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Characterizing Hydrolicer treatment of *Lepeophtheirus salmonis* on farmed salmon

June 2021

Master's thesis

Master's thesis
2021
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MSc Ocean Resources
Submission date: June 2021
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Acknowledgments

This thesis was in cooperation with SMIR Group and written as a part of the project Taskforce Salmon Lice, and is a R&D project organized as a PhD program at NTNU. The project is a collaboration between NTNU and Fiskeri- og havbruksnæringens forskningsfinansiering (FHF), SalMar, SalmoNor, Lerøy, Måsøval Fiskeoppdrett AS, Emilsen fisk AS, MOWI, Midt-Norsk Havbruk, Ervik, Refsnes Laks AS, Skretting, Sinkaberg Hansen, AQS, Aqua Kompetanse AS, and Åkerblå.

A thank you letter in acknowledgments is not enough to express my gratitude to my supervisors Kjell Inge Reitan, Anna Solvang Båtnes, and Maria Guttu. Thank you for allowing me to choose a project I found interesting and giving me support and guidance throughout the project.

I would also like to thank SMIR Grup for making the fieldwork possible even with strict pandemic rules. Especially I would like to thank Frida Ervik for pushing to make the fieldwork possible and Ole Kristian Svartaas for the excellent help during the fieldwork.

Lastly, I must give my gratitude to my fellow student and fieldwork partner, Marit Nersten, for helping me collect half of the data and for good company during the project.

Abstract

The salmon louse, *Lepeophteirus salmonis,* infects salmonids and has since the beginning of salmon aquaculture been a challenge for fish welfare and growth. To protect the wild salmon stocks and the farmed salmon, the salmon aquaculture industry is obliged by law to keep the infestation below 0.5 adult female lice on average. The salmon aquaculture industry has several methods, both preventative actions and delousing. Hydrolicer is a mechanical delousing method used frequently. During Hydrolicer treatment, the salmon is first crowded together with a swipe net or ball line. When the upper layers of the salmon pen have a high density of salmon, the Hydrolicer system starts to pump it through the Hydrolicer pipeline system. The Hydrolicer creates turbulence and negative pressure to detach lice before waterjet flushing removes the lice. The standard velocity through the system is 2 m/s and the treatment time per salmon is 21-25s. The knowledge about the effect of the Hydrolicer on *L. salmonis* is limited, and a field study was conducted to investigate the effect of the Hydrolicer delousing method, where data from three different locations (11 net cages in total) were sampled. The salmon was sampled right before treatment (during crowding), and right after Hydrolicer delousing (20 salmon per sample; in total 28 samples before and 28 samples after treatment).

L. salmonis were registered based on life stage groups and their placement on the salmon body. The Hydrolicer pressure adjustments, salmon weight and water temperature was also registered. The overall Hydrolicer effect (% reduction of *L. salmonis*) was investigated, and the pressure adjustments and effect were tested for correlation. The reduction of lice based on *L. salmonis* placement was also investigated. The results show that Hydrolicer delousing had a significant decrease of lice at all three locations, with a median effect at location 1-3 of 78%, 59%, and 72%, respectively. The correlation between the effect and Hydrolicer pressure adjustment was low. Chalimus was the life stage group with the highest reduction, with a median effect of 100%, and adult female was the life stage group with the lowest and most variable effect, with a median effect of 62%-72%. The Hydrolicer had different effects based on *L. salmonis* placement. Zone A (the head) had the lowest reduction, the median effect at location 1-3 was 33%, 10%, and 0% respectively, and no treatment was significant. Zone B (ventral part of the body) was the zone with the highest reduction of *L. salmonis*. The median effect in zone B at location 1-3 was 92%, 91%, 100% respectively, with all treatments being significant. The adult female was found to prefer zone C (from the anal fin and to the caudal fin) during this study. The distribution of other life stage groups was more spread out. The difference in results between each location could be a result of the difference in *L. salmonis* prevalence and mean intensity before delousing, along with the considerable difference in salmon weight. The results show that the Hydrolicer treatment has different effect on the different life stage groups as well as the placement of *L. salmonis* during treatment.

Sammendrag

Lakselusen, Lepeophteirus salmonis, har siden starten av lakseoppdrett vært en utfordring for fiskevelferd og vekst. For å beskytte villaksbestandene og oppdrettslaksen er lakseoppdrettsnæringen i henhold til loven forpliktet til å holde lusenivået under 0,5 voksne hunnlus. Oppdrettsnæringen har flere metoder, både forebyggende og avlusing. Hydrolicer er en mekanisk avlusingsmetode som brukes ofte. Under Hydrolicer-behandling blir laksen først trengt med et orkast eller kulerekke. Når de øvre lagene av merden har høy tetthet av laks, begynner Hydrolicer-systemet å pumpe laksen gjennom Hydrolicer-systemet. Hydrolicer skaper turbulens og undertrykk for å løsne lus før vannspyling fjerner den. Standardhastigheten gjennom systemet er 2 m/s og behandlingstiden per laks er 21-25s. Kunnskapen om effekten av Hydrolicer på *L. salmonis* er begrenset, dermed ble det utført et feltstudie for å undersøke effekten av Hydrolicer-avlusingsmetoden, der det ble tatt prøver av data fra tre forskjellige lokaliteter (totalt 11 merder). Det ble tatt prøver av laksen rett før behandling (under trenging), og rett etter Hydrolicer-avlusing (20 laks per prøve, totalt 28 prøver før og 28 prøver etter behandling). *L. salmonis* ble registrert basert på livsstadier gruppe og deres plassering på laksekroppen. Hydrolicer trykkjusteringer, laksvekt og vanntemperatur ble også registrert. Den totale Hydrolicer-effekten (% reduksjon av L. salmonis) ble undersøkt, og trykkjusteringene og effekten ble testet for korrelasjon. Reduksjon av lus basert på plassering av *L. salmonis* ble også undersøkt. Resultatene viser at Hydrolicer-avlusing hadde en betydelig reduksjon av *L. Salmonis* ved alle 3 lokalitetene, med en medianeffekt ved lokalitet 1-3 på henholdsvis 78%, 59% og 72%. Korrelasjonen mellom effekten og Hydrolicer trykkjustering var lav. Chalimus var den livsstadier gruppen med den høyeste reduksjonen, med en medianeffekt på 100%, og voksen hunnlus var den livsstadier gruppen med den laveste og mest variable effekten, med en medianeffekt på 62-72%. Hydrolicer hadde forskjellige effekter basert på L. salmonis plassering. Sone A (hodet) hadde den laveste reduksjonen, medianeffekten på lokalitet 1-3 var henholdsvis 33%, 10% og 0%, og ingen av behandlingene var signifikant. Sone B (ventral del av kroppen) var sonen med den høyeste reduksjonen av L. salmonis. Medianeffekten i sone B ved lokalitet 1-3 var henholdsvis 92%, 91%, 100%, der alle behandlingene var signifikante. Den voksne hunnlus ble funnet å foretrekke sone C (fra analfinnen og bakover) under denne studien. Fordelingen av andre livsstadier gruppene var mer spredt. Forskjellen i resultater mellom lokalitetene kan være et resultat av forskjellen i prosentandel laks som har lus (prevalence) og gjennomsnittlig intensitet (mean intensity) av lus før avlusing, sammen med den betydelige forskjellen i laksvekt. Resultatene viser at Hydrolicer-behandlingen har ulik effekt på de forskjellige livsstadier gruppene, samt plasseringen av L. salmonis under behandlingen.

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1. Introduction

1.1 Challenges with sea lice infestation

The salmon aquaculture industry started in the end of the 1960s. Norway's long and sheltered coastline with an extensive amount of freshwater have proven to be well suited for the extensive production of Atlantic salmon. Ever since the beginning of Atlantic salmon (*Salmo salar*) farming, the industry has experienced high growth and, with high growth, numerous challenges. Bacterial and viral infections related to production and environmental conditions have caused problems (Asche et al., 1999). With a significant leap in husbandry, vaccine, and knowledge. The industry has overcome many of these challenges. Today, the most significant challenges that restrict the further increase are the sea lice infestations, escapes, diseases, access to suitable areas, and adequate feed resources (Lekve, 2013).

In 2019, the Norwegian aquaculture industry sold 1 447 531 tones of salmon, an increase of 7,1% since the previous year, and an increase of 54% since 2009 (Directorate of fisheries, 2020). The massive increase in biomass of salmon also has another effect, it creates a vast number of hosts susceptible to the parasitic salmon lice, *Lepeophtheirus salmonis*. The highest reported annual biomass of salmon is usually found in October to December (Serra-Llinares et al., 2014). High biomass and relatively high sea temperatures have led to a high rate of salmon lice infection on both farmed and wild fish populations, leading to serious welfare issues for the fish (Finstad et al., 2001). Salmon lice infections have physiological consequences on the host that lead to erosion of the epidermis with exposure of the dermis. Increased stress, changes in blood glucose or electrolytes, reduced hematocrits, and reduced swimming performance are also reported as consequences of the infections. (Wagner et al., 2008) With severe infections of *L. salmonis*, the main cause of death appears to be an osmoregulatory failure through extensive skin damage or secondary bacterial infections (Wootten et al., 1982). The negative consequences for fish welfare and growth, along with the negative implications for wild fish, has caused the government to make regulatory laws and usher in an initiative to reduce lice infestations in salmon farms. This makes lice infestations the most urgent challenge for the salmon aquaculture industry, and also the *L. salmonis* infestations have a considerable economic impact (Torrissen et al., 2013). In 2014

L. salmonis cost the Norwegian industry 5 billion NOK (Iversen et al., 2017), corresponding to ~9 % of farms revenues (Abolofia et al., 2017).

1.2 Lepeophtheirus salmonis

L. salmonis, is a direct ectoparasite, as it is specific to salmonids, and thus a problem for farmed Atlantic salmon. They only need one host to complete its lifecycle, and *L. salmonis* is a copepod belonging to the Caligidae family with a circumpolar distribution in the Northern Hemisphere (Torrissen et al., 2013). It thrives in temperate to subarctic areas and probably requires temperatures of 4°C or higher to complete its life cycle successfully (Boxaspen and Næss, 2000). In studies conducted at temperatures between 2°C and 10°C, the time of hatching ranged from 45.1 days at 2°C to 8.7 days at 10°C, and a large proportion of the nauplii developed into copepods even at 4°C (Boxaspen and Næss, 2000). A study by (Samsing et al., 2016) examined the success rate of eggs hatching and the further development. Eggs incubated at 20 °C and 15 °C had a 100% success rate. However, eggs incubated at 3°C only had a hatching success rate of 28 ± 4% at hatching. All larvae successfully developed to the copepodid stage except for those incubated at 3 °C., where all nauplii died without molting to the copepodid stage. This shows that water temperatures strongly influence the hatching and development of *L. salmonis* larvae. Thus, *L. salmonis* can develop into the infectious stage during the winter, even though biological processes are slowed down.

1.3 Life cycle

L. salmonis has eight different life stages divided into free-living and parasitic stages (figure 1; Hamre et al., 2013). The eight stages are separated from the preceding stage by a molt exposing a new cuticle underneath (Hayward et al., 2011), and the different developmental stages' duration are directly dependent on the water temperature (Stien et al., 2005). The free-living stages consists of two planktonic stages, known as nauplius 1 and 2. During the planktonic stages the lice do not feed (Boxaspen and Næss, 2000), and the nauplius subsists entirely on endogenous lipid reserves. The nauplius then molts into an infective copepodite that actively searches for hosts. The copepodite uses physical cues such as pressure, salinity, light, and vibrations to locate possible areas where hosts are more likely to be. To improve their success rate further, they also display diurnal vertical migrations that enhance their chances of encountering hosts. The copepodite also uses chemoreceptors to identify if a fish is a suitable host (Mordue and Birkett, 2009). If the lice successfully attach to a host, the lice molt, and the parasitic life stages begin. The parasitic stages start with two nonmotile stages, chalimus 1 and chalimus 2. It clings to the hosts through a front filament and feeds on the skin and mucus during these stages. Chalimus 2 then molt into the preadult 1 and 2 stages, where it can move around on the surface of the salmon and swim in the water column (Hayward et al., 2011). The last stage is an adult male and an adult female, where they have reached sexual maturity. *L. salmonis* exhibit sexual dimorphism, where the female is larger than the male. Adult females procreate paired egg strings from the posterior end of the genital segment, that can hold up to 700 eggs (Wootten et al., 1982).

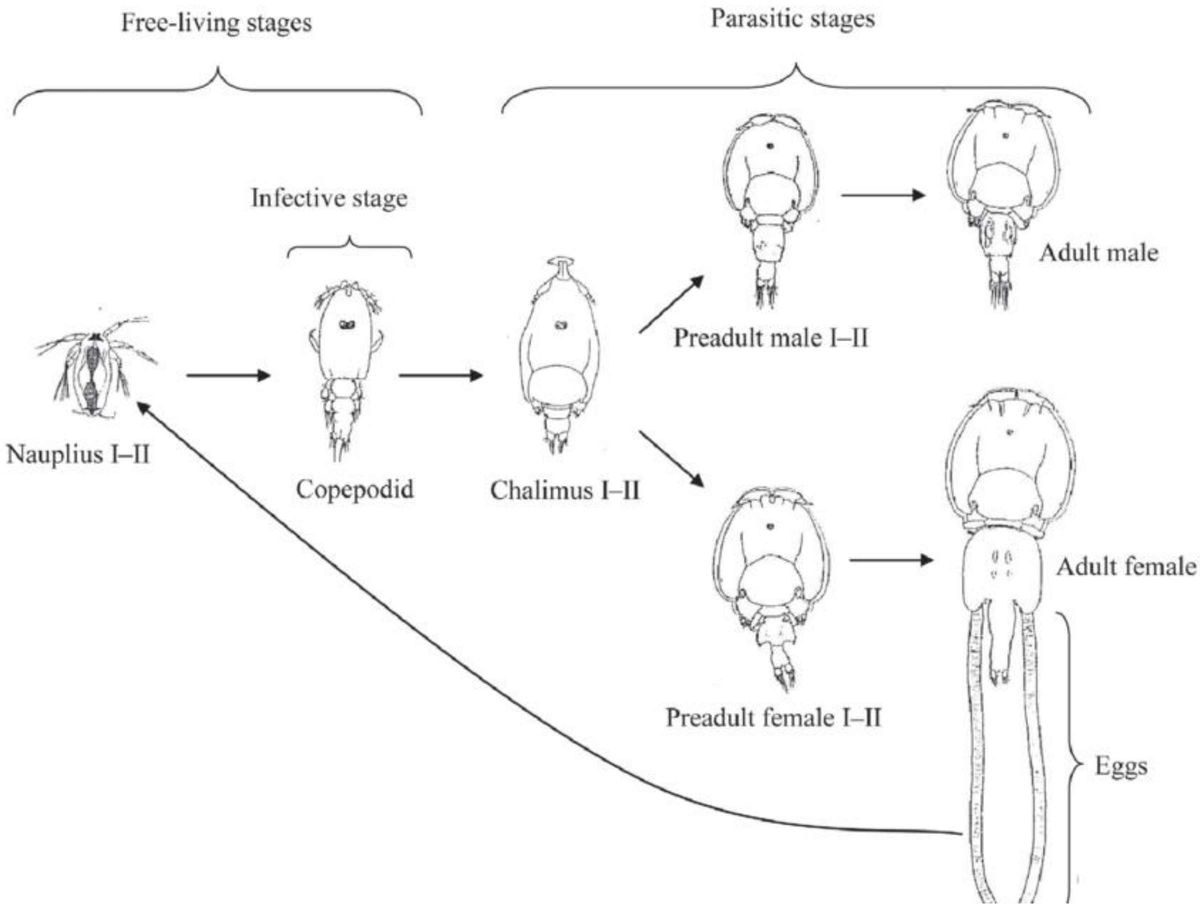

Figure 1 Developmental stages of Lepeophtheirus salmonis (Igboeli et al., 2014)

1.4 *L. salmonis* attachment placement on the salmon

L. salmonis have mobile preadult and adult stages that often move around on the body as well as between hosts. This is especially the case for adult male sea lice (Ritchie, 1997). Their unpredictable movement between hosts varies greatly and seems dependent on males searching for a female. Water current velocity and the salmon swimming behavior have influence on the attachment placement, but little effect on post-settlement survival and dispersal (Samsing et al., 2015), and this complicates the understanding of their prevalence and distribution. Previous studies have investigated the preferred attachment location for the lice (Bui et al., 2020). Showing that the mobile stages tend to prefer the head and the dorsal side of the salmon.

1.5 Regulations on the control of salmon lice in aquaculture facilities

The Norwegian Directorate of fisheries manage the regulatory law for farmed salmon and the lice infestations and are controlled by the Norwegian Food Safety Authority. The salmon aquaculture industry is obliged by law to keep the infestation below 0.5 adult female lice on average. During the annual wild salmon smolt migration, the upper limit of adult female lice per salmon is 0.2 (Directorate of fisheries, 2013). The purpose of the regulations is to reduce the occurrence of salmon lice in aquaculture facilities in order to reduce the harmful effects on wild salmonids stocks are minimized and to, combat the possibility of development of resistance in salmon lice. Each salmon farm must control its lice infestation and have the infrastructure, plans, and measures ready to act should the lice infestation exceed the limits of the regulations. Should a farm exceed the limit, they must engage in delousing. In all treatment, consideration must be given to the welfare of fish, the environment, and food safety. If this is not possible through measures, the fish must be slaughtered or destroyed.

1.6 Delousing treatments

Today the farmers have several methods to combat lice infestation and frequently use both preventative actions and delousing treatments. The delousing methods can be categorized into two groups; Medicinal lice treatments and mechanical lice treatments. Bath and oral treatments are medicinal treatments (Jevne and Reitan, 2019). The mechanical treatments include different physical techniques to dislodge lice. In one of the mechanical treatments, thermic treatment, the salmon is pumped through a pipe-system with heated water 28-34°C (Grøntvedt et al., 2015). Treatment time per salmon varies from 20-30 seconds. The lice will be stunned during the thermic treatment and fall off the host (Jevne and Reitan, 2019). Freshwater is a bath treatment were fish is loaded on board a well-boat and kept in freshwater for 6-10 hours (Gaasø, 2019). The freshwater is used to give the salmon lice osmotic shock (Jevne and Reitan, 2019). The last mechanical treatment is lice flushers. There are several versions available to the market, but in general they use water injectors to create turbulence and negative pressure to remove lice from the salmon (Jevne and Reitan, 2019). One of the flusher methods is the Hydrolicer.

1.7 Hydrolicer delousing

The Hydrolicer delousing treatment is a mechanical, non-medical method for removing salmon lice from farmed fish. Like other delousing methods the salmon is first crowded together with a swipe net or ball line (Nersten, 2021). When the upper layers of the salmon pen have a high density of salmon, the Hydrolicer system starts to pump it through the hydrolicer pipe line system (figure 2). The Hydrolicer system contains three key components: the Hydrolicer, the Hydropump and the Hydrofilter. The Hydrolicer is the main delousing component that creates turbulence and negative pressure to detach lice before waterjet flushing removes the lice. This process is repeated in two Hydrolicer units in each line, and each delousing boat can have several Hydrolicer lines. The Hydroflow is a pump that transports seawater and salmon through the Hydrolicer system. The standard velocity through the system is 2 m/s and the treatment time per salmon is 21-25 seconds. The last part is the Hydrofilter, a drum filter with a filtration grade of 80-micron rectangular mesh, which collects lice of all stages (Hammer, 2021). A previous study reported a Hydrolicer effect (% reduction of lice) of 73-83% for nonmotile lice (copepodite and chalimus 1 and 2), 78-95% for motile lice (preadult 1 and 2 and adult male), and 55-92% for adult female lice (Erikson et al., 2018). During this study, the salmon weight varied from 2.8kg to 4.1kg.

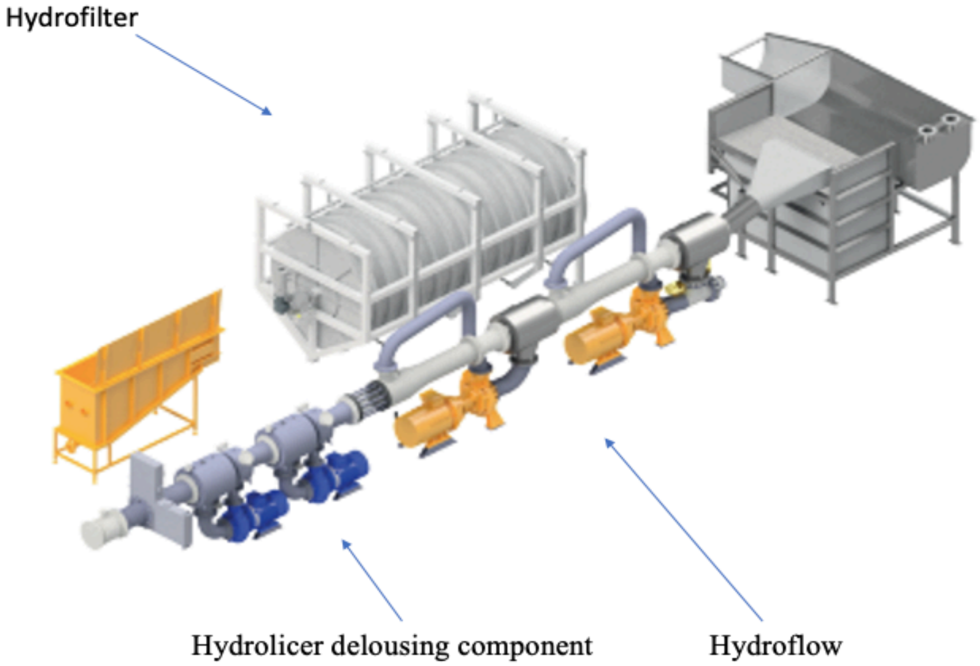

Figure 2 Main components of the Hydrolicer system. The Hydrolicer component creates negative pressure and flushes the lice off the hosts. Hydroflow is a pump that transports the seawater and salmon through the system. The velocity through the treatment is typically 2 m/s, and the treatment time per salmon is 21-25 s. The Hydrofilter filtrate the water from the treatment before going back to the sea. The Hydrofilter collects all the lice that has fallen off during treatment. (Hammer, 2021) Picture source: https://3wwz433myfgt2hx542cnt7s1-wpengine.netdna-ssl.com/wp-content/uploads/2019/09/Hydrolicer.pdf

1.8 Aim

The overall aim of this thesis was to characterize the delousing effect on *L. salmonis with* Hydrolicer treatment. To reach the overall aim, the following research objectives were created.

Thesis research objectives:

1. Characterize the overall delousing effect of Hydrolicer on lice removal.
2. Characterize the delousing effect of using different pressure adjustments on the Hydrolicer.
3. Characterize the delousing effect of using Hydrolicer on chalimus (1 and 2), pre-adult (1 and 2 both female and male), adult male, and adult female.
4. Characterize the preferred sea lice placement on the fish and the effect of Hydrolicer treatment on the attachment distribution of L. salmonis on the fish.

2. Materials and Method

2.1 Study site

The fieldwork was carried out on the delousing boat Hydro Patriot from 6. October 2020 to 20. October 2020 in production area 8 (the coast of Helgeland; figure 3). Data was collected during delousing operations at three different salmon aquaculture locations that will be described as locations 1-3 to protect their anonymity.

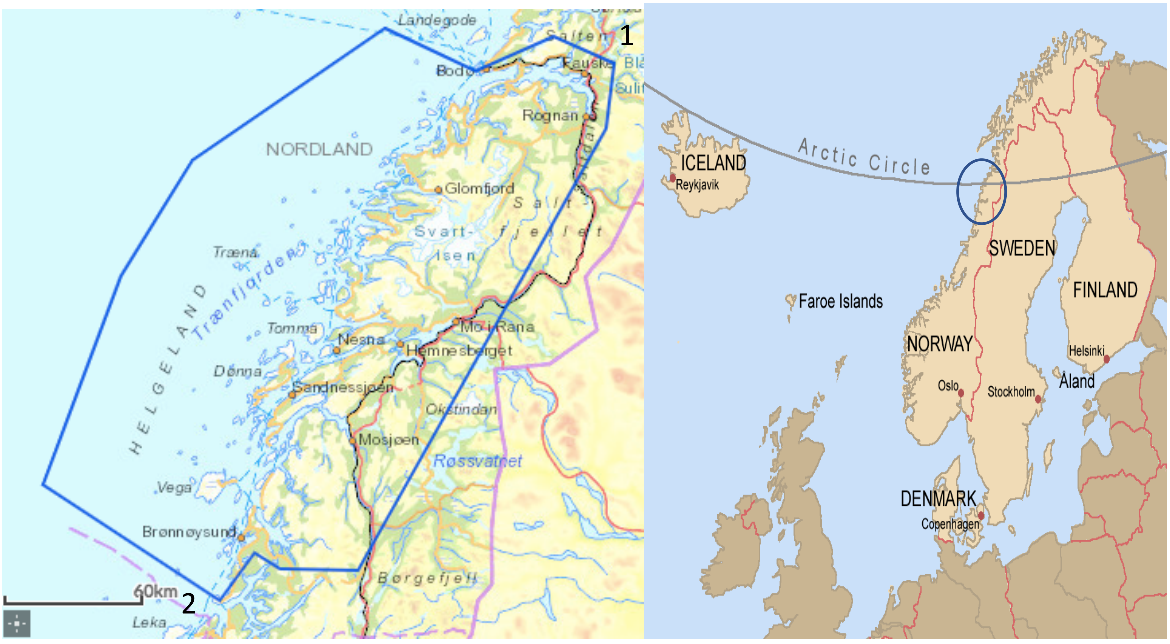

Figure 3 Production area 8 has the boarders 1) Fauske top right [”lat”:”67°18.0333N”,”lon”:”015°41.8315E”] to open sea (west of Helligvær) [”lat”:”67°25.6708N”,”lon”:”013°24.2379E”] and 2) bottom right, Kvaløya [”lat”:”65°12.5720N”,”lon”:”012°01.1859E”

2.2 Hydro Patriot

The Hydrolicer system onboard the Hydro Patriot delousing vessel has 8 treatment lines, each equipped with three Hydroflow pumps. Two placed before the Hydrolicer and one after, and two Hydrolicer version 6.0 with a 4 mm flushing jet. Lastly there are three Hydrofilters in total, that filters out lice from the process water. During sampling, the Hydroflow provided a continuous velocity of 2 m/s. The Hydrolicer pressure adjustment ranged from 0.6 Bar to 1.1 Bar. Changes to the Hydrolicer settings throughout the fieldwork were controlled by the captain and fish health personnel and registered during sampling.

2.3 Data collection

Salmon was sampled from two different stations on Hydro Patriot (figure 4). Station A is the sample station above the entrance of the Hydrolicer line, where salmon were collected under dense crowding. The data for station A will be referred to as pre-Hydrolicer. Sample station B is placed right after the Hydrolicer line, where the salmon was sampled after passing through the delousing system, and the data from station B will be referred to as post-Hydrolicer.

Figure 4 Hydro Patriot delousing vessel, where data was collected. Sample station A (pipes are elevated during sea travel) above the entrance of the Hydrolicer line. Sample station B at the water slide placed after Hydrolicer line.

20 salmon were sampled for pre-Hydrolicer and post-Hydrolicer treatment. The samplings were repeated for each crowding process. The crowding procedure varied between two methods, swipe net and ball line (Nersten, 2021). The salmon aquaculture farms provided the average weight of the salmon for each salmon pen, and sea temperature.

Table 1 Information about location 1-3

	Location 1	*Location 2*	*Location 3*
Sampling date	09.10.20	15.10.20	17.10.20
Water temperature (°C)	11,3	10,3	8,3
N salmon pens	4	4	3
N salmon sampled before Hydrolicer	160	240	160
N salmon sampled after Hydrolicer	*160*	240	160
Salmon weight	$5.49 - 6.24kg$	$3.10 - 3.60kg$	$3.08 - 4.50kg$
Prevalence	*81.2%*	63.3%	43.3%
Mean Intensity	*2.62*	1.91	3.01

Sample procedure at station A

At sampling station A, a foldable drop net connected to a crane was used to catch salmon from the salmon pen (figure 5). The sampling was only done under intense crowding. Salmon caught with drop net was released into the counting station. The collections of salmon at station A were performed by the crew on board. The station contained three different tubs. Tub one was the reception tub, filled with seawater and a grate used to push the salmon over in tub two. Tub two was filled with seawater and Benzoak vet. (15-20ml pr 100L seawater). When the salmon was adequately anesthetized, lice were counted and their position on the salmon body were registered (see chapter 2.4). After registration, the salmon was moved to tub three, where fresh seawater was circulated. The salmon were kept there until showing signs of waking up, then they were moved through a tube that leads back to the salmon pen where it was sampled from (figure 5). After the sampling, the tubs were inspected for lice that had fallen off the host. Then the tubs were emptied through a sieve to catch lice. The tubs were cleaned between each sampling.

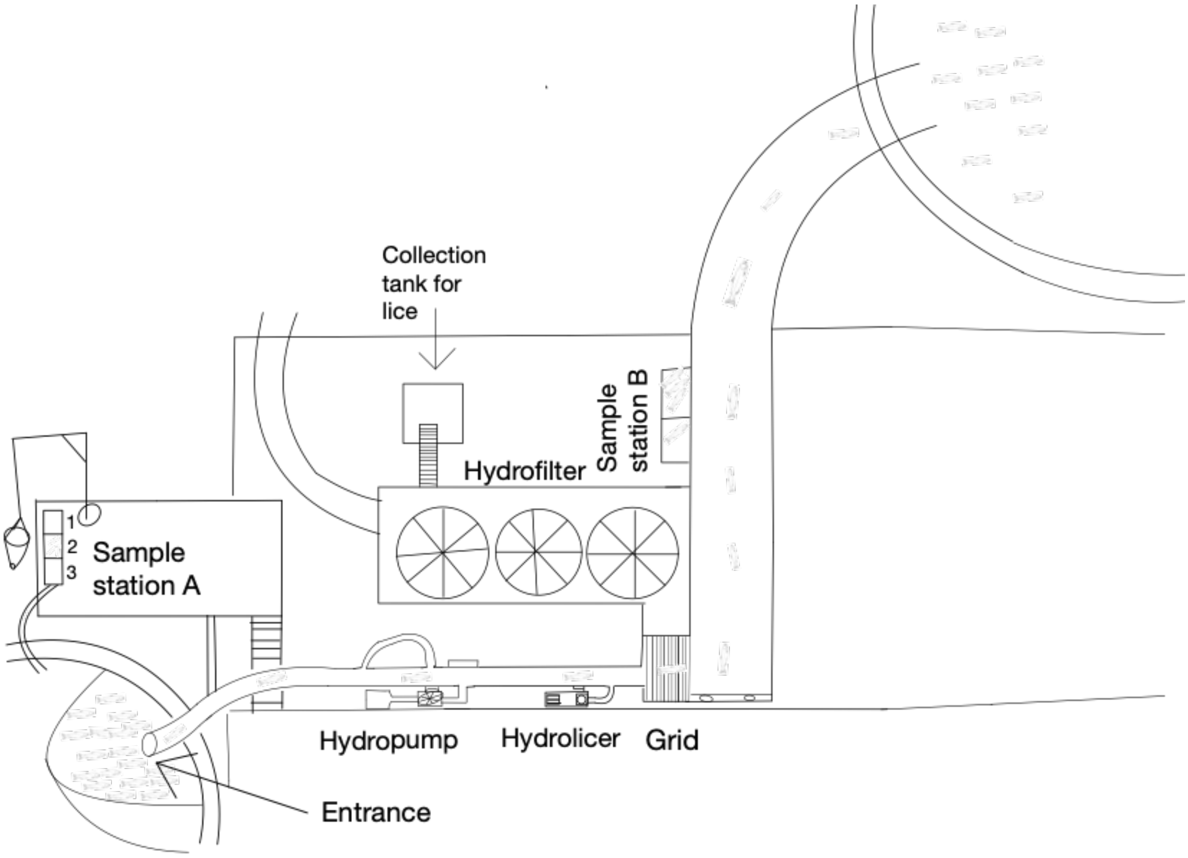

Figure 5 Simplified drawing of the Hydrolicer treatment process. The salmon was pumped in through the entrance of the Hydrolicer line. The treatments start as it gets inside the Hydrolicer line and ends when the salmon slide over the grid. The delousing treatment happens when the salmon goes through the Hydrolicer part of the line. The process water and lice go through the grid. The salmon follows the slide down into an empty salmon pen. The process water goes through three Hydrofilters that collect lice and moves it to a collection tank. Sample station A is placed above the entrance of Hydrolicer and collects samples before the salmon have been deloused. Sample station B is placed after the Hydrolicer treatment lines, the salmon sampled at station B have been deloused.

Sample procedure at station B

Salmon was sampled from the assembly line/slide with a dipnet (figure 5). The sampled salmon was caught from a random hydrolicer line (figure 6).

The counting station had two tubs—one with seawater and Benzoak vet. and the last with circulating seawater. Salmon was collected 1-4 at the time, until 20 salmon had been sampled. The registration process of lice was the same as at station A. When the salmon showed signs of waking up, they were released back into the slide and followed the slide down to a new salmon pen.

Figure 6 Catching salmon with dipnet at sample station B

2.4 Lice Registration

In this study *L. salmonis* were grouped into; Chalimus (chalimus 1-2), pre-adult (pre-adult 1-2, both female and male), adult male, and adult female. The salmon body was divided into four zones (figure 7). Based on those four zones the position of *L. salmonis* was registered. Zone A is the head. Zone B is the ventral part of the body, from the lateral line and up and ends at the adipose fin. Zone C is from the adipose fin to the anal fin and behind to the caudal fin's end. Zone D is from the lateral line and down and stretches from the anal fin to the gills (figure 7).

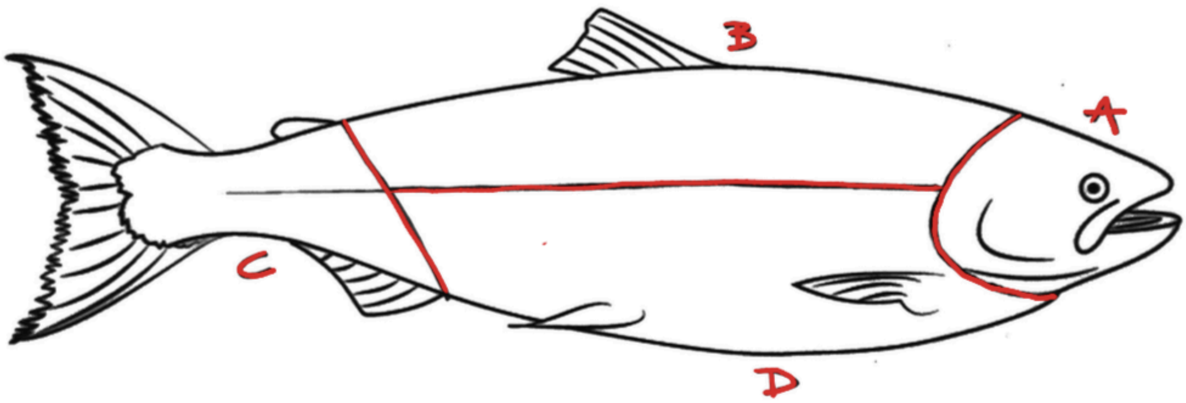

Figure 7 Salmon divided into four zones

2.5 Data treatment and statistic

The analyzing and calculations of the registered data were done in Excel (Microsoft excel for Mac, version 16.48). All statistical analyses were performed with SPSS software (IMB SPSS Statistics, version 26). The data from the three locations were kept separate at all statistical models, calculations, and graphs because the factors influencing the delousing effect had different values. Each data point in the analyses and graphs represents the mean lice infestation per salmon. The normality of data was investigated using the descriptive statistics function in SPSS. The data did not have a normal distribution. Thus the non-parametric test Mann-Whitney U test and Wilcoxon sign rank test was selected to investigate significance. The significance level for all analyses was set to $p<0.01$ instead of 0.05 to reduce the false discovery rate when conducting multiple tests.

3. Results

3.1 Overall Hydrolicer delousing effect

At location 1, the salmon weight varied from 5.49kg to 6,24kg (table 1) and had a *L. salmonis* prevalence of 81% and a mean lice infestation of 2.40 (representing all life stages grouped together) before Hydrolicer delousing treatment. The mean lice infestation was reduced to 0.46 after Hydrolicer treatment (figure 8 A). The reduction in lice was significant compared to the lice infestation before treatment ($p<0.01$; appendix table 2). The median delousing effect (% reduction of *L. salmonis*) was 78% with an interquartile range from 74-87% (Figure 9).

At location 2, the salmon weight varied from 3.1kg to 3.6kg (table 1) and had a prevalence of 63% and a mean lice infestation of 1.37 (representing all life stages grouped together) before Hydrolicer delousing treatment. The mean lice infestation was reduced to 0.57 after Hydrolicer treatment (figure 8 B). The reduction in lice was significant compared to the lice infestation before treatment ($p<0.01$; appendix table 3). The median delousing effect (% reduction of *L. salmonis*) was 59% with an interquartile range from 49-73% (figure 9). The Hydrolicer treatment had lower effect and higher variation in effect compared to the Hydrolicer treatment at location 1.

At location 3, the salmon weight varied from 3.08kg to 4.5kg (table 1) and had a prevalence of 43% and a mean lice infestation of 2.53. (representing all life stages grouped together) before Hydrolicer delousing treatment. The mean lice infestation was reduced to 0.71 after Hydrolicer treatment (figure 8 C). The lice reduction was significant compared to the before treatment ($p<0.01$; appendix table 4). The median delousing effect (% reduction of *L. salmonis*) was 72% with an interquartile range from 61-86% (figure 9). The Hydrolicer treatment had higher effect and lower variation in effect compared to the Hydrolicer treatment at location 2.

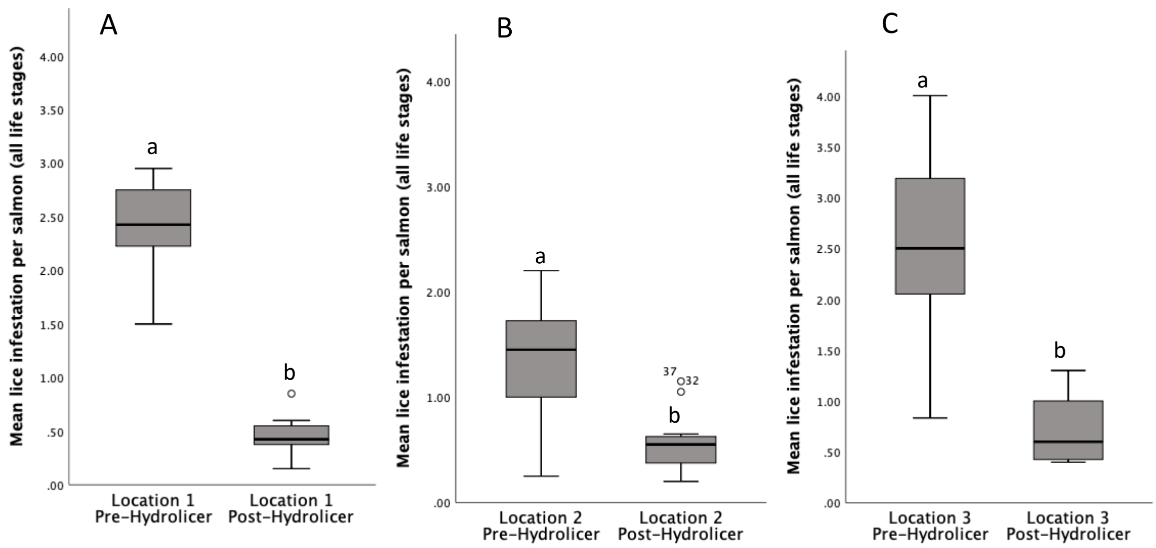

Figure 8 Mean number of L. salmonis per salmon, including all life stages, before and after Hydrolicer treatment at A) Location 1 (20 salmon sampled 8 times), B) Location 2 (20 salmon sampled 12 times), and C) Location 3 (20 salmon sampled 8 times). Different letters (a and b) denote significant difference (p<0.01) in mean lice infestation. Circle symbols show outliers.

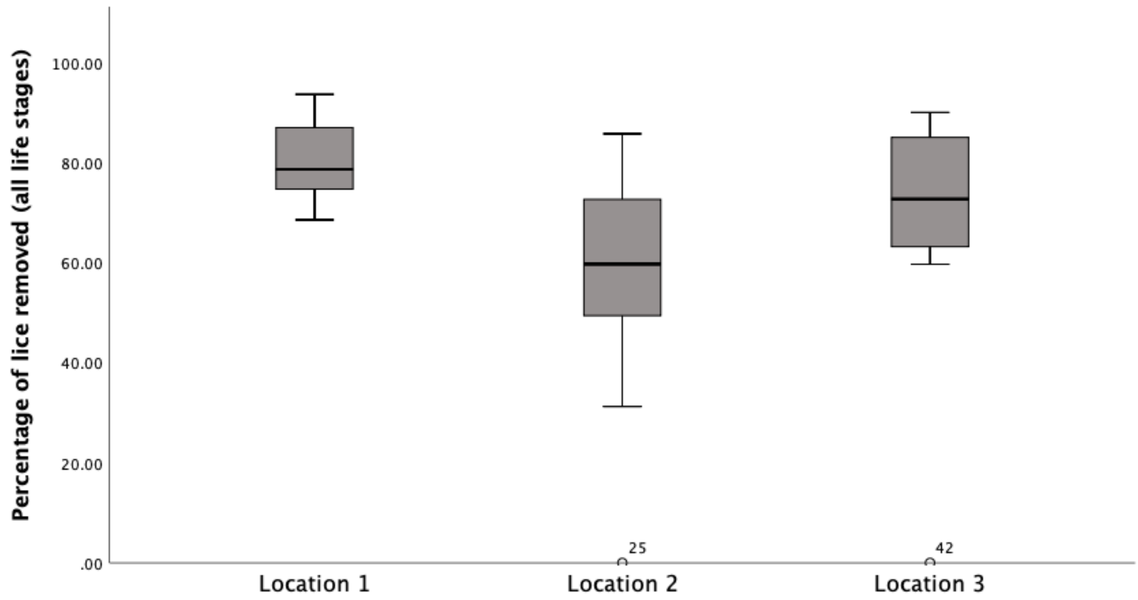

Figure 9 Hydrolicer delousing effect – percentage of L. salmonis removed with Hydrolicer treatment at location 1-3. 20 salmon sampled 8 times at location 1 and 3 and sampled 12 times at location 2 before and after Hydrolicer treatment. Circle symbols show outliers.

3.2 The effect based on pressure adjustment

The delousing effect (% reduction of *L. salmonis*) of different pressure adjustments, 0.6 bar, 0.7 bar, and 1.1 bar at location 1, showed a trend for better effect with higher pressure adjustments. The effect for 0.6 bar varied from 68% to 86%. The effect for 0.7 bar varied from 76% to 80%. The effect for 1.1 bar varied from 87% to 93% (figure 10).

At location 2 the pressure adjustment were 0.7 bar, 0.8 bar, and 0.9 bar. The effect for 0.7 bar ranged from 0% to 56%. The effect for 0.8 bar ranged from 31% to 85%. The effect for 0.9 ranged from 70% to 84% (figure 11). There was a trend for better effect with higher pressure adjustments like the results from location 1.

At location 3 the pressure adjustments was 0.8 bar, 0.9 bar, and 1.0 bar. The effect for 0.8 bar varied from 66% to 82%. The effect for 0.9 bar varied from 59% to 87%. The effect for 1.0 bar varied from 0% to 90% (figure 12). The results from location 3 contradicts the trend for higher delousing effect with higher pressure adjustments, showed at location 1 and 2. A correlation test between effect and pressure adjustment gave an R^2-value of 0.216 (appendix table 6), showing a weak relationship between effect and pressure adjustment.

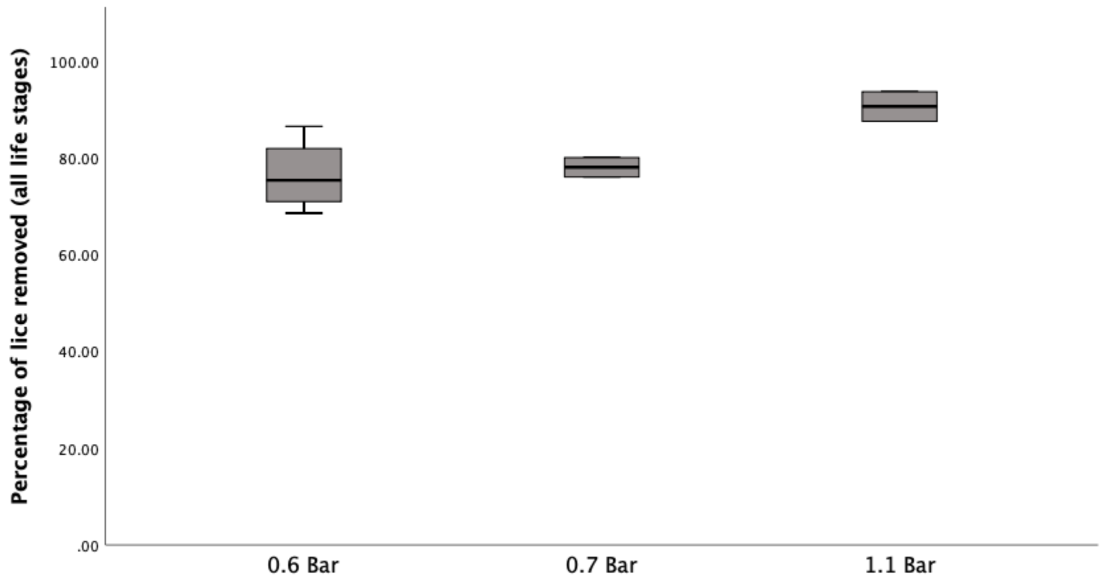

Figure 10 Percentage of L. salmonis removed from farmed salmon at Location 1 with Hydrolicer treatment using 0.6 bar (20 salmon sampled 4 times), 0.7 bar (20 salmon sampled 2 times), and 1.1 bar (20 salmon sampled 2 times) in pressure adjustments.

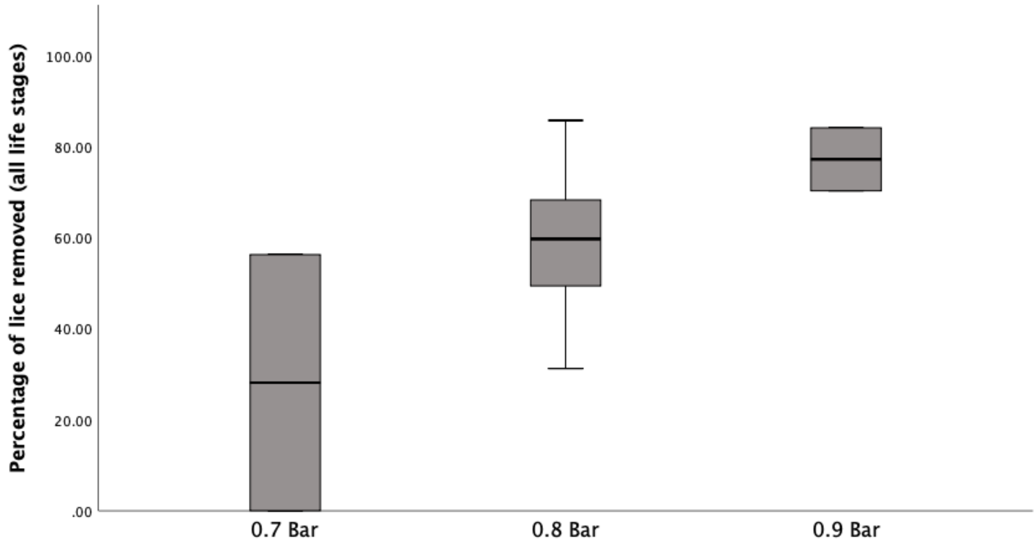

Figure 11 Percentage of L. salmonis removed from farmed salmon at location 2 with Hydrolicer treatment using 0.7 bar (20 salmon sampled 2 times), 0.8 bar (20 salmon sampled 8 times), and 0.9 (20 salmon sampled 2 times) bar in pressure adjustments.

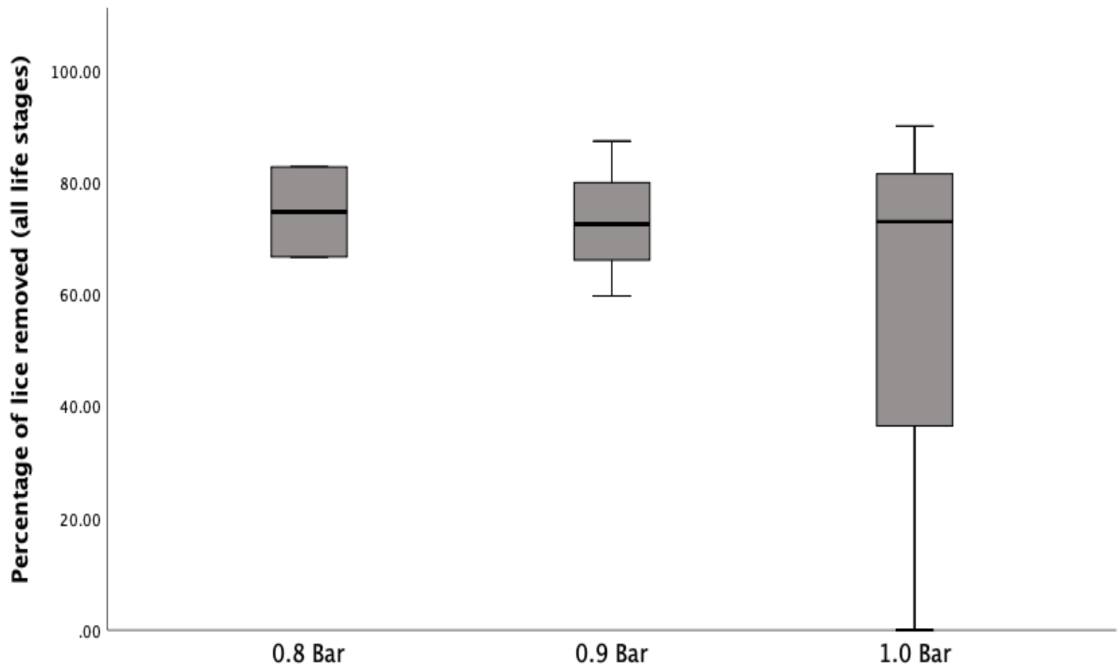

Figure 12 Percentage of L. salmonis removed from farmed salmon at location 3 with Hydrolicer treatment using 0.8 bar (20 salmon sampled 2 times), 0.9 bar (20 salmon sampled 3 times), and 1.0 bar (20 salmon sampled 3 times) in pressure adjustments.

3.3 The Hydrolicer delousing effect on life stage groups of *L. salmonis*

At location 1, the mean infestation of chalimus was 0.43, pre-adult 0.56, adult male 0.88 and adult female 0.52 per salmon before Hydrolicer treatment. The use of Hydrolicer reduced a significant amount of each life stage (figure 13 A-D; p<0.01; Appendix table 6). The Hydrolicer treatment reduced the mean infestation of chalimus to 0, pre-adult to 0.08, adult male to 0.18 and female adult to 0.19. The median delousing effect (% reduction) for chalimus was 100% (figure 14). The median effect on pre-adult was 86%, the median effect for adult male was 78% and the median effect for adult female was 72%

At location 2, the mean infestation of chalimus was 0.03, pre-adult 0.03, adult male 0.59, and adult female 0.71 per salmon before Hydrolicer treatment. The use of Hydrolicer reduced a significant amount of adult male and adult female (figure 15 A-D; p<0.01; Appendix table 7). Mean infestation of chalimus was reduced to 0.004, pre-adult to 0.008, adult male to 0.28, and adult female to 0.28. The median effect for chalimus was 100%. The median effect for pre-adult was 100%. The median effect for adult male was 57%. The median effect for adult female was 62% and varied from 0% to 85% (figure 16).

At location 3, the mean infestation of chalimus was 0, pre-adult 0.07, adult male 1.1, and adult female 1.35 per salmon. The use of Hydrolicer reduced a significant amount of pre-adult and female adult (figure 17 A-D; p=0.01; Appendix table 8) .Hydrolicer treatment reduced the mean lice infestation for pre-adult to 0, adult male to 0.24 and adult female to 0.51 (figure 19 A-D) The median effect for pre-adult was 100%. The median effect for adult male was 77%. The median effect for adult female was 70% and varied from 0% to 88% (figure 18).

The delousing effect for life stage groups of *L. salmonis* at location 1-3 follows the same trend as where the effect decreases, and the variation was increasing from chalimus to adult female. Showing that the L. salmonis is more resistant to the Hydrolicer treatment the more developed it is. To investigate the relationship between effect and life stage groups, the different life stage groups were tested for significant differences in effect. The delousing effect between pre-adult and adult males were significant different (p=0.01; appendix table 9).

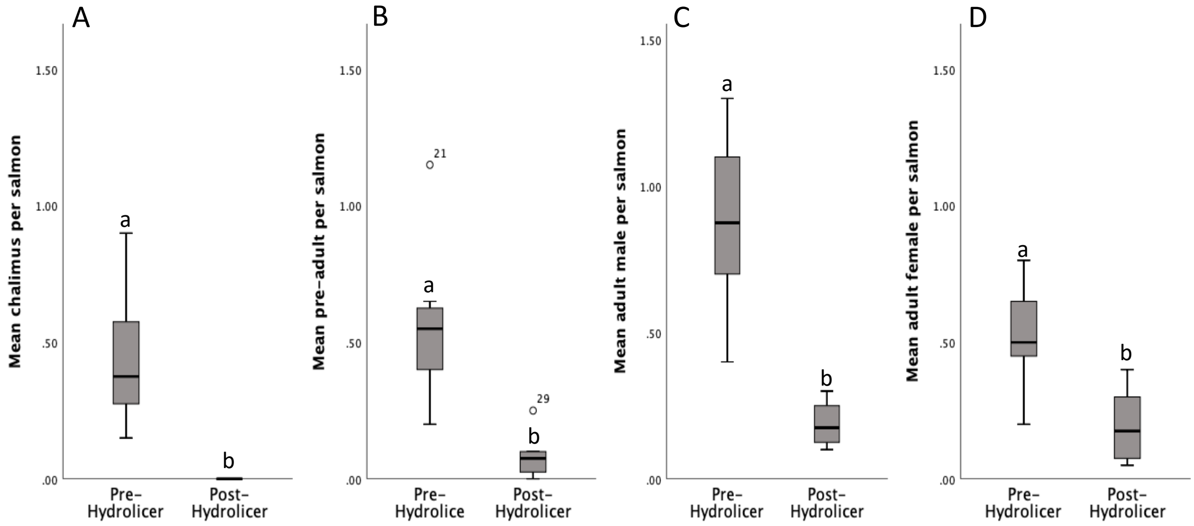

Figure 13 Infestation of the L. salmonis life stage groups A) chalimus, B) pre-adult, C) adult male and D) adult female before and after Hydrolicer treatment on farmed salmon at location 1. 20 salmon were sampled 8 times before and after Hydrolicer treatment. Life stage groups pre and post hydrolicer denoted with different letters (a and b) have significantly different (p<0.01) mean lice infestation.

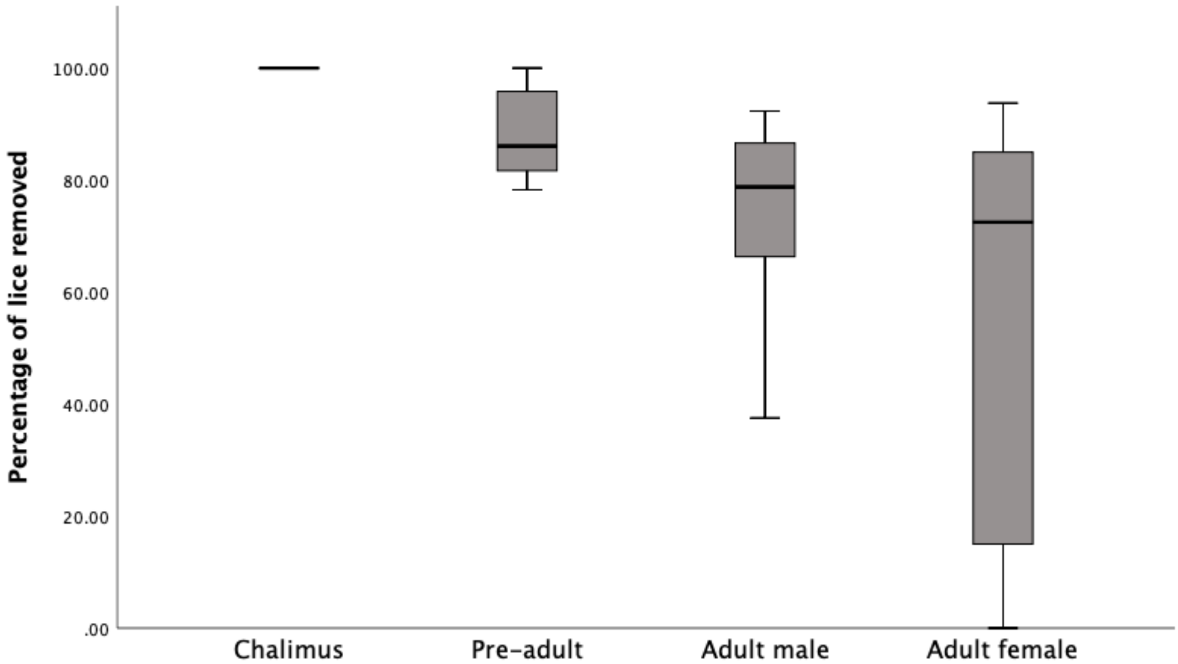

Figure 14 Hydrolicer delousing effect – percentage of Chalimus, Pre-adult, Adult male, and Adult female removed from farmed salmon with Hydrolicer treatment at location 1 (20 salmon sampled 8 times, before and after Hydrolicer treatment).

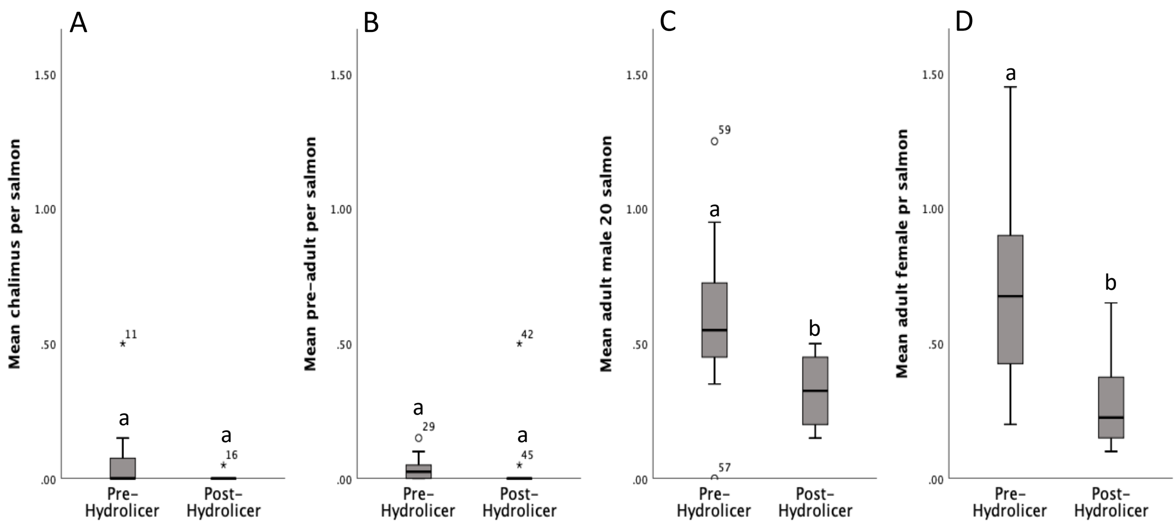

Figure 15 Infestation of the L. salmonis life stage groups A) chalimus, B) pre-adult, C) adult male and D) adult female before and after Hydrolicer treatment on farmed salmon at location 2. 20 salmon were sampled 12 times before and after Hydrolicer treatment. Life stage groups pre and post hydrolicer denoted with different letters (a and b) have significantly different ($p<0.01$) mean lice infestation.

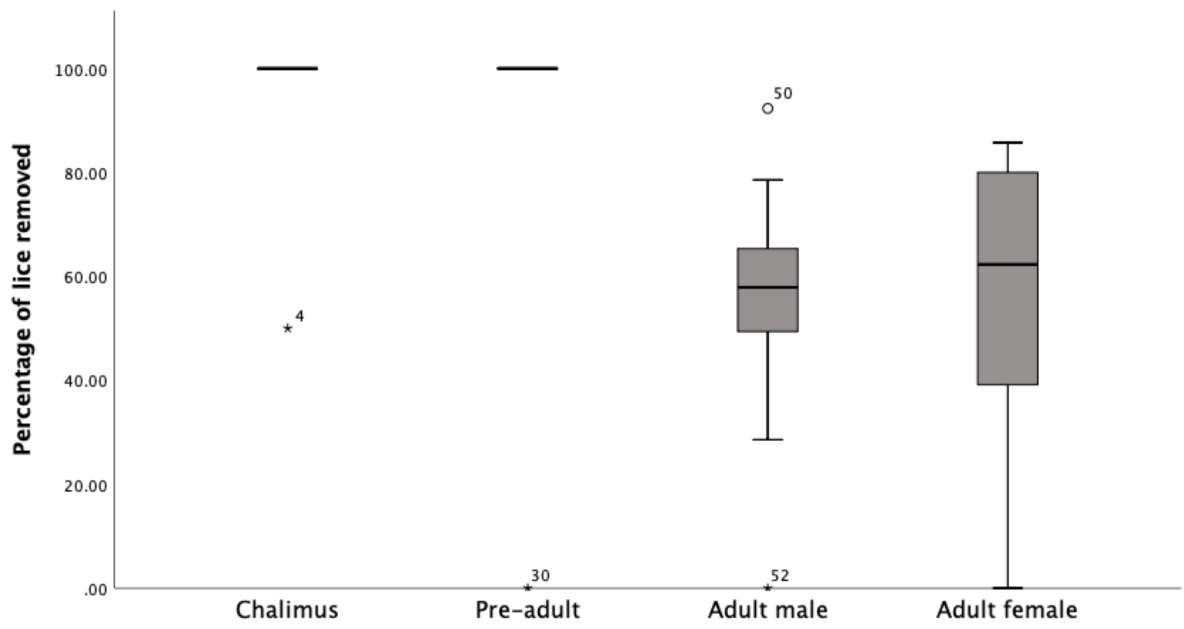

Figure 16 Hydrolicer delousing effect – percentage of Chalimus, Pre-adult, Adult male, and Adult female removed from farmed salmon with Hydrolicer treatment at location 2. (20 salmon sampled 12 times, before and after treatment). Circle symbol show outliers. Star show extreme outliers.

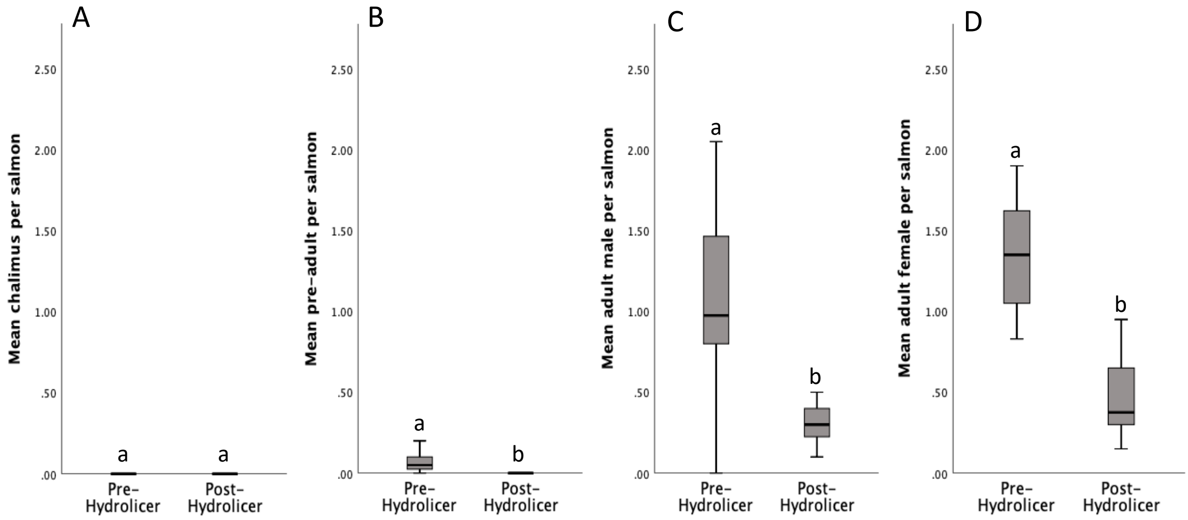

Figure 17 Infestation of the L. salmonis life stage groups A) chalimus, B) pre-adult, C) adult male and D) adult female before and after Hydrolicer treatment on farmed salmon at location 3. 20 salmon were sampled 8 times before and after Hydrolicer treatment. Life stage groups pre and post hydrolicer denoted with different letters (a and b) have significantly different ($p<0.01$) mean lice infestation.

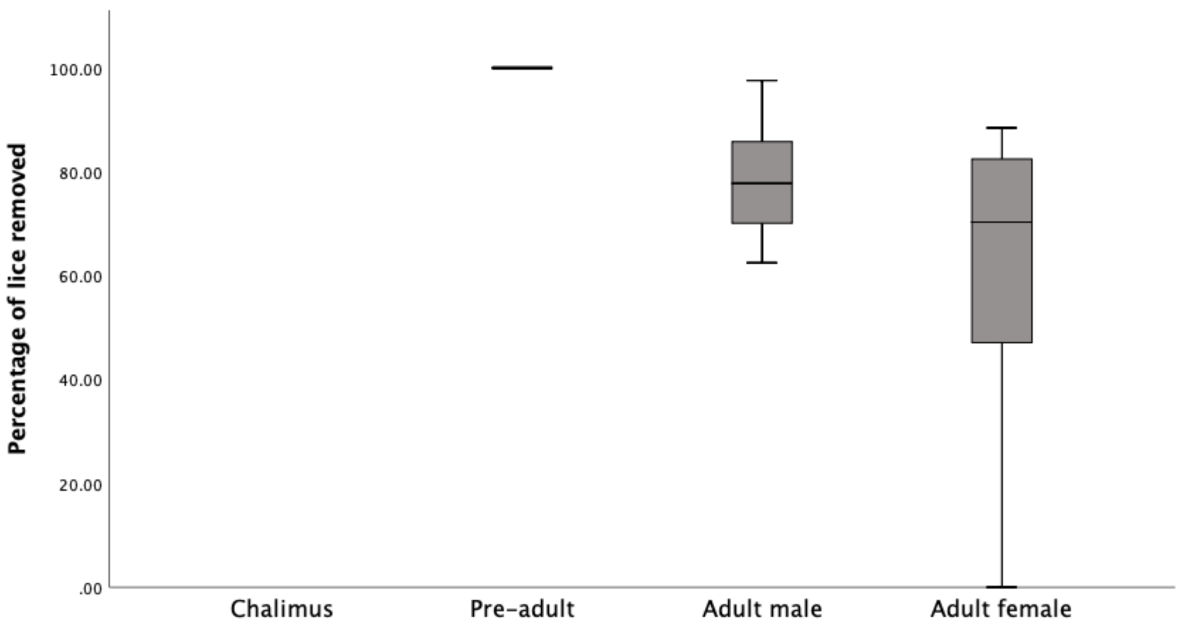

Figure 18 Hydrolicer delousing effect – percentage of Chalimus, Pre-adult, Adult male, and Adult female removed from farmed salmon with Hydrolicer treatment at location 3 (20 salmon sampled 8 times, before and after Hydrolicer treatment).

3.4 The Hydrolicer delousing effect on *L. salmonis* placed on different salmon body zones

At location 1 the mean lice infestation (all life stages grouped together) before treatment was 0.34 lice per fish in zone A, 0.38 in zone B, 1.06 in zone C, and 0.64 in zone D (figure 19 A-D). The Hydrolicer treatment reduced the mean lice infestation in zone A to 0.23, zone B to 0.03, zone C to 0.29, and zone D to 0.01. The Hydrolicer treatment in zone B, C, and D significantly reduced the number of lice ($p=0.01$; appendix table 10). The median effect (% reduction of *L. salmonis*) in zone A was 33%, and varied from 0% to 62% (figure 20). The median effect in zone B was 92% and varied from 92% to 100%, except for one outlier with 50% effect. The median effect in zone C was 79%, and varied from 38% to 96%. The median effect zone D was 100%, and varied from 66% to 100%, showing a clear differences in effect between zones.

At location 2 the mean lice infestation before treatment in zone A was 0.25, in zone B 0.18, zone C 0.93 and zone D 0.07. The Hydrolicer treatment reduced the mean lice infestation in zone A to 0.13, B to 0.05, C to 0.39, and D to 0.02 (figure 21 A-D). Zone B and zone C had a significant reduction in lice after Hydrolicer treatment ($p=0.01$; Appendix table 11). The median effect in zone A was 10% (figure 22). The median effect in zone B was 91% and varied from 50% to 100%, except for one outlier with 0%. The median effect in zone C was 57% and varied from 53% to 86%, except for two outliers at 0%. The median effect in zone D was 75%, and varied from 0% to 100%.

At location 3 the mean lice infestation before treatment in zone A was 0.07, in zone B 0.38, in zone C 0.93, and in zone D 0.1(figure 23 A-D). The Hydrolicer treatment reduced the mean lice infestation in zone A to 0.06, B to 0.02, C to 0.56, and D to 0.03. Only in Zone B the reduction in lice was significant ($p<0.01$; Appendix table 12). The median effect in zone A was 0% and varied from 0% to 25% (figure 24). The median effect in zone B was 100%, and varied from 33% to 100%. The median effect in zone C was 55%, and varied from 33% to 88%. The median effect in zone D was 100%, and varied from 50% to 100%.

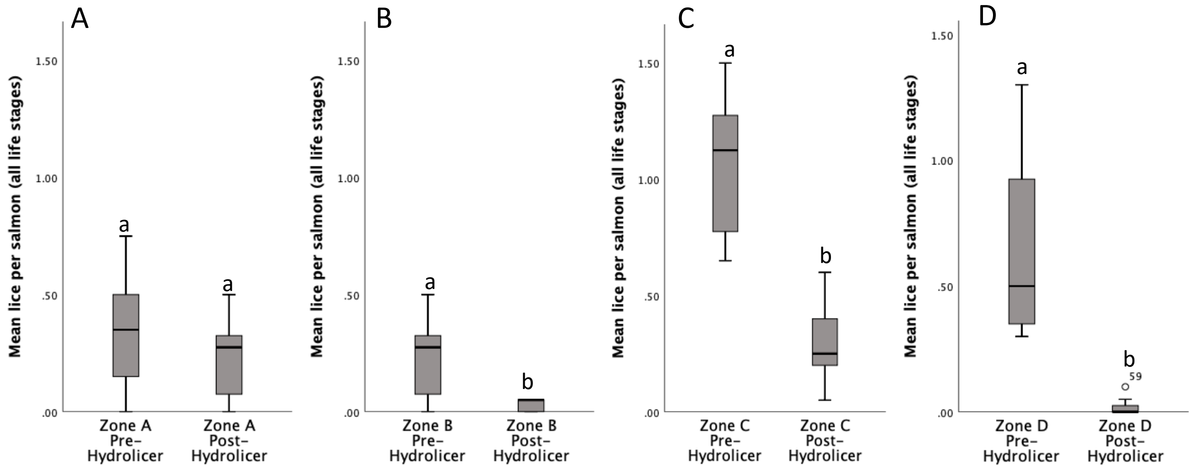

Figure 19 L. salmonis infestation in the different salmon body zones: A) Zone A, B) zone B, C) zone C, and D) zone D before and after Hydrolicer treatment on farmed salmon at location 1. 20 Salmon were sampled 8 times before and after Hydrolicer treatment. Results pre and post Hydrolicer denoted with different letters (a and b) have significantly different ($p<0.01$) mean lice infestation.

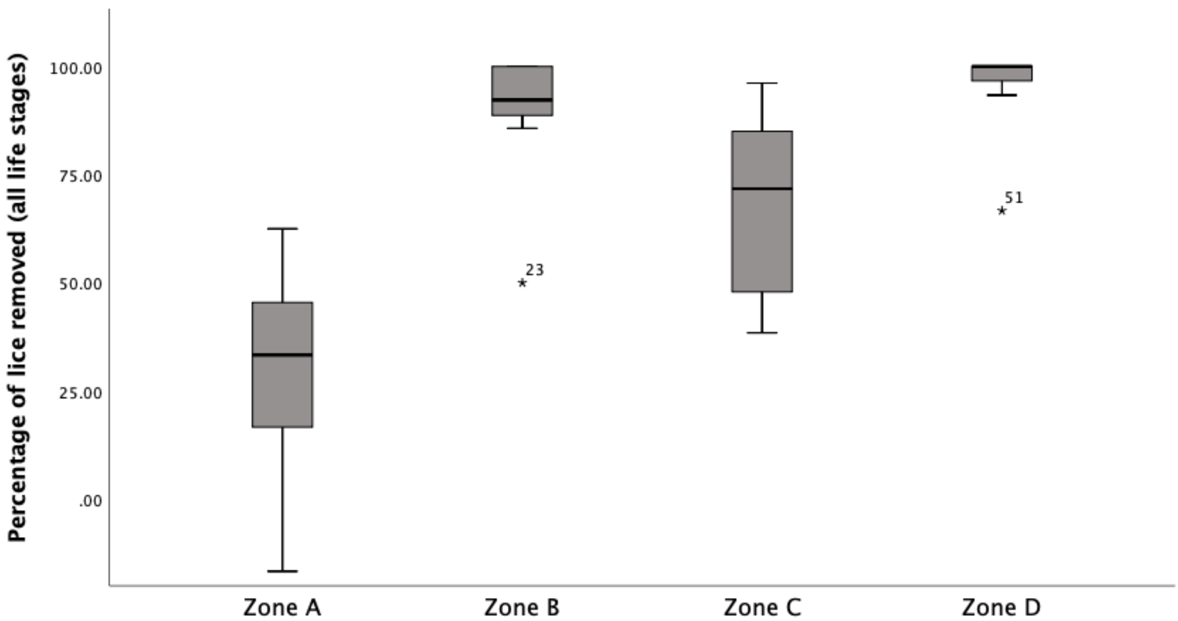

Figure 20 Hydrolicer delousing effect – Percentage of L. salmonis removed from Zone A, B, C and D with Hydrolicer treatment on farmed salmon at location 1 (20 salmon sampled 8 times, before and after Hydrolicer treatment). Star symbol shows extreme outliers.

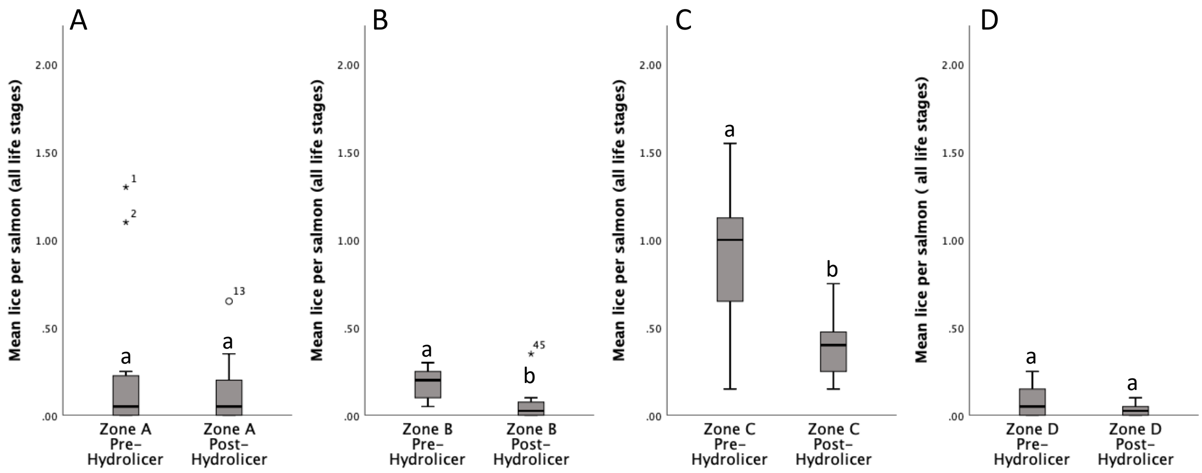

Figure 21 L. salmonis infestation in the different salmon body zones: A) Zone A, B) zone B, C) zone C, and D) zone D before and after Hydrolicer treatment on farmed salmon at location 2. 20 salmon were sampled 12 times before and after Hydrolicer treatment. Results pre and post Hydrolicer denoted with different letters (a and b) have significantly different ($p<0.01$) mean lice infestation.

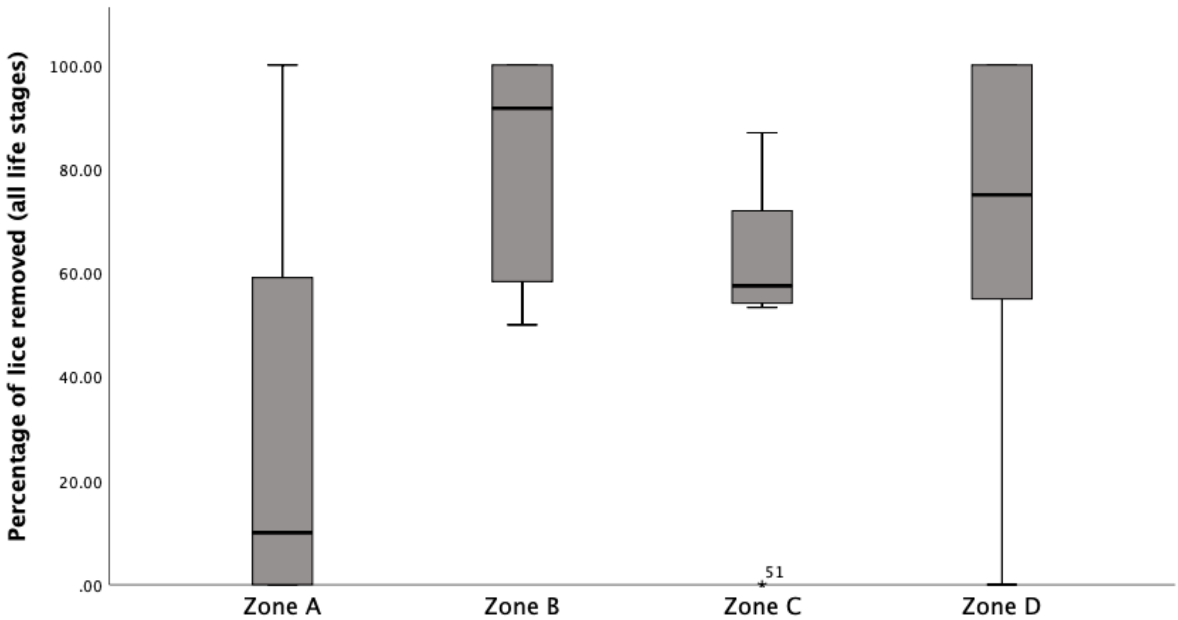

Figure 22 Hydrolicer delousing effect – Percentage of L. salmonis removed from Zone A, B, C and D with Hydrolicer treatment on farmed salmon at location 2 (20 salmon sampled 12 times, before and after Hydrolicer treatment). Star symbol shows extreme outlier

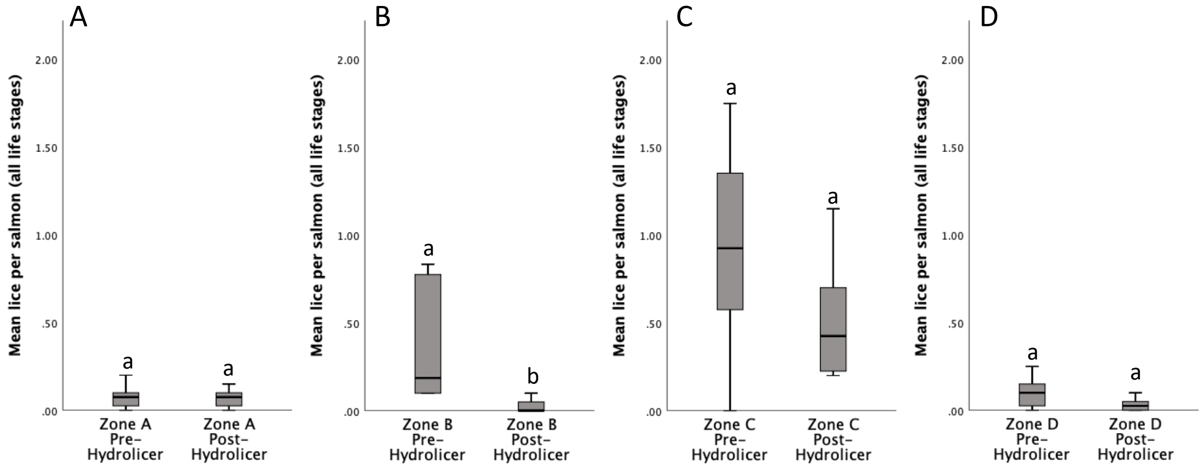

Figure 23 L. salmonis infestation in the different salmon body zones: A) Zone A, B) zone B, C) zone C, and D) zone D before and after Hydrolicer treatment on farmed salmon at location 3. 20 salmon were sampled 8 times before and after Hydrolicer treatment. Results pre and post Hydrolicer denoted with different letters (a and b) have significantly different ($p<0.01$) mean lice infestation

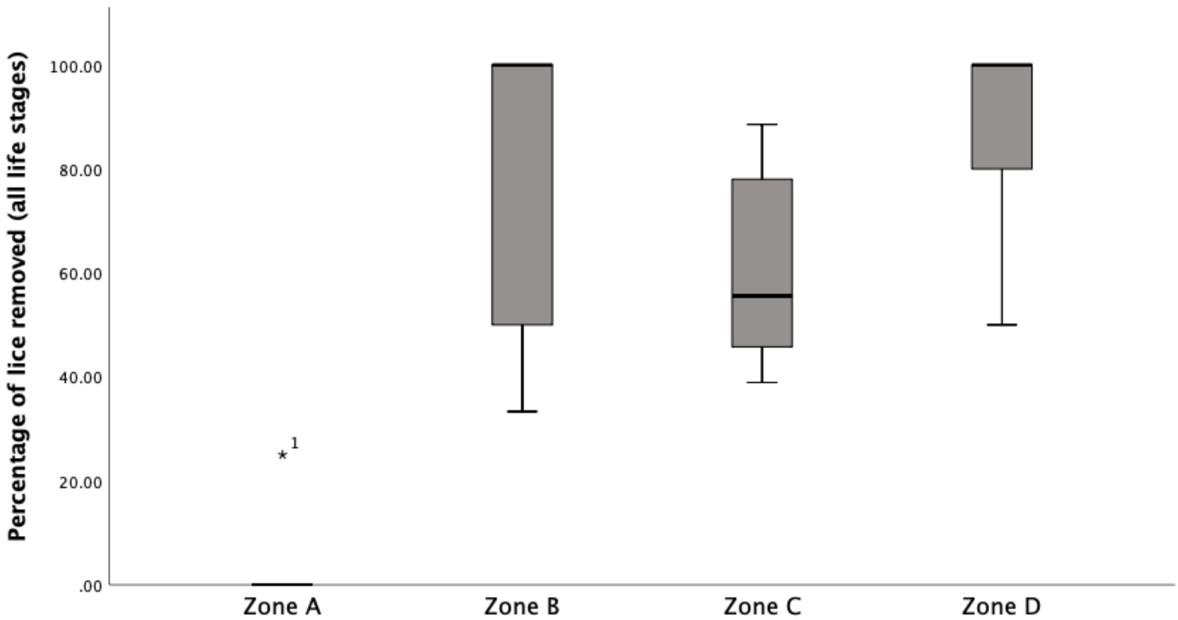

Figure 24 Hydrolicer delousing effect – Percentage of L. salmonis removed from Zone A, B, C and D with Hydrolicer treatment on farmed salmon at location 3 (20 salmon sampled 8 times, before and after Hydrolicer treatment). Star symbol shows extreme outliers.

3.5 The distribution of life stage groups before and after Hydrolicer delousing

The abundance of chalimus was low, but it was found in all zones. Figure 26 A shows that in this study it appeared mostly in zone D and some in Zone C. After Hydrolicer treatment only three samples had chalimus infestation. In those three samples, chalimus was found in zone C and D (figure 26 B).

The abundance of pre-adult was higher than chalimus and was found in all zones. It appeared the most in Zone B, C, and D, showing a trend for Zone D, C, and B as preferred attachment location in that order (figure 27 A). After treatment, the abundance of pre-adult was low. However, the pre-adult life stage was found in zone B, C and D after Hydrolicer treatment (figure 27 A).

The adult male life stage infestation was high and was found in all zones. However, the adult male showed a clear preference for zone C. It also had high infestation in zone B and D (figure 28 A). After Hydrolicer treatment, the abundance was lower, but adult males had a frequencies pattern much like before Hydrolicer treatment. Adult male was found the most in zone C. It was also found in zone B and D, although not so often compared to the situation before Hydrolicer treatment (figure 28 B).

The adult female life stage showed a clear and strong preference for zone C. Still it was found in all zones (figure 29 A). After Hydrolicer treatment, the adult female abundance was lower but still showed the same frequencies pattern (figure 29 B).

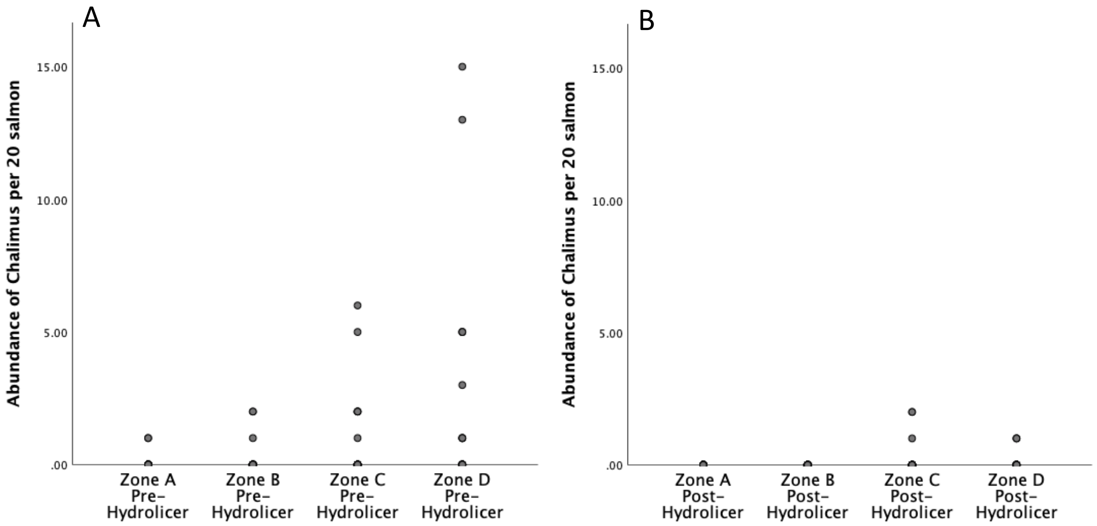

Figure 25 Abundance of chalimus in salmon body zone A-D per 20 salmon A) before and B) after Hydrolicer delousing on farmed salmon. 20 salmon were sampled 28 times before and after Hydrolicer treatment in location 1, 2 and 3.

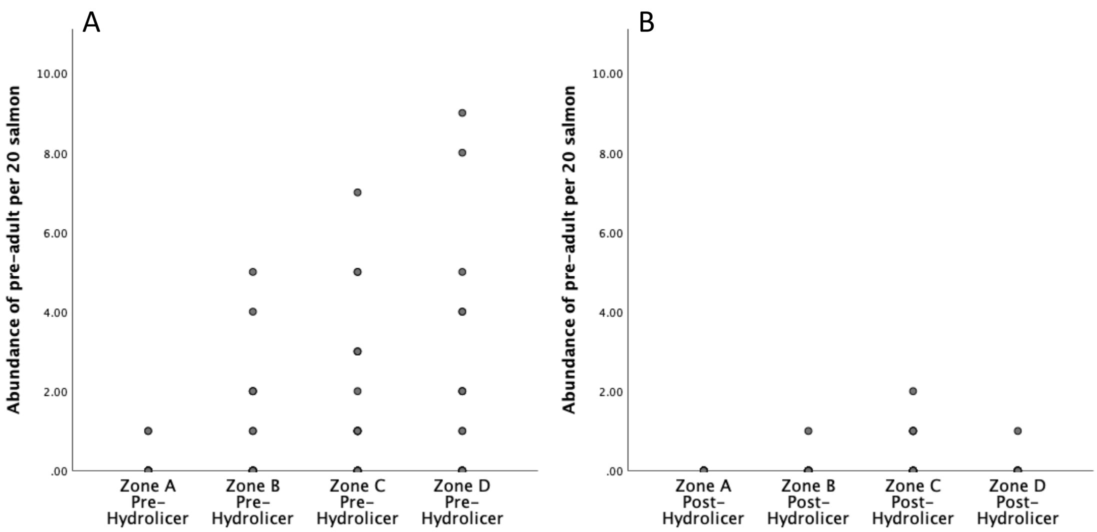

Figure 26 Abundance of pre-adult in salmon body zone A-D per 20 salmon A) before and B) after Hydrolicer delousing on farmed salmon. 20 salmon were sampled 28 times before and after Hydrolicer treatment in location 1, 2 and 3.

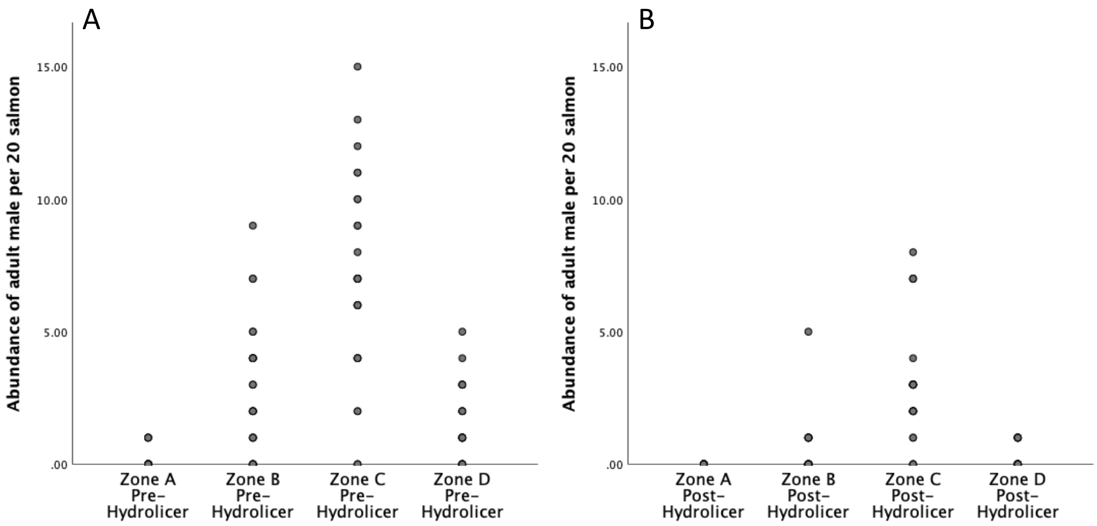

Figure 27 Abundance of adult male in salmon body zone A-D per 20 salmon A) before and B) after Hydrolicer delousing on farmed salmon. 20 salmon were sampled 28 times before and after Hydrolicer treatment in location 1, 2 and 3.

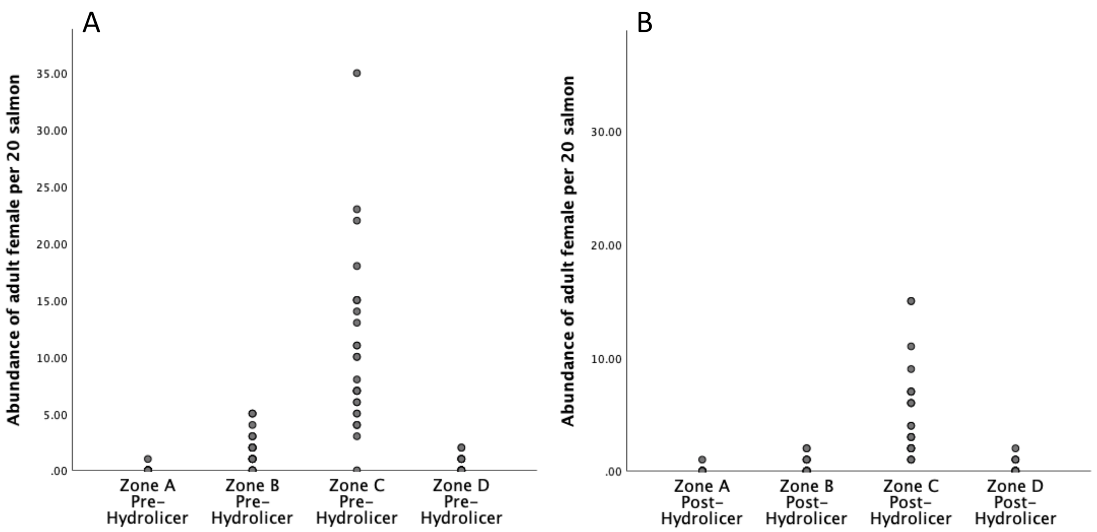

Figure 28 Abundance of adult female in salmon body zone A-D per 20 salmon A) before and B) after Hydrolicer delousing on farmed salmon. 20 salmon were sampled 28 times before and after Hydrolicer treatment in location 1, 2 and 3.

4. Discussion

The research objectives of this thesis were to investigate and characterize delousing effect on *L. salmonis* with Hydrolicer treatment. The main objectives were to characterize the overall delousing effect on *L. salmonis* with Hydrolicer treatment. Characterize the delousing effect of using different pressure adjustments on the Hydrolicer. Characterize the delousing effect of using Hydrolicer on chalimus (1 and 2), pre-adult (1 and 2 both female and male), adult male, and adult female. Characterize the preferred attachment distribution and the effect of Hydrolicer treatment on the attachment distribution of L. salmonis on farmed salmon.

4.1 The overall Hydrolicer effect

The first research objective was to investigate the total Hydrolicer effect. This was calculated for each location and the results showed that the Hydrolicer delousing works, but has variation in effect between and within each location. At location 1, the interquartile effect was 74%-87%. Location 2 49%-73%. Location 3 61%-86% (figure 9). An abundance of factors influences their difference in effect. A main factor might be the difference in salmon weight and prevalence of L. salmonis before treatment.

If the prevalence is low, the chance for the sampled salmon to be without lice is higher. Thus affecting the percentage of removed lice per salmon. Compared to another mechanical delousing method, fresh water delousing (Gaasø, 2019). Is the overall delousing effect a bit lower. However, the time it takes to delouse a salmon pen and location is much faster with Hydrolicer. As a freshwater treatment can take up to 10 hours, and if the salmon biomass is high the well-boat can only delouse half of the salmon pen at the time. Thus the risk for reinfection of the location is lower with Hydrolicer as it will delouse all the salmon at a location faster. Thermal delousing is mechanical method with similar delousing speed as Hydrolicer. The total effect of thermal delousing was 75-100%, but treatments were not statical significant (Grøntvedt et al., 2015). Although the Hydrolicer showed a bit lower percentage in effect, the results were significant. Making it a great option for the aquaculture industry along with the other treatments.

One can see that the effect is higher, and the variation in effect is lower at location 1, where the salmon had the highest weight and highest prevalence, even if the pressure adjustment were lower compared to other locations. Other factors such as the farmers' and the crew on board Hydro Patriot ability to execute the crowding procedure and Hydrolicer treatment in the correct tempo could affect the delousing effect. Another factor that could explain why location 1 had a better effect is that location 1 was the only location where we found an relatively high amount of chalimus. The Hydrolicer had, for the most part, a 100% effect on chalimus, Thus affecting the total delousing effect in a positive way.

4.2 Characterize the delousing effect of using different pressure adjustments on the Hydrolicer

The Hydrolicer effect based on pressure adjustment was different from each location. At location 1 and 2, there seems to be a relation between effect and pressure adjustment, but at location 3 the results had a lot of variation and the effect did not go up when the pressure adjustment did go up. The correlation test gave a weak relationship between pressure adjustment and Hydrolicer effect. This could be because the locations have such different levels of prevalence and mean intensity before treatment, and also the weight of salmon was different between locations that the data is not comparable. Another factor is the sample size. Some of the pressure adjustments had few samplings making the results less credible and more susceptible to being statical outliers.

4.3 Hydrolicer delousing effect on life stage groups chalimus, pre-adult, adult male, and adult female

The effect on chalimus was 100% whit a couple of outliers, showing better results this time than a previous study (Erikson, 2018-12-21). This is somewhat uncertain as we did not find a lot of chalimus before treatment, affecting the statistical power behind the effect. Due to the low number of chalimus before treatment, the effect was only significant at location 1. Chalimus seems to be the life stage most affected by Hydrolicer treatment, and if that is the case, the crowding process could have removed an unknown amount of the chalimus before treatment. On the other hand, most of the lice could have been in the same generation and have developed past the chalimus stage. Furthermore, the sampling procedure was not optimal to detect chalimus that had fallen off during the registration process, as we did not have the equipment with small enough filter to collect chalimus.

The effect on pre-adult at locations 1-3 was 86%, 100%, and 100%, respectively. There was a low abundance of pre-adult just like the chalimus, making the results somewhat uncertain. The pre-adult could have fallen off during dense crowding or during sampling. The Mann-Whitney U test gave only significant reduction of pre-adult at location 1. The low number of pre-adult before treatment could be a factor that caused the delousing at location 2 and 3 to not be significant. However, the overall Hydrolicer effect on pre-adult was high.

The effect on adult male at location 1-3 was 78%, 57%, 77%, respectively, and the effect was only significant for locations 1 and 2. At location 3, there was a high variance in number of adult males, thus affecting the significance test, even though the median shows a high effect. The abundance of adult males was relatively high, thus making the results credible. The trend between locations is consistent with the effect on male adults. The high variation could be because of increasing resistance to treatment with increasing development stage. Overall the results matches the results from (Erikson, 2018-12-21) but they have grouped the lice life stages together differently that makes it somewhat harder to compere.

The effect on adult female at location 1-3 was 0%-93%, 0%-85%, and 0%-88%, respectively. The boxplot shows an extremely high variation in effect. However, the effect was significant for all locations. The high variation is affected by some Hydrolicer treatments with low effect. The median effect for location 1-3 was 72%, 62%, 70% showing that overall the Hydrolicer treatment removes two thirds of the adult female infestation. The boxplot shows that the trend for lower effect as the life stages get more developed, ending with adult females, is consistent with all locations. Showing that the Hydrolicer is more effective the less developed the lice are. The delousing effect on adult female is consistent with previous study of Hydrolicer (Erikson, 2018-12-21) were the adult female also had high variation and the lowest reduction.

4.4 Characterize the preferred attachment distribution and the effect of Hydrolicer treatment on the attachment distribution of L. salmonis on farmed salmon

The third research objective was to characterize the preferred attachment distribution and the effect of Hydrolicer treatment on the attachment distribution of L. salmonis on farmed salmon.

The median effect for zone A-D at location 1 was A: 33% B: 92% C: 79% D: 100%. At location 2 was A: 10% B: 91% C: 57% D: 75%. At location 3 A: 0% B:100% C: 55% D: 100%. This shows a clear trend for effect being higher at zone B and D. The boxplot also show that the variation was extremely high, sometimes ranging from 100% effect to 0%. Only zone B had a significant effect at all three locations. Zone A gave the lowest effect and was not significant at any location. Zone C was significant at locations 1 and 2, but not at 3. Zone D had a high median effect and was significant, but had only significant reduction of lice at location 1. The better effect at zone B and D may be because the salmon is thicker in those two zones than zone A and C, as the head and tail part is a lot thinner and smaller. This could make the zone B and D more affected by the water jets inside the Hydrolicer, compared to zone A and C. We can see the same effect if we compare the results with the total Hydrolicer effect between the locations. The salmon with the highest weight had the best delousing effect, and the salmon with the lowest weight had the lowest delousing effect. Another reason why zone A showed low effect could be that the abundance of lice in zone A was low throughout the investigation, thus making it harder to achieve high affect.

The attachment location of chalimus varied between the zones but shows a trend for preferring zone C and D. Pre-adult were spread evenly between B, C, and D, with some registrations in zone A. Adult male was found in all zones but showed a clear preference for zone C. Adult female had an even clearer and specific preference for zone C, but like adult males, it was found in all zones. Reasons for this distribution of the chalimus and pre-adult could be that the attachment location is more or less random. And as the lice develop, the lice can move to a specific Zone. The male however, is likely to be more mobile and distributed more evenly in search of a mate. However, these results do not correspond to previous studies (Bui et al., 2020), that observed the female adult be more spread out over the salmon body and had more observation of adult females in zone A. In the current study, the zone A was the least occupied zone for all life stages. The difference in results between these two studies could be because the water temperature was different, which could indicate that *L. salmonis* have different preferences for attachment location at different temperatures.

4.5 Limitations

The data sampling was a limiting factor for this thesis. We did not have the equipment to weigh every single individual. The data of fish weight was given as mean salmon weight at salmon pen level, thus losing valuable data during sampling of salmon, we observed that the salmon varied a lot in weight within the same salmon pen. The high variation and uncertainty in weight makes any analysis of the effect based on salmon weight uncertain. The registration process was also a source for possible errors in the data. We did not have the equipment to collect the smallest lice that could have fallen off the salmon body, thus making the registered abundance of chalimus and pre-adult lower than it actually might be. The collection of salmon at station A and B could often be quite challenging and unpredictable. Sometimes we could collect more than 20 salmon. Although we did not count the lice on the salmon when we passed 20 samples, the last ones could have had lice that fell off, thus making the number of lice that have fallen off in the tub higher than it was.

4.6 Recommendations for future studies

I would recommend improving the data sampling; the importance of accurate data cannot be neglected. The fieldwork should have been longer and also with more and better equipment. One should gather accurate data on every individual salmon, both length and weight. This could contribute to figuring out if Hydrolicer is more suitable for salmon that is relatively high in weight or salmon with lower weight and length. A study of fish welfare and mortality correlated to effect in lice reduction would also be of great interest to the industry and could help farmers use new knowledge to increase the welfare and mortality during Hydrolicer treatment. A characterizing of the Hydrolicer effect on the salmon louse Caligus elongatus would be as important as this study.

4.7 Conclusion

The overall Hydrolicer treatment had significant reduction of lice at all locations. However, location 1 had the highest reduction in lice with 78%. The relationship between pressure adjustment and the effect was low in this study, this may be because the results from location 1-3 were too different to compare directly to each other or the effect can be more related to fish weight.

The Hydrolicer treatment removed almost all chalimus and had a median effect of 100%. The effect went down when life stages were more developed. Adult female were the life stage with lowest reduction, 62-72%. Adult female had also extreme variation in effect ranging from 0-93%. Showing that the Hydrolicer have different effect based on what life stage the *L. salmonis* was during treatment.

The Hydrolicer had different effects based on *L. salmonis* placement. Zone A (the head) had the lowest reduction of lice. The highest median effect in zone A was only 33%, and not one significant treatment. Zone B and zone D was the zone with the highest reduction of *L. salmonis*. 91%-100% effect in zone B and 75%-100% effect in zone D. However only zone B had significant reduction at all locations.

Adult female was the life stage with the clearest preference for a specific zone, zone C. Adult male was more spread between zone B, C , and D but appeared mostly in zone C. The younger life stages were spread more evenly between zone B, C, and D. After Hydrolicer treatment, Zone C was standing out as the zone with most remaining lice. However, zone C also had the most registration of lice before treatment.

5. References

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Appendix

Table 1: Test for normality

	Skewness and kurtosis pre hydrolicer	Skewness and kurtosis post hydrolicer
Location 1	S; 1.06 ± 0.19 K;1.30 ± 0.38	S; 3.68 ± 0.19 K; 17.05 ± 0.38
Location 2	S; 0.99 ± 0.15 K; 0.72 ± 0.31	S; 1.78 ± 0.15 K; 2.90 ± 0.31
Location 3	S; 1.69 ± 0.19 K; 2.87 ± 0.381	S; 2.14 ± 0.19 K; 5.21 ± 0.38

Table 2: Mann-Whitney U test for significant reduction of lice at location 1

Test Statistics[a]

Location 1	Mean_lice
Mann-Whitney U	.000
Wilcoxon W	36.000
Z	-3.363
Asymp. Sig. (2-tailed)	.001
Exact Sig. [2*(1-tailed Sig.)]	.000[b]

a. Grouping Variable: Location

b. Not corrected for ties.

Table 3: Mann-Whitney U test for significant reduction of lice at location 2

Test Statistics[a]

Location 2	Mean_lice
Mann-Whitney U	4.000
Wilcoxon W	82.000

Z	-3.930
Asymp. Sig. (2-tailed)	.000
Exact Sig. [2*(1-tailed Sig.)]	.000[b]

a. Grouping Variable: Location

b. Not corrected for ties.

Table 4: Mann-Whitney U test for significant reduction of lice at location 3

Test Statistics[a]

Location 3	Mean_lice
Mann-Whitney U	.000
Wilcoxon W	36.000
Z	-3.363
Asymp. Sig. (2-tailed)	.001
Exact Sig. [2*(1-tailed Sig.)]	.000[b]

a. Grouping Variable: Location

b. Not corrected for ties.

Table 5: Correlation between effect and pressure adjustment

Tests of Between-Subjects Effects

Dependent Variable: Effect

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3251.229[a]	5	650.246	1.213	.336
Intercept	103513.234	1	103513.234	193.100	.000
Pressure	3251.229	5	650.246	1.213	.336
Error	11793.328	22	536.060		
Total	138161.551	28			
Corrected Total	15044.557	27			

a. R Squared = .216 (Adjusted R Squared = .038)

Table 6: Mann-Whitney U test, of significant reduction

Test Statistics[a]

Location 1	chalimus	Pre_adult	Adult_male	Adult_female
Mann-Whitney U	.000	1.000	.000	4.000
Wilcoxon W	36.000	37.000	36.000	40.000
Z	-3.593	-3.275	-3.373	-2.971
Asymp. Sig. (2-tailed)	.000	.001	.001	.003
Exact Sig. [2*(1-tailed Sig.)]	.000[b]	.000[b]	.000[b]	.002[b]

a. Grouping Variable: Locations

b. Not corrected for ties.

Table 7: Mann-Whitney U test, of significant reduction

Test Statistics[a]

Location 2	chalimus	Pre_adult	Adult_male	Adult_female
Mann-Whitney U	46.500	50.000	25.000	20.000
Wilcoxon W	124.500	128.000	103.000	98.000
Z	-1.938	-1.523	-2.737	-3.012
Asymp. Sig. (2-tailed)	.053	.128	.006	.003
Exact Sig. [2*(1-tailed Sig.)]	.143[b]	.219[b]	.006[b]	.002[b]

a. Grouping Variable: Locations

b. Not corrected for ties.

Table 8: Mann-Whitney U test, of significant reduction

Test Statistics[a]

Location 3	chalimus	Pre_adult	Adult_male	Adult_female
Mann-Whitney U	32.000	8.000	8.000	2.000
Wilcoxon W	68.000	44.000	44.000	38.000

Z	.000	-2.910	-2.524	-3.153
Asymp. Sig. (2-tailed)	1.000	.004	.012	.002
Exact Sig. [2*(1-tailed Sig.)]	1.000[b]	.010[b]	.010[b]	.001[b]

a. Grouping Variable: Locations

b. Not corrected for ties.

Table 9: Wilcoxon singed ranks test, of significant difference in effect between life stages

Test Statistics[a]

	Effect_on_adult_male - Effect_on_adult_female	Effect_on_pre_adult - Effect_on_adult_male	Effect_on_chalimus - Effect_on_pre_adult
Z	-.368[b]	-3.061[b]	-1.183[b]
Asymp. Sig. (2-tailed)	.713	.002	.237

a. Wilcoxon Signed Ranks Test

b. Based on negative ranks.

Table 10: Mann-Whitney U, test for significant reduction of lice in each zone

Test Statistics[a]

Location 1	Zone A	Zone B	Zone C	Zone D
Mann-Whitney U	22.000	2.500	.000	.000
Wilcoxon W	58.000	38.500	36.000	36.000
Z	-1.066	-3.193	-3.373	-3.453
Asymp. Sig. (2-tailed)	.286	.001	.001	.001
Exact Sig. [2*(1-tailed Sig.)]	.328[b]	.001[b]	.000[b]	.000[b]

a. Grouping Variable: Location

b. Not corrected for ties.

Table 11: Mann-Whitney U, test for significant reduction of lice in each zone

Test Statistics[a]

Location 2	Zone A	Zone B	Zone C	Zone D
Mann-Whitney U	65.500	18.500	17.000	50.500
Wilcoxon W	143.500	96.500	95.000	128.500
Z	-.388	-3.138	-3.184	-1.327
Asymp. Sig. (2-tailed)	.698	.002	.001	.185
Exact Sig. [2*(1-tailed Sig.)]	.713[b]	.001[b]	.001[b]	.219[b]

a. Grouping Variable: Location

b. Not corrected for ties.

Table 12: Mann-Whitney U, test for significant reduction of lice in each zone

Test Statistics[a]

Location 3	Zone A	Zone B	Zone C	Zone D
Mann-Whitney U	31.500	1.500	19.500	16.000
Wilcoxon W	67.500	37.500	55.500	52.000
Z	-.055	-3.279	-1.319	-1.753
Asymp. Sig. (2-tailed)	.956	.001	.187	.080
Exact Sig. [2*(1-tailed Sig.)]	.959[b]	.000[b]	.195[b]	.105[b]

a. Grouping Variable: Location

b. Not corrected for ties.