FIRUM
knowledge for sustainable aquaculture

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Lumpfish & AkvaNest

Impact on welfare and cleaner fish efficiency (FHF project 901781)

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1 Summary

1.1 Norsk sammendrag

Dødeligheten blant rognkjeks er høy, og deres renseaktivitet er inkonsekvent. Dødelighet skyldes ofte bakterieinfeksjoner, som kan oppstå når fiskens immunsystem svekkes. Dette kan skje dersom hudens fysiske barriere blir skadet som følge av håndtering eller tøffe værforhold. I tillegg kan langvarig stress fra utilstrekkelig tilgang til fôr eller skjul, eller energien som brukes for å håndtere sterke strømmer og bølger, ytterligere svekke immunforsvaret og gjøre fisken mer mottakelig for infeksjoner. Studier på skjul for rognkjeks har ikke kommet til enighet om hvilken type skjul som er best, kun at de bør ha glatte og relativt faste overflater. Naturlige skjul av tang kan redusere bakterieinfeksjonspresset (f.eks. Tenacibaculum) på grunn av deres antibakterielle egenskaper. I tillegg har de fordeler som lavt vedlikehold, myk tekstur og redusert risiko for plastforurensning. Anekdotiske data fra lakseoppdrett viser at rognkjeks foretrekker å oppholde seg i tang som vokser på overflater i merdene, så tareskjul kan fremme aktivitet hos rognkjeks nær dem. På samme måte kan laks være mindre avvisende mot å nærme seg tareskjul, noe som kan gi flere muligheter for rensing.

Forståelse av fordøyelsestiden for lakselus i rognkjeks er avgjørende for å vurdere renseaktivitet. Lus i magen til rognkjeks gir en indikasjon på aktivitet, men forholdet mellom antall lus i magen og antall lus spist per dag er fortsatt uklart.

I dette prosjektet ble et laboratorieforsøk utført for å undersøke fordøyelsestiden for lakselus i rognkjeks. Rognkjeks ble bedøvet og gitt én bevegelig lus eller én voksen hunnlus, enten levende eller frossen. Dette ble gjentatt ved to temperaturer, 6 og 9 grader Celsius. Rognkjeks ble dissekert hver dag i 12 dager, og eventuelle lus som ble funnet, ble vurdert for nedbrytningsgrad. I tillegg ble et feltforsøk i et industrielt lakseoppdrett gjennomført for å sammenligne levende tareskjul med plastskjul. Tre merder med tareskjul og tre med plastskjul ble utstyrt med kameraer nær skjulene, og indikatorer for rognkjeksens velferd samt mageinnhold ble samlet inn annenhver uke i tre måneder. Mikrobiomprøver ble også tatt fra gjeller og hud hos rognkjeks samt fra skjulene.

Fordøyelsestidene var mye lengre enn forventet. Det virket ikke som om vanntemperatur eller lusens friskhet hadde noen effekt, noe som også var uventet. Det var derimot en forventet effekt av lusens utviklingsstadium, der voksne hunnlus tok mye lengre tid å fordøye enn bevegelige lus (henholdsvis 12 og 6 dager i snitt). Disse resultatene står i kontrast til andre resultater publisert mens dette forsøket pågikk, som indikerte litt over én dags fordøyelsestid (Staven et al. 2024).

Tareskjul fremmet verken aktivitet hos rognkjeks, interaksjoner med laks, antall lus i magen eller antall lus på laksen. Når det gjelder rognkjeksens velferd, var det ingen forskjeller i operasjonelle velferdsindikatorer mellom de to typene skjul, men det var forskjeller i mikrobiomet, både på skjulene

og på fisken. Skjul av tang motsto begroing og inneholdt færre sykdomsfremkallende bakterier, noe som potensielt kan redusere infeksjonsrisikoen. Over tid tilpasset mikrobiomet på rognkjeksens hud seg til sin type skjul, noe som antyder at tareskjul kan redusere infeksjonspress under stressende hendelser. Ved å redusere bakterieinfeksjonspress kan naturlige tareskjul redusere dødeligheten, forlenge rognkjeksens renseperiode og derved øke deres samlede effektivitet i oppdrett.

1.2 English summary

Mortality amongst lumpfish is high and their cleaning activity is inconsistent. Mortality is often caused by bacterial infections, which may occur when the fish's immune system is weakened. This can happen if the physical barrier of their skin is compromised due to injuries from handling or harsh weather conditions. Additionally, prolonged stress from inadequate access to feed or shelter, or the energy expended to cope with strong currents and waves, can further suppress their immune defences, making them more susceptible to infections. Studies on lumpfish shelters have not reached a consensus on which type of shelter is the best, only that they need to have smooth and fairly firm surfaces. Natural seaweed shelters may reduce bacterial infection pressure (e.g., Tenacibaculum) due to their anti-bacterial properties. Additionally, they offer benefits such as low maintenance, a soft texture, and reduced plastic pollution risk. Anecdotal data from salmon farms indicates that lumpfish prefer to spend their time in seaweed growing on surfaces in the cages, so seaweed shelters might promote lumpfish activity near them. Similarly, salmon might be less averse to approaching seaweed shelters, which could lead to more opportunities for cleaning.

Understanding the digestion time of salmon lice in lumpfish is key to assessing cleaning activity. Lice in lumpfish stomachs provide a measure of activity, but the relationship between stomach counts and daily lice consumption remains unclear.

In this project, a laboratory experiment was carried out to investigate the digestion time of salmon lice in lumpfish stomachs. Lumpfish were sedated and fed one mobile louse or one adult female louse either alive or frozen. This was repeated at two temperatures, 6 and 9 degrees Celsius. Lumpfish were dissected each day for 12 days and any lice discovered were scored for degradation level. Additionally, a field experiment in an industrial salmon farm was carried out to compare seaweed shelters to plastic shelters. Three cages with seaweed shelters and three cages with plastic shelters were equipped with cameras near the shelters and lumpfish welfare indicators as well as stomach contents were sampled every two weeks for three months. Microbiome samples were also taken from the lumpfish gills and skin as well as the shelters.

Digestion times were much longer than expected. There did not appear to be any effect of water temperature or of louse freshness, which was also unexpected. There was an expected effect of louse developmental stage, with adult females taking much longer to digest than mobile lice (12 and 6 days on average, respectively). These results are contrary to results published while this experiment took place that indicated just over one day in digestion time (Staven et al. 2024).

Seaweed shelters did not promote lumpfish activity levels, interactions with salmon, louse numbers in stomachs, or number of lice on the salmon. Considering lumpfish welfare, while operational welfare indicators also did not differ between the two types of shelters, there were differences in microbiome, both on the shelters and on the fish. Seaweed shelters resisted biofouling and harboured fewer pathogenic bacteria, potentially reducing infection risks. Over time, lumpfish skin microbiomes aligned

with their shelter types, suggesting that seaweed shelters might reduce infection pressure during stress events. By lowering bacterial infection pressure, seaweed shelters could reduce mortality, extending the lumpfish's cleaning period and overall effectiveness on farms.

2 Background

2.1 Scientific background for the project

Lumpfish mortality in salmon farms is high and their cleaning effect is highly variable (Boissonnot et al. 2023; 2022). One contributor to both lumpfish welfare and their behaviour within a salmon cage, is the shelter that is offered to them. The optimal type of inorganic shelter to use in lumpfish cages has been investigated scientifically (Imsland and Conlon 2019; Imsland et al. 2018) and some trials have been conducted to compare seaweed shelters to their plastic counterparts (Svendsen 2021; Vedvik 2021), though with mixed results. However, research has shown that fish living in regular close contact with a host or shelter have a different microbial composition in their skin mucus than those fish living without this contact (Pratte et al. 2018). Also, a pilot study carried out at Firum found that seaweed shelters had a lower relative amount of *Tenacibaculum* on their surface than plastic shelters (Figure 1). Lumpfish are known to be vulnerable to infections by *Tenacibaculum* species (Spilsberg et al. 2022) as well as other bacterial diseases (Erkinharju et al. 2020). Investigating the potential effect of the plastic and seaweed shelters on the lumpfish's skin mucus microbiota could provide knowledge to help mitigate these infections. Additionally, seaweed such as *Saccharina latissima*, which is the natural habitat for juvenile lumpfish, does not require cleaning, and eliminates the risk of plastic pollution from lumpfish shelters when deployed instead of plastic shelters.

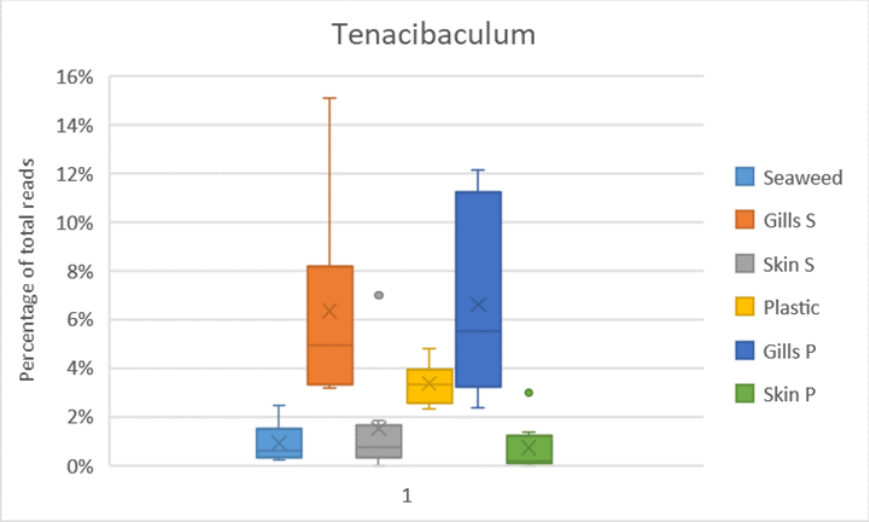

Figure 1 Relative amounts of reads defined as Tenacibaculum Haliotis from sequencing of microbiota extracted from seaweed and plastic shelters and the gills and skin mucus of lumpfish using them.

None of the above studies directly investigated whether natural seaweed shelters might affect lumpfish in such a way that they become more efficient cleaners. Some investigations were done about their operational welfare indicators and their preferences, though not comparing whether the lumpfish preferred natural seaweed over plastic shelter types. If natural seaweed promotes natural behaviour in lumpfish, this could lower stress levels, increase activity levels, and indirectly lead to more interactions

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with salmon. Similarly, salmon might also prefer natural seaweed, which again could increase the chance of interaction and cleaning.

Cleaning efficiency in lumpfish is difficult to investigate for several reasons. The end result – number of lice on the salmon – tells only half the story, as this number is also dependent on the infection pressure at the site. In other words, you may have highly effective cleaners and still have too many lice. The number of lice found in a lumpfish stomach on the other hand is a clear indication of how many lice that fish has consumed – but in how long? There is little good evidence to show the factors that affect how long a salmon louse persists in a lumpfish stomach once consumed. An early study indicates that lice may stay in the stomach for an excess of 72 hours (Eysturskarð, Johannesen, and Eliasen 2017) and while a recent study indicates much shorter digestion times (Staven et al. 2024), no follow up study investigates the effects of temperature, presence of other stomach content, and louse size. Similarly, larger lumpfish are less frequently seen with sea lice in their stomachs, which has led some to conclude that these fish are less effective cleaners. However, larger lumpfish also have a wider digestive tract, and it may simply be that they digest the lice faster, which would lead to fewer lice found in the stomachs.

This project aims to answer questions in relation to FoU challenge "Forebygging og kontroll av lakse- og skottelus", particularly aim number 2 "Fremskaffe kunnskap om hvilke betingelser som gjør rensefisken til en effektiv lusespiser" by seeking to investigate how to improve lumpfish cleaning efficiency using living seaweed shelters (AkvaNest) in salmon cages as opposed to plastic shelters.

In order to thoroughly investigate the actual cleaning efficiency of the lumpfish studied at sea, we aim to carry out a study in tanks investigating digestion time of lice at three different temperatures as well as using three different size classes of lumpfish. This way, we will be better able to determine cleaning activity in the cages studied regardless of sea temperature and lumpfish size.

We also aim to investigate whether AkvaNest, the living seaweed shelters produced by TARI, improve lumpfish welfare through stress reduction, less abrasion, and improved microbial balance on the skin. As lumpfish is the only known cleaner fish native in the Faroe Islands, no other cleaner fish species will be studied in current project. Secondly, we aim to investigate whether through improved welfare or changing behaviour, seaweed shelters may increase cleaning activity in lumpfish. The seaweed species used in this project (*Alaria esculenta* and *Saccharina latissima*) are seaweeds that are already being cultivated in Norway by for example seaweedsoutions.com and leroyseafood.com. They are also species of seaweed that lumpfish are frequently found in

2.2 Project scope

The total budget was 2.713.000 NOK and the planned project duration was two years, though delayed by three months for a total of two years and three months.

2.3 Project organisation

The project was led by Firum in collaboration with Tari and Bakkafrost. The project lead was researcher Ása Johannesen and the project group consisted of Kirstin Eliasen (researcher at Firum), Ása Jacobsen (researcher at Firum), and Agnes Mols Mortensen (researcher and seaweed producer, Tari).

The field work was carried out at Bakkafrost's farm in Froðba. Bakkafrost's contact person was Marner Nolsoe (veterinarian, Bakkafrost).

The project was funded by FHF and the reference group was Eskil Bendiksen, Kjetil Heggen, Eirik Ruud Sigstadstø, Esbern Jóannes Patursson, and Marner Nolsoe.

The work was divided into 5 work packages as follows:

1. Louse digestion, responsible: Kirstin Eliasen, Firum
2. Lumpfish welfare, responsible: Ása Jacobsen, Firum
3. Fish behaviour, responsible: Ása Johannesen, Firum
4. Shelter production and monitoring, responsible: Agnes mols Mortensen, Tari
5. Louse consumption, responsible: Kirstin Eliasen, Firum

3 Aims and objectives

3.1 Objectives and use in industry

The main goal is to investigate the effect of using AkvaNest seaweed shelters on the welfare and cleaning activity and efficiency in lumpfish. This goal is divided into three sub-goals; establishing a reliable louse consumption monitoring method (WP 1), establishing the suitability of live seaweed shelters for lumpfish welfare (WP 2+4), and establishing whether the use of live seaweed shelters increases cleaning activity and efficiency in lumpfish (WP 3 +5). When it comes to decreasing louse burdens on salmon, lumpfish are an important tool in the toolbox available to industry. However, there are still challenges surrounding lumpfish survival and cleaning efficiency, so any potential improvement can have a great impact on the ability of farmers to successfully keep their salmon free from lice. In this project, we hope to clarify the impact of using either living seaweed shelters or plastic shelters for the lumpfish, aiding industry in their decision making.

3.2 Deliverables

Presentations in academic and industry forums, newsletters, and scientific publications. Result about louse digestion rates and the impact of type of shelter will be the main focus of communication to the public, industry, and the scientific community.

4 Project execution

4.1 Structure

The project was divided into work packages, each focusing on specific parts of the main investigation. Each work package had a leading researcher, who was responsible for organising the package execution and collecting the data. Detailed methods are described below split into WP 1, which was a land – based experiment and WP 2-5, which all involved the same field trial.

4.2 WP 1 Digestion of salmon lice in lumpfish stomachs

4.2.1 Research animals

The lumpfish used in this study were collected from salmon cages at a Hiddenfjord farming site, one to two months post deployment. The fish originally came from Benchmark Genetics Iceland hf in Iceland, where the lumpfish were F1 from wild caught broodstock.

At arrival at the research facility, the lumpfish (n = 208, split in two rounds of trials with 144 and 64 lumpfish respectively) were distributed among experimental housing tanks. The tanks were white-bottomed, black-sided fiberglass tanks with a capacity of 125 litres, measuring approximately 50 cm × 50 cm × 50 cm. Maximum biomass in each tank was 10 kg/m3. The tanks had a flow-through system with aerated seawater, maintaining a flow rate of two litres per minute, equating to a full tank exchange every hour. Each tank was equipped with shelters made of black PE drainage pipes cut in half lengthwise and hung vertically in pairs (40 cm long, with a total area of 0.12 m²). The overhead fluorescent lights tubes were set to a 12:12 light:dark schedule. The lumpfish were fed to satiation on commercial feed pellets (Skretting, Clean Lumpfish 3, 3 mm), which was the same feed as the lumpfish were fed at the farming site, once or twice daily throughout the duration of the experiment. To ensure that any salmon lice consumed at the farming site did not affect the study results, and to ensure the lumpfish were acclimatised to their new tank environment, the digestion trial did not start until seven to nine days after their arrival at the research facility.

Lumpfish were intentionally chosen by eye to represent a range in sizes from deployment size up to the size when anecdotally, they decrease their louse cleaning activity (approximately 200g). By the end of the experiment, the lumpfish were weighed and had a mean weight of 94.3 g, with a standard deviation (SD) of 33.3 g, reflecting the size of fish in the early phase of deployment.

Salmon lice used in the study were collected from salmon farms during the mandated sea lice counts, with the collection occurring within days before the start of the trial. The lice were sampled directly from the salmon, placed in buckets filled with seawater, and transported by car to the research facility. To ensure the lice remained alive until the experiment began, aeration was provided to the buckets, and the water was maintained at in situ temperatures. However, in order to compare our results to those of Staven et al. (2024), a subset of the sampled salmon lice was frozen at -80°C immediately upon arrival at the research facility to account for any effect of freezing on digestion time.

4.2.2 Experimental setup and sampling

The study consists of three treatments (freshness, developmental stage and temperature) divided into two trials conducted at separate times.

1. The first trial compared digestion time of frozen and live salmon lice where half the lumpfish (n = 72) were fed live lice and the other half (n = 72) were fed frozen lice. Lumpfish were administered lice in batches of 8. Each batch of lumpfish was assigned to a designated tank (18 tanks in total), representing sampling points at intervals of 0.5, 1, 2, 3, 4, 6, 8, 10, or 12 days after the feeding event with two batches per time point; one batch for frozen and one for live lice totalling 16 fish per time point. Additionally, to compare digestion time of developmental stage of lice, two lumpfish from each batch were administered an adult female salmon louse, while six lumpfish were fed a large mobile salmon louse, i.e. a preadult II or adult male salmon lice (52 adult females and 156 large mobile lice in total).
2. The second trial was carried out at a lower temperature, but using only live salmon lice. The decision to not use frozen lice in the second trial was made in order to minimise the use of animals as the initial trial had clear enough results for frozen lice and establishing the effect of temperature on digestion of live lice had a higher priority. The second trial also did not include the 0.5-day sampling point, as all lice were recovered after 24 hours at the warmer temperature in the previous trial (n = 64, eight tanks in total).

To facilitate handling, lumpfish were lightly sedated using a solution containing 100 mg/L MS-222 (Tjaldurs Apotek, Tórshavn, Faroe Islands), in accordance with the protocol outlined by Skår et al. (2017). The sedated lumpfish were administered a single salmon louse via oral insertion, guiding it past the oesophagus using rounded-tipped forceps.

All lumpfish were offered their usual lumpfish feeds throughout the incubation period to ensure that digestion activity remained normal and like that at a farming site. The selection of sampling points was based on a previous study, which found that 66% of adult male salmon lice could be visually identified three days after being consumed by lumpfish (Eysturskarð, Johannesen, and Eliasen 2017). All fish recovered seamlessly from the sedation, displaying no signs of distress or adverse effects from the procedure, and there were no recorded mortalities throughout the experiment.

Temperature readings were recorded using RBR Solo3 D temperature loggers, capturing data at 10-minute intervals. The experiment was conducted twice, each at a different temperature regime: first, from December 9, 2023, to December 21, 2023, with an average temperature of 9.01°C (minimum: 7.64°C, maximum: 9.52°C) and with an average lumpfish weight of 89.7 g, with a standard deviation (SD) of 20.9 g; and second, from March 1, 2024, to March 13, 2024, with an average temperature of 6.25°C (minimum: 5.14°C, maximum: 6.68°C) and with an average lumpfish weight of 104.5 g, with a standard deviation (SD) of 49.7 g.

At each designated sampling point, lumpfish underwent euthanasia through a 15-minute exposure to a 1 g/L MS-222 solution and their stomachs dissected for content assessment. Time elapsed since feeding, weight, length, and determination of sex were recorded in addition to the presences of lice and lumpfish feed. Each salmon louse discovered was evaluated for signs of degradation, with categorizations of (0) no apparent degradation, (1) slight signs of degradation, and (2) significant signs of degradation (Figure 2). For statistical purposes, when analysing degradation level of lice, a fully digested (not found in the stomach) louse was included at degradation level (3).

Despite being housed at the research facility for at least a week prior to the commencement of the experiment, one lumpfish, which had received a frozen louse, was discovered with 11 adult female salmon lice and one mobile salmon louse in its stomach at four days incubation time (11 days since it was taken from the salmon farm), making it impossible to determine which, if any, of the discovered lice, was the one given to the fish as part of the experiment. This occurrence suggests that the lumpfish retained ingested salmon from the farming site in its stomach for at least 11 days after ingestion, but the fish had to be excluded from the data analysis (n = 207) as it was impossible to determine the degradation state of the experimental louse, whether it was an adult louse or not, or whether it was even still present in the stomach.

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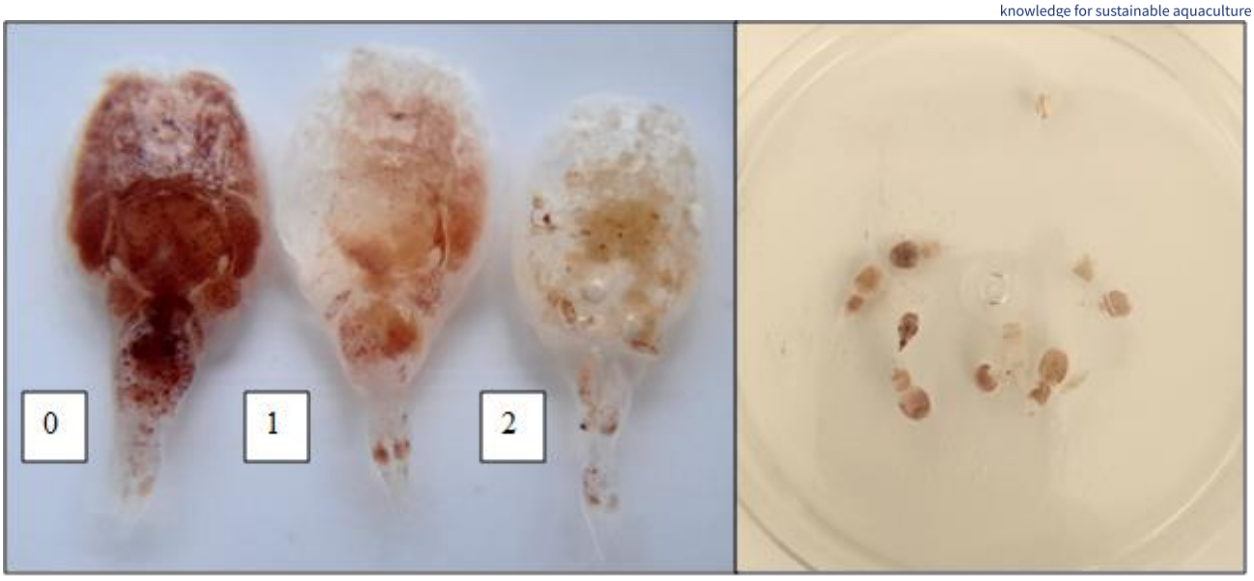

Figure 2 Degradation categories for salmon lice were defined as follows: (0) no apparent degradation, (1) slight signs of degradation, and (2) significant signs of degradation (left). The 11 adult female salmon lice and one mobile salmon louse found in a lumpfish stomach at four days incubation time (right). (Eliasen et al. 2024)

4.3 WP 2-5 Lumpfish cleaning activity and welfare in salmon cages

4.3.1 Project setup

The experiment took place in the Faroe Islands at the Froðba salmon farm owned and operated by Bakkafrost (61°32'27" N, 6°46'05" W). The farming site contained 12 salmon farming cages of which the project had access to sampling and measurements in six, three containing plastic shelters and three containing seaweed shelters (Figure 3). No lumpfish were added to cages without shelters.

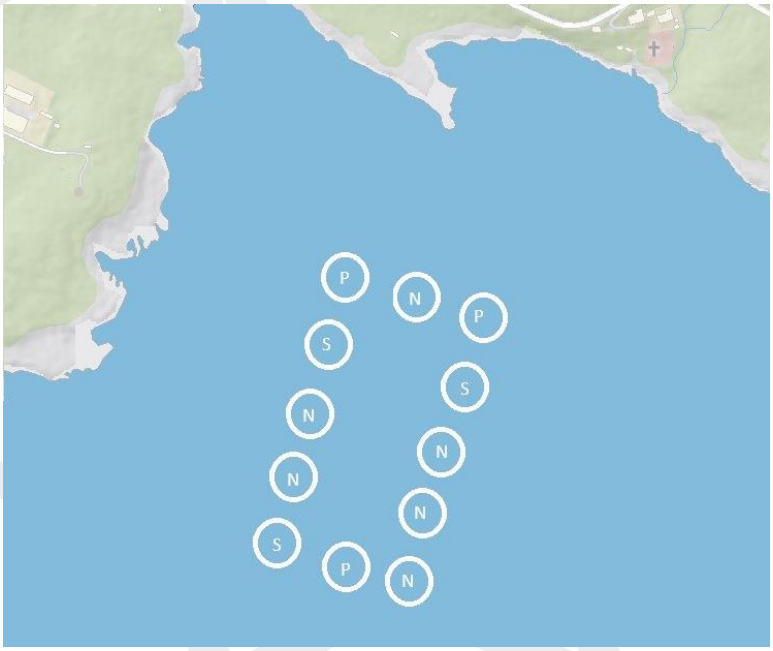

Figure 3 Fieldwork experimental salmon farming cages containing seaweed (S), plastic (P), or no (N) shelters. Cages with no shelters did not have lumpfish. (Jacobsen et al. 2024)

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Lumpfish were added to the cages in April and May 2023, where each cage received lumpfish only once but not all cages on the same date. Shelters were added to cages prior to deployment of lumpfish. The plastic shelters were of the type SeaNest (Imenco, Norway) with a surface area of 30 m2 per shelter. Two shelters were used in each cage with plastic shelters. The species used for seaweed shelters was *Saccharina latissima*, which were propagated onto lines in tanks and grown to appropriate size before deployment in the farming cages approximately 60 meters of lines with seaweed was deployed in each cage. Two main lines (30m) were stretched across the cage diameter with line droppers with seaweed hanging down from the main line every 5 meters. (Figure 4).

Figure 4 Plastic (left) and seaweed (right) shelters used in the cages.

4.3.2 Lumpfish welfare

From deployment in the first cage and throughout the trial period, 10 lumpfish from each cage were sacrificed and scored for Operational Welfare Indicators (OWIs) using the method described in Eliasen et al. (2020).

Fish were weighed to the nearest gram and total length and height were measured to the nearest millimetre. The external indicators for fins, eyes, skin, and sucker were scored from 1-3 following these criteria:

- Fins
  - 1 Indicates very little wear of the fins. Minor tears and signs of regrowth accepted.
  - 2 Indicates some wear with shortened rays and partially missing webbing
  - 3 Indicates fins with wear down to the main body of the fish with open wounds
- Skin
  - 1 Indicates near perfect skin with no to little signs of injury
  - 2 Indicates small signs of injury or bruising, particularly around the spines
  - 3 Indicates open wounds, sometimes connected with a score of 3 in fins
- Eyes
  - 1 Both eyes are in good condition

2 One eye is in poor condition, most likely limiting vision
3 Both eyes are in poor condition. Fish is most likely blind or has very poor eye sight

- Sucker disc
  1 The disc is in good condition and has no deformities
  2 The disc is slightly deformed or the function is compromised by injury
  3 The disc is highly deformed. The fish is unlikely to be able to adhered to surfaces

Fish were dissected and the livers were colour scored from 1 to 6, where 1 indicates a very pale almost yellow liver, 2 is a faintly orange liver, 3 is a fairly bright orange, 4 is very bright orange, 5 is a light brown colour, and 6 is a dark brown colour (Figure 5). The pale livers are considered to indicate low astaxanthin levels, the orange ones high astaxanthin, and the brown livers are an indication of low fat levels in the liver (Eliasen et al. 2020).

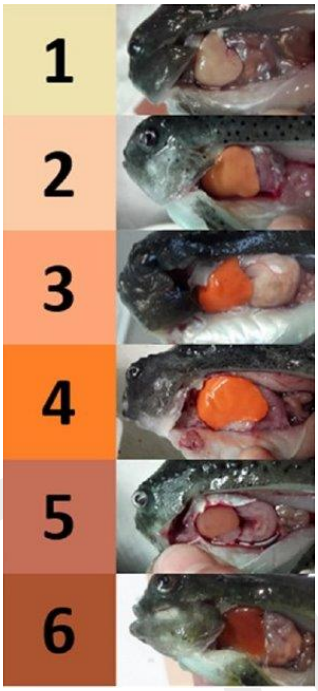

Figure 5 Liver colour scoring scale. (Eliasen et al. 2020)

4.3.3 Microbiome sampling procedure

Sampling for microbiome analyses was performed of lumpfish skin and gills on three occasions using the same individuals that were used for OWI monitoring. Microbiome sampling was performed (A) within ~2 weeks following deployment and (B) again after ~8 weeks and (C) ~14 weeks. In order to allow sampling to follow this strategy, lumpfish from all cages were not sampled at the same date but rather at certain intervals following deployment and the results refer to the sampling point (A-C). Lumpfish retrieved from each cage were kept cool and in separate sterile plastic bags until arrival at the farm's land site within an hour where sampling took place. Sampling of skin and gill mucus was performed using sterile swabs prior to OWI registrations. Care was taken to use clean sterile gloves and a sterilised workbench during sampling and sterile plastic tubes for the swabs. The tubes with swabs were immediately stored in dry ice until arrival at the lab where they were stored at -18 degrees Celsius before DNA extraction within a fortnight.

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Sampling of the shelters was also performed using swabs. Shelters were raised from the cages and triplicate samples were taken from shelters in each cage at each sampling period.

4.3.4 DNA extraction, library preparation, and sequencing

DNA extraction was performed using the Soil DNA Isolation Plus Kit (Norgen). The lysis buffer from the kit was added to the tubes containing the swabs. The samples were incubated at room temperature for 20 minutes with vortex mixing every 5 minutes. The liquid was since transferred to Bead Tubes from the kit before proceeding with the extraction protocol as described by the manufacturer. Elution volume was 80 µl.

Library preparation was performed using the Quick-16S NGS Library Prep Kit (Zymo) but using Nextera Index primers (Qiagen) and the 341f/785r amplicon primers (Herlemann et al. 2011; Klindworth et al. 2013). DNA concentrations were measured using the Qubit dsDNA HS kit (Invitrogen) and Clariostar (BMG Labtech). Libraries were sequenced at Novogene using an Illumina platform producing 2x300 bp paired end reads.

4.3.5 Shelter monitoring

Shelters were monitored for biofouling each sampling period and classified according to level of biofouling (no biofouling = 1, some biofouling = 2, and a lot of biofouling = 3). General condition, including tear, holes, and pests for all shelters, was estimated by the use of ROV imagery and in connection with microbiome sampling. These observations were used to classify the shelters (good condition = 1, okay condition = 2, bad condition = 3). Size and growth of the seaweed shelters was also measured during microbiome sampling.

4.3.6 Monitoring of fish behaviour

An under-water camera (GroCamera, GroAqua) was deployed in each cage at approximately 8 metres depth near a shelter. The cameras were suspended from lines running across each cage and held in an upwards facing position using an attachment developed by GroAqua. The cameras were connected to the farm's video surveillance system and recordings were saved to a local disc and then transferred to Firum.

Recordings were chosen to represent periods close to the microbiome sampling time points and also both periods with strong current and weak current. On each recording day, three sampling points were chosen; 6am, 1pm, and 7pm covering much of the daylight period in addition to times when the salmon were hungry and satiated. Each recording was 10 minutes long and in total 21 recordings were made in each cage (seven sampling days and three recordings per day) totalling 126 video recordings.

Due to weather conditions (algal blooms and resuspension caused by waves), water was murky at times and there were also periods where particularly the seaweed shelters were not very clear in the videos. Therefore, the amount of usable recording time is less than the total recordings gathered.

Each video was scored using "Boris" (Friard and Gamba 2016) with the ethogram in Table 1. The focal behaviours were the visible activities of lumpfish, particularly whether they were swimming freely or attached to shelters and any potential or actual interactions with salmon. Coding for whether a shelter was visible allowed for filtration of the observational data to only that, which was relevant to the shelters. Seaweed grew on many surfaces, so sometimes seaweed was visible in the plastic shelter cages. This was also recorded, meaning that lumpfish might be recorded as active near seaweed in the

cages with plastic shelters. Any interactions with salmon were related to the nature of the nearest shelter, so there may be interactions between lumpfish and salmon near seaweed even though the cage contained a plastic shelter. Anecdotal evidence from salmon farmers on the Faroe Islands suggests that saithe/coley (*Pollachius virens*) can interrupt normal salmon behaviour, so their presence near the shelters was also recorded (as "other fish").

Table 1 Ethogram covering lumpfish activity and interactions between lumpfish and salmon. Continuous behaviours were recorded in seconds and events were counted.

Observation	Type	Description
Plastic shelter	Continuous	A plastic shelter is visible
Seaweed	Continuous	Seaweed (shelter or otherwise) is visible.
Salmon	Continuous	Salmon are near the shelter or non-shelter seaweed
Other fish	Continuous	Shoals of other fish are near the shelter or non-shelter seaweed
Sitting	Continuous	Lumpfish is sitting in the shelter
Swimming	Continuous	Lumpfish is swimming near the shelter or non-shelter seaweed
Near	Event	Lumpfish and salmon are close to each other
Interaction	Event	Lumpfish approach the salmon
Clean	Event	Lumpfish clean the salmon

4.3.7 Lumpfish louse consumption

Lumpfish stomach contents were recorded in connection with the biweekly OWI registrations. The stomach was removed from the lumpfish, emptied into a white plastic container and rinsed. Water was added to the container and swirled around in order to separate any stomach contents. Any visible lice were recorded and removed (Adult salmon louse, motile salmon louse, and sea louse) and water was gently poured out and more added if necessary. Content of salmon- or lumpfish feed was recorded and any other prey species.

Additionally, lice burdens on the salmon were recorded every two weeks according with salmon farming legislation on the Faroe Islands.

4.3.8 Data analysis

Data were primarily analysed in R (R Core Team 2021) with "tidyverse" (Wickham et al. 2019). When possible, linear models were constructed and maximum likelihood ratio tests employed to test for statistical significance. However, some data were specialised in nature and required other statistical methodologies. The particulars are explained in the subsections for the relevant work packages.

4.3.8.1 Louse digestion

Survival analysis was used for estimating the probability of recovering salmon lice over time using the package "survival" (Therneau 2024). The response variable was binary (i.e., identified, or unidentified) at a given time, and the data were a mix of left- and right-censored data, indicating that the true identification times were unknown and could have occurred either before or after a sampling point. The analysis was done in two stages: A non-parametric survival analysis was done to provide an overview of survival probabilities using a Kaplan-Meier estimator. Afterwards, a parametric survival regression using the Weibull distribution was performed to model the survival probabilities over time.

First, a combined survival regression model was fitted to assess the overall significance of developmental stage, temperature, and freshness as predictors. Secondly, the nonsignificant predictors were excluded from the model, and separate regressions for the two developmental stages was fitted. This approach allowed for different scale parameters for each developmental stage, which captured the characteristics of each stage better. For estimation of mean digestion times, the survival curves were integrated.

To determine factors influencing the degradation levels of salmon lice, a cumulative link model (CLM) analysis was used to investigate the interaction between time and freshness (frozen vs live lice), temperature and developmental stage on degradation level. The model included degradation level as ordinal response, and interaction terms between time and each of the factors. The CLM was fitted using the package "ordinal" (Christensen 2019).

4.3.8.2 Microbiome analysis

Raw gene sequence data were processed in Qiime2 (Bolyen et al. 2019) following the methods described in Jacobsen and Johannesen (2023). Once taxonomy, alpha diversity, and beta diversity were obtained, feature tables as well as core metric results and taxonomies were imported into R using the package "qiime2R" (Bisanz 2018). Data were further processed and filtered for plots using the package "metacoder" (Foster, Sharpton, and Grünwald 2017).

In order to compare alpha diversity metrics, mixed effects linear models were constructed using the package "lme4" (Bates 2010) with "lmertest" (Kuznetsova, Brockhoff, and Christensen 2017). First, diversity by sample type was compared with cage as random factor. Then the effect of shelter type was investigated on both diversity on gills and skin again using cage as random factor. Finally, the effect of sample time was investigated using sample type as random effect.

In order to carry out differential abundance analysis, feature tables were filtered to remove unassigned and uncultured OTUs as well as Eukaryota, Mitochondria, and Chloroplasts. The tables were still very large after filtering, so statistical analyses were carried out on the top 30 most abundant taxa. Differential abundance was tested using "corncob's" (Martin, Witten, and Willis 2024) "differentialTest" function, which runs a model using maximum likelihood for the Beta-binomial distribution with a logit link function for each taxon. Because this test is in effect multiple tests, P values were adjusted for multiple testing using the built-in false discovery rate control function. An overall test was constructed comparing all samples from seaweed cages to plastic cages while controlling for sample substrate. Subsequent tests compared abundance on the various substrates separately (gills, skin, and shelters) in cages with plastic and seaweed shelters. Finally, the effect of time on abundance was tested while controlling for sample substrate and shelter type. The output from the models is presented in figures and tables using the expected difference in the logit-transformed relative abundance between the two groups and 95% confidence intervals.

4.3.8.3 Fish behaviour

Data were transformed from raw counts to proportional time expenditure with "shelter visible", be that seaweed or plastic as the total and any behavioural durations as a proportion of that total. For example, duration of lumpfish swimming near seaweed would be counted as a proportion of the time seaweed is visible in that video. This ensures that any behaviours measured are related to a relevant time frame. Similarly, interactions between lumpfish and salmon are adjusted by the length of time that shelters and salmon are visible.

Due to the proportional nature of the resulting data, the data are analysed using Generalised linear models with a binomial family (logistic regression).

5 Results, discussion, and conclusion

5.1 Results

5.1.1 Louse digestion

When looking at the degradation level of the lice found in the stomachs (with empty stomachs recorded as fully degraded lice), the results showed that lice degraded over time ($Z = 8.489$, $N = 207$, $P < 0.001$), higher temperature caused lice to be more degraded ($Z = 2.088$, $N = 207$, $P = 0.037$) and live lice were less degraded ($Z = -3.242$, $N = 207$, $P = 0.001$, Figure 6).

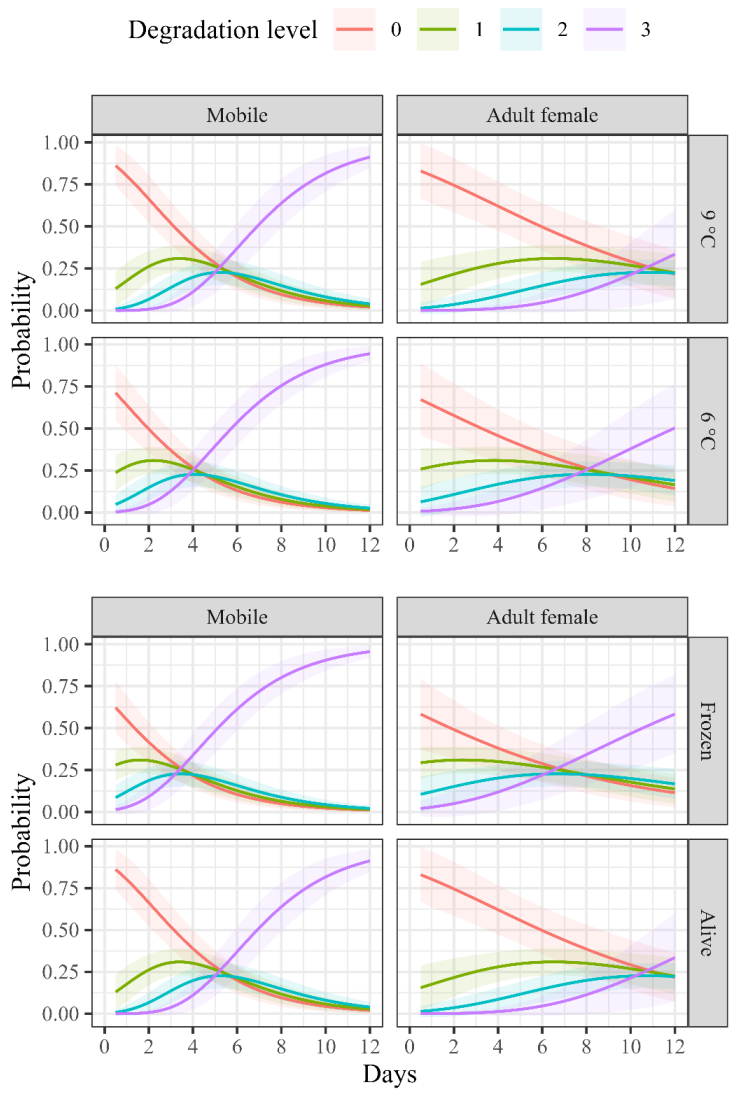

Figure 6 Change of degradation level of lice in lumpfish stomachs over time split by lice life stage (Mobile and Adult) and temperature (upper plot) and freshness state of the louse ingested (lower plot). Degradation levels are: 0) Not degraded, 1) Some soft tissue missing, 2) Most soft tissue missing, 3) No louse found. (Eliasen et al. 2024)

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The interaction between time and sampling method (live vs. frozen lice) was however not significant (Z = 0.493, N = 207, P = 0.622), nor was the interaction between time and temperature (Z = -1.003, N = 207, N = 0.316), indicating that these two variables do not affect degradation over time. The interaction between time and developmental stage was significant (z = -3.540, n = 207, p < 0.001) indicating that mobile lice degraded faster than adult female lice.

A survival regression analysis showed no significant difference in the recovery of sea lice between the two temperatures ($Deviance_{1,202}$ = 1.380, P = 0.240). However, when controlling for temperature, sampling methods differed significantly ($Deviance_{1,202}$ = 4.484, P = 0.034), which was not the case when temperature was not included in the model ($Deviance_{1,203}$ = 1.319, P = 0.250). The analysis showed a significant increase in survival of lice that were mature adults compared to mobile lice ($Deviance_{1,202}$ = 26.198, P < 0.001, Figure 7).

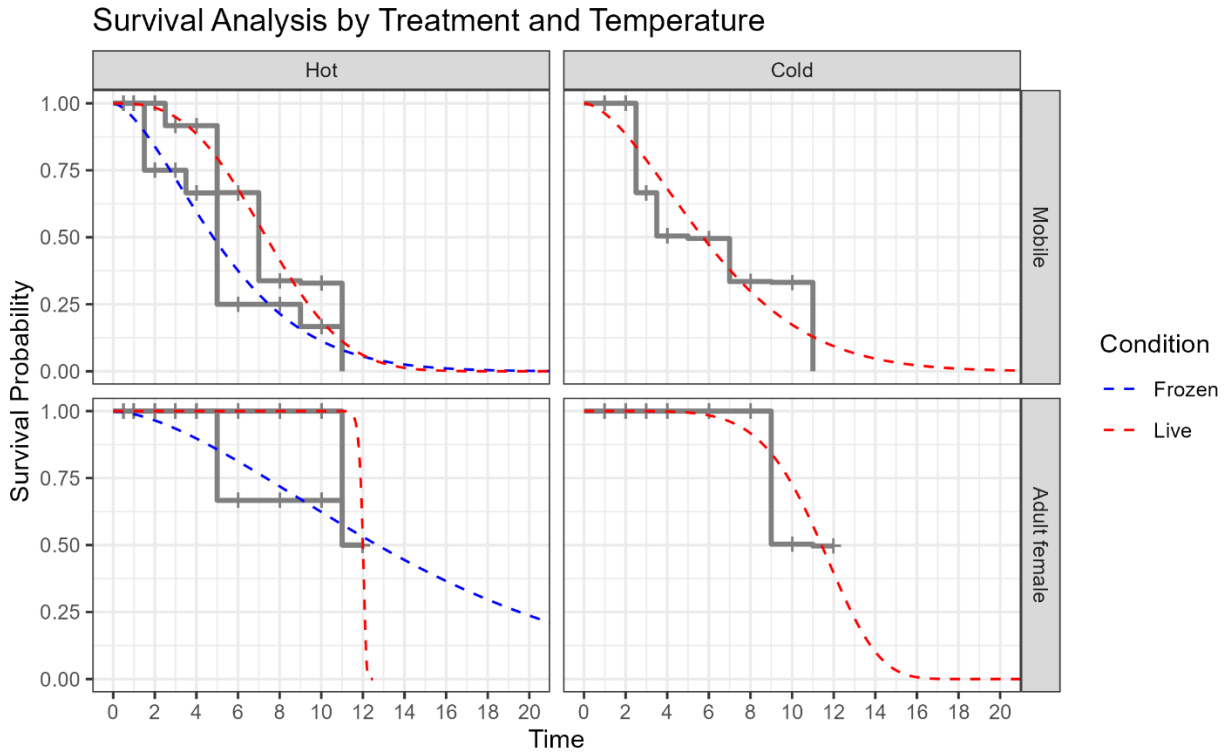

Figure 7 Kaplan-Meyer survival curves (solid) and regression curves for lice in lumpfish stomachs. Crosses are recorded digestion events.(Eliasen et al. 2024)

All lice were recovered within the first day. However, from the second day onward, the proportion of mobile salmon lice steadily decreased, and by day 12, no mobile lice were recovered. In contrast, all adult females were recovered until day four, and by day 12, half of the adult females were still present (Table 2).

Table 2 Average degradation times as integrated from predicted survival curves.

Mobile, mean digestion days		Adule female, mean digestion days	
Live in warm water	7.5	Live in warm water	12

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Live in cold water	6.4	Live in cold water	11.3
Frozen in warm water	5.5	Frozen in warm water	14.3

5.1.2 Welfare

There was no difference in skin or fin condition in the fish from seaweed and plastic shelter cages (Figure 8). There were also no differences in body condition of fish from either shelter type or the proportion of fish with empty stomachs (Figure 9).

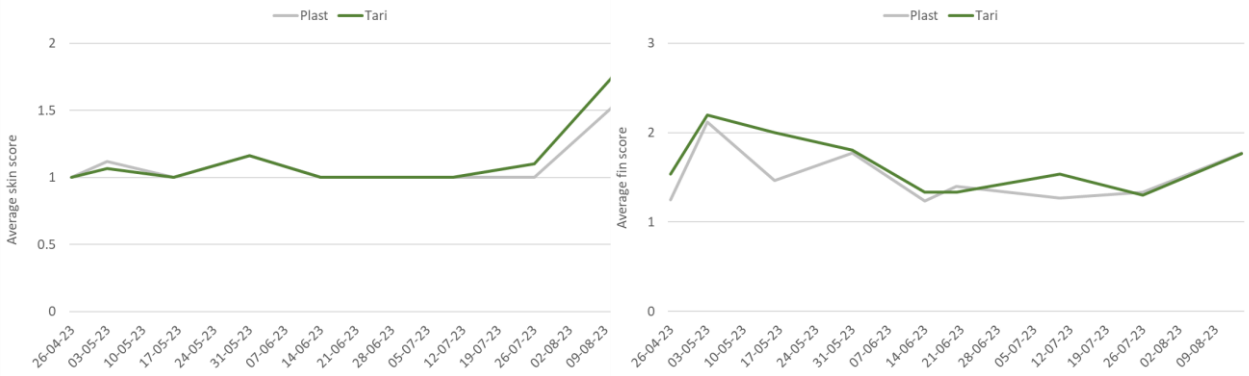

Figure 8 Skin (left) and fin (right) scores of fish living in cages with plastic shelters (light) and seaweed shelters (dark). Figure by Kirstin Eliasen

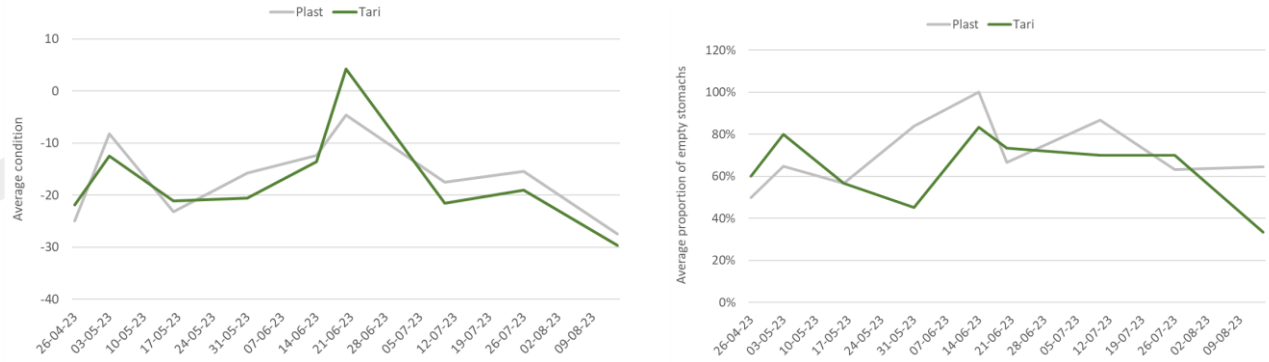

Figure 9 Body condition (left) and proportion of empty stomachs (right) of fish from cages with plastic shelters (light) and seaweed shelters (dark). Figures by Kirstin Eliasen.

5.1.3 Microbiome

Alpha diversity differed depending on sample type (The two tissues; gills and skin, and the two shelters; plastic and seaweed). In terms of Shannon diversity, gills had the lowest and plastic shelters the highest ($F_{3,402.3} = 30.219$, $P < 0.001$) while for phylogenetic diversity, the two shelter types were similar and differed from the two tissue types, which were also similar ($F_{3,399.64} = 23.983$, $P < 0.001$). Additionally, there was an interaction between shelter type and tissue type with phylogenetic diversity being lower on gills in cages with seaweed shelters than in cages with plastic shelters while this was not the case for skin ($F_{1,352} = 13.2818$, $P < 0.001$, Figure 10).

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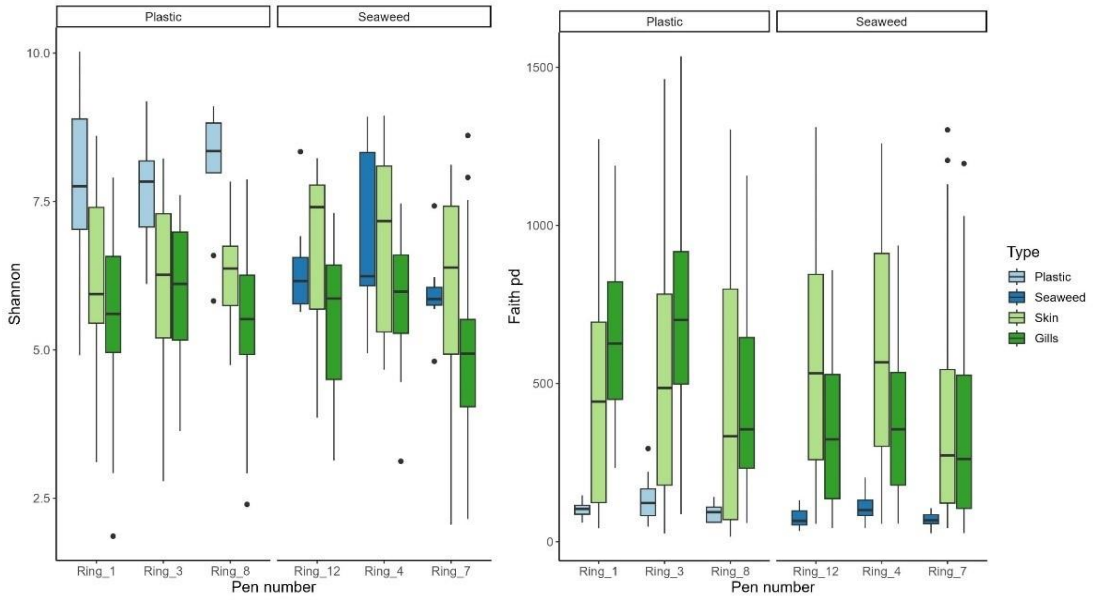

Figure 10 Alpha diversity in sample types split by shelter type and cage. The left panel is Shannon diversity and the right panel is Faith pd.

In terms of changes over time, there was an interaction between the type of shelter available in the cage and sampling time where there was a peak in phylogenetic diversity in the middle of our observation period in seaweed cages and the opposite (a dip) in the plastic cages ($F_{2,405.05} = 21.291$, $P < 0.001$). Conversely, Shannon diversity increased linearly over time in seaweed cages and did not change over time in plastic cages ($F_{2,405} = 24.942$, $P < 0.001$).

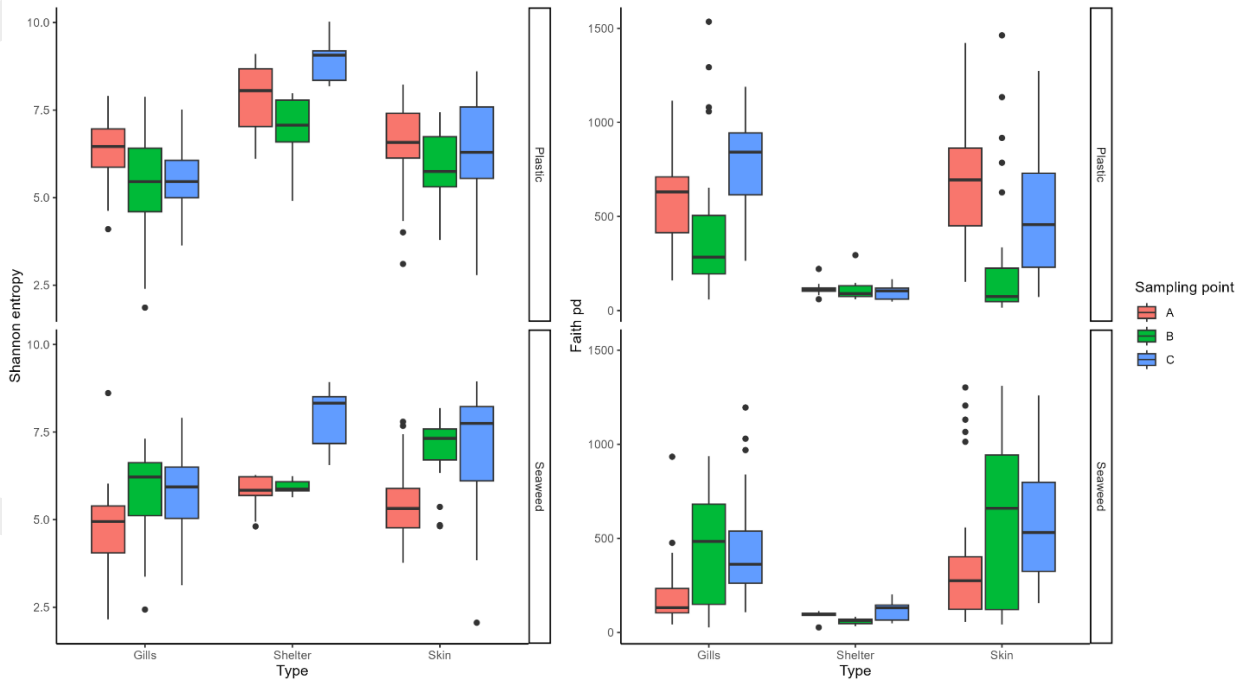

Figure 11 Alpha diversity over time (A-C) split by sample type and shelter type. Left plot is Shannon entropy and right plot is Faith Phylogenetic Diversity.

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The difference in phylogenetic diversity is reflected in differential abundance between the samples. Plastic shelters generally had higher phylogenetic diversity with the most common genera only representing approximately 50% of the reads in the samples. In comparison, the most common genera on seaweed shelters represented more than 70% of the reads in most of the samples (Figure 12). When comparing the relative abundance 30 most abundant genera on plastic and seaweed shelters, 16 of them differed significantly (Figure 12).

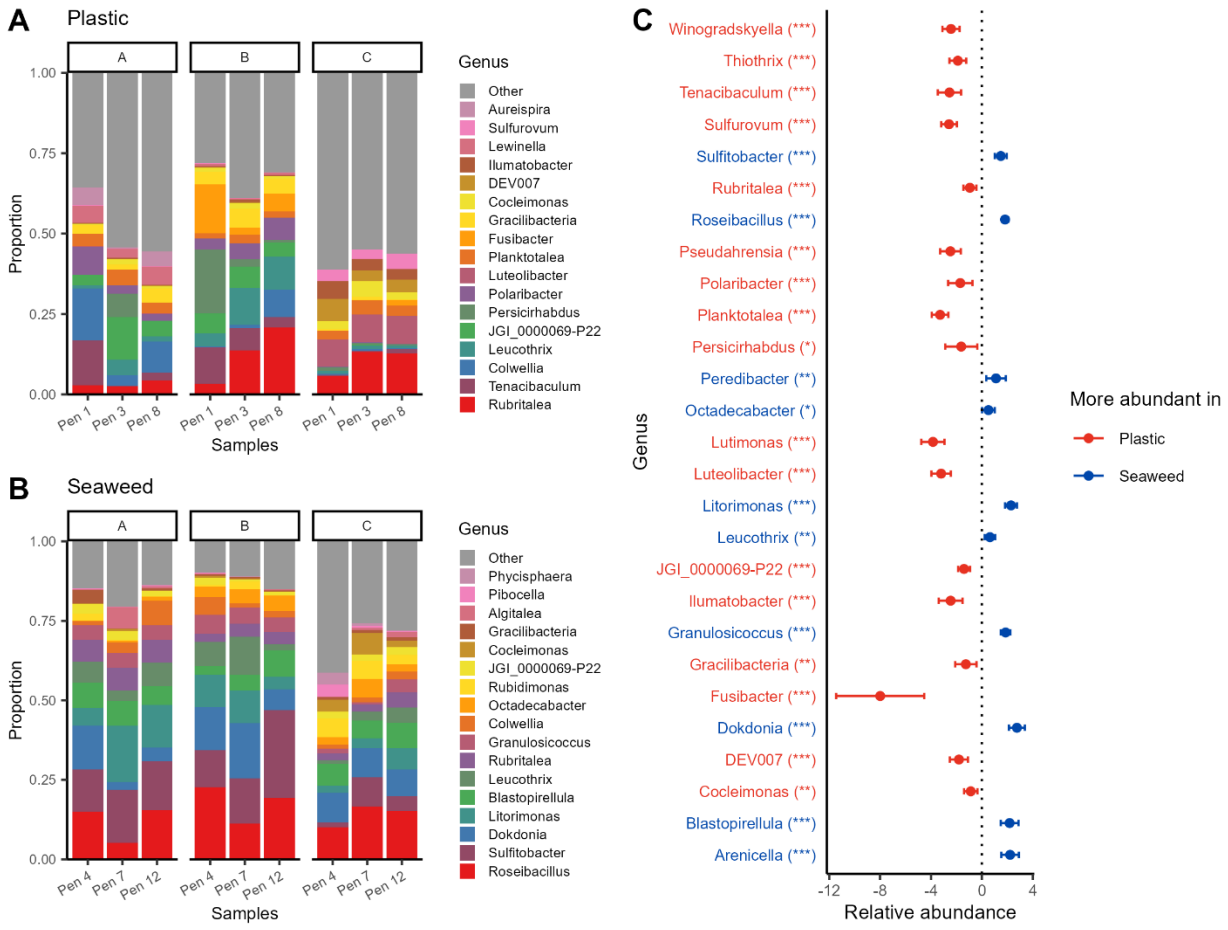

Figure 12 (A and B) Relative abundance of the 17 most abundant genera in plastic shelters (top-left) and seaweed shelters (bottom-left). Facets A-C represent sampling time points and pens refer to the three cages in each of the two treatment groups. (C) Relative abundance of the significantly different genera within the 30 most abundant genera on shelters. Red genera (on the left) are more abundant in plastic shelters and blue genera (on the right) are more abundant in seaweed shelters. (Jacobsen et al. 2024)

When comparing the fish in the cages, the diversity on skin as seen above was fairly similar, but still there were several genera that were differentially abundant between the shelter types that the fish inhabited (Figure 13).

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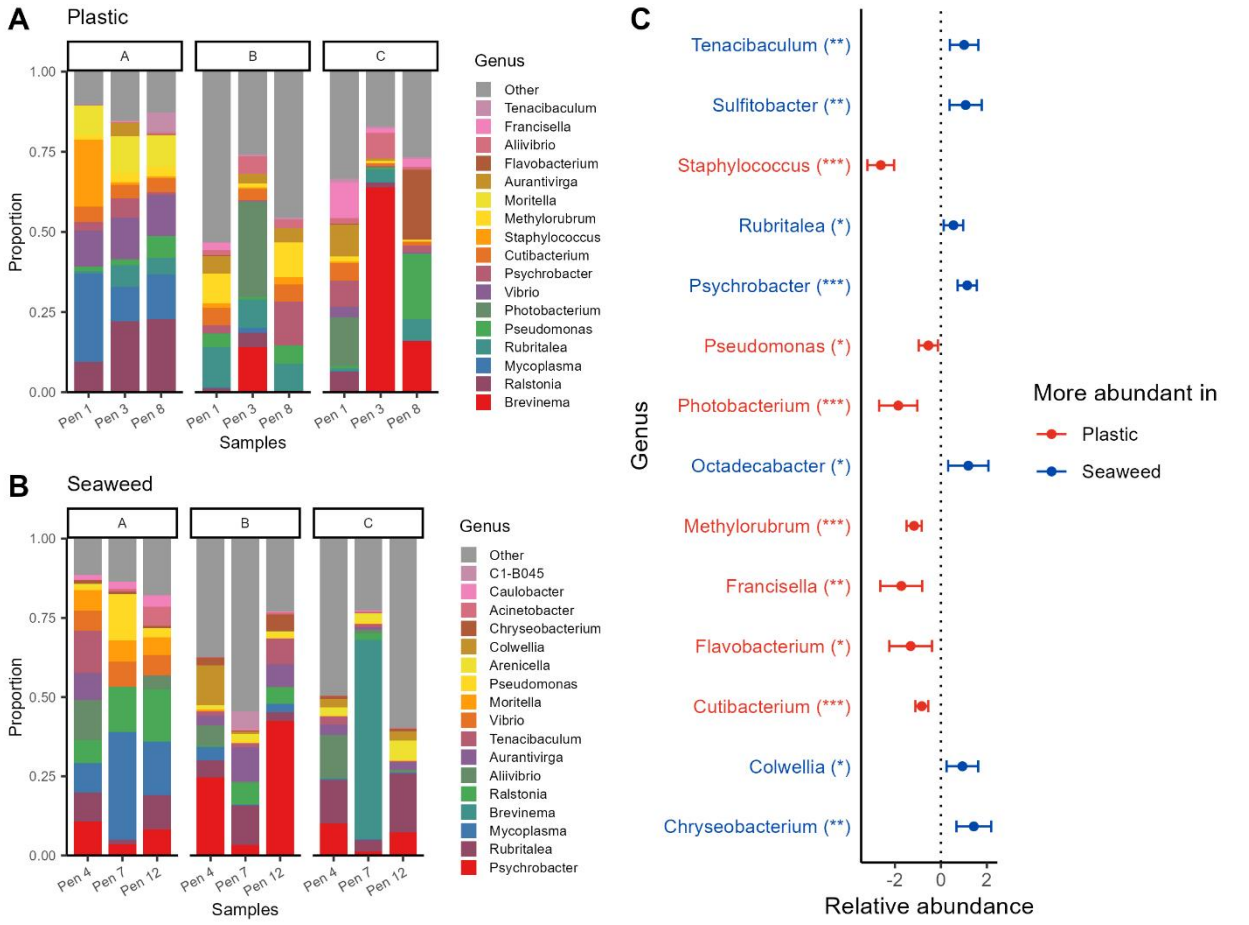

Figure 13 (A and B) Relative abundance of the 17 most abundant genera on lumpfish skin in plastic shelters (top-left) and seaweed shelters (bottom-left). Facets A-C represent sampling time points and pens refer to the three cages in each of the two treatment groups. (C) Relative abundance of the significantly different genera within the 30 most abundant genera on lumpfish skin. Red genera (on the left) are more abundant in plastic shelters and blue genera (on the right) are more abundant in seaweed shelters. (Jacobsen et al. 2024)

Finally, when comparing abundance in gills the fish were much more similar with only four genera significantly differing between the shelter types (Figure 14)

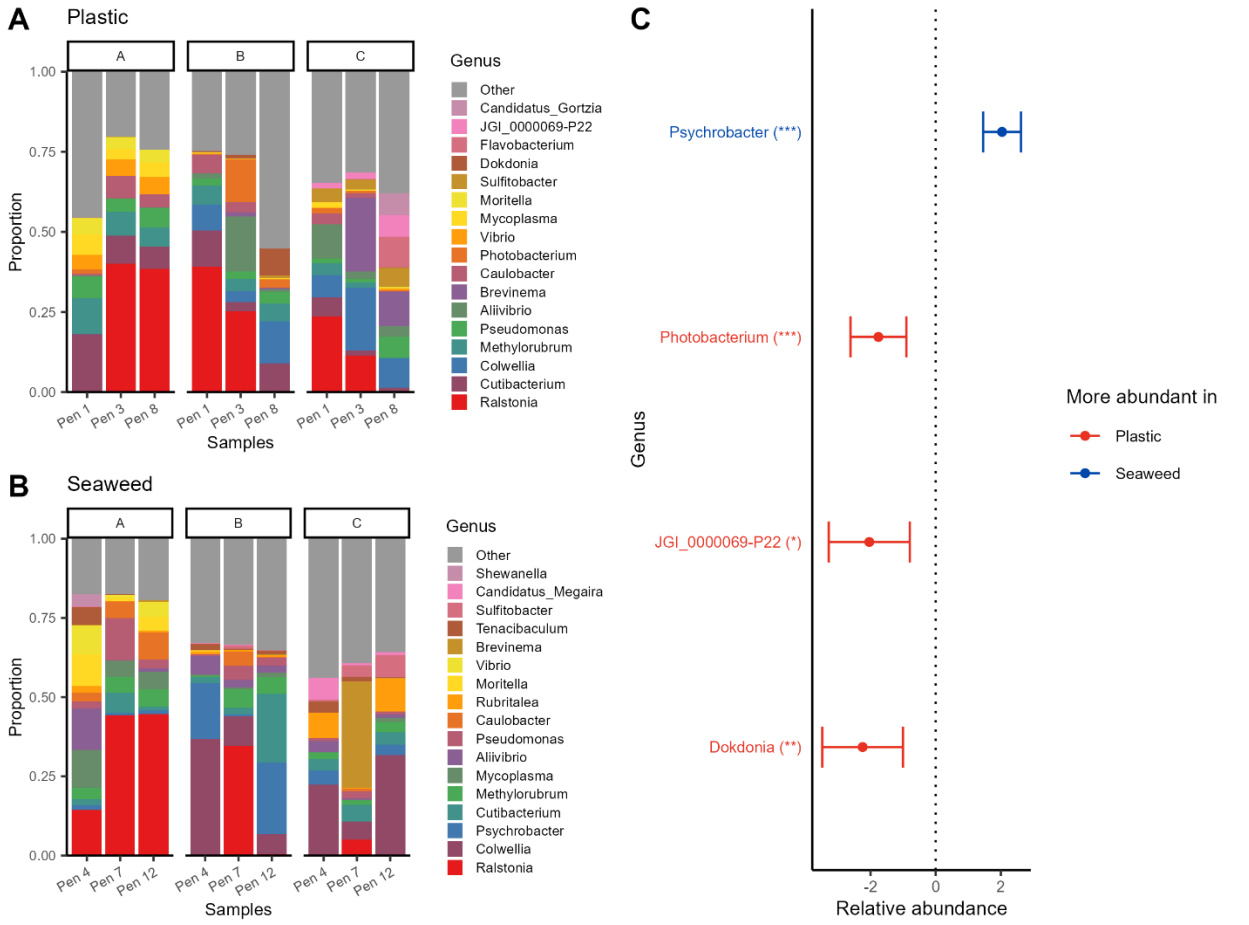

Figure 14 (A and B) Relative abundance of the 17 most abundant genera on lumpfish gills in plastic shelters (top-left) and seaweed shelters (bottom-left). Facets A-C represent sampling time points and pens refer to the three cages in each of the two treatment groups. (C) Relative abundance of the significantly different genera within the 30 most abundant genera on lumpfish gills. Red genera (on the left) are more abundant in plastic shelters and blue genera (on the right) are more abundant in seaweed shelters. (Jacobsen et al. 2024)

Overall, there were many more cases where a potentially harmful bacteria was either more prevalent on plastic shelters than seaweed shelters or on the fish in cages with plastic shelters (Table 3). On the shelters themselves, only *Tenacibaculum* differed significantly with higher abundance in plastic shelters. In fish tissues, particularly skin, several different bacteria were found, and they were generally more prevalent in samples from cages with plastic shelters.

Table 3 Overview of potentially pathogenic bacteria detected amongst the dominating genera in each of the sample types. Significant differences between samples are shown as P) more in plastic shelter cages, S) more in seaweed shelter cages, and X) no difference

Genera	Shelters	Skin	Gills	Reference
Aliivibrio		S	X	(Klemetsen, Karlsen, and Willassen 2021)
Flavobacterium		P	X	(Wahli and Madsen 2018)
Francisella		P		(Colquhoun and Duodu 2011)

Moritella		X	X	(Einarsdottir et al. 2018)
*Photobacterium**		P	P	(Andreoni and Magnani 2014)
Pseudomonas		P	X	(Mjølnerød et al. 2021)
Staphylococcus		P		(Çanak and Timur 2020)
Streptococcus			X	(Toranzo, Magariños, and Romalde 2005)
Tenacibaculum	P	S	X	(Erkinharju et al. 2020)
Vibrio		X	X	(Erkinharju et al. 2020)

5.1.4 Fish behaviour

Fish were more often seen swimming near a shelter in the cages with plastic shelters. This was also true when seaweed was visible in the cages with plastic shelters (Figure 15). In terms of interactions with salmon, more were seen near plastic shelters than near seaweed (Figure 16).

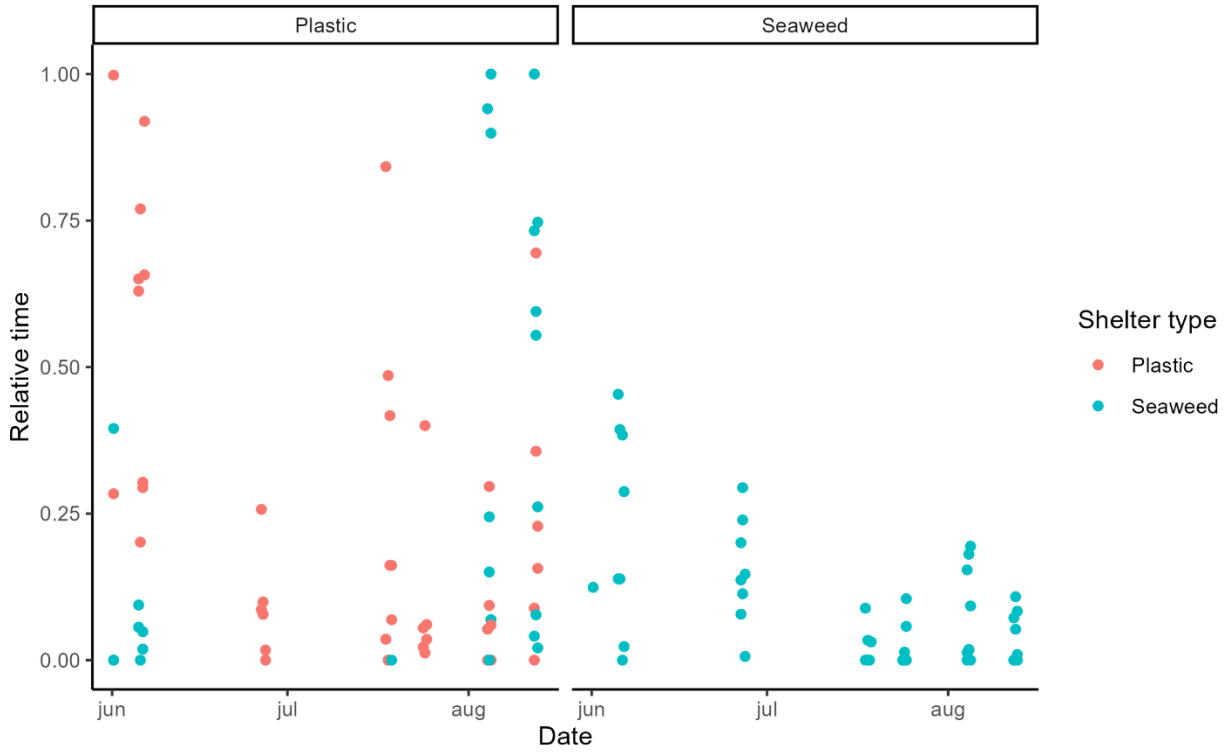

Figure 15 Time spent swimming near a shelter (red dots plastic and blue dots seaweed) relative to the time when the shelters were visible in the video feed. The left panel is cages with plastic shelters and the right is cages with seaweed shelters.

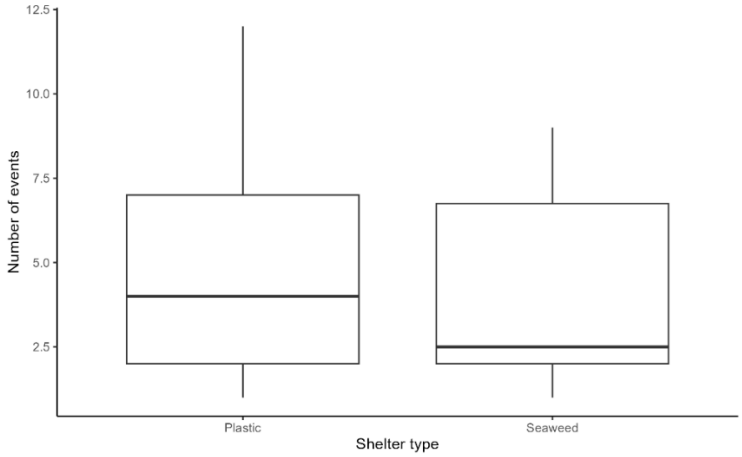

Figure 16 Number of interaction events between lumpfish and salmon near plastic shelters (to the left) and seaweed shelters (to the right). Boxes and whiskers are quartiles.

5.1.5 Shelter condition

The surface areas of the seaweed shelters were estimated based on measurements performed at sampling. In May the seaweed leaves were approximately 95 cm by 9 cm in size, growing to 138 cm by 14 cm in August. This equals a usable surface area of approximately 600 m2 at the start and 1500 m2 at the end of the observation period assuming 170 individual leaves per metre of line (personal communication, Tari) and a realistic estimate of an actual leaf size being approximately 75% of the widest point multiplied by the length and the usable leaf space on a leaf being approximately 50% of the calculated surface. The usable space estimates are based on the necessity of a smooth surface that is large enough for a sucker disc to adhere to. Saccharina leaves are often very wavy at the edges and have a raised "stem" running down the length on one side, which prevent adhesion by lumpfish in certain areas on the leaf (Figure 17).

Figure 17 Saccharina latissima leaf showing "back" and "front" side as well as shape. Image source: seaweedsolutions.com

A cumulative link model showed significant differences between biofouling on the two shelter types ($z = -6.151$, $N = 195$, $P < 0.001$). The plastic shelters had more biofouling than the seaweed shelters at all

three measuring periods (Figure 18) and had heavy biofouling in all three pens at the last measuring. The seaweed shelters on the other hand never had more than light biofouling (2).

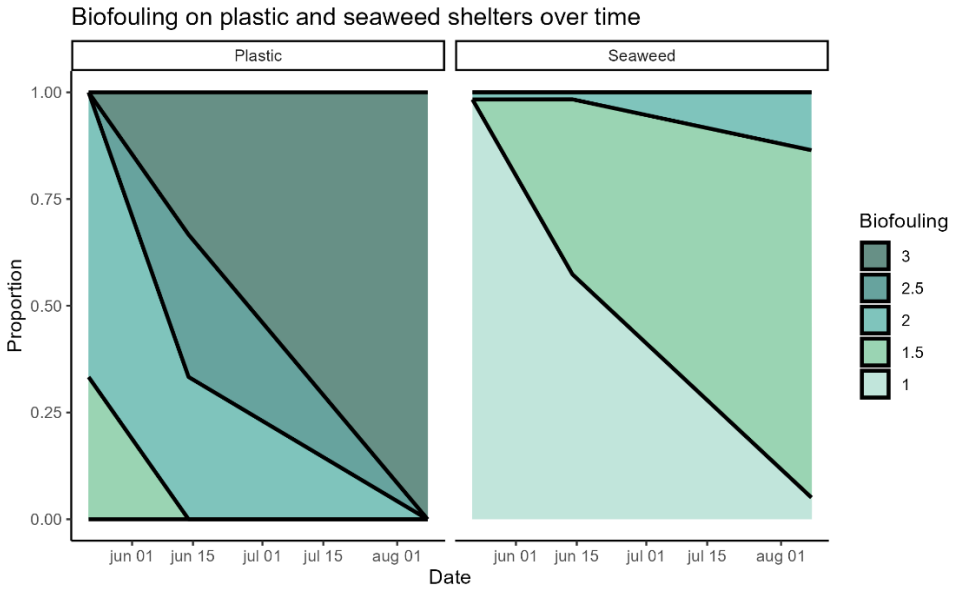

Figure 18 Level of biofouling on the shelters from 1 (no biofouling) to 3 (a lot of biofouling). Plastic shelters are to the left and seaweed shelters to the right. (Jacobsen et al. 2024)

Shelter condition was excellent throughout the trial for plastic shelters with no signs of wear and tear whereas the seaweed shelters did show some wear by the end of the trial (Figure 19). The differences were too small and the data, particularly in terms of plastic seaweed, too uniform to perform meaningful statistics.

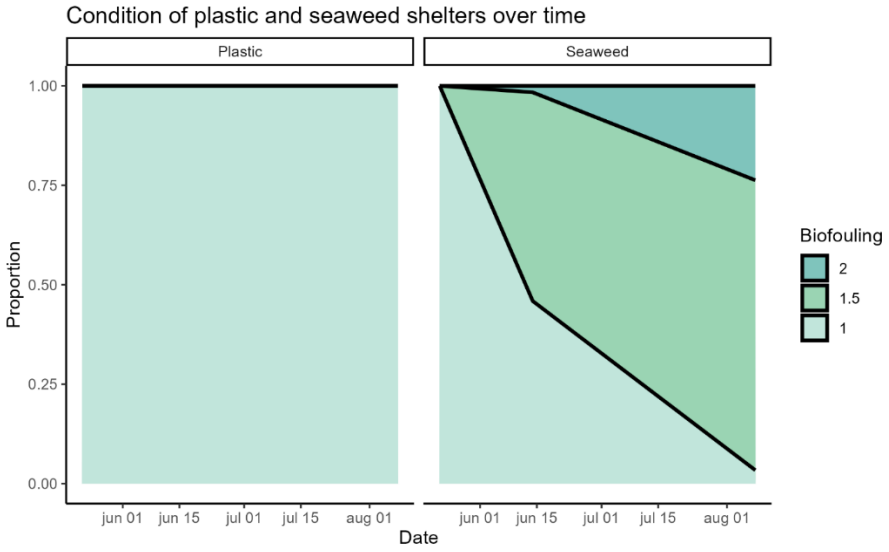

Figure 19 Shelter condition from 1 (good) to 2 (okay). No shelters were scored as poor. Shelters on the left are plastic and on the right are seaweed.

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5.1.6 Louse consumption

No differences were found between louse burdens on salmon in cages with plastic shelters and seaweed shelters (Figure 20).

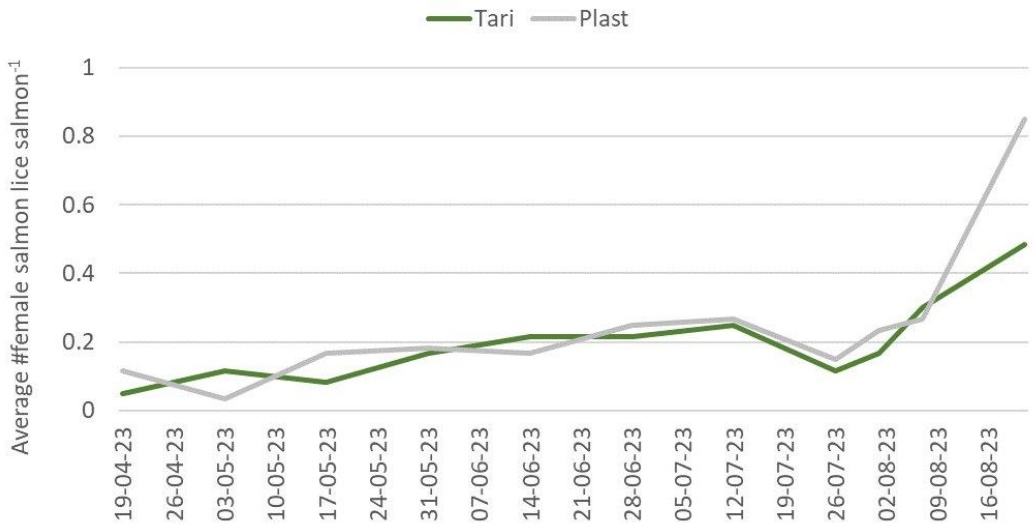

Figure 20 Number of adult female lice on salmon in cages with plastic shelters (light) and seaweed shelters (dark).

There were very few lice found in the lumpfish stomach at all, so it was impossible to determine whether there was any difference between the shelter types (Figure 21).

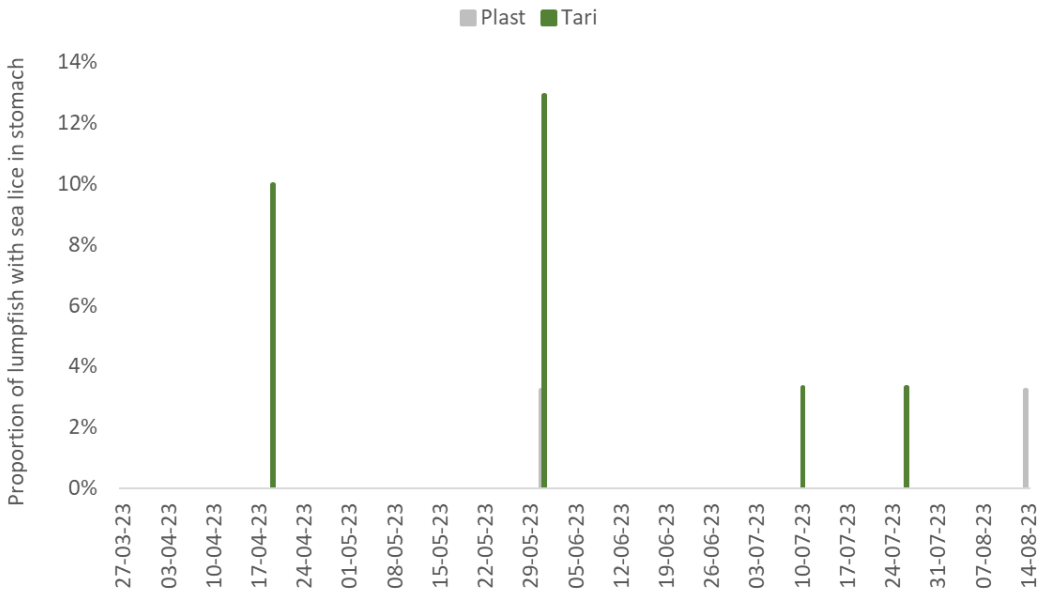

Figure 21 Number of lice found in lumpfish stomachs at each sampling point. Dark bars are fish from cages with seaweed shelters and light from cages with plastic shelters.

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5.2 Discussion

5.2.1 Louse digestion

Digestion of lice took much longer than anticipated and the results suggest an average digestion time of 6-7 days for mobile lice and 11-12 days for adult female lice. This is much longer than reported elsewhere (Staven et al. 2024) and really changes the perspective on how to interpret lumpfish stomach contents. The biggest factor affecting the time it took to digest the lice was the developmental stage of the louse, with adult females taking approximately twice as long to digest as mobile lice. Considering this information, the ratio of adult female lice to mobile lice found in lumpfish stomachs better resembles that found on the salmon (as one can divide the number of adult lice by two to get the "true" ratio consumed in a time window) than previously thought. That being the case, this is an indication that lumpfish are not selective and will just as happily consume mobile lice as adult females.

The digestion results show no effect of temperature and unfortunately, the effect of lumpfish size can not be accurately estimated due to the confounding salmon louse life stage factor. Louse freshness did not show an effect on its own, but when controlling for the otherwise not significant temperature, there was an effect of louse freshness with frozen lice being digested approximately two days faster than live lice (5.5 days and 7.5 days respectively). Unfortunately, we were unable to carry out a balanced design in order to minimise the use of animals, and this is probably why it is difficult to find the true effects of both temperature and louse freshness. How these factors affect digestion times can be seen in Figure 7 and Table 2 shows the estimated degradation times. What is clear, is that life stage completely overwhelms the other factors in effect size.

Degradation over time was also affected by developmental stage, with lice progressing through the stages much more quickly in mobile lice than in adult females. Freshness did not interact with time in degradation level, but did have a significant main effect with frozen lice being more degraded. This indicates that frozen lice either started out a little degraded or degraded slightly more than live lice before the first sampling point and then both degraded at a similar rate over time. This corresponds well with the survival model which found only a small effect of freshness, which probably correspond to the freezing procedure slightly degrading the louse, making it disappear slightly faster as it is already a bit degraded when delivered to the lumpfish.

Future work should focus on lumpfish size in a balanced design with louse life stage. Data from salmon farms indicates that lumpfish vary a lot in their cleaning activity when considering their stomach contents, yet these data are sometimes contradicted by the louse numbers on the salmon. One explanation for this disconnect may be that digestion speed is affected by the activity levels of the lumpfish. In this study, all the fish were inactive, spending the entire trial period sitting attached to the tank wall or a shelter. Future studies ought to focus on manipulating activity levels, for example by introducing currents in the cages and limited resting spaces for controlled periods of time each day throughout the trial to more closely mimic the real activity levels a lumpfish might experience in a salmon farm with tidal currents and net walls.

5.2.2 Welfare

There were no differences to be found in any of the welfare parameters that were measured. Generally, the fish were in good condition, and both types of shelters seemed to provide the necessary surface area for lumpfish to rest and hide. One thing to consider, is that all the cages had some seaweed growing on equipment and the upper most 50cm of the net, so even in cages with plastic shelters, a

proportion of the lumpfish had the option of spending their time in the seaweed instead. This is the case in most commercial salmon farms, as it is difficult to remove seaweed from all of the farming gear in a cage. Whether this was a contributing factor to the good welfare in the cages with plastic shelters or if this is because plastic shelters are adequate is difficult to say. Regardless, considering the OWIs measured in these real production farms, either shelter type is acceptable.

The bacterial communities living on the shelters were very different. The seaweed shelters contained much less *Tenacibaculum* and the plastic shelters generally contained a much more diverse community of bacteria with *Tenacibaculum* being the second most dominant genus (Figure 12). On the fish, there were more genera of potentially harmful bacteria found on the skin of fish with plastic shelters than seaweed shelters (Table 3) while the differences were much smaller in the gills. The gills ought to be more influenced by the water rather than surfaces, so assuming adequate water quality and exchange, the gills would be expected to be quite similar. When looking at the beta diversity on the different sample types, it is also clear that over time, the skin tends to resemble the shelter more and more, which is a good reason to try to maintain pathogen-free shelters.

Given the differences in skin and shelter bacterial communities, it is worth considering whether the lumpfish with plastic shelters were more exposed to infection pressure than those with seaweed shelters. While OWIs were similar and no diseases were registered amongst the lumpfish, it is possible that an event that breaks down the physical barriers of the skin (such as delousing or net cleaning procedures) would disproportionately exposed lumpfish with plastic shelters to infection. Similarly, should long-term stress such as long periods with bad weather compromise the immune system, lumpfish with plastic shelters might be less able to fight of an infection than those with seaweed shelters. Therefore, farms with a history of lumpfish suffering from bacterial infections, or those that anticipate this might be the case in future, might consider using seaweed shelters instead of plastic ones to reduce the exposure of the fish to the potentially pathogenic bacteria associated with the plastic shelters.

5.2.3 Behaviour

There were some differences in terms of the behaviour with more activity seen near the plastic shelters, and even other seaweed in the cages with plastic shelters. However, the camera positioning was such that even when seaweed shelters were visible in the seaweed cages, the viewing angle was fairly poor and the distance greater to that to the plastic shelters. This means that the activity near seaweed shelters is most likely under-reported in these results.

There was an expectation that lumpfish would prefer seaweed to plastic shelters and that there would be more activity near the seaweed shelters than the plastic ones. This turned out not to be the case, and it seems that lumpfish are quite content with spending time in and near plastic shelters. There were many lumpfish observed near seaweed, both shelters and "incidental" seaweed as well, so both types of shelter are suitable as a focal point for lumpfish and salmon to interact near. What was fairly clear from the video observations is that lumpfish do prefer some kind of structure as lumpfish activity was much more prevalent near the shelters than in empty water. Lumpfish were seen both investigating the shelters and simply being near them while not showing any particular shelter related activity. It is unclear why the fish did this, but possible explanations can be that proximity to a shelter provided a sense of safety from predators, a preference for being near a resting place, or perhaps that the water

current was slower near the shelters so the lumpfish did not have to spend as much energy swimming there.

5.2.4 Shelters

Plastic shelters more quickly accumulated biofouling with clear differences in bacterial communities on plastic and seaweed shelters. On the other hand, seaweed shelters were more vulnerable to wear and tear, with most of the seaweed shelters being somewhat worn at the end of the trial period, while the plastic shelters were completely intact. The surface area on the seaweed shelters is not straightforward to calculate, but even with a rather conservative estimate used in this study, the seaweed surface area exceeded that of the plastic shelters by a factor of ten. However, once bad weather events happen in the winter, much of that area may be gone or unusable, so it is important to continually monitor the seaweed shelters to assess them for use as shelters. Comparing this to the continual maintenance necessary to ensure the plastic shelters are clean enough, there is no "maintenance free" easy solution in terms of the type of shelter to use.

5.2.5 Louse consumption

There were no differences to be seen between the shelter types. Lumpfish stomachs contained too few lice to make meaningful statistical comparisons and the lice on the salmon did not differ between the shelter types. This might indicate that the lumpfish did not clean, but when comparing to cages with no lumpfish, there were fewer lice on the salmon where there were lumpfish. Regardless, it does not seem like the type of shelter affects cleaning effect or activity on lumpfish. However, should the differences in bacterial communities cause a mortality event in lumpfish with plastic shelters, one might expect the ones with seaweed shelters to remain active for longer.

5.3 Conclusion

Lumpfish digestion of lice is a much more complex matter than previously thought and our results would suggest that any adult female lice found in a lumpfish stomach may have been consumed up to two weeks ago. This makes estimating lumpfish cleaning efficiency based on stomach contents very difficult. This is also well demonstrated by the fact that we found very few lice in the lumpfish stomach in our field study, yet salmon had fewer lice in cages with lumpfish. More work is needed to better understand the complexities of louse digestion.

Seaweed shelters are a more environmentally friendly lumpfish shelter than plastic shelters. They are also easier to maintain as they do not need cleaning like the plastic shelters, which become covered in biofouling very quickly. In terms of lumpfish welfare, this study found no major differences, but there were differences in microbiome that could lead to problems should the fish experience stressful events or injury. No effects were seen on cleaning activity and effect, suggesting that the use of seaweed shelters should be considered mostly for lumpfish welfare and environmental reasons. However, should lumpfish welfare suffer in the long term due to the differences in bacterial communities, cleaning efficiency would also suffer in those cages.

6 Main findings

- Seaweed shelters are suitable as lumpfish shelters and may have beneficial effects on lumpfish health through effects on the bacterial community on the skin of the lumpfish.

- The type of shelter provided does not affect lumpfish cleaning activity, at least not in the short term.
- Salmon lice found in lumpfish stomachs may have been consumed 1-2 weeks prior to dissection making it difficult to assess cleaning effect from stomach contents.

7 References

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8 Deliverables

8.1 Talks

Ása Jacobsen*, Ása Johannesen, Kirstin Eliasen, Elin Egholm, Agnes Mols Mortensen, **Aqua 2024,** Effect of plastic and seaweed shelters on the skin microbiome of lumpfish Cyclopterus lumpus used as cleaner fish in aquaculture pens. *Copenhagen.*

Kirstin Eliasen*, Ása Jacobsen, Agnes M. Mortensen and Ása Johannesen, **Aqua 2024,** The digestion time for salmon louse *Lepeoptheirus salmonis* in lumpfish *Cyclopterus lumpus* in relation to sampling method, developmental stage, and temperature. *Copenhagen*

Kirstin Eliasen*, Ása Jacobsen, Agnes Mols Mortensen, Sandra Ljósá Østerø, Ása Johannesen, **Rensefisk dialogmøte 2024,** Praksis i andre land – Færøyene. *Trondheim*

Ása Jacobsen*, Kirstin Eliasen, Agnes Mols Mortensen, Ása Johannesen, **Rensefisk Dialogmøte 2022,** Bruk av AkvaNest og effekt og velferd for rognkjeks, *Oslo*

8.2 Publications

Ása Jacobsen, Agnes Mols Mortensen, Kirstin Eliasen, Elin Egholm, Ása Johannesen, 2025, *Effect of plastic and seaweed shelters on the skin microbiome of lumpfish Cyclopterus lumpus used as cleanerfish in aquaculture pens*. (in prep for PlosOne)

Kirstin Eliasen, Sandra L. Østerø, Tróndur T. Johannesen, Esbern J. Patursson, Ása Jacobsen, Agnes M. Mortensen, Marner Nolsøe, Ása Johannesen, 2025, *The digestion time for salmon louse (Lepeoptheirus salmonis) in lumpfish (Cyclopterus lumpus) in relation to freshness, developmental stage, and temperature* (in review at PlosOne, https://www.biorxiv.org/content/10.1101/2024.09.14.613060v1)

Kyst.no, 2023, *Vanskelig å bedømme rensefiskens effektivitet: Store variasjoner i fordøyelsen gjør at forskerne i et nytt FHF-prosjekt sliter med å vurdere lusespising ut fra mageinnhold på rognkjeks*. https://www.kyst.no/fhf-prosjekt-lusespising-rognkjeks/vanskelig-a-bedomme-rensefiskens-effektivitet/1606935

Tentative: Kyst.no, 2024, *Levende tareskjul kan være gunstige for den mikrobielle sammensetningen på huden til rognkjeks,* Press release sent, but no response yet.

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