FIRUM

LiceDetached

The fate of mobile lice detached in the course of salmon crowding processes

Kvantifisering av lakse- og skottelus som løsner fra laksen under trenging i tilknytning til avlusing samt reinfisering av laks på samme eller andre oppdrettslokaliteter

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Sammendrag

Målet med prosjektet var å finne ut av, i hvor høy grad, og under hvilke forhold ulike stadier av lakse- og skottelus, som faller av i forbindelse med håndtering og trengning, kan infisere laks på nytt, både på samme lokalitet og over større avstander.

Det ble talt lus på fisk før, under og etter avlusingsoperasjoner med Optilicer i ni merder i ulike oppdrettsanlegg. Forekomsten av store mobile lakselus (preadult II og voksne hanner) avtok jevnt i løpet av trengningsprosessen hvor 21,6 % av lusene løsnet seg per time. Preadult I og voksne hunner var mer motvillige til å løsne seg. Voksne hunner viste løsrivelsesrater på omtrent 10 % per time, selv om den tidsmessige trenden ikke var statistisk signifikant.

Forekomsten av *skottelus* på fisk viste ikke jevnt avtagende trender under trengningsprosessen, men etter 1,5 timer var den gjennomsnittlige forekomsten av *skottelus* på fisken redusert med 77 %.

Levende frittsvømmende lus ble påvist i vannprøver fra merden med trengt fisk, hvor hanner representerte over 90 % av lusene for begge artene. Bevis for kjønnsspesifikke løsrivelsesatferd ble også observert i nasjonale lakselus data, der lus som løsner under bedøvelse av fisken også er kvantifisert. Store mobile lakselus løsnet oftere enn små mobile lus og voksne hunner, med løsrivelse som økte ettersom lusemengden steg til fire per fisk før den avtok. En lignende trend ble observert for skottelus, med en grense på to voksne lus per fisk. Denne oppførselen kan være knyttet til partnersøkende atferd.

Temperaturen hadde en positiv innvirkning på løsrivelsen av lakselus, spesielt store mobile lus, med rater som økte fra 17 % til 22 % når temperaturen steg fra 6 °C til 12 °C. I motsetning til dette reduserte høyere temperaturer kraftig skottelus løsrivelse, som falt fra 51 % til 34 % over samme område.

Forekomsten av frittsvømmende lus blant den trengte fisk økte under trengningsprosessen. De høyeste mengdene ble funnet i rolig vær, der strømmene ikke spredte lusene raskt. Når det ble tatt høyde for spredning med strømmer og total mengde lus på fisken, var løsrivelsesrater og antall fiskeskjell like, bortsett fra den ene trenging operasjon hvor flere skjell ble funnet, noe som tyder på mer alvorlig trenging. Under disse forholdene økte antallet frittsvømmende skottelus, mens antallet frittsvømmende lakselus forble upåvirket. Men siden dataene er begrenset, bør disse funnene tolkes med forsiktighet. Interessant nok påvirket ikke tøft vær med sterke vindkast og bølger løsrivelse.

Infeksjon av mobile og voksne lakselus på fisk behandlet med Optilicer resulterte i en økning i lusetallet på 44 % for mobile lus og 78 % for voksne hunnlus. Den høyere gjenfestingsraten for voksne hunnlus sammenlignet med mobile lus står i kontrast til

observasjonene i andre deler av studien, der mobile lus generelt viste høyere rater for både avløsning og smitte enn voksne hunnlus.

Indirekte infeksjon av lakselus, enten fra en merd til en annen eller mellom oppdrettsanlegg, er sjelden. Plutselige forekomster av mobile eller voksne lakselus i oppdrettsanlegg kan derfor ikke tilskrives avlusing ved nabooppdrettsanlegg. Undersøkelser av nyutsatt fisk viste at kun i 0,3 % av totalt 1279 6merder ble det påvist indirekte smitte når nærmeste lus var på en vill vert eller et annet oppdrettsanlegg. Når det var lus i andre merder på oppdrettsanlegget var infeksjonene noe høyere da 4,2 % av merdene var indirekte infisert.

Indirekte infeksjon med skottelus derimot var vanlig da 19,5 % av merdene var indirekte infiserte. I de fleste tilfellene var det ingen voksen skottelus på oppdrettsfisken i andre merder på anlegget.

Abstract

The objective of the project was to quantify the detachment of viable mobile and adult salmon lice (*Lepeophtheirus salmonis*) and *Caligus elongatus* during crowding processes and to assess reinfection by these stages at the treated fish farm as well as indirect infection at other farms.

Sea lice were counted on fish before, during and after delousing operations with Optilicer in 9 cages at various fish farms. The abundances of large mobile salmon lice (preadult II and adult males) steadily decreased during the crowding process with an hourly rate of 21.6% while preadult I and adult females were more reluctant to detach, with adult females showing detachment rates of approximately ~10% per hour, although the temporal trend was not statistically significant.

C. elongatus abundances on fish did not show decreasing trends during the crowding process, but after 1.5 hours of crowding the average abundances of *C. elongatus* on the fish had decreased by 77%.

Live free-swimming lice were detected in water samples from within the cage with crowded fish, with over 90% being males for both species. Evidence of gender-specific detachment behaviour was also observed in national sea lice data, where lice that detach during sedation of the fish are also quantified. Large mobile salmon lice detached more frequently than small mobile lice and adult females, with detachment rates increasing as lice abundance increased to 4 per fish before declining. A similar trend was observed for *C. elongatus*, with a threshold of 2 adults per fish. This behaviour may be linked to mate searching.

Temperature positively influenced the detachment rates of salmon lice, particularly large mobile lice, with rates rising from 17% to 22% as temperatures increased from 6°C to 12°C. In contrast, higher temperatures strongly reduced *C. elongatus* detachment rates, which dropped from 51% to 34% over the same range.

The abundance of free-swimming lice in the crowded fish cage increased during the crowding process. The highest abundances were found in calm weather when currents did not disperse them quickly. Accounting for dispersion with currents and total abundance of lice on the fish, detachment rates and the number of fish scales were similar, except during one crowding event where more scales were found, suggesting more severe crowding. Under these conditions, *C. elongatus* detachment rates increased, while salmon lice detachment remained unaffected. However, as the data is limited, these findings should be interpreted cautiously. Interestingly, harsh weather with strong gusts and waves did not influence detachment rates.

Infection by mobile and adult salmon lice on fish treated with Optilicer resulted in a 44% increase in mobile lice and a 78% increase in adult female lice. The higher

reattachment rate for adult female lice compared to mobile lice contrasts with observations in other parts of the study, where mobile lice generally exhibited higher rates of both detachment and infection than adult female lice.

Indirect infection of salmon lice, whether from one cage to another or between farms are rare, thus sudden appearances of large numbers of mobile or adult salmon lice at farms cannot be attributed to delousing operations at neighboring farms. Investigations of newly deployed fish showed that indirect infection was only detected in 0.3% of the total 1279 cages when the nearest louse was on a wild host or another farm. When lice were present in other cages at the farm infections were somewhat higher as 4.2% of the cages were indirectly infected.

Indirect infection with *C. elongatus* on the other hand was more common as 19.5 % of the cages were indirectly infected. In most of the cases, no adult *C. elongatus* were on the farmed fish in other cages at the farm.

Introduction

In salmon aquaculture, the challenge of managing salmon lice is a persistent issue. Infestation of farmed salmon in open net pens has created ideal conditions for salmon lice to reproduce, leading to welfare issues and economic losses (Dempster et al. 2021). Regulations in countries such as Norway and the Faroe Islands mandate control of salmon lice populations to protect both farmed and wild fish. In Norway, management is overseen through a regional traffic light system (Vollset et al. 2017), while the Faroe Islands use a farm-level approach based on their sea lice prevention act (Sea lice regulation act, 2016).

Both salmon lice (*Lepeophtheirus salmonis*) and *Caligus elongatus* are ectoparasites with direct life cycles. These include two planktonic nauplii stages followed by six post-nauplius stages (Hamre et al. 2013, Piasecky and Mackinnon 1995). While *C. elongatus* can complete its life cycle on a single host, studies have shown that adult stages can switch hosts, potentially spreading infestations to new farmed fish populations (Øines et al. 2006, Wootten et al. 1982). Infection pathways for salmon lice are predominantly direct, where copepodites locate hosts and develop into adult stages (Amundrud and Murray 2009). However, handling operations such as delousing are thought to facilitate the detachment of mobile stages, potentially leading to indirect infections.

Various methods exist to limit lice infections, such as biological control, preventive measures and planning. However, lice treatments are by far the most common control method. Treatments are expensive both directly and indirectly due to decreased fish welfare and mortality rates (Overton et al. 2019). It is thus essential to reduce the treatment frequency, while keeping the number of lice at acceptable levels, which requires high levels of efficacy.

Historically, chemical treatments were the primary method for controlling salmon lice infestations. However, due to resistance to chemical agents and the negative environmental impact of these substances non-medical treatments have been implemented. Starting in 2015-2016, both Norway and the Faroe Islands began adopting non-medicinal methods like thermal, mechanical, and freshwater treatments, largely replacing chemical solutions by 2018 (Jevne et al. 2019, Overton et al. 2019, Kragesteen et al. 2021). These methods are costly and require optimization to minimize welfare and economic impacts connected to sea lice treatments.

Non-medicinal treatments are typically carried out using delousing vessels, requiring crowding and pumping of fish. During the crowding phase, the net volume is reduced, and fish are compressed into a smaller space before being transferred to the treatment vessel. While the primary aim is to facilitate efficient delousing, this process can cause lice to detach before the fish enters the delousing vessel. Detachment rates during crowding have been observed to range between 20–40% (Powell et al. 2015, Guttu et al.

2025), and detached lice have been shown to survive for several days (Ritchie 1997, Guttu et al. 2025). These mobile lice can reattach to new hosts, particularly in environments with high host densities, such as farm cages or experimental setups (Ritchie 1997, Bui et al. 2020). Although the extent to which indirect infection contributes to salmon lice populations in natural settings remains largely unknown, sentinel cage studies have suggested that such infections are minimal beyond a 5 km range from infested farms (Pert et al. 2014).

This study employed multiple approaches to address gaps in understanding lice detachment and its impact. Lice counts were conducted before, during, and after Optilicer treatments to quantify detachment rates. Data from the Faroese national monitoring program were analysed to examine species- and stage-specific detachment behaviour, as well as the effects of seasonal changes and lice densities. Water column sampling quantified live lice near treated pens. Population dynamic modelling and particle tracking simulations were used to assess reinfection risks at treated and neighbouring farms, and data from the national sea lice monitoring programme was analysed to detect indirect infection of sea lice on newly deployed fish in a fish farm network.

Study site

Studies were conducted in the Faroe Islands. However, as delousing methods are highly similar in the Faroe Islands and Norway the detachment rates during crowding of the fish are not expected to differ considerably. The seaway distances between farming sites in Norway and the Faroe Islands are comparable (McIntosh et al. 2022). However, the strong tidal currents in the Faroe Islands (Fig. 1) result in significantly higher connectivity of lice larvae between farms compared to findings from model studies conducted in other countries (Kragesteen et al. 2018). Thus, long distance infection rates in the Faroe Islands are expected to be comparable or even higher than in other countries.

The Faroe Islands is divided into farming regions with 1-3 farming sites. Each region typically consists of a fjord and the distance between farm sites in different regions exceeds 5 km (Fig. 1). For all sites, the deployment window for each production cycle is 4 months and there is a minimum requirement of a 2 month fallowing period between cycles. A few farming regions contain multiple farm sites where the distance between farms can be less than 0.5 km, these are legislated to synchronize their production (ICES 2023).

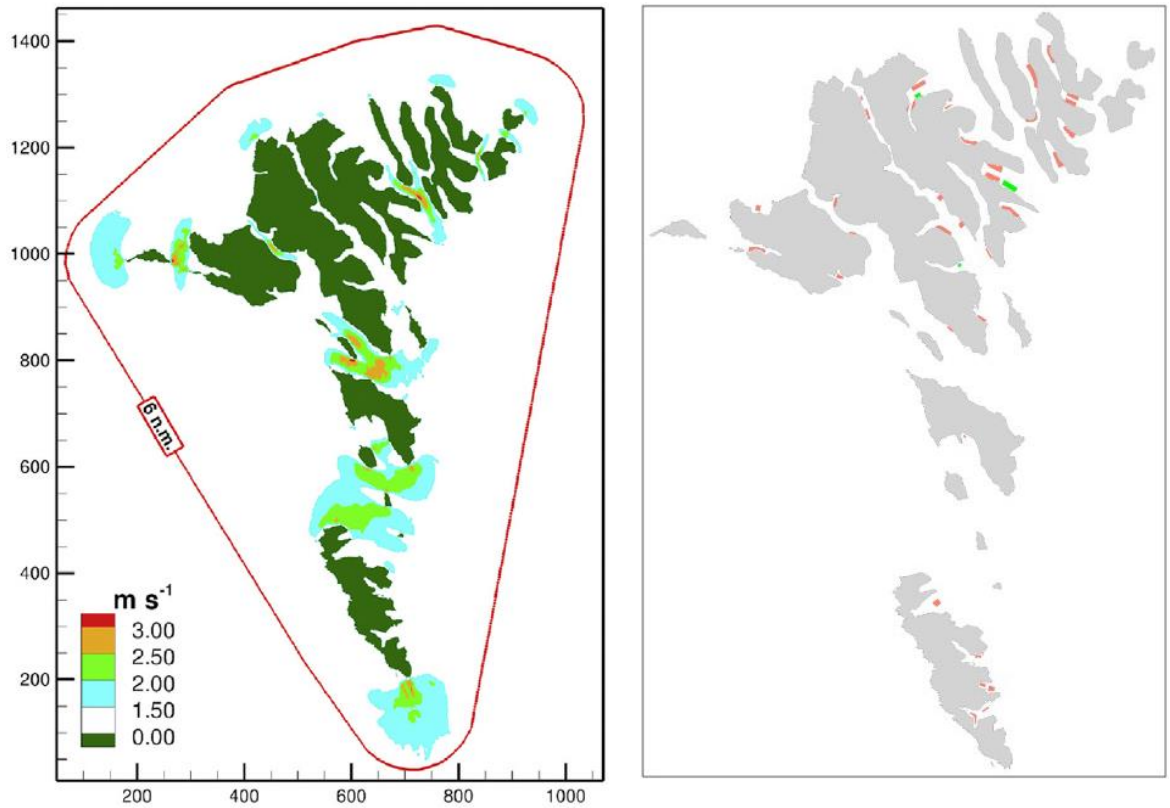

Figure 1. Left - Maximum tidal current velocities around the Faroe Islands (Simonsen and Niclasen 2021). The x and y axis show distances in m and the coloured scale shows velocity in m s^{-1}. Right - Locations of fish farming sites (orange) and seaweed farms (green) in the Faroe Islands (ICES, 2023).

Objectives

The overall objective was to quantify the detachment of viable mobile and adult sea lice in the crowding processes, quantify reinfection by these stages at the treated fish farm, and look at indirect infection at other farms. This was achieved through the following subtasks:

- Quantifying detachment of lice during crowding processes from sea lice counts on fish during delousing operations (WP1)
- Investigating seasonal influence on the species and development stage specific detachment rates (WP1)
- Investigating the amount of various live sea lice stages in the water column near treated net pens (WP2)
- Quantifying reattachment by mobile lice at treated fish farms by combining population dynamic modelling and sea lice counts (WP3)
- Using a particle tracking model approach to assess the likelihood of infection at other fish farms (WP4)
- Investigating indirect infection on newly deployed fish in the data from the national sea lice monitoring programme (WP4)

Project organization

The project was funded by the Norwegian Seafood Research Fund (FHF grant number: 901782). The project was led by Firum in collaboration with the industry partner Bakkafrost. Field work was conducted during delousing operations at Bakkafrost, and the company also provided data from other delousing operations. Access to data from the national sea lice monitoring program was granted from all the Faroese fish farming companies.

Project group

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Problem statement and purpose

Increased knowledge on the circumstances under which detachment of mobile and adult lice is most likely to occur during the crowding process, and whether there is a relationship between environmental conditions and detachment, will enable the industry to optimise treatment efficacy of all non-medicinal treatments since crowding is an essential part of the process. Such knowledge will also enable fish farmers to evaluate to which degree they should collect detached lice with the various commercial options available.

Quantification of the potential problem of reattachment by mobile and adult stages at treated fish farms or even neighbouring farms an overdue task. Information on the possible extent of such issues will provide a stronger knowledge base for a strategic effort to reduce the sea lice problem and the frequency of treatments. Although the studies are conducted in the Faroe Islands, the results are transferable to Norway. This is because the delousing methos are highly similar, and the connectivity, and consequently the potential infection pressure, between farms is comparable or even higher in the Faroe Islands compared to Norway (see section Study site).

Methods

Sea lice monitoring on fish during delousing (WP1)

Data for lice detachment during crowding in connection to non-medical treatments was collected from 9 cages during sea lice treatments with Optilicer onboard the vessel MS Martin at Bakkafrost farming sites (Table 1). Delousing operations were carried out by staff at the various fish farms together with the crew of the delousing vessel.

Sea lice were counted on fish at two stations onboard, i.e. immediately after being pumped onto the delousing vessel, as well as after delousing. For each batch 10 fish were collected using a push net, placed in a sedation tub with Finquel and sedated before visual inspection of sea lice.

The number of sea lice were recorded together with supporting information, i.e. timing of batch start, area of the cage that had been swiped at batch start, as well as behavioural crowding score according to Nobel et al. (2018). A delousing operation typically begins with lining up the net, followed by crowding the fish using a ball line. This operation is performed by staff and boats at the fish farms, and might vary at different sites. To include such diversity in the dataset, sampling was conducted at 6 different farming sites during various seasons. The initial plan was to sample one cage approximately once a month, but logistical challenges and limited activity of MS Martin towards the end of the sampling period made this plan unfeasible.

Sea lice on fish were counted by staff at Firum that normally perform the national sea lice monitoring. During the first monitoring (December 12, 2022) sessile lice were included, and two persons counted lice on fish before delousing and two counted lice on fish after delousing. However, to increase the number of batches, the strategy was

Table 1. Date and location for sea lice counts during treatments with Optilicer, and information on the area of the cage that was swiped at the start of the delousing operation, crowding score, duration of the delousing operation, and number of counted batches of 10 fish that were counted after the fish was pumped onto the vessel before treatment.

Date	Location	Area swiped (%)	Crowding	Duration (h)	Batches
06-12-2022	A 12	75	2	1.13	6
02-05-2023	A 47	70	3	1.10	11
19-06-2023	A 04	60	4	1.36	12
29-06-2023	A 47	70	3	1.17	9
03-07-2023	A 13	60	3	1.97	8
24-08-2023	A 25	70	3	1.35	12
07-12-2023	A 71	55	2	1.08	17
07-12-2023	A 71	60	3	1.32	18
07-12-2023	A 71	65	1	1.82	23

changed, i.e. sessile lice were not counted, and the number of staff increased when possible. Due to lack of available personnel, and to focus on the number of lice on the fish before treatment, lice counts after treatment were not conducted in four cages.

Sea lice were registered in the same groups as in the national sea lice monitoring program. Salmon lice were grouped into adult female, large mobile lice, which include adult males and preadult stage II, and small mobile lice, which include the preadult I stage and preadult II males. For *C. elongatus* adult males and females were grouped as one, as were the chalimus stages of both species.

Batches counted within the first 15 minutes of the delousing operation were assumed representative for uncrowded fish (2-3 batches pr cage). Change in lice number during the subsequent counts were attributed to the crowding operation. National sea lice monitoring was performed 5-12 days prior to the delousing operation and the sea lice levels had typically increased during the days between the national monitoring and the first 15 minutes of the delousing operation.

Sea lice detachment during sedation of fish (WP1)

In the national sea lice monitoring program, lice that detach during fish sedation are registered in addition to lice on the fish. For each cage fish is sedated in the same sedation water, which is then filtered and lice in the filter and on the walls and bottom of the sedation chamber are counted to quantify lice that has detached during sedation. Although the situation does not mirror crowding of fish, the large dataset on detachment during sedation provides insights into behavioral traits that affect detachment.

The dataset includes all sea lice monitoring data for the Faroese farming industry from July 1, 2016, to May 10, 2024, encompassing 47 288 individual sea lice monitoring counts with data collected for each cage. For the analysis of detachment rates, only entries where sea lice were present were used. The data was analyzed per cage, with the percentage of detached lice calculated by dividing the number of lice found in the sedation chamber by the total number of lice on all the fish counted in the cage, including those in the sedation chamber.

Statistical analyses were conducted using the R Statistical Software Environment (R Core Team, 2023), specifically employing the MASS package for negative binomial (NB)

Table 2. Detached lice (%) during sedation of the fish for the registered lice categories.

Louse	mean	variance	% zero counts	n
Adult females	9.9	369	62.1	34575
Large mobile	14.4	424	46.4	39317
Small mobile	11.4	506	66.5	31394
C. elongatus	27.3	981	38.4	25987

models (Venables and Ripley 2002), the pscl package (Zeileis et al. 2008) for zero inflated negative binomial (ZINB) models and the performance package (Lüdecke et al. 2021) to evaluate the performance of various models. For visualization of the influence of parameters on the number of lice that detach during sedation of the fish, the expected values according to the model were calculated in R only including significant estimates and visualized with ggplot2 (Wickham 2016).

The data on the proportion of lice that detached from the salmon during sedation was highly over dispersed and exhibited zero inflation, with variances an order of magnitude higher than the mean and 38 – 67% of the counts being zero (Table 2).

The influence of season and lice abundances on the fish on detachment rates was analysed with temperature, day length and total number of preadult and adult salmon lice and adult *C. elongatus* on the fish as predictors. Poisson models and negative binomial models did not perform well due to overdispersion and zero-inflation (Table 2), which was tested with the performance package in R (Lüdecke et al. 2021). Consequently, zero inflated negative binomial models (ZINB) were used on the data. The Akaike information criterion (AIC) value decreased 81% and 76% when moving from Poisson (PO) to negative binomial (NB) models for the data on adult females and large mobile lice, respectively, and the AIC was reduced by another 9-12% when mowing from NB to ZINB models. For the data on small mobile salmon lice and *C. elongatus* the reduction in the AIC was small when moving from PO to NB, while a significant reduction in AIC appeared in the transition from NB to ZINB models (78% and 83%, respectively). The ZINB model consists of two components: the count part and the zero-inflation part. Initially, all predictors were included in both components. However, in the final analysis, predictors that did not show a significant relationship were

Table 3. Coefficients from the zero-inflated negative binomial model (ZINB) for the various lice categories. – represents predictors that were excluded in the model as they did not show significant relation. All other predictors were significant at ($p<0.01$) *, ($p<0.001$)** or ($p<<0.001$)***

	Adult female	**Large mobile**	**Small mobile**	***C. elongatus***
Count model				
Intercept	3.2108***	3.0602***	3.4266***	4.5013***
Temperature	-	0.0137***	0.0184***	-0.0664***
Day length	0.0202***	-	0.0069***	-0.0063***
Salmon lice/fish	-0.0861***	-0.0603***	-0.0545***	-0.0122***
C. elongatus/fish	-0.0069***	-0.0058***	-	-0.0185***
Zero inflation model				
Intercept	1.4463***	1.3944***	1.2532***	0.7704***
Temperature	-0.0414***	-0.0206*	-0.0205*	-0.0344***
Day length	-	-0.0060*	0.0073**	0.0234***
Salmon lice/fish	-0.3241***	-0.9943***	-0.2487***	0.0429***
C. elongatus/fish	-	-0.0127**	-	-2.8069***

excluded for that component. For small mobile lice, the detachment rate was not influenced by the number of *C. elongatus* on the fish, so this parameter was removed entirely from the analysis of small mobile lice (Table 3).

Collection of free-swimming lice during crowding (WP2)

During the delousing operations plankton samples were collected to detect live free swimming mobile stages. During the project the sampling method was modified from vertical plankton tows at various locations outside the net cage to pumping water samples from inside the net among the crowded fish. In addition, samples were collected on one occasion onboard the vessel (Table 4).

Vertical plankton tows were sampled at various locations outside the crowded fish by towing the plankton net manually from 20 m depth to the surface. The mesh size of the nets was 150 µm and 1 mm, respectively, and equipped with a flow meter at the mouth. However, they did not perform well as very few lice were detected. To enhance efficiency, it was decided to transition to a water pumping method collecting samples among the crowded fish in the cage. Pumping water samples from the cage was, however, time limited, as the equipment had to be removed before space became too constrained, increasing the risk of collision with the vessels pump used to transfer fish onboard.

The pump was attached to a buoy holding it at ~1 m depth and was maneuvered by two connected ropes. The capacity of the pump was 14.4 $m^3 h^{-1}$. From the pump, the seawater was transported through a hose on to the walkway of the cage where it was filtered through a 500 µm mesh net and the material collected. The sampled volume was calculated based on the pumping time and capacity.

On one occasion sampling was conducted from the seawater draining point onboard the delousing vessel with a small handheld net. These samples were not quantitative, but were conducted to explore temporal changes and the duration of each sampling was recorded.

Table 4. Date and location and sampling method for free swimming mobile lice during delousing operations.

Date	Location	Method
06-12-2022	A 12	Vertical plankton tows
02-02-2023	A 05	Vertical plankton tows
02-05-2023	A 47	Vertical plankton tows
29-06-2023	A 47	Pumped from cage
03-07-2023	A 13	Sampled onboard
24-08-2023	A 25	Pumped from cage
07-12-2023	A 71	Pumped from cage

To detect if the collected mobile lice were alive, the filtered sample was swirled and the lice immediately settled on the wall of the container, indicating viable lice. For thorough quantification and identification of species, development stage and gender, the filtered samples were preserved in ethanol and analyzed under a microscope. The number of scales in the sample was also counted.

The current speed and direction were measured at 2 m depth with a Seaguard RCM SW from Aanderaa (www.aanderaa.com).

Modelling sea lice infection in treated cages after delousing (WP3)

One significant challenge in quantification of lice reattachment after delousing operations is to distinguish between newly attached lice and those that have developed from earlier life stages post-treatment. To facilitate distinguishing between indirect infection and development from earlier stages, a salmon lice population dynamic model was applied (https://github.com/TrondurJK/Salmon_lice_simulator). The model is written in Phyton (Rossum, 1995). The model is a fully mechanistic cohort-based model accounting for all salmon lice life cycle stages, seasonal varying temperature and connectivity. The model allows for high resolution of several parameter e.g. internal and external parameters (Kragesteen et al. 2023). However, in the set up for this study, based on extensive experience of Faroese lice data, the model is adjusted or fitted by: i) internal infection pressure, ii) external infection pressure, iii) treatment efficiency, and iiii) cleaner fish efficiency. Internal infection pressure is set to be constant, although this is likely not the case. External infection pressure is divided into two parameters. One is constant while the second parameter is a factor or ratio of the total amount of adult females present in the Faroe Islands. In other words, the ratio approximates the external infection pressure based on the total amount of adult female lice on farmed fish in Faroe Islands. Treatment efficiency has 3 or 4 parameters; one for chalimus stages, one for pre-adult stages, one for adult stages, and one for duration if the treatments is an infeed treatment. Cleaner fish efficiency is estimated for every quarter of a year resulting in one parameter for every quarter cleaner fish are deployed.

Model optimization

The salmon lice population dynamic model is designed to simulate individual cages based on data from the Faroese Food and Veterinary Authority (FFVA), including temperature, lice count, fish numbers, and treatments. The population model is fitted by minimizing the squared absolute difference between model simulation and lice counts with the minimize function from scipy.optimize (Virtanen et al. 2020), the minimize algorithms used were Nelder-Mead and BFGS. The squared absolute difference is summed for three categories: adult stages, preadults stages and chalimus stages. Each category is multiplied by a weight factor before the total difference is summed. Adult stages are multiplied by 10 000, preadults stages are multiplied by 100, and chalimus stages are multiplied by 1. The number of free parameters depends on the number of treatments and duration of cleaner fish deployment; however, the minimum is 3 parameters (one internal and two external).

Data preparation

Data from all the treatments performed on the vessel MS Martin was provided by Bakkafrost. The data was organized in Excel by the farming company and was used to log different data regarding the treatments. For this project the farming site, cage, date, and louse count before treatment and after treatment were the most important registrations. The data was quality controlled using the R Statistical Software Environment (R Core Team, 2023), identifying human error registrations such as farm id and dates. These corrections were scripted making sure that any changes to the raw data were traceable and reproducible.

The data was then harmonized with the FFVA database to which daily operational farming data is reported along with treatment data and the national lice monitoring data. In most cases it was possible to match the cage id in the data from Bakkafrost to the cage id registered in the FFVA database. In cases where no matches were found based on cage id, the fish count registered in the FFVA data was compared to the fish count registered for the cage on the day of treatment in the excel file. By looking at data from the whole farming cycle it was possible to manually match most of the remaining cages using the provided data. This data was then uploaded to a local PostgreSQL database at Firum containing daily updates from the FFVA database, which was further developed to accommodate these new types of sea lice count data, see Table 5 for further details.

Table 5. Summary of the treatment data received from the farming company. Indicating how many treatments were in the original raw data and how many of these passed the quality check and could be uploaded to the local database. The different criteria used for the statistical analysis are also shown, with level 5 highlighted in blue being the criteria level settled on.

Criteria	Treatments data received	all	quality checked
	all	2703	-
	Uploaded to local database	-	1945
1	Included lice counts before and after treatment of both adult females (AF) and mobile stages (PAAM)	-	1737
	Population dynamics simulation performance:		
2	Residual abs(observation – simulation) after treatment < 0.1		1121
3	Number of observations > 5		1097
4	RMSE (observations vs. Simulations) < 2	-	1072
5	*RMSE (observations vs. Simulations) < 1.5*		*1033*
6	RMSE (observations vs. Simulations) < 1		892
7	RMSE (observations vs. Simulations) < 0.5		470

Statistical Analysis

The salmon lice population dynamics was simulated in individual cages using the above mentioned optimization algorithm. The algorithm was set up such that optimization was performed from the start of the cage cycle until the lice count after the Optilicer treatment of interest was reached. Hence, a population dynamic was simulated as many times as there were Optilicer treatments of interest per cage cycle. In the dataset there were on average 3 Optilicer treatments per cage that had lice counts before and after the Optilicer treatment, see figure 2 for further clarification.

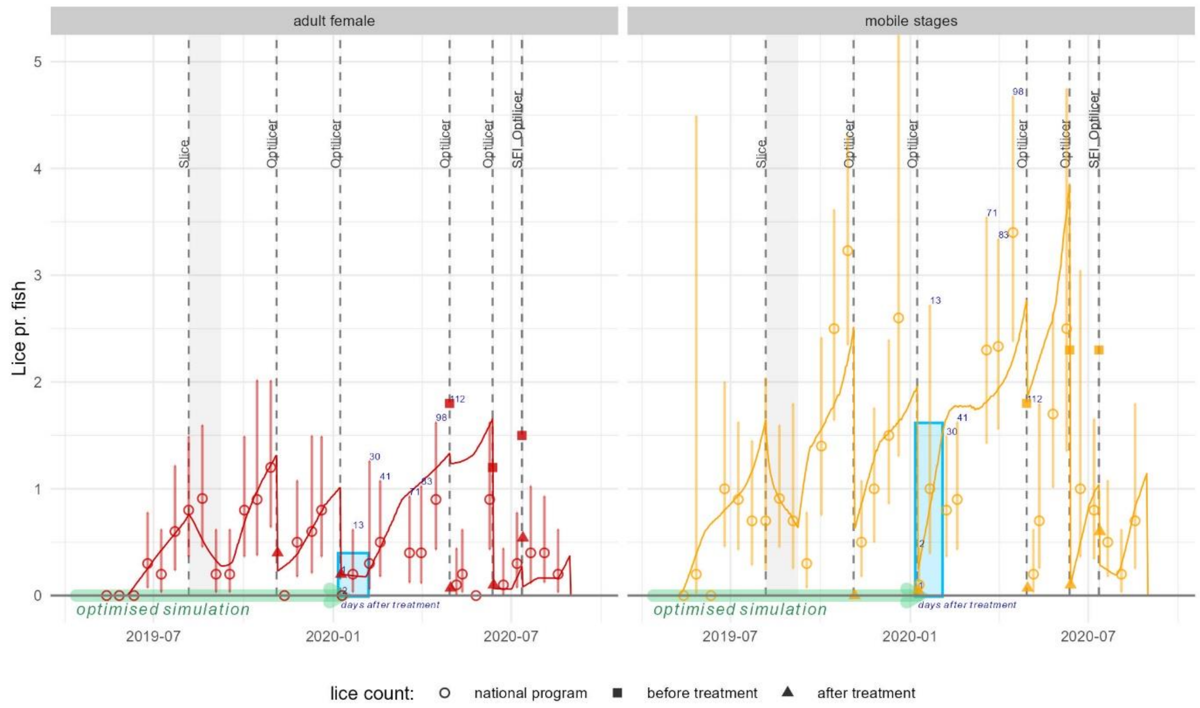


Figure 2. Salmon lice population dynamics simulation, showing the simulation results (adult female and mobile stages in red and orange respectively) and the sea lice counts from the national programme as well as the sea lice counts conducted before and after treatment on the delousing vessel, indicated in the plot by different shapes, circle, square and triangle respectively. The 95 % confidence interval is around the mean negative binomial distribution of the national programme count. The Treatments are indicated by vertical lines, and the treatment duration is indicated by the grey area following a treatment. The green arrows at the bottom of the plot, shows the interval of which this simulation has been optimised. The square blue box indicates the time window used to compare the simulation and observation results. And the blue numbers indicate on which day post treatment that the lice count is from.

Post population dynamic simulations

All work was done in R both statistics and plotting using the Tidyverse library (Wickham et al., 2019). The MASS package was used to calculate the 95 % confidence interval (CI) around the mean of negative binomial distributions for each salmon lice counting

(Venables and Ripley, 2002). The changepoint package was used to determine the changepoint in the simulation after the treatment using the cpt.meanvar function with the “AMOC” method, to determine the time window in which simulation and observation could reliably be compared, indicated by the blue box in Figure 2 (Killick and Eckley 2014).

In the national sea lice monitoring program, lice are counted on a batch of fish and the detached lice left in the sedation bath are also counted as detailed above. The already detached lice were added one by one at random with allowed resampling to the lice count on the individual fish. This was done to keep the count data as integers, making further statistical analysis simpler. The mean and 95 % CIs were calculated using the glm.nb function in the MASS package, in cases where there was no variance observed in the lice count, the geometric mean was used. Because the lice counts performed by the farming company before and after the Optilicer treatment were only reported as means, with no information on how many fish were counted, no CIs were calculated for these means.

To estimate how well the population dynamic simulation was performing, a couple of parameters were used. 1) the absolute residuals (observed – simulation) before and after the treatment of interest was calculated, 2) number of observations within the optimization range, and 3) the root mean square error (RMSE) of observations vs. simulations. Although these are simple measures for model performance for dynamics models of this nature, they were selected for this specific use case, where especially the model performance around the treatment of interest was important so that the model could be used as a baseline reference for reattachment rates.

As the simulation is fitted to the lice count observation conducted on the vessel right after the fish was treated, the residual after treatment was set to 0.1 so that no difference was detected between the simulation and the observation on the day of treatment, ensuring the simulation starting points were comparable. The simulation must include 5 observations, and an RMSE of 1.5 was chosen as sufficient, as this reduced the observation variance by removing large observation outliers, see table 5.

To determine if there was a difference in the mean salmon lice per fish between the observations data and the simulation results post-treatment, hence indicative of a reattachment, a Generalized Linear Mixed Model (GLMM) was deployed using the glmmTMB package in R using the negative binomial distribution (Brooks et al. 2017). The model was set up with the response variable being the mean number of lice per fish. The primary explanatory variable was the data type (simulation or observation). To account for the paired nature of the samples, a random intercept for each sample was added to account for variability among the different samples.

Between farm infection with mobile lice (WP4)

The objective of this work package was to estimate the number of mobile lice that could potentially detach from one farm site, disperse, and reattach at a neighboring farming site. This was achieved using two distinct approaches:

> **Particle Tracking Model**: The first approach estimated how many particles, representing lice, released from one farm site could potentially reach a neighboring site. This was based on a particle tracking model driven by an underlying tidal circulation model.
> **Sea Lice Monitoring Data**: The second approach utilized national sea lice monitoring data to estimate the level of indirect infection. This was done by analyzing whether mobile and adult lice stages were observed on newly deployed fish before it was biologically possible for the lice to develop from a direct infection by copepodites.

Particle tracking model

The particle tracking model is forced by an underlying tidal circulation model which is an implementation of the barotropic mode of the regional oceanic modelling system (ROMS) (Shchepetkin and McWilliams 2005) on a half nautical mile (926 m) grid resolution covering a larger area, and on a 100 m resolution grid for the coastal region (Kragesteen et al. 2018). An open-source GitHub repository is available at https://github.com/Fiskaaling/FO_tidal_particle_tracking.

To assess the potential for detached mobile and adult lice to infect a neighboring farm site, a scenario was simulated in which particles representing mobile lice were released from the farm site A and received by the farm site B, located approximately 13.5 km apart across seaways. Since the particle tracking model was driven by tidal forces release and recipient farms A and B, were chosen as tidal forces are dominating seawater exchange at these sites. Additionally, these two areas are relatively close, which facilitates a strong connection between them. A total of 10 000 particles were released every 30 minutes over a 28-day period. For each particle, ID, release time, and the age of the particle upon entering the receiving area were recorded. The particles were tracked for a simulation period of 14 days. Because mobile lice can infect a new host immediately after detachment, particles were allowed to infect the receiving area from the moment of release up to 14 days after simulation started. A random walk was implemented, and particles had no vertical movement (Kragesteen et al., 2018).

Infection on newly deployed cages

This part of the study has been written as a scientific manuscript so only a short method summary and some major results are provided in this report. The study is based on a sea lice monitoring dataset comprising the entire salmon farming industry from July 1, 2016, to May 10, 2024. The total dataset compromised 1279 cage deployments, where indirect infection was estimated by assuming that the fish was infected by a copepodite immediately after transfer to the sea. If mobile or adult lice were observed in the sea lice monitoring before it was possible according to the temperature dependent development time, these were alleged to be indirect infection. The temperature dependent development time was calculated according to Hamre et al. (2019) and Hamre et al. (2024) for salmon lice and *C. elongatus*, respectively.

Results

Detachment during crowding (WP1)

The average number of lice in the batches counted during crowding was variable but showed a decreasing trend in 6 of the 9 cages for adult females and 8 of the 9 cages for large mobile lice (Fig. 3), but for small mobile lice there was frequently an increasing trend over time. *C. elongatus* shows seasonal variations in abundance on farmed fish in the Faroe Islands and was only registered in 5 of the 9 cages investigated. For 4 of the 5 cages there was a decreasing trend in lice numbers during fish crowding (Fig. 3). Linear regression only showed a significant decrease for large mobile lice in cage 5 (p=0.018), while none of the others were statistically significant. Small mobile lice were only present in small amounts in one of the three batches counted within the first 15

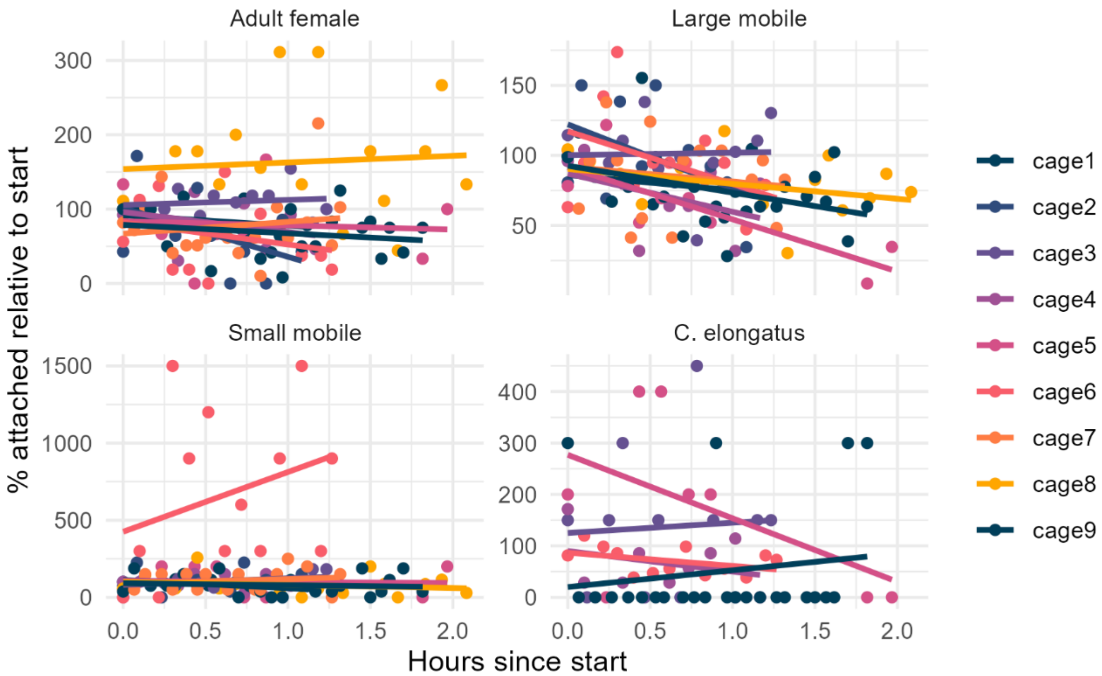


Figure 3. Lice attached to fish during crowding relative to the observed lice abundances during the first 15 minutes of crowding in the 9 investigated cages. Points show average abundances in each batch and lines show the linear temporal trend.

minutes in cage 6, resulting in abnormally high attachment rates relative to the start (Fig. 3 small mobile) and in cage 9 *C. elongatus* was only present in 4 of the 23 counted batches (Fig. 3 *C. elongatus*). In further analysis these two cases were excluded from the dataset.While the analysis of individual cages did not reveal strong statistical significance, pooling the data across all cages might highlight trends that are not apparent at cage level. For all the lice groups the average lice levels in the last 90 minutes of crowding was lower than at the beginning (Fig. 4). However, the lice levels were highly variable during crowding and for small mobile lice and *C. elongatus* the average levels were even higher than at the beginning in some time steps. For large mobile lice the lice levels on the crowded fish steadily decreased and the linear regression analysis revealed a significant decrease with detachment rates of 21.6% per hour ($p<0.001$). When grouping the salmon lice categories the data also shows a significant detachment rate, with an average detachment rate for all post chalimus stages of 14.9% per hour ($p=0.00101$).

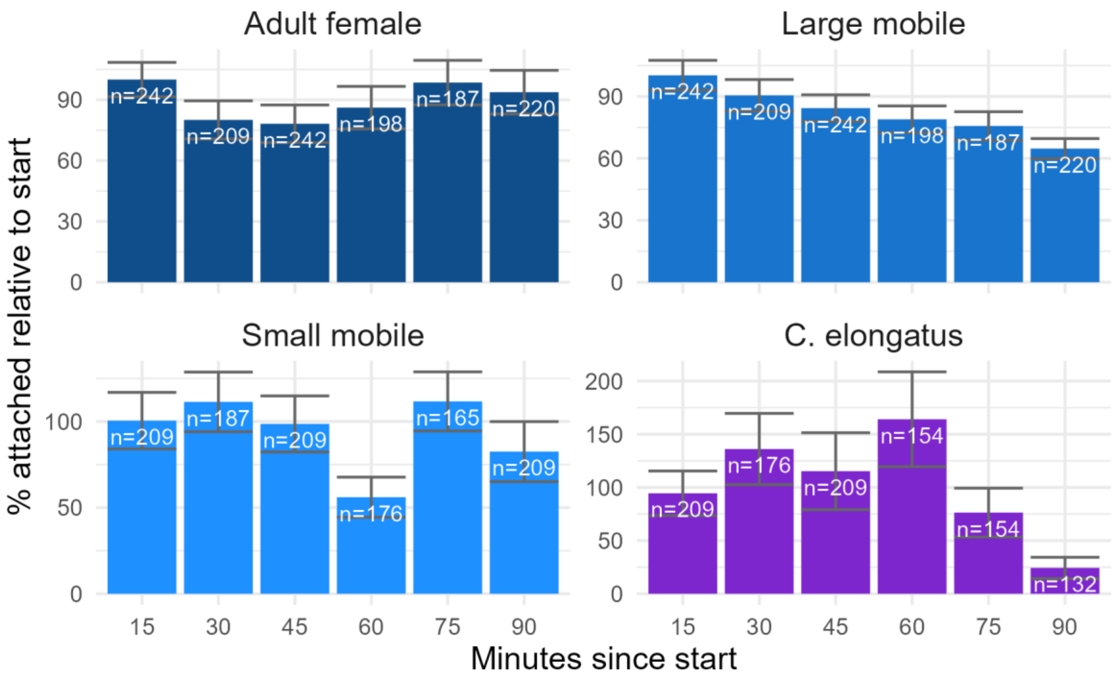


Figure 4. Lice attached to fish during crowding relative to the observed lice abundance during the first 15 minutes. n shows the total number of fish in the group.

Seasonal and density dependent detachment rates (WP1)

An alternative approach to examine detachment rates is to investigate detachment during the sedation of fish in the national sea lice monitoring programme. This extensive dataset allows for the analysis of seasonal and other factors influencing detachment rates.

The detachment rates differed significantly between all the registered lice categories (ANOVA, $p<0.001$). With average detachment rates of 27%, detachment of *C. elongatus* was significantly higher than the detachment rates of the various salmon lice groups (Fig. 5). For salmon lice the detachment rate of large mobile was highest (14% in average) while the average detachment rates of adult females and small mobile lice were 11 and 10%, respectively. The monthly mean values also showed some seasonal variation with the highest *C. elongatus* detachment during winter and highest detachment rates of large mobile salmon lice during autumn (Fig. 5).

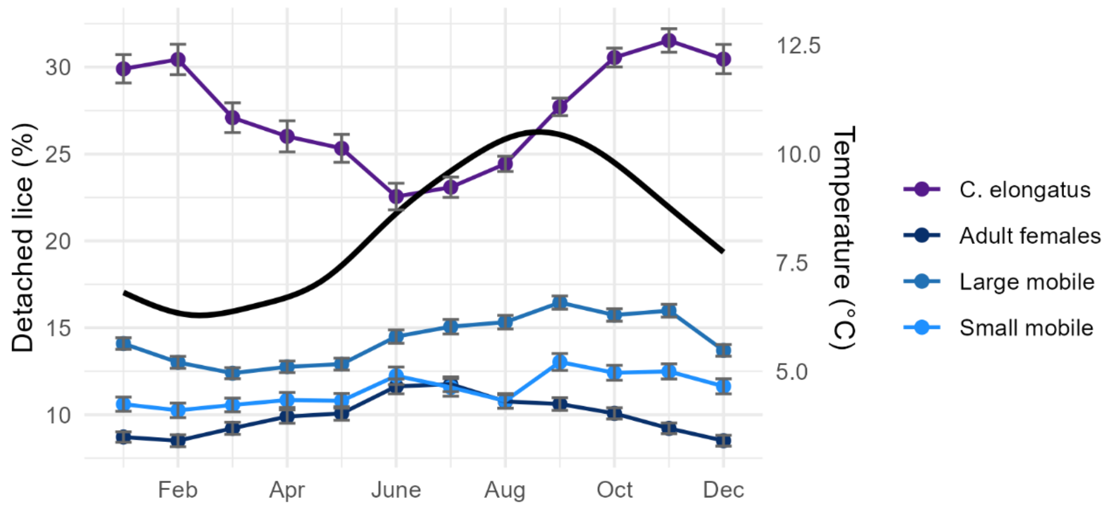


Figure 5. Monthly averaged detachment rates of the various sea lice categories during sedation of fish.

The ZINB model shows that the impact of temperature, day length and abundances of salmon lice and *C. elongatus* on the fish is variable amongst the lice groups (Table 3). Adult female salmon lice were the most reluctant to detach from the fish, where the total number of salmon lice on the fish and day length had the highest influence on detachment rates (Fig. 6). The highest predicted detachment rate was 13.7%, which occurred at the longest day length and at 4 salmon lice per fish (Fig. 7). Detachment rates of small mobile lice were likewise low and showed small influence of most variables. However, the detachment rate increased by 8% from fish with no salmon lice to fish with 7 salmon lice (Fig. 7).

Temperature had a considerable higher influence on detachment rates of large mobile lice than the other salmon lice groups and the influence of salmon lice abundance had an even higher influence. The detachment rate increased from 10% when no salmon lice were present to 23% when 4 salmon lice were on the fish, whereafter the detachment rate decreased slightly with increasing number of salmon lice (Fig. 6). The

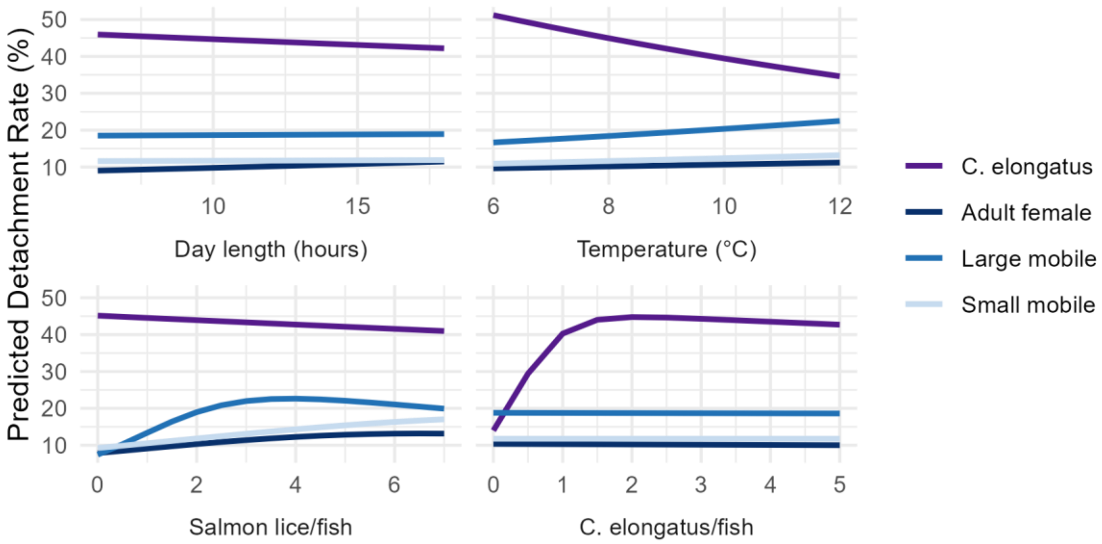


Figure 6. Influence of day length, temperature, and abundance of salmon lice and *C. elongatus* on the fish on detachment rates as predicted by the ZINB model. For each predictor the value is variable while the others were kept at their mean value. For temperature and day length the range was set to the range in the Faroe Islands. For total lice, the number of observations in the dataset decreased exponentially with higher values. The maximum value for the x-axis was set to include 96% of the observed lice counts.

maximum detachment rate of 26.7% was found to be at the highest temperature with 4 salmon lice per fish (Fig. 7). *C. elongatus* detachment rates were influenced by all parameters, but the pattern was opposite to the pattern for salmon lice. Detachment decreased with increasing day length, temperature and increasing numbers of salmon lice on the fish. The abundance of *C. elongatus* on the fish had a similar effect on adult detachment rates as observed with the influence of salmon lice on detachment of large mobile salmon lice. Detachment rates increased rapidly as the number of adult *C. elongatus* rose to 2 per fish, after which the rates declined slightly as the number of lice on the fish continued to increase. (Fig. 6). When 2 *C. elongatus* were present on the fish and the temperature was 6 °C, more than half of the adult *C. elongatus* were expected to detach from the fish according to the ZINB model (Fig. 7).

The nonlinear impact of total salmon lice per fish on the large mobile lice, and the same trend for *C. elongatus,* might indicate mate searching behavior. At lowered densities, increased detachment rates could reflect intensified mate searching effort, but when a certain density threshold is reached, the need for movement decreases, likely due to an increased probability of encountering mates on the fish.

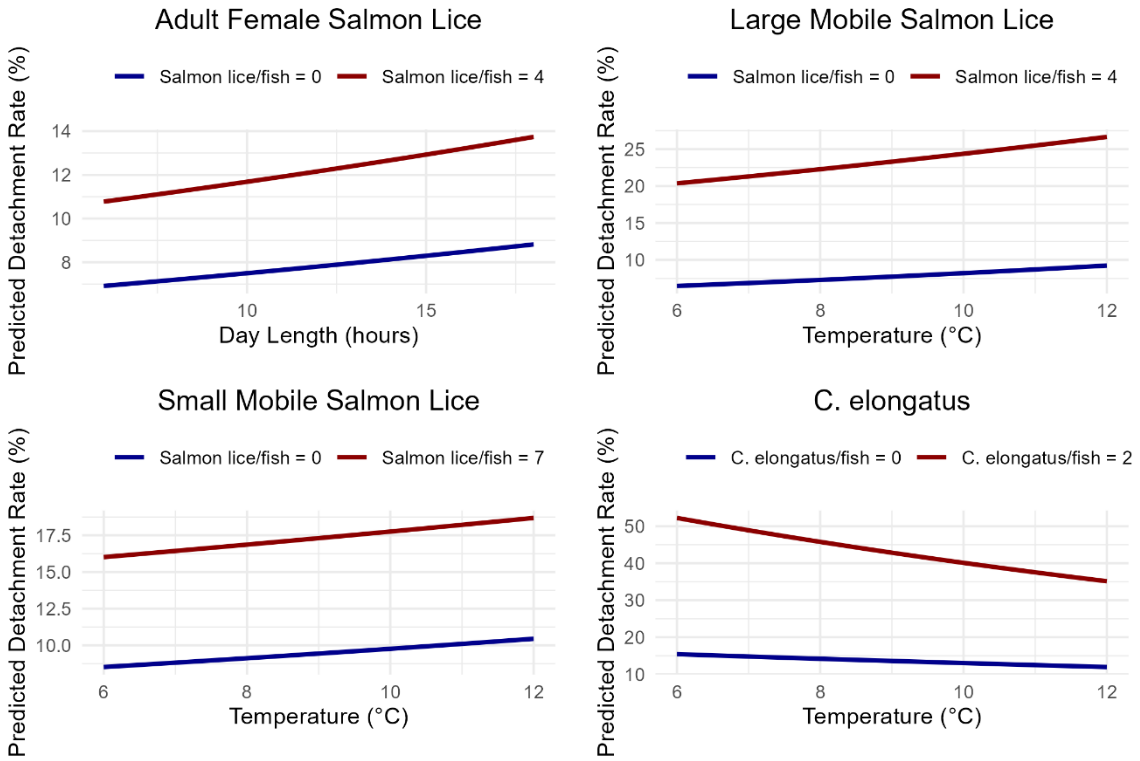
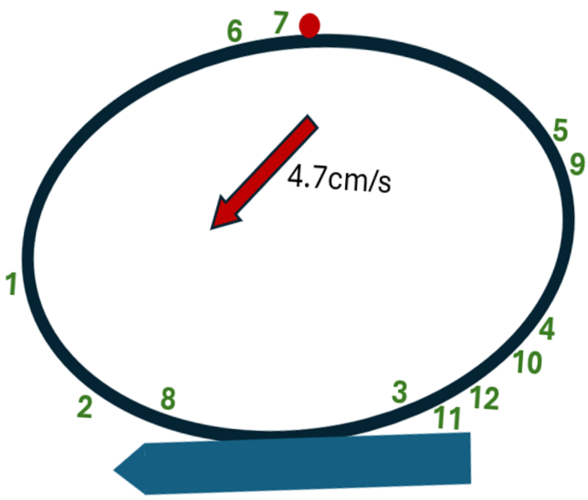


Figure 7. Illustration of the maximum and minimum probability of detachment for each of the four lice groups. Temperature or day length are presented on the x axis while salmon lice/fish or *C. elongatus*/fish are held at the value giving minimum or maximum detachment rate for the lice groups.

Free swimming mobile lice (WP2)

The initial approach to detect free swimming live mobile and adult lice in the proximity of the crowded fish was to conduct vertical plankton tows at various positions outside the net pen. In the three delousing operations where this was done, only two preadult salmon lice and one adult *C. elongatus* were found in the 78 vertical hauls conducted. In addition, the counts of fish scales revealed high variation in abundances, depending on the position of the hauls relative to the position of the ball line.

Some vertical hauls were performed with a 150 µm mesh net to detect nauplii and copepodites. These were more successful, especially during the first attempt where larvae were present in 8 of the 12 hauls. Both nauplii and copepodites were present in the samples downstream the current direction and absent upstream (Fig. 8).



Sample	Nauplii #/m³	Copepodids #/m³
1	0.65	-
2	0.29	-
3	0.27	-
4	0.95	-
5	-	-
6	-	-
7	-	-
8	0.35	-
9	-	-
10	2.39	-
11	-	1.39
12	-	0.26

Figure 8. Sampling locations for vertical hauls with a 150 µm mesh plankton net (numbers in green) and the abundance of larvae in the samples (table). The red dot represents the current speed measurement location, and the arrow represents the current direction. The average current speed was 4.7 cm/s. The position of the delousing vessel is represented by the blue boat in the bottom of the figure.

On one occasion detached lice were collected from the location of seawater drainage after the fish was pumped onboard the ship. This sampling was semi quantitative, with approximately equal sampling volumes, but no quantification of the total volume.

There was no temporal trend in number of lice during the delousing operation and the samples with no lice were equally distributed over the entire sampling period as investigated by a runs test (Fig. 9). In total there were 18 males and 9 females with average counts of 1.13 and 0.56 for males and females, respectively. However, the sex ratio of the detached lice was not significantly different as tested with a binomial test (p=0.061).

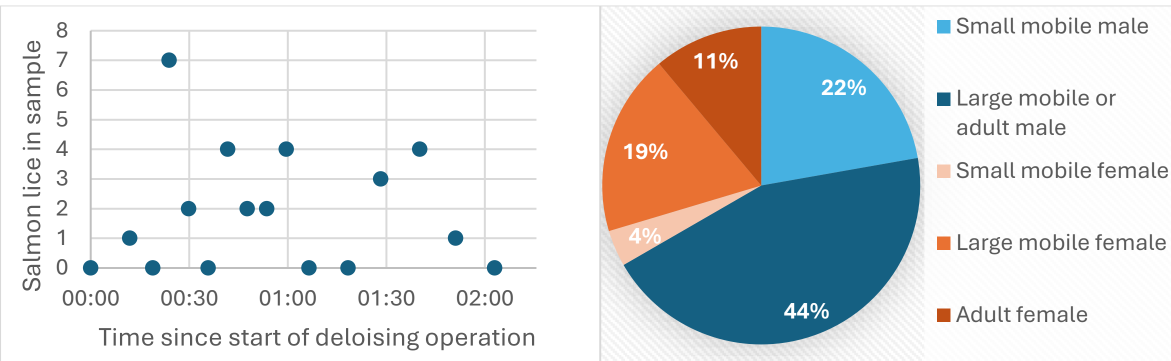


Figure 9. Number of adult and mobile salmon lice in the samples from the seawater drainage onboard the delousing vessel (left). Gender and development stage distribution (right). It was not distinguished between preadult and adult males.

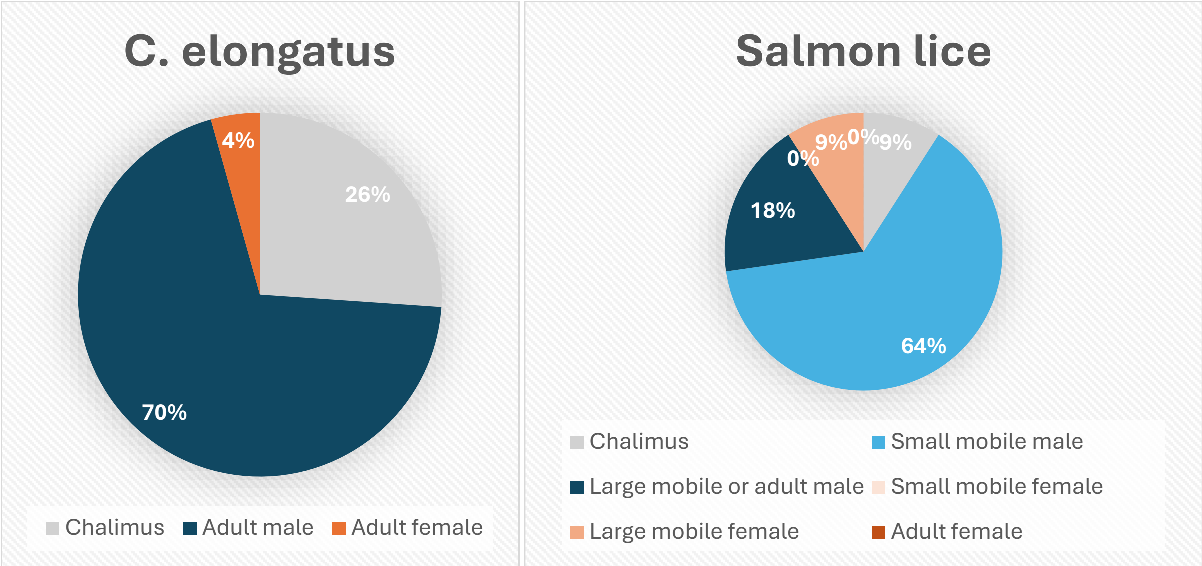


Figure 10. Gender and development stage distribution of all sea lice in the water samples among the crowded fish.

The gender distribution from the water samples collected with a pump among the crowded fish was highly male biased (Fig. 10). The chalimus stages were not gender identified but for all the post chalimus lice 94% of the *C. elongatus* were male and 90% of the salmon lice were male with a dominance of the preadult 1 stage. No adult female salmon lice were found. In this case the male abundance was significantly higher for both species, as tested with a binomial test (p=0.00014 for *C. elongatus* and p=0.0107 for salmon lice).

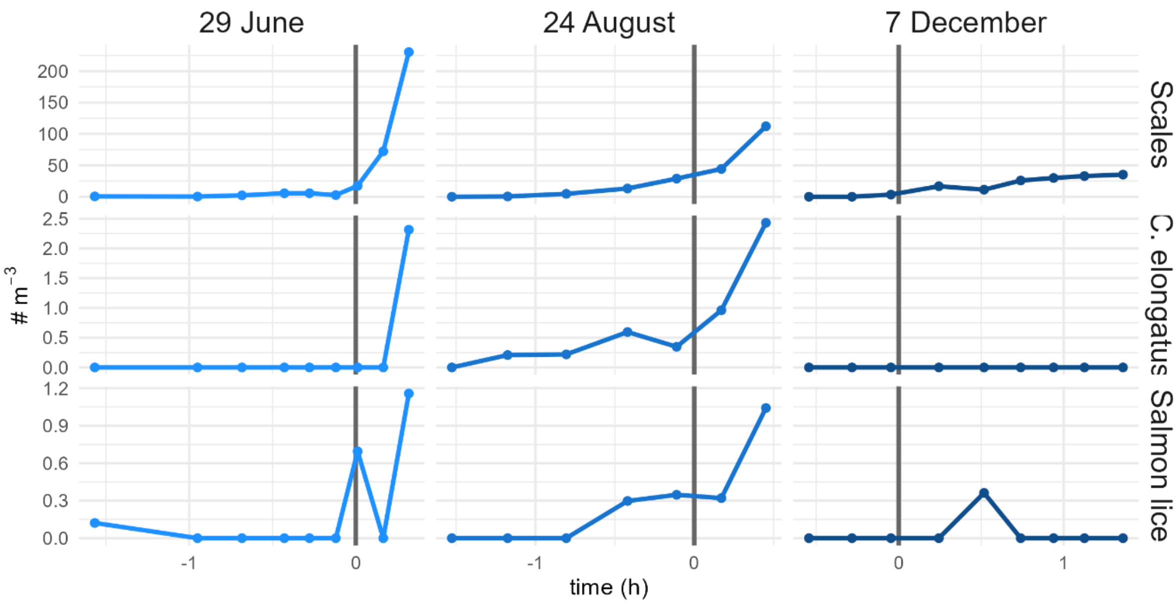


Figure 11 Abundance of fish scales, and live free-swimming adult *C. elongatus* and preadult and adult salmon lice in water samples from the crowded fish. Time zero marks the point when the first fish are pumped onboard the vessel for delousing.

Table 6. The average number of lice on the fish during the first 15 minutes of delousing and number of fish in the net pens at the start of the delousing operation. The main current direction and average current speed at 2 m depth during the delousing operation are also listed.

Date	Adult females #/fish	Large mobile #/fish	Small mobile #/fish	*C. elongatus* #/fish	#fish	Current direction	Current speed cm/s
29-06-2023	0.59 ± 0.14	1.14 ±0.30	0.36 ± 0.14	0.32 ± 0.15	78297	SW - E	5.5
24-08-2023	0.48 ± 0.12	0.58 ± 0.14	0.03 ± 0.03	2.12 ± 0.56	82335	W - S	2.6
07-12-2023	0.41± 0.16	2.09 ± 0.35	0.32 ± 0.12	0 - 0	79749	W - N	6.6

Pumping water samples among the crowded fish was the most successful method for capturing free-swimming mobile, and adult lice. This was done on three sampling occasions with highly variable results (Fig. 11).

Although there were fewer salmon lice on the fish at the start of the delousing operation on August 24, 2023 than on the other two dates, more detached lice were present in the water (Table 6 and Fig. 11). Free swimming lice were found in several samples giving a clear idea of temporal changes in detachment rates (Fig. 11). The two other sampling attempts did not provide such good data, as *C. elongatus* were only found in one sample on June 29, 2023, while it was absent in the last sampling attempt, and salmon lice were only present in 3 samples on June 29, 2023, and in one sample on December 7, 2023.

The weather conditions and current speed at 2 m depth were also variable at the three sampling dates. At the sampling with most free-swimming lice the weather was calm with average winds of 1.2 m s^{-1} and maximum wind gust of 3.6 m s^{-1} at the nearest weather station and the average current speed was less than half of that observed at the two other sampling dates (Table 6). On the 29 June sampling, there was a steady wind with a mean speed of 9.2 m s^{-1} and maximum gust of 12.1 m s^{-1}, while the conditions at the latest sampling were considerably more variable. In addition to the highest measured mean current speed (Table 6), there was also wave action, although not quantified, and the winds were unsteady, with strong gusts. The mean wind speed at the nearest wind station was 4.9 m s^{-1}, with a maximum gust of 19.9 m s^{-1}. One might expect more lice to detach during harsher weather conditions, but on the contrary, fewest lice and fewest fish scales were observed on this day (Fig. 11). However, the dispersion of detached lice is expected to be higher due to the higher current speed and might explain the lower lice concentration in the water.

To account for the variable current speed a simple approximation to obtain more comparable numbers of detachment rates was applied. Fluxes of detached lice (F_{LD}) through a vertical plane in the seawater were estimated by multiplying the measured abundances of free-swimming lice (C) with the average current speed (u), assuming uniform abundances in the seawater surrounding the crowded fish.

$$F_{LD} = Cu$$

After adjusting for variable dispersion due to currents, the fluxes of fish scales were similar in the two cases with the best and worst weather conditions. However, on 29 June, at the onset of delousing, scale fluxes were considerably higher (Fig. 12), potentially reflecting more intense crowding of the fish at that time.

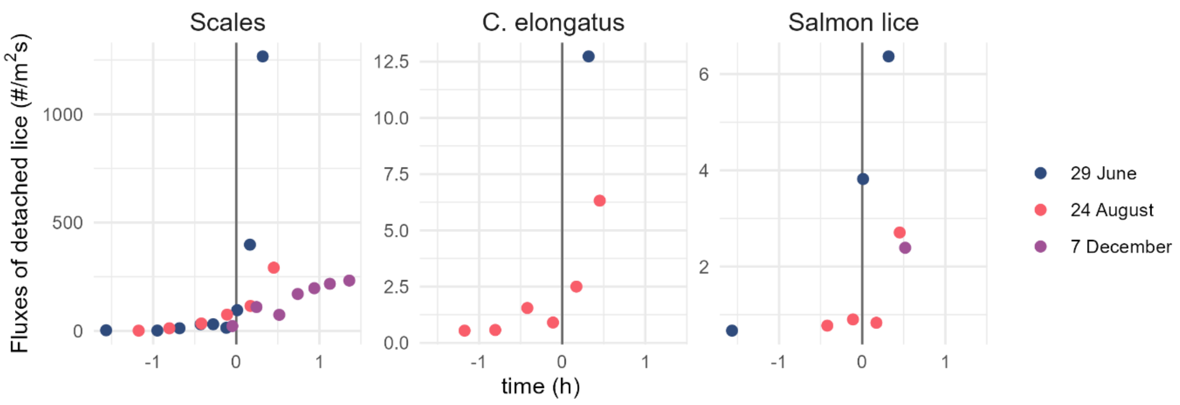


Figure 12 Calculated fluxes of detached lice and fish scales through an imaginary vertical plane within the seawater, positioned among the crowded fish. Datapoints with 0 counts are not included in the figure. Time zero marks the point when the first fish were pumped onboard the vessel for delousing.

On this date, the fluxes of detached lice reached their highest values, 12.7 lice m^{-2} s^{-1} for *C. elongatus* and 6.3 lice m^{-2} s^{-1} for salmon lice. In comparison, on 24 August, the maximum fluxes were considerably lower at 6.3 and 2.7 lice m^{-2} s^{-1}, respectively. Thus, the fluxes of salmon lice were 2.3 times greater on 29 June than on the latter date, closely aligning with a 1.8 higher total abundance of salmon lice on 29 June (Table pump start), when considering both lice and fish counts.

For *C. elongatus*, the flux on 29 June was twice as high as on 24 August, although the total abundance of *C. elongatus* was only about one-seventh of that observed on 24 August. This discrepancy suggests that the potential more intense crowding of the fish on 29 June had considerably higher influence on the detachment of *C. elongatus* than on salmon lice. However, given the limited data points, these findings should be interpreted cautiously.

Indirect infection in treated cages after delousing (WP3)

The observed lice numbers in the first national sea louse monitoring after delousing treatments were significantly higher than predicted by the sea lice population model, which accounts for development from previous stages during the time between delousing and the subsequent sea lice monitoring (Fig. 13). According to the GLMM

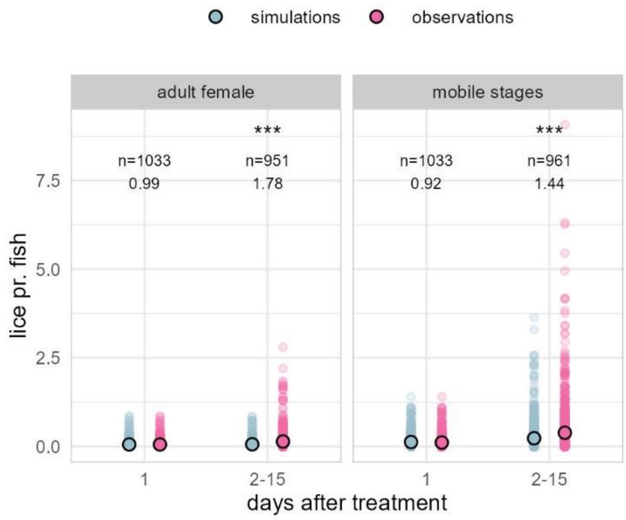


Figure 13. Simulated lice population (blue) and sea lice counts (pink) just after treatment with Optilicer (day 1) and on the day of subsequent sea lice monitoring count of the treated cages (day 2 to 15). The mean and 95 % CI are shown with black outline (circle and error bars). The means are compared by GLMM the statistical significance is indicated on the top by asterisks. The sample size of each group is indicated by n and the modelled estimated difference rate between the simulations and observations is shown below the sample size.

regression model, abundances of mobile lice exceeded predictions by 44%, while adult female lice were 78% more abundant than expected when maturation from previous stages were assumed to be the sole source of lice. These findings suggest considerable reattachment of detached lice following delousing at the farm (Fig. 13 and table 7).

The average number of adult females post treatment was 0.06 per fish and according to the GLMM regression model the average observed number of adult females was 1.78 times higher than predicted by the sea lice population dynamic model, raising the average to 0.11 lice per fish. Similarly, the observed abundance of mobile lice was 1.44 times higher than predicted by the sea lice population model, indicating that reinfection with mobile lice increased the average abundance from 0.23 to 0.33 lice per fish.

To investigate the potential consequence of reattachment post treatment on treatment frequency, a smaller subset of ten simulations were randomly selected, and simulated with adjusted treatment efficiencies on adult females and mobile stages based on the

Table 7. Estimate difference rates (exp(β)) from the GLMM regression models and the *p* value along with the calculated 95 % CI of the estimated difference.

louse	**Days after treatment**	**n**	**Estimated difference rate**	**2.5 % CI**	**97.5 % CI**	***p* value**
adult female	1	1033	0.99	0.77	1.27	0.9254
adult female	2 - 15	951	1.78	1.43	2.21	< 4e-4***
mobile stages	1	1033	0.92	0.77	1.10	0.3598
mobile stages	2 -15	961	1.44	1.28	1.62	< 4e-4***

estimated difference rates respectively, the treatment efficiency on *chalimus* stage was kept constant. It was found that the simulations adjusted for reattachment reached 0.5 adult females per fish on average 7.78 % (95 % CI [3.31, 12.55]) earlier than the simulations with no adjustments for reattachment.

Particle connection between two farm sites (WP4)

The particle tracking model simulations indicate a high potential for particle connectivity between the farming sites A and B. Up to 25% of the particles released from farm A were able to disperse to farm B under the model assumptions that particles were able to infect immediately after release and did not sink on the way. The connection from farm B to farm A was lower with a maximum of 4% of the particles released from farm B reaching Farm A (Fig. 14 and 15). Tidal forcing clearly influenced the connectivity between farms, but further analysis is needed to determine how tides effect this transmission pattern (Fig. 14 and 15).

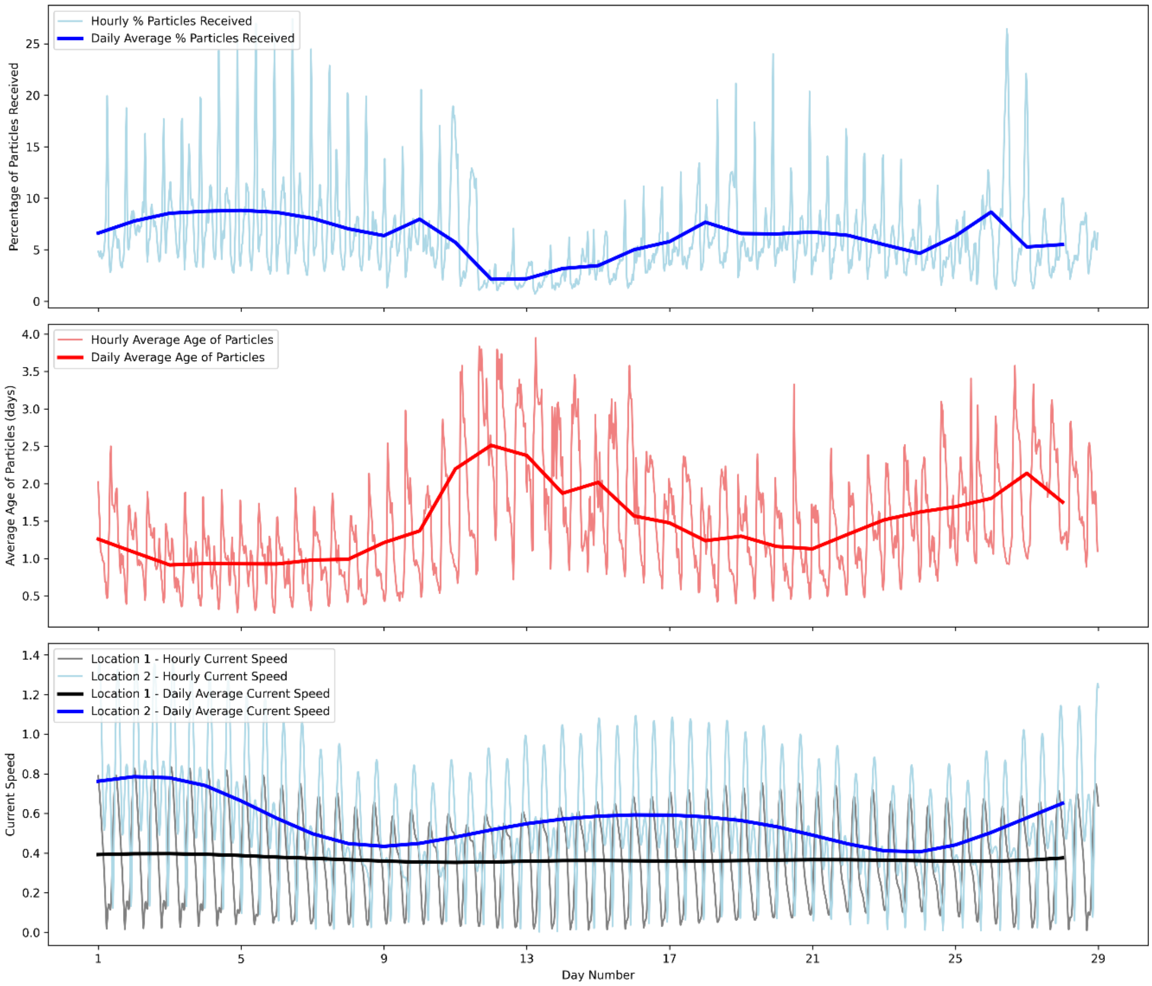


Figure 14. Time series of particle dispersion from farm A to farm B and tidal currents over 29 days, showing hourly and daily averages. The top panel depicts the percentage of particles received at farm B (light blue: hourly, dark blue: daily). The middle panel shows the average age of arriving particles (light red: hourly, dark red: daily). The bottom panel displays current speeds two tidal measurement locations.

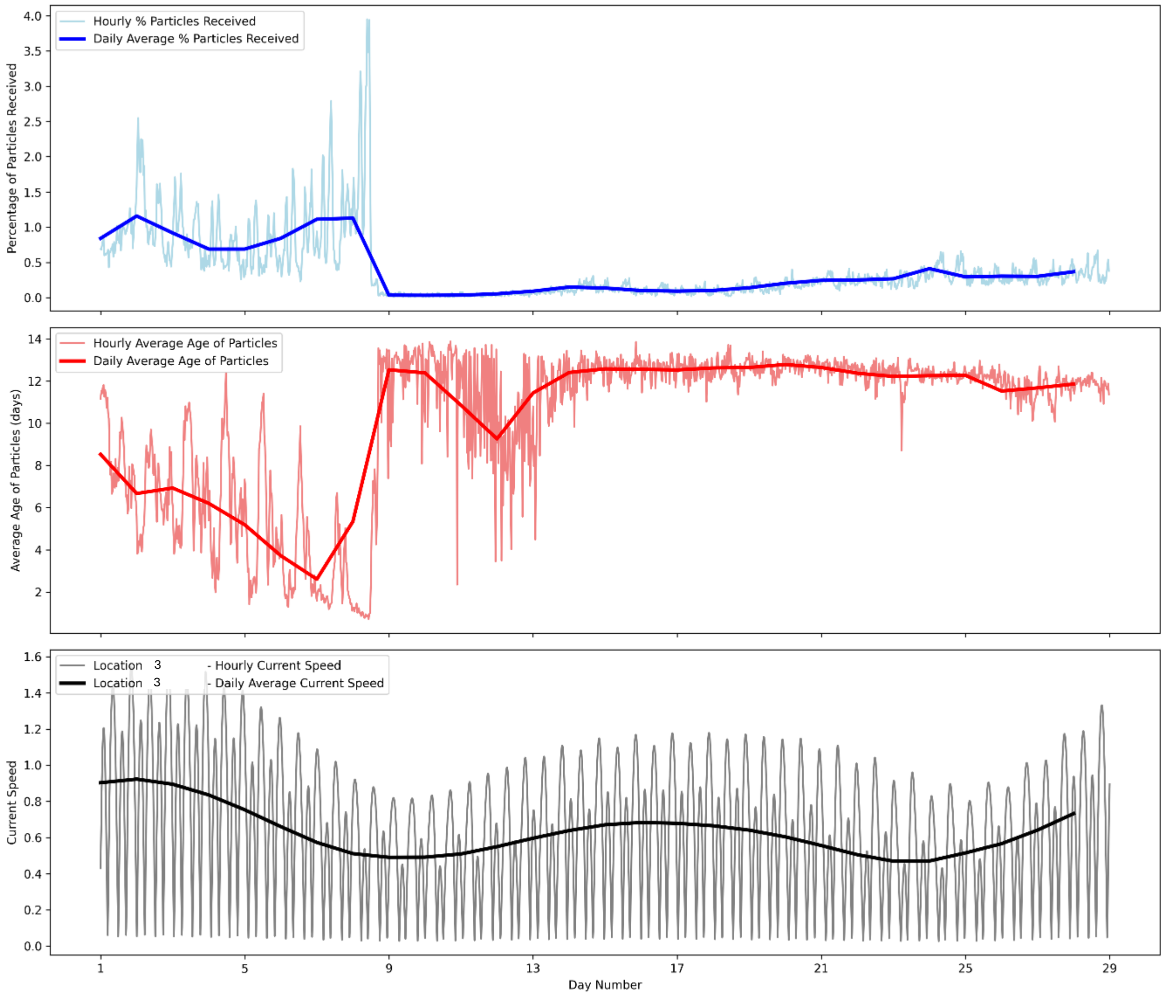


Figure 15. Time series of particle dispersion from farm B to farm A and tidal currents over 29 days, showing hourly and daily averages. The top panel depicts the percentage of particles received at farm A (light blue: hourly, dark blue: daily). The middle panel shows the average age of arriving particles (light red: hourly, dark red: daily). The bottom panel displays current speed a tidal measurement location.

There is a real-life example that could reflect the potential connectivity between farm A and B (Fig. 16 and 17). On December 28, 2021, and January 14, 2022, thermal treatments were performed at farm A, which housed 1 million stocked salmon. One month prior to the former treatment, 750 000 salmon were stocked at farm B. At the onset of the former treatment lice levels at farm A were approximately two mobile and adult salmon lice per fish and approximately 1 adult *C. elongatus* per fish. Assuming that 50% of lice detach during crowding and that 25% (the highest possible connection) or 5% disperse to farm B, it is estimated that 250 000 mobile salmon lice and 125 000 *C. elongatus* could disperse to farm B under the 25% connectivity scenario. Under the 5% connectivity scenario, 50 000 mobile salmon lice and 25 000 C. *elongatus* could potentially disperse to farm B.

In this case, the average lice numbers at farm B could potentially increase by 0.067 to 0.33 mobile salmon lice per fish and 0.033 to 0.167 *C. elongatus* per fish. However, there is no indication of an unexpected increase of mobile salmon lice at farm B in the treatment period (Fig. 16). For C. elongatus, information on population dynamics is limited, as no population dynamic model exists for this species. Notably, lice counts were conducted on the exact same days that treatment were performed (Fig. 17). There is, however, a substantial increase in *C. elongatus* numbers between the lice count on January 14 and the count on January 25, 2022, at farm B.

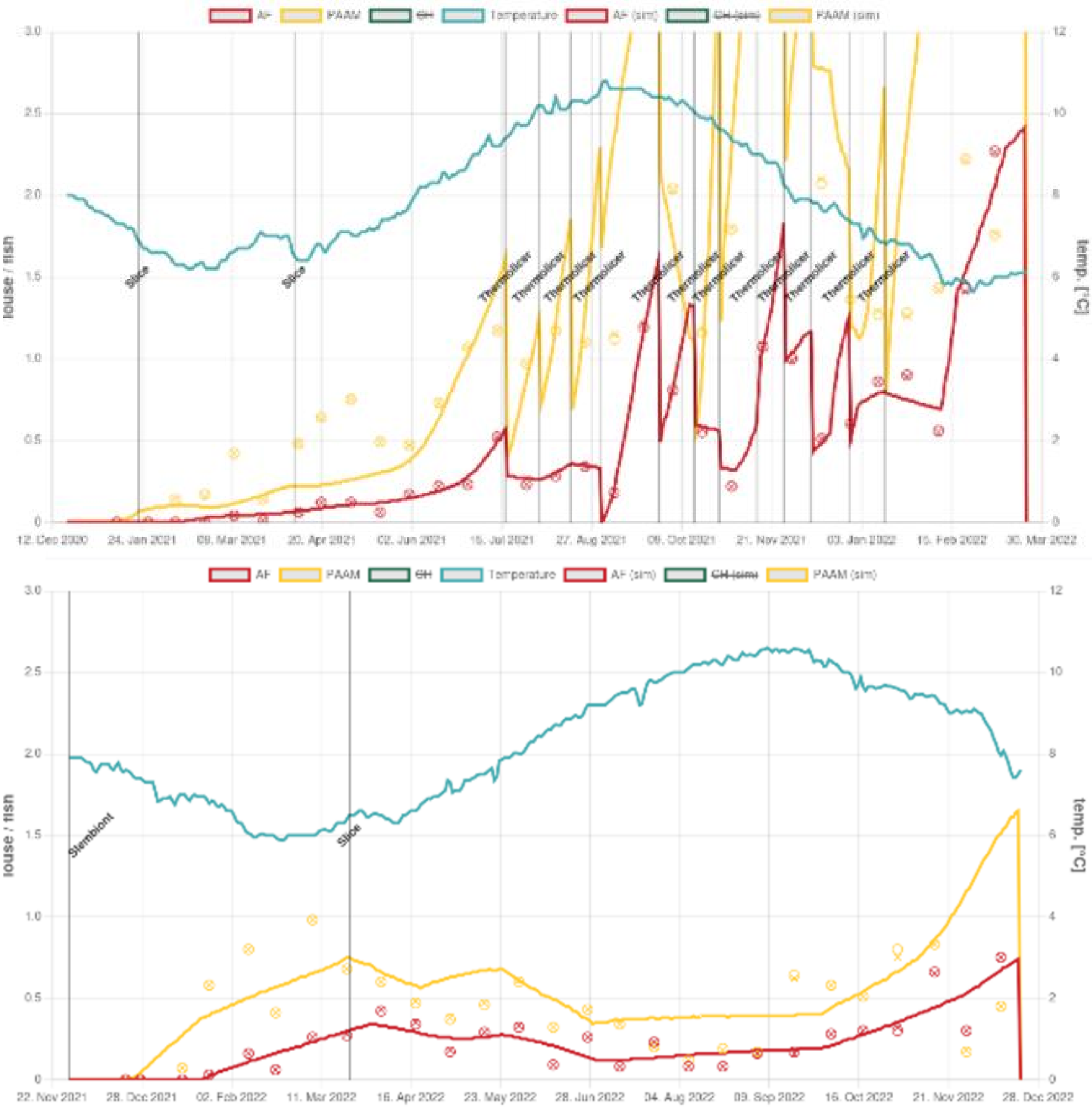


Figure 16. Sea lice populations on farmed fish at farm A (top) and farm B (bottom). The red line shows abundances of adult females, and the yellow line shows abundances of large mobile lice. The vertical grey lines represent treatments with either slice or termolicer and temperature is shown in cyan.

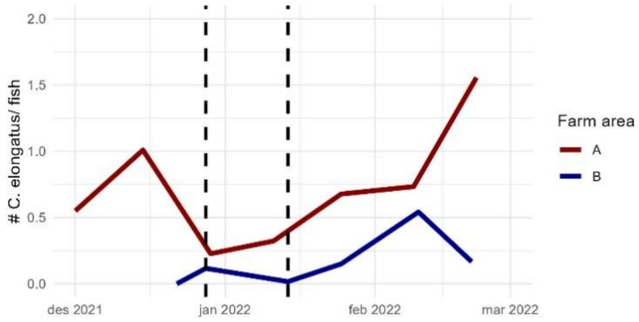


Figure 17. *C. elongatus* abundances at farm A (red) and farm B (blue). The dotted lines indicate thermal treatments at farm A. Fish was deployed at farm B on Nowember, 29, 2021.

Indirect infection at newly deployed cages (WP4)

Indirect infection was investigated on newly deployed fish by identifying the presence of mobile and adult lice stages before these stages could have developed from infective copepodites, under the assumption that the fish were infected immediately after being transferred to the sea. This analysis was performed on data from the national sea lice monitoring programme.

The analysis showed that indirect infection did occur in newly deployed cages, and this was valid for all mobile and adult salmon lice groups and for adult *C. elongatus*.

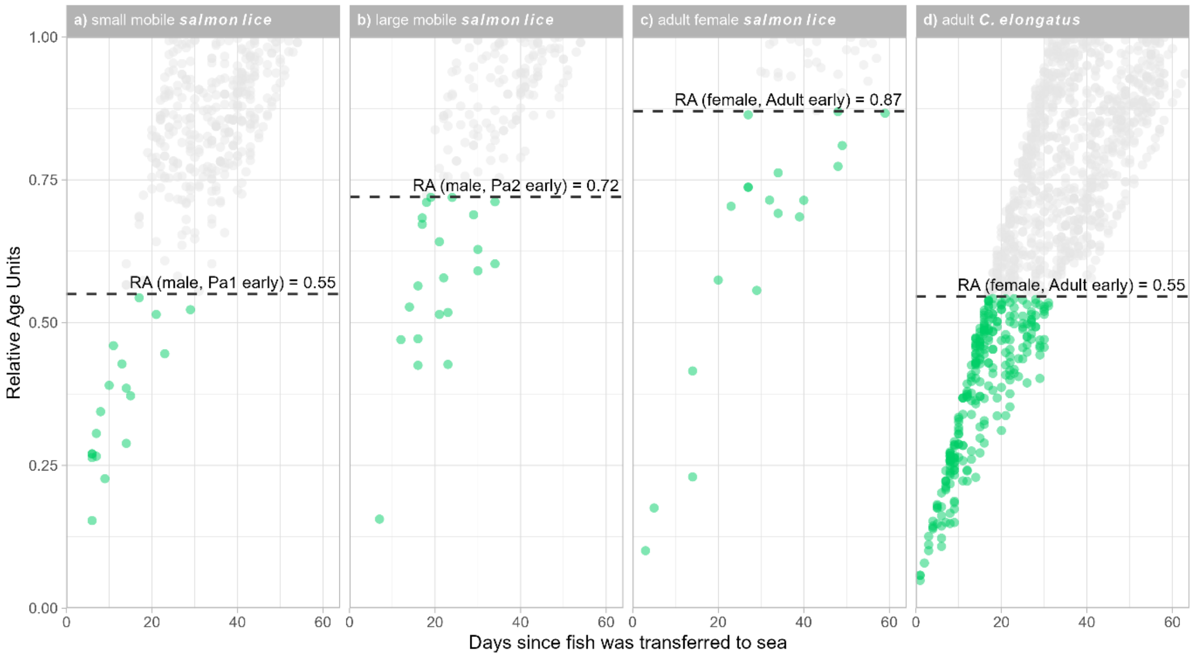


Figure 18. Calculated relative age units (RAU) of salmon lice (a, b, and c) and *C. elongatus* (d) observed on farmed fish when assuming that the fish was infected immediately after transfer to sea. Green dots represent indirect infection routes while grey dots represent direct infection routes. For further information on RAU see Hamre et al. (2019, 2024).

Table 8. Total cages with indirect infection, cages where the sea louse developmental stage was present in other cages prior to infection, proportion of cages with indirect infection relative to the dataset, and cases where the nearest host was from another farm or a wild host.

	Cages with indirect infection	Development stage present at farm	% of total cages with indirect infection	% between farm or wild host to farmed fish transfer
Small mobile salmon lice	17	12	1.3	0.4
Large mobile salmon lice	21	18	1.6	0.2
Adult female salmon lice	19	16	1.5	0.2
All motile salmon lice	54	50	4.2	0.3
Adult *C. elongatus*	250	59	19.5	14.9

However, the indirect infection with salmon lice was substantially lower than for *C. elongatus*. The frequency of observations of mobile and adult salmon lice increased markedly after the period in which direct infection was not possible had ended, as illustrated by the comparison between the green and grey dots in figure 18. On the contrary, the frequency of observations of adult *C. elongatus* showed a continuum with no abrupt changes with increasing relative age units (Fig. 18).

The dataset contained 1279 newly deployed cages in total and indirect infection with mobile or adult salmon lice occurred in 4.2% of the cages (Table 8). However, in most of the occurrences mobile or adult lice were present on fish in other cages at the farm, leaving 4 cages (0.3%) where the nearest host was a wild salmonid or another fish farm. Indirect infection with *C. elongatus* occurred in 19.5% of the cages, where the majority had no adult *C. elongatus* present on farmed salmon in other cages at the farm (Table 8).

Discussion

Detachment during crowding of fish

The study of detachment rates of mobile and adult lice showed that both salmon lice and *C. elognatus* detach during crowding. For *C. elongatus* the data amount was small as the species was only present in 4 of the 9 delousing operations investigated. There was not a clear declining trend in attached lice, but after 1.5 hours of crowding only 23% of the lice remained on the fish (Fig. 4).

For salmon lice the large mobile lice were most susceptible to detach. This group showed a steady decline in lice abundances on the farmed fish during crowding with average lice numbers declining 21.6% per hour. When grouping all mobile and adult salmon lice, there still was a significant decreasing trend in lice numbers, although at a lower rate, i.e. 14.9% per hour. The observations of lice detachment during sedation of the fish in connection to the national sea lice monitoring showed the same trend, where *C. elongatus* were more likely to leave the host than salmon lice, and regarding salmon lice, the large mobile lice were more likely to leave their host compared to adult females and small mobile lice (Fig. 4).

Studies on detachment rates during fish crowding have reported comparable results. Powell et al. (2015) reported average reductions of 14% for adult females and 30% for mobile lice. Guttu et al. (2025) analysed lice numbers before and after crowding during 60 non-medical treatments and found a 29% average reduction in adult females and a 22% reduction in mobile lice (adult males and preadults I and II). In our study, the reduction in adult female lice during crowding was small (~10% after 1.5 hours) and not statistically significant. However, the detachment rate when grouping small and large mobile lice in our study was 22.5%, closely matching Guttu et al. (2025).

Abundances of free-swimming lice

The most successful and reliable method to sample free swimming lice was when water samples were pumped among the crowded fish. However, the results were highly variable, reflecting variable dispersion rates with variable currents. Fewest lice were detected when delousing operations were performed under weather conditions with strong gusts, high current speed and wave action, as the lice most likely were dispersed with the currents. When accounting for the dispersion by currents and total number of lice at the start of the crowding process, abundances of free-swimming lice and abundance of scales were highly comparable in the best and worst weather conditions, while on one occasion (29 June, 2022) the number of fish scales indicated, that the crowding was more severe at the onset of delousing than in the other operations (Fig. 12). Under these conditions the detachment rats of *C. elongatus* were considerably higher than in the other delousing operations, but as this was only one observation, these results must be interpreted with caution. On the other hand, salmon lice detachment rates were not influenced by the potentially more severe crowding and interestingly delousing in harsh weather conditions with strong gusts and wave action did not show higher abundances of free-swimming lice than in operations performed in calm weather.

These studies highlight the need to account for dispersion with currents when sampling free swimming lice from a point source like crowded fish.

Detachment behaviour

Free swimming viable mobile and adult lice found at ~1 m depth in the water column were examined for gender. Although the number of observed lice was small, the pronounced predominance of free-swimming males, exceeding 90% for both species, clearly suggests that males of both species are more likely to: (1) leave a host, (2) swim higher in the water column than females, or (3) be less efficient at locating new hosts compared to detached females. Recent studies on the swimming ability of salmon lice and *C. elongatus* (Barrett, in prep.) found that mobile and adult salmon lice have poor swimming abilities and sink fast, while *C. elongatus* are highly adapted for free swimming. They also found that adult female salmon lice sink somewhat faster than males. Furthermore, another study found that female salmon lice had a slightly higher reattachment rate compared to males (Dalvin, submitted). Faster reattachment rates and slightly higher sinking speeds for females compared to males could partially explain our findings, as the samples were collected near the surface of the water column, where numerous potential hosts were present. The observed reattachment rates for adult female lice following delousing operations, which were higher than for mobile lice, support this explanation. Nevertheless, the most likely explanation for our findings is gender specific detachment behaviour, with males being more predisposed to leave hosts than females.

Studies of host transfer in net cages have found that lice readily transfer hosts. Whereas several studies find that males are significantly more likely to transfer hosts than females (Bui et al. 2020, Connors et al. 2011, Hull et al. 1998), Ritchie (1997) found that 63% of male and 52% of female salmon lice transferred host within 4 days in net pens. Connors et al. (2011) investigated host switching in relation to presence of the opposite sex on the fish and found that for preadult I of both genders host switching was density dependent, whereas preadult II males and adult males were more likely to switch host when females were not present on the current host and adult females mainly remained attached to their host. These findings correlate well with our study on detachment rates during sedation of the fish, as the detachment rate of large mobile lice increased with total number of salmon lice on the fish up to a threshold of 4 lice whereafter it decreased again. For small preadult lice the detachment increased with increasing salmon lice density on the fish (Fig. 6). The increasing detachment rate for large mobile lice with increasing density up to 4 salmon lice per fish might be due to involvement of some chemical cues, where males are more likely to leave the host when sensing lice on nearby fish (Stephenson 2012). The findings that male and female lice show different detachment behaviour aligns with the common findings where mate-searching behaviour is attributed to one gender while the other gender remains stationary (Bandilla et al. 2007).

With detachment rates rising until reaching 2 adult lice per fish, after which the rates decreased slightly, *C. elongatus* also exhibited density-dependent detachment behaviour, probably also linked to the search for mating partners (Fig. 6).

To our knowledge there are no studies on the seasonal influence on detachment rates. In the analysis of the large dataset on detachment rates during sedation of fish in the national sea lice monitoring program, we found temperature to influence detachment rates of all the salmon lice groups and *C. elongatus*, although to a variable degree.

Temperature had little influence on detachment of adult female salmon lice, whereas day length had a more pronounced effect, albeit the detachment rates were generally low. Detachment of large mobile salmon lice was positively correlated to temperature. When keeping the other predictors at their mean value, detachment rates increased from 17% to 22% as temperature increased from 6 °C to 12 °C (Fig. 6), which might be due to higher metabolic rates and hence activity at higher temperatures. However, for *C. elongatus* the pattern differed considerably. Detachment rates decreased sharply from 51% to 34% as temperature increased from 6 °C to 12 °C. This opposing trend suggests species-specific response to environmental variables and might reflect evolutionary adaptations to environmental conditions.

For *C. elongatus* this might be due to migration of hosts. *C. elongatus* abundances on farmed fish exhibit clear seasonal patterns across several regions (McKenzie et al. 2024, Revie et al. 2002, Heuch et al. 2007). In the Faroe Islands, both seasonal and inter-annual variations in abundance occur. Since 2017, abundances have typically begun to rise in August, peaking in September, and then steadily declining towards February (á Norði et al. in prep). This represents a shift towards higher abundances earlier in the year compared to previous patterns documented (á Norði et al. 2015). In the present study, the highest detachment rates of *C. elongatus* was observed during periods with the lowest seawater temperatures, aligning with the seasonal decline in lice numbers on farmed fish.

Indirect infection in treated cages

Salmon lice numbers observed in the national sea lice monitoring following treatment with Optilicer were higher than could be accounted for in the temperature dependent development from previous stages. This part of the study showed higher reattachment rates for adult females compared to mobile lice (Fig. 13). However, the average number of adult females after delousing was considerably smaller than the number of mobile lice. The only similar study that we know of is the study by Guttu et al. (2025) who found that when the fish was deployed in the same cage after treatment the number of mobile lice in the subsequent sea lice monitoring was more than twice as high as after delousing. This is considerably higher than the 44% increase in lice number that could be attributed to reattachment in the present study, but the investigation methods differed as they included lice numbers from monitoring conducted up to 7 days after treatment without accounting for the possible development from earlier stages. For adult females no significant increase in lice numbers was observed in the subsequent sea lice monitoring, which contrasts with the findings of the present study, where the number increased by 78% due to reattachment. While all other results in the present study indicate higher detachment and reattachment rates for mobile lice compared to adult female lice, this particular result shows the opposite trend. This divergence may be attributed to behavioural differences between adult females and mobile lice, as adult females may attach to hosts more quickly than mobile lice. However, further studies are needed to better understand these observed differences.

Modelled long distance indirect infection

The particle tracking simulations indicate that there is a potential for detached lice to be carried with currents and infect neighbouring farms, *e.g.* with the high connectivity from the farm site A to the farming site B. However, in a real-life case, this potential was not evident in salmon lice and *C. elongatus* abundances at farm B after two mechanical treatments performed at farm A (Fig. 16 and 17). The reason for the mismatch between observations and the model is probably that there was no sinking rate of the lice in the model, while resent findings show that mobile and adult salmon lice sink quite fast once they are detached from the host, *i.e.* having sinking rates from ~0.8 to 2 cm s^{-1} depending on development stage (Barret et al. in prep.). However, the same study showed that *C. elongatus* are good swimmers, which aligns with the abundance of *C. elongatus* increasing at farm B when delousing was performed at farm A (Fig.17). But as the fish at farm B had been at sea for a month, the observations of increasing abundances of *C. elongatus* could be lice that had directly infected the fish as copepodites and evolved into adulthood.

Indirect infection in a salmon farm network

As indirect infection occurred in 19.5% of the newly deployed cages, when in most cases, there were no adult *C. elongatus* in other cages at the fish farm, the dataset from the sea lice monitoring programme showed that indirect infection with *C. elongatus* was common (Table 8). However, for salmon lice indirect infection between salmon farms or from wild salmonids was uncommon, as it only was found in 0.3% of the newly deployed cages. This is coplerable to observations in sentinel cages ~5 km away from fish farms where only 0.5 of the infection was due to indirect infection (Pert et al. 2014). However, when salmon lice were present in other cages at the farm, indirect infection levels were somewhat higher adding to 4.2% of the cages. When indirect infection with salmon lice was detected, most often only one salmon louse was observed on the investigated fish, and no salmon lice were observed in 75% of the cases in the subsequent sea lice monitoring count. On the other hand, in observations with indirect infection of *C. elongatus*, more than one louse was observed on the counted fish in more than half of the observations and *C. elongatus* were also detected in the subsequent sea lice monitoring in 67% of the cases.

The higher level of indirect infection with *C. elongatus* corresponds well with the observations of detachment rates for the species in this study and with its documented behaviour. The species is a generalist, and the adult stage can readily transfer between hosts (Pike, 1989), with transitions both within and across different species (Bruno and Stone, 1990, Øines et al. 2006). Free swimming adult *C. elongatus* have also been found in abundances up to 0.4 individuals per cubic meter in plankton samples (Neilson et al. 1987). They demonstrate good swimming ability and can survive without a host for a longer period than salmon lice (Obrestad 2024).

In the few cases were the nearest source of mobile and adult salmon lice was a different farm or a wild salmonid, the development stage at infection was either a preadult I, preadult II or adult male, while no long-distance indirect infection occurred with adult females. However, when the nearest lice were in a different cage at the farming site, indirect infection with adult females also occurred. The higher indirect infection levels at farm level corresponds well with the recent findings that mobile and adult salmon lice tend not to be good swimmers, as they would sink before reaching other farms (Barrett, in prep.).

Implications of the findings on fish farm management

It is often postulated that salmon lice abundances have increased rapidly due to salmon lice treatments at nearby farming sites. However, the findings in this study suggest that indirect infection with salmon lice between farms is highly uncommon and does not significantly influence the population dynamics. Thus, the sudden appearance of large numbers of preadult or adult salmon lice cannot be attributed to delousing operations at neighboring farms. This apparent sudden increase in pre-adult and adult stages is often observed in periods after treatment. Several types of treatments are less effective against chalimus stages, and they don't affect the free-swimming larvae which therefore will be present on the salmon after the treatment or infect the salmon shortly after the treatment. These "hidden" lice eventually molt into preadults and adult stages and can appear almost simultaneously, when considering that there are two weeks between the sea lice counts. This phenomenon can create the impression of an abrupt and unexplained emergence of mobile lice on the farm.

The findings that adult stages of *C. elongatus* readily switch host have considerable implications for management of the parasite. E.g. oral treatment with Emamectin Benzoate is the preferred treatment method in the industry as it is effective for a long period of time, while bath treatments can prove quite ineffective as they only target lice on the fish during the treatment. With a large reservoir of lice on wild fish around the cages, adult *C. elongatus* can readily reinfect the newly treated fish (Imsland 2019).

When crowding fish in connection with non-medical treatments the effectiveness of the effort might be highly variable. Our study found that detachment rates were extremely high towards the end of the crowding operation when the duration was 1.5 hours, and we also found indications of higher fluxes of free-swimming *C. elongatus* when crowding was more severe. Thus, crowding operations should thus be mild and short to keep this lice species on the fish until the fish has been pumped onboard the delousing vessel. Alternatively, a net should be used to catch *C. elongatus* that detach from the fish. On the other hand, preventing *C. elongatus* from leaving the crowded fish and potentially infecting other fish does not prevent indirect infections from wild fish aggregating around the net pens.

Main findings

- Detachment rates of adult *C. elongatus* are considerably higher than for salmon lice
- With detachment rates of 21.6% per hour, large mobile salmon lice are more likely to detach during crowding compared to adult female salmon lice (detachment rate ~10% per hour)
- Indirect infections of salmon lice, whether from one cage to another or between farms, are rare
- Indirect infections with *C. elongatus* are common
- The detachment behaviour of both sea lice species is likely gender specific
- Detachment rates of salmon lice increase with increasing temperature, and for *C. elongatus* the detachment rates decrease substantially with increasing temperature.

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Deliverables

Scientific paper

Title:

Indirect infection of *Lepeophtheirus salmonis* and *Caligus elongatus* in a salmon farm network

Authors:

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Abstract:

Salmon lice (*Lepeophtheirus salmonis*) and *Caligus elongatus* have a direct lifecycle, where infective copepodids attach to their hosts and develop to the adult stage on the same fish. However, with increased attention on salmon lice control and the handling of fish during delousing operations, there has been increasing concern about whether mobile stages of lice, which detach during crowding, might reattach to other fish, thereby following an indirect infection route. Despite these concerns, there is limited documentation on the severity and implications of this potential issue. In the Faroe Islands, sea lice monitoring is conducted by a third party, ensuring consistent documentation across all farms. The data collected is stored in a comprehensive database that includes additional information from fish farms, providing a unique opportunity to investigate the indirect transmission of sea lice between farmed fish or from wild to farmed fish. When fish are transferred to sea, the attached copepodids require a temperature-dependent period to develop into pre-adult and adult stages. If these stages are found on fish that have spent less time at sea than required for development, these lice are likely attached as mobile stages. With only a few lice observed on the fish prematurely, our investigations revealed that indirect salmon lice transmission between salmon farms was highly limited and had no significant impact on the overall epidemiology of salmon lice on farmed fish. In contrast, indirect transmission of *C. elongatus* was more common, as it occurred in 20% of the cages with newly deployed fish, with high numbers of lice, indicating a significant role of indirect infection for this species.

Popular science article

Popular science article submitted to World Aquaculture Magazine

Title:

The Hidden Challenge: Understanding Lice Detachment in Salmon Farms

Authors:

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Summary:

This study explores lice detachment and reinfection dynamics during crowding, providing insights to optimize control strategies. The study revealed significant detachment of lice during crowding, with *C. elongatus* showing the highest detachment rates (77% by the end of crowding) compared to salmon lice (21.6% per hour for large mobile stages). Seasonal changes influenced detachment, with *C. elongatus* detaching more in winter, while salmon lice detachment increased with warmer temperatures. Free-swimming lice, predominantly male, were found in the water post-crowding, highlighting their viability for reinfection. Environmental factors, such as currents, affected lice dispersion, with calmer conditions retaining more lice near farms. These findings emphasize the need for preventive measures and tailored strategies to manage lice mobility and reinfection risks effectively.

Conference presentations

Aliráðstevnan 2024 (Faroese fish farmers association) Tórshavn 01.03.2024

Title:

LiceDetatched – the likelyhood for mobile and adult lice to detatch during crowding and reinfect neighboring farms.

Aqua 2024 (World Aquaculture Society Meeting) Copenhagen 29.08.2024

Title:

The likelihood for mobile lice to leave a host depends on species, temperature and day length

Submitted abstract:

THE LIKELYHOOD FOR MOBILE LICE TO LEAVE A HOST DEPENDS ON SPECIES, TEMPERTURE AND DAY LENGTH

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The most common infection pathway of salmon lice is through the planktonic copepodid stage, but there is an increasing concern that the mobile stages of salmon lice leave/fall off the salmon during crowding operations, potentially re-infecting treated fish or fish in adjacent cages within the same or nearby farms. Knowledge on how many lice fall off during the crowding operation as well as behavioural traits of the various mobile development stages and gender will help to identify the severity of the potential problem.

In this study the number of lice that fell off fish during crowding was quantified at the thermal delousing of 9 individual net pens. In addition, a large dataset from sea lice monitoring of farmed salmon was analysed for behavioural traits regarding lice that fell off. In the Faroese national sea lice monitoring program registrations of lice that fall off during sedation of the fish is imposed. Data from more than 30000 counted net pens from mid-2016 to February 2024 was statistically analysed.

In the dataset sea lice were registered as species (*Lepeophtheirus salmonis* or Caligus *elongatus*) and for salmon lice the development stage was also registered (adult females, large mobile which include preadult stage 2 and adult males, and preadult 1). Temperature and date were also registered and used to analyse potential variations in detachment due to temperature and day length.

C. elongatus were much more prone to detach from the fish than salmon lice and both species showed seasonal variations in the percentage of lice that detached from the fish (Figure 1).

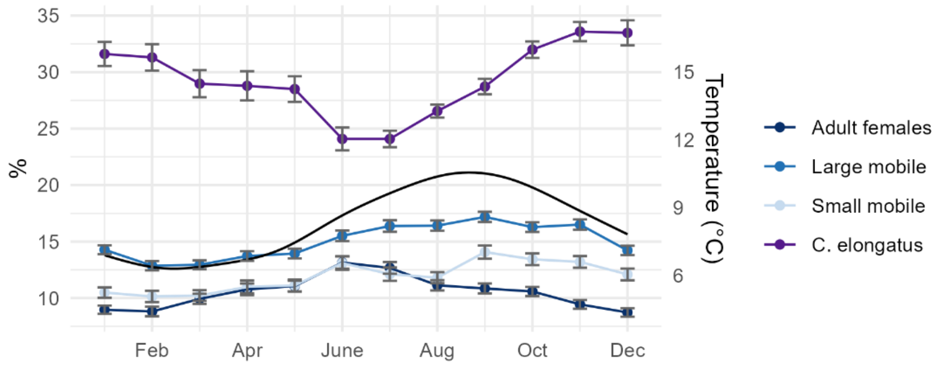

Figure 1 Percentage of lice that fell off during sedation of the fish prior to sea lice monitoring (mean ± SE) and average temperature (black line, secondary y-axis).