Master's thesis

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

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# Assessment of a Passive Plankton Sampler Technology for Monitoring Densities of Planktonic Sea Lice (*Lepeophtheirus salmonis* & *Caligus elongatus*) in the Hardangerfjord

Master's thesis in Ocean Resources
Supervisor: Bengt Finstad (IBI)
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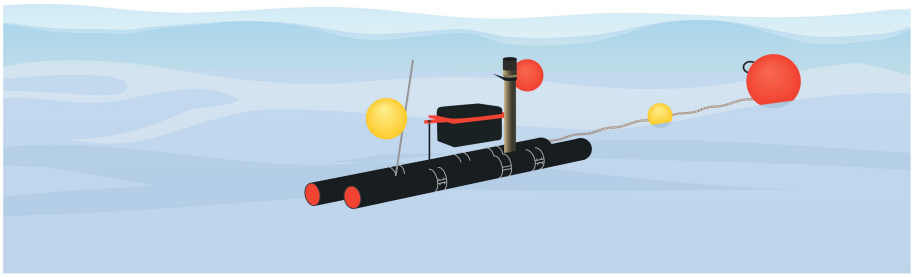

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# Acknowledgements

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This study has been a part of the project "LiceQuest", a collaboration between NTNU and the Norwegian Institute for Nature Research (NINA). The thesis has in addition been a part of Taskforce Salmon Lice (TFSL), a R&D project between NTNU and several Norwegian aquaculture companies.

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Ingvild Tryggestad

# Abstract

The two species of sea lice, *Lepeophtheirus salmonis* and *Caligus elongatus*, are ectoparasitic copepods causing the greatest parasitical threat to salmonid aquaculture and wild salmonid stocks in Norway. The life cycle of the two species begins with three planktonic larvae stages, during which the larvae disperse in open waters dependent on locating and infesting a suitable host for survival. Transmission of sea lice between aquaculture facilities and between domesticated and wild salmonids are mainly occurring in these planktonic stages. Environmental and economic issues related to sea lice outbreaks have developed in line with increased aquaculture production, hence a need for monitoring densities of sea lice along the coast has arisen. Development of efficient and precise techniques for quantifying planktonic sea lice for investigating abundance and distribution of the planktonic stages in fjords and coastal waters have therefore become crucial.

In this context, the ability of a passive plankton sampler (PPS) for collecting planktonic *L. salmonis* and *C. elongatus* was studied in two fjord systems in Hardanger, Vestland County, western Norway. Over a one-month period, planktonic samples were collected from 12 PPS deployed at six separate locations. Simultaneously, environmental parameters (temperature and salinity) were monitored in the water column using sensors attached to a PPS at each study location. Levels of *L. salmonis* and *C. elongatus* in the collected samples were analysed using DNA-based quantification, with species-specific assays combined with ddPCR technology. Statistical analyses were used to assess collected sea lice, variance between localities, the reliability of the PPS and laboratory methodology and effects of temperature and salinity on sea lice collection.

This study demonstrated a successful collection and analyzation of *C. elongatus* and *L. salmonis* larvae using the PPS and DNA-based quantification in combination. The laboratory analysis showed a high level of reliability while PPS measurements exhibited a lower degree of reliability, potentially due to external factors influencing planktonic sea lice collection. The collection of *L. salmonis* larvae exceeded that of *C. elongatus*, possibly attributed to seasonal fluctuations of *C. elongatus*. Significant variations in collection and concentration of *L. salmonis* were discovered between the localities assessed, with higher collection of larvae at localities situated closer to aquaculture farms. Higher lice loads were documented in the northern study area, in line with the modelled larvae abundance of the fjordsystems. No significant effects of salinity or temperature on collection of *L. salmonis* were found in this study. Concludingly, the PPS with implemented suggested enhancements, in combination with DNA-based quantification, was recognized as an applicable methodology for assessing planktonic sea lice in fjords and coastal waters.

# Sammendrag

De to artene av havlus, *Lepeophtheirus salmonis* og *Caligus elongatus*, er ektoparasittiske hoppekreps som utgjør den største parasittiske trusselen i lakseoppdrett og mot bestander av vill laksefisk i Norge. De to artenes livssyklus starter med tre planktoniske larvestadier, der larvene spres i åpent vann, avhengig av å finne og infestere en egnet vert for å overleve. Overføring av lakselus mellom oppdrettsanlegg og mellom domestisert- og vill laksefisk skjer hovedsakelig i disse planktoniske stadiene. Miljømessige og økonomiske problemer knyttet til utbrudd av havlus har utviklet seg i takt med økt akvakulturproduksjon, og det har derfor oppstått et behov for å overvåke tetthet av havlus langs kysten. Utvikling av effektive og presise teknikker for å kvantifisere planktonisk havlus har derfor blitt avgjørende, for å undersøke forekomst og fordeling av de planktoniske stadiene i fjorder og i kystområder.

Evnen til en passiv planktonsamler (PPS) for å samle planktonisk *L. salmonis* og *C. elongatus* ble derfor studert i to fjordsystemer i Hardanger, lokalisert i Vestland fylke, Vest-Norge. Over en måned ble planktoniske prøver samlet inn fra tolv PPS-er satt ut ved seks ulike lokasjoner. Samtidig ble miljøparametere (temperatur og salinitet) overvåket i vannsøylen ved bruk av sensorer festet til en PPS ved hver studielokalitet. Nivåer av *L. salmonis* og *C. elongatus* i innsamlede prøver ble analysert ved hjelp av DNA-basert kvantifisering, med artsspesifikke markører kombinert med ddPCR-teknologi. Statistiske analyser ble brukt for å vurdere innsamlede havlus, variasjon mellom lokaliteter, påliteligheten til PPS-og laboratoriemetodikken, samt effekten av temperatur og salinitet på innsamling av havlus.

Denne studien demonstrerte en vellykket innsamling og analyse av *C. elongatus* og *L. salmonis*-larver ved bruk av PPS og DNA-basert kvantifisering i kombinasjon. Laboratorieanalysen viste et høyt nivå av pålitelighet, mens målingene utført av PPSene viste en noe lavere pålitelighet, muligens forårsaket av eksterne faktorer som kan påvirke innsamling av planktonisk havlus. Det ble samlet inn et større antall larver av *L. salmonis* enn av *C. elongatus*, muligens som følge av sesongmessige forekomster av *C. elongatus*. Signifikante variasjoner i innsamling og konsentrasjon av *L. salmonis* ble oppdaget mellom de ulike lokalitetene, med en høyere innsamling av larver ved lokaliteter nærmere oppdrettsanlegg. Høyere lusenivåer ble dokumentert i det nordlige studieområdet, i tråd med den modellerte luseforekomsten i fjordsystemene. Ingen signifikante effekter av salinitet eller temperatur på innsamling av *L. salmonis* ble funnet. Studien konkluderte med at PPS, med implementerte foreslåtte forbedringer, i kombinasjon med DNA-basert kvantifisering, evalueres som en egnet metode for vurdering av planktonisk havlus i fjordsystemer og kystområder.

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# Abbreviations

| | |
|---|---|
| *C. elongatus* | *Caligus elongatus* |
| ddPCR | Digital droplet Polymerase Chain Reaction |
| IMR | Institute of Marine Research |
| *L. salmonis* | *Lepeophtheirus salmonis* |
| MAB | Maximum Allowable Biomass |
| MTIF | Ministry of Trade, Industry and Fisheries |
| NFSA | The Norwegian Food Safety Authority |
| NINA | Norwegian Institute for Nature Research |
| NOK | Norwegian krone |
| NTNU | Norwegian University of Science and Technology |
| NVI | The Norwegian Veterinary Institute |
| PPS | Passive Plankton Sampler |
| TLS | The Traffic Light System |

# 1 Introduction

The global human population is estimated to exceed 9 billion people by 2050, and along with this is a challenge rising: of feeding the world without compromising our natural resources (FAO, 2022). Aquatic food systems are in this context considered crucial, holding a potential of providing more sustainable animal-protein sources, and constituting a larger proportion of humanity's nutritious food requirements (Gephart et al., 2020). Among the current seafood production strategies, aquaculture systems are evaluated to hold the greatest potential for expansion and development and have since the late 1980s been considered the main driver of growth in the total seafood production worldwide (FAO et al., 2021).

Mariculture, or coastal aquaculture, is the cultivation of aquatic animals and plant organisms in seawater or brackish water. Despite a great diversity of farmed mariculture species, the Atlantic salmon (*Salmo salar* Linnaeus, 1758) has established a leading role as the dominative cultivation species, farmed in open sea-based cages (FAO, 2022). Stated possibilities of an increase in mariculture production has led to a prominent position of mariculture for future sustainable food production (Poore & Nemecek, 2018; Costello et al., 2020; Clawson et al., 2022). However, environmental concerns regarding open net-pen aquaculture production, the leading method for producing Atlantic salmon, have arisen over the past decades (Belle & Nash, 2008; Olaussen, 2018). Finding suitable methods of evaluating and mitigating environmental concerns and implementing necessary managemental tools (Holmer et al., 2008; Samuel-Fitwi et al., 2012), is therefore a prerequisite towards a future sustainable mariculture industry.

## 1.1 Norwegian aquaculture industry

From the early 1970s, the Norwegian aquaculture industry has experienced an outstanding development and growth. It has evolved from a few pioneers striving to make small-scale farming successful, to a massive worldwide industry producing more than half of the worlds farmed Atlantic salmon (Taranger et al., 2015). The long, sheltered coast of Norway has proven to be well fitted for extensive production of salmonids in open sea-based cages, with farming localities currently distributed along the entire coastline (Grefsrud et al., 2023). This has led to Norwegian aquaculture industry constituting a leading role, as the current largest producer of farmed Atlantic salmon worldwide (FAO, 2022). Despite an increase in aquaculture production worldwide (FAO, 2022), the Norwegian aquaculture production has experienced a stagnation over the past decade (The Norwegian Directorate of Fisheries, 2023a). Sustainability issues have developed in line with the expansion, and several bottlenecks are currently preventing further green growth of the industry (Grefsrud et al., 2021). Environmental and economic challenges related to high mortality, infectious disease, parasites, and impaired fish health are severe issues that require solutions to

enable further sustainable growth (Sommserset et al., 2023). Especially important is controlling the infestation of ectoparasitic sea lice (*Caligidae*), a major health hazard for both farmed and wild salmonids in northern waters (Overton et al., 2019).

## 1.2 Sea lice biology and ecology

The two genera *Lepeophteirus* and *Caligus* are common ectoparasitic copepods of the family Caligidae, that parasitize anadromous and marine fish. *Lepeophteirus* and *Caligus* include around 162 and 268 species respectively, whereas the two species *Lepeophteirus salmonis* (Krøyer, 1837) and *Caligus elongatus* (Nordmann, 1832) are constituting the greatest parasitic impact on both farmed and wild salmonids in northern marine waters (Vollset et al., 2018; Thorstad et al., 2022). *C. elongatus* and *L. salmonis*, hereafter referred to as sea lice, are naturally occurring in the northern hemisphere, feeding on mucus, skin, and blood of a host (Pike & Wadsworth, 1999; Frazer et al., 2012). *L. salmonis* is a host-specific parasite, only infesting salmonids, while *C. elongatus* is classified as a generalist, proven to possibly target at least 80 species of fish (Wootten et al., 1982; Kabata, 1992).

In line with the expansion of intensive aquaculture production in open pens at sea, the number of potential hosts for sea lice to parasite has increased, inducing unnaturally high lice infestations levels (Frazer et al., 2012; Johnsen et al., 2016). This development has led to sea lice costing the Norwegian aquaculture industry billions of NOK annually, both in direct costs for preventing and combatting the parasite, as well as with indirect cost related to reduced growth, quality, early harvest, and a series of health and welfare issues (Bowers et al., 2000; Abolofia et al., 2017). Biological issues with sea lice infestations and outbreak of disease are connected and are currently considered one of the main sources of expenditure and biological risk for the industry (Iversen et al., 2017; Misund, 2022).

In addition to causing great economic expenses, sea lice are considered the greatest current parasitical hazard to fish health and welfare, due to the severe negative consequences of infestations on both farmed and wild fish stocks (Forseth et al., 2017; Thorstad et al., 2022; Sommserset et al., 2023). Empirical studies have classified salmon lice (*L. salmonis*) specifically as one of the two largest current threats to the wild salmon population in Norway (Forseth et al., 2017; Bøhn et al., 2020). Sea lice feeding on fish may cause direct abrasion skin, risks of anaemia and disrupted osmoregulation of the host, as well as possibilities of outbreaks of secondary infections (Revie et al., 2002; Barker et al., 2019). Infestations may also negatively affect growth, fecundity, and overall fitness, and could in worst case lead to euthanasia of fish (Grimnes & Jakobsen, 1996; Noble et al., 2018; Overton et al., 2019)

### 1.2.1 Life cycle and development

Sea lice have direct life cycles, needing only one host to complete their full life cycle, from hatched egg to adult. The reproduction of sea lice is sexual, with high fecundity and short generation times (Groner et al., 2014). Each adult female sea lice develop eggs in two egg strings, containing approximately 50 - 1000 eggs per string (Costello, 1993; Brooker et al., 2018). Adult females may produce up to eleven pairs of egg strings throughout a lifetime, dependent on environmental conditions, age, and overall condition of the lice (Heuch et al., 2000; Brooker et al., 2018).

The life cycles of *L. salmonis* and *C. elongatus* are quite similar, with eight developmental stages, all separated by a molt (Hamre et al., 2013). The first two stages of their life cycle consist of free-living planktonic nauplius I and nauplius II stages. These are newly hatched sea lice larvae drifting passively in water without feeding. Depending on temperature, the nauplii molts into an infective copepodid stage, dependent on locating and attaching to a host. The last five stages are parasitic and differ between the species. *L. salmonis* develops from the copepodid phase into two attached chalimus stages, two preadult stages and ends with an adult phase. For *C. elongatus* there is no preadult stage, but four chalimus phases and one final adult stage (Hamre et al., 2013).

### 1.2.2 Behavior and distribution of larvae

In the fluctuating marine environment, copepodid sea lice are dependent on the ability of locating a host for survival (Costello, 2006; Brooker et al., 2018). In the first three planktonic stages, sea lice may scatter with currents over long distances, with a potential horizontal dispersal of 10-50 km (Costello, 2006; Samsing et al., 2017). It is believed that planktonic stages of sea lice are the most important stages for transmission between aquaculture farms, and between farms and wild fish, even though pre-adult and adult lice have the ability to swim in the water column (Amundrud & Murray, 2009). Planktonic sea lice larvae have been documented spending several weeks in its planktonic stages, drifting in the currents of the upper water masses, in search of a suitable host (Stien et al., 2005; Groner et al., 2019). The success rate of this is, however, highly dependent upon host densities, as well as the hydrodynamic conditions of the area (Johnsen et al., 2016; Cantrell et al., 2018).

In addition, several adaptive behavioural traits by using hydrodynamic, physical, and chemical cues, such as changes in light, salinity, pressure, vibration, or chemicals, are thought to play a crucial role of the sea lice larvae direct movement, and hence ability to seek environments where possible hosts are situated (Mordue & Birkett, 2009). With the use of sensillae, pores and antennules, their primary sensory interface, copepodids have a series of sensory structures to detect physical, as well as chemical signals (Bron et al., 1993). Swimming hosts passing sea lice, producing currents, are an example of such

mechanical cues working as a guidance for copepodids to locate possible hosts (Mordue & Birkett, 2009). Another possible facilitating strategy for finding hosts is *L. salmonis* strong diel vertical migration in response to light, migrating upwards towards light and the surface at daytime, while sinking to deeper levels at night. At the same time, salmon smolt migrates in the opposite direction, increasing host-parasite encounters (Heuch et al., 1995). For *C. elongatus* it is suggested that they do not inhibit such strong vertical migration, possibly due to their ability to inhibit a wider habitat as teleost generalists (á Norði et al., 2015). In addition to behaviour based on physical conditions, sea lice larvae may detect and respond to chemical stimuli, such as odors from mucus and blood from fish. Copepodids then responds with "burst swimming", an expected adaptive behaviour for locating hosts (Bron et al., 1993).

Sea lice larvae have a patchy distribution, normally found in quite low densities in the water column (Pike & Wadsworth, 1999). Copepodids are in general found located at or close to the surface of sea water, while nauplii, with more limited swimming and sensory abilities, are thought to be located deeper than copepodids (Penston et al., 2008; Amundrud & Murray, 2009). While there is evidence that sea lice are sourced from and remain near salmon farms, there is also evidence that planktonic sea lice might be able to be distributed several kilometres from a farm (Costelloe et al., 1996; McKibben & Hay, 2004; Penston et al., 2004). Planktonic sampling has revealed a direct connection between sea lice larvae and abundance of planktonic larvae, through documenting a rapid decrease in densities of nauplii with increased distance from aquaculture facilitates (Costelloe et al., 1998; Penston et al., 2004).

### 1.2.3 Effects of abiotic factors

Sea lice are stenohaline organisms, with optimal survival and development in waters with a salinity greater than 27 ‰ (Ljungfeldt et al., 2017). Copepodids have shown to avoid salinities below 27‰, with a compromised survival of copepods below 29 ‰ (Bricknell et al., 2006). Although sea lice survival is strongly dependent on a higher salinity, with a narrow toleration for fluctuations in salinity, planktonic sea lice larvae have been registered congregating in areas with lower salinity, such as in mouth of estuaries during salmon smolt migration in the spring (Costelloe et al., 1998; McKibben & Hay, 2004). This requires an ability to avoid or tolerate lower salinity seawater, as reduced salinity might affect behaviour, fecundity and survival, and their diel vertical migration are thought to be an adaptation in favour of this (Blaylock & Bullard, 2014). *C. elongatus* holds a lower tolerance of a reduction in salinity than *L. salmonis*, where a reduction of salinity has a greater effect on the abundance of *C. elongatus* than a reduction in temperature (Heuch et al., 2007).

Water temperature regulates development, dispersal, and the reproductive output of sea lice (Samsing et al., 2016). As water temperature rise or sink, the sea lice life cycles respond by becoming shorter or longer, respectively (Hamza et al., 2014). In this way,

development and growth of sea lice are highly dependent on temperature. Studies has shown that female lice become adult at 13 days at 21 degrees, versus 72 days at 6 degrees (Hamre et al., 2019). The copepodid can survive without a host for up to 13 days in low temperatures, but the duration of their existence in the planktonic stages is directly influenced by the energy content of the yolk, the consumption of which is highly temperature dependent (Samsing et al., 2016). In warmer water nauplii will develop faster to the infective copepodid stage, but the infective window would be shorter as they consume their yolk sac faster (Pike & Wadsworth, 1999; Tucker et al., 2000).

A seasonal change in the abundance of *C. elongatus* and *L. salmonis* has been documented in previous studies. While *C. elongatus* have been documented to be more dominant during winter months (á Norði et al., 2015), higher densities of *L. salmonis* have been shown in warmer water temperatures as found during summer months (Helland et al., 2015; Hamre et al., 2019). In addition, studies have shown a pattern of inverse correlation of abundance between the *C. elongatus* and *L. salmonis* throughout the year (Revie et al., 2002; á Norði et al., 2015)

## 1.3 The traffic light system (TLS)

To promote a sustainable and predictable growth of the Norwegian aquaculture industry, the Norwegian government, the Ministry of Trade, Industry and Fisheries (MTIF), implemented a production area regulation system called the Traffic Light system (TLS) in 2017 (Karlsen et al., 2016). This indicator-based spatial management system was established for regulating production capacity in Norwegian salmonid aquaculture, through dividing the Norwegian coast into 13 production areas (PA) (Figure B.1, Appendix B) (MTIF, 2017; Sønvisen & Vik, 2021).

The government identified environmental impacts as the primary determinant for the potential of growth in a production area (Nilsen et al., 2017; MTIF, 2017). Among these impacts, effects of salmon lice on wild salmonid stocks were considered one of the most critical limitations for responsible growth of the industry (MTIF, (2014–2015)). Therefore, salmon lice serve as a key sustainability and welfare indicator in the TLS, directly impacting the growth of the aquaculture sector, by determining the maximum allowable biomass (MAB) in each PA. Every other year, each PA is assigned a colour indicating one of three actions: potential of 6 % increase in MAB (green), maintaining current MAB (yellow), or requirements of a 6% reduction in MAB (red). This classification is based on the estimated sea-lice induced mortality rate on wild salmonid smolts in a PA, as the presence of sea lice and its negative effects on wild salmonid stocks is evaluated to be linked to aquaculture production levels (Tveterås et al., 2020).

For evaluating the situation in each PA, the Ministry of Trade, Industry and Fisheries (MTIF) and the Norwegian Food Safety Authorities (NFSA), appointed the Institute of Marine Research (IMR), in collaboration with the Norwegian Veterinary Institute (VI) and the Norwegian Institute for Nature Research (NINA), to establish an expert group for providing annual risk assessments of impacts of sea lice on wild salmonids along the coast (Nilsen et al., 2017; Vollset et al., 2022). These risk assessments are built upon several components, for providing a thorough area evaluation. Firstly, predictions of dispersal of sea lice based on reported lice levels from aquaculture farms, temperature and water currents are evaluated through hydrodynamic dispersion models (Larsen & Vormedal, 2021). Secondly, a national surveillance program for salmon lice on wild salmonids (NALO) provides field data to help validate the model estimations (Nilsen et al., 2019). Based on these assessments, the expert group provides recommendations to the MTIF once every second year, where areas are evaluated with <10% (green), 10-30% (yellow) or >30% (red) sea lice induced mortality. This therefore serves as a primary foundation for the government's determination of assigning either green, yellow or red "traffic lights" for each PA along the Norwegian coast (MTIF, 2017).

### 1.3.1 Sea lice monitoring

To validate estimation models of sea lice levels along the Norwegian coast, the NALO program incorporates various field monitoring efforts aimed for estimating presence and impact of salmon lice on wild salmonids. The following methods, described in Nilsen et al., (2022), comprise the total field monitoring methodologies implemented in the annual NALO surveillance program. Firstly, collection and lice counting of fyke-net- and gillnet-caught trout (*Salmo trutta*) and Arctic charr (*Salvelinus alpinus*) are conducted. Fyke-nets are placed 30-50 meters from a shoreline at depths of 1-2 meters. Routine check-ups with lice counting at least once every 24 hours are implemented, after which the captured fish are released. Multiple gillnets are deployed from the shoreline, extending 30 meters into the fjord, with regular inspections where captured fish are euthanized before lice counting is performed. Secondly, pelagic trawling is conducted in outer parts of larger fjord systems, capturing migrating postsmolt salmonids for registration of lice infestations and other relevant parameters, before the smolt is euthanized. Lastly, sentinel cages with a volume of 1 $m^3$ are submerged 1-2 m below sea surface, holding approximately 30 domesticated Atlantic salmon smolt. These cages are placed in selected monitoring fjords, for assessing lice infestations over a two-week period.

All presented methodologies involve the use of live salmonid fish, where all methods except fyke-nets end with euthanasia of fish. Each of the 13 production areas undergoes assessment using one or several of these monitoring methods within the NALO program annually. These assessments provide crucial knowledge regarding the presence and distribution of salmon lice concentrations along the Norwegian coast, as an integrated part

of the TLS system, in efforts of ensuring a sustainable growth and management of the aquaculture industry in Norway today (Nilsen et al., 2022).

## 1.4 DNA-based quantification

Molecular methods, particularly DNA-based identification techniques, have in recent years been acknowledged as a rapid and accurate methodology to quantify distribution and abundances of planktonic sea lice (Bui et al., 2021). This approach involves species-specific assays and droplet-digital-PCR (ddPCR) in combination, enabling the estimation of number of sea lice DNA-copies within a collected sample (McBeath et al., 2006; Hindson et al., 2011). In contrast to the conventional microscopy, the method has demonstrated its ability to reduce operator dependency and lowering costs, providing a faster and more efficient way of processing large number of samples, independent of sample density (Bui et al., 2021).

The approach has previously been developed and utilized within fish farms, quantifying presence of both *L. salmonis* and *C. elongatus*, and has been validated and benchmarked against other methodologies in a pilot project (LICETECH, NRC-number 254718). Experimental tests involving calibrating the DNA-based quantification of sea lice larvae in relation to other methods, have yielded promising results. The ddPCR methodology demonstrated an accuracy of 85% and exhibited a high precision level (Bui et al., 2021). Consequently, ddPCR has been proposed as a viable solution for quantifying sea lice within samples, and as a tool for studying the dispersal, behaviour, and biology of sea lice in their larval stages (Brooker et al., 2018; Bui et al., 2021). The methodology is expected to provide precise quantification of sea lice abundance within samples, offering a potential validation of the NALO monitoring methodologies and the particle dispersal model forming the basis of TLS (Brooker et al., 2018; Bui et al., 2021). However, it is important to note that the methodology has yet to be tested in combination with large-volume planktonic samples from coastal conditions in fjordsystems.

## 1.5 Aim of study

The project *LiceQuest* aims to develop and test semi-automated sampling and DNA-based quantification technology in combination, as a tool for assessing pelagic sea lice densities in fjords and coastal waters. Through the project, high volume plankton samples coupled with sensitive DNA-methodology will be tested to quantify abundance and dispersal of planktonic sea lice. This technology might provide data applicable for validating dispersal models currently implemented in the TLS system, without capturing, handling, or euthanizing any live fish. In addition, the methodology has been evaluated to be more cost efficient, holding improved efficiency compared to existing methods, and allowing sea lice estimations over larger areas than what is currently achievable.

This master thesis aimed at contributing to the project *LiceQuest* and its goals. The overall aim of this study was to investigate the ability of a passive plankton sampler (PPS), developed by NTNU and NINA, to function as an instrument for measuring densities of planktonic sea lice in the water column. Firstly, by testing the PPS in several locations in the Hardangerfjord, Vestland, western Norway, through collecting planktonic samples and monitoring abiotic factors over a one-month period. Secondly, through analysing presence of *C. elongatus* and *L. salmonis* using DNA-based quantification within the collected samples. On this basis, the present study aimed to investigate the following hypotheses:

1. A passive plankton sampler, combined with DNA-based quantification, can function as an operative method for collecting and analysing levels of *C. elongatus* & *L. salmonis* in marine waters.
   1.1. A higher number of *L. salmonis* than *C. elongatus* will be collected and analysed.
   1.2. A high reliability of both the PPS and laboratory method will be observed.

2. Collection and concentration of *C. elongatus* & *L. salmonis* will vary between investigated i) study areas and ii) localities.
   2.1. Collected levels of *L. salmonis* correlate with model estimated concentrations.
   2.2. Abundance of sea lice are affected by i) sea temperature and ii) salinity within study areas.

# 2 Materials and methods

## 2.1 Study areas

From the 10th of May to the 10th of June 2022, the present study was conducted in two fjord systems in Hardanger, in Vestland county, western Norway (Figure 1). The fjord systems are a part of the middle and outer parts of the Hardangerfjord, located at the southwest coast of Norway. In this study, the two fjord systems were divided into northern and southern study areas, based on their location in the larger Hardangerfjord. The study areas of the project were mainly chosen based on the Hardangerfjords central position in the NALO-program, with the southern study area set as a focus area, in addition to the annual assessment of sea lice levels in the Hardangerfjord system (Nilsen et al., 2022). Other decisive factors were the complexity and variety of the Hardangerfjord, with a high level of aquaculture sites, a protected salmon fjord, and fluctuating oceanographic characteristics. This was crucial qualities of the area to be able to answer the given research hypotheses of the study.

### 2.1.1 The Hardangerfjord

The Hardangerfjord is Norway's second longest fjord, with a total length of approx. 183-km. The fjord stretches in a north-easterly direction between Halsnøy and Huglo in the west, to Odda and Eidsfjord in the east (Figure 1) (Azad et al., 2019; Thorsnæs, 2021). The main fjord consists of four basins, separated by shallower sills. The water depths range from approx. 120 to 800 m, increasing inwards in the fjord (Azad et al., 2019), with a fjord width ranging from approximately 2 to 7 km (Dalsøren et al., 2020).

The fjord consists of several fjord branches and freshwater outlets, forming a complex hydrography in the area (Asplin et al., 2014). Water temperatures varies greatly throughout the year, ranging between ~5 °C to >20°C from March to December (Johnsen, 2011; Asplin et al., 2011). A variety of circulation processes in the fjord system causes transport and mixing of the water masses, from freshwater inputs, tides, local winds, and external fluctuations in stratification on the coastal shelf (Dalsøren et al., 2020). The high number of river outlets affects both salinity and temperature of water masses, creating areas with brackish surface water. The level of freshwater flux discharging to the fjord varies with year, season and on a day-to-day basis (Sjøtun et al., 2015). However, fluxes tend to peak during spring and melting season, where significant freshwater flux stratifies water masses. During this time, a brackish surface layer is created throughout the fjord (Azad et al., 2019). Local tides affect the water column, possibly driving barotropic and baroclinic exchange in the area (Dalsøren et al., 2020). In the outer parts of the fjord system is the dominant wind direction north-south, whereas the wind tends to follow up- and downwind directions in the mid-inner parts of the fjord affecting strength and direction

of currents (Mayer et al., 2020). Currents vary greatly in the fjord, with several forcing mechanisms affecting time, space, and strength of currents (Asplin et al., 2014).

The middle and outer parts of the Hardangerfjord composes one of the densest farming areas of salmonid fish in Norway (Dalsøren et al., 2020). The Hardangerfjord is situated in production area 3 (PA3) in the TLS (Figure 2), reaching from Karmøy in the south, to Sotra in the north. In 2022, 125 active on-growing salmonid aquaculture sites were registered in PA3, with a total production capacity (monthly standing biomass) of >90 000 metric tonnes (Barentswatch, 2023; Norwegian Directorate of Fisheries, 2023). However, several environmental issues are currently limiting sustainable aquaculture production in the area (Grefsrud et al., 2023). PA3 were therefore classified as a red production zone, from 2022 to 2024, with demands of 6% reduction in total production capacity within the area (MTIF, 2022). In addition to being a hotspot for salmonid aquaculture, the Hardangerfjord constitutes an important migration route for wild salmonid stocks (Bøhn et al., 2020). PO3 contains a total of 13 anadromous salmon rivers, including one official national salmon river located in the study area (Figure 2) (Ugedal et al., 2022). Ten of these rivers are situated in the Hardangerfjord system, with a total theoretical smolt production (number of smolt) of ~120 000 smolt (Ugedal et al., 2022).

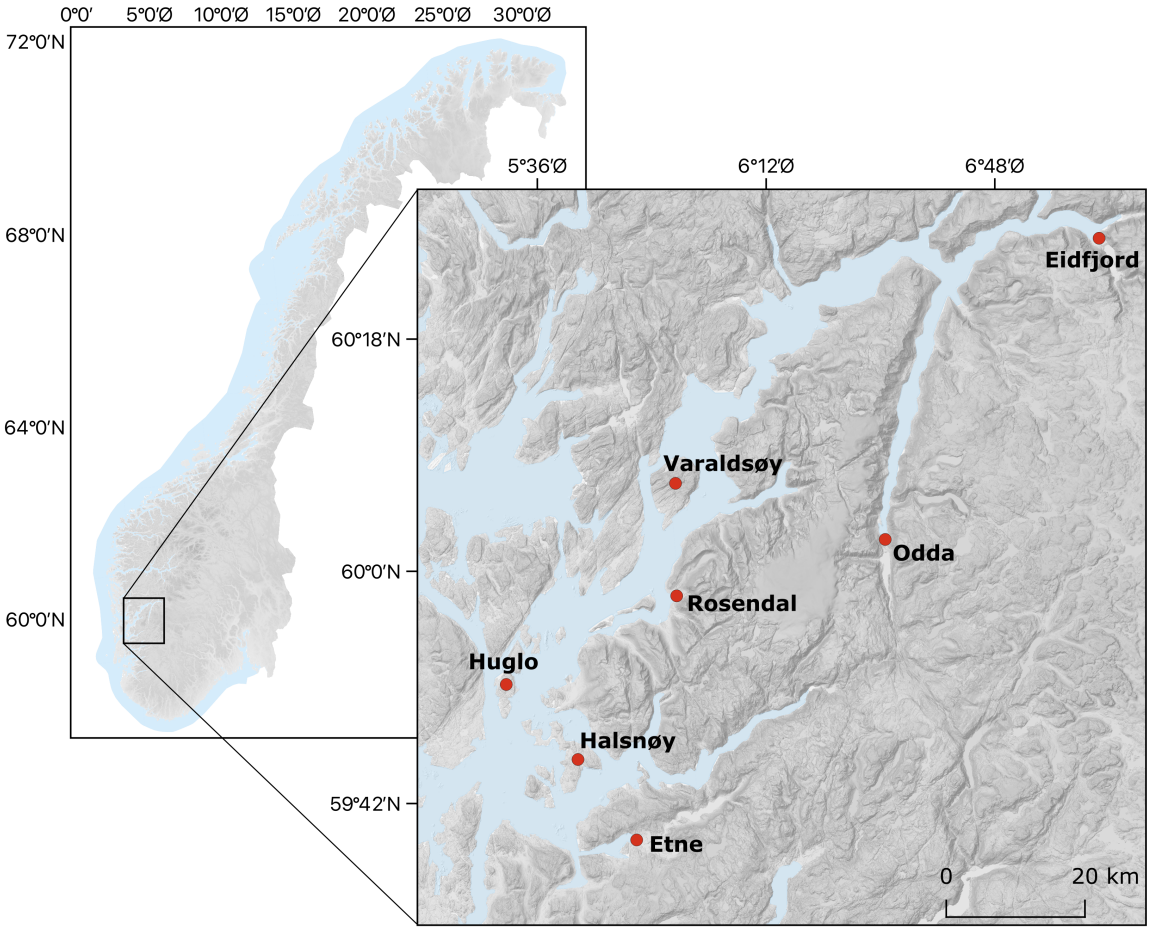


**Figure 1:** The Hardangerfjord in Vestland county, western Norway. Including relevant townships illustrated by red circles, and the fjords geographical position in Norway. Map created in QGIS (QGIS Development Team, 2022). Data retrieved from GeoNorge (2023).

### 2.1.2 Southern study area

The southern study area consists of Melen, Halsnøyfjorden and Etnefjorden (Figure 2). Melen is a short piece of the fjord, reaching 2 km in between Bjoafjorden in the west and Skåneviksfjorden in the east. Halsnøyfjorden is a 13 km long continuation of Bømlafjorden, transferring into Husnesfjorden. Several aquaculture sites are situated in Melen and Halsnøyfjorden, illustrated in Figure 2. Etnefjorden is a sidearm of Ølsfjorden and Hardangerfjorden, in Etne municipality, reaching 8,5 km east to Etnesjøen. In the inner most parts of Etnesjøen drains Etne river, an anadromous, protected national salmon river, with great ecological, industrial, and cultural importance (St.prp.nr.32, 2006-2007; Bjørn et al., 2009). Due to its protected classification, there are no aquaculture sites present in the fjord (Figure 2). The Etnefjord is one of five focus areas with continuous sea lice monitoring over several weeks, as a part of the NALO program (Nilsen et al., 2019). Etnefjorden holds several spawning and nursery areas for wild salmonid fish stocks and is classified as a national salmon fjord (Hordaland-fylkeskommune, 2017).

### 2.1.3 Northern study area

The northern study area comprises Kvinnheradsfjorden and Øynefjorden, located in the outer parts of the Hardangerfjord (Figure 2). Kvinnheradsfjorden is a continuation of Husnesfjorden, in Kvinnherad municipality, outside Rosendal township. The fjord stretches 21 km northeast to the island Varaldsøy, where Kvinnheradsfjorden ends by splitting into Øynefjorden (western fjordarm) and Sildafjorden (eastern fjordarm) (Dannevig et al., 2019; Økland & Todt, 2021). Øynefjorden reaches 11,5 km north, to Kvam municipality, containing 4 aquaculture sites (Figure 2). The study area in total contains several freshwater runoffs, as well as several aquaculture sites as illustrated in Figure 2.

### 2.1.4 Sampling localities

The fieldwork was conducted at six localities in the northern and southern study area (Table A.1, Appendix A), illustrated in Figure 2. The starting points of the northern and southern study area were situated in Rosendal and Etne, respectively. For the northern study area, three localities were selected as specific sampling points: M4, M6 & M7. As for the southern study area, the following localities were selected: M15, M11 & M12. A total of 12 passive plankton samplers were set out, with 2 samplers (A/B) at each locality, providing duplicates. The specific sampling localities in the study areas were selected based on the locations of sentinel cages in the fjordsystem. Hence, all samplers in the study were positioned close to a postsmolt cage, enabling comparisons between the methodologies.

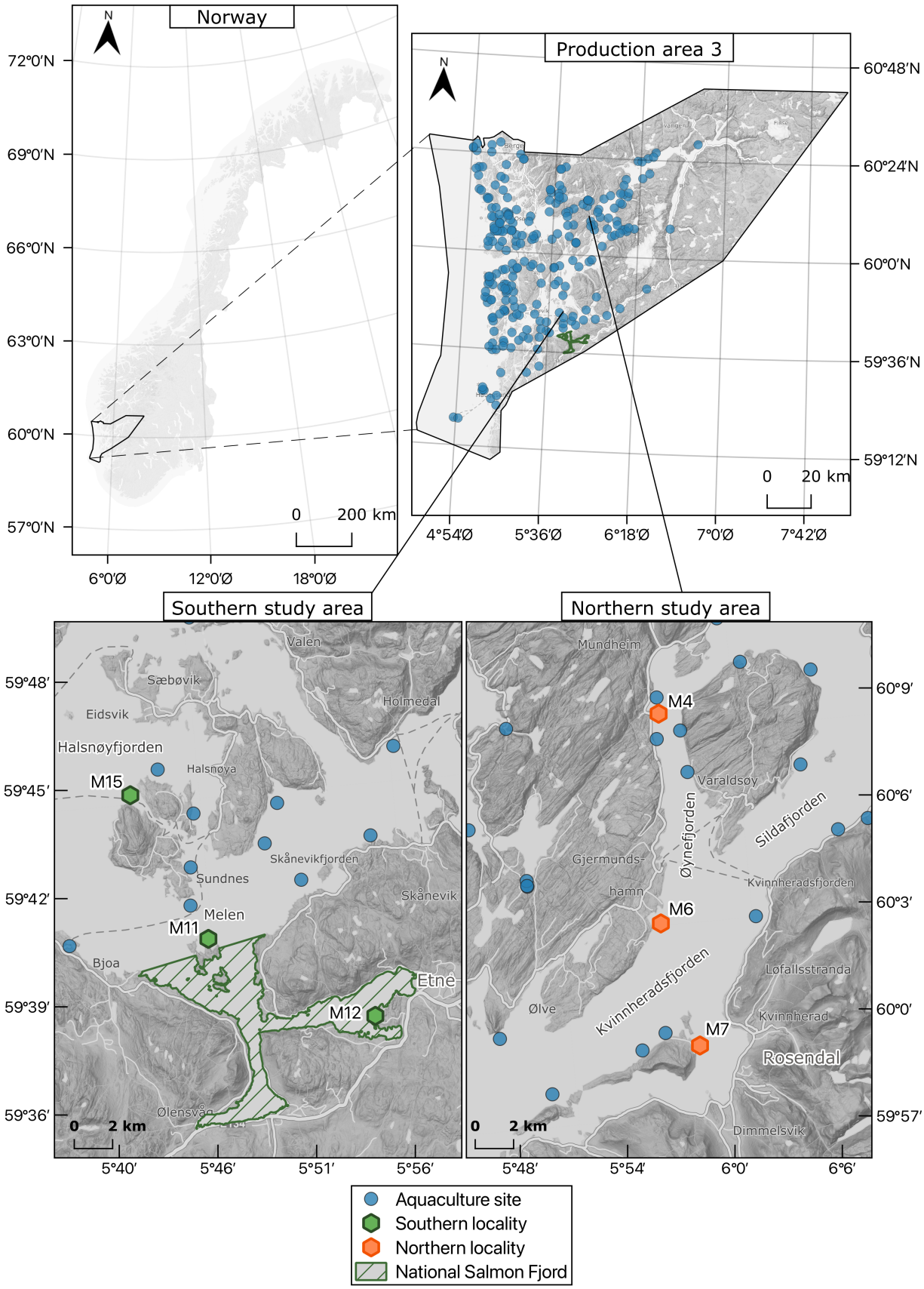


**Figure 2:** Map of Norway including Production Area 3 and study areas within. Active aquaculture sites (2023) (blue circles), National Salmon Fjord (Etnefjorden) (green shaded area) and Southern & Northern study localities (green & orange hexagons) with relevant fjord names illustrated. Map created in QGIS version 3.30.2 (QGIS Development Team, 2023). Data retrieved from GeoNorge (2023) & The Norwegian Directorate of Fisheries (2023b).

## 2.2 Planktonic sampling

### 2.2.1 Passive plankton sampler

Plankton samples were collected with a passive plankton sampler (PPS), designed, and produced by NINA, NTNU and M-Tech (M-Tech, Trondheim, NOR) in collaboration (Figure 3). The PPS was equipped with two round floating tubes at the top for stability in the water. Two small buoys were added to the PPS to strengthen its visibility when submerged in water. Two steel threaded rods were placed vertically down to a plankton tube (98 cm L, 25 cm D). A selection grid (25 cm D) was placed in front of the plankton tube for filtration of small planktonic species passing with currents. This construction led to a collection of plankton at around 65 cm depth, measured from centre of floating tubes to centre of plankton tube. A zooplankton net (135 cm L) with a mesh size of 125 $\mu$m was connected at the end of the plankton tube. A cod end (12 cm D) was placed at the end of the zooplankton net to gather filtered plankton. A digital flow meter was installed in the mouth of the PPS, connected to an electronics box with a battery, wires and a screen, recording volume of water filtered per sampling interval.

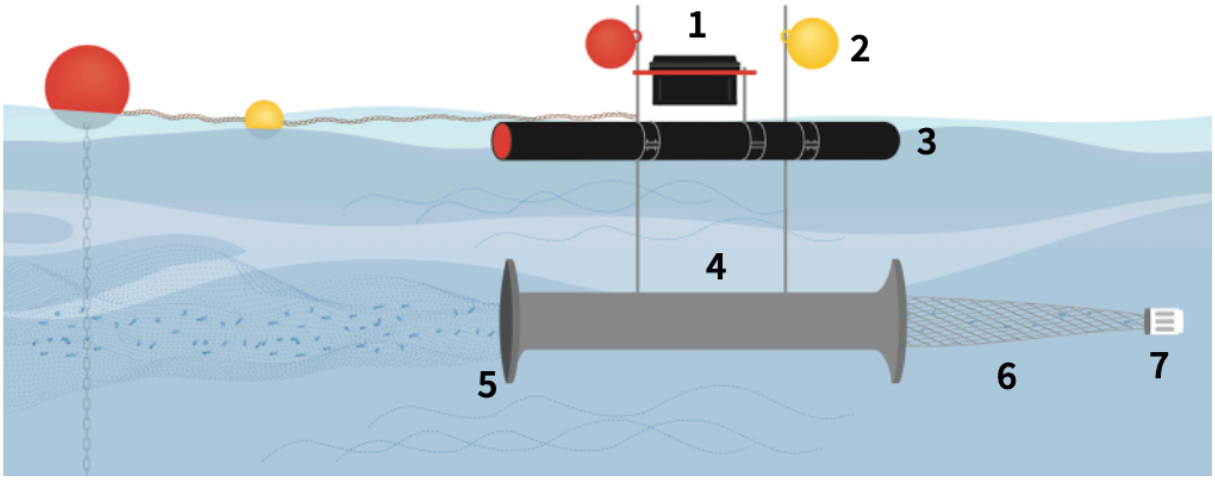


**Figure 3:** Overview of a passive plankton sampler submerged in the water column, connected to a mooring system with two buoys for visibility in water. Components of the passive plankton sampler are presented by numbers: **1.** Electronics box; **2**. Buoys; **3.** Floating tubes; **4.** Plankton tube; **5.** Prefilter & flow meter; **6.** Zooplankton net; **7.** Cod end. Illustration: Astrid Strømmen / NTNU Graphic Centre.

Prior deployment of the PPS, anchoring connected to two buoys were deployed at each site (Figure 3). The PPS were then placed at the selected localities (Table A.1, Appendix A), connected to buoys allowing the PPS to float freely with the natural direction of the currents. The PPS were deployed and kept at sea for the entire duration of the sampling period (see Table A.2, Appendix A for exceptions). Inspection and collection of samples from the individual sites were performed with an interval of 3-6 days, with separate

sampling days for northern and southern study area (Table A.3, Appendix A). An integrated standard procedure for collecting samples from localities was implemented. This included sampling at sites from furthest to closest distance from the respective starting positions (Table A.1, Appendix A).

### 2.2.2 Sampling procedure

The planktonic samples collected in this study were obtained with a standard sampling procedure. Samples were collected by first retrieving a PPS from sea at a given study site. The PPS was detached from buoys and brought into the boat (Figure 4, [A]). The plankton net was first detached from the PPS, before the net was flushed down with sea water using a spraying hose (Figure 4, [B]). The collected plankton material in the cod end was filtered through a 150 µm mesh cup, before any remaining material in the cod end were sprayed with saltwater into the mesh cup. The flushing procedures of plankton net and cod end was included as a procedure to ensure collection of all possible lice larvae collected by the PPS. The material in the mesh cup was then sprayed with 96% ethanol and transferred to 50- or 250-ml polypropylene tubes dependent on sample size. The samples were lastly preserved with 96% ethanol on site, and marked by location, site, and date of sampling. After preservation, samples were temporarily stored in a cooling box, transported by boat to the mainland and kept in a -20°C freezer at the field station during the sampling period.

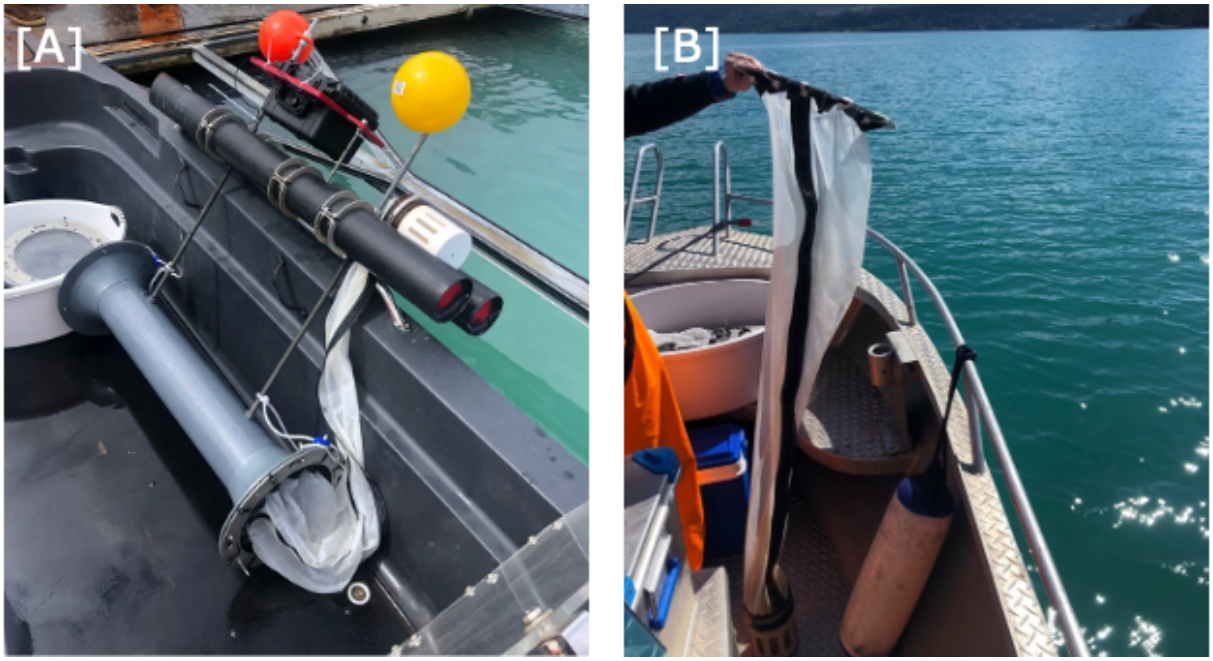


**Figure 4:** [A] Passive plankton sampler retrieved from fjord for sampling. [B] Spraying of net with sea water as a part of the sampling procedure, collecting planktonic samples. Photographs by Ingvild Tryggestad (NTNU).

## 2.3 Laboratory analysis

All collected plankton samples were transported to NINA Trondheim for laboratory analysis at the end of the study period in June 2022. Here, the samples were stored in cooling facilities, at -20 °C, until start of laboratory work in November 2022. The plankton samples were analysed by the Centre for Biodiversity Genetics (NINAGEN) at NINA, with the use of the following protocol.

### 2.3.1 Laboratory protocol

The planktonic material preserved with ethanol were first homogenized in 250-mL or 50-mL tubes, dependent on total plankton volume in a sample. Homogenization was performed using a FastPrep-96 homogenizer at 1600 rpm for 2 minutes (MP Biomedicals, CA, US), with an added mix of Matrix-A and Matrix-D beads (MP Biomedicals, CA, US). From each sample, three subsamples of 500 µl were retrieved and transferred to three 1.5 mL Eppendorf tubes. Subsamples were then dried in a heating cabinet at 56°C. 540 µL ATL-buffer (Qiagen, Hilden, DE) and 60 µl proteinase K (Qiagen, Hilden, DE) were then added to each tube, thereafter vortexed and incubated at 56°C overnight. Subsequently were a MagMax Tissue 2.0 kit (ThermoFisher, MA, USA) eluted in 250 µl AE buffer (Qiagen, Hilden, DE) used on a KingFisher Apex robot (ThermoFisher, MA, USA), for extracting DNA from each of the subsamples. Determination of DNA concentration of *L. salmonis* and *C. elongatus* were conducted by the use of species-specific assays, together with droplet digital PCR (ddPCR) (QX200 AutoDG Droplet Digital PCR System, Bio-Rad Laboratories, CA, US) (McBeath et al., 2006). The PCR included 3.64 µM of forward and reverse primers, 0.86 µM probe, dH2O, 10 µL ddPCR™ Supermix for Probes and 1 µL DNA template, in a total reaction volume of 22 µL. For each ddPCR run, DNA isolated from *L. salmonis* and *C. elongatus* copepodids was used as positive control, and dH2O as negative control. For generating droplets were an AutoDG Instrument (Bio-Rad Laboratories, CA, US) used, and PCR amplification was performed in a Veriti 96-Well Thermal Cycler (Applied Biosystems, MA, US). The following thermal cycling conditions were used in the analysis: an initial denaturation step at 95°C for 10 min, 40 cycles of denaturation at 95°C for 30 s, annealing and extension at 60°C for 1 min, a final step of denaturation at 98°C for 10 min and a final hold at 4°C. Automatic detection of the fluorescent signal in the droplets were performed by transferring the PCR plates to a QX200 Droplet Reader (Bio-Rad Laboratories, CA, US). With the use of the software QX Manager Standard Edition v1.2 (Bio-Rad Laboratories, CA, US), positive droplets were separated from negative, according to the manufacturer's instructions. Calculation of the total number of DNA copies in a sample was conducted based on the concentration estimated by QX Manager, template volume (1 µL), elution volume (250 µL), subsampled volume (500 µl) and total lysis volume (3.500 µL for 50 mL tubes, and 175.000 µL for 250 mL bottles). The number of larvae was then estimated by dividing the total number of DNA-copies by 1.5 million DNA-copies, which is the estimated average DNA-copy number per lice (unpublished findings, NINA).

## 2.4 Estimation of salmon copepodids

Estimations of salmon copepodites in northern and southern study area were provided by The Institute of Marine Research (IMR). This is based on IMRs national operational model, developed for estimating the distribution and numbers of infective salmon lice larvae (copepodites) along the Norwegian coast (Sandvik et al., 2020). The model system is based on hydrodynamics, calculated number of salmon lice reported from active aquaculture facilities, combined with knowledge of larvae behaviour and distribution with currents (Sandvik et al., 2019). This constitutes a salmon lice dispersion model, with high resolution in time and space, enabling estimations of variation in lice distribution along the fjord axis (Myksvoll et al., 2018; Sandvik et al., 2020). Data provided by IMR included the estimated median number of *L. salmonis* copepodites present throughout the water column (down to 20 m depth) per day. The estimates were calculated based on an 8 km * 8 km square area surrounding the sentinel cages located in Rosendal (Northern study area) and Etne (Southern study area) from 1$^{st}$ of May to 30$^{th}$ of June.

## 2.5 Recording of abiotic parameters

Sea temperature (°C) and salinity (‰) were measured at each study site using small-sized Star-Oddi DST CTD loggers (Star-Oddi, Garaðabær, IS). CTD measurements started the 14$^{th}$ of May instead of 10$^{th}$ of May, due to issues with equipment, and this is therefore the applicable study period for the abiotic parameters (Table A.3, Appendix A). A CTD logger was attached to sampler A at each study site, with a total of 6 CTD loggers, providing CTD measurements from all sites. The data loggers were put in self-made protective housing and attached to the bottom of a steel threaded rod of the PPS (see Figure 3), at approx. 0,5 m depth. Recordings were set with an interval of 30 minutes for continuous measurements throughout the study period.

## 2.6 Data treatment and statistical analysis

Statistical analysis and visual presentation of data was performed using R version 4.2.2 (2022-10-31) and R studio (Version 2022.12.0+353) (R Development Core Team, 2022), after treating raw data in Excel (Microsoft® Excel for Mac, Version 16.71). The significance threshold was set at $P < 0.05$ for all statistical analysis. Illustrative figures used for presenting results were made using *ggplot2* package in RStudio, except for repeatability results generated by the *plot* function in base R.

### 2.6.1 Sea lice analysis

Results from sample triplicates from the laboratory analysis provided three individual values for each plankton sample. One large outlying datapoint of a lab triplicate was removed based on visual inspection of the data, likely to represent incomplete crushing of a sample during plankton sample analysis. Mean number of *L. salmonis* and *C. elongatus*

were calculated for each sample, providing one value of number of sea lice collected within a sampling period. Statistical probability of detecting sea lice during the study period were performed using generalized linear models, the *glm* function (binomial family). The presence of *L. salmonis* in individual samples was included as a binomial response variable (presence = 1, absence = 0), while locality, days sampled, and sample date were included as predictor variables. All collected samples analysed for *L. salmonis*, both with and without detected lice, were included in this analysis. A non-parametric Kruskal-Wallis rank sum test, the *kruskal.test* function, was used for analysing variance among study area and localities. This was conducted to determine whether there was a statistically significant difference between the collection of lice between study areas and between localities. Associated pairwise comparisons between the number of collected lice larvae and the different localities were investigated with the non-parametric Wilcoxon rank sum test, with the *pairwise.wilcox.test* function. P-values were adjusted using the Benjamini-Hochberg (BH) method to control the false discovery rate. All collected samples analysed for *L. salmonis*, both with detected lice and without detected lice, were included in both the Kruskal-Wallis rank sum test and the Wilcoxon rank sum test.

### 2.6.2 Repeatability analysis

To quantify the individual reliability of the laboratory measurements and PPS measurements, the R package *rptR* were used, with the *rpt* function (Stoffel et al., 2017, 2020). Through extracting estimates of variance components from mixed effects models, the repeatability was estimated. Uncertainty was quantified through parametric bootstrapping providing the 95% confidence intervals, while P-values were provided by log-likelihood ratio tests. For the laboratory measurements, the number of larvae collected per sample were set as a response variable, while the individual lab samples (Lab sample ID) were used as random effect. For the PPS measurements, number of larvae collected per sample were used as a response variable, and date, location and site combined (e.g., 14.05.22 M11A) were set as random effect. The number of parametric bootstraps and permutation runs were set to 10 000 for both analyses, for achieving stable and accurate results (Kleijnen, 2018). As the response variable represents the number of larvae collected, it was quantified assuming a Poisson error structure (Stoffel et al., 2020). Graphic illustrations of repeatability were made using the *plot* function. All collected samples analysed for *L. salmonis*, both with and without detected lice, were included in reliability analysis for both the PPS and laboratory measurements.

### 2.6.3 Abiotic analysis

Temperature and salinity measurements from CTD recordings were retrieved using SeaStar Application Software (Star-Oddi, Garaðabær, IS). Time recordings for each sampling interval were collected and merged with the abiotic data in Excel for further data treatment. Mean, standard deviation, minimum and maximum level of salinity and temperature in

each sampling period were calculated for all localities. Error in CTD recordings were discovered for locality M12 & M11, based on visual inspection of data, and were removed from the dataset. The error was likely to represent an error in a CTD recorder (M12), combined with horizontal positioning of the PPS and CTD dataloggers during fieldwork (M11) leading to CTD recordings above water. Valid CTD data were merged with PPS data and imported to R studio for further statistical analysis. Impacts of environmental factors on the collection of sea lice larvae were assessed using a generalized linear mixed effects model, the glm function (binomial family). Samples with lice (0=absence of lice, 1 = presence of lice) were set as response variable with mean temperature and mean salinity as predictor variables. For the abiotic glm model, all collected samples analysed for *L. salmonis*, both with and without detected lice, were included. The influence of environmental parameters on collected planktonic sea lice levels was investigated using a linear mixed effects model, the *lm* function, with log-transformed mean larvae as response variable, and mean temperature and mean salinity as predictor variables. The linear mixed model only included samples with presence of *L. salmonis* in the northern study area.

# 3 Results

## 3.1 Collected planktonic sea lice

Of all 77 planktonic samples analysed (following 2.3.1), planktonic sea lice were detected in 43 samples. 34 of the total samples collected and analysed did not contain any detected sea lice larvae.

### 3.1.1 Total collection of *C. elongatus*

Estimations of the total sum of planktonic *C. elongatus* larvae captured by the PPS at both study areas are shown in Figure 5. *C. elongatus* were detected in 10 samples, 8 from locations in southern study area, and 2 from locations in northern study area (Table A.4, Appendix A). The number of *C. elongatus* larvae collected were at a general low level, with only three samples exceeding 1 lice larva in total per sample (Table A.4, Appendix A). The largest sample analysed contained 115 larvae, with an average of 38 larvae per sampling day. Two other samples showed 43.92 and 18.42 larvae present in the samples, with an average of about 11 and 4 larvae per day, respectively. Moreover, the majority of collected samples containing larvae were sampled in the southern area and at the M11 locality, while only 2 out of 10 samples containing *C. elongatus* were collected in localities in northern area. Given the small number of *C. elongatus* larvae in the collected samples, the species was excluded from statistical analysis.

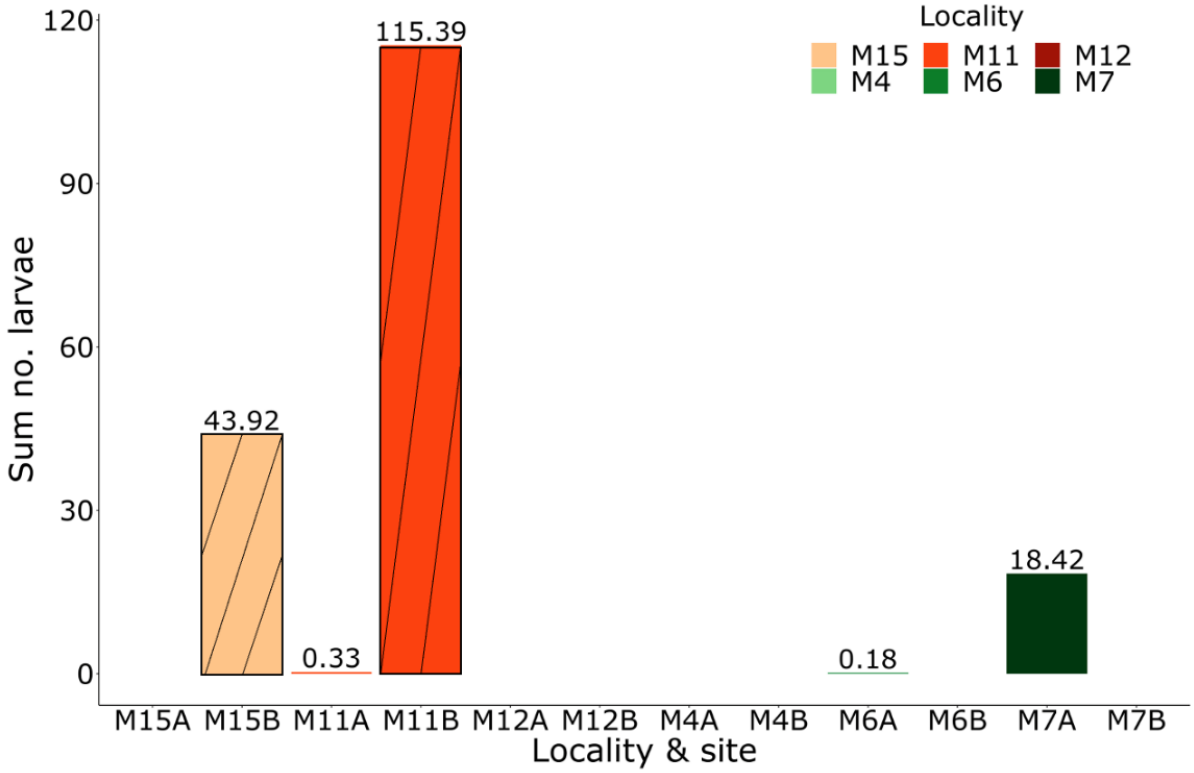


**Figure 5:** Total sum of collected planktonic ***C. elongatus*** larvae from the PPS during the study period (10th of May – 10th of June) from northern and southern study area. Including localities in southern (M15-M12) and northern (M4-M7) study area and study sites A (Colour) & B (Shaded) within all localities.

### 3.1.2 Total collection of *L. salmonis*

Estimations of total sum of planktonic salmon larvae captured showed collection of lice larvae with the use of the PPS at both study areas (Figure 6). *L. salmonis* were detected in 33 samples. 14 of these samples were collected from locations in the southern study area, while 19 samples were collected from northern localities. The PPS succeeded in collecting lice in all but one locality during the study period. However, all other study localities and study sites (A/B) succeeded in collecting planktonic sea lice.

In the southern study area, the PPS collected lice at both sites at M15 and M11, while the two PPS deployed at study locality M12 did not capture any lice larvae during the total study period. Estimations showed that M15 collected around 3 (2.73) lice, while M11 collected 7,5 lice larvae throughout the study period. This resulted in a total of around 10 lice collected from all PPS deployed in the southern study area.

In the northern study area, the PPS collected lice at all study localities and sites. The area showed the highest total number of collected planktonic lice, but with variations within localities and sites. M4 collected the total highest number of lice larvae, with over 14 larvae collected at M4A. A total of 21 lice larvae were collected from M4, while around 6 and 7 lice were collected from M6 and M7, respectively. A total of 34 larvae were collected from all PPS deployed in the northern study area.

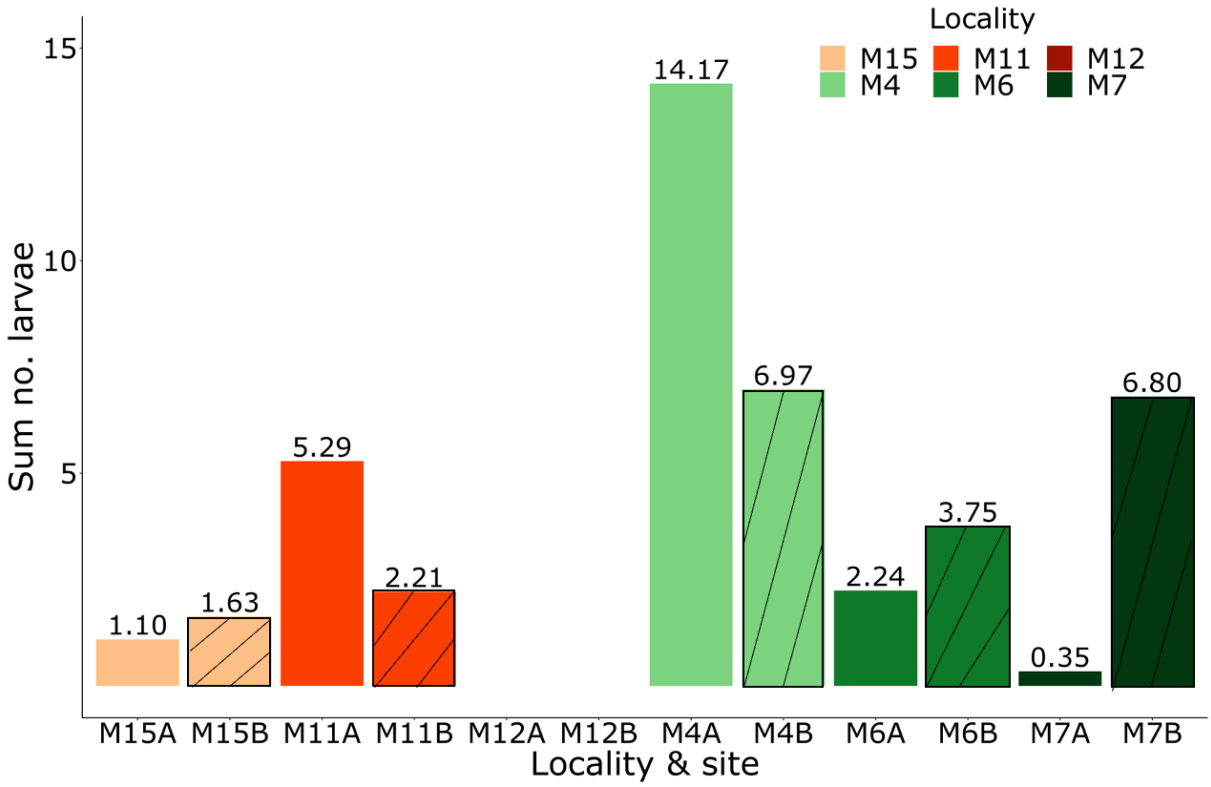


**Figure 6:** Total sum of collected planktonic ***L. salmonis*** larvae from the PPS during the study period. Including localities in southern (M15-M12) and northern (M4-M7) study area and study sites A (Colour) & B (Shaded) within all localities.

### 3.1.3 Southern study area

Analyses of planktonic samples from the PSS show salmon lice (*L. salmonis*) larvae collected from two of three study localities in the southern area (Figure 7). Salmon lice were successfully collected at M15 and M11 at different study periods, while no lice were collected from M12, located in the inner parts of Etnefjorden (see Figure 2). The first collection of lice was found in sampling period 3, from 20th-24th of May. Levels of detected lice varied from <1 (0.09) to > 2 (2.3) per sampling interval. Lice were collected from sites A and B in both M11 and M15. M11 collected lice in 10 samples, with the highest total amount of detected lice in the southern area. As a result of issues with the PPS at both sites at M15, where one PPS and one net were lost, samples and hence data are lacking from site A in study period 2 – 5 (Table A.2, Appendix A). In addition, samples and data from site B are lacking in study period 2, 4 and 5 (Table A.2, Appendix A), due to issues with the net of the sampler.

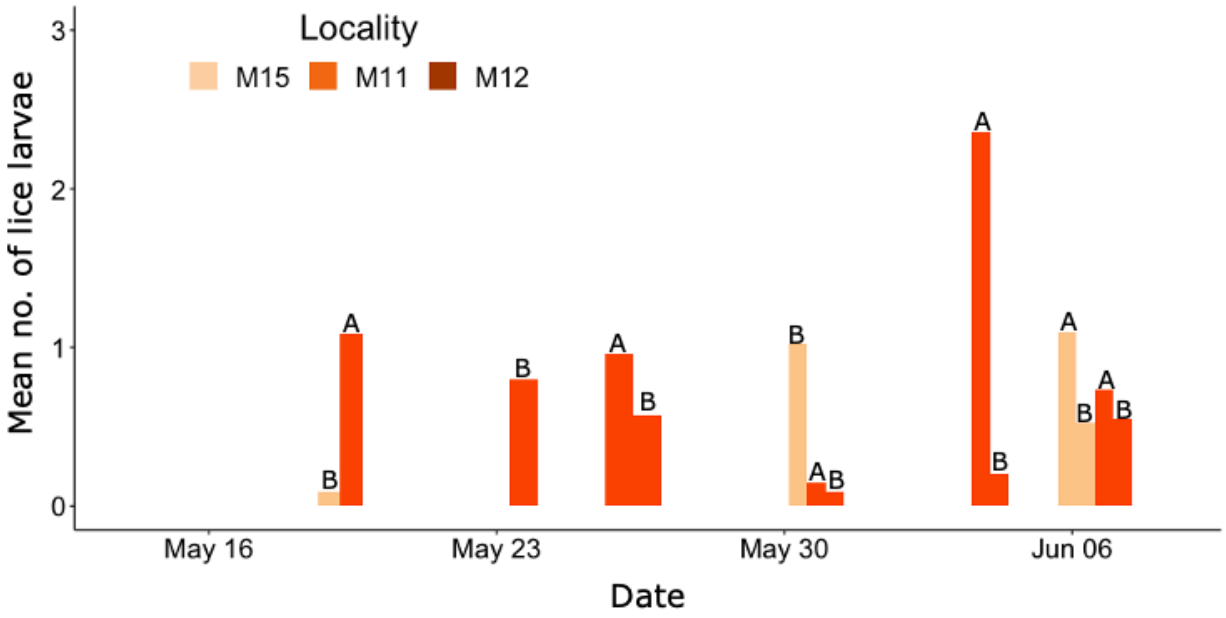


**Figure 7:** Mean number of collected *L. salmonis* lice larvae per study interval, locality, and site (A/B), in **southern** (M15, M11, M12) study area from 10th May to 10th of June 2022. Each bar represents the mean number of collected lice, estimated from triplicate lab analysis, in a planktonic sample from one sampling interval. Y-axis interval ranging from 0 to 3 lice larvae.

### 3.1.4 Northern study area

Measurements of sea lice from planktonic samples show salmon lice (*L. salmonis*) collected in all three localities in the northern study area (Figure 8). The first collection of salmon lice was found in sampling period 1, with continuous/further collection of lice at all study localities through the total study period. Levels of detected lice varied from <1 (0.07) to nearly 8 (7.8) total collected lice larvae per sampling interval. M4 showed the highest frequency of collected lice in the northern area during the study period. The highest observed lice level was observed in locality M4, in study period 5, with 7.8 collected lice. Lice have been collected from sites A and B in all localities in the northern area.

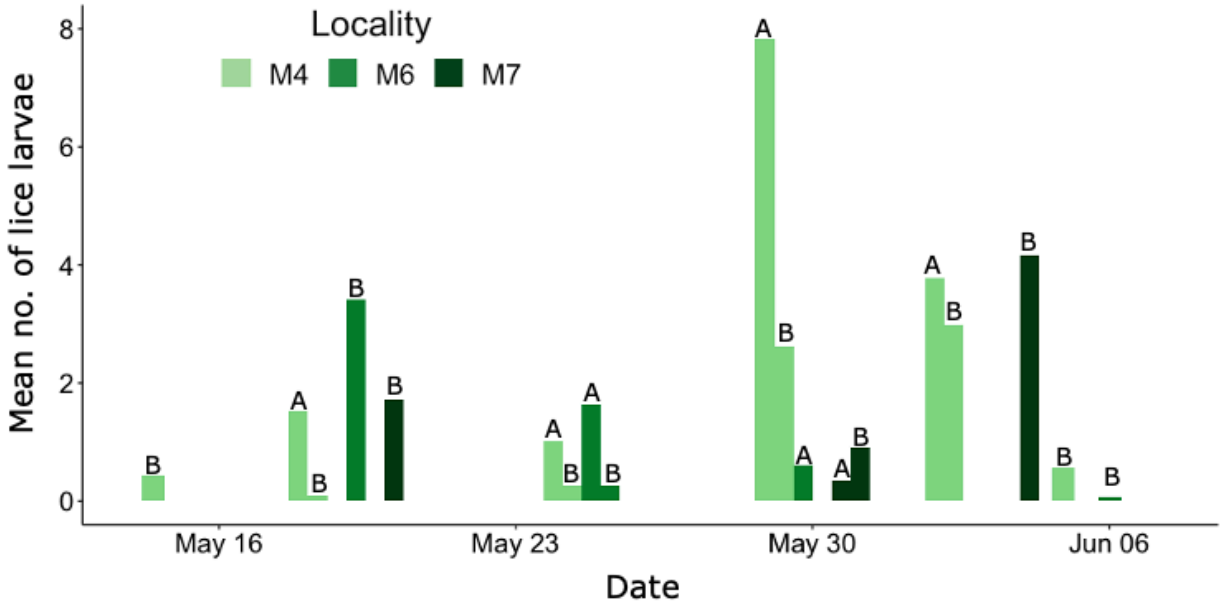


**Figure 8:** Mean number of collected *L. salmonis* lice larvae per study interval, locality, and site (A/B), in **northern** (M4, M6, M7) study area from 10th May to 10th of June 2022. Each bar represents the mean number of collected lice, estimated from triplicate lab analysis, in a planktonic sample from one sampling interval. Y-axis interval ranging from 0 to 8 lice larvae.

## 3.2 Quantifying probability of collecting salmon lice

The probability of detecting salmon lice was investigated using a binomial regression model, with locality M4 as a reference (Table 1). The model included all localities as predictors, days sampled and sample date.

The analysis showed that the probability of collecting lice in locality M6 was significantly different from M4 (p=0.038). A significant difference was also found between M15 and M4 (p=0.039). A highly significant difference in collecting lice was also found between M4 and M7 (p=0.008). No significant differences between M4 and M11, nor between M4 and M12 were detected. However, due to no lice detected at M12, as documented in 3.1.2, the lack of data hindered an effective model run including this locality. The large confidence interval for M12 (-441.135 to 56.711), and the high source of error (1520.428) also supports this, with an elevated level of uncertainty. Moreover, the analysis showed a negative coefficient estimate for all localities, implying that all other localities (M6, M7, M11, M12, M15) have a lower probability of collecting lice than M4.

Effects of number of sampling days and the date of sampling on the probability of collecting lice were investigated. The analysis revealed a significant influence of both the number of days sampled (p=0.045) and the sample date (p= 0.004), on the probability of collecting lice. Both days sampled and sampling date showed a positive coefficient estimate, indicating that with increased date and number of days sampled, the probability of collecting lice in all localities correspondingly increased.

**Table 1:** Summary of a binomial model with M4 as reference, with sampling date, days sampled and localities as predictor variables, and log-transformed number of point intercepts with number of larvae as the response variable. Estimated intercepts, standard error (SE), z-value and 95% confidence intervals and p-values are included.

| Predictor | Estimate | SE | z value | 95% CI | p |
|---|---|---|---|---|---|
| Intercept | -7.76631 | 3.755 | -2.068 | -16.225 to -1.035 | **<0.05** |
| Location M6 | -3.8728 | 1.61319 | -2.401 | -7.800 to -1.226 | **<0.05** |
| Location M7 | -4.2856 | 1.61974 | -2.646 | -8.229 to -1.636 | **<0.01** |
| Location M15 | -3.53072 | 1.71650 | -2.057 | -7.650 to -0.624 | **<0.05** |
| Location M11 | -1.8399 | 1.43179 | -1.285 | -5.270 to 0.681 | 0.198 |
| Location M12 | -21.8199 | 1520.4289 | -0.014 | -441.135 to 56.711 | 0.986 |
| Days sampled | 0.9533 | 0.47597 | 2.003 | 0.092 to 2.014 | **<0.05** |
| Sample date | 0.2293 | 0.08139 | 2.818 | 0.099 to 0.432 | **<0.01** |

## 3.3 Quantifying variance among areas and localities

Investigation of variation between study areas was conducted by estimating the degree of statistical variation between the study areas and number of collected planktonic salmon lice (*L. salmonis*) larvae. No significant variation across the number of larvae collected and study areas were found ($\chi^2$ = 2.7016, df = 1, p = 0.1002) with the use of Kruskal-Wallis rank sum test. In other words, no evidence of a statistically significant difference between the passive plankton samplers per study site, and number of salmon larvae collected per site, was found. No further tests on study sites were therefore performed due to lack of statistical significance.

Investigation of variation between localities was conducted by estimating the degree of statistical variation between localities and the amount of collected salmon lice larvae. Strong evidence of statistically significant differences between individual localities and number of lice larvae collected throughout the total study period were found ($\chi^2$ = 25.038, df = 5, p = 0.000137), using Kruskal-Wallis rank sum test. Therefore, at least one locality was statistically significantly different from the others, in terms of number of collected lice larvae.

Further pairwise comparisons between the number of collected lice and locality showed statistically significant differences between several localities and number of collected salmon lice larvae (Table 2). Significant differences were found between M4 and M6/M7/M12/M15. M12 also showed significant differences from M6, M11, and M15. No significant difference was found between locality M15, M11 and M6 and M7, nor between M4 and M11, related to number of lice larvae collected.

**Table 2:** Statistical differences (p-values) between the six study localities, based on the average number of collected *L. salmonis* larvae throughout the study period (Wilcoxon rank sum test). Significant p-values marked by ***p < 0.001, ** p < 0.01, * p < 0.05.

| Locality | M4 | M6 | M7 | M15 | M11 |
|---|---|---|---|---|---|
| **M6** | 0.02678* | - | - | - | - |
| **M7** | 0.02678* | 0.85146 | - | - | - |
| **M15** | 0.04041* | 0.88089 | 0.72715 | - | - |
| **M11** | 0.08372 | 0.30665 | 0.22750 | 0.46188 | - |
| **M12** | 0.00015*** | 0.02678* | 0.05961 | 0.02678* | 0.00173** |

Data was analysed using Kruskal-Wallis rank sum test (continuous) and pairwise Wilcoxon rank sum test when significant, with adjusted P-values by Benjamini-Hochberg (BH) method. Wilcoxon rank sum test p-values denotes significant differences in mean number of lice larvae collected between localities (*** p < 0.001, ** p < 0.01, * p < 0.05).

## 3.4 Estimated salmon lice concentrations

The salmon lice concentrations in southern (Etne) and northern study area (Rosendal) were estimated based on the IMR salmon lice model (Figure 9). The concentration of both areas was estimated to be low at the start of the study period, where both the northern and southern areas showed an estimated salmon lice concentration below 0.02 at the beginning of May. Thereafter the salmon lice concentration of both areas showed an increase throughout the study period, with some degree of fluctuation. There was estimated to be a higher general level of lice concentration in the northern study area, than in southern study area. A distinct peak in salmon lice concentration was estimated twice. Firstly, in the second and third week of June, in southern and northern study areas, respectively. Secondly, a maximum in salmon lice concentration was estimated in the fourth week of June (outside the study period of this study), with >0.10 copepodites per $m^2$ in the southern area and >0.21 copepodites per $m^2$ in the northern area.

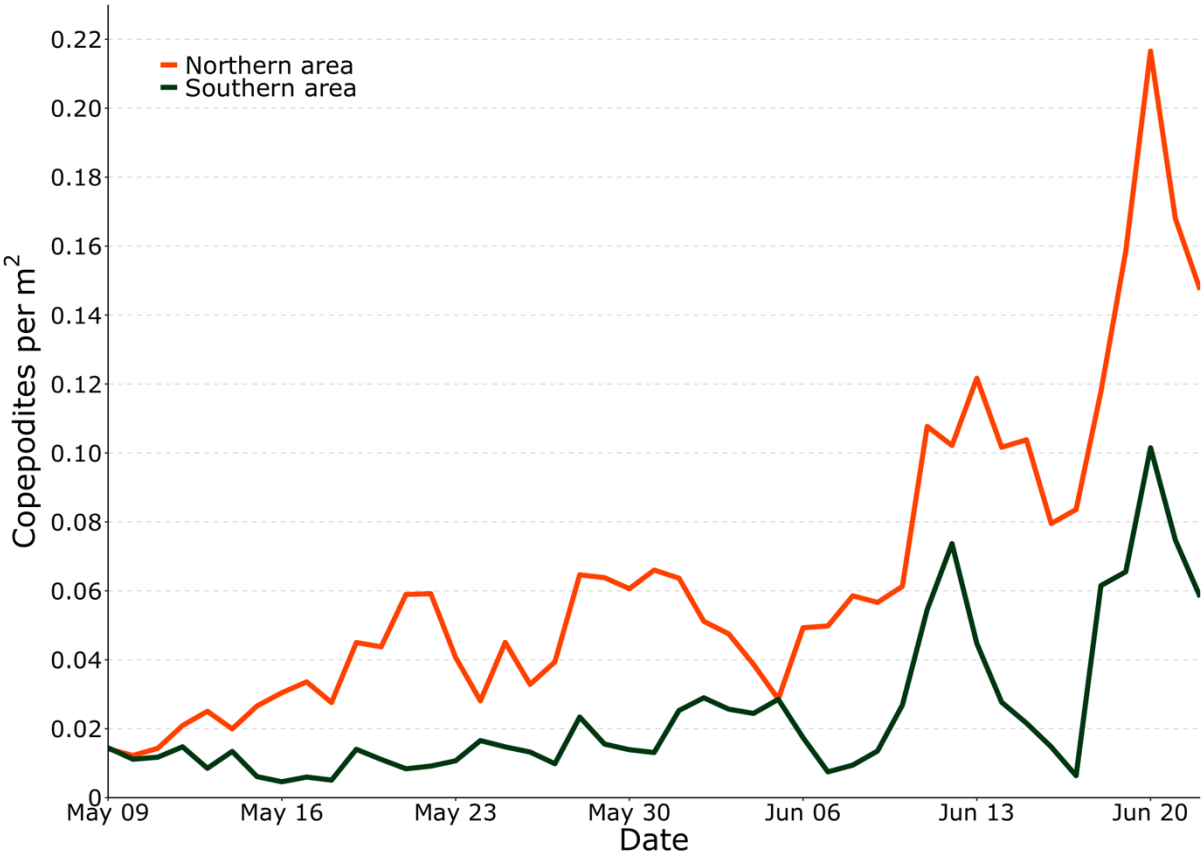


**Figure 9:** Estimated salmon lice level (median value of copepodites per $m^2$) in southern (orange solid line) and northern (green solid line) study area, between 9th of May and 20th of June 2022. Figure based on copepodid estimations provided by Anne D. Sandvik, IMR.

## 3.5 Quantifying reliability

### 3.5.1 Laboratory methodology

Reliability (repeatability) quantification of the laboratory analysis was performed using number of larvae collected as response variable and lab sample ID as random effect. The quantification showed a repeatability for number of larvae within lab samples at 0.81 (SE=0.033), on the link-scale approximation, with a confidence interval of repeatability derived from parametric bootstrapping of [0.738, 0.867] (Figure 10). Level of repeatability indicated that 81% of the variance in number of larvae could be explained by the lab sample ID. The likelihood ratio test (LRT) estimated $p < 0.001$, indicating strong evidence against the null hypothesis of zero repeatability.

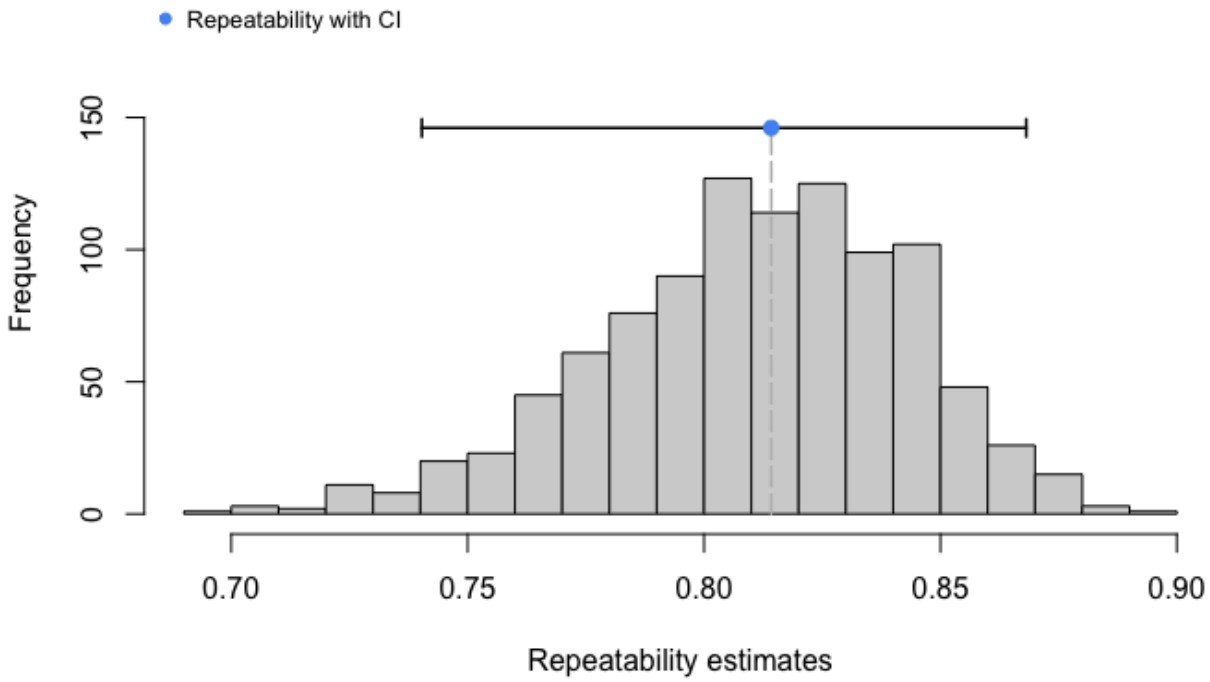


**Figure 10:** Repeatability estimations of number of lice as a response to lab sample ID as random effect and grouping factor, with 10 000 parametric bootstraps and permutation. Including a link-scale approximations repeatability of 0.81(81%), and CI [0.738, 0.867].

### 3.5.2 Passive plankton sampler

Quantifying the individual reliability (repeatability) of measurements from the PPS were performed using the number of larvae collected as response variable, and a combination of date, location, and site as random effect. Repeatability for the measurements of salmon lice, performed by the PPS within the total sampling period (10.05 – 10.06 2022), was 0.32 (SE=0.126) on the link-scale approximation, with a confidence interval of repeatability derived from parametric bootstrapping of [0.052, 0.55] (Figure 11). The level of repeatability therefore indicated that 32% of the variance in number of larvae could be explained by location ID (Locality/Site/Date). A p-value of <0.001 was estimated (likelihood ratio test), suggesting strong evidence against the null hypothesis of zero repeatability.

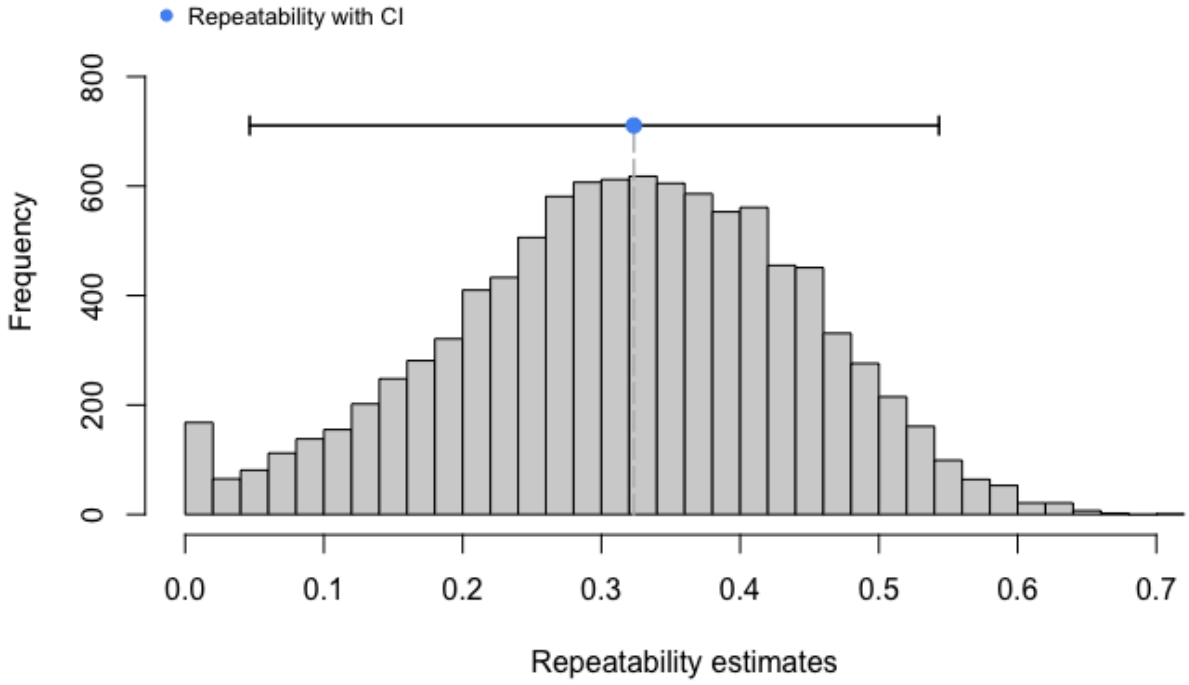


**Figure 11:** Repeatability estimations of number of lice as a response to location date/ID as random effect and grouping factor, with 10 000 parametric bootstraps. Including a link-scale approximations repeatability of 0.323 (32%), and CI [0.052, 0.55].

## 3.6 Recorded abiotic factors

Recordings of sea temperature and salinity showed minor variations, following the same trends, at the different study locations (Figure 12). Water temperature showed a slight increase throughout the study period, in the interval between 10 - 15 °C, peaking at 15 °C towards the end of the study period (June). Salinity measurements at M11 showed a salinity of around 25 ppt from start to end of study period, with a low degree of variation. In the northern study area, a decrease in salinity was recorded, going from around 25 ppt on the 14th of May to 20 ppt on the 10th of June (Figure 12).

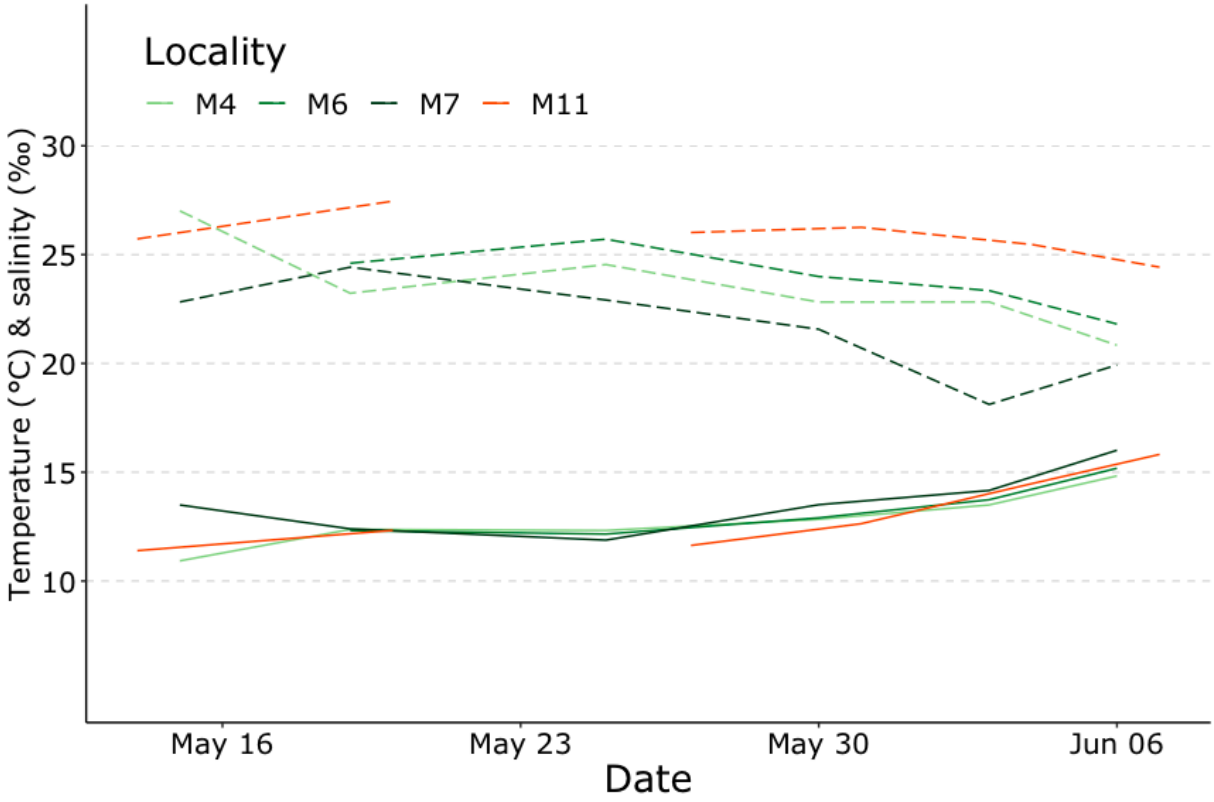


**Figure 12:** Recorded salinity (dashed line) and water temperature (solid line) at 1 m depth in northern study area (M4, M6, M7) and southern study area (M11), from 14th of May to 10th of June 2022.

Measurements of sea temperature and salinity were affected by a series of issues during the study period. In the southern study area, there were issues connected to CTD recordings at all study sites. Only location M11 provided abiotic measurements, but lacked data from 24th to 27th of May, as the recorder was out of water. For M15, the PPS with CTD data logger was lost, hence no environmental data was captured from this study site. For M12, temperature and salinity recordings were not credible, due to an error on the CTD data logger at site. M12 and M15 are therefore excluded from analyses on abiotic factors. For the northern study area, M4 and M7 were measuring credible CTD data continuously throughout the study period. For M6, CTD were not measured until the 19th of May, due to issues with the sampler.

### 3.6.1 Effects of abiotic factors on collection of salmon lice

Effects of salinity and temperature (predictor variables) on the success of collecting *L. salmonis* in northern study area (M4, M6 & M7) were investigated with the use of a generalized linear regression model, the *glm* function. 38 analysed planktonic samples were included, samples both with and without collected lice. Estimates indicated no significant effects of either temperature (p=0.420, Table 3) or salinity (p=0.858, Table 3) on the success of detecting lice in the northern study area (Table 3). Both predictors showed confidence intervals including zero, an additional indication of no significant effect of salinity and temperature on the success of collecting *L. salmonis*.

**Table 3:** Estimated coefficients of model predictors, temperature & salinity, with collection or no collection of salmon lice set as response variable, using generalized linear regression model (glm), family Binomial. Respective standard error (SE), z value, 95% confidence interval (CI) and p-values are included.

| Predictor | Estimate | SE | z value | 95% CI | p |
|---|---|---|---|---|---|
| Intercept | 6.55374 | 12.14665 | 0.540 | -17.292 to 32.0213 | 0.590 |
| Temperature | -0.38485 | 0.47725 | -0.806 | -1.386 to 0.535 | 0.420 |
| Salinity | -0.05101 | 0.28463 | -0.179 | -0.647 to 0.520 | 0.858 |

### 3.6.2 Correlation of abiotic factors and collected salmon lice

Effects of salinity and temperature (predictor variables) on the amount of collected *L. salmonis* (log adjusted) in northern study area (M4, M6 & M7) were assessed using a linear regression model, the *lm* function (Table 4 & Figure 13). The model showed no significant correlation between temperature and log adjusted collected number of sea lice larvae (p = 0.142, Table 4). Nor was any correlation estimated between salinity and collected number of sea lice larvae (p = 0.119, Table 4). In other words, neither temperature nor salinity had a significant effect on the number of lice collected by PPS at localities in the northern study area. Both predictors showed t-values ($-1.96<x<1.96$), which strengthens the indication of no statistically significant effect of temperature and salinity on number of collected sea lice larvae. Wide confidence intervals indicated a considerable amount of uncertainty of the estimates. $R^2$ of 0.1809 indicated that temperature and salinity explained 18.09% of the variance in the response variable.

**Table 4:** Estimated coefficients of model predictors, temperature & salinity, with log of mean collected salmon lice set as response variable, using linear regression model. Respective standard error (SE), z value, 95% confidence interval (CI) and p-values are included.

| Predictor | Estimate | SE | t value | 95% CI | p |
|---|---|---|---|---|---|
| Intercept | 53.042 | 44.3402 | 1.196 | -41.466 to 147.551 | 0.250 |
| Temperature | -3.2725 | 3.2782 | -0.998 | -10.259 to 3.714 | 0.334 |
| Salinity | -1.8191 | 0.1393 | -0.989 | -5.740 to 2.101 | 0.338 |

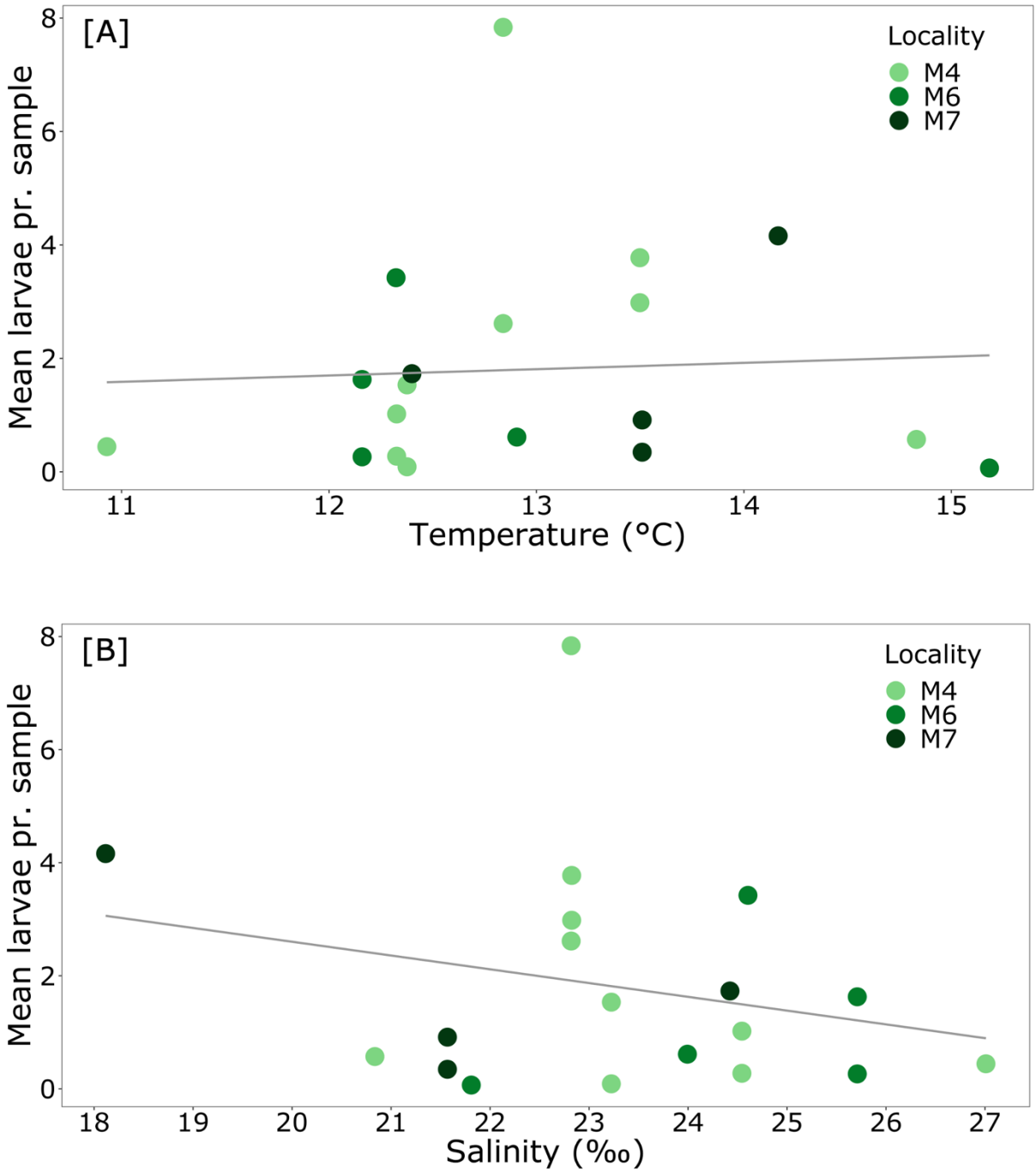


**Figure 13:** Correlation of temperature [A] / salinity [B] and mean number of collected salmon lice larvae per sample with detected lice in localities in northern study area (M4, M6, M7). Regression line (solid line) is the predictive values for the effect of [A] salinity & [B] temperature on mean number of collected larvae. Individual samples collected containing lice are represented by a single dot, with associated colour representing collection locality.

# 4 Discussion

The aim of the present study was to investigate the potential of a passive plankton sampler technology for monitoring densities of planktonic sea lice. The research was conducted by collecting planktonic samples from 12 passive plankton samplers in the Hardangerfjord, over a one-month period. Present DNA concentration of *L. salmonis* and *C. elongatus* within these samples were investigated by species-specific assays combined with ddPCR technology. Sea lice data from IMRs model was used as additional results. In the following chapters, the results of the study will be discussed based on the given research hypotheses and considering previous relevant studies.

The results from the present study must be interpreted with some caution due to difficulties related to the use of the PPS during field work. This resulted in a limited dataset with absence of samples from several sites under several sampling periods (Table A.2, Appendix A). The small number of identified *C. elongatus* did not provide a dataset robust enough for statistical analysis. Lack of valid CTD recordings from one study area limited the overall abiotic assessment of the study. Nevertheless, the presented results can be used for indication of trends in presence and distribution of sea lice in the area, and as an overall evaluation of the PPS' ability and potential as a suitable sampling strategy.

## 4.1 Evaluation of collected planktonic sea lice larvae

The first hypothesis addressed in the present study stated that a passive plankton sampler can function as a method for collecting levels of *C. elongatus* & *L. salmonis* in marine waters. In this study, the total estimation of sea lice levels in the collected samples showed presence of both species (see Figure 5 & Figure 6), with detection of sea lice in 43 of 77 collected samples. This confirms parts of the first hypothesis, as both species were successfully collected with the PPS. However, great variation within lice collection and detected species were discovered between the PPS assessed, as stated in the second hypotheses.

### 4.1.1 *Caligus elongatus*

*C. elongatus* were detected in only 10 of the 77 samples obtained from the different PPS studied, with a general low level of larvae per sample (Table A.4, Appendix A). The three samples containing more than one estimated larva could not indicate any pattern either in location or period of sampling (see Figure 5). However, the low occurrence of the species is supported by studies showing a pronounced seasonal abundance of *C. elongatus*, observing large occurrences in autumn and winter months, to nearly an absence of the species during spring and summer (Øines et al., 2006; Heuch et al., 2007; á Norði et al., 2015).

Despite the limited data basis for evaluating presence, distribution, or variation of *C. elongatus* larva in the present study, a successful collection and laboratory quantification of the species were nevertheless demonstrated. Several studies have reported presence of infective *C. elongatus* copepodites in the water column throughout the year (Karlsbakk et al., 2001; Heuch et al., 2002; Karlsbakk et al., 2003). However, the behaviour and duration of survival of planktonic *C. elongatus* is not well understood nor documented (Neilson et al., 1987; Paulsen, 2018). Consequently, implementation of the PPS holds the potential for advancing further research and enhancing our understanding of planktonic *C. elongatus*. Specifically, for investigating knowledge gaps outlined by Paulsen (2018), pertaining to both the species in general and its implications in Norwegian aquaculture conditions.

### 4.1.2 *Lepeophtheirus salmonis*

In contrast to the low results of *C. elongatus, L. salmonis* were identified in 33 samples, from both northern and southern study area (see Figure 6). The detected higher number of planktonic *L. salmonis* compared to *C. elongatus* aligns with hypotheses 1.1 and previous studies conducted on planktonic sea lice in the water column (á Norði et al., 2015; Harte et al., 2017). A large variability of sampled *L. salmonis* concentrations was demonstrated, both within study sites and across study areas, ranging from an average of 0 to 8 larva within a sampling period (Figure 7 & 8). These findings were in accordance with hypotheses 2 and previous studies displaying a patchy and highly variable distribution of *L. salmonis* in both space and time in fjords and coastal waters (Asplin et al., 2014; Johnsen et al., 2014; Sandvik et al., 2016; Skarðhamar et al., 2019). The highest concentrations of lice observed at M11 and M4 in the southern and northern study area, respectively, aligns with findings from previous studies displaying elevated levels of planktonic sea lice in close proximity to aquaculture facilities (Costelloe et al., 1998; Nelson et al., 2018).

In the southern study area, *L. salmonis* were collected in the two localities in the outer parts of the study area (Figure 2). M11 was the locality with the highest frequency of collected samples containing *L. salmonis*, as well as the highest total number of larvae sampled in the area (see Figure 7). However, the total number of larvae estimated showed a general low lice level, with a maximum mean level of approximately 2.8 lice per sample collected (Figure 7). Yet, these low lice detections are in accordance with previous studies showing generally low lice densities in open-waters (Byrne et al., 2018; Fernandez-Gonzalez et al., 2022). A total lack of sea lice collected from both PPS at M12 were observed throughout the study period, despite a reported presence of lice collected by a towed horizontal plankton net, as well as on smolt placed in the IMRs sentinel cage in the area (Karlsen et al., 2022; Andersson, 2023). However, all evaluated methods by Andersson, 2023, used for lice estimations showed a general low lice level in the southern study area. The higher lice level detected in the outer parts of the southern study area, compared to

the inner parts, is in addition supported by the IMR model estimations of lice levels in the area (Figure B.2, Appendix B) (Karlsen et al., 2022).

Sea lice were collected from all sites in the northern study area (Figure 8). However, compared to M4, both the frequency of samples containing lice and the total number of collected larvae were lower at locality M6 and M7. There are several possible explanations for this observation. Firstly, larvae produced in farms represents a point source with high larvae densities, that combined with onsite factors may be responsible for a maintained amplification of lice larvae densities in and around aquaculture farming (Costelloe et al., 1996; Hogans, 1997; Costello, 2006; Asplin et al., 2014). Hence, the larger amount of larva collected at M4 could be due to higher larvae densities in proximity to aquaculture farms. Secondly, planktonic larvae in high densities close to aquaculture have been found rapidly diluted with distance from farms, a possible explanation of the lower collected larvae in localities further positioned from aquaculture farming in this study (Costelloe et al., 1998; Nelson et al., 2018). However, lice larva is also found distributed several kilometres from a farm, aggregating close to shore and estuary mouths, with suggested origin from lice on wild salmonids, as well as from aquaculture farms (Costelloe et al., 1996; McKibben & Hay, 2004; Penston et al., 2004). Due to several possible explanations of lice dispersal in fjord systems, a further assessment of processes and factors effecting behaviour and spread of sea lice larvae within fjord systems is suggested, for a more in-depth evaluation and understanding of larvae dispersal.

The overall sum of larvae was found to be at a higher level in the northern area than in the southern area (Figure 6). This was supported by the estimated salmon lice concentrations of the areas (see Figure 9) as well as by studies investigating lice levels of the areas (Nilsen, 2016; Karlsen et al., 2022; Andersson, 2023). Moreover, an indication of a higher number of detected sea lice in the later parts of the study period were found, in late May and early June, for both study areas. These findings were in accordance with the model estimated salmon lice concentrations (Figure 9 & Figure B.2, Appendix B). Low lice concentrations were found in both areas in the early May, both within PPS samples, and within the estimated salmon lice model (Figure 7, Figure 8, Figure 9). Consequently, the estimated concentrations and collected sea lice showed comparable trends in southern and northern study area, as stated in hypotheses 2.1. These findings were further supported by Nilsen et al. (2022), who reported a lower infestation rate on smolt placed in the inner parts and the southern side fjords of the Hardangerfjord. In contrast, a moderate lice level was estimated in the middle to outer parts of the Hardangerfjord (Nilsen et al., 2022). Lastly, when evaluating the variation in larvae collection between site A and B, no clear trend was observed for either of the studied localities. This might be explained by the limited number of lice samples, which hindered the interpretation of trends within the study sites (A/B).

## 4.2 Evaluation of probability of collecting lice

To gain insight into the variations in the presence, absence, and concentrations of larvae between the different areas, the study investigated the probability of collecting lice (Table 1). M4 was set as a reference, as this locality collected the overall highest lice quantity, although lice were detected in the same number of samples for both M11 and M4. M4 was found to be significantly different from all localities but M11 and M12 (Table 1). As M11 and M4 collected lice in the same number of samples, the lack of significant difference between the localities was expected. For M12, the total lack of detected lice and hence substantial number of zeros, affected the model in which it could not detect any significant difference from M4. Nevertheless, an observable difference between M4 and M12 was detected, comparing the localities with most and least detected lice. Moreover, this study discovered an increased likelihood of catching lice with longer sampling intervals. As longer intervals accordingly increase the total water volume filtrated through the PPS, a higher likelihood of collecting lice was consequently expected. In addition, a higher probability of lice collection was found throughout the study period, a finding in alignment with several studies stating higher abundance of *L. salmonis* larvae with increased water temperatures throughout spring and summer months (Helland et al., 2015; á Norði et al., 2015; Hamre et al., 2019). The findings in the present study of an increase in *L. salmonis* abundance throughout the study period were also supported by the model estimated abundancies of infective salmon lice larvae in the start and at the end of the study period (Figure B.2, Appendix B).

## 4.3 Evaluation of variance among areas and localities

The second hypothesis acknowledged the anticipated variance within the study areas and the individual localities assessed. Consequently, estimations were carried out to evaluate the extent of variation between areas, localities, and levels of collected larvae (see section 3.3). No significant difference between study areas and number of collected larvae were found, a finding not in accordance with hypotheses 2i) of variation between study areas, nor with previous assessments of lice levels outside Etne (southern area) and Rosendal (northern area) (Nilsen et al., 2019, 2020; Nilsen et al., 2021; Karlsen et al., 2022). However, the present study revealed significant differences in the number of collected sea lice between several of the six study localities (Table 2). This result conforms with hypotheses 2ii) of variation between localities, and is consistent with previous field observations of lice abundance in the Hardangerfjord in the past years (Nilsen et al., 2019, 2020; Nilsen et al., 2021; Karlsen et al., 2022)

Localities near aquaculture sites, M4 and M11, showed a tendency of a higher general lice load compared to the other localities assessed in the respective areas (Figure 7 & Figure 8). The finding of significant differences between the number of collected lice in localities close to aquaculture facilities and localities in more protected areas, further strengthened

this finding (Table 2). These findings were also in alignment with previous studies stating higher planktonic lice abundance near aquaculture farms, with a rapid density decrease with increased distance from farms (Costelloe et al., 1998; Penston et al., 2004). Also, a significant difference was found between M4 and the remaining localities in the northern area, stating a clear distinction between localities and the level of collected lice. Deployment of PPS at localities with varying distance from aquaculture facilities therefore proves its ability for evaluating planktonic sea lice distribution, possibly originating from farms. The PPS might therefore facilitate the evaluation of impacts of aquaculture production on a larger scale. This might help provide insight into the horizontal distribution of larvae, and for further knowledge of its encounter with wild salmonid stocks throughout the season.

In addition, locality M4 in northern study area showed significant differences from the two more protected localities in the southern study area (Table 2), which can be explained by the very low level of collected larvae in both locations in the southern area (Figure 7). The generally low quantity of lice estimated in M6, M7 and M15 could explain the lack of significant differences between these localities (see Figure 6). However, investigation of more samples would be needed to draw any conclusions about the distribution patterns of planktonic sea lice in the area. In the present study, this was not conducted due to the limitations of the master thesis.

## 4.4 Evaluation of repeatability

Hypotheses 1.2 stated an expected high reliability level of the laboratory and PPS methodology used in this study. Estimations of the reliability of measurements run by the laboratory procedure, as well as for measurements obtained by the PPS, allowed for evaluation of level of precision of the methods.

### 4.4.1 Laboratory method

The laboratory analysis performed, described in 2.3.1, using DNA species-specific assays combined with droplet digital PCR (ddPCR), is a relatively new method of extracting and identifying sea lice in planktonic samples (Bui et al., 2021). Conventional methods of detection and identification of larval lice have historically involved sample collection and microscopy for visual inspection in search of sea lice larvae (McBeath et al., 2006). The estimated repeatability level of the laboratory methodology of 0.81, indicating that 81% of the variance in number of larvae estimated can be explained by the sample ID (Figure 10). This implied a high level of consistency of the ddPCR method of detecting sea lice in the planktonic samples. The estimate of an elevated level of repeatability, a low p-value, and confidence intervals including zero, therefore confirms parts of hypotheses 1.2 of a reliable laboratory method. This were supported by studies stating that implementation of methods based on genetic species-specific identification, with the use of real-time PCR

probes, constitutes a more rapid and improved method compared to more traditional methodology (McBeath et al., 2006). Evaluations of applicable methods have in addition documented ddPCR as the only method that exhibited adequate accuracy and precision to reliably enumerate copepodites in a plankton sample, with a minimal risk of sample contamination (Bui et al., 2021). Hence, the elevated level of estimated reliability is supported by previous studies examining the methodology.

### 4.4.2 Passive plankton sampler method

The measurements performed by the PPS were found to have an estimated repeatability of 0.32, which suggests that 32% of the variance in number of larvae estimated can be attributed to the PPS deployed at specific sampling sites (Figure 11). Accordingly, the results from the analysis could not characterize collected larvae to be the single most likely measurement to describe individual variance between passive plankton samplers at the different study sites. However, the results may be considered valuable in terms of stating that variation in collection of larvae in different localities are not singularly affected by the passive plankton samplers as an equipment of measuring lice. In addition, as the variance cannot be explained by a single known factor, the remaining 68% of variance (random noise) is then caused by other influencing factors (Rudeck et al., 2020). As the PPS were collecting passively drifting planktonic lice, several factors such as presence of lice, oceanographic conditions or abiotic factors could possibly affect whether the PPS are successfully collecting lice or not. The collection of lice during this study period is therefore not necessarily representative for evaluating the overall ability of the PPS as a methodology. The repeatability was estimated based on a small data set, collected over a one-month period, possibly leading to results with low credibility. Testing several PPS over a longer period might therefore provide a stronger estimate and result in an increased level of repeatability. This, however, is merely assumptions and was not carried out in the study due to the limitations of the thesis.

## 4.5 Evaluation of effects of abiotic factors

Hypotheses 2.2 of the study were related to the effects of abiotic parameters, with a possible correlation between salinity (‰), temperature (°C) and number of lice collected. The abiotic recordings showed a slight decrease in salinity and a slight incline in temperature from the start to the end of the study period (Figure 12), in accordance with abiotic observations in the Hardangerfjord in previous years (See Figure B.3 & Figure B.4, Appendix B) (Albretsen & Asplin, 2021). Elevated sea water temperatures towards summer, combined with an increase in freshwater run-off found in spring causing lower salinities, further supported the abiotic trends found in the present study (Sjøtun et al., 2015).

This study discovered no significant correlation of salinity nor temperature affecting the success of collecting lice in the northern study area (Table 3). Neither was any significant correlation found between changes in temperature and salinity, and the amount of collected larvae (Table 4). However, the survival of sea lice and the rate at which they deplete their energy reserves have been proven to be strongly influenced by both temperature and salinity (Bricknell et al., 2006). Nevertheless, earlier studies have also stated that sea lice larvae can tolerate a wide range of temperatures (4 to 20 degrees), where higher and lower temperatures shortened or lengthened the larval developmental time, respectively (Johnson & Albright, 1991; Boxaspen & Næss, 2000; Brooks, 2005). In the present study, recorded temperatures ranged from ~10 to ~16 degrees, with a slight increase towards the end of the study period (Figure 12). These temperatures therefore fall within the tolerated range for the present sea lice larvae within the area, a possible explanation for the lack of effects of temperature on lice larvae collection. In addition, an increased temperature has been proven to decrease larvae survival time, with an estimated survival time of 13 days at 10 degrees, and 9.5 days at 15 degrees (Samsing et al., 2016). At the same time, Hamre et al. (2019) demonstrated higher *L. salmonis* egg production in line with higher temperatures, with a suggested elevated local infection pressure, and vice versa with lower temperatures. Hence, there is a need for further investigation on the relationship between temperature, sea lice larvae production, and its survival time, for a more precise evaluation of the effects of temperature on larval distribution.

In regards of salinity, the development of *L. salmonis* nauplii have been proven to only be deleteriously affected by salinities below 26‰ (Johnson & Albright, 1991), while copepod survival is compromised at salinities below 27‰ (Bricknell et al., 2006). In the present study, salinities were solely recorded below 26‰, and due to lack of variation no effect of salinity on collection of *L. salmonis* in the area could be stated (Table 3 & Table 4). Consequently, the lack of demonstrated effects of neither salinity nor temperature on the collection of lice larvae might be explained by the low degree of variation in the recorded abiotic factors in this study. In other words, low variation in salinity and temperature recordings across localities (see Figure 12) could be an explanatory factor of the lack of abiotic effects. Regardless of this, the low sample size, and weak data basis, with lack of data from southern localities, limited the overall evaluation of the influence of abiotic factors on lice levels of the areas. To investigate the actual effects of abiotic factors on the collection of lice in the area, a longer study period for assessing fluctuations in abiotic parameter, in combination with more rapid plankton sampling, is recommended.

## 4.6 Perspectives & improvement of method

This work represents the first successful study on collecting planktonic sea lice of both *C. elongatus* and *L. salmonis* with the use of a new PPS technology in a Norwegian fjord. However, in addition to the sources of error previously discussed are the following

improvements worth consideration, for enhancing the methodology in further practice and studies. Stabilizing tubes of the PPS and electronics box (see Figure 3) were not adequately waterproof in this study, causing a vertical position of the PPS in water, and lack of flow estimation data. Ensuring a consistent flow rate and effective sampling of plankton from all PPS facilitates the computation of larval concentrations per $m^3$, which in turn enables more accurate comparisons of collected larval densities between studies. Moreover, moorings implemented in the study could not withstand environmental conditions of the area, leading to loss of a sampler. Hence, implementation of a secure customized mooring setup is recommended. Other suggested improvements of the method are implementation of a larger plankton net opening, allowing filtration of larger water volumes, as well as attachment of several nets for sampling plankton at different depths within the water column. In the context of the laboratory method, differentiation between presence of nauplii and copepodites in samples would be beneficial to study the presence and dispersal of different life stages of planktonic sea lice in fjord systems. Considering implementation of the suggested enhancements, the PPS combined with DNA-based quantification can be evaluated as an appropriate method, for use in assessments and ground-truthing of model estimations of planktonic sea lice levels along the Norwegian coast.

# 5 Conclusion

The present study aimed to assess the potential of a passive plankton sampler (PPS) technology combined with DNA-based quantification, for monitoring densities of planktonic sea lice in the water column. The research involved collecting planktonic samples from 12 PPS in two study areas in the Hardangerfjord, over a one-month period. Thereafter, DNA concentrations of *L. salmonis* and *C. elongatus* were analysed within collected samples, using species-specific assays and ddPCR technology. The result of this study provides valuable insight into the PPS ability to function as a sampling strategy for evaluating presence and distribution of planktonic sea lice.

A successful collection and analyzation of both *C. elongatus* and *L. salmonis* larvae with the use of the PPS and DNA-based quantification were demonstrated in this study. A high reliability of the laboratory analysis was found, indicating a successful use of DNA-based quantification of sea lice in planktonic samples. However, the PPS measurements showed a lower reliability, suggesting that external factors were influencing the collection of sea lice larvae. Variation between collection of sea lice and concentration of both species were found, both between study areas and localities. *C. elongatus* were detected in only a few samples, indicating a low occurrence of the species, consistent with seasonal abundance. In contrast, *L. salmonis* were more frequently detected in samples, displaying a high variability in concentrations across study sites, with elevated levels closer to aquaculture activity. In addition, similar trends were found between the quantified *L. salmonis* levels with modelled larvae abundance of the area. No effects of salinity or temperature on collection of *L. salmonis* were found, possibly due to the low variation within the recorded abiotic parameters, combined with limited data and sample size.

Consequently, this study suggests the PPS as a potential method for further research on *C. elongatus*, and for assessing distribution of *L. salmonis* in coastal areas. Results from the present study propose that the PPS, in combination with DNA-based quantification, offers a relevant methodology for assessing presence and distribution of planktonic sea lice in fjord systems. Further research on implementation of the methodology on a larger scale for managemental purposes is thus recommended.

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# Appendix A

**Table A.1:** Specific sampling locations in northern and southern study areas including coordinates. The order of sampling procedure is illustrated by an arrow, by first sampling at sites located furthest away from the starting positions.

| Plankton sampling localities | | |
|---|---|---|
| Sampling order | Northern study area | Southern study area |
| ↓ | M4 (60°07.9'N, 5°54.7'E) | M15 (59°45.0'N, 5°39.7'E) |
| | M6 (60°02.1'N, 5°55.2'E) | M11 (59°41.2'N, 5°44.4'E) |
| | M7 (59°58.4'N, 5°58.0'E) | M12 (59°39.1'N, 5°53.5'E) |

**Table A.2:** Overview of sampling periods and presence of PPS for each study locality and site during the fieldwork period. The presence of both PPS is marked by A/B, individual PPS at a locality is marked by site letter (A or B), lack of PPS at a locality is marked by -. Notice study period 8 only relevant for southern study area.

| Study localities | | | | | | |
|---|---|---|---|---|---|---|
| | Southern study area | | | Northern study area | | |
| Study period | M15 | M11 | M12 | M4 | M6 | M7 |
| 1 | A/B | A | A | A | A/B | A/B |
| 2 | - | A/B | A/B | A/B | A | A/B |
| 3 | B | A/B | A/B | A/B | A/B | A/B |
| 4 | - | A/B | A/B | A/B | A/B | A/B |
| 5 | - | A/B | A/B | A/B | A/B | A/B |
| 6 | A/B | A/B | A/B | A/B | A/B | A/B |
| 7 | A/B | A/B | A/B | A | A/B | A/B |
| 8 | A/B | A/B | A/B | | | |

**Table A.3:** Planktonic sampling intervals divided into study periods (SP), for northern study area, from 11th of May to 9th of June, and southern study area, from 10th of May to 10th of June. Start of CTD recordings is marked in bold.

| Sampling intervals & periods | | | |
|---|---|---|---|
| SP | Northern area (Rosendal) | | Southern area (Etne) |
| 1 | 11.05 - 15.05 (4 days) | | 10.05 – 14.05 (4 days) |
| **2** | **15.05 - 19.05 (4 days)** | **Start CTD recordings** | **14.05 - 20.05 (6 days)** |
| 3 | 19.05 - 25.05 (6 days) | | 20.05 – 24.05 (4 days) |
| 4 | 25.05 - 30.05 (5 days) | | 24.05 – 27.05 (3 days) |
| 5 | 30.05 - 03.06 (4 days) | | 27.05 – 31.05 (4 days) |
| 6 | 03.06 - 06.06 (3 days) | | 31.05 – 04.06 (4 days) |
| 7 | 06.06 - 09.06 (3 days) | | 04.06 – 07.06 (3 days) |
| 8 | | | 07.06 – 10.06 (3 days) |

**Table A.4:** Samples of collected *C. elongatus* larvae using the PPS. Including study area, study site, study period (SP), sum levels of larvae per sample and collected larvae per sampling day.

| Study area | Study site | SP | Sum larvae | Larvae per day |
|---|---|---|---|---|
| Southern | M11A | 1 | 0,08 | 0,02 |
| Southern | M15B | 1 | **43,92** | 10,98 |
| Southern | M11A | 4 | 0,07 | 0,02 |
| Southern | M11B | 4 | **115,04** | 38,35 |
| Southern | M11A | 5 | 0,15 | 0,04 |
| Southern | M11B | 5 | 0,31 | 0,08 |
| Southern | M11A | 6 | 0,11 | 0,03 |
| Southern | M11B | 6 | 0,05 | 0,01 |
| Northern | M6A | 4 | 0,18 | 0,04 |
| Northern | M7A | 4 | **18,42** | 3,68 |

# Appendix B


**Figure B.1:** Aquaculture Traffic Light system in Norway per 2022. The 13 aquaculture production areas (PA's) along the Norwegian coast, classified according to lice-induced mortality of wild migrating salmon smolt. Status for each PA is indicated by green (potential of 6% increase in MAB), yellow (maintaining current MAB) and red colour (requiring 6% reduction in MAB) (The Norwegian Directorate of Fisheries, Yggdrasil, 2023).

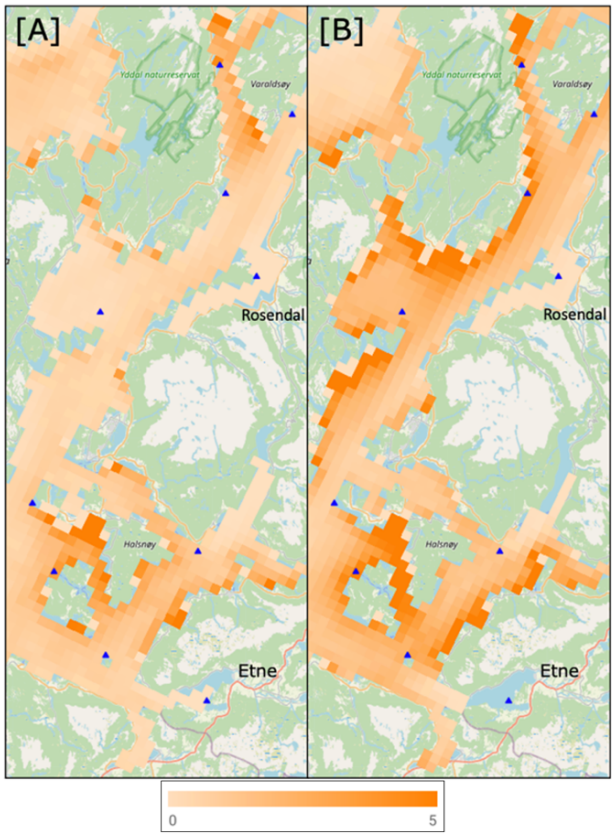



**Figure B.2:** Estimated abundancies of infective salmon lice larvae (from 0 to 5 larvae per m3) in Northern and Southern study area in [A] week 18 and [B] week 23 in 2022 (The Norwegian Directorate of Fisheries, Lakseluskartet, 2023).

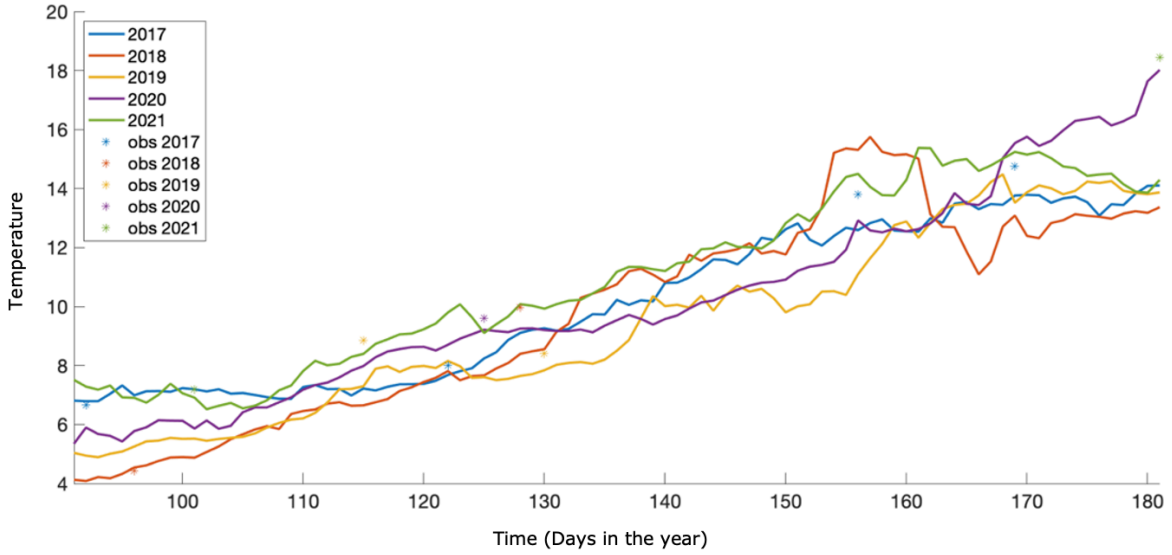



**Figure B.3:** Observed and modelled temperatures at 3 meters depth in the Hardangerfjord, from April to June, in 2017 to 2021 (Albretsen & Asplin, 2021).

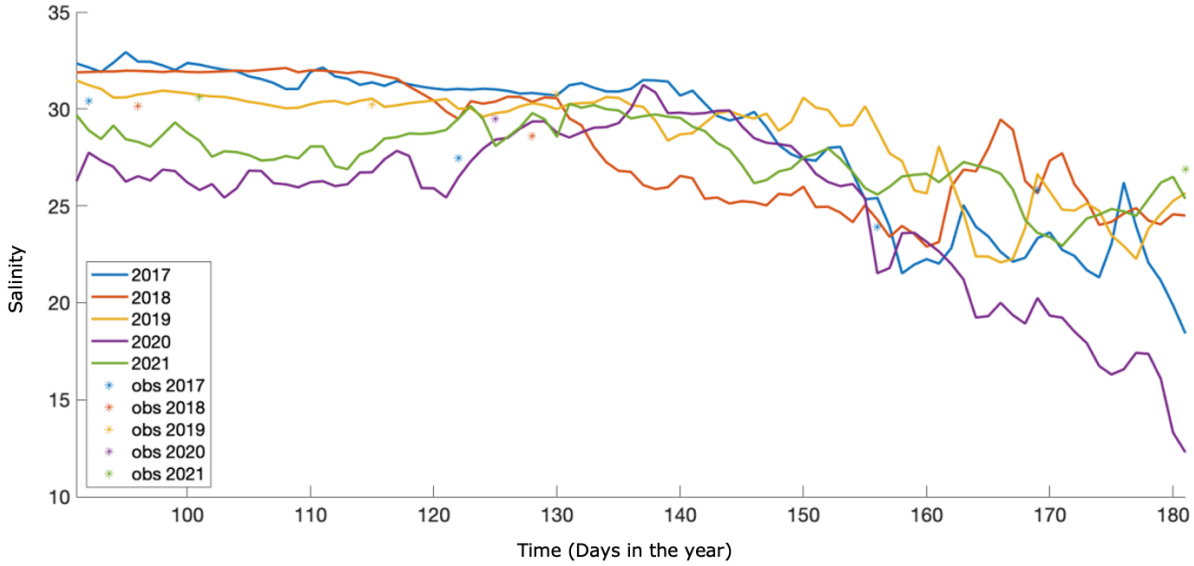
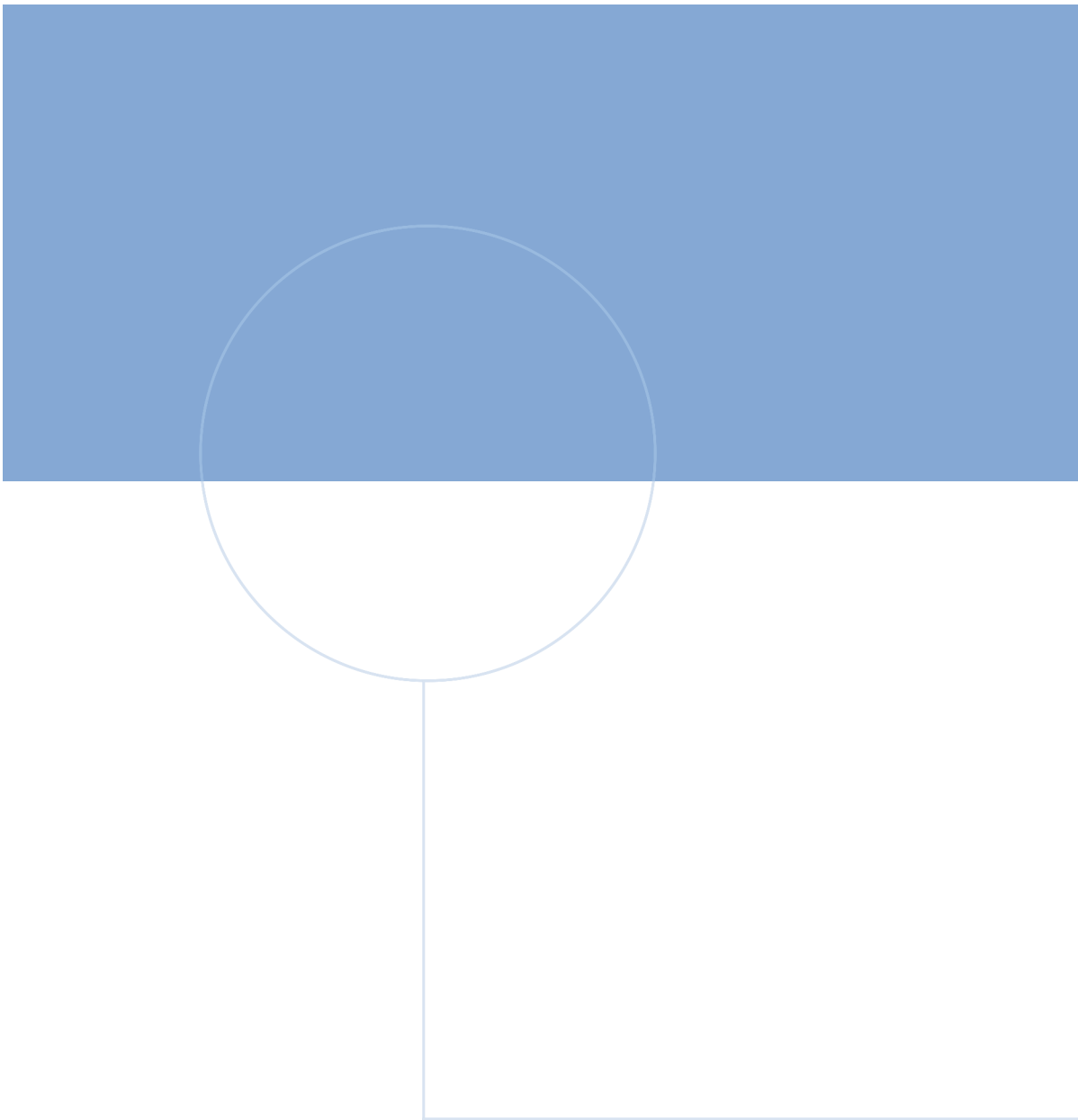



**Figure B.4:** Observed and modelled salinities at 3 meters depth in the Hardangerfjord, from April to June, in 2017 to 2021 (Albretsen & Asplin, 2021).



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