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# Abundance of planktonic sea lice in intensive sea farm locations at Frøya: January-September 2018

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Norwegian University of  
Science and Technology

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Marine Coastal Development

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# Abstract

*Lepeophtheirus salmonis* and *Caligus elongatus* are the two species of sea lice that pose the greatest threat to salmon aquaculture and wild salmonids in Norway. Infections from one location to another mainly occurs in the planktonic stages. Knowledge of the abundance and distribution of these stages in association with salmon farms is crucial to be able to develop strategies and solutions that can reduce the impact of sea lice infection on both sea farms and wild populations of salmonids. There is limited knowledge on the abundance and distribution of planktonic stages of sea lice in and around sea cages. A seasonal study was conducted to investigate the abundance of planktonic stages of sea lice at two separate sea farms over a period of eight months. This was done by conducting plankton tows on the inside and outside of the cages. Abundance and temperature were tested for correlation. Distribution of planktonic lice within and outside cages was also investigated to establish the source of the planktonic sea lice found in the seasonal study. Abundance of planktonic stages inside and outside cages was compared, and biofouling was tested for as a potential retention mechanism keeping the planktonic sea lice from drifting freely out of the cage net. There was a significant increase in abundance of planktonic stages of sea lice in July and August compared to other months. Abundance was strongly correlated with sea temperature. There was also a strong correlation between the abundance of adult females and planktonic stages of *L. salmonis*. Over 99% of the planktonic lice found across all studies were nauplii, fewer than 1% were infective copepodids. Abundance of planktonic stages were higher where the ocean current exited the cage compared to where it entered. The results of the inside and outside abundance comparison as well as the biofouling investigation were inconclusive. The main results are in accordance with previous findings on the topic of sea lice, and demonstrate the importance of improving preventive measures for sea lice in the aquaculture industry.





# Samandrag

*Lepeiotheirus salmonis* og *Caligus elongatus* er dei to artane av havlus som som utgjer det største trugsmålet mot lakseoppdrett og villaks i Norge. Spreiing av lusa skjer hovudsakleg i planktoniske stadium. Kunnskap om mengde og distribusjon av desse stadiene tilknytta oppdrettsanlegg er avgjerande for å kunne utvikle strategiar og løysingar som kan redusere verknaden av havlusinfeksjon på både oppdrettsfisk og ville populasjonar av laksefisk. Det er begrensa kunnskap om mengde og fordeling av planktoniske stadium av havlus inne i og ved rundt merder. Ein sesongstudie blei gjennomført for å undersøke mengde planktoniske stadium av havlus ved to oppdrettsanlegg over ein periode på åtte månader. Dette blei gjort ved å utføre trekk med planktonhåv, på innsida og på utsida av merdene. Mengde lus og temperatur blei testa for korrelasjon. Distribusjon av planktonisk lus inne i og utanfor merdene blei også undersøkt for å etablere kjelda til havlusa ein fann i sesongstudiet. Mengde planktoniske stadium inne i og utanfor merdene blei samanlikna, og det blei sjekka om begroing fungerte som ein mekanisme som hindra havlusa i å flyte ut av nettet i merda. Det var betydeleg auke i mengde planktoniske stadium av lus i juli og august samanlikna med andre månader. Mengde lus var sterkt korrelert med sjøtemperatur. Det var også sterk korrelasjon mellom mengde vaksne holus og planktoniske stadium av *L. salmonis*. Over 99% av lusa som blei funne i planktoniske stadium var nauplii, færre enn 1% var kopepodittar. Mengde planktonisk lus var høgare der havstraumen gjekk ut av merda enn der straumen kom inn. Det gjekk ikkje an å trekke sikre slutningar frå resultata av mengde lus på innsida av merda samanlikna med utsida. Det gjekk heller ikkje an å trekke slutningar angående forholdet påvekst har for å hindre lus i å flyte fritt ut av nettet på merda. Dei viktigste resultata i oppgåva er i tråd med tidligare funn om havlus og viser kor viktig det er å ha forebyggjande tiltak mot lus i akvakulturindustrien.



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# 1. Introduction

## 1.1 Sea lice in Norwegian aquaculture

The aquaculture industry in Norway has experienced a variety of challenges since its beginning in the 1970s. Diseases related to production, environmental conditions, and bacterial and viral infections have caused problems (Asche, Guttormsen, & Tveterås, 1999). Today, however, one of the greatest challenges is *Lepeoptheirus salmonis* (Krøyer 1837). With the increasing production of Atlantic salmon (*Salmo salar*), the number of available hosts for parasites also increases. This has led to high rates of lice infection on both farmed and wild fish populations, which may lead to serious welfare issues for the fish (Finstad, Bjørn, Grimnes, & Hvidsten, 2000). Serious infections may cause reduced growth, higher probability of secondary infections and even death.

Methods of preventing infections as well as treating already infected fish have resulted in large costs for the aquaculture industry (Johnson et al., 2004). The estimated cost of salmon lice to the Norwegian aquaculture industry has increased from around 1 billion NOK in 2011 to 5 billion NOK in 2016 (Iversen, Hermansen, Nystøyl, & Hess, 2017). These estimates do not include the costs associated with reduced growth rates in infected fish. Thus, the real cost is potentially even higher. It is not known if the cost will keep growing, but 2016 compared to 2015 indicated that the costs of treatments have decreased while the costs of pre-emptive measures have increased (Iversen et al., 2017).

Sea lice (Caligidae) is a family of parasitic copepods that naturally occurs in the wild and have the ability to infect both farmed and wild fish. However, because of the immense growth of the salmon farming industry during the last thirty years, the number of potential hosts has increased tremendously. This have resulted in several challenges for the aquaculture industry. Since farmed salmon are strongly affected by sea louse infections, much attention has been given to the impact of sea lice on fish welfare (Johnson et al., 2004).

A relationship between sea lice outbreaks in salmon farms and increased infections in wild salmonid populations have been suggested in Scotland (Butler, 2002; Middlemas, Fryer, Tulett, & Armstrong, 2013), Norway (Bjørn, Finstad, & Kristoffersen, 2001), Ireland (Tully, Gargan, Poole, & Whelan, 1999), Canada (Krkošek, Lewis, Morton, Frazer, & Volpe, 2006). Mainly due to this suggested relationship, the Norwegian government has halted the growth of the salmonid aquaculture industry somewhat. In order to keep growing, the industry must reduce the impact of salmon lice on wild populations of salmon (Vollset et al., 2017). Several measures have been implemented to increase

control of sea louse infections in fish farms, including preventive measures to limit infection pressure and methods for delousing already infected fish. One of the most recently implemented methods is a system called “traffic light system”. In the traffic light system, aquaculture production in Norway is divided into zones, each of which is graded based on the environmental impact of the aquaculture industry on wild salmonid populations. Based on the grade (green, yellow, or red), production may increase, remain at current levels, or be reduced (Karlsen, Finstad, Ugedal, & Svåsand, 2016).

## 1.2 Sea louse biology

Sea lice (*Caligidae*) is a family of parasitic copepods that feed on the blood, mucus, and skin of marine fish. Lesions caused by the lice can result in anaemia, disrupted osmoregulation, and increased risk of secondary infections (Wootton, Smith, & Needham, 1982; Pickering & Pottinger, 1989; MacKinnon, 1993). The two species of sea lice that have the greatest impact on farmed Atlantic salmon are *L. salmonis* and *Caligus elongatus* (Normann 1832). *L. salmonis* is a specific parasite that only infects salmonids, while *C. elongatus* is a generalist that targets a large variety of teleosts (Schram, 2004).

*L. salmonis* and *C. elongatus* have similar life cycles. Both have two planktonic nauplius stages during which they do not feed. They then moult into an infective copepodid stage, where the lice can attach to a host. The chalimus and preadult stages differ between the species. *L. salmonis* has two chalimus stages and two preadult stages, while *C. elongatus* has four chalimus stages. Both species have one adult stage (Hamre et al., 2013). See Figure 1 for an illustration of all life stages of *L. salmonis*. The different stages can be divided into three categories. The first category is the free-living planktonic stages. This category consists of nauplius I and II, as well as the infective copepodid prior to infecting a host. The second category is the sessile stages, where the louse is attached to the fish but unable to move. This category includes the infective copepodid and chalimus stages. The third category is the motile stages, where the louse is able to move on the surface of the fish. This category includes the preadult and adult stages (Costello, 2006). Moults occur between each stage, and the developmental time depends on temperature (Burka, Fast, & Revie, 2011)

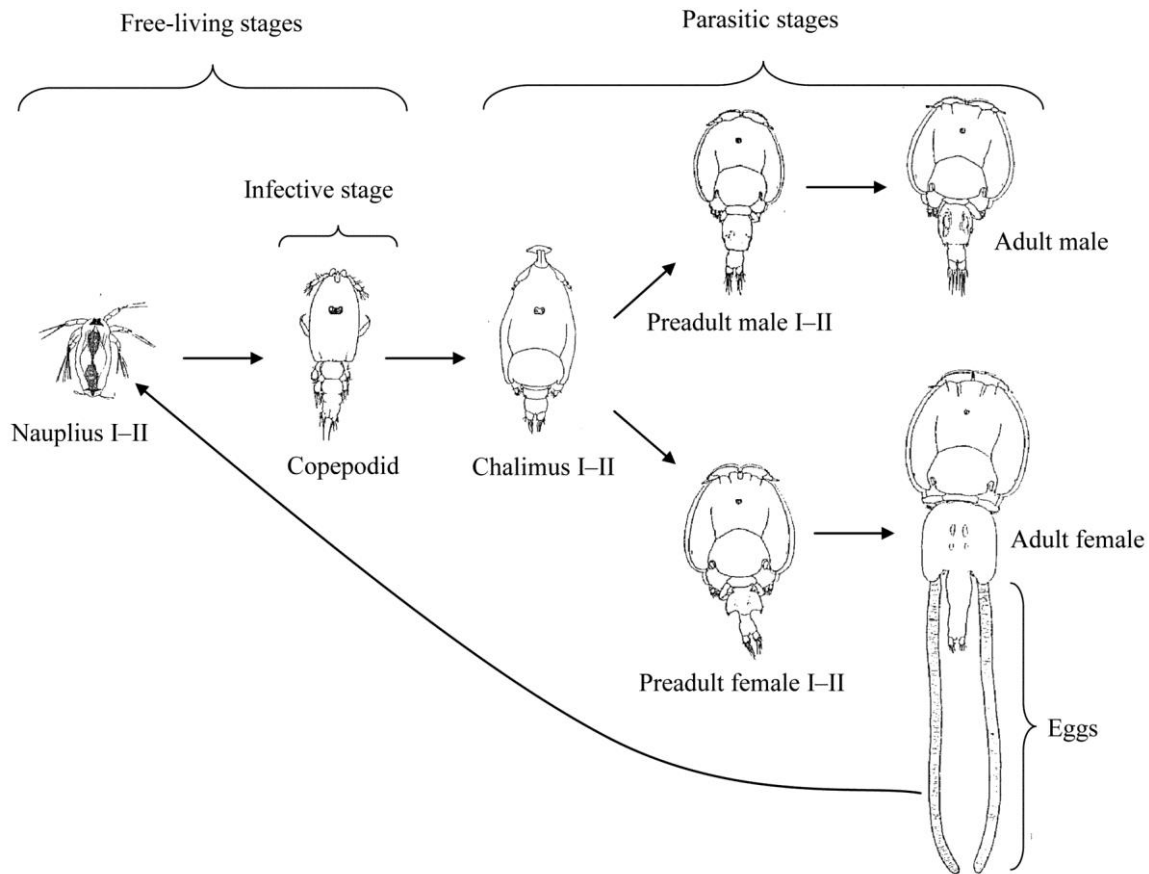


Figure 1: Life cycle of *L. salmonis* (Igboeli, Burka, & Fast, 2014)

Adult *L. salmonis* females can produce up to eleven pairs of egg strings during their lifetime, with each egg string containing 55-704 eggs (P. Heuch, Nordhagen, & Schram, 2000). Boxaspen and Næss (2000) incubated *L. salmonis* eggs at different temperatures (ranging from 2°C to 10°C). Day of hatching was dependant on temperature where eggs incubated at 2°C on average hatched after 45 days, 21.6 days at 5°C and 8.7 days at 10°C. They also measured hatching success and found that eggs hatched at 2°C could develop into infective copepodids, although with lower success rate than at higher temperatures (Boxaspen & Næss, 2000).

Temperature also impacts the developmental time between stages. Developing from nauplii I to the infective copepodid stage takes on average 9.3 days at 5°C, 3.6 days at 10°C, and 1.9 days at 15°C. Copepodid survival without a host ranged from 2 to 8 days in the same range of temperatures (Johnson & Albright, 1991a).

Infective copepodids of *L. salmonis* have a distinct diel vertical migration where they stay at the surface during the day and sink to deeper layers during the night. Nauplii stay at the surface and have small variations in depth during a day (P. A. Heuch, Parsons, & Boxaspen, 1995). Dispersal of *L. salmonis* and *C. elongatus* between potential host populations is believed to occur during the

planktonic stages (Amundrud & Murray, 2009). Due to the low horizontal swimming capabilities of these stages, dispersal potential is highly dependent on surface currents as a means of transportation. Using a particle tracking model, Brooks (2005) estimated that nauplii did not necessarily accumulate at their hatching site, and would under certain conditions be transferred 10-40 km from their hatching sites before becoming infective. They concluded with that local wind conditions was the dominant factor of dispersion. On this basis, depending on environmental conditions, an increase in wild fish infections in the immediate vicinity of the source of sea lice larvae could be not be expected because nauplii would not moult into infective copepodids until dispersed far away from the source. On the other hand, Krkošek, Lewis, and Volpe (2005) criticises this model claiming that several of the parameters used were incorrect resulting in a higher dispersion rate of the planktonic stages compared to real conditions. They propose an alternative model that concludes that the infective stages of sea lice do in fact accumulate close to the source of the larvae.

High temperatures increase the rate of development for each life stage, thereby decreasing the infective window (Tucker, Sommerville, & Wootten, 2000). Surface currents facilitate the potential of dispersal, as strong currents can transport plankton further than weak currents.

Studies have shown that there are seasonal changes in abundance of *C. elongatus* and *L. salmonis*. á Norði et al. (2015) reported that *C. elongatus* was most dominant during the winter months, while Revie, Gettinby, Treasurer, and Rae (2002) showed high abundance in the summer. Both studies observed a pattern of inverse correlation of abundance between the two species.

### 1.3 Species classification

Studies have shown that individual size cannot be used as the sole criteria to separate planktonic stages of *L. salmonis* and *C. elongatus* due to overlapping size distributions of the planktonic stages. Table 1 shows reported lengths and widths of *L. salmonis* and *C. elongatus* from several different studies. Schram (1993) found nauplius I length of *L. salmonis* ranging from 470-575 µm and nauplius II length ranging from 590-620 µm. Estimates of average length of *L. salmonis* also differed between studies. A laboratory experiment conducted by S. Johnson and Albright (1991b) found the average length of nauplius I to be 540 µm and Nauplius II 560 µm, compared to 511 µm and 606 µm reported by Schram (1993). The range of nauplius sizes in *C. elongatus* was also large. Pike, Mordue, and Ritchie (1993) found nauplius I from 404-481 µm and nauplius II from 504-550 µm. Piasecki (1996) found nauplius I from 441-585 µm and nauplius II from 455-533 µm. Width measurements overlap between the two species as well (Schram, 2004).

The best way of separating the two species is by visual assessment of morphological traits and pigmentation. The nauplius I of *L. salmonis* has black pigmentation around the eyes as well as black pigmentation dorsally at the urosome. Brown pigmentation is present in the middle of the body. The nauplii II of salmon lice have a slimmer shape than the nauplii I. There is also black pigmentation dorsally between and around the eyes and in bands at the urosome. Brown pigmentation is present dorsally as two C-shaped figures. The infective copepodid stage also has brown C-shaped figures dorsally, as well as black bands on the urosome. *C. elongatus* has the same differences in body shape between nauplius I and II as *L. salmonis*, but the distribution and colour of the pigmentation differs. *C. elongatus* has red pigmentation present dorsally along the sides of the bodies of both nauplius I and II. Red pigmentation is also present in the copepodid stage. Other common species, such as *Lepeoptheirus pollachius*, can be differentiated by having black pigmentation on the antennules, not present in *L. salmonis* or *C. elongatus* (Schram, 2004). When storing plankton samples in formaldehyde, pigmentation will rapidly bleach, making the differentiation of these species difficult. Alcohol may be a better storing agent to preserve pigmentation, but may cause shrinkage (Schram, 2004).

Table 1: Size ( $\mu\text{m}$ ) of planktonic stages of *L. salmonis* and *C. elongatus* (Schram, 2004)

	Nauplius I	Nauplius II	Copepodid
<i>L. salmonis</i>			
Length $\pm$ SD	540 $\pm$ 40	560 $\pm$ 10	700 $\pm$ 10
Width $\pm$ SD	220 $\pm$ 10	200 $\pm$ 10	280 $\pm$ 10
N	25	16	25
Johnson & Albright, 1991			
Length $\pm$ SD, range	511 $\pm$ 24, 470–575	606 $\pm$ 10, 590–620	684 $\pm$ 16, 658–709
Width $\pm$ SD, range	188 $\pm$ 8, 165–200	205 $\pm$ 10, 195–230	229 $\pm$ 7, 223–252
N	30	22	15
Schram, 1993			
<i>C. elongatus</i>			
Length $\pm$ SD, range, N	448 $\pm$ 5, 441–585, 10	487 $\pm$ 20, 455–533, ?	661 $\pm$ 30, 580–810, 308
Piasecki, 1996			
Length $\pm$ SD			
Early	404 $\pm$ 3		
Mid	444 $\pm$ 3	504 $\pm$ 3	756 $\pm$ 3
Late	481 $\pm$ 1	550 $\pm$ 3	
N	20–24	20–24	20–24
Width $\pm$ SD			
Early	226 $\pm$ 3		272 $\pm$ 2
Mid	208 $\pm$ 3	182 $\pm$ 1	
Late	194 $\pm$ 2	226 $\pm$ 2	
N	20–24	20–24	20–24
Pike et al., 1993			
Length $\pm$ SD, range, N	440 $\pm$ 12, 430–460, 4 451 $\pm$ 13, 431–469, 8 467 $\pm$ 9, 444–482, 27	490 $\pm$ 15, 468–507, 10 515 $\pm$ 12, 490–540, 11 532 $\pm$ 7, 520–545, 12	624 $\pm$ 13, 610–640, 15 702 $\pm$ 10, 690–715, 3
Width $\pm$ SD, range, N	180 $\pm$ 9, 165–190, 8 193 $\pm$ 11, 180–210, 4	182 $\pm$ 6, 175–200, 11 185 $\pm$ 5, 177–190, 10	212 $\pm$ 7, 200–220, 16 240, 3
Present publication	203 $\pm$ 5, 190–209, 27	200 $\pm$ 5, 190–203, 12	



## 1.4 Study aims

The impact of sea lice, on both farmed and wild salmonids is widely regarded as a big issue in Norway. Knowledge is an essential part of understanding and problem solving, and issues regarding sea lice. As a part of the effort of gaining knowledge Taskforce salmon lice was established as a PhD programme with collaboration between Norwegian University of Science and Technology (NTNU), Fiskeri- og havbruksnæringens forskningsfinansiering (FHF) and several different aquaculture companies. The main goal of this collaboration is to gain knowledge on sea lice biology, infection mechanisms and dispersion.

The overarching aim of this thesis is to establish knowledge on both the abundance and the distribution of planktonic sea lice, inside and outside sea cages at salmon farms. This was done by performing a seasonal study. In addition to the seasonal study, a distribution and a storing study were conducted as well. These were added in order to strengthen the results from the main, seasonal study. This thesis will aim to fulfill goal 1 and 2 as listed, while investigating the following hypotheses.

1. Characterize the abundance of planktonic stages of sea lice in association to fish farm installations over time (January to August). I propose the following hypothesis:
  - 1.1 Abundance of planktonic stages of sea lice will be significantly higher during the summer months (June, July and August) compared to the colder winter months (January, February). Temperature is expected to be positively correlated with planktonic sea lice abundance.
  - 1.1 The abundance of infective copepodids will be significantly lower than nauplii.
2. Characterize the distribution of planktonic stages of sea lice inside and outside a cage. Here, I hypothesize that:
  - 2.1 Abundance of planktonic stages of sea lice will be higher on the inside of a cage compared to the outside.
  - 2.2 Biofouling coverage on the cage net is positively correlated with abundance of planktonic stages of sea lice found on the inside of a cage compared to the outside.
  - 2.3 The highest abundance of planktonic stages of sea lice will be found where the current goes out of the cage.

## 2. Materials and methods

Three studies were performed. The first study was a time series for studying the abundance of planktonic stages of sea lice during the period January to August at two fish farm locations (hereafter called the “seasonal study”). The second study was conducted to characterise the distribution of sea lice in and around sea cages (called the “distribution study”). The third experiment was done to determine the effect of ethanol storage on planktonic stages of salmon lice over a period of 3 weeks (henceforth referred to as the “storage study”).

### 2.1 Seasonal study

#### 2.1.1 Fish farming locations



Figure 2: Map of aquaculture localities in northern Frøya. Locality type indicated by colour: Salmonids (red), other fish species (yellow), algae (green). Localities used in this thesis (red star) locality A (Valøyen) and locality B (Mannbruholmen) (Fiskeridirektoratet, 2019).

In the seasonal study, samples were collected from two different fish farms in Trøndelag located north of Frøya (Figure 2). Mannbruholmen (N:63°84.73', E8°53.675') and Valøyen (63°81.73', 8°46.248') are both owned and run by the company Mowi ASA. Frøya municipality has 35 aquaculture localities for production of Atlantic salmon (Fiskeridirektoratet, 2019) and is one of the areas in Norway with

the highest production of Atlantic salmon. Both farms used cages without louse-skirts for the duration of this study.

### 2.1.2 Sea temperature and louse abundance on farmed salmon

All fish farms must report information about salmon lice infection rates, delousing operations, and temperature at the farm weekly. Sea water temperatures (°C) were measured regularly by the fish farmers at depths of 5 and 10 m at all sea farms. The reported temperature was an average of the two measurements. The number of motile, non-motile and adult female lice must also be reported. This is done by sampling 20 fish from each cage on the given farm. All data from the farm was reported to the Food Safety Services and was then uploaded to a Norwegian surveillance and information platform developed by the Norwegian Coastal Administration called barentswatch.no. The information on this website is open to the public (Baretswatch, 2019).

### 2.1.3 Sampling period

Samples were collected once every month from January to August in 2018. Sampling in each location was done within a 48 hour period except in January, when samples from Mannbruholmen were collected on the 17<sup>th</sup> and from Valøyen on the 31<sup>th</sup>. This was necessary because of challenging weather conditions and time constraints. The experiment was concluded in August due to slaughtering in September. Management at the fish farms approved all visits to the farms, and information about delousing and other operations that could affect sampling was considered before deciding when to do the sampling.

To ensure safety and sampling success, sampling was only undertaken when weather conditions were not too extreme. These considerations were taken on a case by case basis. Data from [mærmeldingen.no](http://mærmeldingen.no) was used to help determine the optimal time for sample collection. [Mærmeldingen.no](http://Mærmeldingen.no) uses weather data from the Norwegian meteorological institute and NRK and presents information about temperature, wind and currents for aquaculture localities.

To get the most consistent and representative results, samples were taken from the same cages each month for as long as possible. When this was no longer feasible because of operations on the farm (May for Valøyen and April for Mannbruholmen), samples were instead obtained from other cages at each location for the rest of the study.

### 2.1.4 Sampling places

Samples were collected from three separate cages at each fish farm. Data from [mærmeldingen.no](http://mærmeldingen.no) and onsite visual estimates were used to determine the direction of the ocean current. Onsite visual estimates included looking at macro-algae growing on moorings and the cages, particles in the water, and also using the plankton net and observing in which direction it was pulled. Samples were collected from the floating collar of the sea cage at the spot where the current exited the cage, see Figure 3.

#### Sampling for seasonal study

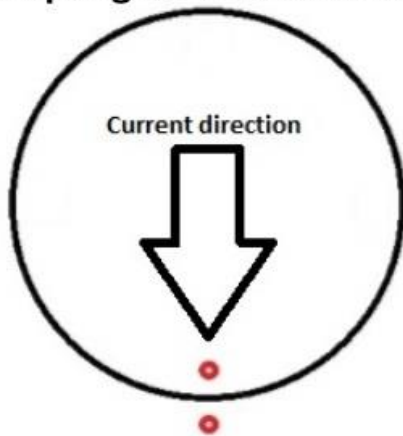


Figure 3: Illustration of where samplings were taken in relation to the current, arrow indicates current direction and red dots indicate sampling spots.

### 2.1.5 Plankton sampling

Samples were collected using a standard conical plankton net, 180 cm from opening to cod end with a 150  $\mu\text{m}$  mesh size and a 50 cm in diameter opening (see Figure 4 for illustration of the plankton net). The plankton net was lowered to the depth of 7 m and subsequently raised at a speed of approximately 1 m/s. Samples were first collected on the outside and then on the inside of the cages to minimize the probability of disturbing the sampling location. Two tows were taken for each sample and two replicates were taken for each spot. Throughout the samplings, the water current direction was carefully observed. If the current shifted, sampling was relocated to the new location where the current exited the cage.

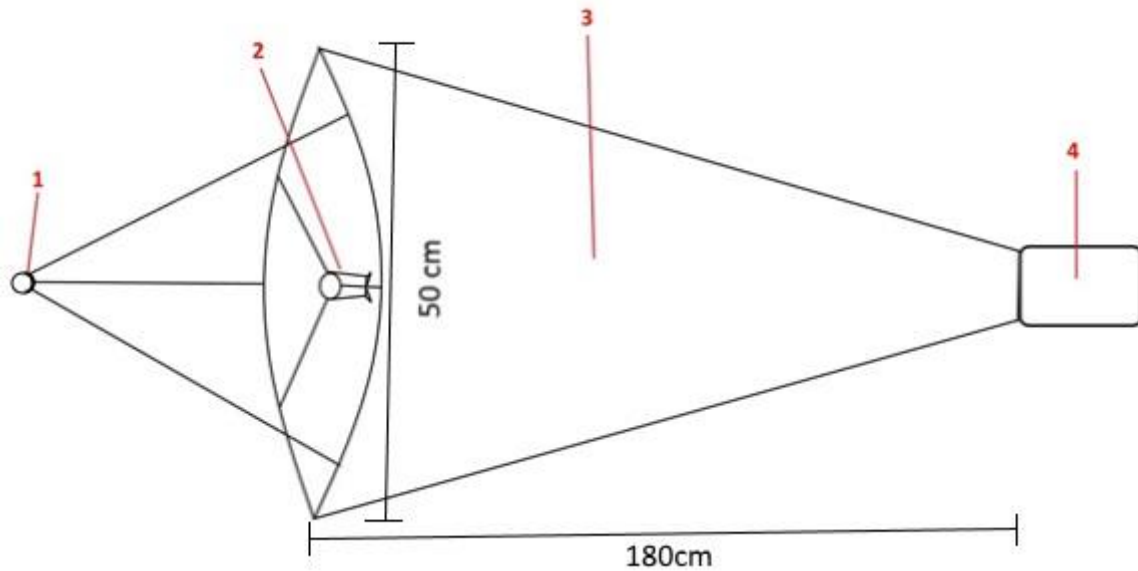


Figure 4: Illustration of a standard conical plankton with triple point connection (1), digital flow meter (2), plankton net mesh size (150 µm) and removable cod end (4), Length of net (180 cm) and width of mouth (50 cm)

To control for overflow and obtain an accurate estimate of the water volume filtered through the net, a digital flow meter with back-run stop was attached at three points at the mouth of the plankton net so that the flow meter was suspended in the middle of the opening. This allowed calculations of total water volume entering the mouth of the net for each tow.

After each tow, the net was rinsed with a seawater pump. All organisms > 150 µm would then be expected to be in the removable cod end (Figure 4). The cod end was then detached and emptied over a rough filter (1000 µm mesh size) to remove large organisms and large pieces of macroalgae. The remaining content was then emptied over a fine filter (150 µm mesh size). All organic content retained in the fine filter was transferred to polyethylene bottles (250 mL) together with ethanol (96%) and stored at 4°C until further analyses. Some seawater was transferred together with the organic material, so the ethanol concentration of the final samples was around 70%. Analyses were further conducted as described in section 2.6

## 2.2 Distribution study

The distribution study was performed twice in August (week 32 and 33) and September (week 38) at Mannbruholmen. Samples were collected at four different locations on the salmon cage based on the ocean current direction, see Figure 5. The four different sampling places in the sea cage was decided with the current direction as basis, as described in section 2.1.4. Sample collection and analyses were done using the method described above for the seasonal study (section 2.1.5), although not all samples could be examined within a week.

## Sampling for distribution study

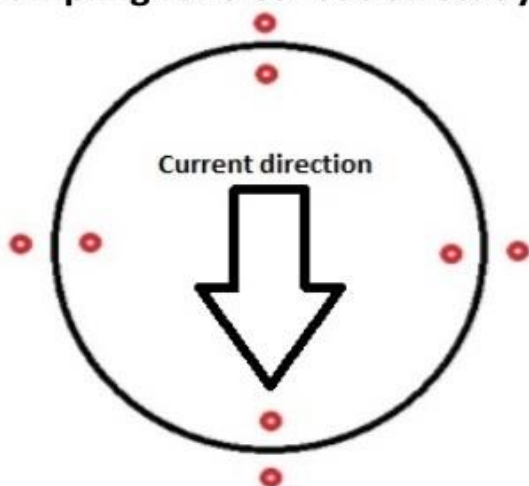


Figure 5: Illustration of where samplings would take place in relation to current, arrow indicate current direction and red dots indicate sampling sites, inside and outside the cage

## 2.3 Storing study

The storing study was conducted with the use of lab-cultured salmon lice. 38 nauplii I, 20 nauplii II and 20 copepodids were used. The lice were first sedated with use of a mixture of Finquel: 100% trikainmesylat. They were then stored in ethanol (70%) at 4°C to imitate the storage conditions of the other experiments (see section 2.1.5). Each louse was stored in an individual Eppendorf tube and examined a total of five times: alive and after 3, 6, 12, and 24 days. The lice were photographed under a stereo microscope, and changes in size parameters were quantified.

## 2.4 Analysis of biofouling

At each sample site, biofouling was recorded by lowering a waterproof camera attached to a rod down to 1 m on both the inside and outside of the net. A screenshot of the video was taken and used to quantify the amount of biofouling occurring on the net. To determine the fouling percentage, a grid net with 60 random dots were placed on top of the image using the picture editing program paint.net. Dots that occurred on top of areas with fouling were registered as fouling occurrences. A percentage was calculated by dividing the number of fouling occurrences on the total number of dots. This percentage was used to categorize the amount of fouling on a scale from one to five (Table 2).

Table 2: Categorization of biofouling based on percentage of coverage.

Category	Amount of fouling	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

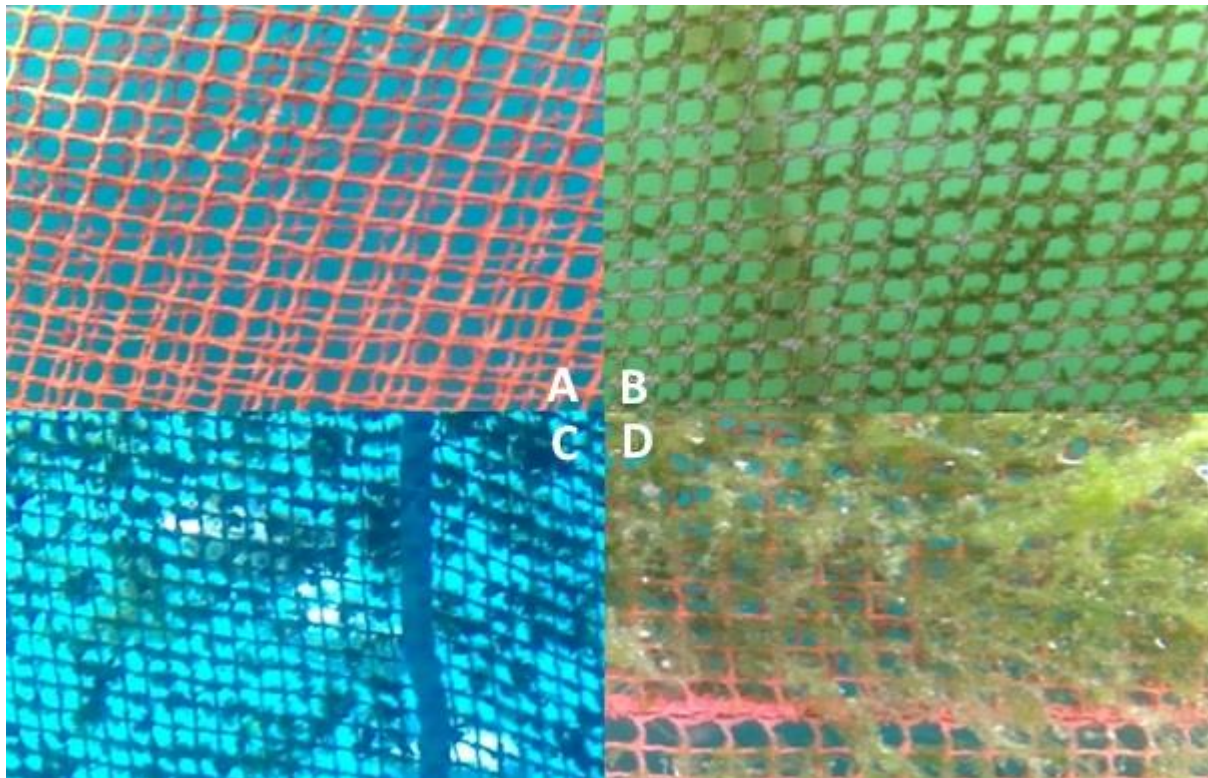


Figure 6: Pictures taken of sea cage net with underwater camera with various degree of biofouling: A (No fouling), B (Low fouling), C (High fouling) and D (Full fouling)

## 2.5 Sample counting and analysis:

Samples were first filtered through a filter with mesh size of 90  $\mu\text{m}$ . Small portions of the contents were then transferred to counting chambers together with filtrated sea water for examination. The organic material in the chambers was analysed under a stereo microscope (Leica M205 C, 0.78-16.0x). Every chamber was thoroughly examined with a magnification of 1.6 to look for lice at the surface, then with a magnification of 2 to look for lice in the rest of the chamber. In samples with high densities of organic content it was not practical to analyse the entire sample. In these cases, half the sample was examined. The remaining half was stored at 4°C for possible future analyses.

To minimize the risk of pigmentation loss making species identification impossible, the first replicates of every sample were analysed within a week of sample collection. The remaining samples were examined as soon as possible, but the time of analysis varied from 6 to 60 days.

When lice were found in the sample, they were transferred to separate wells to be photographed. The lengths and widths of the lice were measured in the program Zen 2012 (blue edition, version 1.1.1.0). The lice were stored in ethanol (96%) in Eppendorf tubes at -22°C.

Photographs of lice were used to determine species and developmental stage. Morphological traits such as pigmentation and shape were used. If species identification was impossible due to degeneration, loss of pigmentation or damage, the individual was classified as “unknown”.

## 2.6 Processing and statistical analysis

Most of the statistical analyses and graphs were done in the statistical software program R (R Core Team, 2018). Data organization, some statistical analyses and some figures was done in Microsoft Excel version 16.0.11. Figures and adjusting of illustrations were done using the photo editing program paint.net. Tables were created in Microsoft Word for Windows. Significance level was set to  $p < 0.05$  unless otherwise specified.

### 2.6.1 Evaluation of data in the seasonal abundance

Normality was tested for with the Shapiro-Wilk test. The Mann-Whitney U test was used to compare the abundance of the mean abundance from each month (McKnight & Najab, 2010). Spearman rank correlation test (Lyerly, 1952) was used to see if there were any monotonic relationship between temperature and the abundance of planktonic sea lice as well as amount of adult female *L. salmonis* in a sea farm. The relationship between adult females of *L. salmonis* and planktonic stages of *L. salmonis* was tested for as well. This is a correlation test that measures the strength and direction of monotonic relationships, not the linear relationship between the variables.

### 2.6.2 Test of distribution of sea lice inside or outside of the sea cage

For analysing the relationship between inside and outside samples, a Mann-Whitney U test was performed. This is a nonparametric test that works with data that are not normally distributed and works well at small sample sizes (McKnight & Najab, 2010). Correlations between amount of biofouling and proportional abundance between inside and outside samples were tested for with Spearman rank correlation (Lyerly, 1952). Error bars in the graphical representations of abundance in both the seasonal study and the distribution study are shown as standard error. This is to illustrate the uncertainty around the estimates of the mean measurements.



### 2.6.3 Evaluation of size differences in the storage study

Normality of the data was tested with use of the Shapiro-Wilk test, which showed that normality could not be assumed for either the length or the width of the lice. A nonparametric test called the Wilcoxon rank sum test was used to determine if there was a significant difference between the means (Wilcoxon, Katti, & Wilcox, 1970). This test is similar to the paired student t-test, but can be used on data that are not normally distributed (Bridge & Sawilowsky, 1999). Standard deviations are presented together with range in the tables in the storage study to show the spread of the data.

### 3. Results

#### 3.1 Sea lice abundance (January-August 2018)

##### 3.1.1 Salmon lice abundance at the farms

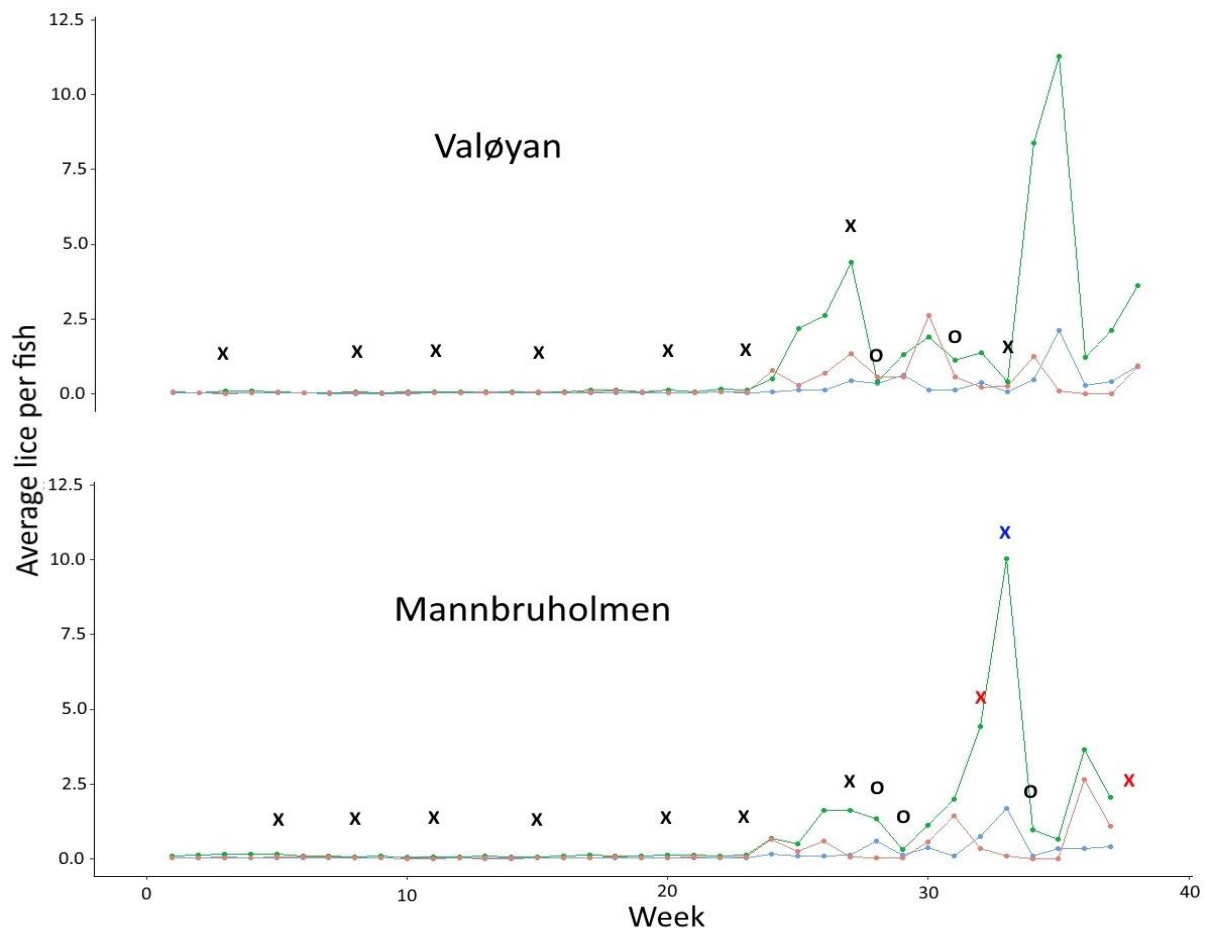


Figure 7 Average number of salmon lice (*L. salmonis*) on farmed fish at Valøyan (upper panel) and Mannbruholmen (lower panel) in January-September 2018. Different stages indicated by colour: Motile stages (green), non-motile stages (red), adult female stage (blue). X indicates time for sampling of planktonic stages. Colour of X-es indicates type of sample: Seasonal sample (black) and distribution sample (red), samples for both experiments (blue). O indicates when delousing operations took place.

Figure 7 shows the average number of different life stages of salmon lice that is reported by the webpage barentswatch.no (Baretswatch, 2019). The numbers of lice at the fish farms are average numbers based on registrations on 20 randomly sampled fish from each sea cage. From week 1 to week 23 neither Mannbruholmen nor Valøyan had lice of any stages exceeding 0.15 lice per fish on average. In weeks 24-27 both farms had an increase in the abundance of all three stages. From week 28 to 33 the abundance of lice fluctuated with a peak of lice in motile stages at Valøyan in week 30 (an average of 1.89 motile lice per salmon) and at Mannbruholmen in week 31 (an average of 1.44 motile

lice per salmon). In August a large peak in both adult female lice and motile stages were observed at Valøyen (week 35), with averages of 11.3 motile lice and 2.11 adult female lice per fish and at Mannbruholmen in week 33 with averages of 10.05 motile and 1.68 adult females per fish. Both farms conducted two delousing operations in short succession in July (within 4 weeks).

### 3.1.2 Temperature

Figure 8 shows the sea temperature (°C) from January to September for the sampling localities. Both localities have similar temperature profile throughout the experimental period. Temperature decreased from Week 1 (7°C) to week 12 (5°C) and then increased until the end of the experiment week 39 (14°C). In week 14 temperature at Mannbruholmen had a peak with 7°C, two degrees higher than the measurements the week prior and week after.

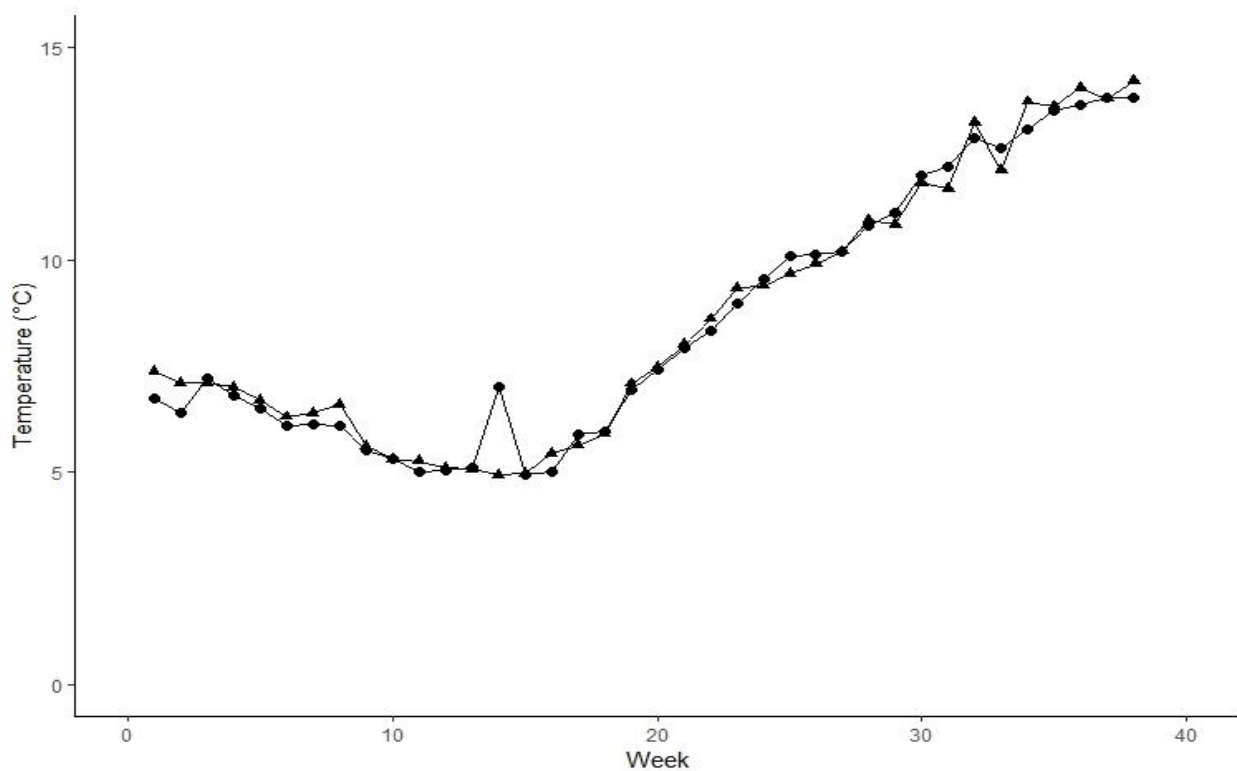


Figure 8: Weekly average sea temperature measured at 5 and 10 m depth (°C) measurements during the sampling period (week 1-38 2018). The temperatures at Mannbruholmen is shown as circles, at Valøyen as triangles.

Figure 9 shows the relationship between the average number of adult female *L. salmonis* that was observed on a farm compared to the average temperature measured. Low average number of adult females throughout the experiment except for August. A strong correlation between adult female *L. salmonis* and temperature was observed ( $R^2 = 0.83$  with  $p = 0.001$ ,  $N = 16$ )

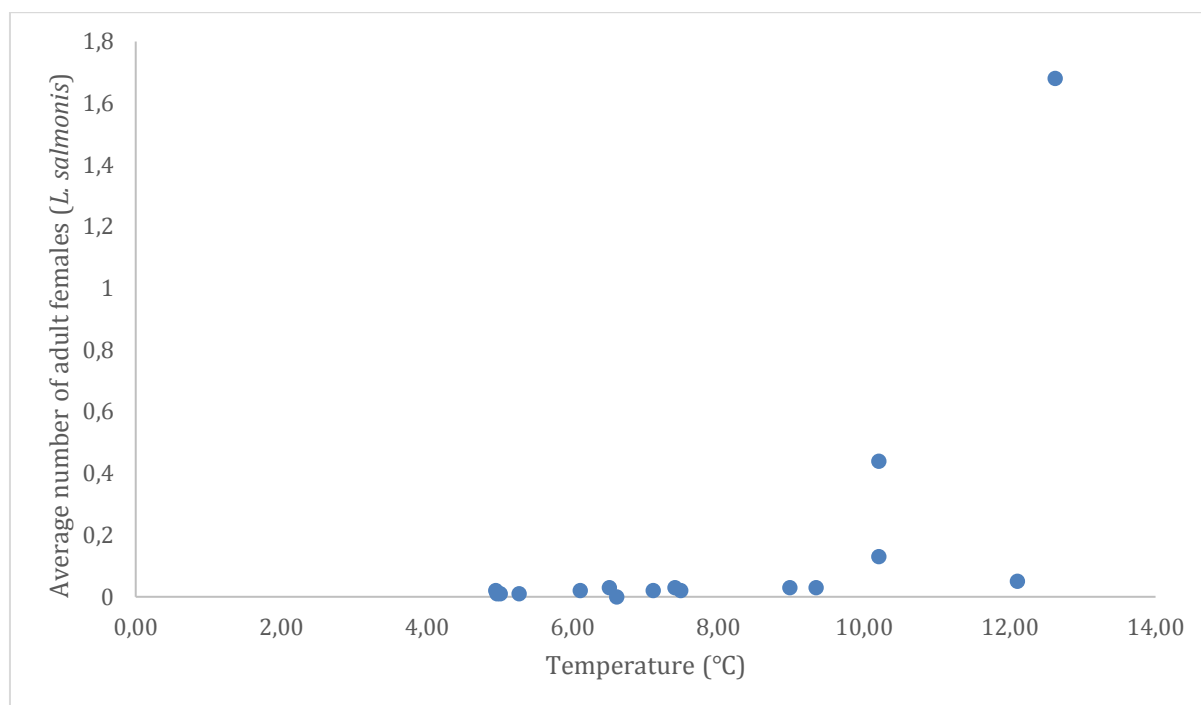


Figure 9: Scatterplot of Average number of female lice (*L. salmonis*) and temperature (°C) during the sampling period.

### 3.1.3 Biofouling in the seasonal study

Table 3: Degree of biofouling at both fish farms during the seasonal experiment. Values presented as percentage and category (1-5), standard deviation for percentage shown for each month.  $N=3$  for each value.

Month	Mannbruholmen		Valøyen	
	%	SD	%	SD
January	7	±4	7	±0
February	9	±4	17	±30
March	14	±17	12	±11
April	86	±16	45	±40
May	67	±27	8	±4
June	53	±32	8	±3
July	36	±44	58	±24
August	81	±12	8	±12

Table 3 shows the degree of biofouling on the net during of the experimental period. Mannbruholmen had overall more fouling than Valøyen throughout the period, although both localities had low degree of biofouling from January to March. Fouling at Mannbruholmen was especially high in April and August. Valøyen had low amounts of biofouling throughout the experimental period except for April

and July where medium to high fouling was observed. No significant correlation between the degree of fouling and the abundance of planktonic sea lice distribution outside and inside the net was observed ( $R^2 = 0.1941$  with  $p > 0.05$ ).

### 3.1.4 Abundance of planktonic sea lice stages over time

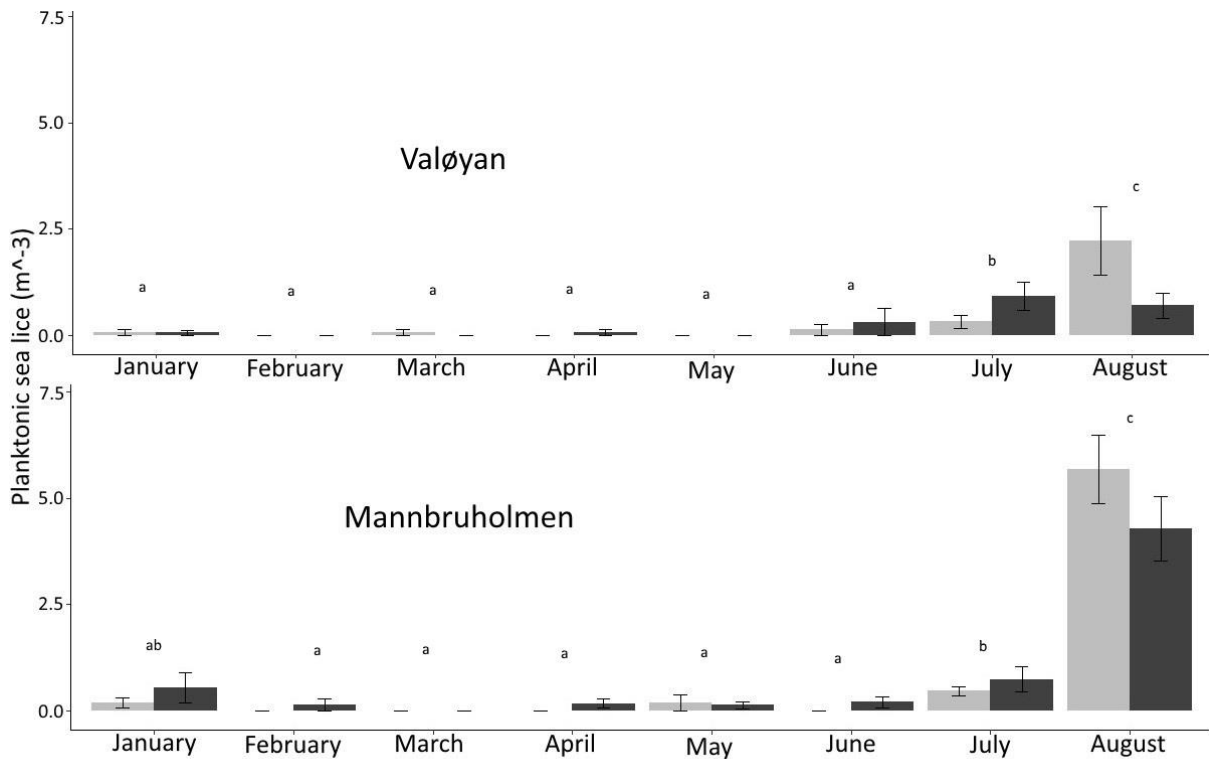


Figure 10: Number of planktonic sea lice  $m^{-3}$  sampled from 0-7 m depth at Valøyan (upper panel) and Mannbruholmen (lower panel) from January-August 2018,  $N = 6$  for each column, colour indicates sample location (gray denotes inside samples, black denotes outside samples). Error bars show standard error. Months denoted with the different letters (a, b and c) have a significantly different ( $p < 0.05$ ) total abundance in lice compared to other months at that site.

Figure 10 shows number of planktonic sea lice ( $m^{-3}$ ) at Valøyan and Mannbruholmen during the sampling period. There were low amounts of lice from January to June at Valøyan, Mannbruholmen had more lice in January compared to Valøyan, but had low abundances from February through June. There was no significant difference in the amount of sea lice from January to June at Valøyan ( $p > 0.05$ ), and the same was the case for abundances from February to June at Mannbruholmen. Significant increase in abundance was present in July and August at Valøyan, and the same for Mannbruholmen with the exception of January that was not significantly different from June samples. Significant increase in abundance of lice occurred from July to August for both farms. No significant difference was found between inside and outside sample locations for any month ( $p > 0.05$ ).

Figure 11 shows the relationship between the abundance of planktonic stages of *L. salmonis* and temperature. A strong correlation between temperature and planktonic sea lice abundance was observed ( $R^2 = 0.71$  with  $p = 0.005$ ,  $N = 16$ ).

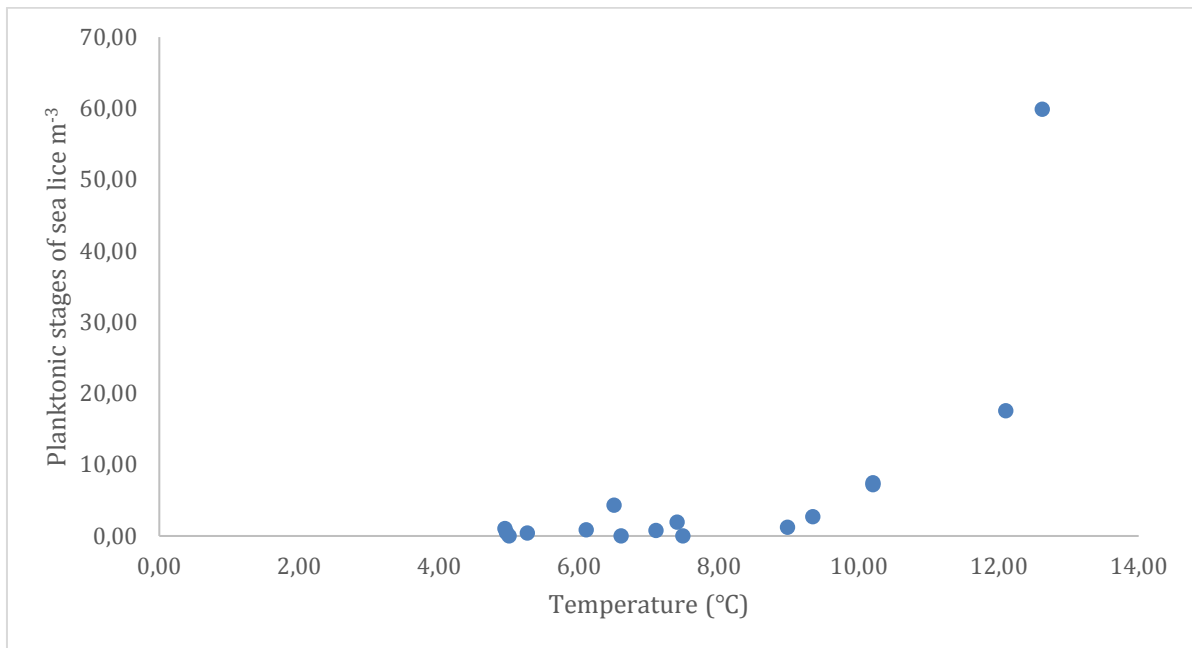


Figure 11: Scatterplot of planktonic stages of sea lice m<sup>-3</sup> and temperature (°C) over the experimental period

### 3.1.5 Species abundance in the seasonal study

Figure 12 shows number of lice of different species per  $m^{-3}$  over the whole sampling period. *L. salmonis* was the most abundant species in August at both farms. *C. elongatus* was found in small numbers throughout the experimental period. Other species were found sporadically at low abundances.

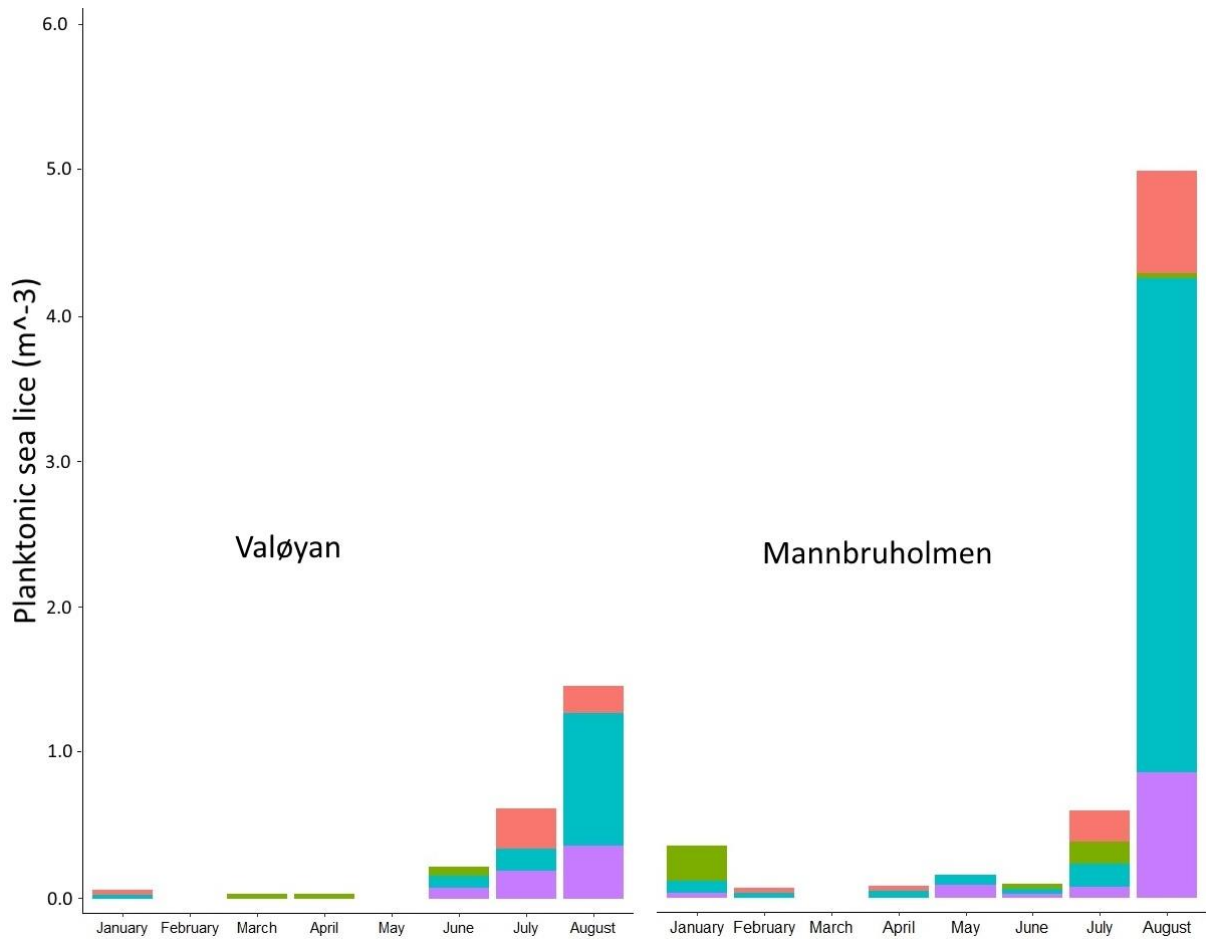


Figure 12: Number of planktonic sea lice ( $m^{-3}$ ) in water samples (0-7 m depth) at Valøy and Mannbruholmen from January-August 2018, colours indicate present species at given months: *C. elongatus* (orange), *L. pollachius* (green), *L. salmonis* (blue) and unknown individuals (pink).

Figure 13 shows a scatterplot of planktonic stages and adult females of *L. salmonis*. Most of the measurements of adult females were low, as well as the abundance of planktonic stages of *L. salmonis*, with the exception of August samples. A strong correlation between Adult females of *L. salmonis* and planktonic stages of *L. salmonis* was found ( $R^2 = 0.93$  with  $p = 0.001$ ,  $N = 16$ ).

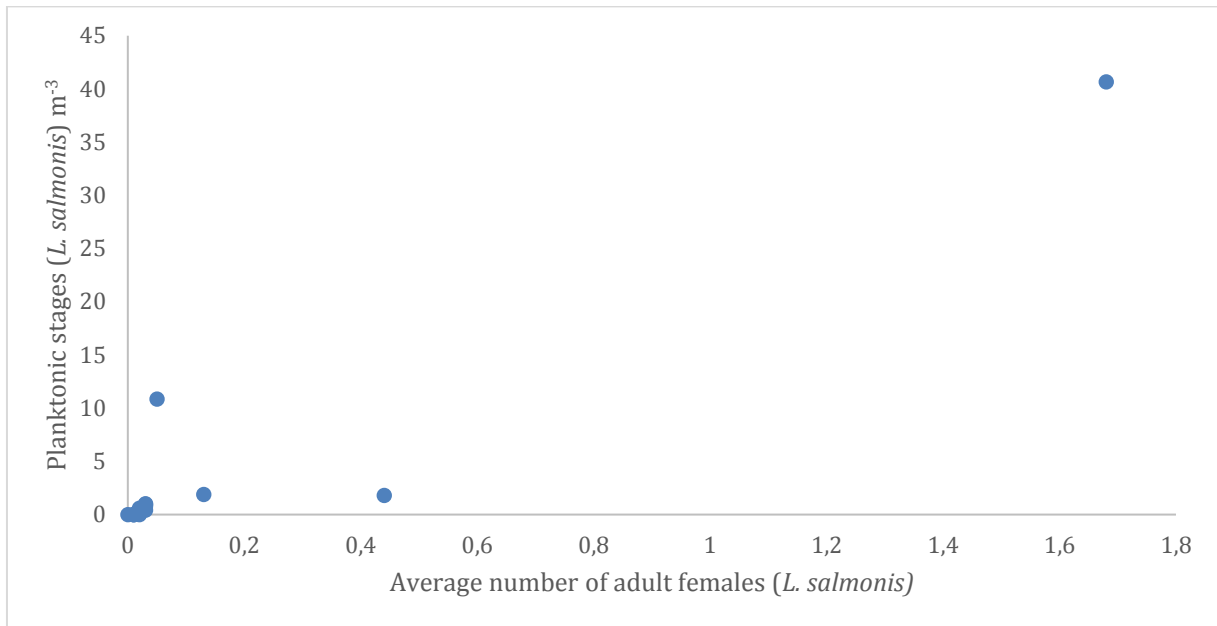


Figure 13: Scatterplot of planktonic stages of (*L. salmonis*) and Adult females (*L. salmonis*)

## 3.2 Sea lice distribution within a cage – distribution study

### 3.2.1 Distribution of sea lice inside and outside the sea cage

Figure 14 shows the average ( $n = 3$ ) proportional abundance of planktonic sea lice m<sup>-3</sup> found in the different sampling locations of the cage (illustrated by the inset). Highest abundance of planktonic sea lice was found at the outflow of the cage. Side 1 had a large abundance as well. Inflow and side 2 had low proportional abundance. Inside samples had more lice than the outside samples at sampling locations where most of the lice were found. The difference between inside and outside samples for Inflow and Side 2 was not significant ( $p > 0.05$ ). There was a significant difference between Outflow and Inflow samples ( $p < 0.01$ ). Significant difference between inside and outside sampling locations were observed for outflow ( $p < 0.01$ ) and side 1 ( $p < 0.05$ ).



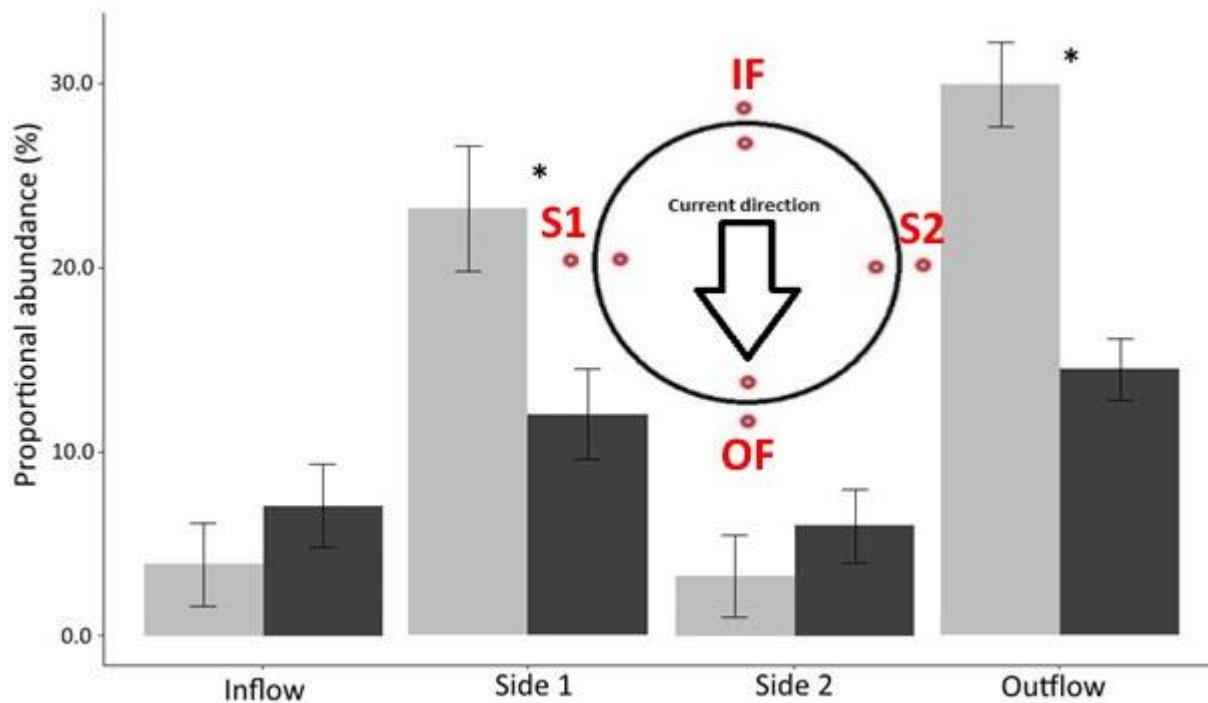


Figure 14: Proportional abundance of planktonic sea lice per m<sup>-3</sup> filtrated water from four different sampling spots on the cage. From three separate samplings collected in August and September 2018 at Mannbruholmen. Grey columns indicate inside samples, black columns indicate outside samples. Error bar shows standard error. Inset denotes sampling locations in relation to water current: Inflow (IF), Side 1 (S1), Side 2 (S2) and Outflow (OF). Asterisk denotes significant difference between inside and outside samples.

### 3.2.2 Species abundance

Figure 15 shows the proportion of sea lice species at the different sampling places. *L. salmonis* was the most abundant species at all places. *C. elongatus* was more common in the outflow samples compared to the other samples. Lice classified as unknown due to lack of pigmentation were present at low abundance at all sampling places.

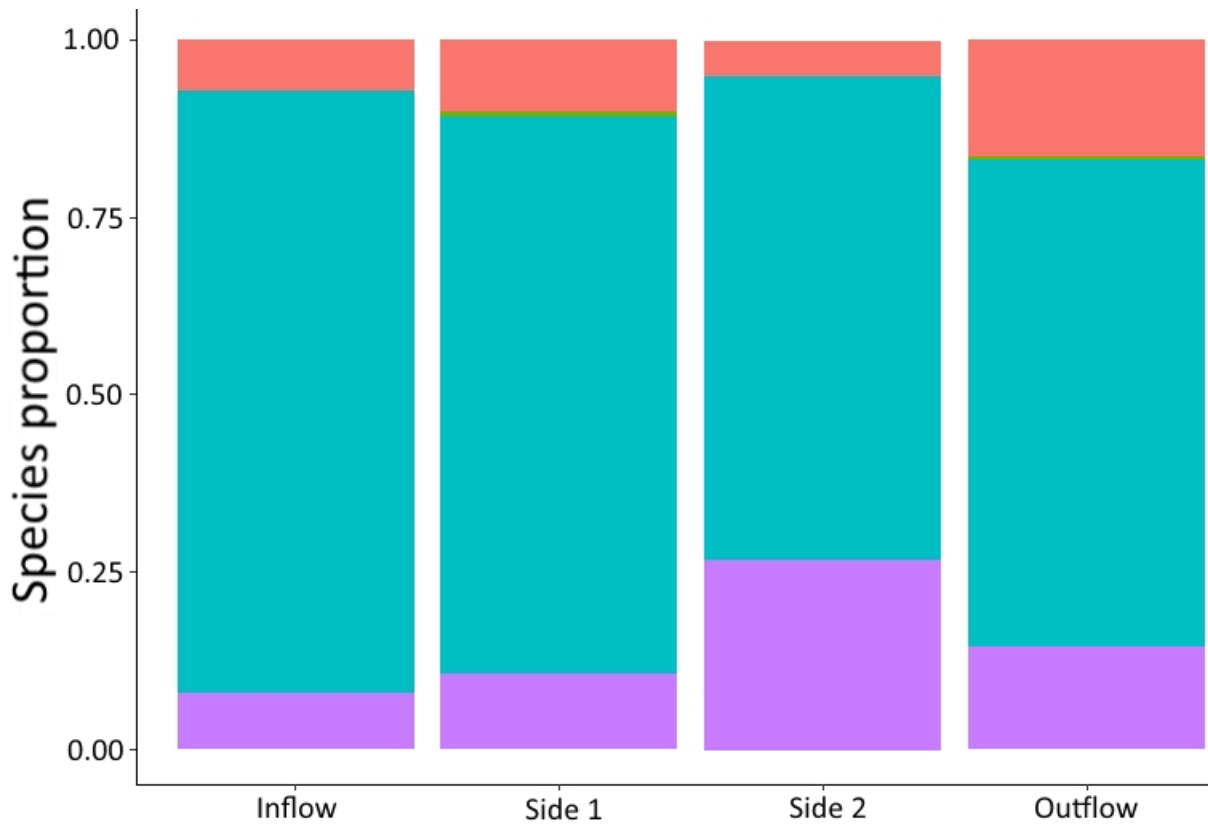


Figure 15: Proportional abundance of planktonic Sea lice species ( $m^{-3}$ ) from different sampling places. Colours indicate species present at given sample spots: *C. elongatus* (orange), *L. pollachius* (green), *L. salmonis* (blue) and unknown individuals (pink). Values represent average of 3 different samplings.

### 3.2.3 Biofouling

Table 4 shows the amount of biofouling from the distribution study. Sampling 1 and 2 had high to full fouling while the third sampling had low to medium fouling. No significant correlation between the relationship between inside and outside and biofouling were found ( $p > 0.05$ )

Table 4: Amount of biofouling from both fish farms during the distribution experiment, values presented as percentage and category (1-5)

	Sampling 1		Sampling 2		Sampling 3	
Sampling place	%	Category	%	Category	%	Category
Inflow	75	4	96	5	7	2
Side 1	90	5	88	5	11	2
Side 2	98	5	93	5	15	2
Outflow	83	5	85	5	30	3

### 3.3 Storing experiment

Table 5 shows the average length and width ( $\mu\text{m}$ ) of *L. salmonis* of three different life stages stored in ethanol (70%). Each individual was measured before storing and four times during storing; after 3, 6, 12 and 24 days. Nauplii I showed significant difference in length on day 3 and day 6 compared to day 0, whereas the width was significantly different at all days (day 3, 6, 12 and 24) compared to day 0. Nauplii II length was significantly different at day 12 and 24, while width difference was not significant. Copepodids had significant change in width and length for all measurements (day 3, 6, 12 and 24). All significant changes in size was an increase from day 0, except for the length of nauplii I that had reduced length on measurements at day 6 and 12.

Table 5: Average length and width ( $\mu\text{m}$ ) of *L. salmonis*  $\pm$ standard deviation of nauplii 1 (N=28), nauplii 2 (N=20) and copepodides (N=20) of *L. salmonis* measured before they were stored (0 days) and days of storing. \* symbol illustrates significant difference from initial measurements ( $p < 0.05$ ).

	0 days	3 days	6 days	12 days	24 days
<b>Nauplii I</b>					
Length $\pm$ SD	547 $\pm$ 22	544 $\pm$ 21	539 $\pm$ 18*	538 $\pm$ 23*	542 $\pm$ 26
Width $\pm$ SD	211 $\pm$ 9	217 $\pm$ 10*	219 $\pm$ 10*	218 $\pm$ 11*	218 $\pm$ 11*
<b>Nauplii II</b>					
Length $\pm$ SD	591 $\pm$ 16	601 $\pm$ 14	599 $\pm$ 14	607 $\pm$ 11*	607 $\pm$ 14*
Width $\pm$ SD	219 $\pm$ 9	219 $\pm$ 11	218 $\pm$ 9	219 $\pm$ 10	220 $\pm$ 8
<b>Copepodid</b>					
Length $\pm$ SD	777 $\pm$ 12	803 $\pm$ 16*	805 $\pm$ 14*	807.0 $\pm$ 18*	801 $\pm$ 24*
Width $\pm$ SD	249 $\pm$ 6	272 $\pm$ 7*	270 $\pm$ 7*	272.8 $\pm$ 6*	270 $\pm$ 8*

### 3.4 Life stages

Table 6 shows the number of nauplii and copepodids sampled during the seasonal study and the distribution study. Most of the lice were in nauplii I or nauplii II stage. Less than 1 percent of the sampled lice were in the copepodid stage. None of the observed copepodids was identified as *L. salmonis*.

Table 6: Number of nauplii and copepodids found in the two studies.

Seasonal study	Copepodid	Nauplii	% copepodid
Mannbruholmen	4	156	2.6
Valøyen	0	62	0
Distribution study	1	415	0.2
<b>Total</b>	5	633	0.8

### 3.5 Nauplii size

Table 7 shows the size of the nauplii found in the different experiments. Nauplii I and nauplii II were combined in the table because of difficulties in differentiating between the two life stages. Average length and width were significantly different ( $p < 0.01$  for both measures) between *L. salmonis* and *C. elongatus*. Some overlap between the size of the different species was evident.

Table 7: Average length and width ( $\mu\text{m}$ )  $\pm$  standard deviation of nauplii of different species (*L. salmonis*, *C. elongatus*, *L. pollachius* and individuals not eligible for identification categorized as unknown) from the different experiments. Individuals with no size measurements were not included. Range in both length and width is presented as maximum and minimum observed values ( $\mu\text{m}$ ).

		Nauplii			
<b>Distribution study</b>		<i>L.s</i>	<i>C.e</i>	<i>L.pol</i>	<i>Unknown</i>
	Length $\pm$ SD	514 $\pm$ 17	450 $\pm$ 24	562 $\pm$ 56	485 $\pm$ 36
	Width $\pm$ SD	201 $\pm$ 8	177 $\pm$ 9	193 $\pm$ 14	198 $\pm$ 21
	N	309	52	2.0	51
<b>Seasonal study</b>					
<b>Valøyen</b>	Length $\pm$ SD	525 $\pm$ 18	465 $\pm$ 19	549.1 $\pm$ 54	501 $\pm$ 39
	Width $\pm$ SD	207 $\pm$ 8	179 $\pm$ 13	218 $\pm$ 5	196 $\pm$ 20
	N	28	13	3	16
<b>Mannbruholmen</b>	Length $\pm$ SD	514 $\pm$ 24	457 $\pm$ 27	503 $\pm$ 42	481 $\pm$ 50
	Width $\pm$ SD	204 $\pm$ 12	176 $\pm$ 10	223 $\pm$ 29	198 $\pm$ 17
	N	101	24	10	19
<b>Total</b>	Length $\pm$ SD	515 $\pm$ 19	454 $\pm$ 24	520 $\pm$ 49	487 $\pm$ 40
	Width $\pm$ SD	202 $\pm$ 9	177 $\pm$ 10	218 $\pm$ 26	198 $\pm$ 20
	N	438	89	15	86
	Range Length	419-621	401-520	419-603	394-595
	Range Width	166-284	156-215	172-274	154-242

## 4. Discussion

The goal of this thesis was to investigate the abundance and distribution of planktonic stages of sea lice over time in association with sea farm installations. Furthermore, abundance was tested against temperature and adult females of *L. salmonis* and distribution was tested against biofouling to see if there were any correlations.

Results from these investigations showed that there was a significant increase in abundance of planktonic stages of sea lice in July and August compared to other months. Abundance had a strong positive correlation with sea temperature measurements. The correlation between the abundance of adult female *L. salmonis* and planktonic stages of sea lice was also strongly positive at both sites. Over 99% of the planktonic stages of lice found in the studies study were nauplii and less than 1% were infective copepodids, none of the copepodids were identified as *L. salmonis*. Abundance of planktonic stages of sea lice were higher at the Outflow of the cage compared to the Inflow. The results regarding abundance on the inside compared to the outside of the cage net were inconclusive as well as results regarding biofouling as a mechanism to hinder the flow of lice out of the cage net.

### 4.1 Abundance of planktonic stages of sea lice and temperature

The seasonal study showed similar development of sea lice abundance for the duration of the experimental period for both sea farms. The abundance of the planktonic stages of sea lice was low from January to June. It was not until July that the abundance was statistically higher than the previous months, with the exception of Mannbruholmen, where the July abundance was not significantly different from the January abundance. Overall the increase in abundance over time corresponds well with hypothesis 1.1, stating that the warmer summer months would have higher abundances than the colder winter months. However, the increase in abundance did not occur until July. Sea temperature was similar for both sea farms, being below 10°C in the end of June and reaching 12°C in July. This might indicate a threshold temperature, where the generation time of the louse reaches a level of reproduction output that results in sudden increase in population growth rate. The effect of temperature on both developmental time of eggs and between stages is well established (S. Johnson & Albright, 1991b ;Boxaspen & Næss, 2000). Other environmental factors may also have influenced the result, but temperatures may not have been high enough prior to June to accumulate high enough abundances of infected fish for the abundance of planktonic stages to be higher than previous months. While temperature data from week 14 at Mannbruholmen showed deviance from the corresponding week at Valøyan, no samplings were performed this week, and is therefore not relevant for this study.

The abundance of both planktonic stages and non-planktonic stages of *L. salmonis* started to increase in July. The abundance was significantly higher in August compared to July for both farms giving further backing to hypothesis 1.1, claiming that abundance is temperature dependant. Species composition in the planktonic samples also changed over time and the increase in *L. salmonis* abundance accounts for most of the increase in total abundance. The highest abundance of planktonic stages was found in the last sampling of the distribution study in September, where sea temperature was measured to be over 14°C. The species composition was similar to the August samples from the seasonal study, with *L. salmonis* being the numerically dominant species. Temperature and abundance of planktonic sea lice showed a strong positive correlation ( $R^2 = 0.71$  with  $p = 0.005$ ) While the Spearman correlation test, showed significance it is still possible that the correlation is due to some random factors, both because of the relatively low number of observations ( $N = 16$ ) in combination with the low number of individuals found in large parts of the experiment. If several months have similar abundances, as was the case in the seasonal study, small variations in abundance may lead to a large difference in rank in the test.

The vast majority of the planktonic stages of sea lice found in both the seasonal and the distribution study were nauplii. Out of all the individuals found from the two studies less than 1% were infective copepodids and none of the copepodids found in the seasonal or the distribution study were categorized as *L. salmonis*. This corresponds well with hypothesis 1.2, stating that abundance of copepodids are much lower than nauplii stages. This is further supported by the literature, which describes an increase in the number of copepodids with distance away from sea farms (Costelloe, Costelloe, & Roche, 1996; Penston, Millar, Zuur, & Davies, 2008). The reason for the low amounts of copepodids may be because when the lice reach the copepodid stage it has an enormous number of potential hosts to infect and therefore attaches as soon as it becomes infective (Gravil, 1996; O'Donoghue, Costelloe, & Costelloe, 1998). It may also be because the pre-infective stages are dispersed far away from the farm before reaching the infective stage (Brooks, 2005).

## 4.2 Characterization of distribution of planktonic stages of sea lice inside and outside a cage.

In both the seasonal study and the distribution study samples were taken on the inside and outside very close to the net wall. According to hypothesis 2.1, I would expect to find more planktonic sea lice on the inside of the cage compared to the outside, with the assumption of a mechanism such as biofouling and the net itself keeping the lice from flowing freely out of the cage (Costelloe et al., 1996). None of the months of sampling in the seasonal study showed any significant difference between the abundance of planktonic stages of sea lice between the outside and inside samples ( $p > 0.05$ ). Despite

the lack of significance, the August samples seemed to have more on the inside than on the outside, especially at Valøy. It is possible that the lack of significance is caused by insufficient data. In the distribution study the abundance showed no significant difference, but the proportional abundance showed statistically significant difference for two of the sampling places: Outflow ( $p < 0.01$ ) and Side 1 ( $p < 0.05$ ).

The lack of significant difference in abundance between the inside and outside samples in the distribution study could be due to the large variation in the number of lice found at each sampling. The abundance of lice was much lower at the first sampling compared to the last. The large differences in variance, in addition to variations within the sample replicates makes it difficult to find statistical backing, especially when combined with a low number of replicates. The use of the proportional abundance negates the discrepancy between the different samplings and gives a better idea of the actual relationship between the sampling places. The significant difference between the proportional abundance of inside and outside samples from the distribution study indicates that there could be a mechanism that hinders the lice from flowing freely out of the cage, but due to only the proportional abundance being significant, not the abundance itself, more data are required before drawing any conclusions.

Biofouling was tested as a possible retention mechanism for keeping planktonic sea lice from flowing freely out of the cage, as stated in hypothesis 2.2. No significant correlation was found in either of the studies. There may be several different reasons for this result. An issue when looking at the potential effect of biofouling was that enough lice had to be present to be able to test the effect. This excludes the months January-June. In July most of the lice at Valøy were found on the outside of the net, with biofouling measurements indicating medium-high fouling with 55% on average. In August most of the lice were found on the inside of the cages, when the biofouling was low, with 8.3% fouling on average. At Mannbruholmen, July samples were collected with medium fouling (36%) on average and most of the lice were found on the outside of the cage. In August it was a high amount of fouling on average (81%) and most of the lice was found on the inside of the cage. The Spearman test for correlation looking at the relationship of abundance between the inside and outside compared with biofouling measurements from each cage did not show any significant correlation ( $p > 0.05$ ). No correlation was found between the amount of biofouling and the relationship in abundance between inside and outside samples at the Outflow in the distribution study.

There are several issues when looking at biofouling as a regulator of distribution. One problem was that the experimental design had a small number of replicates per month (12 for each farm). This, combined with the low number of lice found in the start of the sampling period, and the high within-sample variation in abundance, made it difficult to find significant differences that can be fully

reliable. Another challenge is the fact that ocean current strength and direction varied throughout the experiment, and it was difficult to measure accurately. It is likely that current strength is an important factor when it comes to biofouling retention potential. If biofouling has an effect on the retention of planktonic stages of sea lice within a cage, that effect is likely to vary in relation to factors that were not sufficiently accounted for in this study.

Hypothesis 2.3 suggests that the highest abundance of planktonic stages of sea lice would be found at the Outflow of the cage. The distribution study showed that the Outflow sampling location had significantly higher proportional abundance than the Inflow sampling location ( $p < 0.01$ ). The same significance level is also present when looking at abundance ( $p < 0.01$ ). This indicates that the main source of planktonic stages of sea lice going out of the cage is the infected salmon within the cage, which also supports the choice of sampling location for the seasonal study. The distribution study showed a large proportionate abundance of lice at Side 1 compared to Inflow and Side 2. There may be several reasons for this, but it is likely that spreading of lice from other cages and local current conditions may have caused this. In the second sampling for the distribution study, one of the cages upstream to the one sampled from had raised nets due to preparation for delousing. This could have resulted in an increased amount of newly hatched nauplii in the water current. A different possibility for the higher than expected amount of lice in Side 1 is that the sampling location may not have been correct. The side sampling spots were the hardest locations to decide and if the location chosen for Side 1 was shifted towards the Outflow spot, I would expect to find higher abundances than if the location was correct. Current speed and direction may also have impacted the results, but none of the collected data indicated any deviances in this sampling location compared to the others.

The storing experiment showed change in size for all planktonic stages of *L. salmonis*. The nauplii I length decreased during storage, while the width increased. Nauplii II showed no significant change in width, but the length increased. The copepodids increased in both length and width. This is in contrast to current literature, which suggests that shrinking is the most common problem when storing Caligidae in ethanol (Schram, 2004). Several of the individuals of Nauplii I had severe deformities due to the contents of the body bursting out of the shell. When calculating the length and width, the individuals with burst bodies were sorted out. The deformities were only a problem with the nauplii I. This may be because they have weaker exoskeletons than the other two life stages. It may also be because the nauplii I that were used in this experiment were close to moulting, decreasing the structural integrity of the shell. The internal organs of the lice from all stages seemed to shrink to some degree, but the method used to measure size included the shell, so the shrinking of the internal organs did not have an impact on the length and width. These results seem to indicate that any examination of size should be performed *before* storage, in order to be representative for the size of live individuals.



### 4.3 Suggestions for future studies

To refine the study of the relationship between inside and outside abundance, as well as the seasonal study it would be recommended to have an increased number of replicates per location, and possibly to increase the amount of filtered water for each sample. This would make the statistical analysis of the data more robust. The potential problem with increasing sample number and volume is the increase in workload. If species are going to be determined, samples must be analysed within a week to avoid extensive loss of pigmentation (Schram, 2004). Samples analysed from samplings in August took 3-4 hours on average to analyse, and having a large number of samples may necessitate storage that would in turn negatively affect pigmentation and thus species determination. Another suggestion would be to plan sampling events to periods when there were more lice on the farms, to avoid zero-lice samples.

It may be prudent to collect data on current speed at every sampling site in order to determine if there is an interaction between biofouling and current speed. An alternative method to determine biofouling may also be useful, as the method used in this study had some limitations. Only a snapshot of the net cage was categorized, with the assumption that the fouling was uniformly distributed. A setup where more pictures could be taken at various depths could increase the accuracy of the biofouling estimate. Biofouling can consist of many different species with different sizes and shapes that could affect flow through differently. The method used did not consider what kind of organism the fouling consisted of. A registration of the degree of fouling in addition to type of fouling organism could have improved the data.

A significant difference between the abundance of planktonic stages of lice at the Outflow and Inflow and Side 2. However, the number was also high for Side 1. Due to limited data it is difficult to determine whether this is a true effect, or the result of random factors not accounted for. Further, there was a temporal variation in when samples were taken for each of the four sampling locations, which confounds the data somewhat. In future iterations of this study, it would be advisable to sample all four locations simultaneously, to avoid this confound.

### 4.4 Conclusions

The most significant finding of this study was that the abundance of planktonic stages of sea lice significantly increased from July until August. This is likely due to the increase in temperature, increasing the reproductive output of the adult lice. These findings may suggest that these months are a crucial period for the aquaculture industry to implement measures to prevent the spread of sea lice. Future studies should extend the sampling period to determine how the abundance of planktonic stages develops through the autumn season. Although the findings were inconclusive, the relationship

between the inside and outside samples does suggest that there might be some mechanism causing retention of lice within the cage. Future studies should be conducted to identify these mechanisms. Nauplii were the dominant planktonic stages found. This may be due to the plankton drifting away from the cage before developing into the infective stage, or they may infect fish rapidly after reaching the infective stage. Due to the relatively long time between stages as described by S. Johnson and Albright (1991a), the latter explanation seems most likely. This would lend support to the conclusions of Brooks (2005), that moulting from nauplii to copepodide happens further downstream from the cages. However, more research is needed to settle this question.

The result of the distribution study suggests that salmon cages are a major source of nauplii in the surrounding waters. This may have implications for the spread of salmon louse between farms as well as for the conservation of wild salmon populations (Bjørn et al., 2001; Butler, 2002). Although the study was subject to certain limitations as discussed above, the main results do agree with previous findings. The spread of sea lice from fish farms remains a serious challenge for the aquaculture industry, and more research is needed in order to develop new solutions.

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