Sampling Strategies, Distribution and Concentration of Planktonic Salmon Lice Copepods in the Outer Hardangerfjord and the Altafjord

Margrethe Kristine Nilsen
BIO-3950 Master thesis in Biology
August 2016
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Supervisor
Marit Reigstad, The Arctic University of Norway UiT

External supervisor
Jofrid Skarðhamar, Institute of Marine Research (IMR)
Front photo by Margrethe Kristine Nilsen
Salmon lice under a microscope
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Margrethe Nilsen
UiT, August 15th 2016
Summary

Salmon lice are parasitic copepods with three planktonic larvae stages, consisting of two nauplii stages and a copepodite stage. The parasite spread during these stages as plankton, and with the increased number of host represented by salmonid fish in aquaculture it is important to know the concentrations and ecology of the free-living stages. Both *Lepeophtheirus salmonis* and *Caligus elongatus* are parasitic lice on salmon and trout, and will be referred to as salmon lice through this thesis.

Finding suitable sampling strategies to collect salmon lice copepods makes it possible to obtain field observed concentrations of salmon lice in their infective stage and in open water. Through this thesis, three different kinds of gear (Go-Flo water sampler, net hauls and a provisional bilge pump) and in total seven different strategies (different depths and volumes sampled) have been tested. In total 117 samples were collected and analysed. The vertical net haul proved to be best suited for the task of collecting salmon lice copepods under sub-optimal weather conditions, as often is the case in Norwegian fjords. In the two fjords investigated, the outer Hardangerfjord and the Altafjord concentrations ranged between 0-30 ind. m\(^{-3}\) and between 0-13 ind. m\(^{-3}\), respectively. These field data were compared with results from a hydrodynamic salmon lice model, and concluded that the range of concentrations found in the field was within the same range of concentrations simulated by the model. The concentrations obtained were also similar to concentrations found during previous studies in aquaculture impacted regions around Scotland and the Faroe Islands. This study found that areas less influenced by aquaculture had lower concentrations of salmon lice copepods (<2 ind. m\(^{-3}\)), as seen in the samples from Talvik, situated within a National Salmon Fjord. The copepodite size and their vertical, horizontal and seasonal distribution were also investigated. The size range of the copepodite salmon lice caught during this study was smaller than expected from earlier studies. This could be because the two common salmon lice species, *L. salmonis* and *C. elongatus*, may both have been present in the samples, and *C. elongatus* is normally smaller during their copepodite stage. From the vertical distribution of lice, a patchy aggregation in the upper 5 m was found, while the only observed trend in the horizontal distribution was that the concentrations were lower at stations that were >10 km from the nearest salmonid farm. Due to rough weather during the October cruise and only sampling through the autumn in Altafjord, this thesis had insufficient data to determine any seasonal distribution of salmon lice.

Knowledge on field concentrations and the spatial distribution of salmon lice is important to ensure a sustainable growth and management of the salmon farm industry in Norway.
Table of content

1. Introduction.................................................................................................................. 1
2. Background.................................................................................................................. 3
   2.1 Salmon Lice Biology ............................................................................................. 3
   2.2 Salmon Lice Ecology and Distribution ................................................................. 6
3. Material and methods................................................................................................. 9
   3.1 Study sites .............................................................................................................. 9
      3.1.1 The Hardangerfjord ..................................................................................... 9
      3.1.2 The Altafjord ............................................................................................... 10
   3.2 Sampling methods for salmon lice ....................................................................... 12
   3.3 Sampling periods and strategies ......................................................................... 14
      3.3.1 The Hardangerfjord ..................................................................................... 14
      3.3.2 The Altafjord ............................................................................................... 15
   3.4 Analysis and identification of salmon lice in the laboratory ................................ 16
   3.5 Calculations and equations .................................................................................. 18
   3.6 Software used ....................................................................................................... 19
4. Results ....................................................................................................................... 21
   4.1 Hydrography .......................................................................................................... 21
   4.2 Comparison of sampling strategies ..................................................................... 23
   4.3 Concentrations of salmon lice ............................................................................ 25
      4.3.1 The Hardangerfjord ..................................................................................... 25
      4.3.2 The Altafjord ............................................................................................... 25
   4.4 Standing stock of salmon lice .............................................................................. 30
   4.5 Size distribution .................................................................................................... 31
   4.6 Vertical and horizontal distribution ..................................................................... 34
      4.5.1 Vertical distribution ..................................................................................... 34
      4.5.2 Horizontal distribution ............................................................................... 34
5. Discussion .................................................................................................................. 37
   5.1 Evaluation of sampling gear and strategy ......................................................... 37
   5.2 Range of concentrations of copepodite salmon lice? .......................................... 40
5.3 Field Observations and model output ................................................................. 43
5.4 Size distribution .................................................................................................. 46
5.5 Vertical and horizontal distribution of salmon lice ........................................... 48
  5.4.1 Vertical distribution in the water column ..................................................... 48
  5.4.2 Horizontal distribution ............................................................................... 49
  5.4.3 Seasonality .................................................................................................... 50
5.6 Summary and Perspectives ............................................................................... 51
6. References ............................................................................................................. 53
1. Introduction

Parasitic sea lice represent an economical problem for the salmon farm industry as well as an ecological threat. Norway is the world’s largest provider of farmed salmon, and the high concentration of salmon farms along the Norwegian coast has given rise to numerous fish health complications (Taranger et al. 2015). In this regard there are two parasitic species of sea lice that cause the greatest threat to both farmed and wild salmonids, the *Lepeophtheirus salmonis* (Krøyer 1837) that are specialists on salmonid species, and *Caligus elongatus* (Nordmann 1832) that is a common parasite on over 20 different teleost fish species in Norway (Boxaspen 2006, Heuch et al. 2007). Sea lice larvae found in the Northern hemisphere are likely to be either of these two species (Penston et al. 2004), and they will both be referenced to as salmon lice throughout this thesis. Both species are natural ectoparasites in the Norwegian coastal ecosystem and they feed on the mucus, skin and blood of their host, they are generally not deadly unless in larger numbers. Nevertheless, they may cause decreased fitness in the host, e.g. reduced growth and fecundity as well as secondary infections and osmotic problems (Stien et al. 2005, Boxaspen 2006, Costello 2006, Goater et al. 2013).

Salmon lice have a direct host life cycle, which means that they have no intermediate host and can therefore multiply very quickly within a farm system (Costelloe et al. 1995, Nilsen et al. 2014). On average 3502 sea cages with a maximum of 200 000 Atlantic salmon or rainbow trout in each is operational every month in Norway (Fiskeridirektoratet 2015, Taranger et al. 2015). This high occurrence of salmon farms makes it possible to sustain an unnaturally large population of salmon lice due to the high number of available hosts, furthermore the number of farms along the Norwegian coast increase every year (Asplin et al. 2013, Fiskeridirektoratet 2015). Preventative actions, ranging from fallowing and shielding skirts to vaccines and harder chemical treatments is used to attempt to get rid of the problem and has caused an arms race between the salmon farm industry and the louse (Lien et al. 2014, Liu & Bjelland 2014, Aaen et al. 2015, Hjeltnes et al. 2016). In addition, chemical treatment can affect other crustaceans and thus generate a cascade effect throughout the ecosystem, as they are nutritionally important to higher trophic levels (Torrissen et al. 2013, Liu & Bjelland 2014).

As a copepod, the salmon lice belongs to one of the most successful groups in the ocean, highly numerous and widespread. Copepods can quickly accelerate and achieve high speed due to their torpedo-shaped and muscular body (Kjørboe 2011). Their antennas provide them with accurate
three-dimensional information about their surroundings and the unique escape jump helps them manoeuvre through the hazardous environment of the ocean (Kiørboe 2011). This, and the fact that salmon lice have three planktonic stages which due to wind driven circulation and ocean currents can ensure dispersal on an average of 27 km over 5-15 days makes the louse a highly adapted parasite with both a great chance of surviving and of reaching a host (Costello 2006, 2009).

Previous studies on salmon lice have mainly focused on the attached stages. In later years more studies concerning the biology and ecology of salmon lice in their free-living stages have been requested, and now several have been conducted. Such studies are important in understanding the spatiotemporal distribution and concentration of lice in the vast water masses, and in helping create precise models of the infection prognosis of the lice. This knowledge is key for a sustainable management of our coastal areas and the growing aquaculture industry (Boxaspen 2006), and the connection between the concentration of salmon lice in the water masses and lice found on the farmed fish is important information to have when predicting the infection pressure on the wild salmonid fish (Johnsen et al. 2016). The National Monitoring Program of Salmon Lice (NALO) is an annual surveillance survey of salmon lice infections on wild salmonids along the Norwegian coast. During this surveillance, smolt is deployed in sea cages and left in a given fjord for three weeks, wild fish is caught with twine and ruse fishing is conducted. The number of attached lice and stages is analysed on each fish. In addition to this, the infection pressure of salmon lice is modelled with a hydrodynamic model (IMR 2016), also using the surveillance data (Johnsen et al. 2016). Due to this and the strict duty to report amount of lice within the farms, a lot is known about the infection pressure and the attached salmon lice, but more research is needed to connect the model and the surveillance dataset. In response to this the NordLus project was established, a study to increase the knowledge on the spatiotemporal distribution and field concentrations of planktonic salmon lice in a Northern Norwegian fjord.

As part of the NordLus project, this thesis aims to increase knowledge on three separate problems in relation to the surveillance of salmon lice. First, this study will test and determine suitable sampling strategies for collecting planktonic salmon lice copepods in Norwegian coastal waters. Second, try to obtain observational field concentrations of salmon lice copepods in the study areas, which can be used to check the performance of the hydrodynamic salmon lice models. Third, investigate patterns of vertical and horizontal distribution. A further goal of this thesis is to increase our understanding of the biology, ecology and life history of salmon lice by studying their free living life stages as this knowledge will contribute to the management of salmon lice along the Norwegian coast.
2. Background

2.1 Salmon Lice Biology
Within the subclass Copepoda in the subphylum Crustacea we find the order Siphonostomatoida and the family of Caligidae or simply sea lice. This group include *Lepeophtheirus salmonis* and *Caligus elongatus* (Boxshall & Walter 2015, Walter & Boxshall 2015), the two dominating species of parasitic sea lice in the Northern hemisphere (Penston et al. 2004). Crustaceans have a hard exoskeleton and go through several stages of moulting, they have a segmented body and jointed limbs. Copepods within the order of Siphonostomatoida have a cylindrical tube for a mouth and also possesses a frontal filament that function as an attachment organ during their mobile stages (Gonzalez-Alanis et al. 2001, Goater et al. 2013). All sea lice species have a characteristic dorsoventrally flattened body and their cephalothorax is adapted into a suction cup which creates a vacuum when in contact with the surface of a compatible host. In addition, sea lice have modified their second antennae and maxillipeds as prehensile structures for a better grip (Goater et al. 2013). As a copepod, salmon lice have several distinct characteristics which make them so successful (Kiørboe 2011). First of all, copepods have a torpedo-shaped body which allows them to quickly accelerate and achieve high speed. Their body shape helps them to navigate and move through the water. Their antennas with sensitive setae compliment this by functioning as a sensory devise that give precise information about the nearby environment (Mauchline et al. 1998, Kiørboe 2011). This makes the copepods capable of using their second advantage; their escape or attack jump. When a nearby predator, prey or host approaches, the copepod uses their swimming legs backwards creating a propeller-like forward movement with the potential velocity of 1000 body lengths per second, about an order of magnitude higher than for other similar organisms (Lenz et al. 2004, Kiørboe 2011).

Due to the prevalence of *L. salmonis* and *C. elongatus* in the Northern hemisphere, it is likely that the sea lice larvae found here is either of these two species (Penston et al. 2004). The *L. salmonis* is the most common and therefore the most studied species, but the following traits will apply to *C. elongatus* as well. In their copepodite stage the salmon lice linger motionless in the water, waiting for a chance to attach on a host. Triggered by a response to water flow or mechanical vibrations by the host, they use their attack jump to seize on to the host as they pass them (Bron et al. 1993). Both *L. salmonis* and *C. elongatus* have eight stages of moulting and a direct life cycle where the parasite has no intermediate host (Figure 1, Goater et al. 2013, Hamre et al. 2013).
At all developmental stages *L. salmonis* is mostly larger than *C. elongatus*. This is especially clear at the copepodite stage, but at all stages the size range may overlap, (Table 1, Schram 2004, Danielsen 2013).

Table 1: Size range of *L. salmonis* and *C. elongatus* from earlier studies, (Based on Table 1 in Schram (2004)).

<table>
<thead>
<tr>
<th>Study</th>
<th>Collected</th>
<th>Species</th>
<th>Nauplius I</th>
<th>Nauplius II</th>
<th>Copepodid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schram (1993)</td>
<td>Western Norway</td>
<td><em>L. salmonis</em></td>
<td>0.470-0.575</td>
<td>0.590-0.620</td>
<td>0.658-0.709</td>
</tr>
<tr>
<td>Piasecki (1996)</td>
<td>Laboratory, Canada</td>
<td><em>C. elongatus</em></td>
<td>0.441-0.585</td>
<td>0.455-0.533</td>
<td>0.580-0.810</td>
</tr>
</tbody>
</table>

Figure 1: Life cycles of *Lepeophtheirus salmonis* and *Caligus elongatus*, both containing eight instars. **Light blue:** The free-living stages, which are the stages this thesis focuses on. After hatching, sea lice go through two nauplius stages and then the infective copepodid stage. **Darker blue:** Attached stages, containing chalimus I-II, then the two species separate. *C. elongatus* go through chalimus III-IV, while *L. salmonis* complete two pre-adult stages before both *L. salmonis* and *C. elongatus* develop into adult lice. Male is shown to the right and female to the left for both species. Drawn after figures in Schram (2004), Whelan (2010) and Johnson & Jakob (2012).
Within both species, the adult female carries a pair of egg strings containing between 100 and 1000 eggs (Costello 1993). In total, the female can produce between six and eleven broods (Pike & Wadsworth 1999, Boxaspen 2006), creating a potential of 600-11 000 eggs per female. The generation time for *L. salmonis* doubles from 4 weeks at 18°C to 8-9 weeks at 6°C (Wootten et al. 1982), demonstrating that all development times are highly dependent on the temperature in the surrounding water masses. However, temperatures along the Norwegian coast fluctuates considerably (1-18°C, Breen 1986), which will affect the population dynamics (Costello 2006). After hatching, the eggs develop into free-living nauplii. During the two nauplius stages they are non-feeding and it takes 1.69 days at 20°C and 11.52 days at 5°C from the first nauplii instar to develop into a non-feeding and infective copepodid (Table 2, Samsing et al. 2016). Johnson & Albright (1991) found that the survival rate for nauplii to develop into copepodid is ≈ 50 % at 10°C and salinity of 30 . The copepodite stage is the infective window for salmon lice, and can last between 6.66-10.15 days, also depending on temperature (Table 2).

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Nauplius I and II</th>
<th>Copepodite (infective window)</th>
<th>Larvae stages (total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3°C</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5°C</td>
<td>11.52±1.72</td>
<td>10.15±4.00</td>
<td>21.62±9.12</td>
</tr>
<tr>
<td>7°C</td>
<td>7.05±0.58</td>
<td>12.73±2.85</td>
<td>19.77±2.65</td>
</tr>
<tr>
<td>10°C</td>
<td>3.81±0.66</td>
<td>13.19±2.12</td>
<td>17.00±2.13</td>
</tr>
<tr>
<td>15°C</td>
<td>2.19±0.40</td>
<td>9.68±1.11</td>
<td>11.87±1.09</td>
</tr>
<tr>
<td>20°C</td>
<td>1.69±0.90</td>
<td>6.66±0.90</td>
<td>8.34±0.60</td>
</tr>
</tbody>
</table>

When the copepodid attaches itself to a host by using their modified antennae and maxillipeds, they develop into a sessile chalimus (Costello 2006). Once *L. salmonis* have surpassed the two chalimus stages they become pre-adult. At this stage they can move freely on their host and feed on different areas. *C. elongatus* does not have the pre-adult stages, but instead have four chalimus stages. After the last chalimus stages of *C. elongatus* and the two pre-adult stages of *L. salmonis* they mature into adult lice (Piasecki 1996, Hamre et al. 2013). Research indicates that salmon lice can live up to seven months (210 days, Mustafa et al. 2000), and survive during winter if attached to a host (Boxaspen 2006).
2.2 Salmon Lice Ecology and Distribution

A parasite is an organism that obtains its nutrients at the cost of another organism, the host. It is the most common life history strategy to obtain nutrients through all phyla. They are rarely lethal, but in higher numbers this may be the case (Goater et al. 2013). *L. salmonis* and *C. elongatus* are both ectoparasites, which mean that they feed on the surface of their host, utilizing their mucus, skin and blood (Costello 2006, Goater et al. 2013). *C. elongatus* is a widespread generalist species found on >20 teleost fish species in Norway (Heuch et al. 2007). In contrast, *L. salmonis* is a specialist on salmonid species (Costello 2006). Urquhart (2008) showed that the two species thrived in different areas on their sea trout host. *L. salmonis* where found more of at the dorsal regions and *C. elongatus* more abundant on ventral and caudal locations. In addition they found that both species co-existed and were almost equally abundant on the same fish (Urquhart et al. 2008).

Being a stenohaline organism, salmon lice can only tolerate a narrow set of fluctuations in salinity. Copepods of *L. salmonis* avoid salinity below 27, and the survival of copepods is compromised at salinity below 29 (Bricknell et al. 2006). This inflicts their vertical distribution as they have to reside in the upper water column, but underneath the fresh water layer often found at the surface (Blaylock & Bullard 2014). Previous studies have found that *L. salmonis* has a higher tolerance to lower salinities than *C. elongatus* and that they can survive up to 3 weeks at low salinities when attached to a host. Individuals that are attached survive longer than free-living individuals (Costello 1993, Finstad et al. 1995). This can be viewed as an adaptation to living within an estuarine. In an experiment on copepodite attachment, Genna et al (2005) found that they were most successful in medium light (300 lux), low velocity of the host (0.2 cm s$^{-1}$) and at full salinity (35).

The vertical migration of planktonic *L. salmonis* has been disputed. Empirical data indicate that salmon lice copepods are adapted to the life history of their host and therefore reside in the transit of the wild migrating salmonids in estuaries (Costello 2009). Heuch et al. (1995) suggested that the nauplius and copepods actively swim towards the surface during daylight to ensure dispersion into estuaries by onshore currents. During night time they sink passively down again. Costello (2006, 2009) proposed a model for horizontal distribution based on some of these premises of behaviour within the vertical water masses. Only the lice do not sink downwards, which causes an aggregation of lice in the surface water below the halocline (Costello 2006, 2009). Gillibrand and Willis (2007) showed that when this behaviour was included in their model the results of the horizontal distribution where more accurate to field observations. Nauplii have exhibited less active swimming and phototaxic behaviour (Heuch et al. 1995), but observations have indicated that nauplii may actively seek to the warmest water masses (Johnsen et al. 2014, Nordi et al. 2015). In conclusion, *L. salmonis* seem to be very positively phototaxic in their copepodite stage and migrate vertically.
towards the light. Knowledge on the vertical distribution of *C. elongatus* is limited. It is speculated that the vertical migration towards the surface is not as strong in this species because it has a greater range of hosts species and therefore have a wider habitat it can thrive in (Nordi et al. 2015).

Larvae stages of salmon lice in general have a maximum potential of horizontal dispersion somewhere between 10-50 km, depending on larvae behaviour and the local hydrographic conditions (Siegel et al. 2003, Costello 2006, Penston et al. 2011). In the Faroe Islands spatial distribution of *L. salmonis* copepods is shown to be heavily influenced by the wind direction, where the copepods aggregate in surface water that was pushed towards shore (Nordi et al. 2015). This was not found for *C. elongatus*, and may be due to differences in vertical migration between the two species (Nordi et al. 2015). Studies from both Ireland and Scotland found that salmon lice copepodite concentrations where highest close to shore and the estuary mouth, while the concentration of nauplii rapidly decreased away from the farm site (Costelloe et al. 1995, Penston et al. 2004, Penston et al. 2011). All this results in a patchy and non-predictable distribution of the larval stages, both vertically and horizontally (Amundrud & Murray 2009, Asplin et al. 2013).

In autumn there has been observed larger numbers of *C. elongatus* in central and northern parts of Norway (Øines et al. 2006) which may imply a seasonal distribution of the species, while *L. salmonis* was found throughout the year on the Faroe Islands. Also here, *C. elongatus* were practically absent during summer and the most dominant sea lice species during winter (Nordi et al. 2015).
3. Material and methods

3.1 Study sites
Two fjords were studied in this thesis. The outer Hardangerfjord and the Altafjord, which are both part of the NALO-program (managing program for salmon lice infections on wild salmonid species). The Hardangerfjord was chosen to test the sampling strategies due to the high abundance of salmon farms and therefore an expected high concentration of salmon lice. As a part of the Nordlus project, the Altafjord was selected due to its location in Northern Norway and since this fjord already had an ongoing NALO-study. Furthermore, this area was chosen due to an expected increase of aquaculture activity in and around this fjord, and Altafjord is an important nature reserve as it is a big National Salmon Fjord. The specific locations for investigation were selected after running a hydrodynamic lice model which predicted areas that would have the highest abundance of salmon lice or based on knowledge of the infection pressure in the area.

3.1.1 The Hardangerfjord
As the fourth longest fjord in the world and second longest in Norway, the Hardangerfjord is a complex fjord system. It is located between 59.3 – 60.35°N in Hordaland County in Western Norway (Figure 2), and because of its proximity to both the Norwegian Institute of Marine Research and the University of Bergen, it is a well-studied area. The fjord has a high number of potential hosts for parasitic salmon lice with perhaps the densest concentration of salmon farms in the world (> 100 fish farm for salmon and trout, Fiskeridirektoratet 2016) and a production of > 100 000 tons of trout and salmon per year (Fiskeridirektoratet 2015). This in turn gives opportunity for a substantial population of salmon lice within the fjord (Asplin et al. 2013). The Hardangerfjord is a large fjord system of several fjord branches creating a complex hydrography (Asplin et al. 2013). The fjord is ca. 170 km long with a 170 m sill at the mouth which gives a monthly water replacement in the upper 50 m water masses (Asplin et al. 2013). The main fjord is 2-6 km broad and the deepest point is 850 m (Taranger et al. 2014). Temperatures vary throughout the year, ranging between -6 °C to >20 °C at 3 m depth from June to December (Asplin et al. 2011) and from -5-16 °C from March to June in the upper 10 m (Johnsen 2011). A number of smaller and bigger river outlets within the fjord, provide a large amount of freshwater seasonally as the snow storages melt. In the summer and autumn the brackish water layer may stretch down to about 5 m deep (Johnsen 2011, Taranger et al. 2014). This thick layer of brackish water may influence the salinity down to 15-25 m. In the winter months the
salinity never goes lower than 25. During the melting season (peaks in June) the salinity can be as low as 15 above 5 m depths, creating a halocline in the upper water masses (Johnsen 2011).

Figure 2: Map of the study site in the outer Hardangerfjord, indicating the location of 11 stations and the salmonid fish farm sites in the area. Station 12 is outside the map area at location N 59.56.75, E 5 25.93 (Possvik).

3.1.2 The Altafjord
The Altafjord is located at 70-70.3 °N in Finnmark County in Northern Norway (Figure 3). The fjord contains a large fjord system with several fjord arms, stretching ca. 30 km into the country. The fjord has a width varying between 4-14 km and the deepest point is 450 m (Taranger et al. 2014). Three inlets, Stjernsund, Rognsund and Vargssund are found in the outer part, with sill depths of 190, 60 and 50 m, respectively. The sill of 190 m prohibits the basin water of the Altafjord to have free exchange of water with the open sea. The Alta River is the largest fresh water source, and is situated at the innermost part of the Altafjord (Mankettikkara 2013). The surface salinity varies a lot within the fjord, ranging between 6-35, with the lower salinities found in the inner part closer to the river outlets and the higher values observed in the outer part (Mankettikkara 2013). Surface water temperature ranges from 2-11 °C throughout the year in the middle of the fjord (Eilertsen &
Skarøhamar 2006), and between 6-16 °C during summer in the inner part of the fjord (Mankettikkara 2013). The Altafjord is a National Salmon Fjord and therefore there are no active farming localities in the inner part. Outside the national salmon fjord borders there are >30 salmonid farms (Fiskeridirektoratet 2016), and the industry is growing in the county (20 000 tons in 2007, 90 000 tons in 2013, Taranger et al. 2014).

Figure 3: Map of the study site in the Altafjord, indicating the location of all 9 stations and the salmonid fish farm sites in the area.
3.2 Sampling methods for salmon lice
Salmon lice in their planktonic stages have proved challenging to sample and therefor it has been difficult to obtain data on their concentration and distribution patterns (Costelloe et al. 1999, Tully et al. 2002). Recent studies in the Faroe Islands, Ireland and Scotland have successfully sampled salmon lice larvae with vertical net hauls and horizontal tows, where the horizontal tows seem to be the preferred sampling strategy (Costelloe et al. 1998, Penston et al. 2004, Penston & Davies 2009, Penston et al. 2011, Nordi et al. 2015). A 180-200 μm WP-2 net haul is standard gear used to sample zooplankton, and often a finer mesh (90 μm) is used for the smaller species or younger stages (Anonymous 1968). Due to higher concentration of material and later challenges with enumeration work in the lab the 90 μm mesh size is tiresome to use and the small larvae may slip through the 180 μm mesh, therefore research on salmon lice larvae has created their own tradition with a 150 μm mesh for both net and sieves (Schram 2004, Penston et al. 2011, Nordi et al. 2015). Previous attempts on sampling salmon lice larvae in Norway have largely been unsuccessful (Skarðhamar 2016a), and the uncertainty regarding the spatial distribution and concentrations led to a decision to apply a broad sampling strategy which could withstand rough weather and to include methods applied in recent plankton research.

Go-Flo
A Go-Flo water sampler is an alternative gear used in plankton research, which has proved to increase concentrations in quantifying smaller species and stages (Figure 4A, Svensen et al. 2012). The Go-Flo used had a length of 1.27 m and a volume of 30 L. The selected depth was measured from the middle of the Go-Flo. It was lowered to the given depth using a crane, triggered, hauled up again and emptied on deck. The content of the Go-Flo bottle was emptied through a silicon tube and concentrated over a sieve. Mesh size of the sieve was adjusted during the study period, ranging from 20-180 μm. The Go-Flo sample small but precise volumes at specific depths, and has the benefit of efficiently collecting smaller zooplankton species and stages which are often underrepresented when using 180 μm (or even 90 μm) mesh size (Svensen et al. 2012, Antonsen 2014).

Net hauls
Vertical net hauls and horizontal tows with a WP-2 net with opening area of 0.25 m² areal was added to the sampling strategy (Figure 4B). We had only access to nets with 90 or 180 μm. Both were tested during the study. Vertical net hauls were taken from 10 m depth to the surface, sieving 2 500 L of water, and horizontal tows was dragged 100 m at 4 m depth, filtering 25 000 L of water. Both methods required a crane and were pulled at 0.5 m/s to avoid clogging and ensure efficient filtering of water. The net was hosed with sea water after each haul to collect all the organisms in the
removable filtering-cod. This strategy was assumed to be easy to use in the field and to efficiently filter through large volumes. The drawbacks include dense samples which had to be diluted substantially before analyses, and vertical hauls provides no depth specific data beyond that the lice reside within 10-0 m depth, which result in less quantitative data. The horizontal tows provided depth specific data, but shallow sampling close to the surface was impossible during windy conditions.

**Pump**

Finally a provisional pump was added as a method (Figure 4C). The pump used was originally a bilge pump, connected to two thick hoses with valves at each end to ensure water was pumped upwards. 1000 L of water was pumped from the preferred depths and then sieved through a WP-2 net. As with the net hauls the filtering-cod end of the WP-2 net contained the concentrated sample. The water faucet was used to fill the pump with water to get it started and to clean it after use. This method precisely samples through a moderately large volume of water and give depth specific information, ensuring robust quantitative data. In addition this equipment did not require a crane. The volume was measured based on repeated measures of time to fill 50 L of water to estimate pump efficiency in L/second.

Regardless of which method was applied, the concentrate was transferred to a measuring beaker with the use of a wash bottle. This made a 200 ml sample, and then added 50 ml Zoofix (buffered formaldehyde, hexamethylenetetramine and propanediol, 20 %, Unstad & Tande 1991) for preservation in a 250 ml PVC plastic bottle, resulting in a 4 % concentration of formaldehyde.
3.3 Sampling periods and strategies

During this study, two investigations were conducted in the Hardangerfjord and three in the Altafjord. Research was conducted during periods expected to have high concentration of salmon lice, and all samples were taken during daylight. Methods were added and adjusted throughout the study period to optimize the sampling of copepodite salmon lice.

3.3.1 The Hardangerfjord

Two studies took place around Halsnøya in the Hardangerfjord (59.7°N) summer and fall of 2014, with the boat Fangst and Brattstrøm. The first cruise was conducted 21.06.14-23.06.14. Twelve stations were investigated with merely the use of a 30 L Go-Flo at 3 m depth and a 20 μm sieve was used to get rid of the excess water, and ensure that no copepods were lost. Due to transport complications a Niskin bottle rosette of 50 L replaced the Go-Flo during the second cruise, 04.09.14-06.09.14. Since it was used the same way, and to make the comparison to the other expeditions easier, it is still labelled as Go-Flo in Table 3 and in the result chapter. The Niskin bottle rosette was applied two times, making a 100 L sample, at 3, 5 and 10 m depth. The water collected was emptied bottle by bottle into a 90 μm sieve (5 bottles of 10 L, preformed two times, 100 L in total). The mesh size was changed to avoid clogging when filtering through larger volumes and to ensure basis for comparison to the vertical hauls and horizontal tows. In addition, vertical hauls from 10 m to the surface and 100 m horizontal tows at 4 m depth was conducted using a WP-2 net with a mesh size of 90 μm. See Table 3 for an overview of methods used at the different stations. In total 31 samples were collected throughout the second cruise, and eight stations were investigated. The samples were preserved with a 4 % final concentration of formaldehyde in a zoofix mix.
Table 3: Overview of stations and methods used for samples done in the outer Hardangerfjord summer and autumn 2014, and in the Altafjord autumn 2015. In total 117 samples were collected and analysed.

<table>
<thead>
<tr>
<th>Location</th>
<th>Date (sampled)</th>
<th>Stations</th>
<th>Method</th>
<th>Depth (m)</th>
<th>Mesh size (m)</th>
<th>Sampled volume (L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hardangerfjord</td>
<td>21.-23.06.14</td>
<td>1-12</td>
<td>Go-Flo</td>
<td>3</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>Hardangerfjord</td>
<td>04.-06.09.14</td>
<td>1, 2, 6, 7, 11</td>
<td>Go-Flo (Niskin)</td>
<td>3</td>
<td>90</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Go-Flo (Niskin)</td>
<td>5</td>
<td>90</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Go-Flo (Niskin)</td>
<td>10</td>
<td>90</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Horizontal net haul</td>
<td>4</td>
<td>90</td>
<td>25 000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Vertical net haul</td>
<td>10-0</td>
<td>90</td>
<td>2 500</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3, 5, 9</td>
<td>Go-Flo (Niskin)</td>
<td>3</td>
<td>90</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Vertical net haul</td>
<td>10-0</td>
<td>90</td>
<td>2 500</td>
</tr>
<tr>
<td>Altafjord</td>
<td>22.-24.08.15</td>
<td>1-9</td>
<td>Go-Flo</td>
<td>3</td>
<td>180</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pump</td>
<td>1</td>
<td>180</td>
<td>1 000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pump</td>
<td>3</td>
<td>180</td>
<td>1 000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Vertical net haul</td>
<td>10-0</td>
<td>180</td>
<td>2 500</td>
</tr>
<tr>
<td>Altafjord</td>
<td>18.-20.09.15</td>
<td>1-9</td>
<td>Pump</td>
<td>1</td>
<td>90</td>
<td>1 000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pump</td>
<td>3</td>
<td>90</td>
<td>1 000</td>
</tr>
<tr>
<td>Altafjord</td>
<td>08.-10.10.15</td>
<td>1, 2, 4-9</td>
<td>Pump</td>
<td>1</td>
<td>90</td>
<td>1 000</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>Pump</td>
<td>3</td>
<td>90</td>
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<td>Pump</td>
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<td></td>
<td>Pump</td>
<td>3</td>
<td>90</td>
<td>1 000</td>
</tr>
</tbody>
</table>

### 3.3.2 The Altafjord

Three cruises were carried out in the Altafjord during the fall of 2015, with the boats KV Heimdal, KV Farm and a Selfa Arctic speedsjark, 10 m. In addition to the methods used in the Hardangerfjord, a provisional pump was tried out to increase sampling volume compared to the volumes achieved with the Go-Flo. The horizontal net haul was omitted as a suitable method because there were very few lice collected by this method in the Hardangerfjord and it was weather dependent. In the August cruise a vertical net haul, Go-Flo sample (150 L) at 3 m depth and two samples of 1000 L at depths 1 and 3 m with the pump were conducted (Table 3). Mesh size for all methods were 180 μm as the copepodite lice were assumed too large to be under-sampled by 90 μm, and to avoid all small non-lice plankton. Nine stations in total were investigated at different parts of the fjord: Korsfjord, Øksfjord and in Talvik. All methods were applied at each station, making 36 samples in total. During both the September and October cruise only the pump was used and sieved through a 90 μm WP-2 net. Later the samples were used to check for potential loss using a 180 μm mesh, to confirm that no
lice were lost during the August cruise. In total this resulted in 18 samples for each of the two expeditions, and during the October cruise 2 extra samples were taken to check the accuracy of the methods. All the concentrates were preserved in a similar way as the cruises in the Hardangerfjord.

3.4 Analysis and identification of salmon lice in the laboratory
The samples were sieved through a 20 and 90 μm sieve, depending on which sieve was used in the field and transferred to a beaker filled with filtered sea water. To reduce the fumes from the formaldehyde all samples were aerated approximately 24 hours prior to analysis. All lab work with samples before aeration and during was conducted within a ventilation hood, using appropriate gloves. Residues sieved of the sample were transferred to an appropriate waste container to secure suitable future handling of the chemicals. Since the amount of salmon lice were relatively low compared to other copepod species, the whole sample had to be analysed. To homogenise the sample it was stirred in a figure eight, ten times using a stirring rod. This was to distribute the organisms evenly before subsampling. Subsamples were then transferred to a counting chamber. Identification and enumeration was conducted using a stereo microscope (Leica MZ 16, 40 – 100 x magnification). The same procedure was used on the concentrate from all methods applied. The copepod stage of the salmon lice was identified, counted and measured. The prosome length of the copepods were measured, as this is a standard measure in copepod research (Figure 5, Mauchline et al. 1998), and this measure is also less sensitive to potential shrinkage caused by fixation. The characteristic pigmentation of *L. salmonis* and *C. elongatus* is used as taxonomic identification to separate the two species. Formaldehyde removes the pigmentation of the lice, therefore making it difficult to separate *L. salmonis* from *C. elongatus* in preserved samples. The copepods were therefore identified down to family: Caligidae (sea lice). Given the prevalence of these species in the Northern hemisphere, the sea lice larvae identified was likely to be either *L. salmonis* or *C. elongatus* (Penston et al. 2004). They will be referenced to as salmon lice, as they both are parasites on salmonid species and a hazard for wild and farmed salmon and trout in Norway. To identify the species, a taxonomic description by Danielsen (2013) and Schram (2004) was used. The two nauplius stages were too difficult to identify from other nauplius, and thus not included in the present study.

Because of the risk of clogging with the use of 90 μm mesh size when filtering through large volumes of water during the late summer bloom (Schram 2004), a 180 μm was applied during the August cruise in the Altafjord. To make sure no lice were lost due to the coarse mesh size in the plankton net, the sample from the September cruise was counted after using first a 90 μm sieve then a 180 μm sieve.
Figure 5: Drawing of *Lepeophtheirus salmonis* and *Caligus elongatus*, and demonstration on how the prosome length was measured. Drawn after (Schram 2004).
3.5 Calculations and equations

To estimate the sampling efficiency for each method and to calculate the mean concentration of copepodite salmon lice for a specific fjord/location the following equation was used for each method or fjord.

\[
\text{Mean concentration (ind. m}^{-3}) = \frac{\sum \text{copepods (ind.)}}{\text{Liter sampled at station or in fjord/1000 (L/1000)}}
\]  

(1)

To compare the field data from Altafjord to the estimates from the hydrodynamic model given as ind. m\(^2\), the concentrations (ind. m\(^3\)) from this study was integrated over a given depth. This was done by integrating the field concentrations (ind. m\(^3\)) for each of the sampling strategies over a depth from 0-5 m resulting in a standing stock for the abundance of copepodite salmon lice (ind. m\(^2\)). Sampling methods with only one measured value (Go-Flo and vertical net haul) was assumed to be homogenously distributed within the five upper meters and therefore multiplied by 5 m (Equation 2). The samples from the vertical net haul is already integrated through the method, the concentration estimated is a mean from the water column 10-0 m. The pump gave concentration at two specific depths (1 m, 3 m) and was integrated by letting the concentration at 1 m represent the depth interval of 0-2 m, and the concentration estimated at 3 m was representative for the 3-5 m depth interval (Equation 3). The depth chosen reflected earlier results and literature (Penston et al. 2004, Penston et al. 2008, Nordi et al. 2015) stating that most of the copepodite salmon lice would reside in the upper 5 m of the water column.

Go-Flo and vertical net haul: \[\text{ind. m}^{-2} (0 - 5 \text{ m}) = \text{ind. m}^{-3} \times 5 \text{ m}\]  

(2)

Pump (1 and 3 m): \[\text{ind. m}^{-2} (0 - 5 \text{ m}) = (\text{ind. m}^{-3}_1 \times 2 \text{ m}) + (\text{ind. m}^{-3}_3 \times 3 \text{ m})\]  

(3)
In studies of salmon lice the total length of the lice is the standard measurement. To facilitate comparison with earlier literature a ratio between the prosome length and the total body length was established. 15 lice had their prosome length and the total length measured to establish an aspect ratio between the prosome length and the total length, so that a total length could be estimated for all the measured salmon lice copepods (Equation 4).

\[
\text{Length ratio} = \left( \frac{\text{Prosome length}}{\text{Total length}} \right)
\]  

(4)

3.6 Software used
Calculations were done in Microsoft® Excel® for Windows (Microsoft Corp. Redmond, WA, USA). Graphs and statistical analysis was done by using SYSTAT 13 for Windows (Crane Software International Ltd, Chicago, USA). Maps were made by using ArcGIS Desktop Advanced 10.1 (Esri Inc. USA). CTD data analysed by MiniSoft SD200W (SAIV A/S Bergen, Norway). Paint® for Microsoft® Windows (Microsoft Corp. Redmond, WA, USA) used for simple picture and figure handling and other figures and pictures was made with Adobe® Photoshop® Elements software (Adobe Systems Incorporated, CA, USA).
4. Results

4.1 Hydrography

Results from the CTD-measurements showed that during the June cruise in the outer Hardangerfjord both salinity and temperature at the depth (3 m) were salmon lice were sampled was relatively stable (see example in Figure 6A). Salinity ranged from 27 to 28 and temperature was between 15-16°C at 3 m depth at all 12 stations. The deeper water masses had temperatures as low as 7°C. The halocline in the Hardangerfjord was positioned between 4-11 m depth, which means that all samples during this cruise were collected above the halocline.

Another cruise was conducted in September during the autumn bloom, and the CTD-profiles showed the same tendencies as the June cruise (Figure 6B). Salinity for the whole water column measured (down to 10 m) ranged between 23 and 32 for all stations. Station 2 stood out here with a minimum salinity of 23 (data not shown), while none of the other stations had salinity <26. Below 3 m, the salinity ranged from 24 (Station 2) to 30 at the 8 sampled stations. The temperature was fairly stable, ranging from 15-17°C through the whole water column. The halocline was positioned between 3 m and 7 m, which means that samples were taken both over and under the haloclines.

Figure 6: Selected example multigraph of the hydrographical profile of the measured water column, illustrating the general trends in the water for the sampled stations during the A) June and B) September 2014 cruise to the Hardangerfjord. Note that the scales for density (t, s, p), temperature (°C) and salinity differ between the two graphs.
Figure 7: Selected example multigraph of the hydrographical profile from station 1 in the Korsfjord, illustrating the general trends in the water for the sampled stations during the A) August, B) September and C) October 2015 cruise to the Altafjord. Note that the scales for density (t, s, p), temperature (°C) and salinity differ between the three graphs.

The CTD-profiles from the Altafjord shows that in general the temperature was lower compared to the stations in the outer Hardangerfjord, ranging from 8-14°C in August, 9-10°C in September and 8-10°C in October (Figure 5). None of the stations had salinity <30 from 1 m depth and only Station 5 (Talvik) had a fresh water layer (August and September). The position of the halocline varied more from area to area than between months. In the Korsfjord, the haloclines in general were located between 4 and 6 m depth as seen in Figure 7 A and B. In the two stations in Talvik, the halocline was situated above 3 m depth and in the Øksfjord it was found generally below 9 m. Because of wind gusts of hurricane force and over all bad weather throughout the October cruise, the CTD-profiles are jagged, the shallow halocline is broken and the water column mixed down to ~15 m depth (Figure 7C), which is a common situation in the autumn.
4.2 Comparison of sampling strategies
The strategies have been refined during this study to find a sampling strategy which met three criteria; large enough volume sampled to get actual concentrations, applicable under sub-optimal conditions and samples which are manageable to analyse within the time span of a master thesis.

Even though the vertical net haul collected the highest number of salmon lice copepods during the September investigation in the outer Hardangerfjord, the Go-Flo used at 3 m depth, with a mean sampled concentration of 7.5 ind. m$^{-3}$, is by far the most efficient sampling strategy when volumes are taken into consideration (Figure 8). Further, this is supported in the results from the Altafjord, as the Go-Flo applied at 3 m is the most efficient strategy accompanied by the vertical net haul, both with a mean sampled concentration of 2.2 ind. m$^{-3}$. With this, the Go-Flo proves to be an effective method for sampling copepodite salmon lice. However, when applied at depths 5 m and 10 m no lice are present in the sample. In light of this it is clear that it is not the Go-Flo as a method that is inadequate, but rather that there is little or no copepodite salmon lice found at these depths.

![Figure 8: The mean concentration of copepodite sea lice illustrating the sampling efficiency for each of the sampling strategies. Calculations based on Equation 1. The Go-Flo applied at 3 m depth is in both study sites the most efficient method providing highest concentrations, and in the Altafjord it is equivalent to the vertical net haul. Only the data from the September cruise in the Hardangerfjord and the August cruise in the Altafjord is used.](image-url)
The horizontal net haul applied at 4 m depth tested in the outer Hardangerfjord also proved to be an inefficient sampling strategy with a sampling efficiency of 0.01 salmon lice copepod ind. m\(^{-3}\). Presumably because no copepodite salmon lice were distributed this deep, and the horizontal net haul at shallower depths was not feasible due to waves. This resulted in the removal of the three sampling strategies; Go-Flo applied at 5 and 10 m depth and the horizontal net haul in later investigations. In the Altafjord the pump applied at 1 and 3 m depth, had a mean sampling efficiency of 1.2 and 1.1 ind. m\(^{-3}\), respectively. This is approximately half of the sampling efficiency of the Go-Flo (3 m) and the vertical net haul (0-10 m) from the same cruise.

The variation within the measured concentrations of salmon lice copepods is high for all methods applied. There are several samples that have no copepodite salmon lice and some samples have high numbers. This becomes especially clear when looking at the results from the vertical net hauls from the August cruise conducted in the Altafjord (Figure 9). Several of the samples have no copepodite salmon lice or very few (median of 1.2 ind. m\(^{-3}\)), and then 32 of the copepod salmon lice individuals were found in one vertical net haul sample resulting in a 12.8 ind. m\(^{-3}\) concentration for Station 6, Øksfjord. The patchiness and variability thus seem to be large.

![Figure 9: Box plots demonstrating the variance of copepodite salmon lice sampled by each of the methods. The median concentration for both the Go-Flo and the Pump (1 m) is 0 m\(^{-3}\), the Pump (3 m) has a median of 1 m\(^{-3}\) and the vertical net haul from the Hardangerfjord and the Altafjord has a median of respectively 0.2 and 1.2 m\(^{-3}\). Only the data from the September cruise in the Hardangerfjord and the August cruise in the Altafjord is used. n=number of stations sampled.](image-url)
4.3 Concentrations of salmon lice
To survey the infection pressure of salmon lice in their infective stage, the copepod individuals of the two salmon lice species sampled in the field were used to calculate the concentrations (ind. m$^{-3}$) for each location, and to integrate the standing stock. The concentrations of copepodite salmon lice found in the two fjords are based on few individuals due to relatively low concentrations. The numbers of lice sampled with many of the tested strategies were too low to allow any statistical analysis, and therefore the uncertainty in concentrations is potentially high. The salmon lice copepods sampled and counted from each sample ranged from 0-32 ind. despite adjustment of methodology to increase the sample volumes. These low numbers causes the precision of concentration to be low, resulting in an uncertainty between 35-100 % (Harris et al. 2000).

4.3.1 The Hardangerfjord
During the June cruise in the outer part of the Hardangerfjord, no lice were found in the samples. The sampled volume of 30 L for each station and a total sample volume of 360 L were too small to catch any lice or to obtain the actual concentrations of parasitic salmon lice. Accordingly, during the September cruise the total sampling volume was increased to 146 800 L. The calculated number of copepodite salmon lice per cubic meter (ind. m$^{-3}$) at the eight stations in outer the Hardangerfjord and the respective methods is shown in Figure 10. Sampled stations ranged from 0-30 copepodite salmon lice ind. m$^{-3}$, and they were identified at six out of eight stations. The mean concentration obtained by Go-Flo at 3 m and the vertical net hauls was 0.7±8.8 ind. m$^{-3}$ (n=8 stations).

4.3.2 The Altafjord
Nine stations were studied in three different parts of the Altafjord (Korsfjord, Talvik and Øksfjord), and in three different months (August, September and October) throughout the autumn of 2015. The success rate of the different sampling methods, the concentrations of salmon lice copepods and their regional distribution, during the August investigation are shown in Figure 11. Out of the nine stations, copepodite salmon lice were found in eight, concentrations ranging from 0-12.8 ind. m$^{-3}$ for the whole Altafjord. Talvik as an area distinguishes from the Korsfjord and the Øksfjord as a low concentration zone, with Station 5 that had no copepodite salmon lice. In general, Talvik had lower concentrations of salmon lice ranging between 0-0.8 ind. m$^{-3}$. The mean concentration sampled in Talvik was 0.2±0.3 ind. m$^{-3}$ (n=2). Salmon lice were found within all sampled stations in the Korsfjord, and the samples from the four different sampling strategies had concentrations ranging from 0-6.7 ind. m$^{-3}$, with a mean sampled concentration of 1.2±2.4 ind. m$^{-3}$ (n=3). Also in the Øksfjord lice were...
found at all stations and the concentrations ranged between 0-12.8 ind. m$^{-3}$, and a mean of 3.0±3.6 ind. m$^{-3}$ (n=4). Here the vertical net haul at station 6 in the Øksfjord stands out, as 32 salmon lice copepods (12.8 ind. m$^{-3}$) were found in this single sample.

Figure 10: Concentration of salmon lice copepods (ind. m$^{-3}$), results from each sampling strategy applied during the autumn Hardangerfjord cruise, and grouped by station. For station localisation, see map in Figure 2. Station 3, 5 and 9 had no horizontal net haul and the Go-Flo was only used at 3 m.
Figure 11: Concentration of copepodite salmon lice (ind. m$^{-3}$) obtained during the August cruise in the Altafjord, sorted by station, sampling gear and strategy. Talvik is within the border of the National Salmon Fjord Reserve. Both the Korsfjord and the Øksfjord contain several salmon fish farms. For station and salmon farm localisation, see map in Figure 3.
In September a second cruise was conducted in the Altafjord, and this time only the bilge pump was used due to practical reasons (Figure 12).

Figure 12: Concentration of copepodite salmon lice (ind. m\(^{-3}\)) obtained during the September cruise in the Altafjord, sorted by station and strategy. The pump was the only gear used during this expedition. Talvik is within the border of the National Salmon Fjord Reserve. Both the Korsfjord and the Øksfjord contain several salmon fish farms. For station and salmon farm localisation, see map in Figure 3.
Similarly to the August cruise, Talvik stands out as an area of lower concentrations of salmon lice copepods in September. Once again it represents the only area with one station (Station 4) without any sampled copepods and generally lower concentrations ranging from 0-2 ind. m$^{-3}$, and the low mean concentration of 0.75±0.96 ind. m$^{-3}$ (n=2). In the Korsfjord, all three stations had copepodite salmon lice in at least one of the two sampled depths, and concentrations were between 0-8 ind. m$^{-3}$. The mean concentration was 3.67±3.50 ind. m$^{-3}$ (n=3). Within all three stations in the Korsfjord the bilge pump samples from 1 m depth contained salmon lice copepods. A different pattern was found in the Øksfjord. Still all the sampled stations from the Øksfjord contained copepodite lice at least one depth, but all stations had salmon lice copepods at 3 m depth and Station 7 had no copepodite salmon lice at 1 m depth. The concentration of copepodite salmon lice in the Øksfjord samples was found to be between 0 and 9 ind. m$^{-3}$ and have a mean of 2.88±2.90 ind. m$^{-3}$ (n=4). Contradictory to the results from the August cruise where the Øksfjord had the highest mean concentration with 2.4±3.6 ind. m$^{-3}$ compared to 1.8±2.4 ind. m$^{-3}$ in the Korsfjord, the Korsfjord had the highest mean concentration with 3.7±3.5 ind. m$^{-3}$ in September, compared to a concentration of 2.9±2.9 ind. m$^{-3}$ in the Øksfjord.

During analysis it was observed that some of the lice had small characteristic differences, but because of the preservation (formaldehyde) the pigments were too deteriorated to distinguish the species from each other. Both species were counted as salmon lice since the other probable species was *Caligus elongatus*. The last cruise took place in October, 08.10.15-10.10.15. During analysis of these samples only one copepodite salmon lice was found. In one of the duplicate samples from Station 3 in the Korsfjord (3 m).
4.4 Standing stock of salmon lice

The concentrations of salmon lice resulting from simulations by the hydrodynamic model are given per square meter (m$^2$). To be able to compare results, the field based concentrations were integrated over depth (m$^3$ to m$^2$; Table 4). This was done according to Equation 2 and 3 in the chapter about material and methods. The chosen integration depth was 5 m, based on the results from the outer Hardangerfjord and from literature, stating that most of the copepodite salmon lice will reside in the surface layers above 5 m (Penston et al. 2004, Penston et al. 2008, Nordi et al. 2015).

The standing stock of copepodite salmon lice caught with Go-Flo and the vertical net haul during the September Hardangerfjord cruise ranges from 0-15 ind. m$^{-2}$, and had a mean value of ≈5 ind. m$^{-2}$. Station 11 is removed from the table as there was not collected any lice here in the Hardangerfjord and there was no station 11 in the Altafjord, but it is included in the calculations. The abundance of copepodite salmon lice collected during the August cruise in the Altafjord ranges from 0-64 ind. m$^{-2}$ for the nine stations examined, with a mean of ≈9 ind. m$^{-2}$. The September cruise standing stock was between 0-40 ind. m$^{-2}$, with a mean of ≈13 ind. m$^{-2}$. In October there were only caught lice at Station 3, with a standing stock of 3 ind. m$^{-2}$.

Table 4: Overview of the integrated abundance of salmon lice copepods (ind. m$^{-2}$, 0-5 m) for the all stations investigated in the outer Hardangerfjord and the Altafjord.

<table>
<thead>
<tr>
<th>Cruise and method</th>
<th>Station 1</th>
<th>Station 2</th>
<th>Station 3</th>
<th>Station 4</th>
<th>Station 5</th>
<th>Station 6</th>
<th>Station 7</th>
<th>Station 8</th>
<th>Station 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hardangerfjord</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Go-Flo</td>
<td>0 m$^2$</td>
<td>15 m$^2$</td>
<td>0 m$^2$</td>
<td>-</td>
<td>0 m$^2$</td>
<td>0 m$^2$</td>
<td>5 m$^2$</td>
<td>-</td>
<td>10 m$^2$</td>
</tr>
<tr>
<td>Vertical</td>
<td>15 m$^2$</td>
<td>0 m$^2$</td>
<td>10 m$^2$</td>
<td>-</td>
<td>15 m$^2$</td>
<td>0 m$^2$</td>
<td>0 m$^2$</td>
<td>-</td>
<td>5 m$^2$</td>
</tr>
<tr>
<td>Altafjord August</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Go-Flo</td>
<td>0 m$^2$</td>
<td>33.33 m$^2$</td>
<td>33.33 m$^2$</td>
<td>0 m$^2$</td>
<td>0 m$^2$</td>
<td>0 m$^2$</td>
<td>0 m$^2$</td>
<td>0 m$^2$</td>
<td>33.33 m$^2$</td>
</tr>
<tr>
<td>Pump</td>
<td>3 m$^2$</td>
<td>3 m$^2$</td>
<td>4 m$^2$</td>
<td>0 m$^2$</td>
<td>0 m$^2$</td>
<td>7 m$^2$</td>
<td>27 m$^2$</td>
<td>2 m$^2$</td>
<td>6 m$^2$</td>
</tr>
<tr>
<td>Vertical</td>
<td>6 m$^2$</td>
<td>8 m$^2$</td>
<td>8 m$^2$</td>
<td>4 m$^2$</td>
<td>0 m$^2$</td>
<td>64 m$^2$</td>
<td>0 m$^2$</td>
<td>4 m$^2$</td>
<td>6 m$^2$</td>
</tr>
<tr>
<td>Altafjord September</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pump</td>
<td>2 m$^2$</td>
<td>12 m$^2$</td>
<td>40 m$^2$</td>
<td>0 m$^2$</td>
<td>7 m$^2$</td>
<td>7 m$^2$</td>
<td>3 m$^2$</td>
<td>16 m$^2$</td>
<td>33 m$^2$</td>
</tr>
<tr>
<td>Altafjord October</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pump</td>
<td>0 m$^2$</td>
<td>0 m$^2$</td>
<td>3 m$^2$</td>
<td>0 m$^2$</td>
<td>0 m$^2$</td>
<td>0 m$^2$</td>
<td>0 m$^2$</td>
<td>0 m$^2$</td>
<td>0 m$^2$</td>
</tr>
</tbody>
</table>
4.5 Size distribution
During laboratory analysis of the samples, the prosome length of all salmon lice copepods was measured. For an easier comparison to other studies on salmon lice where the total length is measured, the total lengths of 15 copepodite salmon lice sampled were measured and a ratio of ≈0.65±0.03 was established, calculated with the use of Equation 4 (Table 5).

Table 5: The prosome length and total length of 15 lice was measured to estimate a relationship between the two numbers that can be used to determine the approximately total length of the lice in this investigation. The prosome length/total length is between 0.60 and 0.69, with a mean of 0.65±0.03.

<table>
<thead>
<tr>
<th>Prosome length (mm)</th>
<th>Remaining length (mm)</th>
<th>Total length (mm)</th>
<th>Ratio (Prosome length/Total length)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.40</td>
<td>0.18</td>
<td>0.58</td>
<td>0.69</td>
</tr>
<tr>
<td>0.41</td>
<td>0.23</td>
<td>0.64</td>
<td>0.64</td>
</tr>
<tr>
<td>0.52</td>
<td>0.26</td>
<td>0.78</td>
<td>0.67</td>
</tr>
<tr>
<td>0.34</td>
<td>0.17</td>
<td>0.51</td>
<td>0.67</td>
</tr>
<tr>
<td>0.37</td>
<td>0.18</td>
<td>0.55</td>
<td>0.67</td>
</tr>
<tr>
<td>0.33</td>
<td>0.17</td>
<td>0.50</td>
<td>0.66</td>
</tr>
<tr>
<td>0.42</td>
<td>0.20</td>
<td>0.62</td>
<td>0.68</td>
</tr>
<tr>
<td>0.27</td>
<td>0.18</td>
<td>0.45</td>
<td>0.60</td>
</tr>
<tr>
<td>0.40</td>
<td>0.22</td>
<td>0.62</td>
<td>0.65</td>
</tr>
<tr>
<td>0.44</td>
<td>0.22</td>
<td>0.66</td>
<td>0.67</td>
</tr>
<tr>
<td>0.34</td>
<td>0.16</td>
<td>0.50</td>
<td>0.68</td>
</tr>
<tr>
<td>0.31</td>
<td>0.19</td>
<td>0.50</td>
<td>0.62</td>
</tr>
<tr>
<td>0.44</td>
<td>0.22</td>
<td>0.66</td>
<td>0.67</td>
</tr>
<tr>
<td>0.37</td>
<td>0.24</td>
<td>0.61</td>
<td>0.62</td>
</tr>
<tr>
<td>0.40</td>
<td>0.23</td>
<td>0.63</td>
<td>0.63</td>
</tr>
</tbody>
</table>

Mean: 0.38±0.06 0.20±0.03 0.59±0.09 ≈ 0.65 (±0.03)

Based on this, a distribution frequency of the estimated total length for the copepodite salmon lice collected in the outer Hardangerfjord was made (Figure 13). Only 16 copepods were caught in total at this site and therefore this is not a valid representation of the size distribution for all the salmon lice within the Hardanger fjord system. Sampled copepodite salmon lice from the Hardangerfjord shows a big variance in their measured size. The prosome length ranges from a minimum of 0.26 mm to a maximum of 0.52 mm, which is a twofold increase and is reflected in the estimated total length. The mean prosome length was 0.34±0.09 mm, whereas the mean estimated total length was 0.53±0.14 mm (n=16 copepodite salmon lice).
Prosome length was also measured on all 123 salmon lice copepods found through the three cruises conducted in the Altafjord. Distribution of copepodite salmon lice within the size categories for the estimated total length is shown in Figure 14. The prosome length of the copepods collected here ranges from 0.26 mm to 0.55 mm, with a mean of 0.38±0.06 mm, which is somewhat longer than the salmon lice copepods from the Hardangerfjord. The range of the estimated total length is 0.40-0.85 mm and the mean 0.58±0.1 mm (n=123).

Figure 13: Number of copepodite sea lice individuals pr. size category collected in the Hardangerfjord, September 2014. The estimated total length size range is divided into nine size categories; each 0.05 mm. Mean value is 0.53±0.14 mm (n=16 copepodite salmon lice).
Figure 14: Number of copepodite sea lice individuals pr. size category collected in the Altafjord, autumn 2015. Mean value for the dataset is 0.58±0.1 mm (n=123 copepodite salmon lice).
4.6 Vertical and horizontal distribution

4.5.1 Vertical distribution
Results from both fjord regions show that the copepodite salmon lice reside in the upper water layers. Results from the outer Hardangerfjord show that the three sampling strategies applied solely below three meter depth had little or no lice in their corresponding samples (Figure 8). On the other end, the Go-Flo used at 3 m depth had clearly the highest concentration of salmon lice. This was taken into consideration when the study in the Altafjord was conducted. A method sampling at 1 m (Pump (1 m)) was added, to check if the copepods reside further up in the water column in the absence of a freshwater layer with salinity below 27. In the hydrography results from the Altafjord we can see that none of the sampled stations had salinities below 30 from 1 m and down, and here the samples from the pump applied at 1 m depth had some more copepodite salmon lice than the one at 3 m depth (Figure 8). Figure 12 show that 5 out of 9 stations had more copepodite salmon lice at 1 m, but in total the samples from the September cruise in the Altafjord had the same amount of copepods in both the pump applied at 1 m and at 3 m (24 lice each). The results from the Altafjord do show the same tendencies as in the Hardangerfjord and that the copepods of salmon lice may prefer even shallower water than 3 m depth.

4.5.2 Horizontal distribution
A distribution trend for the concentration of copepodite salmon lice with distance from nearest farm could be seen from the observations (Figure 15A). When the distance is >10 km a reduction in concentrations is found, as seen in the Talvik area that had a much lower presence of salmon lice copepods than both the Øksfjord and the Korsfjord. The distance from nearest farm within the Korsfjord and the Øksfjord ranged from 0.25-2.85 km, and no specific trend of distribution was observed. To explore if there could be a pattern with distance from shore the stations within the Korsfjord and the Øksfjord were sorted after distance from land as well (Figure 15B), but no clear trend was observed.
Figure 15: Graphs arranged after A) distance from nearest farm and B) distance from land for the concentrations found during the August Altafjord cruise, 2015. (By Ørjan Garfjell).
5. Discussion

The results from this study must be interpreted with caution due to the difficulty capturing salmon lice in their planktonic stages resulting in a limited dataset. The few individuals identified from each station did not provide a dataset robust enough for statistical analysis. Nevertheless, the results can be used as indicators of trends in concentration, distribution and size range, as well as suggesting the most suitable sampling strategy for the Norwegian fjords.

5.1 Evaluation of sampling gear and strategy
During this study three different kinds of gear and in total seven different strategies have been tested. The strategies have been refined throughout this study to find a sampling strategy which met the three criteria; large enough volume sampled to obtain actual concentrations, applicable under sub-optimal weather conditions and samples which are manageable to analyse. Water bottles, nets and pumps are all established methods for zooplankton sampling, but with different strengths, weaknesses and suitability dependent on purpose (Harris et al. 2000). For free-living salmon lice, vertical and horizontal tows have previously been the preferred method (Penston et al. 2004, Nordi et al. 2015). Penston et al. (2004) tested the use of a pump and vertical and horizontal net hauls in Loch Shieldaig, Scotland, and found that horizontal tows at the surface proved to be the best suited method out of the three to collect planktonic salmon lice. Nordi et al. (2015) also had success in sampling free-living salmon lice with horizontal tows applied ~0.25 m below the surface in the Faroe Islands. However, previous attempts to sample planktonic salmon lice in Norway have failed (Skarðhamar 2016a) and the uncertainty regarding their spatiotemporal distribution led us to use an open-minded approach when planning this survey. Following is a detailed discussion on each sampling strategy, along with a table where all methods tested are compared.

A 30 L Go-Flo bottle applied at 3 m depth was by far the most efficient sampling strategy collecting copepodite salmon lice during the September cruise in the outer Hardangerfjord and equally as good as the vertical net hauled from 10 m to the surface in the August Altafjord cruise (Figure 8). Go-Flo samples are easy to analyse but the bottles are impractical to use in the field due to the low volumes sampled and the heavy gear (Table 6). During this study, volumes of 30 L, 100 L and 150 L (Table 3) were collected using Go-Flo bottles. These volumes are undesirably small to obtain reliable estimates of abundance in relatively low concentrations, such as both L. salmonis and C. elongatus (Nordi et al. 2015). Further, at the sampled depths of 5 m and 10 m no copepodite salmon lice were found. All of this indicates that it is not the Go-Flo as a method that is failing here, but the depth sampled.
Table 6: Overview of method used during this study, with comments on usability in field and during analysis following a conclusion for each method.

<table>
<thead>
<tr>
<th>Method</th>
<th>Volume</th>
<th>In field</th>
<th>During analysis</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Go-Flo</td>
<td>Small</td>
<td>Impractical to use when sampling larger volumes because it has to be applied several times making it time consuming and impractical. Also a crane is required.</td>
<td>The easiest samples to analyse.</td>
<td>A good method to establish more accurate where the copepodite salmon lice reside in the water column, but inconvenient to use in the field.</td>
</tr>
<tr>
<td>Horizontal tow</td>
<td>Very large</td>
<td>Easy to use in the field, but need a crane. Time effective and samples huge volumes. Has to make sure it stays in the right depth at all times. Sensitive to waves when sampling close to the surface.</td>
<td>The hardest samples to analyse. Had to be diluted several times and still the samples were highly concentrated with plankton making it very time consuming and difficult to distinguish the plankton species from each other.</td>
<td>A useful method if applied at suitable depth (surface). Time consuming to analyse, so more time or more people are needed to go through the samples.</td>
</tr>
<tr>
<td>Vertical net haul</td>
<td>Large</td>
<td>Easiest to use in the field, but a crane is required. Very time effective and easy to get larger volumes.</td>
<td>Moderately easy to analyse, but quite time consuming. The sample is concentrated and has to be diluted some but not nearly as much as the samples from the horizontal tows.</td>
<td>In all consideration this is the most time effective and most practical method to use.</td>
</tr>
<tr>
<td>Pump</td>
<td>Large to very large</td>
<td>Easy to use in the field, and no crane required. Samples strictly at narrow depth interval and easy but somewhat time consuming to get larger volumes.</td>
<td>Easy and time effective to go through during analysis.</td>
<td>Both easy to use in field and easy to analyse the samples of. But sampling at narrow and specific depth makes it vulnerable to the experienced patchiness when sampling copepodite salmon lice.</td>
</tr>
</tbody>
</table>

The horizontal tows were largely unsuccessful during this study. This may be due to the method itself or the sampled depth. The sampling strategy resulted in a concentration of 0.01 ind. m\(^3\), and consequently the horizontal tow applied at 4 m depth proved to be an inadequate approach. The horizontal tow strategy filtered a total sampled volume of 125 000 L, the largest of all strategies tested. Therefore, it is hard to imagine that it is a coincidence that almost all the samples collected with this method had no copepodite salmon lice. It was not tested on shallower depths in our studies due to waves and windy conditions, and it is therefore hard to know for sure if it was the chosen depth or other factors that led to the failure of collecting salmon lice copepods. Factors such as the water failing to flow through the net due to the high density of plankton, which could have led to
clogging and caused a bottle effect as the net was towed forward, which could hinder proper sampling. Even though it is convenient to use in the field, the effort to analyse the samples were tremendous due to the density of total plankton obtained (Table 6). The application of horizontal tows are weather dependent, which was an important factor to consider in the Northern Norwegian fjord, especially in autumn and winter time when conditions often are rough. Thus this method was not proceeded with in later studies in the Altafjord, even though it was the preferred method in earlier studies.

Vertical net hauls were a lot easier to use in the field compared to the Go-Flo, less weather sensitive than the horizontal tows and easily sampled through large volumes which has been demonstrated a necessity when studying free-living salmon lice. The samples were harder and quite time consuming to analyse, owed to the higher density of plankton and a greater diversity (Table 6). One of the samples in the Altafjord (Station 6, Øksfjord) had a concentration of 12.8 ind. m$^{-3}$ (Figure 9). In this one sample, 32 individuals of copepodite salmon lice was found, which is over half of the lice collected in total by the vertical net haul in the Altafjord (total of 50 ind.) during the August cruise. If this outlier had been removed, the Go-Flo would have showed to be the most efficient sampling method in the Altafjord as well, in terms of individuals caught per volume sampled. In addition to this, the vertical net haul sampled all the way from 10 m to the surface. It represents an undesirable effect when trying to decide where in the water column the L. salmonis and C. elongatus reside, but at the same time the vertical net haul integrates across patches in depth specific layers.

The provisional bilge pump was added as a method to ensure a higher sampled volume size and in addition get depth specific data. This sampling strategy proved to underestimate the concentration of copepodite salmon lice, when compared to the Go-Flo (3 m) and the vertical net haul applied at the same time. The bilge pump sampled at one precise depth (1 and 3 m), which may have caused the sampled concentrations to be lower than anticipated. Previous studies have shown that the patchiness of both the L. salmonis and C. elongatus is high, and by sampling at one specific depth it is easy to miss the lice (Asplin et al. 2013).

The patchiness of the salmon lice distribution is a challenging feature when it comes to sampling methodology, and both the horizontal and the vertical patchiness had to be taken into consideration when deciding on a sampling strategy. The vertical net haul samples through all the depths where it is plausible to collect both L. salmonis and C. elongatus, resulting in point specific concentrations at a given time and without needing to consider changes in the vertical distribution. This sampling strategy is possible to conduct under sub-optimal conditions. A horizontal tow applied at the surface, where the highest concentration of L. salmonis is thought to reside, can easily sample through
enormous water masses and therefore compensate for some of the patchiness in the horizontal distribution of copepodite salmon lice. Horizontal tows do however not take vertical distribution into account, like alterations due to the presence of a fresh water layer. In addition, this method is highly weather dependent. The fjords sampled in this study were non-local and the cruises had to be planned ahead. The weather conditions within this limited time frame were impossible to foresee and could have a large effect when applying this method.

All this considered, our experience and results concluded that the vertical net haul is the most reasonable method to use when trying to check the performance of the hydrodynamic salmon lice models. With both this study and earlier studies in mind, a possible better method would be to focus sampling in the upper 5 m using a vertical net haul, since both conclude that copepods of salmon lice are located above 5 m (Heuch et al. 1995, Costello 2006, Nordi et al. 2015). This way one avoids sampling through water masses where it is unlikely to collect either L. salmonis or C. elongatus, and at the same time collect less of the unwanted plankton, thus making the further analysis easier. To compensate for the smaller volume sampled compared to the horizontal tow, one could apply the vertical net haul several times at the same location and add the concentration to the same sample bottle. Mesh size varied through the testing period, from 20-180 μm. To make sure that no lice are lost and at the same time avoid some of the smaller unwanted plankton, one should use a mesh size between 90-180 μm. Preferably a 150 μm net and sieve, which will ensure less clogging than a 90 μm sieve and at the same time not lose any copepodite salmon lice.

5.2 Range of concentrations of copepodite salmon lice?
To know something about the infection pressure of parasitic salmon lice it is important to know the concentrations in the given area. Compared to other free-living copepod species, L. salmonis and C. elongatus appear in relatively low concentrations. In comparison, species like Microsetella norvegica and Calanus finmarchicus have been found in concentrations of >200 000 ind. m$^{-3}$ in Northern Norwegian fjords (Antonsen 2014) and >30 000 ind. m$^{-3}$ on the North Norwegian Shelf (Halvorsen et al. 1999), respectively. In this study, concentrations of salmon lice copepodites have ranged from 0-30 ind. m$^{-3}$, where the highest concentrations were observed in the outer Hardangerfjord (Table 7). In the Altafjord the highest concentration found was 12.8 ind. m$^{-3}$. Earlier observed concentrations in Scotland and the Faroe Islands have ranged from 0-534.3 ind. m$^{-3}$ (Table 7, Penston et al. 2004, Penston et al. 2011, Nordi et al. 2015).
Table 7: Overview of concentrations observed in three earlier studies and the concentrations found during this study. The horizontal tow and the Go-Flo applied at 5 and 10 m depth is not included for the Hardangerfjord data. In addition, the Altafjord concentrations are given separately for each of the sampled locations (Korsfjord, Talvik and Øksfjord). After thorough testing Penston (2004), Penston (2011) and Nordi (2015) choose horizontal tows at the surface as the preferred method, thus their concentrations are given for the minimum and maximum of the sampled site. While for this study, where several sampling strategies have been conducted, the minimum and maximum is concentrations is given with the specific sampling strategy at each location. The mean is the average concentration at given location and time.

<table>
<thead>
<tr>
<th>Study</th>
<th>Study area</th>
<th>Season</th>
<th>Species</th>
<th>Min. (ind. m$^{-3}$)</th>
<th>Max. (ind. m$^{-3}$)</th>
<th>Mean (ind. m$^{-3}$)</th>
<th>Sampling strategy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penston (2004)</td>
<td>Loch Shieldaig Site A, Scotland</td>
<td>Spring/Summer</td>
<td>Caligidae</td>
<td>0</td>
<td>11.2</td>
<td>0.5</td>
<td>Horizontal tow, surface</td>
</tr>
<tr>
<td>Penston (2004)</td>
<td>Loch Shieldaig Site S, Scotland</td>
<td>Spring/Summer</td>
<td>Caligidae</td>
<td>0</td>
<td>543.3</td>
<td>≈ 58</td>
<td>Horizontal tow, surface</td>
</tr>
<tr>
<td>Penston (2011)</td>
<td>Loch Torridon, Scotland</td>
<td>Full year</td>
<td><em>L. salmonis</em></td>
<td>0</td>
<td>4.2</td>
<td>-</td>
<td>Horizontal tow, 0-0.5 m</td>
</tr>
<tr>
<td>Nordi (2015)</td>
<td>Sundalagið, Faroe Islands</td>
<td>November - June</td>
<td><em>L. salmonis</em></td>
<td>0</td>
<td>≈ 3.2</td>
<td>-</td>
<td>Horizontal tow 0.25-0.75 m</td>
</tr>
<tr>
<td>Nordi (2015)</td>
<td>Sundalagið, Faroe Islands</td>
<td>November - June</td>
<td><em>C. elongatus</em></td>
<td>0</td>
<td>1.0</td>
<td>-</td>
<td>Horizontal tow 0.25-0.75 m</td>
</tr>
<tr>
<td>Hardangerfjord</td>
<td>Outer Hardangerfjord</td>
<td>September</td>
<td>Caligidae</td>
<td>0</td>
<td>30.0</td>
<td>0.7±8.8</td>
<td>Go-Flo (3 m), Vertical (10-0 m)</td>
</tr>
<tr>
<td>Altafjord</td>
<td>Korsfjord</td>
<td>August</td>
<td>Caligidae</td>
<td>0</td>
<td>6.7</td>
<td>1.2±2.4</td>
<td>Go-Flo (3 m), Pump (1 and 3 m), Vertical (10-0 m)</td>
</tr>
<tr>
<td>Altafjord</td>
<td>Talvik</td>
<td>August</td>
<td>Caligidae</td>
<td>0</td>
<td>0.8</td>
<td>0.2±0.3</td>
<td>Go-Flo (3 m), Pump (1 and 3 m), Vertical (10-0 m)</td>
</tr>
<tr>
<td>Altafjord</td>
<td>Øksfjord</td>
<td>August</td>
<td>Caligidae</td>
<td>0</td>
<td>12.8</td>
<td>3.0±3.6</td>
<td>Go-Flo (3 m), Pump (1 and 3 m), Vertical (10-0 m)</td>
</tr>
<tr>
<td>Altafjord</td>
<td>Korsfjord</td>
<td>September</td>
<td>Caligidae</td>
<td>0</td>
<td>8.0</td>
<td>3.7±3.5</td>
<td>Pump (1 and 3 m)</td>
</tr>
<tr>
<td>Altafjord</td>
<td>Talvik</td>
<td>September</td>
<td>Caligidae</td>
<td>0</td>
<td>2.0</td>
<td>0.8±1.0</td>
<td>Pump (1 and 3 m)</td>
</tr>
<tr>
<td>Altafjord</td>
<td>Øksfjord</td>
<td>September</td>
<td>Caligidae</td>
<td>0</td>
<td>9.0</td>
<td>2.9±2.9</td>
<td>Pump (1 and 3 m)</td>
</tr>
<tr>
<td>Altafjord</td>
<td>Korsfjord</td>
<td>October</td>
<td>Caligidae</td>
<td>0</td>
<td>1.0</td>
<td>0.1±0.4</td>
<td>Pump (1 and 3 m)</td>
</tr>
</tbody>
</table>
A patchy and therefore complicated distribution is expected, since salmon lice copepods have showed tendency to aggregate, especially close to shore and estuary mouths (Penston et al. 2004, Amundrud & Murray 2009, Penston et al. 2011, Asplin et al. 2013). This is demonstrated by the high variation of the sampled copepodite salmon lice concentrations both within the different study sites and across the studies. In light of this, the high concentrations found, such as 534.3 ind. m$^{-3}$ in Scotland and 30 ind. m$^{-3}$ in the Hardangerfjord, can be viewed as extreme values and thus outliers, caused by the copepodite salmon louses patchy distribution. When removing these extreme values, the general concentration range found in the present study is between 0-13 ind. m$^{-3}$, against 0-11 ind. m$^{-3}$ in the earlier studies from other regions (Table 7). Another important observation is that all 13 study sites had a minimum concentration of 0 ind. m$^{-3}$. This indicates that the concentrations estimated in the present study are realistic, despite them being based on few individuals. In addition, it underlines the expected patchy distribution of copepodite salmon lice.

When comparing the concentrations found in the outer Hardangerfjord and the Altafjord, the same patchiness can be seen. The outer Hardangerfjord is a high concentration area for salmon lice from mid-June, according to the hydrodynamic model and earlier observations (Taranger et al. 2014). The concentration obtained here in September by Go-Flo (3 m) and vertical net hauls (10-0 m) had a higher maximum than found in all the Altafjord locations, but were highly variable with a low mean of 0.72±8.76 ind. m$^{-3}$ (Table 7). Patchiness was also seen when the results from the Altafjord was studied as a whole, with a minimum concentration of 0 ind. m$^{-3}$ to a maximum of 12.8 ind. m$^{-3}$, and mean concentrations from 0.22±0.28 to 3.67±3.50 ind. m$^{-3}$. Talvik was stable as a low concentration site during the investigations conducted through this project. The highest mean concentration in the Altafjord change from August to September. The Øksfjord had the highest mean concentration in August and the Korsfjord had the highest in September, demonstrating that the concentrations are constantly changing and are point specific.
5.3 Field Observations and model output

The standing stock of salmon lice found in the Altafjord varied between 0-64 ind. m$^{-2}$, with a mean of 9.9 and 13.1 ind. m$^{-2}$ for August and September, respectively. Separating the fjords from each other, we get a range of 0-33 ind. m$^{-2}$ for the Korsfjord and 0-64 ind. m$^{-2}$ in the Øksfjord for the August cruise, with a mean of 11.1 ind. m$^{-2}$ in the Korsfjord and 12.1 ind. m$^{-2}$ in the Øksfjord from the present study. The hydrodynamic salmon lice model used by the Institute of Marine Research simulates the distribution of salmon lice, determined by currents, temperature and salinity (IMR 2016). Comparing the field data from this study to the hydrodynamic model output in Figure 16 (Skarðhamar 2016b), it can be seen that the model predicts a much higher variance and abundance of copepodite $L.\ salmonis$ in the Øksfjord than in the Korsfjord during the August sample period (indicated with vertical lines). This is comparable with the standing stock range and means found in the field. Further, the mean simulated by the model in the Korsfjord is below 3 ind. m$^{-2}$, while in the Øksfjord it is between 10-20 ind. m$^{-2}$. Even though the field data contains both $L.\ salmonis$ and $C.\ elongatus$, and they can be expected to approximately have a 50/50 distribution (Urquhart et al. 2008), the model output mean in the August sample period in the Korsfjord is lower compared to the observed field data, while in the Øksfjord the mean abundance for both the model output and the field data is within the same range.

When comparing the September field data to the model output, the model predicts a lower abundance. In the Korsfjord the range of the standing stock were 2-40 ind. m$^{-2}$, with a mean of 18 ind. m$^{-2}$, while a 3-33 ind. m$^{-2}$ range was found in the Øksfjord, with a mean of 15 ind. m$^{-2}$ from the field data. As in August, the model predicts an abundance of $L.\ salmonis$ below 3 ind. m$^{-2}$ in the Korsfjord, which is much lower than the observed mean of 18 ind. m$^{-2}$ estimated from the field data. Even when the 50/50 distribution of the two species is taken into consideration, the prediction of the model is low. In the Øksfjord the model expected 10 ind. m$^{-2}$ $L.\ salmonis$ for the same sample period, which is well within the same range as seen in the field data with 15 ind. m$^{-2}$ for the two species. When taking into consideration that during the September cruise only the pump was used, which later proved to underestimate the concentration of copepodite salmon lice, this indicates that the model estimated a low abundance for both fjords in September.

In conclusion the hydrodynamic model output seems to be within the same range or lower when estimating the abundance of copepodite salmon lice as found in the field, when comparing results from the specific time of sampling to the corresponding day given by the model. The models have their weaknesses and have the same issues predicting patchiness as seen in the field data and may therefore not be time and point specific. It is perhaps better to compare the concentration range of the model output to the range observed in the field. Over all the observed field concentrations is
within the same range as the mean concentrations the model simulates. The model predicts a max concentration of 120 ind. m$^2$ in the Øksfjord, which is much higher than the maximum found in the field, 64 ind. m$^2$. There was not sampled in the field during the peaks simulated by the model, therefore it is not possible to say that the model over all predicts low concentrations.
Figure 16: Hydrodynamic lice model output for the Korsfjord and the Øksfjord during the August and September cruise to the Altafjord in 2015. The data in the figure is integrated by day fields and is therefore not completely accurate, but the relative change over time is correct. Vertical lines indicate the sample periods for the two expeditions. By Skarðhamar (2016b).
5.4 Size distribution

The length of all copepodite salmon lice found in the samples were measured. This was done to be able to compare the size range to other studies and for the possibility of differentiating between *L. salmonis* and *C. elongatus*. Compared to the mean total length from previous studies (Schram 2004), the copepodite salmon lice sampled in both the outer Hardangerfjord and the Altafjord during this study were smaller (Table 8). The maximum of the size range for both the Hardangerfjord and the Altafjord is approximately twice as large as the minimum, which is not the case for the measurements of *L. salmonis* or *C. elongatus* by neither Schram (1993) nor Piasecki (1996). This could be because the sampled lice from this study were captured as free-living plankton from the ocean, while the measured copepodite salmon lice from earlier studies are grown in cultures and under optimized conditions. And that the preservation applied (formaldehyde) in this study may lead to some shrinking.

<table>
<thead>
<tr>
<th>Table 8: Overview of the size range of the measured copepodite salmon lice in present study and the established size range of both <em>L. salmonis</em> and <em>C. elongatus</em> from two earlier studies.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Study</strong></td>
</tr>
<tr>
<td>Results Hardangerfjord (2014)</td>
</tr>
<tr>
<td>Results Altafjord (2015)</td>
</tr>
<tr>
<td>Schram (1993), <em>L. salmonis</em></td>
</tr>
<tr>
<td>Piasecki (1996), <em>C. elongatus</em></td>
</tr>
</tbody>
</table>

During analysis some small differences in body shape and urosome (the bottom segments) on the copepodite lice were observed, but due to the preservation used (4% formaldehyde) it was not possible to distinguish the two probable species from each other (*L. salmonis* and *C. elongatus*) as the characteristic pigmentation disappears. Could the size distribution hint at the fact that there were two species present in the Altafjord at the time of sampling? This may be the case, as *C. elongatus* has a wider total length range (0.580-0.810 mm, Piasecki 1996), and the mean total length of the lice collected in the Altafjord (0.577±0.095 mm) is just below this. Especially at the copepodite stage the two species may differ in size, where *C. elongatus* is often smaller than *L. salmonis* (Danielsen 2013). It is further known that *C. elongatus* thrive in autumn and winter months (Øines et al. 2006), and can be practically absent during late spring and summer (Nordi et al. 2015).
Throughout the September cruise in the Altafjord, smolt was deployed in sea cages and during the October cruise they were collected and sent to Bergen to analyse the attached lice. The analyses found that two different species of sea lice were attached to the fish, both *Lepeophtheirus salmonis* and *Caligus elongatus*. To investigate further, 15 smolt were taken out and analysed to establish the proportions of the two different species, shown in Table 9. Note that here all stages of attached lice found on the smolt were counted, not only the copepodite instar. Out of 104 attached lice 40 of them was *C. elongatus*, which is ≈39 %.

Table 9: Amount of attached lice for each of the two different species found on the salmon smolt deployed in the Altafjord autumn 2015 (Data from Sussie Dalvin, Institute of Marine Research (IMR)).

<table>
<thead>
<tr>
<th>Smolt</th>
<th><em>Lepeophtheirus salmonis</em></th>
<th><em>Caligus elongatus</em></th>
<th>Total number of attached lice</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>14</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>9</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>10</td>
<td>7</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>11</td>
<td>3</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>12</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>13</td>
<td>18</td>
<td>9</td>
<td>27</td>
</tr>
<tr>
<td>14</td>
<td>3</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>15</td>
<td>9</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td><strong>Total number:</strong></td>
<td><strong>64</strong></td>
<td><strong>40</strong></td>
<td><strong>104</strong></td>
</tr>
</tbody>
</table>

Furthermore, the size distribution for all 123 copepodite lice collected in the Altafjord (Figure 14) has a clear drop in amount of lice found with a total length between 0.55-0.60 mm. This could indicate that the size distribution found in the Altafjord is indeed of *L. salmonis* and *C. elongatus* overlapping. This is only speculation, as the copepods found in the samples were only identified down to family, *Caligidae*. Together with the distribution of the two species found on the smolt and earlier observations on the seasonal abundance of *C. elongatus*, it is highly plausible that the samples contained both *L. salmonis* and *C. elongatus*.
5.5 Vertical and horizontal distribution of salmon lice

5.4.1 Vertical distribution in the water column

The vertical distribution of planktonic salmon lice copepods is expected to be patchy and influenced by salinity, temperature, light and currents, as well as the copepods own movement (Johnsen et al. 2014). Sampling below 3 m depth was less successful in collecting copepodites of salmon lice, suggesting that the copepods reside in the upper water layers. This corresponds with previous studies, which also conclude that L. salmonis reside even shallower (0-1 m depth) during the copepodite stage in the absence of a fresh water layer (Costelloe et al. 1998, Penston et al. 2004), like most of the sampled sites during this study. As knowledge on the vertical distribution of C. elongatus is limited, it is only speculated that the two species may have different vertical distribution (Nordi et al. 2015). The shallowest investigated depth (3 m) in the Hardangerfjord had the highest abundance of copepodite salmon lice. In the Altafjord, samples taken by the bilge pump at 1 m had a higher concentration of copepods than at 3 m. This may indicate that the copepodite salmon lice reside all the way up at the surface, also in Northern Norway. It is expected that L. salmonis during their copepodite stage will reside as shallow as possible, avoiding salinities below 27 (Bricknell et al. 2006). Through the two nauplius stages they will stay further down in the water column (Heuch et al. 1995). Penston et al. (2008) demonstrated this with taking samples at 0 and 5 m depth. Here nauplii were most abundant at 5 m depth, while the copepods were most abundant at the surface. As stated in the hydrography chapter, none of the stations in the Altafjord had salinity values below 30, and consequently the L. salmonis copepods could reside all the way up to the surface. This may be an adaption by the copepods to ensure dispersal by the onshore currents, as well as residing in the part of the water column where the salmonids feed (Costello 2006). Salmonids will remain in the shallow waters of the littoral zone where the salmonids feed (Costello 2006). The pump sample strategy underestimated the concentration of copepodite salmon lice. As this method samples very depth specific, the underestimation gives support to the suggested patchy vertical distribution.

Nordi et al (2015) suggested that the vertical migration towards the surface is not as strong for C. elongatus since it has more possible hosts and therefore have a wider habitat it can thrive in. This may cause the two species to have different horizontal distribution, as seen in the study from the Faroe Islands (Nordi et al. 2015).
5.4.2 Horizontal distribution

A trend of the concentration of copepodite salmon lice can be seen when the distance from nearest farm was over 10 km, as found in the Talvik area (Figure 15A). Talvik is inside the National Salmon Fjord reserve and is supposed to be an area with no salmon lice. Still, low concentrations were found in both August and September. Penston et al. (2011) investigated the concentrations of planktonic *L. salmonis* before and after relocation of a salmon farm in Loch Torridon, Scotland. They found that even if the relocation led to a significant drop in the production of *L. salmonis* nauplii at the vacated site, some larvae were transported from somewhere else. Most likely from other farm sites that was ~5-8 km away. Relating this to the Talvik area, this may be the case here for the low concentrations observed. Here the distance to nearest farm was ~10-13.5 km, which is a distance within the documented potential horizontal dispersion of salmon lice larvae of 10-50 km (Siegel et al. 2003, Costello 2006, Penston et al. 2011). The hydrodynamic salmon lice model simulated that the lice did not disperse from the Øksfjord to the Korsfjord (Skarðhamar 2016a), a distance between 36 and 72 km.

Earlier studies have suggested three different patterns of dispersion for *L. salmonis*, which facilitates the patchy distribution observed. First, that *L. salmonis* larvae are most abundant within 100 m from the nearest farm site and decrease in concentrations as the distance increases (Costelloe et al. 1999). In this study there were not observed any clear trends of decreasing amount of copepodite salmon lice for the stations sampled within the Korsfjord and the Øksfjord which had a distance between 0.250-2.85 km from nearest farm site. After hatching, it is the nauplii salmon lice that will aggregate close to the farms (Penston et al. 2004, Æ Norði et al. 2016). Since this stage was not identified during this study it can only be guessed that this may explain why there was not found any clear tendencies of decreasing amount of salmon lice copepods away from nearest farm site. Second, that copepods would accumulate close to shore (Johnsen 2011, Nordi et al. 2015). When sorting the stations in the Korsfjord and the Øksfjord after distance from land (Figure 15B), there was not observed any clear pattern of decrease. Thirdly, it is suggested that copepods will be transported toward river outlets and aggregate here. This is especially clear for Site S in the study conducted in Loch Shieldaig, Scotland (Table 7, Penston et al. 2004). Here a maximum >500 ind. m\(^{-3}\) and a minimum of 0 ind. m\(^{-3}\) were found with an average of ~ 58 ind. m\(^{-3}\). Even in an area where such high concentrations are found at one time and space, there may later be no copepodite salmon lice sampled. This underlines the non-predictable and complicated distribution. During this study it was not sampled close to the river outlets, thus this thesis does not have data to support or refute this.
The distribution of sea lice, especially *L. salmonis*, is highly influenced by salinity, currents and temperature (Johnsen 2011). The salmon lice’s adaptation to vertically migrate enables it to influence their horizontal distribution. Along the Norwegian coast line and in the water column the temperature varies, which will increase or decrease the maximum potential horizontal dispersion since lower temperatures will increase the duration of the different instars (Table 2). This will result in a longer period for the planktonic salmon lice to disperse in, potentially leading to a more widespread horizontal distribution. Consequences like this can be of negative effect for both the salmon farm industry and the wild salmon.

5.4.3 Seasonality
The Hydrodynamic salmon lice model output (Figure 16) illustrates the seasonal fluctuations of copepodite salmon lice abundance through the autumn of 2015 in the Korsfjord and the Øksfjord. This illustrates an example of seasonal distribution, where several peaks in abundance can be seen and then periods of lower abundance. The model only includes the *L. salmonis*. It has however been observed on the smolt from the Altafjord and in earlier studies from Scotland (Urquhart et al. 2008) that *C. elongatus* has a prevalence of ≥ 39 % when compared to *L. salmonis*. As *C. elongatus* is also a vital parasite on salmonid species, this is important to take into consideration. Especially in autumn, *C. elongatus* has proved to be a dominating species in central and Northern Norway (Øines et al. 2006). Why there was only found one salmon lice copepodite during the October cruise is difficult to establish. During the October cruise to the Altafjord, only one of three salmon farms in the Korsfjord and two of seven salmon farms in the Øksfjord were operational (Fiskeridirektoratet 2016). As the same amount of salmon farm sites were operational in September, it is unlikely that this is the reason for the lack of salmon lice copepodes. The multigraph of the hydrographical profile for the Altafjord (Figure 7C) show that the October CTD-profile is jagged and the halocline is broken because of bad weather. This may have caused the sampling to be less successful, and explain why there was not collected more than one copepodite salmon louse.
5.6 Summary and Perspectives

Under Norwegian coastal conditions vertical net hauls (10-0 m) proved to be the best suited sampling strategy, robust against both weather conditions and the vertical distribution of the copepodite salmon lice. Further it was established that the copepodite salmon lice would reside above 5 m depth. Therefore it would be better to sample with a vertical net hauled from 5 m depth to the surface, this way one would avoid sampling through water masses where it is unlikely to collect any copepodite salmon lice. To compensate for the smaller volume sampled, the vertical net haul could be applied several times at the same location and added to the same sample bottle. This way both ensuring larger volume sampled and sampling through the appropriate areas of the water column.

Disregarding outliers, the concentrations observed in the field was similar to the range seen in earlier studies, with ~0-13 ind. m$^{-3}$ for present study against ~0-11 from earlier studies from the Faroe Islands and Scotland (Table 6). This indicates that the concentrations in the open water are quite similar between the different regions. The distribution is patchy and complicated, as expected. Also when comparing the field data from this study to the hydrodynamic model output estimating the abundance of copepodite salmon lice, the field data seems to be within the same range. Due to the uncertainty of why so few salmon lice were sampled during the October Altafjord cruise, it is not possible to determine any seasonality of salmon lice copepods from the data collected during this study.

As the salmon lice has a potential maximum dispersal between 10-50 km (Siegel et al. 2003, Costello 2006, Penston et al. 2011) it is possible that parasitic salmon lice can reside all over both the Altafjord and the Hardangerfjord (Johnsen 2011), in various concentrations and patchily distributed. With this in mind in addition to the narrow vertical distribution, Figure 17 illustrates the infection pressure a salmonid fish could experience when leaving the Alta River.

Figure 17: Conceptual illustration of the distance and infection pressure a salmonid fish leaving the Alta River could encounter.
Traveling approximately 60 000 m from the river outlet to the fjord mouth it might every meter encounter concentrations of parasitic salmon lice between 0-12.8 ind. m$^{-3}$. Within the National Salmon Fjord (0-25 000 m) the concentrations are expected to be between 0-2 ind. m$^{-3}$, but in the middle and outer region the predicted concentration can be as high as 12.8 ind. m$^{-3}$. Whether low concentrations, like those found in Talvik, are enough to constitute harmful infections on wild salmonids, is not known. More knowledge on the critical doses of sea lice to infect salmonid fish is needed (Johnsen 2011), to know whether the lower doses within the National Salmon Fjord is potentially harmful. The best solution would be to ensure that the life cycle of the parasite is not completed, resulting in overall lower concentrations as the salmon lice can easily spread inside the fjord system.
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