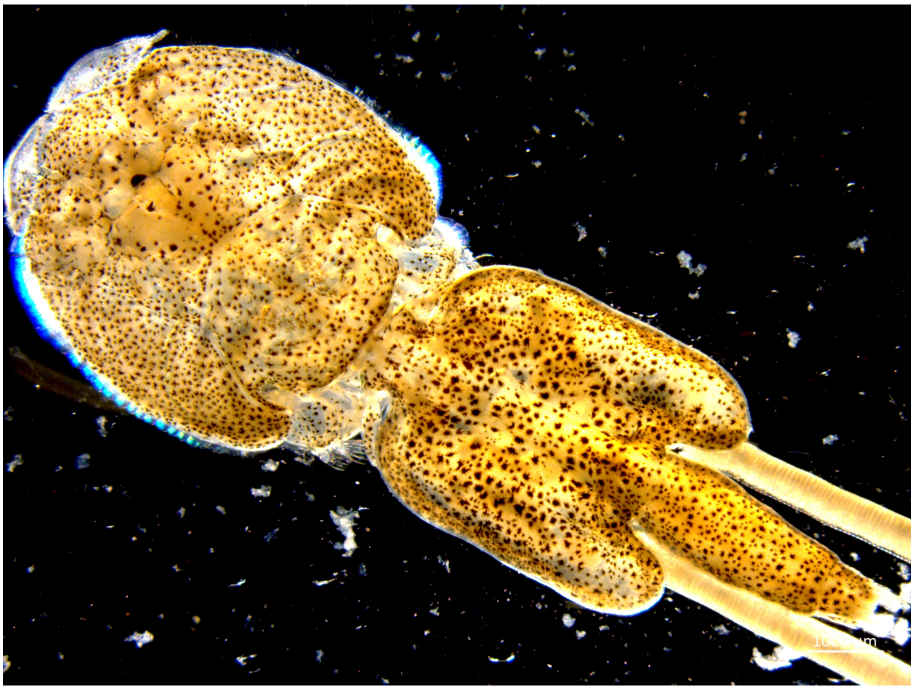
Master's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Natural Sciences
Department of Biology

Maria Gaasø

Sea lice *(Lepeophtheirus salmonis* and *Caligus elongatus)* during freshwater treatment

Master's thesis in Ocean Resources
Supervisor: Yngvar Olsen
December 2019



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Preface

This thesis has been completed at the Department of Biology at the Norwegian University of Science and Technology (NTNU) Trondheim between September 2018 and December 2019 parts of the Taskforce salmon Lice project.

Taskforce salmon lice is an R&D project organised as a PhD program at NTNU. The project consists of three parts; one founded by Fiskeri- og havbruksnæringens forskningsfinansiering (FHF), one by NTNU and one part funded by the aquaculture companies 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. The overall objective to the project is to establish fundamental knowledge on how sea lice infest farmed salmon and the mechanisms of how the parasites spread within and between farmed and wild populations of salmonids.

First, I would like to thank my main supervisor Yngvar Olsen – the help and support of the thesis, and the good feedback during the writing process. To my co-supervisor Anna Solvang Båtnes, for good feedback, advice and guidance. Furthermore, to my second co-supervisors Maria Guttu for assisting in the pilot field trip and for the quick feedback and good help.

I wish to acknowledge the good help provided by Solveig Gaasø (Frøy) during fieldwork and planning. I wish also to say thanks to Marit Stormoen (NMBU) for good help with the literature on freshwater treatment and *Caligus elongatus*.

I would also like to thank all the staff on board the well-boats and on the aquaculture sites for your good salmon sampling help, transportation and the good company and food we got served while on fieldwork. A big thank you to Margrét for the assistant help on the field trip – the thesis would not be finished if it was not for you.

I would like to thank all my fellow students and staff at Sealab (NTNU Centre of Fisheries and Aquaculture). A special thanks to all my family and friends for their support and understanding for my lacking social life the last semester(year), and a big shout-out to the Seababes and the HiST 'family'.

Maria Gaasø, Trondheim, December 2019

Abstract

Since the beginning of the aquaculture farming industry that started in Norway in the 1960s, sea lice have been a problem for the fish welfare. With an economic impact more significant than any other parasite. The Norwegian Scientific Advisory Committee for Atlantic Salmon have identified *Lepeophteirus salmonis* as the second largest threat to Norwegian salmon. In more recent years, *Caligus elongatus* has also become a problem for the salmon industry in Norway. With *L. salmonis* increased resistance, changes in legislation, use of new non-medical treatments (NMT), increased media focus and improved knowledge on the sea lice have reduced the use of the traditional medical treatments. A new NMT method for delousing the salmon is with the use of freshwater baths onboard well boats. The salmon is first crowded in the pen and then transferred over a dewatering unit into the wells filled with freshwater. Treatment time is dependent on the lice load per pen and on the different sea lice stages on the fish. The knowledge about the effect of the freshwater treatment on the sea lice is limited, and a field study was conducted to investigate the effect and efficiency of the freshwater delousing method. In total five full-scale treatments were followed, where countings during different stages of treatment were conducted. The salmon were weighed, length measured, and the different lice stages were registered on each sampled fish. Total lice numbers of *L. salmonis* and *C. elongatus* on each fish and the salmons' condition factor, K, and the length were tested for correlation. The crowding method was compared against lice numbers from the week prior to treatment, before treatment and after treatment. Lice reduction during each treatment was investigated. The results showed a rapid decrease in lice numbers from the start of the freshwater delousing to the 1st counting in the wells. After ~5 hours little change in the lice, reduction was registered for both *L. salmonis* and *C. elongatus.* The mechanical delousing effect from crowding, pumping and grading may cause the louse to detach from the salmon and therefore might explain parts of the rapid decrease, in addition to the effect of freshwater itself. Attached stages of *L. salmonis* and the *C. elongatus* showed the highest reduction in numbers after the freshwater treatment. Adult female and male had a low reduction compared to the other lice stages, and they have a higher tolerance toward freshwater by taking up ions from their host to maintain their osmolarity. Crowding method used in the pen before the treatment showed to have an impact on the lice numbers on each fish. When a swipe net was used to crowd the salmon, the lice numbers on the salmon were lowest. There was no correlation between the K-factor of the salmon and the number of lice and between the length of the salmon and the number of lice attached. The main results from the treatment showed generally a high reduction in all stages of *L. salmonis* and for *C. elongatus.* Adult male of *L. salmonis* had the lowest total reduction, 77.7% at the end of treatment, while *C. elongatus* had the highest reduction effect, 99.6%, at the end of the treatment.

Sammendrag

Siden begynnelsen på 1960-tallet, har lus vært et problem for fiskevelferden for akvakultur næringen i Norge. Lusen har en større økonomisk påvirkning enn noen annen parasitt, og *Lepeophteirus salmonis* har blitt indentifisert som den nest største trusselen mot norsk laks av Vitenskapelig råd for lakseforvaltning i Norge. I de senere år har skottelusen, *Caligus elongatus*, blitt et problem for laksenæringen i Norge. Økt resistens, endringer i lovgivningen, bruk av nye ikke-medikamentelle metoder, økt media fokus og økt kunnskap om sjø-lusen redusert bruken av de tradisjonelle medikamentelle behandlingsmetodene. En ny ikke-medikamentell metode for avlusning er bruk av ferskvannsbad om bord i brønnbåter. Laksen blir først trengt i merden, og deretter pumpet over en rist og inn i brønnene som er fylt mer ferskvann. Behandlingstiden er avhengig av antall lus per merd og fordelingen av lusestadier. Kunnskapen om effekten av ferskvannsbehandling på sjø-lus er begrenset, og en feltundersøkelse be utført for å undersøke effekten og effektiviteten av ferskvannsbehandlingen. Totalt ble det fem fullskala ferskvannsbehandlinger fulgt, hor det ble gjort tellinger på forskjellige tidspunkt under behandlingen. Hver enkelt laks undersøkt ble veid, målt lenge og antall lus og stadier ble registrert. Det totale antallet av *L. salmonis* og *C. elongatus* på hver fisk og laksens kondisjons faktor, K, samt laksens lengde ble testet for korrelasjon. Trengemetode ble sammenlignet med lusetall fra uken før behandling, under trenging og uken etter behandling. Reduksjonen av lus under hver behandling ble undersøkt. Resultatene viste en rask nedgang i lusetall fra begynnelsen av ferskvannsbehandlingen til den første tellingen i brønnene. Etter ~5 timer var det liten endring i reduksjonen for både *L. salmonis* og *C. elongatus*. Mekanisk avlusningseffekt fra trengsel, pumping og gradering kan føre til at lusen løsner fra laksen og kan være en årsak til den raske nedgangen. Faste stadier av *L. salmoni*s og *C. elongatus* hadde den høyeste reduksjonen i ved slutten av ferskvannsbehandlingen. Voksne hunner og hanner av *L. salmonis* hadde en lav reduksjon sammenlignet med de andre lusestadiene. Trengningsmetoden som ble brukt i merden før behandlingen viste å ha innvirkning på lusetallene på hver fisk. Bruken av orkast for å samle laksen viste seg å gi det laveste lusetallet på hver laks før behandlingen. Korrelasjonstesten viste ingen sammenheng mellom K-faktoren til laksen og antall lus og mellom lengden på laksen og antall lus som var fast. Hovedresultatet fra behandlingen viste generelt en høy reduksjon i alle luste stadiene til *L. salmonis* og for *C. elongatus.* Voksen hann av *L. salmonis* hadde den laveste totale reduksjonen på 77.7% etter behandlingen, mens *C. elongatus* viste den høyeste effekten på 99.6% etter behandlingen.

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INTRODUCTION

1.1. BACKGROUND

Aquaculture in Norway started in the 1960s, and the Atlantic salmon (*Salmo salar)* farming industry has had a rapid growth during the last decades and has become one of the biggest industries in the country. Norway's long and sheltered coastline have proven to be well suited for extensive production of Atlantic salmon (Philis, Gracey et al. 2018). Salmon farms are distributed along the entire coast of Norway, with varying degree of production density. The South-Western part of the Norwegian coast has the highest production density. The production of Atlantic salmon has increased from 600 t in 1974 to 1 3500 000t in 2018 (Asche and Bjorndal 2011, Statistics Norway 2019) and accounts for more than 80 % of the aquaculture production in Norway (Cole, Cole et al. 2009, Philis, Gracey et al. 2018). The highest reported biomass is normally found in the period October to December (Serra-Llinares, Bjørn et al. 2014).

The rapid growth of the farming industry has also brought challenges, like diseases and parasites. A major threat is sea lice, both the salmon louse (*Lepeophteirus salmonis* (Krøyer)) and *Caligus elongatus* (Normann), that have been a problem for the industry since it started in the ’60s. The Norwegian Scientific Advisory Committee for Atlantic Salmon have recently identified salmon lice as the second largest threat to Norwegian salmon (Thorstad and Forseth 2017). Different experiments from 1996 and up to 2014, done with naturally infested post-smolt salmon and infested salmon, showed that salmon lice, especially when the salmon lice grow from the nonmotile stage to the motile stage, can stress the salmon, cause problems with its osmotic balance and degrade its immunological capacity (Karlsen, Asplin et al. 2018).

The high production of Atlantic salmon has created good conditions for the sea lice populations to grow and spread. The economic impact of *L. salmonis* is bigger than any other parasite (Torrissen, Jones et al. 2013). In 2014 *L. salmonis* cost the Norwegian industry 5 billion NOK (Iversen, Hermansen et al. 2017), corresponding to ~9 % of a farms revenues (Abolofia, Asche et al. 2017).

1.2. SEA LICE

Sea lice in aquaculture is a collective term that covers several species of parasitic crustaceans. Crustacean parasites on fish belong to the classes Copepoda and Branchiura, and the orders Amphipoda and Isopoda in the Malacostraca. Some have a high host specificity and can only live on one or a few host species, such as the salmon louse*,* while others have a lower host specificity and a big range of hosts to live on, such as *C. elongatus.*

Both *L. salmonis* and *C. elongatus* belong to the class Copepoda and the family Caligidae (Poppe, Bergh et al. 1999).

1.2.1. Lepeophteirus salmonis

Since *L. salmonis (*Krøyer) only needs one host to complete its life cycle, it classifies as a direct parasite. It has a circumpolar distribution in the northern hemisphere and develops in high salinity waters (Torrissen, Jones et al. 2013), and consumes skin, epidermis, mucus and blood from the salmon (Karlsen, Asplin et al. 2018).

The lifecycle consists of eight stages (Hamre, Eichner et al. 2013) (Figure 1). The louse hatch from the egg strings as a nauplius larva and drift in the water masses. The planktonic stage consists of two stages, the Nauplius 1 and Nauplius 2. In the infective stage, the copepodid larva finds its host and attaches to it with grip claws. The larva subsists entirely on endogenous lipid reserves and displays positive phototaxis, semiotaxis and rheotaxis, which means that the copepodids display diurnal vertical migrations, respond to waterborne gradients of host-derived chemicals and move towards vibrations of host origin (Torrissen, Jones et al. 2013). The nonmotile stages include two chalimus stages, Chalimus 1 and Chalimus 2, where the lice are attached to the host through frontal filaments and

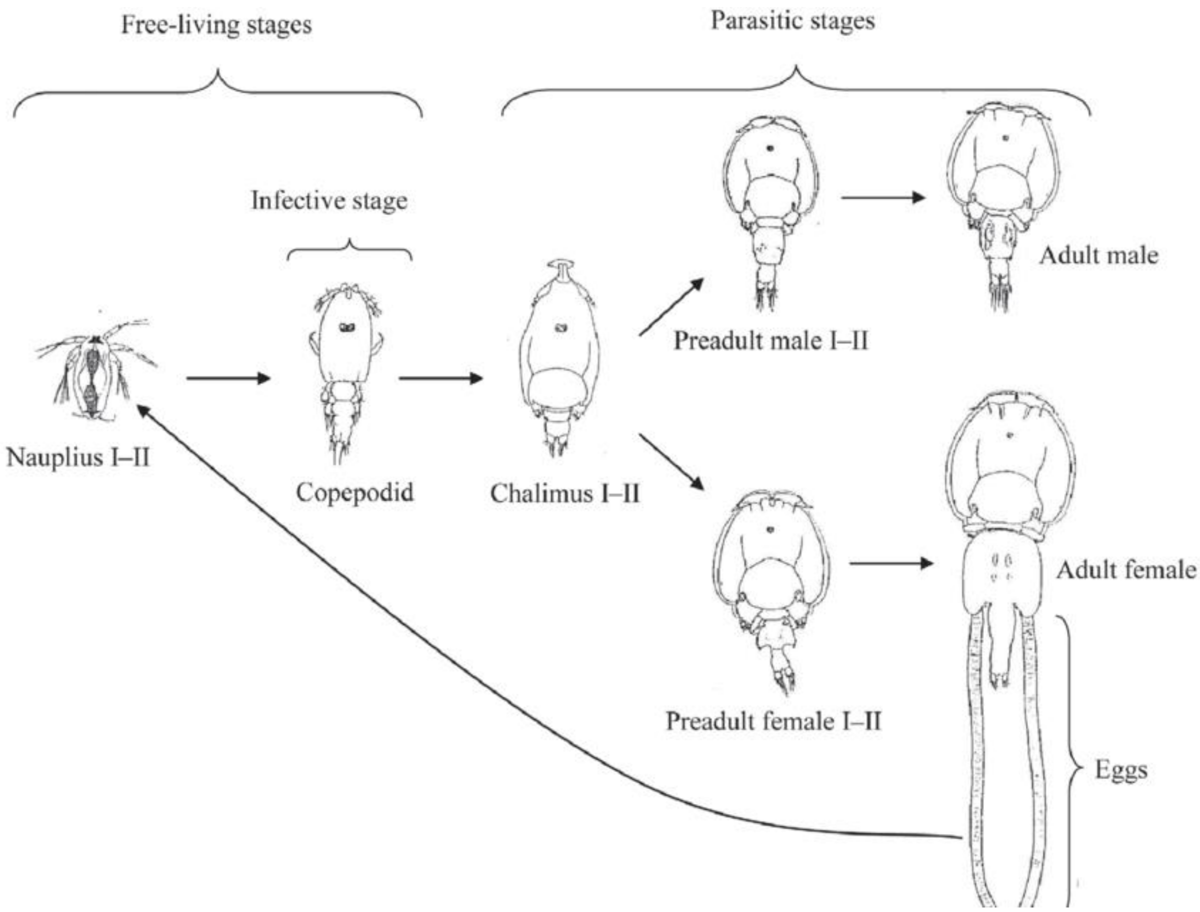


Figure 0-1 Developmental stages of Lepeophtheirus salmonis (diagram, not to scale, modified by Igboeli from (Schram 2006))

are feeding on the host. During the next stages, pre-adult stage 1 & 2, the lice can move around on the host and between hosts. The preadult stages are followed by the adult stages, in which the egg strings are produced. The different lice stages vary greatly in size, where the Nauplius 1 stage are half a millimetre and the Adult F stage are over one centimetre (Table 1-1). The pre-adult and adult F are generally bigger than the pre-adult and adult M. The main problem for the industry is not the free-swimming adult lice, but those that are attached to the host, chalimus, pre adult and adult stages.

During its life span, one female louse can produce ten to eleven pairs of egg strings. Under lower temperatures, the egg strings tend to be longer and have a higher fecundity (Hayward, Andrews et al. 2011, Torrissen, Jones et al. 2013). The louse's generation time changes with sea-temperatures. At higher temperature, the generation time becomes shorter (Wootten, Smith et al. 1982, Samsing, Oppedal et al. 2016).

Table 0-1: Mean length (mm) for each lice stage(Schram 2006, Hamre, Eichner et al. 2013)

Lice stage	Mean Length (mm)
N I	0.51
N II	0.61
C – free sw.	0.68
C - attached	0.79
Ch I	1.19
Ch II	2.26
Pre adult M I	3.35
Pre adult M II	4.31
Pre adult F I	3.59
Pre adult F II	5.18
Adult M	6.22
Adult F	9.96

Salmon louse and salinity

Wootten (1982) found that *L. salmonis* eggs will not hatch at a salinity of 10 PSU at 12 °C, while Johannessen (1977) reported that outside the range of 5-12 °C most eggs aborted at 11.5 PSU (Johannessen 1977). The attached salmon louse can rely on host-dependent mechanisms where they gain ions from the host to maintain homeostasis during exposure to freshwater. The free-swimming adult louse starts to die within 9 hours due to dilution by osmosis in their haemolymph (Hahnenkamp and Fyhn 1985). The copepodid stage have a low tolerance for freshwater, and after 1 hour for exposure shows a mortality of 96-100%. Attached stages that are older than the copepodid stage show a higher tolerance against freshwater (Wright, Oppedal et al. 2016). The detrimental effect of exposure to lower salinities is presumably related to osmotic stress. In brackish water with a salinity of >12 PSU, the adult female lice can osmoregulate independent of their host (Hahnenkamp and Fyhn 1985).

1.2.2. *Caligus elongatus*

C. elongatus was first described by Nordmann in 1832. It may have up to 80 different hosts and is much smaller than the salmon louse (Tables 1-1 and 1-2; (Poppe, Bergh et al. 1999). Sequencing of the CO1-gene (mitochondrial cytochrome C oxidase subunit I gene)

– used as an universal marker for species identification(Pentinsaari, Salmela et al. 2016), has shown that there are two genotypes of *C. elongatus* in Norwegian waters (Øines and Heuch 2005) that appear to be different and it may perhaps be two different species (Øines and Schram 2008)? The distribution of the genotypes seems to vary with host species, season, and how *C. elongatus* meets the host (Øines and Heuch 2007).

C. elongatus has a direct life cycle that consists of eight stages separated by moults. The first two nauplius stages, nauplii I and nauplii II, hatch from the egg strings. The copepodid is the infective stage and attach to the fish with grip-hooks and stay attached with a frontal filament. A copepodid can live freely in the water masses with salinity above 25 PSU for 31 days at 12 °C (Andersen 2006).

During the copepodid stage, the louse becomes attached, and the next stages are chalimus I, II, III and IV. Unlike the salmon louse, *C. elongatus* does not have a preadult stage (Piasecki 1996, Paulsen 2018). The adult males and females become sexually mature at the same time, but the males become mobile earlier and start to search on the fish for a mate. The male mates with a young adult female, but they can also attach to a female in the chalimus IV stage and wait for it to become sexually mature. The males die after mating. On average a female produces 89 eggs per egg string (Hogans and Trudeau 1989). *C. elongatus* are generally more mobile than the salmon louse, it moves faster on the host and it can transfer between hosts (Wootten, Smith et al. 1982).

As for the salmon louse, the generation time of *C. elongatus* are heavily influenced by temperature. A higher temperature will give a shorter generation time (Costello 2006), and at 10 °C, will it take 8 days from the production of egg strings to hatching (Piasecki and MacKinnon 1995). The most common hosts for *C. elongatus* are lumpfish (*Cyclopterus lumpus)*, pollock (*Pollachius pollachius)*, sea trout *(Salmo trutta)*, herring *(Clupea harengus)*, saithe (*Pollachius virens)* and cod *(Gadus morhua)* (Heuch, Øines et al. 2007).

Table 0-2: Mean length for each lice stage of C. elongatus.

Lice stage	Mean length (mm)
N I	0.45
N II	0.49
C	0.66
Ch I	0.82
Ch II	1.34
Ch III	2.34
Ch IV M	3.36
Ch IV F	3.79
Adult M	4.33
Adult F	5.38

C. elongatus and salinity

Andersen (2006) found that copepodites died immediately at 10 PSU while half the population died within 4-8 hours at 15 PSU. At 20 PSU, half the population was still alive after 48 hours, and at 25 PSU, half of the copepodites survived for 25 days. He also found that the copepodites activity level increased with the salinity and that copepodites of *C. elongatus* survive longer in salinities higher or equal to 15 PSU compared to salmon lice copepodites (Andersen 2006).

1.3. Treatments

The regulation of salmon lice in aquaculture in Norway is governed by "Regulations on combating salmon lice in aquaculture facilities" (Directorate of Fisheries 2013), which is implemented to *"reduce the prevalence of salmon lice so as to minimize the adverse effects on fish in aquaculture facilities and in wildlife populations of salmonids, and to reduce and combat the development of salmon lice".* The regulation applies for aquaculture sites with salmonids in the sea and state that the aquaculture sites should have a coordinated plan for control and combat of salmon lice (Directorate of Fisheries 2013).

Treatments against sea lice infection have been performed since 1997 to reduce the harmful effects on both farmed and wild salmonids (Heuch, Bjørn et al. 2005). Due to changes in legislation, increased resistance, use of new non-medical treatments (NMT), increased media focus and research on the salmon lice, the use of medical treatments has changed over the last years (Remen and Sæther 2018).

The increase in resistance or reduced sensitivity against medical treatment have led to an increase in the use of NMT. A common denominator for NMT's are that the fish need to be crowded before being pumped through the delousing systems, and the crowding has shown to be a large well-fare problem for fish. The most commonly used NMT-methods are mechanical cleaning(brushing/flushing), short-time increase in temperature and freshwater treatments (Jensen, Gu et al. 2019). Medical- and NMT's are often used in a combination with one or several preventative methods to combat the louse and are mentioned as Integrated Pest Management (IPM). Preventative methods can be such as skirts in lice-tight material around the cages, the use of health-feed to prevent spreads, breeding for more lice-resistant fish and the use of cleaner fish in the pen (Norwegian Veterinary Institute 2019).

With the increasing use of NMT's came a concern of the potential of sea lice to evolve tolerance against these methods, as it did to the 'traditional' medical control strategies (Bale, Van Lenteren et al. 2007).

In 2017 the Food Safety Authority in Norway announced a new salmon lice regulation for aquaculture sites (Djupevåg 2017). The background for the new regulation was the situation with increased resistance against medicaments, development of new non-medical methods for treatments against salmon lice, increased fish welfare problems related to treatments, and the new regulations for the production capacity at the aquaculture sites.

In the fourth quarter of 2019, the new regulations will be established by Ministry of Trade, Industry and Fisheries (Djupevåg 2017).

1.4. Freshwater treatment

There are several methods available for using freshwater to delouse salmon. One method is a sea pen with snorkel, that has a freshwater layer in the upper part, whereas the most used and efficient is freshwater baths in well boats, with or without reuse of freshwater (personal comment S. Gaasø, 2019).

1.4.1. General description of delousing method with freshwater bath-treatment

To be able to load the fish into the boat during freshwater treatment, the fish must be crowded in the pen. Different companies have their own procedures for crowding. Two methods for crowding the salmon can be with the use of a ball line ("kulerekke"), and another is with a swipe net ("orkast"). The two methods differ in use and effectiveness. During crowding with swipe net, a seine is used inside the pen to collect the salmon. The ball line is pulled under the pen and tightened to 'crowd' the salmon in one part of the pen.

The crowded salmon is pumped onboard through big tubes, over a dewatering unit/grid to remove seawater and thus ensure the quality of the freshwater with ~zero salinity. Sometimes, if ordered, the salmon are sorted by size before separated into wells.

Any cleaner fish in the pen before treatment start are handled according to Aquaculture operation regulations (Directorate of Fisheries 2008). Seawater that is filtered out on the dewatering unit or grading station is pumped over a filter to collect lice and other biological particles that are in the water. The time required for transferring the salmon from the pen to the well boat differs with the method used to 'crowd' the salmon and is also dependent on the size and the volume of salmon, and the capacity of the well boat.

The treatment time, the time salmon are held in the freshwater bath, start when the last salmon are transferred from the pen and into the well. The duration varies and is dependent on the lice load per pen and different sea lice stages, but typically ranges from six to 10 hours. The freshwater is often reused and is filtered continuously during and between each treatment to avoid the possibility of detached lice resettling on the salmon.

When emptying the boat, the freshwater is filtered, and the biological material is delivered to the aquaculture site and becomes silage. The exact method differs with well boats and equipment available and the filter type may vary (personal comment S. Gaasø, 2019).

Freshwater baths on well boats with reuse of freshwater was the method used in all five treatments in this study. The well boats get their freshwater from a known reservoir. The freshwater parameters that are collected are salinity, oxygen level, temperature, pH, TAN, NH_3, N_2, TGP and CO_2. Depending on the water quality, the water is then treated to obtain the quality needed to ensure the best possible result regarding fish welfare and reduction of sea lice.

1.5. AIM

This thesis is a part of Taskforce Salmon Lice R&D project where the non-chemical delousing method of Freshwater bath was investigated. The main aim of this thesis was to investigate the effect and the efficiency that freshwater treatment may have on *C. elongatus* and *L. salmonis.*

Research questions of the thesis were as follows:

1. What is the development of sea lice on the salmon over the course of freshwater treatment?

2. Will the crowding method have an impact on lice numbers before treatment start and on the total effect?

3. Is there a correlation between the condition factor K of the salmon and the number of lice attached?

2. MATERIALS AND METHOD

2.1. STUDY SITE

Fieldwork was carried out in 2019 between week 35 and 37, at three different location; in this thesis given as Location 1, 2 and 3 for anonymity, in production area 6: Nordmøre and Sør-Trøndelag (Figure 2-1) (Directorate of Fisheries 2017). Production area 6 has the boarders; 1) Takneset (Fræna) N 62° 59.28′ Ø 7 ° 06.30′ to open sea N 63° 34.80′ Ø 6° 31.20′ and 2) County border at Skjemta, Flatanger, N 64° 25.74′ Ø 10° 30.60′ to open sea N 64° 49.80′ Ø 8° 58,20′ (Directorate of Fisheries 2017).

2.2. DATA COLLECTION

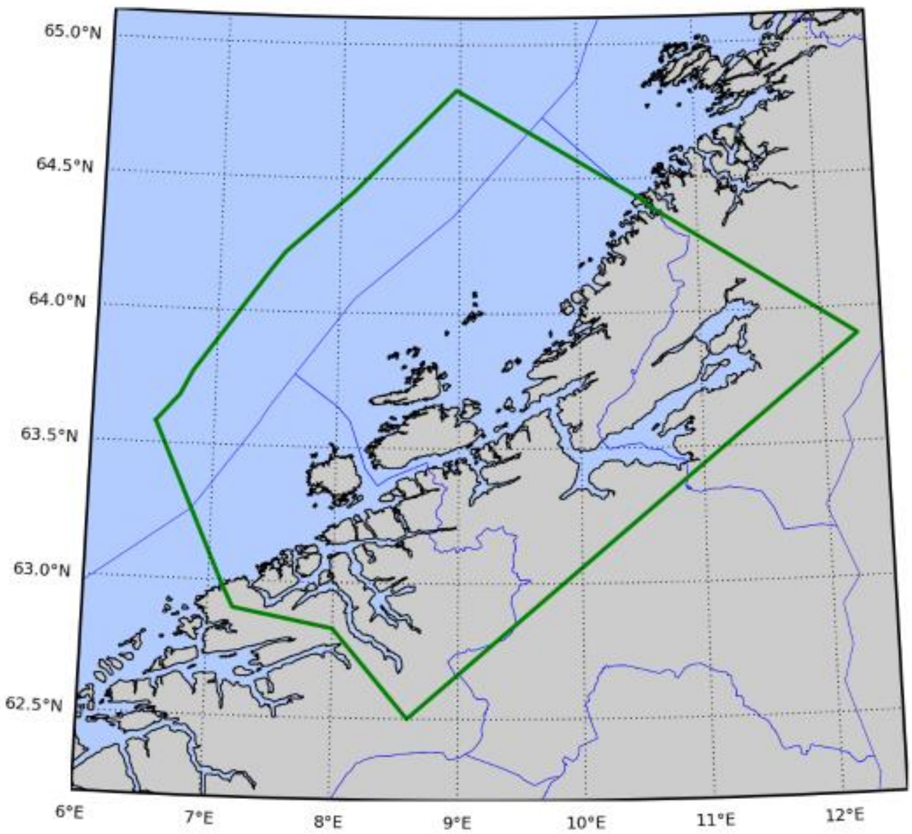


Figure 2-1: Production area 6 outlined with green boarder. The latitude and longitude are listed in the text (Directorate of Fisheries 2017).

Collection of salmon from the pen and wells was done by dipnet, and the salmon were anaesthetized in a 200L or 300L tub filled with seawater, Benzoak veterinary (15-20ml per 100L water), and a lice fabric (a cloth that hinders the lice escaping from the tray). Weight, length and number of sea lice (Chapter 2.2.2 and 2.2.3) were registered for each fish, and the fish was immediately returned to where it was sampled from. In each sampling, 20 fish were analysed. When the last fish was analysed, any remaining lice in the tray was counted and registered. Before the next sampling, the tub and the lice fabric were cleaned, and new water and sedation were added.

2.2.1. Sea temperatures

The regulation of salmon lice states that the temperature at an aquaculture site should at least be measured once per week at 3m deep if the temperature is ≥ 4 °C. The seawater temperatures at the locations during the sampling period were obtained from barentswatch.no/fiskehelse at the respective sites and time (Table 3-1).

2.2.2. Lice Registration

The *"Regulations on combating salmon lice in aquaculture facilities" (Attachment 1) Requirements for routine counting of salmon lice*; specifies how the stages of salmon lice should be grouped during counting. The regulation separates the lice stages in three groups, a) adult female lice, b) motile stages and c) attached stages, where b) motile stages include adult male lice and preadult male and female lice and c) attached stages includes chalimus I & II and copepodids. It also specifies the allowed threshold value of adult female lice per fish at each region and season. Requirements for routine counting of salmon lice there are also stated, and what stages of salmon lice that should be counted. The number of fish counted each season are also specified (Directorate of Fisheries 2013).

In the present study, all *L. salmonis* in all counting's were registered in the following four categories; attached, pre-adult, adult male and adult female. The presence or absence of egg strings attached to the adult female was also registered. All the different stages of *C. elongatus* were counted in one group.

Based on a preliminary investigation of the freshwater treatment, five sample points during the freshwater treatment were chosen; one during crowding of the salmon in net pens, three times during the treatment time - in each well, port- (PW) and starboard (SbW), and one final during the unloading of the salmon (Figure 2-2).

To ensure that there was enough time for each counting, an estimate of one hour per counting at each well was set. To be able to finish counting in each well before the fish was unloaded into the pen, the last counting had to start two hours before unloading. Five freshwater treatment experiments were totally conducted at three different locations.

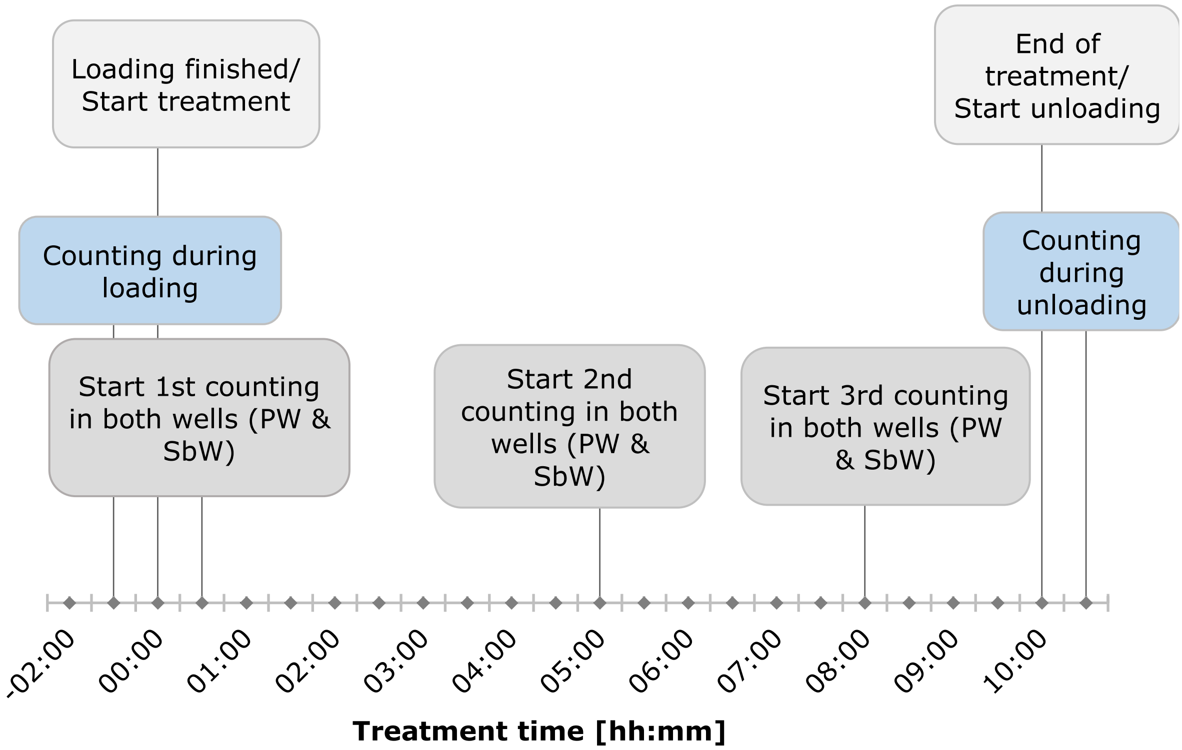


Figure 2-2: Illustrative timeline of a freshwater treatment, indicating time at which the lice countings were carried out during treatment.

The lice numbers at the locations from the week prior to and the week after the treatment were collected from the respective sites with help from the site managers.

L. salmonis from the locations were registered in the following categories: adult female lice, motile stages (sometimes in two groups; small motile and big motile; also includes adult males), attached lice and *C. elongtaus.*

2.2.3. Weight and length of salmon

The counting and the measurements were carried out on a counting-table (Figure 2-3), that had engraved length dimensions on one side.

The salmon was measured using a kitchen weight that could measure weight up to 10 kg. On top of the weight, a plate was used to balance/distribute the salmon's weight (Figure 2-3).

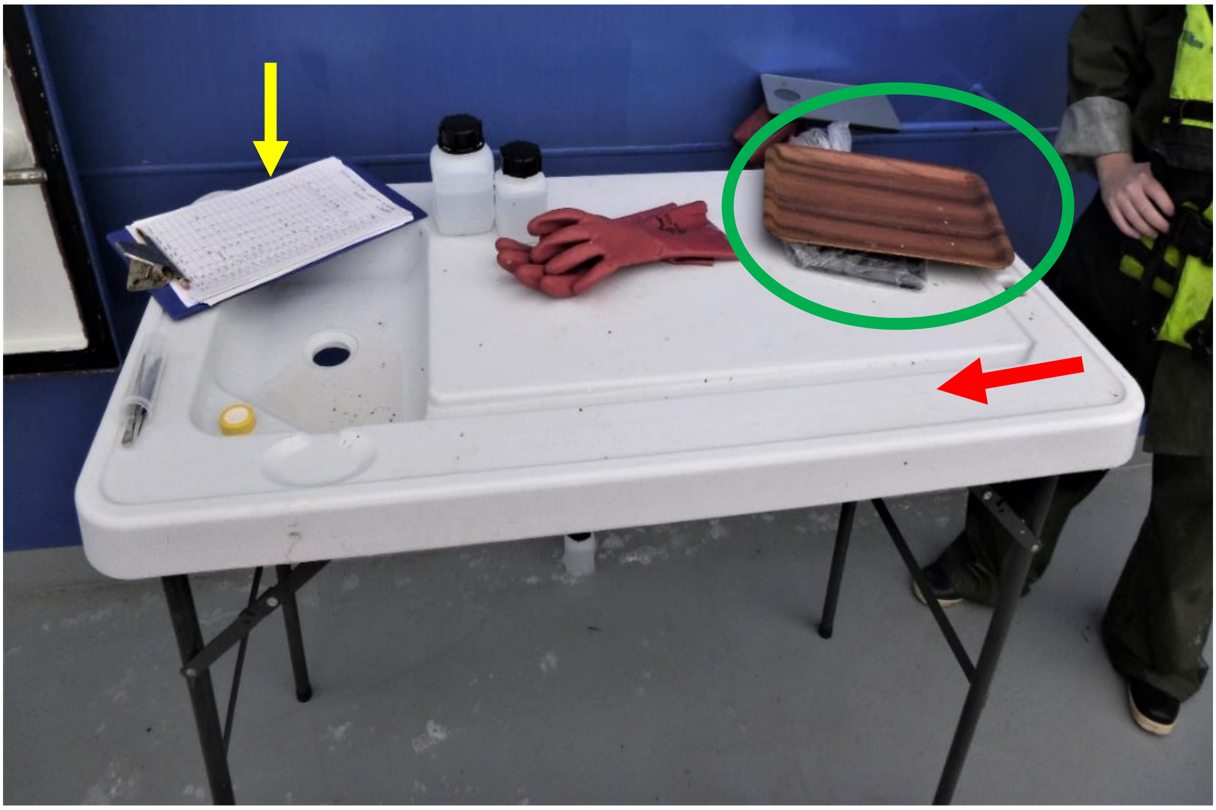


Figure 2-3: Working place; a lice counting-table with registration form (yellow arrow), gloves, weight and plate (green ring) and length measurement line (red arrow). Photo: Maria Gaasø

The maximum standard length of salmon was measured (Figure 2-4).

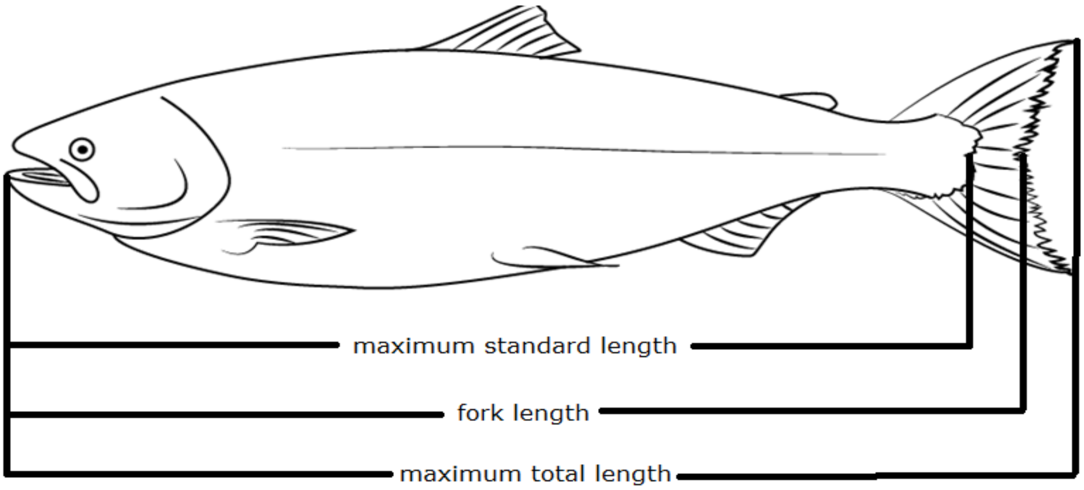


Figure 2-4: Measuring length's; Maximum standard length, fork length and maximum total length.
Pictures source: https://www.drawingtutorials101.com/how-to-draw-a-salmon

In fisheries and general fish biology studies, Fulton's condition factor, K, is widely used as a measure of the fish "condition" (Nash, Valencia et al. 2006). The value of K is calculated from the weight and the length of the fish using the formula (Ricker 1975);

$$K = \frac{W}{L^3} \qquad \textit{Equation 1}$$

Where K is the condition factor, W is the weight of the fish (g), and L is the length of the fish (mm) (Nash, Valencia et al. 2006). Since the weight of the fish are used in grams and the length in mm a factor of N is often used, to get the number closer to 1.

In this thesis the formula used to calculate the condition factor was;

$$K = \frac{10^N W}{L^3} \qquad \textit{Equation 2}$$

where *W* is the weight of the fish in grams (g), *L* is the standard length of the fish in millimetres (mm), N is set to 5 so the K factor falls in the rage close to 1 (PSM and Baxter 1998).

2.2.4. Characteristics of freshwater

Water treatment temperature (°C), salinity (PSU) and oxygen (%), fish density (kg/m^3) in the wells, loading time, treatment time and unloading time was obtained from the well boats. Fish density was calculated from the total biomass (kg) and the volume in the wells (1600m^3).

The temperature of the treatment water should have a maximal variation of ± 3 °C from the seawater temperature at the given location, and should never be below 5 °C.

Optimal oxygen saturation levels are 100% and it should be kept between 80 – 120 %. The salinity of the freshwater has a maximum level of 3 PSU for lice treatment.

2.3. STATISTICAL ANALYSIS

All statistical analyses were performed either using Excel (Version 1911, Microsoft Office 365 ProPlus) or R-studio (Version 1.1.442).

Lice reduction between countings were investigated by ANOVA and T-test.

The variables used in Spearman's correlation test was tested in R studio and found to be non-normally distributed.

The standard error is written as ±SE, any other variables as standard deviation will be explained.

2.3.1. Spearman's correlation test

The Spearman's correlation coefficient is well suited for use at analyses of ordinal variables and non-normally distributed variables. There is no requirement of a linear relationship between the observation data, as long the range-values give a linear relationship. The coefficient (rho) assesses how well the relationship between two variables can be described using a monotonic function. For a correlation between two variables x and y, the formula for calculating the coefficient, ρ (rho), is given by;

$$r_s = 1 - \frac{6\sum_{i=1}^{n} d_i^2}{n(n^2-1)} \qquad \textit{Equation 3}$$

where d_i is the difference in ranks for x and y (Mukaka 2012).

Rho (ρ) will be high when observations have a similar rank between the two variables, and low when the observations have a dissimilar rank between the two variables. If the two variables have an entirely opposed correlation, rho will come close to a value of -1 (Table 2-1) (Hinkle, Wiersma et al. 2003).

Table 2-1: Spearman's correlation - size and interpretation (Hinkle, Wiersma et al. 2003)

Strength of Correlation	**Interpretation**
.90 to 1.00 (-.90 to -1.00)	Very high positive (negative) correlation
.70 to .90 (-.70 to -.90)	High positive (negative) correlation
.50 to .70 (-.50 to -.70)	Moderate positive (negative) correlation
.30 to .50 (-.30 to -.50)	Low positive (negative) correlation
.00 to .30 (-.00 to -.30)	Negligible correlation

Spearman's ρ (Rho) was examined by using the `cor.test (x, y, method = "spearman")` function available in base R.

3. RESULTS

3.1. WATER MEASUREMENTS

The temperature of the treatment-water(°C), the -salinity (PSU) and the saturation of the oxygen (%) were recorded in each well through all five treatments. Location 1 had a temperature difference between seawater and treatment water of 3 °C, location 2 a temperature difference of 2.5 °C and location 3 a temperature difference of 2 °C. The oxygen saturation was 110 % in both wells for all five treatments.

The highest salinity level was 1 PSU in port well (PW) at treatment 3.2, and 0.9 in starboard well (SbW) (Table 3-1). Treatment 2.1 had the lowest salinity level of 0.2 PSU in both wells. Treatment 1 had a salinity of 0.7 PSU in each well, Treatment 2.2 0.3 PSU in each well and Treatment 3.1 had 0.5 PSU in SbW and 0.4 PSU in PW (Table 3-1).

Table 3-1: Water characteristics; fish density, treatment-water temperature, salinity and oxygen obtained from the well boat. Sea temperature was taken from https://www.barentswatch.no/fiskehelse/2019 at each location and the respective week of treatment.

Treatment:	**1 PW**	**1 SbW**	**2.1 PW**	**2.1 SbW**	**2.2 PW**	**2.2 SbW**	**3.1 PW**	**3.1 SbW**	**3.2 PW**	**3.2 SbW**
Fish density (kg/m^3)	68.4	67.8	76.4	51	72.3	51.3	67.4	67.2	61.8	63.1
Treatment water temperature	10.5	10.5	11	11	11	11	11.3	11.3	11.3	11.3
Salinity max. (PSU)	0.7	0.7	0.2	0.2	0.3	0.3	0.5	0.4	1	0.9
Oxygen (%)	110	110	110	110	110	110	110	110	110	110
Sea water Temperature	13.9	13.9	13.6	13.6	13.6	13.6	13.3	13.3	13.3	13.3

3.2. LICE NUMBERS DURING TREATMENT

The lice numbers from each counting in each well are plotted in a timeline, where loading time and unloading time are shaded in the graphs. *L. salmonis* in all counting's were registered in the following four categories; attached, pre-adult, adult male and adult female, while for *C. elongatus* all stages were counted as one. Each treatment has different loading-, treatment- and unloading time. SbW had five counting's, while PW had four, this can be seen in Figure 3-1 to 3-5 as points in the lines.

3.2.1. Treatment 1

Mean number of *C. elongatus* on each fish was 4.1±0.79 during loading, while the mean number of total *L. salmonis* was 14.9±1.1 lice per fish.

Attached, pre-adult and *C. elongatus* had a strong reduction from start treatment to the 1st counting in SbW (Figure 3-1). After the 1st counting, there were found no egg strings, while the total numbers of *C. elongatus* first are reduced to zero after the 2nd counting. The last two hours during the treatment did not have any strong effect on the numbers of lice.

Pre-adult attached and *C. elongatus* showed a strong reduction in numbers from the treatment start to the 1st counting in PW. Egg strings were reduced to zero after the first counting in PW.

An ANOVA test, with recline, between counting during loading and the 3rd counting in both wells (SbW and PW) showed that there were was a significant reduction in lice numbers ($p<0.01$).

An ANOVA test, with recline, between 3rd counting in SbW (8 hours and 40 min after treatment start) and unloading showed no significant reduction in numbers of lice between these two counting times ($p=0.64$)

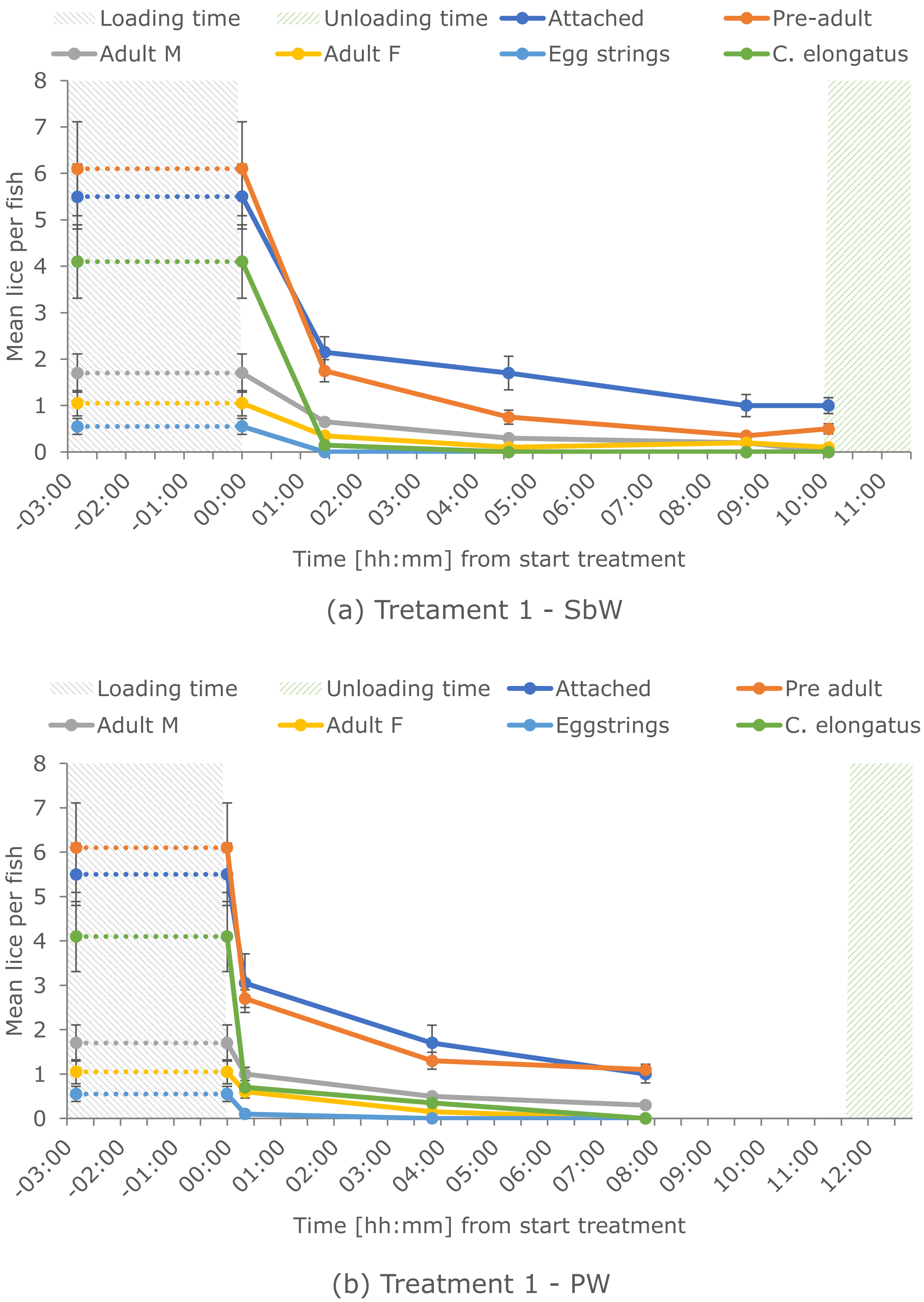


Figure 3-1: Mean number L. salmonis; attached, pre-adult, adult M, adult F, egg strings and C. elongatus per fish during the treatment at location 1, SbW (a) and PW (b). The white field between loading and unloading represent the freshwater exposure time (SbW = 10 hours, PW = 11 hours and 35 minutes). The standard error is represented by the 1SE bars.

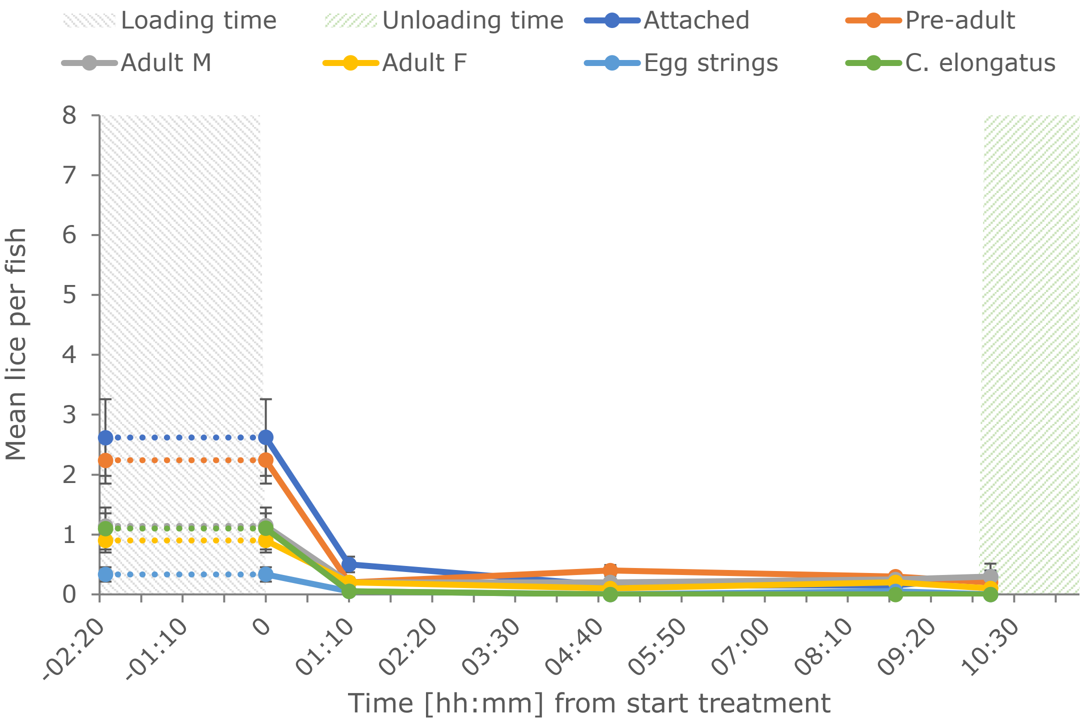
3.2.2. Treatment 2.1

Mean number of *C. elongatus* on each fish was 1.1±0.35 during loading, while mean number of total *L. salmonis* was 7.25±0.86 lice per fish.

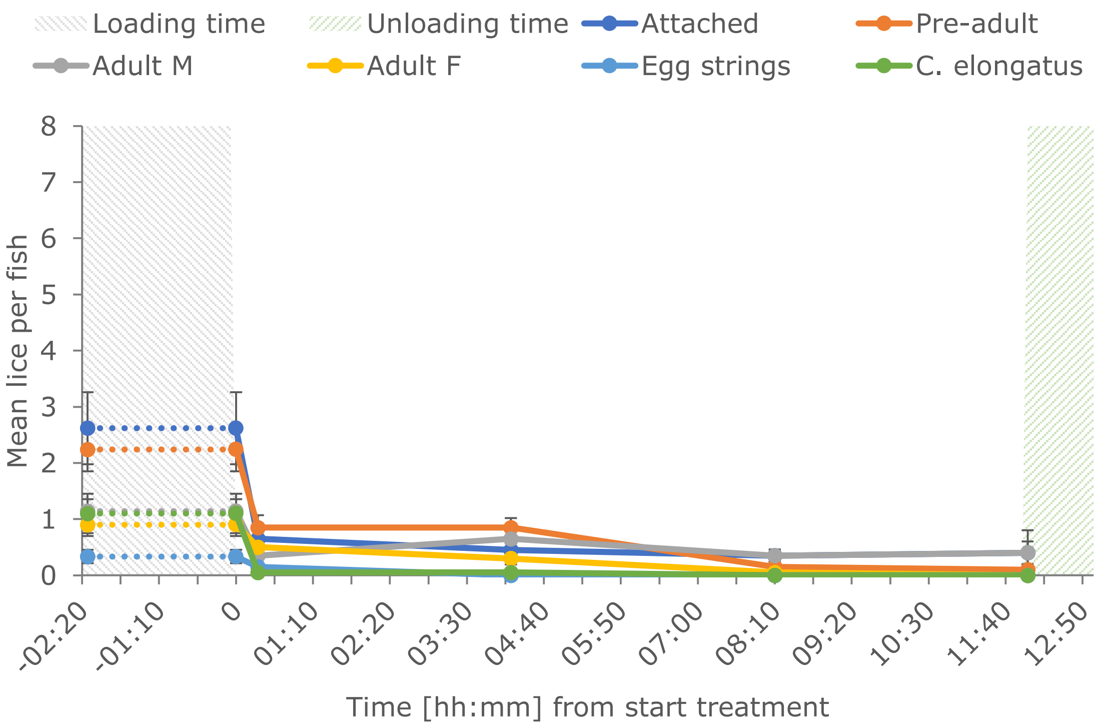
Attached pre-adult and *C. elongatus* showed a strong reduction from start of treatment to the 1st counting in SbW (Figure 3-2 (a)). All egg strings were released after the 1st counting, while the total number of *C. elongatus* first was reduced to zero after the 2nd counting. The last four hours during the treatment did not show any significant effect on lice numbers. All lice groups showed a decline immediately after the start of treatment in PW (Figure 3-2 (b)).

An ANOVA test, with recline, between counting during loading and the 3rd counting in both wells (SbW and PW) showed that there are a significant reduction in lice numbers in both wells ($p<0.01$).

An ANOVA test, with recline, between the 3rd counting in both wells (SbW and PW) and unloading of both wells (SbW and PW) showed that there was no significant reduction in lice numbers ($P=0.92$ and $P=0.88$).



(a) Treatment 2.1 - SwB



(b) Treatment 2.1 - PW

Figure 3-2: Mean number of L. salmonis; attached, pre-adult, adult M, adult F, egg strings and C. elongatus per fish during treatment 2.1, SbW (a) and PW (b). The white field between loading and unloading represent the freshwater exposure time (SbW = 10 hours, PW = 12 hours). The standard error is represented by the 1 SE bars.

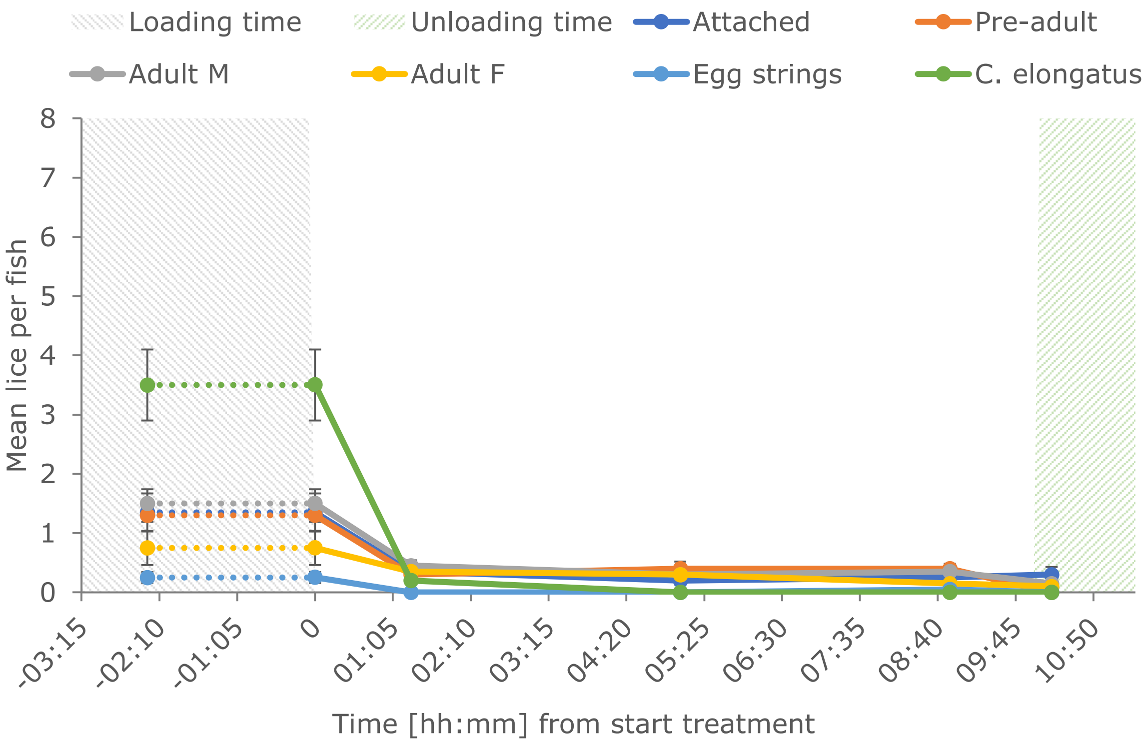
3.2.3. Treatment 2.2

Mean number of *C. elongatus* on each fish was 3.5±0.6, while mean number of total *L. salmonis* was 4.9±0.77 during loading.

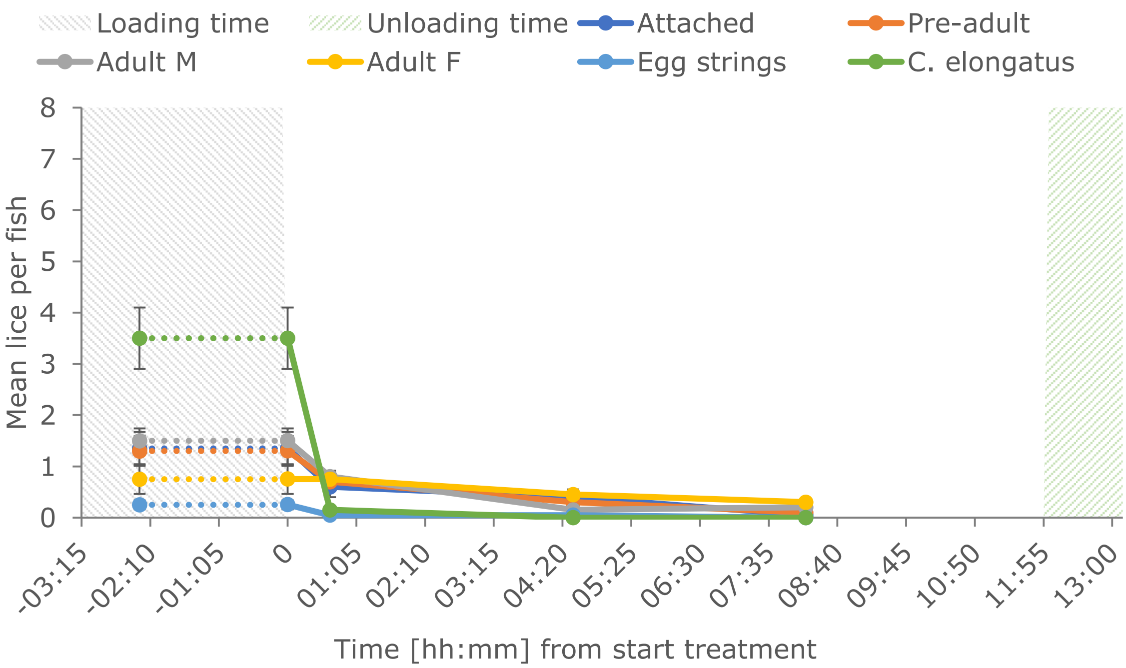
Attached pre-adult and *C. elongatus* showed a significant reduction from the start of treatment to the 1^{st} counting in SbW (Figure 3-3 (a)). All egg strings were released after the 1^{st} counting, while the total number of *C. elongatus* was reduced to zero after the 2^{nd} counting. The last four hours during the treatment had an effect on the lice numbers. All lice groups showed a decline immediately after the start of treatment in PW, (Figure 3-3 (b)).

An ANOVA test, with recline, between counting during loading and the 3rd counting in both wells (SbW and PW) showed that there was a significant reduction in lice numbers ($p<0.01$).

An ANOVA test, with recline, between 3rd counting in well SbW and unloading, showed a significant difference in lice numbers ($p<0.01$).



(a) Treatment 2.2 - SbW



(b) Treatment 2.2 - PW

Figure 3-3: Mean number of L. salmonis; attached, pre-adult, adult M, adult F, egg strings and C. elongatus per fish during treatment 2.2, SbW (a) and PW (b). The white field between loading and unloading represent the freshwater exposure time (SbW = 10 hours, PW = 12 hours). The standard error is represented by the 1SE bars.

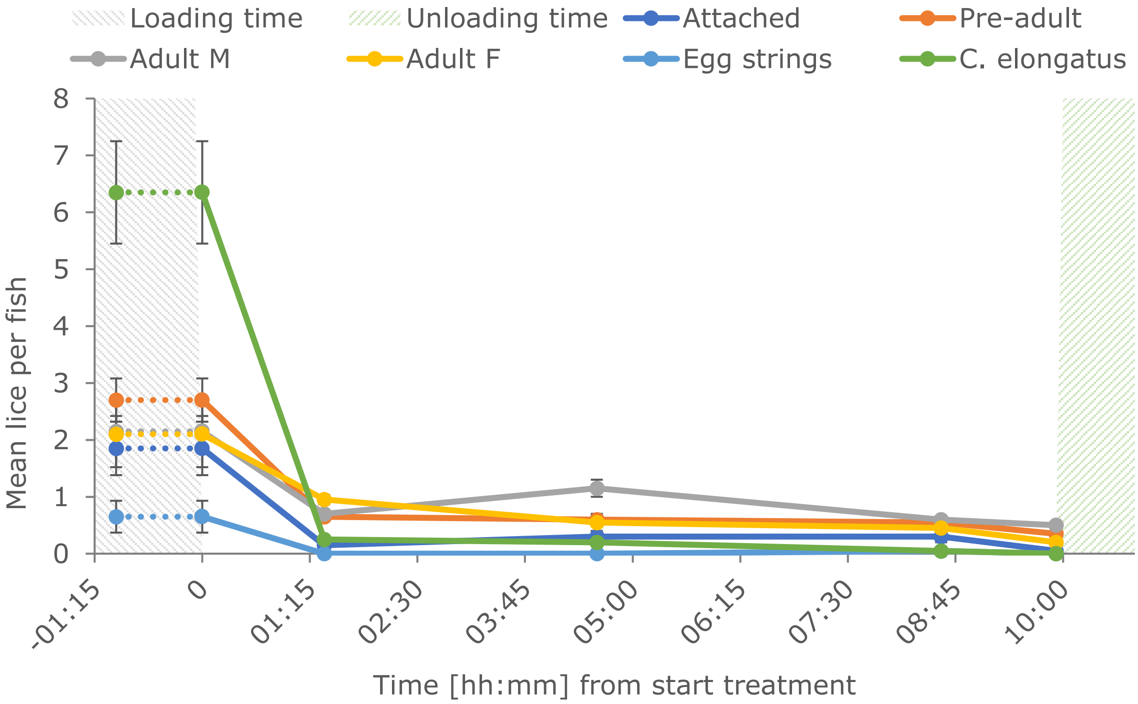
3.2.4. Treatment 3.1

Mean number of *C. elongatus* on each fish was 6.35 ±0.9 during loading, while mean number of *L. salmonis* was 8.8±1.19 lice per fish.

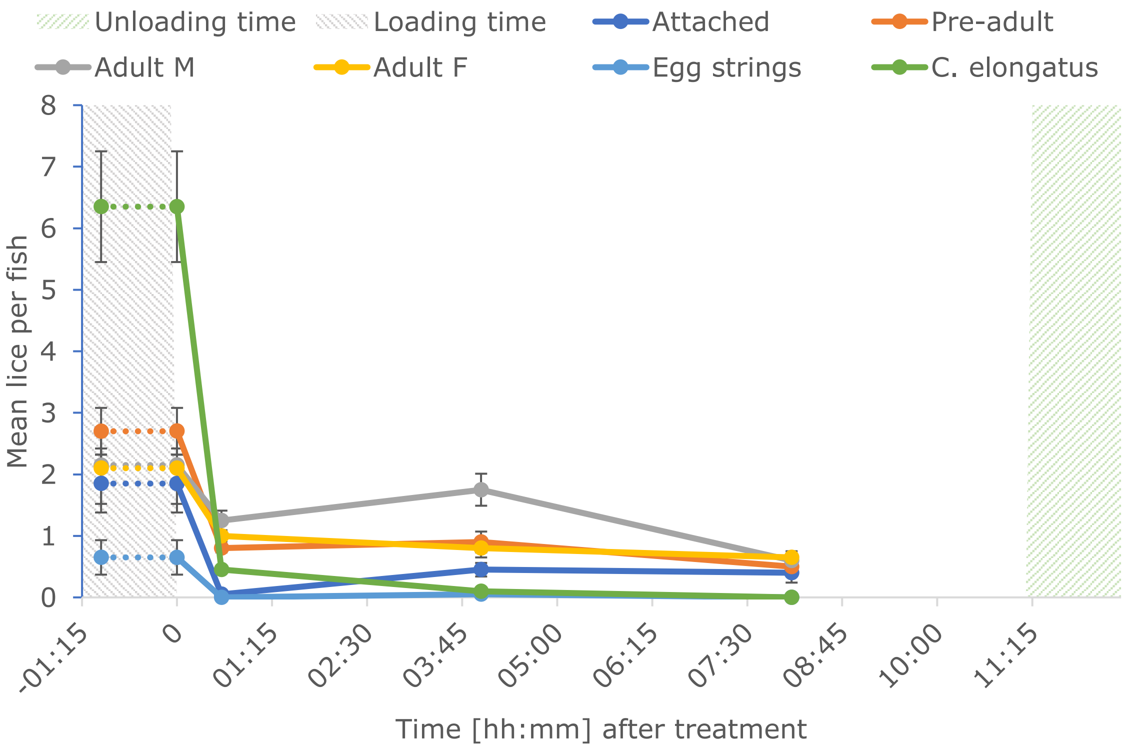
Attached, pre-adult and *C. elongatus* showed a reduction from start treatment to the 1st counting in SbW (Figure 3-4 (a)). All egg strings were released after the 1st counting, while the total number of *C. elongatus* was first reduced to zero after the 2nd counting. The last four hours during the treatment showed an effect on the lice numbers. All lice groups showed a decline immediately after the start of treatment in PW (Figure 3-4 (b)).

An ANOVA test, with recline, between counting during loading and the 3rd counting in both wells (SbW and PW) showed that there was a significant reduction between in lice numbers ($p<0.01$).

An ANOVA test, with recline, between 3rd counting in well SbW and unloading, showed a significant difference in the two countings ($p<0.01$)



(a) Treatment 3.1 - SbW



(b) Treatment 3.1 - PW

Figure 3-4: Mean number of L. salmonis; attached, pre-adult, adult M, adult F, egg strings and C. elongatus per fish during treatment 1 at location 3, SbW (a) and PW (b). The white field between loading and unloading represent the freshwater exposure time (SbW = 10 hours, PW = 11 hours and 13 minutes). The standard error is represented by the 1SE bars.

3.2.5. Treatment 3.2

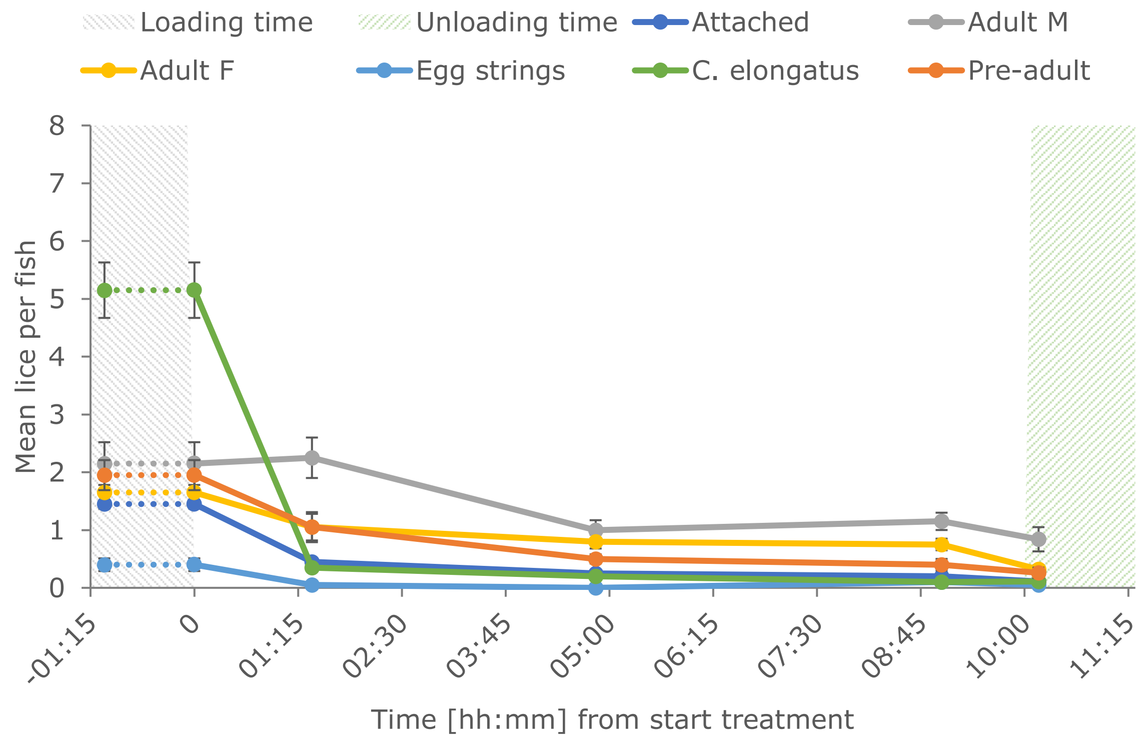
Mean number of *C. elongatus* on each fish was 5.15±0.48 lice during loading, while the total number of *L. salmonis* was 7.2±0.79 per fish.

Attached, pre-adult and *C. elongatus* had a significant reduction from the start of treatment to the 1st counting in SbW (Figure 3-5 (a)). All egg strings was released after the 1st counting, while the total number of *C. elongatus* first was reduced to zero after the 2nd counting. The last four hours during the treatment showed a significant effect on the lice numbers.

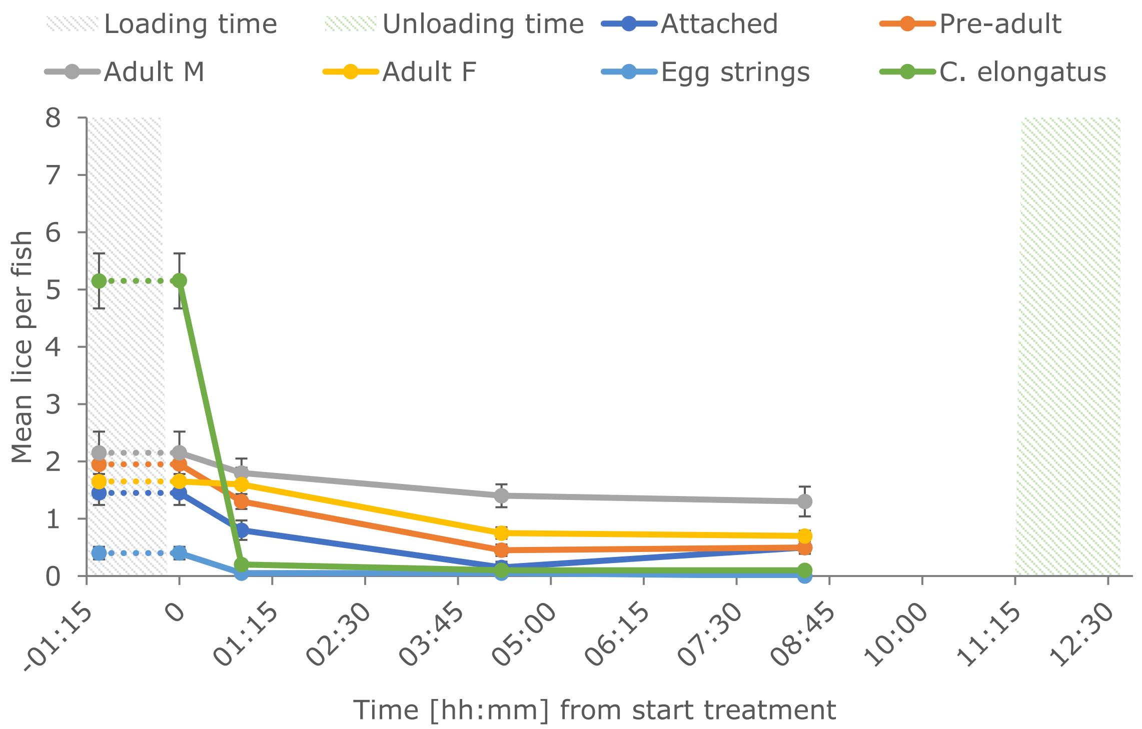
There was a clear reduction in lice numbers from treatment start to the 2nd counting in PW. From 2nd to 3rd counting the lice numbers was stable, except for attached lice, that had a small increase.

AN ANOVA test, with recline, between counting during loading and the 3rd counting in both wells (SbW and PW) showed that there was a significant reduction in lice numbers ($p<0.01$).

An ANOVA test, with recline, between 3rd counting in well SbW and unloading, showed a significant difference in the two countings ($p<0.01$)



(a) Treatment 3.2 - SbW



(b) Treatment 3.2 - PW

Figure 3-5: Mean number of L. salmonis; attached, pre-adult, adult M, adult F, egg strings and C. elongatus per fish at each counting during treatment 3.2, SbW (a) and PW (b). The white field between loading and unloading represent the freshwater exposure time (SbW = 10 hours, PW = 11 hours and 26 minutes). The standard error is represented by the 1SE bars.

3.3. Treatment effect

The treatment effect expresses the percentage of lice removed through the different treatment stages relative to the numbers during loading. The numbers are mean values for all five treatment, and both wells (1st, 2nd and 3rd counting) (Figure 3-6).

All lice stages showed a high reduction in numbers for all five treatments, from start to end of the procedure. The overall reduction of total *L. salmonis* numbers for all five treatments was 86.3%±1.55, where Treatment 1 showed the highest total effect of 88.9% reduction of *L. salmonis (*Appendix Table 0-8). Treatment 3.2 showed the lowest reduction of *L. salmonis,* at 78.8%. The total mean effect on *C. elongatus* for all five treatments was a reduction of 99.6%±0.36, where Treatment 3.1 has the lowest reduction, 97.9%, (Appendix Table 0-8). All *C.* elongatus individuals were removed in the other four treatments, showing a 100% reduction.

The highest treatment effect of the attached stages of salmon lice was found in Treatment 3.1, showing a reduction of 97.3%. The treatment with the lowest effect was Treatment 2.2 showing a reduction of 77.8% (Appendix Table 0-8).

An ANOVA test, with recline, showed that there was a significant reduction in lice numbers in the wells from 1st to the 2nd counting ($p<0.01$), from the 2nd to the 3rd counting ($p<0.05$) and from the 3rd counting to during unloading ($p<0.01$) (Figure 3-11).

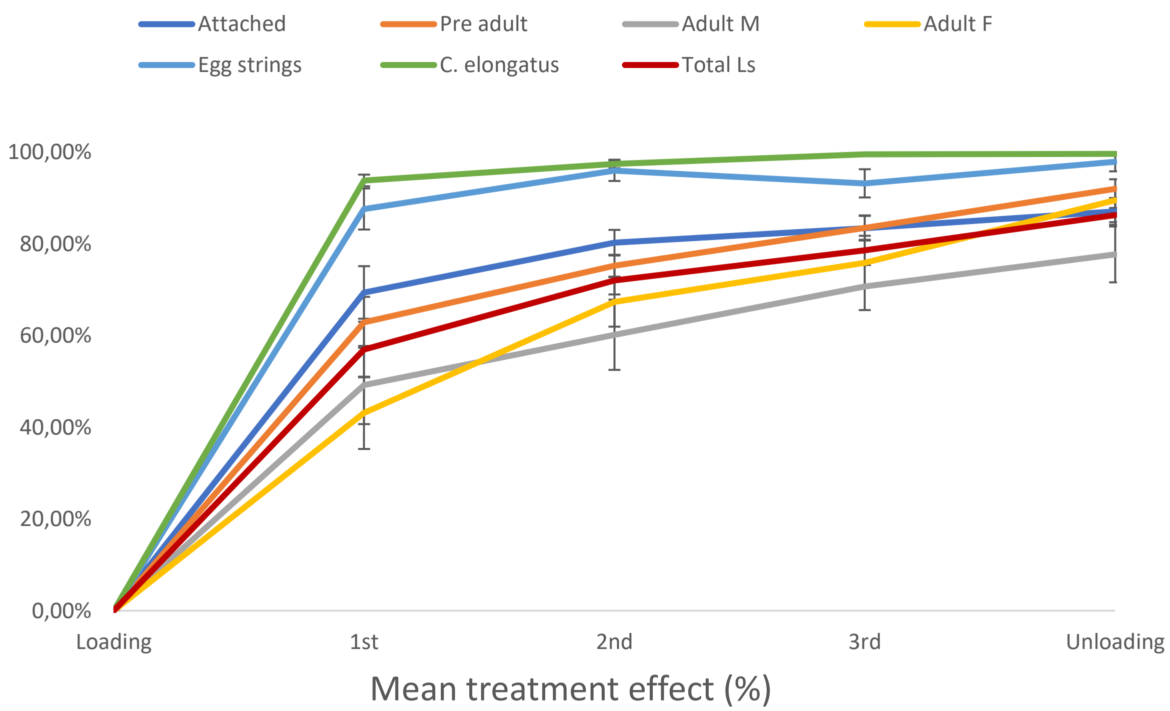


Figure 3-6: Mean lice reduction (%) for all 5 treatments in both SbW and PW shown for each counting point, during loading, 1st, 2nd, 3rd counting in the wells and during unloading. The standard error is represented by the error bars.

3.3.1. Lice numbers weeks prior and after treatment

At location 1, two pens; pen A and pen B, were treated with freshwater at the same time, and then merged into one pen after the treatment. The mean lice numbers of the two pens are illustrated in Figure 3-7, pen A and B. The % reduction from the week prior for Pen A to the week after treatment for attached, motile, adult F, *C. elongatus* and total *L. salmonis* stages was 100%, 97.4%, 100%, 96.4% and 98%, respectively. The % reduction for attached, motile, adult F, *C. elongatus* and total *L. salmonis* stages from pen B was 100%, 84.6%, 100%, 70% and 96.4%, respectively (Figure 3-7).

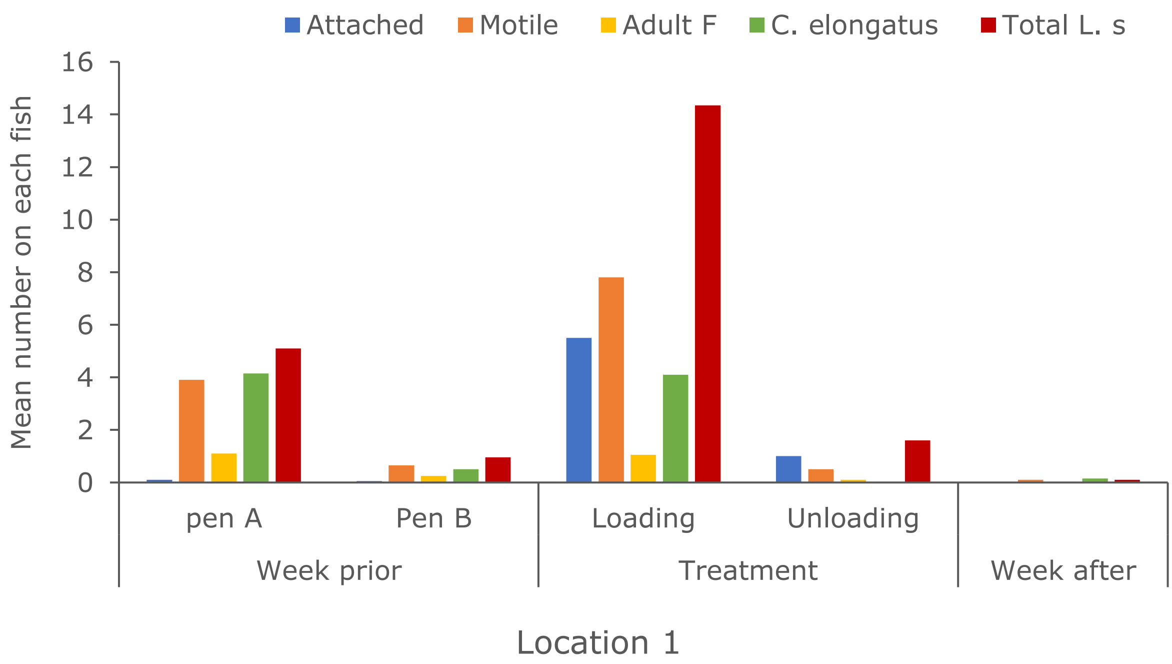


Figure 3-7: Mean lice numbers at Location 1 from the week prior (pen A and B) treatment, during loading and unloading, and the week after.

At Location 2, fish from one pen were sorted by size during the treatment and then unloaded into two separate pens. The lice numbers was counted the week prior, and two countings at the week after treatment (pen C and pen D) (Figure 3-8). The % reduction from the week prior to the week after in pen C for attached, motile, adult F, *C. elongatus* and total *L. salmonis* stages was 100%, 76.9 %, 100%, 13.3% and 89.3%, respectively. The % reduction from the week prior to the week after in pen D for attached, motile, adult F, *C. elongatus* and total *L. salmonis* stages was 50%, 69.2%, 92.3%, 40% and 78.6%, respectively.

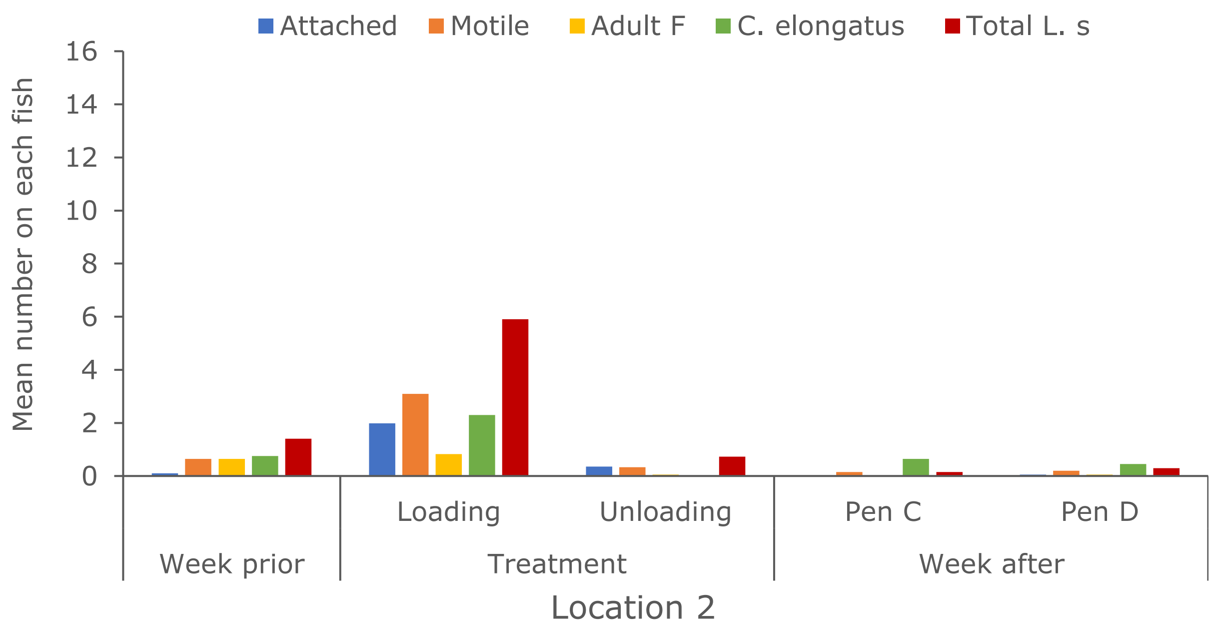


Figure 3-8: Mean lice numbers at Location 2 from the week prior treatment, during loading and unloading, and the week after.

For Location 3, the % reduction from the week prior to the week after freshwater treatment for attached, motile, Adult F and *C. elongatus* stages were 100%, 70.8%, 66.7% and 81.8% respectively (Figure 3-9).

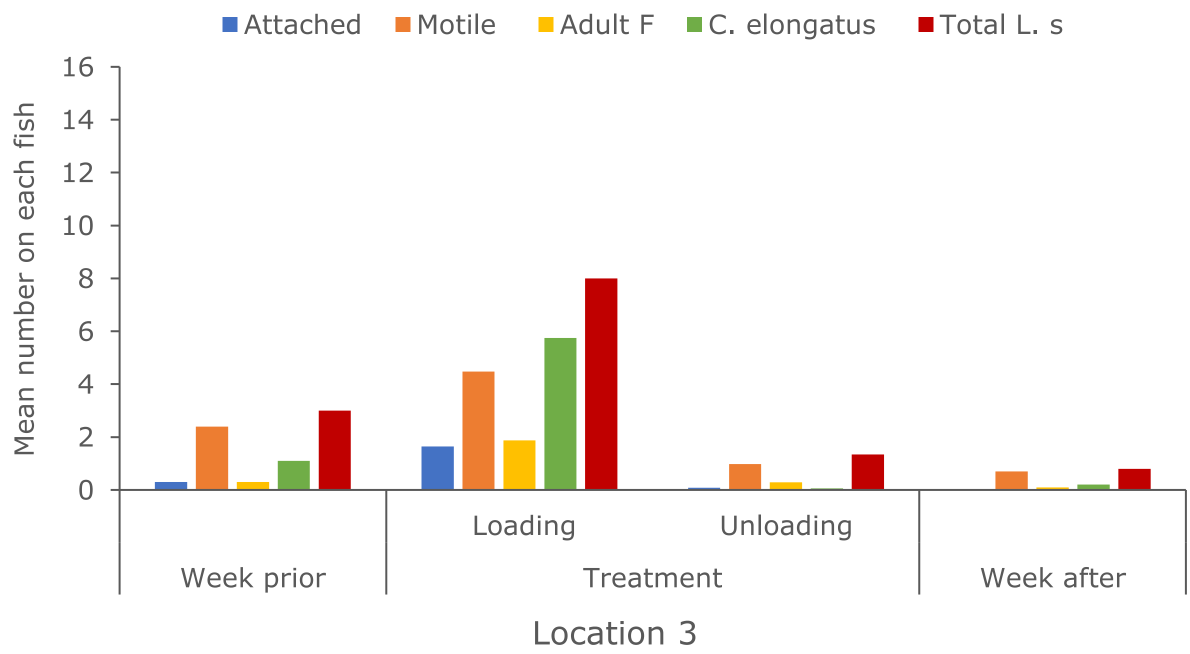


Figure 3-9: Mean lice numbers at Location 3 from the week prior treatment, during loading and unloading, and the week after.

3.4. CROWDING METHOD

The different crowding methods, swipe net and ball line, are plotted against the mean lice numbers of *L. salmonis* and *C. elongatus* from the week prior treatment (Figure 3-10 (a) and (b)). Three different crowding methods were used; ball line noted as K, two swipe net hauls noted as O2, and four swipe net hauls noted as O4.

The % increase in mean lice numbers of *L. salmonis* from the week prior to during crowding/loading were, 276 % for Treatment 1, 518 % for Treatment 2.1, 350 % for Treatment 2.2, 293 % for Treatment 3.1 and 240 % for Treatment 3.2. Treatment 1, 3.1 and 3.2 used method K for crowding the salmon, while Treatment 2.1 used O2 and Treatment 2.2 used O4 (Figure 3-10 (a)).

The % increase in mean lice numbers of *C. elongatus* from the week prior to during crowding/loading were, 176 % for Treatment 1, 153 % for Treatment 2.1, 467 % for Treatment 2.2, 569 % for Treatment 3.1 and 468 % for Treatment 3.2.

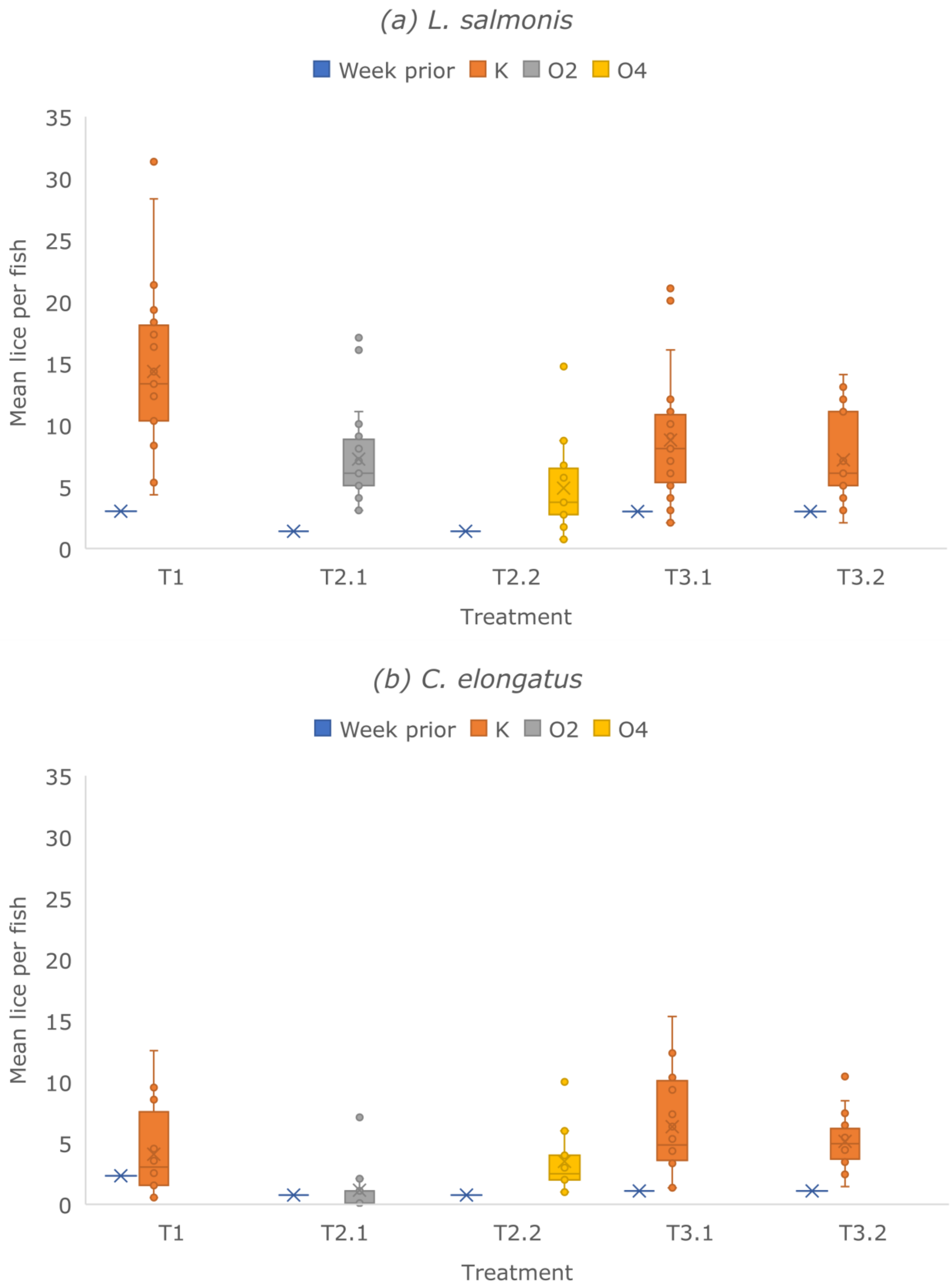


Figure 3-10: The number of L. salmonis (a) and C. elongatus (b) during the loading/crowding of the different Treatments; T1, T2.1, T2.2, T3.1 and T3.2.Treatment T1, T3.1 and T3.2 used crowding method 'K', while T2.1 method 2O and T2.2 method 4O. 4O, 2O and K are the different crowding methods, and stands for four purse seine, two purse seine' and float line, respectively. Week prior shows mean lice numbers the week prior to each treatment. Mean values are indicated with cross, error bars represent standard error, and outliers are included.

3.5. Measurements

The measurements of the weight and length that was conducted during the fieldwork are listed in Table 3-2.

The weight measured by the automatic fish counter onboard the ship was obtained together with water measurements for each treatment. These weights were generally in good agreement with the results from the kitchen weights, measurement performed manually. The fish counter - weights exceeded mean ± 1SE of the kitchen weight in all the 10 cases. They exceeded the mean ± 2SE (~95% CI) in 9 out of 10 cases. The differences were generally small, the fish counter is likely good enough method for weight measuring.

Table 3-2: Mean weight (kg) and length (mm) per fish measured by the fish counter vs. weight measured (kg) with kitchen weight. Standard deviation and standard error are listed.

Treatment	Fish counter	Kitchen weight	SD	SE	Length	SD	SE
1 PW	4.65	4.72	1.18	0.13	688	48.4	5.41
1 SbW	4.67	4.61	1.13	0.13	680	51.7	5.17
2.1 PW	2.92	3.10	0.67	0.07	602	37.4	3.95
2.1 SbW	3.61	2.82	0.58	0.06	586	37.9	3.99
2.2 PW	2.84	3.14	0.63	0.08	615	32.9	3.68
2.2 SbW	3.66	2.71	0.53	0.06	588	33.5	3.35
3.1 PW	2.91	2.54	0.71	0.08	582	43.6	4.88
3.1 SbW	2.87	2.49	0.63	0.06	573	41.0	4.10
3.2 PW	2.88	2.54	0.64	0.07	568	45.8	5.12
3.2 SbW	2.89	2.70	0.60	0.06	580	39.9	4.01

3.5.1. Biometry vs. lice numbers

The Spearman's correlation test was conducted on each counting to see if there was a correlation between the salmon's K-factor and the number of total lice *L. salmonis* and *C. elongatus* attached to the salmon and between maximum standard length (MSL) and the total number of lice.

The only results that did show a correlation were Treatment 3.1 for counting during crowding, it showed a moderate (.30<rho>.50), positive correlation between K-factor and number of *C. elongatus* (Appendix Table 0-10). Treatment 2.2 showed for MSL and the total number of *L. salmonis*, a moderate (.30<rho>.50), positive correlation (Appendix Table 0.-11).

All counting's that did not show a significant (p<0.1)/negligible (.00<rho>.30) positive (negative) correlation for either K-factor or MSL are listed in Appendix (Appendix Table 0-8 to 0-12).

4. DISCUSSION

The aim of this study was to investigate what effect and efficiency the freshwater delousing method may have on *C. elongatus* and *L. salmonis*. The research was conducted by sampling the salmon weight, length and lice numbers during freshwater treatment. Data from well boats and the respective aquaculture sites was gathered and used as results.

4.1. THE EFFECT OF THE FRESHWATER DELOUSING METHOD

The first question in the present study addresses the development of sea lice on the salmon over the course of freshwater treatment. The total treatment effect showed a reduction in all stages of *L. salmonis* and *C. elongatus,* where the lowest reduction was 77.7% and the highest at 99.6% (Appendix Table 0-8). The stage that showed the lowest % reduction from the freshwater treatment was the adult male stage of *L. salmonis*, while *C. elongatus* had the highest % reduction after the treatment. A general pattern of a rapid decrease between the lice counting prior to delousing and to the 1st counting in the wells can be seen (Figure 3-1 to 3-5). This pattern of decrease can be seen for all the lice stages (Figure 3-6), the graph has the steepest % reduction between the lice counting prior to delousing and 1st counting in wells. Because of mechanical procedures during freshwater treatments as crowding, pumping and grading there could still be expected reductions in chalimus to the adult stages (Wright, Oppedal et al. 2016). The mechanical delousing effect from the freshwater treatment might be an explanation, aside from the effect of the freshwater, for the general pattern of a rapid decrease in all stages between the lice counting prior to delousing to the 1st counting in the wells. The detachment of the lice stages can influence the regulation of the osmolarity in the louse and therefore cause the louse to die from dilution (Wright, Oppedal et al. 2016).

L. salmonis

The mean of all five treatments showed a reduction effect on the total number of *L. salmonis*. At the unloading process, a total of 86.3% (78.8-88.9%) of the salmon lice was released from the salmon. The highest effect on all stages of *L. salmonis* was at Treatment 2.2, with 88.9%. The adult stages of L. salmonis can survive in low salinity waters with a host-dependent mechanism, where they take up ions from the host to replace those lost to the environment, this mechanism increases their tolerance towards freshwater (Hahnenkamp and Fyhn 1985, Johnson and Albright 1991). This is supported by the results, where the mean % reduction of adult male and female are low compared to the other stages, (Figure 3.6, Appendix Table 0-8).

Johnson and Albright (1991) state that the younger stages of *L. salmonis* start to die within 9 hours after being exposed to freshwater. This is evident from the case that the attached lice showed the highest mean % reduction of all *L. salmonis* stages (Figure 3-6). Wright, Oppedal et al. (2016) showed that the earliest attached (copepodid) stage of *L. salmonis* was highly susceptible to freshwater, with a mortality rate of 96-100% after only 1 hour of freshwater exposure. While a freshwater exposure up to 8 days had only a partial effect to the attached chalimus and pre-adults, and it did not affect the attached adult survival. Even though the attached stage had the highest mean % reduction of all the *L. salmonis* stages, it did not show a reduction as Wright, Oppedal et al. (2016) reported. The attached group in this study consists of both the copepodid stage and chalimus 1 and 2. The reason for the 'low' % reduction might be that the attached chalimus was borrowing ions and was burrowed into the mucosal layer on the host in order to reduce the surface area exposed (Hahnenkamp and Fyhn 1985, Sievers, Oppedal et al. 2019). Another reason might be that even though attached copepodids die quickly in freshwater, they remain attached to the hosts after death (Wright, Oppedal et al. 2016). Most eggs on the female adult *L. salmonis* will be aborted at 11.5 PSU and below (Johannessen 1977), and the results from all the five treatments showed that the reduction of egg strings took place already during the 1st and the 2nd counting in the wells (Figure 3-6).

When comparing the amount of the different lice stages of *L. salmonis* on each fish at each treatment, a clear difference in the lice numbers could be seen. At Location 1 (Figure 3-7), the younger stages of *L. salmonis* was the majority, while at Location 3 (Figure 3-9), the adult stages were the majority. Wootten, Smith (1982) state that a higher sea temperature will give *L. salmonis* a shorter generation time. The study of Tucker, Sommerville et al. (2000) found that <70% of *L. salmonis* had developed to chalimus 2 by 10 days post-hatching at 11-12 °C. The sea temperatures at Location 1, 2 and 3 was 13.3, 13.6 and 13.9 °C, respectively (Table 3-1). The sea temperature may explain the differences in the stages of L. salmonis between Location 1 and 3 and that there was a two-week difference in the countings, and thus resulting in that the younger stages, attached and pre-adult, at Location 1 are the same generation as the pre-adult and adult stages at Location 3.

C. elongatus

Andersen found that *C. elongatus* have a higher level of activity and survival at higher salinities and that copepodites dies immediately at and below 10 PSU (Andersen 2006). In all five treatments at the present study, the total number of *C. elongatus* showed an immediate strong reduction in numbers (Figure 3-1 to 3-5). This is also revealed from (Figure 3-6), where the mean % reduction of *C. elongatus* are high at the 2nd counting in the wells. Compared to the mean % reduction of total *L. salmonis* at the 1st counting in wells and after treatment, the reduction of *C. elongatus* was higher. The treatment that showed the highest % reduction of *C. elongatus* during the 1st counting was Treatment 2.1 with 95.5 % (89.6 – 95.5%). A suggestion for the difference in reduction can be that since *C. elongatus* can have up to 80 different hosts, it is not that dependent to stay attached on the salmon as *L. salmonis* is. Another suggestion is that *C. elongatus* are not that attached to the salmon as *L. salmonis* and will therefore easily detach during crowding. This can be supported by the mean numbers of *C. elongatus* during the crowding during the different treatments (Figure 3-10). The mean number of *C. elongatus* was a lot smaller than the numbers of *L. salmonis (*Appendix Table 0-2 to 0-6). Alternatively, *C. elongatus* may be more sensitive to exposure to low salinities.

Treatment time

The main treatment time, time from the last salmon are pumped into the wells and to the first salmon are pumped back into the pen, was 10 hours for each of the five treatments 1, 2.1, 2.2, 3.1 and 3.2. Since the well boats can only unload salmon from one well at a time, there will be one well, in this study, which is PW, that have a slightly longer time with salmon exposed to freshwater, (Appendix Table 0-1). Figure 3-1 to 3-5 also shows that after ~5 hours of exposure there was little change in the lice reduction, for both *L. salmonis* and *C. elongatus* in all five treatments*.* An ANOVA test showed that there was a significant ($p<0.05$) reduction in lice numbers between all five counting's (Figure 3-6). The significance of the reduction of lice numbers between the last two counting's varied between the five treatments. Treatment 1 and 2.1 showed no significant reduction, while Treatment 2.2, 3.1 and 3.2 showed a significant ($p<0.01$) reduction. The variation in the significance of the last two counting in all the five treatments shows that the treatment time after ~5 hours does not influence the total reduction effect as it might thought to be. When looking at the effect on the different stages there seems to be a higher reduction on the pre-adult, adult male and female of *L. salmonis* with longer treatment time (Appendix Table 0-8). The treatment time should therefore be decided by the industry, after the need of each location and after what lice stages that dominates in the pens at the location.

4.2. CROWDING

The second question addressed in this study was if the different crowding method used to collect the salmon in the pen before loading onto the well boat would have an impact on lice numbers before treatment start and on the total effect. Treatment 2.2 had the lowest total number of *L. salmonis* during crowding compared with the other treatments. The second-lowest numbers were found for Treatment 2.1 (Figure 3-10 (a)). Treatment 2.1 and 2.2 also had a smaller mean number of *C. elongatus* compared to the other treatments. This may suggest that the crowding method used at Location 2, have an impact on the total lice numbers during crowding. The crowding method used at Treatment 2.1 and 2.2 were the swipe net. Wright, Oppedal et al. (2016) reported that the freshwater delousing effect might be increased due to mechanical dislodgement during crowding, pumping and grading procedures. When a ball line is dragged under the pen, all the salmon are crowded in one operation. With a swipe net there it may be needed more than one haul to be able to collect all the salmon. The repeated use of swipe net in the pen will most likely have a mechanical dislodgement effect on the attached sea lice and therefore fewer sea lice will remain attached on the salmon before being pumped onboard the well boat. The lice that have been dislodged may stay in the water masses and reattach to the salmon after the treatment. If this was the case the lice numbers in the week after treatment would be higher at Location 2, compared to Location 1 and 3 (Figure 3-7 to 3-9). The location that had the highest lice numbers the week after treatment was Location 3 (Figure 3-9). Treatment 3.1 and 3.2 used the ball line to crow the salmon in the pen before treatment. Despite using the ball line Treatment 3.1 and 3.2 hade the lowest % reduction in total *L. salmonis* and *C. elongatus* compared to the other treatments (Appendix Table 0-7). Since Location 3 had a higher percentage of adult stages than the other locations during treatment, the lice numbers the week after may be explained by that.

Development of lice numbers after treatment

All the locations showed a reduction on different louse stages a week following freshwater treatment (Figure 3-7 to 3-9). In Location 1 and 2, *C. elongatus* had the lowest % reduction (Figure 3-7 and 3-8). One possible explanation for this can be that since *C. elongatus* have so many host species and can infect the salmon after treatment by transfer from other hosts species that are in the area around an aquaculture site. The motile *L. salmonis* have the highest numbers in the week after the treatments (Figure 3-7 to 3-9). Wright and Oppedal et al. (2016) suggest that pre-adult also may be accessing the host-dependent osmoregulatory mechanisms, and gain ions from feeding (Wright, Oppedal et al. 2016), while the report of Sievers and Oppedal et al. (2019) suggests that copepodids may also be accessing that method (Sievers, Oppedal et al. 2019). The adult female had a generally low presence the week after treatment at all three locations (Figure 3-7 to 3-9).

4.3. THE CORRELATION BETWEEN THE TOTAL NUMBER OF LICE AND K-FACTOR/MSL

The third question in this research was if there is a correlation between the condition factor, K, of the salmon and number of lice attached, and between the length of the salmon and number of lice attached. The correlation test for *L.* salmonis showed only two countings that had a moderate, 0.50<*rho*>0.70, positive correlation between the K-factor and the total number of *L. salmonis.* The maximum standard length (MSL) vs. *L. salmonis* showed more countings that had a moderate correlation than the K-factor. Not one of the countings that had a high correlation, 0.70<*rho*>1.00 (Appendix Table 0-11). Only one counting had a moderate, 0.50<rho>0.70, positive correlation between the K-factor and the total number of *C. elongatus (*Appendix Table 0-10)*.* Between the length and the total number of *C. elongatus* there was no correlation. Condition factors such as Fulton's, have been developed to provide standardised measures of fish health. Fulton's condition factor assumes that the heavier the fish are for a given weight the better the condition the fish has. However, the validity of Fulton's condition factor, K, have been questioned due to doubtful results (Morton and Routledge 2006). Factors that can have an impact on the results is the crowding method, the health of each salmon and the use of IPM's at the aquaculture site. The crowding method showed an effect on the number of lice that are attached to the salmon before a freshwater treatment, and therefore might impact the correlation. *Rho* and the p-value from the correlation at each treatment showed no results that would indicate an effect from the different crowding method (Appendix Table 0-9 to 0-12).

4.4. FUTURE WORK AND PERSPECTIVES

A lesson learned is that the countings in the wells should have been more distributed/spread evenly during the exposure time in each well. One error source for the lice countings are the lice that are counted in the tub at the end of each counting. Since the lice are distributed on all 20 fishes counted in one sample and therefore would be an error source in the distribution explanation of lice between the fishes.

The data obtained from the aquaculture sites should contain every single counting and not just the mean of each lice stage. This way there would be a way of normalising the lice numbers form the week prior treatment against the lice numbers in each crowding method. To get a better evaluation on the possible effect the different crowding methods might have on the lice numbers before treatment, countings before, and several times during crowding would give a better understanding of the effects.

To be able to see the long-term effect of the freshwater treatment have on the salmon louse there should be a cultivation project on egg strings that have been through a freshwater treatment to check the viability of the nauplii hatching from those egg strings.

Research in the aquaculture industry are constantly developing, and the amount done one sea lice are increasing for each year. The research on *C. elongatus* is limited, but it is increasing, and the research on *L. salmonis* is well documented (Groner, Rogers et al. 2016). For both species there are research gaps in the understanding of evolutionary processes and research is needed to understand the mechanisms and variation in freshwater tolerance fully. Since *L. salmonis* populations show considerable family-level genetic variation, with links to temperature and salinity tolerance (Ljungfeldt, Quintela et al. 2017) any commercial treatment should aim for a high reduction of the louse population as possible. Sievers, Oppedal et al. (2019) said that the freshwater treatment should be incorporated within a cyclical treatment regime whereby different treatment types are applied in succession so that the louse would not be able to develop resistance to any treatment. They also pointed out that proper louse control measures are critical to ensure continued efficacy of the treatment and for the protection of both cultured and wild fish (Sievers, Oppedal et al. 2019).

5. CONCLUSION

The results showed generally a high reduction in all stages of *L. salmonis* and for *C. elongatus.* Adult male of *L. salmonis* had the lowest reduction, 77.7% at the end of treatment, while *C. elongatus* had the highest reduction effect, 99.6%, from the treatment. The results showed a rapid decrease in lice numbers from the start of the delousing to the 1st counting in the wells. After ~5 hours little change in the lice reduction were shown for all stages of *L. salmonis* and *C. elongatus.* Mechanical procedures as crowding, pumping and grading may contribute to the rapid decrease in lice numbers from treatment start. The freshwater treatment showed to have a good % reduction effect on the pre-adult of *L. salmonis* and on *C. elongatus* inn all five treatments. The low % reduction effect on the adult stages (adult male and female) of *L. salmonis* can be explained by that they borrow ions from their host to replace those lost to the environment and therefore increase their tolerance toward freshwater. The 10-hour exposure time would therefore not be enough to have the same effect on the adult stages as it has on the younger ones. However, more research on the gaps in the understanding of evolutionary processes and research are needed to fully understand the mechanisms and variation in freshwater tolerance.

Crowding method showed an impact on the lice numbers during crowding. Repeated use of swipe net may cause a mechanical delousing effect (Wright, Oppedal et al. 2016) in the pen, and therefore treatments that used swipe net to crowd the salmon had a lower mean number of lice attached to the fish before being pumped onto the well boat.

The results showed no correlation between the K-factor of the salmon and the number of *L. salmonis* and *C. elongatus.* Correlation between the salmon's length and the number of *L. salmonis* and *C. elongatus* nor was found. The results can be concluded with that Fulton's condition factor is not a reliable method for estimating the parasite load on a salmon.

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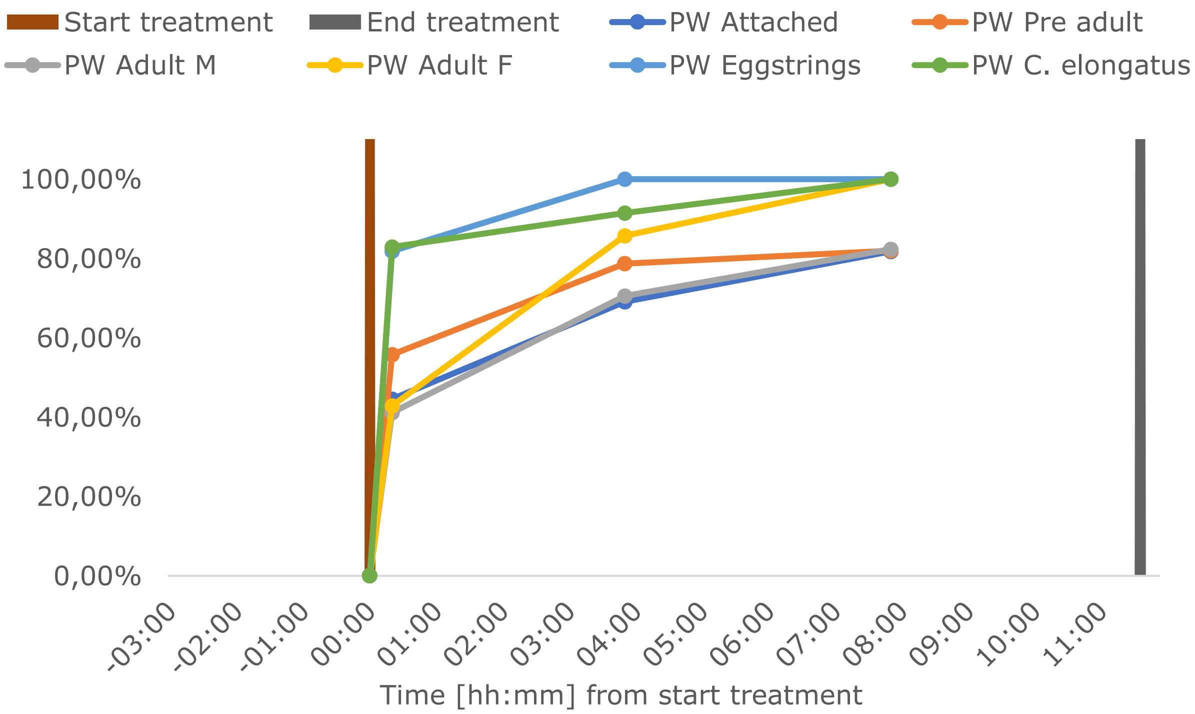
APPENDIX

1. TREATMENT TIME

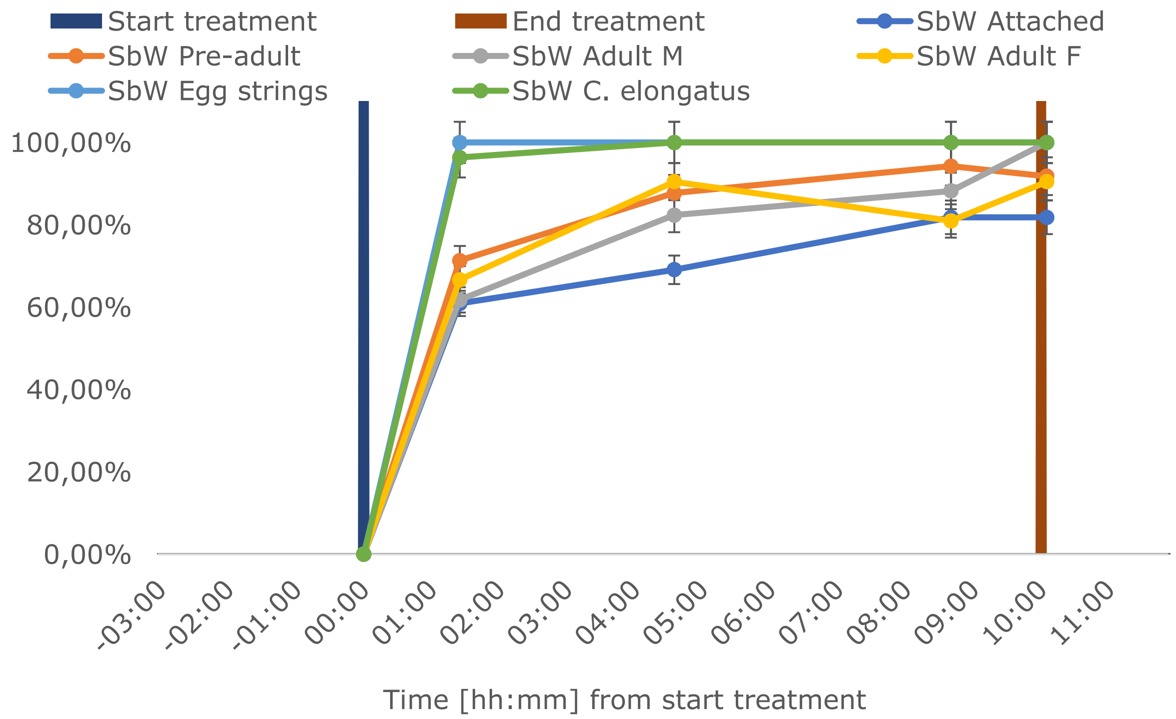
Table 0-1: Loading time, freshwater exposure time and unloading time for each well (SbW and PW) at each treatment.

	Loading time	Freshwater exposure time	Unloading time
T1 SbW	3 h	10 h	1 h 30 min
T1 PW	3 h	11 h 35 min	1 h 15 min
T2.1 SbW	2 h 17 min	10 h	1 h 25 min
T2.1 PW	2 h 17 min	12 h	1 h
T2.2 SbW	3 h 13 min	10 h	1 h 25 min
T2.2 PW	3 h 13 min	12 h	1 h 20 min
T3.1 SbW	1 h 15 min	10 h	50 min
T3.1 PW	1 h 15 min	11 h 13 min	1 h 11 min
T3.2 SbW	1 h 16 min	10 h	1 h 20 min
T3.2 PW	1 h 16 min	11 h 26 min	1 h 25 min

2. Effect of treatment

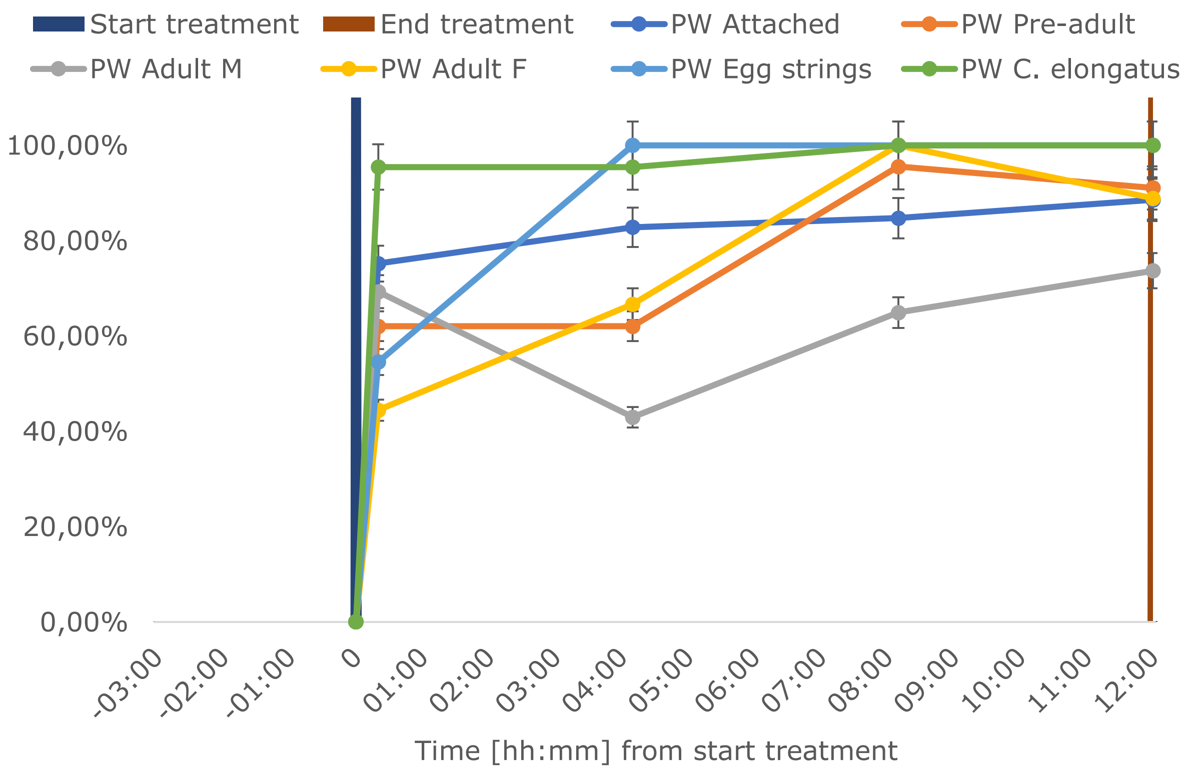


(a) 1 - PW

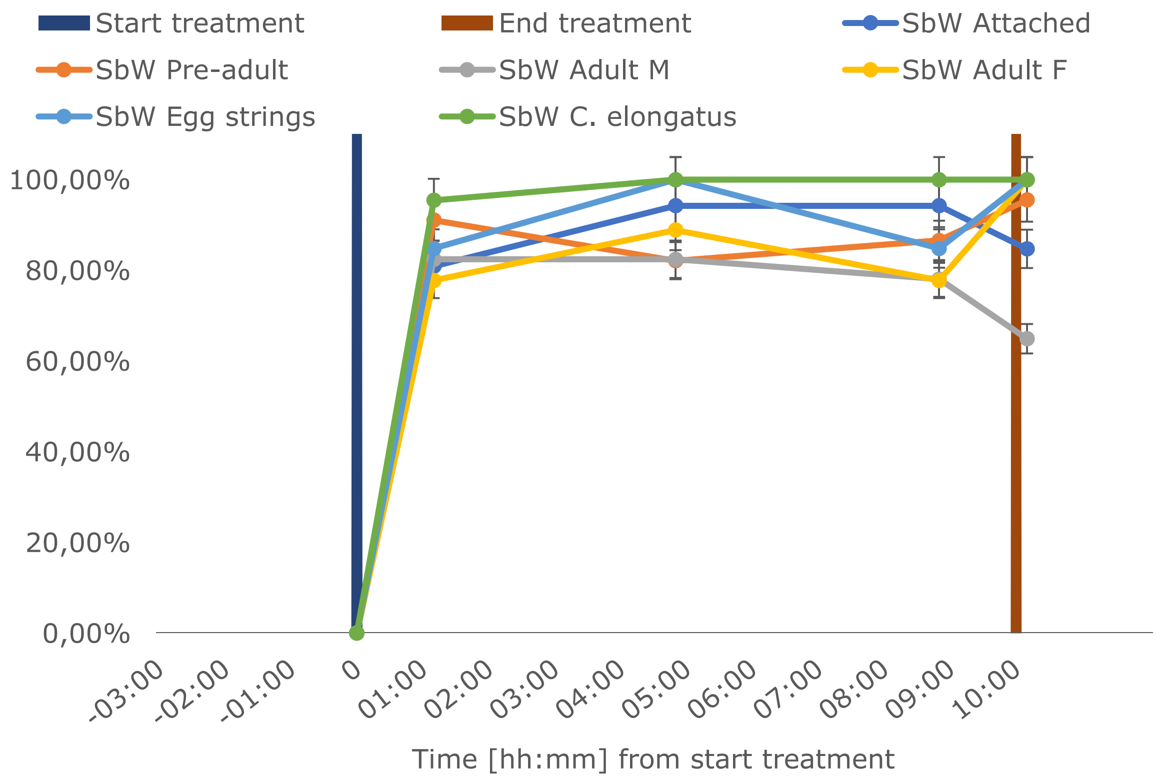


(b) 1 - SbW

Figure 0-1:The mean % reduction of L. salmonis stages attached, pre adult, adult M, adult F and egg strings, and C. elongatus during Treatment 1 in (a) PW and (b) SbW. The standard error is represented by the 1SE bars.

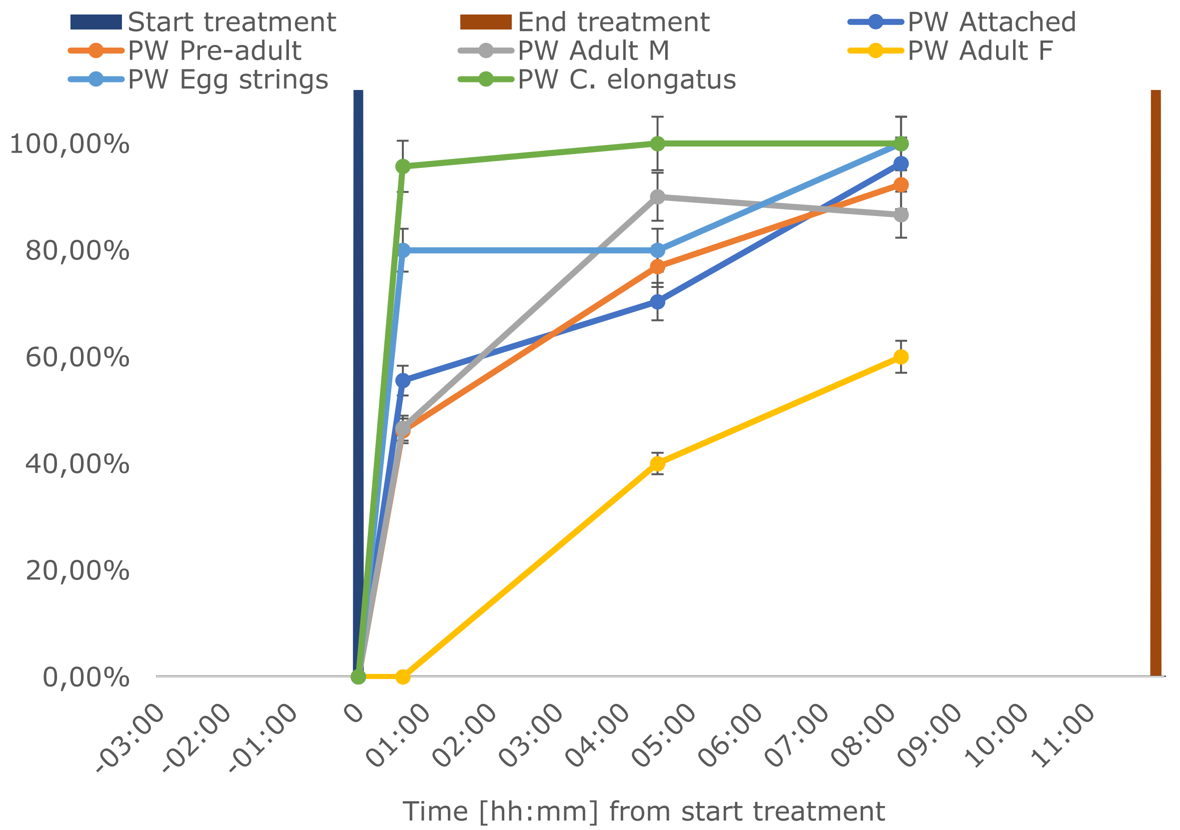


(a) 2.1 - Pw

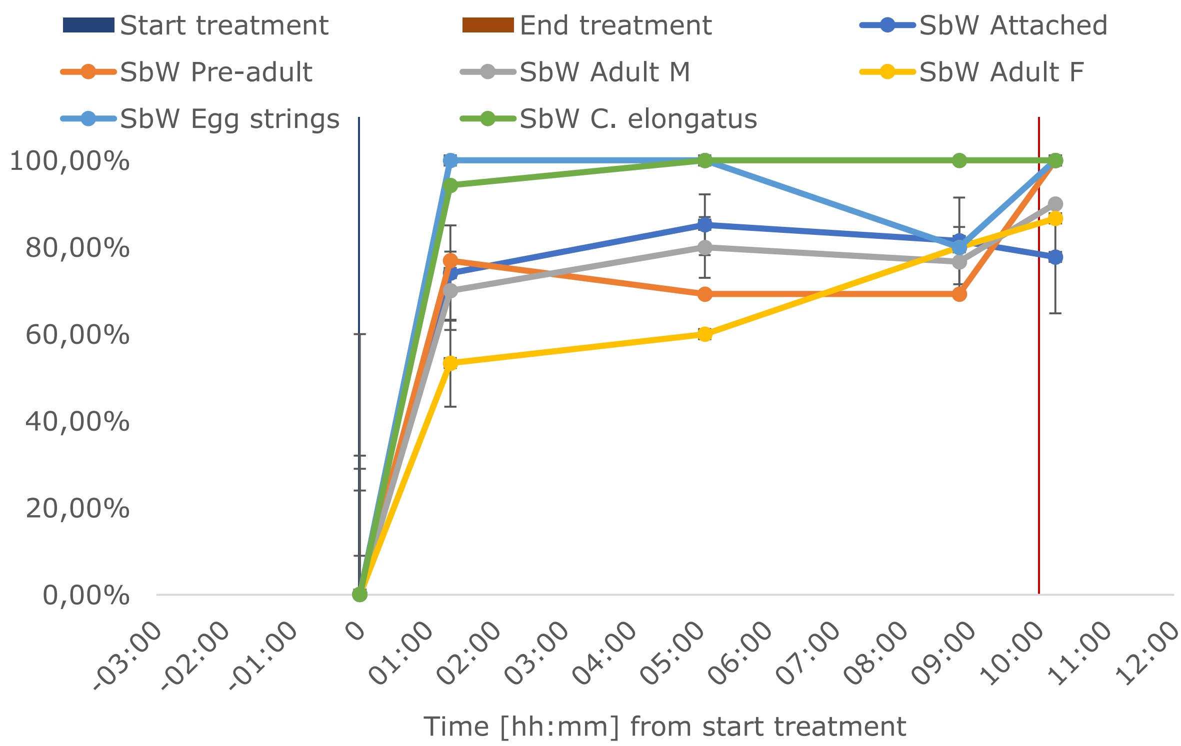


(b) 2.1 - SBw

Figure 0-2: The mean % reduction of L. salmonis stages attached, pre adult, adult M, adult F and egg strings, and C. elongatus during Treatment 2.1 in (a) PW and (b) SbW. The standard error is represented by the 1SE bars.

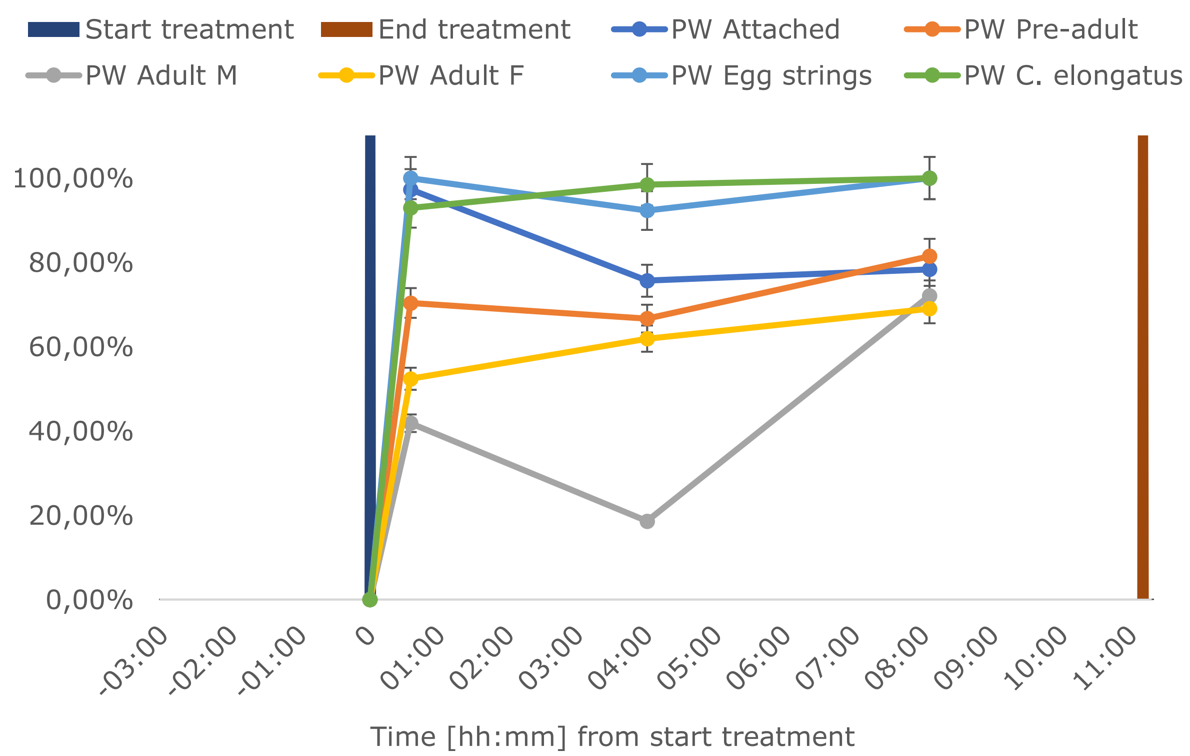


(a) 2.2 - PW

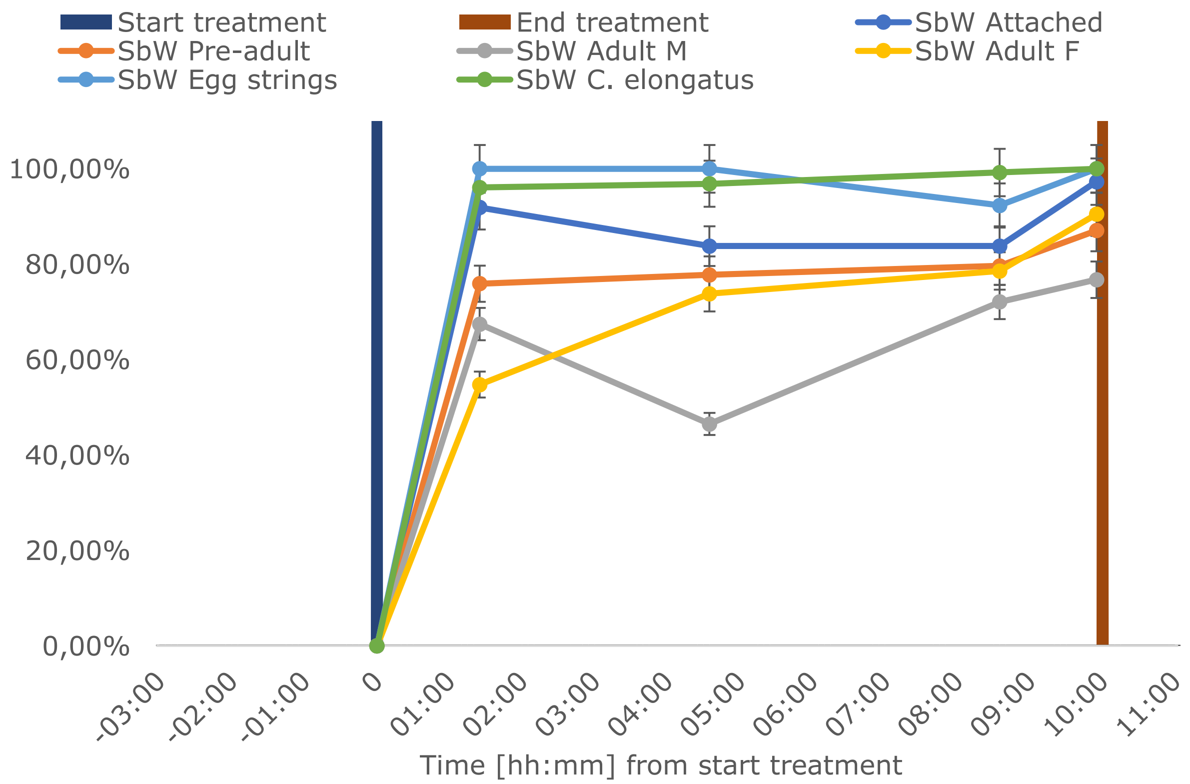


(b) 2.2 - SbW

Figure 0-3: The mean % reduction of L. salmonis stages attached, pre adult, adult M, adult F and egg strings, and C. elongatus during Treatment 2.2 in (a) PW and (b) SbW. The standard error is represented by the 1SE bars.

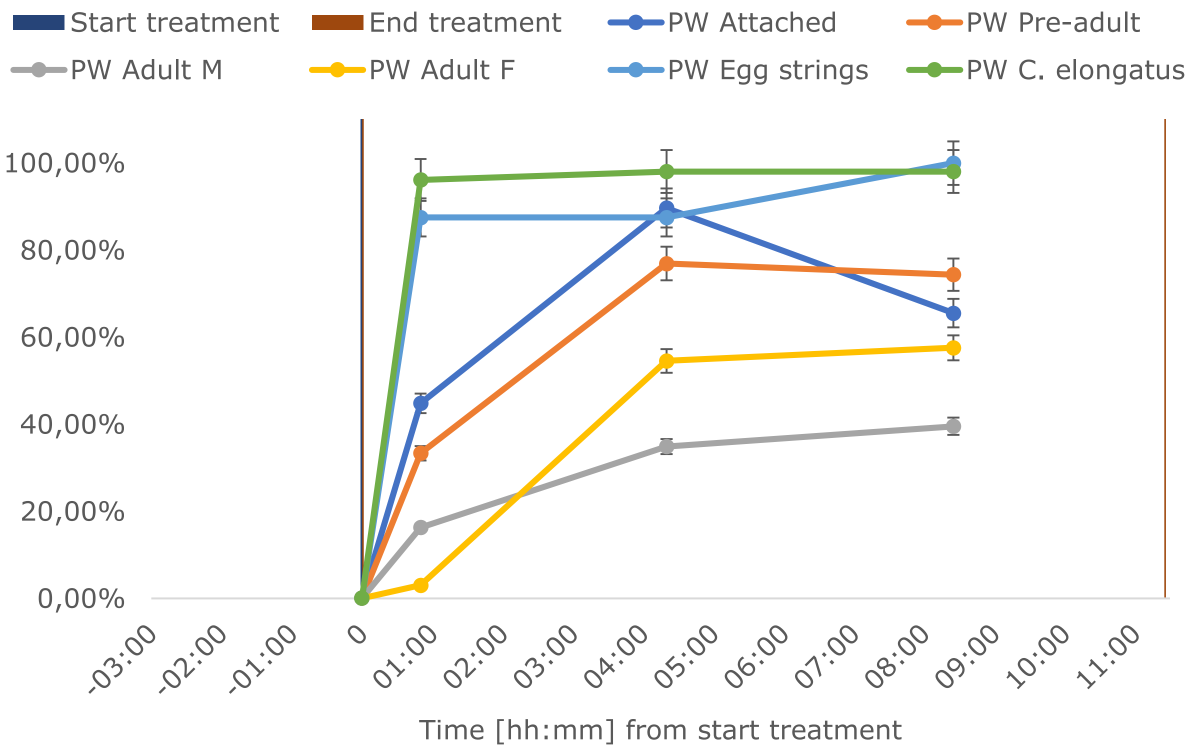


(a) 3.1 - PW

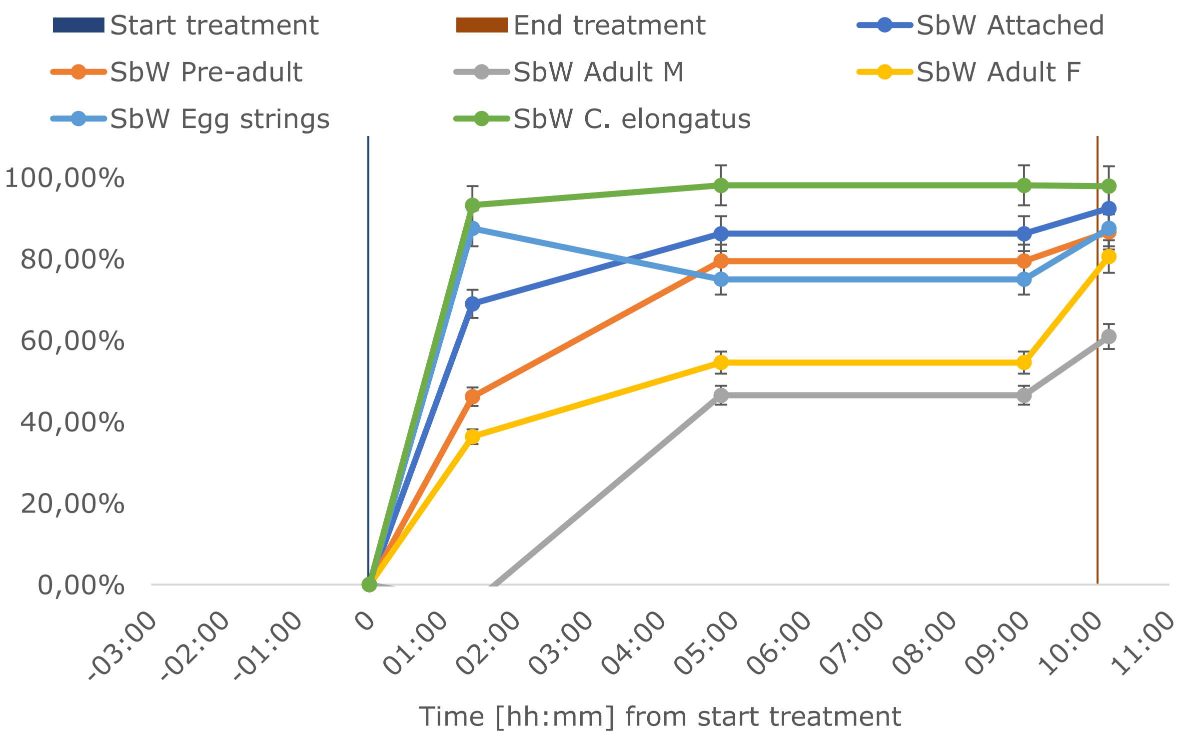


(b) 3.1 - SbW

Figure 0-4: The mean % reduction of L. salmonis stages attached, pre adult, adult M, adult F and egg strings, and C. elongatus during Treatment 3.1 in (a) PW and (b) SbW. The standard error is represented by the 1SE bars.



(a) 3.2 - PW



(b) 3.2 - SbW

Figure 0-5: The mean % reduction of L. salmonis stages attached, pre adult, adult M, adult F and egg strings, and C. elongatus during Treatment 3.2 in (a) PW and (b) SbW. The standard error is represented by the 1SE bars.

3. Mean lice Numbers during treatments

Table 0-2: Mean values of lice per salmon at each well, PW and SbW, at Treatment 1 at the different counting's

Treatment 1 PW	-02:50 Crowding	00:20 1st PW	03:50 2nd PW	07:50 3rd PW
Attached	5.5	3.05	1.7	1
Pre adult	6.1	2.7	1.3	1.1
Adult M	1.7	1	0.5	0.3
Adult F	1.05	0.6	0.15	0
Egg strings	0.55	0.1	0	0
C. elongatus	4.1	0.7	0.35	0

Treatment 1 SbW	-02:50 Crowding	01:25 1st SbW	04:35 2nd SbW	08:40 3rd SbW	10:05 Unloading
Attached	5.5	2.15	1.7	1	1
Pre adult	6.1	1.75	0.75	0.35	0.5
Adult M	1.7	0.65	0.3	0.2	0
Adult F	1.05	0.35	0.1	0.2	0.1
Egg strings	0.55	0	0	0	0
C. elongatus	4.1	0.15	0	0	0

Table 0-3: Mean values of lice per salmon at each well, PW and SbW, at Treatment 2.1 at the different counting's

Treatment 2.1 PW	-02:15 Crowding	00:20 1st PW	04:10 2nd PW	08:10 3rd PW	11:50 Unloading
Attached	2.62	0.65	0.45	0.35	0.4
Pre adult	2.24	0.85	0.85	0.15	0.1
Adult M	1.14	0.35	0.65	0.35	0.4
Adult F	0.90	0.5	0.3	0.05	0
Egg strings	0.33	0.15	0	0	0
C. elongatus	1.10	0.05	0.05	0	0

Treatment 2.1 SbW	-02:15 Crowding	01:10 1st SbW	04:50 2nd SbW	08:50 3rd SbW	10:10 Unloading
Attached	2.62	0.5	0.15	0.15	0.3
Pre adult	2.24	0.2	0.4	0.3	0.2
Adult M	1.14	0.2	0.2	0.25	0.3
Adult F	0.90	0.2	0.1	0.2	0.1
Egg strings	0.33	0.05	0	0.05	0
C. elongatus	1.10	0.05	0	0	0

Table 0-4: Mean values of lice per salmon at each well, PW and SbW, at Treatment 2.2 at the different counting's

Treatment 2.2 PW	**-02:20 Crowding**	**00:40 1st PW**	**04:30 2nd PW**	**08:10 3rd PW**
Attached	1.35	0.6	0.4	0.05
Pre adult	1.3	0.7	0.3	0.1
Adult M	1.5	0.8	0.15	0.2
Adult F	0.75	0.75	0.45	0.3
Egg strings	0.25	0.05	0.05	0
C. elongatus	3.5	0.15	0	0

Treatment 2.2 SbW	**-02:20 Crowding**	**01:20 1st SbW**	**05:05 2nd SbW**	**08:50 3rd SbW**	**10:15 Unloading**
Attached	1.35	0.35	0.20	0.25	0.3
Pre adult	1.3	0.30	0.40	0.40	0
Adult M	1.5	0.45	0.30	0.35	0.15
Adult F	0.75	0.35	0.30	0.15	0.1
Egg strings	0.25	0	0	0.05	0
C. elongatus	3.5	0.20	0	0	0

Table 0-5: Mean values of lice per salmon at each well, PW and SbW, at Treatment 3.1 at the different counting's

Treatment 3.1	**-01:00 Crowding**	**00:35 1st PW**	**04:00 2nd PW**	**08:05 3rd PW**
Attached	1.85	0.05	0.45	0.4
Pre adult	2.7	0.8	0.9	0.5
Adult M	2.15	1.25	1.75	0.6
Adult F	2.1	1	0.8	0.65
Egg strings	0.65	0	0.05	0
C. elongatus	6.35	0.45	0.1	0

Treatment 3.1	**-01:00 Crowding**	**01:25 1st SbW**	**04:35 2nd SbW**	**08:35 3rd SbW**	**09:55 Unloading**
Attached	1.85	0.15	0.3	0.3	0.05
Pre adult	2.7	0.65	0.6	0.55	0.35
Adult M	2.15	0.7	1.15	0.6	0.5
Adult F	2.1	0.95	0.55	0.45	0.2
Egg strings	0.65	0	0	0.05	0
C. elongatus	6.35	0.25	0.2	0.05	0

Table 0-6: Mean values of lice per salmon at each well, PW and SbW, at Treatment 3.2 at the different counting's

Treatment 3.2 PW	-01:05 Crowding	00:50 1st PW	04:20 2nd PW	08:25 3rd PW
Attached	1.45	0.8	0.15	0.5
Pre adult	1.95	1.3	0.45	0.5
Adult M	2.15	1.8	1.4	1.3
Adult F	1.65	1.6	0.75	0.7
Egg strings	0.4	0.05	0.05	0
C. elongatus	5.15	0.2	0.1	0.1

Treatment 3.2 SbW	-01:05 Crowding	01:25 1st SbW	04:50 2nd SbW	09:00 3rd SbW	10:10 Unloading
Attached	1.45	0.45	0.25	0.2	0.11
Pre adult	1.95	1.05	0.5	0.4	0.26
Adult M	2.15	2.25	1	1.15	0.84
Adult F	1.65	1.05	0.8	0.75	0.32
Egg strings	0.4	0.05	0	0.1	0.05
C. elongatus	5.15	0.35	0.2	0.1	0.11

4. Reduction (%) of lice for each Treatment

Table 0-7: Mean % reduction for C. elongatus and the attached, pre adult, adult M, adult F stage and egg strings for L. salmonis at each counting in each well during each treatment.

		Total *L. salmonis*	**Attached**	**Pre adult**	**Adult M**	**Adult F**	**Egg strings**	***C. elongatus***
Treatment 1 PW	Loading	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %
	1 PW	48.8 %	44.6 %	55.7 %	41.2 %	42.9 %	81.8 %	82.9 %
	2 PW	74.6 %	69.1 %	78.7 %	70.6 %	85.7 %	100 %	91.5 %
	3 PW	83.3 %	81.8 %	82.0 %	82.4 %	100 %	100 %	100 %
Treatment 1 SbW	Loading	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %
	1 SbW	65.9 %	60.9 %	71.3%	61.8 %	66.7 %	100 %	96.3 %
	2 SbW	80.1 %	69.1 %	87.7 %	82.4 %	90.5 %	100 %	100 %
	3 Sbw	87.8 %	81.8 %	94.3 %	88.2 %	81.0 %	100 %	100 %
	Unloading	88.9 %	81.8 %	91.8 %	100 %	90.5 %	100 %	100 %
Treatment 2.1 PW	Loading	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %
	1 PW	65.9 %	75.2 %	62.1 %	69.3 %	44.4 %	54.6 %	95.5 %
	2 PW	67.4 %	82.8 %	62.1 %	43.0 %	66.7 %	100 %	95.5 %
	3 PW	87.0 %	84.7 %	95.5 %	64.9 %	100 %	100 %	100 %
	Unloading	87.0 %	88.6 %	91.1 %	73.7 %	88.9 %	100 %	100 %
Treatment 2.1 SbW	Loading	0.00 %	0.00 %	0,00 %	0.00 %	0.00 %	0.00 %	0.00 %
	1 SbW	84.1 %	80.9 %	91.1 %	82.5 %	77.8 %	84.9 %	95.5 %
	2 SbW	87.7 %	94.3 %	82.1 %	82.5 %	88.9 %	100 %	100 %
	3 Sbw	87.0 %	94.3 %	86.6 %	78.1 %	77.8 %	84.9 %	100 %
	Unloading	87.0 %	84.7 %	95.5 %	64.9 %	100 %	100 %	100 %
Treatment 2.2 PW	Loading	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %
	1 PW	41.8 %	55.6 %	46.2 %	46.7 %	0.00 %	80.0 %	95.7 %
	2 PW	73.5 %	70.4 %	76.9 %	90.0 %	40.0 %	80.0 %	100 %
	3 PW	86.7 %	96.3 %	92.3 %	86.7 %	60.0 %	100 %	100 %
Treatment 2.2 SbW	Loading	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %
	1 SbW	70.4%	74.1 %	76.9 %	70.0 %	53.3 %	100 %	94.3 %
	2 SbW	75.5 %	85.2 %	69.3 %	80.0 %	60.0 %	100 %	100 %
	3 Sbw	76.5 %	81.5 %	69.3 %	76.7 %	80.0 %	80.0 %	100 %
	Unloading	88.8 %	77.8 %	100 %	90.0 %	86.7 %	100 %	100 %

		Total *L. salmonis*	**Attached**	**Pre adult**	**Adult M**	**Adult F**	**Egg strings**	***C. elongatus***
Treatment 3.1 PW	Loading	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %
	1 PW	64.8 %	97.3 %	70.4 %	41.9 %	52.4 %	100 %	92.9 %
	2 PW	55.7 %	75.7 %	66.7 %	18.6 %	61.9 %	92.3 %	98.4 %
	3 PW	75.6 %	78.4 %	81.5 %	72.1 %	69.1 %	100 %	100 %
Treatment 3.1 SbW	Loading	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %
	1 SbW	72.2 %	91.9 %	75.9 %	67.5 %	54.8 %	100 %	96.1 %
	2 SbW	70.5 %	83.8 %	77.8 %	46.5 %	73.8 %	100 %	96.8 %
	3 Sbw	78.4 %	83.8 %	79.6 %	72.1 %	78.6 %	92.3 %	99.2 %
	Unloading	87.5 %	97.3 %	87.1 %	76.7 %	90.5 %	100 %	100 %
Treatment 3.1 PW	Loading	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %
	1 PW	23.61 %	44.8 %	33.3 %	16.3 %	3.03 %	87.5 %	96.1 %
	2 PW	61.8 %	89.7 %	76.9 %	34.9 %	54.6 %	87.5 %	98.1 %
	3 PW	58.3 %	65.5 %	74.4 %	39.5 %	57.6 %	100 %	98.1 %
Treatment 3.1 SbW	Loading	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %	0.00 %
	1 SbW	33.3 %	69.0 %	46.2 %	-4.65 %	36.4 %	87.5 %	93.2 %
	2 SbW	64.6 %	82.8 %	74.4 %	53.5 %	51.5 %	100 %	94.2 %
	3 Sbw	65.3 %	86.2 %	79.5 %	46.5 %	54.6 %	75.0 %	98.1 %
	Unloading	78.8 %	92.4 %	86.7 %	60.9 %	80.6 %	87.5 %	97.9 %

5. Mean % reduction of lice

Table 0-8: Mean % reduction for C. elongatus and the attached, pre adult, adult M, adult F stage and egg strings for L. salmonis at each counting during each treatment.

Counting	Total *L. s*	Attached	Pre adult	Adult M	Adult F	Egg strings	*C. elongatus*	Treatment
Loading	00.0 %	00.0 %	00.0 %	00.0 %	00.0 %	00.0 %	00.0 %	T1
	00.0 %	00.0 %	00.0 %	00.0 %	00.0 %	00.0 %	00.0 %	T2.1
	00.0 %	00.0 %	00.0 %	00.0 %	00.0 %	00.0 %	00.0 %	T2.2
	00.0 %	00.0 %	00.0 %	00.0 %	00.0 %	00.0 %	00.0 %	T3.1
	00.0 %	00.0 %	00.0 %	00.0 %	00.0 %	00.0 %	00.0 %	T3.2
	00.0 %	00.0 %	00.0 %	00.0 %	00.0 %	00.0 %	00.0 %	Total
1st	57.3 %	52.7 %	63.5 %	51.5 %	54.8 %	90.9 %	89.6 %	T1
	75.0 %	78.1 %	76.6 %	75.9 %	61.1 %	69.7 %	95.5 %	T2.1
	56.1 %	64.8 %	61.5 %	58.3 %	26.7 %	90.0 %	95.0 %	T2.2
	68.0 %	73.1 %	54.7 %	53.6 %	100 %	94.5 %	94.5 %	T3.1
	28.5 %	39.7 %	58.0 %	19.7 %	87.5 %	94.7 %	94.7 %	T3.2
	57.0 %	69.4 %	62.9 %	49.2 %	43.2 %	87.6 %	93.9 %	Total
2nd	77.4 %	69.1 %	83.2 %	76.5 %	88.1 %	100 %	95.7 %	T1
	77.5 %	88.5 %	72.1 %	62.7 %	77.8 %	100 %	97.7 %	T2.1
	74.5 %	77.8 %	73.1 %	85.0 %	50.0 %	90.0 %	100 %	T2.2
	67.6 %	79.7 %	72.2 %	32.6 %	67.9 %	96.2 %	97.6 %	T3.1
	63.2 %	86.2 %	75.6 %	44.2 %	53.0 %	93.8 %	96.1 %	T3.2
	72.0 %	80.3 %	75.2 %	60.2 %	67.4 %	96.0 %	97.4 %	Total
3rd	85.5 %	81.8 %	88.1 %	85.3 %	90.5 %	100 %	100 %	T1
	87.0 %	89.5 %	91.1 %	71.5 %	88.9 %	92.4 %	100 %	T2.1
	81.6 %	88.9 %	80.8 %	81.7 %	70.0 %	90.0 %	100 %	T2.2
	77.0 %	81.1 %	80.6 %	72.1 %	73.8 %	96.2 %	99.6 %	T3.1
	61.8 %	75.9 %	76.9 %	43.0 %	56.1 %	87.5 %	98.1 %	T3.2
	78.6 %	83.4 %	83.5 %	70.7 %	75.8 %	93.2 %	99.5 %	Total
Unloading	88.9 %	81.8 %	91.8 %	100 %	90.5 %	100 %	100 %	T1
	87.0 %	86.6 %	93.3 %	69.3 %	94.4 %	100 %	100 %	T2.1
	88.8 %	77.8 %	100 %	90.0 %	86.7 %	100 %	100 %	T2.2
	87.5 %	97.3 %	87.0 %	76.7 %	90.5 %	100 %	100 %	T3.1
	78.8 %	92.4 %	86.7 %	60.9 %	80.6 %	87.5 %	97.9 %	T3.2
	86.3 %	87.1 %	92,0 %	77.7 %	89.5 %	97.9 %	99.6 %	Total

6. K-FACTOR VS. *L. SALMONIS*

Table 0-9: Results from the correlation test between K-factor and the number of L. salmonis at each counting in each well at each Treatment

Counting	**rho**	**S**	**P-value**
T1_1	-0.230811	1637	0.3276
T1_2PW	**0.530006**	**535.79**	**0.01959**
T1_2SbW	0.2893152	203.26	0.3617
T1_3PW	-0.01446625	1156.5	0.9531
T1_3SbW	-0.1984587	1594	0.4016
T1_4PW	0.1918662	1074.8	0.4177
T1_4SbW	-0.0317833	1372.3	0.8942
T1_5	Weight missing		
T2.1_1	-0.02091342	1357.8	0.9303
T2.1_2PW	-0.1619142	1545.3	0.4952
T2.1_2SbW	0.06638815	1241.7	0.7809
T2.1_3PW	0.1648988	1110.7	0.4872
T2.1_3SbW	0.01078828	1315.7	0.964
T2.1_4PW	-0.250745	1663.5	0.2863
T2.1_4SbW	0.221338	1035.6	0.3483
T2.1_5PW	-0.3820466	228.04	0.2759
T2.1_5SbW	0.05820252	155.4	0.8731
T2.2_1	0.02740914	1293.5	0.9087
T2.2_2PW	0.04696851	1267.5	0.8441
T2.2_2SbW	0.1847322	1084.3	0.4356
T2.2_3PW	-0.1206045	62.754	0.7967
T2.2_3SbW	Weight missing		
T2.2_4PW	0.1825417	1087.2	0.4411
T2.2_4SbW	0.3338775	885.94	0.1502
T2.2_5	-0.1705979	1556.9	0.4721
T3.1_1	0.07450841	1230.9	0.7549
T3.1_2PW	0.1232149	1166.1	0.6048
T3.1_2SbW	0.01191357	1314.2	0.9602
T3.1_3PW	**0.3832524**	**820.27**	**0.09532**
T3.1_3SbW	-0.3032055	1733.3	0.1938
T3.1_4PW	0.1308979	1155.9	0.5823
T3.1_4SbW	0.3003608	930.52	0.1982
T3.1_5	-0.0036374	1334.8	0.9879
T3.2_1	0.310061	917.62	0.1834
T3.2_2PW	-0.08907195	1448.5	0.7088
T3.2_2SbW	0.258479	986.22	0.2712
T3.2_3PW	0.2223096	1034.3	0.3462
T3.2_3SbW	0.1446315	1137.6	0.5429
T3.2_4PW	0.283605	952.81	0.2256
T3.2_4SbW	-0.244389	1655	0.2991
T3.2_5	0.239434	867.05	0.3235

7. K – FACTOR VS. *C. ELONGATUS*

Table 0-10: Results from the correlation test between the K-factor and number of C. elongatus at each counting in each well at each treatment. NA means that there was no C. elongatus

Counting	**rho**	**S**	**P-value**
T1_1	0.07125507	1235.2	0.7653
T1_1PW	-0.2585393	1434.7	0.2852
T1_2SbW	NA	NA	NA
T1_3PW	NA	NA	NA
T1_3SbW	NA	NA	NA
T1_5	NA	NA	NA
T2.1_1	-0.0008066967	1340.7	0.9731
T2.1_2PW	NA	NA	NA
T2.1_2SbW	NA	NA	NA
T2.1_5	NA	NA	NA
T2.2_1	-0.135467	1510.2	0.569
T2.2_2PW	NA	NA	NA
T2.2_2SbW	NA	NA	NA
T2.2_5	NA	NA	NA
T3.1_1	**0.5723735**	**568.74**	**0.008356**
T3.1_2PW	0.1581431	1119.7	0.5055
T3.1_2SbW	NA	NA	NA
T3.1_5	NA	NA	NA
T3.2_1	-0.1386581	1514.4	0.5599
T3.2_2PW	NA	NA	NA
T3.2_2SbW	NA	NA	NA
T3.2_5	NA	NA	NA

8. Length vs. *L. salmonis*

Table 0-11: Results from the correlation test between length of the salmon and number of L. salmonis at each counting in each well at each Treatment.

Counting	**rho**	**S**	**P-value**
T1_1	-0.1579559	1540.1	0.506
T1_2PW	0.1900892	1077.2	0.4221
T1_2SbW	0.1912174	1075.5	0.4191
T1_3PW	**0.3775089**	**827.91**	**0.1008**
T1_3SbW	0.1415581	1141.7	0.5516
T1_4PW	0.1182787	1172.7	0.6194
T1_4SbW	0.2421119	1008	0.3037
T1_5	-0.1529533	1533.4	0.5197
T2.1_1	0.193221	1073	0.4144
T2.1_2PW	-0.1804887	1570	0.4464
T2.1_2SbW	-0.07455893	1429.2	0.7547
T2.1_3PW	-0.02809684	1367.4	0.9064
T2.1_3SbW	-0.02363127	1361.4	0.9212
T2.1_4PW	0.1204601	1169.8	0.6129
T2.1_4SbW	-0.09326947	1454	0.6957
T2.1_5PW	0.4057513	98.051	0.2447
T2.1_5SbW	-0.05820252	174.6	0.8731
T2.2_1	0.3759611	829.97	0.1023
T2.2_2PW	-0.1911662	1584.3	0.4195
T2.2_2SbW	**0.3883129**	**813.54**	**0.09066**
T2.2_3PW	0.1166505	1174.9	0.6243
T2.2_3SbW	-0.2960511	1723.7	0.205
T2.2_4PW	**0.3909265**	**810.07**	**0.08833**
T2.2_4SbW	-0.05651975	1405.2	0.8129
T2.2_5	0.1598809	1117.4	0.5007
T3.1_1	0.1777612	1093.6	0.4534
T3.1_2PW	0.2307982	1023	0.3276
T3.1_2SbW	0.3697461	838.24	0.1086
T3.1_3PW	-0.1276894	1499.8	0.5916
T3.1_3SbW	0.03085415	1289	0.8973
T3.1_4PW	0.04402928	1271.4	0.8538
T3.1_4SbW	-0.2743137	1694.8	0.2418
T3.1_5	-0.3657808	1816.5	0.1127
T3.2_1	0.08066148	1222.7	0.7353
T3.2_2PW	**0.4629172**	**714.32**	**0.03984**
T3.2_2SbW	0.09625429	1202	0.6864
T3.2_3PW	0.2671863	974.64	0.2548
T3.2_3SbW	-0.008968438	1341.9	0.9701
T3.2_4PW	0.1908737	1076.1	0.4202
T3.2_4SbW	0.1378402	1146.7	0.5622
T3.2_5	-0.08171263	1233.2	0.7395

9. LENGTH VS. *C. ELONGATUS*

Table 0-12: Results from the correlation test between length of the salmon and number of C. elongatus at each counting in each well at each Treatment.

Counting	**rho**	**S**	**P-value**
T1_1	0.02498097	1296.8	0.9167
T1_2PW	NA	NA	NA
T1_2SbW	NA	NA	NA
T1_3PW	NA	NA	NA
T1_3SbW	NA	NA	NA
T1_5	NA	NA	NA
T2.1_1	0.02021316	1303.1	0.9326
T2.1_2PW	NA	NA	NA
T2.1_2SbW	NA	NA	NA
T2.1_5	NA	NA	NA
T2.2_1	0.1672027	1107.6	0.4811
T2.2_PW	NA	NA	NA
T2.2_2SbW	NA	NA	NA
T3.2_5	NA	NA	NA
T3.1_1	0.004189027	1324.4	0.986
T3.1_2PW	-0.2567245	1671.4	0.2746
T3.1_2SbW	NA	NA	NA
T3.1_5	NA	NA	NA
T3.2_1	-0.03259479	1373.4	0.8915
T3.2_2PW	-0.2567245	1671.4	0.2746
T3.2_2SbW	NA	NA	NA
T3.2_5	NA	NA	NA

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