NTNU

Norwegian University of
Science and Technology

Characterization of planktonic sea lice distribution and association to fish farm installations

Margrete Slåtsve Øvrelid

Marine Coastal Development
Submission date: May 2017
Supervisor: Kjell Inge Reitan, IBI

Norwegian University of Science and Technology
Department of Biology

Acknowledgment

This thesis was written at the Department of Biology, NTNU, Trondheim, under the guidance of Professor Kjell Inge Reitan. The thesis was a part of the project *Taskforce lakselus* witch is a collaboration between NTNU and the aquaculture industry, including following companies; SalMar, Marine Harvest, Måsøval-gruppen, Bjørøya, Lerøy Midt, Midt-Norsk Havbruk, Salmonor, Sinkaberg-Hansen, Ervik Laks og Ørret, Emilsens fisk and Åkerblå. The work has taken place at NTNU Centre of Fisheries and aquaculture (Sealab), and at field stations at Frøya, Flatanger and Namsos, from June 2016 to April 2017.

First, I would like to thank my supervisors Kjell Inge Reitan and Lone Sunniva Jevne for constructive guidance and wise suggestions through the writing process. Lone also deserve a warm thank you for guidance through planning and completion of fieldtrips and laboratory work. I am thankful for Dag Altin, Tora Bardal, Siv Anina Etter, and others at Sealab who helped me with the technical aspects of the thesis. I would also like to thank the staff at Måsøval Fishfarm AS, Lerøy Midt AS and Salmar Farming AS, both for letting me collect the samples at their farms, and for helping me whenever needed.

Tanks to all my fellow students at Sealab for making the last two years memorable and enjoyable, and my family for all support. Finally, I would like to thank my great friends, especially Lisa Marie, for motivational support and the ability to sustain a social life during the master period.

Trondheim, May 2017

Margrete Slåtsve Øvrelid

Abstract

Lepeoptheirus salmonis (Krøyer 1837) and *Caligus elongatus* (Normann 1832) are the sea lice species posing the biggest threat to both farmed and wild salmonid stock in the Northern hemisphere today. The three first stages are planktonic, and the transmission of sea lice within farms, and between farms and wild fish happens mainly in these stages. Today it is limited observation on planktonic sea lice distribution in and around fish farms, and it is a need for more knowledge on how the planktonic stages of sea lice are distributed in association to sea cages. Such knowledge is important to get a better understanding of sea lice dispersion and infection mechanisms at salmon farms.

In this regard distribution of planktonic stages of sea lice inside and outside of sea cages in a fish farm were investigated by conducting horizontal plankton tows. The plankton tows were taken at two different farms, one wearing skirts and the other one without. How biofouling and washing activities may affect the distribution of sea lice were documented by taking plankton tows in relation to fouling on the net, and in different times of the washing cycle. A hatching experiment were conducted to characterize the planktonic stages of *L. salmonis* and *C. elongatus*, which was further used to differentiate between species in the plankton tows.

The results showed that live planktonic *L. salmonis* and *C. elongatus* had characteristic pigmentation, and differed in size. However, due to loss of pigmentation, individuals fixated on formaldehyde (4%) could not be specified further than to family (Caligidae). Regarding the prevalence of *L. salmonis* and *C. elongatus* in Norway, it is likely that the planktonic sea lice found in the plankton samples belonged to these two species. The result from the plankton surveys showed that nauplii were the most abundant planktonic life stage in the water column at fish farms. Planktonic sea lice was shown to be retained inside the sea cages wearing skirts. No such effect of sea cages not wearing skirts were detected, and sea cage nets did not seem to hinder the transport of planktonic sea lice to the surrounding water to a very high degree. Biofouling of the net could to some extend retain sea lice inside of the cage, most possible due to reduced water flow. It was not found an elevation of sea lice larva in the water masses immediately after cleaning of the nets, thus it did not seem that the planktonic stages of sea lice stay in the biofouling, and it is not likely that cleaning activities of the sea cage could be a source of infection by releasing pre-infective and infective larvae.

Sammendrag

Lepeoptheirus salmonis (Krøyer 1837) og *Caligus elongatus* (Normann 1832) er de to artene av havlus som gjør størst skade på både oppdrettslaks og vill laksefisk på den nordlige halvkule. De tre første stadiene av lakselus er planktoniske, og overføring av lakselus mellom oppdrettsanlegg og mellom oppdrettsanlegg og vill laksefisk skjer i hovedsak her. I dag er det bare et begrenset antall observasjoner om hvordan de planktoniske stadiene er fordelt i vannmassene ved oppdrettsanlegg, slik kunnskap er viktig for å kunne få en bedre forståelse av lakselusas spredning og infeksjonsmekanisme på oppdrettsanlegg.

Basert på dette ble fordelingen av planktoniske stadier av lakselus på innsiden og utsiden av oppdrettsmerder utforsket ved hjelp av horisontale planktonhåvtrekk. Prøvene ble tatt på to forskjellige oppdrettsanlegg, der det ene anlegget brukte luseskjørt. Hvordan begroing av nøter og vasking av not påvirket fordeling og spredning av luselarver ble også dokumentert ved å ta prøver ved merder med ulik mengde begroing på nettene, og ved forskjellige tider i vaskesyklusen. Et klekkeforsøk ble også utført med hensikt å klekke *L. salmonis* og *C. elongatus*, der individene ble brukt til å lage en enkel karakterisering av nauplius I, nauplius II og kopepoditt stadiene. Denne karakteriseringen skulle videre brukes til å kunne skille mellom de to artene funnet i planktonprøvene.

Resultatene viste at skottelus og lakselus hadde karakteristiske pigmenteringer, og at lakselus generelt var større enn skottelus i alle de tre førstestadiene. Når lusene ble fiksert på formaldehyd (4%) mistet de pigmenteringen, og grunnet stor variasjon i størrelse var det ikke mulig å artsbestemme luselarvene fra oppdrettsanleggene. Luselavene ble derfor i denne oppgaven bare bestemt til familien Caligidae, men grunnet utbredelsen av *L. salmonis* og *C. elongatus* tilhører de mest sannsynligvis en av disse to artene. Resultatene fra planktonundersøkelsene viste at nauplii var det vanligste planktoniske livsstadiet i vannmassene rundt oppdrettsanlegg. De planktoniske stadiene ble også i stor grad holdt igjen på innsiden av luseskjørt. Det ble ikke sett den samme effekten på merder som ikke brukte skjørt, og her var tettheten av luselarver tilnærmet den samme rett på innsiden som på utsiden av nettet. Begroing på nøtene kan føre til at luselarver produsert inne i nøtene blir holdt inne i nøtene til en større grad, mest sannsynligvis grunnet redusert vann flyt gjennom maskene. Det ble ikke funnet flere planktoniske lakselus i vannmassene rett etter at nøtene ble vasket, og det er lite sannsynlig at de planktoniske stadiene oppholder seg i groen og at spyling av not kan være en kilde til infeksjon ved å frigjøre nauplii og kopepoditter.

Table of Contents

1 Introduction 1

1.1 Salmon lice in aquaculture 1

1.2 Sea lice biology and ecology 2

1.2.1 Life cycle 2

1.2.2 Behavior of larvae 4

1.2.3 Dispersal and distribution of planktonic sea lice 5

1.3 Lice skirts as method against sea lice infestations 5

1.4 Biofouling in marine finfish aquaculture 6

1.5 Study aims and approach 7

2 Material and Method 8

2.1 Characterization of planktonic stages of sea lice 8

2.1.1 Sampling areas and collection 8

2.1.2 Hatching and characterization of planktonic stages 9

2.2 Distribution of planktonic sea lice inside and outside sea cages 10

2.2.1 Sampling sites 10

2.2.2 Sea lice infections on fish farm sites 11

2.2.3 Sampling period 11

2.2.4 Sample collection 12

2.2.5 Analysis and identification of plankton samples in the laboratory 14

2.2.6 Data analysis for calculating the amount of biofouling 14

2.3 Processing of data and statistical analysis 16

2.3.1 Length and width measurements 16

2.3.2 Distribution of planktonic sea lice 16

3 Results 17

3.1 Characterization of *L. salmonis* and *C. elongatus* larvae 17

3.1.1 External morphology and pigmentation of live pelagic sea lice larvae 17

3.1.2 Length and width of pelagic stages 19

3.2 Distribution of planktonic stages inside and outside sea cages 21
3.2.1 Temperature measurements 21
3.2.2 Biofouling 22
3.2.3 Number of Sea lice on farm 23
3.2.4 Abundance of planktonic sea lice 25
3.2.5 Size distribution of planktonic sea lice at fish farms 26
3.2.6 Larval densities on the inside and outside of the cage 27
3.2.7 Effects of biofouling on distribution of sea lice 29
3.2.8 Effect of washing of the net on dispersal of sea lice 30
4 Discussion 32
4.1 Overview 32
4.2 Identification of planktonic sea lice 32
4.2.1 Hatching and characterization of planktonic stages 32
4.2.2 Comparison of hatched nauplii with nauplii in plankton samples 34
4.3 Distribution of planktonic sea lice at salmon farm 36
4.3.1 Concentration of planktonic sea lice at farms 36
4.3.2 Comparison of the two locations 37
4.4 Distribution of planktonic stages inside and outside sea cages 37
4.5 Biofouling as a source for reinfection at farms 39
4.5.1 Effects of biofouling on distribution of sea lice 39
4.5.2 Effect of washing of the net on dispersal of sea lice 39
4.6 Summary 40
5 Conclusion 41
6 References 42
Appendix I 47
Appendix II 48
Appendix III 49
Appendix VI 51

Abbreviations

Copepodid	The third and final planktonic stage
Nauplius (plural nauplii)	The first and second life stages after hatching
NI	Nauplius I
NII	Nauplius II
Planktonic stages	Nauplius I and II, and copepodid stages of sea lice
Salmon lice	*Lepeoptheirus salmonis*
Sea lice	In this thesis used as a collective term for *L. salmonis* and *C. elongatus*.
Sea lice larvae	Nauplius I, nauplius II, and copepodid stages of *L. salmonis* and *C. elongatus*.

1 Introduction

1.1 Salmon lice in aquaculture

Sea lice is one of the main problems in the Norwegian aquaculture industry today. High occurrence of sea lice leads to a significant economic loss for the salmon aquaculture industry and reduction in farmed salmons health and welfare. In 2014 the total cost of sea lice infections at fish farms in Norway were estimated be around 3-4 Billion NOK (Iversen & Hermansen *et al.*, 2015; Johnson & Bravo *et al.*, 2004; Liu & Bjelland, 2014). The increase in salmon lice infections in fish farms the last two years have further increased the cost to ~6 Billion NOK (personal communication Kjell Inge Reitan). Sea lice are naturally occurring parasites, but as industrialization of salmonid aquaculture has intensified, so has the number of available hosts in the ocean. This has led to high lice infestations on both farmed and wild fish (Finstad & Bjørn *et al.*, 2000; Heuch & Mo, 2001; Pike & Wadsworth, 1999).

Sea lice are a family (Caligidae) of parasitic copepods that, normally infect external surfaces of marine fish, feeding of the blood, skin and mucus of their host (Frazer & Morton *et al.*, 2012; Pike & Wadsworth, 1999). Sea lice infections have several negative effects on the hosts health and welfare, and could be lethal at high infection rates. The feeding activities of sea lice can cause lesions in the skin, anemia, osmoregulatory failure and increase susceptibility of secondary infections (Grimnes & Jakobsen, 1996; Wootten & Smith *et al.*, 1982). In the Northern Hemisphere the two most common caligid species infecting salmonids are *Lepeoptheirus salmonis*, common name salmon lice (Krøyer 1837) and *Caligus elongatus* (Normann 1832) (Pike & Wadsworth, 1999). *L. salmonis* is a host specific parasite which only infects salmonids, whereas *C. elongatus* are a teleost generalist (á Norði & Simonsen *et al.*, 2016), known to infect more than 80 different fish species (Schram, 2004).

In addition to cause problems at farms, epizootics of sea lice on wild salmonids have been observed in coastal marine areas where salmon farms operate (Peet, 2007). Correlations between outbreaks at fish farms and increased infection on wild salmon populations have been reported in Scotland (Butler, 2002), Ireland (Tully & Whelan, 1993; Tully & Gargan *et al.*, 1999), Canada (Martin & Mark *et al.*, 2006; Morton & Routledge *et al.*, 2005) and Norway (Bjørn & Finstad *et al.*, 2001; Bjørn & Finstad, 2002). There have been are a great deal of concerns about how the sea lice affects the wild salmonid populations, and this concern is a

main reason for limitation in further sustainable increase in salmonid production (Karlsen & Johnsen *et al.*, 2017).

1.2 Sea lice biology and ecology

1.2.1 Life cycle

The life cycle of *L. salmonis* consists of eight stages (Figure 1.1), where each stage is separated by a molt. Like most other parasitic copepods, they have a direct life cycle, meaning they only need one host to complete their life cycle, although more than one host individual may be involved (Hayward & Andrews *et al.*, 2011). Sea lice have a temperature-dependent life cycle, where an increase in temperature reduces the minimum developmental time (Stien & Bjoern *et al.*, 2005). The generation times for *L. salmonis* are 7-13 weeks and 6-12 weeks for *C. elongatus* at temperatures from 10-16 °C (Johnson & Albright, 1991a; Tully, 1989). See Table 1.1 for the effect of water temperature on the duration of development time for planktonic stages of *L. salmonis*.

After hatching, *L. salmonis* are released into the water column and proceed through three planktonic larval stages: nauplius I, nauplius II, and copepodid. Newly hatched nauplii drifts passively with the currents and do not feed. After the nauplius stages they molt into the infective copepodid stage, where they have to find and infect a host. Following the copepodid stage there are to sessile chalimus stages (I and II), which are attached to the host by a frontal filament (Hamre & Eichner *et al.*, 2013; Pike & Wadsworth, 1999; Tully & Nolan, 2002). When the salmon lice reaches the preadult (I and II) and adult stages they become mobile and can move over the surface of fish, and swim in the water column (Hayward & Andrews *et al.*, 2011; Johnson & Albright, 1991a, 1991b). Here the sea lice detach from the frontal filament, and use the bulk of their body to produce suction to remain on the surface of their host. Adult females produce a pair of egg strings holding ~100-1000 eggs (Costello, 1993), and may reproduce multiple times. An illustration of the whole life cycle of *L. salmonis* is shown in Figure 1.1.

C. elongatus also have a progression of eight life cycle stages, they have the same free-living stages as *L. salmonis* (two nauplius-stages and one copepodid stage) (Hamre & Eichner *et al.*, 2013), but instead of the two preadult stages, they have 4 chalimus stages and molts straight to the adult stage (Piasecki, 1996).

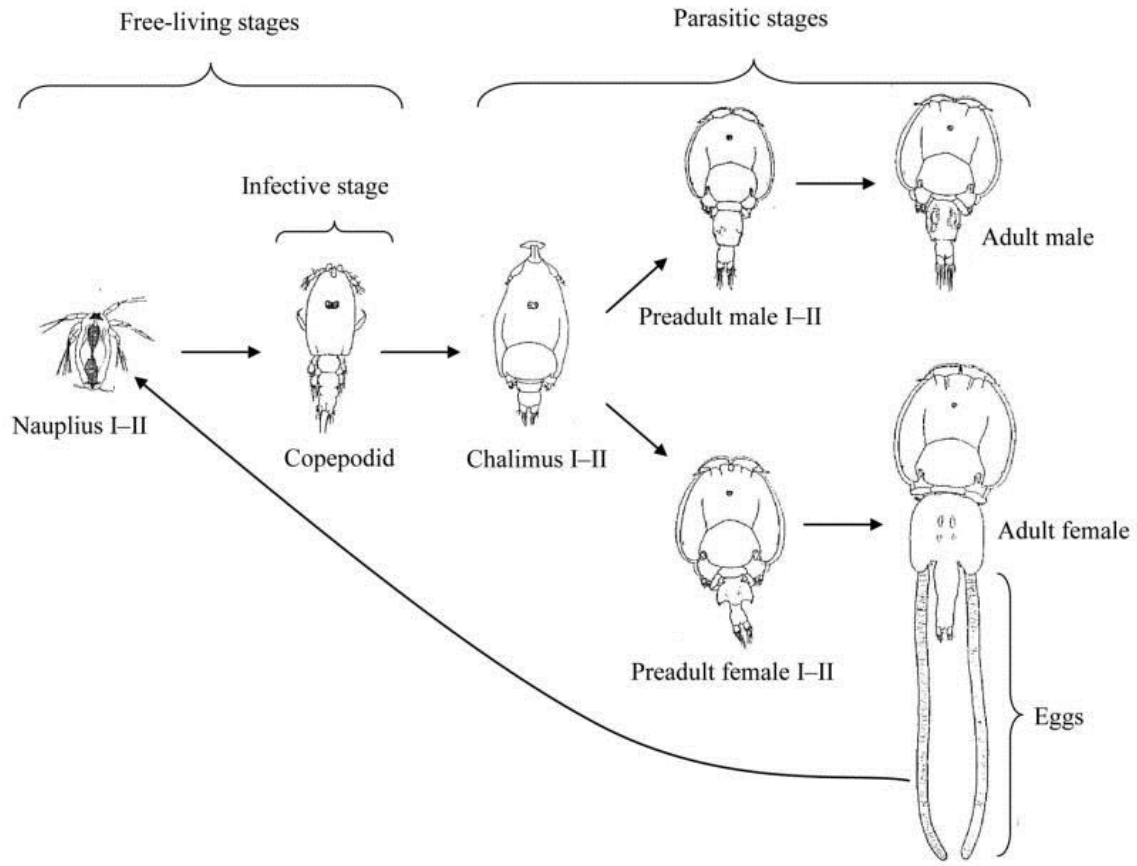

Figure 1.1 - Life cycle of L. salmonis consisting of eigth stages (Igboeli & Burka et al., 2014).

Table 1.1 -Effect of water temperatures on the duration of the free-living stages of *L. salmonis* (Samsing & Oppedal *et al.*, 2016).

Temperature (°C)	**Development times in days**		
	NI and NII	Copepodid	Planktonic stages
20	1.69±0.90	6.66±0.90	8.34±0.60
15	2.19±0.40	9.68±0.11	11.87±1.09
10	3.81±0.66	13.19±2.17	17.00±2.13
7	7.05±0.58	12.73±2.85	19.77±2.65
5	11.52±1.72	10.15±4.00	21.62±9.12
3	-	-	-

1.2.2 Behavior of larvae

The planktonic stages of sea lice have the ability to swim vertically in the water column, and orient themselves at desirable depth. The benefits of vertical movements are probably the possibility to seek host rich environments, and avoidance of predators and undesirable environments (Johnsen & Asplin *et al.*, 2016; Mordue & Birkett, 2009).

Planktonic sea lice are found in the upper surface waters, which are the natural environment for the salmonid host (Mordue & Birkett, 2009). Laboratory experiments carried out with *L. salmonis* showed that copepodids reacted to pressure equivalent to depths greater than 4 meters, by upward vertical movement (Bron & Sommerville *et al.*, 1993). The copepodid stage of *L. salmonis* are positively phototactic and exhibit a daily vertical migration, rising during the day and sinking down at night (Heuch & Parsons *et al.*, 1995). Salmon exhibit a reverse migration, and transmission can take place when sea lice and salmon cross each other in the water column. *L. salmonis* nauplii do not show the same diel vertical distribution patterns (Bron & Sommerville *et al.*, 1993), but may have the ability to sense temperature and actively seek the depth with the highest achievable temperature within the water column (á Norði & Simonsen *et al.*, 2015; Johnsen & Fiksen *et al.*, 2014). The knowledge about vertical distribution patterns of *C. elongatus* are not as comprehensive as the *L. salmonis*, but it is suggested that they do not have an strong vertical migration like the one observed in planktonic *L. salmonis* (á Norði & Simonsen *et al.*, 2015). The reason for this could be because they are a teleost generalist, thus inhabit a broader habitat (á Norði & Simonsen *et al.*, 2015).

Copepodids are also able to alter their position in the water column in response to salinity. Sea lice are a stenohaline organism and reduced salinity affects sea lice behavior, fecundity and survival. Experiments done on free-living *L. salmonis* established that salinities below 29 ppt reduced the survival, and that the copepodids actively avoids salinities below 27 ppt (Bricknell & Dalesman *et al.*, 2006; Mordue & Birkett, 2009). The infective copepodid stage also respond to mechanical vibrations and chemical stimuli with “burst-swimming” and “looping”, behavior believed to be used for finding and encounter hosts (Bron & Sommerville *et al.*, 1993).

1.2.3 Dispersal and distribution of planktonic sea lice

Even though preadult and adult sea lice are able to swim in the water column, it is believed that planktonic life stages are the most important for transmission between farms, and between farms and wild fish (Amundrud & Murray, 2009). Sea lice have a relatively long larval stage (Amundrud & Murray, 2009; Samsing & Oppedal *et al.*, 2016), and could in this period travel as far as 10-50 km (Costello, 2006). The dispersal of sea lice is strongly determined by environmental factors, where in addition to currents, temperature is especially important for the dispersal and infective window. As mentioned, they do not feed during the planktonic stages and are therefore dependent on their yolk sac to be able to detect and infect a host (Tucker & Sommerville *et al.*, 2000). In warmer water nauplii will develop faster to the infective copepodid stage, but the infective window would be shorter because they consume their yolk sac faster (Pike & Wadsworth, 1999; Tucker & Sommerville *et al.*, 2000). Vertical distribution, affected by behavioral traits can also affect dispersal (Asplin & Boxaspen *et al.*, 2011).

Planktonic sea lice have a patchy and non-predicable distribution, and they are normally found in very low densities in the water column (Pike & Wadsworth, 1999; Woll, 2012). Plankton sampling have revealed that the density of nauplii decrease rapidly in relation to distance from fish farms (Costelloe & Costelloe *et al.*, 1998; Penston & McKibben *et al.*, 2004). The ratio of copepodids to nauplii increased with distance from the farm, due to the decrease in density of nauplii (á Norði & Simonsen *et al.*, 2015; Costelloe & Costelloe *et al.*, 1996; Morton & Routledge *et al.*, 2011; Penston & McKibben *et al.*, 2004). Copepodids have shown a tendency to aggregate close to shore and estuary mouths (á Norði & Simonsen *et al.*, 2015; Amundrud & Murray, 2009; McKibben & Hay, 2004; Penston & McKibben *et al.*, 2004). Norði et al. (2015) also showed that wind driven currents had a strong influence on the dispersal of planktonic *L. salmonis*, pushing the copepodids towards shallow waters (á Norði & Simonsen *et al.*, 2015). The spatial distribution of *C. elongatus* were not as affected by the wind driven currents as the *L. salmonis* and Norði et al. (2015) suggested that this is because of the difference in vertical migration patterns between the two species, where *L. salmonis* tends to aggregate in surface waters, and dispersal is mainly affected by winds.

1.3 Lice skirts as method against sea lice infestations

Chemical delousing of sea lice is the most commonly used method for combating sea lice. However, resistance and reduced sensitivity against drugs have been widely reported and

alternative methods to control and prevent sea lice at fish farms have been of great interest in recent years (Igboeli & Burka *et al.*, 2014). One of these methods are the lice skirts, that enclose the upper parts of open pens (Næs & Heuch *et al.*, 2012). The skirts can be made of tarpaulin or preamble fabric and are placed vertically around the upper few meters of the sea cage. The water flow through the skirt are depending of the fabric the skirts are made of, where permeable fabric allow some water flow through the cage. Lice skirts are placed in that part of the water column where the infection rate is expected to be highest, and prevent sea lice infection by forming a physical barrier between the parasitic copepodid stage and the salmon (Stien & Nilsson *et al.*, 2012). Lice skirts are shown to reduce infection of sea lice at salmon farms when the skirts are 10 meters deep (Næs & Heuch *et al.*, 2012).

1.4 Biofouling in marine finfish aquaculture

The large surface area, and increased dissolved nutrients from salmons, makes the net structures on salmon cages ideal substrate for fouling (Hodson & Lewis *et al.*, 1997). Biofouling of nets is a significant problem for marine aquaculture, because a highly fouled net restrict the water flow through the occlusion of the net and can dramatically decrease the amount of dissolved oxygen available to the fish (De Nys & Guenther, 2009). Heavily fouled nets also increase the drag of the net, which alters the cage structure and behavior in rough seas (Dürr and Watson 2010). Fouling of the net could also host pathogens or parasites, which could cause disease (De Nys & Guenther, 2009; Dürr & Watson, 2010).

The amount of biofouling are shown to affect the distribution and dispersion of sea lice larva produced in the cage, where highly fouled nets can act as an physical barrier and the planktonic stages could be retained inside of the net for a longer period (Costelloe & Costelloe *et al.*, 1996). High densities of planktonic sea lice larvae have also been reported larvae in the water column shortly after cleaning of the cage, and it is possible that the free-living stages of sea lice could be associated with the organisms fouling the net (Woll, 2012).

To reduce the amount of biofouling, the net pens are washed regularly (Guenther & Misimi *et al.*, 2010), most Norwegian fish farms clean their nets at least once a month during the summer (personal communication with employees at fish farm). The washing devices used for *in situ* washing of the nets consists of a rig with rotating discs with nozzles. The rig is lowered inside the cage, and seawater with high pressure are passed through the nozzles to dislodge and remove the biofouling (AkvaGroup, 2017; Guenther & Misimi *et al.*, 2010).

1.5 Study aims and approach

It is limited observations on planktonic sea lice distribution in and around fish farms. It is a need for more knowledge on how the planktonic stages of sea lice are distributed in association to sea cages. Such knowledge is important to get a better understanding of sea lice dispersion and infection mechanisms at salmon farms. The present study was conducted to investigate the distribution of planktonic stages of sea lice inside and outside of sea cages in a fish farm. The main hypothesis (1) was that the sea lice was to a strong extent maintained inside the sea cages, and the sea cage nets hinder the transport of planktonic sea lice to the surrounding waters.

- A sub-goal was to characterize the possible association of sea lice to the bio-fouling of the net. The hypothesis (2) for this was that sea lice larvae may be associated with the fouling of the nets, and fouling may delay dispersal of sea lice out of the net.
- In addition, the effect of cleaning of the net was investigated on the dispersal of larval stages of sea lice. This hypothesis (3) was that cleaning activities of the sea cage could be a source of infection by the releasing pre-infective and infective lice larvae, from fouling on the nets.
- Another aim was to see if there were differences in densities of planktonic sea lice between location with and without skirt, and make a simple characterization of planktonic stages of *L. salmonis* and *C. elongatus*.

These goals have been approach by:

- Collecting gravid sea lice known to be *L. salmonis* and *C. elongatus* in order to cultivate egg-strings until copepodids, to make a simple characterization of the larval stages, and attempt to distinguish between species in the plankton samples.
- Taking plankton samples on the inside and outside of salmon cages and calculated the concentration in the samples
- Taking plankton samples through the washing cycle of the net; before, after and one day after the nets were cleaned.
- Taking plankton samples at two different sites, one with and the other one without a skirt.

2 Material and Method

Two experiments were performed. In the first experiment eggs from *L. salmonis* and *C. elongatus* were hatched in the laboratory and the development of nauplius I, nauplius II, and copepodid stages were characterized. The second experiment was conducted in the field around Inntian where plankton tow samples were taken in the water column at fish farms.

2.1 Characterization of planktonic stages of sea lice

2.1.1 Sampling areas and collection

A hatching experiment was conducted in order to make a simple characterization of the nauplii and copepodid stages of *L. salmonis* and *C. elongatus*. Collection of gravid sea lice females were carried out at two Salmar Farming AS fish farm locations in Namsos and Flatanger, which were known to have *L. salmonis* and *C. elongatus* infections at that time. Location A was located at Årnes, Namsos (N:64º35.763':E:11º16.406') and location B at Makrellskjæret (N: 64º32.956':E:10º43.985'). The positions of the farms are shown in Figure 2.1.

The collection of sea lice was carried out by catching farmed Atlantic salmon from the net pen, which were anaesthetized in a bath of benzoak. During anesthesia, attached gravid sea lice with egg strings were collected and placed in containers filled with seawater. Twenty five gravid *L. salmonis* were collected at location A, and 15 *C. elongatus* at location B. The sea lice were transported back to the laboratory in cooled seawater for hatching and further characterization.

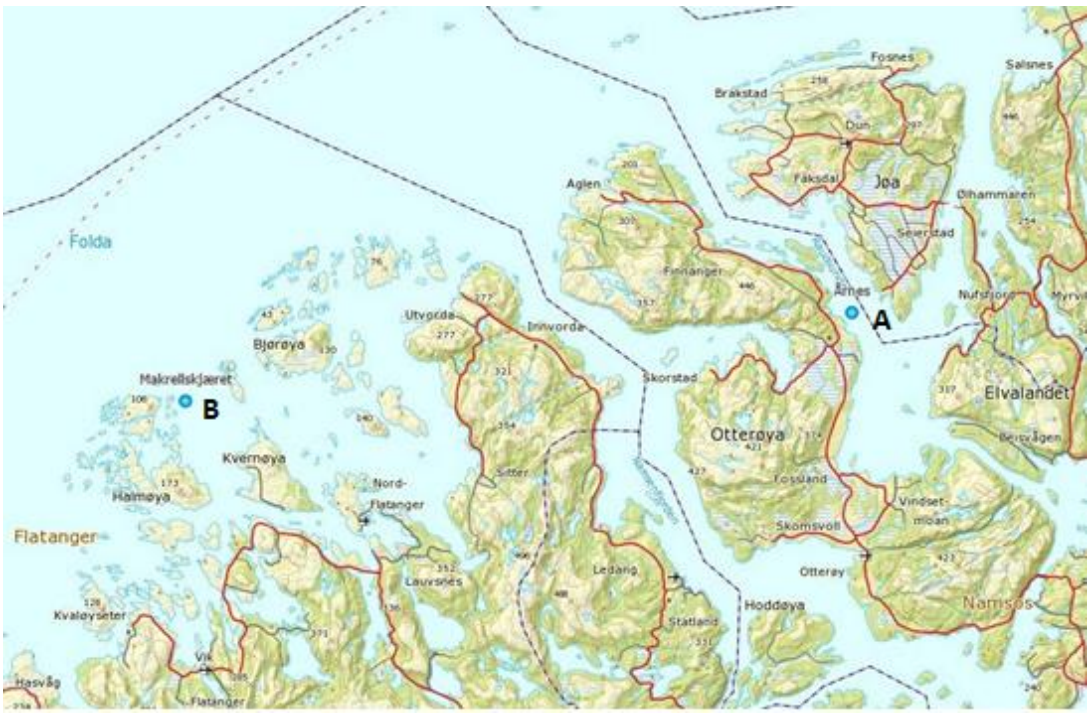

Figure 2.1 - Map of the two locations in Namsos and Flatanger region, localities A (Årnes) and B (Makrellskjæret) are marked with blue point (Fiskeridirektoratet).

2.1.2 Hatching and characterization of planktonic stages

At the lab, egg strings were detached from the female sea lice using a scalpel. Each egg string was placed in individual containers at 10 ºC, for hatching and maturation. The containers were filled with maturated and filtrated seawater, to reduce the risk of bacterial growth. Every day 50 % of the water was changed to maintain good water quality. The development of the egg strings and hatched larvae were observed in a stereomicroscope Leica MS05 C, 0.78-16.0x. Individuals of nauplius I, nauplius II and copepodids of *L. salmonis* were sedated with MS-222 for examination of pigmentation and length measurements of live individuals. Individuals of both *L. salmonis* and *C. elongatus* were fixated with 4-5% formaldehyde solution to be able to compare live and formaldehyde fixated specimens. Photos for length measurements were taken with Carl Zeiss Microscopy Gmbtt camera (2011) at 5.0 magnification. The software Zen Digital Imaging for Light Microscopy (2012) was used to conduct the length and width (greatest width in the middle of the body) measurements. The pictures measured later, were measured with the software ImageJ. Size measurement and photo work were done in cooperation with another master student; Henriette Ingebrigtsen.

2.2 Distribution of planktonic sea lice inside and outside sea cages

2.2.1 Sampling sites

Plankton tows were carried out at two different locations. Location C was a Måsøval Fiskeoppdrett AS salmon farm located at Bukkholmen, Frøya (N:63°43,432'E:8°54,749') and location D was a Lerøy Midt AS site situated at Hofsøya, Frøya (N:63°43,329'E:8°54,769'). The positions of the two farms are shown in Figure 2.2. These farms were chosen because they were located close to each other in an area known have sea lice infection.

Bukkholmen consisted of eight circular net pens, in two rows east-west direction. Five of the net pens contained salmon, and the fish were deployed in the sea from the period of March to May 2016. The total biomass ranged from 252 924 kg in June to 493 350 kg in August when the last samples were taken. Farm C used sea lice skirts, and as Farm D used no skirts, the influence of lice skirts could be studied. The lice skirts used at location C were a 10 meters deep tarpaulin with no water flow through the skirt. Hofsøya consisted of nine net pens, in two rows in east-west direction. There were salmon in all of the cages, and the salmonid were deployed in the end of March 2016. Total biomass at location D ranged from 759 094 kg in June 25 to 1548 889 kg in August.

Figure 2.2 - Map of the two locations in the Frøya region, localities C (Bukkholmen) and D (hofsøya) are marked with blue points (Fiskeridirektoratet).

Location C is positioned between two islands, Inntian to the east and Frøya to the west. The two islands seemed to give some shelter from waves and wind, and the location seemed more sheltered than location D, which was placed on the outside of the island Inntian (personal observation). The main current at both sites were from southeast to northwest.

There are no freshwater runoff in close proximity of the farms, thus it was expected that the salinities were relatively constant around 32-34 ppt. This was deduced from personal communication with employees at the company. At location C no sea lice treatment had been done prior to or during sampling. At location D, the whole farm had been treated with emamectin benzoate in week 24 and mechanical delousing in week 33 and 35.

2.2.2 Sea lice infections on fish farm sites

The fish farming company provided data on abundance of *L. salmonis* present on the salmon. The fish farm personnel counted sea lice on a minimum of 20 fish in each cages weekly, in accordance with legislated sea lice monitoring. *L. salmonis* present on the fish were grouped into gravid females, pre-adults and chalimus.

2.2.3 Sampling period

Plankton samples were taken in the summer of 2016 in two different periods, the first one in June and the second in August. Total number of plankton samples taken at each date, for the two locations, are summarized in Table 1. Plankton samples were taken at different times through in the washing cycle of a net; before, immediately after and 1 day after the nets were cleaned.

Table 2.1 -Overview of sampling dates and number of plankton samples taken at Bukkholmen and Hofsøya.

Date	Bukkholmen (C)		Hofsøya (D)	
	Inside	**Outside**	**Inside**	**Outside**
17.06.2016	-	-	2	2
20.06.2016	-	-	8	8
21.06.2016	-	-	8	8
24.06.2016	6	6	-	-
27.06.2016	14	14	-	-
28.06.2016	6	6	-	-
09.08.2016	6	6	-	-
10.08.2016	6	6	-	-
11.08.2016	6	6	-	-
14.08.2016	-	-	8	8
15.08.2016	-	-	6	6
18.08.2016	6	6	-	-

2.2.4 Sample collection

The presence and density of sea lice larvae at each site was investigated with samples from plankton tows. A standard conical plankton net with a 200 µm mesh size and a 50 cm mouth diameter was lowered to 7 meters depth and vertically pulled up to the surface at a speed approximately 1 m/sec. Samples were taken by hand from the floating collar of the sea cage. To get the direction of the current, a four-meter long thread with a weight attached to the end was lowered into the seawater, and the movement of the thread were observed. The samples were taken downstream of the cage, as shown in Figure 2.3.

Plankton samples was taken outside, and then on the inside of the net pen, in order to eliminate the possibility of disturbing the site before a sample were collected. Two tows were pooled for each sample, and two replica was taken at each site.

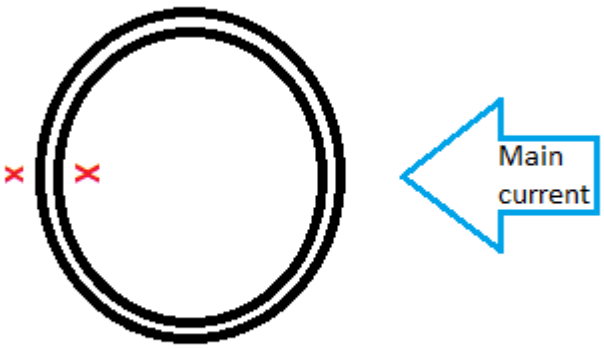

Figure 2.3 – Sampling position of the cage in relation to current, sample sites are indicated by red crosses.

Presence of phytoplankton in the sea water could clog the net at high densities, and the sample volume was corrected for this, using a digital Flow Meter with back-run stop (DC-Denmark) to detect the flow of water through the net. The flow meter was suspended 10 cm down in the middle of the net. Accurate sample volume was calculated for each sample according to equation 1.

$$\text{Nr. of revolutions x 0,3 x net opening area } (m^2) \text{x } 1000 = \text{water volume} \quad (1)$$

The average sample volume in June and August was 1.62 (±0.36) m^3 and 2.16 (±0.31) m^3 respectively. The variations in volume of plankton samples, were due to variation in the natural plankton densities, with most plankton in the spring, inhibiting water flow through the plankton net. Upon finishing a tow, the plankton net was washed down using seawater, in order to collect all the organisms in the removable cod end. The cod end was removed and the content was filtrated trough a 1300-µm sieve, to eliminate large materials. The plankton less than 1300 µm were transferred to square bottles, containing formaldehyde solution (sodium tetraborate 20 g/L formaldehyde) for fixation. After the plankton samples were added the concentration of formaldehyde solution were corrected to be 4-5 %. Samples were stored at 4.0 ºC until analysis. The samples were stored for a minimum of 10 days before they were analyzed.

2.2.5 Analysis and identification of plankton samples in the laboratory

At the lab, samples were washed trough a 500-µm sieve to remove coarse material and larger zooplankton. Then the remaining fraction of the samples was filtrated trough a 70- µm to retain the smaller fraction of the samples and washed to remove the formaldehyde solution. Filtrated material were diluted with seawater to 20-85 ml, dependent on the densities of the samples. At first, fractions of the sample were analyzed, but due to low amount of sea lice the whole samples were analyzed for the remaining samples. The samples were stirred with a pipette to distribute the organisms evenly before subsamples were transferred to 5 ml counting chambers. The bigger fraction (>500 µm) of multiple samples were analyzed for sea lice, to ensure no sea lice were lost in the filtering process.

Identification and counting were conducted using a stereomicroscope Leica MZ05 C (0.78-16.0x). Sea lice in each sample were counted, taken pictures of, and length and width were measured. The pictures were taken with a Carl Zeiss Microscopy Gmbtt camera (2011) at 5.0 magnification, and the length and width measurements were made in the program Zen Digital Imagining for Light microscopy (2012). The analyzed samples (<500 µm) and the bigger fraction (>500 µm) were preserved separate in 96 % ethanol. The nauplii and copepodids from the plankton samples were identified as sea lice larvae by using Practical identification of pelagic sea lice larvae by Schram (2012). It was not possible to distinguish between species, so the identification of sea lice only went as far as the family Caligidae.

2.2.6 Data analysis for calculating the amount of biofouling

To document the amount of biofouling on each pen, a waterproof camera was used, and videos of the net were taken on the inside and outside approximately one meter from the net. Pictures for further analysis were retrieved from the video by using Windows Movie Maker for Windows 10. The pictures from each cage were further used to quantify the amount of biofouling on the net, and each cage were placed into categories from null fouling to full fouling depending on the amount of biofouling on the pictures (Table2.2). For each picture a net grid with 60 random points were placed over the pictures (Figure 2.4) using the software Adobe Photoshop CC 2017. Number of points touching the biofouling on the net were counted, and the percent of points touching biofouling was calculated relative to the total number of points (60). From the percent of biofouling on the net, each pen were placed into categories. The areas from each pen investigated were 37.65 (SD± 31.55) cm^2 (Appendix III).

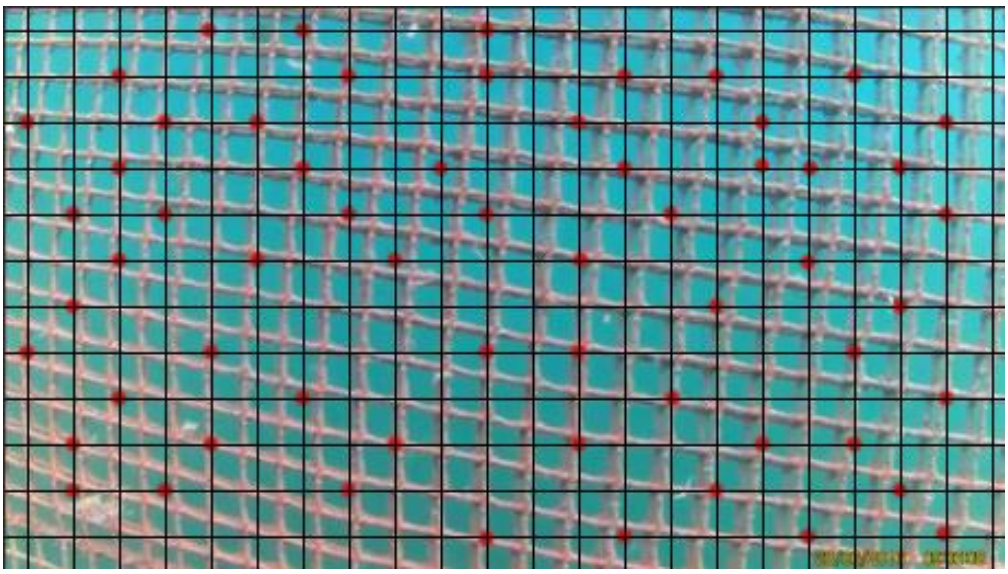

Figure 2.4 - Demonstration of how the amount of biofouling were measured. Black net grid, with 60 random points (red dots).

Table 2.2 - Categorization of percentage biofouling.

Nr	Category	Biofouling (%)
1	No Fouling	0-4
2	Low fouling	5-25
3	Medium fouling	26-40
4	High fouling	41-80
5	Full fouling	81-100

2.3 Processing of data and statistical analysis

The program SigmaPlot version 13.0 for Windows was used for statistical analyses and creation of graphs. The statistical analysis for the plankton survey data was conducted using the software programming language for statistical computing and graphics R, version 3.3.3 trough RStudio, 1.0.143. Tables were created in Microsoft Word for Windows, and the initial data processing and transformation were performed in Microsoft Excel for Windows. Unless otherwise specified, the significant level is set to p=0.05, and if not otherwise specified, values are given with corresponding standard deviation (±SD).

2.3.1 Length and width measurements

A Shapiro-Wilk test was used to test for normality of size data, at significant level of $P \leq 0.05$. To test for equality of variance between the measurements a Brown-Forssythe test was used, also with a significant level of $P \leq 0.050$. The data showed a non-normal distribution and the sample sizes were low. Therefor a non-parametric test was chosen to compare the significant differences in size distribution in different groups. A Mann-Whitney test are an alternative test to the independent sample t-test (McKnight & Najab, 2010). It is non-parametric test and was used to test whether two sample means was equal or not.

2.3.2 Distribution of planktonic sea lice

The calculated densities for each sample used in this master thesis, are the average of the two parallel samples. The average and standard deviation for each sample can be found in appendix III. The distribution data were shown to be over-dispersed, and followed a negative binomial distribution.

Count data with volume as offset variable were used in a general linear model. Since negative binomial distribution and poisson distribution are both used for count data (Hinkelman, 2012), it was tested if the data fitted both distributions. The poisson model assumes that the variance of the data are equal to the mean, and were shown not to fit the data by a Goodness of fit test (chi-square, $p>0.05$). By using a chi-square test the negative binomial distribution were shown to fit the data well with a Goodness of fit with higher than $p<0.05$. The key properties for this distribution is that it expects the sample variance to exceed the sample mean (Hinkelman, 2012), thus fitting this data well. The negative binomial distribution were used when- analyzing the relationships and significance between variables.

3 Results

3.1 Characterization of *L. salmonis* and *C. elongatus* larvae

3.1.1 External morphology and pigmentation of live pelagic sea lice larvae

Live *L. salmonis* are oval and almost transparent, with some black and brown pigmentation. Nauplius I has brown pigmentation on the middle of the body, and some around the eyes. They have black pigmentation around the eyes and at the posterior end of the body (Figure 3.1, 1a). Nauplius II have a more slender body shape and less brown pigmentation (Figure 3.1, 2a). They are more heavily pigmented with black pigmentation around and between eyes. Black pigmentation can also be seen as two C-shaped figures dorsally. In later larvae, black pigmentation (3-4 band) can be seen on the posterior end of the nauplii. The copepodid are heavily pigmented around the eyes, and have two contiguous black pigmented eyes (Figure 3.1, 3a).

Live *C. elongatus* are also oval and almost transparent with red and yellow pigmentation. The Nauplius I have red pigmentation placed along the sides of the body, in addition to at the anterior and posterior end, like shown in Figure 3.2 (1a). The nauplius II have the same red pigmentation dorsally and ventrally as the nauplius I. The pigmentation alongside the body is divided into six patches, three on each side of the body (Figure 3.2, 2b). The copepodid shows the same pigmentation patterns as Nauplius II, and the eyes are dark pigmented and easily seen (Figure 3.2 3a). When fixated with 4 % formaldehyde solution all pigmentation disappeared from both species, it were only possible to see a small difference in body shape between nauplius I and nauplius II (Figure 3.1 1b, 2b, 3b & Figure 3.1 1b, 2b, 3b).

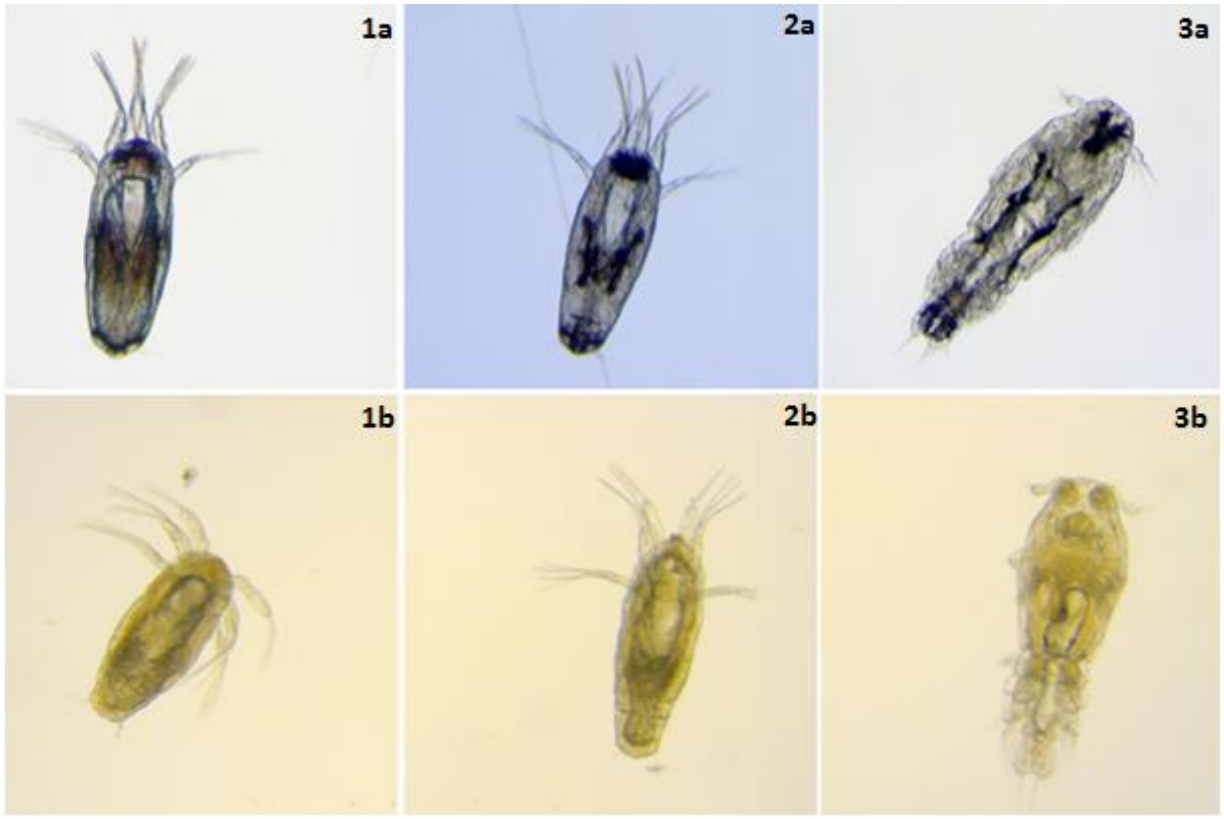

Figure 3.1 - Pigmentation of planktonic stages of *L. salmonis* 1: nauplius I, 2: nauplius II, 3: Copepodid, a: live, b: fixated in formaldehyde.

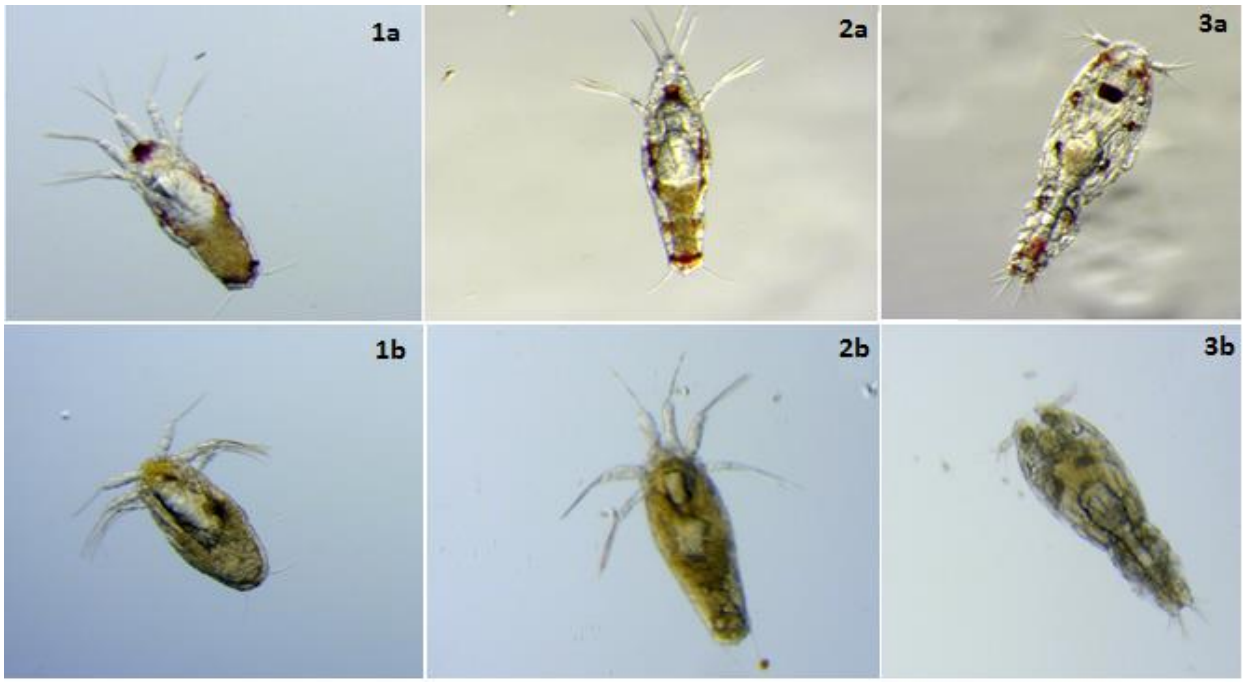

Figure 3.2 - Pigmentation of planktonic stages of C. elongatus where 1: nauplius I, 2: nauplius II, 3: Copepodid, a: live, b: fixated in formaldehyde.

3.1.2 Length and width of pelagic stages

Length and width of pelagic *L. salmonis* and *C. elongatus* (live and fixated) are shown in table 3.1. Live *L. salmonis* did not show a significant increase in length from nauplius I to nauplius II ($p>0.05$), but showed a significant increase in length from nauplius II to copepodid stage ($p<0.001$). The nauplius II stage was the slimmest stage, where both nauplius I and copepodid were found to be significantly broader ($p<0.001$). *C. elongatus* showed a significant increase in length from nauplius I through nauplius II ($p<0.001$) to the copepodid stage ($p<0.001$). The width of *C. elongatus* did not show a significant increase from the nauplius I stage to the nauplius II stage ($p>0.05$), but the copepodid were significantly longer than the two previous stages ($p<0.001$).

The overall length and width of all the live planktonic stages of *L. salmonis* were somewhat greater than for *C. elongatus*. The length and width of live *L. salmonis* Nauplius I were significantly longer than of *C. elongatus* (both $p<0.001$). The *L. salmonis* nauplii II was slightly longer and wider than *C. elongatus* but this difference was not significant ($p>0.05$). The copepodid of *L. salmonis* was significantly longer ($P<0.05$) and wider ($P<0.001$) than *C. elongatus*.

Table 3.1– Length and width (µm) of nauplius I, nauplius II and copepodids of *L. salmonis* and *C. elongatus*, alive and fixated on formaldehyde.

	Nauplius I		**Nauplius II**		**Copepodid**	
	Live (µm)	***Fixated (µm)***	***Live (µm)***	***Fixated (µm)***	***Live (µm)***	***Fixated (µm)***
L. salmonis						
Length ± SD	515±14	467±10	526±21	527±12	696±22	687±18
With ± SD	211±7	198±8	188±8	193±6	244±6	250±12
(N)	(29)	(15)	(18)	(20)	(10)	(9)
C. elongatus						
Length ± SD	470±10	380±12	519±14	498±13	673±14	667±14
With ± SD	185±10	155±6	186±6	181±6	223±8	233±7
(N)	(11)	(14)	(12)	(17)	(12)	(11)

Individuals fixated on formaldehyde showed shrinking for some of the life stages in both species. The shrinkage were most apparent in the nauplius I stage, and was significant for both length (48 µm, 90 µm, $p < 0.001$) and width (13 µm, 30 µm, $p < 0.001$) for *L. salmonis* and *C. elongatus* respectively. No significant shrinkage was found in *L. salmonis* nauplius II, which were found to increase in width fixated on formaldehyde ($P<0.05$) compared to live individuals. The *C. elongatus* nauplius II showed a significantly shrinkage in length ($p<0.001$), but not width ($p>0.05$). No statistically significant differences were observed in *L. salmonis* copepodids. The same was found for *C. elongatus* copeopdids, but here the fixated specimens were wider than the live individuals (10 µm, $P< 0.05$).

3.2 Distribution of planktonic stages inside and outside sea cages

3.2.1 Temperature measurements

The personnel working at the fish farm measured the sea temperature every week at 5 meters depth at both locations (Figure 3.3). The temperatures in the sea were approximately the same at both localities, in both June and August. In June, the water temperature ranged from 9-11 °C and in August the water temperature were higher, ranging from 13-14 °C.

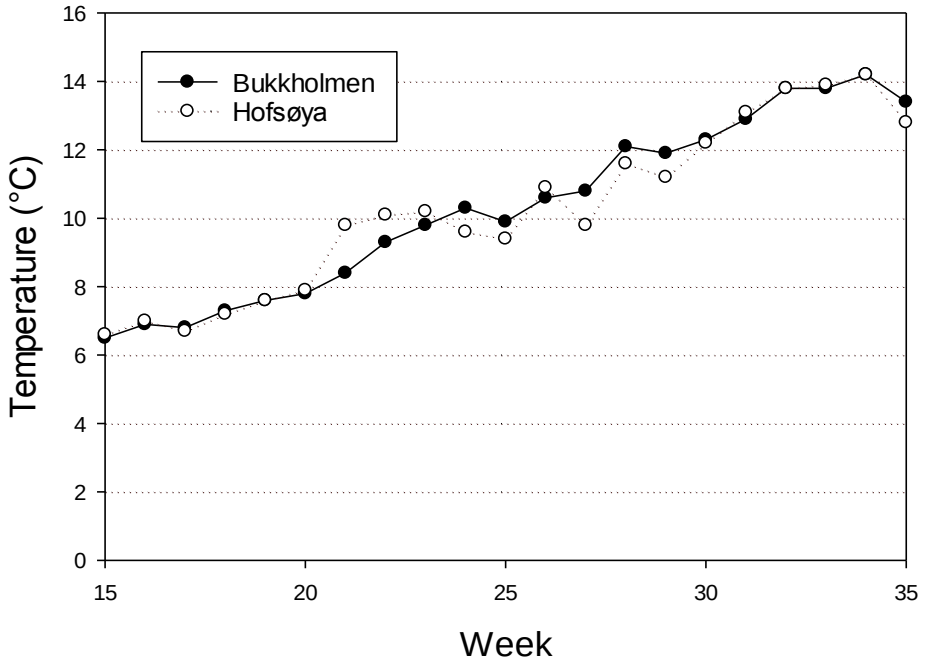

Figure 3.3 - Weekly sea temperature measurements from week 15-35 in 2016.

3.2.2 Biofouling

Figure 3.4 shows the biofouling category of the net before, immediately after and one day after washing. The amount of biofouling of the net was only documented at Bukkholmen (C). The fish cage netting had a low biofouling coverage at both sites during the sampling periods, and only one cage were categorized to full fouling. Before wash, the nets ranged from no fouling to full fouling, with most of the nets categorized to medium fouling. Cleaning of the nets with high water pressure were an effective way to dislodge the biofouling, and after washing the nets were categorized to low degree of fouling to no fouling at all ($p<0.001$). One day after wash, the nets were in the same condition as immediately after washing. Illustration on the amount of biofouling on nets in each category can be seen in Appendix I.

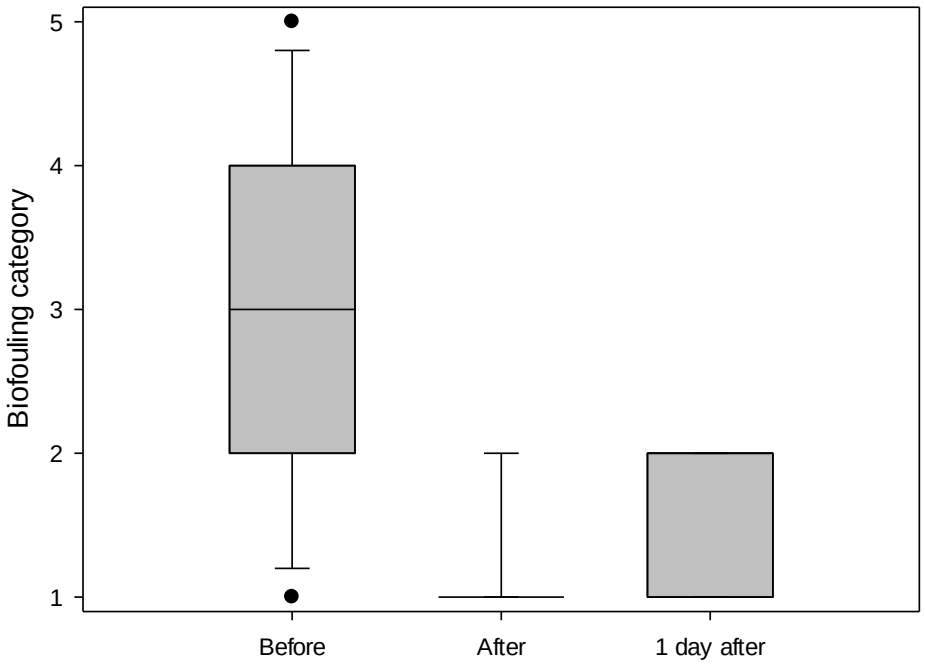

Figure 3.4 - Amount of biofouling on the net before, after and one day after wash. The boxplots shows the maximum and minimum values (whiskers), the lower and upper quartiles (box), the median (horizontal line) and outliers (dots).

3.2.3 Number of Sea lice on farm

The fish farm employees counted the number of attached *L. salmonis* on the salmon at each locality every week. Figure 3.5 and 3.6 shows number of attached salmon lice, sampling periods, and which week the localities were treated against sea lice for locality Bukkholmen and Hofsøya, respectively. Number of gravid salmon lice was below the threshold level of 0.5 gravid lice per fish through the period of sampling on both farms. In general, there were more attached *L. salmonis* in all stages at both sites in August compared to June ($P<0.05$). The number of gravid *L. salmonis* present on the fish was also higher at Hofsøya (D), compared to Bukkholmen (C) which used skirts, both in June ($p<0.05$) and in August ($p<0.01$). Gravid salmon lice present at Bukkholmen (C) were estimated (based on number of salmon in cages and sea lice counts) to be 60 000-67 000 individuals in June, and 140 000-67 000 in August. At Hofsøya (D) gravid salmon lice present on salmon at farms were estimated to be 180 000-115 600 individuals in June and 240 000 in August. The number of *C. elongatus* on fish at Bukkholmen is shown in Table 3.2. At Bukkholmen no sea lice treatment were carried out prior to or during sampling. Hofsøya were treated with emamectin benzoate in week 24 and mechanical delousing in week 33 and 35, both times the whole farm was treated.

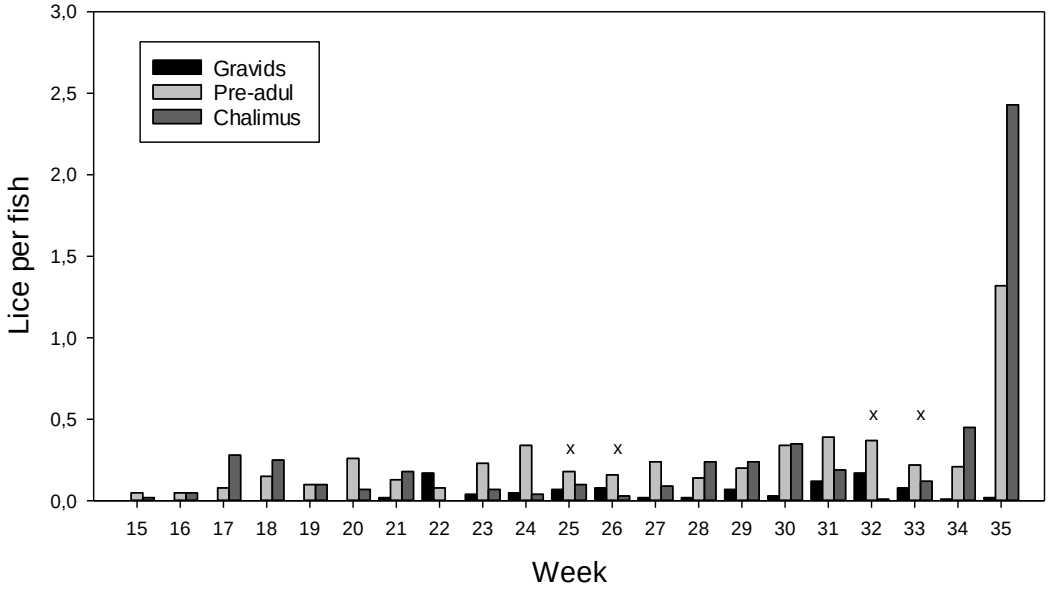

Figure 3.5 - Number of Sea lice *(L. salmonis)* (gravids, Pre-adult, Chalimus) per fish present at Bukkholmen fish farm from week 15 to week 35. The weeks when the plankton samples were taken at the fish farm are marked by x.

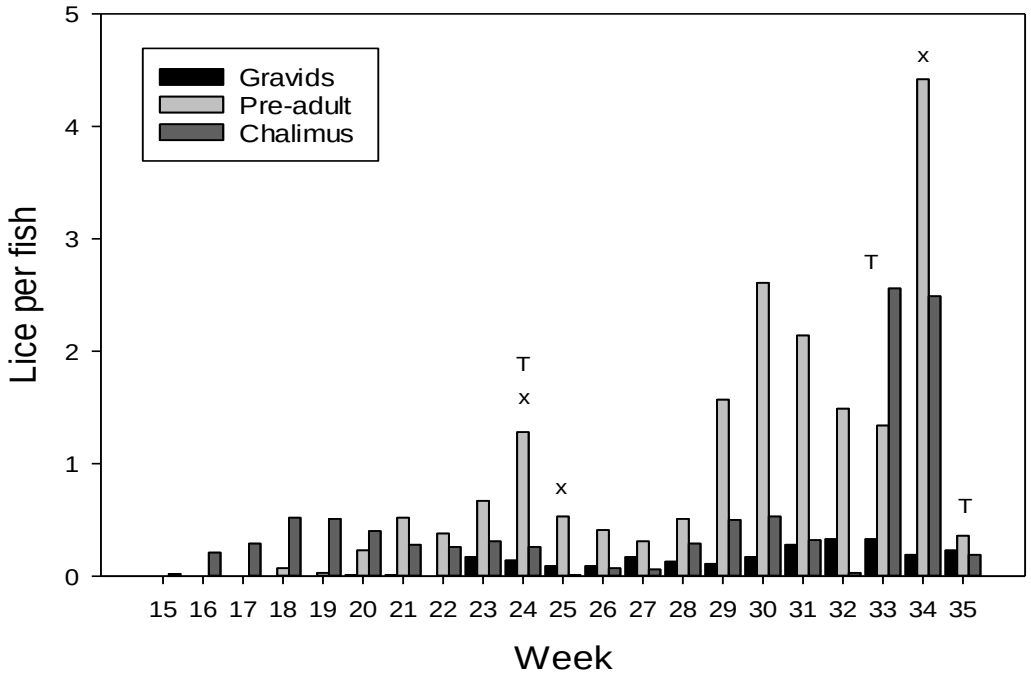

Figure 3.6 - Number of Sea lice (*L. salmonis*) (gravids, pre-adult and chalimus) per fish present at Hofsøya fish farm from week 15 to week 35. Weeks when plankton samples were taken at the fish farm are marked by x and time of lice treatment (delousing) are marked by T.

Table 3.2 - Average number of attached *C. elongatus* fish at Bukkholmen (C).

Attached C. elongatus all stages		
Week	**Lice per fish**	**SD**
25	0.14	0.232916
26	0.05	0.111803
32	0.266	0.282896
33	0.668	0.82781

3.2.4 Abundance of planktonic sea lice

The number of sea lice in the plankton tows were used to calculate the concentration of sea lice larvae (Ind. m^{-3}) in each sample. The nauplius was the dominant stage of planktonic sea lice (Figure 3.9) at both locations. The relative proportion of planktonic sea lice stages at Bukkholmen showed that 98.7% of the lice retrieved were nauplius, and only 1.3% copepodids. At Hofsøya the proportion of planktonic sea lice stages were 80 % nauplius and 20 % copepodids. All copepodids were found on the inside of the net. The site with most planktonic sea lice were Bukkholmen and accounted for 90.5% of the total nauplii found in all the plankton samples. Only 9.5% of the individuals were found at Hofsøya. Because almost no copepodids were found, the following results apply mainly to the nauplii stages.

The variation within the measured densities of planktonic sea lice in the different samples were high at both sampling sites, ranging from 0-22.25 ind. m^{-3}. There were mainly observed low densities of planktonic sea lice, where 64% of the samples contained no planktonic sea lice. The average density found at the fish farms were 0.80(±2.57) m^{-3}. Density of planktonic sea lice in each sample can be seen in Appendix IV.

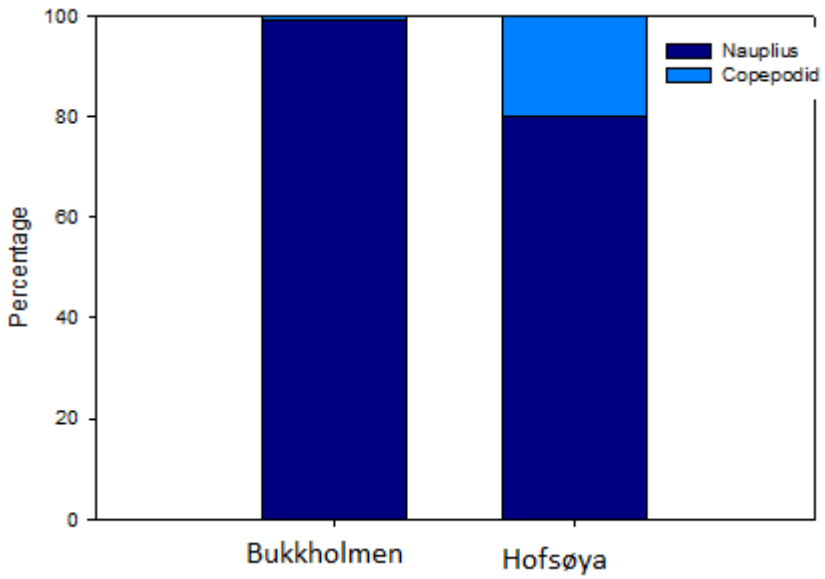

Figure 3.7 – Relative proportion of nauplii and copepodids of total planktonic sea lice at Bukkholmen and Hofsøya.

3.2.5 Size distribution of planktonic sea lice at fish farms

Figure 3.3 shows the length and width distribution of the planktonic sea lice retrieved in the plankton tow. The nauplii retrieved, ranged from 353-621 µm in length and 149-242 µm in width. Copepodids ranged from 569-750 µm in length and 208-243 in width.

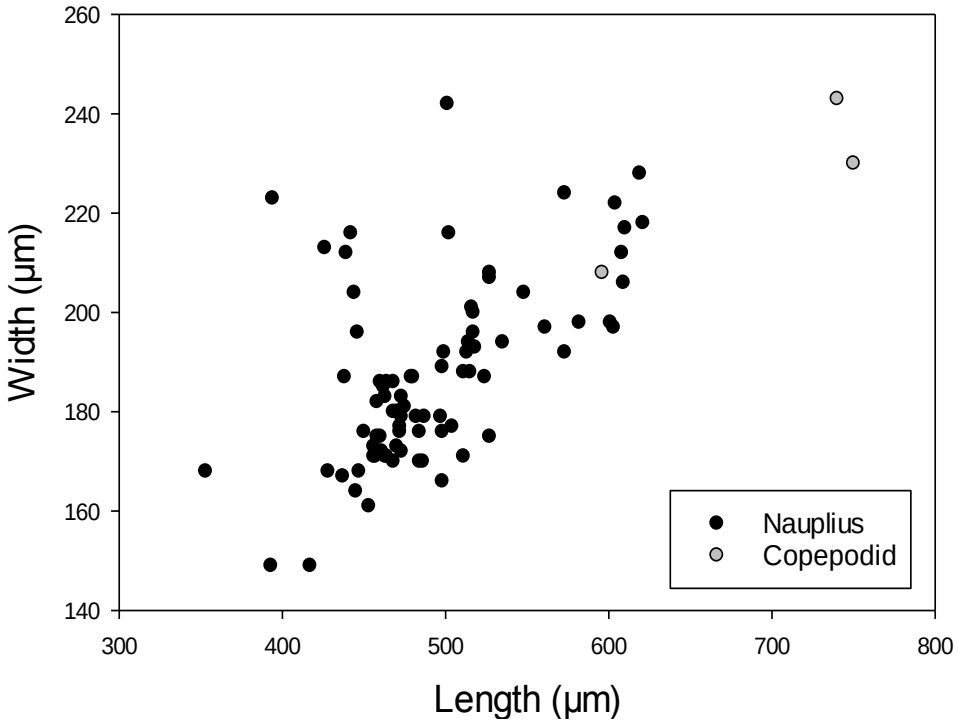

Figure 3.8 – Scatter plot of length and width measurements of the individuals found in the plankton samples.

3.2.6 Larval densities on the inside and outside of the cage

Planktonic sea lice larvae were found on the inside and outside of the net pen on both sites. Figure 3.8 shows the density of planktonic sea lice on the inside and outside of the pen for all samples. A Mann-Witney Rank Sum Test revealed a statistical significant difference in density of sea lice on the inside and outside of cages, with a significant higher density of sea lice on the inside (1.52 ± 3.58m^{-3}) of the cage compared with the outside (23 ± 0.47 m^{-3}), (P<0.01).

The difference in planktonic sea lice densities on the inside and outside of the pen at Bukkholmen are shown in Figure 3.9. There was a statistically significant (Mann-Whitney test, P=<0.001) higher concentration of sea lice on the inside compared to on the outside of the net, with an average concentration of 2.26 (±4.38) larvae m^{-3} and 0.14 (±0.39) larvae m^{-3}, respectively. At Hofsøya the average density on the inside were 00.17(±0.34) larvae m^{-3} and 0.25 (±0.10) larvae m^{-3} on the outside. At Hofsøya, no significant difference in density of planktonic sea lice on the inside compared to the outside was found (Figure 3.10).

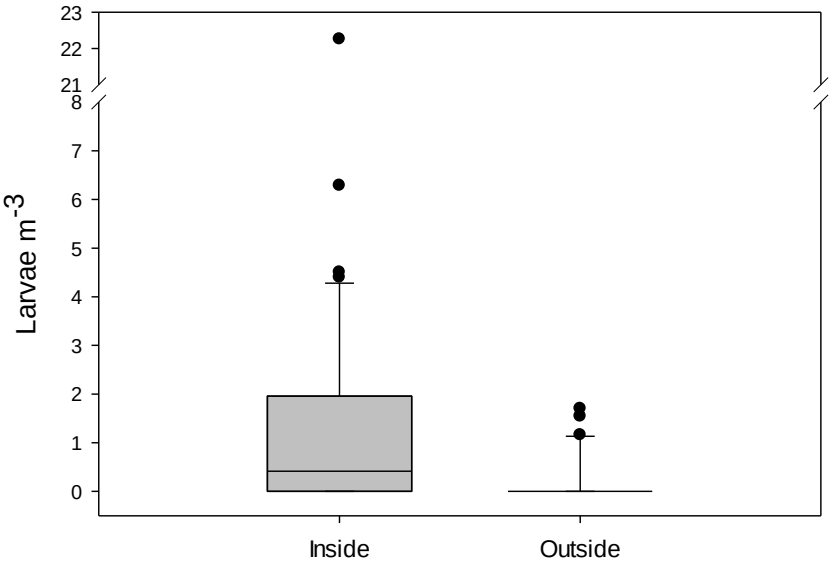

Figure 3.9 - Boxplot showing the density of planktonic sea lice (m^{-3}) found in the plankton samples on the inside and outside of the net pen. The boxplot shows the lower and upper quartiles (box), maximum and minimum values (whiskers), the median (Horizontal line inside the box) and outliners (dots).

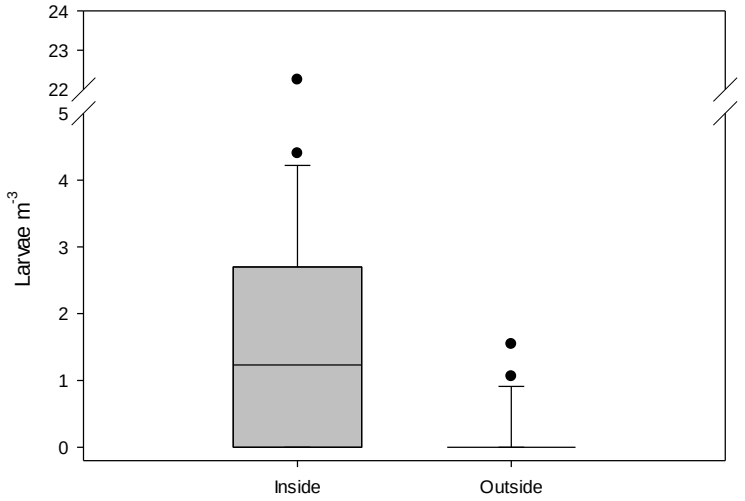

Figure 3.10 - Difference in average abundance of planktonic sea lice larvae found in the plankton samples on the inside and outside of the net pen at Bukkholmen. The boxplot shows the lower and upper quartiles (box), maximum (and minimum) value (whiskers), the median (Horizontal line inside the box) and outliners (black marks/dots).

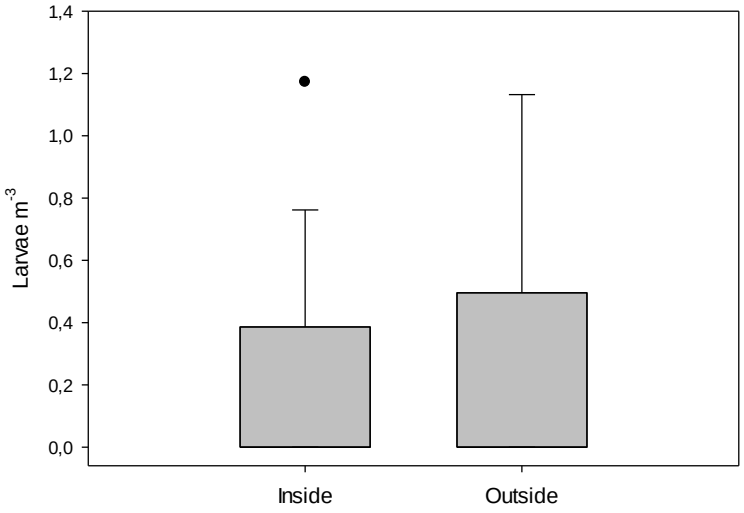

Figure 3.11 - Difference in average abundance of planktonic sea lice larvae found in the plankton samples on the inside and outside of the net pen at Hofsøya (D). The boxplot shows the lower and upper quartiles (box), maximum (and minimum) value (whiskers), and the median.

3.2.7 Effects of biofouling on distribution of sea lice

Planktonic sea lice were found inside the cages for every biofouling category at Bukkholmen (Figure 3.13). Although no statistically significant difference in density of sea lice inside the cages in relation to biofouling were found ($p>0.05$), there was observed a trend where more lice was retained inside of the cage at higher biofouling. The planktonic sea lice densities on the outside of the cage did not show any significant differences in relation to fouling on the net ($p>0.05$).

Cages with low amount of biofouling showed a significantly higher amount of sea lice on the inside of the cage, compared to the outside ($p<0.050$), the cages in the other biofouling categories did not show any significant differences in planktonic sea lice densities between inside and outside ($p>0.050$).

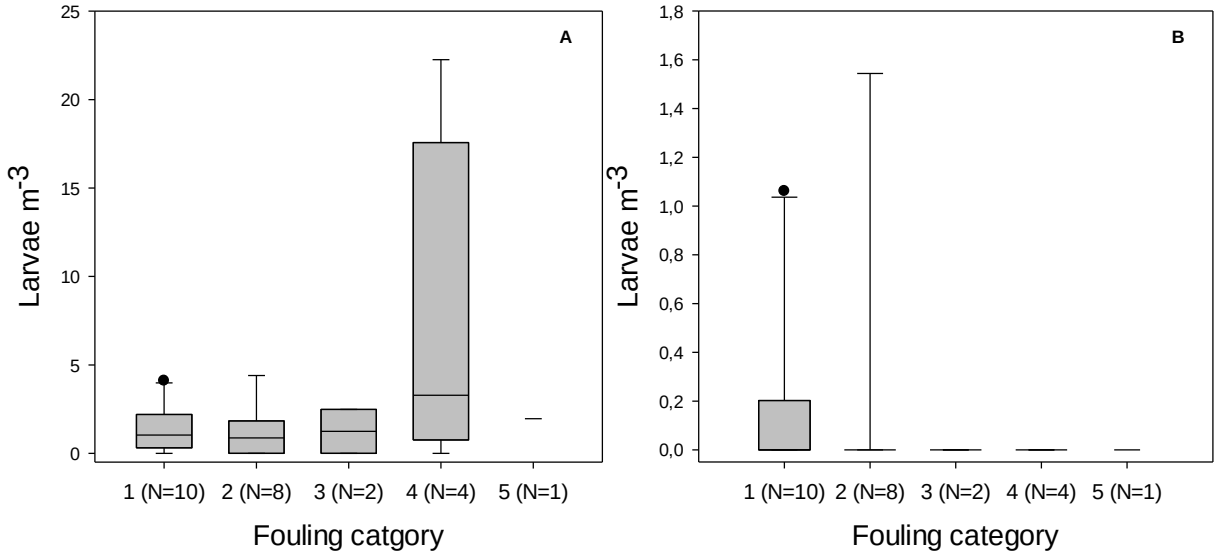

Figure 3.12 - Boxplot of the density of planktonic sea lice (m^{-3}) found in the plankton samples related to biofouling category of the net. The boxplot shows the lower and upper quartiles (box), maximum (and minimum) value (whiskers), the median (Horizontal line inside the box) and outliners (black marks/dots). A: Inside, B: Outside.

3.2.8 Effect of washing of the net on dispersal of sea lice

At Bukkholmen sea lice larvae were more abundant on the inside of the cage throughout the whole washing cycle ($p<0.05$) (Figure 3.11). The data suggests that the planktonic sea lice densities were a little lower on the inside of the cage immediately after wash, but no significant differences in densities in relation to washing cycle were found ($p>0.05$). It did not appear to be any changes in larvae densities of sea lice on the outside of the net throughout the washing cycle (Figure 3.11).

At Hofsøya the density on the inside of the cages were somewhat higher immediately after wash, compared to before and one day after wash, but there were no statistically significant differences ($p>0.05$). On the outside, no apparent differences in planktonic sea lice densities related to washing of the cage netting were detected (Figure 3.12).

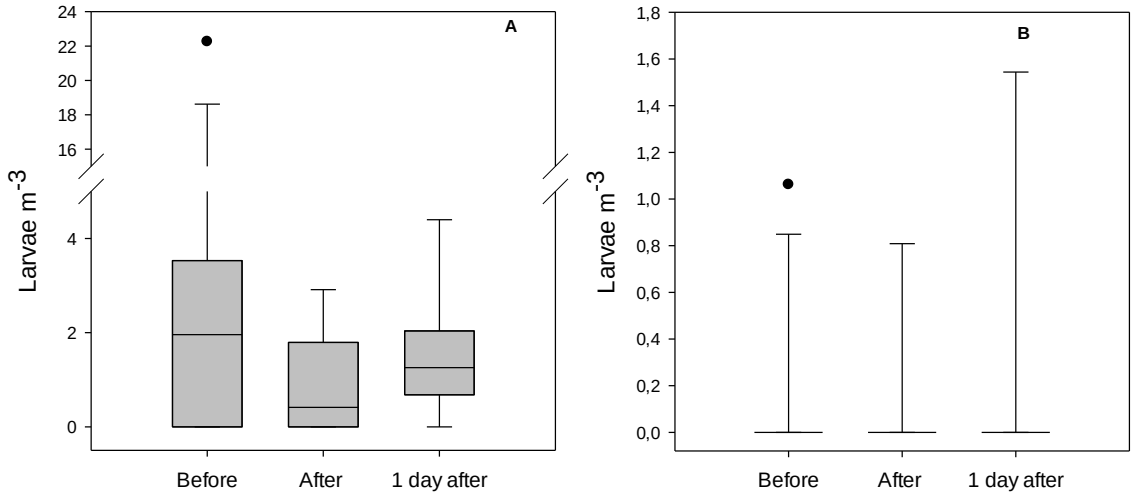

Figure 3.13 - Boxplot of the density of planktonic sea lice (m^{-3}) found in the plankton samples on the inside and outside of the net pen at Bukkholmen. The boxplot shows the lower and upper quartiles (box), maximum (and minimum) value (whiskers), the median (Horizontal line inside the box) and outliners (black marks/dots). A: Inside, B: Outside.

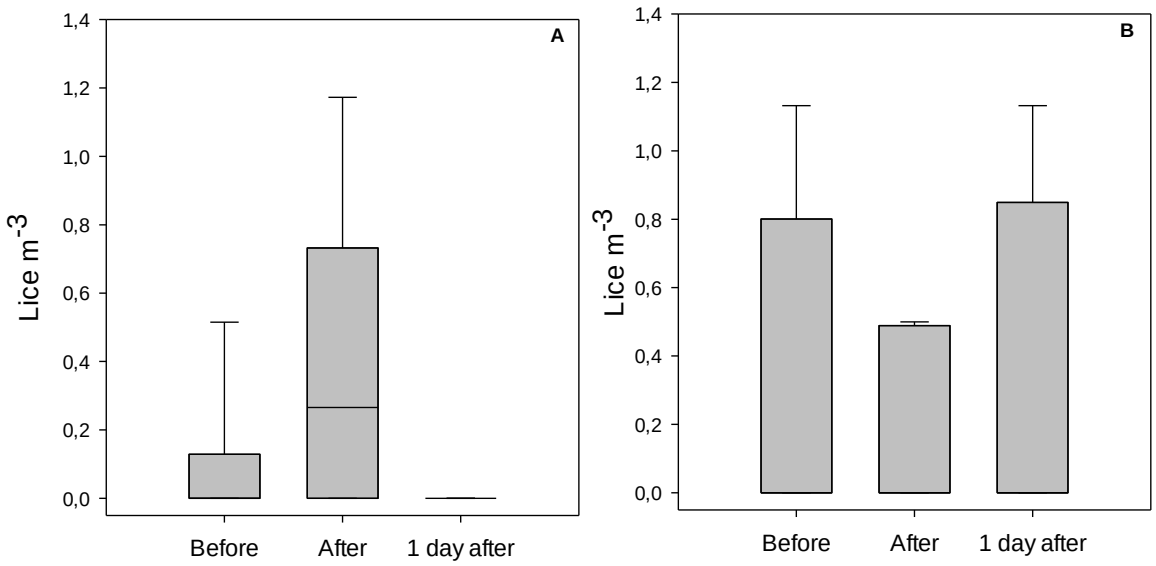

Figure 3.14 - Boxplot of the density of planktonic sea lice (m^{-3}) found in the plankton samples on the inside and outside of the net pen at Hofsøya. The boxplot shows the lower and upper quartiles (box), maximum (and minimum) value (whiskers), the median (Horizontal line inside the box) and outliners (black marks/dots). A: Inside, B: Outside.

4 Discussion

4.1 Overview

The goal for this thesis was to investigate how the net caging and associated biofouling affected the distribution and density of planktonic stages of sea lice inside and outside of sea cages at fish farms. The sample collection was done at two fish farm facilities with different practices and therefore considered as two different cases, instead of two replicates.

The findings demonstrate that the pigmentation color and pattern of *L. salmonis* and *C. elongatus* is a key factor in identification of live pelagic larvae. When fixated on formaldehyde (4%), the pigmentation was lost, and length and width measurements were not safe enough to distinguish between the two different species. The nauplii stages were the most abundant planktonic life stages near fish farms. The highest density of planktonic sea lice were consistently found inside sea cages wearing skirts, here the sea lice were retained to a strong degree on the inside of the cages. At cages not wearing skirts, there was not found clear evidence of sea lice retaining inside sea cages, and the density of sea lice were approximately the same on the inside and outside of the cage. The net alone may hinder the transport of planktonic sea lice out of the net, but not to a very high degree. However, high amount of fouling on the net could delay dispersal of sea lice out of the net. There was not observed any elevation in density of sea lice in the water column immediately after washing the net, and based on this it is not likely that cleaning activities could be a source of infection by releasing pre-infective and infective larvae.

4.2 Identification of planktonic sea lice

4.2.1 Hatching and characterization of planktonic stages

The hatching of *L. salmonis* and *C. elongatus* in the laboratory showed that the two different species had characteristic pigmentation, which differed in both pattern and color. *L. salmonis* had black and brown pigmentation, whereas *C. elongatus* had red pigmentation (Figure 3.1 and 3.2). There were observed some variation in pigmentation of individuals of both species, but the general pigmentation patterns presented in results were constant for all individuals in the presented stage. The body shape also showed some individual differences within stages of both species, especially in nauplius I, where they were more round and egg shaped immediately after

hatching compared to later on in the stage. However, when pigmentation pattern and body shape were combined they were distinct enough to differentiate between nauplius I and nauplius II, for both species. In live nauplii and copepodids, the color of pigmentation was shown to be a good way to distinguish between the two species, and nauplius stage within species. These findings are in accordance with Scham (2004), where color of pigments and its distribution were listed as a key factor in identification of pelagic stages of caligids. When the planktonic lice stages were fixated on formaldehyde, fading of pigmentation was observed, and the characteristic pigmentation was lost (Figure 3.1 and 3.2).

The length and width measurements in the hatching experiment showed the same trend as size data of the planktonic *L. salmonis* and *C. elongatus* from other publications (Johnson & Albright, 1991b; Piasecki, 1996; Pike & Mordue *et al.*, 1993; Schram, 1993, 2004). *L. salmonis* was larger than *C. elongatus*, and the sea lice became longer in each stage. *L. salmonis* nauplius I and copepodid stage were in this study found to be statistically longer and wider than equivalent stages of *C. elongatus* ($p<0.5$). In the nauplius II stage *L. salmonis* were also longer and wider, but here the difference was not significant. The results from this study shows that there are size differences between species (Table 3.1), however the differences in size were not always significant ($p>0.05$), and between some stages the differences were not as apparent.

Comparing present size measurements with published size measurements it comes apparent that size vary between different studies (Johnson & Albright, 1991b; Piasecki, 1996; Pike & Mordue *et al.*, 1993; Schram, 1993, 2004). The most apparent difference is that *L. salmonis* nauplius II stage in present study are smaller than previous publications. As the studies are conducted in different countries and possibly, at different seasons, variation between studies could be because the size of planktonic stages of sea lice can be affected by environmental parameters, thus vary with season and location (Gravil, 1996; Pike & Wadsworth, 1999; Schram, 1993). The variation in published and present data, suggest that size measurements alone could be an unreliable way to distinguish between species, even if the specimens are live and fresh. These findings are in accordance with Schram (2004), which concluded that the dimension of sea lice nauplii and copepodid stages are overlapping, and that size data is not a reliable mean to differentiate between species.

Shrinkage of several organisms fixated on formaldehyde has been recorded, and in copepodids formaldehyde have been shown to cause a 20 % weight loss (Hopkins, 1968; Omori, 1969, 1978; Põllupüü, 2007; Thibault-Botha & Bowen, 2004). When marine individuals are fixated on formaldehyde solution (4%) the osmotic pressure are over twice as high as in sea water, and

shrinkage appear because the osmotic pressure drive water out of the organism (Steedman, 1976; Thibault-Botha & Bowen, 2004). In this study there were found some shrinkage in the planktonic sea lice, but the shrinkage were not consistent for all stages (Table 3.1). Nauplius I stage of both species showed a significant shrinkage when fixated on formaldehyde. In the nauplius II stage however, only the length of *C. elongatus* showed a significant shrinkage. The copepodid stage did not seem to be affected by formaldehyde, and no shrinkage was observed in both species.

The formaldehyde-induced shrinkage of individuals in this study seemed to decrease as the sea louse developed through the planktonic stages. The reason for this may be that the sea lice shell becomes more rigid after each molt and as the larvae grows the more rigid shell hinder water to diffuse out of the larva. Another reason for no consistent shrinkage could be due to the use of different individuals for size measurements in live and fixated animals. Planktonic sea lice larvae grow within each stage (Piasecki, 1996; Pike & Mordue *et al.*, 1993), and if individuals for size measurements were taken at different times within each stage the initial size would vary. Because the individuals fixated on formaldehyde and the live ones were not the same, individual size differences prior to fixation may have affected the results.

4.2.2 Comparison of hatched nauplii with nauplii in plankton samples

The pelagic stages of *L. salmonis* and *C. elongatus* were morphologically very similar, and the hatching experiment showed that pigmentation color and pattern would be the best characteristic to differentiate between species and nauplii stages. However, the plankton samples were preserved on formaldehyde, and the planktonic sea lice larvae found in the plankton samples had lost their color and pigmentation pattern. During analysis, some small differences in body shape were observed in both nauplii and copepodids from the plankton sample. Due to the lack of pigment, it was not possible to distinguish between the two probable species, even with shape differences present.

Figure 4.1 shows the size and width measurement of all nauplii retrieved in the plankton samples compared to individuals from the hatching experiment. The hatching experiment showed that there were size differences between the planktonic stage of *L. salmonis* and *C. elongatus*. However, nauplii from the plankton tow varied greatly in size, and it was not possible to differentiate between the species in the plankton tows based on their size. Even though size

measurements were not a reliable source to differentiate between the species and nauplii stages, they could give an indication of which species and stages present in the plankton tows. The nauplii from the plankton tow ranged from 353-621 µm in length and 149-242 µm in width. This size range could possibly be a product of the presence of both species in the water column. Sea lice data from Bukkholmen showed the presence of *L. salmonis* and *C. elongatus*, so it is possible that planktonic sea lice in the water column could belong to both species. Some of the individuals are also relatively large, and exceeds the size measurements of all hatched sea lice. Other publications have found a mean length of *L. salmonis* nauplii II to be ~ 600 µm (Schram, 1993), and the larger individuals may be *L. salmonis* nauplii II.

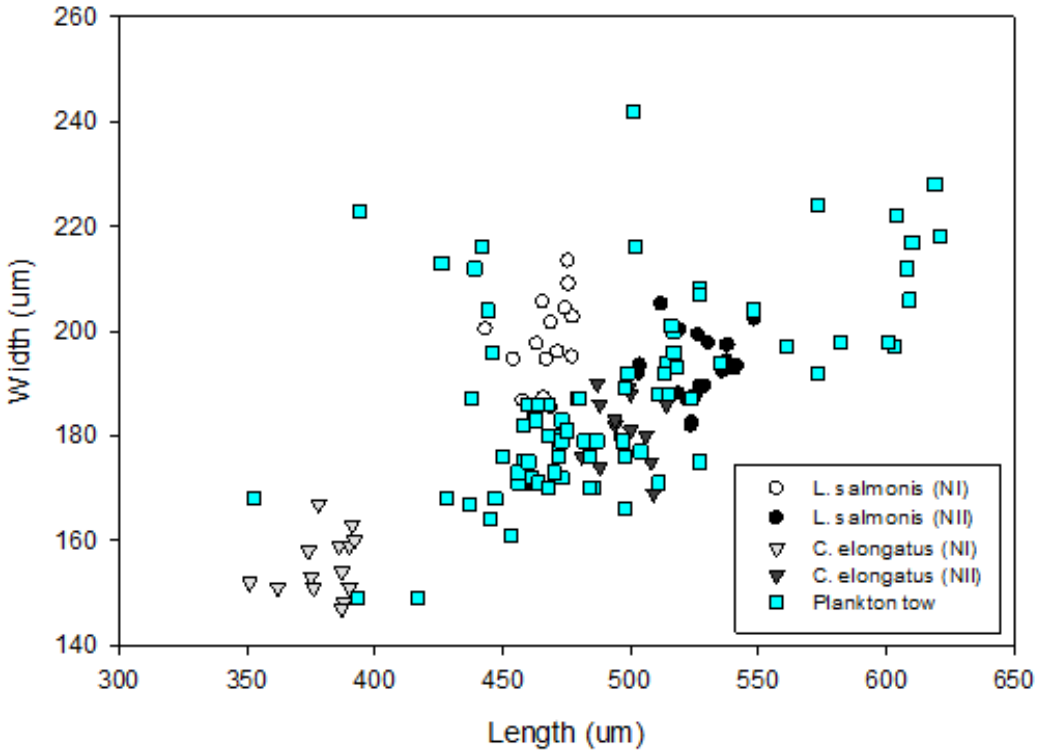

Figure 4.1 - Length and width measurements of *C.elongatus* and *L. salmonis* NI and NII hatched at the lab and fixated on formaldehyde compared to the length measurements from the individuals found in the field.

Formaldehyde buffered with borax is regarded as the best general fixative for plankton samples when for taxonomic and morphological purposes (Harris & Wiebe *et al.*, 2000; Low, 1977;

Schram, 2004). The reason for using buffered formaldehyde in this thesis was to preserve the planktonic sea lice, and keep the pigmentation for the longest time. However, the pigmentation was lost during fixation and formaldehyde (4%) was not beneficial, as the easiest way to identify sea lice nauplii would be through their pigmentation. Other studies, which were able to determine the species using stereomicroscopic investigations, used 99% ethanol as preservation, and the samples were counted within a week (á Norði & Simonsen *et al.*, 2016; Noroi & Simonsen *et al.*, 2015). To see if ethanol was a better fixative in regard of preserving pigmentation, some plankton samples on ethanol (99%) was investigated (See Appendix II). In the plankton samples preserved on ethanol the pigmentation seemed to be better preserved, and for future work it would be beneficial to use ethanol instead of formaldehyde if the purpose is to differentiate between species. Danielsen (2014) proposed that fixation with formaldehyde (8-10%) for one day only, before preserving the plankton samples in ethanol (96%) would be the best method for keeping sea lice pigmentation. In addition, real-time PCR assay can be used in identification of *L. salmonis* and *C. elongatus* (Noroi & Simonsen *et al.*, 2015).

It was not possible to determine the planktonic sea lice larvae down to species. This is because the larval stages of *L. salmonis* and *C. elongatus* were very similar, making the species identification after preservation on formaldehyde solution difficult. Therefore in these thesis it was only possible to determine the nauplius and copepodid stages to family: Caligidae, not to genus or species. In further work it be beneficial to do a correct identification of species, as it would give a better interpretation of the data due to possible differences in infection reservoir and dispersal mechanisms between the two species (Penston & McKibben *et al.*, 2004).

4.3 Distribution of planktonic sea lice at salmon farm

4.3.1 Concentration of planktonic sea lice at farms

The density of planktonic sea lice ranged from 0-22 m^{-3}, which indicates a patchy and uneven distribution in the water column at fish farms (Costelloe & Costelloe *et al.*, 1996). Nauplius were the dominant stages of planktonic sea lice at both locations. In total, 98.7% of the sea lice found in the water column were nauplii, compared to only 1.3% copepodids. This is in accordance with other studies, where nauplii are found to be the most abundant life stage in the water column near fish farms (á Norði & Simonsen *et al.*, 2015; Costelloe & Costelloe *et al.*, 1996; Gravil, 1996; Morton & Routledge *et al.*, 2011). In this work, the maximum observed concentration of copepods was 0.5 m^{-3}, a concentration comparable to other studies conducted

in Faroe Islands and Ireland (á Norði & Simonsen *et al.*, 2016; Costelloe & Costelloe *et al.*, 1996). Here the maximum density of *L. salmonis* copepodids near farms were found to be 0.3 m^{-3}. The maximum copepodid density in this study was higher than observed in Faroe Islands and Ireland, but is still in the density range generally observed in open water (á Norði & Simonsen *et al.*, 2015; Costelloe & Costelloe *et al.*, 1996; Morton & Routledge *et al.*, 2011). Gravil (1996) and O'Donoghue et al. (1998) proposed that as the nauplii molts in to copepodids, they are immediately ready to infect a host, and will do so at the earliest available opportunity. At salmon farms, there is a high host density, and copepodids have a high possibility to find and infect a host. If this is the case it could be an explanation for the low concentration of copepodids obtained in the plankton tows at fish farms (Gravil, 1996; O'Donoghue & Costelloe *et al.*, 1998). Another explanation could be that nauplii produced at the farm were transported away from the farm prior to molting into copepodids. The survival from nauplii to copepodid stage could also have been poor, contributing to a low density of copepodid (Morton & Routledge *et al.*, 2011; Penston & McKibben *et al.*, 2004).

4.3.2 Comparison of the two locations

The site with most planktonic sea lice were Bukkholmen and accounted for 90.5% of the nauplii found in the plankton samples. The remaining 9.5% were found at Hofsøya. This does not correspond with the estimated number of gravid females present at the fish farms, where Hofsøya had a higher number of gravid sea lice compared to Bukkholmen. Hofsøya had been treated for lice prior to sampling. This could have contributed to a low production of nauplii at the farm, thus reducing the density of planktonic sea lice in the water column. This is in correspondence with Morton et al. (2011) and Penston et al. (2011) who found an reduction of planktonic nauplii nearby farms assosiated whith chemical treatments. Another contributor to lower density at farm Hofsøya could be because the location did not use skirts, and that nauplii produced at the fishfarm were transported out of the cage with the water current.

4.4 Distribution of planktonic stages inside and outside sea cages

Highest densities of nauplii (max 22.25 m^{-3}) were consistently recorded on the inside of cages at Bukkholmen, which used skirts. Here the average density of planktonic sea lice larvae were significantly higher on the inside (2.26±4.38 m^{-3}) compared to the outside (0.137 ±0.393 m^{-3})

($p<0.001$). At Hofsøya there was not a significant difference in the density recorded inside (0.17 ± 0.342 m^{-3}) and outside (0.246 ± 0.413 m^{-3}) of the cage ($p>0.05$).

At Bukkholmen less than 10 % of the larval density recorded on the inside was found on the outside. Samples were taken downstream, which may indicate that the larvae hatched inside the cage to a strong extent retained inside sea cages with lice skirts. Skirts are made to form a physical barrier between the salmon inside sea cages, and the infective copepodid stage in the water masses (Næs & Heuch *et al.*, 2012). In this study, the skirt had the opposite function, contributing to retaining sea lice produced at the cage inside of that cage. Costelloe et al. (1996) also observed high retention time, but here heavy fouling of the net were speculated to be the cause of reduced water flow inside of the net. At Hofsøya there were on average found 40% more sea lice on the outside, downstream of the cage compared to the inside, although this was not significant ($p>0.05$). The lower density difference at Hofsøya could be because sea lice were not retained to the same degree as in Bukkholmen, and that sea lice produced inside of the cage were to a higher degree transported away with the currents. If assuming that all sea lice found in the plankton samples originates from the fish farm, this is in contradiction the hypothesis (1). As no apparent change in planktonic sea lice density on the inside and outside of the cage was found, it did not seem that the sea cage nets hinder transport of planktonic sea lice to the surrounding waters. From this data, it would seem that sea cages wearing skirts hinder the transport of planktonic sea lice to a higher degree than the netting alone, and that it is a higher possibility of reinfection at Bukkholmen because nauplii are retained inside of the cages for a longer time. At the same time 40 % of the larvae retrieved at Hofsøya were found on the inside of the sea cage, and there is a possibility that they could stay inside of the cage until molting into copepodids, which could possibly lead to re-infection of the salmonids in the pen.

Due to the delousing activities at Hofsøya, it cannot be said with certainty that rapid dispersion of planktonic sea lice produced at the farm was the main reason for low density of planktonic sea lice at Hofsøya. It could also have been a product of low production of sea lice, and a possible retention of sea lice inside of cages were not detected because there was no hatching of sea lice inside of the cages.

4.5 Biofouling as a source for reinfection at farms

4.5.1 Effects of biofouling on distribution of sea lice

The amount of biofouling on the cages did not seem to affect the distribution of planktonic sea lice at fish farms. The density of sea lice were relatively similar inside and outside sea cages independent of the amount of biofouling on the net. There were not found any significant relationship between the density of sea lice at the farms, and the amount of biofouling on the net ($p>0.05$). However, a trend where more sea lice retained inside of the cage at higher biofouling could be seen (Figure 3.11). This trend is in accordance with the hypothesis (2) stating that sea lice larvae may be associated with the fouling of the nets, and fouling may delay dispersal. Costelloe (1996) examined the density of sea lice from cages which were heavily fouled, and found that the biofouling had a large effect on the dispersal of nauplii produced inside of the cage. Throughout this study, the nets were relatively clean even immediately prior to washing. Only one net was categorized to full fouling, and 72% of the nets examined were in the category no fouling or low fouling. It is possible that planktonic sea lice could be retained to a higher degree inside of fish cages with highly fouled nets, but that the amount of biofouling at the cages was too low to affect the dispersal of sea lice out of these sea cages. The relatively clean nets also resulted in a low sample size from net pens with high fouling, and the low amount of samples could be the reason for no statistically significant effects. At the same time all the nets examined for biofouling were from Bukkholmen. They used skirt, so the skirts could have masked a possible effect of the biofouling on the larval dispersal. It would have been favorable to check for effects of biofouling on nets without skirts, to see if the skirts cold have been a reason for no apparent effect.

4.5.2 Effect of washing of the net on dispersal of sea lice

There were not observed any significant differences in planktonic sea lice densities throughout the washing cycle at Bukkholmen and Hofsøya. At Bukkholmen, the highest densities of sea lice were found on the inside of cages before washing (Figure 3.11). At Hofsøya the highest densities were found on the inside immediately after washing (Figure 3.12). However, the differences were not great enough to be significant ($p>0.05$), and are probably due to their patchy distribution.

Cleaning activities of the sea cage nettings have been speculated to be a source of infection by releasing pre-infective and infective larvae, which were attached to the biofouling. If this is

correct, it would be expected to see an elevated density of planktonic sea lice in the water masses immediately after washing (Woll, 2012). The amount of biofouling on the net in this experiment was significantly reduced on cages immediately after washing ($p<0.001$, Figure 3.4), so if cleaning activities affected the dispersal it would be expected to see this in the results. This was not the case at Bukkholmen. At Hofsøya, there could be seen an elevation of sea lice density on the inside of the net immediately after wash, but the differences were not significant compared to other periods in the washing cycle ($p>0.05$). These findings suggest that cleaning activities of the sea cage did not affect the distribution of planktonic sea lice in the water column and corresponds well with a previous study investigating sea lice association to biofouling on the net done by Leithet et al. (2017). Here they found only one nauplius in the biofouling on net pens even though nauplii were present in the water column (Leitet & Hagemann *et al.*, 2017). These findings contradict both hypothesis (2) and hypothesis (3), and indicates that planktonic sea lice do not stay in the biofouling. Because of this, it is not likely that cleaning activities on the net could be a source of infection by releasing pre-infective and infective larvae.

4.6 Summary

The present study demonstrate that planktonic sea lice larvae are present both on the inside and outside of the cages at fish farms. The density of sea lice was found to be highest inside cages wearing skirts and it is likely that sea lice nauplii produced inside the cages are retained to a higher degree inside of cages wearing skirts. No such effect was detected at sea cages not wearing skirts, here the densities of sea lice were approximately the same on both sides.

Although, the amount of biofouling did not show a significantly impact on the distribution of planktonic sea lice inside and outside the cage, there could be seen a trend for higher retention of sea lice inside of the cages with highly fouled nets. There were not detected any significant differences between samples taken before, immediately after, and one day after wash. The results indicate that sea lice could to some extend be maintained inside the sea cage, if the nets are heavily fouled. None of the findings suggests that sea lice stay in the biofouling growing on the net pens. Because of this, it would also be unlikely that washing of the cage could increase the infection risk by releasing pre-infective and infective larvae.

5 Conclusion

- Live planktonic *L. salmonis* and *C. elongatus* had characteristic pigmentation, but when fixated on formaldehyde it was not possible to distinguish between free-living *L. salmonis* and *C. elongatus* in plankton samples.
- Planktonic sea lice seemed to be maintained inside the sea cages wearing skirts. No such effect of sea cages not wearing skirts were detected, and it is not likely that sea cages nets hinder the transport of planktonic sea lice to the surrounding water to a very high degree.
- Biofouling of the net could to some extent retain sea lice inside of the cage, most likely due to reduced water flow.
- Cleaning activities of the net pens did not seem to affect the dispersal of planktonic sea lice larvae, and it is not likely that cleaning activities of the sea cage are a source of infection by releasing pre-infective and infective larvae staying in the biofouling.

6 References

á Norði, G., Simonsen, K., Danielsen, E., Eliasen, K., Mols-Mortensen, A., Christiansen, D., *et al.* (2015). Abundance and distribution of planktonic Lepeophtheirus salmonis and Caligus elongatus in a fish farming region in the Faroe Islands. *Aquaculture Environment Interactions, 7*(1), 15-27.

á Norði, G., Simonsen, K., & Patursson, Ø. (2016). A method of estimating in situ salmon louse nauplii production at fish farms. *Aquaculture Environment Interactions, 8*, 397-405.

AkvaGroup. (2017). Idema notvaskere, http://www.akvagroup.com/produkter/merdbasert-akvakultur/vaskesystemer/notvasker-rigger

Amundrud, T., & Murray, A. (2009). Modelling sea lice dispersion under varying environmental forcing in a Scottish sea loch. *Journal of fish diseases, 32*(1), 27-44.

Asplin, L., Boxaspen, K. K., & Sandvik, A. D. (2011). Modeling the Distribution and Abundance of Planktonic Larval Stages of Lepeophtheirus salmonis in Norway. In S. Jones & R. Beamish (Eds.), *Salmon Lice: An integrated approach to understanding parasite abundance and distribution* (pp. 29-50): Wiley-Blackwell.

Bjørn, P. A., Finstad, B., & Kristoffersen, R. (2001). Salmon lice infection of wild sea trout and Arctic char in marine and freshwaters: the effects of salmon farms. *Aquaculture Research, 32*(12), 947-962. doi: 10.1046/j.1365-2109.2001.00627.x

Bjørn, P. A., & Finstad, B. (2002). Salmon lice, Lepeophtheirus salmonis (Krøyer), infestation in sympatric populations of Arctic char, Salvelinus alpinus (L.), and sea trout, Salmo trutta (L.), in areas near and distant from salmon farms. *ICES Journal of Marine Science: Journal du Conseil, 59*(1), 131-139.

Bricknell, I. R., Dalesman, S. J., O'Shea, B., Pert, C. C., & Luntz, A. J. M. (2006). Effect of environmental salinity on sea lice Lepeophtheirus salmonis settlement success. *Diseases of aquatic organisms, 71*(3), 201-212.

Bron, J. E., Sommerville, C., & Rae, G. H. (1993). Aspects of the behaviour of copepodid larvae of the salmon louse Lepeophtheirus salmonis (Krøyer, 1837). In G. A. Boxhall & D. Defaye (Eds.), *Pathogens of wild and farmed fish: sea lice* (pp. 125-142): Ellis Horwood Limited.

Butler, J. R. A. (2002). Wild salmonids and sea louse infestations on the west coast of Scotland: sources of infection and implications for the management of marine salmon farms. *Pest management science, 58*(6), 595-608.

Costello, M. J. (1993). Review of methods to control sea lice (Caligidae: Crustacea) infestations on salmon (Salmo salar) farms. In G. A. Boxhall & D. Defaye (Eds.), *Pathogens of wild and farmed fish: sea lice* (pp. 219-252): Ellis Horwood Limited.

Costello, M. J. (2006). Ecology of sea lice parasitic on farmed and wild fish. *Trends in parasitology, 22*(10), 475-483.

Costelloe, M., Costelloe, J., & Roche, N. (1996). Planktonic dispersion of larval salmon-lice, Lepeophtheirus salmonis, associated with cultured salmon, Salmo salar, in western Ireland. *Journal of the Marine Biological Association of the United Kingdom, 76*(01), 141-149.

Costelloe, M., Costelloe, J., Coghlan, N., O'donohoe, G., & O'connor, B. (1998). Distribution of the larval stages of Lepeophtheirus salmonis in three bays on the west coast of Ireland. *ICES Journal of Marine Science: Journal du Conseil, 55*(2), 181-187.

Danielsen, E. (2014). Identification of the free swimming larval stages of *Caligus elongatus* and *Lepeoptheirus salmonis*. *Fiskaaling rit 2013-5, Technical Report , Fiskaaling, Faroe Islands*.

De Nys, R., & Guenther, J. (2009). The impact and control of biofouling in marine finfish aquaculture. In C. Hellio & D. Yebra (Eds.), *Advances in Marine Antifouling Coatings and Technologies* (pp. 177-207): Woodshead Publishing: Cambridge, UK.

Dürr, S., & Watson, D. I. (2010). Biofouling and antifouling in aquaculture. *Biofouling*, 267-287.

Finstad, B., Bjørn, P., Grimnes, A., & Hvidsten, N. (2000). Laboratory and field investigations of salmon lice [Lepeophtheirus salmonis (Krøyer)] infestation on Atlantic salmon (Salmo salar L.) post-smolts. *Aquaculture Research, 31*(11), 795-803.

Fiskeridirektoratet. Fiskeridirektorates kart for akvakultur, from http://www.fiskeridir.no/Kart

Frazer, L. N., Morton, A., & Krkošek, M. (2012). Critical thresholds in sea lice epidemics: evidence, sensitivity and subcritical estimation. *Proceedings of the Royal Society of London B: Biological Sciences*, rspb20112210.

Gravil, H. R. (1996). Studies on the biology and ecology of the free swimming larval stages of Lepeophtheirus Salmonis (Kroyer, 1838) and Caligus Elongatus Nordmann, 1832 (Copepoda: Caligidae).

Grimnes, A., & Jakobsen, P. (1996). The physiological effects of salmon lice infection on post-smolt of Atlantic salmon. *Journal of Fish Biology, 48*(6), 1179-1194.

Guenther, J., Misimi, E., & Sunde, L. M. (2010). The development of biofouling, particularly the hydroid Ectopleura larynx, on commercial salmon cage nets in Mid-Norway. *Aquaculture, 300*(1–4), 120-127. doi: http://dx.doi.org/10.1016/j.aquaculture.2010.01.005

Hamre, L., Eichner, C., Caipang, C., Dalvin, S., Bron, J., Nilsen, F., *et al.* (2013). The Salmon Louse Lepeophtheirus salmonis (Copepoda: Caligidae) Life Cycle Has Only Two Chalimus Stages. *PLoS One, 8*(9). doi: 10.1371/journal.pone.0073539

Harris, R., Wiebe, P., Lenz, J., Skjoldal, H.-R., & Huntley, M. (2000). *ICES zooplankton methodology manual*: Academic press.

Hayward, J. C., Andrews, M., & Nowaq, F. B. (2011). Introduction: Lepeoptheirus salmonis-A Remarkable Success Story In S. Jones & R. Beamish (Eds.), *Salmon lice: an integrated approach to understanding parasite abundance and distribution* (pp. 1-20): John Wiley & Sons.

Heuch, P. A., Parsons, A., & Boxaspen, K. (1995). Diel vertical migration: a possible host-finding mechanism in salmon louse (Lepeophtheirus salmonis) copepodids? *Canadian Journal of Fisheries and Aquatic Sciences, 52*(4), 681-689.

Heuch, P. A., & Mo, T. A. (2001). A model of salmon louse production in Norway: effects of increasing salmon production and public management measures. *Diseases of aquatic organisms, 45*(2), 145-152.

Hinkelman, T. (2012). Count data and GLMs: Choosing among Poisson, negative binomial, and zero-inflated models

Hodson, S. L., Lewis, T. E., & Burkea, C. M. (1997). Biofouling of fish-cage netting: efficacy and problems of in situ cleaning. *Aquaculture, 152*(1), 77-90. doi: http://dx.doi.org/10.1016/S0044-8486(97)00007-0

Hopkins, T. L. (1968). Carbon and Nitrogen Content of Fresh and Preserved Nematoscelis dijficilis, a Euphausiid Crustacean. *Journal du Conseil, 31*(3), 300-304.

Igboeli, O. O., Burka, J. F., & Fast, M. D. (2014). Lepeophtheirus salmonis: a persisting challenge for salmon aquaculture. *Animal Frontiers, 4*(1), 22-32. doi: 10.2527/af.2014-0004

Iversen, A., Hermansen, Ø., Andreassen, O., Brandvik, R. K., Marthinussen, A., & Nystøyl, R. (2015). Kostnadsdrivere i lakseoppdrett.

Johnsen, I. A., Fiksen, Ø., Sandvik, A. D., & Asplin, L. (2014). Vertical salmon lice behaviour as a response to environmental conditions and its influence on regional dispersion in a fjord system.

Johnsen, I. A., Asplin, L., Sandvik, A. D., & Serra-Llinares, R. M. (2016). Salmon lice dispersion in a northern Norwegian fjord system and the impact of vertical movements. *Aquaculture Environment Interactions, 8*, 99-116.

Johnson, S., & Albright, L. (1991a). Development, growth, and survival of Lepeophtheirus salmonis (Copepoda: Caligidae) under laboratory conditions. *Journal of the Marine Biological Association of the United Kingdom, 71*(02), 425-436.

Johnson, S., & Albright, L. (1991b). The developmental stages of Lepeophtheirus salmonis (Krøyer, 1837)(Copepoda: Caligidae). *Canadian Journal of Zoology, 69*(4), 929-950.

Johnson, S. C., Bravo, S., Nagasawa, K., Kabata, Z., Hwang, J., Ho, J., *et al.* (2004). A review of the impact of parasitic copepods on marine aquaculture. *Zool. Stud., 43*(2), 229-243.

Karlsen, Ø., Johnsen, I., Skarðhamar, J., Sandvik, A. D., Albertsen, J., Nilsen, R., *et al.* (2017). Risikorapport norsk fiskeoppdrett 2017. In T. Svåsand, E. S. Grefsrud, Ø. Karlsen, B. O. Kvamme, K. Glover, V. Husa & T. S. Kristiansen (Eds.).

Leitet, M., Hagemann, A., Blöcher, N., Steinhovden, K. B., & Olsen, L. (2017). Forekomst av lakselus i groe på not og rensefiskskjul. *Norsk Fiskeoppdrett*.

Liu, Y., & Bjelland, H. v. (2014). Estimating costs of sea lice control strategy in Norway. *Preventive Veterinary Medicine, 117*(3–4), 469-477. doi: http://dx.doi.org/10.1016/j.prevetmed.2014.08.018

Low, C. (1977). Zooplankton Fixation and Preservation. *Journal of the Fisheries Board of Canada, 34*(8), 1254-1254.

Martin, K., Mark, A. L., Alexandra, M., Frazer, L. N., & John, P. V. (2006). Epizootics of wild fish induced by farm fish. *Proceedings of the National Academy of Sciences, 103*(42), 15506. doi: 10.1073/pnas.0603525103

McKibben, M. A., & Hay, D. W. (2004). Distributions of planktonic sea lice larvae Lepeophtheirus salmonis in the inter-tidal zone in Loch Torridon, Western Scotland in relation to salmon farm production cycles. *Aquaculture Research, 35*(8), 742-750.

McKnight, P. E., & Najab, J. (2010). Mann-Whitney U Test. *Corsini Encyclopedia of Psychology*.

Mordue, A. J., & Birkett, M. A. (2009). A review of host finding behaviour in the parasitic sea louse, Lepeophtheirus salmonis (Caligidae: Copepoda). *Journal of Fish Diseases, 32*(1), 3-13. doi: 10.1111/j.1365-2761.2008.01004.x

Morton, A., Routledge, R. D., & Williams, R. (2005). Temporal Patterns of Sea Louse Infestation on Wild Pacific Salmon in Relation to the Fallowing of Atlantic Salmon Farms. *North American Journal of Fisheries Management, 25*(3), 811-821. doi: 10.1577/M04-149.1

Morton, A., Routledge, R., McConnell, A., & Krkošek, M. (2011). Sea lice dispersion and salmon survival in relation to salmon farm activity in the Broughton Archipelago. *ICES Journal of Marine Science: Journal du Conseil, 68*(1), 144-156.

Noroi, G., Simonsen, K., Danielsen, E., Eliasen, K., Mols-Mortensen, A., Christiansen, D., *et al.* (2015). Abundance and distribution of planktonic Lepeophtheirus salmonis and Caligus elongatus in a fish farming region in the Faroe Islands. *Aquaculture Environment Interactions, 7*(1), 15-27. doi: 10.3354/aei00134

Næs, M., Heuch, P., & Mathisen, R. (2012). Bruk av «luseskjørt» for å redusere påslag av lakselus Lepeophtheirus salmonis (Krøyer) på oppdrettslaks. *Vesterålen Fiskehelsetjeneste (in Norwegian).*

O'Donoghue, G., Costelloe, M., & Costelloe, J. (1998). Development of a management strategy for the reduction/elimination of sea lice larvae, Lepeophtheirus salmonis, parasites of salmon and trout.

Omori, M. (1969). Weight and chemical composition of some important oceanic zooplankton in the North Pacific Ocean. *Marine Biology, 3*(1), 4-10.

Omori, M. (1978). Some factors affecting on dry weight, organic weight and concentrations of carbon and nitrogen in freshly prepared and in preserved zooplankton. *International Review of Hydrobiology, 63*(2), 261-269.

Peet, C. R. (2007). *Interactions between sea lice (Lepeoptheirus salmonis and Caligus clemensii), juvenile salmon (Oncorhynchus keta and Oncorhynchus gorbuscha) and salmon.* University of Victoria.

Penston, M. J., McKibben, M. A., Hay, D. W., & Gillibrand, P. A. (2004). Observations on open-water densities of sea lice larvae in Loch Shieldaig, Western Scotland. *Aquaculture Research, 35*(8), 793-805.

Piasecki, W. (1996). The developmental stages of Caligus elongatus von Nordmann, 1832 (Copepoda: Caligidae). *Canadian Journal of Zoology, 74*(8), 1459-1478.

Pike, A., Mordue, A., & Ritchie, G. (1993). The development of Caligus elongatus Nordmann from hatching to copepodid in relation to temperature. *Pathogens of wild and farmed fish: sea lice*, 51-60.

Pike, A. W., & Wadsworth, S. L. (1999). Sealice on Salmonids: Their Biology and Control. *Advances in Parasitology, 44*, 233-337. doi: http://dx.doi.org/10.1016/S0065-308X(08)60233-X

Põllupüü, M. (2007). Effect of formalin preservation on the body length of copepods. *Proc. Estonian Acad. Sci. Biol. Ecol, 56*(4), 326-331.

Samsing, F., Oppedal, F., Dalvin, S., Johnsen, I., Vågseth, T., & Dempster, T. (2016). Salmon lice (Lepeophtheirus salmonis) development times, body size, and reproductive outputs

follow universal models of temperature dependence. *Canadian Journal of Fisheries and Aquatic Sciences, 73*(12), 1841-1851.

Schram, T. A. (1993). Supplementary descriptions of the developmental stages of Lepeophtheirus salmonis (Krøyer, 1837)(Copepoda: Caligidae). *Pathogens of wild and farmed fish: sea lice, 1*, 30-47.

Schram, T. A. (2004). Practical identification of pelagic sea lice larvae. *Journal of the Marine Biological Association of the UK, 84*(01), 103-110.

Steedman, H. (1976). Osmotic pressure in fixation and preservation. *Monographs on oceanographic methodology, 4*, 186-188.

Stien, A., Bjoern, P., Heuch, P., Elston, D., & Stien, A. (2005). Population dynamics of salmon lice Lepeophtheirus salmonis on Atlantic salmon and sea trout. *Marine Ecology Progress Series, 290*, 263-275.

Stien, L. H., Nilsson, J., Hevrøy, E. M., Oppedal, F., Kristiansen, T. S., Lien, A. M., *et al.* (2012). Skirt around a salmon sea cage to reduce infestation of salmon lice resulted in low oxygen levels. *Aquacultural Engineering, 51*, 21-25.

Thibault-Botha, D., & Bowen, T. (2004). Impact of formalin preservation on Pleurobrachia bachei (Ctenophora). *Journal of experimental marine biology and ecology, 303*(1), 11-17.

Tucker, C. S., Sommerville, C., & Wootten, R. (2000). An investigation into the larval energetics and settlement of the sea louse, Lepeophtheirus salmonis, an ectoparasitic copepod of Atlantic salmon, Salmo salar. *Fish Pathology, 35*(3), 137-143.

Tully, O. (1989). The succession of generations and growth of the caligid copepods Caligus elongatus and Lepeophtheirus salmonis parasitising farmed Atlantic salmon smolts (Salmo salar L.). *Journal of the Marine Biological Association of the United Kingdom, 69*(02), 279-287.

Tully, O., & Whelan, K. F. (1993). Production of nauplii of Lepeophtheirus salmonis (Krøyer) (Copepoda: Caligidae) from farmed and wild salmon and its relation to the infestation of wild sea trout (Salmo trutta L.) off the west coast of Ireland in 1991. *Fisheries Research, 17*(1), 187-200. doi: 10.1016/0165-7836(93)90018-3

Tully, O., Gargan, P., Poole, W., & Whelan, K. (1999). Spatial and temporal variation in the infestation of sea trout (Salmo trutta L.) by the caligid copepod Lepeophtheirus salmonis (Kroyer) in relation to sources of infection in Ireland. *Parasitology, 119*, 41-51.

Tully, O., & Nolan, D. (2002). A review of the population biology and host–parasite interactions of the sea louse Lepeophtheirus salmonis (Copepoda: Caligidae). *Parasitology, 124*(07), 165-182.

Woll, A. K. r. (2012). Miljødokumentasjon Nordmøre. *Samlerapport for fase 1 og 2. Møreforskning, rapport nr. MA 14-06.*

Wootten, R., Smith, J. W., & Needham, E. (1982). Aspects of the biology of the parasitic copepods Lepeophtheirus salmonis and Caligus elongatus on farmed salmonids, and their treatment. *Proceedings of the Royal Society of Edinburgh. Section B. Biological Sciences, 81*(03), 185-197.

Appendix I

Illustration of the amount of biofouling nets had in each biofouling category.

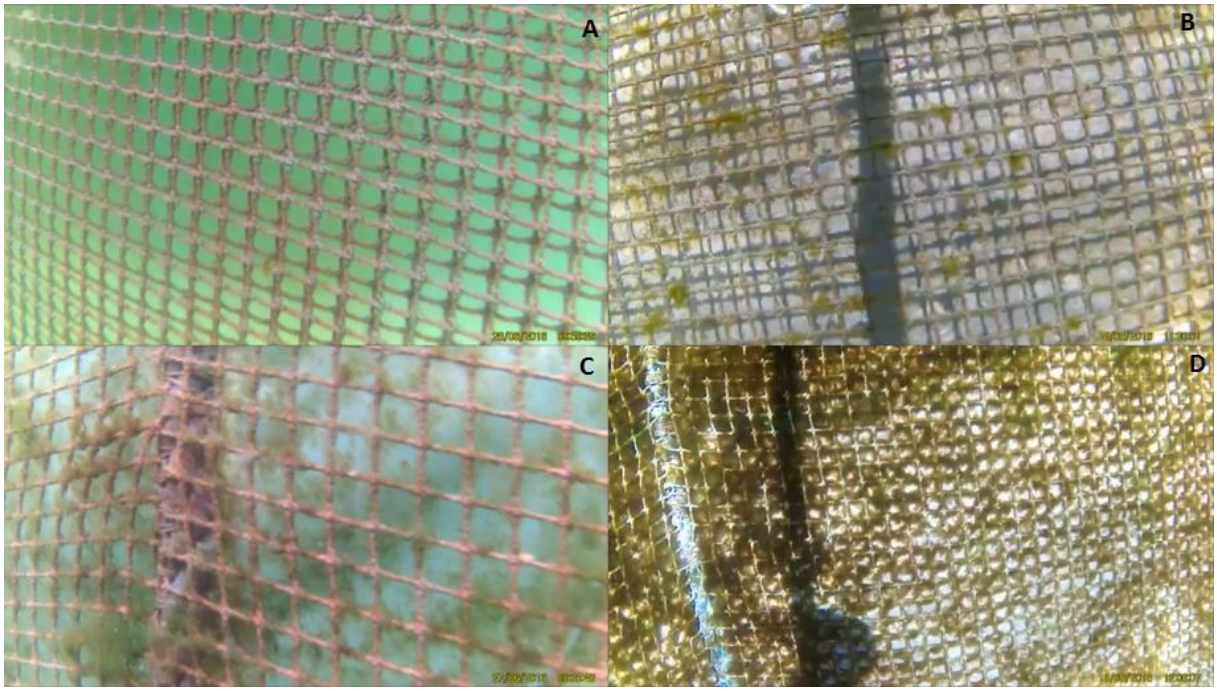

Figure A1 – Biofouling on net pens in category no fouling (A), low fouling (B), medium fouling (C) and high fouling (D)

Appendix II

Illustrations of sea lice retrieved in plankton samples fixated on formaldehyde (4%) and Ethanol (98%)

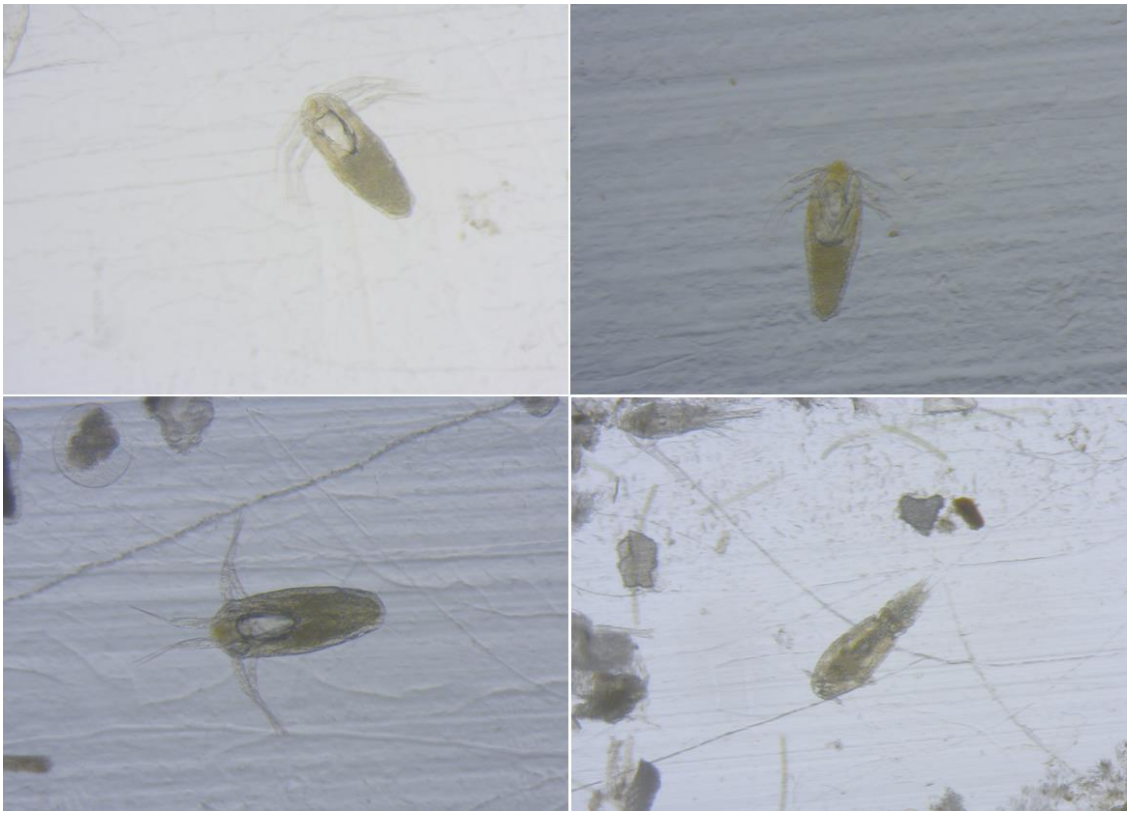

Figure A.2 – Illustration of planktonic sea lice found in the plankton samples fixated on formaldehyde.

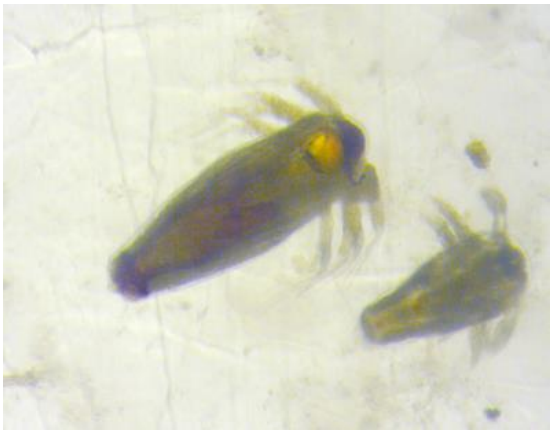

Figure A.3 - Illustration of sea lice nauplii from plankton sample fixated on ethanol.

Appendix III

Biofouling category and analyzed area

Table A1 – Biofouling category and analyzed area (cm^2) of the pens analyzed.

Date	Locality	Pen	When	Area analyzed (cm^2)	Biofouling (%)	Category
2016-06-24	Bukk	2	Before	20.93	41.67	4
2016-06-24	Bukk	5	Before	43.68	1.67	1
2016-06-24	Bukk	8	Before	14.96	88.33	5
27.06.2016	Bukk	5	Before	35.978	16,67	2
27.06.2016	Bukk	4	Before	18.380	40,00	3
27.06.2016	Bukk	1	After	17.598	0,00	1
27.06.2016	Bukk	2	1DayAfter	24.637	0,00	1
27.06.2016	Bukk	3	After	39.106	5,00	2
27.06.2016	Bukk	4	After	24.767	0,00	1
27.06.2016	Bukk	5	After	15.251	0,00	1
28.06.2016	Bukk	5	1DayAfter	13.557	11,67	2
28.06.2016	Bukk	4	1DayAfter	15.773	5,00	2
28.06.2016	Bukk	3	1DayAfter	31.024	0,00	1
09.08.2016	Bukk	8	Before	34.190	38,33	3
09.08.2016	Bukk	7	Before	26.331	51,67	4
09.08.2016	Bukk	2	Before	45.233	56,67	4
10.08.2016	Bukk	7	After	17.598	0,00	1

10.08.2016	Bukk	8	After	155.755	3,33	1
10.08.2016	Bukk	2	After	20.596	3,33	1
11.08.2016	Bukk	8	1DayAfter	52.291	0,00	1
11.08.2016	Bukk	7	1DayAfter	48.622	6,67	2
11.08.2016	Bukk	2	1DayAfter	15.512	11,67	2
18.08.2016	Bukk	8	Before	57.207	16,67	2
18.08.2016	Bukk	2	Before	83.818	73,33	4
18.08.2016	Bukk	7	Before	35.196	14,00	2

Appendix VI

Density of lice in each sample

Table A2 – Averages lice (m^{-3}) at each sampling site and corresponding standard deviation (m^{-3}) at Bukkholmen.

Bukkholmen				
Date	**When**	**Where**	**Avarage (m^{-3})**	**SD (m^{-3})**
2016-06-24	Before	Inside	22.253	12.874
2016-06-24	Before	Outside	0.000	0.000
2016-06-24	Before	Inside	4.100	5.798
2016-06-24	Before	Outside	1.062	1.501
2016-06-24	Before	Ins	1.960	2.772
2016-06-24	Before	Out	0.000	0.000
2016-06-27	After	Ins	0.000	0.000
2016-06-27	After	Out	0.000	0.000
2016-06-27	Before	Ins	1.096	1.550
2016-06-27	Before	Out	0.000	0.000
2016-06-27	After	Ins	0.000	0.000
2016-06-27	After	Out	0.000	0.000
2016-06-27	Before	Ins	0.000	0.000
2016-06-27	Before	Out	0.000	0.000
2016-06-27	After	Ins	0.000	0.000
2016-06-27	After	Out	0.000	0.000
2016-06-27	1DayAfter	Ins	0.679	0.961
2016-06-27	1DayAfter	Out	0.000	0.000
2016-06-27	After	Ins	0.809	1.144
2016-06-27	After	Out	0.000	0.000
2016-06-28	1DayAfter	Ins	2.038	2.882

2016-06-28	1DayAfter	Out	0.000	0.000
2016-06-28	1DayAfter	Ins	4.400	4.221
2016-06-28	1DayAfter	Out	1.544	2.184
2016-06-28	1DayAfter	Ins	1.258	0.000
2016-06-28	1DayAfter	Out	0.000	0.000
2016-08-09	Before	Ins	3.033	0.286
2016-08-09	Before	Out	0.000	0.000
2016-08-09	Before	Ins	3.531	2.397
2016-08-09	Before	Out	0.000	0.000
2016-08-09	Before	Ins	2.486	1.172
2016-08-09	Before	Out	0.000	0.000
2016-08-10	After	Ins	0.414	0.586
2016-08-10	After	Out	0.000	0.000
2016-08-10	After	Ins	1.793	0.133
2016-08-10	After	Out	0.809	1.144
2016-08-10	After	Ins	2.913	1.885
2016-08-10	After	Out	0.000	0.000
2016-08-11	1DayAfter	Ins	0.000	0.000
2016-08-11	1DayAfter	Out	0.000	0.000
2016-08-11	1DayAfter	Ins	1.231	0.443
2016-08-11	1DayAfter	Out	0.000	0.000
2016-08-11	1DayAfter	Ins	1.957	0.366
2016-08-11	1DayAfter	Out	0.000	0.000
2016-08-18	Before	Ins	0.000	0.000
2016-08-18	Before	Out	0.000	0.000
2016-08-18	Before	Ins	0.000	0.000

2016-08-18	Before	Out	0.000	0.000
2016-08-18	Before	Ins	0.653	0.924
2016-08-18	Before	Out	0.000	0.000

Table A3 – Averages lice (m^{-3}) at each sampling site and corresponding standard deviation (m^{-3}) at Hofsøya.

Hofsøya				
Date	**Net**	**Where**	**Average (m^{-3})**	**SD (m^{-3})**
2016-06-17	Before	Ins	0.000	0.000
2016-06-17	Before	Out	1.132	1.601
2016-06-20	After	Ins	1.173	0.057
2016-06-20	After	Out	0.500	0.706
2016-06-20	After	Ins	0.000	0.000
2016-06-20	After	Out	0.485	0.686
2016-06-20	Before	Ins	0.000	0.000
2016-06-20	Before	Out	0.690	0.976
2016-06-20	Before	Ins	0.515	0.728
2016-06-20	Before	Out	0.000	0.000
2016-06-21	1DayAfter	Ins	0.000	0.000
2016-06-21	1DayAfter	Out	1.132	1.601
2016-06-21	1DayAfter	Ins	0.000	0.000
2016-06-21	1DayAfter	Out	0.000	0.000
2016-06-21	After	Ins	0.000	0.000
2016-06-21	After	Out	0.000	0.000
2016-06-21	After	Ins	0.586	0.828

2016-06-21	After	Out	0.000	0.000
2016-08-14	After	Ins	0.000	0.000
2016-08-14	After	Out	0.000	0.000
2016-08-14	Before	Ins	0.000	0.000
2016-08-14	Before	Out	0.000	0.000
2016-08-14	Before	Ins	0.000	0.000
2016-08-14	Before	Out	0.000	0.000
2016-08-14	Before	Ins	0.000	0.000
2016-08-14	Before	Out	0.000	0.000
2016-08-15	1DayAfter	Ins	0.000	0.000
2016-08-15	1DayAfter	Out	0.000	0.000
2016-08-15	1DayAfter	Ins	0.000	0.000
2016-08-15	1DayAfter	Out	0.000	0.000
2016-08-15	After	Ins	0.531	0.751
2016-08-15	After	Out	0.000	0.000