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Fate of *Lepeophtheirus salmonis* and *Caligus elongatus* During Farmed Salmon Crowding

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Abstract

The spread and propagation of sea lice, including *Lepeophtheirus salmonis* and *Caligus elongatus*, have been a particular economic and ecological problem, jeopardizing the wild salmon population as well as the farmed salmon production. Crowding, increasing the fish density, is an important part of salmon handling and delousing management. Both marine biologists and fish-farmers question how stress response and mechanical impact from crowding affect lice detachment, eluding lice from delousing and spreading into free water masses. Therefore, it is of interest to investigate the fate of *Lepeophtheirus salmonis* and *Caligus elongatus* during farmed salmon crowding.

Atlantic salmon (*Salmo salar*) were sampled during a pre-crowding situation and during crowding at three fish farm locations in Northern Norway, October 2020. The pre-crowding situation registrations were done by the locations, as part of obligatory, weekly lice countings. The mean number of sessile *L. salmonis*, mobile *L. salmonis*, adult female *L. salmonis* and *C. elongatus* per fish for each net pen was registered in the pre-crowding situation. The salmon body was split into four zones, and the numbers of the different stages of *L. salmonis* and the numbers of *C. elongatus*, were registered for each body zone during the crowding registration.

The crowding operations did not affect the mean numbers of sessile and mobile *L. salmonis*, but the mean numbers of adult female *L. salmonis* and *C. elongatus* were significantly higher during crowding. This increase might be explained by adult female *L. salmonis* and *C. elongatus* attaching to the fish during crowding, development to other stages and/or natural variations in the lice distribution. The change from pre-crowding situation to during crowding in the mean number of sessile *L. salmonis*, mobile *L. salmonis* and *C. elongatus* per fish, showed a significant positive correlation to fish size. Adult female *L. salmonis* and fish size was not significantly correlated. The correlations might be influenced by location-specific factors e.g. temperature and number of days between the samples in addition to the fish's exposure time in the sea water.

Neither the number nor the placement of *L. salmonis* and *C. elongatus* were significantly different for the different numbers of crowding operations or the different crowding methods - swipe net and ball line. However, with the current study design, it is not known whether the lice stay on the host, or detach and reattach during the crowding. 0.28% of adult female *L. salmonis* were observed on the host's head (zone A) during crowding.

Sammendrag

Økt reproduksjon og spredning av *Lepeophtheirus salmonis* og *Caligus elongatus* hindrer videre vekst i oppdrettsnæringen og truer den naturlige bestanden av villaks. Trenging vil si å øke tettheten av fisken og er en viktig del av både avlusning og behandling av oppdrettslaks. I forbindelse med trengingen mistenker både marinbiologier og oppdrettsnæringen at lusa hopper/skubbes av fisken og dermed unngår avlusning og sprer seg videre i vannmassene til annen oppdrettslaks og villaks. Denne studien har derfor til hensikt å kartlegge *Lepeophtheirus salmonis* og *Caligus elongatus* under trenging av oppdrettslaks.

Prøver av atlantisk laks (*Salmo salar*) ble tatt før trenging og under trenging på tre ulike oppdrettslokasjon på Helgelandskysten, høsten 2020. Data for lusetall før trenging er hentet fra de ukentlige, obligatoriske lusetellingene som ble gjort av lokale røktere. Gjennomsnittlig antall fastsittende *L. salmonis*, bevegelige *L. salmonis*, voksen hunnlus *L. salmonis* og *C. elongatus* per fisk ble registrert i prøvene før trenging. Laksen ble delt inn i fire soner og antallet av de ulike stadiene av *L. salmonis* og antallet *C. elongatus* ble registrert for hver sone under trenging.

Trengeoperasjonene påvirket ikke gjennomsnittlig antall fastsittende og bevegelige *L. salmonis*, men antall *L. salmonis* voksen hunnlus og *C. elongatus* var i snitt signifikant høyere under trenging. Mulige forklaringer er at lusa fester seg til fisken under trenging, at lusa utvikler seg til andre stadier og/eller naturlige variasjoner i lusepopulasjonen. Endringen i gjennomsnittlig antall lus mellom før trenging og under trenging for fastsittende og bevegelige *L. salmonis* og *C. elongatus* viste en signifikant positiv korrelasjon til fiskestørrelse. *L. salmonis* voksen hunnlus og fiskestørrelse hadde ingen signifikant korrelasjon. Korrelasjonen kan være påvirket av lokasjonsspesfikke faktorer som temperatur og antall dager mellom prøvetakingene samt hvor lenge fisken har vært eksponert for sjøvann.

Antall og plassering av *L. salmonis* og *C. elongatus* var ikke signifikant forskjellig i de ulike antall trengingene og i de ulike trengetypene, avkastnot og kulerekke. Måten studien er gjennomført på gir likevel ikke informasjon om lusa hopper/skubbes av fisken og deretter hopper på igjen under trengingen. Det ble funnet 0,28% *L. salmonis* voksen hunnlus på vertens hode (sone A) under trenging.

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1 Introduction

1.1 Background

For over forty years, salmonid aquaculture has been a growing industry in Norway. Among the production countries, Norway is in the lead, producing more than half the farmed salmonids in the world. In 2020, the export value of Norwegian farmed salmon was 70 069 million NOK (Statistics Norway, 2021). In order to meet the growing population and the growing demand for food and fish, it is essential to keep the continued high growth rates in aquaculture (Jansen et al., 2012). However, the spread and propagation of sea lice, including *Lepeophtheirus salmonis* Krøyer 1837 and *Caligus elongatus* Nordmann 1832, represent economical and ecological problems in particular, jeopardizing the wild salmon population as well as the farmed salmon production (Jansen et al., 2012; Costello, 2009; Krkošek et al., 2005).

Sea lice refers to the parasitic copepods of the family Caligidae, and exist as a natural part of the brackish and marine environment (Boxaspen, 2006). The parasite has affected the farmed salmon industry since the beginning of 1970s, being responsible for disease outbreaks in the aquaculture industry (Boxaspen, 2006; Johnson et al., 2004). Both *L. salmonis* and *C. elongatus* are found in the northern hemisphere (Boxaspen, 2006) and feed on mucus, skin and blood, attached to the host (Pike and Wadsworth, 1999). This may cause serious damage as the feeding leads to an abrasion of the skin, making the fish vulnerable to bacterial and viral infections (Barker et al., 2019; Revie et al., 2009). As a result of infestations in farms, wild stocks have also shown to be negatively affected (Revie et al., 2009). Thus, a successful control of sea lice is a prerequisite to get a sustainable growth in the aquaculture industry.

1.2 Ecological and Economic Effects of Sea Lice

Sea lice have always been a natural component in the marine environment. The changed situation the recent decades, is the increased density of farmed fish, functioning as potential hosts. Frazer et al. (2012) pointed out how host density can influence parasite transmission; if there are more host individuals in the vicinity, a pathogen has a better chance to encounter a host. In this way, salmon farms consisting of high densities of fish at a limited space make perfect conditions for *L. salmonis* and *C. elongatus* (Torrissen et al., 2013).

An increase in sea lice abundance in and around fish farms is worrisome, as it elevates the risk of spreading to the wild populations of Atlantic salmon and sea trout. The possible consequences are increased lice infestations and increased stress levels, skin erosions and secondary infections, all of which reduce the condition of the host. A possible fate of the wild population is a slower host growth, increasing the risk to be captured by predators, and reduced swimming ability. The latter may be followed by several negative ecological consequences, including slower migration rates through coastal waters which might elevate the risk of infestation by sea lice copepodids, further increasing the effects (Revie et al., 2009).

As a response, the Norwegian fish farming industry and regulators have developed a National Action Plan against *L. salmonids* on salmonids. Norwegian fish farms are obligated to regularly report lice levels. If the number of lice exceeds 0.5 adult females or more than 3 mobile lice on average per fish, lice treatment is legally required (The Food Act, 2009). For both ecological and economic reasons, the fish farm industry aims to find the most optimal and effective lice treatment.

Growth reduction, low feed efficiency and increased mortality rate may be the consequences of increased sea lice infestation in salmon fish farms. Seen from an economic view, sea lice control has been essential both to avoid production losses and to secure the quality of marketable products, out of risk for having a lower marked price (Liu and Bjelland, 2014). Even though there are great costs associated with lice treatment, the alternative economic consequences of mortality and production loss due to infestation, would probably be greater (Bowers et al., 2000; Abolofia et al., 2017).

1.3 Biology of the Sea Lice

1.3.1 *L. salmonis*

Of the Caligidae family, *L. salmonis* has been the most increasing and serious problem in the salmonid industry, affecting the Atlantic salmon (Brandal and Egidius, 1979). The morphology of the development stages of *L. salmonis* has been characterized by, among others, Schram et al. (1993) and Johnson and Albright (1991b) (Figure 1.1). The life cycle of caligid copepods typically has five life phases, and includes eight stages; two free-swimming nauplius stages, one infective copepodid phase, two attached chalimus stages, two preadult stages and an adult phase (Hamre et al., 2013).

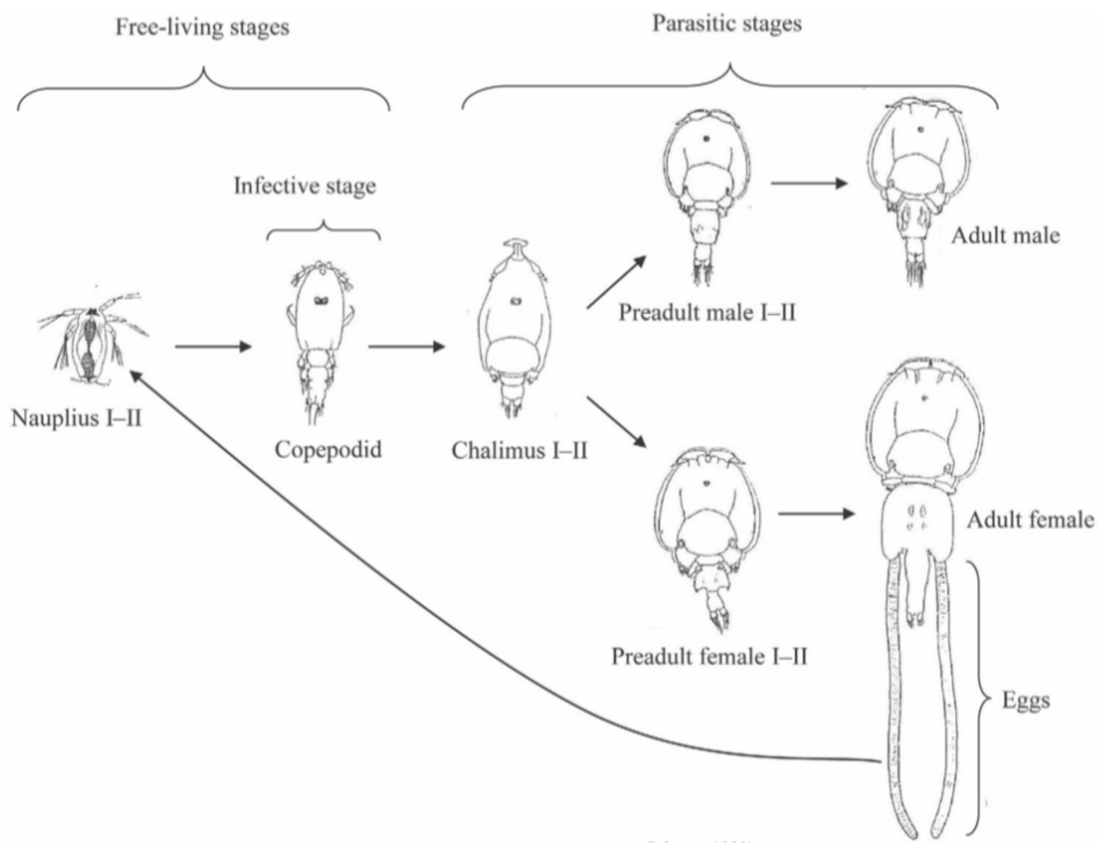

Figure 1.1: Developmental stages of *L. salmonis*. Including five phases; free swimming nauplius (two stages); free swimming copepodid (one stage); chalimus (two stages); pre-adult (two stages) and adult (one stage).

Source: Igboeli et al., 2014, modified from Schram et al., 1993.

1.3.2 *C. elongatus*

The life cycle of *C. elongatus* consist of two nauplius, one copepodid, four chalimus and one adult stage (Piasecki, 1996). The copepodid is infective, and together with the nauplius stage, free-swimming. The subsequent stages are parasitic and live on the fish. Similar to *L. salmonis*, *C. elongatus* causes skin lesions when abundant (Revie et al., 2002b), however, *C. elongatus* are golden-brown or yellow in appearance and females are notably smaller than *L. salmonis* females. The *C. elongatus* females are only slightly larger than the *C. elongatus* males (Piasecki, 1996). Unlike *L. salmonis*, *C. elongatus* are not host-specific, and has been reported from more than 80 species of marine fishes (Hogans, Trudeau et al., 1989).

1.3.3 Distribution on the Host Body

A study done on *L. salmonis* distribution on artificially infected sea trout showed that chalimus larvae preferred to stay on the fins and the gills, with the main preference for the dorsal fin (Bjørn and Finstad, 1998). Both pre-adult male and female preferred to settle on the head and dorsal areas. According to another study on wild salmon, adult males and females predominated along the posterior dorsal mid-line between the dorsal and caudal fins (Todd et al., 2000). Adult males and females were also found in the area around the anal fin and on the sides of the head, respectively. However, Bjørn and Finstad (1998) found that the distribution pattern of the lice changed gradually, from overdispersed at the chalimus stages towards more random patterns in the later stages. The reason for this may be active host rejection and/or the fish physically removing the lice by rubbing their bodies against the tank. While the chalimus larvae are attached to the host body until they reach the pre-adult stage, pre-adult and adult sea lice are mobile and have the ability to transfer positions.

C. elongatus are not known to be restricted to any specific body area of the fish (Hogans, Trudeau et al., 1989). Based on *C. elongatus* mobility and tendency to leave the host when it is disturbed, makes distribution data on *C. elongatus* particularly unreliable (Pike and Wadsworth, 1999).

1.3.4 Lice Number and Stress Levels

Speilberg et al. (2018) demonstrated handling and overcrowding to induce considerable stress and risk trauma to the fish. A study on the effect of handling and overcrowding, combined with a restricted access to the surface on the attachment of *L. salmonis* copepodids to Atlantic salmon, found that long-term overcrowding had no effect on *L. salmonis* attachment and did not influence the first stage of the infestation behaviour of the sea louse: recognition and attachment. However, the study found that handling procedures increase the susceptibility of Atlantic salmon to *L. salmonis*. The increase in plasma cortisol is suggested as a possible aetiology (Delfosse et al., 2020).

1.3.5 The Impact of Abiotic Factors on Sea Lice

The entire life cycles of sea lice are situated in marine environments which in the northern hemisphere involve salinity around 35‰ (Blindheim et al., 2000). However, adults of *L. salmonis* are found to live for 9.5 days at 10‰ salinity (Johnson and Albright, 1991a). The development and reproduction rate for *L. salmonis* is also found to be greatly influenced by seawater temperature (Hamre et al., 2019). The study found that the lice development increased in the temperature range from 6°C to 21°C. 21°C gave significantly faster growth rate, and female lice became adults at 13 days post infection (compared to 72 days at 6°C).

1.4 Delousing Management

As the amount of sea lice has been expanding both inside and outside the salmon farms, different methods to control the sea lice infestation have been developed. Chemotherapeutants, freshwater treatment, mechanical and thermal delousing systems, cleaner fish and lice skirts are the strategies used today.

1.4.1 Chemotherapeutants

There are five main chemotherapeutants used for salmon louse control by the industry (Overton et al., 2019). The chemotherapeutants are mainly used through bath treatments, or as in-feed additives (Burridge et al., 2010). One way of performing a bath treatment is by increasing the fish density by lining a sea-cage with a tarpaulin and reducing the water volume, then adding the chemotherapeutant to the cage (Volent et al., 2017). Another method is to increase the fish density by crowding and pumping the fish into a wellboat. The chemotherapeutants used for bath treatment are organophosphates, pyrethroids or hydrogen peroxide. The chemotherapeutant is added to the water and the salmon are held in the bath for the recommended treatment time. When the treatment is done, the tarpulin is removed or the fish are pumped out of the wellboat. The chemotherapeutant is then released into the surrounding water. In-feed additives are different chemotherapeutants and avermectins are used for these kinds of treatments. Due to increased drug resistance in the sea lice population, the drug consumption against lice has decreased after 2015 (Norwegian Food Safety Authority, 2018).

1.4.2 Freshwater Treatment

Freshwater treatment is an alternative to the chemotherapeutant bath treatment. The fish are crowded before they are pumped into a wellboat where both fish and lice are exposed to fresh water. The treatment time is usually 4-8 hours, and the fish are pumped back to the cage after treatment. Freshwater treatment disturbs the osmotic balance of the sea lice, which will paralyze and eventually kill the lice (Holan et al., 2017). A study showed that freshwater treatment is effective on copepodids, but pre-adult and mature females are more tolerant (Reynolds, 2013). This study reported a reduction in average number of chalimus, pre-adults and mature females to be 100%, 97% and 92% respectively, compared to the pre count. However, the reduction is not necessarily caused by the freshwater treatment only (Holan et al., 2017).

1.4.3 Thermal Delousing

Thermal delousing treatment involves exposing the fish to warm water at 28-34 °C in 20-30 seconds. The warm water inactivates the sea lice and removes their ability to stick to the fish skin surface. There are currently two commercial thermal delousing methods, Optilice (Roth, 2016) and Thermolicer (Grøntvedt et al., 2015). Thermolicer is based on a water circulation system where the fish is crowded and pumped into a processing loop. Within the system, the fish is briefly bathed in lukewarm seawater for 25-30 seconds. The sudden change in temperature causes the lice to fall of the fish. The lice is then collected and destroyed (Scale AQ, 2014).

The Optilice treatment system is based on an open tub system. The fish is pumped from the cage to the tub, and bathed in temperature-controlled water (up to 36 °C) in a controlled and time-adjusted treatment. After the treatment, the fish flows back to the cage and all sea lice are filtered out and destructed (Optimar, 2021). The thermal delousing methods are only effective on mobile lice, not chalimus. A delousing effect up to 94-98% on mobile and mature lice has been reported (Roth, 2016).

1.4.4 Mechanical Delousing Methods

Mechanical delousing is another non-drug delousing method used in the aquaculture industry today. Mechanical treatment technologies require the fish to be crowded and then pumped up into a treatment system, where the lice are mechanically removed from fish (Overton et al., 2019). There are three commercial mechanical delousing methods; Hydrolicer (Hydrolicer®), SkaMik (SkaMik As) and FLS delousing system (Flatsetsund Engineering AS).

Hydrolicer delousing technology pumps fish through the Hydrolicer unit at a controlled speed. The lice is detached by underpressure and turbulence followed by low pressure flushing. The specific water formation causes the sea lice to be "vacuumed" off the fish. The process happens in a closed water column at a controlled flow speed, and repeated in two Hydrolicer units in each transport line (Smir AS, 2019). A study on stress and welfare during delousing treatment by Erikson et al. (2018) reported that 66-82% of the lice were removed by the Hydrolicer delousing process. For this study, the delousing efficiency was 80%, 82% and 65% for chalimus, mobile and mature lice, respectively.

The SkaMik delousing system (SkaMik 1.5) involves four different treatments in different chambers. The treatment process starts with the fish being pumped into a delousing vessel before it is separated onto transport lines to the SkaMik 1.5 unit. Inside the unit, the fish goes through a drainage chamber, a flushing chamber, a brush chamber and at last a final flushing chamber. After the final flushing, the fish is returned to the cage. The process time inside the SkaMik 1.5 device is 1.5 second. The sea lice are collected in a filter system before they are destroyed and all process water is filtered (SkaMik as, 2020). The SkaMik-system producers have reported a 97.0% reduction of the total number of lice after SkaMik 1.5 treatment.

The FLS delousing system is based on a closed flushing system. The fish is pumped into the system via a funnel with two low pressure washers within. The lice are flushed/sprayed off as the fish passes through the pipe. After treatment, the water is filtered to prevent the lice being released into the surrounding waters (Overton et al., 2019). The total treatment time is approximately 10 second. The recommended fish size is up to 3.7 kg. The reduction effect is reported to be 81-100 % for mobile lice and 76-91% for mature lice (Gismervik et al., 2017). Even though the reduction efficiency on sea lice is high, the mechanical delousing methods are not free of problems. A report found that scale loss and skin bleeding are particularly associated with mechanical delousing using water jets (Hjeltnes et al., 2019).

1.4.5 Alternative Lice Control Strategies

Cleaner fish (e.g. *Labris bergylta* known as ballan wrasse and *Cyclopterus lumpus* known as lumpfish) are functioning as a biological control measure, and have since 2010 been the most widely adopted alternative pest control strategies (Brooker et al., 2018). A study indicated that the use of cleaner fish can delay the time it takes to reach 0.1 adult female lice per salmon in the beginning of a production cycle (Jevne and Reitan, 2019). The study also suggests that the timing of cleaner fish deployment into salmon cages is important, as this can influence its effectiveness in controlling salmon lice. In 2019, over 61 million cleaner fish individuals were deployed into Norwegian salmon cages (Directorate of Fisheries, 2020a). Even though cleaner fish is considered an important tool in the sea lice management, a report from the Norwegian

Food Safety Authority (2019) shows that the aquaculture industry does not satisfy cleaner fish welfare criteria, and the cleaner fish mortality is high. In order to continue the use of cleaner fish in the aquaculture industry, a significant improvement in cleaner fish health and welfare must be documented. As an alternative to cleaner fish, underwater lasers that shoot lice off the fish (Optical Delousing™ and Stingray Marine Solutions AS) have been introduced. However, the laser delousing method is still under development and Bui et al. (2020a) reported a non-existing difference in lice abundance on fish in cages with lasers present compared to cages without lasers.

Shielding skirts around the sea cages are also an alternative sea lice control strategy used today. The skirts are placed around the first few meters below the water surface, where salmon lice larvae are most prevalent (Heuch et al., 1995). In this way, the upper part of the ocean water column (with salmon lice) is prevented from interacting with the water inside the fish cages. The control strategy has not been optimal, however. The reduced water exchange might have a negative impact on the water quality and oxygen levels within the cage (Stien et al., 2012).

1.5 Crowding as Part of Salmon Handling

Slaughter, sorting (fish are transferred to different net pens according to size), splitting (fish are transferred out of the net pen if the biomass exceeds a certain size) and delousing involve moving the salmon out of the net pen. This is done by pumping the fish through one or more pipes connected to a wellboat or a treatment boat. In order to load the fish to the boat, the pipes must get access to all the fish in the net pen. At this point, crowding is involved.

Crowding is the process of increasing the fish density by forcing it tighter together using a net and/or floating elements. The main purpose of crowding is to increase the fish density and to "force" the fish closer to the pipes. In this way, the fish are easily accessed and may be loaded onto the the wellboat or treatment vessel. The process is usually conducted by first raising the net wall and bottom in gradual steps, then applying smaller crowding nets to facilitate the final increase in fish density (Føre et al., 2018).

Crowding is an essential part of the farmed fish industry, and has to be managed with carefulness. Speilberg et al. (2018) showed that salmon crowding initiates a stress response in the fish. The intensity of the crowding seems to be proportional to the amount of initiated stress. The fish welfare might also be affected by the reduction of the cage volume, increasing the chance of fish colliding/hitting other fish or cage components (Føre et al., 2018). As a result of the fish stress response to crowding, following is stated in The Aquaculture Act (2008): *"Handling, including vaccinating, crowding, hauling and pumping, should be carried out carefully in a justifiable pace, in order to avoid harm and unnecessary stress"*.

The Norwegian Regulation is purposed to maintain fish welfare and makes the baseline for how fish farm operations are done in practice. In order to lower the stress levels of the fish, several crowding operations in a row is conducted. In this way, the fish are exposed to crowding in a shorter period of time, and avoid being crowded more than once. The fish farmers follow the same principles and regulations for crowding operations, but the number of operations, duration and type of method varies and depend upon fish health and amount of fish in the net pen (pers. comm., E. Grøntvedt, 12.4.2021). It is also questioned by marine biology scientists and the fish farmers (pers. comm., E. Grøntvedt, 12.4.2021) if the stress response and/or mechanical impact caused by the crowding, affect the lice detachment, making more lice elude the delousing process and spread in the free water masses.

1.6 Aims of this study

The primary aim was to investigate the fate of *L. salmonis* and *C. elongatus* during farmed salmon crowding. The study was based on three research questions:

i) How does the crowding operations affect the number of lice compared to a pre-crowding situation?

ii) What effect does the size of the fish have on the number of lice in a pre-crowding situation compared to during crowding?

iii) How does the different crowding methods affect lice detachment and placement on fish body?

2 Materials and Method

2.1 Study Site

Fieldwork was carried out in autumn 2020 between week 41 and 43, at three different Atlantic salmon (*Salmo salar*) farm locations at Helgelandskysten in Northern Norway (Figure 2.1). For anonymity, the data collection sites were denoted as Location A, B and C. The data was collected within the production area 8 called "Helgeland to Bodø" shown in blue.

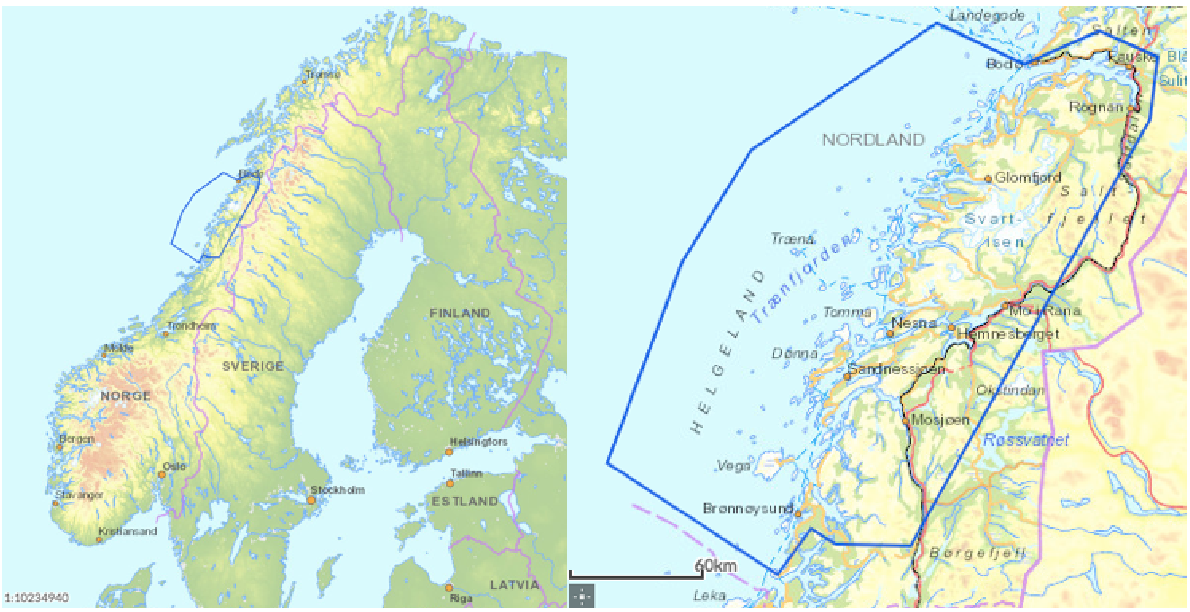

Figure 2.1: Production area 8, Helgelandskysten to Bodø, outlined with blue boarder (Directorate of Fisheries, 2020b).

2.2 Net Pen Arrangement

Fish were sampled during a pre-crowding situation and during crowding. A pre-crowding situating is characterized by a maximum net pen volume and a normal swimming behaviour and fish density, e.g. no crowding (Figure 2.2 A). Bottom weights were distributed along the bottom circumference of the net. Maximum 24 hours before crowding operations, the net was raised towards the water surface, decreasing the net pen volume. This operation is called *lining up the net pen*. The net closest to the pipes loading the fish were not fully raised. In this way, a "pocket" of fish in the loading area is made (Figure 2.2 B and C). By raising more of the net, the size of the pocket was decreased before crowding operation with ball line. The lining up process must be performed properly to avoid pockets or shallow areas where the fish might be stuck (Norwegian Food Safety Authority, 2014).

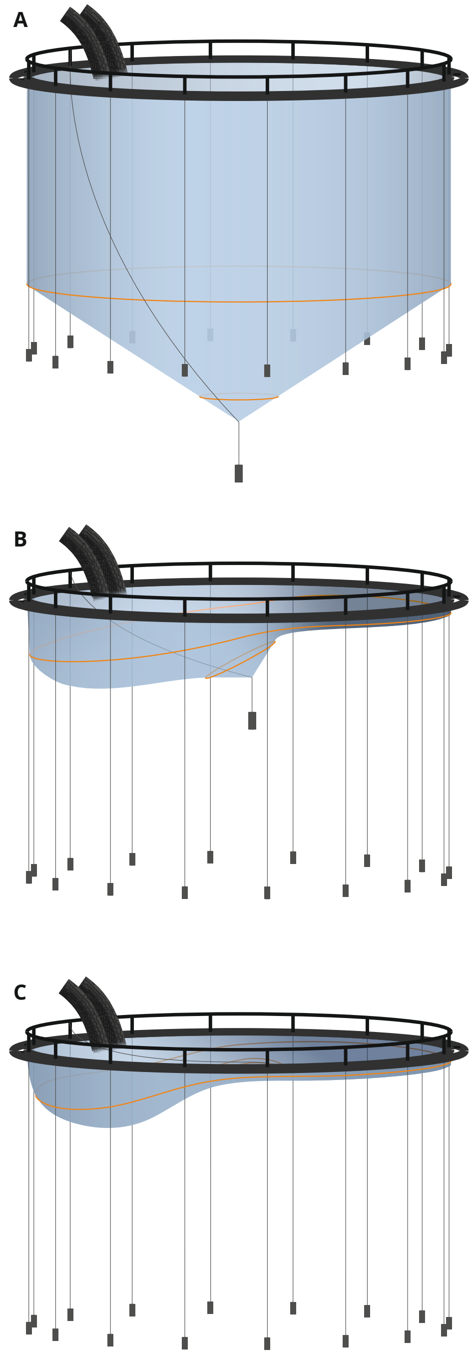

Figure 2.2: Lining up the net pen. **A**, net is not lined up (pre-crowding situation); **B**, net is lined up (during swipe net crowding). By not fully raising the net associated with 8-10 bottom weights, a pocked is made; **C**, net is lined up (during ball line crowding). By not fully raising the net associated with 6-8 bottom weights, a (smaller) pocked is made.

2.3 Crowding Operations

The main purpose of crowding is to increase the fish density in order for the pipes to load the fish out of the pen. There are two crowding methods used in aquaculture today:

1) crowding by using a swipe net
2) crowding by using a ball line

A more detailed description of the two methods is given in Chapter 2.3.1 and 2.3.2. The additional components involved in crowding of the fish are a wellboat with pipes, winches and service vessels (Figure 2.3). During crowding, the function of the wellboat is to transport the fish from the net pen into wells, using one ore more pipes placed 1-1.5 m below the water surface in the net pen (pers. comm., E. Grøntvedt, 12.4.2021). For the current study, the number of pipes were six. The pipes are adjusted during crowding according to the fish location in the water masses. The function of the crane and winches are to adjust the tension of the ropes connected to the swipe net or ball line. By pulling the swipe net/ball line, the fish density increases, and the fish are crowded towards the pipes. The main function of the service vessel is to monitor the crowding and pull back the swipe net or ball line when the crowding is finished, or needs to be stopped immediately (e.g. strong currents, too low oxygen levels, high levels of reduces fish welfare etc.). The swipe net or ball line is withdrawn by pulling the center rope (Figure 2.3).

To load all the fish with minimal stress during ordinary fish farm handling, 1-3 crowding operations with swipe net are performed before one last crowding operation with ball line (pers. comm., E. Grøntvedt, 12.4.2021). A crowding operation begins when the swipe net/ball line is placed in the water and the pipes are loading fish, and ends when the swipe net is pulled back or all the fish is loaded. The number of crowding operations depends on the biomass. Only one crowding operation with ball line is performed if the biomass is small and the loading (and crowding) can be performed within 1.5 hours. If the biomass and loading time exceeds this time limit, more operations are performed. An individual fish is not crowded more than once. For the delousing and crowding operations investigated in this study, the fish were starved for 72 hours prior to the Hydrolicer treatment to avoid additional stress during the delousing and crowding operations.

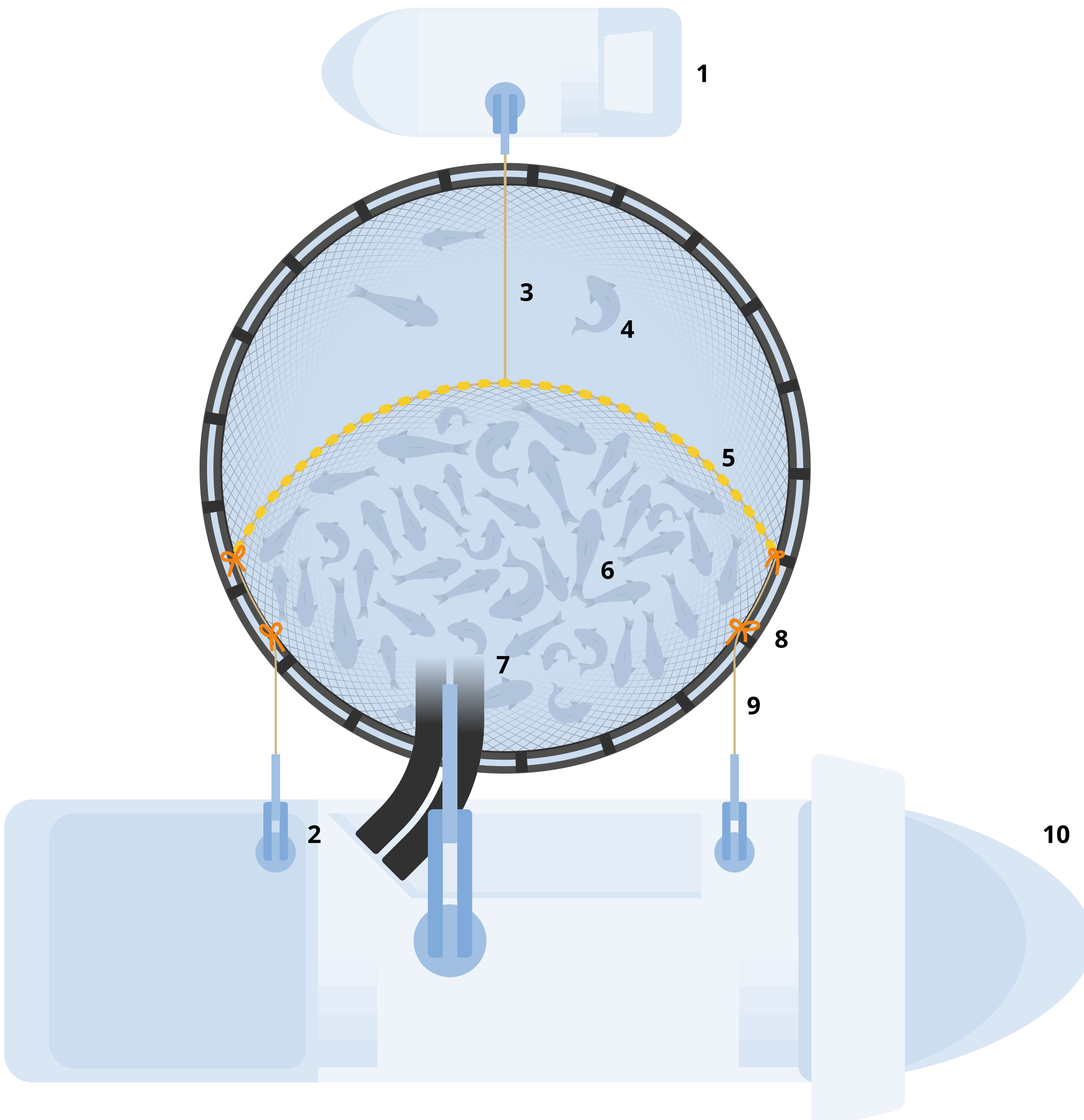

Figure 2.3: Overview of a crowding operation with swipe net. **1**, service vessel; **2**, crane with winch; **3**, center rope; **4**, not crowded fish; **5**, swipe net; **6**, crowded fish; **7**, pipes; **8**, tie point; **9,** rope; **10**, wellboat/treatment vessel.

2.3.1 Crowding Operation Using a Swipe Net

Different sizes of swipe nets are used according to the depth and size of the net pen. The swipe net (Figure 2.4) is designed to be wider than the net pen diameter and to have approximately the same depth as the net pen when it is lined up. In this way, the fish is "trapped" between the swipe net and the loading pipes. The swipe net is forced to unfold vertically in the water masses by a line of floating elements (netbuoys) attached to the top and heavy lead ropes attached to each side. Prior to the crowding, the swipe net is placed in the water inside the net-pen-end opposite to the pipes (Figure 2.5).

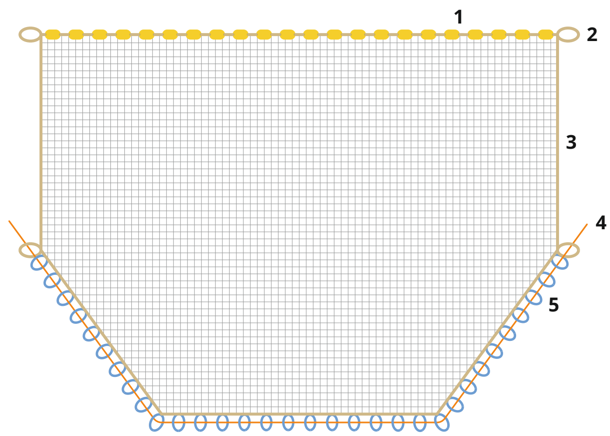

Figure 2.4: Swipe net. **1**, netbuoys; **2**, lifting eye; **3** lead rope; **4**, steel rings; **5**, purse rope (Illustration based on Mørenot Aquaculture AS, 2021).

Figure 2.5: Crane on service vessel places the swipe net in the water.

After the swipe net is unfolded along with the net pen inside, the purse rope in the bottom of the swipe net is pulled in each end, making a U-shape both horizontally and vertically, trapping the fish additionally. The swipe net is then pulled carefully along the inside of the net pen using a rope attached to the lifting eyes. At the beginning of the swipe net crowding, this rope is tied to the cage fence with smaller ropes at several points (Figure 2.6). As the swipe net is pulled closer to the pipes, the tie points are cut. In this way, the swipe net is kept as close to the net pen inside as possible.

When the swipe net reaches the pipe end of the net pen, the crane at the wellboat/treatment vessel is used to pull the swipe net vertically in the air (Figure 2.7), decreasing the net pen volume additionally. The swipe net is used until there is one load left. One load is defined as the amount of fish emptying the net in maximum 1.5-2 hours (pers. comm., E. Grøntvedt, 12.4.2021). In this study, crowding operation with swipe net was performed 1-3 times for each net pen (see Table 2.1 for detailed crowding operation information).

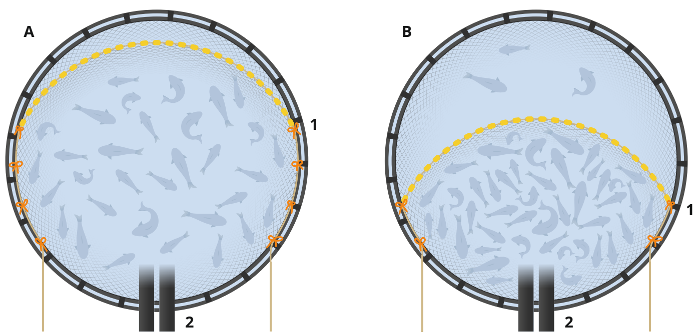

Figure 2.6: Crowding operation with swipe net seen from above. **A**, in the beginning of crowding. No tie points are cut; **B**, in the middle of crowding. Some of the tie points are cut. **1**, tie point; **2**, pipes.

Figure 2.7: The ending of a crowding operation with swipe net. The swipe net is lifted vertically in the air.

2.3.2 Crowding Operation Using a Ball Line

Crowding with a ball line works differently from swipe net crowding by not involving the application of a smaller net. The ball line consists of a number of floating, round fenders threaded along a long rope (Figure 2.8). The ball line may be compared to an enlarged pearl necklace. Crowding with ball line is the final crowding operation and ensures all the fish left are loaded. The ball line is placed outside the net pen on the opposite side of the pipes and wellboat/treatment vessel. The ball line rope ends are attached to the cranes at the wellboat/treatment vessel. By pulling the ball line ends, the fenders are dragged underneath the net towards the pumps and wellboat/treatment vessel. As the ball line is pulled underneath, the net is "forced" over the balls/fenders and decreasing the net pen volume. The fish have nowhere to go except towards the loading area and pipes (Figure 2.9).

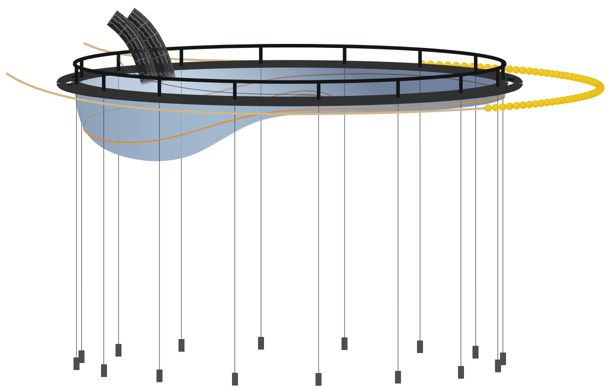

Figure 2.8: Ball line placed outside the net pen opposite to the pipes.

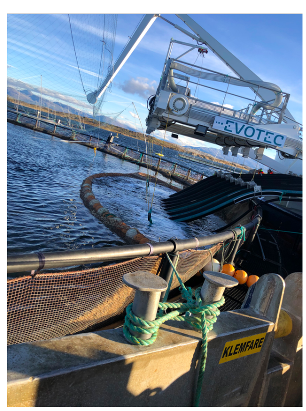

Figure 2.9: The ending of a crowding operation with ball line. Only a few fish are left in the net pen.

For both ball line and swipe net crowding, the speed of the lining (pulling the swipe net/ball line against the pipes) is important. The speed must be fast enough for the crowding to be effective, but slow enough to avoid additional stress to the fish. An optimal and smooth speed of the lining process is also important to avoid pockets in the net during crowding. In addition, fish health and welfare is an important factor during crowding. Weak and diseased fish handle crowding poorly (Speilberg et al., 2018; Snieszko, 1974). Increasing the number of crowding operations to decrease the duration of each crowding should be considered if the fish is weak.

For the current study, the number of crowdings, type of crowding, crowding duration and mean oxygen saturation were registered (Table 2.1).

Table 2.1: Crowding operation information for each net pen (1-4) at all locations (A-C).

Net pen	Crowding number	Type of crowding	Crowding duration (min)	Mean oxygen saturation (%)
A1	1	Swipe net	65	100.6
	2	Ball line	128	86.9
A2	1	Swipe net	60	93.0
	2	Ball line	118	96.3
A3	1	Swipe net	60	92.5
	2	Ball line	105	95.5
A4	1	Swipe net	195	106.8
	2	Ball line	111	107.0
B1	1	Swipe net	93	90.0
	2	Swipe net	62	91.8
	3	Ball line	80	89.1
B2	1	Swipe net	76	86.0
	2	Swipe net	44	88.3
	3	Ball line	90	115.6
B3	1	Swipe net	69	91.0
	2	Swipe net	43	94.0
	3	Ball line	120	95.2
B4	1	Swipe net	71	127.2
	2	Swipe net	47	116.7
	3	Ball line	141	90.8
C1	1	Swipe net	35	87.0
	2	Swipe net	32	95.0
	3	Ball line	103	94.4
C2	1	Swipe net	59	91.0
	2	Swipe net	33	91.0
	4	Ball line	185	95.0
C3	1	Swipe net	41	88.5
	2	Swipe net	39	89.0
	3	Ball line	40	97.5

2.4 Fish Sampling: During Crowding

For locations A and B, four net pens were analyzed, denoted by A1-A4 and B1-B4 (Table 2.2). Three net pens were analyzed on location C, denoted by C1-C3. The biomass data for each net pen was obtained from the fish farms. The average fish weight was based on the total biomass and number of fish in each net pen. The temperature was measured in the net pen 1-1.5 m below the water surface. All locations had cylinder nets of the type Polarcirkel. Lice skirts were removed during summer 2020.

Table 2.2: Net pen information for each net pen (1-4) at all locations (A-C) during crowding.

Net pen	Water temperature (°C)	Biomass (tons)	Net pen circumference (m)	Average fish weight (kg)	Total number of crowdings	Days between samples
A1	11.4	473.5	120	6.23	2	4
A2	11.2	425.7	120	5.94	2	5
A3	11.4	313.2	120	5.66	2	5
A4	11.3	448.3	120	5.49	2	6
B1	10.4	320.2	120	3.20	3	1
B2	10.3	349.6	120	3.60	3	2
B3	10.3	297.4	120	3.10	3	2
B4	10.1	292.0	120	3.10	3	3
C1	9.7	350.0	160	4.33	3	4
C2	8.3	475.0	160	3.08	4	5
C3	8.7	37.00	160	4.50	3	6

Note: "Days between samples" is the number of days between pre-crowding situation and crowding.

Lice registration was performed during Hydrolicer delousing process on the ship Hydro Patriot. Fish were collected from 11 net pens, distributed on Location A, B and C (Table 2.2). 20 fish from each net pen during every crowding were collected and analyzed. Due to short crowding operations, only 35 fish at C1 and 49 fish at C2 (instead of 60) were analyzed. The net pens contained salmon i ranging from 3.08 and 6.20 kilograms. The fish were collected with a big landing net as shown in Figure 2.10 a). The big landing net was only able to collect fish close to the loading pipes. It was not possible to collect the fish from the delousing vessel unless the fish were crowded. To ensure the lice registration was finished in time, the fish collection started early in the crowding process. The average time between first collection and last lice registration was 36 minutes, ranging from 25 to 75 minutes.

Collected fish were anesthetized in a 200 liter (L) tub (Figure 2.10 b) filled with seawater and Benzoak vet. (15-20 ml/100L water) and lice registration was performed (see Chapter 2.5 for detailed description). The fish were then transferred to the recovery tub and returned to the net pen as quickly as possible. Before the next sampling, the tub was cleaned and any remaining lice were collected and registered using a sieve at the tub drain, and new water and sedation were added.

(a)

(b)

Figure 2.10: a) Big landing net controlled by a winch used to capture fish during crowding and b) lice registration tub containing three departments; **right**, newly collected fish; **middle**, water containing anaesthesia; **left**, fresh sea water, recovery tub.

2.5 Lice Registration: During Crowding

Shortly after being anesthetized, the fish were lifted out of the tub and analyzed one by one. The body surface, including fins and gills, was split up into a total of 4 zones (Figure 2.11). The zone classification is based on Bjørn and Finstad (1998) research on infected sea trout post smolts. For each salmon, the number of *L. salmonis* and *C. elongatus* was registered for each body zone. For *L. salmonis*, lice life stage was registered in the following five categories; sessile, pre-adult, adult male and adult female with and without eggstrings.

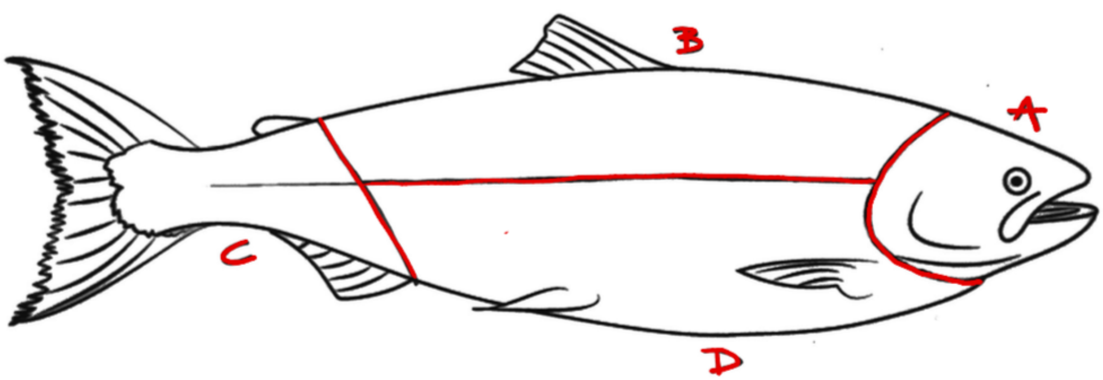

Figure 2.11: Zone classification of salmon body for lice registration. **A**, head, operculum and gills; **B**, dorsal area of the body above the lateral line from the head to anterior end of the soft dorsal fin; **C**, posterior dorsal and ventral area of the body including soft dorsal, caudal and anal fin; **D**, ventral area of the body below the lateral line from the head to the anterior end of anal fin. (Drawing Tutorials, 2020).

2.6 Fish Sampling and Lice Registration: Pre-Crowding Situation

Fish sampling and analysis of the pre-crowding situation were performed by the employees at each location, 1-6 days prior to delousing and crowding for the respective net pens (Table 2.2). In each net pen, 20 fish were collected using a hand net. The total number of *L. salmonis* and *C. elongatus* was registered. In addition, the mean number of sessile, mobile and females (with and without eggstrings) was registered for *L. salmonis*. Lice position according to body zones was not registered for the pre-crowding situation. Data from lice registration during the pre-crowding situations was obtained from each location.

2.7 Statistical Analysis

All calculations were performed in excel and the data was analyzed using SPSS Software (IBM® SPSS® Statistics, version 27). The significance threshold was set at 0.05.

A Wilcoxon Signed Rank Test was performed to compare the mean between mean numbers of lice in the pre-crowding situation and during crowding. Pearson Correlation Coefficient was used to measure the linear relationship between fish size and the change in the mean number of *L. salmonis* and *C. elongatus* between pre-crowding situation and during crowding.

Prevalence and mean intensity (Table 2.3) of *L. salmonis* and *C. elongatus* were calculated for each crowding operation in every net pen analyzed (Appendix). In addition, prevalence and mean intensity for each lice stage, and each lice stage at each zone was calculated for *L. salmonis*. All calculations were performed in Excel (Microsoft® Excel for Mac, version 16.16.10). All variables were checked for normality and were found to have a non-normal distribution. A Mann-Whitney test was performed to check differences in mean values between swipe net and ball line crowding for the mean intensity and prevalence of *C. elongatus* and the different lice stages of *L. salmonis*.

Table 2.3: Variables, definitions and associated formulas based on Bush et al., 1997.

Variable	Definition	Formula
Mean number of lice (M)	The total number of parasites of a particular species observed in a sample (N_{po}) divided by the number of fish analyzed in that sample (N_{fa}).	$M = \frac{N_{po}}{N_{fa}}$
Change in mean number of lice (ΔM)	The mean number of lice during crowding (M_{dc}) minus the mean number of lice in the pre-crowding situation (M_{pc}).	$\Delta M = M_{dc} - M_{pc}$
Prevalence (P)	The number of fish infected with 1 or more individuals of a particular species (N_{fi}) divided by the number of fish analyzed in that sample (N_{fa}).	$\mathrm{P} = \frac{N_{fi}}{N_{fa}}$
Mean intensity (MI)	The total number of parasites of a particular species observed in a sample (N_{po}) divided by the number of hosts infected with that parasite (N_{fi}).	$MI = \frac{N_{po}}{N_{fi}}$
Average change in Mean number of lice ($\overline{\Delta M}$)	The sum of changes in Mean number of lice (ΔM), divided by the number of net pens (n)	$\overline{\Delta M} = \frac{\sum_{i=1}^{n} \Delta M_i}{n}$

3 Results

3.1 Mean Number of Lice: Pre-crowding Situation and During Crowding

To match the pre-crowding situation registrations, samples of adult female *L. salmonis* with and without eggstrings during crowding are categorized as one category (female) in the current analysis. The mean number of female *L. salmonis* (p-value = 0.013) and of *C. elongatus* (p-value = 0.003) were significantly higher during crowding compared to the pre-crowding situation (Figure 3.1). The mean number of sessile *L. salmonis* (p-value = 0.154) was higher during crowding, but the difference was not significant. For mobile *L. salmonis* (p = 0.929), the mean number differed only slightly between the pre-crowding situation and during crowding. However, the variation in mean number per fish for mobile *L. salmonis* was notably higher compared to the other stages.

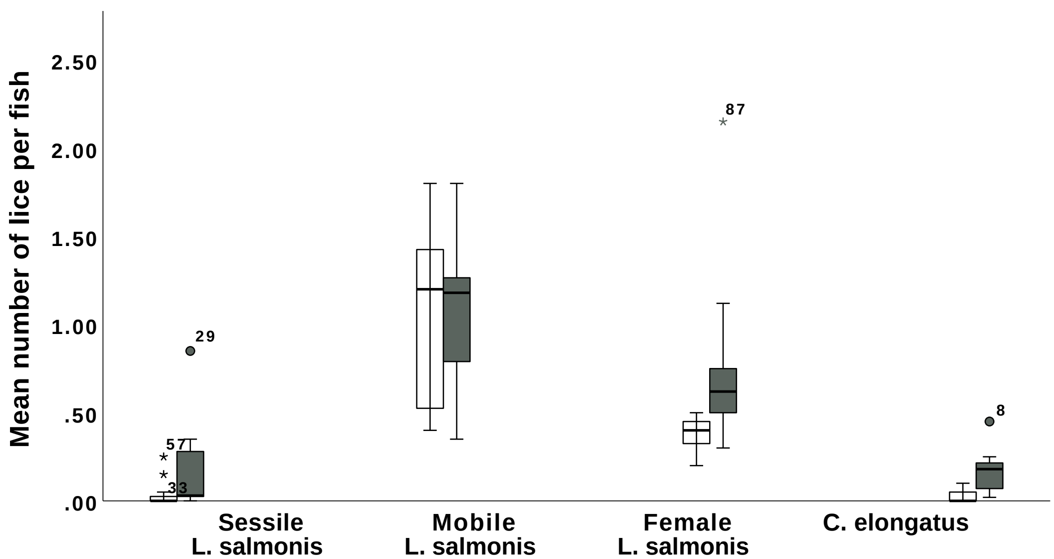

Figure 3.1: Mean number of *L. salmonis* and *C. elongatus* per fish in pre-crowding situation (open boxes, n = 11 samples, one sample from each net pen, 220 fish in total) and during crowding (grey boxes, n = 29 samples, 2-3 samples from each net pen, 524 fish in total) for all locations, given as the 25th, median, and 75th percentile together with outliers (o) and high extreme values (*).

On location A and C, the mean number of sessile *L. salmonis* per fish was 0 in the pre-crowding situation, meaning this stage was not present at the given locations in those samples (Figure 3.2). In the pre-crowding situation on location B however, the mean number of sessile *L. salmonis* ranged from 0.05-0.25 per fish. During crowding, sessile *L. salmonis* was found on all locations and the mean number ranged from 0 - 0.85 per fish. The mean number of mobile *L. salmonis* was generally higher on location A and C compared to location B during crowding. The mean number of female *L. salmonis* and *C. elongatus* was higher during crowding for all net pens. Overall, there was on average 0.9 more lice (*L. salmonis* and *C. elongatus*) individuals per fish during crowding compared to the pre-crowding situation, but this difference was not significant.

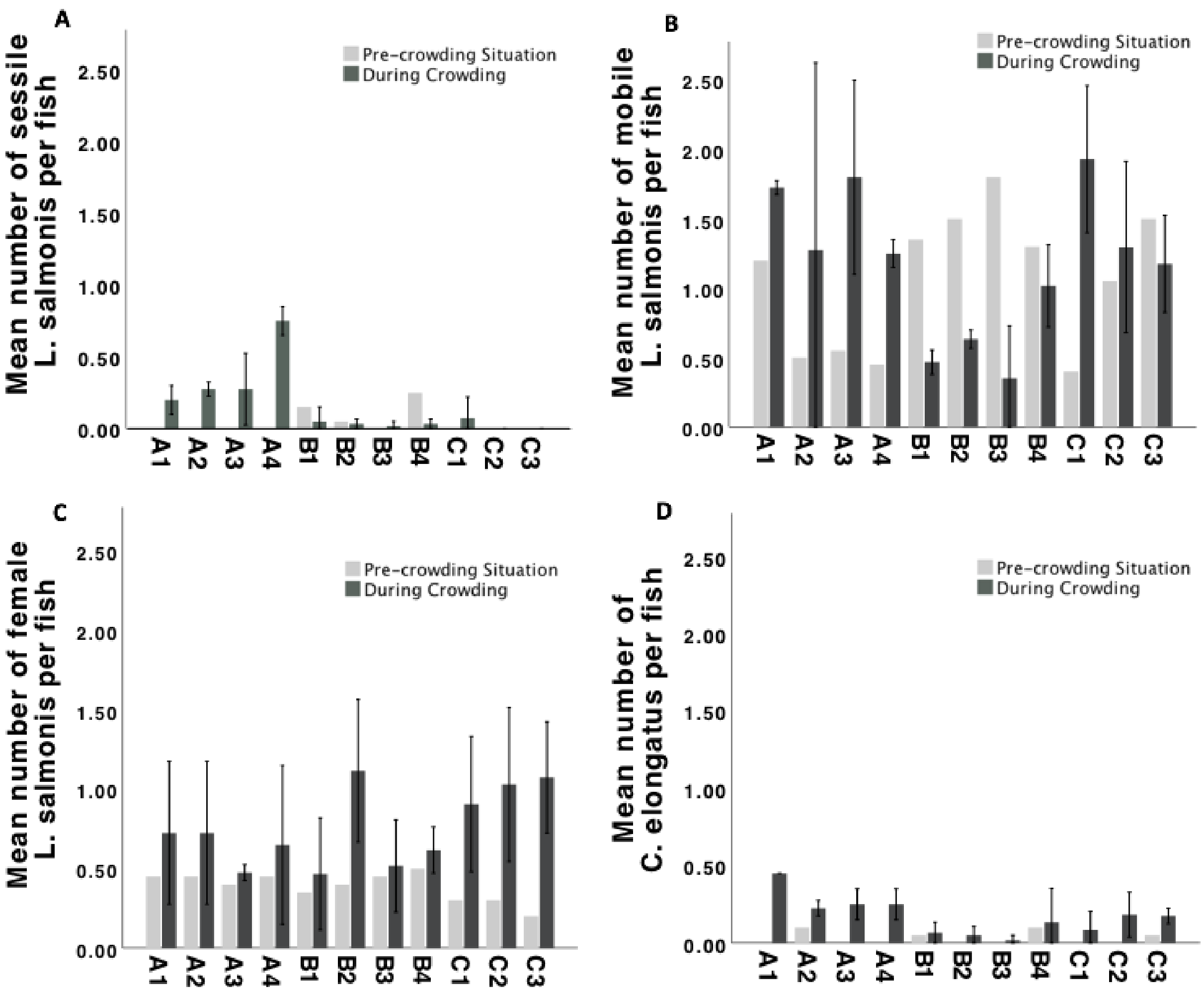

Figure 3.2: Mean number of **A**, sessile *L. salmonis*; **B**, mobile *L. salmonis*: **C**, female *L. salmonis*; **D**, *C. elongatus* per fish in pre-crowding situation and during crowding. For the pre-crowding situation, n = 11 samples, one sample from each net pen, 220 fish in total and for samples during crowding, n = 29 samples, 2-3 samples from each net pen, 524 fish in total. Error bars are ± SE.

The average change in the mean number of lice ($\overline{\Delta M}$) from pre-crowding situation to during crowding was positive for all stages of *L. salmonis* and *C. elongatus* (Figure 3.3 **A**). Female *L. salmonis* had the most notable change with an average of 0.38 more lice per fish during crowding. The change in mean number of mobile *L. salmonis* was positive for all net pens at location A and net pen C2. For net pen C2, C3 and location B, the change in mobile

L. salmonis was negative, ranging from -1.45 to -0.28. 72 % of the four mean number cases (sessile, mobile, female and *C. elongatus*) at each 11 net pen were positive (Figure 3.3 **B**), finding more lice during crowding compared to the pre-crowding situation.

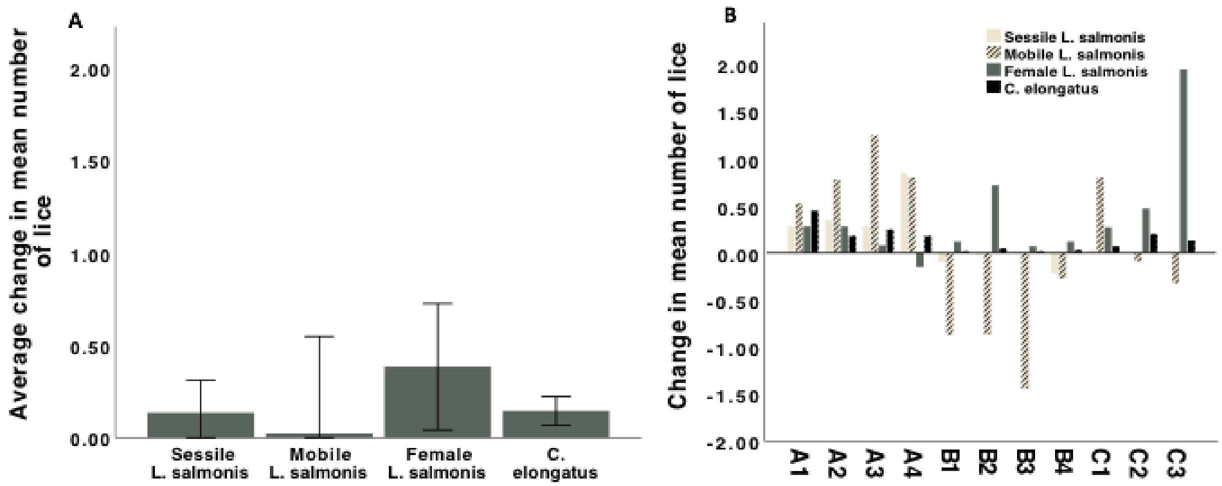

Figure 3.3: **A**, the average change in mean number of lice according to stage/species between pre-crowding situation and during crowding; **B**, the change in mean number of lice according to net pen between pre-crowding situation and during crowding. For the pre-crowding situation, n = 11 samples, one sample from each net pen, 220 fish in total and for samples during crowding, n = 29 samples, 2-3 samples from each net pen, 524 fish in total. Error bars are ± SE.

3.2 Lice Numbers and Fish Size

The change from pre-crowding situation to during crowding in mean number of sessile *L. salmonis*, mobile *L. salmonis* and *C. elongatus* per fish showed a positive significant correlation to fish size (Table 3.1). There was no significant correlation between change in mean number of female *L. salmonis* and fish size. The correlation coefficient and scatterplots of the correlating variables indicate that the change from pre-crowding situation to during crowding in mean number of sessile *L. salmonis*, mobile *L. salmonis* and *C. elongatus* is increasing as the fish size increases (Figure 3.4). That means the bigger fish, the more lice is found during crowding relative to the same net pen in the pre-crowding situation. Mobile *L. salmonis* showed the steepest fitted line and had the strongest correlation coefficient (0.795).

Table 3.1: Pearson correlation of fish size and change in mean number of lice between pre-crowding situation (n = 11 samples, one sample from each net pen, 220 fish in total) and during crowding (n = 29 samples, 2-3 samples from each net pen, 524 fish in total).

		Sessile *L. salmonis*	Mobile *L. salmonis*	Female *L. salmonis*	*C. elongatus*
Fish size	Pearson Correlation	0.745	0.795	-0.065	0.768
	Sig. (2-tailed)	0.009	0.003	0.849	0.006
	n	11	11	11	11

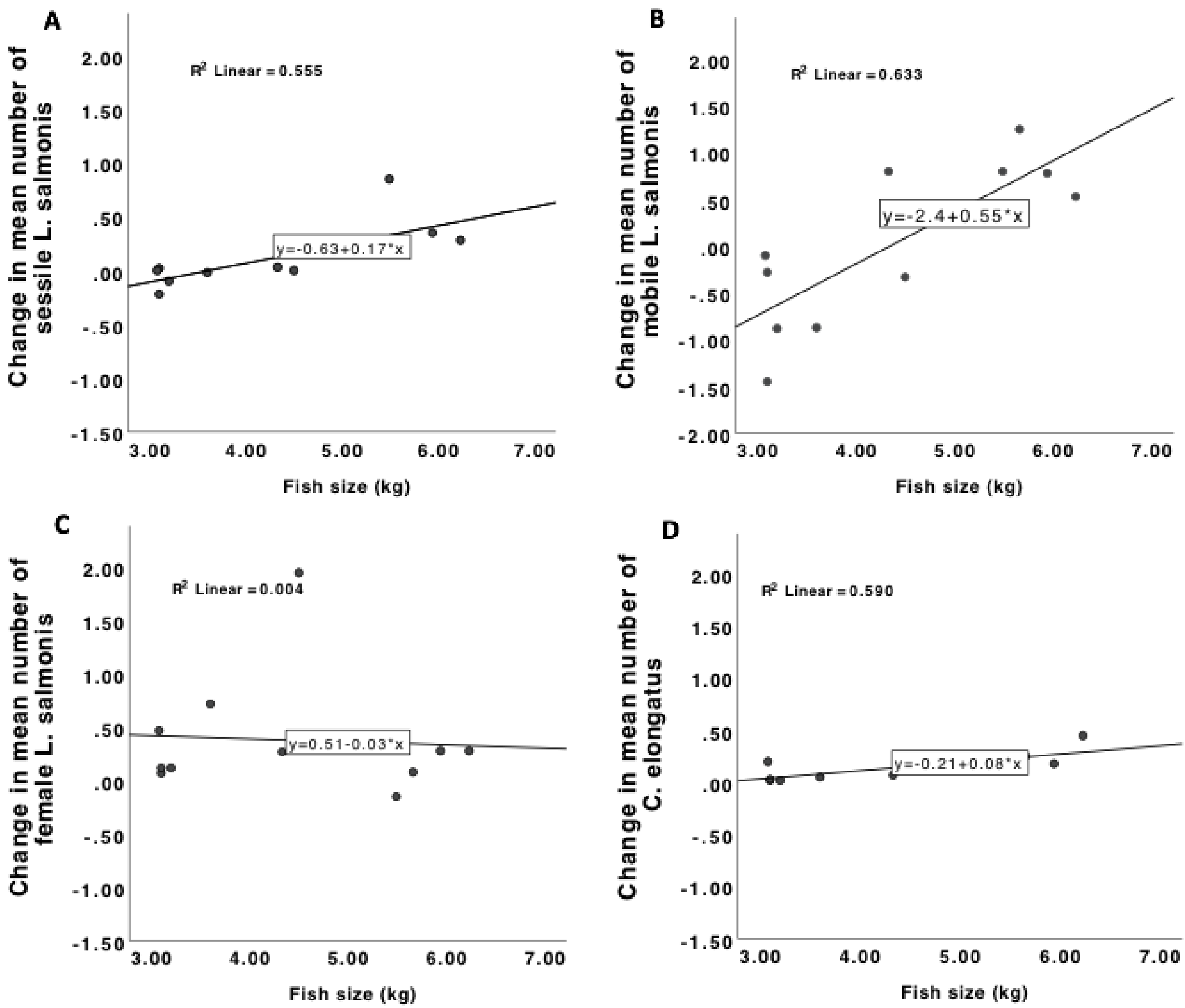

Figure 3.4: The relationship between change in mean number of **A**, sessile *L. salmonis*; **B**, mobile *L. salmonis*; **C**, female *L. salmonis*; **D**, *C. elongatus* and fish size. R^2 and formula of best fitted line are shown in each of the plots.

3.3 Effect of Number of Crowding Operations and Crowding Method

Results from the Mann-Whitney test showed for *L. salmonis* and *C. elongatus* that neither the mean number of lice, prevalence nor mean intensity for each lice stage were significantly different during swipe net crowding and ball line crowding. See Table A1-A6 in Appendix for numeric mean number of lice, mean intensity and prevalence. For 7 out of 11 (64%) net pens, the mean number of *L. salmonis* per fish increased or did not change between the first and second crowding operation (2 out of 4 net pens with two crowding operations and 5 out of 7 net pens with three crowding operations) (Figure 3.5). 2 of 7 net pens had an increase between second and last crowding operation. The change in the mean number of lice per fish did not seem to be affected by the number of crowding operations or by different crowding methods.

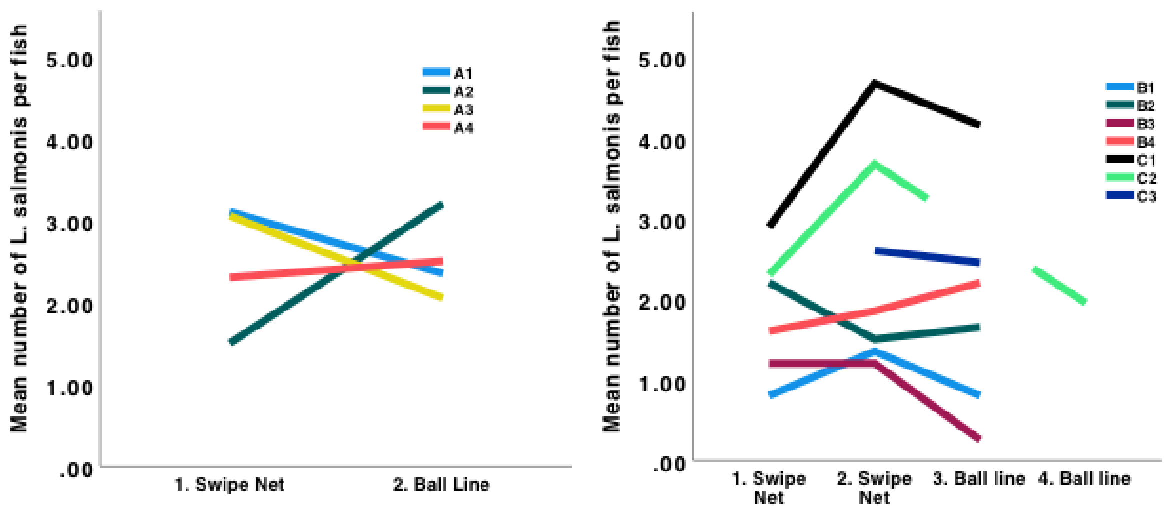

Figure 3.5: Mean number of *L. salmonis* per fish in 1st, 2nd, 3th and 4th crowding during swipe net and ball line crowding operations. Location A (left) had two crowding operations and location B and C (right) had three crowding operations. For net pen C2, lice data from 3th crowding with swipe net was not registered.

3.4 Effect of Crowding Method on Lice Placement

The results included no significant differences in mean intensity or prevalence of *L. salmonis* and *C. elongatus* in the different body zones, A-D, during swipe net crowding and ball line crowding (Figure 3.6). For both lice species, mean intensity and prevalence were relatively low and stable in zone A. The prevalence of *C. elongatus* was close to 0 for all zones, indicating that only a small amount of the fish were infected with *C. elongatus* compared to the total number of fish examined.

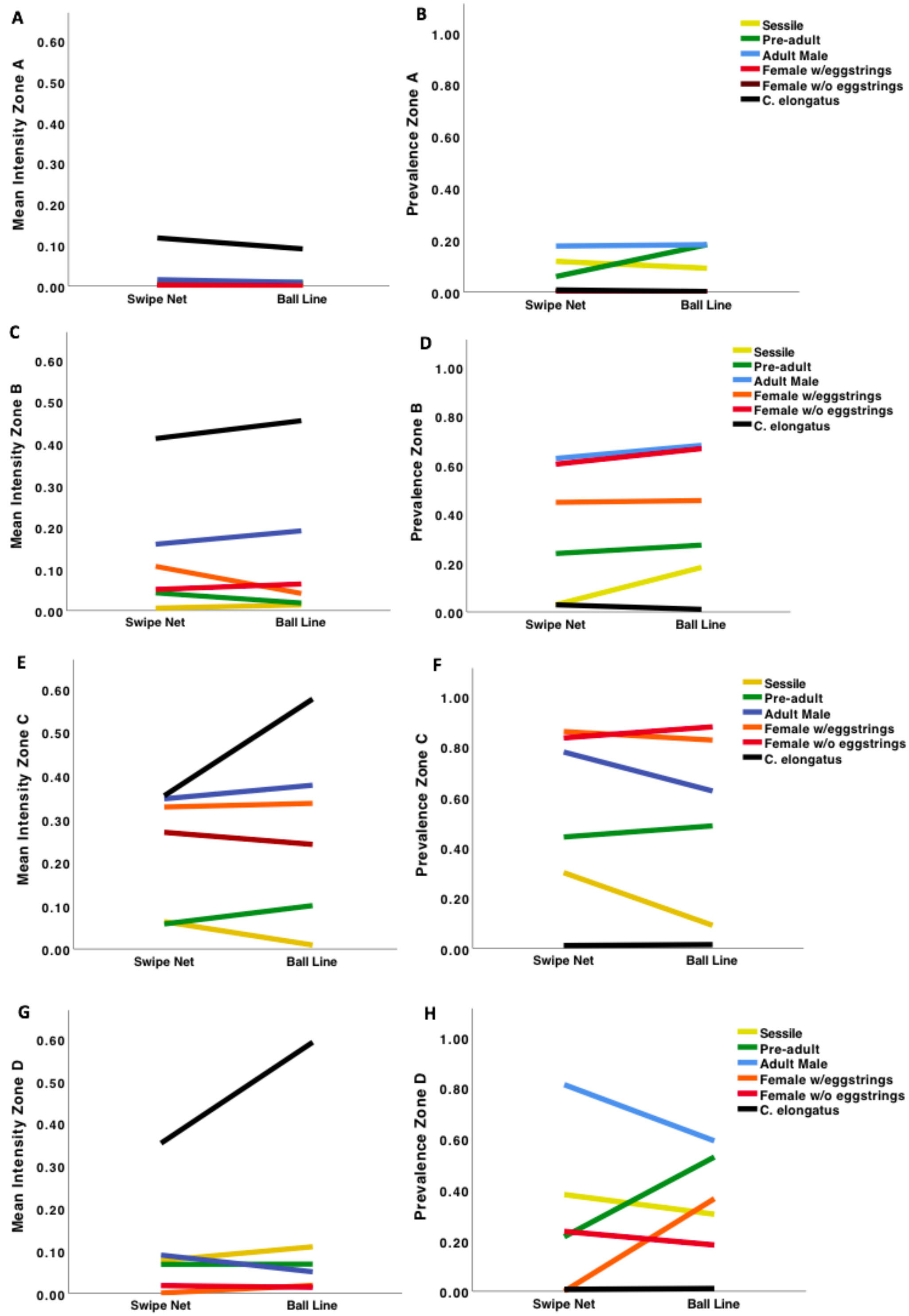

Figure 3.6: Mean intensity (**A**, **C**, **E** and **G**) and prevalence (**B**, **D**, **F**, and **H**) of *L. salmonis* and *C. elongatus* in Zone A-D during swipe net and ball line crowding. n = 29 samples, 2-3 samples from each net pen, 524 fish in total.

For *L. salmonis*, 63.3% of the sessile lice observed on the fish were found in zone D on the ventral area of the fish body (zone classification is given in Figure 2.11). Adult male (59.7%), female w/eggstrings (86.2%) and female w/o eggstrings (76.7%), showed a preference for zone C, the posterior of the fish body. Pre-adult lice were mainly found in zone C (38.9%) and zone D (40%). 0.28% of the total number of female *L. salmonis* observed on the fish body was found in zone A. For the *C. elongatus* observed on the fish, no specific body zone was preferred.

Of all the lice observed, 1.25% of the sessiles, 22.1% of the pre-adults, 31.5% of the adult males, 16.4% of the adult females w/eggstrings, 20% of the adult females w/o eggstrings and 28.4% of *C. elongatus* were dislodged from the host and found in the anesthesia tub.

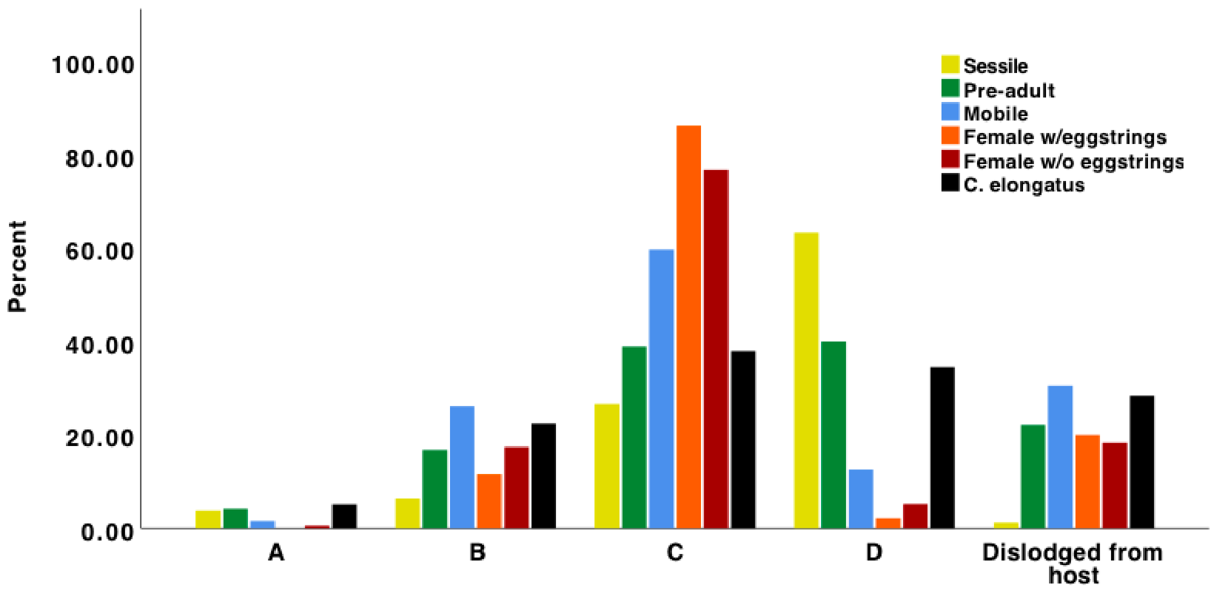

Figure 3.7: Percentage of lice placement observations on the fish (zone A-D) and of lice dislodged from the host. n = 29 samples, 2-3 samples from each net pen, 524 fish in total.

4 Discussion

The primary aim of this study was to investigate the fate of *L. salmonis* and *C. elongatus* during farmed salmon crowding. It is questioned by marine biologists and the fish farmers if the stress response and/or mechanical impact caused by the crowding affect the lice detachment, making more lice elude the delousing process and spread in the free water masses.

4.1 Pre-Crowding Situation and During Crowding

The mean number of lice in the pre-crowding situation was compared to the mean number of lice during crowding, to see if there was a significant difference. The results indicate that crowding had no significant effect on mobile lice or sessile lice. However, for adult female *L. salmonis* and *C. elongatus*, the mean number of lice was significantly higher during crowding, possibly indicating that more adult female *L. salmonis* and *C. elongatus* attach to the fish during crowding compared to normal swimming behavior in the pre-crowding situation. Another interpretation of this result is that mobile *L. salmonis* develop into adult females between the samples, making the mean number of adult female *L. salmonis* higher during crowding.

For *C. elongatus*, the development from copepodid to chalimus and adult stage might explain some of the significant differences in mean levels between the pre-crowding situation and during crowding. Hogans, Trudeau et al. (1989) report that increased water temperatures in general, resulted in greater numbers of parasites and *C. elongatus* exhibit shorter generation times in response to high water temperatures (5 week generation time at 10°C). However, the result of the analysis in terms of more *C. elongatus* attaching to the fish during crowding, deviates from the trend suggested in the literature by Pike and Wadsworth (1999) where *C. elongatus* tend to leave the host body when it is disturbed. If this trend is the case and crowding is considered to be a disturbance, the attachment of *C. elongatus* and large variations in lice numbers in general, might be explained by host transfer, intrinsic mortality, new infections and/or recruitment from nearby cages (Bui et al., 2020b).

Another interesting result is the reduction in the mean number of mobile *L. salmonis* on location B. The reduction could be explained by mobile lice leaving their hosts during crowding, but a similar reduction on location A and C would then have been expected. One explanation for this may be that some of the mobile lice have developed into adult females between the two samples. Nevertheless, one should note that for location B, it was only 1-3 days between the samples and the development to adult female probably does not explain the majority of the reduction of mobile lice. If this was the case, a greater increase in female lice on location B would have been expected. It is difficult to give an exact answer to what factors are causing the reduction, but as similar cases are not found on location A and C, the influencing factors are probably location-specific.

An average of 0.9 more lice (*L. salmonis* and *C. elongatus*) individuals per fish during crowding, indicates an overall higher lice density during crowding. Some of this increase in the number of lice might be caused by nauplii development to chalimus (sessile). Location A had the greatest difference in sessile *L. salmonis* registrations (0,3 sessiles per fish during crowding versus 0 in pre-crowding situation) in addition to the highest registered temperatures. The temperature on Location A ranged from 11.2-11.4 °C. The development of nauplii to sessile *L. salmonis* takes 3.6 days at 10°C (Johnson and Albright, 1991a) and the development time increases as the temperature increases (Hamre et al., 2019). This is interesting as the temperature was 0.9-2°C lower on location B and C, where the number of sessile *L. salmonis* registered was lower during crowding. In addition, the number of days between the two samples at location A was 4-5, giving the nauplii more time to develop compared to location B with a shorter time between the samples.

One concern about the absence of sessile *L. salmonis* on location A and C in the pre-crowding situation is that many fish farmers find small, sessile lice the most difficult to detect during lice counting (Thorvaldsen et al., 2019). It might be the case that the real number of sessile lice are higher and that the current lice numbers are affected by the knowledge and training of employees who perform the counts.

In general, it is important to note that there was only taken one sample (20 fish) from each net pen in the pre-crowding situation. Replication is important for the appropriate testing application of significance testing and few replicates leave more data to happen by chance (Wester, 1992). In addition, the study by Bui et al. (2020b) found variation in lice distribution at the same research site over time and according to developmental stage and sex of the louse. This might also explain some of the variation between lice numbers in the pre-crowding situation and during crowding. Bui et al. (2020b) suggest further investigations to map preferences as the lice are mobile and can easily move across the host's surface. In addition, another study found that increased lead time between counting and treatment (which is crowding in this study) events affects the estimated baseline value of pre-treatment sea lice abundance in Atlantic salmon (Gautam et al., 2016). They report that counting events that occurred on the day of treatment provided the highest baseline estimate of sea lice abundance during late summer and autumn than other days. This may have had an impact on this study and one should keep in mind that lice abundance in one sample may not be able to predict the abundance in the same sample a time after.

4.2 Change in Lice Numbers and Fish Size

The correlation test with fish size and change in lice number between pre-crowding and crowding indicates that for sessile *L. salmonis*, mobile *L. salmonis* and *C. elongatus*, more lice attach between the pre-crowding situation and during crowding as the fish size increases.

The reason for the attachment could be associated with the handling procedures, increasing the susceptibility of Atlantic salmon to *L. salmonis*. Delfosse et al. (2020) suggest the increase in plasma cortisol as a possible reason. They propose that the plasma cortisol is modifying the release and/or the composition of semiochemicals and thereby increasing the susceptibility of the salmon. On the other side, a study by Hemre and Krogdahl (1996) found that the cortisol peak after handling was independent of fish size (0.523 kg versus 2.917 kg). However, it is found that *L. salmonis* levels increase the longer the salmon are exposed in seawater (Saksida et al., 2007; Revie et al., 2002a). The larger fish has probably spend more time in seawater than the smaller fish. Despite this fact, much lower levels of *C. elongatus* infestation are observed in the second year, late in the production cycle (Revie et al. (2002b)).

Discussing this correlation, it is important to recognize that the fish sizes are highly associated with location A, B and C. Potential influence could therefore be location-specific factors e.g. temperature and/or the days between pre-crowding situation and crowding. Location A had fish in the highest weight class, the highest temperatures and 4-6 days between the samples. Compared to location B and C, the combination of high temperatures and more days between the samples may have caused good growth conditions for *L. salmonis* and *C. elongatus*. This might explain some of the positive difference between the pre-crowding situation and during crowding as sea lice development is dependent upon temperature (Hamre et al., 2019). One should also note that female *L. salmonis* develops slower than males (Hamre et al., 2019) and might be the reason it did not show any correlation to fish size (temperature and days between samples).

4.3 Effect of Number of Crowding Operations and Crowding Method

The results showed no specific effects of the number of crowding operations and crowding method on *L. salmonis* and *C. elongatus.* The results indicate that the different crowding methods do not have a different effect on the detachment, attachment and the position of *L. salmonis* and *C. elongatus* on the host body. However, one study limitation should be acknowledged. With the current study design, it is not known if the lice detach from the host and then reattach during the crowding operation.

The main goal of the crowding operations is to increase the fish density which is the result of both swipe net and ball line in this study. For ball line crowding, the net pen is lined up additionally, decreasing the volume further which might have an impact on the sea lice. Nonetheless, compared to the swipe net crowding, the biomass of the fish is smaller and the fish density will therefore be approximately the same for the two crowding methods. Also, even though there is done more than one crowding operation per net pen, it is important to note that an individual fish is not crowded more than once. This means the fish (and lice) is only experiencing dense fish environment once, which might explain why the number of crowding procedures does not show an effect on the associated *L. salmonis* and *C. elongatus.*

4.4 Lice Placement on Host Body

Compared to previous studies on *L. salmonis* distribution on fish host body, very few adult females (0.28 %) were found on the head, corresponding to zone A. Two studies reports 53% and 26% of the females preferred the head (Bui et al., 2020b; Bjørn and Finstad, 1998). The effect of crowding, indicating that female lice are moving to the dorsal back of the fish body as the fish is crowded, can not be directly excluded to be the reason for this. However, the temperature in the study by Bui et al. (2020b), was notably higher at an average of 14.3°C.

A more specific division of the dorsal side in zone B and ventral side in zone D could be more informative because it shows in more detail what part of the fish the lice prefer. Hogans, Trudeau et al. (1989) found that parasites in general congregate to a specific area of the fish. Congregation on those areas should, among other things, reduce the chance of being physically pushed off the host. In the data collection of the study, *L. salmonis* were generally observed behind the dorsal fin, the adipose fin and the anal fin. This however, was not emphasized with the current study design.

This study did not indicate that *C. elongatus* preferred a specific site on the host body which agrees with the literature (Hogans, Trudeau et al., 1989). The study by Hogans, Trudeau et al. (1989) reports no direct competition between *C. elongatus* and *L. salmonis* which also seems to be the case in this study because the two lice species are found in the same sites on the host.

Overall, the study of the mean number of lice in the pre-crowding situations versus during crowding does not indicate that lice detach from the salmon body during crowding contradicting the fish farmers' and marine biologists' suspicions. However, the absence of registration of lice position on the host body in the pre-crowding situation limits the study because it is not possible to know if the lice have stayed in the same position between the samples. Also, possible detachment and reattachment between the two samples are uncertain with the current study design.

5 Conclusion and Further Work

5.1 Conclusion

Comparing the mean numbers of lice per fish before and during crowding, the crowding operations did not affect the mean number of mobile and sessile *L. salmonis*. However, the mean number of female *L. salmonis* and *C. elongatus* was significantly higher during crowding compared to the pre-crowding situation. The increase might be explained by attachment during crowding, natural variations in the lice distribution and/or the lice developing to other stages due to a relatively long time interval between the samples.

The change from pre-crowding situation to during crowding in the mean number of sessile *L. salmonis*, mobile *L. salmonis* and *C. elongatus* showed a significant positive correlation to fish size. There was no significant correlation between the change in the mean number of adult female *L. salmonis* and fish size. The correlations might be influenced by location-specific factors e.g. temperature and the amount of days between the samples in addition to the fish's exposure time to seawater.

Neither the number nor the placement of *L. salmonis* and *C. elongatus* were significantly different for the different numbers of crowding operations or the different crowding methods - swipe net and ball line. However, with the current study design, it is not known whether the lice stay on the host, or detach and reattach during the crowding. 0.28% of adult female *L. salmonis* were observed on the host's head (zone A) during crowding.

5.2 Further Work

The duration of the crowding for the analyzed fish was 15-45 minutes, but the fish may be crowded up to 1.5 hour (pers. comm. E. Grøntvedt, 12.4.2021). It would therefore have been interesting to study the effect of crowding on lice numbers and position in a more detailed way. The current study design did not allow for the registration of the individual fish's crowding exposure time. As pointed out earlier, the fish's stress level is shown to affect the susceptibility to sea lice (Delfosse et al., 2020). It is therefore conceivable that lice numbers and positions on host body in combination with crowding exposure time could be more informative in order to understand the effect of crowding on *L. salmonis* and *C. elongatus*.

The study is also limited by the time interval between the pre-crowding situation and the crowding. It would have been optimal to do one sample as close to the lining up as possible and one sample between the lining up and the crowding operation. The current study does not have any information on lice numbers and position moments before crowding. With up to 6 days between the samples, it is difficult to determine if the lice numbers and position are due to crowding or other influencing factors.

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Appendix: Numeric Data of the Mean Number, Mean Intensity and Prevalence of *L. salmonis* and *C. elongatus*

Table A1: Prevalance (P), Mean Intensity (MI) and Mean number (M) of *L. salmonis* during crowding in net pen A1-B1. Zones A-D are denoted by A, B, C, and D. Sessile, Pre-adult, Adult male, Adult Female with eggstrings and Adult Female without eggstrings are denoted by S, PA, AM, AF and AFX respectively. − = not detected or less than <0.01.

Net pen	A1	A1	A2	A2	A3	A3	A4	A4	B1	B1	B1
Crowding method	Swipe net	Ball line	Swipe net	Ball line	Swipe net	Ball line	Swipe net	Ball line	Swipe net	Swipe net	Ball line
P	0.90	0.80	0.70	0.75	0.90	0.75	0.90	0.85	0.50	0.50	0.50
MI	3.44	2.94	2.14	4.27	3.39	2.73	2.56	2.94	1.60	2.70	1.60
P_A_S	-	-	1.00	1.00	1.00	-	-	-	-	-	-
P_B_S	-	1.00	-	-	0.50	-	-	1.00	-	-	-
P_C_S	0.60	-	0.50	-	-	-	0.50	1.00	0.50	-	-
P_D_S	0.67	-	1.00	0.60	1.00	1.00	0.79	0.73	1.00	-	-
MI_A_S	-	-	0.05	0.05	0.05	-	-	-	-	-	-
MI_B_S	-	0.10	-	-	0.10	-	-	0.05	-	-	-
MI_C_S	0.25	-	0.30	-	-	-	0.10	0.10	0.10	-	-
MI_D_S	0.15	-	0.05	0.25	0.25	0.15	0.70	0.75	0.05	-	-
P_A_PA	-	-	-	1.00	1.00	1.00	-	-	-	-	-
P_B_PA	0.75	-	-	1.00	0.80	-	-	-	-	-	-
P_C_PA	1.00	1.00	-	0.67	1.00	1.00	-	0.67	-	-	-
P_D_PA	1.00	1.00	0.75	1.00	0.89	1.00	1.00	0.80	-	-	-
MI_A_PA	-	-	-	0.05	0.10	0.05	-	-	-	-	-
MI_B_PA	0.20	-	-	0.10	0.25	-	-	-	-	-	-
MI_C_PA	0.15	0.15	-	0.30	0.25	0.35	-	0.15	-	-	-
MI_D_PA	0.10	0.20	0.20	0.10	0.45	0.10	0.40	0.25	-	-	-
P_A_AM	-	-	-	1.00	0.00	-	-	-	-	1.00	1.00
P_B_AM	0.50	1.00	-	1.00	0.75	0.75	1.00	0.67	1.00	0.00	1.00
P_C_AM	0.86	0.73	0.50	0.64	0.78	0.58	0.71	0.38	0.83	0.44	0.33
P_D_AM	1.00	0.50	1.00	1.00	1.00	1.00	0.80	1.00	-	-	-
MI_A_AM	0.00	-	-	0.05	-	-	-	-	-	0.05	0.05
MI_B_AM	0.50	0.15	-	0.35	0.20	0.20	0.10	0.15	0.50	-	0.20
MI_C_AM	0.35	0.75	0.10	0.55	0.45	0.60	0.35	0.40	0.30	0.45	0.15
MI_D_AM	0.20	0.10	0.05	0.15	0.05	0.05	0.25	0.05	-	-	-
P_A_AF	-	-	-	-	-	-	-	-	-	-	-
P_B_AF	-	-	1.00	-	1.00	1.00	-	-	-	-	-
P_C_AF	0.69	0.86	0.60	0.63	1.00	0.75	1.00	1.00	1.00	0.80	1.00
P_D_AF	-	-	-	-	-	-	-	1.00	-	-	-
MI_A_AF	-	-	-	-	-	-	-	-	-	-	-
MI_B_AF	-	-	0.50	-	0.10	0.10	-	-	-	-	-
MI_C_AF	0.80	0.35	0.25	0.40	0.30	0.20	0.20	0.10	0.05	0.25	0.20
MI_D_AF	-	-	-	-	-	-	-	0.05	-	-	-
P_A_AFX	-	-	-	-	-	-	-	-	-	-	-
P_B_AFX	1.00	-	-	1.00	1.00	1.00	-	0.33	-	1.00	1.00
P_C_AFX	1.00	1.00	1.00	0.75	1.00	1.00	-	1.00	1.00	0.71	1.00
P_D_AFX	-	-	-	1.00	-	-	-	-	-	-	-
MI_A_AFX	-	-	0.05	-	-	-	-	-	-	-	-
MI_B_AFX	0.05	-	-	0.15	0.05	0.10	-	0.15	-	0.10	0.05
MI_C_AFX	0.10	0.05	0.05	0.20	0.05	0.05	-	0.10	0.15	0.35	0.15
MI_D_AFX	0.00	-	-	0.10	-	-	-	-	-	-	-
P_CH	0.25	1.00	0.25	0.20	0.35	0.15	0.60	0.70	0.10	-	-
P_PA	0.40	0.35	0.15	0.45	0.95	0.50	0.40	0.30	-	-	-
P_AM	0.75	0.75	0.10	0.90	0.55	0.55	0.55	0.30	0.30	0.25	0.30
P_AF	0.55	0.30	0.20	0.25	0.40	0.25	0.20	0.15	0.05	0.20	0.20
P_AFX	0.15	0.05	0.10	0.40	0.10	0.15	-	0.15	0.15	0.35	0.20
MI_CH	1.60	1.50	1.60	1.50	1.14	1.00	1.33	1.29	0.15	-	-
MI_PA	1.63	1.14	1.33	1.44	1.53	1.00	1.50	1.33	-	-	-
MI_AM	1.47	1.73	4.00	1.44	1.27	1.73	1.27	2.67	1.50	2.20	1.33
MI_AF	1.45	1.50	1.50	2.00	1.00	1.20	1.00	1.00	1.00	1.50	1.00
MI_AFX	1.00	1.00	2.00	1.13	1.00	1.00	-	1.67	1.00	1.43	1.00
M	3.10	2.35	1.50	3.20	3.05	2.05	2.30	2.50	0.80	1.35	0.80

Table A2: Prevalance (P), Mean Intensity (MI) and Mean number (M) of *L. salmonis* during crowding in net pen B2-B4. Zones A-D are denoted by A, B, C, and D. Sessile, Pre-adult, Adult male, Adult Female with eggstrings and Adult Female without eggstrings are denoted by S, PA, AM, AF and AFX respectively. − = not detected or less than <0.01.

Net pen	B2	B2	B2	B3	B3	B3	B4	B4	B4
Crowding method	Swipe net	Swipe net	Ball line	Swipe net	Swipe net	Ball line	Swipe net	Swipe net	Ball line
P	0.80	0.70	0.85	0.65	0.65	0.20	0.75	0.75	0.70
MI	2.75	2.14	1.94	1.85	1.85	1.25	2.13	2.47	3.14
P_A_S	-	-	-	-	-	-	-	-	-
P_B_S	-	-	-	-	-	-	-	-	-
P_C_S	1.00	-	-	-	1.00	-	-	-	-
P_D_S	-	-	1.00	-	-	-	1.00	1.00	-
MI_A_S	-	-	-	-	-	-	-	-	-
MI_B_S	-	-	-	-	-	-	-	-	-
MI_C_S	0.05	-	-	-	0.05	-	-	-	-
MI_D_S	-	-	0.05	-	-	-	0.05	0.05	-
P_A_PA	-	-	-	-	-	-	-	-	-
P_B_PA	-	1.00	-	-	-	-	-	-	-
P_C_PA	1.00	1.00	1.00	-	1.00	-	-	1.00	1.00
P_D_PA	-	-	-	-	-	-	-	-	-
MI_A_PA	-	-	-	-	-	-	-	-	-
MI_B_PA	-	0.05	-	-	-	-	-	-	-
MI_C_PA	0.05	0.05	0.05	-	0.05	-	-	0.05	0.10
MI_D_PA	-	-	-	-	-	-	-	-	-
P_A_AM	-	-	-	-	-	-	-	-	-
P_B_AM	-	1.00	0.60	1.00	1.00	-	0.80	1.00	1.00
P_C_AM	0.86	0.83	1.00	1.00	0.50	-	1.00	0.64	0.67
P_D_AM	1.00	1.00	1.00	1.00	-	-	1.00	1.00	-
MI_A_AM	-	0.00	-	-	-	-	-	-	-
MI_B_AM	-	0.15	0.25	0.10	0.10	-	0.25	0.20	0.10
MI_C_AM	0.35	0.30	0.20	0.40	0.20	-	0.35	0.55	0.30
MI_D_AM	0.10	0.05	0.05	0.05	-	-	0.15	0.15	-
P_A_AF	-	-	-	-	-	-	-	-	-
P_B_AF	1.00	-	1.00	-	-	-	-	-	1.00
P_C_AF	0.56	0.80	0.90	0.83	0.78	1.00	1.00	0.67	0.62
P_D_AF	-	-	-	-	-	-	-	-	1.00
MI_A_AF	-	-	-	-	-	-	-	-	-
MI_B_AF	0.10	-	0.05	-	-	-	-	-	0.05
MI_C_AF	0.45	0.25	0.50	0.30	0.45	0.05	0.05	0.15	0.65
MI_D_AF	-	-	-	-	-	-	-	-	0.05
P_A_AFX	-	-	-	-	-	-	-	-	-
P_B_AFX	0.50	1.00	-	1.00	-	1.00	-	1.00	1.00
P_C_AFX	0.60	0.63	0.88	1.00	1.00	1.00	1.00	1.00	0.70
P_D_AFX	1.00	-	1.00	-	-	-	1.00	-	-
MI_A_AFX	-	-	-	-	-	-	-	-	-
MI_B_AFX	0.10	0.10	-	0.05	-	0.10	-	0.05	0.05
MI_C_AFX	0.75	0.40	0.40	0.20	0.25	0.10	0.30	0.20	0.50
MI_D_AFX	0.10	-	0.05	-	-	-	0.05	-	-
P_CH	0.05	-	0.05	-	0.05	-	0.05	0.05	-
P_PA	0.05	0.10	0.05	-	0.05	-	-	0.05	0.10
P_AM	0.40	0.45	0.40	0.55	0.20	-	0.70	0.70	0.30
P_AF	0.35	0.20	0.50	0.25	0.35	0.05	0.05	0.10	0.50
P_AFX	0.60	0.35	0.40	0.25	0.25	0.20	0.35	0.25	0.40
MI_CH	1.00	-	1.00	-	1.00	-	1.00	1.00	-
MI_PA	1.00	1.00	1.00	-	1.00	-	-	1.00	1.00
MI_AM	1.38	1.33	1.38	1.18	1.75	-	1.36	1.79	2.33
MI_AF	1.57	1.50	1.10	1.20	1.43	1.00	2.00	1.50	0.30
MI_AFX	1.67	1.42	1.13	1.00	1.00	1.00	1.43	1.40	0.30
M	2.20	1.50	1.65	1.20	1.20	0.25	1.60	1.85	2.20

Table A3: Prevalance (P), Mean Intensity (MI) and Mean number (M) of *L. salmonis* during crowding in net pen C1-C3. Zones A-D are denoted by A, B, C, and D. Sessile, Pre-adult, Adult male, Adult Female with eggstrings and Adult Female without eggstrings are denoted by S, PA, AM, AF and AFX respectively. − = not detected or less than <0.01.

Net pen	C1	C1	C1	C2	C2	C2	C3	C3
Crowding method	Swipe net	Swipe net	Ball line	Swipe net	Swipe net	Ball line	Swipe net	Ball line
P	1.00	0.83	0.90	0.45	0.78	0.50	0.85	0.60
MI	2.89	5.60	4.61	5.11	4.71	3.90	3.00	4.08
P_A_S	-	-	-	-	-	-	-	-
P_B_S	-	-	-	-	-	-	-	-
P_C_S	1.00	-	-	-	-	-	-	-
P_D_S	-	-	-	-	-	-	-	-
MI_A_S	-	-	-	-	-	-	-	-
MI_B_S	-	-	-	-	-	-	-	-
MI_C_S	0.22	-	-	-	-	-	-	-
MI_D_S	-	-	-	-	-	-	-	-
P_A_PA	-	-	-	-	-	-	-	-
P_B_PA	1.50	-	1.00	-	-	-	-	1.00
P_C_PA	-	0.50	-	-	-	-	1.00	-
P_D_PA	-	-	-	-	-	1.00	-	1.00
MI_A_PA	-	-	-	-	-	-	-	-
MI_B_PA	0.22	-	0.05	-	-	-	-	0.05
MI_C_PA	-	0.33	-	-	-	-	0.05	-
MI_D_PA	-	-	-	-	-	0.05	-	0.05
P_A_AM	-	1.00	-	-	-	-	1.00	-
P_B_AM	0.60	-	0.46	-	1.00	1.00	1.00	-
P_C_AM	1.00	1.00	0.86	1.00	0.57	0.67	0.71	1.00
P_D_AM	2.00	-	1.00	1.00	1.00	-	1.00	1.00
MI_A_AM	-	0.17	-	-	-	-	0.05	-
MI_B_AM	0.44	-	0.65	-	0.11	0.05	0.05	-
MI_C_AM	0.22	0.33	0.70	0.05	0.78	0.30	0.35	0.20
MI_D_AM	0.11	-	0.05	0.10	0.11	-	0.15	0.10
P_A_AF	-	-	-	-	-	-	-	-
P_B_AF	2.00	0.60	1.00	-	1.00	1.00	1.00	-
P_C_AF	2.00	0.38	0.54	1.00	0.50	0.78	1.00	1.00
P_D_AF	-	-	-	-	-	1.00	-	1.00
MI_A_AF	-	-	-	-	-	-	-	-
MI_B_AF	0.11	0.83	0.20	-	0.11	0.05	0.05	-
MI_C_AF	0.11	1.33	0.64	0.10	0.22	0.45	0.30	0.15
MI_D_AF	-	-	-	-	-	0.05	-	0.05
P_A_AFX	-	-	-	-	-	-	-	-
P_B_AFX	2.00	-	1.00	0.75	-	-	1.00	1.00
P_C_AFX	1.00	1.00	0.71	1.00	0.80	1.00	0.44	0.62
P_D_AFX	-	-	-	1.00	-	-	1.00	-
MI_A_AFX	-	-	-	-	-	-	-	-
MI_B_AFX	0.11	-	0.05	0.20	-	-	0.05	0.05
MI_C_AFX	0.56	0.10	0.35	0.10	0.56	0.10	0.45	0.65
MI_D_AFX	-	-	-	0.10	-	-	0.05	-
P_CH	0.56	-	-	-	-	-	-	-
P_PA	0.67	0.17	0.05	-	-	0.05	0.05	0.10
P_AM	0.78	0.50	0.95	0.15	0.67	0.25	0.50	0.30
P_AF	0.67	1.00	0.55	0.10	0.22	0.45	0.35	0.20
P_AFX	1.00	0.33	0.30	0.35	0.44	0.10	0.30	0.45
MI_CH	0.40	-	-	-	-	-	-	-
MI_PA	0.83	3.00	1.00	-	-	1.00	4.00	1.00
MI_AM	1.14	3.00	2.42	7.33	2.83	3.20	2.30	3.00
MI_AF	0.67	0.17	0.55	3.50	2.50	0.44	0.57	0.75
MI_AFX	0.78	1.00	2.33	2.00	2.00	4.00	2.33	2.44
M	2.89	4.67	4.15	2.30	3.67	1.95	2.60	2.45

Table A4: Prevalance (P), Mean Intensity (MI) and Mean number (M) of *C. elongatus* during crowding in net pen A1-B1. Zones A-D are denoted by A, B, C, and D. − = not detected or less than <0.01.

	A1	A1	A2	A2	A3	A3	A4	A4	B1	B1	B1
Crowding method	Swipe net	Ball line	Swipe net	Ball line	Swipe net	Ball line	Swipe net	Ball line	Swipe net	Swipe net	Ball line
P	0.03	0.02	0.01	0.02	0.02	0.01	0.01	0.01	0.01	-	0.01
MI	1.00	1.50	1.00	1.00	1.00	1.00	1.00	1.00	1.00	-	1.00
P_A	-	-	-	-	0.01	-	-	-	-	-	-
P_B	0.01	-	0.01	0.01	-	-	-	0.01	0.03	-	-
P_C	0.05	0.04	0.03	0.03	0.04	0.01	0.01	0.03	-	-	0.01
P_D	0.04	0.03	0.01	0.03	0.03	0.04	0.01	0.01	-	-	0.01
MI_A	-	-	-	-	1.0	-	-	-	-	-	-
MI_B	1.00	-	1.00	1.00	-	-	-	1.00	1.00	-	-
MI_C	1.00	1.33	1.00	1.00	1.00	1.00	1.00	1.00	-	-	1.00
MI_D	1.00	1.5	1.00	1.00	1.00	1.00	1.00	1.00	-	-	1.00
M	0.45	0.45	0.20	0.25	0.30	0.20	0.15	0.20	0.10	-	0.10

Table A5: Prevalance (P), Mean Intensity (MI) and Mean number (M) of *C. elongatus* during crowding in net pen B2-B4. Zones A-D are denoted by A, B, C, and D. − = not detected or less than <0.01.

	B2	B2	B2	B3	B3	B3	B4	B4	B4
Crowding method	Swipe net	Swipe net	Ball line	Swipe net	Swipe net	Ball line	Swipe net	Swipe net	Ball line
P	-	-	0.01	-	-	-	-	0.01	0.01
MI	-	1.00	1.00	1.00	-	-	1.00	1.00	1.25
P_A	-	-	-	-	-	-	-	-	0.01
P_B	-	0.01	0.01	-	-	-	-	-	0.03
P_C	-	-	-	-	-	-	-	0.01	-
P_D	-	-	0.01	-	-	-	0.01	0.03	-
MI_A	-	-	-	-	-	-	-	-	1.00
MI_B	-	1.00	1.00	-	-	-	-	-	1.00
MI_C	-	-	-	-	-	-	-	1.00	-
MI_D	-	-	1.00	-	-	-	1.00	1.00	-
M	-	0.05	0.10	0.05	-	-	0.05	0.15	0.25

Table A6: Prevalance (P), Mean Intensity (MI) and Mean number (M) of *C. elongatus* during crowding in net pen C1-C3. Zones A-D are denoted by A, B, C, and D. − = not detected or less than <0.01.

	C1	C1	C1	C2	C2	C2	C3	C3
Crowding method	Swipe net	Swipe net	Ball line	Swipe net	Swipe net	Ball line	Swipe net	Ball line
P	0.11	0.33	0.05	0.1	-	-	0.05	-
MI	1.00	1.5	1.00	1.00	-	1.00	1.00	-
P_A	0.11	-	-	-	-	-	-	-
P_B	-	0.33	-	0.05	-	0.05	0.05	-
P_C	-	-	0.05	0.05	-	-	-	-
P_D	-	-	-	-	-	-	-	-
MI_A	1.00	-	-	-	-	-	-	-
MI_B	-	1.00	-	1.00	-	1.00	1.00	-
MI_C	-	-	1.00	1.00	-	-	-	-
MI_D	-	-	-	-	-	-	-	-
M	0.11	0.5	0.05	0.10	0.11	0.35	0.15	0.20