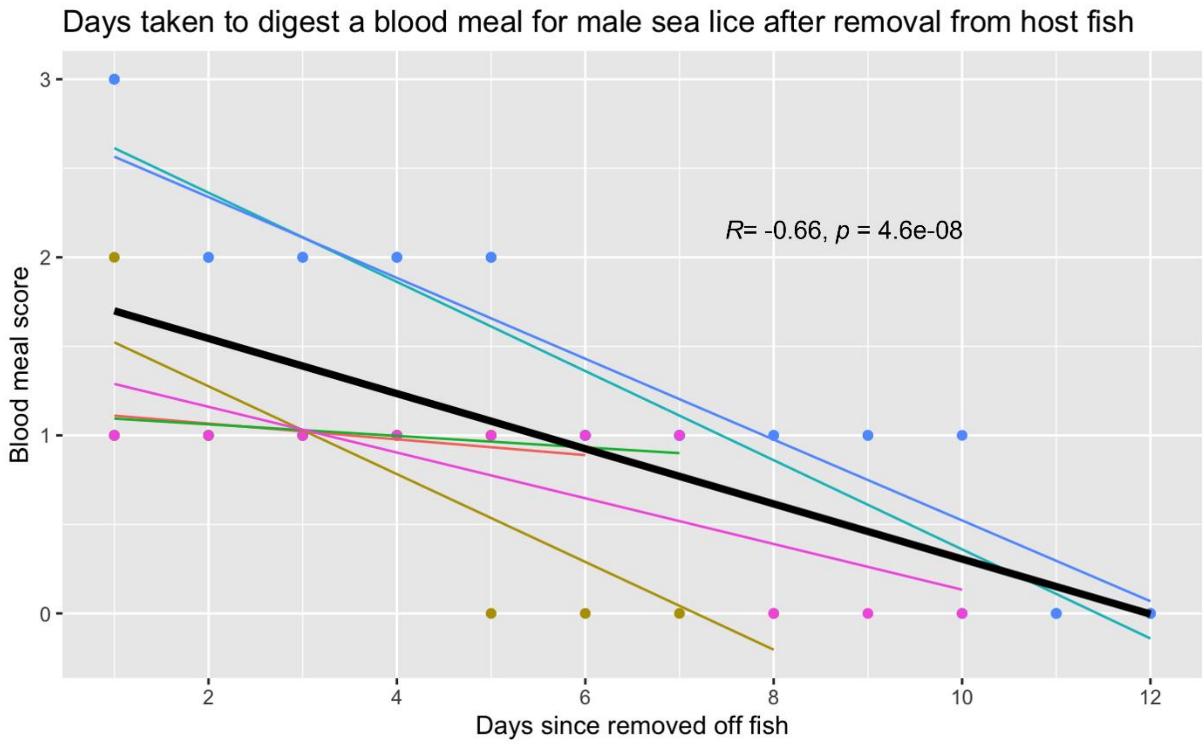


Figure 18. *Relationship between blood meal score of adult male salmon lice and days since taken off the host fish, for six individuals. The thick black line is the overall regression line.*

Discussion

This master's thesis sought to answer four concerns regarding the behaviour of dispersal in male salmon lice. If male dispersal is affected by sex ratio, pheromones in the water and male body size determine the reproductive strategies males should employ. Lastly, consider male-male competition through mate guarding. This was accomplished by the compilation of ParAnthropE data, single host pheromone experiment, multiple host pheromone experiment, and digestion time experiment.

The study had little noticeable difference in jumping off host based on pheromones alone and results were not statistically significant even when another potential host was added to the tank. The study conflicts with hypothesis and a previous preformed study of signals, but had a slightly different method. Males had a sporadic movement pattern on host and on choice of new host, though successful switch always hosted occurred toward a host with at least one salmon louse of the opposite sex. Size in males is normally distributed and seems to have no advantage to either end of spectrum in normal distribution when seeing effect on size in male-male competition and on dispersal. Males that mate guard do not stay on the same female until she is fertilized which contradicts expected findings, and the experiment shows that there is a feeding cost that could weaken the male for those who successfully mate guard. It should be mentioned that it is difficult to draw reliable conclusion from a small data set, and it should have been repeated with a different population of fish and salmon lice to check for repeatability. Nevertheless, the sample used is large enough that one can draw indicating results and find some significant levels although the factors looked at rarely gave significant values of tested effect.

1. Sex-ratio influence on dispersal patterns

Male salmon lice are the sex that mostly disperse to reproduce (Timothee Michaux, Master thesis in prep., University of Liège, Belgium), this is risky for the male, because it risks not finding another host or if it found another host could risk that there were no females on it. For question 1 in this master thesis, I looked at when a male switch host, what is on the other host, and if it stays, what is on the original host?

Studies show that males can sense other potential host, female salmon lice and other male salmon lice (Devine et al., 2000). It responded more on males than adult females (Stephenson, 2012). This was suggested as a way for males to adjust sex ratio on hosts so that it would not switch to a host where reproductive possibilities were very limited due to high number of males (Stephenson, 2012). From data collected on male-female ratio the males who decided to disperse moved toward lower sex ratios. This could be an indicator that males are very good at regulating themselves and knowing the proportion of males on other fish, but in the proportion of the males that did disperse we see that the ratio of males to females have a large variation going from where there were almost only males to going to where there was only females.

To create a greater understanding of this I found that dispersal patterns of salmon lice, and it seemed that fish has a larger effect on dispersal than the salmon lice population. This pattern is supported by previous studies that have seen the same effect of hosts that they have different rates of infection (Uglevik et al., 2017). The host is a living habitat for the salmon lice, it is therefore naturally that it also have different effect on the parasites occupying the skin. Some salmon are naturally more adapted against parasitic attach than others (Bush et al., 2002). Though host might have the largest effect on dispersal patterns, in individually looking at host that has a male-female ratio, fish with few females had more male lice switch host than those who had an adequate number of females. Only fish ID9760, ended up with a higher proportion of males then females. The rest are either same or more females.

2. Effect of pheromones in water on dispersal

After finding no significant effect of treatment water on the dispersal of male salmon lice in single host pheromone experiment, we included another potential host and different type of treatment water (multiple host pheromone experiment) as a variable to see if this would prompt dispersal. It seemed to lead to more jumping, which suggests that higher ratios of lice move to new fish when there is another host present and there are more pheromones in the water and also pre-adult females. However, no significant treatment differs greatly from the other treatments though pre-adults present promote more dispersal. The hypothesis was not found significant and therefore one cannot conclude based on this alone that pheromones decide male behaviour in dispersal.

In Ingvarsdottir et al. (2002) free swimming male salmon lice were placed in a y-tube with sea water and a stimulus for 10 minutes, significant results were found in that pre-adult females attracted males in their direction. Males can detect potential mates in water, strengthen theory of male's detection of chemoreception distinguishing female developmental stages presented by Ritchie et al.(1996). These results were not represented in the experiment in this thesis. Though its been seen that male swim more towards pre-adult females, the male will continue to switch host to look for mates when there are no more pre-adult females (appendix 6). Comparing results with Ingvarsdottir et al. (2002) both experiments used a short time set up, but their salmon lice were free swimming and used extract from water containing female salmon lice. This differs from my set up which used a host and females on another host. Hence potentially more pheromones were present in Ingvarsdottir et al. (2002) experiment than the set up I had, especially in single host pheromone experiment.

From these findings it seems unlikely that disrupting semiochemical signals alone could solve overpopulation of salmon lice in fish farms. From previously then Competitive attraction seems the most optimal to use as that would require a small set up and could collect the males, but method is density-dependent. Camouflage is density-independent, but there is a knowledge gap in knowing if a large amount of semiochemical signals could affect other species around or be effective (El-Shafie & Faleiro,2017). Most critically, we do not yet know all the mechanisms required to change the dissemination of a male salmon louse, or if pheromones from the female are sufficient. As this study do not support that it is.

I hypothesized that when in contact with pheromones from an unfertilized female salmon, the male lice would be more prone to dispersal from their original host fish to a new host fish. My study did not find strong support for this hypothesis. Instead, I observed movement of lice on the original host, which could indicate that the male lice kept searching on its original host for a mate instead of moving to a new host, and given more time they would understand that the female was not on their host (appendix 3).

Methodological considerations

An advantage of our experimental approach using single tanks is that the activity of individual lice can be recorded in a highly controlled environment. In addition these observations can easily be handled without handling the fish, and the procedure is relatively easy. In the planning phase for this experiment we discussed that salmon might be too large to comfortably stay in single tanks without them being restricting. Yet having them stay in larger tanks with other salmon would cause more difficulties in recording and gaining information needed. It was therefore decided that temporarily staying in the single tanks could be possible as long as it never lasted longer than 48h, and ideally less. Staying too long in an unknown environment and not being able to adapt due to size would most likely create discomfort for the fish, which might then respond with stress. It was therefore decided to give fish in the treatment group significant time (early morning, before experiment started) ahead of the start of the trials to calm down, but not long enough to discomfort the fish too much by keeping it in a single tank.

The salmon with the response (male salmon lice on host) had less time in single tanks and was never more than 8h in the single tank before transported back to its original group. Stress might therefore have been more prominent in the first hour as it had just been anesthetized for transfer. Perhaps for further studies smaller fish and more time should be added. The fish was also handled by me whenever a salmon louse needed to be placed back on, although all fish received this same type of slight handling before next trial started, and this should likely not bias the results.

In the second setup with two hosts per tank, the treatment group stayed in their original, bigger tank and experienced less stressors because of that. Here the treatment groups have been handled slightly different. Four treatment tanks were in use, of which three experienced slight stress before the experiment started by some of the fish being removed from those tanks to be used in the experiment, and some being anesthetized to collect parasites. The fourth tank (containing preadult females) is a part of a larger experiment in ParAnthropE and from that tank I only borrowed water without disturbing the fish. All in all, our setups though not perfect are unlikely to have caused any bias in our results, and it thus seems that males were more enclined to jump towards the scent of preadult females.

3. Relationship between male body size and dispersal

Salmon louse size have been found to be plastic and vary with temperature (Nordhagen et al,. 2000). Additionally female salmon lice seem to have changed strategy in reproduction when in salmon farms versus wild salmon, they produce more eggs in first clutch then females not affected by salmon farming areas, but have lower survival rate later (Mennerat et al,. 2017). Will size in males change behaviour when all have been reared at the same temperature?

This study found no correlation between male body length and proportion dispersal from week to week (6-8 weeks). Average length of male remained approximately the same. Size was also looked at from the perspective if length varied based on weeks they could be recorded (1-3 weeks low and 6-8 weeks high) in appendix 4. Here similar results were found. There is no significant difference between the average length of those who survived 6-8 weeks and those who did not. These findings are unexpected as it has previously been found that smaller males might be less successful in dispersal (fall into filter) and that might be that they have to disperse due to being out competed by males that are larger on its original host (Steinar Trengereid, Master thesis, 2020, UiB). This was not found either as male-male competition can be represented in part through mate guarding and the size average for males that mate guard and those who do not are the same. Only minimum and maximum differs from the two, were non mate guarding males have more variation in size than those who mate guard. Length of males therefore in this thesis do not seem to affect dispersal or competition on host.

Where size did have a slight variation was in males' reproductive investment. Though not significantly so. Males that mate guard a female have a slightly larger genital proportionate to size then those who do not. This can be further supported by the correlation plot on genital investment on proportion of dispersal from males. There we see that it's a weak negative correlation, although not significantly so. These findings might suggest that length of males is not linked to any of the tested behaviours.

4. Male-Male competition costs

Once a male salmon louse had gripped onto a pre-adult female, I hypothesised that males would remain attached to the female for the rest of the mate guarding period, until the female became an adult and male salmon lice could fertilise her. One unanticipated finding was that males do not always stay grasped on to female until she has reached adulthood. The thought is that it's inefficient for the male to spend time clutching to the female if there is no competition because it prevents him from finding food or other potential mates during that period of time led to the development of the hypothesis. The finding was unexpected and suggests that mate guarding might have a bigger cost to the male then first anticipated. Mate guarding is an evolved behaviour to monopoly access to females, an outcome from male-male competition (Davies et al., 2012). A male must decide if it is to mate guard and use time and resources to secure high chance of paternity or copulate with already adult females. Mate guarding is a fitness trade-off for the males. Time spent mate guarding could be spent copulating with another female (Komdeur, 2001).

Though very interesting results, it could be misrepresented. Females could only be identified if not moulted, hence some of those that mate guarded in week 1 could have stayed on until female reached adulthood copulated and then was not mate guarding in week two because it had been successful (appendix 5). By mate guarding the male has a high chance of being the father of the female he has clutched onto, but could miss out on more mating's with already available adult females. From this it has been suggested that males mate guard for longer when more competition for females are present (Steinar Trengereid, Master thesis, 2020, UiB). What could disrupt the hypothesis is handling method of the salmon lice. They are picked off its host and photographed, this might put extra strain on the male to hold on to the female during this time. Parasitic copepods are a very diverse group, some morphological changes have been adapted to its parasitic life style. It has specialized in hanging on to the surface of its fish host using its cephalothorax which is concave and can help to stay on the surface, but it also uses antennae (Bush et al., 2002). The part collected in ParAnthropE should be repeated with improvements to ID to female and not disrupt precopula by removing them temporarily off host.

Lice are less active and mortality rates are higher if lice stays off a host for more than 7 days (Ingvarsdottir et al., 2002). Hence risking mate guarding a female that will spend more the 7 days to reach adult stages might be too costly for a male salmon louse as his own mortality increases greatly and the cost of risking mating only once to the mate guarded female or let go and feed before mating with other potentials mate is a cost the male needs to weigh against each other. Males that mate guard do not have as high a score in stomach content as those who do not. This is expected results as they are clasped onto a female and can not feed during this period. Males digest their blood meal inn less then 12 days. After that they do not have more nourishment and become weaker until a new meal have been had. There no surprise that there was a strong negative correlation between blood meal score and days since salmon louse was removed off host. This could produce that it is indeed costly for males to start mate guarding and especially if they have to do it earlier due to strong male-male competition. For improvement to the digestion time experiment set up it would be interesting to be able to include only those who started of a score on 3 and have more males included, but this was only a small part of this master thesis and more resources into this part of the project was not available (lack of males). All males used in this set up was also males that had been included in the larger pheromone experiments, that could have been an additional stressor that should be avoided.

In pheromones effect on dispersal either data collection used found no significant to the treatment. Although this study focuses heavily on pheromones from females on dispersal in males, the findings may well have a bearing on the complexity of male behaviour on dispersal, as it conflicts with literature (Ingvarsdottir et al., 2002) findings on pheromones alone when salmon lice are not on a host.

Conclusion

The present study was designed to test the extent to which male dispersal relates to mating opportunities, and complete our previous knowledge on mate guarding behaviour in male salmon lice. I found indication that males might detect the presence of females on other fish, as shown by their dispersing to fish with lower sex ratios. Pheromones released from females to signal males are perhaps not reason enough alone to disperse. Pheromones do, however, create movement and some dispersal off males. Male dispersal does not seem to be linked to their phenotype (body or genital size). One of the more unexpected findings from this study might be that males do not guard consistently until the point when females are adult and can be fertilised. This might be explained by the need for foraging as their guts seem to be empty after a few days.

This has been the first attempt to examine the complexity of male dispersal in a parasite species. Despite relatively small sample sizes I find intriguing patterns that would have deserved further study, given more time. This study could have large implication for our understanding of parasite behaviour, and additionally contribute to a more environmentally friendly solution to high abundances of salmon lice in aquaculture. This small case study should therefore be expanded into a larger evolutionary ecology study, which would allow to answer several questions.

Areas for future study

One avenue for improvement would be to optimise the method used for testing the effect of pheromones on dispersal of *L. salmonis*, and separate age classes more clearly, as well as testing separately the effect of male pheromones on male dispersal. This would provide valuable information on whether females continuously release pheromones or do so only in some stages (in particular, the preadult stage).

The outcomes of this study also open a variety of ramifications. An interesting aspect is the costs and benefits of mate guarding, and how these might be altered by intensive farming (leading to high parasite loads) as compared to wild salmonid populations. One might expect that males that have not secured a mate on a fish might be more enclined to disperse, but this would depend on the number of hosts and lice present around.

During our experiment observations of males jumping and landing back on the same fish were done. Males were also seen to be very mobile, almost running on the surface of their host, sometimes running after each other. Direct observations of male-male competition on the fish (e.g. by filming them) might provide additional insight into the reasons why males did not consistently guard the same female prior to fertilisation in our study.

Male salmon lice do jump, as seen in the larger ParAnthropE project where they clearly move much from week to week. One hour might have been too short to observe significant jumping, and longer time in the tanks could produce more significant differences between the treatment groups.

Studying how sex ratio on one fish host affects male and female behaviour might inform about the environmental conditions that may make it beneficial for males to disperse. In this study the location of male lice on their host was recorded, but I did not link it to individual males, hence their individual movement could unfortunately not be tracked. My observations, however, were consistent with previous studies showing that most adult lice stayed dorsally (Pike & Wadsworth, 1999). There seems to be extensive searching on the as the positions of males recorded each hour rarely were located in the same area of the fish. Previous observations within ParAnthropE (S. Trengereid, A. Mennerat) as well as from previous studies suggest that females may actively meet or avoid males on the fish by using various locations, depending on whether they are available and interested in mating or not (Pike & Wadsworth, 1999).

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Appendices

Appendix 1.

Table 2. Male salmon lice from the Single host pheromone experiment (22.3-23.3), Multiple host pheromone experiment (29.3-01.4) and Digestion time experiment (01.4). All males were from same population and some was reused during the experiments marked in x is if in use.

	22.3.22	23.3.22	29.3.22	30.3.22	01.4.22
P1	x	x			
P2	x	x			
P3	x	x	x		
P4	x	x	x		
P5	x	x			
P6	x				
P7	x	x			
P8	x	x			
P9	x	x			
P10	x				
P11	x	x	x	x	x
P12	x	x	x	x	
P13	x				
P14	x				
P15	x	x			
P16	x				
P17		x			
P18		x	x	x	x
P19			x	x	x
P20			x	x	
P21				x	x
P22				x	x
P23				x	
No ID		**3x**	**x**	**2x**	**3x**

Appendix 2.

Cut off for males that could represent pre-adult stage 2

```
second <- measures |>
  filter(Day != "8", Day != "9", Day != "22")
```

Days cut out as they represent repeats of same males.

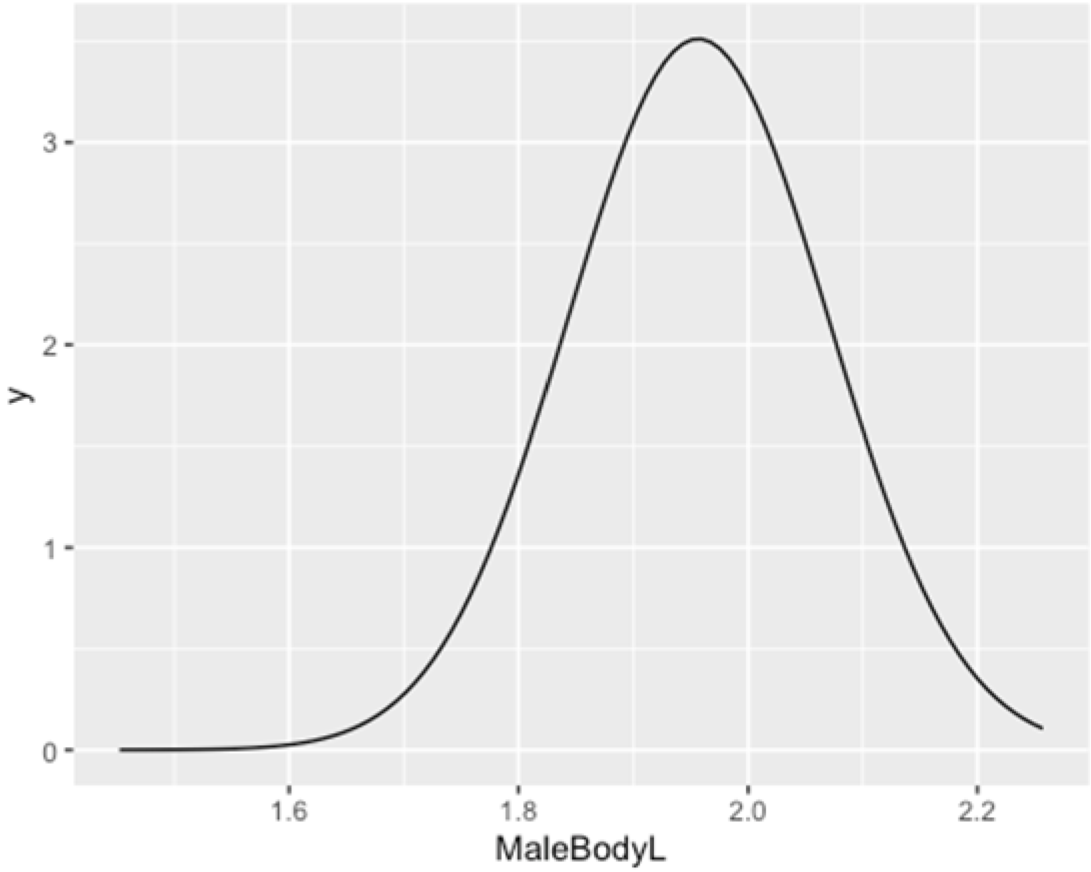
```
ggplot(second, aes(x = MaleBodyL)) +

stat_function(

fun = dnorm,

args = with(second, c(mean = mean(MaleBodyL, na.rm=TRUE), sd = sd(MaleBodyL, na.rm=TRUE)))

)
```



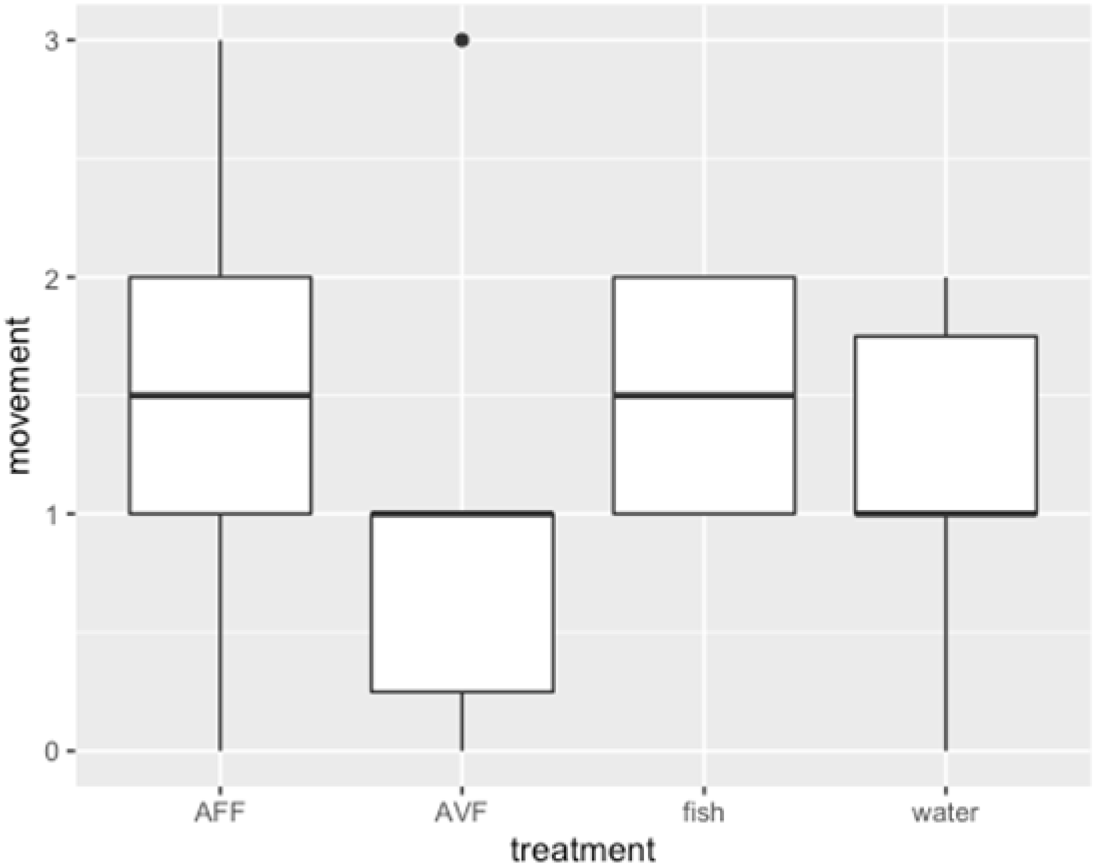

```
shapiro.test(second$MaleBodyL)
##
## Shapiro-Wilk normality test
##
## data: second$MaleBodyL
## W = 0.85702, p-value < 2.2e-16
meanmale = mean(second$MaleBodyL, na.rm=TRUE)

sdmale = sd(second$MaleBodyL, na.rm=TRUE)
```

meanmale + (2*sdmale)
[1] 2.183665
meanmale - (2*sdmale)
[1] 1.729211
Normal size distribution: [1] 2.183665 [1] 1.729211
Higher value do not necessarily need to be cut off as max length do not affect stage confusion.
Lower limit set at 2sd.

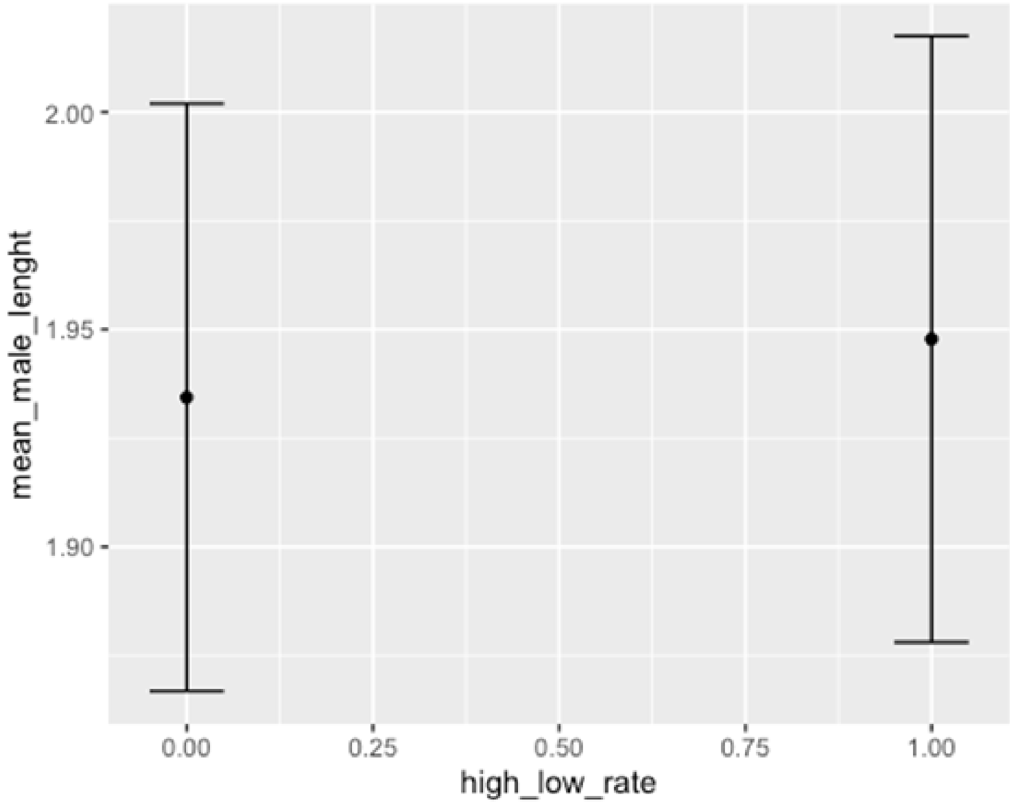
Appendix 3.

Movement on host:



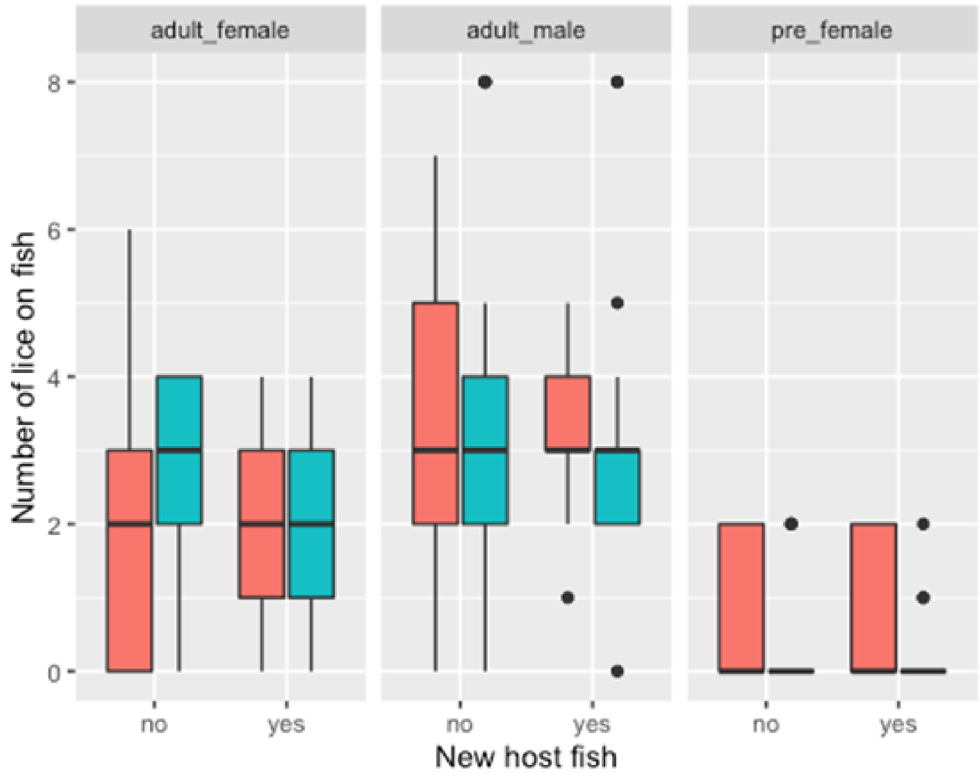
This figure is the measured movement of salmon lice on its host, The fish was divided into zones and once the salmon lice moved over to another zone it was given a score of 1. All the lice had movement, but finding an accurate way or representing this with the data I recorded could only give indications as individual lice was not ID yet. Hence some lice might have switched zones, but it would be read as no switching.

Appendix 4.



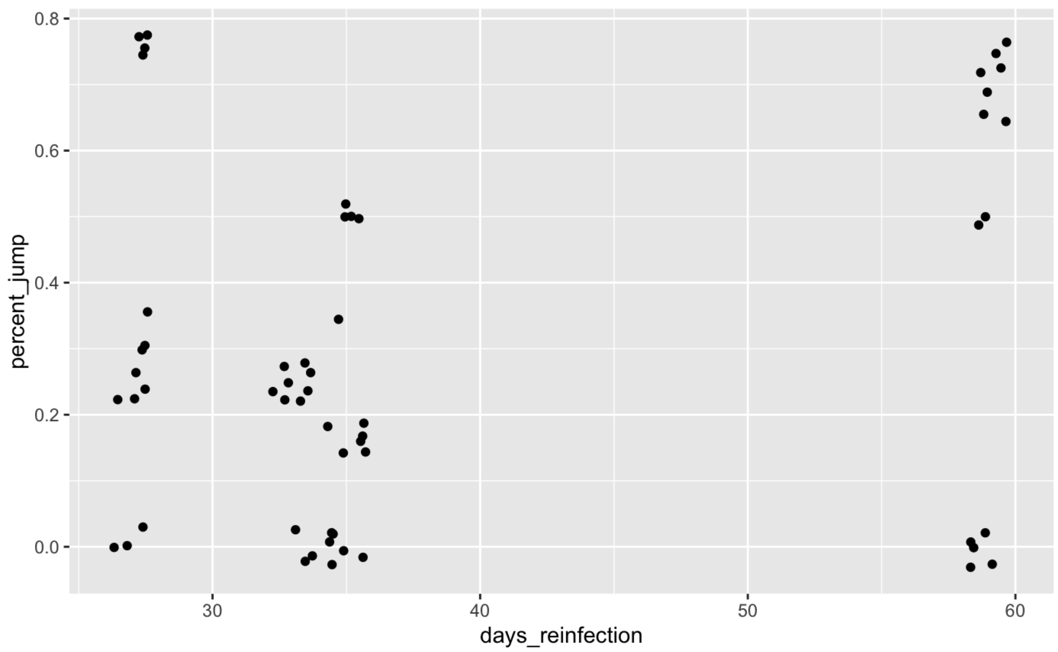
Means of lenght for males for two different categories. First low is the ones that only lasted 1-3 weeks and was then lost. High means that they lasted to week 6-8.

Appendix 5.



Focus only on pre_female column. This represent pre-adult 2 females that was counted. Red is week 1, blue is week 2. As we can see in this dataset, between the weeks chosen most females grew up in this particular week.

Appendix 6.



A rough figure showing that dispersal also occur when all pre-adult females have grown up. Because still many male lice jump even though its been almost 60 days since last reinfected.