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# Comparison of a passive plankton sampler technology with existing methods to estimate salmon lice (*Lepeophtheirus salmonis*) levels

Master's thesis in Ocean Resources

Supervisor: Bengt Finstad

Co-supervisor: Frode Fossøy, Nathan Mertz

May 2023



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Science and Technology



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Department of Biology





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Trondheim, May 2023

Amanda Andersson

# Abstract

Salmon lice (*Lepeophtheirus salmonis*) is considered one of the biggest obstacles for industrial growth of the Norwegian aquaculture industry. It is difficult to determine the exact number of salmon lice in the sea due to their dynamic nature. Therefore, there is need for a broad variation of methods to be able to manage the balance between nature and industry. The passive plankton sampler (PPS) is a new technology, enabling collection of bulk plankton samples over several days.

The purpose of this study was to compare the PPS to a selection of methods that are being used today to estimate lice levels in the sea. The methods that are being compared are a horizontally towed plankton net, the Institute of Marine Research's (IMR) model and IMR's sentinel cages. The collection of plankton was performed in the period May and June of 2022 in the Hardangerfjord.

Through the study, the ability of the PPS has been investigated when it comes to collecting plankton in the field. The methods compared are different, but there are some signs of correlation and similar trends. The PPS correlates most with the IMR model with a correlation coefficient ( $\rho$ ) of 0,46. With the plankton net there was a  $\rho$  of 0,33 and with the sentinel cages a  $\rho$  of 0,28. This study shows some promising indications that the PPS has potential for this kind of field sampling but there is still need for more development and testing to determine how the PPS can contribute. Potentially it could support the other methods used today as a managing tool when it comes to the Norwegian surveillance program for salmon lice (NALO) programme or the Traffic Light System.

# Sammendrag

Lakselus (*Lepeophtheirus salmonis*) er sett på som en av de største utfordringene for industriell vekst innen Norsk akvakultur industri. Det er vanskelig å bestemme eksakte lakselus-nivåer i sjøen på grunn av den dynamiske naturen. Det er derfor behov for en bred variasjon av metoder for å kunne administrere balansen mellom natur og industri. Den passive planktonsamleren (PPS), er en ny teknologi som gjør det mulig å samle inn bulkplanktonprøver over flere dager.

Hensikten med dette studiet var å sammenligne PPS med et utvalg metoder som brukes i dag for å estimere lusenivåer i sjøen. Metodene som blir sammenlignet er et horisontalt slept planktonnett, Havforskningsinstituttet (HI) sin spredningsmodell og HI sine vaktbur. Innsamlingen av plankton foregikk i perioden mai og juni i 2022 i Hardangerfjorden.

Gjennom studiet er det undersøkt evnen PPS innehar når det gjelder å samle inn plankton i felt. Metodene det sammenlignes med er ulike, men det er fortsatt noen tegn til korrelasjon og like trender. PPS korrelerte mest med HI sin modell med en korrelasjons koeffisient ( $\rho$ ) på 0,46. Med planktonnettet var det en  $\rho$  på 0,33 og med HI sine vaktbur en  $\rho$  på 0,28. Denne studien viser noen lovende indikasjoner på at PPS har potensial for denne typen feltprøvetaking, men det er fortsatt behov for mer utvikling og testing for å finne ut hvordan PPS kan bidra. Den kan potensielt bidra med å støtte de andre metodene brukt som et reguleringsverktøy i dag når det kommer til den nasjonale lakselusovervåkingen (NALO) programmet eller Trafikklyssystemet.





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# 1 Introduction

## 1.1 Aquaculture

### 1.1.1 Aquaculture production in a global perspective

The human population are continuously growing. It's estimated that the world population has passed 8 billion people in 2022 and will reach 9,7 billion by 2050. By 2100 it will reach 10,4 billion (Gaigbe-Togbe et al., 2022). More people will result in increased food demand around the world. It is estimated that the food demand will increase with 58-98% between 2005-2050 (Keating et al., 2014). The increased food demand requires increased production of food. Aquaculture has grown three times faster than agriculture since 1970, with a rate of 8,3 % per year. To sustain the population of 9 billion people, there is need of 50% more food production overall to have good quality of life for humans. Agriculture will be limited due to land, freshwater and nutrients. Future population will be dependent on the increase of aquaculture to ensure enough food worldwide (Diana et al., 2013).

Approximately 80% of the salmon that is harvested is farmed salmon. And fish accounts for 7% of all protein consumption by humans produced. If the consumption per capita stays the same and the human population reaches 9 billion people, global fish production will need to reach 43 million tonnes. The land cannot sustain the increase that is demanded, so fish products must be doubled to be able to provide enough protein for the population. In 2020 the total supply of salmon exceeded 2,65 million tonnes gross weight globally, so the supply is increasing (MOWI, 2021).

### 1.1.2 Aquaculture production in a Norwegian perspective

Most of the farmed fish in the world is farmed in Norway, Scotland, Chile, and Canada (MOWI, 2021). It's estimated that the seafood production will double by 2030 and increase by five times by 2050 in Norway (Misund & Tveterås, 2019; Olafsen et al., 2012). The salmon farm industry began in 1970, and throughout the years it has become one of the largest and most important industries we have and is one of Norway's largest export industries by economic value. There are several species being cultivated, but Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) is by far the ones we rely on the most (Taranger et al., 2015). In 1994,

200 000 tonnes of farmed fish worth 10 billion NOK were sold in Norway. In 2020 approximately 1,5 million tonnes farmed fish worth 69 billion NOK were sold. There has been an expansion on 59 billion NOK in that timeframe. The export value of farmed salmon was 74 billion NOK in 2020. Nearly 70% farmed fish were exported to EU and there are close to 100 countries that received farmed salmon from Norway (Regjeringen, 2021).

### 1.1.3 Environmental aspects of aquaculture

The salmon farming industry is threatening the wild fish, and as a result it can keep the industry from growing if we cannot come up with better solutions to secure the wild fish, environment, and ecosystem overall (Taranger et al., 2015). Sea lice is one major problem and are considered one of the biggest obstacles of the growth of the industry. The management of lice has two goals, one is to conserve wild Atlantic salmon (*Salmo salar*), sea trout (*Salmo trutta*) and Arctic char (*Salvelinus alpinus*), and the other is to ensure the growth of the aquaculture industry. The current management system in Norway focuses on preservation at the river population level to maintain biodiversity and a sustainable fishery to protect wild salmon. The Norwegian government have become stricter over the years and are limiting the increase of aquaculture and the granting of licenses for production. And to get a license the company need to follow much stricter regulations for sea lice management. Also new regulatory frameworks have taken place, in 2017 the coast was divided in to 13 independent production zones, to better manage each zone. To be able to calculate and manage what decisions will be made for each zones the traffic light system was implemented (Vollset et al., 2018).

## 1.2 Sea lice

### 1.2.1 *Lepeophtheirus salmonis*

*Lepeophtheirus salmonis* is an ectoparasite dependent on a host to live and reproduce. It has eight life stages, nauplius 1 and 2 and the ineffective copepodite stage being planktonic stages. The size of the copepodite is 0,8 mm. Chalimus 1 and 2 are when the lice are attached to the exterior parts of the fish. In the preadult 1 and 2 stages the lice can start to move around on the fish. The last stage is the adult stage. This is when the male and female can reproduce. The female has a pair of egg strings where each string contains 150-400 eggs. When the egg hatches, they are released to the water as free floating nauplii (á Norði et al., 2016; Bui et al., 2021; Havforskningsinstituttet, 2018). Heuch et al. (2000) investigated the number of egg strings *L. salmonis* produced during the lifespan. The 44 females they had in the experiment produced

218 pairs of egg strings at 7,2 °C. This gives an average of 4,9 reproductive cycles. Three of them made 11 pairs of egg strings during their life span. The size of the female excluding the egg string can reach over 18 mm in length while males can reach up to 7 mm (Pike, 1989). The development of the eggs and each life stage are dependent on water temperature. The lifecycle goes faster with higher temperature. The development for the different stages at 10 °C: eggs take 8,6 days, nauplii larvae to infectious copepodite takes 3,6 days, and from egg to an adult male it takes 40 days and from an egg to a female adult it takes 52 days (Johnson & Albright, 1991).

The *L. salmonis* mostly feed on exterior parts of the fish like skin and mucus. In large numbers it can eat through the skin and start to feed on flesh and blood. In severe cases where the fish gets open wounds, they are in danger of getting secondary diseases as a result (Braden et al., 2015). The severity of sea lice infection can range from mild skin damage to stress induced mortality (Costello, 2006).

### 1.2.2 *Caligus elongatus*

*Caligus elongatus* is next to *L. salmonis* as the most common sea lice affecting the farmed and wild Atlantic salmon in Norway and Scotland. As with *L. salmonis*, *C. elongatus* is also an ectoparasite, feeding on mostly exterior parts (McBeath et al., 2006). Through the years the *L. salmonis* has been viewed as the biggest threat for the fish, and as a result there has been less focus on *C. elongatus*. But as the industry has expanded, it's observed that *C. elongatus* can also be a big issue for the salmon and the lumpfish (*Cyclopterus lumpus*) which are being used to reduce levels of *L. salmonis* in the net pens. *C. elongatus* as an adult is smaller than the adult female *L. salmonis*. It has the size of 5-6 mm (AkvaplanNiva, 2019). The female can reach 6 mm in length while the male reaches 5 mm in length (Pike, 1989). *C. elongatus* are less host specific than *L. salmonis*. *C. elongatus* also have 8 stages in the life cycle. It lacks preadult stages. The life cycle consists of nauplius I and II, copepodite, chalimus I, II, III, IV and adult. The first three stages are also planktonic (Hemmingsen et al., 2020; Piasecki, 1996; Piasecki & MacKinnon, 1995).

## 1.3 Atlantic salmon and sea trout

The Atlantic salmon (*Salmo salar*) is an anadromous fish species most common in the north Atlantic oceans. The life cycle of Atlantic salmon consists of egg, alevin, fry, parr, smolt, post smolt, adult and spawning adult. The female returns to the childhood river between October

and January to spawn. The parr can live from one to six years in the river until it becomes a smolt and migrates out to sea in late spring or summer. The salmon can stay in the sea from 1 to four years before becoming a spawning adult (Hansen & Quinn, 1998; Hoff, 2020). Many salmon only reproduce once due to exhaustion, but some of them survive and can reproduce several times (Havforskningsinstituttet, 2019b). The sea trout (*Salmo trutta*) is also an anadromous fish species. Sea trout's starts to migrate to the sea from April-May, and in some cases as late as the fall. It migrates to the sea at the age from 2-5 years and lives out in the sea for 1-3 years before it returns to the rivers to spawn. Many of the sea trout survives the breeding and can go back and forth from the river and the sea to spawn for many seasons. The sea trout belongs naturally in Europe, and can occur in every country and some nearby countries in Africa and Asia (Jonsson & Finstad, 1995; Pethon et al., 2022; Sæter, 2009)

## 1.4 Consequences of sea lice

### 1.4.1 Sea lice and interactions with wild fish

On marine and farmed fish in the sea, the most common ectoparasite are the copepods *L. salmonis* and *C. elongatus* – sea lice belonging to the family Caligidae (McBeath et al., 2006; Mordue & Birkett, 2009). Both species occur naturally in low numbers on the northern hemisphere on wild salmon where there are no fish farming, but since the 1980's and the expansion of fish farming it has been observed outbreaks of sea lice in larger numbers in Norway, Ireland, Canada and Scotland (Thorstad & Finstad, 2018). Due to the growth in production of salmon and because of increased host numbers, the number of parasites feeding on the salmon has grown in huge numbers (Taranger et al., 2015; Torrissen et al., 2013). This contributes to overall more sea lice in the sea and especially in areas near fish farms. Sea lice from fish farming increases the mortality of wild salmon (Taranger et al., 2015) and can cause osmoregulatory dysfunction, physiologically stress responses, anaemia, reduced feeding and growth, reduced diseases resistance, increased mortality, and increased susceptibility to secondary microbial infections. Sea trout can also suffer from fungal and bacterial infections when returning to freshwater (Thorstad & Finstad, 2018).

Studies has also shown that fish infested with sea lice are more susceptible to predators (Vollset et al., 2018). It is estimated that more than 10-11 adult lice per salmon smolt causes mortality (Heuch et al., 2005; Thorstad & Finstad, 2018). The fish can have stress responses due to the chalimus stages, but the biggest effect on the fish is caused when the lice become mobile, increase in numbers and develops to later life stages (Vollset et al., 2018).

Salmon farms have negative effects on wild populations, this has been shown in different studies. Consequences of increased salmon farming and increased sea lice levels has on occasions resulted in declining populations in Norway, Scotland, Ireland, and Canada (Krkosek et al., 2007; Marty et al., 2010; Thorstad & Finstad, 2018).

#### 1.4.2 How it is affecting the industry when it comes to production and cost

*L. salmonis* and *C. elongatus* have a major economic impact on the salmonid fish farming industry and are the most damaging parasites for the industry (Costello, 2006). For Norway it's estimated that the sea lice cost for the industry is near to 5 billion NOK per year (Iversen et al., 2017). Norway and Chile accounts for most of the production and are responsible for most of the cost (Costello, 2009). Taking inflation into account and the increasing production of salmonids, the cost today is most likely to be much larger, if assumed that the cost will increase as production increases.

Measures against sea lice consist of prevention, control, and reduction (Lusedata, 2020). Many of the methods used are a threat to the health of the salmon/sea trout, cleaner fish, the environment, and it can even make the lice resistant (Holan et al., 2017). This is costly for the industry and negative for the biological aspect. There is therefore a need for developing technology that prevents infestation of sea lice, in a larger scale than today (Almås & Ratvik, 2017).

### 1.5 Regulations in aquaculture regarding sea lice

In 2017 there was issued a production area regulation on behalf of Ministry of Industry and Fisheries (NFD). This regulation stated that the Norwegian coast was divided into 13 production areas (Figure 1) (Lovdata, 2017). The areas were divided in such way to ensure as little spread of *L. salmonis* as possible between the areas. The purpose of the production areas was to regulate the production in each area based on sustainability indicators to ensure the health of the wild salmon (Vollset et al., 2019). The production in each area was determined by the traffic light system (Fagerbakke, 2020).



**Figure 1:** Geographical division of the 13 production areas from the Swedish border to eastern Finnmark. Map: [www.hi.no](http://www.hi.no).

The traffic light system came into use in 2017 and appears in the Production Area Regulation. (Trafikklyssystemet, 2021). The traffic light evaluates the environmental impact in each zone and determines the production volume in each area every second year (Vollset et al., 2018). NFD determines if each production area will be given green, yellow or red colour. The decision is based on the judgement of the steering group and expert group based on aspects within natural science and socio-economic consequences (Trafikklyssystemet, 2021). The expert group has assessed the categorisations of limits for *L. salmonis* induced wild fish mortality for post smolts in the 13 production areas in these categories: Low: < 10% *L. salmonis*-induced wild fish mortality, Moderate: 10-30% *L. salmonis*-induced wild fish mortality and High: >30% *L. salmonis*-induced wild fish mortality (Vollset et al., 2019). The goal is to keep the impact of mortality of wild salmon at a minimum and up to 10%. If the induced mortality is between acceptable limits, the given production area gets green light and can grow production by 6% (Myksvoll et al., 2020). If not, the given production area gets a red light and must decrease production with 6 %, and with yellow the given production area can produce the same amount (Fagerbakke, 2020).

The Lice Regulation is an important part of the regulation of sea lice. It has the purpose to reduce the number of *L. salmonis* and prevent resistance among the *L. salmonis*. It described the acceptable limits for *L. salmonis* on the fish farms (Fiskeridepartementet, 2012). Under normal production the fish handlers count the sea lice present every 7 or 14 days depending on the sea temperature. The limits for acceptable *L. salmonis* are 0,2 and 0,5 adult female lice per fish on average depending on the weeks and season (Lovdata, 2012, 2017). The limits are 0,2



in those periods where wild post smolt are wandering out from the rivers to the sea (Mattilsynet, 2022). The time when the post smolt wanders out to sea from the river varies between rivers and years (Johnsen, 2020).

## 1.6 Monitoring methods

In the national surveillance program monitoring *L. salmonis* on wild salmon (NALO) the infection pressure on wild salmon is monitored by trawling, fish nets, fish traps and sentinel cages (Vollset et al., 2021). IMR in Norway is conducting this program on a mission from The Norwegian Food Safety Authority (MT) and NFD. IMR shall coordinate the surveillance, the research connected to this field and counselling related to the infestation of *L. salmonis* (Nilsen et al., 2021). In addition, there are models to estimate and monitor sea lice infestation. IMR, The Veterinary Institute and SINTEF all has developed one model each (Vollset et al., 2021), and there are also Virtual Post smolt (VPN) (Johnsen, 2020). The different models have been verified with comparison with the methods mentioned in the beginning of the chapter. The purpose of these models is to estimate when and where the density of infectious copepodites is so high that it is damaging the wild salmon by estimating the infestation on post smolt that migrates out from the salmon fjords (Vollset et al., 2021).

### 1.6.1 IMR's hydrodynamic model

The model estimates how many lice larvae that are hatched every hour on each farming facility in the whole country, based on the reports of *L. salmonis* and water temperature. The estimation is based on how many adult female lice there are at the farming sites based on what the farmers report (Havforskningsinstituttet, 2019a). The model uses nauplii production from every farming facility. It is a hydrodynamic model, that uses principals for transport in water. The models estimate the water currents, water temperature and the salinity, and based upon that calculates the spread of infectious copepodites in the first two meters of the water column along the Norwegian coast. The amount of copepodites in an area will describe the infection pressure for the post smolt (Vollset et al., 2021).

### 1.6.2 Sentinel cages

Thirty salmon smolts are placed in a sentinel cage for fourteen days. The cages are 1 m<sup>3</sup> and closed and are placed in the upper water column from 0,5-1,5 m depth. After fourteen days the smolts are taken out and counted for lice. In every fjord system that are under surveillance, 18-

20 cages are used. They use farmed salmon smolt (80-100g), so the size will be bigger than wild salmon smolt (15-25g). The threshold for low infestation is < 2 lice/fish/14 days, moderate from 2-6 lice/fish/14 days and high > 6 lice/fish/14 days (Vollset et al., 2021).

### 1.6.3 Plankton collection

Traditionally plankton is collected by using some type of planktonic net that can be towed horizontally or dragged up the water column vertically. It's a commonly used method and has been used a long time (Langford, 1953). In this master project a passive plankton sampler (PPS) technology will be tested. It will be placed out in the sea for several days collecting plankton. The advantage with this PPS is that it collects plankton in a passive way, and the handlers can come back after a few days to pick it up, process the sample and place it out again.

## 1.7 eDNA background

DNA barcoding is a method that uses a standardized DNA-region as a tag for a species to be able to identify it quickly fast and accurately (Valentini et al., 2009). Polymerase Chain Reaction (PCR) methods are molecule-based methods. They are limited when it comes to identifying the difference in larval stages but are on the other hand capable of processing numerous samples (Bui et al., 2021). Droplet digital PCR (ddPCR) are the third generation of PCR. With ddPCR exact quantification of nucleic acids within a sample are enabled, this allows for accurate detection and quantification of low abundance targets (Nyaruaba et al., 2019). With ddPCR the samples are divided into droplets containing a single target molecule (Hindson et al., 2011). A specific segment of DNA gets copied up to a billion times. PCR uses a short synthetic DNA fragment called primers to be able to select a segment of the genome. This makes it possible to copy the segment repeatedly (Smith, 2022). Environmental DNA (eDNA) metabarcoding takes a sample from the environment such as water, sediment, or air. From these samples, the DNA is extracted. With primers the DNA gets amplified. The primers can be universal or general in PCR and sequenced using next generation sequencing to generate thousands to a million of reads. These data will give the presence of different species and give the knowledge of the biodiversity in that given area (Ruppert et al., 2019). The difference in barcoding and metabarcoding, is that the barcoding focuses on a specific organism in a sample, and metabarcoding focuses on the species composition in a sample (Cristescu, 2014). With eDNA ddPCR it is possible to take a sample from the environment and target one single species (Nyaruaba et al., 2019; Ruppert et al., 2019).

## 1.8 Aim of study

The aim of this study was to compare existing methods used today to monitor and estimate number of *L. salmonis* in Hardanger, with the passive plankton sampler (PPS), a new low cost, high volume, bulk plankton collection system, combined with molecular quantification tools. The method will be compared with methods such as hydrodynamic model, sentinel cages and horizontal net taws. The study aims to test these hypotheses:

1. Is it possible to collect *L. salmonis* in Hardanger using the PPS and determine lice levels in the samples using ddPCR.
2. The PPS will show same trends and correlates with the i) plankton net, ii) IMR model, iii) sentinel cages.

## 2 Materials and methods

### 2.1 Study area

The collection of data was conducted during May and June 2022. The length of the fieldwork was approximately 6-8 weeks with planning and preparation. The fieldwork took place the Hardangerfjord in the west coast of Norway (Figure 2) close to Etne and Rosendal. Our place for collection was conducted at different fjord systems in the outer part of the Hardangerfjord: Etnefjorden (59°65'N, 5°87'E), Melen (59°68'N, 5°74'E), Halsnøyfjorden (59°75'N, 5°65'E), Øynefjorden (60°13'N, 5°91'E) and Kvinnheradsfjorden (60°00'N, 5°96'E). Due to distance between the fjord systems, Etne and Rosendal were visited twice a week by combination of car, ferry, and small boat transport. The sample period for Etne started 10.05.2022 and ended 10.06.2022. For Rosendal the sample period started 11.05.2022 and ended 09.06.2022.



**Figure 2:** Map of Norway, showing the location for the fieldwork. Map: [www.barenswatch.no](http://www.barenswatch.no).

There were six sampling sites, three in Etne and three in Rosendal. Each site had two samplers. The names used for the sampling sites are as follows: M12, M11, M15, M4, M6 and M7. Each sites had two samplers which is marked A and B. The samplers were placed next to IMR sentinel cages and are named after that ID. The positions for the IMR cages and the sampling sites are displayed in Table 1.

**Table 1:** Coordinates for each sampling site.

| Location ID | Location | Coordinates             |
|-------------|----------|-------------------------|
| M12         | Etne     | °59 39.1' N, °5 53.5' E |
| M11         | Etne     | °59 41.2' N, °5 44.4' E |
| M15         | Etne     | °59 45.0' N, °5 39.7' E |
| M4          | Rosendal | °60 07.9' N, °5 54.7' E |
| M6          | Rosendal | °60 02.1' N, °5 55.2' E |
| M7          | Rosendal | °59 58.4' N, °5 58.0' E |

Figure 3 shows the two different sampling areas Rosendal (Figure 3A) and Etne (Figure 3B) and each sampling site. M12, M11 and M15 are in the Etne area. M12 is located inside the Etne fjord in the national salmon fjord close to the outlet of the national salmon river. M11 is in Melen close to the border of the national salmon fjord, and M15 further out and more exposed in Halsnøyfjorden, both M11 and M15 are close to aquaculture facilities. M4, M6 and M7 are in the Rosendal area. M4 is located inside Øynefjorden and are close to several facilities. M6 is in Kvinnheradsfjorden in the middle of sampling area in Rosendal, the width of the fjord is between this location and nearby aquaculture facilities. M7 is closest to Rosendal town centre in the Kvinnheradsfjord, and lays close to an island, on the other side of the island there are some aquaculture facilities.

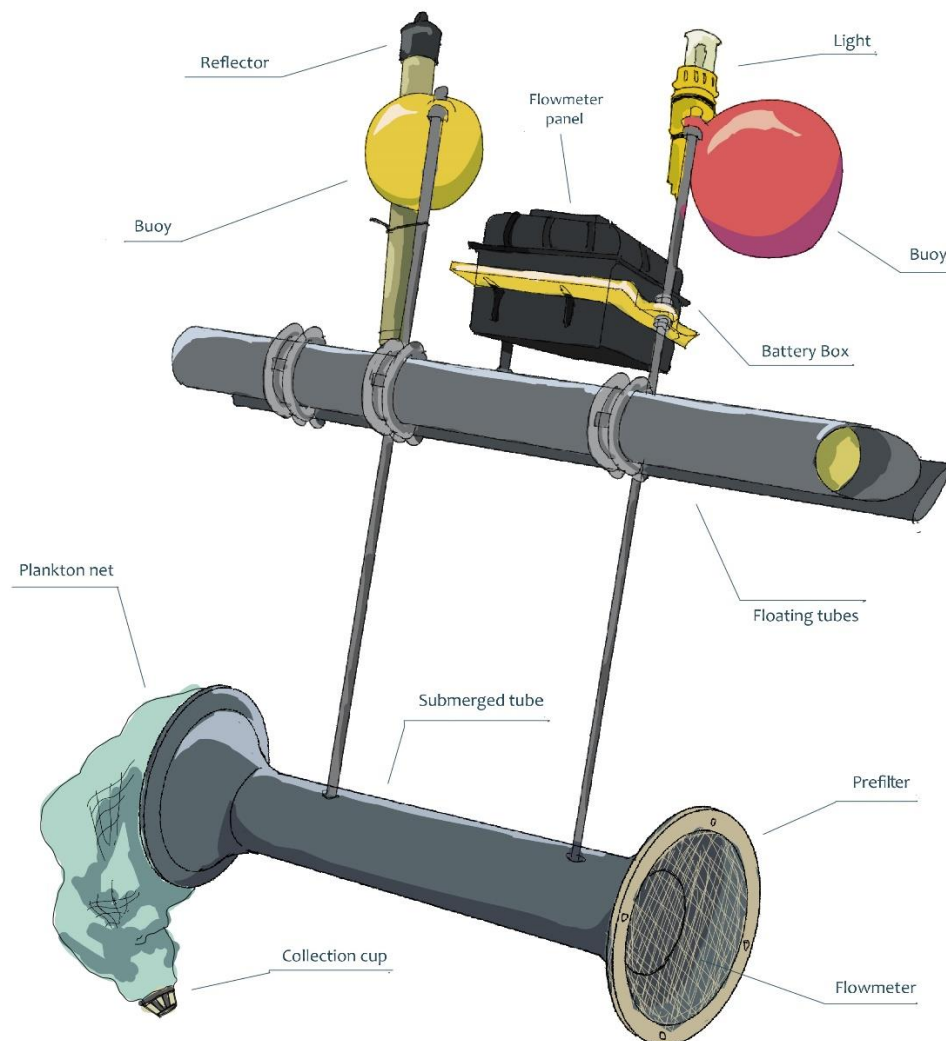


**Figure 3:** Map over Rosendal (A) and Etne (B), showing each position for each sampling site and the farming sites in the area. The national salmon fjord in Etne is shown and salmon river outlet as well as anadromous river outlets. Orange spots: sampling sites, black/blue spots: farming facilities, green/purple point: anadromous/ salmon river outlet, blue-green coloured area: national salmon fjord. Map: www.barenswatch.no.

## 2.2 Field methods

### 2.2.1 The passive plankton sampler (PPS)

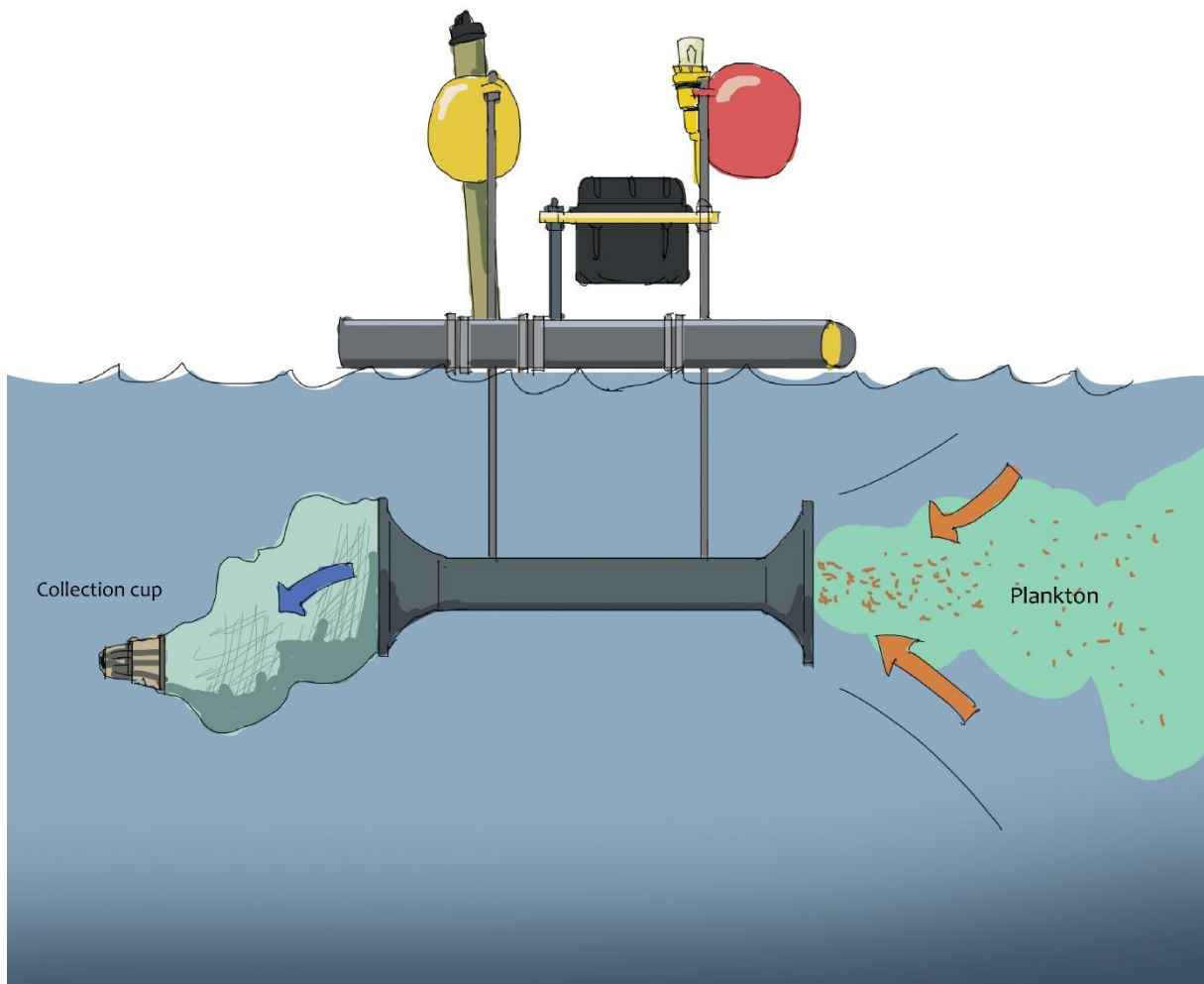
The PPS (Figure 4 and 5) consists of a 98 cm long plastic tube from mouth to mouth that is submerged 65 cm in the sea from the centre to the centre of two floating tubes. The submerged tube has a mouth diameter of 25 cm. On the submerged tube there is a flow meter at the inlet point where the water enters, and a pre filter to prevent larger particles for entering the tube. In the other end there is a 135 cm long zooplankton net with a mesh size of 125  $\mu\text{m}$  attached with a cup that collects plankton. The cup has a diameter of 12 cm. On top of the sampler, there are buoys to make it easier to see where the sampler is, light and reflector for other vessels, and a box to put a battery in with a panel to read how much flow the flow meter has registered.



**Figure 4:** Overview of the passive plankton sampler and the components. Illustration: Vegard Andersson.

### 2.2.1.1 Placing it out in the fjord system

The PPS floats in the water and is anchored to the sea bottom (Figure 5 and 6). It is attached with a hook to the front of the sampler with a long rope that goes down to a chain at the sea bottom where there is an anchor hooked to the bottom. On the rope there is two buoys that makes some of the rope float at the surface. The length of the rope makes it possible for the sampler to move around in the water depending on the current. This makes it possible for the water to enter the front of the sampler.



**Figure 5:** How the passive plankton sampler behaves and floats in the sea. Illustration: Vegard Andersson.



**Figure 6:** The passive sampler placed in the water at M11 in Etne. Photo: Amanda Andersson.

#### 2.2.1.2 Collecting of samples

Each Monday and Thursday it was collection day at Etne, and each Tuesday and Friday it was collection day at Rosendal. This was the general rule, but the weather and other unexpected issues could cause change in schedule, making the sampler stay in the sea from 3 days at shortest to 6 days at longest.

The samplers were unhooked from the rope and taken into the boat. Plankton nets were taken of the sampler, and then rinsed down to the cup at the end using seawater (Figure 7B and 7C). Then the plankton in the cup was rinsed out into another cup, with a mesh bottom (Figure 7D and 7E). Then it was rinsed over into bottles using ethanol and a sieve (Figure 7A). The bulk plankton samples were preserved in 50ml tubes or 250ml bottles using absolute ethanol, depending on the size of the plankton samples (Figure 7F). Flow meters were checked, and battery changed before assembling and putting the PPS back into the sea.

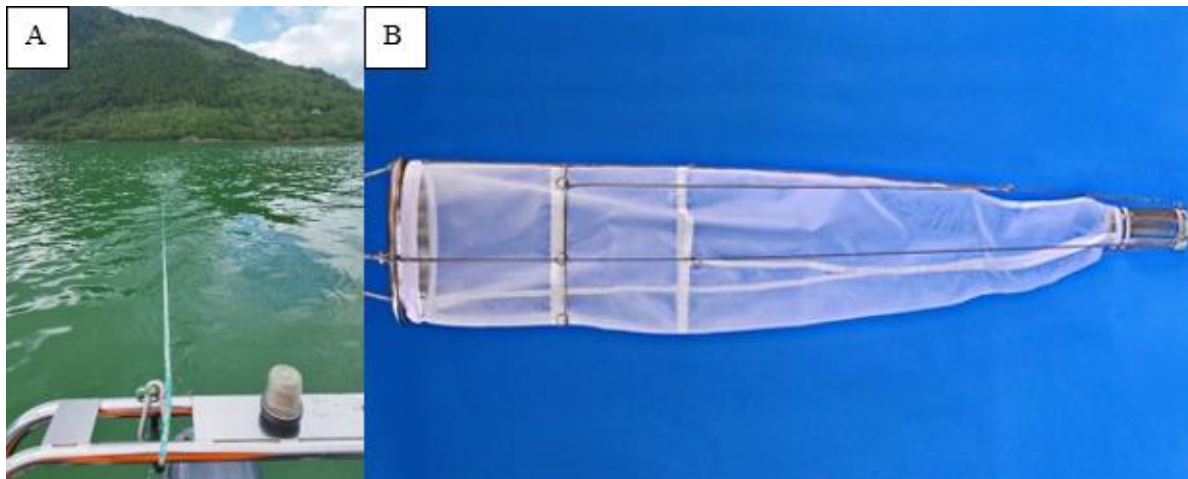




**Figure 7:** Plankton processing in the field. **A:** The equipment used for plankton processing. Ethanol, spray bottle for ethanol, spray bottle for water, sieve, and funnel. **B:** Rinsing down the plankton net with saltwater before taking the collection cup of. **C:** Spraying down the cup from the outside to get as much of the plankton growth of the mech net before taking the cup off. **D:** Rinsing out plankton out of the collection cup into the sieve using saltwater. Photo: Amanda Andersson. **E:** Plankton sample before rinsing it over into the sieve. **F:** Finished sample ready for storage. Pictures: Amanda Andersson/Ingvild Tryggestad.

### 2.2.2 Trawling with the plankton net after the boat

Plankton samples was taken using a WP2 net (Figure 8B) from KC-Denmark with a mesh size of 150  $\mu\text{m}$ , length of 2 m and a mouth width of 57 cm (KC-Denmark, 2023). The net contained an analog flow meter with back-run stop centred 10 cm inside the mouth of the net. This made it possible to know the volume of water passing through for each sample. The plankton net was towed behind the boat horizontally in between preservation of plankton collections (Figure 8A). It was attached to the back of the boat and towed with low speed. It was towed when we processed each sample and taken out of the sea in between pick up of the PPS. One bottle of plankton was collected at each site, and the method of processing these samples was similar with the process for the PPS.



**Figure 8:** **A:** Thawing the plankton net behind the boat in Hardanger 2022. Photo: Amanda Andersson. **B:** WP2 plankton net. Photo: [www.kc-denmark.dk](http://www.kc-denmark.dk)

### 2.3 Containing the samples

Plankton samples of 50ml tubes/250ml bottles were topped with absolute ethanol after arriving from fieldwork. They were placed in the freezer at the cabin each evening. They stayed in the freezer the entire trip, and at the end of the trip they were shipped to NINA lab in Trondheim for processing (Figure 9). At NINA they were stored in the freezer at  $-20^{\circ}\text{C}$ . The lab analyses were started in September 2022 with the 50 ml tubes. And continued with the 250 ml bottles in October 2022. The analyses continued during the fall and was finished in January 2023.

### 2.4 Laboratory protocol

The following laboratory protocol were used for analysing the plankton samples collected in Hardanger by the Centre for Biodiversity Genetics (NINAGEN) at NINA in Trondheim:

Depending on the size of the plankton samples stored in ethanol, the samples were first homogenized in 50 ml tubes or 250 ml bottles containing a mix of Matrix-A and Matrix-D beads (MP Biomedicals) (Figure 9), at 1600 rpm for 2 min using a FastPrep-96 homogenizer (MP Biomoedicals). From every sample three subsamples of 500  $\mu\text{l}$  were collected and then transferred to three 1,5 mL Eppendorf tubes and dried in heating cabinet at  $56^{\circ}\text{C}$ . Following, every tube was added with 540  $\mu\text{l}$  ATL buffer (Qiagen) and 60  $\mu\text{l}$  proteinase K (Qiagen), and then vortexed and incubated at  $56^{\circ}\text{C}$  overnight. Extraction of DNA from every subsample was done using a MagMax Tissue 2.0 kit (ThermoFisher) on a KingFisher Apex robot (ThermoFisher) and eluted in 250  $\mu\text{l}$  AE buffer (Qiagen).

The DNA concentration of *L. salmonis* and *C. elongatus* was established using species-specific assays (McBeath et al., 2006) and a droplet digital PCR (QX200 AutoDG Droplet Digital PCR System, Bio-Rad Laboratories). In a total reaction volume of 22  $\mu\text{L}$ , the PCR included 3.64  $\mu\text{M}$  of forward and reverse primers, 0.86  $\mu\text{M}$  probe, dH<sub>2</sub>O, 10  $\mu\text{L}$  ddPCR™ Supermix for Probes and 1  $\mu\text{L}$  DNA template. As a positive control isolated DNA from *L. salmonis* and *C. elongatus* copepodids was used in every ddPCR run and dH<sub>2</sub>O as a negative control in every ddPCR run. Using the AutoDG Instrument (Bio-Rad Laboratories) droplets were generated, and PCR amplification was conducted in a Veriti 96-Well Thermal Cycler (Applied Biosystems). The sequential thermal cycling conditions were used: 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. To a QX200 Droplet Reader (Bio-Rad Laboratories) the PCR plates were transferred for automatic detection of the fluorescent signal in the droplets. The software QX Manager Standard Edition v1.2 (Bio-Rad) was used to segregate positive from negative droplets according to the manufacturer's instructions.

Based on the concentration estimated by QX Manager, template volume (1  $\mu\text{L}$ ), elution volume (250  $\mu\text{L}$ ), subsampled volume (500  $\mu\text{L}$ ) and total lysis volume (3.500  $\mu\text{L}$  for 50 mL tubes, and 175.000  $\mu\text{L}$  for 250 mL bottles) the total number of DNA copies in a sample was calculated. By dividing the total number of DNA-copies by 1.5 million DNA-copies, which is the estimated average DNA-copy number per lice, the number of larvae was estimated.



**Figure 9:** Processing of plankton samples at the lab at NINA. Photo: Ida Pernille Øystese Andersskog.

## 2.5 Data treatment and statistical analyses

The statistical analyses and visual presentation of the data was conducted using R 4.2.3 and RStudio. The raw data was treated in excel.

77 samples were collected for the passive sampler and 20 samples for the plankton net. During inspection of visual data, some outliers was removed from the triplicates. The decision was made on the ground that it looked like something had gone wrong during lab analyses. If outliers hadn't been removed, they would probably have disrupted further analyses. In this study, the focus was comparisons of *L. salmonis* with different methods, therefore *C. elongatus* were removed from the database. Additional reasons were the small amount of *C. elongatus* found in the collected samples (Tryggestad, 2023), combined with lack of measurements of the species in methods of comparison.

The data from the plankton sampler and plankton net are treated the same way. The mean larvae are taken from the three subsamples and presented as the amount of *L. salmonis* found in each sample. The raw data for the IMR model and sentinel cages are provided from IMR. For the IMR model, the data is the estimate of *L. salmonis* in each location (M12, M11, M15, M4, M6 and M7). To compare the data, the IMR data was summarized based on number of days the PPS stood out in the sea. During our fieldwork there was two periods with smolt in the sentinel cages. The data for the sentinel cages are the average number of *L. salmonis* per fish for each location, divided into smolt cage period 1 (SCP 1) (12.05.22-26.05.22) and smolt cage period 2 (SCP 2) (26.05.22-09.06.22). The data from the IMR model and sentinel cages are a representation of the data paired up with the given sample (77 samples in total) taken by the PPS on given time and location (Table A1 and A2)

The significance level was set to  $p < 0,05$ . During visual look at the data with histograms and histograms with log transformations, it was clear that the data was not normally distributed. Because of this Spearman's correlation rank was chosen for further analyses. For visual presentation ggplot and multiplot were used.

## 3 Results

### 3.1 Database

For each of the methods there were variations in how much data there was available (Table 2). For the PPS there were 77 samples in total, and out of them 43% of the samples contained *L. salmonis*. The plankton net had 70% of the samples containing *L. salmonis* out of 20 samples. IMR model had no data for M12 (Table A3), and based on the 77 data points, 80,5% of the data provided information about *L. salmonis*. For the sentinel cages 96,1% of the 77 data points were providing information about *L. salmonis* during the fieldwork period. The three data points lacking data for the sentinel cages represent one cage of fish with no data and this was due to a hole in the sentinel cage in SCP 1 on location M15 (Table A1). This resulting in 3 datapoints out of the 77 samples where there was no information about average lice per fish.

**Table 2:** Amount of data for each of the methods during the fieldwork period from 10.05.2022 to 10.06.2022. Data representation based on the 77 datapoints. PPS representing total amount of *L. salmonis* found in the 77 samples. Plankton net representing total amount of samples with *L. salmonis* out of 20 samples. IMR model representing data available for the 77 samples. Sentinel cages representing data based on average lice per fish available for the 77 samples taken by the PPS.

|                                 | <i>L. salmonis</i> | No<br><i>L. salmonis</i> | No<br>data | N  | % Samples<br>of 0<br><i>L. salmonis</i> | % Samples<br>with no data | % Samples<br>with<br><i>L. salmonis</i> |
|---------------------------------|--------------------|--------------------------|------------|----|---|---------------------------|---|
| <b>Passive plankton sampler</b> | 33                 | 44                       | -          | 77 | 57%                                     | 0%                        | 43%                                     |
| <b>Plankton net</b>             | 14                 | 6                        | -          | 20 | 30%                                     | 0%                        | 70%                                     |
| <b>IMR model</b>                | 62                 | -                        | 15         | 77 | 0%                                      | 19,5%                     | 80,5%                                   |
| <b>Sentinel cages</b>           | 74                 | -                        | 3          | 77 | 0%                                      | 3,9%                      | 96,1%                                   |

### 3.2 Number of *L. salmonis* during the sample period

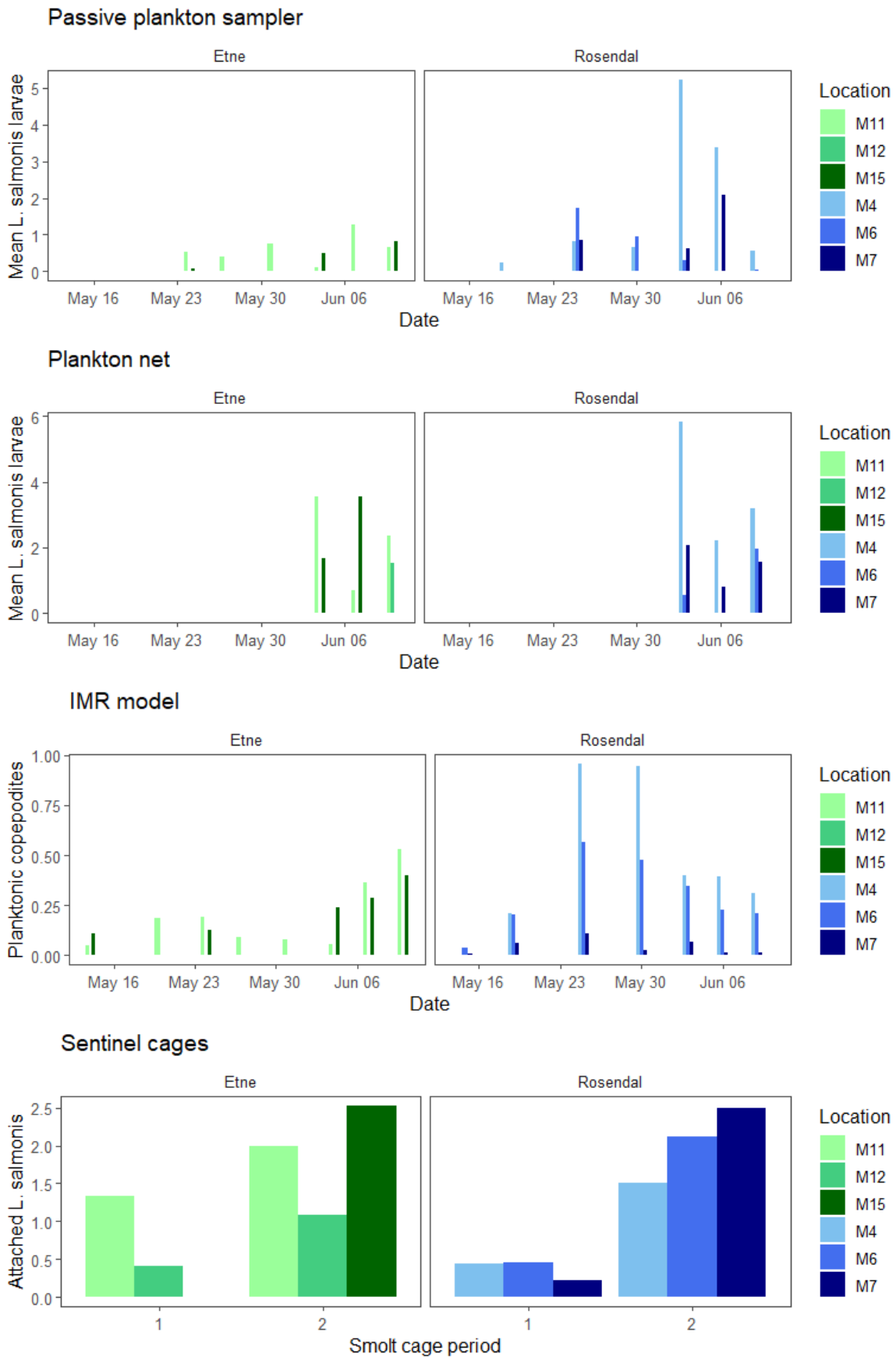
In Figure 10 Passive plankton sampler, the average of *L. salmonis* for sampler A and B for each location collected during the fieldwork period are visually displayed for each of the sampler periods (Table A1 and A2) for Etne and Rosendal. For Rosendal there was collected overall more *L. salmonis* over the course of the fieldwork compared to Etne. The highest amount collected was ~7,84 *L. salmonis* on M4 in sampler period 5 in Rosendal (Table A2) compared to ~2,36 *L. salmonis* for M11 as the highest amount measured in Etne in sampler period 7 (Table

A1). Sample period 5 for Rosendal lasted for 4 days, and the same goes for sample period 7 for Etne. The highest amount collected for M15 was ~1,09, for M6 it was ~3,42 and for M7 it was ~4,1. The PPS did not collect any *L. salmonis* for M12 located in the national salmon fjord in the area. There can also be seen a trend where there are collected more *L. salmonis* in the second half of the field work in Rosendal.

In Figure 10 Plankton net, the collected *L. salmonis* are shown. There were only samples taken with the plankton net in the second half of the total fieldwork period, from 31.05.2022 to 10.06.2022. The plankton net collected *L. salmonis* at every location. The highest amount collected was 03.06.2022 with ~5,83 *L. salmonis* in Rosendal at M4. In Etne the highest value was ~3,55 at M11, with M15 being very close with ~3,54. The only collection that showed *L. salmonis* at M12 was on 10.06.2022 with ~1,52 mean *L. salmonis*. At M6 the highest value was ~1,96 and at M7 ~2,08. The plankton net shows regular collection with only three samples having 0 *L. salmonis*.

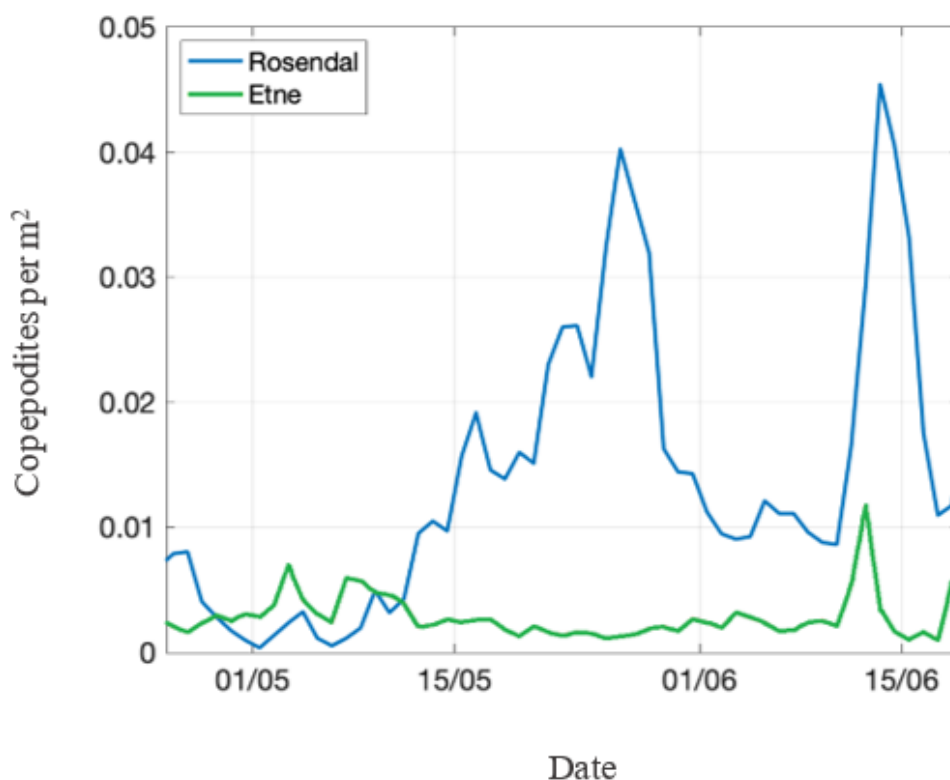
The IMR model data displayed in Figure 10 IMR model, shows evenly distributed data with gradual increase, and decrease during the sampling period. There were no values with 0 predicted *L. salmonis*, but there lacks data for M12. In the start of June there were an increase in *L. salmonis* for Etne. In Rosendal it's an increase at the end of May and a slow decrease in the start of June. The values sticking out the most are at M4 with ~0,94 *L. salmonis* predicted. The lowest predicted value is at M7 with ~0,01.

In Figure 10 Sentinel cages, the average number of *L. salmonis* per fish for SCP 1 and SCP 2 for the sentinel cages are presented. The average number of *L. salmonis* per fish are showing for each of the locations in the different periods. There can be seen that for both Etne and Rosendal there is more lice per fish in SCP 2. In SCP 1 there are more *L. salmonis* in Etne compared to Rosendal, while in SCP 2 there are a total amount of more *L. salmonis* in Rosendal, but not very far from how it is in Etne in SCP 2. The highest single value is measured for M15 with ~2,53 *L. salmonis* per fish, M7 measures highest value of ~2,5 *L. salmonis* per fish, so very close to M15. In SCP 1 in Rosendal there are overall little *L. salmonis* for all the locations. There are no data for M15 in SCP 1 due to hole in the sentinel cage (Table A3).



**Figure 10:** Amount of *L. salmonis* for each method during the fieldwork period from 10.05.2022 to 10.06.2022.

Figure 11 shows the mean value of infectious copepodites per m<sup>2</sup> in the top two meters of the water column within a square area that covered 8km x 8km around the smolt cages positions IMR has in Etne and Rosendal. The top two meters were chosen for better comparison due to the PPS floating in the first top meter. In the beginning of May there was little variations in copepodites per m<sup>2</sup> both Etne and Rosendal. There are overall more copepodites and higher variations and peaks for Rosendal than Etne (Figure 11). The highest point for Rosendal and Etne happens around the same time, but the values differ with over 0,03 copepodites per m<sup>2</sup>. Rosendal has two high peaks, one at the end of May and one in the middle of June after the fieldwork period.



**Figure 11:** Linear graph of copepodites per m<sup>2</sup> for Rosendal and Etne in the sampling period 2022. Data from Anne D. Sandvik, IMR.

### 3.3 Correlation

For investigation of the correlation between the different methods, Spearman's correlation rank was used (Table 3). Spearman's correlation rank was chosen because when looking at the data, it was not normally distributed or had a linear distribution, the variables for the methods also have different scale. Spearman is a good choice in this situation, due to its ability to discover monotonous relationships between variables even when the data is not linear and assesses the strength and direction of the correlation regardless of the scale of the variables.



The PPS and plankton net had a correlation coefficient (rho) at 0,33. That indicates a weak to moderately positive correlation between the variables. This indicates that a higher value of one variable, will be associated with a higher value of the other variables. The p value shows 0,04, this indicates a significant association between the two variables. This means that the probability of observing an association of this magnitude or greater by chance is less than 5%. That means that it is unlikely that the result is due to chance, and that there is a relationship between the two variables. The p value gives enough evidence to reject the null hypothesis. The correlation between the PPS and IMR model show the most correlation with a rho of 0,47, indicating a moderately positive relationship. The p value is also the one showing most significance, with <0,001. This indicates less than 0,1% probability of observing this association by chance. The PPS and sentinel cages have a rho at 0,28, indicating a weak to moderately positive correlation and a p value of 0,016. This indicates that there is less than 1,6% of observing an association like this by chance.

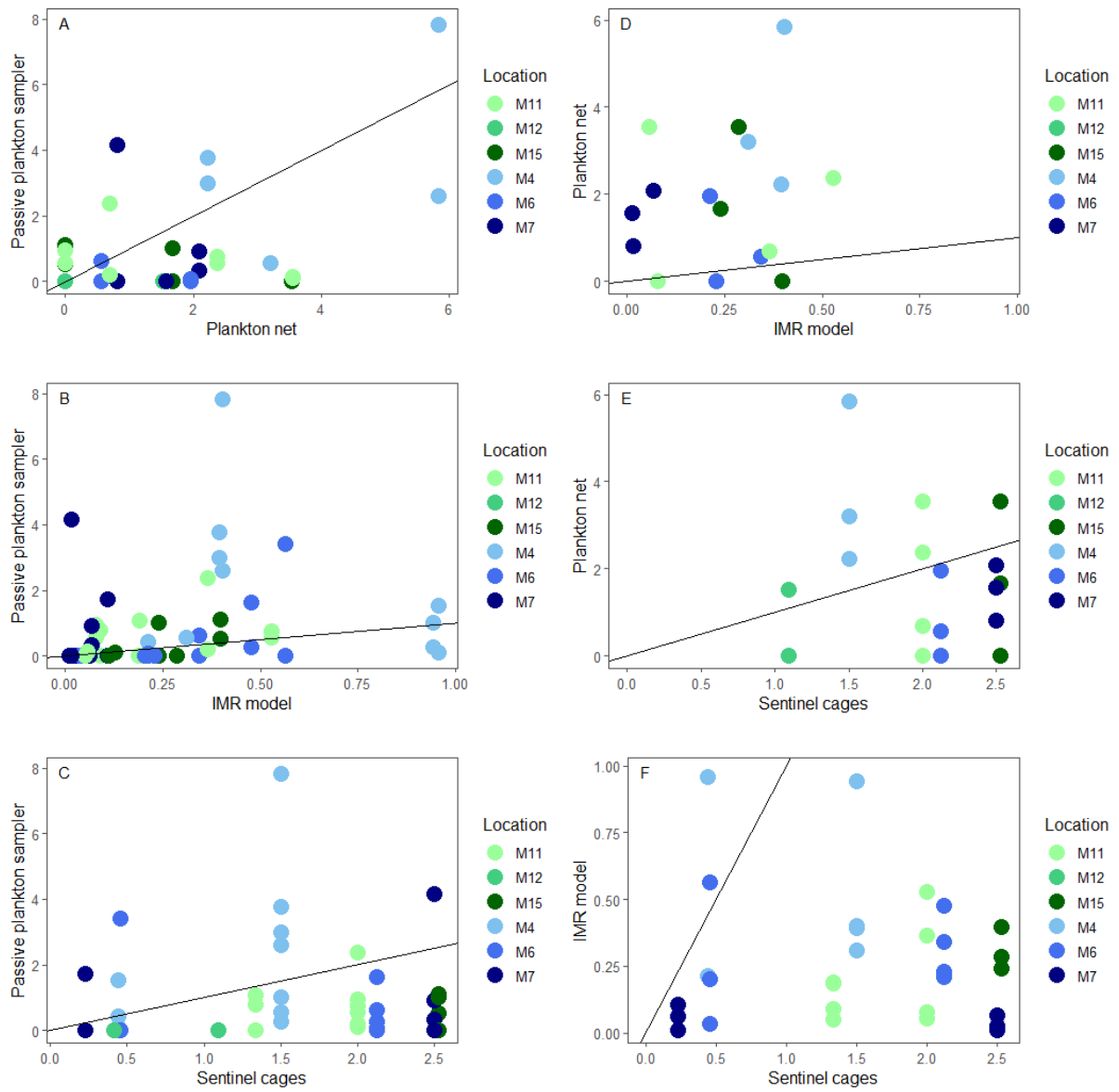
The plankton net has a rho of 0,16 with both IMR model and sentinel cages. This is a very weak positive correlation, indicating that there is not much association between those variables. Both p values do not show any significance at 0,4 and 0,34. This indicates that there is not enough evidence to reject the null hypothesis. The results do not provide strong evidence for real association between the methods. IMR model and sentinel cages are the two methods with the lowest rho being 0,03, this is almost 0, and indicates no correlation between those two. The p value is 0,85, showing that there is no statistical significance between the two variables. The p value tells us that the probability to observe this correlation again or greater correlation by chance is 85%, so there is not enough evidence for the presence of real association.

**Table 3:** Spearman's rank correlation. Samples size (N), sum of observations (S), correlation coefficient (R) and p-value (p) are displayed.

|                | Passive plankton sampler                                | Plankton net                                 | IMR model                                   |
|----------------|---|--|---|
| Plankton net   | N = 20<br>S = 6617,7<br>R = 0,33<br><b>p = 0,04</b>     |  |   |
| IMR model      | N = 77<br>S = 2110<br>R = 0,47<br><b>p = &lt; 0,001</b> | N = 20<br>S = 4188<br>R = 0,16<br>p = 0,4    |   |
| Sentinel cages | N = 77<br>S = 48606<br>R = 0,28<br><b>p = 0,016</b>     | N = 20<br>S = 8324,4<br>R = 0,16<br>p = 0,34 | N = 77<br>S = 33342<br>R = 0,03<br>p = 0,85 |

The methods use different measurements such as lice/time, lice/m<sup>2</sup>, and lice/fish, therefore the scale is different (Figure 12), and that is why there was used correlation rather than a direct comparison of values. In Figure 12 A the relationship between the PPS and plankton net are plotted for visual interpretation. The pattern of the plot shows somewhat weak linear pattern, so there can be seen that there is some correlation like the rho coefficient at 0,33 indicates (Table 3). Some of the variables sticks out for M4, being much higher than the average values, this is also shown in Figure 12 A. The plot for the PPS and IMR model is shown in Figure 12 B. There are linear tendencies, but there can also be seen some outliers that differ from the line. The outlier sticking out the most are from M4. There is high density of plotted values around the line from 0 to 0,5. The overall plot shows there are moderately positive correlation like the rho coefficient at 0,47 indicates (Table 3). The PPS and sentinel cage method are plotted in Figure 12 C. There is a linear pattern showing, and M4 is sticking out the most with one outlier. There are not many plotted values along the line, but there can be seen a positive correlation. The rho coefficient is weak to moderately positive being 0,28 (Table 3), and that shows in the pattern.

The plankton net and IMR model are plotted in Figure 12 D. There can be seen no linear pattern, the pattern goes more in a vertical direction and the line goes in a more horizontal direction. Here there can also be seen an outlier from M4 sticking out. The rho for these methods is 0,16 (Table 3), this shows in the plot since there cannot be seen any specific correlation between plankton net and IMR model. For the plankton net and sentinel cages plotted in Figure 12 E, there are few plotted values close to the line, and there cannot be shown any clear or specific linear pattern, this is what should be expected with the rho coefficient being 0,16 (Table 3). There are more of a square pattern happening in lower right corner, with one value sticking out for M4. For the IMR model and sentinel cage plotted in Figure 12 F, there is clear that there is no specific correlation since the pattern is not linear. The line goes more vertically while most of the plotted values are to be find in lower right corner. The rho for these two methods is the lowest rho of all the comparisons being 0,03 (Table 3), and that is well shown in the plot that there are little to no correlation.



**Figure 12:** Correlation plot for each of the methods.

## 4 Discussion

The aim of this master project was to compare the ability of the Passive Plankton Sampler (PPS) to other methods that are being used to estimate and measure sea lice levels in the sea. The PPS has been compared separately with the other methods which were the WP2 plankton net, the IMR model and IMR sentinel cages.

### 4.1 Database

The database is small and have some lacking data (Table 2). The plankton net only has data for the second half of the fieldwork, there is no IMR model data for M12, there is no data for sentinel cages for M15 in SCP 1 due to holes in the cage (Table A3). The PPS has lacking data for different times and locations during the fieldwork period (Table A1 and A2). This contributes to a database with holes in it for the different methods, this makes the database used for the comparison less robust and can have some effect on the overall results. With only 77 datapoints and even less for the plankton net the results can be limited (Table 2). With the Spearman correlation coefficient there is possible to calculate the exact probability of rho. But even if that is possible, a small database can potentially result in bigger uncertainties and wrong conclusions. So, there is need of caution. With bigger sample size there will be less variability for rho, and the result will be more reliable (De Winter et al., 2016). This is a pilot study so to provide perfect data in the first round would have been difficult to achieve. In the first round it is possible to see indications on whether the PPS is a technology that can work as a method for measuring sea lice levels in the sea.

### 4.2 Number of *L. salmonis* during the sample period

The number of *L. salmonis* varied during the fieldwork period for the PPS (Figure 10). It did not evenly increase and decrease at the different data points. This could be due to the amount of current passing through and the difference in days the PPS stood out in the ocean (Table A1 and A2). The *L. salmonis* distribute itself in a very variable way in the fjord and coastal areas (Sandvik et al., 2019), this can help explain the non-consistent increase and decrease in *L. salmonis* levels (Figure 10) for the PPS. Because there was too little data collected when it comes to the flow meter due to failure in field, there is not a way to prove if the big variations

between the different point was due to current or the days. But when comparing it to the Plankton net, that was only towed in a short amount of time, both measured high values for M4 in sample period 5 (Table A2). There were some similarities with the measurements, but because of the limited data for the plankton net it is not a clear trend here. The plankton net collected *L. salmonis* more consistently and has fewer samples with 0 larvae than the PPS (Table 2). This was not expected since the time of the net tows was much less than the time the sampler stood in the sea. And for the samples where we successfully have the flow meter data, the flow was much larger for the PPS. Plankton nets in different varieties is a well-known method for collecting plankton samples, shown in previous studies either by horizontal or vertical net tows (á Norði et al., 2015; á Norði et al., 2016; Jevne et al., 2021; Nelson et al., 2018).

The IMR model gave more continuous data through the sampling period, this made it easier to see if there were trends with the PPS. It showed that there were overall more *L. salmonis* in Rosendal than Etne for both methods. It also showed very clearly that the PPS provided more fluctuating data than the IMR model. Due to the lack of samplers present at M15 (Table A1) it is hard to know how it would have looked in the start of the fieldwork, but for the IMR model there was little *L. salmonis* at that time but increased at the end of the period. Since the model is based on counting of *L. salmonis* on the fish at fish farms, manually by humans (Havforskningsinstituttet, 2019a), there are room for human error, and this causes uncertainties (Sandvik et al., 2020). The small number of fish they count compared to the total amount in the net pens, results in an estimate of copepodites in the sea that may vary greatly. The good thing with the model is that it gives an overview throughout the year on how the *L. salmonis* situations are and has been possible to scale to cover all of Norway. The model has been tested based on other methods, and there are other models predicting the same (Vollset et al., 2021). It has also been quality assured and validated against oceanographic measurement data (Vollset et al., 2022) and it is based on existing and well documented methods (Sandvik et al., 2020). This probably makes the model one of the most accurate estimates out of the methods that has been compared. However, salmon lice copepodites can spread through the currents in many directions due to the many different current components. This makes it hard to determine the spread of lice larvae (Asplin et al., 2014) and there are several knowledge gaps when modelling the spread of sea lice (Karlsen et al., 2016). There has also been posed that there are more uncertainties connected to modelling lice than using sentinel cages, trawling and fish traps for sea trout given that time and space is covered (Boxaspen et al., 2019). The PPS could potentially

help give a picture in combination with models on how the distribution of sea lice varies. Since the PPS can cover different parts of the fjord, and monitor over time, it could possibly discover variations and patches of sea lice that is not that easy to predict with a model since the model predicts quite even values throughout the season (Figure 10).

The sentinel cages visually look very different due to the nature of the method. This method showed the least difference between Etne and Rosendal. It showed lower values in SCP 1 and higher in SCP 2 and this is consistent with what Nilsen et al. (2022) has concluded. The infectious pressure is also higher at M15, M6 and M7 (Figure 10). It had no smolt at M15 in SCP 1, but when looking at how similar patterns the data showed between SCP 1 and SCP 2 (Figure 10), it can be assumed that M 15 would show same pattern in SCP 1 and have higher values than M12 and M11. The sentinel cages also measured *L. salmonis* at M12 in Etne in both periods. This could indicate that being a fish in the sea is different than having a net in the sea. The sentinel cages could possibly give a good picture taking the fish into account, even when smolt placed in a cage do encounter less sea lice than actively swimming smolt (Vollset et al., 2021). The *L. salmonis* are specialised for host selection, it locates, identifies, and settles on a host using a variety of senses in areas where the host are not always continuously present (Mordue & Birkett, 2009). This strengthens the sentinel cage method and is an aspect the PPS and the other methods does not have. This could be the reason the sentinel cage has more *L. salmonis* at M12 than the other methods (Figure 10). The PPS and sentinel cage showed the same trend regarding more lice for Rosendal in the end of May and start of June. For Etne it is harder to tell. Compared to the other methods, the sentinel cages were the one method that did not have more lice at M4 in Rosendal. It is interesting how it has lower values for M4 compared to the rest, but at the same time measures values for M12 when no other except for one net tow for the plankton net does.

In Figure 11, copepodites per m<sup>2</sup> for Rosendal and Etne is displayed. This figure does not consider the different locations like the IMR model does (Figure 10), but as we can see for Figure 11, the pattern showed the same trend for the PPS. For Etne it showed low values with a peak in the start of May, and we can see that there is a similar pattern for Rosendal, two peaks in the end of May and early June. With the plankton net there was not enough data points to see similar trends (Figure 10). The sentinel cages have a whole different pattern to be able to see clear trends other than that there also were more lice at the end of the fieldwork period for Rosendal (Figure 10). What can be seen in Figure 11 is that there is a high peak after our

sampling period ended. There would have been useful to have an extended sampling period for the PPS, to see if this trend would be similar.

There can also be seen a trend where there was on average more *L. salmonis* to be found near aquaculture facilities (Figure 3 and 10). These indications are consistent with IMR, NINA and MT, that expresses that there is a threat to wild fish in areas where there are fish farming and potential for escaped farmed fish (Bjørkan & Hauge, 2019). A study conducted by Nelson et al. (2018) investigated two methods for sampling sea lice larvae. A submersible pump and a traditional round zooplankton net was used around aquaculture facilities and reference sites. The study was carried out from 2012 to 2016, and they found during the study that there were more sea lice around the aquaculture facilities than the reference sites. This is also consistent with the trend found with the PPS and the other methods (Figure 3 and 10), as well as the indicated variation in collected *L. salmonis* found within sites by Tryggestad (2023). Nelson et al. (2018) also found that there are low densities of lice for both sampling methods in his study and argues that the choice of sampling gear should be based on the research question. Some methods are better suited for certain areas. This applies to the general need of several methods to give a good picture on the sea lice situation (Vollset et al., 2018). In different studies (á Norði et al., 2015; á Norði et al., 2016; Nelson et al., 2018) the sea lice level found is often less than 1 lice per m<sup>3</sup>, so that the PPS was able to collect *L. salmonis* through the fieldwork is a good sign and proves the ability, and the number of samples with 0 *L. salmonis* (Table 2) were then not an abnormal finding.

### 4.3 Correlation

The PPS showed positive correlation between all the methods, but the correlation varied. There were indications of weak to moderately positive correlation between the PPS and the plankton net, and the same was seen for the PPS and sentinel cages. The PPS and the IMR model had the highest indication of correlation, indicating a moderately positive correlation. The correlation between these methods is also supported by a significance that indicates there was evidence to reject the null hypothesis. But the size of the database is quite small, and that could result in source of error, so even when the test is showing this result, it can't be said for sure (De Winter et al., 2016). There could be interpreted that when one of the values for each of the methods goes up, it is likely that it could happen for the PPS as well. There was also the aspect of the data being quite different for some of the methods, this could also be source of error, and not

give the accurate picture of the situation regardless of the test. Since all the methods had in common the location and time of year, there could be a causal connection between the methods.

The plankton net, IMR model and sentinel cages when compared to each other showed almost none to no correlation. There was also no significance shown (Table 3). It is interesting how these were so different compared to each other, but when comparing the PPS, it shows correlation with all the methods. This is something that gives more reason to interpret the results with caution.

#### 4.4 The comparison

The different types of measurements and different life stages of *L. salmonis* for each method, resulted in different scale. Because of this, the option was to look at trends and correlation rather than direct comparisons of values. The PPS and plankton net collected planktonic lice (Langford, 1953), the IMR model estimated the planktonic copepodite levels (Sandvik et al., 2019) and the sentinel cages looked at the *L. salmonis* from attached copepodite stage and further development (Vollset et al., 2022). When a method is difficult to compare to the rest, it is hard to find out whether a method is trustworthy and reliable. But with the correlation test it was possible to investigate at some extent. To be able to manage fish farming based upon the knowledge about how much of an impact the sea lice have on wild fish, it should be possible to validate the methods that were being used. Several methods and models would be needed to be able to know the full picture of the dynamic nature, as mentioned by Vollset et al. (2018).

#### 4.5 Challenges

During this pilot study, errors occurred. Problems that were faced included: battery failures, insufficient waterproofing of electronics, a disappearing PPS, drifting PPS, weather conditions such as waves, rain, wind and fog, a shortage of replacement parts, and defect floating tubes which needed to be glued and resulting in lack of PPS at some sites. Due to the environment and shifting weather, the PPS was tested in many ways. This made it possible to find errors in the design and room for improvement. Due to the low reliability of the batteries and the electronics that were not well waterproofed we failed to get consistent flowmeter data. Due to the weather conditions, it made it difficult to collect the PPS on certain days and too risky on other days, resulting in different time intervals the PPS stood out in the sea (Figure A1 and A2). If the PPS should be managed manually, this will make it difficult to maintain equal intervals



of days of collection. In some locations the depth was much deeper and the locations more exposed, this resulted in the loss of one sampler, and the drifting of another one due to the anchor becoming dislodged (Table A1 and A2). Some of the floating tubes on the samplers was not glued properly, making the start of the fieldwork having to take up some of the PPSs for repair (Table A1 and A2). There was not much backup in numbers of samplers and cups, making some sites lack these. Regarding the design, there are many aspects that needs to be improved.

When it came to the handling of the PPS in the water it was a job for two, and much easier with three people. There was need for one to drive the boat and one person to pick up the sampler. If some later versions of the sampler where to be placed alongside the coast in the future, there will be two people needed unless there is some change in design of some sort. Regarding the WP2 plankton net there was possible to manage this as one person since you thaw it behind the boat and can stop the speed of the boat as you handle the net. This makes the plankton net require less staff, at the same time, most jobs out on the sea should have two people in the boat for safety reasons.

#### 4.6 Strength and weaknesses

The different methods, all has strength and weaknesses (Table 4). If looking past the challenges faced during fieldwork, the PPS's ability to stay out for several days is an advantage. This makes it possible to have big volumes of water passing through and collect large samples, but to estimate correct numbers there is need of the flowmeter to work as it should. Many studies use lice per cubic meter (á Norði et al., 2015; á Norði et al., 2016; Nelson et al., 2018) or lice per square meter (Asplin et al., 2020), so to be able to determine lice levels like that, would make better comparisons for the PPS with other studies. When viewing the PPS alongside the plankton net, there was clear evidence of the advantages the plankton net poses with the simple design and easy to use in field. This showed that the PPS is maybe too complicated and possibly made the process of collecting plankton more time consuming than it needed to be. The traditional flowmeter for the plankton net was reliable, but to collect samples of large volumes would possibly be more time consuming than for the PPS since it's operated manually. The model is a good method to get continuous data and overview of the *L. salmonis* infestation. It has been through validations (Vollset et al., 2021) and are maybe one of the most secure methods. But it is based on counting of lice at the fish farms, and that causes uncertainties (Sandvik et al., 2020). Also, there is the aspect of the ocean being unpredictable, and there is

hard to model nature and give exact measurements (Skarðhamar et al., 2018). The sentinel cages use living smolt. This can be a strength and a weakness, depending on the angle. To use fish gives the advantage of simulating more closely how the sea lice would seek the fish and attached in the given area where the cages are placed. At the same time, there is the animal welfare aspect (Lovdata, 2010) with placing 30 salmon smolt in a cage for fourteen days and after that period discharge of them (Vollset et al., 2022). During the SCP 1 there was also many sentinel cages in the area that lacked fish due to high mortality due to the transport (Nilsen et al., 2022). It has also been reported previous years, that some cages have been lost (Nilsen et al., 2019). This causes loss of data, loss of fish and a lost effort. To handle living fish requires most likely more cost and effort to put out in the sea, transport and to grow the stock of fish, there is also required staff for maintenance. This potentially makes the total cost much higher than for the PPS and gives the PPS an advantage. Still the sentinel cages are one of the most certain methods used to look at lice infestations (Boxaspen et al., 2019), and maybe why it is chosen even when the effort and costs are quite high.

**Table 4:** Strengths and weaknesses with the methods.

| <b>Method</b>                   | <b>Strength</b>   | <b>Weaknesses</b>  |
|---------------------------------|---|--|
| <b>Passive plankton sampler</b> | Collects plankton without human presence. Can stay out in the sea for several days.                                   | Issues with design causing problems. Complicated design.   |
| <b>Plankton net</b>             | Easy to use. Simple design that works well. Can collect horizontally and vertically.<br>Many studies to compare with. | Can be time consuming to sample large volumes. Collects small amounts.                             |
| <b>IMR model</b>                | Continuous information. Verified by other methods.  | Nature is unpredictable, and human error is common, the model is based on variables within nature. |
| <b>Sentinel cages</b>           | Realistic aspect of the sea lice seeking the fish. One of the more certain methods.                                   | Animal welfare aspect. Potentially high cost and maintenance.                                      |

#### 4.7 Future work and perspectives

During the project, it is found that the PPS does collect plankton, and the sea lice present in the sample can be analysed and determined. To be able to compare them in a good way, the PPS should be developed further. Since this is a pilot study, there is not much similar experiments being done. Suggestions for improvement is waterproof boxes for the battery, there should be used more reliable batteries than power banks, and the PPS should be floating regardless of the weather. The flow meter system should be developed to be able to store the data in case of

something going wrong with the battery and there should be sensors at every PPS to ensure enough data. There should be done further testing to get enough data to do robust analyses and the volume of water should be considered. Then there will be more ground of deciding what usage the plankton sampler has in a management perspective regarding the sea lice issue.

None of the methods has the answer on how many sea lice there is in the sea, since no single model and parameter can provide datapoints that reflects the complexity of nature (Vollset et al., 2018). Karlsen et al. (2016) points out that there are several knowledge gaps when it comes to modelling the spread of sea lice, predicting the habitat use of wild fish, and predicting lice on wild fish by linking predictions to observe levels of lice on wild fish. Several of the methods are different and look at sea lice in different stages and scales. There should be a broad variation of methods used, and different angles to approach how to measure sea lice to give a big perspective. With traditional field methods alone, there is not possible to get a total overview of the *L. salmonis* infestation. But the IMR model is developed to fill out those observations in time and space (Sandvik et al., 2019). But it cannot be expected to give exact measurements in time and space due to the variable conditions in the ocean and fjords (Skarðhamar et al., 2018). For management use after solid testing of the PPS, it could contribute to managing sea lice. On its own it would not give enough basis to regulate the aquaculture industry, but used in perspective with all the other methods it could contribute to a part of the traffic light system, to ensure a broad base for making decisions. The PPS could contribute to give more insight to the complexity of the *L. salmonis* issue, and the potential of the PPS could be great, if it is placed in much larger scale along the Norwegian coast, both in sheltered areas, exposed, near aquaculture facilities and in salmon fjords. There should also be placed more samplers at one location to avoid errors like we experienced (Table A1 and A2).

## 5 Conclusion

The study has shown that there was possible to collect plankton with the new PPS technology and determine lice levels in each sample using ddPCR. This was consistent with what the first hypothesis states. There were not enough evidence and enough data to make a solid conclusion, but the study showed indications of correlation and by testing out this technology there is taken a step in the right direction by gaining insight to the PPS and different methods used for sea lice purposes. The study showed how difficult all the methods were to compare due to the different scales of each method. A lot of the methods that are used for estimating and measuring lice in the sea are all very different, and they predict but can't tell for sure how much salmon lice there is.

The results indicated that the PPS had weak to moderately positive correlation with the plankton net and sentinel cages. With the IMR model it indicated moderately positive relationship. This showed that there were some connections with the measurements and estimation of *L. salmonis*. The trend for the different methods showed that there were overall more *L. salmonis* in Rosendal than Etne during the sampling period, and this was something all the methods had in common (Figure 10). Hypothesis 2 cannot be concluded for either of the methods compared for sure, but the project has shown promising indications.

There should be further testing of the PPS. There should be a better database, with more data and different measurement like water flow, so there is possible to say something about how much sea lice there is by volume. This could potentially make the comparison with the plankton net and IMR model more solid. There should be tests over longer periods and during different seasons. That would make it easier to see trends and give more base for statistical testing. If further testing is being done, and there is a basis to determine the ability of the plankton sampler, it could potentially contribute to research and monitoring of sea lice. It could potentially work as an additional monitoring method in the NALO program or the Traffic light system to give broader grounds for decision making.



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## Appendix A

**Table A1:** Each sample period for the PPS and where there was lacking a PPS for each location in Etne during the fieldwork period.

| ETNE                        |         |         |            |         |            |            |            |
|-----------------------------|---------|---------|------------|---------|------------|------------|------------|
| DATO                        | PERIODE | M11A    | M11B       | M12A    | M12B       | M15A       | M15B       |
| 10.05-14.05                 | 1       | Sampler | No sampler | Sampler | No sampler | Sampler    | Sampler    |
| 14.05-20.05                 | 2       | Sampler | Sampler    | Sampler | Sampler    | No sampler | No sampler |
| 20.05-24.05                 | 3       | Sampler | Sampler    | Sampler | Sampler    | No sampler | Sampler    |
| 24.05-27.05                 | 4       | Sampler | Sampler    | Sampler | Sampler    | No sampler | No sampler |
| 27.05-31.05                 | 5       | Sampler | Sampler    | Sampler | Sampler    | No sampler | No sampler |
| 31.05-04.06                 | 6       | Sampler | Sampler    | Sampler | Sampler    | Sampler    | Sampler    |
| 04.06-07.06                 | 7       | Sampler | Sampler    | Sampler | Sampler    | Sampler    | Sampler    |
| 07.06-10.06                 | 8       | Sampler | Sampler    | Sampler | Sampler    | Sampler    | Sampler    |
| <b>Periods with sampler</b> |         | 8/8     | 7/8        | 8/8     | 7/8        | 4/8        | 5/8        |

**Table A2:** Each sample period for the PPS and where there was lacking a PPS for each location in Rosendal during the fieldwork period.

| ROSENDAL                    |         |         |            |         |            |         |         |
|-----------------------------|---------|---------|------------|---------|------------|---------|---------|
| DATO                        | PERIODE | M4A     | M4B        | M6A     | M6B        | M7A     | M7B     |
| 11.05-15.05                 | 1       | Sampler | No sampler | Sampler | Sampler    | Sampler | Sampler |
| 15.05-19.05                 | 2       | Sampler | Sampler    | Sampler | No sampler | Sampler | Sampler |
| 19.05-25.05                 | 3       | Sampler | Sampler    | Sampler | Sampler    | Sampler | Sampler |
| 25.05-30.05                 | 4       | Sampler | Sampler    | Sampler | Sampler    | Sampler | Sampler |
| 30.05-03.06                 | 5       | Sampler | Sampler    | Sampler | Sampler    | Sampler | Sampler |
| 03.06-06.06                 | 6       | Sampler | Sampler    | Sampler | Sampler    | Sampler | Sampler |
| 06.06-09.06                 | 7       | Sampler | No sampler | Sampler | Sampler    | Sampler | Sampler |
| <b>Periods with sampler</b> |         | 7/7     | 5/7        | 7/7     | 6/7        | 7/7     | 7/7     |

**Table A3:** Showing the methods that is compared with the PPS and the lack of data for each of the method during the fieldwork period.

|            | <b>IMR model</b> |             | <b>Sentinel cages</b> |             | <b>Plankton net</b> |             |
|------------|------------------|-------------|-----------------------|-------------|---------------------|-------------|
|            | 10.05-27.05      | 31.05-10.06 | 10.05-27.05           | 31.05-10.06 | 10.05-30.05         | 31.05-10.06 |
| <b>M11</b> | Data             | Data        | Data                  | Data        | No data             | Data        |
| <b>M12</b> | No data          | No data     | Data                  | Data        | No data             | Data        |
| <b>M15</b> | Data             | Data        | No data               | Data        | No data             | Data        |
| <b>M4</b>  | Data             | Data        | Data                  | Data        | No data             | Data        |
| <b>M6</b>  | Data             | Data        | Data                  | Data        | No data             | Data        |
| <b>M7</b>  | Data             | Data        | Data                  | Data        | No data             | Data        |





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