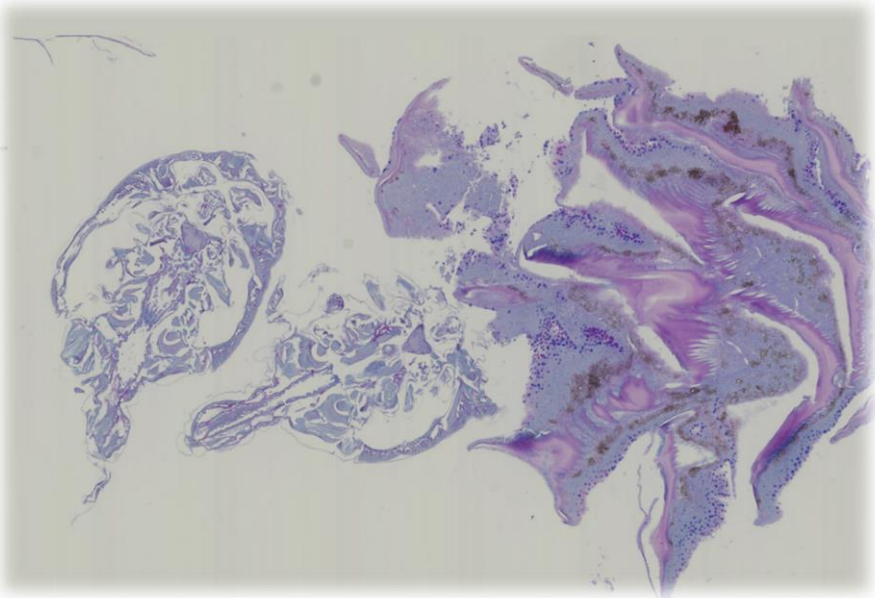
Mucosal responses in post-smolt Atlantic salmon (*S. salar*) following salmon lice (*L. salmonis*) infections



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Abstract

The aquaculture sector is becoming increasingly important both for food production and economic reasons. The main problem today in Norwegian aquaculture is the copepod ectoparasite *Leopeophtheirus salmonis* which limits the growth of the sector in Norway. It is important to limit the amount of sea lice in the industry because it affects wild stocks of Atlantic salmon (*Salmo salar*) and Brown trout (*Salmo trutta trutta*) negatively, especially in early post-smolt stages. Breeding for salmon lice resistance in aquaculture is one of the proposed solutions, and the breeding companies are currently breeding for traits connected to resistance to salmon lice. There are likely many genes affecting resistance to salmon lice, and Atlantic salmon is relatively susceptible to it compared to other salmonids. In order to make breeding for this trait feasible, better marker for innate immune responses are needed and this thesis will look at the possibility of using mucosal mapping in mapping the mucosal dynamics of sea lice infections, and compare families in a large-scale challenge test. The trial was carried out by the breeding company Salmobreed at the Norwegian institute of marine research (IMR) station at Matre, Norway. Weight turned out to have a large effect on mucosal dynamics, at least in the tail of the fish. The tail was also the favoured site for sea lice in this study. Increasing weight seemed to correlate with a weakening of the mucosal quality of the tail, and this could be a result of breeding for increased growth, and not immune function. Family data was not of good enough quality to do any statistics, but some observations were made. They tended to develop in the same way that the general population did in terms of mean weight etc.

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1. Introduction

1.1 Aquaculture

Aquaculture is a growing food industry worldwide, and it is becoming increasingly important for meeting future food demands. While the total volume of food produced by the wild fisheries has remained static the last years, an increase in seafood consumption has been based on an increased yield from aquaculture. Like other forms of food production this sector also struggles with pathogens, parasites and pests, which require new technology, pharmaceuticals and breeding strategies to be used and to be further developed. Aquaculture in Norway has not only been a source of increased food production, but it has also turned out to be of ever increasing economic importance (FAO, 2014). In Norway, this industry has mostly been based around the farming of salmonids, and mainly Atlantic salmon (*Salmo salar*) (Figure 1). Norwegian aquaculture's current biggest problem and environmental issue is the ectoparasite copepod Salmon lice (*Lepeophtheirus salmonis*), which has halted the growth in production. Increases in production locally and nationally will only be possible once this problem have been handled (Svåsand et al., 2016).

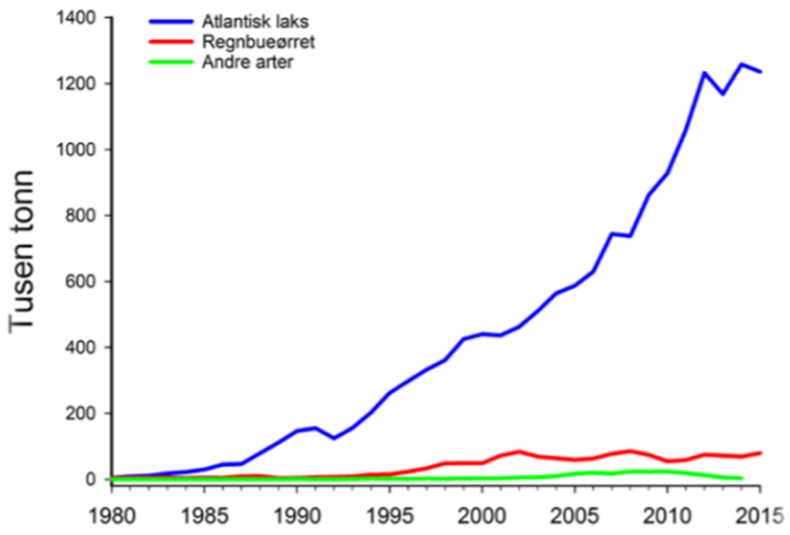


Figure 1

Growth of the aquaculture sector in Norway by species: *The amount of Atlantic salmon (Salmo salar) (blue), rainbow trout (Oncorhynchus mykiss) (red), and other species (green) produced yearly in Norway, measured in metric tons. The production has plateaued since 2012 until today, mostly due to salmon lice. Source: Directorate of Fisheries, cited in Svåsand et al. (2016)*

1.2 The Atlantic salmon: biology and breeding

Atlantic salmon *(S. salar)* are found in the north Atlantic along both North American and European coastlines (Jones, 2004). They belong to the ray-finned Salmonidae family of carnivorous anadromous fishes. Adults will, usually, return to the river they were born in after 1-2 years in the sea. Large amounts of energy are invested by the Atlantic salmon for reproduction. This is mainly due to the upstream migration in the river, and the energy/nutrition investment in each egg. After hiding their eggs in the river gravel, many die from starvation and disease. However, some will return and spawn more than once. The alevin life-stage are born with yolk-sacks, which makes them able to grow without feeding for the first months of their lives, after which they feed on a diet primarily of insects and other small invertebrates. Characteristic markings on their sides gives this fish in this life stage the name fingerlings, or parr. Usually after 2-5 years they will leave the river during the life stage referred to as smolt. Characteristic of the smolt stage is physiological and morphological changes usually referred to as "smoltification", making them adapted to seawater. Changes in gills and other organs to compensate for the higher sea water salinities, change in shape for long distance swimming and becoming silvery in colour for better ocean camouflage as well as behavioural changes more adapted to life in the ocean (Purser and Forteath, 2003, Bigelow, 1963). During the emigration from rivers towards the ocean, the Atlantic salmon is vulnerable to infections from salmon lice due to the higher densities of infectious free-living stages of salmon lice close to coastal systems. In Norway this is a problem further substantiated by the high intensity production of Atlantic salmon in the fjords and along the coast (Svåsand et al., 2016). Atlantic post-smolt were not found to have large issues with the chalimus stages of sea lice, but after the sea lice reach pre-adult stages the post-smolt will experience an increase in mortality caused by lesions and osmoregulatory issues, ultimately causing death in the fish. It was therefore concluded that 30+ salmon lice were potentially enough to kill post-smolt during the early post smolt life-stage (Grimnes and Jakobsen, 1996), a number further reduced to 10 by Holst et al. (2003).

Selective breeding for useful traits in Norwegian salmonid aquaculture is regarded to be one of the reasons of its current success. A breeding program based on the collection of 40 wild populations started as early as the 1970s and was led by Akvaforsk (Thodesen and Gjedrem, 2006). At the same time and during the 80s, private companies started competing in this field.

Advances were done in traits such as improved growth, quality, reduction of early maturation and disease resistance. This has e. g. led to a doubling in growth estimates compared to wild salmon, while at the same time reducing feed requirements by 25%. These improvements have been calculated to reduce feed costs by about US$ 230 million per year. From these breeding programs, two breeding companies were born. (Thodesen and Gjedrem, 2006) First Aquagen in 1992 from the national breeding program, and later in 1999 Salmobreed was founded from the breeding program at Akvaforsk genetics center (Salmonbreed, 2017). Marine Harvest also carries out breeding through the Mowi breeding program (Marine Harvest, 2016).

Breeding programs have in the past decades led to vast improvements for factors like growth, but a well targeted program for the innate immune function of the fish have been harder to do in the past. This could have led to possible reduction in e.g. lice resistance being the result of the innate immune response not having had the same targeted focus. Salmobreed have made breakthroughs in the selection for disease resistance related to certain diseases, and which they incorporated into their breeding program (Gonen et al., 2015). But it is probably harder to target general innate immunity, and previous studies in other food industries have found that there is a link between breeding for growth and a reduced immune function, likely as a result of more energy going towards growth. (van der Most et al., 2011).

In terms of Salmon lice resistance in relationship to breeding and genetics, some studies have been done on the subject. Glover et al. (2004) and the following Glover et al. (2005) concluded that these traits were heritable, but that it probably was linked to more genes than the trait Salmonbreed identified in the case of PD. This makes it harder to target especially for this quality. In a larger study Kolstad et al. (2005) investigated the genetic variation of Atlantic salmon in their susceptibility to salmon lice. They looked at 300 full sibling families in the 2000- and 2001-year classes, and 50 in the 2001-year class. They also looked at a 2002-year class while including data from a challenge test. The challenge test is designed to have a large enough amount of sea lice that all individuals will be affected, thus limiting the random factor of infections and making every individual in the study interesting to look at. The method was described in Kolstad et al. (2005), and in this case has the same amount as the later study (Gjerde et al., 2011) which found the ca. optimum amount of lice for this scenario to be 36. A large variation in lice numbers was observed between individuals in the trials, and a test of correlation between genetics and lice infestations of various lice stages was very high

($r_g \geq 0.98$), which also correlated with weight increases (0.32-0.37). They did, however, find that the heritability was stronger on first infection and weaker in reinfection trials (correlation of 0.26-0.35). They conclude that there was a strong genetic correlation between the challenge test (high density of lice infection) and natural infection ($r_g=0.88$), leading them to conclude on the potential of this kind of selective breeding. To counter the natural variation in natural infections, they recommend that challenge tests were preferable in this endeavour.

A later study by Gjerde et al. (2011) further dealt with the topic of salmon lice resistance. Looking at 2206 individuals, from 154 full-sib families in two tanks under different levels of lice pressure (74 and 36 per fish, the latter being close to the optimal number required to infect almost every fish). Ten days after infection a count of sessile lice (chalimus 2-4) was done. They had a mean bodyweight of 260g in both populations of fish. Fish with larger observed weight, and thus a larger body surface, correlated with higher lice numbers, but the authors thought that breeding for slower growing fish to combat salmon lice would be counter-intuitive as it would increase production time until harvest size. They, therefore, suggested that breeding with low lice density (Lice count/Bodyweight$^{2/3}$) as the desired trait was a better strategy, as it took lice per weight unit (gram) of fish into concern and thus bred for resistance, not smaller fish. Genetic correlation between harvest body weight and lice density were not found, and the authors conclude that the breeding for increased growth rates should not increase susceptibility to salmon lice. Repeated breeding projects aimed at improving sea lice resistance in Atlantic salmon have been done and looking at new types of data could improve the accuracy of this endeavour.

1.3 Salmon Lice

There seem to be differences in how well different salmonid species can fend off this parasite, and Fast et al. (2003) found that Atlantic salmon seems to be very susceptible and unable to defend itself effectively compared to other salmonid species. Fast et al. (2004) found that salmon lice produce secrete that manipulates the immune-response of the host to the benefit of the parasite. As mentioned in section 1.2, there have been found strong indications of heritable resistance to this by MacKinnon (1998), and later by Kolstad et al. (2005), Gjerde et al. (2011), Glover et al. (2005), (Glover et al., 2004).

Salmon lice feed on skin, blood and mucus, and ultimately this will lead to sores. These can again lead to secondary infections, problems with osmoregulation and ultimately death (Tully and Nolan, 2002). These effect do not seem to be too pronounced in the chalimus stages though (Grimnes and Jakobsen, 1996), but that doesn't mean that there isn't an early reaction to infections, which was demonstrated by Tadiso et al. (2011). Tadiso et al. (2011) found that there was a response, although not good enough to ward of infections of sea lice. This could be changed in the future by targeted breeding.

1.4 Fish skin and mucus in salmonids

The outer barriers of the skin of fish (Figure 2) have been developed to handle challenges posed by the surrounding aquatic environment. The skin of fish can be considered a "living barrier", in some respects more akin to the barrier of the intestinal tract of land animals, than the skin of land animals. Mucus from mucous cells in the surface barriers of the fish plays a vital role in the innate immune function, acting as the first barrier towards pathogens and parasites, which the aquatic environment is rich in. (Ángeles Esteban, 2012). The skin is a complex structure surrounding the whole fish, which serves as a barrier against disease and parasites, as well as being important in terms of ions, nutrients etc. and therefore being critical in keeping the chemical homeostasis in the fish. The skin consists of different layers, some overlapping. The outermost layer, epidermis, consists of mucus (in mammals usually referred to as goblet cells) which produce mucus, epithelium (multicellular squamous epithelium) and part of the fish scales (which is found within a scale pocket). Underneath the epidermis we find the dermis. This layer consists of two layers named the stratum spongiosum and, beneath that, the stratum compactum and lastly the hypodermis. These layers consist of connective tissues, capillaries, lower part of scales, pigments cells. Being a living barrier, the skin of fish produces mucus through mucous cells in the epidermis.

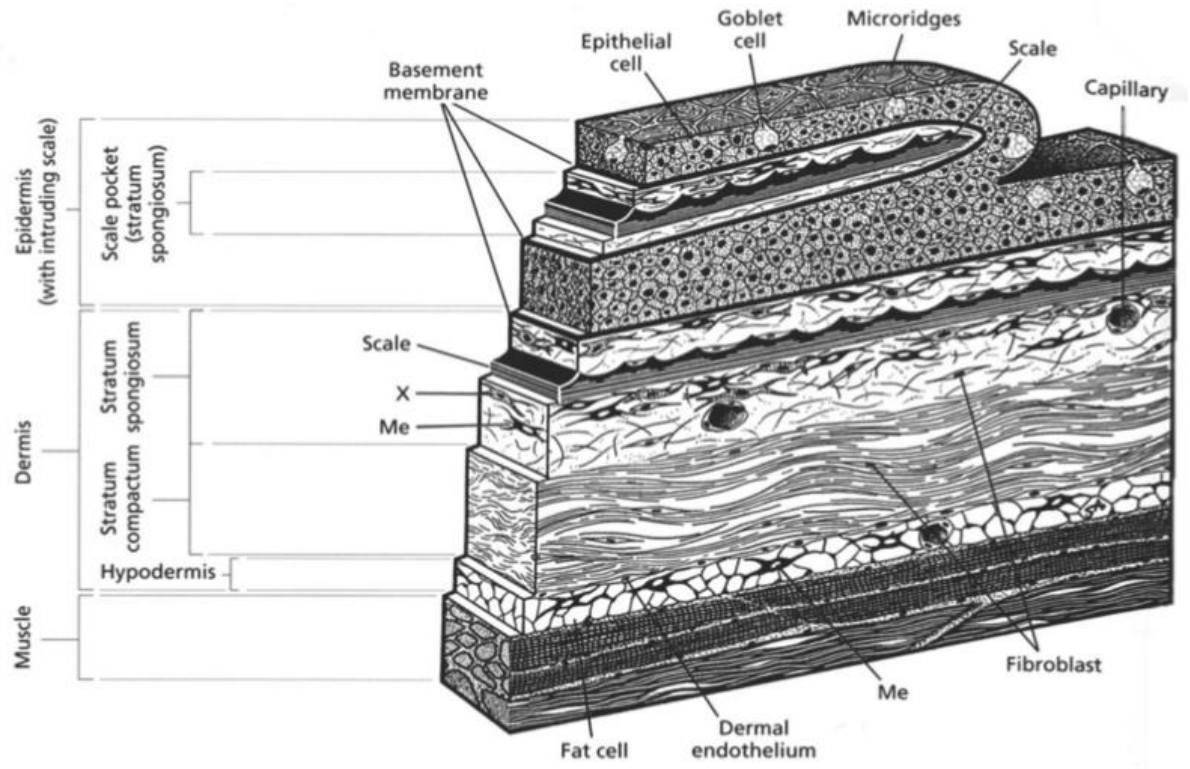
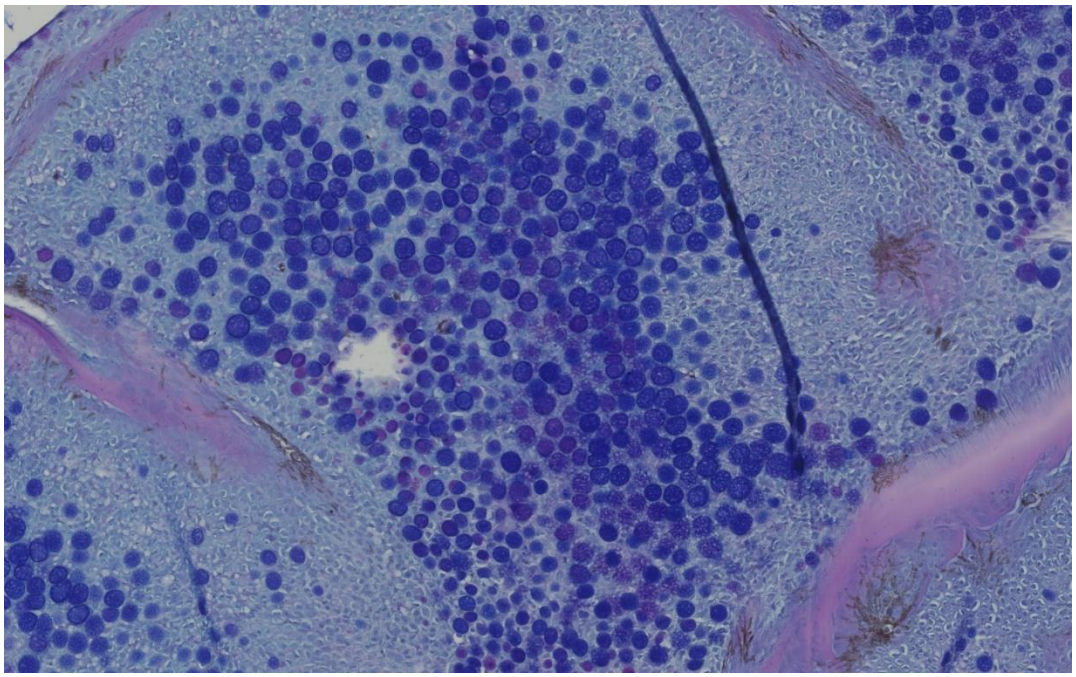


Figure 2 Fish skin section drawing: *A section of fish skin, showing the main types of tissues and single cells usually found in the skin of most teleosts. The mucus producing mucous cell, are found in the epidermis among the epithelial cells closest to the surface. X: xanthophore, Me: melanophore (source: Elliott (2011))*

In ectoparasite infections, both the innate and adaptive immune system are involved in order to minimize the infection, this can be done by altering the quality and/or quality of the mucus. (Beck and Peatman, 2015). The response in the mucus layer in the skin can vary a lot, and examples of high and low mucous cell densities can be found in figure 3 and figure 4.



Fig**ure 3 High mucus cell density in dorsal epithelium.**

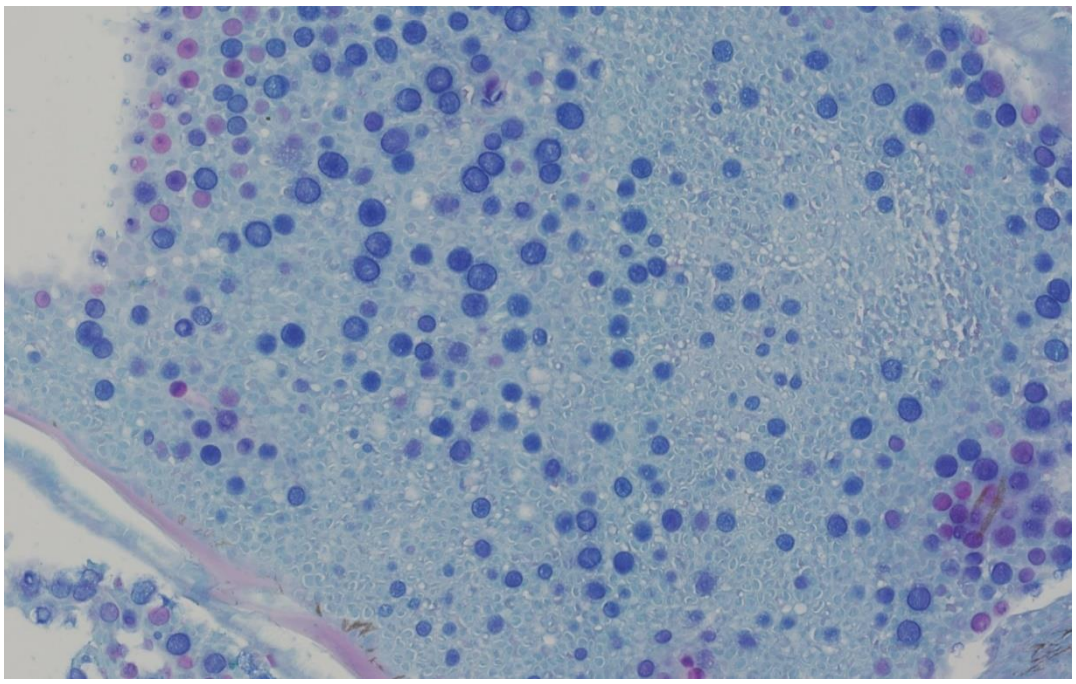


Figure 4 Low mucus cell density in dorsal epithelium.

1.5 Stereology

1.5.1 Background

Traditional histology has usually been based on a two-dimensional relatively small section of a tissue, where placement and amount of certain structures and cells often have been the focus of study. Anatomical structures, pathology etc. have been studied using regular histological

methods, but its weakness is its limited area looked at. Real tissues are three-dimensional, relatively large (as compared to a single section) and not necessarily homogenous in structure. Describing and measuring three-dimensional features (like densities etc.) have been hard to do using traditional histology for these reasons. Stereology has been developed for some decades to deal with this problem. Rather than focus on describing exact positions of cells and tissues, it deals, in my case, with something closer to the three-dimensional properties of one or more tissues. This is possible through a combination of mathematical and histological analyses covering a larger part of the tissue, reducing the number of slices while staying accurate. This approach leads to a decrease in cost and time.

1.5.2 Mucosal mapping

The method of mucosal mapping was first described in Pittman et al. (2011)Pittman et al. (2011). They found evidence for consistent, and repeatable, results when working with tangential sections of tissues. This method gave larger areas of epithelium and mucous cells, without changing the size estimates or ratio between them compared to regular transverse sections. This was important to get more repeatable and significant values when extrapolating from these measurements. The article concluded optimistically that the method could "... quantitatively addressing questions relevant to the efficacy of modulating mucosal production, the effect of interventions against salmon lice and for investigating the quantitative ontogeny of teleost mucosal defences."

A later article by Pittman et al. (2013) dealt with further evaluations of the method, and body site variation. Establishing that if there is a difference in a tissue, with regards to mucosal size and density, it was measurable. They found data tended to normalize at about 100 random mucus cell area estimations, indicating that the true area mean was likely reached. It was further suggested that salmon could show a repeatable pattern in regard to variation in seasonal, dietary, sex, strain differences.

Goal of study:

This study will look at the different mucous defence response to sea lice in different families of fish, when possible, and other factors when not. Other factors are the two categories of infection rates. Fish with high salmon lice infection rates, and fish with low salmon lice infection rates. The state of the mucus cell layer will be evaluated using mucosal mapping, and this will be linked to other relevant factors including weight, sex, specific growth rate (SGR), the effect of local infections.

The main hypotheses:

Are there measurable mucosal differences in high and low sea lice infection groups of Atlantic salmon:

H0: Mucosal dynamics do not interact with the infection rate in different individuals and families of Atlantic salmon.

H1: Mucosal dynamics do interact with the infection rate in different individuals and families of Atlantic salmon.

In bred farmed salmon, most fish are selected for fast growth, does this affect how much energy is invested in the mucosal immune function of the skin?

H0: Mucosal dynamics are not affected by how fast the fish have reached a certain weight.

H2: Mucosal dynamics are affected by how fast the fish have reached a certain weight.

2. Materials and methods

2.1 Experimental setup and initial data collection

The trial was conducted at the Institute of Marine Research's research station at Matre, Matredal, Norway. The Atlantic salmon (Salmo salar) in the experiment was supplied by the breeding company Salmobreed and consisted of their 2015-year class of 300 distinct full-sib families, forming a total of 3624 fish. The fish had originated from Akvaforsk Genetics Center AS (AFGC) at Sunndalsøra, Norway. The fish was fed a standard formulation feed through the trial, and the freshwater had a temperature of 15 ^{o}C. They stayed at Akvaforsk during their early freshwater phase and were injected with PITtags (Passive Integrated Transponder) June 22.-24. 2015 so that individuals were later identifiable. At the date of tagging the fish in my sample group had a mean weight of 29 grams (±12). The fish were treated with a standard short photoperiod (winter signal, usually less than 10-12 hours of daylight). This is a typical procedure done to start and synchronize the smoltification process (Bjerknes et al., 2007).

Following the short photoperiod, they were transported to the research station at Matre on Sep. 19. 2015. They were randomly distributed into two tanks (2 x 5,3m^3), and from then on exposed to a full day photoperiod. The fish had a mean weight of about 60 grams. After a month, when they reached a weight of about 80-100 gram, Oct. 18. 2015, they were introduced to sea water for the smoltification process to complete.

The next stage of the trial started 22 days later, On Nov. 9 2015. 125 000 cultured salmon lice (*Lepeophtheirus salmonis*) copepodites were released into the fish tanks. The number of lice was chosen to reach a level of ~36 lice pr. fish, which is estimated to be the ideal number if the point of the trial is to infect every individual fish. This trial design have been used earlier described as a "challenge test." (Gjerde et al., 2011)

15 days after the infection, at 23. and 24. November, the salmon was terminally anesthetized and collected for salmon lice counting, and further skin sampling. Gradually fish were collected from the tanks and brought to a nearby lab where 5-6 people counted the lice on each fish. The people were instructed to handle the fish with a high level of care so that skin samples remained as untouched and intact as possible. The weight was measured, and the pit tag scanned before samples were made from 49 fish having a high and 46 fish low level of salmon lice at the time of sampling. See table 3 and 4 for an overview of weight and lice loads

in high and low infestation This selection was done gradually which made the selection of individuals a constant evaluation of infection rate based what appeared to be a high and low level during the trial as compared to the other fish which we chose not sample skin from. During sampling we collected lice numbers, which were later changed to lice density (lice/weight $(g)^{2/3}$), which are terms describing their degree of lice infection.

The sampling sites on the fish skin were a 2x2 cm area on the dorsolateral side of the dorsal fin (fish facing left), dorsal tail, ventral belly and head. Data relating to individual weight, lice count, location of lice infection, sex, comments and identification number was collected for all individuals and later digitized.

2.2 creation of histological sections

The skin samples were stored in labeled histo-casettes (Simport) which were shortly after sampling immersed in formalin-containing bottles. For time and budget reasons we later chose only to create slides of, and analyse, the dorsoventral and tail samples gathered. These samples are made by tangentially sectioning the epithelium, and this process is closer explained in the next section.

2.2.1 Fixation and creation of sample slides

The sections that were made contained the epidermis, dermis and sometimes parts of the skeletal muscles. To make these the raw samples were fixated in 10% buffered formalin and stored for further processing. Formic acid was later applied for 5 hours for decalcification, with a following 45-minute rinse in running water. The embedding (pressure modified embedder) were carried out with formalin (1h) x2, ethanol 80 (1h) x1, ethanol 100 (1h) 4x, Xylene (1h) 3x, Paraffin (1h) x3. When the samples were embedded they were sliced into 3 μm thick sections.

These were then stained using Alcian Blue for 4-6 minutes with a following 2-4 minutes of water rinsing, then periodic acid (10%) for 9-11 minutes, a following rinse using distilled water, Schiff's reactive for 29-31 minutes, running water for 9-11 minutes, Harris

Haemotoxylin for 15 seconds then finishing with a 4-6 minutes rinse using running water. The samples were than mounted using ethanol (100%) for 5 minutes 2x, Xylene for 5 minutes 2x, and lastly using DPX (synthetic resin).

The samples were digitally scanned at 200x optical magnification and saved as digital images (Hamamatsu NDPI files) containing the whole slide.

2.3 Analysis of digitized sections

The digitized sections of the collected samples from dorsal- and tail skin was analysed following the method described in Pittman et al. (2013), Pittman et al. (2011). Stereological analysis of the digitized sections of skin was carried out with the software VIS (Visiopharm AS, Integrator System, Hoersholm, Denmark. Version 6.5.0.2303). Mean time spent on each section varied from 40-70 minutes depending on factors like mucus cell number and density per section. Each skin section was measured for mucus cell sizes and number, estimation of total epithelium to mucus cell densities, using stereological methods developed and adapted for the mucosal mapping method. This data is then used to calculate mucus density in the tissue. This creates objective data that can be tested statistically for differences in skin immune responses to stimuli such as lice. The main type of data gathered is in this study:

- **Mucus cell area:**
  The estimated mean mucus cell size in the epithelium analysed, measured in μm^2.
- **Mucus cell density:**
  The estimated density of mucus cells in the epithelium analysed.
- **1 / Mucus cell area / Mucus density**
  An estimated ratio that describes the relative size of mucus cells pr. area unit of epithelium in the tissue analysed. Also described as epithelium tensegrity.

2.4 Data sets and statistics

For statistical analysis, the integrated development environment (IDE) R-Studio (Version 1.0.136) for R were used. Graphs were mainly created with the graphics package ggplot2 (Wickham, 2009) or R's standard plot functions.

We collected data regarding lice numbers and infection site for every individual, as well as sex, weight and relevant comments. The full dataset for 80 individuals with known family ID was used to generate this first attempt at studying this kind of data in relationship to individual and family differences in mucus cell layers.

The complete data from the trial contains sensitive data for the breeding company and therefore statistics are not based on complete information about the population at large.

We focused on dorsal samples, shown to be a statistically reliable site for measuring both cell area means and mucus densities (Pittman et al., 2013) in individuals. I also analysed a smaller selection of tail epithelium from 15 of the highest and lowest performers in term of lice infection rates. This did not give as statistically valuable data as the bigger dataset for dorsal skin but gives some indication of the link between these two sites in the same fish, as well as general differences between sites.

Weight differences between high and low infection groups are estimated with binomial logistic regression. The goodness of fit is reported with three different types of logistic pseudo R2 analyses; “Nagelkerke”, “Hosmer-Lemeshow” and “Cox-Snell”. Odds ratio is useful as it is equivalent to the b-coefficient,Akaike’s information criterion (AIC) is used together with null deviance etc as a way of comparing models (in Generalized Linear Model at least). A Chi square test is done to compare models.

For the remaining data, linear regression was used for comparing correlation between continuous variables. For categorical data with continuous outcomes a two-sample t-test was used if there were two categories, ANOVA if there was more than two. When trying to identify important factors and predictor variables both a scatterplot matrix were used and a stepwise backward/forward regression method were used.

3. Results

3.1 Main Findings

97 skin samples were collected from a total of 3624 fish. Of these 97, 80 had known family ID and were linked to families and other vital data, making them the main dataset. It was not possible to link any measure of lice infection (infection rate group, lice count or lice density) to a mucus cell response in either tail or dorsal epithelium. The mean weight of high infection individuals was significantly higher than low infection groups (132.56 ± 40.94 g and 92.80 g ± 25.94 g respectively) ($p < 0.0005$). Tail mucus cell density, size of the mucus cells and 1/mucus cell area/mucus cell (1/MCA:MCD) was significantly lower than in the dorsal skin (table 4). Epithelium in the tail having local infections of sea lice trended towards having lower mucosal cell densities, but adding final weight to the statistical model showed that an increase in final weight was the main contributor to decreasing mucosal density and 1/MCA:MCD. This effect of increasing weight had a significant effect on increasing 1/MCA:MCD ($p <0,05$), and was associated with an insignificant decrease mucus cell density in the dorsal epithelium of low infection individuals ($p = 0.12$). This increase could mean that an increase in weight correnspponds with fewer mucus cells relative to the amount of epithelium in the skin.

Of the 300 families in this experiment, 69 families were part of my data, and of these 9 had more than one individual. There were not enough individuals in each family to do statistical testing, but the final weight trend in relationship to lice infection rate was found here as well. In general, a larger variation in mucosal response to infections were found in the low infection families, as compared to mixed or high infection families.

3.2 Fish weight and growth

The mean weight of all sampled fish were, at date of tagging 28.7 grams (± 11.9 g). 152 days later at the date of sampling the population had reached a mean weight of 112.8 grams (± 39.5 g) (figure 5). The mean daily specific growth rate (SGR) in this period was of 0.94 (± 0.17) (figure 6). Tank and sex had no significant effect on tag weight, final weight or SGR (table 1). The mean final weight of female fish in the trial was 112,7 g, for males the mean end weight

was 114,5 g (table 1). No significant interaction between sex and weight were found (p = 0.85). This was also the case for mucus density (p=0.38) and average cell area (p=0.62) in dorsal and tail samples.

Table 1: Individuals, tag Weight, Final weight and SGR by Sex, tank and infection rate.

	Female	Male	NA	Tank 9	Tank 10	Low infection	High infection
n	38	39	3	20	60	37	43
Tag weight (g)	29 ± 12	29 ± 12	26 ±8	29 ± 11	29 ± 12	21 ± 11	34 ± 11
Final weight (g)	113 ± 35	115 ± 45	92 ±8	111 ± 39	114 ± 40	93 ± 26	133 ± 41
SGR	0.94 ± 0.18	0.94 ± 0.16	0.86 ± 0.17	0.91 ± 0.16	0.95 ± 0.17	1.01 ± 0.17	0.88 ± 0.15

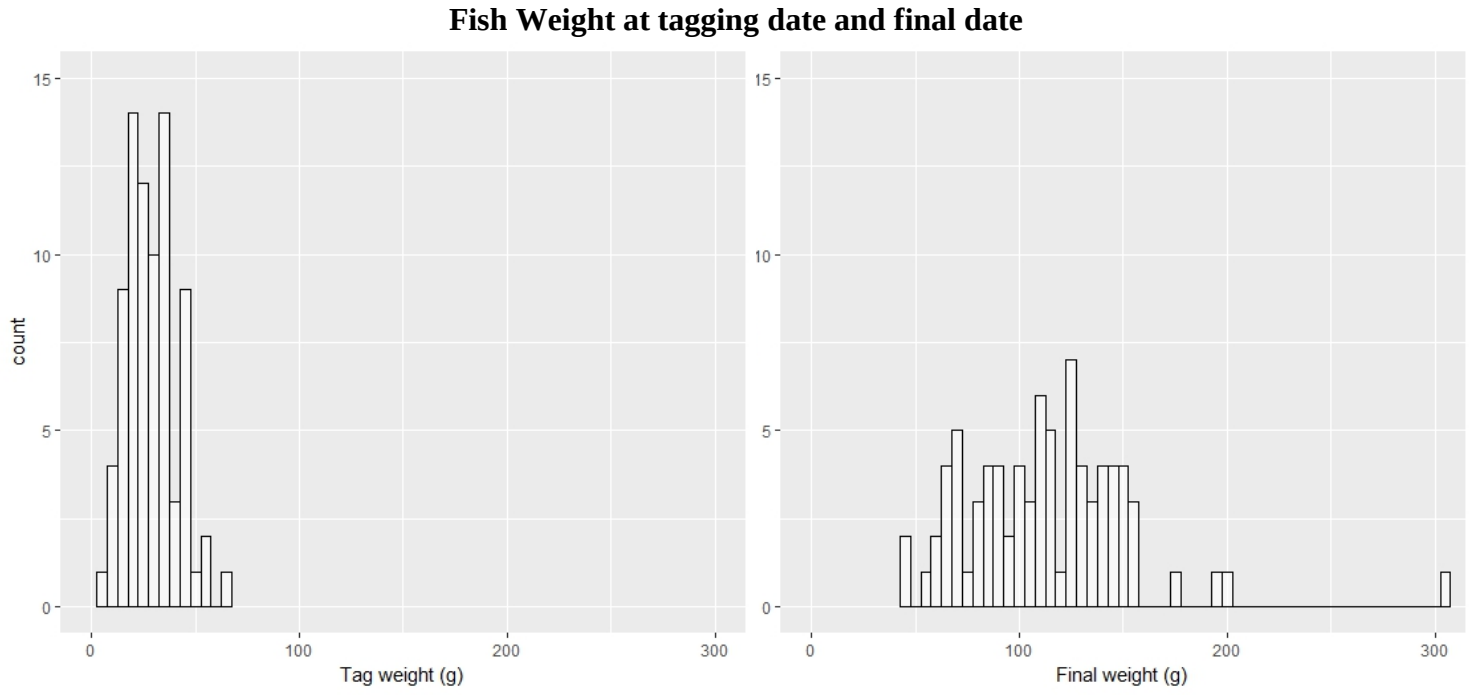


***Figure 5**: **Fish Weight at tagging date and final date:** Left: Weight distribution of all sampled fish (n=80) at date of PIT tagging (22-24. June 2015). The mean weight was 28.7 grams (± 11.9 g). Right: Weight distributions of all sampled fish (n=80) at date of skin sampling (23.-24. November 2016). The mean weight of all individuals was 112.8 grams (± 39.4 g).*

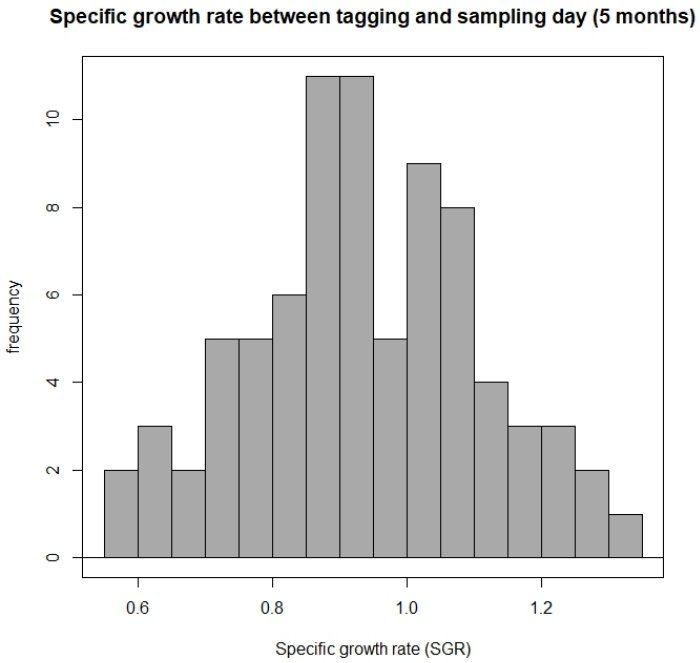


Figure 6:

Specific growth rate distribution*: The mean daily SGR between tagging date and sampling date in the whole sample population were 0.94 (± 0.17), n= 80. The lowest observed specific growth were 0.58, and the highest were 1.33.*

3.3 Lice

The lice had infected the fish for 15 days at the day of sampling, and were mostly found to be in the pre-adult stages. This is in accordance with the lice development speed at 15 °C, which predicts that the lice would be in the pre-adult 1 stage, and possibly pre-adult 2 for males. All the fish in my sample group were chosen based on having high (n=43, 2-12 lice) or low (n=37, 28-79 lice) lice numbers, these were in total n = 80.

The number of lice in the low infection group ranged from 2-12, with a mean of 6.83. The high infection group ranged from 28-79 lice and had a mean of 45.89 (figure 7). Sex did not significantly alter the chance of being in either of the groups, neither did tank. As predicted by count data usually having a Poisson distribution, the sea lice count interval was larger the high infection group (51) than the low infection group (10).

The mean weight significantly varied between the “high” and “low” infection rate categories, with the high infection group being significantly heavier than the low infection individuals ($p < 0.0005$). The high infection rate group had a mean weight of 132.56 ± 40.94 g, and the low infection group had a mean weight of 92.80 g ± 25.94 g (Figure 8) (table 1). There was a significant negative correlation between weights and specific growth rate. The low infection group had a significantly higher daily specific growth rate between date of tagging and sampling day, than the heavier high infection group individuals ($p=0.001$).

Table 2 Overview of mean lice counts, interval, sex ratio and number of individuals in the high and low infection groups of salmon.

Infection level	n	Lice # interval	Mean lice #	Female/male/NA
Low	37	2-12	7 (± 2)	18/17/2
High	43	28-79	46(± 10)	20/22/1

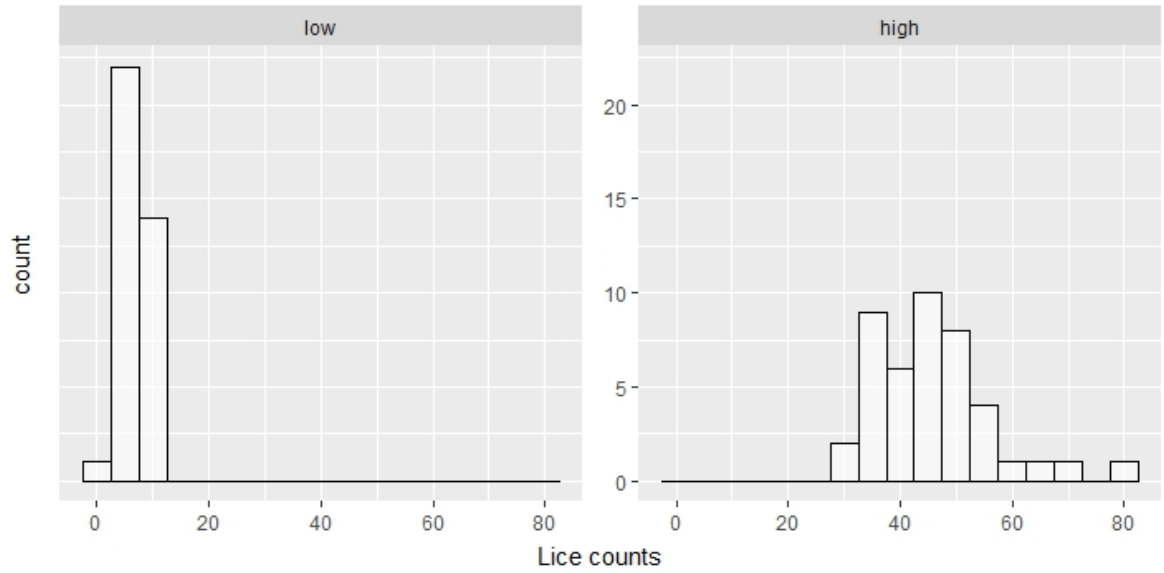
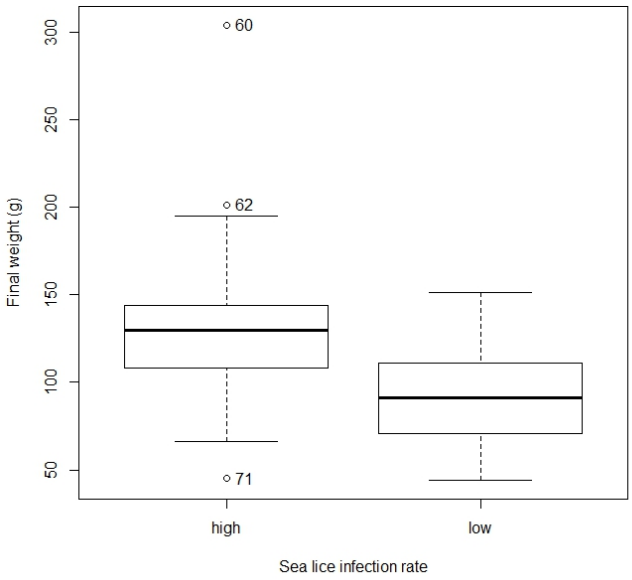


Figure 7 Lice count distribution in the "low" infection group, and lice count distribution in the "high" infection group. The x-axis indicates lice number pr. individual, and the y-axis indicates the frequency of fish with that lice count. The number of lice in the low infection group ranged from 2-12, with a mean of 6.83. The high infection group ranged from 28-79 lice and had a mean of 45.89. Sex did not significantly alter the chance of being in either of the groups, neither did tank.

Figure 8 Final weight by salmon lice infection rate
The difference in weight (grams) between the highly-infected individuals (28-79 lice, a mean of 45.89) and the low infection group (2-12 lice pr. individual, a mean of 6.83). The high infection group had a mean weight of 132.56 ± 40.94 g, and the low infection group had a mean weight of 92.80 g ± 25.94 g. Weight was significantly different for the two groups. The high infection group was significantly heavier (Binomial GLM: p=5.16e-05). "Low" n=37. Sex ratio 18 females. 17 males, 2 unknown. "High" n=43, Sex ratio 20 females, 22 males, 1 unknown.



3.3.1 Body Sites and proximity of infections

Dorsal samples were analysed from all 80 individuals sampled. The data from the tail samples were collected from 30 individuals. 15 were high infection individuals, and 15 were low infection individuals. Tail appear to be the preferred site for sea lice to attach in this experiment. The mean count of salmon lice on the tail samples was 0.89 ± 1.54, for dorsal samples 0.19 ± 0.59, for belly samples 0.21 ± 0.63, and head samples 0.39 ± 0.64 (Table 3).

The high occurrence of local infections on the tail skin made it possible to analyse these for local effects on the mucosal cell layer caused by the proximity of the lice. Some signs of local infection influencing the mucus layer were initially found, but further analysis showed that the most prominent and significant factor in this regard is variables linked to weight, or size, like final weight, tagging weight and SGR (Figure 11).

Table 3 ***Mean body site lice counts:*** The mean lice counts for salmon lice found on, or close to, the sampling sites in the trial. Tail (dorsal part), and to a lesser degree head (dorsal), are far more likely to have sea lice on them than the belly and head sampling sites. n=80.

Tissue	Lice #	Mean (n=80)	HI group mean (n=43)	LI group mean (n=37)	HI/LI ratio
Belly	17	0.21 (± 0.63)	0.33 (± 0.78)	0.08 (± 0.36)	4.13
Head	31	0.39 (± 0.65)	0.6 (± 0.72)	0.14 (± 0.42)	4.29
Dorsal	15	0.19 (± 0.60)	0.30 (± 0.77)	0.05 (± 0.23)	6.00
Tail	71	0.89 (± 1.54)	1.32 (± 1.88)	0.38 (± 0.76)	3.47

3.3.2 Mucus cell area:

Dorsal mucus cells are significantly bigger (209 $\mu m^2 \pm 42$) than the mucus cells in the tail epithelium (158 $\mu m^2 \pm 35$) ($p < 0.005$). There's no significant correlation between final weight on Mucus cell area in tail samples. A trend is found in low infection dorsal samples, but is not significant ($p = 0.14$) (figure 11). Infection rate, lice count or lice density did not cause a significant difference in mucus cell area in dorsal or tail samples. In the dorsal epithelium the high infection rate group had a mean MCA of 210.7 ± 45.1, the low infection rate group had a mean MCA of 207.4 ± 39.6.

3.3.3 Mucus cell density:

Dorsal mucus cell density was 0.19 ± 0.06, which was significantly higher than in the tail at 0.09 ± 0.03 ($p < 0.05$) (figure 10). Final weight of the fishes had a significant negative correlation with tail epithelium mucus cell density ($p < 0.005$). In the tail epithelium there was a trend towards low infection individuals having a lower mucus cell densities ($p = 0.06$) (figure 10), but this effect is not found when controlling for weight differences which seem to be the most important factor. Local sea lice infections were controlled for as well, but weight was found to be the main factor here as well (figure 11). No significant effect of infection rate, lice count or lice density on mucus cell densities were found in dorsal. In dorsal the high infection rate had a mean MCD of 0.19 ± 0.06 the low infection rate group had a mean MCD of 207.4 ± 0.07.

3.3.4 Mucus area to density ratio (1/ Area:Density)

Tail had significantly higher 1/MCA:MCD, (0.08 ± 0.03), than dorsal epithelium (0.03 ± 0.01) ($p < 0,05$), signifying that the ratio of mucus cells to general skin epithelium area in the dorsal sample is higher than in the tail. There is a significant positive correlation between weight and 1/MCA:MCD ($p < 0.05$) in the tail skin indicating that the number of mucus cells haven't increased at the same rate as the amount of total epithelium in the tail (Figure 12). Controlling for lice count, or lice density (lice / weight (g) $^{2/3}$), did not improve the statistical model. This positive correlation between weight and 1/MCA:MCD is also seen in the dorsal epithelium of fish in the low infection rate group, but here it is not significant ($p=0.12$). No significant effect of lice infection rate, lice density (lice / weight (g) $^{2/3}$) or lice number was

found on 1/ MCA:MCD in tail epithelium or dorsal epithelium. The dorsal epithelium had a mean 1/MCA:MCD in high infection group of 0.03 ± 0.01, and the low infection group had 0.03 ± 0.01.

Table 4 ***Mucosal dynamics by tissue type***

The dorsal tissue have significantly bigger cells (MCA), more cells (1 /MCA : MCD) which makes for a higher density of mucus cells (MCD). MCA: Mucus cell area, MCD: Mucus cell density.

	Tail	Dorsal	Two-sample t-test
Mucus cell area	158 ± 35	209 ± 42	P < 0.05
Mucus cell density	0.09 ± 0.03	0.19 ± 0.06	P < 0.05
1 / MCA : MCD	0.08 ± 0.03	0.03 ± 0.01	P < 0.05

Table 5: Means, standard deviation and p-values of low infection rate and high infection rate individuals:

	Low infection rate	**High infection rate**	**Significantly different: p-values**
n	37	43	
Mean lice count	6.84 ± 2.36	45.89 ± 10.38	
Lice # interval	2-12	28-79	
Dorsal MCA	209.7 ± 40.5	211.5 ± 46.9	0.73
Dorsal MCD	0.20 ± 0.07	0.19 ± 0.06	0.29
Dorsal 1/MCA:MCD	0.03 ± 0.01	0.03 ± 0.01	0.64
Tail MCA	207.4 ± 39.6	210.7 ± 45.1	0.8
Tail MCD	0.10 ± 0.04	0.08 ± 0.02	0.06
Tail 1/MCA:MCD	0.08 ± 0.03	0.09 ± 0.03	0.31
Tag Weight	21 ± 11	34 ± 11	**p < 0.0005**
Final Weight	92.80 ± 25.94	132.56 ± 40.94	**p < 0.0005**
SGR	1.01 ± 0.17	0.88 ± 0.15	**p < 0.005**

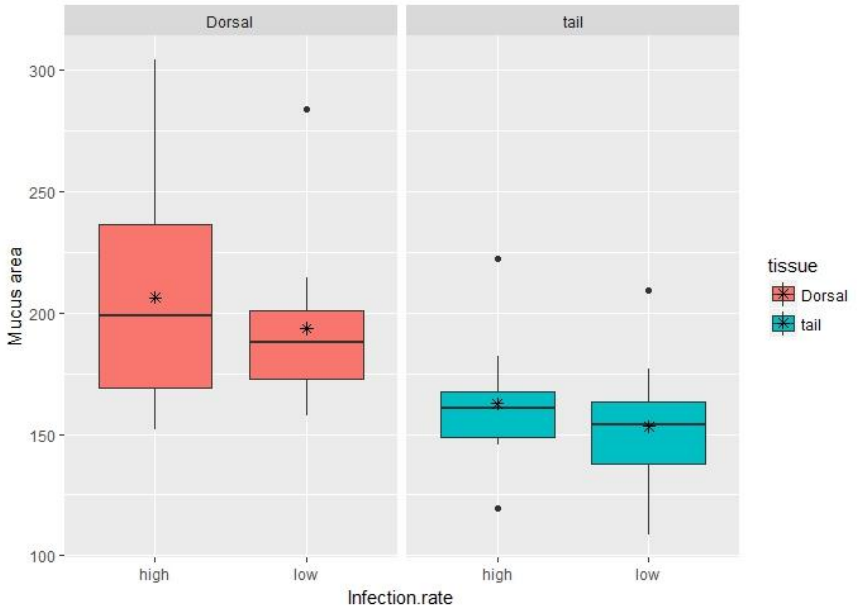


Figure 9 ***Mucus area in relationship to both salmon lice infection categories (high or low) and body site in Atlantic salmon****Average mucus cell area in dorsal and tail epithelium. The difference between the body sites are highly significant ($p < 0.005$), dorsal epithelium having generally larger mucus cells. Significant differences within tissues based on infection rates were not found. Dorsal: "high" n = 43, "low" n =37. Tail "high" n = 15, "low" n = 15.*

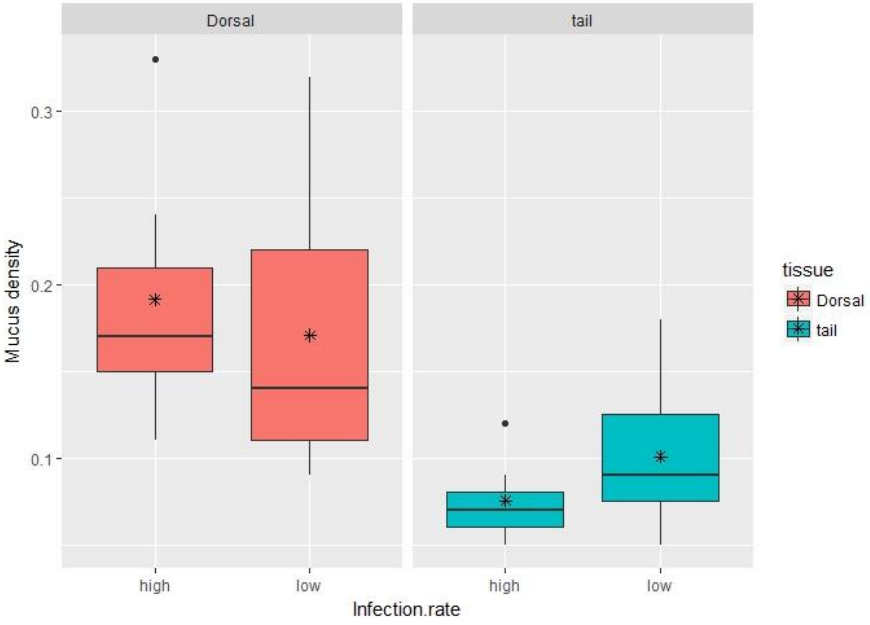


Figure 10 Mucus density in relationship to both infection category and body site in Atlantic salmon

Average mucus cell density in dorsal epithelium is significantly higher than in dorsal tail samples ($p < 0.005$). Low infection individuals in the tail trended towards a higher mean mucus densities than the high infection group ($p = 0.06$), but this effect is not found when controlling for weight differences ($p=0.89$) when controlling for weight..

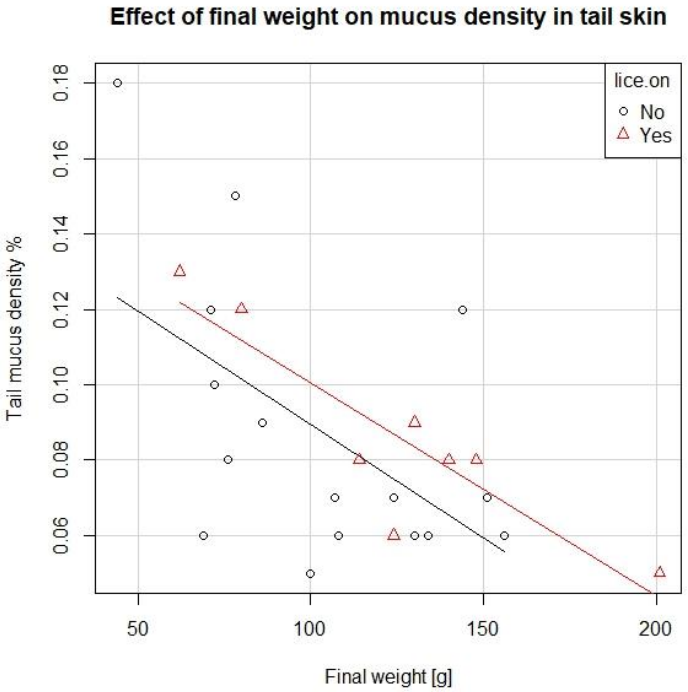


Figure 11 Effect of final weight on mucus density in tail epithelium based on localized or non-localized lice infections. The correlation between weight and mucus density in tail samples is significant (p=0.01). Local infections do not affect this significantly, but there is a non-significant trend towards higher weights in the localized infection group.

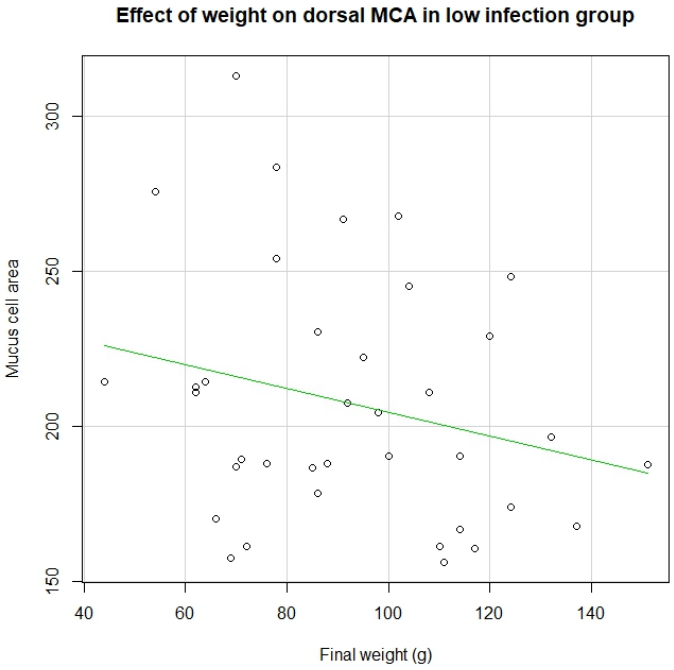


Figure 12 The effect of final weight (g) on dorsal mucus cell area (MCA) in "low" infection individual). *Shows a similar trend to tail samples, but is not significant (p=0.14) (n=37)*

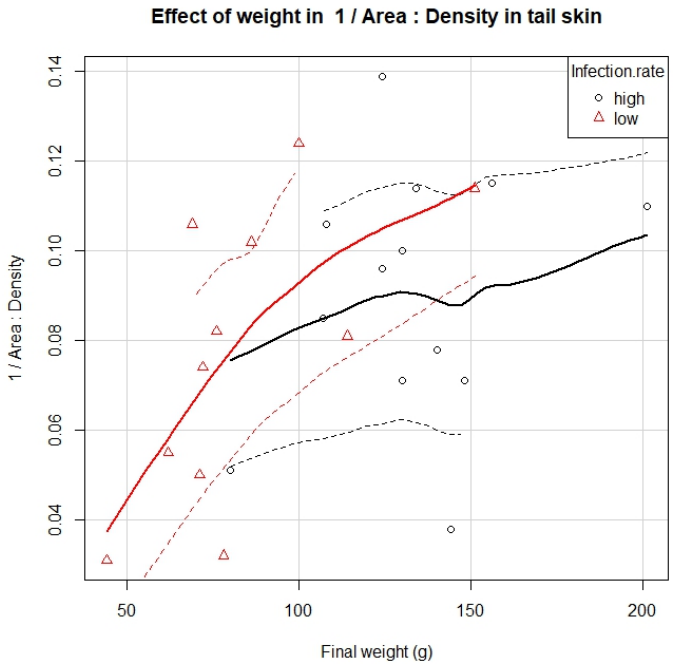


Figure 13 Effect of Final weight on 1/MCA:MCD.in the tail epithelium: *An increase in weight corresponds with a decrease in the number of mucus cells relative to the amount epithelium in the skin. (n=30)*

3.4 Families: Observational data

3.4.1: The families

Only 9 families with 2-3 individuals in each made proper statistical testing impossible, but some general observations about these were made. The families with more than one individual found were sorted based on the family being an only low infection rate family, containing both (mixed infection rate families) or only high infection families (table 4). As we can see in Figure 25, the few families that had replicates hints at a general, but currently untestable, pattern. The high infection family individuals had a mucus density mean value of 16 %, and the average mucus cell area was 207,3 (n=6), for the mixed infection rate families the individuals (n=7) had a mean of 22% mucus density and 219,5 average cell areas. For the low infection group, the individuals (n=7) had a mean of mucus density of 22% and 227,9 average cell area. Families with low infection rates seem to have a higher variability in their response in either direction (Figure 25). These families had higher levels of inter-family variation (Table 4).

Table 6

Families: number, sex ratio, weight, Lice count, Lice density, infection rate category mucous cell density, cell mean area and ranges for both. *Abbreviations: HiR: High infection rate#, LiR: Low infection rate #, DorMCA: dorsal mucous cell area, DorMCA range: The variation of DorMCA in a family in a family, DorMCD: Dorsal mucous cell density, DorMCD range: Variation in mucous cell density in a family.*

Family	#fish	♀	♂	Weight (g)	Lice#	Lice Density	HiR	LiR	DorMCA	DorMCA range	DorMCD	DorMCD range
803	2		2	93 (75-110)	7 (5-9)	33,6 (27,9-39,2)	0	2	174,6 (161,5-188,0)	26,5	19% (13%-25%)	12 %
916	2	2		81,5 (72-91)	6 (5-7)	31,8 (72-91)	0	2	214,1 (161,5-266,7)	105,2	20% (9%-31%)	22 %
601	3	1	2	80 (70-92)	15,7 (4-36)	79,9 (21,9-176,6)	1	2	272,7 (187,2-347,1)	159,9	26% (18%-37%)	19 %
404	2		2	127 (124-130)	31 (6-56)	121,15 (24,1-218,2)	1	1	208,7 (168,8-248,5)	79,7	22,5% (18%-27%)	9 %
920	2		2	106 (102-110)	27 (9-45)	118,6 (41,2 - 196,0)	1	1	246,3 (224,7-267,9)	43,2	29% (23%-35%)	12 %
506	3	1	1	150 (92-201)	52(9-79)	171,7 (44,2-272,6)	2	1	208,8 (170,3-248,3)	78	17,7% (12%-21%)	9 %
205	2	1	1	226 (148-304)	46,5 (39-54)	139,7 (86,3-193,0)	2	0	197,3 (195,5-198,7)	3,2	17% (15%-19%)	4 %
219	2	1	1	139 (130-148)	52 (52-52)	194,2 (185,8-202,6)	2	0	241,8 (235,2-248,4)	13,2	20,5% (17%-24%)	7 %
907	2	1	1	110,5 (104-117)	45 (45-45)	195,8 (188,1-203,5)	2	0	182,8 (177,4-188,2)	10,8	10,5% (10%-11%)	1 %

3.4.2 Mucosal responses in families:

Generally, most individuals found in the upper right corner of Figure 16 are individuals with low infection rates, but they are also found in the lower left area where highly infected individuals are found as well. This means that some of them have both bigger cell areas and density of mucus than the high infection rate group, while some are more like the high and

mixed infection families. Two families were found to have both high and low infection rates, these scored relatively high in mucus density and cell area for both infection rates. This trend was not found when looking at all (n=80) dorsal samples. In the high infection group (n=43) the mean mucus density and cell area were 18.6% and 210,7um, and in the low infection group (n=37) the density was 20,1% and cell area 207,4 µm. There was no significant difference between these two groups. There was not found any significant correlation between infection rate either mucus cell area (p=0.73) or mucus density (p=0.29), perhaps hinting at a large variation in response from different families. The effect of local infections on the sample was not possible on a family basis as there was only one example for tail and dorsal.

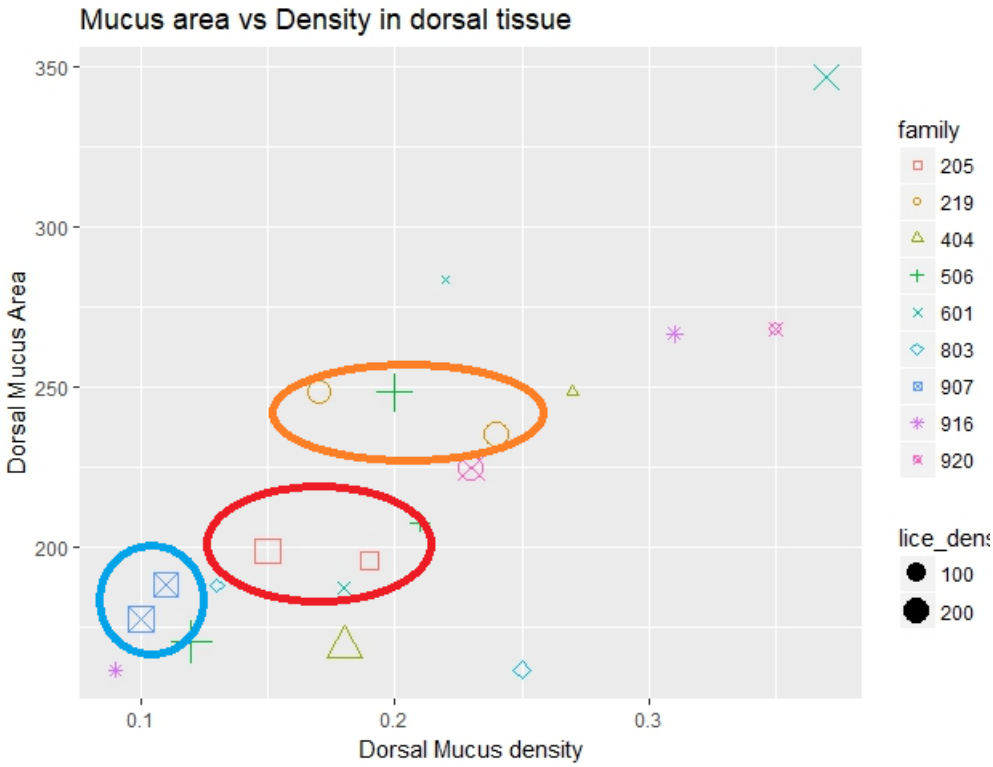
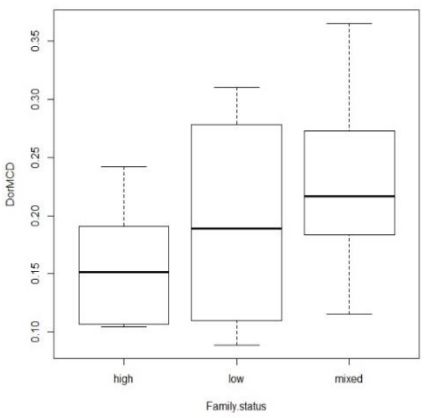


Figure 14: Proposed clustering area of dorsal mucous cell area and density vulnerable, mixed and resistant families of Atlantic salmon : *The relationship between dorsal mucus density and dorsal mucus area shown with the families that had replicates. Families are represented by different symbols, and the size of the symbols indicate lice density on the individual. The families with higher lice densities cluster somewhat on the lower left. The families with lower lice densities do not seem to show any clear patterns. Mean values and intervals are included in Table 5*



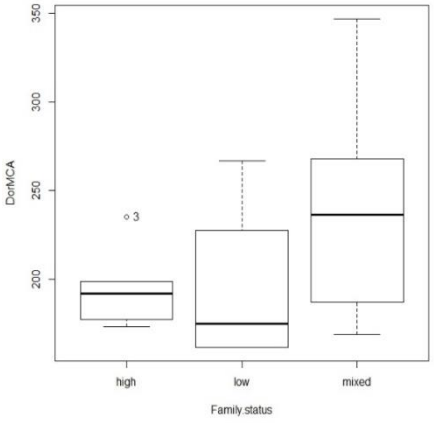
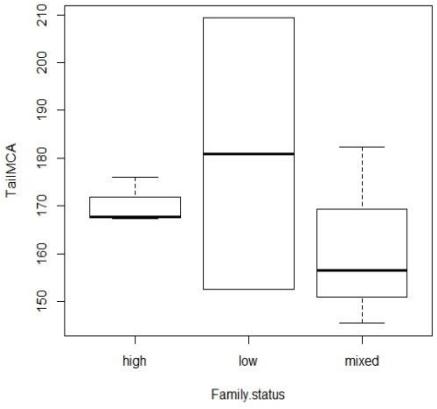
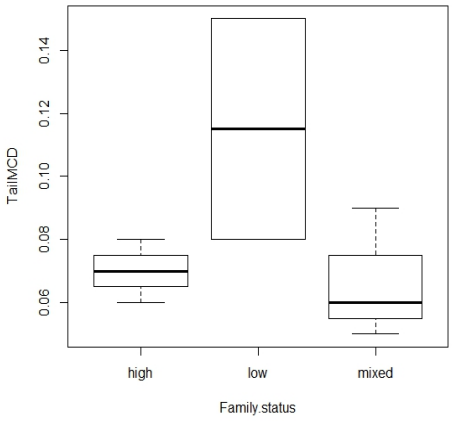


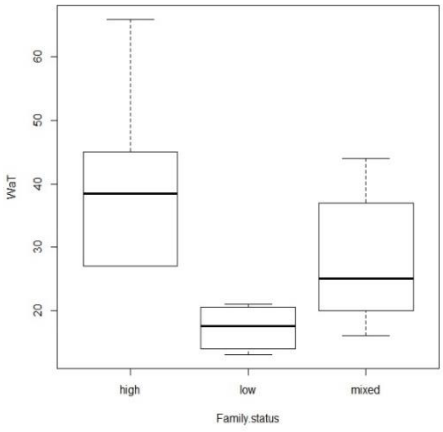
Figure 15 1-6: Family status and relationship to mucous cell densities, cell area and weight

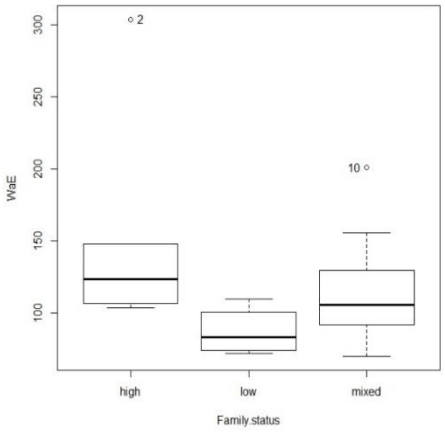
1-2: *Differences in dorsal mucous cell density (left) and dorsal mucous cell area (right) based on family status (low, high, or mixed salmon lice (Lepeophtheirus salmonis) infection rates within a population of families with more than one individual in each family*





3-4: *Differences in tail mucous cell area (left) and tail mucous cell densities based on family status family*





5-6*: Difference in mean weight during weight at tagging (WaT) (left) and 5 months later at final weight (WaE: Weight at end) (right) based on family group status (low infection, high infection or mixed infections infection rates within a population of families with more than one individual in each.*

4. Discussion

In this study we tried to develop a better understanding of the mucosal response to sea lice in individuals having high or low infection rates of sea lice. We wanted to see if it was possible to distinguish this response between different distinct families of fish when possible, and other factors when not. Growth being a energy investment for the fish, we also wanted to look at the connection between how much the fish have grown during its lifetime, and its mucus response.

We could not find any signs that sea lice infections rates, lice numbers or lice densities (Lice count/ weight (g) $^{2/3}$) had any significant effect on the mucosal parameters we have looked at. It was not even found in local infections.

Weight had a strong correlation with decreasing quality of the mucus cell layer in the epithelium, especially in the tail epithelium. It is however hard to conclude on the breeding being at fault, or if this is the normal development of growth in the fish.

4.1 The mucus layer response to sea lice

None of the mucosal parameters (Mucus cell density, mucus cell area and 1/mucus cell area/mucus cell density) are significantly different based on infection levels (infection rate, lice number or lice density). Highly infected individuals had a mean dorsal mucus cell area of 211.5 μm^2 ± 46.9, which is very similar to the low infection group individuals 209.7 μm^2 ± 40.5. This is also found for dorsal mucus cell density (high infection group: 0.19 ± 0.06, low infection group: 0.20 ± 0.07), and 1/MCA/MCD (high infection group: 0.03 ± 0.01, low infection group: 0.03 ± 0.01). (table 5). These findings are also consistent with the lack of difference in tail lice response (table 5).

Most of the sea lice sampled were in late sessile (chalimus II) early pre-adult stages (pre-adult stage I female/male). The varying stages have different effects on the host, and the effect that the lice have on the host usually increases drastically when they reach the pre-adult and adult stages (Holst et al., 2003). Before this it has been found that the chalimus stages causes little to no inflammation response in the skin of Atlantic salmon (Jones et al., 1990, Jonsdottir et al., 1992), but (Jonsdottir et al., 1992) had found that there could be observed an inflammation

circling the landing site of the lice, and one would think that this could initiate a systemic response in the fish if the infection level is high enough. This effect does surprisingly not seem to create a systemic response in fish with many sea lice in this study. This could be the effect of a systemic immunological regulation caused by the sea lice, the lice infection stage being too early or the fish not showing those kinds of responses.

One study found a gene expression change in the salmon between the copepod and chalimus stage (Tadiso et al., 2011), although this response were deemed too weak to have any chance of fending of the intruder. When the parasite moults from the sessile chalimus II-stage they will, as pre-adults, initiate a mild inflammation (Johnson and Albright, 1992) which makes them much more interesting in terms of studying mucosal health and sea lice resistance. There was however not any significant difference in mucosal reaction due to lice infection rate (figure 9, 10, 30 (appendix), 30 (appendix)) or lice counts, or lice density was not found in this study. We could therefore not in this study conclude that there is a measurable response to sessile and motile stages in terms of measurable mucus dynamics in the epithelium. This is in accordance with earlier findings using the same methodology (Thorsen, 2016), but seeing as the amount of sea lice in the high infection group was so large (28-79 lice) as compared to the low infection group (28-79) it was still somewhat surprising. However, this is consistent with other studies conclusion of the response of Atlantic salmon to salmon lice (Fast et al., 2003, Wagner et al., 2008, Fast et al., 2002). The salmon lice is known do produce secretions that lowers the salmon's ability to respond properly to the infection (Fast et al., 2007, Fast et al., 2004), and the lack of an observable response, even to high numbers of motile sea lice could be a reflection of them keeping the response down. Not having a control group without sea lice makes us unable to compare these data to the mucus cells in naïve fish, which could be useful as the number of lice in the "low" infection group could be seen as relatively high to the fish in the low infection group, as they only had a mean weight of 92.80 g ± 25.94 g.

4.2 Weight and loss of innate immune capability

Weight was the main factor for mucus density and 1/MCA:MCD (Figure 12), especially in tail. This means that the relative ratio of mucus cells to amount of epithelium decreased rapidly with increasing fish weight. It is also associated with a general weakening of the tissue streght. A metaphor for this effect could be a certain number of spots on a balloon, and when someone blows air into the balloon the inflation of the surface makes the density of mucus cells much lower as the number of cells does

not increase. This “epithelium inflation” in fish of increasing sizes in this study could explain some of the reason why larger fish in this study tend to get more salmon lice while the mucus response did not improve with size. This could be linked to targeted growth and reduced feed requirement programs that have been done in the last decades (Thodesen and Gjedrem, 2006) not targeting immunological factors like e.g. the mucus cell production sufficiently. A meta study by van der Most et al. (2011) looked at many studies done on this negative correlation between growth focus and loss of immune function in poultry and turkey. They concluded that there was a large and significant link between breeding for growth and reduction of the immune function. In the same meta study it was found, but not as clearly, that the opposite, namely breeding for immune function, did not have the opposite effect. It could therefore be that breeding for immune function could be done to improve immune function without decreasing the improvements done in growth. This can’t necessarily be extrapolated towards epithelium mucus immune function, as energy requirements for different types of immune responses and immune maintenance could vary but could be interesting to look further into. Gjerde et al. (2011) argued that looking at lice infection pressures as lice density (lice/weight (g) $^{2/3}$) would counteract the negative effect that breeding only for sea lice resistance could have on growth.

My study did not control for how growth affected mucus density independent of sea lice infections, as there was no control group without sea lice. We did however include various measures of lice pressure in the models used when looking at MCD, 1/MCA/MCD and MCA in relationship to weight -model.

4.3 Tissue differences

As observed in earlier studies using this stereological methodology Pittman et al. (2013), Maxwell (2015), Rantty (2016), Torrecillas et al. (2011) and Thorsen (2016) there was observed a significant difference between the tail and dorsal epithelium in terms of mucus cell area and density ($p > 0.005$). Following the data in this study, there could be an increasing difference in regards to 1/MCA:MCD and Mucus cell density with increasing size of the fish.

4.5 Family observations

As the quality of the data concerning families were poor because of few individuals in each family (2-3), these findings are purely observational and not of any real scientific value. It could however serve as a proto-study on this topic.

The most interesting finding when looking at families were the similarity in mucus area/density (Figure 25) within high infection families. Individuals with lower degrees of infection showed a much more variable response. Especially the family “601” (table 6) (figure 15) containing both high and low infection individuals shows an interesting response. Where two of the individuals have low lice numbers, the one that belongs to the high lice infection group have a strong mucus response. This could indicate that this family has a strong response to sea lice, but this would only be testable if the more members of that family was present in my data. The family 209 are also interesting in its response to infections as it is the family of the high infection salmon which have kept a strong response, and comparing them to the rest of the dataset could be interesting to see if the other fish of this family do better than average.

As mentioned earlier, the salmon louse suppresses the immune response in the skin, and it would therefore be of great interest to see if increasing the amount of mucus cells relative to the surface epithelia would improve the response at all. Earlier studies (Kolstad et al., 2005, Glover et al., 2005, Glover et al., 2004, Gjerde et al., 2011, Gjerde and Saltkjelvik, 2009) have consistently shown that there is a heritable component to sea lice susceptibility, but this study failing to be able to link families with different mucus cell states to resistance leaves this question unanswered for now. A study looking at many more individuals from each family could maybe be able to link a strong mucus response to increased heritable sea lice resistance.

4.6 Experimental design and statistics

Two main errors were done before and during the experiment which weakened the experiment. The number of families used (300) made the sample size (80) way too small to get sufficient family members, and only after taking part of the sampling was it discovered that the number of families was higher than initially thought. The initial design that was planned had far fewer families in mind and was supposed to be setup in such a way that we would pick the top and bottom families in terms of lice infections (which might have mitigated the problem of having too many families there). Collecting samples from whole families would also be hard as we had collected the samples right after anesthetization and lice counting. This means that we would have to do the initial sampling on all individuals in the trial to go back and pick whole families.

Weight turned out to be a huge factor in my data. During sampling, it would be wise to

include the length of the fish so an estimate for the surface area can be made. This is probably a better estimate for lice infestation than only looking at lice density (Lice/Weight $(g)^{2/3}$).

Because of time constraints, mostly linear models analysed with ANOVA, were used to analyse data. For e. g. dorsal density, a ratio, it might have been better to utilise quasibinomial family in glmPQL. Another issue is the nature of the data, where, if looking at the whole population you are in the case of sea lice infection numbers looking at the two ends of a Poisson distributed population (Gjerde et al., 2011, Kolstad et al., 2005) as the outcome variable. This were taken into account in the binomial glm used for testing the effect of weight on infection rate categories.

4.7 Future recommendations

To further investigate the link between weight, growth and the mucus layer, a study could be designed to look at the mucus cell dynamics when individual fish reach a certain weight. If they grow at different paces this would lead to e.g. 200 gram post smolt at different ages, establishing if growth rate is a factor in decreasing mucus cell ratio to epithelium in the tail. This way one could see if the fish develop more mucus cells and higher mucus cell densities at different growth rates, and if possible single out genetically distinct families having “high growth” in both weight and innate immune fuction.

For future breeding projects I would recommend that the researchers should know about which families are most worth looking into from previous experiments before gathering data. It would be nearly impossible to establish which families were the best, and worst, performers in terms of lice resistance during the sampling days while still being able to effectively store the samples properly. Doing this would counteract the problem of there being 300 families as it would narrow populations down to specific families, instead of all the fish in the trial.

An alternative to this could be to sample 300+ fish in order to have more replicates in each family studied. In this case, there should be a system for calculating lice densities during sampling, so that the fish sampled would be the fish with high and low lice densities instead of lice counts, which as discussed earlier in the article is a better estimate for more directly heritable lice resistance.

To get a better understanding of the effects of local infections on mucus densities and cell area more tail samples should be analysed in later, similar, trials. These are far more likely to be areas with *L. salmonis* attached to them.

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7. Appendix

7.1 Unused tables:

Tank #	n	Female/male/NA	High infection rate	Low Infection rate
Tank 9	20	11/9/0	11	9
Tank 10	60	27/30/3	32	28

Table 7

Infection Rate~weight output Table: *Binomial GLM (binomial logistical regression). R^2: .25 (Hosmer-Lemeshow), .29 (Cox-Snell), 0.39 (Nagelkerke). 1: p < 0.01, 2: p <0.01. AIC: 83.09 (adding sex as a covariable increases AIC to 85.01, and does not add anything to null deviance. It also has a very high p-value (p=0.79). ANOVA shows that the differences between the models are also very low (0.07, with a chi square probability of 0.78)*

	B (SE)	**Lower CI**	**95% CI for odds ratio**	**Upper CI**
Constant	-4.67[1] (1.22)			
Weight	0.04[2] (0.01)	1.02	1.04	1.07

7.2 Unused Figures

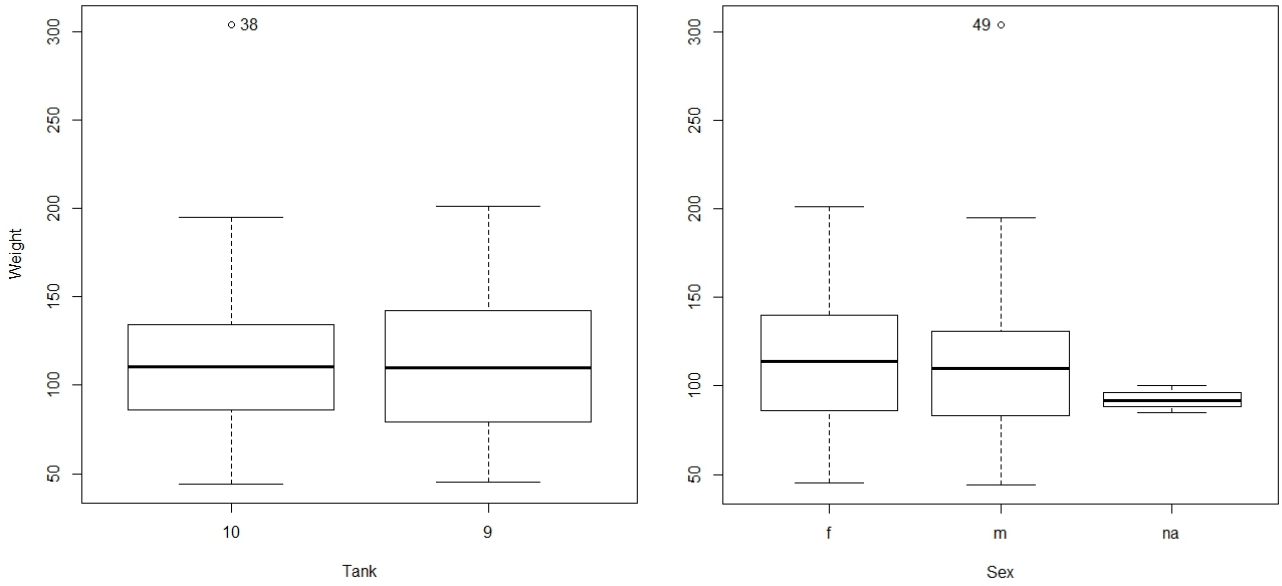


Figure 16

A (on the left) The difference in end weight of the Atlantic salmon (Salmo salar) in the experiment due to tank.

B (on the right) The effect of sex on end weight of the Atlantic salmon in the experiment.

A: There is no difference observed in mean weight between the tanks at the end of the study when the salmon are caught and weighted before sampling. (p=0.79, linear model using ANOVA)

B: There is no observed effect of sex on the observed weight (g) of individuals in the trial. (p=0.65, Linear model using anova)

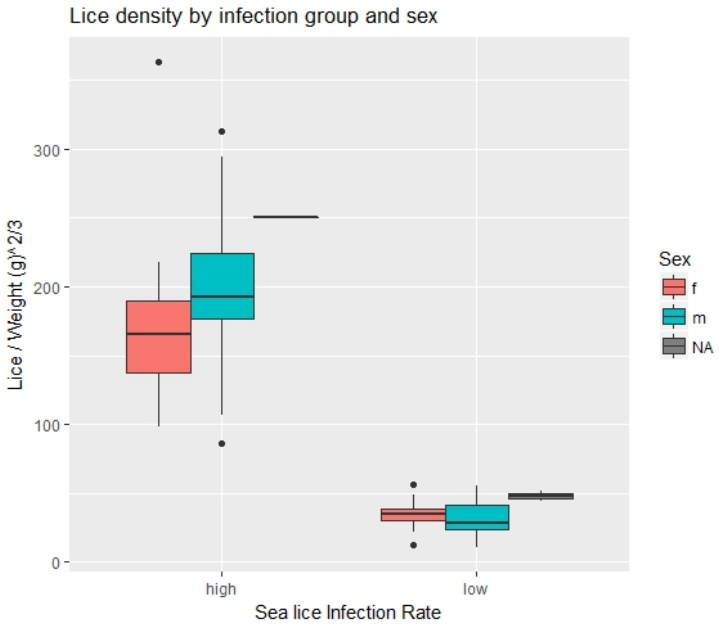


Figure 17

Lice density (Lice / Weight $^{2/3}$) by infection group and sex. The difference found in the high infection group are not significant (p = 0.1984). "low" n = 37, "high" n = 43.

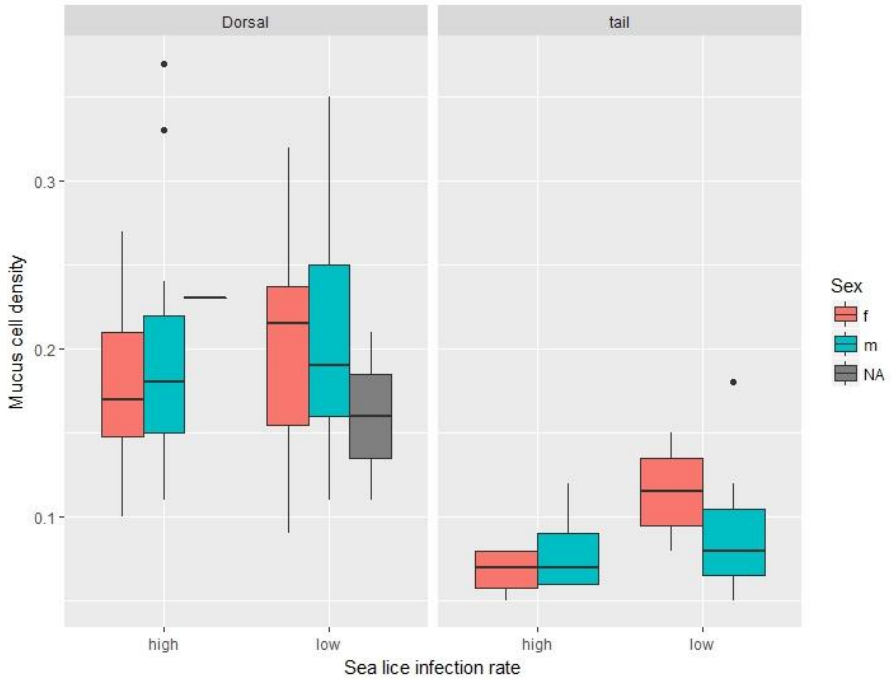
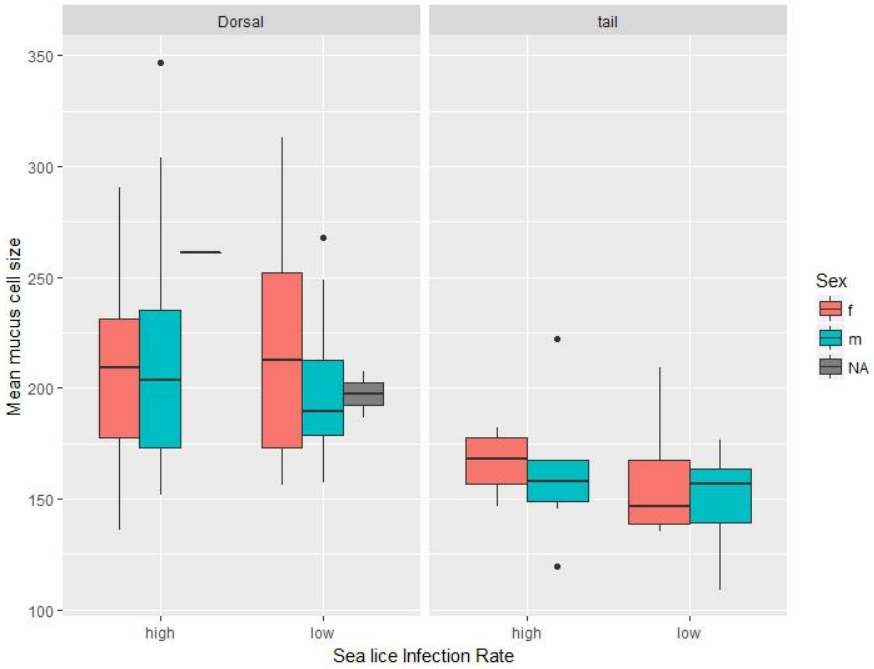
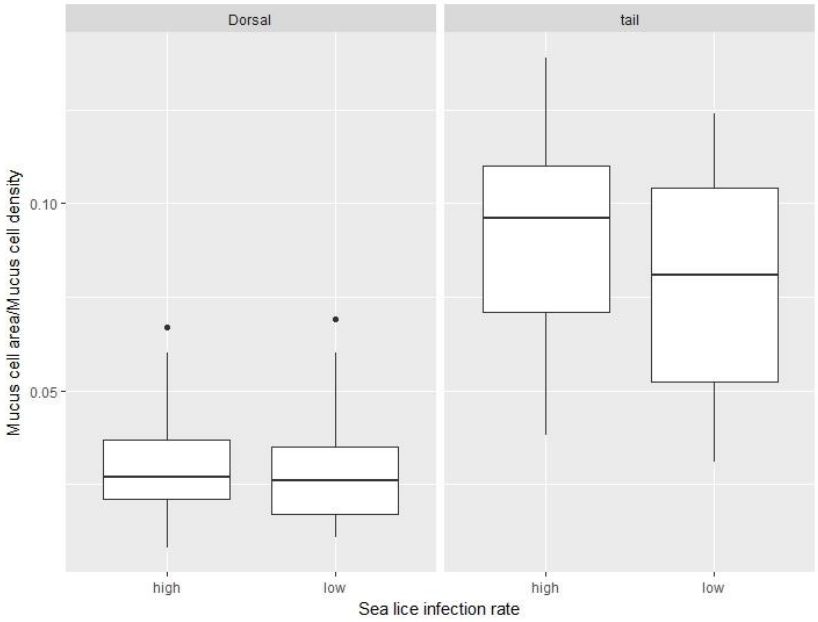


Figure 18 Sex difference in tail low is not significant


Dorsal
tail
Mean mucus cell size
350
300
250
200
150
100
high
low
high
low
Sea lice Infection Rate
Sex
f
m
NA


Dorsal
tail
Mucus cell area/Mucus cell density
0.10
0.05
high
low
high
low
Sea lice infection rate

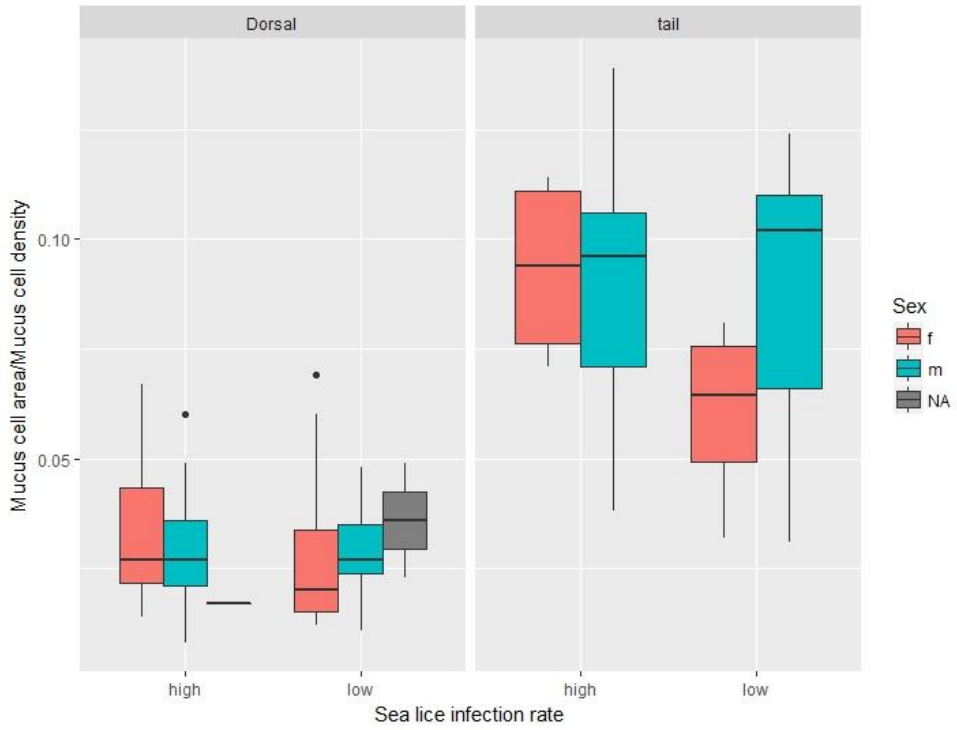


Figure 19 *Observed difference in tail low~sex not significant (p=0,41), also few individuals (n=11)*

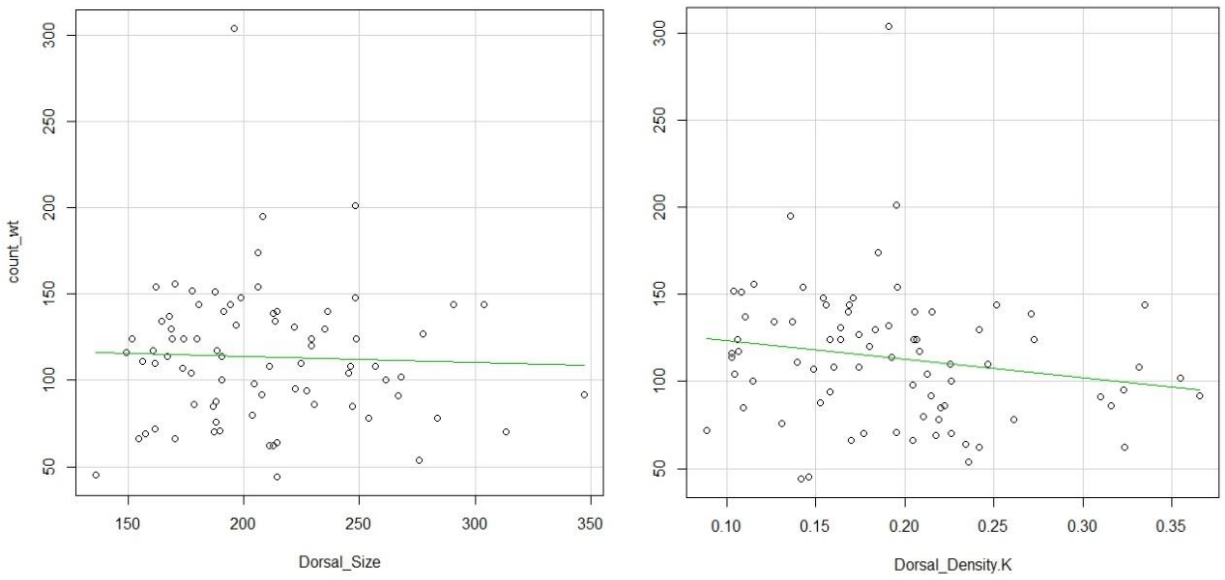


Figure 20

A The correlation between dorsal mucous cell area and the final weight of the fish (g)

B The correlation between dorsal mucous cell density and the end weight in Atlantic salmon (Salmo salar)

No significant correlation was found between the mean cell area of mucous cells in the dorsal samples of the Atlantic salmon (*S. salar*) in the trial based on weight (g) (Figure 8A). There appear to be a

weak negative correlation between the weight (g) of the salmon in this trial, and dorsal mucous cell densities (Figure 8B).

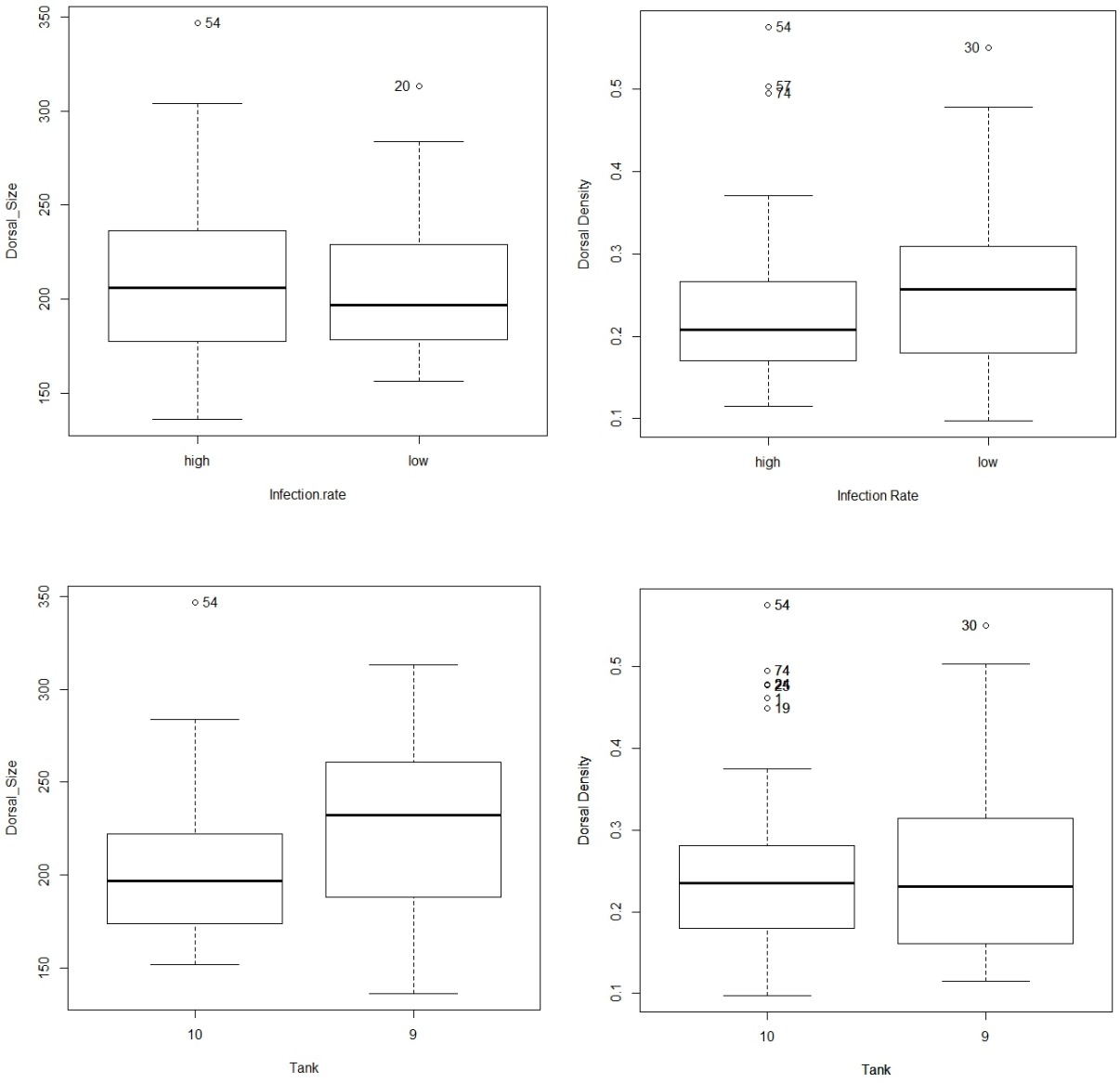


Figure 21

The effect of infection rate (top) and tank (bottom) on the mucus layer of Atlantic salmon following an infection trial.
Top: The effect of infection rate (high or low numbers of salmon lice (L. salmonis)) on dorsal mucous cell area (p=0.73, linear model, anova) (top left), dorsal mucous cell density (top right)
Bottom: The effect of tank on dorsal mucous cell area (p=0.04, linear model, anova) (bottom left), dorsal mucous cell density (bottom right)

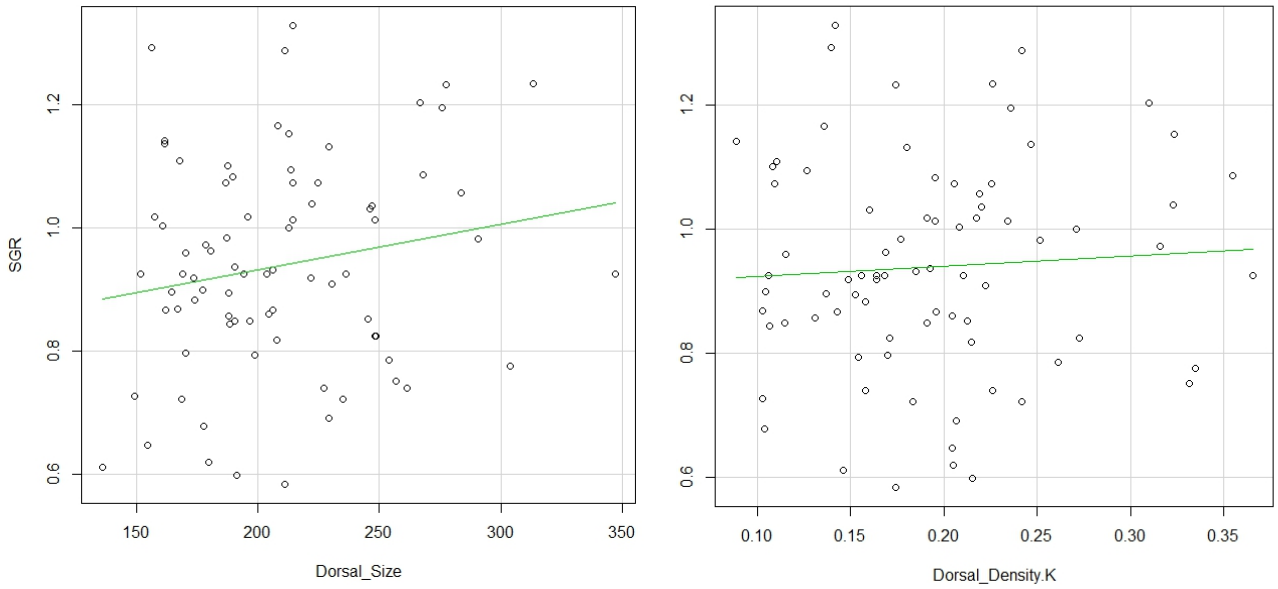


Figure 22

A The correlation between SGR (specific growth rate) and average dorsal mucous cell area (left)

B The correlation between SGR (specific growth rate) and Dorsal mucous cell density (right)

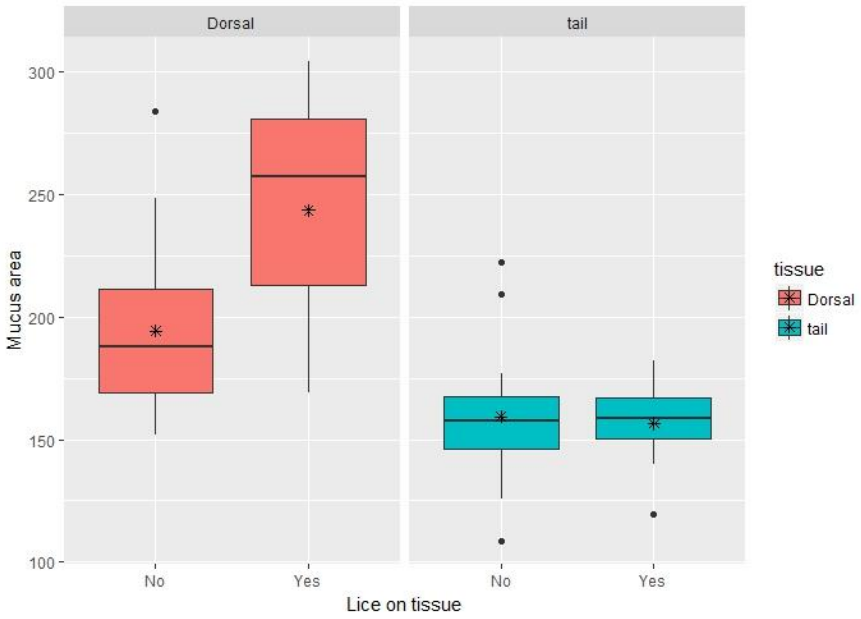


Figure 23

Mucus area by body site and local (yes) or non-local (no) infection

The effect of local infections on mucous cell area. For dorsal samples analysed (n=80) there are far fewer samples with local infections (n=10). Tail samples stereologically analysed (n=24) have a higher rate of local infections (n=8).

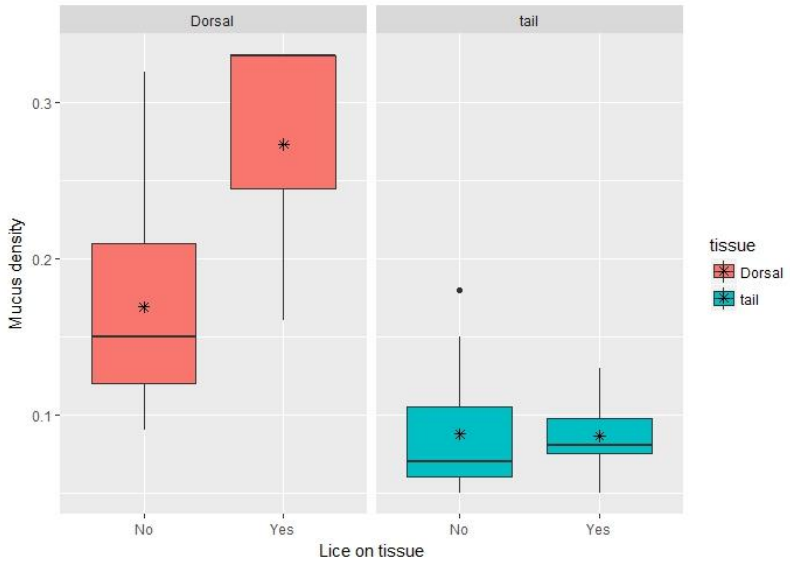


Figure 24

Mucus density by local or non-local infection, and by body site

Figure showing the effect of having lice on tissue sampled on mucus density. For dorsal samples analysed (n=80) there is far fewer samples with local infections (n=10). Tail samples stereologically analysed (n=24) have a higher rate of local infections (n=8).

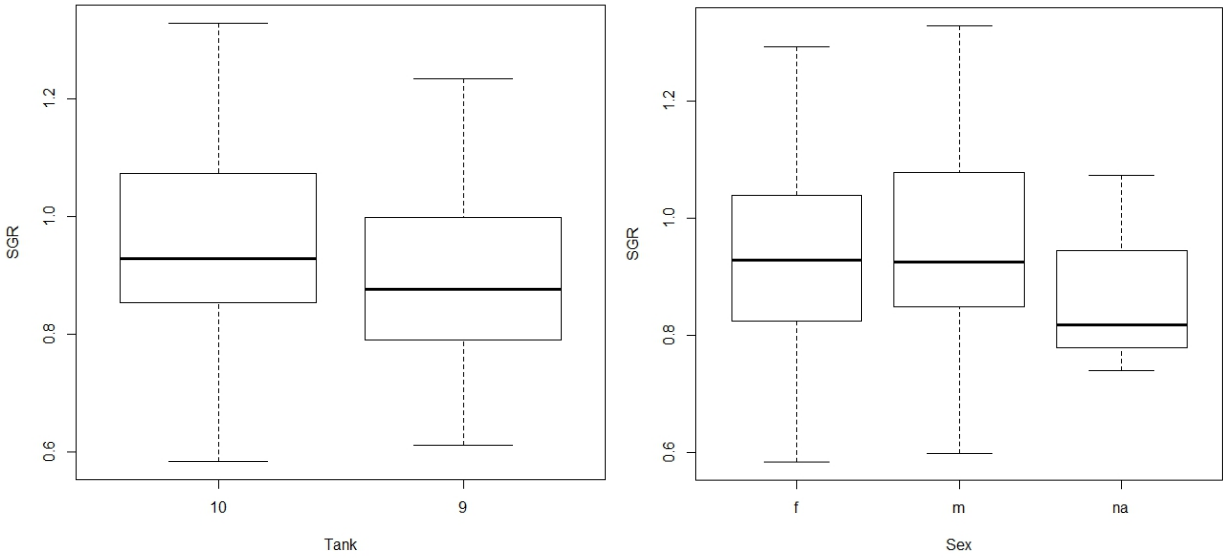


Figure 25

A: The effect of tank on SGR (Specific Growth rate over 5 months) in Atlantic salmon (p=0.37)

B: The effect of sex on SGR (Specific growth rate over 5 months) in Atlantic salmon (p=0.80)

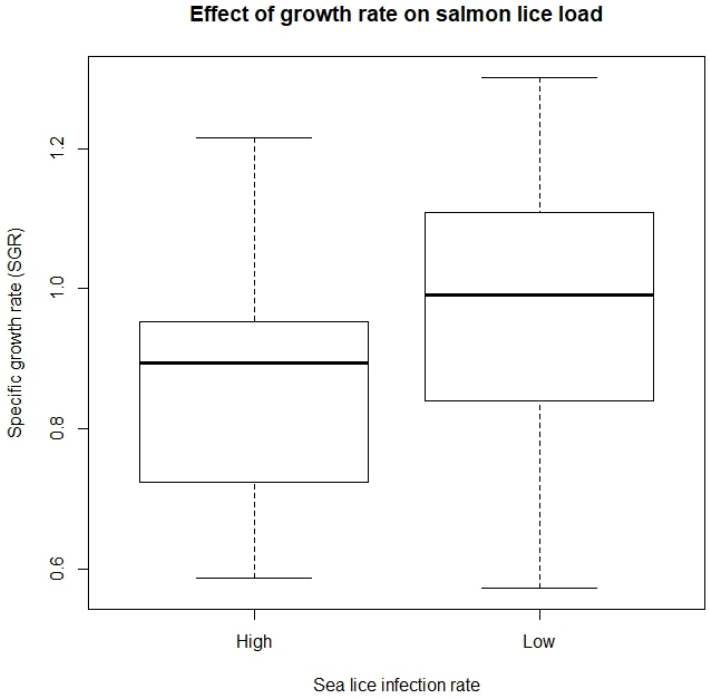


Figure 26

The effect of Specific growth rate in Atlantic salmon (Salmo salar) over 5 months on salmon lice (Lepeophtheirus salmonis) load (p=0.001)

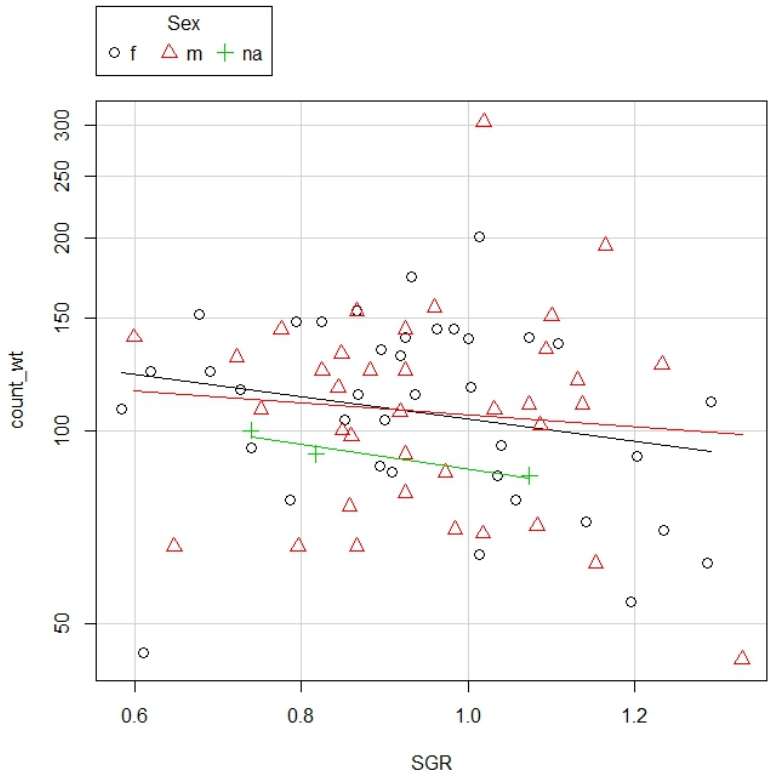


Figure 27 The relationship between count weight (the weight at the end of the trial) and SGR (specific growth rate)

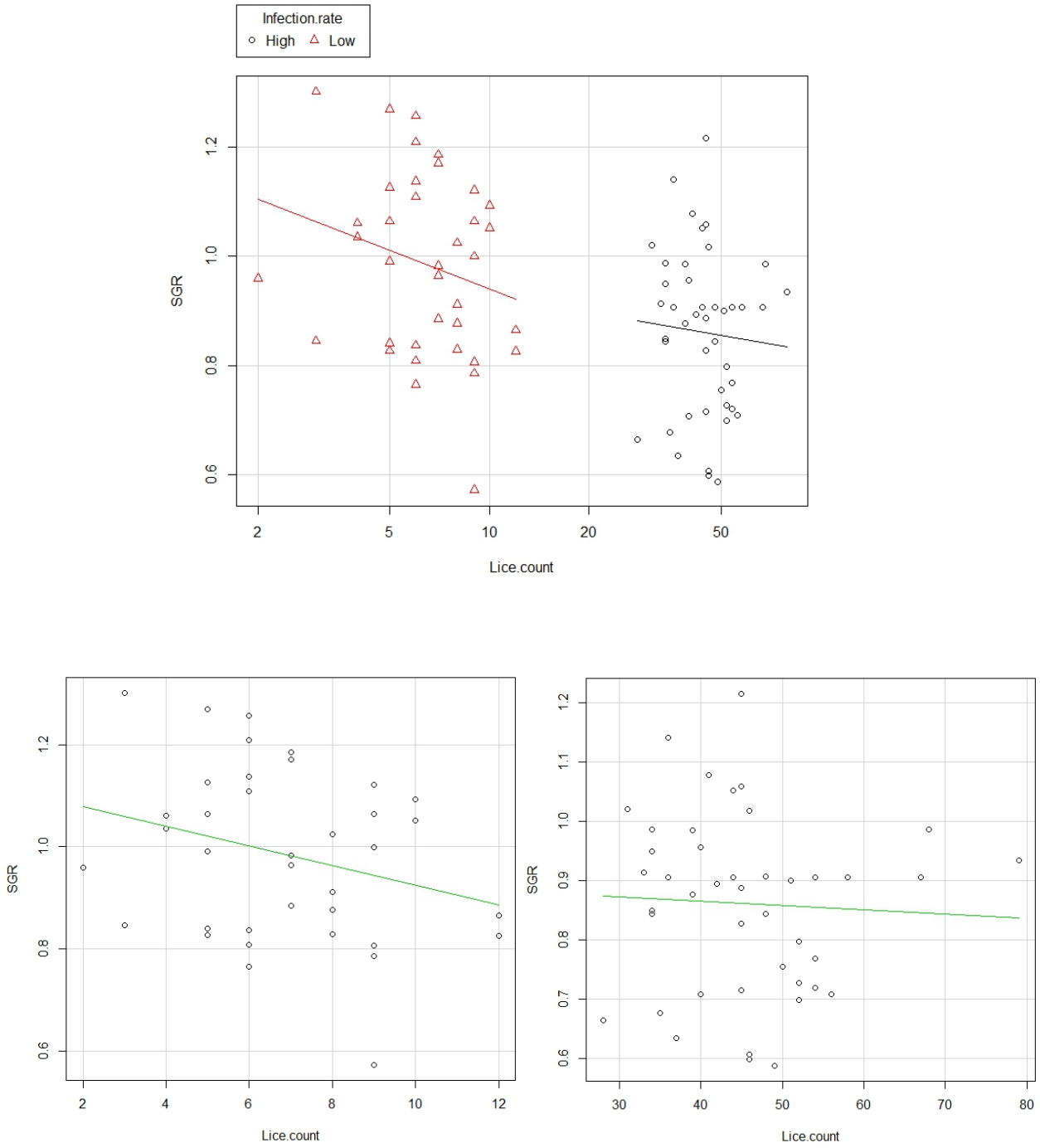


Figure 28

AB & C The correlation between SGR (specific growth rates) and total lice counts in both groups (top), the low infection rate group (lower left) and high infection rate group (lower right)

The data seem to demonstrate a negative correlation between SGR and Lice counts in the high and low infection group. Where the clearest trend is found in the low infection group (left)

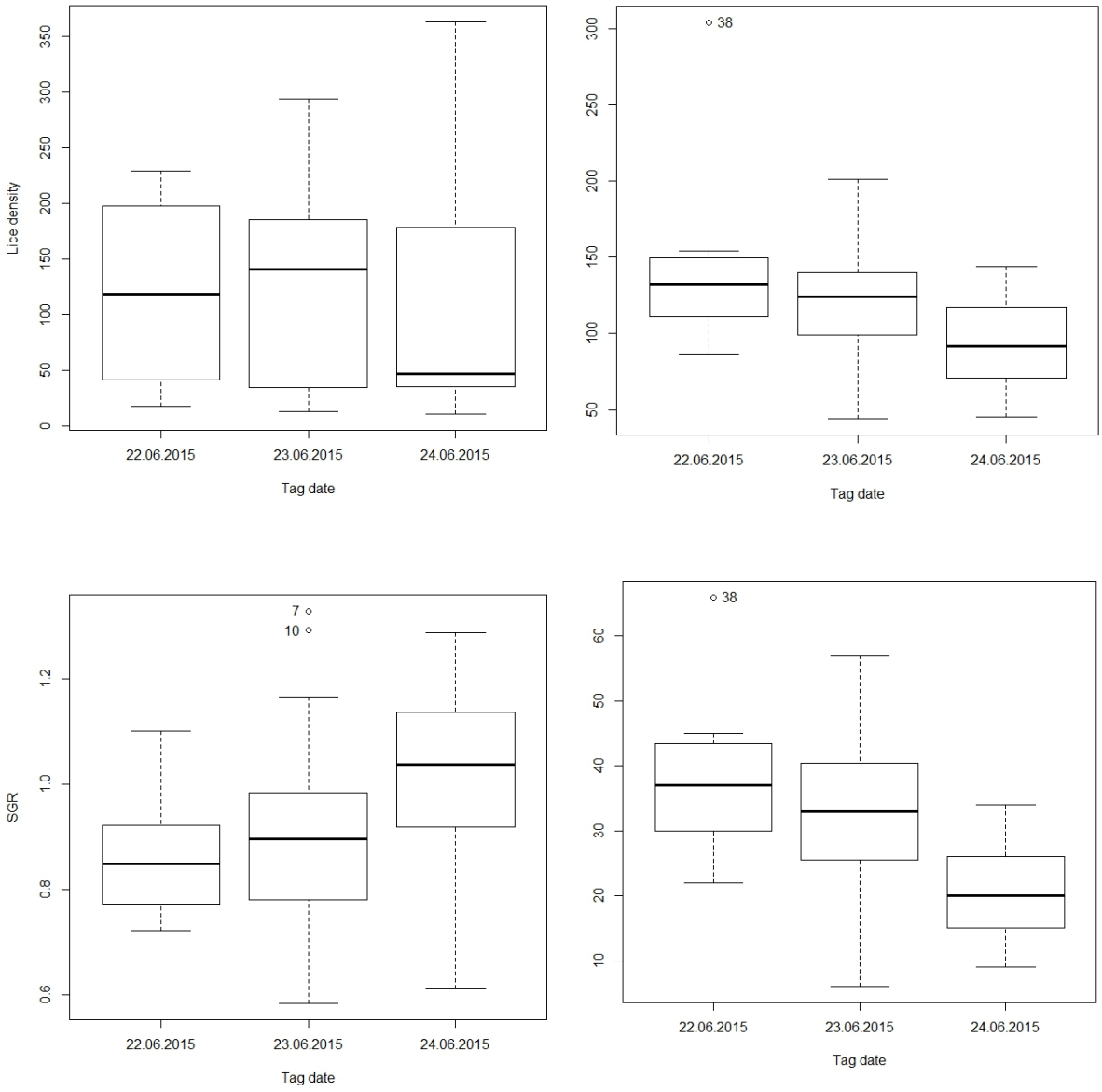


Figure 29

The effect of date of tagging on Atlantic salmon in the trial

A: Tag date vs Lice density

B: Tag date vs End weight (g)

C: Tag date vs Specific Growth Rate (SGR)

D: Mean weight found at each tag date.

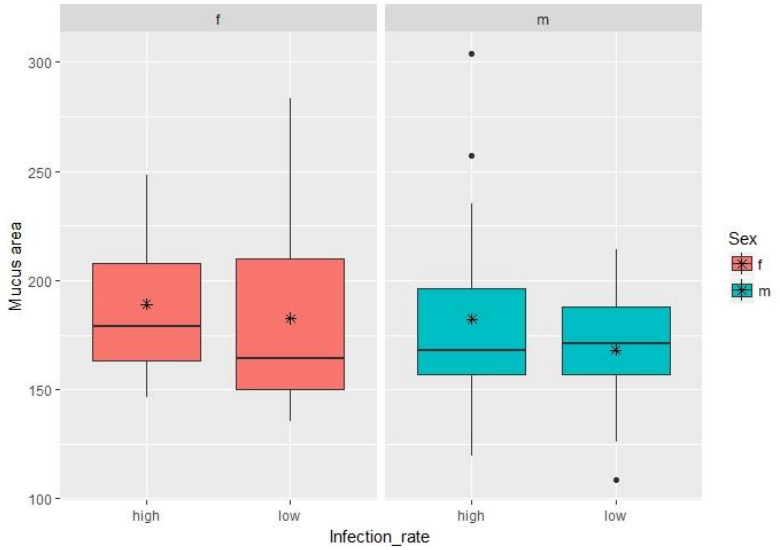


Figure 30

Dorsal mucous cell area by sex and infection rate

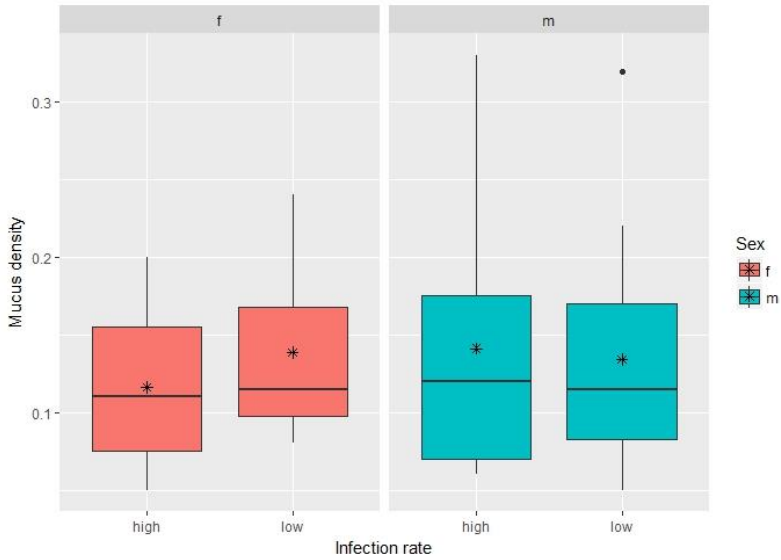


Figure 31

Dorsal mucous cell density by sex and infection rate

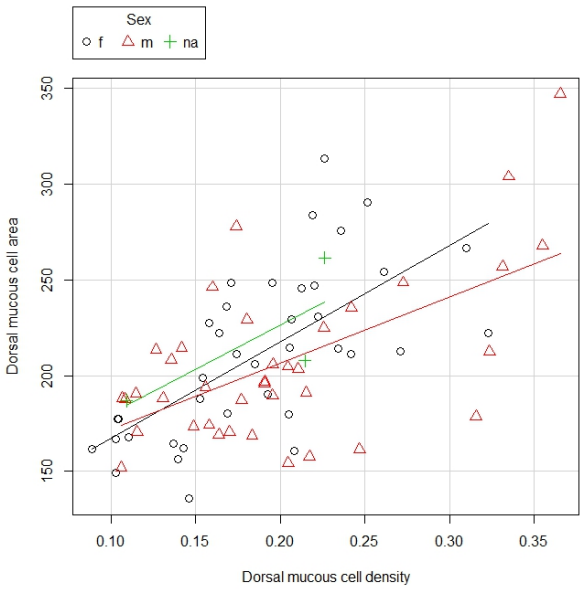
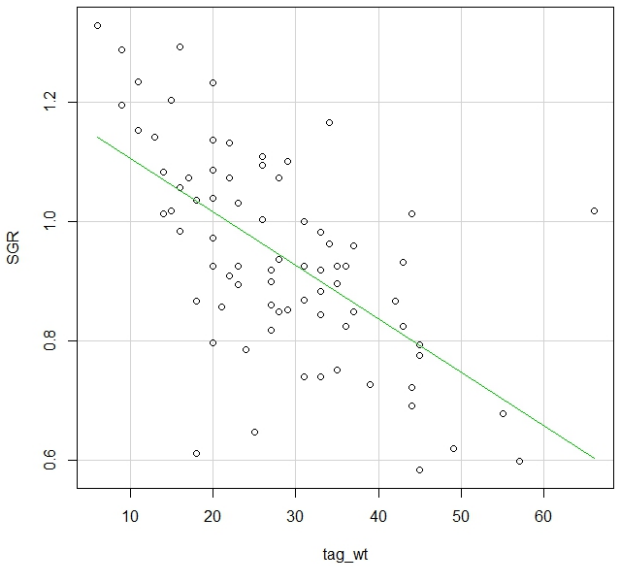


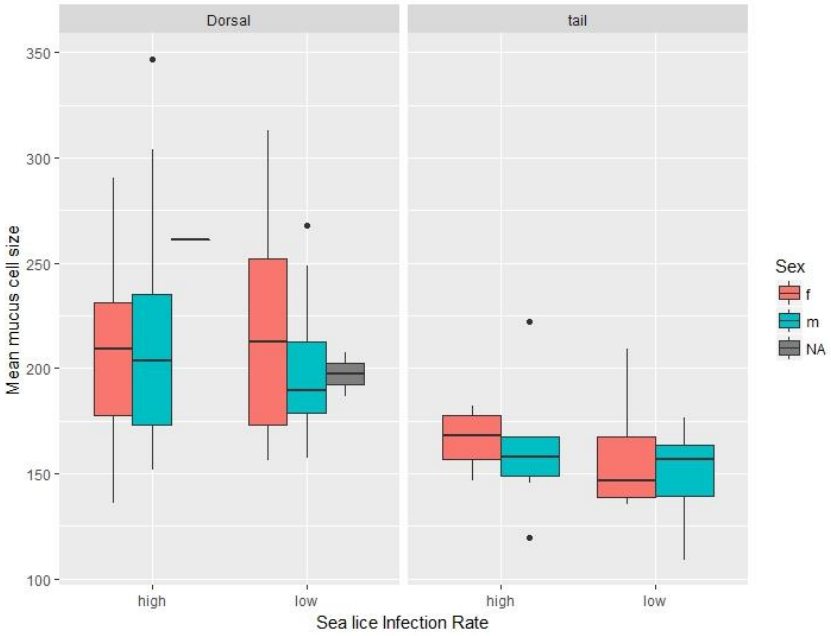
Figure 32

The linear relationship between Dorsal mucous cell density and area, by sex



19D 1

The correlation between specific growth rate between tagging and the end of the trial, and the weight at tag day.



7.3 The dataset:

The whole dataset (Semicolon-divided CSV-file) is available at: goo.gl/dWkzzo, or contact Trygve Hallberg at trygvehallberg@gmail.com