Fishy business, Atlantic salmon (*Salmo salar*) skin mucus microbiome dynamics during salmon louse (*Lepeophtheirus salmonis*) infection

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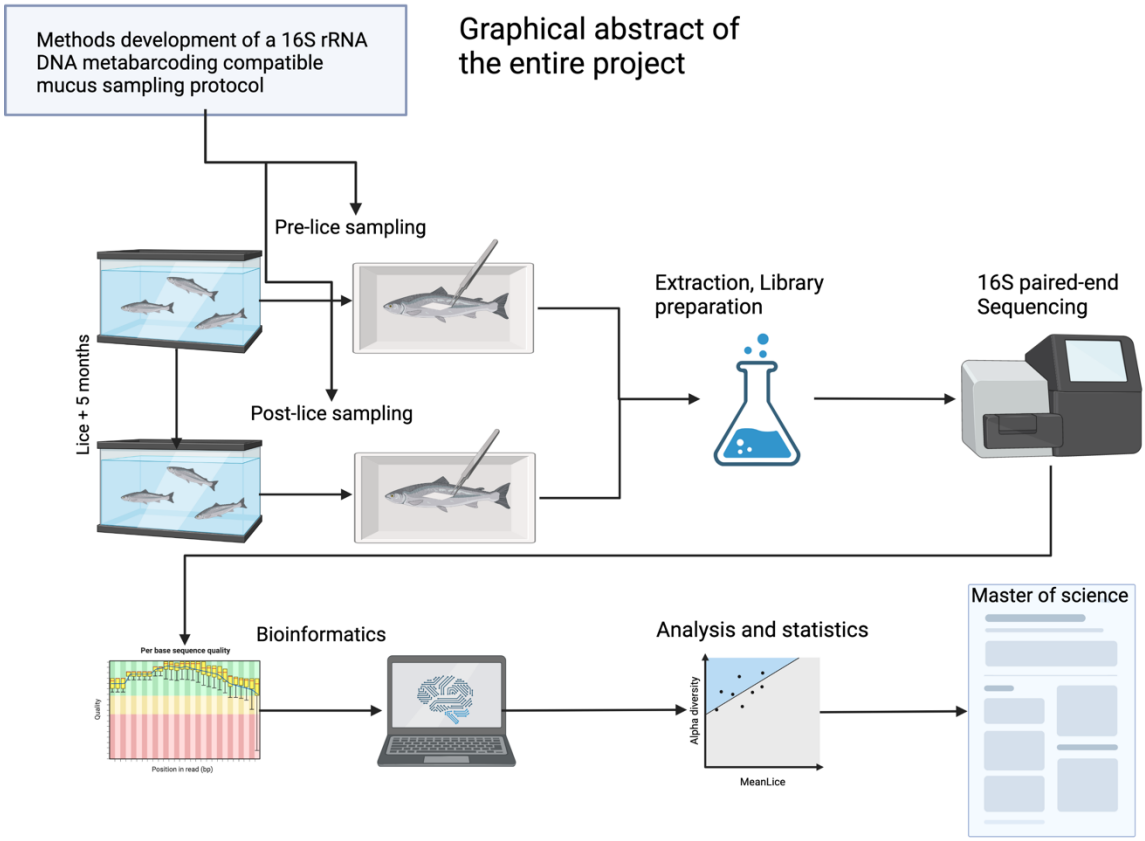
Abstract

Atlantic salmon is crucial for Norway's aquaculture industry, with over 300 million individuals put into pens in 2022. The salmon louse, an ectoparasitic copepod that parasitizes the mucus surface and skin of salmonids, poses a critical threat to both farmed and wild salmon. Research since the 1950s using both culture-dependent and, more recently, culture-independent methods have revealed that fish surface mucus is not only a mix of biomolecules but also a habitat for a diverse set of microorganisms. This microbiome in fish surface mucus has been shown to have crucial implications for the function of the mucus-skin barrier.

In this study, 60 post-smolt Atlantic salmon were exposed to varying levels of salmon louse over five months to investigate the long-term effects on the microbiome of the Atlantic salmon's mucus surface. The microbial community was analyzed using 16S rRNA gene metabarcoding. To enable a 16S rRNA gene metabarcoding workflow, a rapid and efficient method for sampling microbial DNA in fish mucus was developed. Applying this method, I found no significant linear relationship between increasing salmon lice intensity and changes in alpha diversity of the mucus microbiome. However, there was a correlation between beta diversity and increasing lice load. The envfit test of whether the mucus biome composition affected mean lice load explained 14 % of the lice intensity variation but were insignificant. Specific genera of bacteria showed higher abundance with increasing lice intensity, including opportunistic and potentially pathogenic genera like *Tenacibaculum* sp. This bacterium has been associated with wounds, stress, and pathogen/parasite presence in fish, including the disease known as winter ulcers in Atlantic salmon. Additionally, commensal genera like *Oleispira* sp. increased with higher lice intensities and have been suggested to play an essential symbiotic role in salmon mucus, suggesting a potential return to an original steady state or an alternative steady state in the mucus microbiome after long-term exposure to the salmon louse.

This study observed a beta diversity and taxonomic composition shift in the Atlantic salmon mucus microbiome with increasing lice loads. To mechanistically explain this observation, a holistic multi-omics approach is required. Therefore, this study advocates for adopting a parasite-holobiont perspective in future research, emphasizing the need for a comprehensive understanding of the interactions between parasites and host systems.

Graphical abstract



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Abbreviations

16S rRNA – 16S ribosomal RNA

ACE – Abundance-based coverage estimators

Adj. – Adjusted p-value

AQUA - Section for Aquatic biology and toxicology

ASV – Amplicon sequence variant

CI – Confidence interval

DNA – Deoxyribonucleic acid

ESS - effective sample size

GLLVM – General linear latent variable models

IBV – Department of Biosciences

log – logarithm

ND – Nano Drop

NMDS – Non-metric Multi-dimensional Scaling

NSC – Norwegian Sequencing Centre

PCR – Polymerase chain reaction

PERMANOVA – Permutational analysis of variance

PG – PicoGreen assay

PIT - passive integrated transponder

QB – Qubit assay QBT

Adj. – Adjusted p-value

QPS – Qiagen PowerSoil Pro kit

R2 – R squared (coefficient of determination)

Rhat – Potential scale reduction factor

RNA – Ribonucleic acid

Rpm – Rounds per minute

UiB - University of Bergen

UiO – University of Oslo

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1 Introduction

1.1 Salmon louse (*Lepeophtheirus salmonis,* Krøyer, 1837) and Atlantic salmon (*Salmo salar*, Linnaeus, 1758)

The study of parasite-host interactions is a well-established field within terrestrial ecology, veterinary and medical sciences (Poulin, 2002; Poulin et al., 2020). Although there has been some recent interest in aquatic parasite-host interactions, this area of research is still in its nascent stages (Khan, 2012; Poulin et al., 2020; Poulin and Morand, 2000). Fish are the most diverse and oldest group of vertebrates, with an estimated 30,000 taxa (Nelson et al., 2016). Therefore, as reported by (Carlson et al., 2020; Poulin et al., 2020), compared to other parasite-host interactions in other vertebrate groups, there are relativity fewer publications on fish parasites. The difference in publication numbers for fish versus other vertebrate groups is somewhat paradoxical and suggests a publication bias towards certain phylogenetic groups. This is especially true if the assumptions by (Carlson et al., 2020; Windsor, 1998) are remotely accurate.

The aquatic environment is also a vital source of nutrition and income for a significant portion of the growing human population (FAO, 2022). As a result, most of the available research on parasite-host interactions in aquatic ecology focuses on parasites that affect commercially essential species. This is illustrated in the number of hits a Google Scholar search generated for "parasites Atlantic salmon" versus "parasites three-spined stickleback" on the 9th of May 2023. The former search generated 59,600 hits, while the latter generated 16,000 hits. Both fish species are well-studied, which makes the comparison more equitable. One of the most studied parasite-host interactions in aquatic ecology is the interaction between lice and salmonids, particularly the interaction between Atlantic salmon (*Salmo salar,* for the remaining of this thesis referred to as Atlantic salmon) and salmon louse (*Lepeophtheirus salmonis,* Krøyer, 1837, for the remaining of this thesis referred to as salmon louse).

There are numerous reasons for the interest in the Atlantic salmon and salmon louse interaction. For example, salmon louse cost the Norwegian Atlantic salmon farming industry an estimated US$436 million in 2011 (Abolofia et al., 2017). The cost estimates vary from 4

to 12 billion NOK (Misund, 2022). To put this in perspective, (Abolofia et al., 2017) estimated that salmon louse account for a biomass loss of between 3.62% to 16.55%. The report from the Veterinary Institute (Sommerset et al., 2023) indicates that 300 million post-smolts were introduced to the sea in 2022. Therefore, the lower estimate of 3.62% would result in a loss of 10.8 million fish, more than twice the size of the Norwegian human population in 2023.

Another important reason for the interest in this study system is the substantial and harmful ecological impacts of salmon louse on wild salmonids, as stated by (Forseth et al., 2017; Thorstad et al., 2022). Finally, the third reason for the increasing number of studies published on Atlantic salmon and salmon louse interactions is the steep increase in parasite load on both farmed and wild salmonids observed since the 1980s (Torrissen et al., 2013).

Most studies and surveys have focused on how salmon louse affects Atlantic salmon's health individually or at the population level, as exemplified in (Torrissen et al., 2013). Lice attach themselves to the mucus surface of fish, directly affecting this vital defense mechanism (Grimnes and Jakobsen, 1996; Kabata, 1974; Pike, 1989). An integral component of this defense system is the microorganisms residing in the mucus, collectively referred to as the mucus microbiome. Numerous studies have demonstrated the significance of the mucus microbiome for fish health (Boutin et al., 2013b; Gomez et al., 2013; Legrand et al., 2020). However, besides its importance for host health, the microbiome has scarcely been studied concerning parasitism. In particular, to my knowledge, the salmon mucus microbiome in relation to salmon louse has only been studied in one instance (Llewellyn et al., 2017).

1.2 Interactions between salmon louse and the skin-mucosal microbiome of salmonids

Salmon louse is ectoparasites that inhabit and exploit the skin-mucosal barrier of salmonids (Kabata, 1974; Pike, 1989) alongside the mucosal microbiome, which is an integral part of the mucus matrix that forms the skin barrier in fish (Benhamed et al., 2014; Gomez et al., 2013; Gomez and Primm, 2021; Kelly et al., 2017; Kelly and Salinas, 2017; Legrand et al., 2020; Minniti et al., 2019). The skin barrier is one of the most effective defenses against environmental threats, particularly in aquatic organisms (Bakshani et al., 2018; Esteban, 2012;

Hawkes, 1974; Shephard, 1994). The fish skin and mucus microbiota play critical roles in protecting against injury, infections, and hazardous substances and in signaling, sensing, and maintaining the fish's health (Legrand et al., 2020; Reverter et al., 2018). Research on the microbial community in fish skin mucus is not new, and it has been investigated using culture-dependent methods since at least the 1950s (Cahill, 1990; Horsley, 1977, 1973; Liston, 1957). However, most of these studies focused on bacteria that caused food decay and less emphasized the synergistic significance of the microbiome in fish skin mucus. Furthermore, culture-dependent methods were biased, leading to the conclusion that the microbiomes in water and fish skin mucus were similar (Cahill, 1990; Cipriano and Dove, 2011; Horsley, 1977, 1973; Liston, 1957), which is not the case since only a tiny fraction of bacteria can be cultured on standard growth media (Pedrós-Alió and Manrubia, 2016; Rappé and Giovannoni, 2003).

With the advent of high-throughput culture-independent methods mediated by next-generation sequencing (NGS) techniques, new questions can be posed, and old questions can be answered in new ways. The perspective has shifted gradually from viewing bacteria as a problem concerning skin surface to recognizing their importance to the host (Trivedi, 2012). Despite the apparent importance of a well-functioning mucus barrier for fish health, the microbial community of fish skin mucus is relatively less studied than that of the fish gut microbiome. A search on Google Scholar yielded 34,300 results for the former and 96,000 for the latter (searching "skin microbiome fish" versus "gut microbiome fish" on the 10th of May, 2023).

Earlier studies have revealed the high complexity of the fish skin mucus microbiome, its importance in pathogen protection (Boutin et al., 2013b; Lowrey et al., 2015), sensitivity to lifestyle and external drivers (Hamilton et al., 2023; Larsen et al., 2015; Sylvain et al., 2020; Xavier et al., 2019), and its distinctness from the water microbiome (Boutin et al., 2013b; Carlson et al., 2015; Chiarello et al., 2018, 2019; Larsen et al., 2015; Llewellyn et al., 2017; Minniti et al., 2017; Schmidt et al., 2015; Sylvain et al., 2016, 2020) and between different fish species (Chiarello et al., 2018; Hamilton et al., 2023; Larsen et al., 2013). It has also been demonstrated that the fish skin mucus microbiome can respond within hours to disturbances (Minniti et al., 2017).

One of the most significant and ecologically relevant disturbances affecting the microbiome of fish skin mucus is the presence of parasites or pathogens, such as salmon louse. To date, only one study has directly investigated the impact of salmon louse on the microbiome of fish skin mucus (Llewellyn et al., 2017). As far as I am aware, studies have yet to be conducted investigating the long-term effects of salmon louse on the microbiome of fish mucus. The long-term effects of lice on the microbiome of fish mucus are of high importance, as both secondary infection and treatment of lice are identified as primary concerns for fish health (Sommerset et al., 2023).

1.3 Parasite - holobiont perspective on salmon louse infected Atlantic salmon

The traditional perspective on species interactions in ecology has focused mainly on pairwise species-to-species interactions (Barraclough, 2015). In addition, species interactions have been divided into three distinct categories: competition, mutualism, and parasitism. This is changing since novel approaches allow for studying complex systems with many interactions (Garcia and Cochrane, 2005). At the same time, the reality is that an individual from a given species usually represents a unique biome comprising many different species that forms a holobiont (Bordenstein and Theis, 2015; Dheilly, 2014; Zilber-Rosenberg and Rosenberg, 2008). Various entities of the microbiome typically represent a continuum from weak to strong interactions with the host. The interactions can be symbiotic, commensal, or parasitic (Berg et al., 2020). In essence, an individual of a given species is more than just the individual; it is a sum of all its parts.

Parasites are typically orders of magnitude smaller than their host (Poulin and Morand, 2000). Therefore, a plethora of parasites have a lifestyle where they live on or in the host itself and can have direct interactions with the host's microbiota. It can be imagined that the four-node system (host, parasite, microbiome, environment) can comprise multiple interactions (Figure 1). One of the most direct interactions in this system is that the parasite negatively affects host biology, for example, disruption of osmoregulation in Atlantic salmon after infection with lice (Grimnes and Jakobsen, 1996; Wagner et al., 2003). As a repercussion, the host has a narrower environmental niche, which again changes the salinity experienced by the skin mucus microbiome (Lokesh and Kiron, 2016; Schmidt et al., 2015), leading to community changes in the microbiome that again might have repercussions for the host's physiological

state (Boutin et al., 2013b; Brosschot and Reynolds, 2018; Hahn et al., 2022; Peachey et al., 2017; Zaiss and Harris, 2016). This system of multilevel feedbacks can again lead to changing conditions for the parasite. The main point is that when assessing parasite-host interactions, we should investigate it from a parasite-holobiont perspective to gain a better understanding of the complex biological interactions that are possible in this system (Dheilly, 2014).

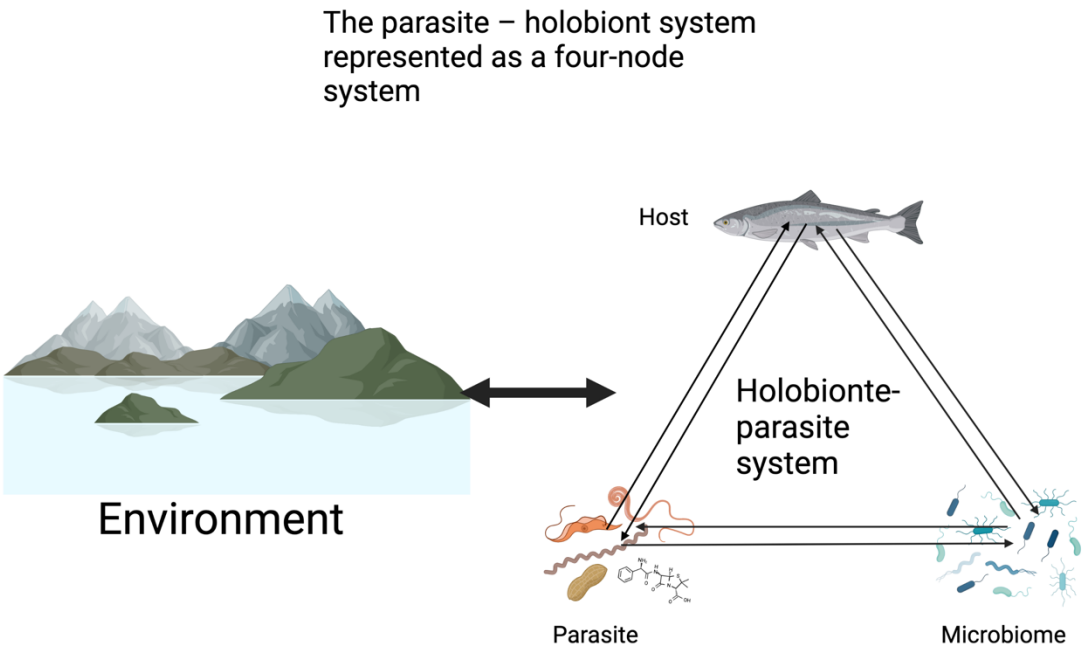

Figure 1: The Parasite – holobiont system is illustrated as a four-node system (host, parasite, microbiome, environment) comprising multiple interactions. Arrows indicate possible interactions within the 4-node system.

1.4 Objectives and aims

This project employed an experimental setup where post-smolt Atlantic salmon was exposed to different loads of salmon louse for five months. To investigate long-term microbiome dynamics in fish mucus while influenced by an ecologically relevant parasite. A 16S rRNA gene metabarcoding approach was used to examine mucus microbiome dynamics in this system. The overarching goal of this study was thus to describe and investigate microbial community dynamics through alpha and beta diversity measures, as well as taxonomic structure in the mucus microbiome of Atlantic salmon after five months of exposure to lice. Additionally, this project aims to promote a holobiont perspective by including the effects of the microbiome as a crucial aspect for further exploration of parasite-host systems in fish. To achieve these overarching objectives, it was deemed necessary to develop a method that allows for precise, rapid, and non-invasive sampling of the Atlantic salmon mucus microbiome while being compatible with a 16S rRNA gene metabarcoding workflow.

Specifically, this study aims:

- To investigate the long-term effects of varying lice intensities on the microbial community residing on the mucus surface of Atlantic salmon.
- To examine the statistical significance of changes in community structure in terms of alpha and beta diversity due to increasing lice intensity.
- To investigate whether there are significant changes in the taxonomic structure of the community with increasing lice intensity, which could indicate a shift in the ratio between commensal and opportunistic genera.
- To advocate for the necessity of including a holobiont perspective in future studies of parasite-host interactions.
- To develop a precise, rapid, non-invasive sampling method for the Atlantic salmon mucus microbiome compatible with 16S rRNA gene metabarcoding.

2 Materials and methods

2.1 Method development of fish mucus sampling, extraction, sequencing, and bioinformatics workflow

The main objective of this master's thesis is to investigate and characterize the bacterial community within Atlantic salmon mucus under the influence of varying lice intensity levels using a 16S rRNA gene metabarcoding workflow similar to the one described by (Liu et al., 2020). To achieve this, it is crucial to collect samples that precisely capture the organisms residing in this mucosal matrix while minimizing any extraneous material. In the study by (Ivanova et al., 2018), different mucus sampling methods, such as scraping, rubbing, and absorption with medical wipes, were compared. The mucus absorption protocol with medical wipes described in (Ivanova et al., 2018) was chosen for this study based on its proven reproducibility in analyzing metabolomic samples and its reduced likelihood of obtaining contaminants. However, further testing was required as the protocol was initially designed for collecting small metabolites rather than microbial DNA, which is relevant to this study. Detailed information on the tests conducted for mucus sampling protocol, extraction procedures, PCR methods, sequencing, and analysis can be found in the subsequent sections on method development (2.1.1 - 2.2.4). The experimental design to address the main objectives of this thesis is described in sections 2.3 and onwards—schematic abstract of the development of the methods in Figure 2.

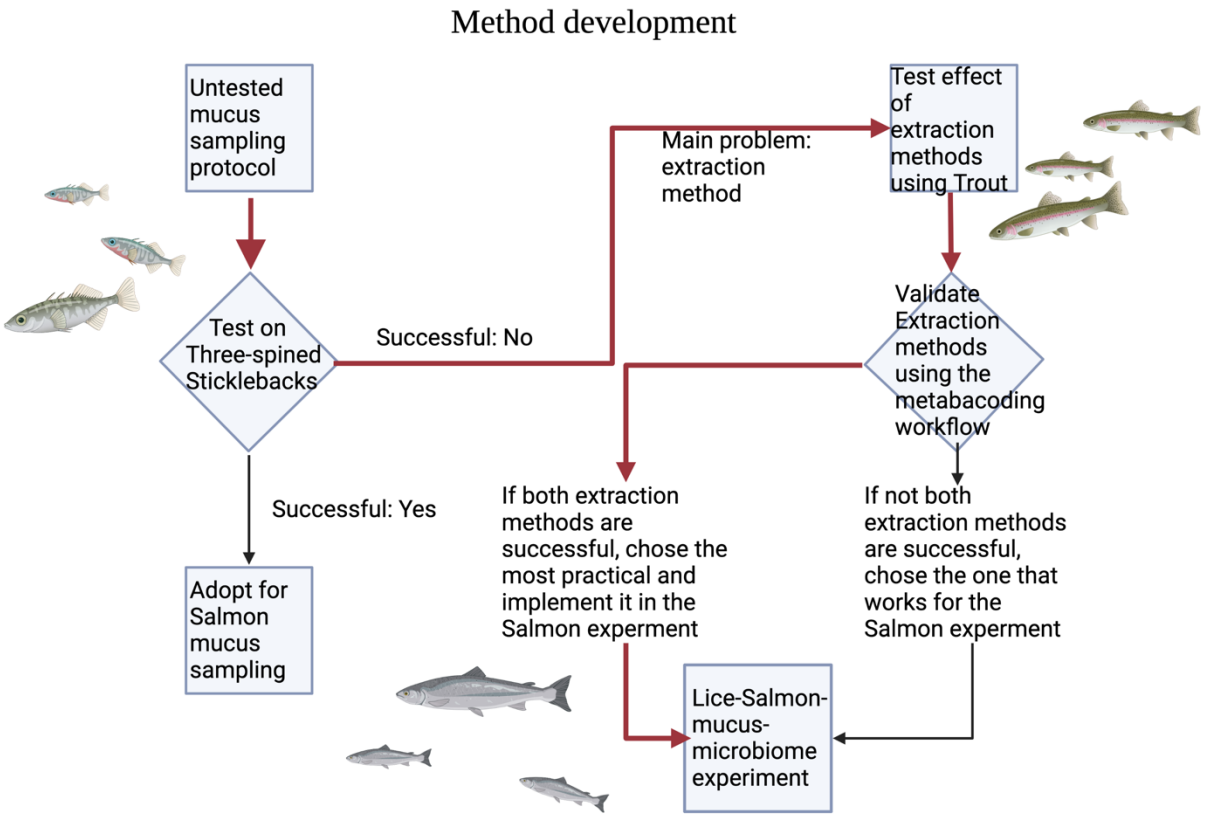

Figure 2: A schematic overview of the thought process behind the method development phase that was necessary before conducting the Atlantic salmon-Lice experiment. The red and bold arrows illustrate the paths chosen during this method development.

2.1.1 Method development using Three-spined Sticklebacks *(Gasterosteus aculeatus,* Linnaeus, 1758*)*: Mucus sampling and microbial DNA extraction

The first adaptation of the absorption with medical wipes protocol (Ivanova et al., 2018) was tested on samples of collected three-spined sticklebacks. On the 11th of September 2021, 30 three-spined sticklebacks were sampled using a beach seine in Drøbak, Norway. Individual fish were caught with an aquarium net (10 x 15 cm) and euthanized with a hard snap to the head.

To collect mucus samples, first, a portable gas burner was used to sterilize two tweezers and scissors for 5 seconds. Next, six plastic trays were washed with 70% EtOH and 10% Cl solution, and gamma sterilized cotton wipes were cut into pieces of 1 cm width and 3 cm

length with the sterilized scissors. Then 14 cotton strips were placed on one of the plastic trays. Finally, two 2 mL cryotubes were placed on each of the remaining plastic trays.

A new pair of clean nitrile gloves were put on. A euthanized stickleback was put on a sterile plastic tray with one of the lateral sides facing up using a flame-sterilized tweezer. A prepared cotton wipe was carefully placed on the side of the fish facing up. After 1-3 seconds of absorbing mucus, the cotton wipe was gently moved around the side of the fish to collect as much slime as possible while avoiding the head and anal opening. The mucus-rich wipe was placed in a cryotube using the same tweezers. The fish was then rotated so that the downwards-facing lateral side faced up, and the procedure was repeated with a new cotton wipe. The procedure was repeated by washing the aquarium net with EtOH and Cl. Selecting a new fish from the beach seine, euthanizing it, then moving it to one of the remaining clean trays for a repeat of the procedure. Gloves were changed regularly, and tweezers were sterilized between each fish. After every fifth fish, all the plastic trays had been used and therefore needed to be rewashed with EtOH and Cl. Everything was repeated until all 30 fish was sampled. The entire mucus sampling process took approximately 10-20 seconds per fish. Cryotubes were immediately stored in a dry shipper on site.

Back at the lab, the frozen wipes were transferred to clean 0.5 mL Eppendorf tubes, and a small hole was made in the bottom of each tube using a sterilized and heated needle. After that, the 0.5 mL Eppendorf tubes were placed into clean 2 mL Eppendorf tubes and centrifuged at 10 000 rpm for 10 minutes to form a bacterial pellet at the bottom of the tube. It was decided to follow the Qiagen Blood & Tissue protocol for the DNA extraction for gram-positive bacteria based on previous work on fish skin microbiomes that used a similar methodology (Chiarello et al., 2019; Minniti et al., 2017; Sylvain et al., 2016). However, only 10 out of 30 samples produced a visible pellet. Then the pellet-forming samples were processed with the Qiagen protocol for gram-positive bacteria, following the manufacturer's instructions.

To quantify the DNA extracts, an in-house protocol with the Quant-itTM PicoGreen® assay and a microplate reader (SynergyTM MX), following the medium-range PicoGreen protocol (Eiler, 2023), was used. The DNA concentration ranged from 0.1 to 3 ng/µL.

2.2 Method development on Brown Trout *(Salmo trutta,* Linnaeus, 1758*)*: Mucus sampling and complete metabarcoding workflow

The primary goals of this Method Development section are as follows: 1) To validate the complete 16S rRNA gene metabarcoding workflow, encompassing sampling, extraction, library preparation, sequencing, and bioinformatics, as outlined by (Liu et al., 2020).
2) To assess the impact of two different extraction kits on microbial composition. 3) To address and enhance the limitations identified in the initial method test.
The ultimate aim is to establish a reliable and robust workflow to implement in the salmon louse- Atlantic salmon-microbiome experiment (sections, 2.3 - 2.7).

2.2.1 Method development on Brown Trout: Mucus sampling and microbial DNA extraction

The first method test described in section 2.1.1 was considered partially successful. The practical feasibility of the method was deemed high, as the concept of absorbing microbial mucus and subsequent extraction worked in several cases. However, several weaknesses were identified. The first weakness was the occasional failure to form a bacterial pellet during DNA extraction. The second weakness was that the size of three-spined sticklebacks differs from that of post-smolt Atlantic salmon. As a result, new test samples were required from fish similar in size to the smallest fish in the Atlantic salmon experiment, around 25.5 cm in length. However, acquiring Atlantic salmon of this size was impossible, so brown trout from a local river (Lysakerelven, Norway) was used instead. On the 20th of August 2022, 20 brown trout were collected using a flyrod, and mucus was collected using a slightly modified version of the protocol described in 2.1.1. The main modification was increasing the size of the cotton strips to 3 cm in width and 5-6 cm in length to suit Atlantic salmon mucus sampling.

Back at the lab, all the (Extraction and library preparation) proceeding work was done either in a laminar flow cabinet or on clean lab benches in pre- and post-PCR labs. Pipets, racks, markers, tips, tubes, and PCR water were sterilized using a UV cabinet and/or washed with 70 % lab-grade EtOH and 10 % Cl. Nitrile gloves were changed regularly and cleaned with EtOH before use. Tubes were spun down to avoid splashing of droplets.

A second alternative extraction kit, not dependent on pellet formation, was chosen to address the issues with pellet formation from the first DNA extraction test. The Qiagen PowerSoil Pro kit was selected due to its successful use in extracting skin-swabbed microbial communities in fish mucus (Pratte et al., 2018; Uren Webster et al., 2018; Xavier et al., 2019). The Qiagen Blood and Tissue kit (QBT) and the Qiagen PowerSoil Pro kit (QPS) were tested on the 20 mucus samples from trout.

Following the procedure described in 2.1.1, 9 out of 10 samples produced a bacterial pellet and were extracted using the QBT protocol for Gram-positive bacteria. The remaining 9 samples were successfully extracted. For the QPS kit, the frozen wipes were placed in the PowerBead Pro Tube using a sterile tweezer, and the remaining extraction process followed the manufacturer's instructions. 9 samples were successfully extracted using the QPS kit. This gave 18 test samples (9 extracted with QBT and 9 with QPS).

DNA quantification was done using three different assays: nanodrop (ND) (NanoDrop® ND-1000 Spectrophotometer), qubit (QB) (Invitrogen, Qubit® 3.0 fluorometer), and PicoGreen (PG) (Eiler, 2023). The QPS kit produced DNA concentrations ranging from 2-11 ng/µL, measured by both QB and PG assays. The QBT kit produced DNA concentrations ranging from 0.1-2 ng/µL, measured by both QB and PG assays. The absorption ratios from the nanodrop were as expected for DNA.

2.2.2 Method development on Brown Trout: Library preparation and sequencing

In this study, the focus was to amplify the V3 and V4 variable regions of the bacterial 16s rRNA gene sequence through a two-step amplification protocol described by (Juottonen et al., 2020) with modifications for bacteria. The first round of PCRs used the bacterial primers 341F (Herlemann et al., 2011) and 805NR (Apprill et al., 2015). In addition, a positive control containing a premade bacterial mock community (ZymoBIOMICS® Gut Microbiome Standard) and a negative control containing only PCR water were included to ensure the reliability of the results. The PCR reactions followed the protocol's instructions using a Mastercycle ep gradient S (Eppendorf) system.

Primer sequences:

Illumina adapter-N4-341F:

5'-
ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCCTACGGGNGGCWGCAG-
3'

Illumina adapter-805NR:

5'-AGACGTGTGCTCTTCCGATCTGACTACNVGGGTATCTAATCC- 3'

After running the first PCR with 20 cycles, mixed results were observed when visualizing the samples on a 1% agarose gel. Therefore, an increase to 25 cycles was implemented. The increase in cycles yielded satisfactory results when visualized. The second PCR followed the protocol employed by (Juottonen et al., 2020). The PCR products were then purified using the Agencourt AMPure XP purification protocol with magnetic AMPure XP beads.

Upon visualizing the PCR products on a gel, it was noticed that the QBT extraction method group gave better bands for every sample than the QPS extraction method group. Furthermore, the negative control showed no bands, whereas the positive control (premade bacterial mock community) had the brightest band. The PCR products were quantified and pooled into a single library based on the measured concentrations. The DNA concentration in the final pool after the second PCR (15 cycles) and purification was 5.45 ng/µL.

To ensure the quality of the library, the A280/A260 and A260/A230 ratios were measured using a nanodrop (NanoDrop® ND-1000 Spectrophotometer). Finally, the pool was visualized on a 1% agarose gel to check for primer dimers. Primer dimers were not observed.

The pooled samples were submitted to the Norwegian Sequencing Center (NSC) at the Department of Medical Genetics in Ullevål, Oslo, Norway, for sequencing on the Illumina MiSeq v2 nano 250 paired-end platform. After demultiplexing, quality control assessments were performed on the raw data, which consisted of FastQ files of forward and reverse reads. On average, each sample yielded 25 000 reads.

The negative control had approximately 20 000 reads. The quality scores of the reads were high, with an average of 35-40 for the first 200 BP but decreased to 20 towards the end of the sequences. The forward reads had a better mean quality score than the reverse reads.

2.2.3 Method development on Brown Trout: Handling of raw and processed sequence data

After downloading the FastQ files to a computer cluster (Sigma2/NRIS, Saga), the primers were trimmed using Cutadapt V.2.10 (Martin, 2011). The processed sequences were then subjected to quality control and filtering using R (4.2.1) and the "*DADA2*" R package (Callahan et al., 2016). The quality threshold of Q = 20 and length threshold of 220 was applied to both forward and reverse reads, with error models and 1e7 bases used to learn the error rates. The sequences were dereplicated, and chimeras were filtered out. However, the initial merging of the reads yielded low amounts of merged reads, which led to the implementation of a concatenation step that added 10 N bases, significantly improving the merging of the reverse and forward reads. ASVs were assigned using the Silva database (v.138) (Yilmaz et al., 2014), and ASVs and taxonomy tables were created. Overall, a mean of 7000 sequences per sample was obtained, with 20 samples in the dataset, 9 belonging to the QPS extraction method and 9 to the QBT extraction method, along with one negative and one positive control.

Metadata, ASV table, and the tax table were merged into a phyloseq object by the "*phyloseq*" R package (McMurdie and Holmes, 2013). The phyloseq object contained 1723 ASVs, 20 samples, and four variables (sample_id, sample_name, treatment, Env). After removing sequences, not including bacteria, chloroplast, and mitochondria, the phyloseq object contained 1423 ASVs, n = 20, and four sample variables.

Since the negative control contained a substantial amount of reads, the R package "*Decontam*" (Davis et al., 2018), with the most stringent contamination threshold of 0.5, was chosen to decontaminate the sequence data. Five ASVs were flagged as contaminants. These were removed from all samples. The following ASVs were identified as contaminants *Staphylococcus, Endozoicomonas, Faecalibacterium, Escherichia/Shigella, Roseburia,* and *Prevotella,* and subsequently removed.

Rarefaction was conducted to obtain an equal amount of reads in each sample to ensure a representative comparison of alpha diversity estimates between samples without introducing bias from varying sample sizes (Willis, 2019). The rarefaction was based on the sample with the lowest number of reads in this experiment (the read number was 1100).

2.2.4 Method development on Brown Trout: Evaluation of DNA extraction method and metabarcoding workflow

To evaluate the effect of the DNA extraction methods and the metabarcoding workflow (2.2.1 – 2.2.3) on microbial mucus community outputs. Four alpha diversity estimates were generated from the rarefied sequence data. Common to all the alpha diversity estimates is their attempt to describe the community structure at a given location. In this case, the location refers to the mucus of individual fish. Different alpha diversity metrics emphasize different aspects of community structure. The Abundance-based Coverage Estimator (ACE) focuses on species richness within the community. For more information, see (Chao and Lee, 1992). The Shannon alpha diversity index combines measures of richness and evenness, with evenness accounting for the relative abundance of different species within the community. For a better understanding, see (Shannon, 1948). The Simpson alpha diversity index, like the Shannon index, considers species richness and the relative abundance of species in relation to each other. However, the calculation method differs slightly between them. For the mathematical background, see (Simpson, 1949). The final alpha diversity index generated is Fisher's index, which incorporates both richness and evenness but employs a different calculation method more suitable for small sample sizes. For details on this index, see (Fisher et al., 1943). ACE, Shannon, Simpson, and Fisher´s alpha diversity estimates were combined with a pairwise Student's t-test to compare the effects of the two extraction methods (QBT and QPS) on alpha diversity in the microbiomes. Boxplots were used for visual inspection.

Beta diversity describes changes in community structure between different biological communities (Anderson et al., 2011) and can be divided into turnover and variation. Turnover characterizes the number of species replaced over a temporal or spatial gradient, while variation primarily focuses on changes in community composition and species abundance. This study used the v3-v4 approach (Anderson et al., 2011) to investigate the variation in species composition and abundance within the mucus microbiome related to the different

extraction methods (QBT vs. QPS kits). Bray Curtis distance was used to calculate beta diversity between bacterial communities of the two extraction methods, and the results were visualized in a non-metric multidimensional scaling (NMDS) plot. In addition, using the R package "*vegan*" (Oksanen et al., 2022), a pairwise Adonis was used to test for differences in beta diversity between the two extraction kits.

2.3 The salmon louse and Atlantic salmon experiment

The main objective of this study, as introduced in section 1.4, is to investigate the effect of increasing salmon louse intensity on the microbial mucus community structure and taxonomic composition of Atlantic salmon over time. To address this objective, an experimental setup is required where Atlantic salmon is subjected to controlled exposure to different levels of lice intensity while minimizing the impact of other external factors. It is also necessary to be able to count the number of salmon louse per fish at multiple time points during the experiment and easily sample the mucus surface of the fish without causing unnecessary disturbance to the fish. Therefore, the experimental system depicted in Figure 3 and described in Section 2.3.2 was chosen. The workflow developed in sections 2.1.1 – 2.2.4 was implemented for the experiment.

2.3.1 Animal welfare

The following animal welfare statement is the one provided by Dr. Adele Mennaret (Main contact UiB) regarding the conducted salmon louse and Atlantic salmon experiment:
The salmon lice used in this study were obtained from Atlantic salmon (*Salmo salar*) hosts maintained in the lab under a permit from Mattilsynet (FOTS 23917) and handled according to Norwegian animal welfare regulations. Once a day, the fish were fed with commercial pellets, and the water flow and oxygen level in the water were checked. Fish were monitored to ensure they were all gaining weight and did not display signs of acute stress or injury (which did not happen during the course of this study). Those fish that needed to be replaced when their size exceeded 1 kg, were humanely euthanized by anesthesia in 1mL/L metomidate and 0.3mL/L benzocaine until unresponsive followed by a blow to the head.

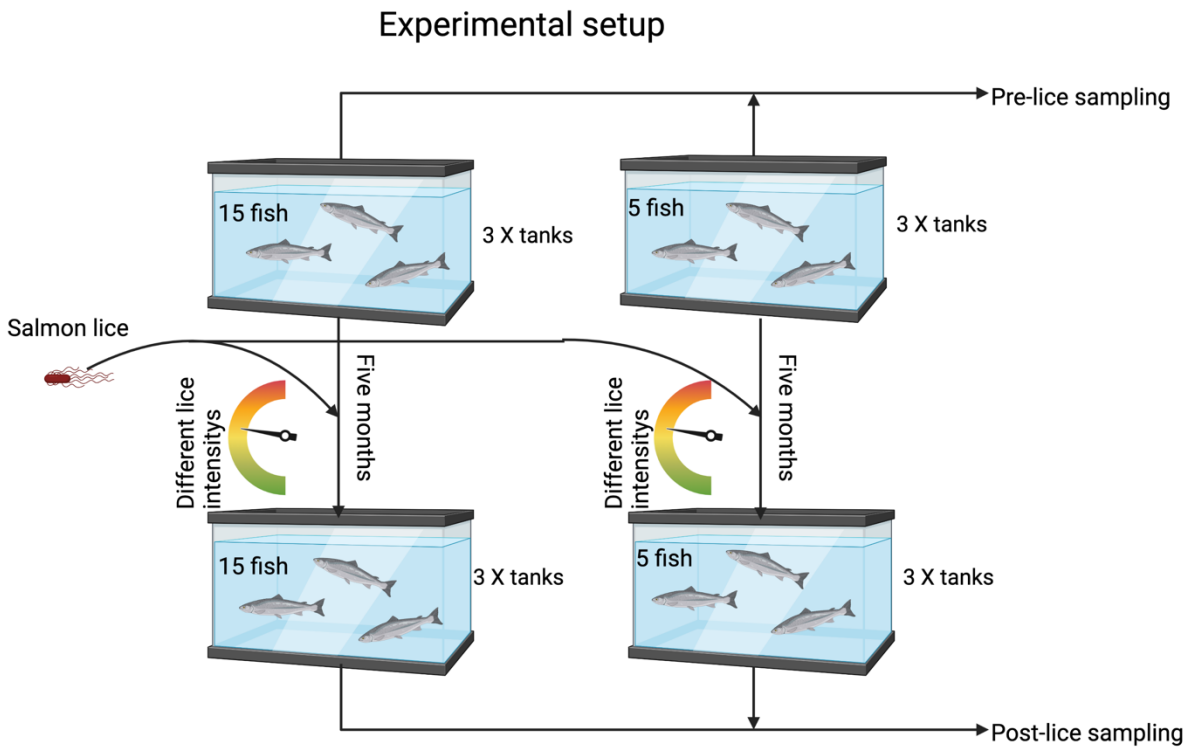

Figure 3: Visual representation of the experimental setup employed in this master's thesis: 6 tanks were used, with 3 tanks housing 5 fish each and 3 tanks housing 15 fish each. After the initial mucus sampling, salmon lice larvae were introduced into the respective tanks. Different densities of lice larvae were added to create a gradient of lice intensity per fish. The tanks with salmon louse were closely monitored for five months. The final mucus sampling was conducted after completing the five months with varying lice regimes. Subsequently, the fish were humanely euthanized.

2.3.2 The experimental setup and mucus sampling

The experiment was conducted over five months (starting on the 24th of November 2021 and ending on the 26th of April 2022). The total number of Atlantic salmon at the start of the experiment was 60. At arrival, the post-smolts had an average length of 26.9 cm (25.5 cm to 28.0 cm). Fish were allowed to acclimate for 7 days in 6 separate tanks at the UiB fish facility. There were three tanks with 15 fish and three tanks with 5 fish. All tanks were kept in the same room. Every fish tank had a unique water supply from a main pipeline that carried seawater from a depth of 110 m from the nearby fjord. The water was UV sterilized and filtered before entering the fish tanks. The temperature in all tanks was kept constant throughout the experiment with minor variations from 8.9 to 10.0 degrees Celsius. All tanks had different nets, buckets, and other gear regularly washed with disinfectant (Virkon S,

Lilleborg) to minimize contamination between tanks. After the acclimation period of 7 days, the first mucus sampling was carried out on the 24th of November 2021. During the next five months, all of the Atlantic salmon was regularly monitored, and the number of lice was counted on each individual after the start of the infection and at the end of the experiment. The counting of lice included the total number of lice per fish and the count of male versus female lice per fish. After five months, the second and final mucus sampling was carried out (26th of April 2022).

Pre-lice mucus sampling included three main steps performed over three days during the first sampling campaign. Step one included anesthesia and mucus sampling (Figure 4). Step two included measuring length, weight, and passive integrated transponder tagging (PIT-tagging). Step three included recovery and infection of salmon lice larvae. All fish were closely monitored during all three phases to prevent unnecessary stress, pain, or suffering. In addition, all fish were closely monitored for the next week to look for adverse effects of the handling. Unfortunately, eight fish died/were euthanized after the stress of the procedure and thus were replaced. A total number of 60 Atlantic salmon remained for the rest of the experiment.

Post-lice mucus sampling was the second and final sampling. It was performed in three distinct steps. Step one included anesthesia and mucus sampling (Figure 4); step two included measurement of length (mean = 38.38 cm) and weight (mean = 678.55 g) and final counting of parasites on each fish. The third step included euthanasia with a club while the fish was still under anesthesia. Water samples (2.3.3) from the holding tanks and blanks (2.3.3) from the equipment used were taken at multiple points during pre- and post-lice samplings.

I refer to supplementary section 12 for a detailed description of anesthesia, PIT-tagging, measurement, and salmon lice infection procedures.

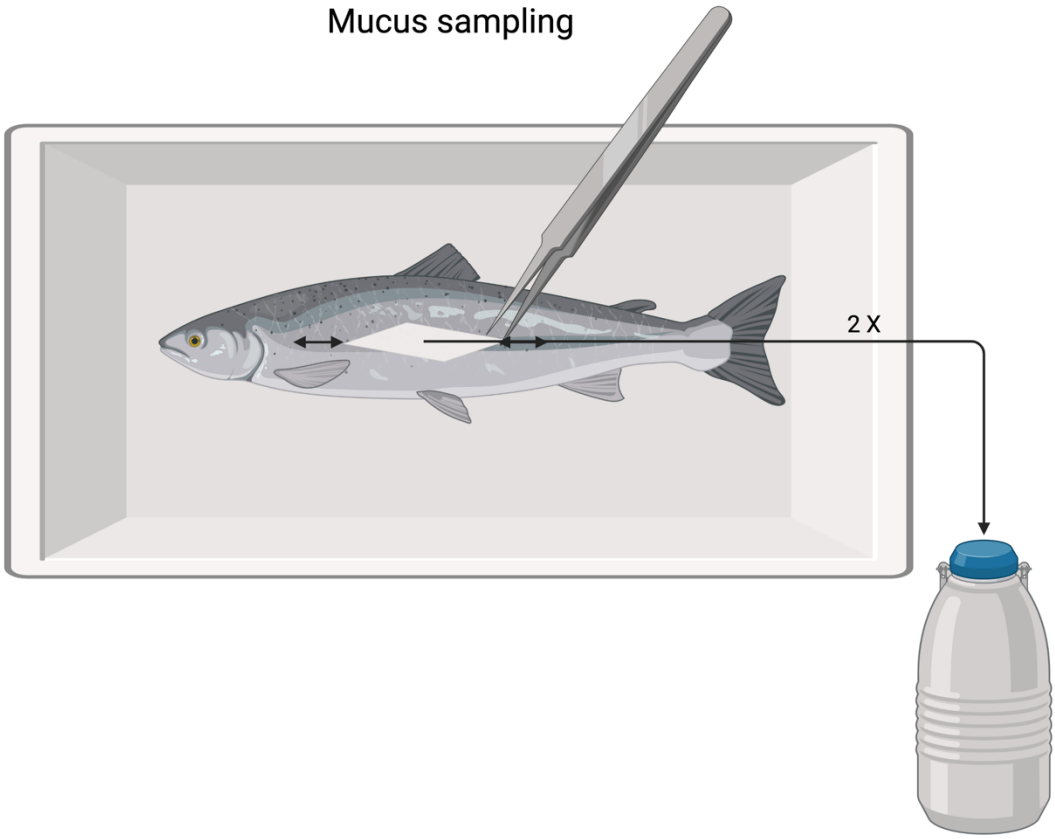

Figure 4: Visualization of how the mucus samples were taken from individual fish: After the fish were anesthetized, they were placed on a plastic tray with one side facing up. A clean medical wipe measuring 3 x 5 cm was placed in the middle of the fish using sterilized tweezers to absorb the fish's mucus. Next, the mucus-rich wipe was transferred with the same tweezers into a 2 ml cryotube and flash-frozen in liquid nitrogen. Finally, the fish was rotated 180 degrees to repeat the procedure on the unsampled side with a new wipe.

2.3.3 Collection of blanks and water samples

The reasoning behind taking blanks from equipment, cotton wipes, and water samples is to distinguish between the microbiome of the individual fish and their surroundings. It is worth mentioning that the sampling was done in a wet lab, so blanks are also necessary for identifying possible contaminants that can later be filtered out bioinformatically.

Blanks were collected daily during the two sampling campaigns. Clean cotton wipes were dampened with PCR-grade water and wiped against freshly cleaned plastic trays, sterilized scissors, and tweezers to obtain blank samples. Additionally, blank samples of the cotton wipes were obtained by adding PCR-grade water directly to the wipes without any contact with other objects.

Sterivex 0.22 µm filters (Millipore® Sterivex™), nitrile gloves, and 60 mL sterile syringes were used to collect water samples from the tanks. The syringes were rinsed three times with water from the sampled tank, and then 60 mL of water was collected and passed through the filter. This process was repeated 16 times, resulting in 960 mL of water passing through the filter. Next, the syringe was filled with air and used to push out any remaining water from the filter. The filters were labeled with the tank's name (T1, T2, T3, etc.) and placed in a dry shipper until transferred to – 80 Celsius freezers. Water samples were only collected from tanks containing experimental fish.

2.3.4 Final notes

In the final mucus sampling on the 26th of April 2022, I sampled fish 1-45. Fish 46-60 and their lice required two more weeks to grow before they could be sampled, so Dr. Adele Mennerat followed the same protocol and sampled these fish on the 10th of May, 2022. The samples and one water sample from the remaining fish tank were stored in -80 degrees Celsius freezers for long-term storage before being shipped overnight on dry ice to UiO. Upon arrival, the samples were immediately frozen and stored in the -80-degree Celsius freezers.

2.4 The salmon louse and Atlantic salmon experiment – sample processing

A DNA metabarcoding approach was selected to describe the dynamics of lice-salmon-microbiome interactions. DNA metabarcoding has proven to be an effective tool for studying biodiversity (Taberlet et al., 2012), making it suitable for investigating the dynamics of the fish mucus microbiome under parasitic influences (Llewellyn et al., 2017; X. Zhang et al., 2018). As illustrated by (Liu et al., 2020), a metabarcoding investigation normally follows the subsequent steps, sampling, DNA extraction and amplification, DNA sequencing, and bioinformatics analysis. Therefore, following the method developed and described in methods 2.1.1- 2.2.4, the same workflow was implemented for processing the Atlantic salmon samples.

2.4.1 Extraction, library preparation, and sequencing

For extraction of DNA from the sterivex filters (Millipore® Sterivex™), the Qiagen PowerWater kit was used. This kit was selected based on previous successful experience with similar work at AQUA (IBV, UiO), and the extraction followed the manufacturer's protocol without deviation. However, the filter from tank 5 (T5) had a leak that affected the DNA extraction performance. All filters were processed on the 11th of October 2022 except for 2T8, which was processed on the 25th of November 2022 due to the later arrival time mentioned in 2.3.4 Final notes.

Based on the testing in sections 2.1.1 - 2.2.4, the Qiagen Power Soil Pro kit was chosen for mucus DNA extraction. The manufacturer's protocol was followed with minor deviations. The only deviation was transferring the mucus wipes from the 2 ml cryo-tube to the homogenization tubes using sterile tweezers. The mucus-rich wipes were then positioned carefully in the beat-beating tubes to allow the beads to move freely. Gloves were washed with ethanol between samples and changed every three samples. Twelve samples were processed simultaneously, with the first extracted on the 11th of October 2022 and the last on the 25th of November 2022

The first library preparation followed the procedure outlined in section 2.2.2, with the first PCR having 25 cycles and the second PCR step following the original protocol (Juottonen et al., 2020). Library preparation of 150 samples commenced on the 25th of November 2022 and was completed on the 27th of November 2022. Quantifying the processed samples was done using qubit (Invitrogen, Qubit® 3.0 fluorometer) HS assay according to manufacture protocol, Picogreen assay (Eiler, 2023), and nanodrop (NanoDrop® ND-1000 Spectrophotometer), but the DNA concentration of the samples was below the required threshold for sequencing. Therefore, the library preparation had to be redone. However, testing different PCR settings for all samples was impractical and uneconomical. Thus, the first eight samples (F1-F8) were selected for testing the number of PCR cycles needed in the first PCR. Three test runs were performed using 25, 30, and 35 cycles. The test run with 35 cycles showed the most promising results with robust and consistent bands on the 1% agarose gel. However, some of the bands on the gel were still unsatisfactory, prompting another test to determine the effect of template DNA volume on PCR reliability.

The new test involved selecting PCR programs with either 30 or 35 cycles and two volumes of template DNA, 3 µl and 5 µl, respectively, resulting in 32 different reactions. The results showed that the bands were most robust and concise for the samples run on 35 cycles with 5 µL of DNA. The different PCRs were tested between the 18th of December 2022 and the 22nd of December 2022. Based on the new test, the modified library preparation procedure involved a first PCR step consisting of 35 cycles with 5 µL of template DNA added to each reaction. The final PCRs included two negative controls and two mock communities.

All PCR reactions were performed in duplicates, and PCR reagents and samples were kept on ice during preparations. The final library preparation started on the 22nd of December 2022 and finished on the 01st of January 2023. After the final bead cleaning step, the DNA concentration of the samples was quantified using the PicoGreen assay. Then, the samples were pooled into a final pool with a concentration of 3.2 ng/µL and a volume of 958 µL. The final pool was visualized on a 1% agarose gel, and two strong bands were observed. One band consisted of fragments between 5-600 bp, and the other band consisted of 1-200 bp fragments (primer-dimer complexes).

Primer dimer complexes can negatively influence sequence results; therefore, the Qiagen Gel Purification (QGP) Kit was selected to remove primer-dimer complexes. However, based on previous experiences from colleagues in the lab, it was known that using the QGP Kit could result in a significant loss of genetic material. This was problematic since I already had a relatively low concentration in my final pool (3.2 ng/µL).

An additional Agencourt AMPure XP purification step was implemented to address the relatively low DNA concentration issue. This step was not meant to eliminate the dimers but rather to increase the overall DNA concentration. The beads in the purification step selectively bind to DNA fragments that are 100 bp and longer, which includes the dimers in the pool that was 1-200 bp in length. To increase the DNA concentration, 520 µL of the final pool was added at the start and then eluated to 120 µL at the end of the cleaning protocol, following the manufacturer's instructions. The resulting DNA concentration in the final pool was now 12.3 ng/µL, deemed sufficient for the Qiagen gel purification Kit procedure.

Two wells of a 1% agarose gel were loaded with 60 µL of the final pool each, and the gel was run for 50 minutes under 120 V and 140 mA. The bands with a length of 5-600 bp were cut out with surgical precision, and the QGP protocol was followed to produce two gel-purified pools with 30 µL each. The DNA concentration in both pools was quantified using the Qubit (Invitrogen, Qubit® 3.0 fluorometer) HS assay according to manufacturers protocol, and both pools had DNA concentrations of around 1.4-1.7 ng/µL. Due to the low DNA concentration, the volume of the pool was reduced from 48 to 22 µL using vacuum drying with 800 rpm under 50 degrees Celsius (Savant DNA 120 SpeedVac Concentrator, Thermo Scientific), and the DNA concentration in the pool was 2.7 ng/µL, with acceptable absorption ratios from the nanodrop (NanoDrop® ND-1000 Spectrophotometer) for sequencing.

A second backup pool was created by combining the leftovers from the first pool before bead cleaning with approximately 300 µL with products from a second pooling of the PCR 2 products, resulting in a final volume of 738 µL. This volume was then reduced to 180 µL by vacuum drying (Savant DNA 120 SpeedVac Concentrator, Thermo Scientific) and gel-purified according to the procedure. The DNA concentration in the resulting sample ranged from 1-1.5 ng/µL. Finally, the four pools were combined into a final pool with a volume of 120 µL, which was further reduced to 45 µL using vacuum drying (Savant DNA 120 SpeedVac Concentrator, Thermo Scientific). This final backup pool was quality controlled using gel electrophoresis, nanodrop (NanoDrop® ND-1000 Spectrophotometer), and qubit (Invitrogen, Qubit® 3.0 fluorometer) HS assay according to manufactures protocol, and the final DNA concentration was 2.38 ng/µL, with a volume of 37 µL.

The samples were sequenced on the Illumina MiSeq V3 2x300 bp paired-end platform. The Norwegian sequencing center used a 10 % phiX solution during sequencing (09th of January 2023).

The data was received on the 27th of January, 2023. The library consisted of 156 samples, with 150 belonging to this study. The raw data consisted of demultiplexed FastQ files representing forward and reverse reads and quality control assessments. Based on the quality control, short reads dominated the samples, and the quality score dropped significantly after 100 bp. The average amount of fragments was around 70 000 reads per sample.

2.4.2 Raw data handling - first sequencing attempt

The FastQ files were downloaded to a computer cluster (Sigma2/NRIS, Saga), and the primers were trimmed using Cutadapt V.2.10 (Martin, 2011). Cutadapt removed nearly all the sequences with standard settings, allowing a maximum error rate of 10% mismatch with the predefined primers and a minimum fragment length of 200 bp. N bases were excluded from error counting. However, a new run of Cutadapt was queued up with non-standard settings to test its ability to identify primer sequences. This new run allowed a maximum error rate of 0.5, equivalent to a 50% error rate, while keeping the minimum read length the same, which yielded significantly more sequences.

Subsequently, the sequences were processed using R (4.2.1) and the "*DADA2*" R package (Callahan et al., 2016). In DADA2, a quality score of 0 was selected, and the threshold for read length was set at 250 bp (Fwd) and 200 bp (Rev). The filtering step removed 45 of the samples. Error models were applied, and 1e7 bases were selected to learn the error rates. The sequences were dereplicated, merged, and chimeras were filtered out. Close to 50% of the sequences were identified as chimeras, and many of the non-chimeric sequences were unable to merge. The taxonomy was assigned using the SILVA database version 138 (Yilmaz et al., 2014), and ASV and taxonomy tables were created. Only 4 samples remained.

2.4.3 When life gives you lemons, you make lemonade

Based on the preliminary analysis of the sequence data, it was deemed that the data was unusable. Therefore, it was decided to deliver the backup pool to sequencing (2.4.1).
The backup pool was delivered to sequencing on the 02nd of February 2023. This time, an extra bead cleanup was decided at the sequencing facility. This reduced short fragments to 3 % of the entire library. In addition, a 20 % phiX solution was also applied. The library was sequenced on the Illumina MiSeq v3 2x300 bp platform.

The sequence data was received on the 13th of February, 2023. The second sequence attempt was more successful. The average amount of reads in each sample was 100 000 reads. Some samples had an excess of 270 000 reads, while others had only 1000 reads. The mean quality of the reads had a quality score of 35-40 for the first 200 BP and decreased rapidly at the end

of the sequences. The forward read had a better mean quality score than the reverse reads. The conclusion was still that the data was of poor quality compared to previous work done for the AQUA section. However, the data was still deemed usable for the resulting analysis.

2.5 Bioinformatics and processed data analysis

2.5.1 Handling of raw and processed sequence data

Primers and adapters were removed with Cutadapt V.2.10 (Martin, 2011). The sequenced were filtered, dereplicated, concatenated, merged, filtered for chimera reads, and assigned taxonomically using the DADA2 pipeline. The quality threshold selected was Q = 0, and the length threshold was 220 for both forward and reverse reads. The set thresholds resulted in unreliable sequences and significant losses of non-chimeric reads.

A different approach was needed to minimize the loss of non-chimeric reads and ensure more reliable sequences. A **mock community comparison and sequence length optimization** were implemented to achieve this. This protocol utilizes relative abundance, taxonomic composition, and sequences from a supplied mock community (ZymoBIOMICS® Gut Microbiome Standard). The protocol compares the sequenced mock community with the real mock community using the DADA2 pipeline. This is done sequentially with different forward and reverse read length combinations, focusing on the 170-250 bp range for both reads. The length is increased by 10 bp in each subsequent run, e.g., the first run checked 170 fwds and 170 revs, the second run checked 170 forward, and 180 reverse, and so on, for all combinations within the 170 to 250 bp range for both reverse and forward reads. During each run, all steps in the DADA2 pipeline are applied, including filtering, dereplication, concatenation, merging, filtering for chimeric reads, and taxonomic assignment. This is only done for the mock community, and the result is compared to the ZymoBIOMICS® Gut Microbiome Standard using Bray-Curtis distance matrices. The number of non-chimeric reads, and artificial ASVs were also recorded.

Based on the optimizing procedure, the following thresholds were chosen: quality score of 0, forward read length of 200 bp, reverse read length of 170 bp, and concatenation with 10 N bases. The DADA2 pipeline is known for its ability to handle lower-quality reads by

considering the quality profile in its error model (Callahan et al., 2016). Therefore, the processed sequence data was now deemed sufficient for downstream analysis.

In order to investigate lice variables in the metadata that could not be used simultaneously, the growth of the fish during the experiment and tank effects. The metadata was examined using autocorrelation analysis and visualized. The average lice intensity per fish (MeanLice) was selected as the most descriptive lice variable and was used throughout the remainder of the study. The method for analyzing the metadata is described in supplementary section 2.

The sequence data analysis involved merging the metadata, ASV table, and tax table using the "*Phyloseq*" R package (McMurdie and Holmes, 2013) to create a phyloseq object containing 10133 ASVs, 145 samples, and 29 sample variables (Table 1 in supplementary, section 1 metadata). After filtering out non-bacterial sequences (i.e., Archaea, eukaryotic, chloroplast and mitochondria), the phyloseq object was reduced to 6028 ASVs, n = 145, and 29 sample variables.

To increase the likelihood of analyzing only ASVs associated explicitly with the fish mucus microbiome. The bacteria from water samples, blanks, and negative controls were removed. The "*Decontam*" R package (Davis et al., 2018) then identify 621 ASVs as contaminants based on their prevalence in the negative control, water samples from the second sampling, and blank samples. The most stringent threshold of 0.5 was selected according to the developer's recommendations. As a result, the cleaned phyloseq object now contained 4192 ASVs and 131 samples. Data with water microbiome included has also been analyzed and is shown in supplementary sections 9 - 11.

Close to all samples corresponding to the first sampling, as such small fish, had close to zero or zero reads left. While the fish samples corresponding to the second sampling, as such fully grown fish, had a good number of reads. Therefore, it was decided to subset the data only to include fish from the second sampling. The resulting phyloseq object contained 4192 ASVs and 60 samples.
To ensure a representative comparison of alpha diversity estimates between samples without introducing bias from varying sample sizes (Willis, 2019), rarefaction was conducted to obtain an equal amount of reads in each sample. After rarefication, 1559 ASVs remained, and the number of samples was 42.

An additional dataset with unrarefied data was also produced for comparison of the effect of rarefaction. Samples with less than 50 ASVs were removed to avoid introducing bias in alpha diversity and beta diversity calculations. This data frame contained 4192 ASVs and n = 58. The unrarefied results are presented in supplementary sections 6-8.

2.6 Statistics, salmon louse effect on microbiome community structure

The alpha diversity was estimated using the same approach outlined in section 2.2.4. The resulting alpha diversity estimate was employed as the response variable in the subsequent linear models. Both ACE and Simpson estimates were utilized in the ensuing linear models. The research question only pertains to alterations in alpha diversity resulting from increasing lice loads without explicitly focusing on the alpha diversity metric (i.e., richness and/or evenness). Thus, only the abundance-based coverage estimator ACE was selected for further analysis. The results obtained from the Simpson index are presented in supplementary section 5. The ACE index solely considers the presence of species/ASVs, starting from 0, with a value of ACE = 1 denoting the presence of a single species. Higher ACE values correspond to greater diversity in terms of richness. For a more detailed description of ACE, see (Chao and Lee, 1992)

2.6.1 Linear effects of salmon louse on alpha diversity

To test the hypothesis that an increase in lice intensity alters the alpha diversity of the Atlantic salmon surface microbiome, linear models were chosen as the analytical approach. Given the experimental design, it is natural to consider the tank as a random effect that needs to be accounted for. Fish within the same tank are expected to be less independent of each other than fish between tanks. Biologically, it can be postulated that bacteria present in the mucus of one fish could be transmitted to neighboring fish but not to a third fish in another tank. Therefore, not all the 42 remaining fish are necessarily independent of each other. The "*GlmmTMB*" (Brooks et al., 2017) R package was used to fit two mixed linear models. Both models had tanks as a random effect, MeanLice as the explanatory variable, and log(ACE) as the response variable. The random effect had six levels, as there were six tanks in the experimental setup.

The first model had a random intercept (formula: ~1 | Tank). The random intercept model allows the line's starting point to vary across tanks, but the slope remains the same across all tanks, meaning expected increases in MeanLice would have the same effect across all tanks. The second model had a random intercept and slope (formula: ~MeanLice | Tank). Besides expecting the intercept to vary due to the random effects of the tanks, it is also anticipated that the effect of MeanLice on the response variable differs by the tank. Therefore, estimating both a random intercept and slope necessitates more data. However, the random intercept and slope model encountered convergence issues due to the lack of data points.

Bayesian-based mixed linear models might exhibit greater robustness when handling smaller data sets and those with fewer levels. Utilizing an alternative method to maximum likelihood is advantageous when assessing the Maximum likelihood Mixed linear model results. In this study, two Bayesian-based mixed linear models were fitted to the data utilizing the R package "*BMRS*" (Bürkner, 2021, 2018, 2017). Priors were established using the "get_prior" command on the original data, with the family set to Gaussian. Both fitted models included Tank as a random effect. The first model comprised a random intercept solely (log(ACE) ~ MeanLice + (1 | Tank)), whereas the second model included both a random intercept and slope (log(ACE) ~ MeanLice + (MeanLice |Tank)). The random intercept and slope model displayed effective sample size (ESS) values below the recommended threshold of 1000 (Bürkner, 2017), therefore it was discarded. However, both models were within the Rhat values threshold recommended by (Vehtari et al., 2021).

2.6.2 Salmon louse effect on beta diversity

An essential part of microbial community dynamics is how it changes with either time, location, or an experimental gradient. The microbial community change is commonly described through beta diversity measures as turnover in species or variation in abundance. To investigate the alterations in abundance variations of ASVs in the lice-salmon-mucus system. The beta diversity calculation was conducted per the methodology described in section 2.2.4, albeit with three modifications. Firstly, the explanatory variable was changed from comparing two extraction procedures (QBT and QPS-kits) to the gradient in mean lice intensity. Secondly, Adonis2, a form of Permutational Multivariate Analysis of Variance

(PERMANOVA) using distance matrices, was utilized instead of pairwise Adonis. The decision to use Adonis2 over pairwise adonis was motivated by the desire to analyze the data holistically rather than in pairs. Finally, the "strata" option was employed to adjust for tanks' influence on the data, as recommended by (Oksanen et al., 2022). The formula used was: bray Curtis distances ~ MeanLice, strata = Tank. The beta diversity output was subsequently presented in an NMDS plot.

In the lice-holobiont system, lice's presence likely impacts the Atlantic salmon's mucus microbiome (Llewellyn et al., 2017). It is also conceivable that the microbiome may exert an influence on the parasites (Peachey et al., 2017; Scheifler et al., 2022; Zaiss and Harris, 2016). To investigate possible effects of the microbiome on lice, the envfit function included in the R package "*vegan*" (Oksanen et al., 2022) was utilized. This analytical technique is particularly beneficial when there is a supposition that interactions can proceed in both directions. An NMDS plot subsequently visualized the results of this investigation.

2.6.3 Taxonomic structure – visualization and statistical analysis

So far, alpha diversity has been investigated using linear models, while beta diversity has been described using Bray distance matrices and analyzed with PERMANOVA models, envfit function, and NMDS plots. The remaining step is to examine the actual taxonomic structure of the bacterial community to provide a comprehensive description of the impact of lice intensity on the Atlantic salmon surface microbiome. To investigate the taxonomic structure, the taxonomic composition of the different fish samples was visualized using bar plots at the phylum and genus levels, utilizing the R package "*ggplot2*" (Wickham, 2016). In addition, the top fifteen most abundant ASVs per sample were selected for visualization at the phylum and genus levels.

An essential concept that several studies on the microbiome of fish mucus have investigated is whether an external driver leads to community changes in the taxonomic structure, specifically whether an external driver leads to an imbalance between opportunistic and commensal taxa (Boutin et al., 2014, 2013b, 2013a, 2012; Brown et al., 2019; Llewellyn et al., 2017; Lowrey et al., 2015; Stevens et al., 2016). More specifically aimed at the system at

hand, analysis aimed to investigate whether the abundance of different ASVs was either over or under-expressed with increasing mean lice intensity. To investigate this, a Generalized Latent Linear Variable Model (GLLVM) was fitted to the data using the R package "*gllvm*" (Niku et al., 2019b). The model was fitted with random intercept due to the tank effect described by the package vignette (Niku, 2023). For a deeper description of the model itself, see (Niku et al., 2019a). A threshold only including ASVs with an abundance of more than 200 reads was set. This was partly done to minimize computation time and increase statistical robustness, and if the number of unique ASVs was too high, the coefplot function (Base R) did not work correctly. The model results were visualized using the coefplot function.

2.7 Graphical summary of workflow - the salmon louse and Atlantic salmon experiment.

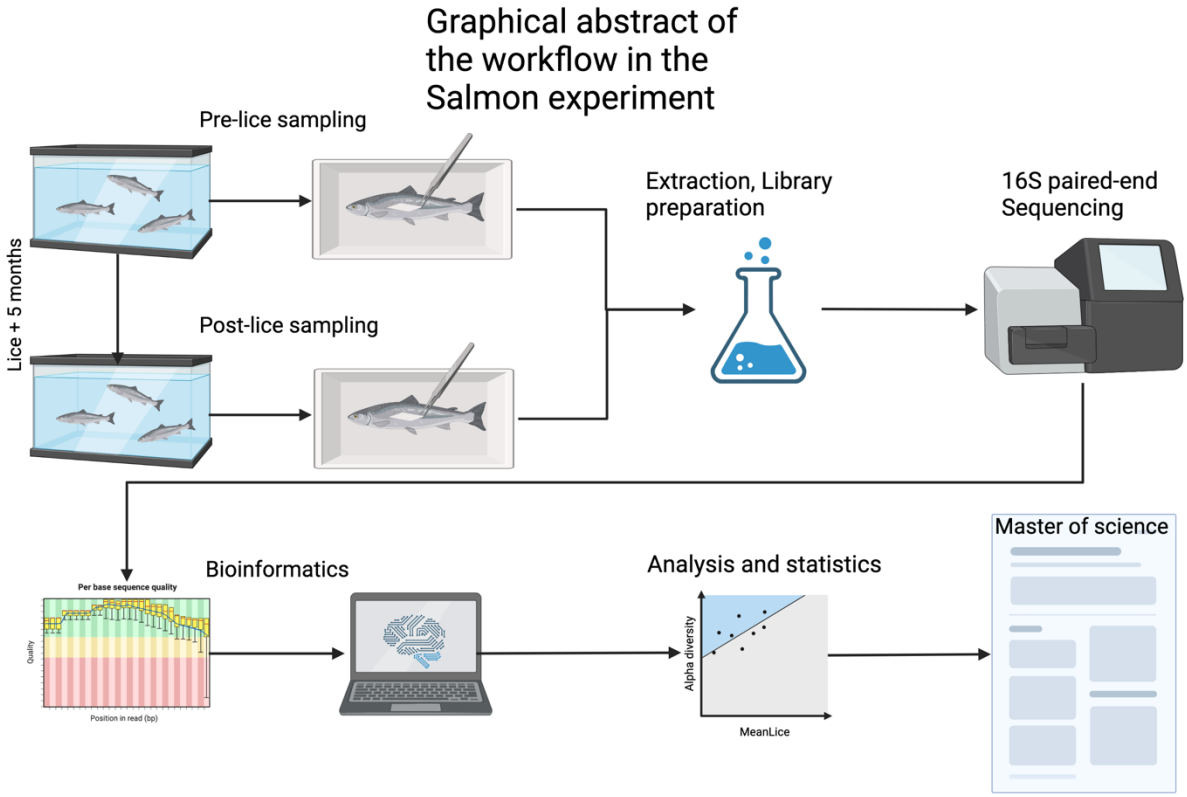

Figure 5: Graphical summary of the workflow used in the salmon louse and Atlantic salmon experiment.

3 Results

3.1 Results of method development on Brown trout *(Salmo trutta)*

The microbiome diversity of trout between the extraction methods was evaluated to determine which extraction method to use for processing the samples from the Atlantic salmon-lice experiment. Estimated alpha diversities of the microbial communities in the individuals did not exhibit any significant differences between extraction methods using a pairwise Student's t-test, with p-values for the comparisons ranging between 0.269 and 0.598 between the two tested extraction methods (Qiagen Blood and Tissue kit (QBT) and Qiagen Power Soil kit (QPS), n of 9 each). Based on the boxplots in Figure 6, there was a high degree of overlap in the estimated alpha diversity of the mucus microbiome among the different fish samples for the two extraction methods tested on trout. The substantial overlap between the boxplots for the different extraction methods highlights that the choice of extraction method had a minimal impact on the resulting alpha diversity estimates.

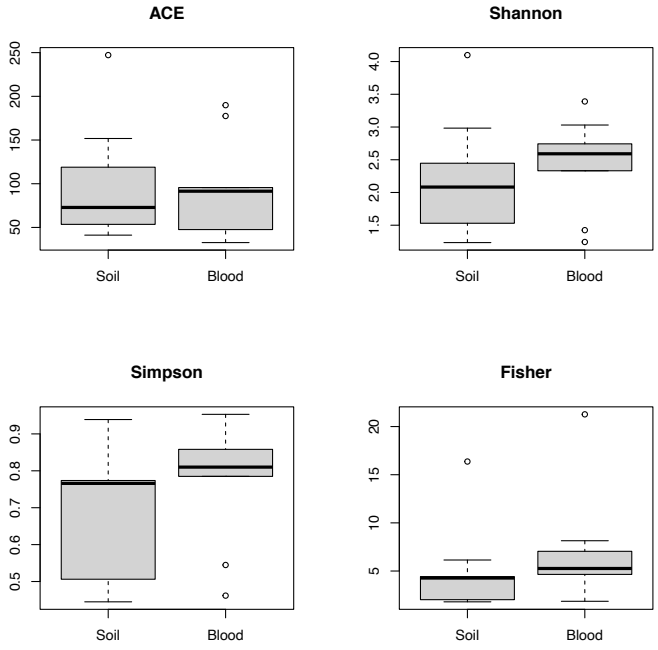

Figure 6: Boxplots of four different alpha diversity estimates compared between treatments. ***X-axis*** *is the treatment (soil = samples extracted with the QPS - kit, Blood = samples extracted with the QBT - kit).* ***Y-axis*** *alpha diversity estimates.* ***1)*** *Top left, ACE alpha diversity estimator. Higher values indicate a more diverse bacterial community.* ***2)*** *Top right, Shannon alpha diversity estimator. Higher values represent a more diverse bacterial community.* ***3)*** *Bottom left, Simpson alpha diversity estimator. A lower number indicates a more diverse bacterial community.* ***4)*** *Bottom right, fisher diversity estimator. A lower number means a more diverse bacterial community.*

The estimated beta diversity of the microbiomes from the brown trout was visualized in an NMDS plot (Figure 7). In the NMDS plot, 12 out of 18 samples (6 QBT and 6 QPS) clustered together, illustrating that the extraction method had little to no effect on beta diversity. This observation is supported by the pairwise Adonis analysis, which also indicated that the extraction method did not significantly impact beta diversity (p-value = 0.563).

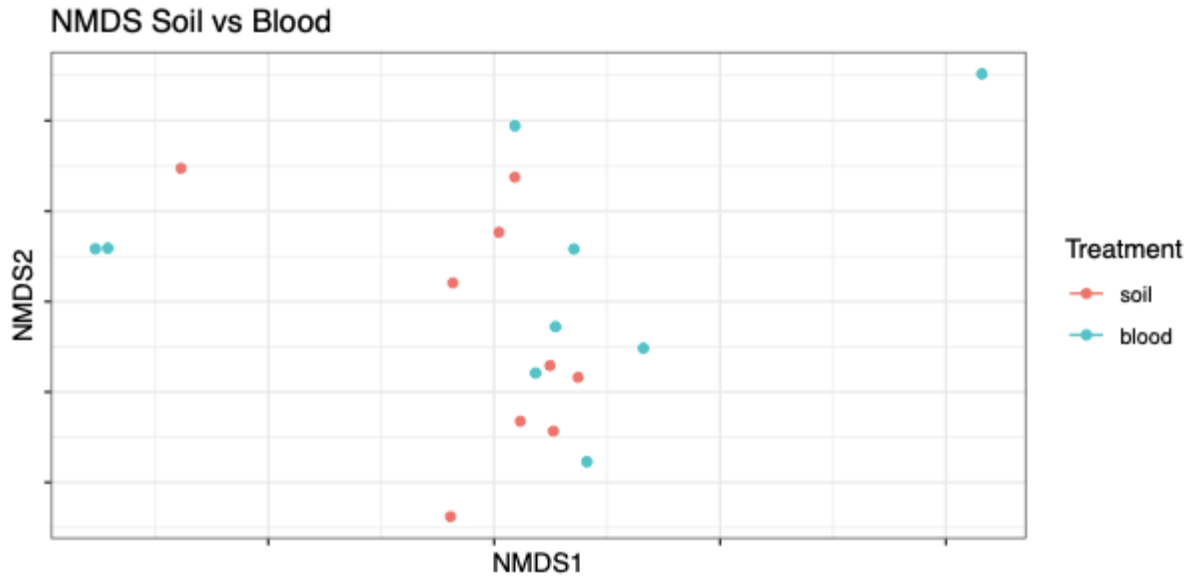

Figure 7: NMDS plot of beta diversity between individual samples. The red dots represent samples extracted with the QPS - kit. Cyan dots represent samples extracted with the QBT - kit. The more grouped the dots are, the more similar the bacterial communities are.

Based on the results shown above, i.e., no effect of extraction method, the Qiagen Power Soil kit was selected for the Atlantic salmon-lice experiment due to its user-friendly nature.

3.2 Results of the salmon louse and Atlantic salmon experiment

3.2.1 Experiment

The objective of the Salmon louse and Atlantic salmon experimental setup was to examine the long-term impact of increasing lice intensity on the dynamics of skin mucus microbial communities in Atlantic salmon. The experiment lasted for five months. In total, 60 fish were grown from a mean start weight of 194.8 g to a mean end weight of 678.5 g. Eight fish died after the first sampling but were immediately replaced. The fish were successfully infected with salmon lice larvae that grew on the fish during the entire experiment. Among the fish studied, the minimum number of mean lice (MeanLice) per fish was 0.5, while the maximum reached 13. On average, the MeanLice value for all fish was 5.9, with a median value of 6.0. A summary table of the ranges, means, and medians for the metadata is in Table 5, supplementary section 2. The result of the metadata analysis is presented in supplementary section 2. However, the take-home message is that tank influenced MeanLice, but not the clustering of individual fish (PCA plot shown in supplementary, section 2, Figure 2). Overall, the experimental setup was regarded as a success.

3.2.2 Results mock community comparison and sequence length optimization

Because of the problem with low sequence quality from second and final sequencing, there was a need to incorporate a sequence length optimization procedure using the commercial mock community as a benchmark to enhance the credibility of the data while minimizing unnecessary data loss. The outcome of this sequence length optimization procedure is illustrated in Figure 8. The plot demonstrates that a sequence length of 200 basepairs (bp) for the forward (fwd) read (Y-axis) and 170 bp for the reverse (rev) read (X-axis) resulted in the lowest dissimilarity between the sequenced mock and the actual mock (Bray distance = 0.46). When using a sequence length threshold of 200 bp for fwd reads and 170 bp for rev reads, a substantial proportion of non-chimeric reads (as shown in Supplementary Figure 4), and a minimal number of artificial ASVs (as shown in Supplementary Figure 5) were obtained. Based on the presented results, a read length of 200 bp fwd and 170 bp reverse was chosen as the sequence length thresholds for the rest of this study.

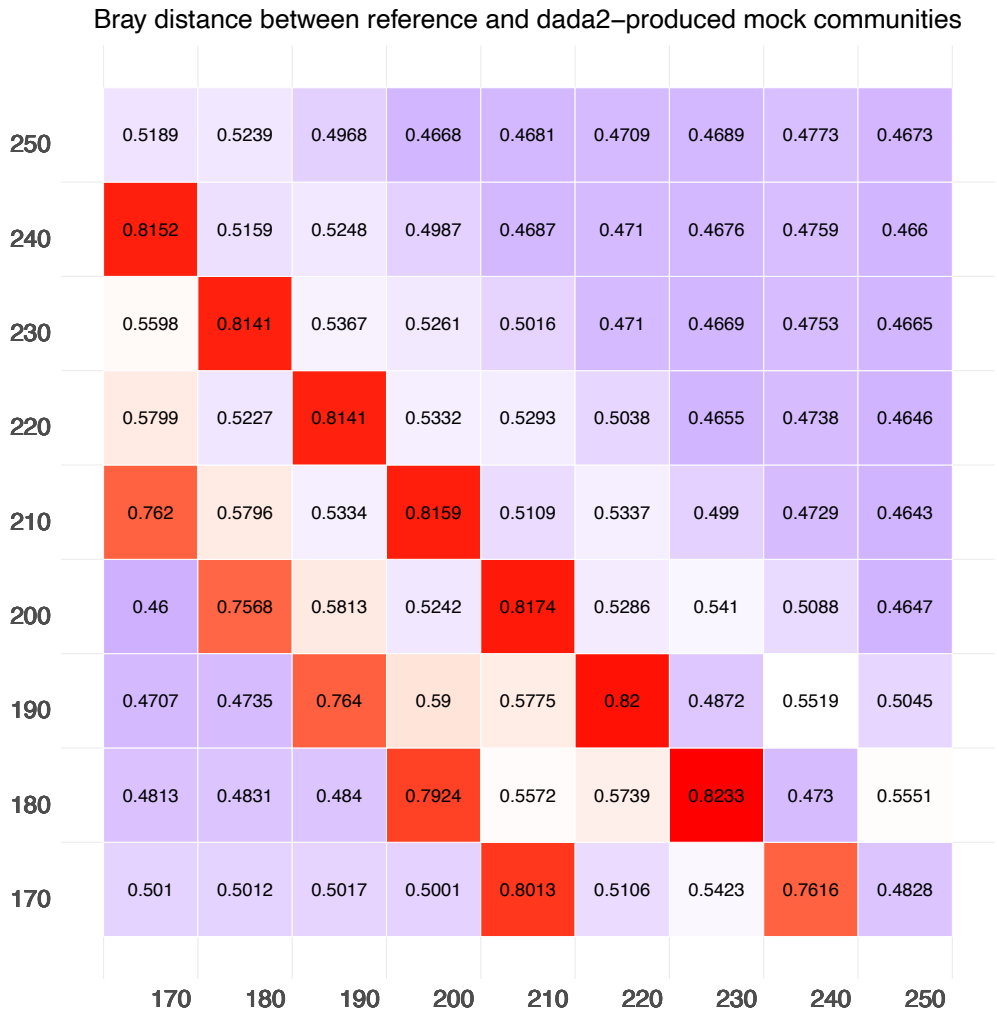

*Figure 8: Heatmap illustrating bray distance between reference mock and the mock yielded from the dada2 pipeline. The **X-axis** is the reverse read length in base pairs, and **Y-axis** is the forward read length. Lower numbers and colder colors indicate a higher degree of similarity.*

3.2.3 Mixed effects linear models results - effect of lice on alpha diversity

In order to investigate the long-term effects of salmon louse intensity on the dynamics of skin mucus microbial communities in Atlantic salmon, the alpha diversity of the infected salmon's mucus microbiomes was assessed. The resulting estimates of alpha diversity richness, using the Abundance-based Coverage Estimator (ACE), exhibited variation across the samples. The lowest richness estimate of 30.41 was observed in sample 2f3, while the highest of 265.95 was observed in sample 2f56. The mean richness estimate was calculated to be 93.74, with a median value of 58.91. Correspondingly, the log-transformed ACE richness estimates were determined as 3.41, 5.58, 4.31, and 4.076 for the respective samples.

To investigate the effect of increasing mean lice intensity (MeanLice) on the estimated alpha diversity richness (log(ACE)) of the mucus microbiomes in Atlantic salmon and control for the expected tank effect in this experimental setup, one maximum likelihood mixed linear model and one Bayesian mixed linear model were applied. Both models included random intercept only.

The results of the maximum likelihood mixed linear model (log(ACE) ~ MeanLice + (1 | Tank)) explained 27 % of the variance (conditional R2 = 0.27), while the fixed effects alone only explained 0.0715 %. The intercept that corresponds to MeanLice = 0 was estimated to be 4.24 (95% CI [3.66, 4.81], p < .001). The effect of MeanLice is statistically non-significant (beta = -6.13e-03, 95% CI [-0.11,0.10], p = 0.908; Std. beta = -0.02, 95% CI [-0.21, 0.17])

The Bayesian mixed linear model (log(ACE) ~ MeanLice + (1 | Tank))) explained 32 % of the variance (R2 = 0.32, 95% CI [0.11, 0.51], adj. R2 = 0.24), and the fixed effects alone explained only 2 % of the variance (95% CI [1.05e-10, 0.13]). The intercept is estimated to be 4.26 (95% CI [3.64, 5.00], has a 100 % probability of being higher than zero, 99,1% of being significant (> 3.50), and 0 % of being large (< 21.00). The indices are reliable with an ESS of 1442 and converged with a Rhat of 1.006. The effect of MeanLice has an estimated median of -0.02 (95% CI [-0.12, 0.09]), a 61.27 % chance of being negative, and is non-significant. The estimates are reliable, with a Rhat of 1,006 and an ESS of 1716. In Figure 9, the model estimates are plotted, post-posterior checks are shown, and the estimated effect size is plotted.

The model outputs above and the resulting Figure 9 (maximum likelihood mixed linear model results is shown in supplementary section 4, Figure 6) shows that an increase in mean lice intensity has no significant linear effect on alpha diversity in the mucus microbiome of the Atlantic salmon in this experiment.

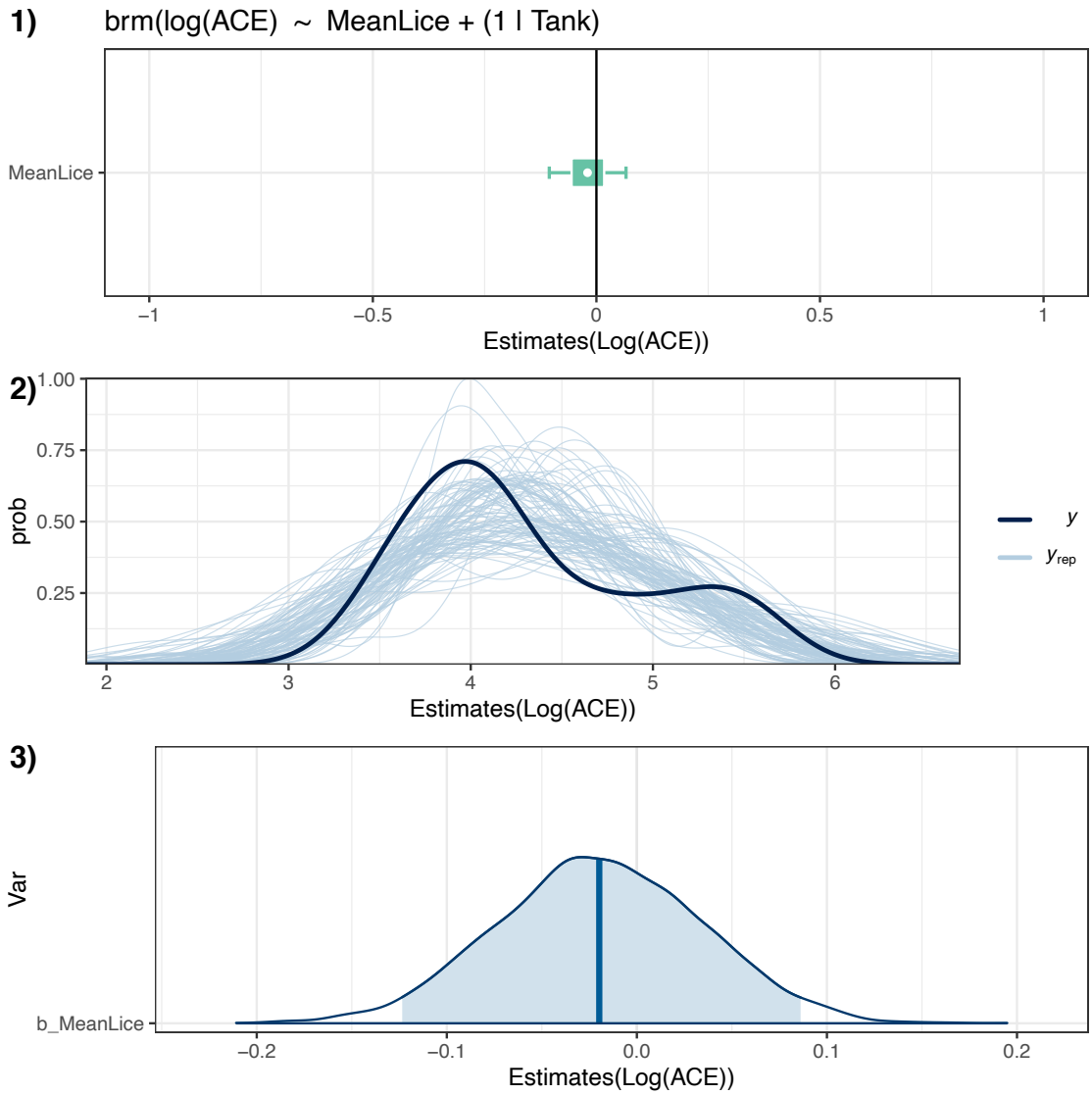

Figure 9: Plots showing the results from the linear Bayesian mixed effect model with random intercept (brm(log(ACE) ~ MeanLice + (1 | Tank)). 1) Point estimate of the effect of MeanLice (slope) on log(ACE). The confidence interval of the point estimate and confidence interval of the slope crosses zero. It, therefore, has a high chance of containing zero, meaning that MeanLice has no significant effect on Log(ACE). 2) A plot showing the results from the post-posterior check. The thick blue line is the actual distribution of the data, while the light blue lines are the predictions for the data distribution made by the model. It's a high degree of overlap, meaning the model fits the data well. 3) The last plot indicates the probability density of the point estimate for the effect of MeanLice on log(ACE). Again, the effect has a high probability of being zero.

3.2.4 Effect of lice on beta diversity

To comply with the primary objective of describing skin mucus microbial community dynamics in Atlantic salmon under various lice intensity regimes, beta diversity was calculated using Bray-Curtis distance matrices. This distance matrix was used in the Adonis2 models (a type of Permutational Multivariate Analysis of Variance (PERMANOVA) using distance matrices) to check for covariations between ASV abundances and increasing mean lice intensity. In addition, to keep in line with the parasite-holobiont perspective, an envfit function was used to investigate the effect of the mucus microbiome on lice intensity.

The results of the Adonis2 presented in Table 1 indicate that beta diversity, as described by variation in ASV abundances, correlated significantly (p-value = 0.001 non-strata model, p-value = 0.024 strata model) with increasing lice intensity. The effect of lice was not substantial, as the variation in lice intensity explained only 12% of the abundance variation in ASVs. Similarly, the results (Table 1) of the envfit function on the Bray Curtis derived NMDS determined that variation in lice loads was explained by 14% by the variation in the microbiome community on each Atlantic salmon. The effect of the microbial community on mean lice intensity was insignificant (p-value = 0.066). The tanks explained 36% of the variation in fish microbiome community structure according to the non-strata Adonis2 model (Table 1: p = 0.001). In the envfit model, the tank explained 70% of the variation in mean lice intensity. The results from the NMDS plot in Figure 10 showed that the microbiomes in the fish clustered clearly by tank, with tanks 8 and 9 exhibiting a high degree of clustering.

The abovementioned results illustrate that increasing mean lice intensity has a weak but significant effect on the beta diversity of the mucus microbiomes in this experiment. The mucus microbiome may affect lice load, but since it is non-significant, it’s unclear. Tank has a substantial and highly significant effect on beta diversity in the fish mucus microbiomes.

Table 1: Summary of results for the two PERMANOVA models and the Envfit function: ***Model:*** *The model setup being used,* ***R2(MeanLice)****: The amount of variation in the data described by the MeanLice variable,* ***P-value (MeanLice)****: The p-value of the tested effect of MeanLice on beta diversity,* ***R2 (Tank)****: The amount of variation in the data described by the Tank variable,* ***P-value (Tank)****: The p-value of the tested effect of Tank on beta diversity,* ***NA:*** *values not returned form a model,* ******* *Not significant value.*

Model	R2(MeanLice)	P-value (MeanLice)	R2 (Tank)	P-value (Tank)
(dist.bray ~ MeanLice + Tank)	0.119	0.001	0.355	0.001
(dist.bray ~ MeanLice, strata = metadata$Tank)	0.119	0.024	NA	NA
envfit	0.142	0.066*	0.695	0.001

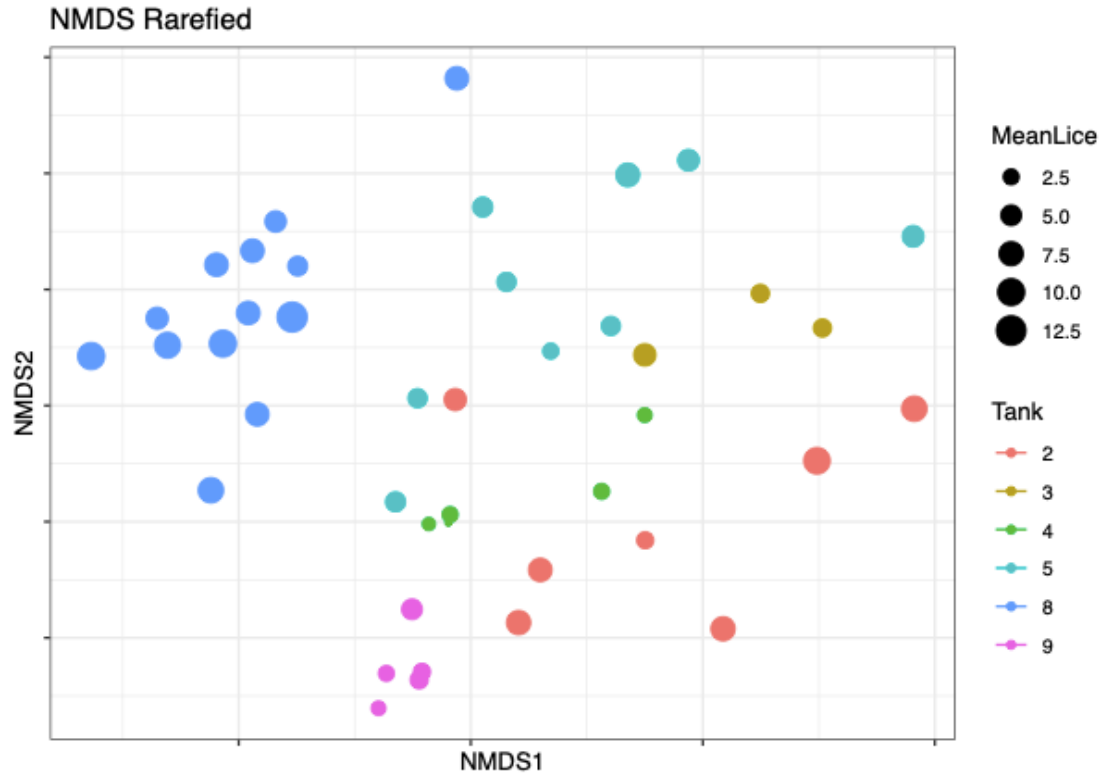

Figure 10: NMDS plot of Bray-Curtis dissimilarity matrix of the variation of ASV abundance in each fish. The size of the dots indicates the mean lice intensity of each fish. The color indicates the different tanks. The closer dots are to each other, the more similar the bacterial community. The farther apart the dots are, the more dissimilar the bacterial community.

3.2.5 Taxonomic composition visualization with bar plots

A critical part of the microbiome dynamics of Atlantic salmon under a parasitic influence is the taxonomic composition of bacteria in the mucus matrix. The taxonomic composition of the bacteria was visualized in bar plots. The result of the phylum bar plot (Figure 11) shows that the bacterial community was predominantly composed of *Proteobacteria* at the phylum level, accounting for 25-100% of reads across all samples. *Bacteroidota* was the second most abundant phylum (0-50%), followed by *Verrucomicobiota* (0-25%) and *Actinobacteriota* (0-15%). The dominant phyla are in line with previous studies on skin mucus microbiomes in fish (Baumgärtner et al., 2022; Chiarello et al., 2018, 2015; Lokesh and Kiron, 2016; Minniti et al., 2017; Reid et al., 2017; Uren Webster et al., 2020, 2018; Wilson et al., 2008)

The results from genus level barplot (Figure 12) show that *Oleispira* sp was found to dominate the samples, which were observed in previous surface mucus microbiome studies on post-smolt Atlantic salmon (Lokesh and Kiron, 2016; Reid et al., 2017). Furthermore, high abundances of *Tenacibaculum* sp and *Acinetobacter* sp were occasionally observed in this study, which is consistent with findings from other surface mucus microbiome studies. (Boutin et al., 2013b; Ghosh et al., 2022; Horsley, 1973; Llewellyn et al., 2017; Miyake et al., 2020; Reid et al., 2017; Tapia-Paniagua et al., 2018).

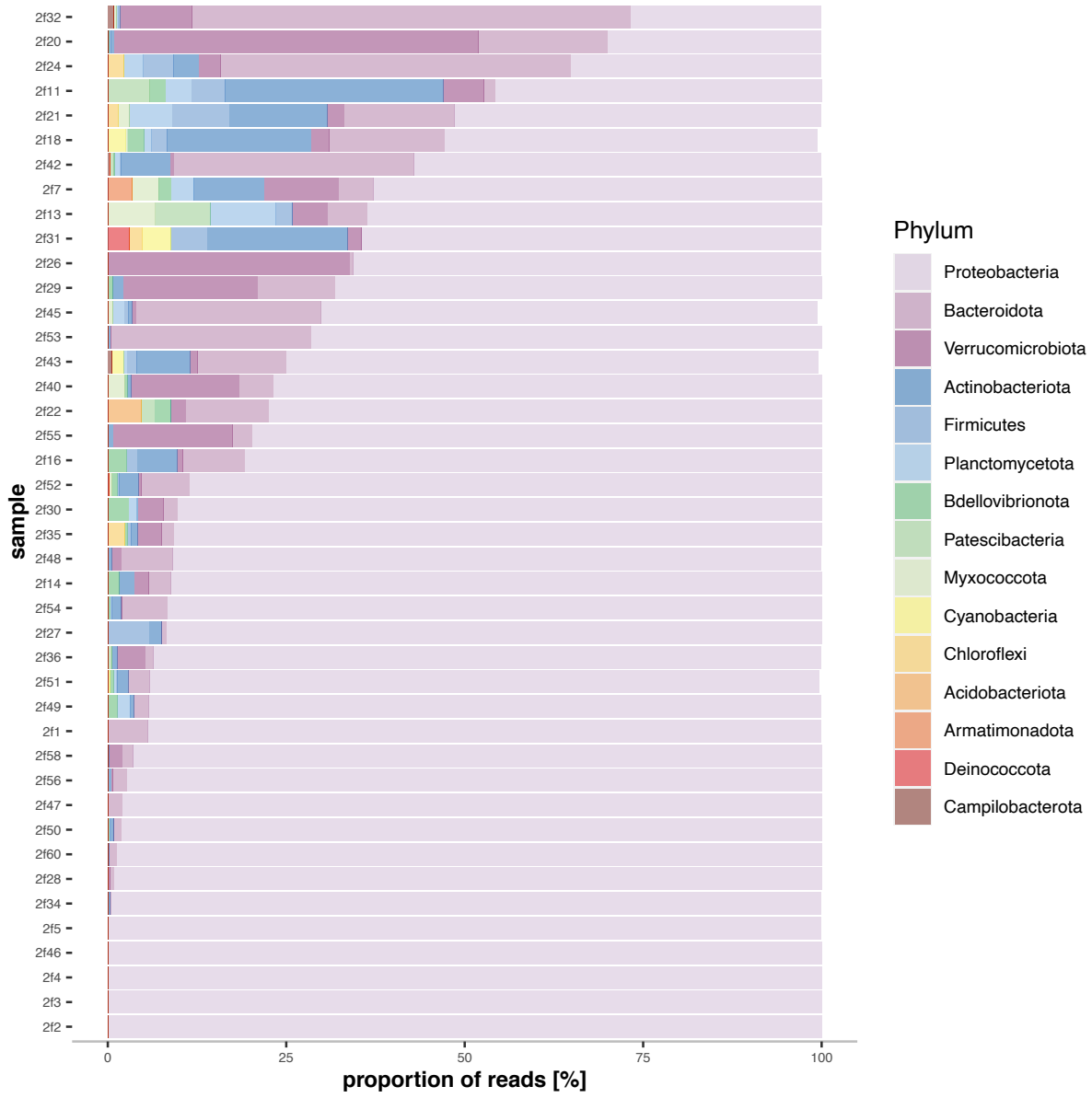

*Figure 11: A bar plot showing taxonomic composition on the phylum level. The **X-axis** describes the proportion of reads in each sample corresponding to a given phylum. **Y-axis** shows the different samples corresponding to the different fish. Different colors correspond to unique phyla, with the color code at the top of the figure. The missing percentages filled by the white areas symbolize ASVs that were not belonging to the 15 most abundant phylum shown here.*

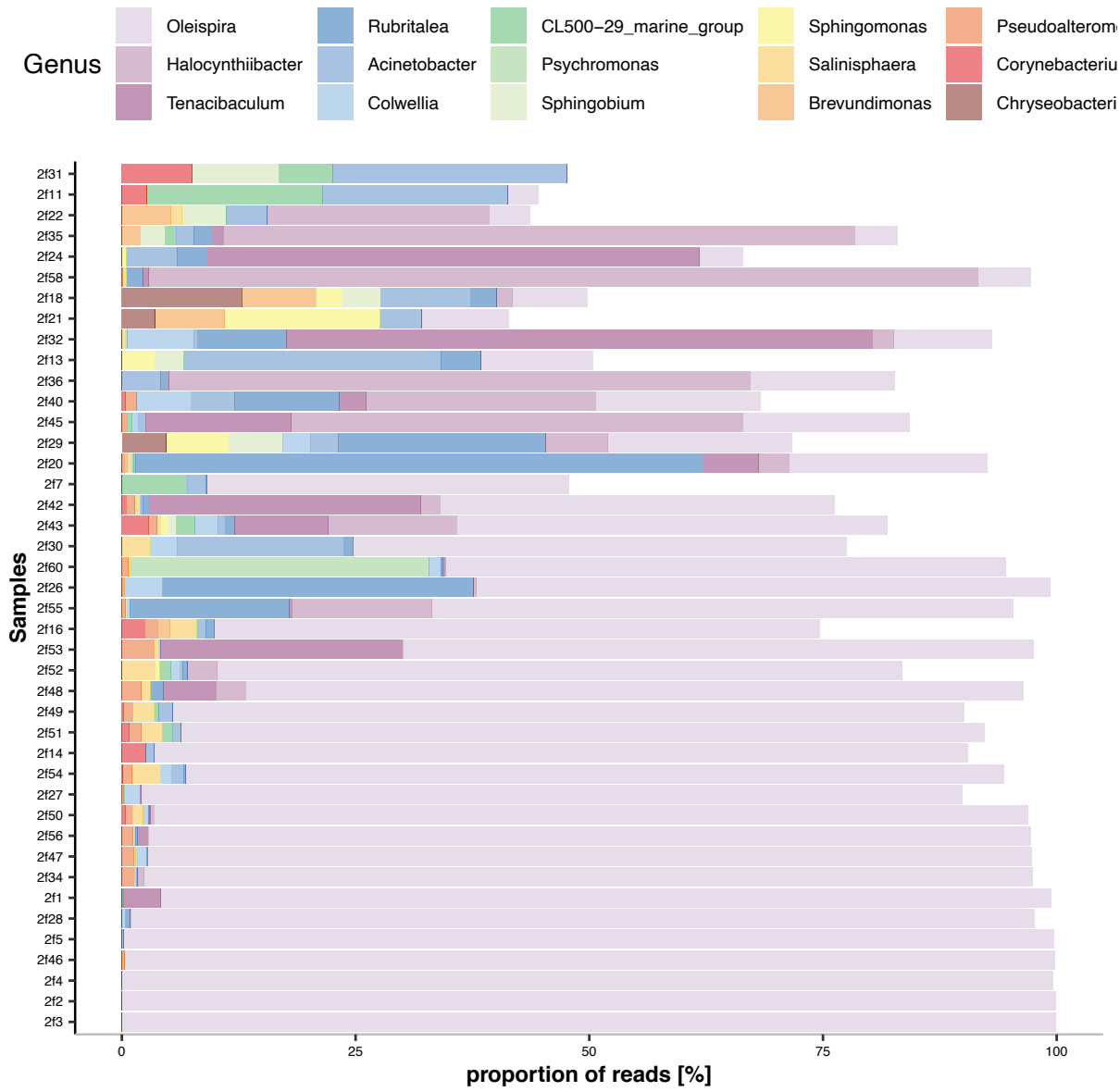

*Figure 12: A bar plot showing taxonomic composition on the genus level. The **x-axis** describes the proportion of reads in each sample corresponding to a given genus. **Y-axis** shows the different samples corresponding to the different fish. Different colors correspond to unique genera, with the color code at the top of the figure. The missing percentages filled by the white areas symbolize ASVs that were not belonging to the 15 most abundant genera shown here.*

3.2.6 Result of taxonomic composition with increasing lice intensity

The final aim of this project was to investigate bacterial taxonomic relationships to increasing lice loads. The result of the random intercept Generalized Latent Linear Variable Model (GLLVM) visualized in Figure 13 shows that four genera (*Corynebacterium* sp*, Acinetobacter* sp*, Tenacibaculum* sp*,* and *Oleispira* sp) generally increased with lice load. Oleispira sp was the only genus with ASVs showing both significant negative and positive relationships with lice load (MeanLice).

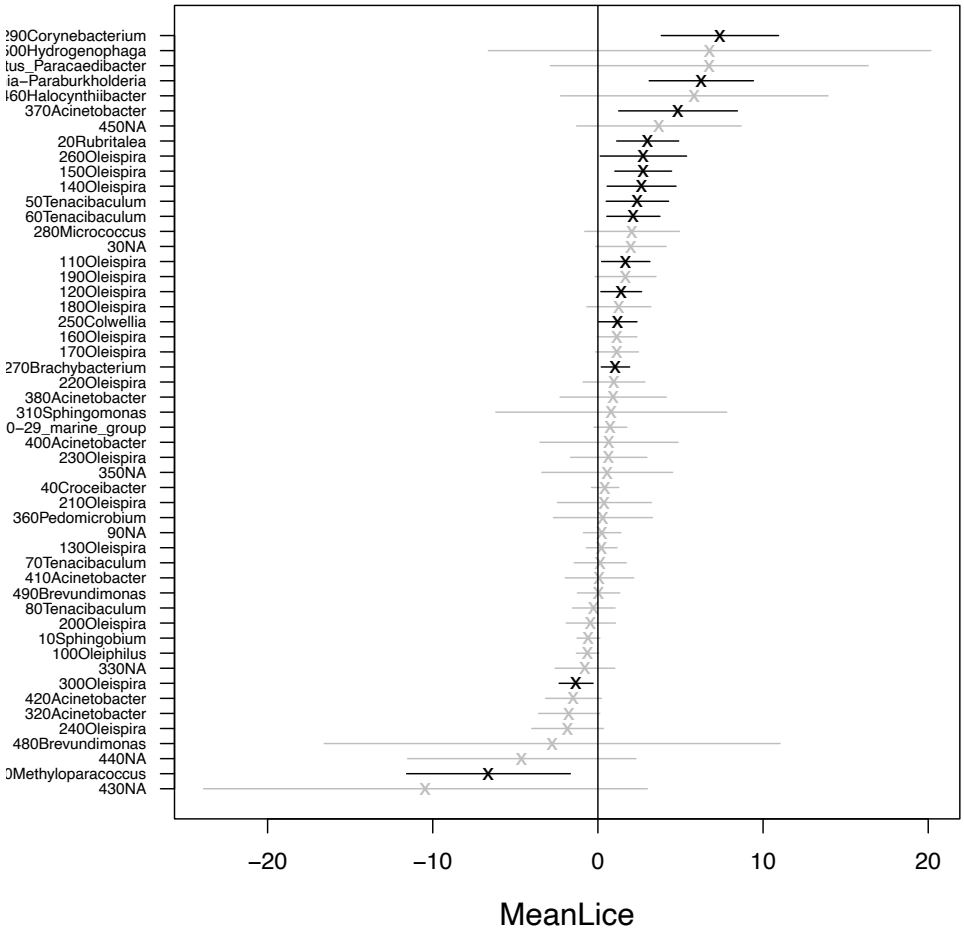

Figure 13: Plot of coefficients of the abundance of the 51 most abundant bacteria in the experiment as a general latent linear function of mean lice intensity. The X is the point estimate of the coefficients, while the line is the confidence interval. Black coloring means that the confidence interval doesn't contain zero and that the bacteria genus is either over or under-represented with increasing mean lice intensity. Coefficient estimates to the left side of the zero line are under-represented with increasing mean lice intensity, while the opposite is true for the right side. The abundance coefficients are ordered from the highest positive abundances at the top to the lowest at the bottom.

4 Discussion

4.1 The effect of salmon louse intensity on alpha diversity in Atlantic salmon

No significant linear correlation between salmon louse intensity and alpha diversity in the mucus microbiome was revealed by either of the mixed linear models with random intercepts (glmmTMB and BMRS). Therefore, it can be concluded that lice load does not seem to alter alpha diversity in this experimental setup. This finding contrasts with a similar investigation (Llewellyn et al., 2017) of salmon louse's effect on the surface microbiome of Atlantic salmon, which reported a significant correlation between lice presence and changes in alpha diversity (Shannon) and richness (Chao1) (Llewellyn et al., 2017). There are also numerous other investigations exploring the interplay between a pathogen/parasite and the mucus microbiome, which indicate a marked shift in alpha diversity and/or richness (Llewellyn et al., 2017; Miyake et al., 2020; Reid et al., 2017; She et al., 2017; X. Zhang et al., 2018)

The divergent results between these studies (Llewellyn et al., 2017) and mine may be due to several factors, including the sampling interval (Larsen et al., 2015; Minniti et al., 2017). In contrast to my study's sampling scheme, (Llewellyn et al., 2017) collected samples at four-time points over 35 days. Several studies indicate that the temporal factor plays a crucial role in the composition of mucus microbiomes in fish (Larsen et al., 2015; Legrand et al., 2018; Minniti et al., 2017). The work of (Legrand et al., 2018) revealed that the alpha diversity of infected fish differed significantly from that of healthy fish shortly after infection but gradually returned to a structure more comparable to that of healthy fish later in the disease's course. However, there appear to be no studies from the Atlantic salmon-salmon louse system that extends longer than 35 days. Therefore, the result of no change in alfa diversity with higher lice loads observed after five months in this study may indicate that the microbial community in fish mucus can stabilize after a certain amount of time. Furthermore, (Llewellyn et al., 2017) examine the impact of salmon louse compared to a control, that is, the effect of lice presence on community structure. A possible biological explanation for the absence of significant changes in alpha diversity in the present study is that salmon louse can secrete immunosuppressive substances (Lewis et al., 2014; Øvergård et al., 2022), which may inhibit long-term microbial community changes.

Additionally, a notable disparity between my study and that of (Llewellyn et al., 2017) concerns the mean number of lice per fish, which was 5.88 and 23, respectively. However, other studies examining Atlantic salmon under infection conditions have also reported non-significant alterations in alpha diversity that correlated with the pathogen dose (Reid et al., 2017). In the former study, *Oleispira sp*. was also found to dominate the community, akin to my findings. A certain threshold of lice intensity may be necessary to induce long-term changes in the fish mucus microbiome. I have yet to learn of any controlled studies examining threshold values regarding mean salmon louse intensity and long-term structural changes in the mucus microbiome of salmonids.

Other explanations for the non-significant effect of lice on mucus microbiome alpha diversity include biases introduced by the metabarcoding method. 16S rRNA gene studies are fraught with various biases, a need for more standardization, and varying sequence data quality, including issues with extraction, PCR, bioinformatic methods, and the selection of statistical tools (Birtel et al., 2015; D'Amore et al., 2016; Noecker et al., 2017; Shakya et al., 2013; Vasileiadis et al., 2012; J. Zhang et al., 2018). In addition, it is conceivable that the PCR and the quality of the sequences contributed to a result that differs from others, as detailed in the methods section above. Finally, publication bias, where positive results are more likely to be published (Easterbrook et al., 1991), presents challenges in comparing the prevalence of parasites that have no impact on alpha diversity in fish mucus research.

4.2 The effect of lice intensity on beta diversity in Atlantic salmon

Beta diversity is a concept that describes the changes in community structure between different biological communities (Anderson et al., 2011). This concept can be broadly categorized into two groups: turnover and variation. Turnover characterizes the number of species replaced over a given temporal or spatial gradient. At the same time, variation primarily focuses on the changes in community composition and species abundance over time, geographic scale, experimental gradient, external factors, or a combination thereof. In this study, the v3-v4 (Anderson et al., 2011) approach was employed to examine the variation in ASVs composition and abundance within the mucus microbiome over an experimental gradient ranging from mean lice of 0.5 to 13. This study found that lice intensity had a weak but significant impact on beta diversity ($p = 0.024$), explaining 12% of the variation in observed ASVs abundance (Table 1, Results).

In their study, (Llewellyn et al., 2017) found a significant effect of lice on beta diversity, which is consistent with the results of this study. However, the strength of the effects/significance of lice intensity on the variation in beta diversity of the mucus microbiome was more pronounced in (Llewellyn et al., 2017) compared to this study, which aligns with the trend in alpha diversity mentioned earlier. Other studies have reported similar findings, indicating that the presence of a pathogen/parasite can significantly impact beta diversity in the surface microbiome structure of fish (Miyake et al., 2020; Reid et al., 2017; She et al., 2017; X. Zhang et al., 2018). In the study conducted by (Legrand et al., 2018), it was observed that the change in community structure of the diseased fish became less distinct from the control fish over time. This finding suggests that the alteration in beta diversity exhibits a temporal component.

Various mechanisms can elucidate the observed alterations in beta diversity, many of which are explained directly or indirectly by immune responses. For instance, (Easy and Ross, 2009) reported a change in the protein composition of Atlantic salmon mucus due to infection by salmon louse, while (Fast et al., 2002) demonstrated through their experimentation with salmonids that there are significant variations in the quantity of lysozyme, alkaline phosphatases, and histological parameters in the mucus barrier of salmonids after lice infection. In addition, as previously mentioned, (Lewis et al., 2014; Øvergård et al., 2022)

indicated that salmon louse can secrete immune-suppressing substances. The changes in immune responses due to lice infection (Gallardo-Escárate et al., 2019) can, in turn, lead to changes for the bacteria living in the mucus. Research has shown that immune substances in fish mucus can aid in attaching bacteria to the mucus surface (Kelly et al., 2017) and that bacteria residing on the mucus surface are enveloped in immunoglobulins (Xu et al., 2013). The immune system's role in selecting for or against specific bacteria is emphasized by (Gomez et al., 2013) and (Kelly et al., 2017). Therefore, it may be presumed that the influence of salmon louse modifies the immune response in Atlantic salmon, which, in turn, alters the environment for bacteria residing in the Atlantic salmon mucus. This environmental impact, in turn, can influence the relationship between beneficial and opportunistic bacteria, resulting in beta diversity changes with lice intensity. Although (X. Zhang et al., 2018) shows that this interaction is possible, this was done in a somewhat different system than in my study.

Research on salmonids and other fish species has indicated, with varying degrees of success, that surface microbiomes can act as protectors against pathogens/parasites (Boutin et al., 2013b, 2013a, 2012; Brown et al., 2019; Lowrey et al., 2015; Stevens et al., 2016). Therefore, the envfit function was selected to examine whether Atlantic salmon's microbial community influenced lice infection intensity. The results of the envfit analysis revealed that the microbial community could explain 14% of the variation in lice intensity (Table 1, Results). This suggests that the composition of the fish mucus microbiome may have some influence on lice infection. However, it is important to interpret these findings cautiously as the test result is just outside the significance threshold of 0.05 (Table 1, Results). Thus, this study cannot definitively conclude that microbial composition directly affects lice intensity. Nonetheless, in line with a more holistic approach, this study highlights the importance of considering how the microbiome may impact pathogens/parasites and recommends further investigation of this in future studies.

4.3 Taxonomic composition, the balance between opportunistic and commensal ASVs

At the phylum level (Figure 11), the bacterial community in the Atlantic salmon skin mucus was dominated by *Proteobacteria*, comprising 25-100% of reads across all samples. *Bacteroidota* was the second most abundant phylum (0-50%), followed by *Verrucomicobiota* (0-25%) and *Actinobacteriota* (0-15%). Previous studies on Atlantic salmon and other fish have reported the prevalence of Proteobacteria as the dominant phylum, as well as the high abundance of *Bacteroidota* and *Actinobacteriota* (Baumgärtner et al., 2022; Chiarello et al., 2018, 2015; Lokesh and Kiron, 2016; Minniti et al., 2017; Reid et al., 2017; Uren Webster et al., 2020, 2018; Wilson et al., 2008).

At the genus level (Figure 12), *Oleispira* sp was the dominant taxon in 50% of the samples, comprising 70-100% of reads and approximately 10% in the remaining samples. Previous studies on post-smolt Atlantic salmon have also reported a high abundance of *Oleispira* sp *(Lokesh and Kiron, 2016; Reid et al., 2017)*. *Tenacibaculum* sp and *Acinetobacter* sp were found in several studies, with occasional high abundances, and are often regarded as an opportunistic group (Boutin et al., 2013b; Ghosh et al., 2022; Horsley, 1973; Llewellyn et al., 2017; Miyake et al., 2020; Reid et al., 2017; Tapia-Paniagua et al., 2018). The second-highest abundance was observed for *Halocynthiibacter* sp, which contrasts with previous literature on the surface microbiome of Atlantic salmon. The presence of this bacterium may be attributed to its occurrence in the diet or the water, as it is associated with hard bottoms and has been observed in sediments under a fish farm (Keeley et al., 2021; Xie et al., 2020). Alternatively, an error in taxonomic identification may have occurred due to the short sequences and poor quality, as highlighted in the results and methods section. In other studies, *Rubritalea* sp has been reported to be abundant in the gill and surface mucus of Atlantic salmon (Lorgen-Ritchie et al., 2022; Minniti et al., 2019; Slinger et al., 2021).

The results obtained from the General Linear Latent Variable Model (GLLVM) indicate that there are several bacteria with higher abundances at higher lice intensity (Figure 12).
Of particular interest are three genera (*Oleispira* sp*, Tenacibaculum* sp*,* and *Acinetobacter* sp) due to their higher quantity, as mentioned above. Several studies have described *Tenacibaculum* sp and *Acinetobacter* sp as opportunistic pathogens. In particular,

Tenacibaculum sp contains several suspected fish pathogens that are problematic for Atlantic salmon (Olsen et al., 2011; Rahman et al., 2015; Småge et al., 2016; Spilsberg et al., 2022; "Tenacibaculose," 2023). Furthermore, it is increasingly evident that *Tenacibaculum* sp increases in abundance when fish in marine environments experience stress or suffer from wounds across different species (Avendaño-Herrera et al., 2006b; Karlsen et al., 2017; Llewellyn et al., 2017; Olsen et al., 2011; Rahman et al., 2015; Tapia-Paniagua et al., 2018; Wynne et al., 2020). Despite the overrepresentation of *Tenacibaculum* sp in infections, wounds, and stress, *Tenacibaculum* sp has also been found to be a natural part of the Atlantic salmon's mucus microbiome (Wynne et al., 2020). Therefore, it cannot be ruled out that *Tenacibaculum* sp naturally belongs to the Atlantic salmon and becomes more prevalent when the function of the mucus microbiome is under pressure, a condition known as dysbiosis. This can be explained by growth experiments showing that *Tenacibaculum maritimum*, for example, struggles to survive when other bacteria are present in the growth medium (Avendaño-Herrera et al., 2006a).

The *Oleispira* sp abundance exhibited non-significant changes with salmon louse intensity, significant increases, and significant decreases. The general trend in this experiment is not a general decrease in *Oleispira* sp abundance. This contrasts with similar studies (Reid et al., 2017). If *Oleispira* sp is a genus associated with a functioning post-smolt mucus microbiome, it may indicate that the microbial community stabilizes after a disturbance, as indicated by (Cipriano and Dove, 2011; Legrand et al., 2018).

Corynebacterium sp exhibited the highest observed increase in abundance with higher lice intensity, mainly driven by samples 2f31 and 2f11 (Figure 12). Previous studies have reported a connection between *Corynebacterium* sp and Atlantic salmon, including salmon louse (Bergh, 2019; Cipriano and Dove, 2011). However, recent evidence suggests that *Corynebacterium* sp is primarily associated with the gut microbiome of Atlantic salmon (Dehler et al., 2017; Li et al., 2021; Rudi et al., 2018).

Burkholderia-Caballeronia-Paraburkholderia sp. exhibited the subsequent highest increase in lice intensity. The author is unaware of any previous reports of an increase in this genus in Atlantic salmon under stress. A study on loach *(Paramisgurnus dabryanus)* reported this genus in fish, but this remains a rare finding (Wang et al., 2023).

Additionally, *Methyloparacoccus* sp. demonstrated a substantial reduction with increasing salmon louse intensity, but this species has been found in the gut microbiome of farmed tilapia and not Atlantic salmon (Bereded et al., 2021).

The detection of genera not previously identified in studies on Atlantic salmon or originating from the Atlantic salmon gut microbiome may result from errors in taxonomic assignment with the SILVA database. Or these errors may stem from short sequences with occasional low quality and/or contamination from the fish gut microbiome in some samples. Studies have demonstrated that fish's gut and skin microbiomes differ (Hamilton et al., 2023; Lowrey et al., 2015; Sylvain et al., 2020; Xavier et al., 2019). Nonetheless, one of the limitations of this study is that the gut microbiome was not mapped, and therefore, it remains to be seen where the atypical genera originate from.

4.4 Ecological importance – Mucus bacteria matter

It is widely accepted that the surface system of fish, including the skin and surface mucus, serves as the primary and most crucial line of defense against external stressors (Esteban, 2012; Hawkes, 1974; Shephard, 1994). In addition, the mucus layer of fish is not only a protective barrier but also a habitat and source of nutrients for a diverse community of microorganisms (Benhamed et al., 2014; Gomez et al., 2013; Gomez and Primm, 2021; Kelly et al., 2017; Kelly and Salinas, 2017; Legrand et al., 2020; Minniti et al., 2019).

Numerous studies have reported changes in the composition and diversity of the microbiome on fish mucus under varying conditions, including pH (Sylvain et al., 2016), salinity (Hamilton et al., 2023, 2019; Lokesh and Kiron, 2016; Schmidt et al., 2015), season (Larsen et al., 2015; Wilson et al., 2008), location (Uren Webster et al., 2018; Xavier et al., 2019), wild versus aquaculture fish (Tarnecki et al., 2019; Uren Webster et al., 2018), treatment (Carlson et al., 2015; Minniti et al., 2019; Mohammed and Arias, 2015), stress (Boutin et al., 2013b), prebiotics (Baumgärtner et al., 2022; Boutin et al., 2013a, 2012), temperature (Ghosh et al., 2022), and pathogens/parasites (Legrand et al., 2018; Llewellyn et al., 2017; Miyake et al., 2020; Reid et al., 2017; She et al., 2017; X. Zhang et al., 2018).

Furthermore, the microbiome on the fish surface mucus appears to be partly host-specific, as it differs from the surrounding water (Boutin et al., 2013b; Carlson et al., 2015; Chiarello et

al., 2019, 2018; Larsen et al., 2015; Llewellyn et al., 2017; Minniti et al., 2017; Schmidt et al., 2015; Sylvain et al., 2020, 2016) and varies depending on the host species and location (Chiarello et al., 2018; Hamilton et al., 2023; Larsen et al., 2015). Some studies have explored potential causal links between the fish microbiome and factors such as lifestyle (Chiarello et al., 2018; Xavier et al., 2019), immune system (Gomez et al., 2013; Kelly and Salinas, 2017; X. Zhang et al., 2018), and genetics (Boutin et al., 2014).

The author of this study has the impression that the mucus layer coating the microbiome of fish represents an extension of the fish itself, thereby justifying the use of a holobiont perspective. Specifically, it is recommended that the interactions between the fish, its microbiome, and an external driver be considered as a holobiont, as opposed to an external factor system, as proposed by (Limborg et al., 2018). This approach is particularly relevant to classic parasite-host interactions, as advocated by (Dheilly, 2014; Dheilly et al., 2017, 2015), and is supported by several studies, including those by (Brosschot and Reynolds, 2018; Glendinning et al., 2014; Hahn et al., 2022; Peachey et al., 2017; Scheifler et al., 2022; Zaiss and Harris, 2016)

Concerning salmon louse, it is established that they impact the host's immune system (Gallardo-Escárate et al., 2019; Lewis et al., 2014; Øvergård et al., 2022), and there is a causal link between the fish's immune system, and it's surface microbiome (Gomez et al., 2013; Kelly et al., 2017; Kelly and Salinas, 2017; Xu et al., 2013; X. Zhang et al., 2018). It is conceivable that the fish's microbiome may likewise influence the success of salmon louse. For instance, two studies have indicated that kairomones are produced not by the fish but as a byproduct of the microbiome´s activity (Beklioglu et al., 2006; Ringelberg and Van Gool, 1998). (Mordue (Luntz) and Birkett, 2009) describe how salmon louse locates their hosts and highlight the critical role of kairomones in this process. Whether there is a relationship between kairomones originating from the fish's microbiome and the identification of salmon louse hosts is conjectured.

In addition, research has demonstrated that antimicrobial agents in fish mucus are involved in developing frontal filaments in another species of lice (Núñez-Acuña et al., 2016). Multiple studies indicate that bacteria in the surface microbiome of fish can generate antimicrobial compounds (Benhamed et al., 2014; Lowrey et al., 2015; Stevens et al., 2016; Tarnecki et al., 2019). It is an area that warrants further investigation from a more comprehensive perspective

of the parasite (holobiont 1) and host (holobiont 2) interaction as to whether there exists a correlation between the antimicrobial compounds produced by bacteria in the surface mucus and salmon louse.

The significance of considering the parasite as a holobiont in addition to the host has been argued by several researchers (Dheilly, 2014; Dheilly et al., 2017, 2015). A study on salmon louse by (Gonçalves et al., 2020) has demonstrated that the microbiome of salmon louse can act as a reservoir for fish pathogens. This implies that the harm caused by lice infection may not solely stem from the parasite but also a range of potentially pathogenic bacteria inherent to its microbiome. This becomes particularly intriguing given that, as discussed earlier, various disruptions can alter the microbiome from a potentially stable state to a potentially unstable one, and salmon louse can simultaneously modulate the immune system of the host fish. This, in turn, can create an imbalance between commensal and opportunistic bacteria, as elaborated in multiple publications (Boutin et al., 2014, 2013b, 2013a, 2012; Brown et al., 2019; Llewellyn et al., 2017; Lowrey et al., 2015; Stevens et al., 2016).

A comprehensive exploration of the ecological principles underlying the formation of microbial communities reveals their intricacies. To further this discussion, I refer to the following seminal works (Bell et al., 2005; Brooks et al., 2016; Burke et al., 2011; De Roy et al., 2013; Jousset et al., 2017; Sale, 1978; Vellend, 2010; Wittebolle et al., 2009). To delve deeper into the application of these principles to fish mucus microbiomes, I suggest examining the following articles (Boutin et al., 2014; Chiarello et al., 2019, 2018; Ghosh et al., 2022; Leonard et al., 2014).

4.5 General improvements

There exist various opportunities for improvement within the context of this study.

The primary weakness of this study is the experimental design. The author believes that having an equal number of fish in each tank would have improved the study's validity. In this way, the impact of lice would have been the sole controlling factor, thereby minimizing extraneous variables. Using different lice intensity regimes in each tank is appropriate for a gradient study such as this one; therefore not considered a limitation. Nonetheless, this study's dependence on a more extensive study with logistical, economic, and animal welfare constraints made it impossible to maintain equal fish densities. Another limitation of this study is the temporal distance between sampling time points. Although a 5-month interval between the first and last samplings was employed, more frequent sampling, including water samples, would have enabled a more comprehensive examination of the microbial system's temporal dynamics resulting from salmon louse. The previous study by (Llewellyn et al., 2017) supports including multiple sampling time points in this system but acknowledges a lack of data for periods exceeding 35 days. While more sampling time points could have been beneficial, it may have also introduced confounding effects, as handling the fish can impact the microbiome (Minniti et al., 2017) and mask the effects of salmon louse. Nonetheless, the author believes that more frequent sampling would have been beneficial. However, given the considerable number of samples already generated, which required significant processing, further sampling was not deemed feasible. Control fish were also lacking in this study, and this is a big limiting factor to assess the effect of presence of lice itself on the skin mucus microbiome of Atlantic Salmon.

Another limitation of the experimental design is the failure to map the microbial community of the salmon louse, which reduces the strength of the argument for the inclusion of a holobiont perspective in parasite-host systems. Moreover, the study should have included the fish's health parameters, precluding an assessment of whether different microbial communities impacted various health parameters in the fish relative to lice intensity. In addition, the gut microbiome of the fish was not mapped, thereby limiting the ability to explain the presence of different bacteria known to be gut-associated that had occasional high abundances. Again, these limitations were partially attributable to logistical and capacity

constraints. Lastly, the study did not investigate whether different lice doses led to varying community structure thresholds. It is plausible that the average of 5.88 lice per fish was too small to elicit a persistent response.

In addition to the weaknesses dicussed above, the present study suffered from technical challenges encountered in the laboratory. Specifically, the amplification of samples in the 2-step PCR reaction presented a significant obstacle. The PCR amplification difficulties resulted in a high number of PCR cycles being required (35 cycles for the first step and 15 cycles for the second step). It is widely recognized that PCR reactions may introduce various biases in the community structure. However, the present study encountered an additional problem in the form of lengthy (100-200 bp) primer-dimer complexes, which necessitated further sample treatments, including gel purification. This step resulted in a loss of genetic material and required a subsequent stage of vacuum drying and centrifugation prior to sequencing. It is plausible that these steps could introduce biases and contribute to DNA fragmentation (e.g., UV light from gel purification, temperature from vacuum drying, and centrifugation). These technical difficulties could account for the unsuccessful initial sequencing attempt and the lower-than-expected quality of the fragments from the second sequencing. Therefore, the resulting data for analysis may be suboptimal, and as such, the conclusions of the following analysis should be interpreted with caution. Moreover, the technical challenges rendered the original plan for pairwise comparison between the start and end microbiomes unfeasible due to the loss of most of the samples from the first collection.

Several factors could account for the significant challenges encountered in PCR amplification. For example, the choice of extraction protocol, as described in the methods and results sections, could be a contributing factor. Additionally, inhibitory substances in the mucus matrix of the salmon used in this experiment may explain the difficulties encountered. Diluting the DNA extract with sterile PCR water is a standard solution to address this issue. Although this was considered, it was not performed due to the significant delays that the project had already experienced. Different PCR reactions with varying numbers of cycles and amounts of template DNA were tested, and negative and positive controls were included at each step, indicating no issues with the PCR reagents. The extraction protocol employed in this study is generally effective at handling inhibitory substances, albeit from soil samples. It is also possible that the microbiome of wild fish is more diverse than farmed fish, resulting in the possibility of amplifying more bacteria with greater ease. The literature supports this

claim, indicating significant differences between the microbiomes of wild and farmed fish (Tarnecki et al., 2019; Uren Webster et al., 2020, 2018).

The present analysis has several potential weaknesses that need to be addressed. Firstly, the sequences used in this study were at the lower end of the quality scale. As a result, quality values and sequence lengths had to be significantly reduced to obtain enough data. Although mock communities were used as a baseline for the decision on the length of the sequences used, it can be argued that the mock community used is a commercial product and may not necessarily represent the microbiome in this system studied. A self-made mock based on fish mucus bacteria could have been more appropriate.

Secondly, sequences belonging to water, negative controls, mock communities, and blanks were filtered out using the "*decontam*" R package to ensure that only the sequences that had a high probability of belonging to and being essential for the fish were analyzed. However, it should be noted that the filtering process may have excluded some genera that should have been included. It has been shown that the water and surface microbiome of fish differs from each other (Boutin et al., 2013b; Carlson et al., 2015; Chiarello et al., 2019, 2018; Larsen et al., 2015; Llewellyn et al., 2017; Minniti et al., 2017; Schmidt et al., 2015; Sylvain et al., 2020, 2016), but some genera are found in both water and on the surface of the fish. Hence, filtering out all water sequences may have resulted in the loss of some relevant ASVs. Analysis of the mucus microbiome has been conducted without excluding the water microbiome, and the results are shown in supplementary sections 9 - 11. The outcomes of this analysis were similar, offering the same general trends, including no linear correlation between the mucus microbiome´s alpha diversity and increasing lice load (Figures 16 and 17, supplementary) and a significant covariation between lice loads and mucus beta diversity (Figures 18, 19 and Table 6, supplementary). Lastly, the same genera of bacteria were overrepresented and underrepresented with increasing lice loads (Figure 20, supplementary). However, the explanatory power from lice load was weaker on beta diversity, and other not previously observed ASVs of bacteria were observed in the GLLVM model output (Figure 20, supplementary).

Thirdly, the analysis was conducted on rarefied data, which can be seen as a potential weakness. While rarefying data is a common practice in microbial ecology, it has advantages and disadvantages, as discussed in the literature (Cameron et al., 2021; Gloor et al., 2017;

McMurdie and Holmes, 2014; Willis, 2019). This study conducted all analyses with rarefied and non-rarefied data (shown in supplementary section 6 - 8), but the results did not differ significantly. However, there was more noise with non-rarefied data, and processing time on a MacBook Air 2012 took longer. Furthermore, the choice of ACE as the alpha diversity index may also be discussed, as other diversity measures such as Chao, Shannon, and Simpson could have been used instead. However, the results were similar (Simpson result shown in supplementary section 5, Figures 7 and 8), and ACE was chosen as the most straightforward measure, despite lacking an evenness criteria.

Finally, the choice of analysis for beta diversity may also be a potential area for improvement. This study used a Bray-Curtis matrix on the abundances of different ASVs and a PERMANOVA, providing only a p-value, indicating a significant change in beta diversity with increasing lice intensity. However, whether the variation in ASV abundance with increasing mean lice increases or decreases is unclear. It's also hard to quantify if the change in beta diversity is biologically relevant, i. e I don’t have an estimate of how substantial the change is. A beta diversity analysis that would produce confidence intervals would be beneficial. However, the GLLVM model indicates that the primary trend in this experiment is that the majority of ASVs tend to be overrepresented with higher lice loads, which means that variation in ASVs abundance increases with increasing lice loads.

4.6 The way forward

There exist various opportunities for improvement, discoveries, and a better understanding of the field as a whole.

Regarding potential improvements within the field, including more frequent sampling time points and mapping the microbial community of the salmon louse are areas that require attention. Additionally, incorporating the fish's health parameters and mapping the gut microbiome would provide a more comprehensive understanding of the relationship between salmon louse and their host. Finally, developing methods that permit the manipulation of lice dose would be beneficial to investigate whether different quantities elicit varying thresholds of community structure changes. Nonetheless, such improvements would require significant resources and expertise and pose ethical and logistical challenges.

For future research, it is imperative to consider incorporating a more comprehensive range of omics tools. (Riiser et al., 2019), has, for instance, demonstrated the potential of utilizing a metagenomics-based approach to achieve a higher degree of resolution in the taxonomic composition of the gut microbiome of cod (*Gadus morhua*). This method may prove necessary for concluding the role of suspected opportunistic/pathogenic taxa as the causative agents of observed phenomena. Moreover, characterizing the functional attributes of the microbial community associated with the fish surface is crucial in establishing its significance. Key features that may aid in drawing meaningful conclusions about the fish surface microbiome include immunoregulatory genes, mucin degradation metabolic pathways, antimicrobial compounds, attachment mechanisms to mucus, microbial communication, and virulence potential/pathogenicity. A noteworthy study on fish mucus by (Carda-Diéguez et al., 2017) employed a metagenomic approach, identifying a high abundance of *Vibro sp* and confirming the predominance of Proteobacteria from previous studies. Furthermore, this approach allowed for identifying six species of *Vibro sp* and suggesting that fish slime may select for virulence factors due to the overexpression of virulence genes relative to water. A similar study on *Tenacibaculum sp* could offer valuable insights into the potential overexpression of opportunistic virulence/pathogenicity genes.

Utilizing a metatranscriptomics approach to analyze changes in gene expression in a parasite - holobiont system, as in the study under consideration, would enable a more comprehensive description of the possible interactions between the different entities in the system. In addition, it may allow the characterization of transient interactions between the fish surface microbiome and the fish itself, such as those arising from environmental disturbances or immunological interactions during infection, as demonstrated by (X. Zhang et al., 2018). This study made several noteworthy findings through the combination of 16S metabarcoding and transcriptomics data, including elevated levels of several immune-related compounds during infection and a correlation between immune compounds and changes in community structure from symbionts to more opportunistic ASVs.

Furthermore, exploring the significance of small metabolites in the interactions between the fish system, the fish surface microbiome, and the environment may prove critical. Employing metabolomics tools to investigate the role of various metabolites in these interactions is a possible avenue for further research. One potential hypothesis that may be tested using

metabolomics is whether kairomones are produced by the fish or the microbial community on the fish, as mentioned earlier in the discussion. One problem with the investigation of the origins of kairomones would be the assignment of the kairomones to either fish or the microbiome. A possible solution to this would be the use of multi-omics, especially metagenomics, that could have the power to map if either bacteria or the fish had the metabolic pathways to produce suspected kairomones.

The primary advantage for future fish mucus microbiome research lies in utilizing a multi-omics approach, whereby various techniques synergistically complement one another. For instance, in this investigation, it is feasible to conclude that there has been a potential community transformation associated with an escalating intensity of salmon louse. However, employing terminologies such as dysbiosis would be inappropriate since it remains unclear whether this alteration leads to diminished functionality or has negative implications. By adopting a multi-omics approach, such interactions could be elucidated. For example, metabolomics could provide a glimpse into the chemical ecology, metagenomics could shed light on the community structure and identify members with the potential to produce certain compounds, and transcriptomics could indicate the actual synthesis at a given moment. This could offer valuable insights into the holobiont system under investigation.

Furthermore, future studies should incorporate other microbiome constituents, including archaea, fungi, and eukaryotic organisms. The various phylogenetic groups may have intriguing connections that could significantly enhance fish mucus microbiome dynamics comprehension.

Lastly, it is worth noting that there is a growing need for standardization among different studies to enable comparative analysis between studies, as discussed in the review by (Gomez and Primm, 2021).

5 Conclusion

The present study did not reveal a significant linear correlation between changes in alpha diversity in the mucus microbiome of Atlantic salmon with increasing loads of salmon louse. Nonetheless, it revealed a weak but statistically significant correlation between salmon louse intensity and changes in beta diversity in the mucus microbiome of Atlantic salmon. The taxonomic structure analysis also suggests a potential shift from commensal bacteria to more opportunistic bacteria due to increased salmon louse intensity in the mucus microbiome of Atlantic salmon. Notably, *Tenacibaculum sp*. was among the genera with significantly higher abundance, which aligns with existing literature on Atlantic salmon and other fish affected by a stressor. This raises the possibility that an increase in *Tenacibaculum sp.* abundance could serve as a bioindicator of declining fish health. However, further multi-omics studies must verify this proposition and provide a mechanistic evidence burden for this possibility.

This study also found that prolonged disturbance to the Atlantic salmon skin mucus microbial community over longer timescales may lead to an alternative steady state or resetting to the original steady state. Moreover, the study identifies several arguments for adopting a more holistic approach to describing parasite-host interactions. However, it cannot be considered a fully holistic examination of such interactions, as more powerful multi-omics tools are needed, and the microbiome of the salmon louse was not sequenced. Finally, despite encountering significant challenges, this study demonstrated that metabarcoding is a relatively resilient technique. It identified many of the same trends as previous research regarding community structure in the Atlantic salmon surface microbiome system.

References

Abolofia, J., Asche, F., Wilen, J.E., 2017. The Cost of Lice: Quantifying the Impacts of Parasitic Sea Lice on Farmed Salmon. Mar. Resour. Econ. 32, 329–349. https://doi.org/10.1086/691981

Anderson, M.J., Crist, T.O., Chase, J.M., Vellend, M., Inouye, B.D., Freestone, A.L., Sanders, N.J., Cornell, H.V., Comita, L.S., Davies, K.F., Harrison, S.P., Kraft, N.J.B., Stegen, J.C., Swenson, N.G., 2011. Navigating the multiple meanings of β diversity: a roadmap for the practicing ecologist. Ecol. Lett. 14, 19–28. https://doi.org/10.1111/j.1461-0248.2010.01552.x

Avendaño-Herrera, R., Irgang, R., Magariños, B., Romalde, J.L., Toranzo, A.E., 2006a. Use of microcosms to determine the survival of the fish pathogen *Tenacibaculum maritimum* in seawater. Environ. Microbiol. 8, 921–928. https://doi.org/10.1111/j.1462-2920.2005.00981.x

Avendaño-Herrera, R., Toranzo, A., Magarinos, B., 2006b. Tenacibaculosis infection in marine fish caused by *Tenacibaculum maritimum*: A review. Dis. Aquat. Organ. 71, 255–66. https://doi.org/10.3354/dao071255

Bakshani, C.R., Morales-Garcia, A.L., Althaus, M., Wilcox, M.D., Pearson, J.P., Bythell, J.C., Burgess, J.G., 2018. Evolutionary conservation of the antimicrobial function of mucus: a first defence against infection. Npj Biofilms Microbiomes 4, 1–12. https://doi.org/10.1038/s41522-018-0057-2

Barraclough, T.G., 2015. How Do Species Interactions Affect Evolutionary Dynamics Across Whole Communities? Annu. Rev. Ecol. Evol. Syst. 46, 25–48. https://doi.org/10.1146/annurev-ecolsys-112414-054030

Baumgärtner, S., James, J., Ellison, A., 2022. The supplementation of a prebiotic improves the microbial community in the gut and the skin of Atlantic salmon (*Salmo salar*). Aquac. Rep. 25, 101204. https://doi.org/10.1016/j.aqrep.2022.101204

Beklioglu, M., Telli, M., Gozen, A.G., 2006. Fish and mucus-dwelling bacteria interact to produce a kairomone that induces diel vertical migration in *Daphnia*. Freshw. Biol. 51, 2200–2206. https://doi.org/10.1111/j.1365-2427.2006.01642.x

Bell, T., Newman, J.A., Silverman, B.W., Turner, S.L., Lilley, A.K., 2005. The contribution of species richness and composition to bacterial services. Nature 436, 1157–1160. https://doi.org/10.1038/nature03891

Benhamed, S., Guardiola, F.A., Mars, M., Esteban, M.Á., 2014. Pathogen bacteria adhesion to skin mucus of fishes. Vet. Microbiol. 171, 1–12. https://doi.org/10.1016/j.vetmic.2014.03.008

Bereded, N.K., Abebe, G.B., Fanta, S.W., Curto, M., Waidbacher, H., Meimberg, H., Domig, K.J., 2021. The Impact of Sampling Season and Catching Site (Wild and Aquaculture) on Gut Microbiota Composition and Diversity of Nile Tilapia (*Oreochromis niloticus*). Biology 10, 180. https://doi.org/10.3390/biology10030180

Berg, G., Rybakova, D., Fischer, D., Cernava, T., Vergès, M.-C.C., Charles, T., Chen, X., Cocolin, L., Eversole, K., Corral, G.H., Kazou, M., Kinkel, L., Lange, L., Lima, N., Loy, A., Macklin, J.A., Maguin, E., Mauchline, T., McClure, R., Mitter, B., Ryan, M., Sarand, I., Smidt, H., Schelkle, B., Roume, H., Kiran, G.S., Selvin, J., Souza, R.S.C. de, van Overbeek, L., Singh, B.K., Wagner, M., Walsh, A., Sessitsch, A., Schloter, M., 2020. Microbiome definition re-visited: old concepts and new challenges. Microbiome 8, 103. https://doi.org/10.1186/s40168-020-00875-0

Bergh, S.K., 2019. Effect of salmon lice treatment and lice infection on bacterial colonization on Atlantic salmon skin (Master of Science). NTNU, Trondheim.

Birtel, J., Walser, J.-C., Pichon, S., Bürgmann, H., Matthews, B., 2015. Estimating Bacterial Diversity for Ecological Studies: Methods, Metrics, and Assumptions. PLOS ONE 10, e0125356. https://doi.org/10.1371/journal.pone.0125356

Bordenstein, S.R., Theis, K.R., 2015. Host Biology in Light of the Microbiome: Ten Principles of Holobionts and Hologenomes. PLOS Biol. 13, e1002226. https://doi.org/10.1371/journal.pbio.1002226

Boutin, S., Audet, C., Derome, N., 2013a. Probiotic treatment by indigenous bacteria decreases mortality without disturbing the natural microbiota of *Salvelinus fontinalis*. Can. J. Microbiol. 59, 662–670. https://doi.org/10.1139/cjm-2013-0443

Boutin, S., Bernatchez, L., Audet, C., Derôme, N., 2013b. Network Analysis Highlights Complex Interactions between Pathogen, Host and Commensal Microbiota. PLOS ONE 8, e84772. https://doi.org/10.1371/journal.pone.0084772

Boutin, S., Bernatchez, L., Audet, C., Derôme, N., 2012. Antagonistic effect of indigenous skin bacteria of brook charr (*Salvelinus fontinalis*) against *Flavobacterium columnare* and *F. psychrophilum*. Vet. Microbiol. 155, 355–361. https://doi.org/10.1016/j.vetmic.2011.09.002

Boutin, S., Sauvage, C., Bernatchez, L., Audet, C., Derome, N., 2014. Inter Individual Variations of the Fish Skin Microbiota: Host Genetics Basis of Mutualism? PLOS ONE 9, e102649. https://doi.org/10.1371/journal.pone.0102649

Brooks, A.W., Kohl, K.D., Brucker, R.M., Opstal, E.J. van, Bordenstein, S.R., 2016. Phylosymbiosis: Relationships and Functional Effects of Microbial Communities across Host Evolutionary History. PLOS Biol. 14, e2000225. https://doi.org/10.1371/journal.pbio.2000225

Brosschot, T.P., Reynolds, L.A., 2018. The impact of a helminth-modified microbiome on host immunity. Mucosal Immunol. 11, 1039–1046. https://doi.org/10.1038/s41385-018-0008-5

Brown, R.M., Wiens, G.D., Salinas, I., 2019. Analysis of the gut and gill microbiome of resistant and susceptible lines of rainbow trout (*Oncorhynchus mykiss*). Fish Shellfish Immunol. 86, 497–506. https://doi.org/10.1016/j.fsi.2018.11.079

Burke, C., Steinberg, P., Rusch, D., Kjelleberg, S., Thomas, T., 2011. Bacterial community assembly based on functional genes rather than species. Proc. Natl. Acad. Sci. 108, 14288–14293. https://doi.org/10.1073/pnas.1101591108

Bürkner, P.-C., 2021. Bayesian Item Response Modeling in R with brms and Stan. J. Stat. Softw. 100, 1–54. https://doi.org/10.18637/jss.v100.i05

Bürkner, P.-C., 2018. Advanced Bayesian Multilevel Modeling with the R Package brms. R J. 10, 395–411. https://doi.org/10.32614/RJ-2018-017

Bürkner, P.-C., 2017. brms: An R Package for Bayesian Multilevel Models Using Stan. J. Stat. Softw. 80, 1–28. https://doi.org/10.18637/jss.v080.i01

Cahill, M.M., 1990. Bacterial flora of fishes: A review. Microb. Ecol. 19, 21–41. https://doi.org/10.1007/BF02015051

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nat. Methods 13, 581–583. https://doi.org/10.1038/nmeth.3869

Cameron, E.S., Schmidt, P.J., Tremblay, B.J.-M., Emelko, M.B., Müller, K.M., 2021. Enhancing diversity analysis by repeatedly rarefying next generation sequencing data describing microbial communities. Sci. Rep. 11, 22302. https://doi.org/10.1038/s41598-021-01636-1

Carda-Diéguez, M., Ghai, R., Rodríguez-Valera, F., Amaro, C., 2017. Wild eel microbiome reveals that skin mucus of fish could be a natural niche for aquatic mucosal pathogen evolution. Microbiome 5, 162. https://doi.org/10.1186/s40168-017-0376-1

Carlson, C.J., Dallas, T.A., Alexander, L.W., Phelan, A.L., Phillips, A.J., 2020. What would it take to describe the global diversity of parasites? Proc. R. Soc. B Biol. Sci. 287, 20201841. https://doi.org/10.1098/rspb.2020.1841

Carlson, J.M., Hyde, E.R., Petrosino, J.F., Manage, A.B.W., Primm, T.P., 2015. The host effects of *Gambusia affinis* with an antibiotic-disrupted microbiome. Comp. Biochem. Physiol. Part C Toxicol. Pharmacol., Aquatic animal models of human disease - 7th Conference 178, 163–168. https://doi.org/10.1016/j.cbpc.2015.10.004

Chao, A., Lee, shen-M., 1992. Estimating the Number of Classes Via Sample Coverage. J. Am. Stat. Assoc. 87, 210–217. https://doi.org/10.1080/01621459.1992.10475194

Chiarello, M., Auguet, J.-C., Bettarel, Y., Bouvier, C., Claverie, T., Graham, N.A.J., Rieuvilleneuve, F., Sucré, E., Bouvier, T., Villéger, S., 2018. Skin microbiome of coral reef fish is highly variable and driven by host phylogeny and diet. Microbiome 6, 147. https://doi.org/10.1186/s40168-018-0530-4

Chiarello, M., Paz-Vinas, I., Veyssière, C., Santoul, F., Loot, G., Ferriol, J., Boulêtreau, S., 2019. Environmental conditions and neutral processes shape the skin microbiome of European catfish (*Silurus glanis*) populations of Southwestern France. Environ. Microbiol. Rep. 11, 605–614. https://doi.org/10.1111/1758-2229.12774

Chiarello, M., Villéger, S., Bouvier, C., Bettarel, Y., Bouvier, T., 2015. High diversity of skin-associated bacterial communities of marine fishes is promoted by their high variability among body parts, individuals and species. FEMS Microbiol. Ecol. 91, fiv061. https://doi.org/10.1093/femsec/fiv061

Cipriano, R.C., Dove, A., 2011. Far From Superficial: Microbial Diversity Associated With The Skin And Mucus Of Fish. Khaled Bin Sultan Living Oceans Found. 156–7.

D'Amore, R., Ijaz, U.Z., Schirmer, M., Kenny, J.G., Gregory, R., Darby, A.C., Shakya, M., Podar, M., Quince, C., Hall, N., 2016. A comprehensive benchmarking study of protocols and sequencing platforms for 16S rRNA community profiling. BMC Genomics 17, 55. https://doi.org/10.1186/s12864-015-2194-9

Davis, N.M., Proctor, D.M., Holmes, S.P., Relman, D.A., Callahan, B.J., 2018. Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. https://doi.org/10.1101/221499

De Roy, K., Marzorati, M., Negroni, A., Thas, O., Balloi, A., Fava, F., Verstraete, W., Daffonchio, D., Boon, N., 2013. Environmental conditions and community evenness determine the outcome of biological invasion. Nat. Commun. 4, 1383. https://doi.org/10.1038/ncomms2392

Dehler, C.E., Secombes, C.J., Martin, S.A.M., 2017. Environmental and physiological factors shape the gut microbiota of Atlantic salmon parr (*Salmo salar* L.). Aquaculture, Cutting Edge Science in Aquaculture 2015 467, 149–157. https://doi.org/10.1016/j.aquaculture.2016.07.017

Dheilly, N.M., 2014. Holobiont–Holobiont Interactions: Redefining Host–Parasite Interactions. PLOS Pathog. 10, e1004093. https://doi.org/10.1371/journal.ppat.1004093

Dheilly, N.M., Bolnick, D., Bordenstein, S., Brindley, P.J., Figuères, C., Holmes, E.C., Martínez Martínez, J., Phillips, A.J., Poulin, R., Rosario, K., 2017. Parasite Microbiome Project: Systematic Investigation of Microbiome Dynamics within and across Parasite-Host Interactions. mSystems 2, e00050-17. https://doi.org/10.1128/mSystems.00050-17

Dheilly, N.M., Poulin, R., Thomas, F., 2015. Biological warfare: Microorganisms as drivers of host–parasite interactions. Infect. Genet. Evol. 34, 251–259. https://doi.org/10.1016/j.meegid.2015.05.027

Easterbrook, P.J., Gopalan, R., Berlin, J.A., Matthews, D.R., 1991. Publication bias in clinical research. The Lancet, Originally published as Volume 1, Issue 8746 337, 867–872. https://doi.org/10.1016/0140-6736(91)90201-Y

Easy, R.H., Ross, N.W., 2009. Changes in Atlantic salmon (*Salmo salar*) epidermal mucus protein composition profiles following infection with sea lice (*Lepeophtheirus salmonis*). Comp. Biochem. Physiol. Part D Genomics Proteomics 4, 159–167. https://doi.org/10.1016/j.cbd.2009.02.001

Eiler, A., 2023. DNA quantification with PicoGreen. dx.doi.org/10.17504/protocols.io.6qpvrbz3lmkn/v1

Esteban, M., 2012. An Overview of the Immunological Defenses in Fish Skin. ISRN Immunol. 2012. https://doi.org/10.5402/2012/853470

FAO, 2022. The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation. Rome, FAO. https://doi.org/10.4060/cc0461en

Fast, M., Ross, N., Mustafa, A., Sims, D., Johnson, S., Conboy, G., Speare, D., Johnson, G., Burka, J., 2002. Susceptibility of rainbow trout *Oncorhynchus mykiss*, Atlantic salmon *Salmo salar* and coho salmon *Oncorhynchus kisutch* to experimental infection with sea lice *Lepeophtheirus salmonis*. Dis. Aquat. Organ. 52, 57–68. https://doi.org/10.3354/dao052057

Fisher, R.A., Corbet, A.S., Williams, C.B., 1943. The Relation Between the Number of Species and the Number of Individuals in a Random Sample of an Animal Population. J. Anim. Ecol. 12, 42–58. https://doi.org/10.2307/1411

Forseth, T., Barlaup, B.T., Finstad, B., Fiske, P., Gjøsæter, H., Falkegård, M., Hindar, A., Mo, T.A., Rikardsen, A.H., Thorstad, E.B., Vøllestad, L.A., Wennevik, V., 2017. The major threats to Atlantic salmon in Norway. ICES J. Mar. Sci. 74, 1496–1513. https://doi.org/10.1093/icesjms/fsx020

Gallardo-Escárate, C., Valenzuela-Muñoz, V., Núñez-Acuña, G., Carrera, C., Gonçalves, A.T., Valenzuela-Miranda, D., Benavente, B.P., Roberts, S., 2019. Catching the complexity of salmon-louse interactions. Fish Shellfish Immunol. 90, 199–209. https://doi.org/10.1016/j.fsi.2019.04.065

Garcia, S.M., Cochrane, K.L., 2005. Ecosystem approach to fisheries: a review of implementation guidelines1. ICES J. Mar. Sci. 62, 311–318. https://doi.org/10.1016/j.icesjms.2004.12.003

Ghosh, S.K., Wong, M.K.-S., Hyodo, S., Goto, S., Hamasaki, K., 2022. Temperature modulation alters the gut and skin microbial profiles of chum salmon (*Oncorhynchus keta*). Front. Mar. Sci. 9. https://doi.org/10.3389/fmars.2022.1027621

Glendinning, L., Nausch, N., Free, A., Taylor, D.W., Mutapi, F., 2014. The microbiota and helminths: sharing the same niche in the human host. Parasitology 141, 1255–1271. https://doi.org/10.1017/S0031182014000699

Gloor, G.B., Macklaim, J.M., Pawlowsky-Glahn, V., Egozcue, J.J., 2017. Microbiome Datasets Are Compositional: And This Is Not Optional. Front. Microbiol. 8. https://doi.org/10.3389/fmicb.2017.02224

Gomez, D., Sunyer, J.O., Salinas, I., 2013. The mucosal immune system of fish: The evolution of tolerating commensals while fighting pathogens. Fish Shellfish Immunol. 35, 1729–1739. https://doi.org/10.1016/j.fsi.2013.09.032

Gomez, J.A., Primm, T.P., 2021. A Slimy Business: the Future of Fish Skin Microbiome Studies. Microb. Ecol. 82, 275–287. https://doi.org/10.1007/s00248-020-01648-w

Gonçalves, A.T., Collipal-Matamal, R., Valenzuela-Muñoz, V., Nuñez-Acuña, G., Valenzuela-Miranda, D., Gallardo-Escárate, C., 2020. Nanopore sequencing of microbial communities reveals the potential role of sea lice as a reservoir for fish pathogens. Sci. Rep. 10, 2895. https://doi.org/10.1038/s41598-020-59747-0

Grimnes, A., Jakobsen, P. j., 1996. The physiological effects of salmon lice infection on post-smolt of Atlantic salmon. J. Fish Biol. 48, 1179–1194. https://doi.org/10.1111/j.1095-8649.1996.tb01813.x

Hahn, M.A., Piecyk, A., Jorge, F., Cerrato, R., Kalbe, M., Dheilly, N.M., 2022. Host phenotype and microbiome vary with infection status, parasite genotype, and parasite microbiome composition. Mol. Ecol. 31, 1577–1594. https://doi.org/10.1111/mec.16344

Hamilton, E.F., Element, G., van Coeverden de Groot, P., Engel, K., Neufeld, J.D., Shah, V., Walker, V.K., 2019. Anadromous Arctic Char Microbiomes: Bioprospecting in the High Arctic. Front. Bioeng. Biotechnol. 7. https://doi.org/10.3389/fbioe.2019.00032

Hamilton, E.F., Juurakko, C.L., Engel, K., Neufeld, J.D., Casselman, J.M., Greer, C.W., Walker, V.K., 2023. Environmental Impacts on Skin Microbiomes of Sympatric High Arctic Salmonids. Fishes 8, 214. https://doi.org/10.3390/fishes8040214

Hawkes, J.W., 1974. The structure of fish skin. Cell Tissue Res. 149, 147–158. https://doi.org/10.1007/BF00222270

Horsley, R.W., 1977. A review of the bacterial flora of teleosts and elasmobranchs, including methods for its analysis. J. Fish Biol. 10, 529–553. https://doi.org/10.1111/j.1095-8649.1977.tb04086.x

Horsley, R.W., 1973. The Bacterial Flora of the Atlantic Salmon (*Salmo salar* L.) in Relation to its Environment. J. Appl. Bacteriol. 36, 377–386. https://doi.org/10.1111/j.1365-2672.1973.tb04119.x

Ivanova, L., Tartor, H., Grove, S., Kristoffersen, A., Uhlig, S., 2018. Workflow for the Targeted and Untargeted Detection of Small Metabolites in Fish Skin Mucus. Fishes 3, 21. https://doi.org/10.3390/fishes3020021

Jousset, A., Bienhold, C., Chatzinotas, A., Gallien, L., Gobet, A., Kurm, V., Küsel, K., Rillig, M.C., Rivett, D.W., Salles, J.F., van der Heijden, M.G.A., Youssef, N.H., Zhang, X., Wei, Z., Hol, W.H.G., 2017. Where less may be more: how the rare biosphere pulls ecosystems strings. ISME J. 11, 853–862. https://doi.org/10.1038/ismej.2016.174

Juottonen, H., Fontaine, L., Wurzbacher, C., Drakare, S., Peura, S., Eiler, A., 2020. Archaea in boreal Swedish lakes are diverse, dominated by Woesearchaeota and follow deterministic community assembly. Environ. Microbiol. 22, 3158–3171. https://doi.org/10.1111/1462-2920.15058

Kabata, Z., 1974. Mouth and Mode of Feeding of Caligidae (Copepoda), Parasites of Fishes, as Determined by Light and Scanning Electron Microscopy. J. Fish. Res. Board Can. 31, 1583–1588. https://doi.org/10.1139/f74-199

Karlsen, C., Ottem, K.F., Brevik, Ø.J., Davey, M., Sørum, H., Winther-Larsen, H.C., 2017. The environmental and host-associated bacterial microbiota of Arctic seawater-farmed Atlantic salmon with ulcerative disorders. J. Fish Dis. 40, 1645–1663. https://doi.org/10.1111/jfd.12632

Keeley, N., Laroche, O., Birch, M., Pochon, X., 2021. A Substrate-Independent Benthic Sampler (SIBS) for Hard and Mixed-Bottom Marine Habitats: A Proof-of-Concept Study. Front. Mar. Sci. 8. https://doi.org/10.3389/fmars.2021.627687

Kelly, C., Salinas, I., 2017. Under Pressure: Interactions between Commensal Microbiota and the Teleost Immune System. Front. Immunol. 8. https://doi.org/10.3389/fimmu.2017.00559

Kelly, C., Takizawa, F., Sunyer, J.O., Salinas, I., 2017. Rainbow trout (*Oncorhynchus mykiss*) secretory component binds to commensal bacteria and pathogens. Sci. Rep. 7, 41753. https://doi.org/10.1038/srep41753

Khan, R.A., 2012. Host-Parasite Interactions in Some Fish Species. J. Parasitol. Res. 2012, e237280. https://doi.org/10.1155/2012/237280

Larsen, A., Tao, Z., Bullard, S.A., Arias, C.R., 2013. Diversity of the skin microbiota of fishes: evidence for host species specificity. FEMS Microbiol. Ecol. 85, 483–494. https://doi.org/10.1111/1574-6941.12136

Larsen, A.M., Bullard, S.A., Womble, M., Arias, C.R., 2015. Community Structure of Skin Microbiome of Gulf Killifish, *Fundulus grandis*, Is Driven by Seasonality and Not Exposure to Oiled Sediments in a Louisiana Salt Marsh. Microb. Ecol. 70, 534–544. https://doi.org/10.1007/s00248-015-0578-7

Legrand, T.P.R.A., Catalano, S.R., Wos-Oxley, M.L., Stephens, F., Landos, M., Bansemer, M.S., Stone, D.A.J., Qin, J.G., Oxley, A.P.A., 2018. The Inner Workings of the Outer Surface: Skin and Gill Microbiota as Indicators of Changing Gut Health in Yellowtail Kingfish. Front. Microbiol. 8. https://doi.org/10.3389/fmicb.2017.02664

Legrand, T.P.R.A., Wynne, J.W., Weyrich, L.S., Oxley, A.P.A., 2020. A microbial sea of possibilities: current knowledge and prospects for an improved understanding of the fish microbiome. Rev. Aquac. 12, 1101–1134. https://doi.org/10.1111/raq.12375

Leonard, A.B., Carlson, J.M., Bishoff, D.E., Sendelbach, S.I., Yung, S.B., Ramzanali, S., Manage, A.B.W., Hyde, E.R., Petrosino, J.F., Primm, T.P., 2014. The Skin Microbiome of *Gambusia affinis* Is Defined and Selective. Adv. Microbiol. 2014. https://doi.org/10.4236/aim.2014.47040

Lewis, D.L., Barker, D.E., McKinley, R.S., 2014. Modulation of cellular innate immunity by *Lepeophtheirus salmonis* secretory products. Fish Shellfish Immunol. 38, 175–183. https://doi.org/10.1016/j.fsi.2014.03.014

Li, Y., Bruni, L., Jaramillo-Torres, A., Gajardo, K., Kortner, T.M., Krogdahl, Å., 2021. Differential response of digesta- and mucosa-associated intestinal microbiota to dietary insect meal during the seawater phase of Atlantic salmon. Anim. Microbiome 3, 8. https://doi.org/10.1186/s42523-020-00071-3

Limborg, M.T., Alberdi, A., Kodama, M., Roggenbuck, M., Kristiansen, K., Gilbert, M.T.P., 2018. Applied Hologenomics: Feasibility and Potential in Aquaculture. Trends Biotechnol. 36, 252–264. https://doi.org/10.1016/j.tibtech.2017.12.006

Liston, J., 1957. The Occurrence and Distribution of Bacterial Types on Flatfish. Microbiology 16, 205–216. https://doi.org/10.1099/00221287-16-1-205

Liu, M., Clarke, L.J., Baker, S.C., Jordan, G.J., Burridge, C.P., 2020. A practical guide to DNA metabarcoding for entomological ecologists. Ecol. Entomol. 45, 373–385. https://doi.org/10.1111/een.12831

Llewellyn, M.S., Leadbeater, S., Garcia, C., Sylvain, F.-E., Custodio, M., Ang, K.P., Powell, F., Carvalho, G.R., Creer, S., Elliot, J., Derome, N., 2017. Parasitism perturbs the mucosal microbiome of Atlantic Salmon. Sci. Rep. 7, 43465. https://doi.org/10.1038/srep43465

Lokesh, J., Kiron, V., 2016. Transition from freshwater to seawater reshapes the skin-associated microbiota of Atlantic salmon. Sci. Rep. 6, 19707. https://doi.org/10.1038/srep19707

Lorgen-Ritchie, M., Clarkson, M., Chalmers, L., Taylor, J.F., Migaud, H., Martin, S.A.M., 2022. Temporal changes in skin and gill microbiomes of Atlantic salmon in a recirculating aquaculture system – Why do they matter? Aquaculture 558, 738352. https://doi.org/10.1016/j.aquaculture.2022.738352

Lowrey, L., Woodhams, D.C., Tacchi, L., Salinas, I., 2015. Topographical Mapping of the Rainbow Trout (*Oncorhynchus mykiss*) Microbiome Reveals a Diverse Bacterial Community with Antifungal Properties in the Skin. Appl. Environ. Microbiol. 81, 6915–6925. https://doi.org/10.1128/AEM.01826-15

Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal 17, 10–12. https://doi.org/10.14806/ej.17.1.200

McMurdie, P.J., Holmes, S., 2014. Waste Not, Want Not: Why Rarefying Microbiome Data Is Inadmissible. PLOS Comput. Biol. 10, e1003531. https://doi.org/10.1371/journal.pcbi.1003531

McMurdie, P.J., Holmes, S., 2013. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLOS ONE 8, e61217. https://doi.org/10.1371/journal.pone.0061217

Minniti, G., Hagen, L.H., Porcellato, D., Jørgensen, S.M., Pope, P.B., Vaaje-Kolstad, G., 2017. The Skin-Mucus Microbial Community of Farmed Atlantic Salmon (*Salmo salar*). Front. Microbiol. 8, 2043. https://doi.org/10.3389/fmicb.2017.02043

Minniti, G., Rød Sandve, S., Padra, J.T., Heldal Hagen, L., Lindén, S., Pope, P.B., Ø. Arntzen, M., Vaaje-Kolstad, G., 2019. The Farmed Atlantic Salmon (*Salmo salar*) Skin–Mucus Proteome and Its Nutrient Potential for the Resident Bacterial Community. Genes 10, 515. https://doi.org/10.3390/genes10070515

Misund, B., 2022. Kostnadsutvikling i oppdrett av laks og ørret: Hva koster biologisk risiko? (No. 41–2022, H& S). NORCE Helse og Samfunn.

Miyake, S., Soh, M., Azman, M.N., Ngoh, S.Y., Orbán, L., Seedorf, H., 2020. Insights into the microbiome of farmed Asian sea bass (*Lates calcarifer*) with symptoms of tenacibaculosis and description of *Tenacibaculum singaporense* sp. nov. Antonie Van Leeuwenhoek 113, 737–752. https://doi.org/10.1007/s10482-020-01391-9

Mohammed, H.H., Arias, C.R., 2015. Potassium permanganate elicits a shift of the external fish microbiome and increases host susceptibility to columnaris disease. Vet. Res. 46, 82. https://doi.org/10.1186/s13567-015-0215-y

Mordue (Luntz), A.J., Birkett, M.A., 2009. A review of host finding behaviour in the parasitic sea louse, Lepeophtheirus salmonis (Caligidae: Copepoda). J. Fish Dis. 32, 3–13. https://doi.org/10.1111/j.1365-2761.2008.01004.x

Nelson, J.S., Grande, T.C., Wilson, M.V.H., 2016. Fishes of the World. John Wiley & Sons. https://doi.org/10.1002/9781119174844

Niku, J., 2023. Analysing multivariate abundance data using gllvm [WWW Document]. URL https://jenniniku.github.io/gllvm/articles/vignette1.html (accessed 5.14.23).

Niku, J., Brooks, W., Herliansyah, R., Hui, F.K.C., Taskinen, S., Warton, D.I., 2019a. Efficient estimation of generalized linear latent variable models. PLOS ONE 14, e0216129. https://doi.org/10.1371/journal.pone.0216129

Niku, J., Hui, F.K.C., Taskinen, S., Warton, D.I., 2019b. gllvm: Fast analysis of multivariate abundance data with generalized linear latent variable models in r. Methods Ecol. Evol. 10, 2173–2182. https://doi.org/10.1111/2041-210X.13303

Noecker, C., McNally, C.P., Eng, A., Borenstein, E., 2017. High-resolution characterization of the human microbiome. Transl. Res., Microbiome and Human Disease Pathogenesis 179, 7–23. https://doi.org/10.1016/j.trsl.2016.07.012

Núñez-Acuña, G., Marambio, J.P., Valenzuela, T., Wadsworth, S., Gallardo-Escárate, C., 2016. Antimicrobial peptides from *Salmon salar* skin induce frontal filament development and olfactory/cuticle-related genes in the sea louse *Caligus rogercresseyi*. Aquaculture 464, 171–177. https://doi.org/10.1016/j.aquaculture.2016.06.023

Oksanen, J., Simpson, G.L., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., Caceres, M.D., Durand, S., Evangelista, H.B.A., FitzJohn, R., Friendly, M., Furneaux, B., Hannigan, G., Hill, M.O., Lahti, L., McGlinn, D., Ouellette, M.-H., Cunha, E.R., Smith, T., Stier, A., Braak, C.J.F.T., Weedon, J., 2022. vegan: Community Ecology Package.

Olsen, A.B., Nilsen, H., Sandlund, N., Mikkelsen, H., Sørum, H., Colquhoun, D.J., 2011. *Tenacibaculum* sp. associated with winter ulcers in sea-reared Atlantic salmon *Salmo salar*. Dis. Aquat. Organ. 94, 189–199. https://doi.org/10.3354/dao02324

Peachey, L.E., Jenkins, T.P., Cantacessi, C., 2017. This Gut Ain't Big Enough for Both of Us. Or Is It? Helminth–Microbiota Interactions in Veterinary Species. Trends Parasitol. 33, 619–632. https://doi.org/10.1016/j.pt.2017.04.004

Pedrós-Alió, C., Manrubia, S., 2016. The vast unknown microbial biosphere. Proc. Natl. Acad. Sci. 113, 6585–6587. https://doi.org/10.1073/pnas.1606105113

Pike, A.W., 1989. Sea lice — Major pathogens of farmed atlantic salmon. Parasitol. Today 5, 291–297. https://doi.org/10.1016/0169-4758(89)90020-3

Poulin, R., 2002. Qualitative and quantitative aspects of recent research on helminth parasites. J. Helminthol. 76, 373–376. https://doi.org/10.1079/JOH2002141

Poulin, R., Morand, S., 2000. The Diversity of Parasites. Q. Rev. Biol. 75, 277–293. https://doi.org/10.1086/393500

Poulin, R., Presswell, B., Jorge, F., 2020. The state of fish parasite discovery and taxonomy: a critical assessment and a look forward. Int. J. Parasitol., Special issue on 'Fish Parasitology' 50, 733–742. https://doi.org/10.1016/j.ijpara.2019.12.009

Rahman, T., Suga, K., Kanai, K., Sugihara, Y., 2015. Infection Kinetics of *Tenacibaculum maritimum* on the Abraded Skin of Japanese Flounder *Paralichthys olivaceus*. 魚病研究 50, 44–52. https://doi.org/10.3147/jsfp.50.44

Rappé, M.S., Giovannoni, S.J., 2003. The Uncultured Microbial Majority. Annu. Rev. Microbiol. 57, 369–394. https://doi.org/10.1146/annurev.micro.57.030502.090759

Reid, K.M., Patel, S., Robinson, A.J., Bu, L., Jarungsriapisit, J., Moore, L.J., Salinas, I., 2017. Salmonid alphavirus infection causes skin dysbiosis in Atlantic salmon (*Salmo salar* L.) post-smolts. PLOS ONE 12, e0172856. https://doi.org/10.1371/journal.pone.0172856

Reverter, M., Tapissier-Bontemps, N., Lecchini, D., Banaigs, B., Sasal, P., 2018. Biological and Ecological Roles of External Fish Mucus: A Review. Fishes 3, 41. https://doi.org/10.3390/fishes3040041

Riiser, E.S., Haverkamp, T.H.A., Varadharajan, S., Borgan, Ø., Jakobsen, K.S., Jentoft, S., Star, B., 2019. Switching on the light: using metagenomic shotgun sequencing to characterize the intestinal microbiome of Atlantic cod. Environ. Microbiol. 21, 2576–2594. https://doi.org/10.1111/1462-2920.14652

Ringelberg, J., Van Gool, E., 1998. Do bacteria, not fish, produce 'fish kairomone'? J. Plankton Res. 20, 1847–1852. https://doi.org/10.1093/plankt/20.9.1847

Rudi, K., Angell, I.L., Pope, P.B., Vik, J.O., Sandve, S.R., Snipen, L.-G., 2018. Stable Core Gut Microbiota across the Freshwater-to-Saltwater Transition for Farmed Atlantic Salmon. Appl. Environ. Microbiol. 84, e01974-17. https://doi.org/10.1128/AEM.01974-17

Sale, P.F., 1978. Coexistence of coral reef fishes — a lottery for living space. Environ. Biol. Fishes 3, 85–102. https://doi.org/10.1007/BF00006310

Scheifler, M., Sanchez-Brosseau, S., Magnanou, E., Desdevises, Y., 2022. Diversity and structure of sparids external microbiota (Teleostei) and its link with monogenean ectoparasites. Anim. Microbiome 4, 27. https://doi.org/10.1186/s42523-022-00180-1

Schmidt, V.T., Smith, K.F., Melvin, D.W., Amaral-Zettler, L.A., 2015. Community assembly of a euryhaline fish microbiome during salinity acclimation. Mol. Ecol. 24, 2537–2550. https://doi.org/10.1111/mec.13177

Shakya, M., Quince, C., Campbell, J.H., Yang, Z.K., Schadt, C.W., Podar, M., 2013. Comparative metagenomic and rRNA microbial diversity characterization using archaeal and bacterial synthetic communities. Environ. Microbiol. 15, 1882–1899. https://doi.org/10.1111/1462-2920.12086

Shannon, C.E., 1948. A mathematical theory of communication. Bell Syst. Tech. J. 27, 379–423. https://doi.org/10.1002/j.1538-7305.1948.tb01338.x

She, R., Li, T.-T., Luo, D., Li, J.-B., Yin, L.-Y., Li, H., Liu, Y.-M., Li, X.-Z., Yan, Q., 2017. Changes in the Intestinal Microbiota of Gibel Carp (*Carassius gibelio*) Associated with *Cyprinid herpesvirus* 2 (CyHV-2) Infection. Curr. Microbiol. 74, 1130–1136. https://doi.org/10.1007/s00284-017-1294-y

Shephard, K.L., 1994. Functions for fish mucus. Rev. Fish Biol. Fish. 4, 401–429. https://doi.org/10.1007/BF00042888

Simpson, E.H., 1949. Measurement of Diversity. Nature 163, 688–688. https://doi.org/10.1038/163688a0

Slinger, J., Wynne, J.W., Adams, M.B., 2021. Profiling Branchial Bacteria of Atlantic Salmon (*Salmo salar* L.) Following Exposure to Antimicrobial Agents. Front. Anim. Sci. 2. https://doi.org/10.3389/fanim.2021.756101

Småge, S.B., Brevik, Ø.J., Duesund, H., Ottem, K.F., Watanabe, K., Nylund, A., 2016. *Tenacibaculum finnmarkense* sp. nov., a fish pathogenic bacterium of the family *Flavobacteriaceae* isolated from Atlantic salmon. Antonie Van Leeuwenhoek 109, 273–285. https://doi.org/10.1007/s10482-015-0630-0

Sommerset, I., Wiik-Nielsen, J., Oliveira, V.H.S., Moldal, T., Bornø, G., Haukaas, A., Brun, E., 2023. Fiskehelserapporten 2022 (No. ISSN nr 1893-1480 (elektronisk utgave)), Veterinærinstituttets årlige oversikt over fiskehelsen i Norge. Veterinærinstituttet 2023.

Spilsberg, B., Nilsen, H.K., Tavornpanich, S., Gulla, S., Jansen, M.D., Lagesen, K., Colquhoun, D.J., Olsen, A.-B., 2022. Tenacibaculosis in Norwegian Atlantic salmon (*Salmo salar*) cage-farmed in cold sea water is primarily associated with *Tenacibaculum finnmarkense* genomovar *finnmarkense*. J. Fish Dis. 45, 523–534. https://doi.org/10.1111/jfd.13577

Stevens, J.L., Jackson, R.L., Olson, J.B., 2016. Bacteria associated with lionfish (*Pterois volitans/miles* complex) exhibit antibacterial activity against known fish pathogens. Mar. Ecol. Prog. Ser. 558, 167–180. https://doi.org/10.3354/meps11789

Sylvain, F.-É., Cheaib, B., Llewellyn, M., Gabriel Correia, T., Barros Fagundes, D., Luis Val, A., Derome, N., 2016. pH drop impacts differentially skin and gut microbiota of the Amazonian fish tambaqui (*Colossoma macropomum*). Sci. Rep. 6, 32032. https://doi.org/10.1038/srep32032

Sylvain, F.-É., Holland, A., Bouslama, S., Audet-Gilbert, É., Lavoie, C., Val, A.L., Derome, N., 2020. Fish Skin and Gut Microbiomes Show Contrasting Signatures of Host Species and Habitat. Appl. Environ. Microbiol. 86, e00789-20. https://doi.org/10.1128/AEM.00789-20

Taberlet, P., Coissac, E., Pompanon, F., Brochmann, C., Willerslev, E., 2012. Towards next-generation biodiversity assessment using DNA metabarcoding. Mol. Ecol. 21, 2045–2050. https://doi.org/10.1111/j.1365-294X.2012.05470.x

Tapia-Paniagua, S.T., Ceballos-Francisco, D., Balebona, M.C., Esteban, M.Á., Moriñigo, M.Á., 2018. Mucus glycosylation, immunity and bacterial microbiota associated to the skin of experimentally ulcered gilthead seabream (*Sparus aurata*). Fish Shellfish Immunol. 75, 381–390. https://doi.org/10.1016/j.fsi.2018.02.006

Tarnecki, A.M., Brennan, N.P., Schloesser, R.W., Rhody, N.R., 2019. Shifts in the Skin-Associated Microbiota of Hatchery-Reared Common Snook *Centropomus undecimalis* During Acclimation to the Wild. Microb. Ecol. 77, 770–781. https://doi.org/10.1007/s00248-018-1252-7

Tenacibaculose [WWW Document], 2023. . Tenacibaculose. URL https://www.vetinst.no/sykdom-og-agens/tenacibaculose (accessed 5.1.23).

Thorstad, E.B., Forseth, T., Fiske, P., 2022. Status of wild Atlantic salmon in Norway 2022, Rapport fra Vitenskapelig råd for lakseforvaltning;17. Vitenskapelig råd for lakseforvaltning.

Torrissen, O., Jones, S., Asche, F., Guttormsen, A., Skilbrei, O.T., Nilsen, F., Horsberg, T.E., Jackson, D., 2013. Salmon lice – impact on wild salmonids and salmon aquaculture. J. Fish Dis. 36, 171–194. https://doi.org/10.1111/jfd.12061

Trivedi, B., 2012. Microbiome: The surface brigade. Nature 492, S60–S61. https://doi.org/10.1038/492S60a

Uren Webster, T.M., Consuegra, S., Hitchings, M., Garcia de Leaniz, C., 2018. Interpopulation Variation in the Atlantic Salmon Microbiome Reflects Environmental and Genetic Diversity. Appl. Environ. Microbiol. 84, e00691-18. https://doi.org/10.1128/AEM.00691-18

Uren Webster, T.M., Rodriguez-Barreto, D., Castaldo, G., Gough, P., Consuegra, S., Garcia de Leaniz, C., 2020. Environmental plasticity and colonisation history in the Atlantic salmon microbiome: A translocation experiment. Mol. Ecol. 29, 886–898. https://doi.org/10.1111/mec.15369

Vasileiadis, S., Puglisi, E., Arena, M., Cappa, F., Cocconcelli, P.S., Trevisan, M., 2012. Soil Bacterial Diversity Screening Using Single 16S rRNA Gene V Regions Coupled with Multi-Million Read Generating Sequencing Technologies. PLOS ONE 7, e42671. https://doi.org/10.1371/journal.pone.0042671

Vehtari, A., Gelman, A., Simpson, D., Carpenter, B., Bürkner, P.-C., 2021. Rank-normalization, folding, and localization: An improved R_hat for assessing convergence of MCMC. Bayesian Anal. 16. https://doi.org/10.1214/20-BA1221

Vellend, M., Agrawal, A., 2010. Conceptual Synthesis in Community Ecology. Q. Rev. Biol. 85, 183–206. https://doi.org/10.1086/652373

Wagner, G.N., McKinley, R.S., Bjørn, P.A., Finstad, B., 2003. Physiological impact of sea lice on swimming performance of Atlantic salmon. J. Fish Biol. 62, 1000–1009. https://doi.org/10.1046/j.1095-8649.2003.00091.x

Wang, Z., Li, S., Zhou, Q., Zhang, J., Li, Yongan, Li, Youjie, Yuan, Z., Huang, G., 2023. Effects of different protein and lipid levels on the growth performance and intestinal microflora of loach (*Paramisgurnus dabryanus*). Anim. Nutr. 13, 229–239. https://doi.org/10.1016/j.aninu.2023.02.007

Wickham, H., 2016. ggplot2, Use R! Springer International Publishing, Cham. https://doi.org/10.1007/978-3-319-24277-4

Willis, A.D., 2019. Rarefaction, Alpha Diversity, and Statistics. Front. Microbiol. 10. https://doi.org/10.3389/fmicb.2019.02407

Wilson, B., Danilowicz, B.S., Meijer, W.G., 2008. The Diversity of Bacterial Communities Associated with Atlantic Cod *Gadus morhua*. Microb. Ecol. 55, 425–434. https://doi.org/10.1007/s00248-007-9288-0

Windsor, D.A., 1998. Controversies in parasitology, Most of the species on Earth are parasites. Int. J. Parasitol. 28, 1939–1941. https://doi.org/10.1016/S0020-7519(98)00153-2

Wittebolle, L., Marzorati, M., Clement, L., Balloi, A., Daffonchio, D., Heylen, K., De Vos, P., Verstraete, W., Boon, N., 2009. Initial community evenness favours functionality under selective stress. Nature 458, 623–626. https://doi.org/10.1038/nature07840

Wynne, J.W., Thakur, K.K., Slinger, J., Samsing, F., Milligan, B., Powell, J.F.F., McKinnon, A., Nekouei, O., New, D., Richmond, Z., Gardner, I., Siah, A., 2020. Microbiome Profiling Reveals a Microbial Dysbiosis During a Natural Outbreak of Tenacibaculosis (Yellow Mouth) in Atlantic Salmon. Front. Microbiol. 11. https://doi.org/10.3389/fmicb.2020.586387

Xavier, R., Mazzei, R., Pérez-Losada, M., Rosado, D., Santos, J.L., Veríssimo, A., Soares, M.C., 2019. A Risky Business? Habitat and Social Behavior Impact Skin and Gut Microbiomes in Caribbean Cleaning Gobies. Front. Microbiol. 10. https://doi.org/10.3389/fmicb.2019.00716

Xie, S., Wei, D., Tan, B., Liu, Y., Tian, L., Niu, J., 2020. *Schizochytrium limacinum* Supplementation in a Low Fish-Meal Diet Improved Immune Response and Intestinal Health of Juvenile *Penaeus monodon*. Front. Physiol. 11, 613. https://doi.org/10.3389/fphys.2020.00613

Xu, Z., Parra, D., Gómez, D., Salinas, I., Zhang, Y.-A., von Gersdorff Jørgensen, L., Heinecke, R.D., Buchmann, K., LaPatra, S., Sunyer, J.O., 2013. Teleost skin, an ancient mucosal surface that elicits gut-like immune responses. Proc. Natl. Acad. Sci. 110, 13097–13102. https://doi.org/10.1073/pnas.1304319110

Yilmaz, P., Parfrey, L.W., Yarza, P., Gerken, J., Pruesse, E., Quast, C., Schweer, T., Peplies, J., Ludwig, W., Glöckner, F.O., 2014. The SILVA and "All-species Living Tree Project (LTP)" taxonomic frameworks. Nucleic Acids Res. 42, D643–D648. https://doi.org/10.1093/nar/gkt1209

Zaiss, M.M., Harris, N.L., 2016. Interactions between the intestinal microbiome and helminth parasites. Parasite Immunol. 38, 5–11. https://doi.org/10.1111/pim.12274

Zhang, J., Ding, X., Guan, R., Zhu, C., Xu, C., Zhu, B., Zhang, H., Xiong, Z., Xue, Y., Tu, J., Lu, Z., 2018. Evaluation of different 16S rRNA gene V regions for exploring bacterial diversity in a eutrophic freshwater lake. Sci. Total Environ. 618, 1254–1267. https://doi.org/10.1016/j.scitotenv.2017.09.228

Zhang, X., Ding, L., Yu, Y., Kong, W., Yin, Y., Huang, Z., Zhang, Xuezhen, Xu, Z., 2018. The Change of Teleost Skin Commensal Microbiota Is Associated With Skin Mucosal Transcriptomic Responses During Parasitic Infection by *Ichthyophthirius multifillis*. Front. Immunol. 9. https://doi.org/10.3389/fimmu.2018.02972

Zilber-Rosenberg, I., Rosenberg, E., 2008. Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. FEMS Microbiol. Rev. 32, 723–735. https://doi.org/10.1111/j.1574-6976.2008.00123.x

Øvergård, A.-C., Midtbø, H.M.D., Hamre, L.A., Dondrup, M., Bjerga, G.E.K., Larsen, Ø., Chettri, J.K., Buchmann, K., Nilsen, F., Grotmol, S., 2022. Small, charged proteins in salmon louse (*Lepeophtheirus salmonis*) secretions modulate Atlantic salmon (*Salmo salar*) immune responses and coagulation. Sci. Rep. 12, 7995. https://doi.org/10.1038/s41598-022-11773-w

Supplementary

1 Metadata

Table 1 metadata: Part 1 of the complete metadata collected for this study includes the following information: ***DateSampling****: The date when the samples were collected,* ***TankFrom****: Holding tanks used during the acclimatization period,* ***Tank****: Main holding tanks used during the experimental period,* ***FishName****: Unique name assigned to each fish; a number in front indicates the second sampling,* ***FishRank****: Order of the sampling conducted each day,* ***FishTag****: Pit tag number assigned to the fish for identification,* ***Length****: Length of the fish at the beginning of the experiment (in centimeters),* ***Weight****: Weight of the fish at the beginning of the experiment (in grams),* ***Comments****: Additional comments or observations about the fish,* ***Replacement****: If a fish died, the pit tag number of the replacement fish,* ***Pair****: Pairing of fish names from the first and second samplings based on pit number,* ***StartLiceF****: Number of female lice on each fish at the start of the experiment,* ***StartLiceM****: Number of male lice on each fish at the start of the experiment,* ***EndLiceF****: Number of female lice on each fish at the end of the experiment.*

DateSamplin	TankFrom	Tank	FishName	FishRank	FishTag	Length	Weight	Comments	Replacemen	Pair	StartLiceF	StartLiceM	EndLiceF
24.11.2021	1	5	f1	1	8448	27	183	euthanised 3	notag	f012f39	0	2	7
24.11.2021	1	5	f2	2	9094	26,5	187			f022f42	1	3	3
24.11.2021	1	5	f3	3	8753	26,5	201	euthanised 3	8368	f032f34	1	0	5
24.11.2021	1	5	f4	4	57	26,5	191	(real tag number 0057)		f042f36	4	3	4
24.11.2021	1	5	f5	5	9728	25,5	165			f052f33	1	2	6
24.11.2021	1	5	f6	6	8328	26	180	euthanised 3	8321	f062f44	1	3	2
24.11.2021	1	5	f7	7	8542	26,5	183			f072f35	2	2	3
24.11.2021	1	5	f8	8	8726	27,5	192	euthanised 3	8709	f082f31	0	3	2
24.11.2021	1	5	f9	9	8468	28	216			f092f43	0	0	1
24.11.2021	1	5	f10	10	9454	26,5	185			f102f37	0	0	4
24.11.2021	1	5	f11	11	9444	28	218			f112f41	0	0	4
24.11.2021	1	5	f12	12	9108	26,5	189	euthanised 3	8409	f122f45	0	0	6
24.11.2021	1	5	f13	13	9602	27	214	euthanised 3	8561	f132f38	0	0	3
24.11.2021	1	5	f14	14	9654	27,5	215			f142f40	0	0	5
24.11.2021	1	5	f15	15	8688	26,5	185			f152f32	0	0	6
24.11.2021	1	4	f16	16	8652	27	198	euthanised 3	8424	f162f29	1	3	NA
24.11.2021	1	4	f17	17	9681	26	181			f172f26	0	0	0
24.11.2021	1	4	f18	18	8882	26	180			f182f28	3	2	0
24.11.2021	1	4	f19	19	9193	27,5	211			f192f30	1	4	0
24.11.2021	1	4	f20	20	8275	28	223			f202f27	0	2	1
25.11.2021	6	3	f21	1	8603	26	191			f212f23	4	4	1
25.11.2021	6	2	f22	2	8794	26,5	187			f222f19	4	10	1
25.11.2021	6	3	f23	3	9526	27,5	212			f232f21	2	2	2
25.11.2021	6	2	f24	4	8202	26,5	188			f242f10	6	7	0
25.11.2021	6	2	f25	5	8586	26,5	179			f252f16	1	2	0
25.11.2021	6	2	f26	6	9623	28	236			f262f13	4	2	0
25.11.2021	6	2	f27	7	8673	26	165			f272f17	1	4	0
25.11.2021	6	3	f28	8	8230	26	163			f282f24	5	3	0
25.11.2021	6	2	f29	9	9102	26	170			f292f14	3	4	2
25.11.2021	6	2	f30	10	8359	26,5	185			f302f08	4	6	1
25.11.2021	6	2	f31	11	9316	27	179			f312f15	5	6	1
25.11.2021	6	2	f32	12	9170	28	215			f322f11	5	8	1
25.11.2021	6	2	f33	13	8750	27,5	222			f332f06	4	3	0
25.11.2021	6	2	f34	14	9130	26,5	172			f342f12	8	2	0
25.11.2021	6	2	f35	15	8786	27,5	241			f352f20	3	5	1
25.11.2021	6	2	f36	16	8437	28	214			f362f18	5	4	0
25.11.2021	6	2	f37	17	8268	27	197			f372f07	2	1	3
25.11.2021	6	3	f38	18	8559	26,5	195			f382f22	3	2	1
25.11.2021	6	3	f39	19	8614	28	223	euthanised 2	notag	f392f25	3	3	1
25.11.2021	6	2	f40	20	8498	26,5	187			f402f09	5	4	2
25.11.2021	7	8	f41	1	9963	26,5	170			f412f52	7	6	1
25.11.2021	7	8	f42	2	8719	26	174			f422f58	3	4	5
25.11.2021	7	9	f43	3	8775	26,5	194			f432f05	3	2	0
25.11.2021	7	9	f44	4	8628	26,5	184			f442f01	4	3	2
25.11.2021	7	8	f45	5	8253	27	186			f452f48	4	4	3
25.11.2021	7	8	f46	6	8426	28	228			f462f60	5	5	6
25.11.2021	7	9	f47	7	9044	28,5	239	euthanised 3	8577	f472f03	3	4	0
25.11.2021	7	8	f48	8	8255	27,5	199			f482f53	8	6	3
25.11.2021	7	8	f49	9	8638	27,5	204			f492f55	2	1	3
25.11.2021	7	8	f50	10	9438	26,5	174			f502f46	3	2	7
25.11.2021	7	8	f51	11	8417	27,5	222	euthanised 3	9947	f512f56	7	6	2
25.11.2021	7	8	f52	12	9527	27,5	208			f522f51	10	10	1
25.11.2021	7	8	f53	13	8431	26	160			f532f47	4	3	2
25.11.2021	7	8	f54	14	8835	26,5	180			f542f54	2	6	3
25.11.2021	7	8	f55	15	9133	27,5	223			f552f49	4	5	0
25.11.2021	7	9	f56	16	8138	26	170			f562f02	3	2	0
25.11.2021	7	8	f57	17	9287	26,5	173			f572f57	5	4	1
25.11.2021	7	8	f58	18	9175	25,5	165			f582f50	6	4	0
25.11.2021	7	8	f59	19	8598	27,5	246			f592f59	5	4	4
25.11.2021	7	9	f60	20	9705	25,5	168			f602f04	4	0	0
26.04.2022	1	5	2f39	39	8448	38	640	obs! fish hos	notag	f012f39	0	2	7
26.04.2022	1	5	2f42	42	9094	37,5	608			f022f42	1	3	3
26.04.2022	1	5	2f34	34	8753	37	628	obs! fish hos	8368	f032f34	1	0	5
26.04.2022	1	5	2f36	36	57	38,5	735			f042f36	4	3	4
26.04.2022	1	5	2f33	33	9728	35	521			f052f33	1	2	6
26.04.2022	1	5	2f44	44	8328	37	575	obs! fish hos	8321	f062f44	1	3	2
26.04.2022	1	5	2f35	35	8542	38	706			f072f35	2	2	3
26.04.2022	1	5	2f31	31	8726	39	670	obs! fish hos	8709	f082f31	0	3	2
26.04.2022	1	5	2f43	43	8468	37	580			f092f43	0	0	1

Table 2 metadata continued: Part 1 of the complete metadata collected for this study includes the following information: ***DateSampling****: The date when the samples were collected,* ***TankFrom****: Holding tanks used during the acclimatization period,* ***Tank****: Main holding tanks used during the experimental period,* ***FishName****: Unique name assigned to each fish; a number in front indicates the second sampling,* ***FishRank****: Order of the sampling conducted each day,* ***FishTag****: Pit tag number assigned to the fish for identification,* ***Length****: Length of the fish at the beginning of the experiment (in centimeters),* ***Weight****: Weight of the fish at the beginning of the experiment (in grams),* ***Comments****: Additional comments or observations about the fish,* ***Replacement****: If a fish died, the pit tag number of the replacement fish,* ***Pair****: Pairing of fish names from the first and second samplings based on pit number,* ***StartLiceF****: Number of female lice on each fish at the start of the experiment,* ***StartLiceM****: Number of male lice on each fish at the start of the experiment,* ***EndLiceF****: Number of female lice on each fish at the end of the experiment.*

26.04.2022	1	5	2f37	37	9454	37	604			f102f37	0	0	4
26.04.2022	1	5	2f41	41	9444	39,5	714			f112f41	0	0	4
26.04.2022	1	5	2f45	45	9108	38	531	obs! fish hos	8409	f122f45	0	0	6
26.04.2022	1	5	2f38	38	9602	37	650	obs! fish hos	8561	f132f38	0	0	3
26.04.2022	1	5	2f40	40	9654	37,5	606			f142f40	0	0	5
26.04.2022	1	5	2f32	32	8688	38	627			f152f32	0	0	6
26.04.2022	1	4	2f29	29	8652	38	635	obs! fish hos	8424	f162f29	1	3	NA
26.04.2022	1	4	2f26	26	9681	38,5	669			f172f26	0	0	0
26.04.2022	1	4	2f28	28	8882	36	515			f182f28	3	2	0
26.04.2022	1	4	2f30	30	9193	41	779			f192f30	1	4	0
26.04.2022	1	4	2f27	27	8275	40	728			f202f27	0	2	1
26.04.2022	6	3	2f23	23	8603	37	620			f212f23	4	4	1
26.04.2022	6	2	2f19	19	8794	41	811			f222f19	4	10	1
26.04.2022	6	3	2f21	21	9526	40	782			f232f21	2	2	2
26.04.2022	6	2	2f10	10	8202	38	669			f242f10	6	7	0
26.04.2022	6	2	2f16	16	8586	39	632			f252f16	1	2	0
26.04.2022	6	2	2f13	13	9623	40,5	830			f262f13	4	2	0
26.04.2022	6	2	2f17	17	8673	36	553			f272f17	1	4	0
26.04.2022	6	3	2f24	24	8230	38	601			f282f24	5	3	0
26.04.2022	6	2	2f14	14	9102	37	605			f292f14	3	4	2
26.04.2022	6	2	2f8	8	8359	37,5	632			f302f08	4	6	1
26.04.2022	6	2	2f15	15	9316	40,5	763			f312f15	5	6	1
26.04.2022	6	2	2f11	11	9170	39,5	789			f322f11	5	8	1
26.04.2022	6	2	2f6	6	8750	40	841			f332f06	4	3	0
26.04.2022	6	2	2f12	12	9130	39,5	699			f342f12	8	2	0
26.04.2022	6	2	2f20	20	8786	31	338			f352f20	3	5	1
26.04.2022	6	2	2f18	18	8437	40,5	766			f362f18	5	4	0
26.04.2022	6	2	2f7	7	8268	38,5	707			f372f07	2	1	3
26.04.2022	6	3	2f22	22	8559	39	732			f382f22	3	2	1
26.04.2022	6	3	2f25	25	8614	36,5	542	obs! fish hos	notag	f392f25	3	3	1
26.04.2022	6	2	2f9	9	8498	38	659			f402f09	5	4	2
02.06.2022	7	8	2f52	7	9963	40	720			f412f52	7	6	1
02.06.2022	7	8	2f58	13	8719	37	608			f422f58	3	4	5
26.04.2022	7	9	2f5	5	8775	39,5	705			f432f05	3	2	0
26.04.2022	7	9	2f1	1	8628	37	578			f442f01	4	3	2
02.06.2022	7	8	2f48	3	8253	40	778	eye infection		f452f48	4	4	3
02.06.2022	7	8	2f60	15	8426	41	865			f462f60	5	5	6
26.04.2022	7	9	2f3	3	9044	36	532	obs! fish hos	8577	f472f03	3	4	0
02.06.2022	7	8	2f53	8	8255	38	650			f482f53	8	6	3
02.06.2022	7	8	2f55	10	8638	42	892			f492f55	2	1	3
02.06.2022	7	8	2f46	1	9438	41	815			f502f46	3	2	7
02.06.2022	7	8	2f56	11	8417	38,5	640	obs! fish hos	9947	f512f56	7	6	2
02.06.2022	7	8	2f51	6	9527	41	887			f522f51	10	10	1
02.06.2022	7	8	2f47	2	8431	39	694			f532f47	4	3	2
02.06.2022	7	8	2f54	9	8835	41,5	896			f542f54	2	6	3
02.06.2022	7	8	2f49	4	9133	40,5	861			f552f49	4	5	0
26.04.2022	7	9	2f2	2	8138	39	737			f562f02	3	2	0
02.06.2022	7	8	2f57	12	9287	43	938			f572f57	5	4	1
02.06.2022	7	8	2f50	5	9175	37,5	692	itag lost		f582f50	6	4	0
02.06.2022	7	8	2f59	14	8598	28,5	258			f592f59	5	4	4
26.04.2022	7	9	2f4	4	9705	38	675			f602f04	4	0	0
24.11.2021	1	1	T1	1	0	0	0	0	0	0	0	0	0
24.11.2021	2	2	T2	2	0	0	0	0	0	0	0	0	0
24.11.2021	3	3	T3	3	0	0	0	0	0	0	0	0	0
24.11.2021	4	4	T4	4	0	0	0	0	0	0	0	0	0
24.11.2021	5	5	T5	5	0	0	0	0	0	0	0	0	0
24.11.2021	6	6	T6	6	0	0	0	0	0	0	0	0	0
24.11.2021	7	7	T7	7	0	0	0	0	0	0	0	0	0
24.11.2021	8	8	T8	8	0	0	0	0	0	0	0	0	0
26.04.2022	2	2	F2T2	1	0	0	0	0	0	0	0	0	0
26.04.2022	10	10	ROM2f2T2	2	0	0	0	0	0	0	0	0	0
26.04.2022	3	3	F2T3	3	0	0	0	0	0	0	0	0	0
26.04.2022	4	4	F2T4	4	0	0	0	0	0	0	0	0	0
26.04.2022	5	5	F2T5	5	0	0	0	0	0	0	0	0	0
02.06.2022	8	8	f2t8	6	0	0	0	0	0	0	0	0	0
24.11.2021	0	0	b1	0	0	0	0	blank table	0	0	0	0	0
24.11.2021	0	0	b2	0	0	0	0	blank wipes	0	0	0	0	0
25.11.2021	0	0	b3	0	0	0	0	blank table	0	0	0	0	0
25.11.2021	0	0	b4	0	0	0	0	blank wipes	0	0	0	0	0
26.04.2022	0	0	b1f2	0	0	0	0	blank table	0	0	0	0	0
26.04.2022	0	0	b2f2	0	0	0	0	blank wipes	0	0	0	0	0
27.12.2022	0	0	n1	0	0	0	0	negative con	0	0	0	0	0
27.12.2022	0	0	n2	0	0	0	0	negative con	0	0	0	0	0
27.12.2022	0	0	NEG	0	0	0	0	negative con	0	0	0	0	0
27.12.2022	0	0	m1	0	0	0	0	Mock	0	0	0	0	0
27.12.2022	0	0	m2	0	0	0	0	Mock	0	0	0	0	0
27.12.2022	0	0	MOC	0	0	0	0	Mock	0	0	0	0	0
27.12.2022	0	0	Combined_N	0	0	0	0	Mock	0	0	0	0	0

Table 3 metadata continued: Part 2 of the complete metadata collected for this study includes the following information: ***EndLiceM****: Number of male lice on each fish at the end of the experiment,* ***MeanLiceF****: Mean number of female lice on each fish during the experiment,* ***MeanLiceM****: Mean number of male lice on each fish during the experiment,* ***MaxLiceF****: Maximum number of female lice observed on each fish,* ***MaxLiceM****: Maximum number of male lice observed on each fish,* ***LiceStart****: Total number of lice on each fish at the start of the experiment,* ***LiceEnd****: Total number of lice on each fish at the end of the experiment,* ***TotLice****: Total number of lice observed on each fish during the entire experiment,* ***MeanLice****: Average number of lice during the entire experimental period on each fish,* ***DaysInTank****: Total number of days a fish spent in a given tank,* ***TotalGrowth****: Total weight gain (in grams) of the fish during the experimental period,* ***GrowthPerDay****: Weight gain (in grams) per day during the experiment,* ***VolumeFiltered****: Amount (ml) of water filtered through the Sterivex filters****, Env****: Indicates whether it is a negative control (blank and negative control), positive sample (water sample or mucus sample), or mock sample (positive control),* ***Type****: Type of sample, including fish first sampling, fish second sampling, blank, water sample, negative control, or positive control.*

EndLiceM	MeanLiceF	MeanLiceM	MaxLiceF	MaxLiceM	LiceStart	EndLice	TotLice	MeanLice	DaysInTank	TotalGrowth	GrowthPerD	volumefilter	env	Type
5	NA	NA	NA	NA	2	12	14	7	153	457	2,98692811	0	positive	fish_01
1	1,32	1,5	4	7	4	4	8	4	153	421	2,75163399	0	positive	fish_01
3	NA	NA	NA	NA	1	8	9	4,5	153	427	2,79084967	0	positive	fish_01
3	1,82	2,23	4	6	7	7	14	7	153	544	3,55555556	0	positive	fish_01
1	1,77	1,77	6	6	3	7	10	5	153	356	2,32679739	0	positive	fish_01
6	NA	NA	NA	NA	4	8	12	6	153	395	2,58169935	0	positive	fish_01
4	1,5	1,77	4	5	4	7	11	5,5	153	523	3,41830065	0	positive	fish_01
6	NA	NA	NA	NA	3	8	11	5,5	153	478	3,12418301	0	positive	fish_01
4	2,14	2,55	6	6	0	5	5	2,5	153	364	2,37908497	0	positive	fish_01
3	2,05	2,45	5	6	0	7	7	3,5	153	419	2,73856209	0	positive	fish_01
6	3,82	3,09	6	9	0	10	10	5	153	496	3,24183007	0	positive	fish_01
2	NA	NA	NA	NA	0	8	8	4	153	342	2,23529412	0	positive	fish_01
7	NA	NA	NA	NA	0	10	10	5	153	436	2,8496732	0	positive	fish_01
3	1,68	2,14	5	6	0	8	8	4	153	391	2,55555556	0	positive	fish_01
3	2,54	2,73	7	9	0	9	9	4,5	153	442	2,88888889	0	positive	fish_01
NA	NA	NA	NA	NA	4	0	4	2	153	437	2,85620915	0	positive	fish_01
1	0,14	0,68	2	2	0	1	1	0,5	153	488	3,18954248	0	positive	fish_01
0	0,41	0,36	3	2	5	0	5	2,5	153	335	2,18954248	0	positive	fish_01
0	0,14	0,59	1	4	5	0	5	2,5	153	568	3,7124183	0	positive	fish_01
0	0,55	0,5	3	2	2	1	3	1,5	153	505	3,3006536	0	positive	fish_01
3	1,62	1,86	4	4	8	4	12	6	152	429	2,82236842	0	positive	fish_01
9	1,76	3,57	4	11	14	10	24	12	152	624	4,10526316	0	positive	fish_01
1	1,81	2,24	4	7	4	3	7	3,5	152	570	3,75	0	positive	fish_01
7	0,76	1,53	6	7	13	7	20	10	152	481	3,16447368	0	positive	fish_01
3	1	1,81	5	5	3	3	6	3	152	453	2,98026316	0	positive	fish_01
13	1,48	3,9	6	13	6	13	19	9,5	152	594	3,90789474	0	positive	fish_01
2	1,1	1,19	3	4	5	2	7	3,5	152	388	2,55263158	0	positive	fish_01
4	0,62	2,42	5	5	8	4	12	6	152	438	2,88157895	0	positive	fish_01
5	1,57	1,61	4	5	7	7	14	7	152	435	2,86184211	0	positive	fish_01
3	0,95	1,76	4	6	10	4	14	7	152	447	2,94078947	0	positive	fish_01
8	1,52	2,38	5	8	11	9	20	10	152	584	3,84210526	0	positive	fish_01
3	2,76	4,33	5	11	13	4	17	8,5	152	574	3,77631579	0	positive	fish_01
5	2,38	3	7	6	7	5	12	6	152	619	4,07236842	0	positive	fish_01
9	1,24	2,52	8	9	10	9	19	9,5	152	527	3,46710526	0	positive	fish_01
3	1,33	2,05	5	5	8	4	12	6	152	97	0,6381579	0	positive	fish_01
6	1,48	3,71	6	6	9	6	15	7,5	152	552	3,63157895	0	positive	fish_01
9	2,19	2,81	8	9	3	12	15	7,5	152	510	3,35526316	0	positive	fish_01
1	2,05	1,48	5	3	5	2	7	3,5	152	537	3,53289474	0	positive	fish_01
2	NA	NA	NA	NA	6	3	9	4,5	152	319	2,09868421	0	positive	fish_01
9	1,05	2,48	5	9	9	11	20	10	152	472	3,10526316	0	positive	fish_01
0	1,17	1,85	7	6	13	1	14	7	189	550	2,91005291	0	positive	fish_01
2	1,25	1,4	5	5	7	7	14	7	189	434	2,2962963	0	positive	fish_01
1	0,71	1,76	3	4	5	1	6	3	152	511	3,36184211	0	positive	fish_01
1	1,14	0,33	4	3	7	3	10	5	152	394	2,59210526	0	positive	fish_01
3	0,68	2,11	4	7	8	6	14	7	189	592	3,13227513	0	positive	fish_01
4	2,26	2,6	6	8	10	10	20	10	189	637	3,37037037	0	positive	fish_01
0	NA	NA	NA	NA	7	0	7	3,5	152	293	1,92763158	0	positive	fish_01
3	1,74	1,55	8	6	14	6	20	10	189	451	2,38624339	0	positive	fish_01
3	1,02	1,96	5	4	3	6	9	4,5	189	688	3,64021164	0	positive	fish_01
5	0,94	2,11	7	7	5	12	17	8,5	189	641	3,39153439	0	positive	fish_01
3	NA	NA	NA	NA	13	5	18	9	189	418	2,21164021	0	positive	fish_01
5	0,64	1,92	10	10	20	6	26	13	189	679	3,59259259	0	positive	fish_01
3	1,62	2,41	4	5	7	5	12	6	189	534	2,82539683	0	positive	fish_01
3	0,79	1,32	4	6	8	6	14	7	189	716	3,78835979	0	positive	fish_01
2	1,13	2,22	5	5	9	2	11	5,5	189	638	3,37566138	0	positive	fish_01
0	0,67	0,52	3	2	5	0	5	2,5	152	567	3,73026316	0	positive	fish_01
2	1,92	1,89	5	6	9	3	12	6	189	765	4,04761905	0	positive	fish_01
4	1,36	1,7	6	4	10	4	14	7	189	527	2,78835979	0	positive	fish_01
3	1,47	1,43	5	5	9	7	16	8	189	12	0,06349206	0	positive	fish_01
0	0,38	0,62	4	2	4	0	4	2	152	507	3,33552632	0	positive	fish_01
5	NA	NA	NA	NA	2	12	14	7	153	457	2,98692811	0	positive	fish_02
1	1,32	1,5	4	7	4	4	8	4	153	421	2,75163399	0	positive	fish_02
3	NA	NA	NA	NA	1	8	9	4,5	153	427	2,79084967	0	positive	fish_02
3	1,82	2,23	4	6	7	7	14	7	153	544	3,55555556	0	positive	fish_02
1	1,77	1,77	6	6	3	7	10	5	153	356	2,32679739	0	positive	fish_02
6	NA	NA	NA	NA	4	8	12	6	153	395	2,58169935	0	positive	fish_02
4	1,5	1,77	4	5	4	7	11	5,5	153	523	3,41830065	0	positive	fish_02
6	NA	NA	NA	NA	3	8	11	5,5	153	478	3,12418301	0	positive	fish_02
4	2,14	2,55	6	6	0	5	5	2,5	153	364	2,37908497	0	positive	fish_02

Table 4 metadata continued: Continuation of Part 2 of the complete metadata collected for this study includes the following information: ***EndLiceM****: Number of male lice on each fish at the end of the experiment,* ***MeanLiceF****: Mean number of female lice on each fish during the experiment,* ***MeanLiceM****: Mean number of male lice on each fish during the experiment,* ***MaxLiceF****: Maximum number of female lice observed on each fish,* ***MaxLiceM****: Maximum number of male lice observed on each fish,* ***LiceStart****: Total number of lice on each fish at the start of the experiment,* ***LiceEnd****: Total number of lice on each fish at the end of the experiment,* ***TotLice****: Total number of lice observed on each fish during the entire experiment,* ***MeanLice****: Average number of lice during the entire experimental period on each fish,* ***DaysInTank****: Total number of days a fish spent in a given tank,* ***TotalGrowth****: Total weight gain (in grams) of the fish during the experimental period,* ***GrowthPerDay****: Weight gain (in grams) per day during the experiment,* ***VolumeFiltered****: Amount (ml) of water filtered through the Sterivex filters****, Env****: Indicates whether it is a negative control (blank and negative control), positive sample (water sample or mucus sample), or mock sample (positive control),* ***Type****: Type of sample, including fish first sampling, fish second sampling, blank, water sample, negative control, or positive control.*

3	2,05	2,45	5	6	0	7	7	3,5	153	419	2,73856209	0	positive	fish_02
6	3,82	3,09	6	9	0	10	10	5	153	496	3,24183007	0	positive	fish_02
2	NA	NA	NA	NA	0	8	8	4	153	342	2,23529412	0	positive	fish_02
7	NA	NA	NA	NA	0	10	10	5	153	436	2,8496732	0	positive	fish_02
3	1,68	2,14	5	6	0	8	8	4	153	391	2,55555556	0	positive	fish_02
3	2,54	2,73	7	9	0	9	9	4,5	153	442	2,88888889	0	positive	fish_02
NA	NA	NA	NA	NA	4	0	4	2	153	437	2,85620915	0	positive	fish_02
1	0,14	0,68	2	2	0	1	1	0,5	153	488	3,18954248	0	positive	fish_02
0	0,41	0,36	3	2	5	0	5	2,5	153	335	2,18954248	0	positive	fish_02
0	0,14	0,59	1	4	5	0	5	2,5	153	568	3,7124183	0	positive	fish_02
0	0,55	0,5	3	2	2	1	3	1,5	153	505	3,3006536	0	positive	fish_02
3	1,62	1,86	4	4	8	4	12	6	152	429	2,82236842	0	positive	fish_02
9	1,76	3,57	4	11	14	10	24	12	152	624	4,10526316	0	positive	fish_02
1	1,81	2,24	4	7	4	3	7	3,5	152	570	3,75	0	positive	fish_02
7	0,76	1,53	6	7	13	7	20	10	152	481	3,16447368	0	positive	fish_02
3	1	1,81	5	5	3	3	6	3	152	453	2,98026316	0	positive	fish_02
13	1,48	3,9	6	13	6	13	19	9,5	152	594	3,90789474	0	positive	fish_02
2	1,1	1,19	3	4	5	2	7	3,5	152	388	2,55263158	0	positive	fish_02
4	0,62	2,42	5	5	8	4	12	6	152	438	2,88157895	0	positive	fish_02
5	1,57	1,61	4	5	7	7	14	7	152	435	2,86184211	0	positive	fish_02
3	0,95	1,76	4	6	10	4	14	7	152	447	2,94078947	0	positive	fish_02
8	1,52	2,38	5	8	11	9	20	10	152	584	3,84210526	0	positive	fish_02
3	2,76	4,33	5	11	13	4	17	8,5	152	574	3,77631579	0	positive	fish_02
5	2,38	3	7	6	7	5	12	6	152	619	4,07236842	0	positive	fish_02
9	1,24	2,52	8	9	10	9	19	9,5	152	527	3,46710526	0	positive	fish_02
3	1,33	2,05	5	5	8	4	12	6	152	97	0,6381579	0	positive	fish_02
6	1,48	3,71	6	6	9	6	15	7,5	152	552	3,63157895	0	positive	fish_02
9	2,19	2,81	8	9	3	12	15	7,5	152	510	3,35526316	0	positive	fish_02
1	2,05	1,48	5	3	5	2	7	3,5	152	537	3,53289474	0	positive	fish_02
2	NA	NA	NA	NA	6	3	9	4,5	152	319	2,09868421	0	positive	fish_02
9	1,05	2,48	5	9	9	11	20	10	152	472	3,10526316	0	positive	fish_02
0	1,17	1,85	7	6	13	1	14	7	189	550	2,91005291	0	positive	fish_02
2	1,25	1,4	5	5	7	7	14	7	189	434	2,2962963	0	positive	fish_02
1	0,71	1,76	3	4	5	1	6	3	152	511	3,36184211	0	positive	fish_02
1	1,14	0,33	4	3	7	3	10	5	152	394	2,59210526	0	positive	fish_02
3	0,68	2,11	4	7	8	6	14	7	189	592	3,13227513	0	positive	fish_02
4	2,26	2,6	6	8	10	10	20	10	189	637	3,37037037	0	positive	fish_02
0	NA	NA	NA	NA	7	0	7	3,5	152	293	1,92763158	0	positive	fish_02
3	1,74	1,55	8	6	14	6	20	10	189	451	2,38624339	0	positive	fish_02
3	1,02	1,96	5	4	3	6	9	4,5	189	688	3,64021164	0	positive	fish_02
5	0,94	2,11	7	7	5	12	17	8,5	189	641	3,39153439	0	positive	fish_02
3	NA	NA	NA	NA	13	5	18	9	189	418	2,21164021	0	positive	fish_02
5	0,64	1,92	10	10	20	6	26	13	189	679	3,59259259	0	positive	fish_02
3	1,62	2,41	4	5	7	5	12	6	189	534	2,82539683	0	positive	fish_02
3	0,79	1,32	4	6	8	6	14	7	189	716	3,78835979	0	positive	fish_02
2	1,13	2,22	5	5	9	2	11	5,5	189	638	3,37566138	0	positive	fish_02
0	0,67	0,52	3	2	5	0	5	2,5	152	567	3,73026316	0	positive	fish_02
2	1,92	1,89	5	6	9	3	12	6	189	765	4,04761905	0	positive	fish_02
4	1,36	1,7	6	4	10	4	14	7	189	527	2,78835979	0	positive	fish_02
3	1,47	1,43	5	5	9	7	16	8	189	12	0,06349206	0	positive	fish_02
0	0,38	0,62	4	2	4	0	4	2	152	507	3,33552632	0	positive	fish_02
0	0	0	0	0	0	0	0	0	0	0	0	960	positive	water_sampl
0	0	0	0	0	0	0	0	0	0	0	0	960	positive	water_sampl
0	0	0	0	0	0	0	0	0	0	0	0	960	positive	water_sampl
0	0	0	0	0	0	0	0	0	0	0	0	960	positive	water_sampl
0	0	0	0	0	0	0	0	0	0	0	0	960	positive	water_sampl
0	0	0	0	0	0	0	0	0	0	0	0	960	positive	water_sampl
0	0	0	0	0	0	0	0	0	0	0	0	960	positive	water_sampl
0	0	0	0	0	0	0	0	0	0	0	0	960	negative	water_sampl
0	0	0	0	0	0	0	0	0	0	0	0	960	negative	water_sampl
0	0	0	0	0	0	0	0	0	0	0	0	960	negative	water_sampl
0	0	0	0	0	0	0	0	0	0	0	0	960	negative	water_sampl
0	0	0	0	0	0	0	0	0	0	0	0	960	negative	water_sampl
0	0	0	0	0	0	0	0	0	0	0	0	960	negative	water_sampl
0	0	0	0	0	0	0	0	0	0	0	0	960	negative	water_sampl
0	0	0	0	0	0	0	0	0	0	0	0	0	positive	blank
0	0	0	0	0	0	0	0	0	0	0	0	0	positive	blank
0	0	0	0	0	0	0	0	0	0	0	0	0	positive	blank
0	0	0	0	0	0	0	0	0	0	0	0	0	positive	blank
0	0	0	0	0	0	0	0	0	0	0	0	0	negative	blank
0	0	0	0	0	0	0	0	0	0	0	0	0	negative	blank
0	0	0	0	0	0	0	0	0	0	0	0	0	negative	neg
0	0	0	0	0	0	0	0	0	0	0	0	0	negative	neg
0	0	0	0	0	0	0	0	0	0	0	0	0	negative	neg
0	0	0	0	0	0	0	0	0	0	0	0	0	mock	mock
0	0	0	0	0	0	0	0	0	0	0	0	0	mock	mock
0	0	0	0	0	0	0	0	0	0	0	0	0	mock	mock
0	0	0	0	0	0	0	0	0	0	0	0	0	mock	mock

2 Metadata collection, analysis, and results.

Metadata was collected during both the first and second sampling. The data collected included physical measurements (length and weight) at the start and end, pit tag number, fish name, number of lice per fish after infection, number of lice per fish at the end of the experiment, gender of the lice, and other comments such as death. Table 5 provides a summary of the ranges, means, and medians for the metadata variables used in various parts of the analysis.

The aim of conducting a metadata analysis was to investigate potential confounding effects, identify clear outliers, and detect any errors. First, the metadata was loaded into R (4.2.1) for simple auto-correlation analysis using the "*ggcorrplot*" R package. A simple Principal Component Analysis (PCA) was then performed on the metadata (using the "*ggfortify*" package in R). Boxplots (base R) were also created for parts of the data, focusing on how average lice varied with tank as a factor and how average weight varied with tank.

Table 5: Summary of metadata variable ranges, note 1 = start of the experiment, 2* = end of the experiment. Additional note: The entire set of variables is not represented here for readability purposes.* ***TotalGrowth****: Total weight gain (in grams) of the fish during the experimental period, Weight gain (in grams) per day during the experiment,* ***LiceStart****: Total number of lice on each fish at the start of the experiment,* ***LiceEnd****: Total number of lice on each fish at the end of the experiment,* ***MeanLice****: Average number of lice during the entire experimental period on each fish*

Variable	Min	Max	Mean	Median
Length1* (cm)	25.50	28.50	26.85	26.50
Length2* (cm)	28.50	43.00	38.38	38.25
Weight1* (g)	160.0	246.0	194.8	188.5
Weight2* (g)	258.0	938.0	678.5	669.5
TotalGrowth (g)	12.0	765.0	483.8	484.5
GrowthPerDay (g/day)	0.06349	4.10526	2.99879	3.04610
LiceStart (Lice/fish)	0.000	20.000	6.267	6.000
LiceEnd (Lice/fish)	0.000	13.000	5.533	6.000
MeanLice ((LiceStart + LiceEnd)/2)	0.500	13.000	5.900	6.000

Auto-correlation analysis, as presented in Figure 4, reveals a strong correlation among growth parameters, indicating their interdependency. Most notably, there are strong correlations among lice parameters. Tank correlates only very weakly with the other variables in this meta-data examination.

In the PCA plot, as presented in Figure 5, PC1 explains 44.68% of the dataset's variation and primarily comprises variables correlated with lice parameters, e. i lice parameters seem to be autocorrelated. It's observed that start weight and length are autocorrelated weakly with lice variables. PC2 explains 22.97% of the variation and only encompasses growth parameters, meaning that end weight, length, growth per day, and total growth have strong correlations. No clear clustering of individual fish because of tanks was observed.

Figure 6 illustrates the distribution of lice per tank and the corresponding end weight per tank. The analysis demonstrates a correlation between the number of lice and the tanks, indicating a tank-related effect. However, no significant correlation is observed between the weight of the fish and the tanks.

The results of the metadata analyses consistently demonstrate strong correlations among certain variables. Specifically, at the end of the experiment, the different growth parameters exhibit a strong correlation with each other. The metadata analysis confirms the expected interconnections among several lice parameters. Furthermore, the analysis indicates that the tank does not affect fish growth but reflects the anticipated variations in lice intensity across different tanks.

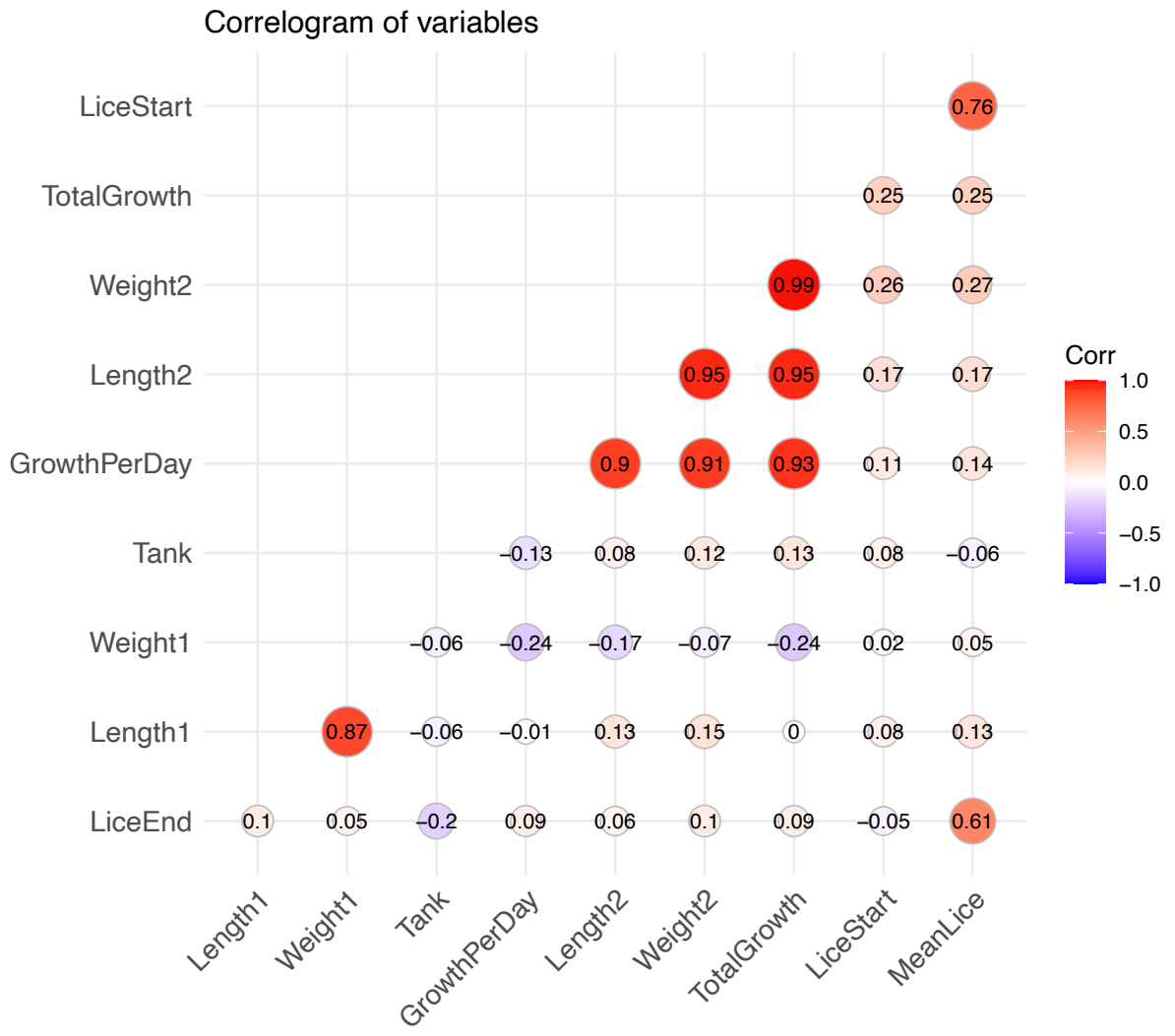

Figure 1: Correlogram of variables in metadata excluding sex-effect variables. The intensity of colors and higher numbers indicate the strength of the correlation. The warmer colors indicate positive correlations, while the colder colors indicate negative correlations.

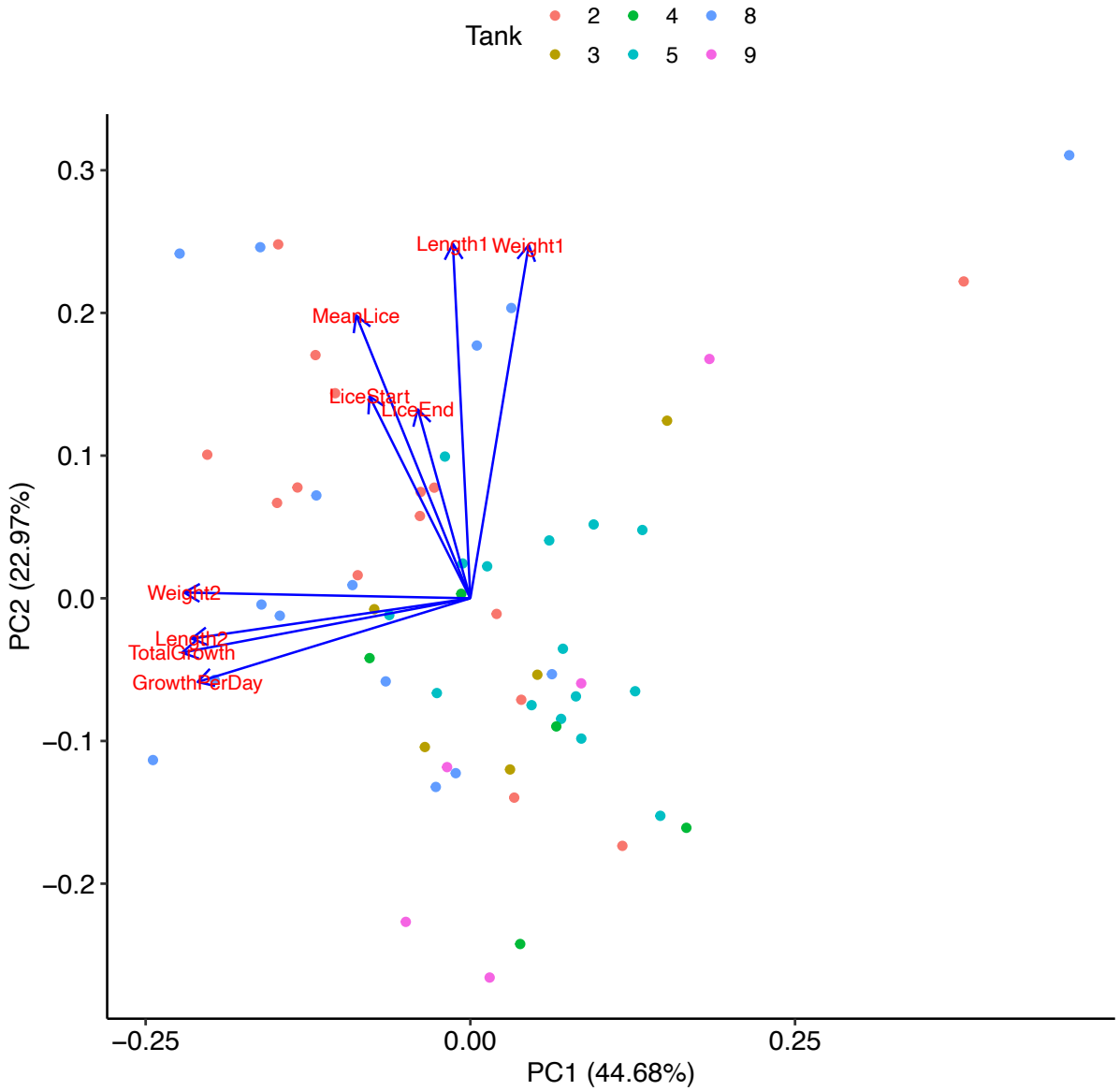

Figure 2: PCA plot of metadata variables excluding sex-effect variables. Dots are color coded to reflect the tank the fish lived in, and dots are individual fish. Arrows are the effect of the variables. Lice variables mainly explain the first ***axis (PC1),*** *and the second* ***axis (PC2****) is primarily described by growth parameters.*

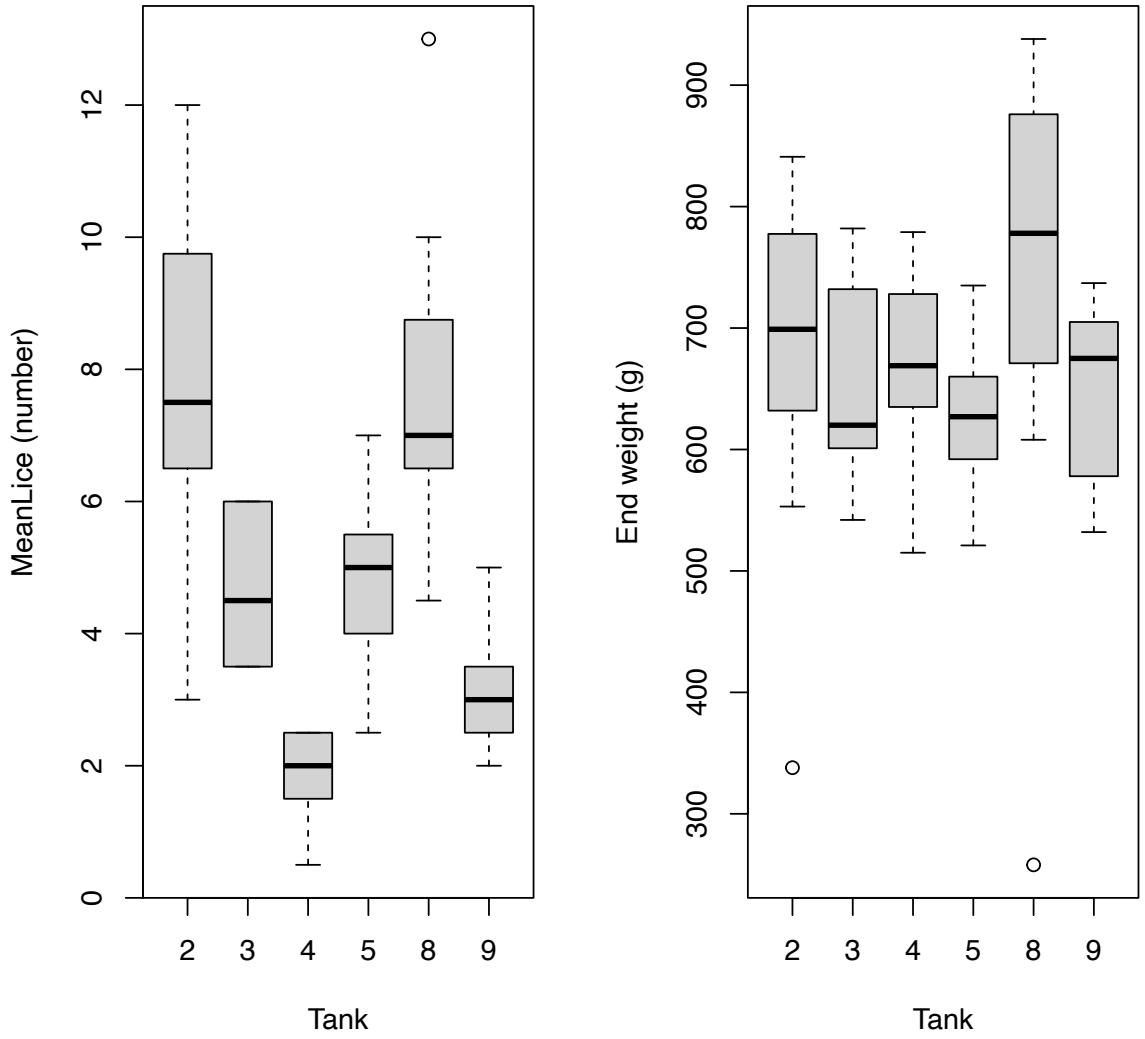

Figure 3: (Left) Boxplot shows the distribution of mean lice per fish per tank. ***X -axis*** *is the tank´s name,* ***Y-axis*** *is the mean number of lice per fish. (Right) Boxplot illustrates the end weight per fish per tank.* ***X -axis*** *is the tank's name, and* ***Y-axis*** *is the end weight of the fish.*

3 Mock – community optimization procedure additional figures

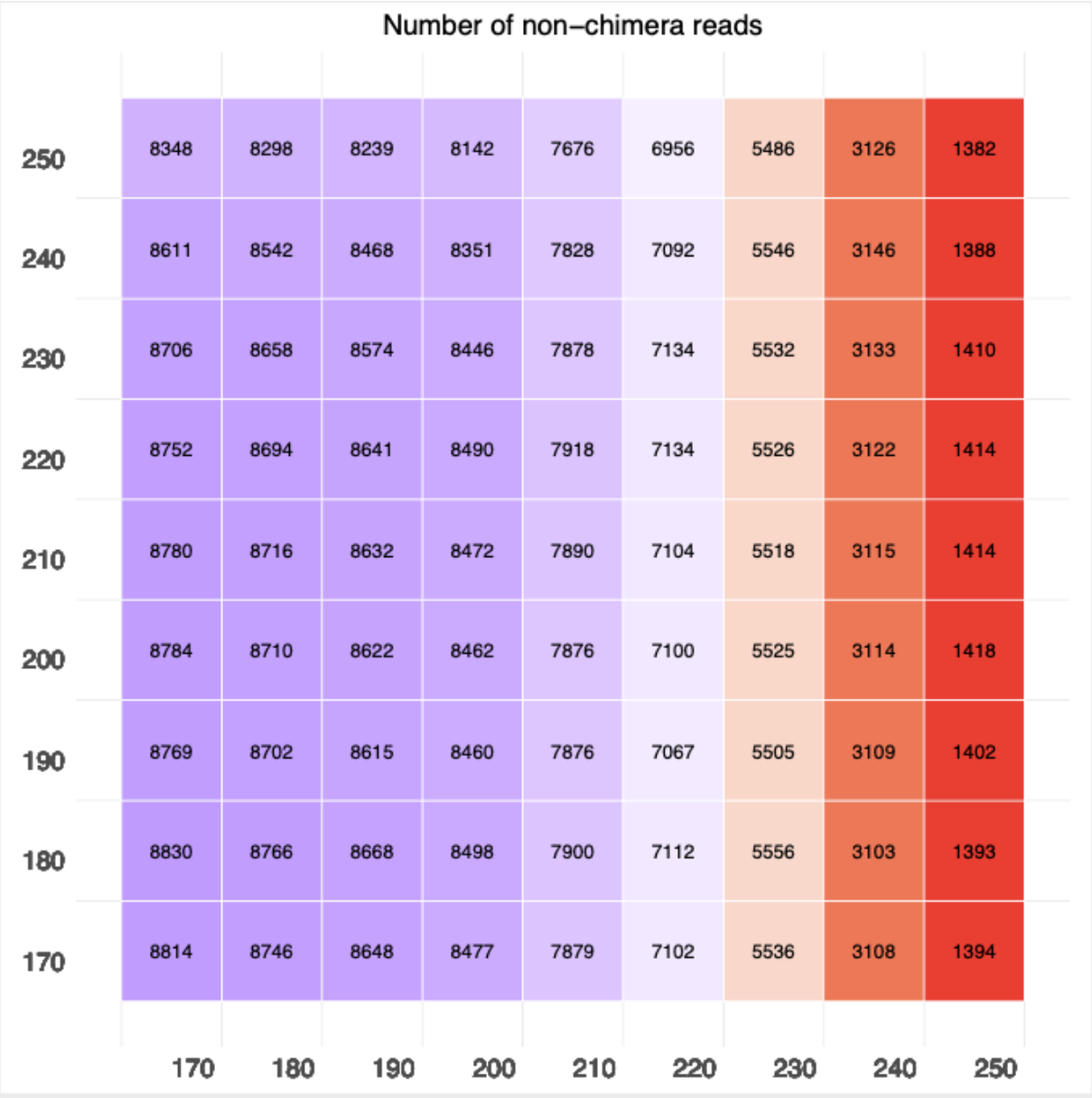

*Figure 4: Heatmap illustrating the number of non-chimera reads yielded for different length combinations of forward and reverse reads. The **X-axis** is the reverse read length in base pairs, and **Y-axis** is the forward read length in base pairs. Higher numbers and colder colors indicate a higher number of non-chimera reads.*

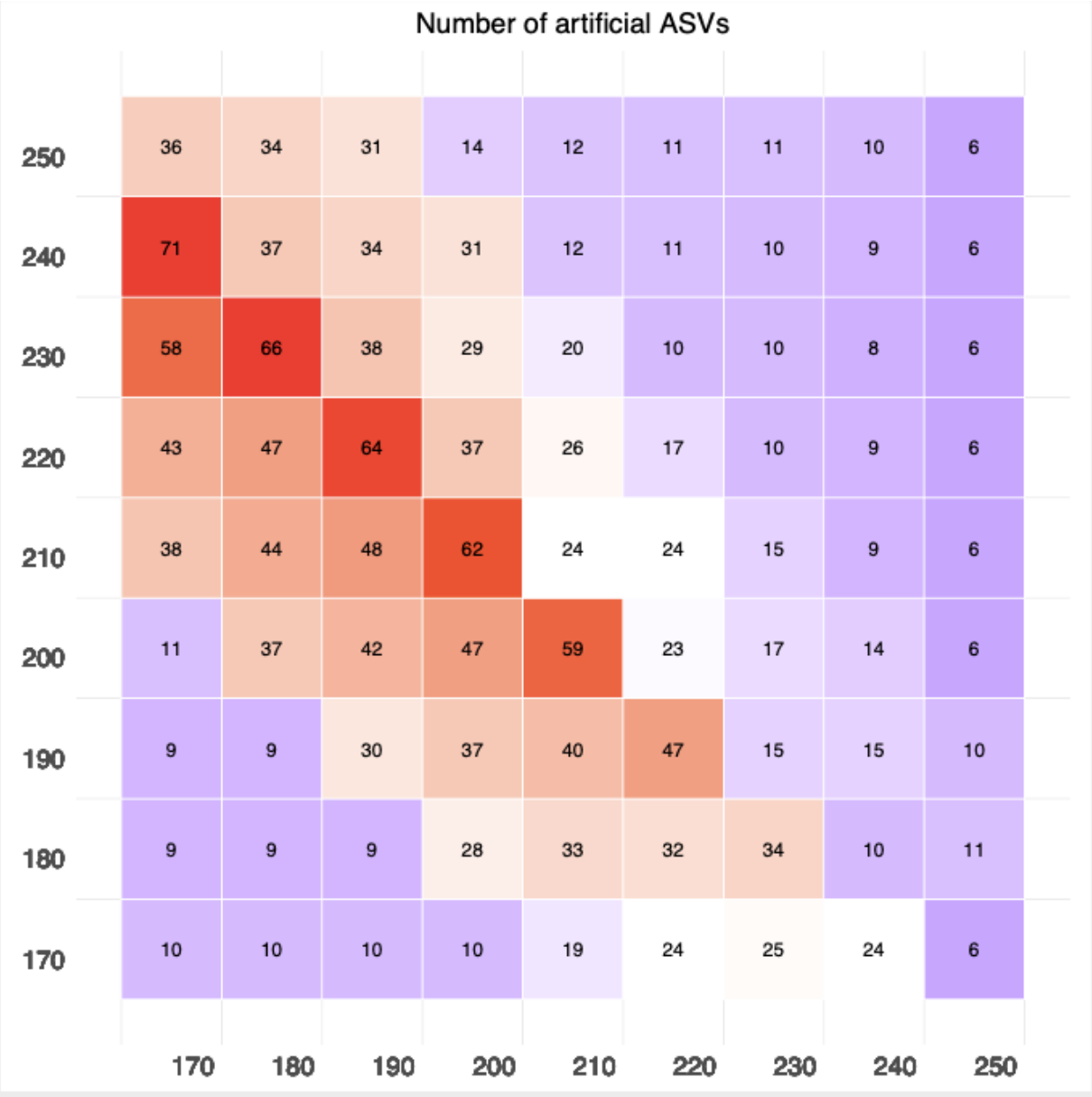

Figure 5: Heatmap illustrating the number of artificial ASVs, yielded for different length combinations of forward and reverse reads. The ***X-axis*** *is the reverse read length in base pairs, and* ***Y-axis*** *is the forward read length in base pairs. Lower numbers and colder colors indicate a lower number of artificial ASVs.*

4 Maximum likelihood mixed linear model figure: Mean lice effect on alpha diversity(Log(ACE)).

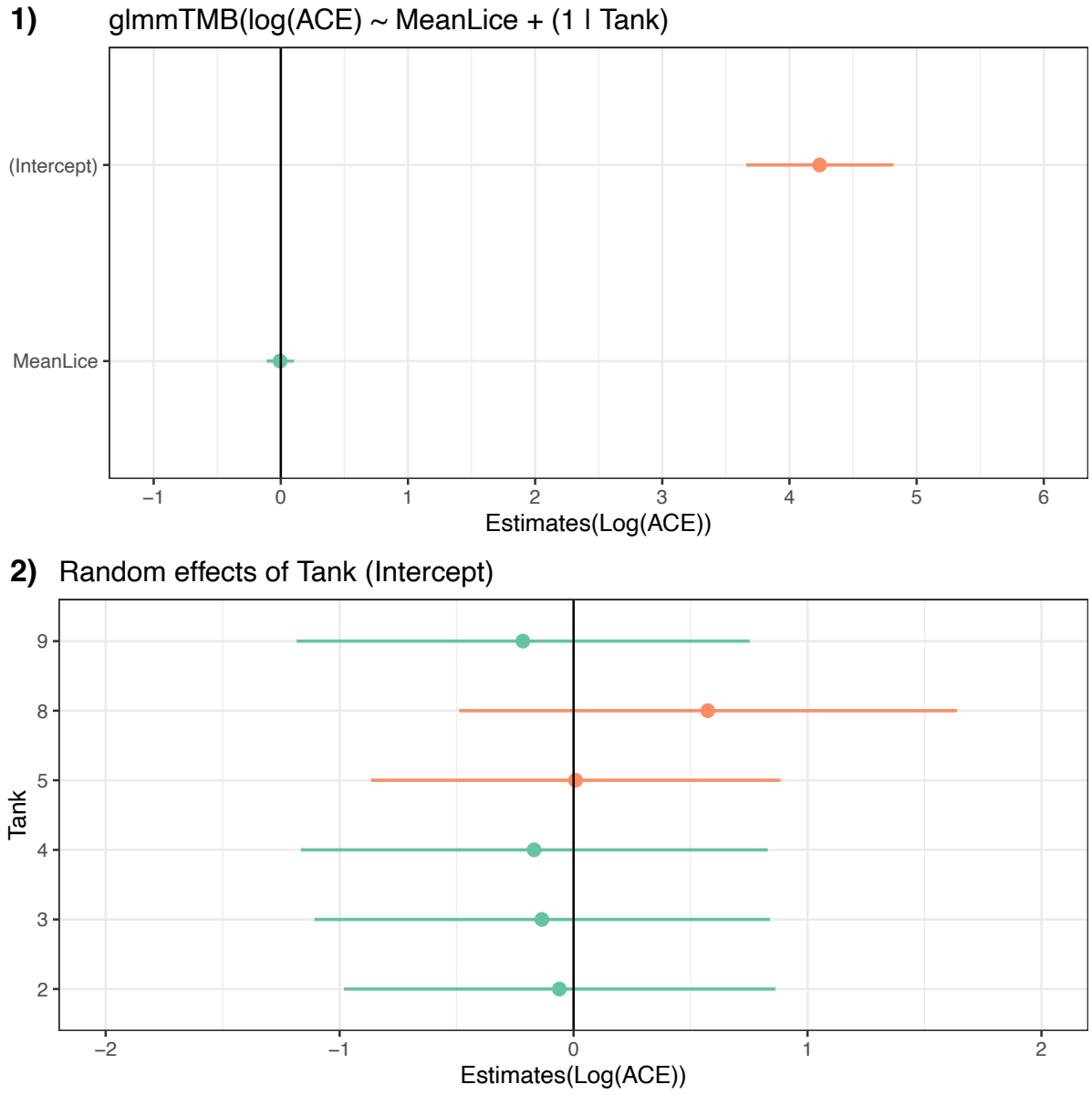

Figure 6: The plot shows the point estimates and confidence intervals for fixed and random effects. Orange coloring indicates positive numbers and greenish colors indicate negative numbers for the point estimate. 1) Plot of fixed effects. The intercept is the mean log(ACE) of all fish without lice. The slope is the effect of MeanLice on log(ACE). The confidence interval of the MeanLice effect overlaps the zero line and is non-significant. 2) Plot of random effects of the tanks.

5 Mixed linear models (Maximum likelihood and Bayesian) figures: Mean lice effect on alpha diversity Simpson.

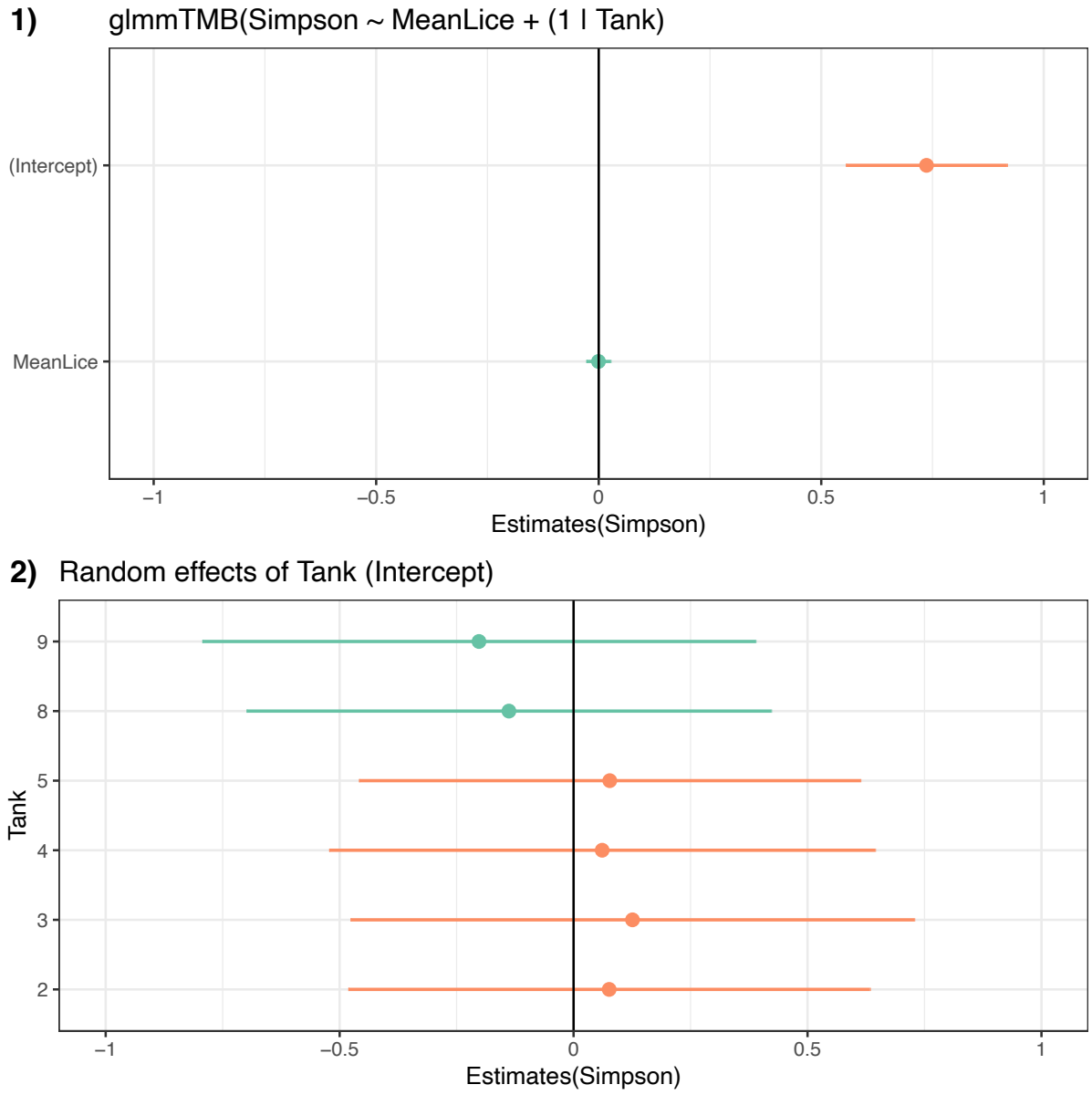

*Figure 7: The plot shows the point estimates and confidence intervals for fixed and random effects. Orange coloring indicates positive numbers and greenish colors indicate negative numbers for the point estimate. 1) Plot of fixed effects. The intercept is the mean **Simpson** of all fish without lice. The slope is the effect of MeanLice on **Simpson**. The confidence interval of the MeanLice effect overlaps the zero line and is non-significant. 2) Plot of random effects of the tanks.*

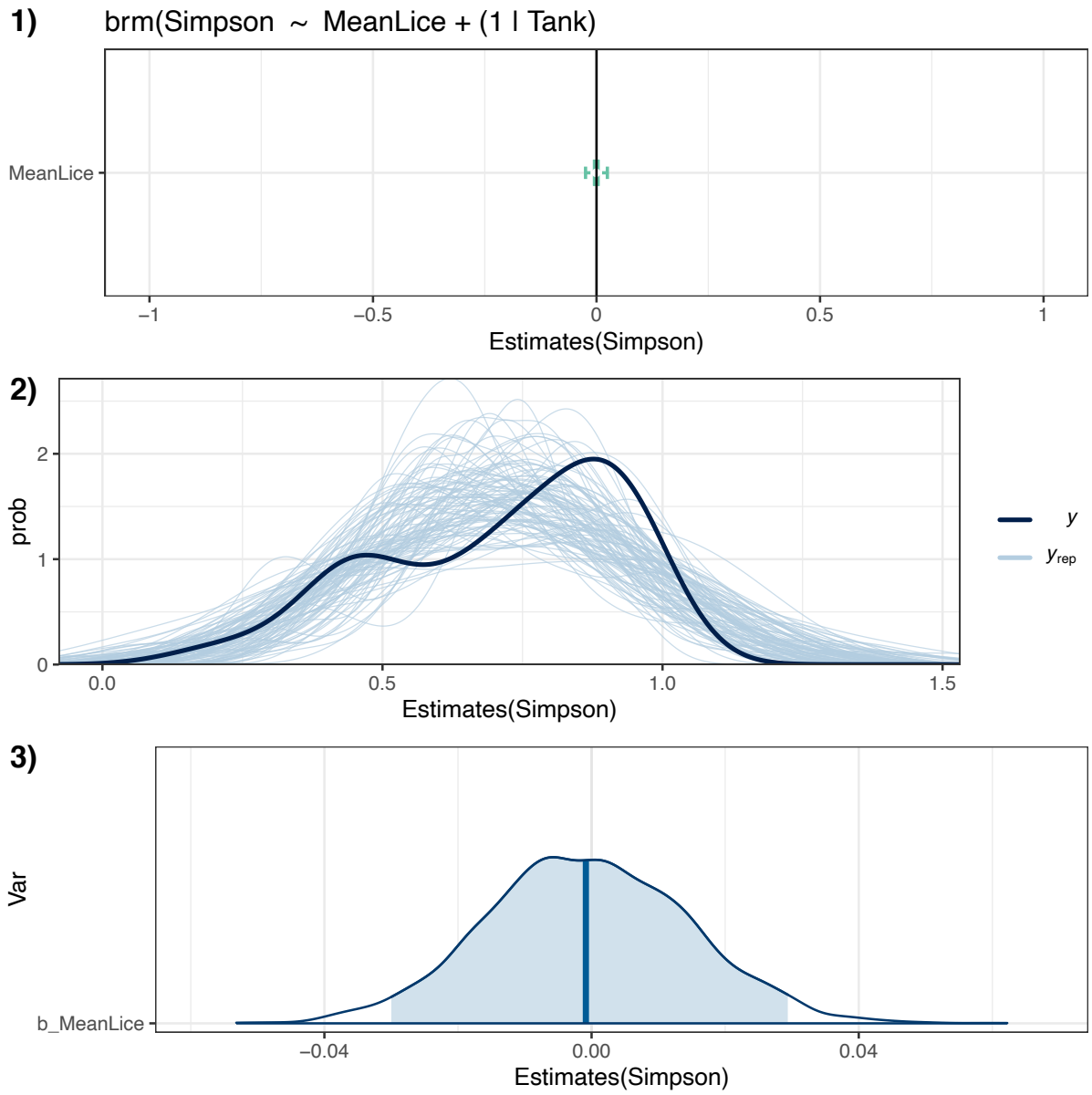

*Figure 8: Plots showing the results from the linear Bayesian mixed effect model with random intercept (brm(**Simpson** ~ MeanLice + (1 | Tank)). 1) Point estimate of the effect of MeanLice (slope) on **Simpson**. The confidence interval of the point estimate and confidence interval of the slope crosses zero. It, therefore, has a high chance of containing zero, meaning that MeanLice has no significant effect on **Simpson**. 2) A plot showing the results from the post-posterior check. The thick blue line is the actual distribution of the data, while the light blue lines are the predictions for the data distribution made by the model. It's a high degree of overlap, meaning the model fits the data well. 3) The last plot indicates the probability density of the point estimate for the effect of MeanLice on **Simpson**. Again, the effect has a high probability of being zero.*

6 Unrarefied data: Mixed linear model figure (Maximum likelihood and Bayesian): Mean lice effect on alpha diversity (Log(ACE)).

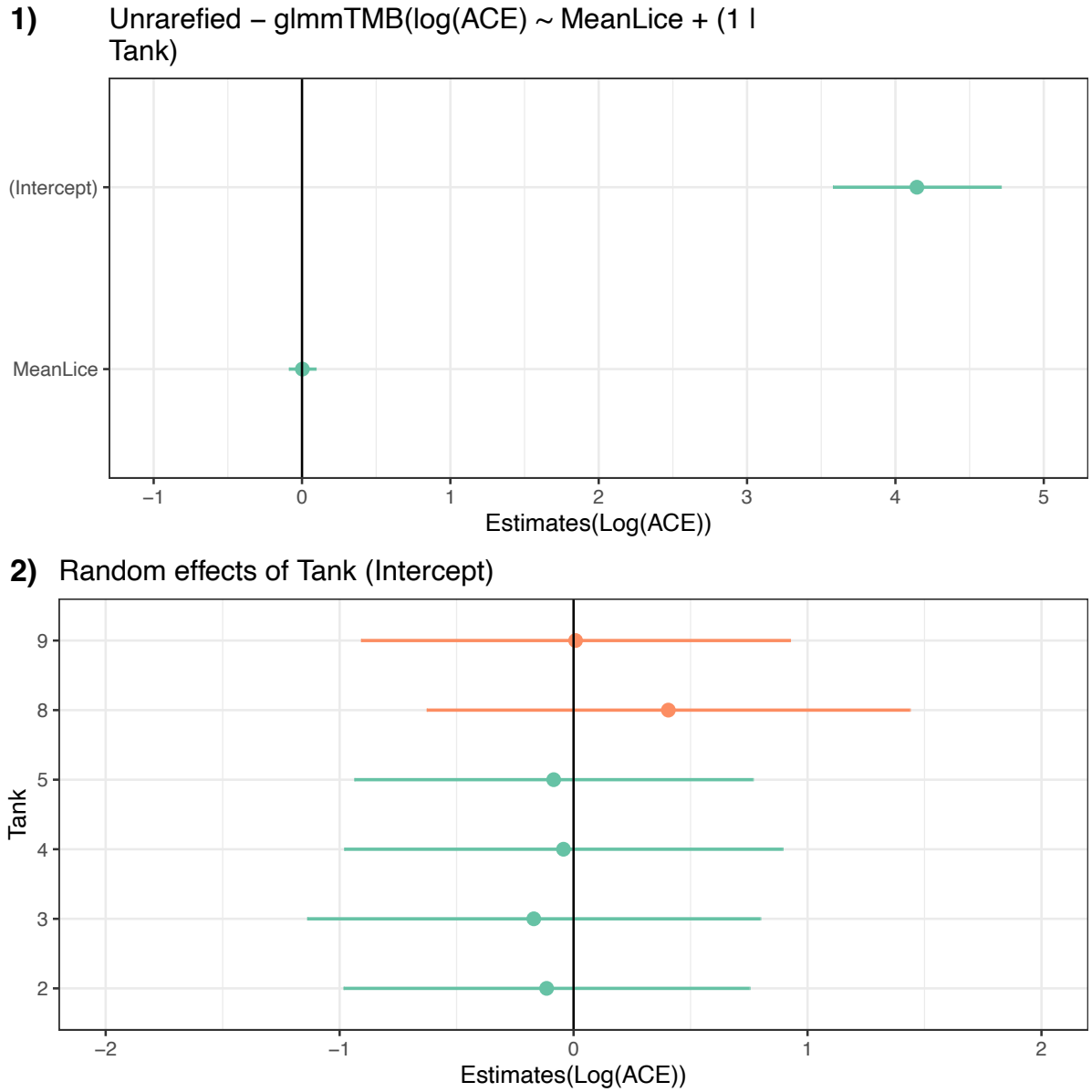

Figure 9: The plot shows the point estimates and confidence intervals for fixed and random effects. Orange coloring indicates positive numbers and greenish colors indicate negative numbers for the point estimate. 1) Plot of fixed effects. The intercept is the mean log(ACE) of all fish without lice. The slope is the effect of MeanLice on log(ACE). The confidence interval of the MeanLice effect overlaps the zero line and is non-significant. 2) Plot of random effects of the tanks.

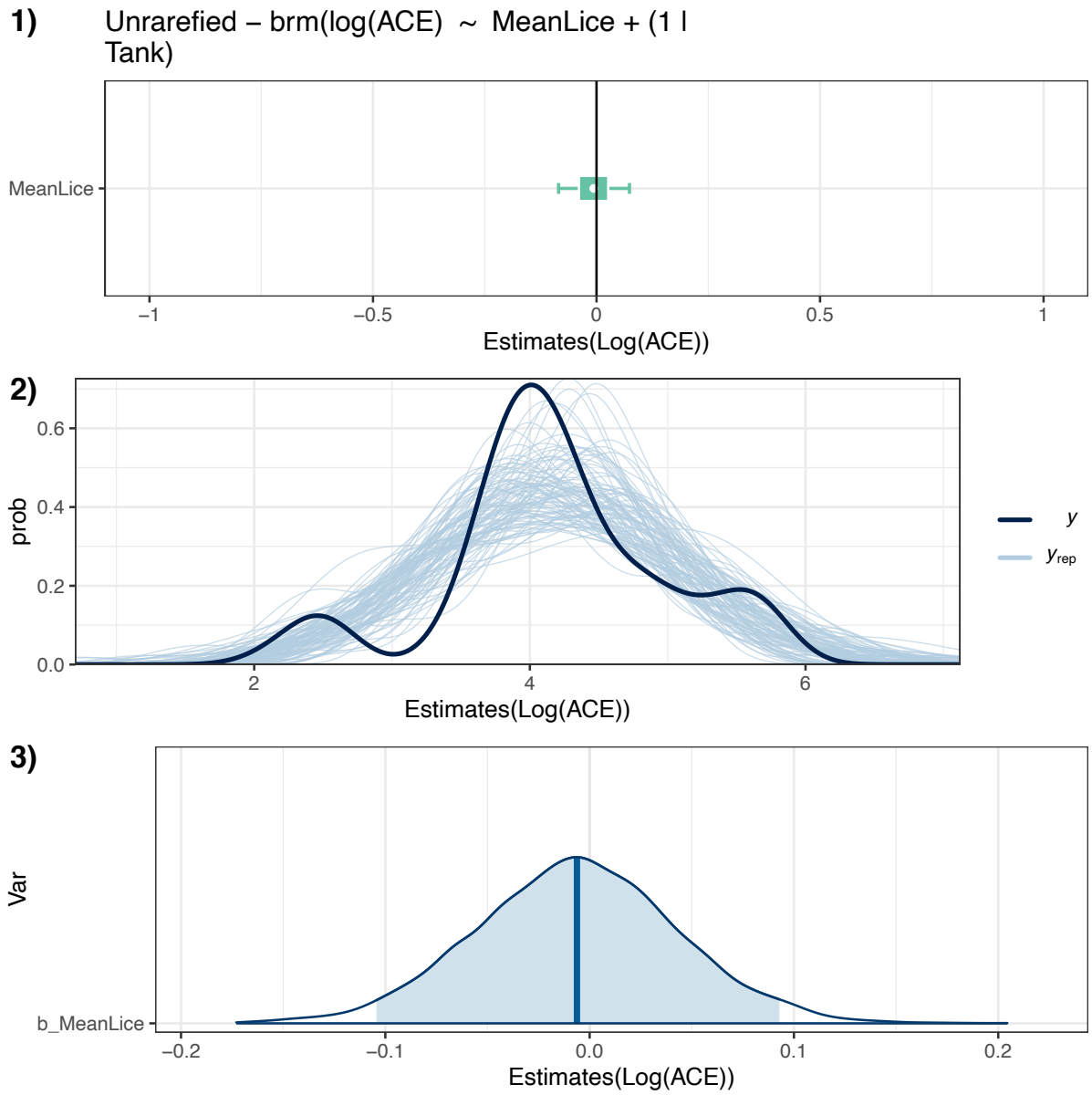

Figure 10: Plots showing the results from the linear Bayesian mixed effect model with random intercept (brm(log(ACE) ~ MeanLice + (1 | Tank)). 1) Point estimate of the effect of MeanLice (slope) on log(ACE). The confidence interval of the point estimate and confidence interval of the slope crosses zero. It, therefore, has a high chance of containing zero, meaning that MeanLice has no significant effect on Log(ACE). 2) A plot showing the results from the post-posterior check. The thick blue line is the actual distribution of the data, while the light blue lines are the predictions for the data distribution made by the model. It's a high degree of overlap, meaning the model fits the data well. 3) The last plot indicates the probability density of the point estimate for the effect of MeanLice on log(ACE). Again, the effect has a high probability of being zero.

7 Beta diversity analysis on un-rarefied data figures.

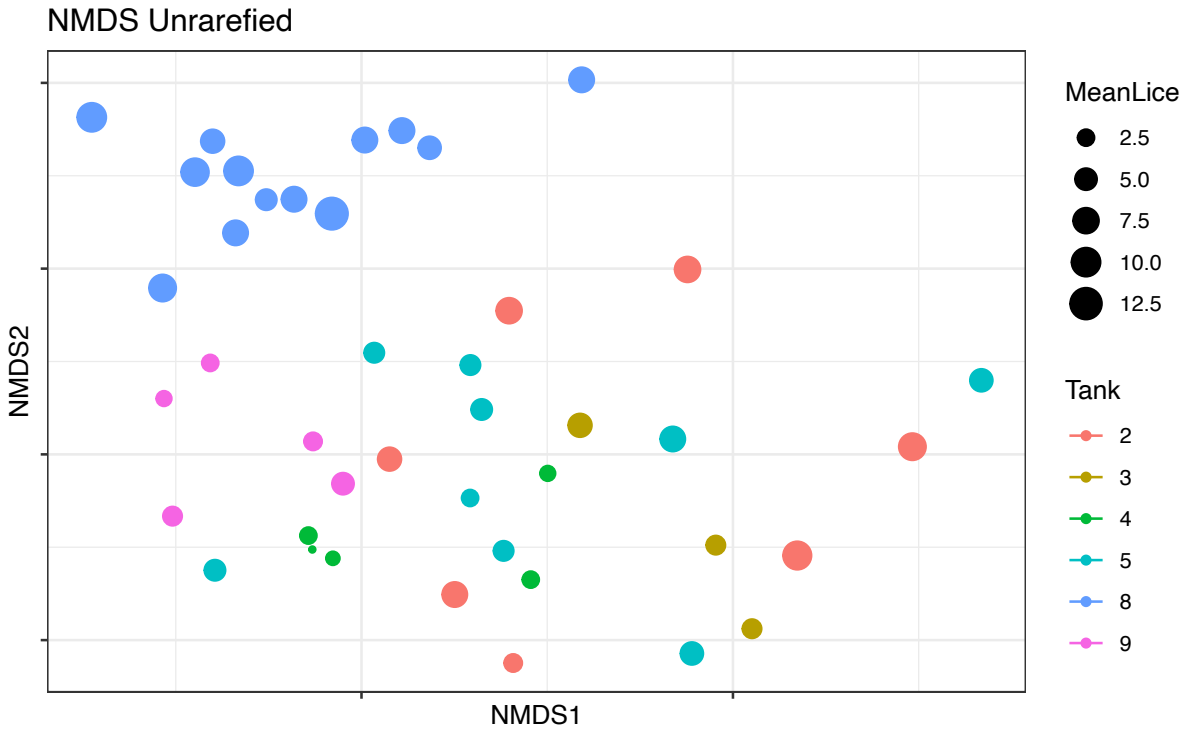

Figure 11: NMDS plot of Bray-Curtis dissimilarity matrix of the variation of ASV abundance in each fish. The size of the dots indicates the mean lice intensity of each fish. The color indicates the different tanks. The closer dots are to each other, the more similar the bacterial community. The farther apart the dots are, the more dissimilar the bacterial community

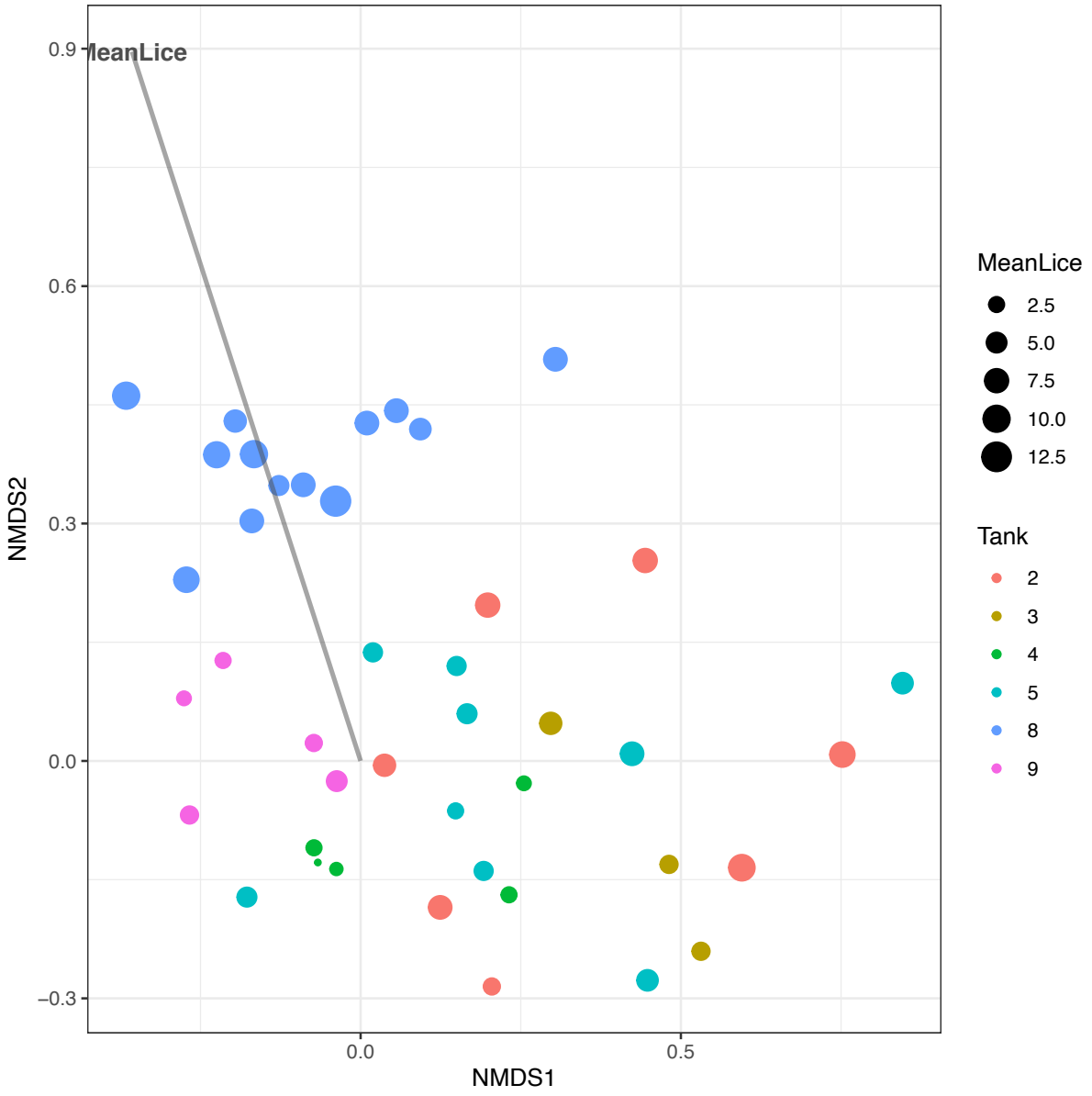

Figure 12: Plot showing the results of the envfit function. The size of the dots indicates the mean lice intensity of each fish. The color indicates the different tanks. The closer dots are to each other, the more similar the bacterial community. The farther apart the dots are, the more dissimilar the bacterial community. The line indicates which axis in the NMDS plot has the most substantial effect on mean lice.

8 Taxonomic composition of un-rarefied data figures

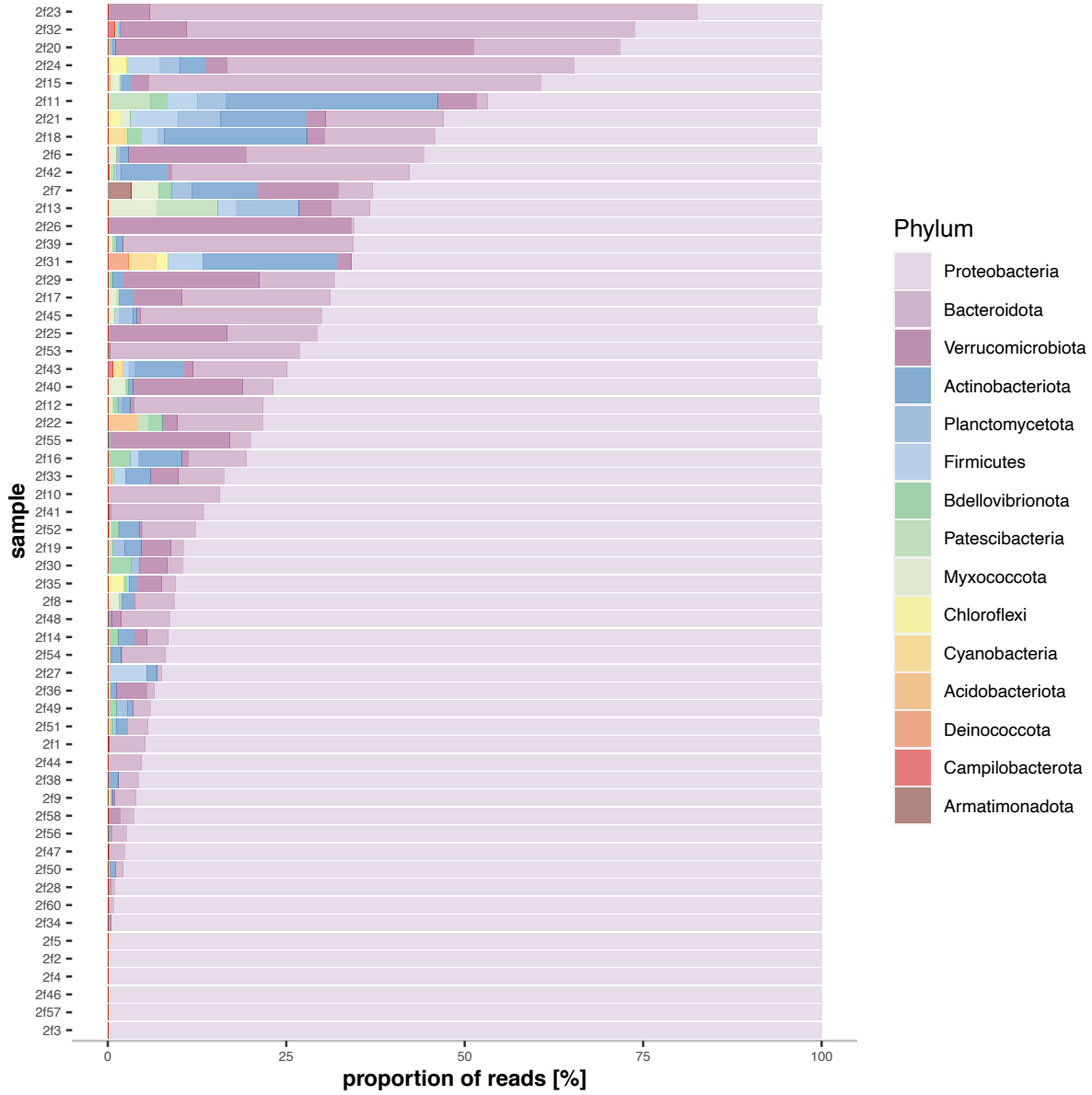

*Figure 13: A bar plot showing taxonomic composition on the **phylum** level. **The x-axis** describes the proportion of reads in each sample corresponding to a given phylum. **Y-axis** shows the different samples corresponding to the different fish. Different colors correspond to unique phyla, with the color code at the top of the figure. The missing percentages filled by the white areas symbolize ASVs that were not belonging to the 15 most abundant phylum shown here.*

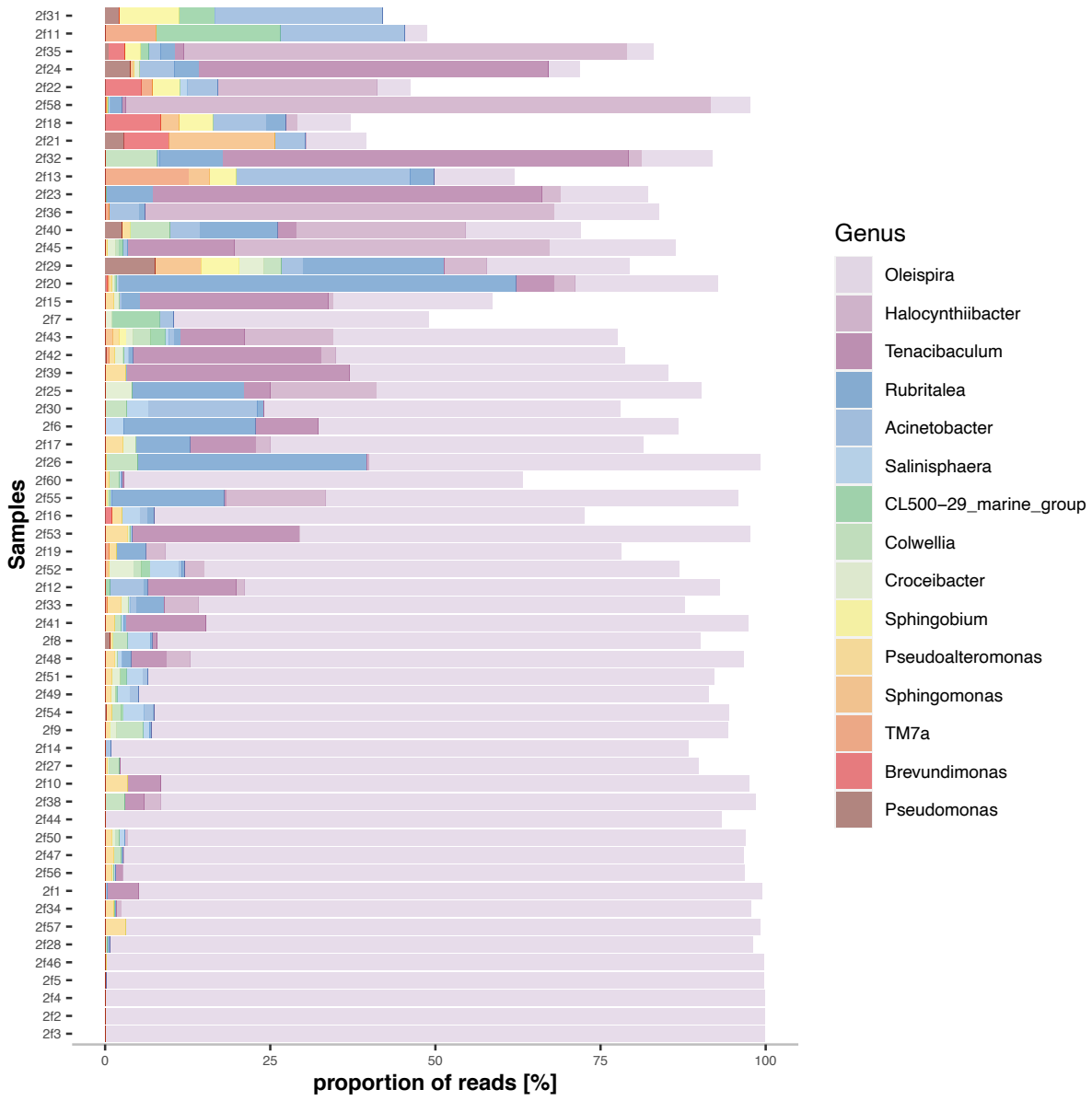

*Figure 14: A bar plot showing taxonomic composition on the **genus** level. The **x-axis** describes the proportion of reads in each sample corresponding to a given genus. **Y-axis** shows the different samples corresponding to the different fish. Different colors correspond to unique ASVs, with the color code at the top of the figure. The missing percentages filled by the white areas symbolize ASVs that were not belonging to the 15 most abundant genera shown here.*

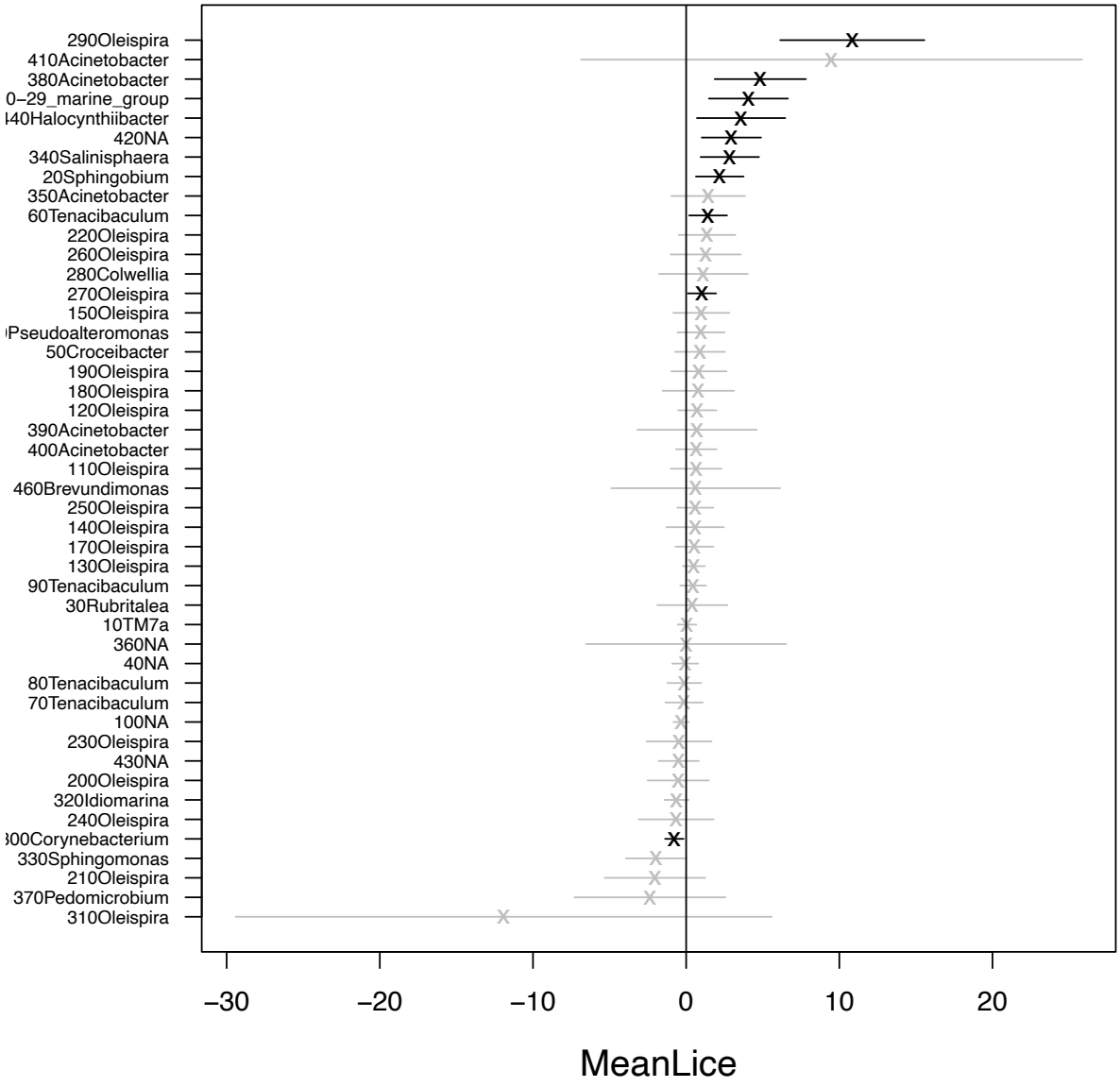

Figure 15: Plot of coefficients of the abundance of the 51 most abundant bacteria in the experiment as a general latent linear function of mean lice intensity. The X is the point estimate of the coefficients, while the line is the confidence interval. Black coloring means that the confidence interval doesn't contain zero and that the bacteria genus is either over or under-represented with increasing mean lice intensity. Coefficient estimates to the left side of the zero line are under-represented with increasing mean lice intensity, while the opposite is true for the right side. The abundance coefficients are ordered from the highest positive abundances at the top to the lowest at the bottom.

9 Water microbiome not removed: Mixed linear model figure (Maximum likelihood and Bayesian): Mean lice effect on alpha diversity (Log(ACE))

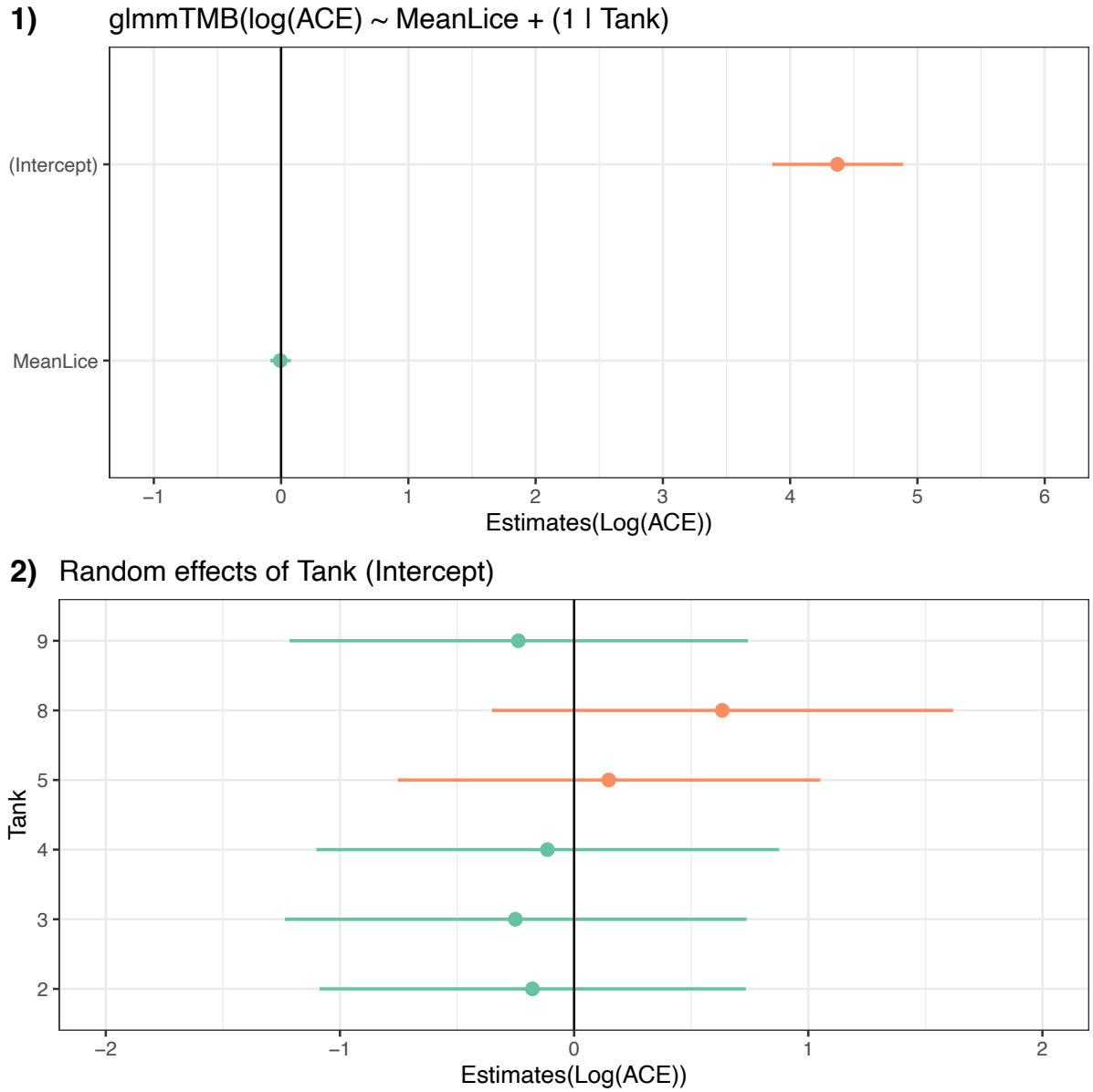

Figure 16: The plot shows the point estimates and confidence intervals for fixed and random effects. Orange coloring indicates positive numbers and greenish colors indicate negative numbers for the point estimate. 1) Plot of fixed effects. The intercept is the mean log(ACE) of all fish without lice. The slope is the effect of MeanLice on log(ACE). The confidence interval of the MeanLice effect overlaps the zero line and is non-significant. 2) Plot of random effects of the tanks.

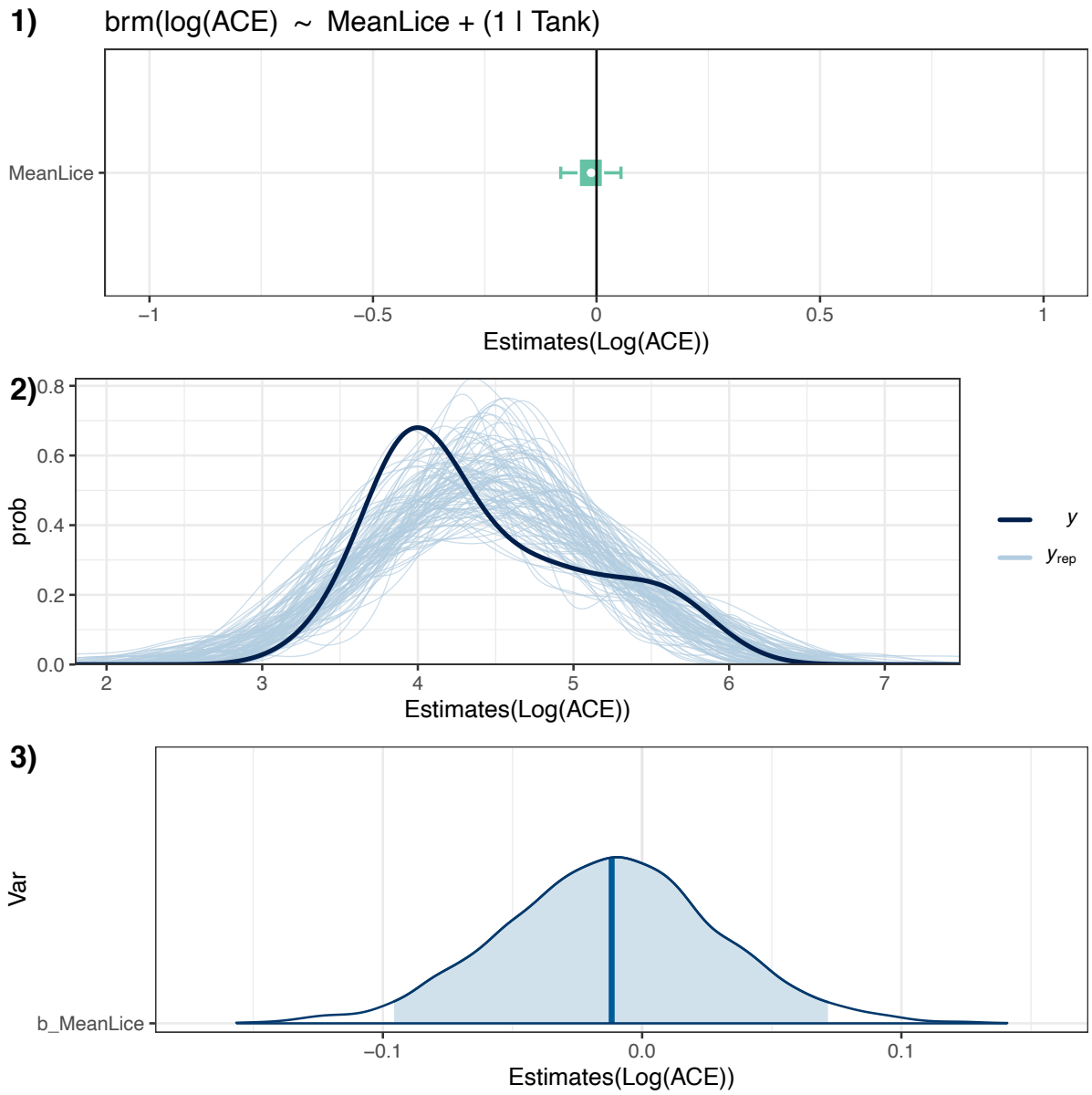

Figure 17: Plots showing the results from the linear Bayesian mixed effect model with random intercept (brm(log(ACE) ~ MeanLice + (1 | Tank)). 1) Point estimate of the effect of MeanLice (slope) on log(ACE). The confidence interval of the point estimate and confidence interval of the slope crosses zero. It, therefore, has a high chance of containing zero, meaning that MeanLice has no significant effect on Log(ACE). 2) A plot showing the results from the post-posterior check. The thick blue line is the actual distribution of the data, while the light blue lines are the predictions for the data distribution made by the model. It's a high degree of overlap, meaning the model fits the data well. 3) The last plot indicates the probability density of the point estimate for the effect of MeanLice on log(ACE). Again, the effect has a high probability of being zero.

10 Water microbiome not removed: Beta diversity analysis figures and table

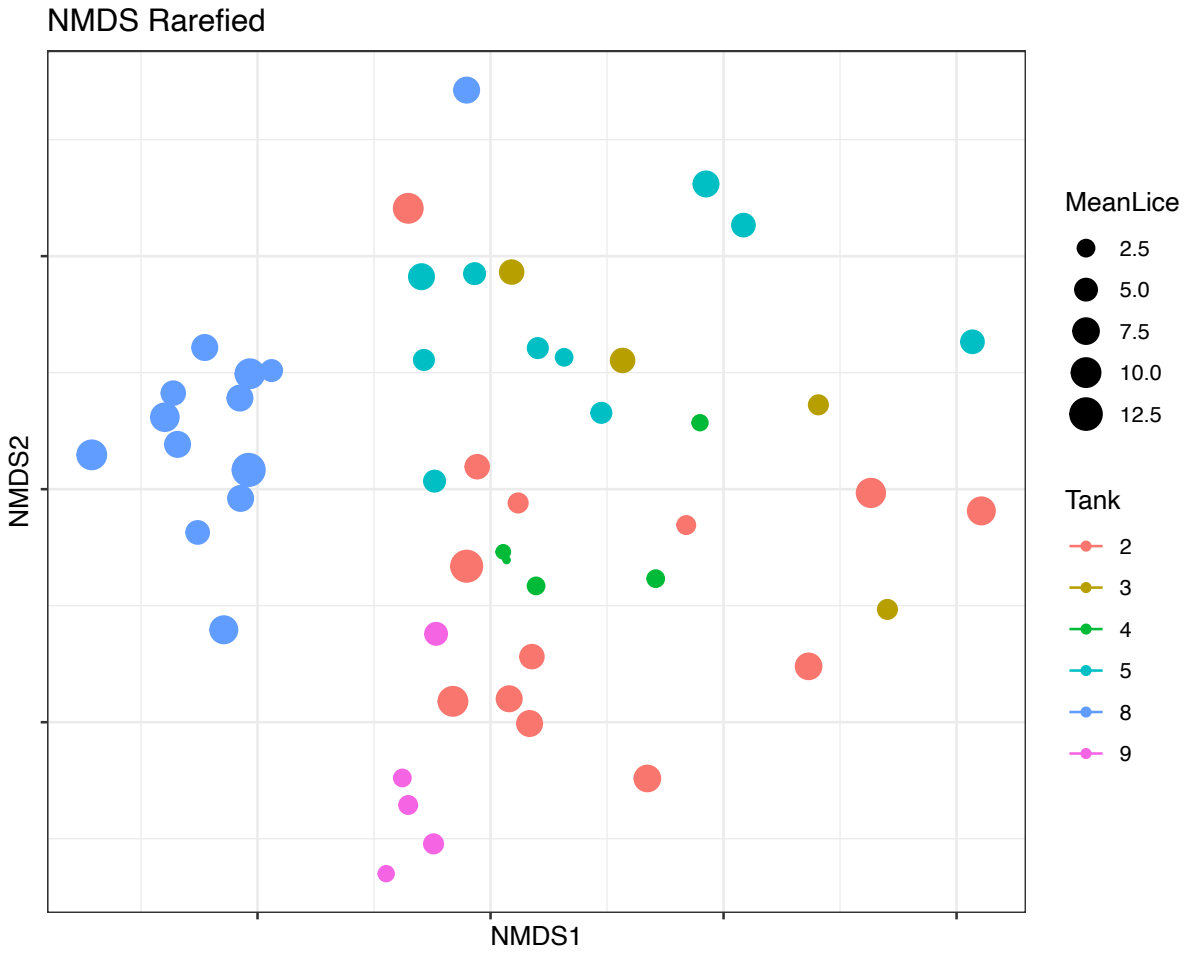

Figure 18: NMDS plot of Bray-Curtis dissimilarity matrix of the variation of ASV abundance in each fish. The size of the dots indicates the mean lice intensity of each fish. The color indicates the different tanks. The closer dots are to each other, the more similar the bacterial community. The farther apart the dots are, the more dissimilar the bacterial community

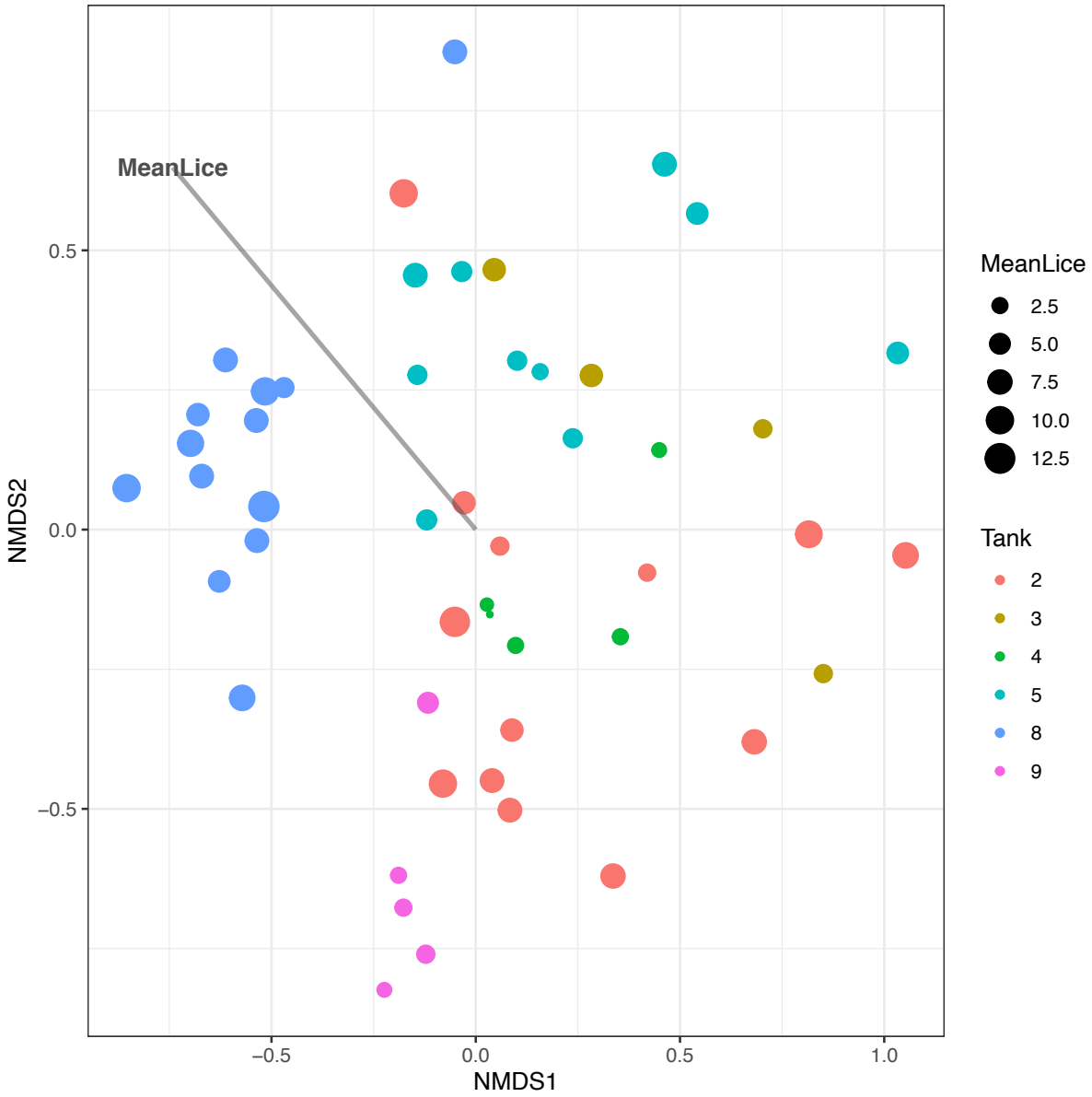

Figure 19: Plot showing the results of the envfit function. The size of the dots indicates the mean lice intensity of each fish. The color indicates the different tanks. The closer dots are to each other, the more similar the bacterial community. The farther apart the dots are, the more dissimilar the bacterial community. The line indicates which axis in the NMDS plot has the most substantial effect on mean lice.

Table 6: Summary of results for the two PERMANOVA models and the Envfit function: ***Model:*** *The model setup being used,* ***R2(MeanLice)****: The amount of variation in the data described by the MeanLice variable,* ***P-value (MeanLice)****: The p-value of the tested effect of MeanLice on beta diversity,* ***R2 (Tank)****: The amount of variation in the data described by the Tank variable,* ***P-value (Tank)****: The p-value of the tested effect of Tank on beta diversity,* ***NA:*** *values not returned form a model,* ******* *Not significant value*

Model	**R2(MeanLice)**	**P-value (MeanLice)**	**R2 (Tank)**	**P-value (Tank)**
(dist.bray ~ MeanLice + Tank)	0.080	0.001	0.408	0.001
(dist.bray ~ MeanLice, strata = metadata$Tank)	0.080	0.050	NA	NA
envfit	0.092	0.109*	0.594	0.001

11 Water biome not removed: Taxonomic composition figures

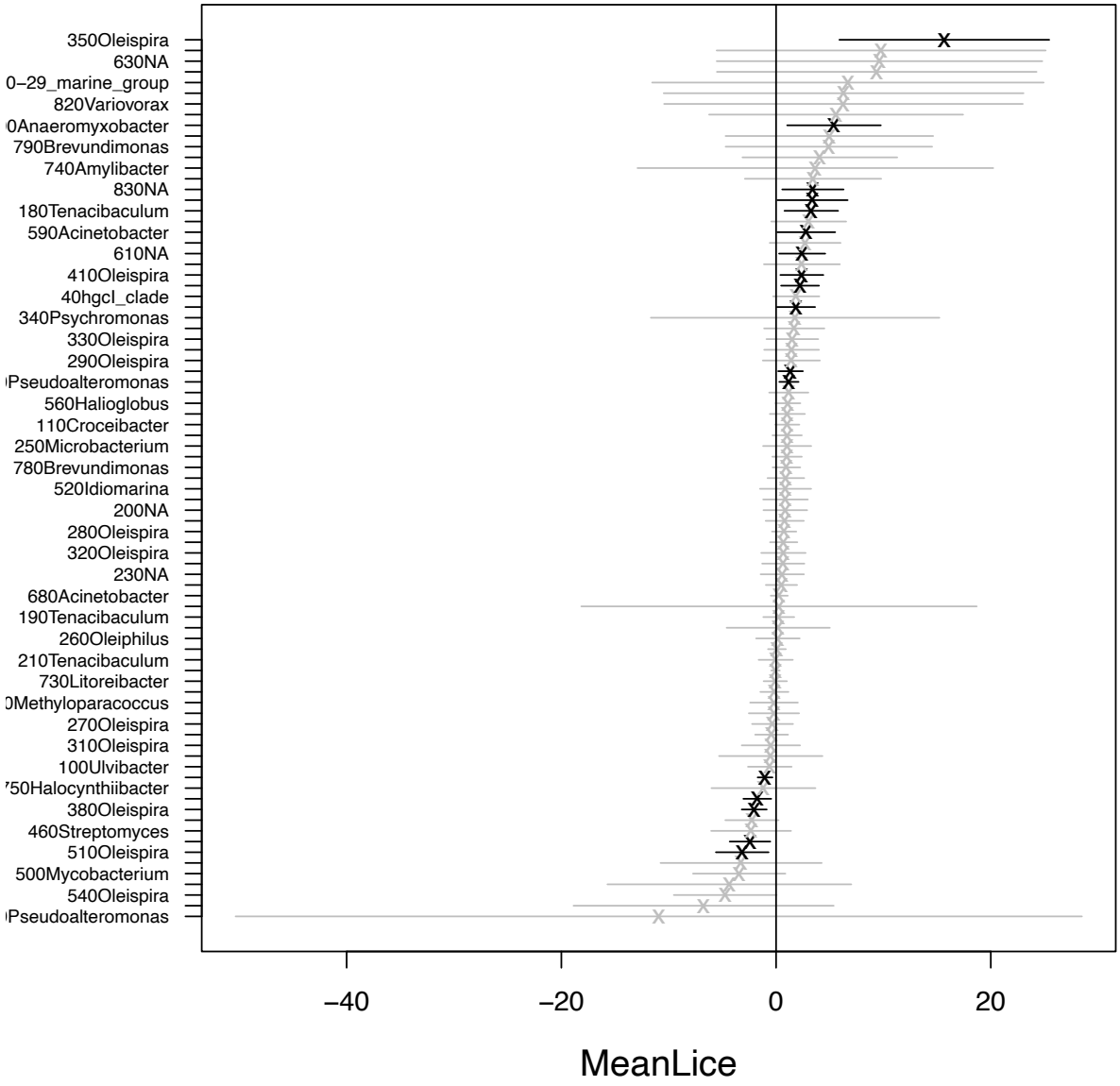

Figure 20: Plot of coefficients of the abundance of the 51 most abundant bacteria in the experiment as a general latent linear function of mean lice intensity. The X is the point estimate of the coefficients, while the line is the confidence interval. Black coloring means that the confidence interval doesn't contain zero and that the bacteria genus is either over or under-represented with increasing mean lice intensity. Coefficient estimates to the left side of the zero line are under-represented with increasing mean lice intensity, while the opposite is true for the right side. The abundance coefficients are ordered from the highest positive abundances at the top to the lowest at the bottom.

12 Information about the experiment and how it was done, the material supplied by Dr. Adele Mennaret (UiB) (Including information on lice infection, measurement of fish, PIT-tagging, and disinfection):

Experimental setup and lice infection

This study was conducted in collaboration with the larger ParAnthropE (Anthropogenic Parasite Evolution) project led by Dr. Adele Mennerat and funded by the Research Council of Norway (FRIPRO 287405). This project consists in testing theoretical models developed by evolutionary biologists (ref) to predict how parasites will evolve when their transmission is made easier by gathering a high number of hosts in a limited space (as is the case with major human activities including urban life and intensive farming and aquaculture). The approach consists in experimental evolution, where ectoparasitic salmon lice originally sampled from various areas along the Norwegian coast are brought into the lab to start evolutionary lines that are maintained throughout 10 cycles of reinfection (10 generations of parasites), under two contrasting sets of conditions. These two experimental treatments, called "*low transmission*" and "*high transmission*" respectively, differ in the density of hosts (1 fish / 100 L *versus* 3 fish / 100 L) as well as in the density of infective larvae, *i.e.* the contact rate between hosts and parasites upon infection (0,15 larvae / L *versus* 3 larvae / L, *i.e.* a 20-fold difference achieved by adjusting the volume of water relative to the amount of infective larvae.

The project started by sampling eggs from at least 100 female lice (representing well over 200 individuals per parental generation as this parasite reproduce sexually and females are known to mate with multiple males, ref) from each of three distinct locations along the Norwegian. These locations are located in Oppedal in Sognefjord, Austevoll in Hordaland, and Fosså in Rogaland (see Figure 21 below).

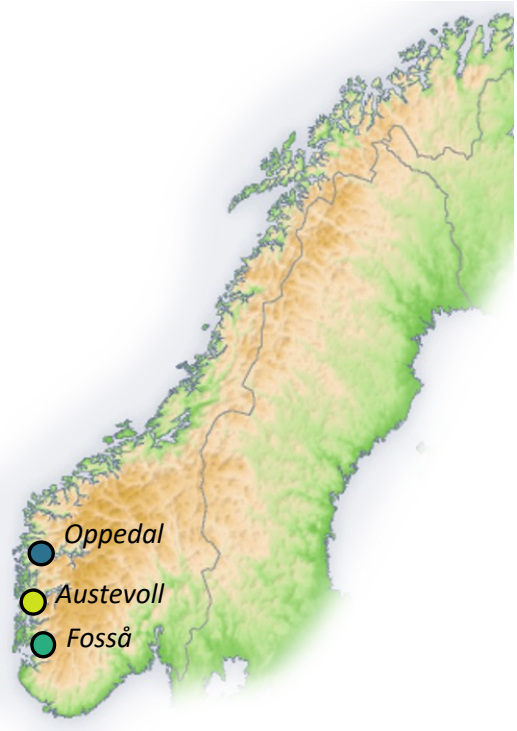

Figure 21: Map of salmon lice locations.

The eggs from parental lice sampled at each of these three locations were incubated for 14 days in the lab, following protocol described in Hamre et al. (2009). After hatching, the initial pool of infective larvae from each of the three location was used to infest a set of two populations of Atlantic salmon (Salmo salar) hosts housed in 1m x 1m, 500L tanks supplied with filtered and UV-treated seawater (hence founding three "*low transmission*" lines and three "*high transmission*" lines that serve as replicates for each of the two experimental treatments). The aim is to monitor salmon lice populations during ~2 years (~ 10 generations). The lack of acquired host resistance in this system [41] allows in principle to keep the same host individuals through consecutive infection cycles; salmon hosts, however, need to be replaced whenever they outgrow 1 kg.

About 40 to 50 days post-infection the lice reach their pre-adult stage. From that point onwards they are monitored weekly following a standard capture and anesthesia procedure: each salmon was individually netted and anaesthetized in 1mL/L metomidate and 0.3mL/L benzocaine until unresponsive, after which it was inspected for salmon lice in a tray filled with anethesia seawater. All adult lice were carefully removed with fine curved forceps and

placed onto a moistened paper label in a petri dish, after which the salmon was placed into a holding tank to recover. Female salmon lice were tagged with p-chips and registered (see below), photographed, and placed back onto their original host salmon, which was returned to its tank.

PIT-tagging

All salmon hosts were tagged prior to the study by performing a small incision under anesthesia in their ventral side and inserting an passive transponder (iso 162 iTag, BTS-ID®) into their abdominal cavity, which were read using a R-560 reader (BTS-ID®).

Disinfection

During all the time between first data collection and the second one, we disinfected net, buckets, trays, between each tank, to avoid bacterial contamination from tank to tank. To do so, we sprayed all equipment with a disinfectant solution (Virkon S, Lilleborg).

Measurement of fish:

Under anesthesia the fish were placed in a clean tray and weighted to the nearest gram using waterproof scales (Ohaus®). Their length was taken to the nearest 0,5 cm using a 50 cm-long fish ruler. Both tray and ruler were disinfected between each measuring session and wetted with seawater prior to measurement to avoid damage to the mucus, skin, or scales of the fish that may be caused by contact with on a dry surface.