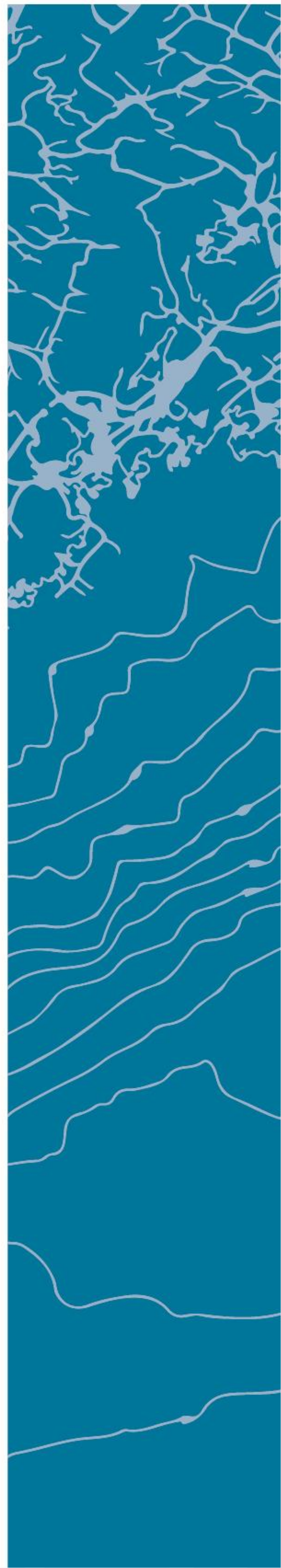

Bacterial diversity within and outside the premises of a South Norwegian salmon fish farm.

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"Life on earth is such a good story you cannot afford to miss the beginning. Beneath our superficial differences we are all of us walking communities of bacteria. The world shimmers, a pointillist landscape made of tiny living beings" – Lynn Margulis, 1997.

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Abstract

Norwegian salmon farming is a rapidly expanding sector and the Norwegian Government have a set goal to facilitate a five-fold growth towards 2050. Organic enrichment of the surrounding waters and the sediment below the cages due to the inputs of organic matter from uneaten food, tissue and faecal matter etc., affect the macro -and microfauna. To monitor the effect of these organic inputs, fish breeding companies must perform periodic controls. Traditionally, these periodic controls are based on time-consuming and expensive methods, and the necessary taxonomic knowledge is declining.

In this study, we evaluated the usefulness of marine bacterial communities as bioindicators. Benthic samples were collected from within the premises of a fish breeding facility and compared with samples from outside the facility based on metabarcoding of the 16S rRNA gene. Water samples were also collected from the same sites, and from different depths representing different water layers. The bacterioplankton composition in both benthic and water samples showed significant shifts from within the facility compared to stations outside the fish farm. The bacterial composition also varied greatly between the different water layers. Our results demonstrate the potential for bacterioplankton composition diversity as bioindicators, and that this methodology could be a useful asset in the periodic monitoring controls.

Norsk lakseoppdrett er en raskt voksende sektor, og den norske regjeringen har satt som mål å legge til rette for en femdobling av veksten frem mot 2050. Organisk berikelse av det omkringliggende vannet og sedimentet under merdene på grunn av tilførsel av organisk materiale påvirker makro-og mikrofauna. For å overvåke dette, må oppdrettsselskaper utføre periodiske kontroller. Tradisjonelt er disse kontrollene basert på tidkrevende og dyre metoder, samtidig som den nødvendige taksonomiske ekspertisen som kreves, avtar.

I denne studien vurderte vi nytten av marine bakteriesamfunn som bioindikatorer. Bentiske prøver ble samlet inn fra lokaliteter tilhørende et oppdrettsanlegg og sammenlignet med prøver fra utenfor anlegget, basert på metabarkoding av 16S rRNA-genet. Vannprøver ble også samlet fra de samme stedene og fra ulike dyp som representerer ulike vannlag. Bakterioplanktonsammensetningen i både sediment og vannprøver viste betydelige endringer fra innenfor anlegget sammenlignet med lokaliteter utenfor. Sammensetningen varierte også betydelig mellom vannlagene. Våre resultater viser potensialet for at mangfoldet av bakterioplanktonsammensetning kan benyttes som bioindikatorer, og at denne metoden kan være en nyttig ressurs i periodiske overvåkingskontroller.

Table of contents

ABSTRACT 4

PREFACE 7

1. INTRODUCTION 8

1.1 BACKGROUND 8
1.2 BACTERIA AS ENVIRONMENTAL INDICATORS 9
1.3 MARINE MICROBES 10
1.4 FUNCTIONS AND DIVERSITY 10
1.4.1 PROTEOBACTERIA 11
1.4.2 DESULFOBACTEROTA 12
1.4.3 PLANCTOMYCETOTA 12
1.4.4 CYANOBACTERIA 13
1.4.5 BACTEROIDETES 13
1.4.6 VERRUCOMICROBIOTA 13
1.5 16S RRNA GENE METABARCODING 14

2. OBJECTIVES 16

3. MATERIALS AND METHODS 17

3.1 SAMPLING SITES 17
3.2 BENTHOS SAMPLES 18
3.3 WATER SAMPLES 18
3.3.1 FILTERS 20
3.4 PCR AND GEL ELECTROPHORESIS 21
3.5 NANODROP ANALYSIS 21
3.6 SEQUENCE DATA PROCESSING AND TAXONOMIC ASSIGNMENT 22
3.6.1 IMPORTING AND PRIMER REMOVAL 22
3.6.2 DENOISING READS INTO AMPLICON SEQUENCE VARIANTS (ASVS) 22
3.6.3 TAXONOMIC ASSIGNMENT AND BOX PLOTS 22
3.6.4 FILTERING RESULTANT TABLES 22
3.6.5 RAREFACTION 23
3.6.6 PHYLOGENETIC TREE AND DIVERSITY METRICS 24

4. RESULTS 26

4.1 Samples overview 26
4.2 Comparing eluate and filter samples 26
4.3 Removal of water B-samples 27
4.4 Bacterial/Bacterioplankton community variations 28
4.4.1 Comparing layers 28
4.4.2 Water samples 29
4.4.3 Benthos samples 30
4.5 Alpha diversity analysis 32
4.6 Beta diversity metrics 35

5. DISCUSSION 38

5.1 Benthic bacterial diversity variation by distance 38
5.2 Bacterioplankton diversity variation by distance 38
5.3 Bacterial/Bacterioplankton diversity variation in different layers 39
5.4 Bacteria as bioindicators 40
5.5 DNA isolation from water samples 41
5.6 Sample purity and DNA concentration 41

6. CONCLUSIONS 42

6.1 Concluding remarks 42
6.2 Limitations and future studies 42

7. APPENDIX 43

7.1 Legend to taxonomical bar charts at taxonomic rank 4 (order) 43
7.2 Feature table overview 44
7.3 Comparing A and B samples 45
7.4 QIIME2 input 46
7.5 Overview of all sample sites and results from nanodrop analysis 50
7.6 Retrieved CTD data 2020 53
7.7 CTD data from MFS station 2019 53

8. REFERENCES 54

Preface

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I would also like to thank all the knowledgeable and enthusiastic staff working at the Department of Science at Universitetet i Agder for making my years as a student to one of the most interesting periods in my life. No matter which door I have knocked on, I have always been met with a smile and a genuine wish to help.

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I would also like to thank my fellow students for all the great (and frustrating) moments we have shared.

Lastly, a warm thank you to my wife and family who have always supported and encouraged me along the way. I would have never started this journey if it wasn’t for your support, let alone finished it.

1. Introduction

1.1 Background

It is well known that humans trough time have often chosen coastal areas or places near rivers as preferred locations for their settlements. These regions generally have a high biological production among other benefits, and as a result more than half of the human population live within 60 km of the shoreline. This entails a high exposure to anthropogenic pressures towards the environment originating from sources such as overexploitation, eutrophication, pollution, introduction of invasive species, sewage among others (Halpern et al., 2008, Turner et al., 1996). Furthermore, marine ecosystems have, and still are, degrading due to a number of offshore activities (Duarte et al., 2015).

One such activity is fish farming which have experienced a significant growth during the past 40 years, this is especially evident in the Norwegian salmon production. In 1979 the production of salmon reached 4.390 tonnes, and in 2018 this number was close to 1.3 million tonnes (Fiskeridirektoratet, 2021). Moreover, the Norwegian Government has a set goal to increase this industry five-fold within 2050 (Olafsen et al., 2012).

Among the environmental impacts imposed by this activity is nutrient enrichment of the benthos and waters neighbouring the aquaculture facilities due to discharged waste food, faecal matter and other excretory products (Buschmann et al., 2006). A built up of organic material in relation to fish farms have been reported (Bannister et al., 2014, Carroll et al., 2003, Holmer et al., 2005). However, a recent review by Price et al. (2015) concludes that modern operating fish farms have negligible impacts on the marine water quality surrounding the facilities, but highlights the importance of having the necessary tools available to guide this industry through its expected expansion.

To assess the impacts of anthropogenic pressures and secure a healthy balance between exploitation and ecosystem services, Marine Strategy Framework Directives (MSFD) was implemented in 2008 by the European Commission (van Leeuwen et al., 2014). In the implementation process of these directives, member states agreed to develop and improve innovative and cost-effective monitoring systems with an overall goal to achieve good environmental status of marine waters by 2020.

The evaluation of the marine ecological conditions depends on Biological Quality Element (BQE) assessments, commonly using fish or invertebrates as indicator species (Goodwin et al., 2017). In Norway, all off-shore fish breeding facilities are required by law to perform periodic trend monitoring programs (Fiskeridirektoratet, 2021). The national standard program for conducting

these analyses was introduced in 2000 and is largely based on the Modelling-Ongrowing fish farms-Monitoring (MOM) system (Ervik et al., 1997, Hansen et al., 2001a). The mission of these programs is to monitor how emissions from the facilities affect the area below and surrounding the facility. The MOM-system measures, among other things, the benthos's chemistry, composition and fauna. Rather than conducting the test at certain points in the production cycle, it considers variables such as the density of fish per unit area, feeding rate and the composition of the feed to assess the maximum holding capacity of the facility. Different levels of testing are performed depending on the level of exploitation (Hansen et al., 2001b). However, some of the disadvantages to this traditional method is highlighted by the need for special taxonomical expertise to verify the visual identification of the benthic species involved. This is a labour demanding, slow and costly procedure (Goodwin et al., 2017). In some cases, morphological identification may be impossible, which is often the case with microbial species such as bacteria (Danovaro et al., 2016).

Polymerase Chain Reaction (PCR) techniques have for several decades been used to detect the presence and diversity of the microbiome, allowing for identification of previously unknown bacteria with key roles in important processes such as the degradation of carbon and ammonia oxidation (Head et al., 1998, Azam and Worden, 2004). Alongside the improvements in PCR techniques, the time required and costs involved with sequencing have been notably reduced (Pettersson et al., 2009). This, together with the advancements in the tools used for bioinformatics and sequence technology, it is argued that this field is now sufficiently mature to be included in marine monitoring endeavours (Goodwin et al., 2017).

1.2 Bacteria as environmental indicators

Prokaryotes, including Bacteria and Archaea dominate the marine habitats by numerically measurements with an estimated abundance of 12 x 10^{28} cells (Whitman et al., 1998). Furthermore, unicellular organisms, especially bacteria, with their large population size, short generation times and fast growth rate shows promising indications to function as a powerful option for pollution monitoring (Belkin, 2003, Aylagas et al., 2017, Caruso et al., 2016). Moreover, bacteria responds quickly to environmental changes such as changes in pH (Lauber et al., 2009), temperature (Hall et al., 2008) and environmental contaminants such as uranium and nitrate (Smith et al., 2015) which further strengthen the belief in bacteria as an important asset in environmental monitoring.

In all marine habitats, microbes have a key role in the degradation processes and nutrient cycling, but Marine Strategy Framework Directives largely ignores the microbial assemblages, and their role as environmental indicators (Caruso et al., 2016). So far, the focus have mainly been towards a narrow set of pathogens that are known to infect humans through seawater or seafood (Caruso et

al., 2016), but studies on bacteria as environmental indicators are scarce (Nogales et al., 2011). This has to do with bacterial communities being very complex, and their role as functional groups in marine ecosystems are difficult to determine compared with macro-organisms (Aylagas et al., 2017).

Fortunately, the rapid development in molecular methods together with next generation sequencing technologies have produced new cognizance into bacterial communities composition and their roles in different marine environments (Aylagas et al., 2017). This have provided scientists with an opportunity to identify key microorganisms engaged in important ecosystem processes and expanded the scientist's capability to characterize bacterial communities in multiple samples simultaneously at a low cost (Ferrera and Sanchez, 2016, Tan et al., 2015).

1.3 Marine microbes

Marine microbes (Bacteria, Archaea, viruses, protists, and fungi) exist in enormous numbers and are distributed all throughout the ocean, even in the most extreme environments (Zinger et al., 2011). They are crucial components in the function of all ecosystems and are the engineers behind the Earth's biogeochemical cycles (Falkowski et al., 2008). Ever since the ground-breaking discovery by Hobbie et al. (1977) that polycarbonate nucleopore filters retained all bacteria and thus made them available for counting, most bacteria had dodged discovery because they are so incredible small, and many are uncultivable. This finding inspired microbial oceanographers, and after decades of research in the field of marine microbial ecology it is now acknowledged that pelagic bacteria alone make up most of the oceanic biomass (Azam and Malfatti, 2007, Zehr et al., 2017).

1.4 Functions and diversity

The domain Bacteria consist of procaryotic cells with bacterial rRNA and membrane lipids. Most of the marine bacteria are Gram-negative, as this group of bacteria have a cell wall better adapted for the harsh marine environment with high pressures, lack of light, salinity, temperature extremes and more (Das et al., 2006). Most bacteria inhabiting the oceans are heterotrophic and function as recyclers of organic matter deriving from primary producers, they break down all organic matter and return them into the components they derived from (Sherr and Sherr, 1996). One of the largest assemblies of organic carbon in the biosphere is in the form of dissolved organic matter (DOM). Heterotrophic bacteria, being the dominant organisms in aquatic habitats utilizing DOM, are a critical component in the carbon budgets and cycles (Kirchman, 2002). Even single strains of bacteria can inhabit significant potential in the recycling of marine dissolved organic carbon (DOC), as reported by Pedler et al. (2014). Even though most of the oceans bacterial communities still awaits discovery and analysis, recent research endeavours like the Tara Oceans project have contributed to unravel many previous unknowns (Sunagawa et al., 2020). 210 sampling stations with a global distribution was visited and over 35.000 samples were collected using standardized protocols. With

this vast amount of data, researchers have been able to contribute to important questions in relation to microbial community compositions (Sunagawa et al., 2020), and also gain insight into how microbes adapt to different global environmental conditions (Salazar et al., 2019).

The seasonal changes in phytoplankton populations have shown to be key contributors to the bacterioplankton composition and growth (Bunse and Pinhassi, 2017). Phytoplankton blooms usually takes place during the spring months when light availability combined with rising temperatures and elevated nutrients levels due to seasonal mixing events, creates favourable conditions for the growth of phytoplankton (Buchan et al., 2014). The bacterioplankton composition also change during a bloom event (Teeling et al., 2012), as different molecules are released from the phytoplankton at different stages of the bloom. In the early stages of a bloom, low molecular weight molecules (LMW) are released (carbohydrates, organic acids, sugar alcohols, etc.), and towards the end of the bloom, high molecular weight (HMW) molecules (polysaccharides, proteins, nucleic acids, etc.) are released (Bunse and Pinhassi, 2017).

The domain Bacteria is extremely diverse and complex (Aylagas et al., 2017). For relevance and practical reasons, only a few phyla are included in this thesis. They are chosen based on previous findings in similar projects (Kawahara et al., 2009, Stoeck et al., 2018) and because of their global distribution (Pommier et al., 2007).

1.4.1 Proteobacteria

Proteobacteria is the most extensive phylum within the domain Bacteria and by analysis of the 16S rRNA gene it is divided into 5 classes: Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Epsilonproteobacteria and Zetaproteobacteria. Morphology and physiology vary among the different species within each class, but they are all gram-negative and constitutes most of the known gram-negative bacteria (Rizzatti et al., 2017). In this thesis the focus will be on the two most dominating classes: Alpha- and Gammaproteobacteria.

To date more than a dozen different orders of Alphaproteobacteria is known and have received valid published names (Hördt et al., 2020). They have a global distribution and are represented in a wide array of habitats ranging from soil, pelagic and benthic regions of the ocean and fresh water. Alphaproteobacteria are reportedly the most abundant taxa of microbes, whereas Rhodobacterales and SAR11 is recognized as the dominating clades within the class and together represents approximately 40 % of all bacteria present in surface waters (Giovannoni et al., 2005).

In general, the clade Rhodobacterales are heterotrophic and most are highly versatile (Brinkhoff et al., 2008). Previous studies have shown that Rhodobacterales are identified in high abundances both in temperate (Dang et al., 2008) and sub-artic waters (Fu et al., 2010).

The SAR11 clade is believed to be the most abundant class of bacteria in the marine environment (Morris et al., 2002). They are highly concentrated in the photosynthetic euphotic zone, and in some localized areas the SAR11 clade represents more than 50 % of the total surface microbial community (Morris et al., 2002). The SAR11 is a group of aerobic heterotrophs carbon-oxidizing bacteria and reach their highest abundances in oligotrophic environment, all though some are also found in oxygen-low environments (Giovannoni, 2017). However, anaerobic metabolism is not yet identified within this group (Tsementzi et al., 2016). The SAR11 clade is known to have a small genome (approximately 1,3 Mb) (Giovannoni et al., 2005), and it is hypothesized that the low gene content gives reason to believe that the clades main contribution to the ocean biogeochemistry is aerobic oxidation of organic carbon (Tsementzi et al., 2016).

The largest class of bacteria within the phylum Proteobacteria is Gammaproteobacteria which consists of 15 orders (Williams et al., 2010). This class needs almost exclusively hydrocarbons as their source for energy to thrive (Yakimov et al., 2019). It has recently been documented that various groups of Gammaproteobacteria account for large portions of carbon fixation and the oxidation of sulphur in marine sediments (Dyksma et al., 2016).

1.4.2 Desulfobacterota

Phylum Desulfobacterota is a novel phylum which includes several orders from the class previously known as Deltaproteobacteria, and includes the orders Desulfobacterales, Desulfaculales, Desulfovibrionales, Desulfuromonadales, Syntrophobacterales and Thermodesulfobacteria (Waite et al., 2020). Phylum Desulfobacterota consists of sulphate-reducing bacteria (SRB), which are anaerobic bacteria that reduce sulphate to sulphide with oxidation of hydrogen and organic compounds as their energy source (Waite et al., 2020, Pereira et al., 2007).

1.4.3 Planctomycetota

Bacteria of phulym Planctomycetota are globally distributed and found in a variety of habitats ranging from fresh, brackish, and marine waters in addition to terrestrial soil habitats (Fuerst, 1995). They are moderately thermophilic with an optimal growth temperature at 41 °C (Fuerst, 1995). Several genera within the phylum are key players in the process of anaerobic oxidizing of ammonium gas to dinitrogen gas with nitrite as electron acceptor (abbreviated “anammox reaction”) (Schmidt et al., 2012).

Research have indicated that the anammox reaction is a fundamental and key process in the global nitrogen cycle, and that it may account for up to 80 % of total marine N_2 production (Arrigo, 2005, Schmid et al., 2005). The anammox reaction has been identified in several aquatic ecosystems,

including marine sediments (Hietanen and Kuparinen, 2008, Rich et al., 2008) and anoxic environments (Kuypers et al., 2003).

1.4.4 Cyanobacteria

The phylum Cyanobacteria contain photosynthetic prokaryotes, and they all synthesize chlorophyll *a*. Most of the species produce the phycobilin pigment, phycocyanin, which give them the appearance of being blue in coloration and is the reason behind the popular name "blue-green" algae (Whitton and Potts, 2012).

Two genera of cyanobacteria, *Prochlorococcus* and *Synechococcus* are among the two most important contributors to the primary production in the oceans worldwide, and in some regions they jointly supply approximately 50 % of the fixed carbon (Zwirglmaier et al., 2007). Cyanobacteria are also considered to be the main N_2-fixing microorganism in the ocean (Zehr, 2011). The process of fixing atmospheric N_2, which is one of the most important external sources of N to the surface waters in the oceans, contributes to the export of carbon to the seabed by providing a stoichiometric nutrient flux, which in turn contributes to keep down the atmospheric levels of CO_2 (Zehr, 2011).

1.4.5 Bacteroidetes

Bacteroidetes are a phylum of heterotrophic, gram-negative, unicellular bacteria which are characterized by both phenotypic traits and their 16S rRNA sequence (Kirchman, 2002). They are found in almost all habitats in the biosphere ranging from hydrothermal vents (Sievert et al., 2000) to rocks and sea-ice in Antarctica (Smith et al., 2000) and in oceanic benthos (Llobet-Brossa et al., 1998).

Apart from having an ecological importance, not only because of their abundance and their wide distribution, the group of Bacteroidetes called *Cytophaga-Flavobacterium* functions in a special niche-role in the degradation of complex biomacromolecules (Cottrell and Kirchman, 2000). They are especially adept to degrade different biopolymers, namely cellulose, chitin and pectin (Balows et al., 2013). These biopolymers are part of the high molecular weight fraction (HMW) of DOM. In the oceans, the concentrations of HMW DOM are high, and thus the interaction between *Cytophaga-Flavobacterium* and these biopolymers should expectedly play a significant role in the carbon cycle (Amon and Benner, 1996, Kirchman, 2002), but the evidence for this specialist-role is still limited (Laura et al., 2005).

1.4.6 Verrucomicrobiota

Members of the phylum Verrucomicrobiota are commonly associated with being ubiquitous in soil, although few studies have investigated the diversity and abundance in detail (Bergmann et al.,

2011). Some studies have documented the presence of the phylum in marine habitats, both in the water column (Zaikova et al., 2010, Bano and Hollibaugh, 2002) and the sediment (Urakawa et al., 1999). One study with samples collected from a wide array of different environmental conditions and locations was conducted by Freitas et al. (2012), in which they found that Verrucomicrobiota was represented in 98 % of the analysed samples and thus indicating an ubiquitous distribution in the oceans. Another study conducted a few years later by Cardman et al. (2014), proposed that Verrucomicrobiota phylotypes are involved in the breakdown of polysaccharides. Some years prior to this, three independent studies reported that bacterium belonging to phylum Verrucomicrobiota was aerobic methanotrophs, previously attributed only to members belonging to phylum Proteobacteria (Islam et al., 2008, Dunfield et al., 2007, Pol et al., 2007). These two findings together with the biogeography suggests that this phylum may be important in biogeochemical cycles in the ocean.

1.5 16S rRNA gene metabarcoding

Genomic approaches to species identification utilize the diversity among DNA to identify organisms. In 1987 a new method for extracting microbial DNA from benthos samples was developed by Ogram et al. (1987). This discovery made it possible to identify bacteria that was not cultivable in the laboratory. This method, combined with the analysis and sequencing of the small 16S subunits of rRNA found in all Bacteria have made it possible to identify and infer relationships among the bacterial organisms (Head et al., 1998). The DNA sequences encoding the rRNA molecules are slow to evolve and highly conserved among different species, surrounded by more variable regions (Van de Peer et al., 1996), which makes them an effective target when working with identification.

The term “barcode”, when addressing organisms, was first coined by Hebert et al. (2003). They acknowledged the limitations of morphology-based identification and the decreasing accessibility to taxonomical expertise and called for an identification system that use a small part of each taxon’s DNA sequence as their own specific “barcode”.

When investigating bacterial communities, the metabarcoding (aiming to identify several species at the same time) approach using the 16S rRNA marker gene have been frequently used (Hamady and Knight, 2009). The 16S rRNA gene is commonly used for identifying bacteria because the gene is relatively short, about 1600 base-pairs, and it has nine (V1-V9) hypervariable regions with varying conservation of genes (Kim et al., 2011). These hypervariable regions are accompanied by 10 regions that are highly conserved and thus common among most bacteria (Fukuda et al., 2016) See Figure 1 for a visualisation of the ribosome complex.

Work focussing on integrating microbes such as bacteria into Environmental Impact Assessments (EIA's) has begun, and recently Aylagas et al. (2017) proposed a bacterial community-based index (microgAMBI) to assess the ecological status of marine environments. In their study, they analysed the bacterial composition at 51 coastal and estuarine locations along the Basque coast using high-throughput sequencing of the 16S rRNA gene. The "microgAMBI" is based on Azti's Marine Biological Index (AMBI), an index formulated on the response of macroinvertebrate to different types of stressors (https://ambi.azti.es/). Their results showed that the bacteria community composition can be used to provide an overview of the ecological status and to create an index capable of inferring ecological perturbations in marine systems.

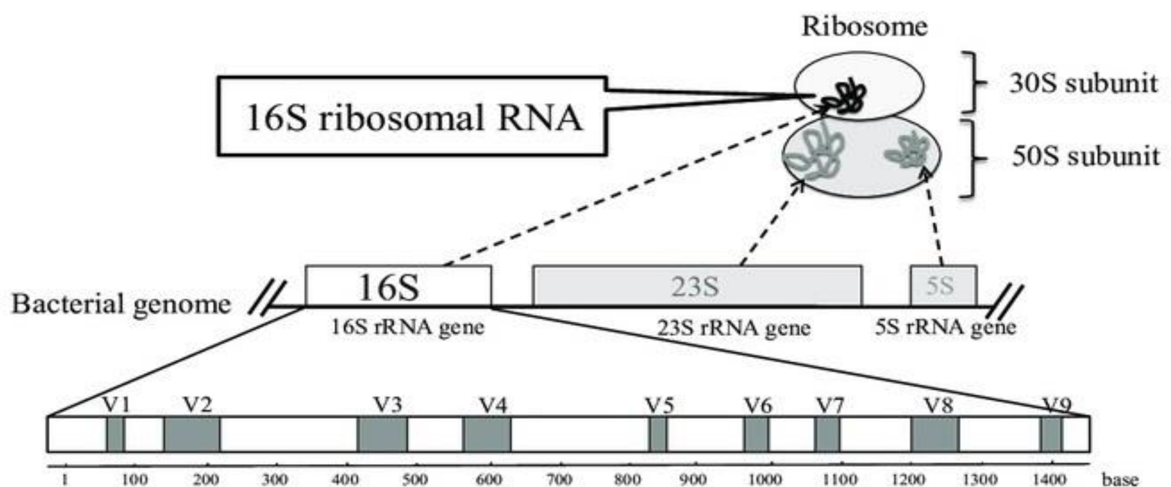


Figure 1: A visual representation of the 30S ribosomal complex. The hypervariable regions (V1-V9) are represented by grey color, and the highly conserved regions are represented in white color. Adapted from Fukuda et al., 2016).

In a follow-up study, Borja (2018) further expanded the index, as well as tested it in different areas of the world. They demonstrated that microgAMBI have the potential to work under different sets of environments and pressures, and that the index provides an efficient system to establish the ecological status based on bacterial communities.

Another recent study by Stoeck et al. (2018) used DNA metabarcoding of benthic bacterial composition to evaluate the ecological footprint from Norwegian salmon farms. In their study they compared their findings with standard macrofaunal biomonitoring surveys and found strong correlations supporting that bacterial communities reacts in a similar way to environmental stressors as do macrofauna communities.

2. Objectives

The usefulness of metabarcoding of marine bacterial communities based on the 16S rRNA gene as bioindicators was assessed in relation to a salmon farm outside Farsund, southern Norway (owned by Korshavn havbruk). To do so, two main research questions were addressed:

1. How does the marine benthic and pelagic bacterial diversity change with distance from "Korshavn havbruk" aquaculture facilities as base?

2. To what degree do marine bacterioplankton communities vary at different depths in the water column and how does this compare to the benthic communities?

3. Materials and methods

3.1 Sampling sites

The different water bodies along the coast are closely connected due to circulation patterns (Figure 2). The water entering the North Sea originates from the Atlantic current. 70 % of the water entering the North Sea also enters the Skagerrak, which is the source for the Norwegian Coastal Current (NCC). The NCC flows along the Norwegian coast and into the Barents Sea in the north (Sætre et al., 2007).

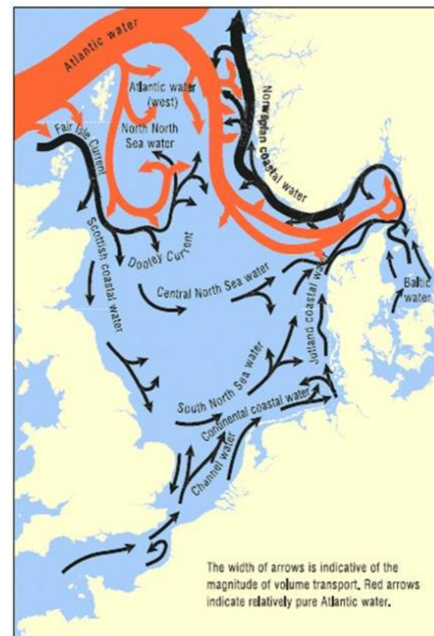


Figure 2: Overview of the main circulation patters along the Norwegian coast (Sætre and Havforskningsinstituttet, 2007).

The sampling area is situated outside the city of Farsund, southern Norway. It is characterized by scattered skerries. The water depth in the area varies, but the sampling sites are all around 60 meters except from the MFS station which extends to 106 meters. The fish cages are located to the east of Langøy, a medium sized island in the Farsund archipelago. The sampling sites Skarvøyflaket and Leirsholmen are located near the fish cages. The MFS (Midtfjordsskjær) station is the main station of the LOBStER (Lister Oceanographic Biologic Station for Education and Research) time series station which have been sampled monthly since 2019. It is located further inshore and is protected by several sills with depths around 30 meters (Figure 4). Norwegian fjords can generally be divided into three layers; Surface, intermediate and basin, each of which are influenced by different environmental conditions (Figure 3). The surface layer typically reaches only a few meters and are influenced by local freshwater input (the river "Lygna" in our case), while the intermediate layer reaches down to the sill level, and the basin (deep) layer is located beneath the sill-depth (Stigebrandt, 2001). This classification of layers is adopted in this thesis.

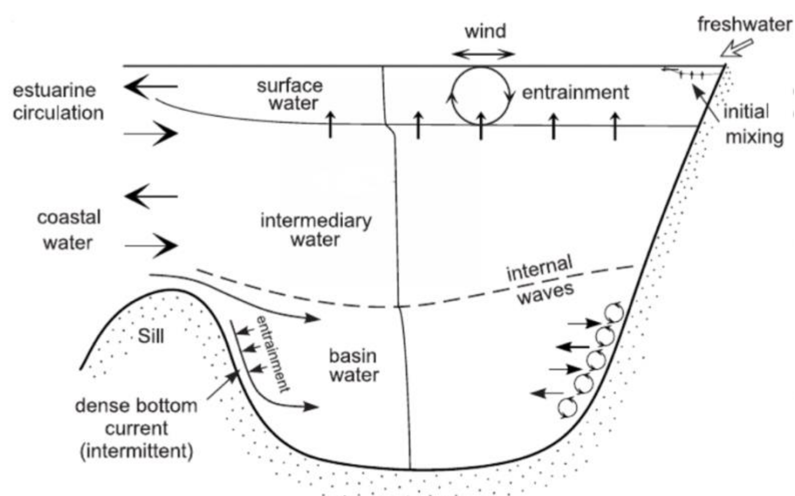


Figure 3: Basic features of a typical fjord system. Adapted from Stigebrandt (2001).

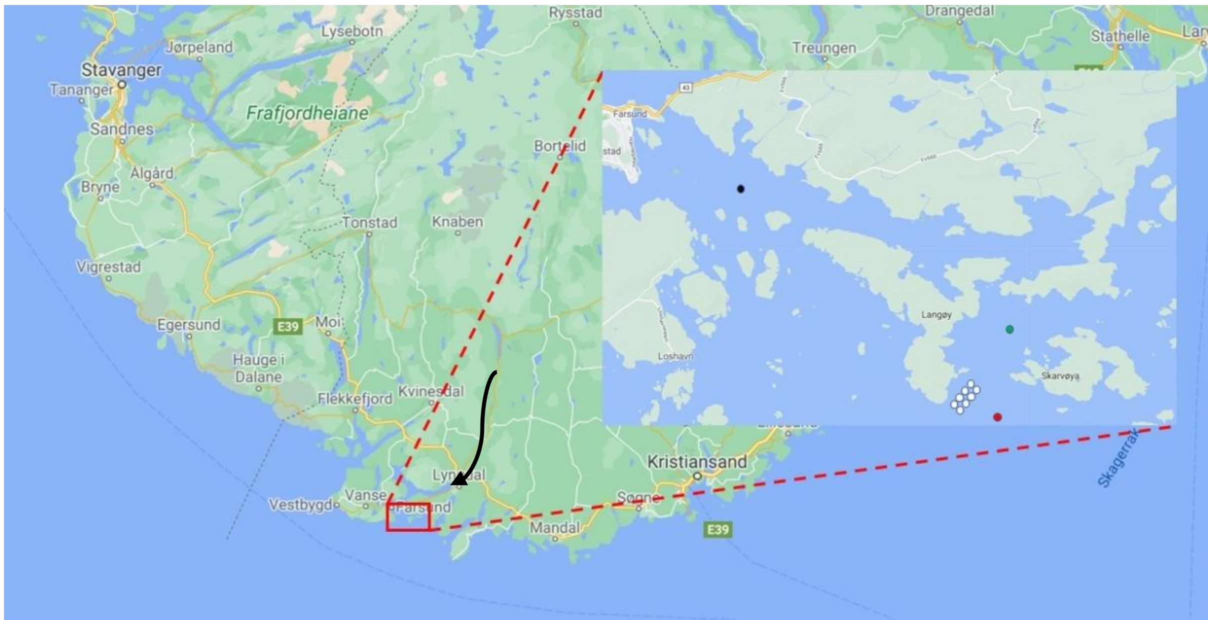


Figure 4: Red dot = Leirsholmen (depth 63,5 m), green dot = Skarvøyflaket (depth 63,5 m), black dot = MFS (depth 106 m), white dots = fish farm "Korshavn Havbruk" (depth ~50 m). In regard to distance Korshavn Havbruk was labelled as "Within", Leirsholmen and Skarvøyflaket as "Close" and MFS as "Far". The black arrow indicates the river "Lygna". Map extracted from maps.google.com

3.2 Benthos samples

For the benthos samples a Van Veen grab (1000 cm^2) with four top lids was used. For each grab, four sub-samples were taken from each of the top lids using a sterile sampling spoon. The spoon was thoroughly washed with 70 % ethanol between each sample. Samples were transferred to 15ml Nunc tubes and stored on ice in a styrofoam box. Back in the lab the samples were transferred to -80 C° freezer awaiting further analysis.

Samples were thawed overnight in a refrigerator prior to further processing. DNeasy Powersoil Pro Kit (Qiagen GmbH, Hilden, Germany) was used to extract DNA from the samples following manufacturer's recommendations with one modification: The lysate was pipetted back into the MB Spin Column and centrifuged at 15,000 x *g* a second time to ensure the spin column was thoroughly rinsed. For each sample 150 mg of sediment was used.

3.3 Water samples

Water samples were collected using a Niskin bottle. Samples were transferred to 1 litre bottles. The bottles were rinsed with sample water from the Niskin bottle 3x before adding the sample. They were then stored on ice in a Styrofoam box. Around 1.6L of seawater was filtered onto 0,22 µm Sterivex™ filters using a Master Flex L/S® (Cole-Parmer, Illinois, USA) peristaltic pump. Filters were stored at -80 C°.

The DNeasy® Plant Mini Kit (Qiagen GmbH, Hilden, Germany) was used to extract DNA from the Sterivex™ filters. The manufacturer's recommendations were followed, with two modifications: 800 µl AP1 buffer was used instead of 400 µl. This to ensure that the entire filter was covered in buffer when vortexed. The 800 µl was extracted with a syringe and treated onwards as two separate samples with 400 µl each (labelled Water (A) and (B)), except for the "St5-M4-5m" sample (Table 1). An error occurred with the syringe, so only 400 µl was extracted and this sample were not labelled with either A or B. One modification was done: The lysate was pipetted back into the DNeasy Spin column and centrifuged again at 6000 x *g* during the last stage of the protocol to ensure all lysate had passed through the filter.

Note: The A and B water samples are to be regarded as technical replicates, and after reviewing the taxonomy for both A and B samples, it was decided to remove the B samples as they were not significantly different from the A samples. A two-tailed type 3 students T-test with 95 % confidence interval was calculated in Microsoft Excel (=TTEST(matrise1;matrise2;2;3)) to support the decision to remove B-samples (see results chapter 4.3). Ideally, the t-test should have been performed on total number of ASVs in the samples as well, but the output from QIIME2 does not provide this data until diversity metrics are calculated. As some of the A and B samples was cut during the rarefaction process prior to diversity metrics calculation, as an alternative, a student t-test was performed using the percentage of the 10 most dominant ASVs from the A and B samples (see appendix 7.3 for a complete list). The samples with date as sample-id was collected and isolated by Chaturi Pabasara Weeraman in summer 2019.

Table 1: Relevant information for all samples before rarefication. Samples are divided into three distance-categories where "Within" is Korshavn havbruk, "Close" is Skarvøyflaket and Leirsholmen and "Far" is MFS station. Samples are also categorized in layers "Benthos", "Surface" (0-2m), "Intermediate" (5-15m) and "Deep" (retrieved approximately 10 meters above seafloor). Note that three water samples collected from empty grab also is in the "Deep" category (St1-M5-A-bv, St1-M5-Fi-bv and St6-M3-A-bv). Number of reads after deblur and removal of chloroplast and mitochondrial sequences together with total ASVs are listed to the far left. Marked in red are the samples that was cut during rarefication (cut-off at 2500 reads) and dismissed for further downstream alpha and beta diversity analysis.

Sample-id	Samplingdate	Coordinates	Sampledepth	Distance	DNA (ng/µl)	A260/A280	A260/230	Sampletype	Layer	Reads	ASVs
Skarvoy-A	25_06_2020	**58°03.288 N**	63,5 m	Close	36,40	1,84	0,36	Benthos	Benthos	5710	461
Skarvoy-B	25_06_2020	**6°53.446 Ø**	63,5 m	Close	26,80	1,83	0,14	Benthos	Benthos	2958	399
Skarvoy-C	25_06_2020		63,5 m	Close	53,10	1,83	0,40	Benthos	Benthos	2534	411
Skarvoy-D	25_06_2020		63,5 m	Close	50,50	1,82	0,74	Benthos	Benthos	2574	407
MFS-A	25_06_2020	**58°05.137 N**	103 m	Far	22,10	1,79	1,47	Benthos	Benthos	3402	514
MFS-B	25_06_2020	**6°49.842 Ø**	103 m	Far	20,80	1,78	0,73	Benthos	Benthos	2467	
MFS-C	25_06_2020		103 m	Far	14,70	1,70	0,07	Benthos	Benthos	2577	387
MFS-D	25_06_2020		103 m	Far	21,40	1,72	1,26	Benthos	Benthos	1426	
Leirs-A	25_06_2020	**58°03.288 N**	63,5 m	Close	17,60	1,69	0,13	Benthos	Benthos	3845	475
Leirs-B	25_06_2020	**6°53.446 Ø**	63,5 m	Close	36,70	1,84	0,36	Benthos	Benthos	1352	
Leirs-C	25_06_2020		63,5 m	Close	36,50	1,79	0,45	Benthos	Benthos	1602	
Leirs-D	25_06_2020		63,5 m	Close	32,00	1,77	0,42	Benthos	Benthos	1667	
St1-M5-A	25_06_2020	**58°03.679 N**	50,3 m	Within	67,40	1,82	1,24	Benthos	Benthos	3673	329
St1-M5-B	25_06_2020	**6°53.188 Ø**	50,3 m	Within	29,00	1,85	0,25	Benthos	Benthos	4547	379
St1-M5-C	25_06_2020		50,3 m	Within	63,70	1,84	1,29	Benthos	Benthos	2473	
St1-M5-D	25_06_2020		50,3 m	Within	45,90	1,86	0,98	Benthos	Benthos	4664	372
St5-M4-A	25_06_2020	**58°03.553 N**	55,1 m	Within	98,30	1,86	1,09	Benthos	Benthos	1725	
St5-M4-B	25_06_2020	**6°53.131 Ø**	55,1 m	Within	110,70	1,85	0,70	Benthos	Benthos	1985	
St5-M4-C	25_06_2020		55,1 m	Within	182,40	1,82	0,54	Benthos	Benthos	2262	

St5-M4-D	25_06_2020		55,1 m	Within	122,70	1,84	2,32	Benthos	Benthos	2120	
MFS-A-2m	25_06_2020	**58°05.137 N**	2 m	Far	7,40	1,48	-2,19	Water	Surface	4206	200
MFS-A-5m	25_06_2020	**6°49.842 Ø**	5 m	Far	9,80	1,58	37,81	Water	Intermediate	3548	94
MFS-A-90m	25_06_2020		90 m	Far	5,60	1,54	-1,12	Water	Deep	2573	138
MFS-Fi-2m	25_06_2020		2 m	Far	4,10	1,61	-0,90	Water	Surface	3000	186
MFS-Fi-5m	25_06_2020		5 m	Far	3,80	1,13	-2,58	Water	Intermediate	1539	
MFS-Fi-90m	25_06_2020		90 m	Far	3,70	1,32	-1,02	Water	Deep	3050	128
St1-M5-A-5m	25_06_2020	**58°03.679 N**	5 m	Within	29,00	1,92	1,73	Water	Intermediate	9328	119
St1-M5-A-45m	25_06_2020	**6°53.188 Ø**	45 m	Within	11,70	1,58	60,09	Water	Deep	5099	164
St1-M5-A-bv	25_06_2020		50,3 m	Within	39,4	1,88	3,58	Water	Deep	3232	293
St1-M5-Fi-5m	25_06_2020		5 m	Within	12,6	1,76	3,39	Water	Intermediate	3271	106
St1-M5-Fi-45m	25_06_2020		45 m	Within	4,70	1,23	-1,08	Water	Deep	5747	167
St1-M5-Fi-bv	25_06_2020		50,3 m	Within	10,30	1,66	5,75	Water	Deep	5897	215
St5-M4-5m	25_06_2020	**58°03.553 N**	5 m	Within	29,60	1,94	2,81	Water	Intermediate	2011	
St5-M4-A-45m	25_06_2020	**6°53.131 Ø**	45 m	Within	6,70	1,42	-1,86	Water	Deep	6201	168
St5-M4-Fi-5m	25_06_2020		5 m	Within	15,1	1,80	2,14	Water	Intermediate	2686	87
St5-M4-Fi-45m	25_06_2020		45 m	Within	1,60	1,11	-0,25	Water	Deep	29398	185
St6-M3-A-bv	25_06_2020	**58°03.698 N**	52 m	Within	62,20	1,94	2,69	Water	Deep	2845	298
St6-M3-Fi-bv	25_06_2020	**6°53.153 Ø**	52 m	Within	13,0	1,61	4,82	Water	Deep	1812	
St7-M2-A-5m	25_06_2020	**58°03.625 N**	5 m	Within	29,70	1,91	2,27	Water	Intermediate	3801	108
St7-M2-A-45m	25_06_2020	**6°53.188 Ø**	45 m	Within	20,00	2,00	3,15	Water	Deep	7618	257
St7-M2-Fi-5m	25_06_2020		5 m	Within	9,90	1,95	6,43	Water	Intermediate	3412	100
St7-M2-Fi-45m	25_06_2020		45 m	Within	9,10	1,98	4,13	Water	Deep	3252	198
31may-0m	31_05_2019	**58°05.137 N**	0 m	Far	N/A	N/A	N/A	Water	Surface	6613	205
31may-90m	31_05_2019	**6°49.842 Ø**	90 m	Far	N/A	N/A	N/A	Water	Deep	6093	230
26june-0m	26_06_2019		0 m	Far	N/A	N/A	N/A	Water	Surface	5699	73
12june-15m	12_06_2019		15 m	Far	N/A	N/A	N/A	Water	Intermediate	4344	152
12june-90m	12_06_2019		90 m	Far	N/A	N/A	N/A	Water	Deep	4511	209
26june-15m	26_06_2019		15 m	Far	N/A	N/A	N/A	Water	Intermediate	1581	
12june-0m	12_06_2019		0 m	Far	N/A	N/A	N/A	Water	Surface	1331	
29july-0m	29_07_2019		0 m	Far	N/A	N/A	N/A	Water	Surface	1204	
12july-0m	12_07_2019		0 m	Far	N/A	N/A	N/A	Water	Surface	1670	
26june-90m	26_06_2019		90 m	Far	N/A	N/A	N/A	Water	Deep	2269	
29aug-90m	29_08_2019		90 m	Far	N/A	N/A	N/A	Water	Deep	1677	
29july-15m	29_07_2019		15 m	Far	N/A	N/A	N/A	Water	Intermediate	1167	
12aug-90m	12_08_2019		90 m	Far	N/A	N/A	N/A	Water	Deep	1406	
31may-15m	31_05_2019		15 m	Far	N/A	N/A	N/A	Water	Intermediate	962	
12july-90m	12_07_2019		90 m	Far	N/A	N/A	N/A	Water	Deep	947	
29july-90m	29_07_2019		90 m	Far	N/A	N/A	N/A	Water	Deep	683	
12july-15m	12_07_2019		15 m	Far	N/A	N/A	N/A	Water	Intermediate	646	
29aug-0m	29_08_2019		0 m	Far	N/A	N/A	N/A	Water	Surface	530	
12aug-15m	12_08_2019		15 m	Far	N/A	N/A	N/A	Water	Intermediate	489	
12aug-0m	12_08_2019		0 m	Far	N/A	N/A	N/A	Water	Surface	142	

3.3.1 Filters

To test how the well the washing (vortexing) of the filters performed, DNA from the filters were also isolated. The filters were carefully removed from the sleeve using a sterile scalpel. The filter was placed in a petri dish with the exposed side turned up. The same scalpel was used to then cut the filter into 8 equally sized pieces. The filter pieces were then placed in a microcentrifuge vial and approximately 0.3 grams of Biospec Zirconia/Silica beads (ThermoFisher Scientific Inc, UK) and 700 µl AP1 buffer was added. The vials containing the filter pieces were then treated with two rounds à 45 sec at 2800 rpm in a MagNA Lyser Rotor (Roche Molecular Systems Inc, Switzerland). Using a set of sterile tweezers, the filter pieces was carefully removed from the tube. This had to be done because the pipette would not reach the eluate with the filter pieces still in the tube. With the filter pieces out, the lysate (around 500 µl) was carefully pipetted out. The rest of the procedure was performed following the same protocol as with the water samples.

3.4 PCR and gel electrophoresis

The extracted DNA was also tested with PCR prior to shipping samples to Integrated Microbiome Resource, Dalhousie University, Halifax, for library preparation and MiSeq sequencing. For sequencing of the V3-V4 region the bacterial specific primers Bakt_341F (CCTACGGGNGGCWGCAG) and Bakt_805R (GACTACHVGGGTATCTAATCC) (Herlemann et al., 2011) were used. The preparations for PCR were the same for both the benthos and water samples. A 300 bp fragment of the V4 region of the 18S rRNA gene was amplified using the eukaryote V3-V4 primers (10 µM) 341F-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG[CCAGCASCYGCGGTAATTCC] and 805R-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG[ACTTTCGTTCTTGATYRATGA] (Piredda et al., 2016). Each sample was amplified in a total volume reaction of 25 µl using 12,5 µl 2x DreamTaq Green PCR Master Mix (ThermoFisher Scientific Inc, UK), 0,5 µl of each forward and reverse primers and 2 µl of DNA. PCR cycle regime were as follows: 95 °C for 3 min, 35 cycles of 95 °C for 1 min, 52 °C for 30 seconds, 72 °C for 1 min and a final extension of 5 min at 72 °C. Negative controls were added to all reactions.

The PCR-product were then run on gel electrophoresis in a 500 mg/50 mL agar/TAE buffer (1x). Run voltage was set to 80 V. Gel runs was then inspected on NuGenius imaging system (Synoptics Ltd., Cambridge, UK).

3.5 Nanodrop analysis

All samples were analysed in a spectrophotometer (NanoDrop™ One, ThermoFisher Scientific Inc, UK) to measure DNA concentration and sample purity. Sensors was cleaned before adding 1 µl of AE buffer as blanking sample. 1 µl of each sample was measured. DNA concentration in ng/µl indicates how much DNA is in the sample. The ratio of the absorbance at wavelengths 260 nm over 280 nm (A260/280) shows the purity of nucleic acids, where 1.8 is optimal. Low values indicate contamination at 280 or less. For the ratio between the absorbance at 260 nm over 230 nm (A260/230), 2.0-2.2 is optimal. Low values indicate contamination at absorbance 230 nm or less. Contamination may be due to proteins and phenols at around 280 nm, and humus acids or carbohydrates at around 230 nm.

3.6 Sequence data processing and taxonomic assignment

3.6.1 Importing and primer removal

The sequence data was visually quality checked using FASTQC (version 0.11.0-Java-11). After inspection, FASTQs files were imported to QIIME2 (Rideout et al., 2016) as type "SampleData[PairedEndSequencesWithQuality] with input format "CasanovaOneEightSingleLanePerSampleDirFmt". A metadata file was produced using Excel and validated with the add-on Keemei (Rideout et al., 2016). All further downstream analyses were done in QIIME2/2020.11 and all code was written in BBEdit.

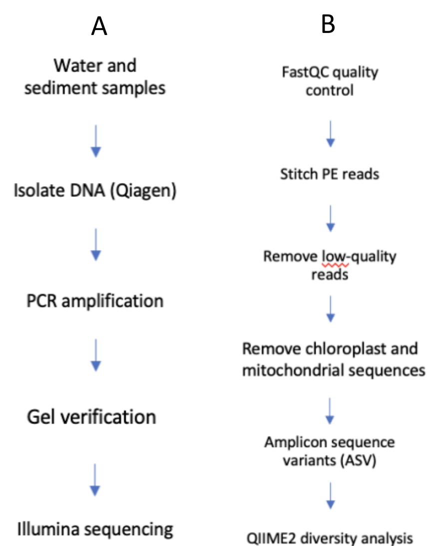


Figure 5: Simplified workflow diagram showing the on-bench procedure (A) and bioinformatic pipeline (B).

Primers were removed with plugin "qiime cut adapt trim-paired" (Martin, 2011) and then summarized with plugin "qiime demux summarize" to make a visual inspection that the primers were successfully removed.

3.6.2 Denoising reads into amplicon sequence variants (ASVs)

Forward and reverse reads was joined with plugin "qiime vsearch join-pairs" before low-quality reads were filtered out using plugin "qiime quality-filter q-score" and summarized with "qiime demux summarize". To correct reads and get ASVs the plugin "qiime deblur denoise-16S" was run. Prior to running deblur, sequences needed to be trimmed to the same length. This was determined after inspection of the quality filter summarization and set with *–p-trim-length 427*. The deblur data was then summarized. An overview of the number of reads is supplied in appendix 7.2.

3.6.3 Taxonomic assignment and box plots

The taxonomic classification was run with plugin "qiime feature-classifier classify-sklearn" (Bokulich et al., 2018) with the latest classifier "silva-138-99-nb-classifier.qza" as reference database. All taxonomic box plots from chapter 4.2 and onwards were produced at taxonomic rank 4 (order). This rank was chosen because it provides sufficient insight into the diversity within each phylum, and simultaneously avoid creating too much noise in the box plots. From each "Layer" and "Distance" category, the overall most represented orders were chosen as basis of comparison.

3.6.4 Filtering resultant tables

ASVs with a frequency lower than 0.1% of the mean sample depth was filtered out using plugin "qiime feature-table filter-features" with *–p-min-frequency* set to 4. Further, contaminants and noise based on taxonomic labels was filtered out with plugin "qiime taxa filter-table". The resulting feature

table was then summarized to be able to determine the maximum depth across all samples. Max depth was set with *–p-max-depth 29398.*

A rarefaction curve was then produced (Figure 6) using plugin "qiime diversity alpha-rarefaction" to inspect where the correct cut-off should be set.

Following inspection of the rarefaction curve, two final feature tables was produced with "qiime feature-table filter-samples": One where cut-off was set to 1 for retaining all samples for taxonomical boxplot analysis, and one rarefied table with sequences cut-off set at 2500 (all samples with a lower read frequency than 2500 were discarded). The latter was summarized with representative sequences using plugin "qiime feature-table filter-seqs" and used for further downstream alfa and beta diversity analyses.

Table 2: Two feature tables was produced for downstream analysis. Left showing information about the data set before rarefication where all samples are retained. The right table are data set information after rarefaction with cut-off at 2500 reads (meaning all samples with a lower read frequency than 2500 reads were discarded).

Deblur summary (cut-off: 1)	
Number of samples	*62*
Number of ASVs	*2.676*
Total frequency	*212.745*
Minimum frequency	*142*
1st quartile	*1.610*
Median frequency	*2.574*
3rd quartile	*4.104*
Maximum frequency	*29.398*
Mean frequency	*3.431*

Rarefied data summary (cut-off: 2500)	
Number of samples	*34*
Number of ASVs	*2.521*
Total frequency	*171.680*
Minimum frequency	*2.534*
1st quartile	*3.091*
Median frequency	*3.817*
3rd quartile	*5.706*
Maximum frequency	*29.398*
Mean frequency	*5.049*

3.6.5 Rarefaction

The cut-off to decide for which samples to exclude for further downstream analyses was based on the rarefaction curve (Figure 6). This approach of rarefying data is a commonly used method, as it has proved to be useful for many other microbial community analyses as samples with low sequencing depth often produce noise (Weiss et al., 2017). Despite the usefulness, it also has its drawbacks, one example being that it may discard large amounts of information depending on where the cut-off is set (McMurdie and Holmes, 2014).

3.6.6 Phylogenetic tree and diversity metrics

A phylogenetic tree for the ASVs was produced using the SEPP method with the plugin "qiime fragment-insertion sepp" (Janssen et al., 2018). As reference database "sepp-refs-gg-13-8.qza" was used.

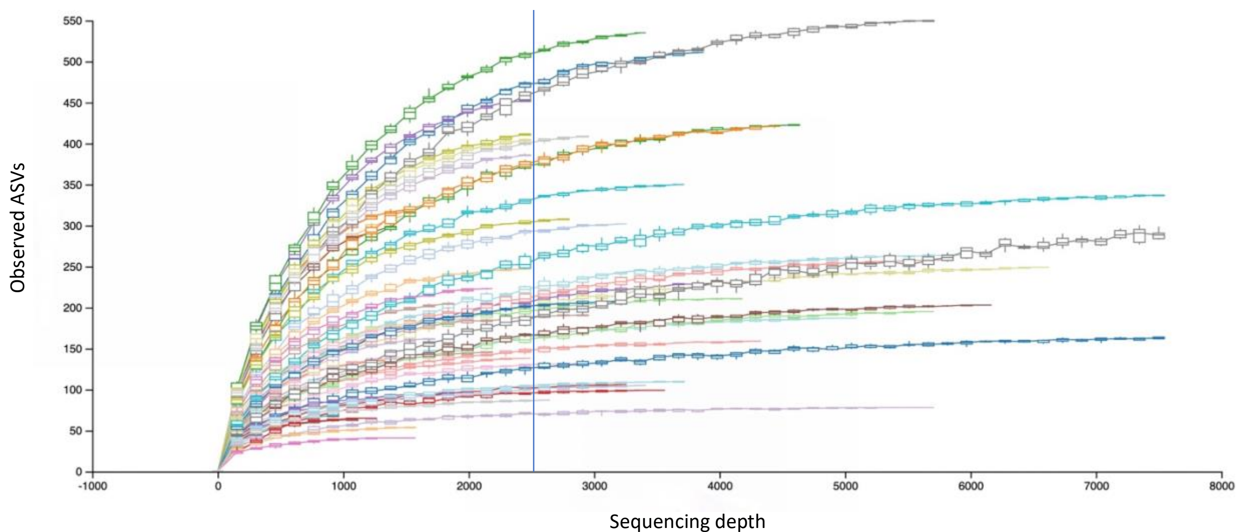


Figure 6: Rarefaction curve showing all samples and their sequencing depth after deblur. Max sampling depth was set to 7500 and the cut-off for retaining samples was set at 2500.

Microbiome data are predominantly gauged utilizing alpha and beta diversity. Alpha diversity measures the variation within one sample, and this can be compared across sample groups. Beta diversity measures the change between samples.

There are numerous methods that measure both alfa and beta diversity. Choosing the correct one for the data in question is important. As a deep understanding of all the different methodologies currently encompassing the field of diversity analysis can be overwhelming (Moreno et al., 2017), but the implemented diversity analysis solutions in QIIME2 are the most frequently used in microbiome analysis (Estaki et al., 2020, Moreno et al., 2018). The implemented solutions include, but are not limited to, Shannon and Observed features indexes for alpha diversity measures, and Bray-Curtis index for beta diversity measures. These have been utilized on similar data (Stoeck et al., 2018), and are also included in the analysis in this thesis.

Normalized data summary	
Number of samples	*34*
Number of ASVs	*2.438*
Mean frequency	*2.500*
Total frequency	*85.000*

Table 3: The number of samples and unique ASVs retained after normalization of dataset at sequencing depth 2500.

To calculate diversity metrices and generating ordination plots the plugin "qiime diversity core-metrics-phylogenetic" was used. Samples were normalised at sequencing depth 2500 with "--p-sampling-depth 2500" (Table 3). This plugin supplies

a range of different alpha and beta diversity analysis. For this thesis Shannon's Index (accounting for evenness and abundance) and Observed Features was produced for inspection of alfa-diversity. These plugins also produce a Kruskal-Wallis analysis which is a ranked non-parametric one-way ANOVA that is used for comparing two or more independent samples with equal or different sample sizes (McKight and Najab, 2010). Bray-Curtis dissimilarity was produced measuring the beta-diversity. For visualisation the "EMPeror" tool was used (Vázquez-Baeza et al., 2017, Vázquez-Baeza et al., 2013).

The SOP followed in this study were based on the SOP provided by Langille Lab, Dalhousie University, Halifax, Nova Scotia, Canada, and can be viewed at https://github.com/LangilleLab/microbiome_helper/wiki/Amplicon-SOP-v2-(qiime2-2020.8). All code is supplied in appendix 7.4.

4. Results

4.1 Samples overview

A total of 62 samples were analysed with reads ranging from 142 to 29398 after filtering and deblur procedures. Samples with reads below 2500 were dismissed as low-read samples are prone to create undesired noise (Weiss et al., 2017). Complete legend to all bar charts at taxonomic rank 4 (order) are supplied in appendix 7.1.

After rarefaction and removal of the B samples, a total of 34 samples normalized at 2500 reads were retained for alfa and beta analysis (Table 3), these samples included a total of 2438 unique ASVs ranging from 73 to 504 ASVs.

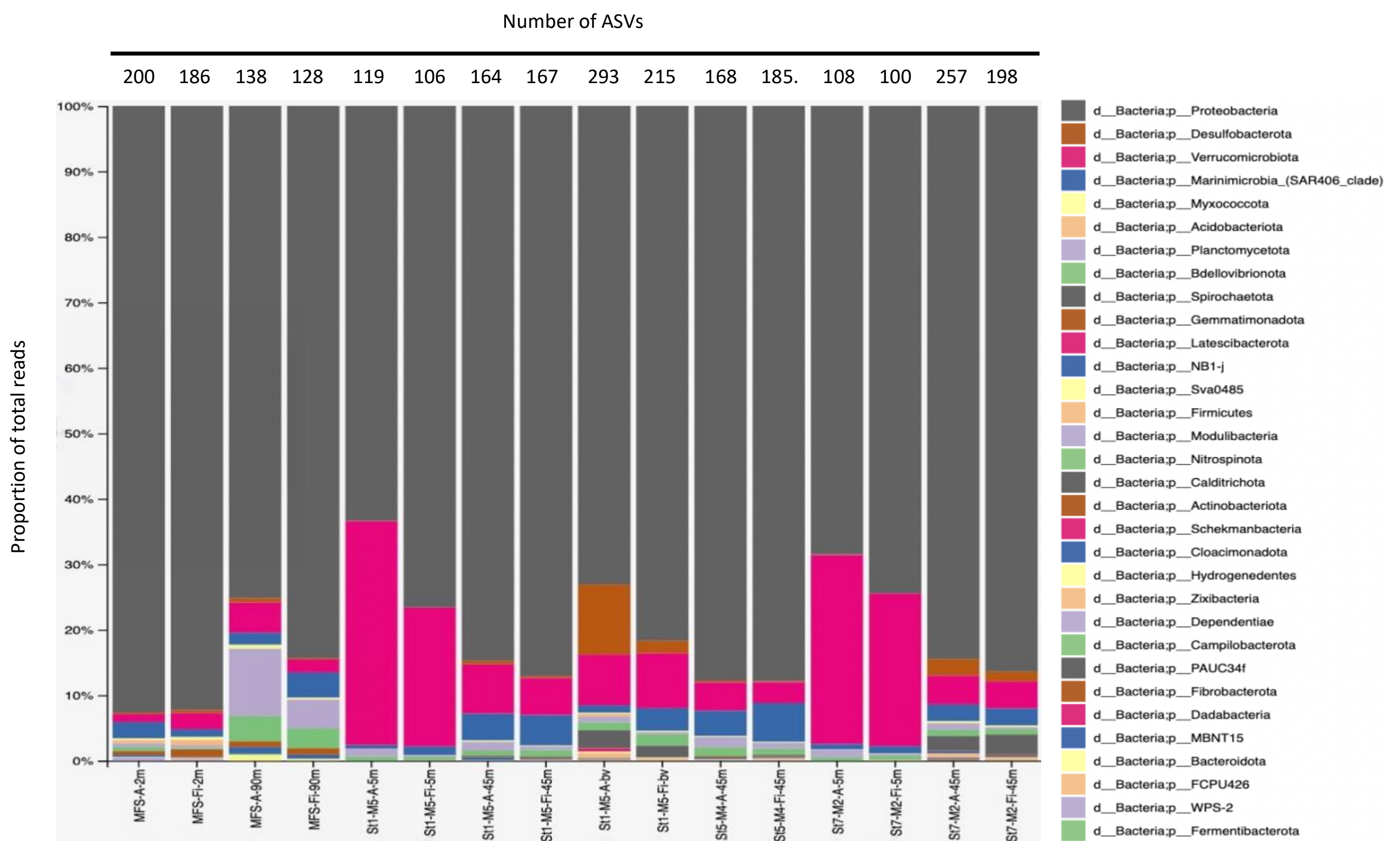


Figure 7: Box plot displaying a comparison at taxonomic rank 2 (phylum) between A-samples (eluate) and Fi-samples (filters). Samples are paired next to each other, and the total number of ASVs are shown on the top. The colors in the box plot corresponds to the legend on the right.

4.2 Comparing eluate and filter samples

The taxonomic bar chart at phylum level (Figure 7) showed a clear relationship between the eluate and filter samples, and no significant difference in the number of ASVs was found based on the two different DNA extracts (p-value = 0,48; Table 3)

Table 3: Number of ASVs in eluate and filter samples and results from student t-test. "+" means more ASVs was observed in filters samples than in eluate.

Sample-id	ASVs Eluate	ASVs Filter	Difference	Student t-test
MFS-A-2m / MFS-Fi-2m	200	186	14	
MFS-A-90m / MFS-Fi-90m	138	128	10	H0: No observed difference in eluate and filter samples
St1-M5-A-5m/ St1-M5-Fi-5m	119	106	13	H1: Observed difference in eluate and filter samples
St1-M5-A-45m / St1-M5-Fi-45m	164	167	+3	
St1-M5-A-bv / St1-M5-Fi-bv	293	215	78	α = 0,05
St5-M4-A-45m / St5-M4-Fi-45m	168	185	+17	p-value = 0,48
St7-M2-A-5m / St7-M2-Fi-5m	108	100	8	
St7-M2-A-45m / St7-M2-Fi-45m	257	198	59	P > 0,05 → H0 is not rejected
Total	1628	1446	202	

4.3 Removal of water B-samples

P-values from a two-side student t-test ranged from 0.90 to 0.99 for all tests with a mean value of 0.97 (Table 4) and confirmed that there is no significant difference between the two water samples extracted from the same filter. Further, inspecting the taxonomic bar chart comparing the A and B water samples supported this by showing very similar content within each pair (Figure 8). A complete list over the 10 most dominating orders within each sample used in evaluating the similarity between A and B samples are supplied in appendix 7.3.

Bacterial community variation in A and B samples

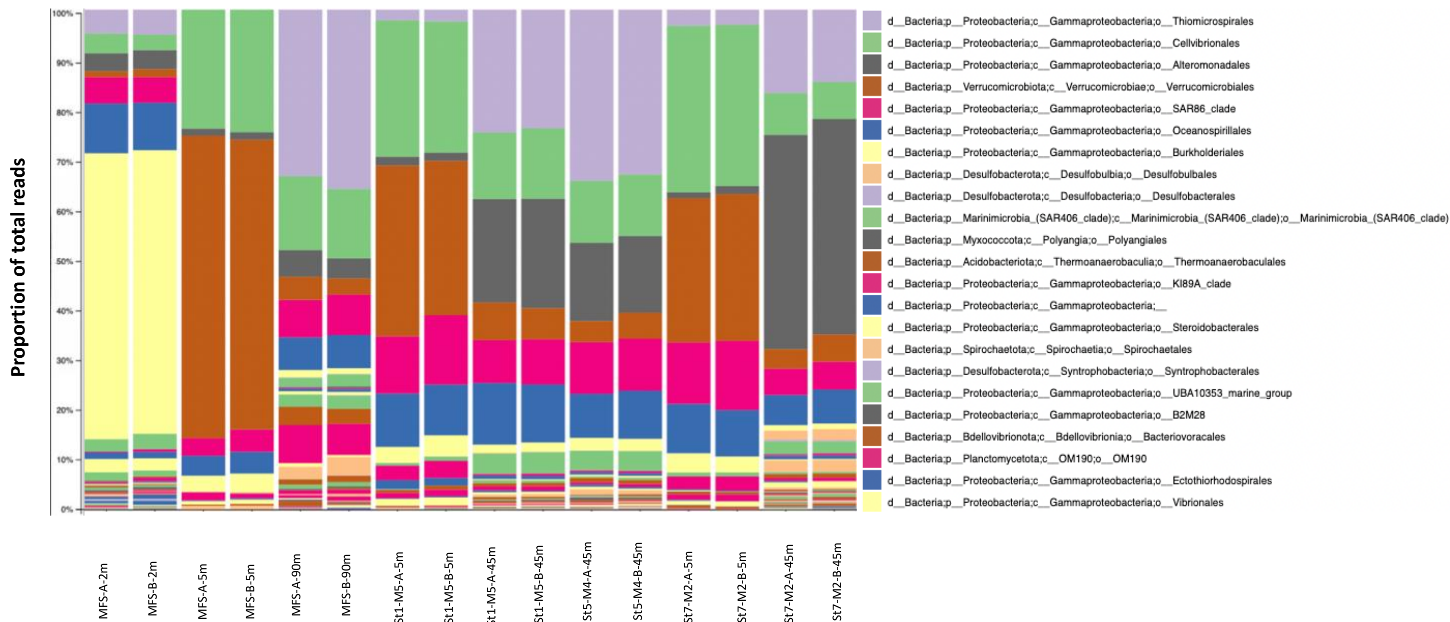


Figure 8: Box plot displaying a comparison between A and B samples at taxonomic rank 4 (order). The colors in the box plot corresponds to the legend on the right. Sample-id on the x-axis.

Table 4: P-value calculated based on the 10 most dominating orders between the A and B samples.

Samples compared	P-value	Students t-test
MFS-A-2m / MFS-B-2m	0,987	
MFS-A-5m / MFS-B-5m	0,995	H0: No observed difference between A and B samples
MFS-A-90m / MFS-B-90m	0,963	H1: Observed difference between A and B samples
St1-M5-A-5m / St1-M5-B-5m	0,991	
St1-M5-A-45m / St1-M5-B-45m	0,992	α = 0.05
St5-M4-A-45m / St5-M4-B-45m	0,997	Mean p-value = 0.97
St7-M2-A-5m / St7-M2-B-5m	0,991	
St7-M2-A-45m / St7-M2-B-45m	0,901	P > 0.05 → H0 is not rejected

4.4 Bacterial/Bacterioplankton community variations

4.4.1 Comparing layers

The surface layer is mainly dominated by Gammaproteobacteria which collectively made up approximately 88 % of the total richness (Table 5). The two orders Cellvibrionales and Burkholderiales are the most represented with 22.5 % and 27.8 % of the total reads. The orders Oceanospirillales and SAR86 also shows a high presence with 15.9 % and 14.1 % of total reads, respectively.

Bacterial community variation in all samples separated by layer

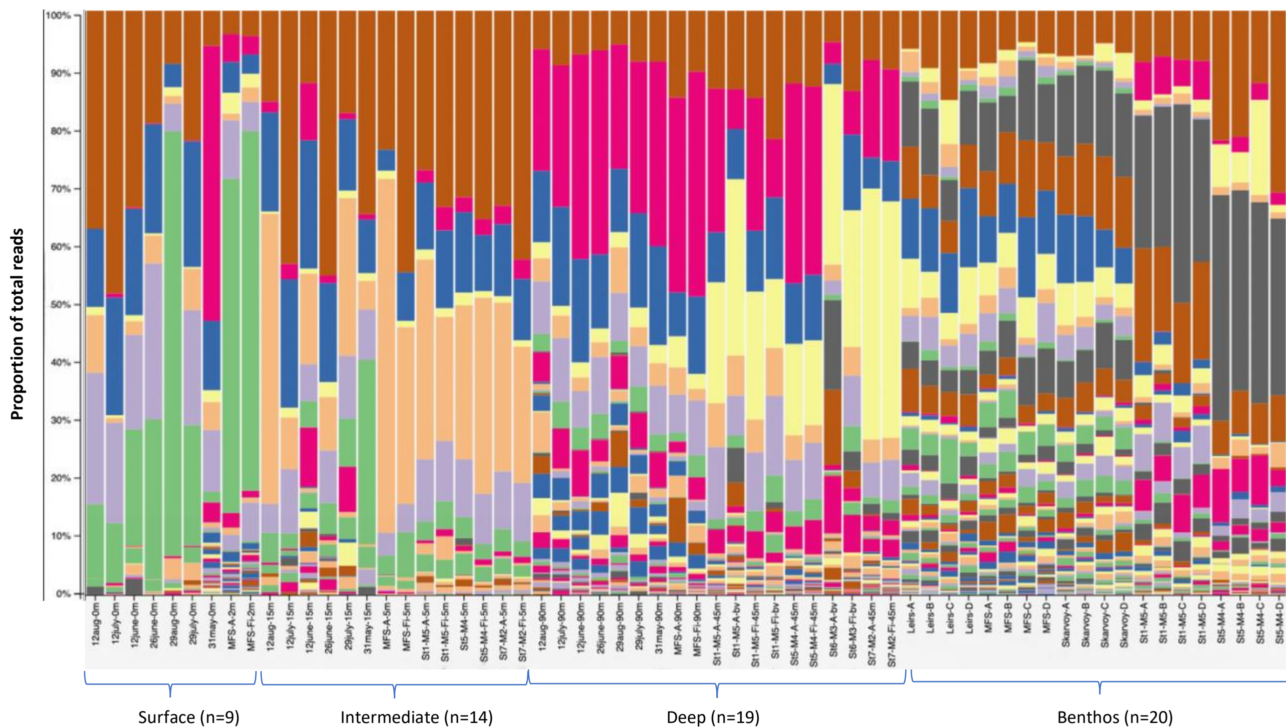


Figure 9: Bar chart visualizing the bacterial community variation across all samples at taxonomic rank 4 (order) sorted by layer. Water samples are categorized as follows: "Surface" = 0-2m depth, "Intermediate" = 5-15m depth, "Deep" = 10 meters above seafloor and "Benthos" which are sediment samples. Each color represents a different ASV. Some colors are repeated but they still represent different ASVs. Legend is provided in Appendix 7.1.

Table 5: The most dominant orders by percentage of total reads in the surface layer (all Gammaproteobacteria + Verrucomicrobiota) compared with the other layers. For the Benthos samples, the 3 most represented Desulfobacterota was choosen as basis of comparison to the other layers. X = negligible coverage.

	Order	% of total reads Surface	Intermediate	Deep	Benthos
Gammaproteobacteria	Cellvibrionales	22,5 %	29,7 %	8,4 %	10,9 %
	SAR86 clade	14,1 %	12,3 %	14,3 %	x
	Alteromonadales	1,3 %	1,4 %	18,6%	3,0 %
	Oceanospirillales	15,9 %	8,3 %	10,7 %	0,7 %
	Burkholderiales	27,8 %	10,0 %	3,7 %	0,5 %
	Thiomicrospirales	6,6 %	2,5 %	29,3 %	1,6 %
	Steroidobacterales	x	x	x	2,5 %
	Verrucomicrobiales	4,0 %	25 %	5,6 %	1,4 %
Desulfobacterota	Desulfobulbales	x	x	x	19,0 %
	Desulfobacterales	x	x	x	10,2 %
	SVA_1033	x	x	x	2,2 %
	Other	7,8 %	10,7 %	9,5 %	48 %

The intermediate layer is also dominated by Gammaproteobacteria with approximately 64 % of the total richness (Table 5). As with the surface layer, the order Cellvibrionales are strongly represented with 29.7 % of the total reads. Order Burkholderiales accounts for 10 % which is 17.5 % less than in the surface layer. Order Verrucomicrobiales (phylum Verrucomicrobiota) represents a quarter of the total reads.

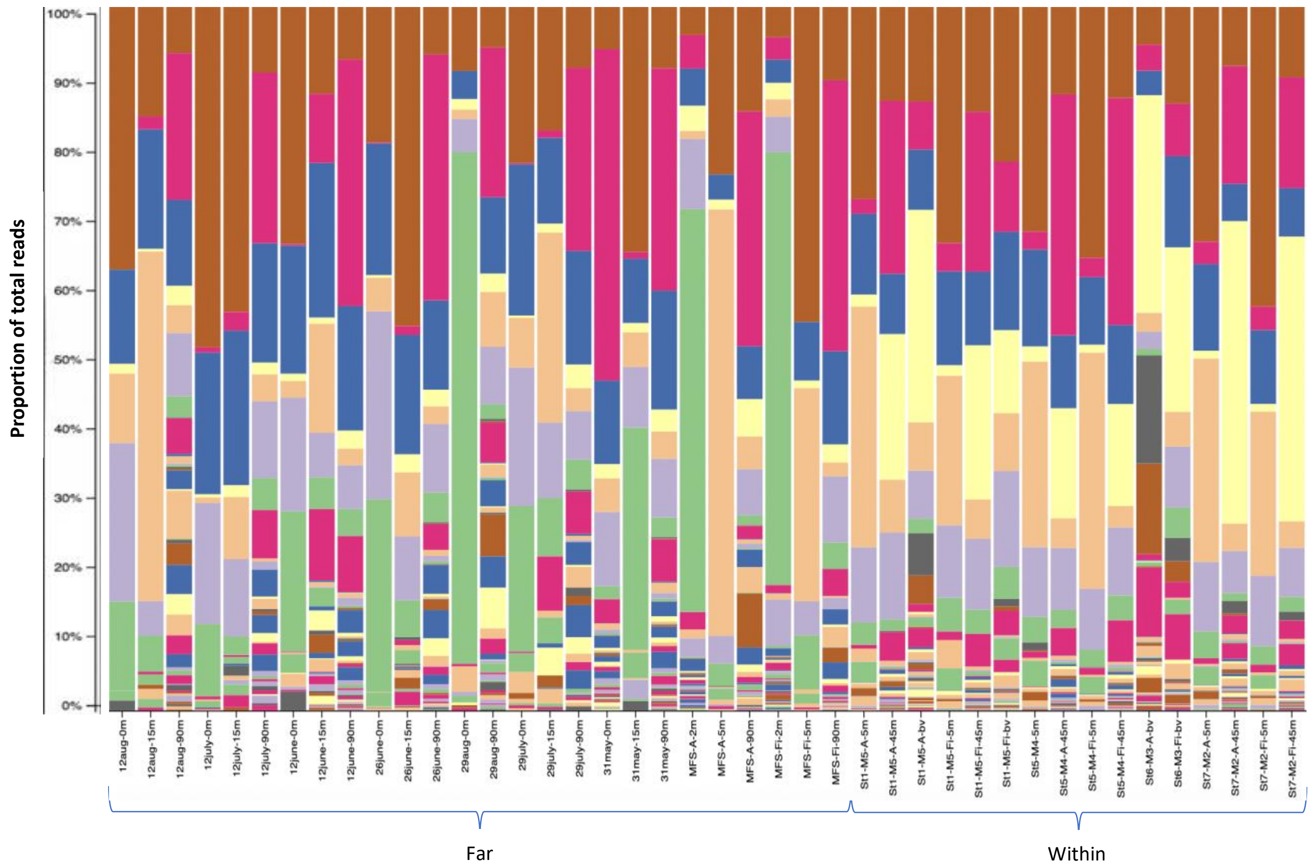
The deep layer is also dominated by the class Gammaproteobacteria with the orders Thiomicrospirales and Alteromonadales being the most represented with 29.3 % and 18.6 % respectively (Table 5). These two orders were not present to any significant degree in either surface or intermediate layers. Order Verrucomicrobiales (phylum Verrucomicrobiota) did only represent 5.6 % of the total reads in the deep layer compared to 25 % in the intermediate layer.

In the benthos layer Gammaproteobacteria was much less dominating, with only 19 % of total reads with order Cellvibrionales accounting for 10.9 % (Table 5). All other orders which are dominating the surface, intermediate and deep layers are almost non-existent in the benthos layer. The class Desulfobacterota with the orders Desulfobulbales (19 %), Desulfobacterales (10.2 %) and SVA_1033 (2.2 %) are not represented in other any other layers than benthos (Table 5). The group "Other" makes up almost half of the represented richness.

4.4.2 Water samples

Figure 9 shows a visualization of the bacterioplankton community variation in the water samples. When comparing the water samples from "Far" and "Within", Gammaproteobacteria is the dominating class in both categories (Table 6). The orders Cellvibrionales and SAR85 clade are represented to a similar extent in both categories, while Alteromonadales have a noticeably higher presence in "Within" than "Far" (16.3 % compared to 1.9 %). The order Burkholderiales show the opposite trend, with a larger presence in "Far" than in "Within", 14.7 % and 2.9 % respectively.

Bacterioplankton community variation in all water samples separated by distance to Korshavn havbruk aquaculture facility



Figur 10: Box plot showing the bacterioplankton community diversity at taxonomic rank 4 (order) in all water samples separated by distance. "Within" are samples from Korshavn havbruk aquaculture facility outside Langøy and "Far" are samples from MFS. Sample-id on the x-axis. Legend is provided in Appendix 7.1.

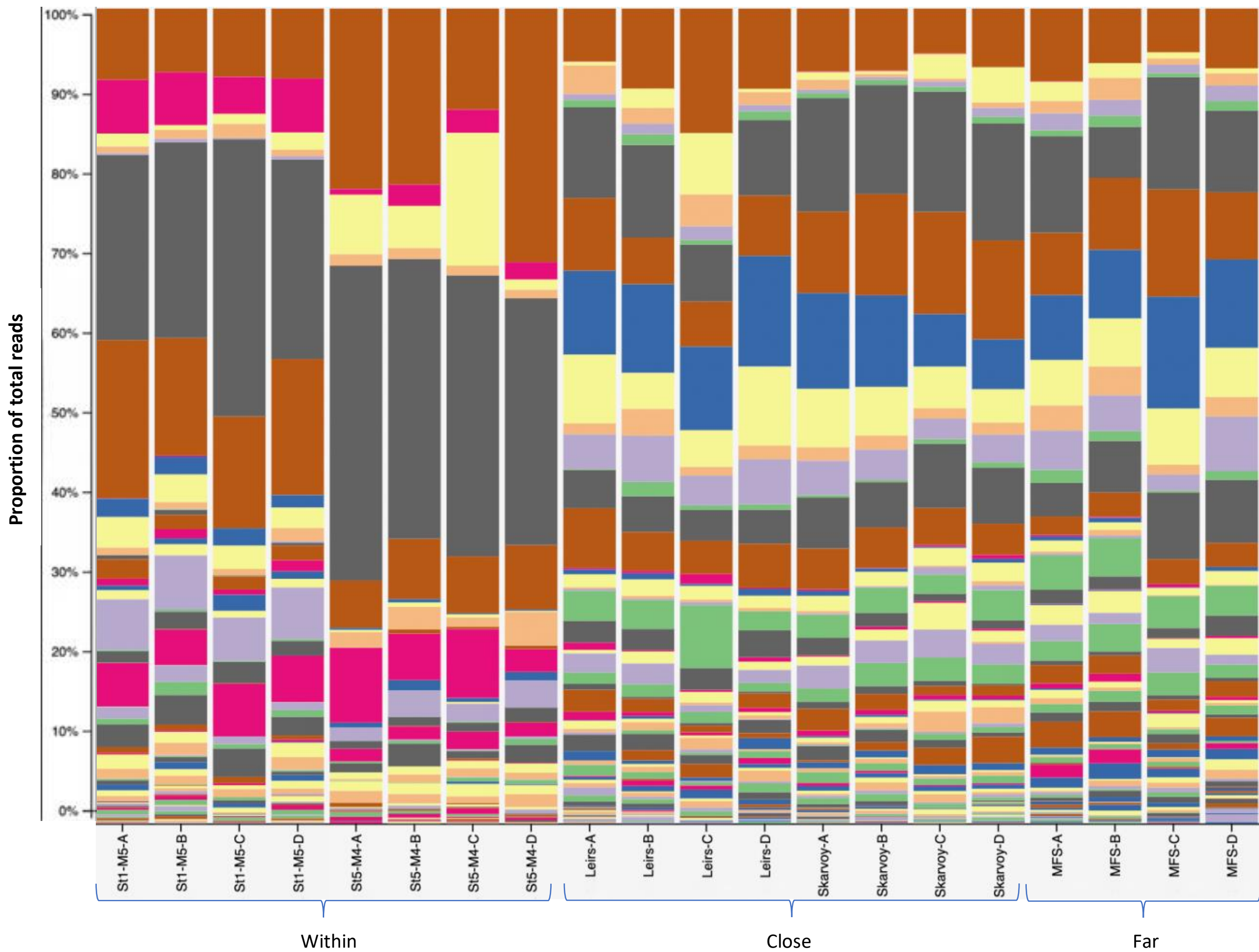
4.4.3 Benthos samples

Distance comparison of the benthos samples are visualized in Figure 11 and summarized in Table 7. All Gammaproteobacteria shows a declining rate of presence from "Within" towards "Far" except from the order Steroidobacterales. The two orders Syntrophobacterales and SVA_1033 (class Desulfobacterota) show a similar trend with an increasing presence with increased distance to "Within". The order Desulfobulbales shows the opposite trend, with a substantial representation of 30.3 % of total reads found "Within", and a lower presence in both "Close" and "Far" (11.5 % and 10.4 % respectively).

Table 6: Percentage coverage of the dominating orders in water samples from the categories "Within" and "Far" compared with each other. Corresponds to Figure 9.

		Within	Far
	Order	**% of total reads**	
Gammaproteobacteria	Cellvibrionales	19,8 %	12,2 %
	SAR86 clade	10,1 %	13,5 %
	Alteromonadales	16,3 %	1,9 %
	Oceanospirillales	9,0 %	10,2 %
	Burkholderiales	2,9 %	14,7 %
	Thiomicrospirales	12,0 %	13,0 %
	Verrucomicrobiales	13,6 %	20,5 %
	Other	16,4 %	24,0 %

Bacterial community variation in all benthos samples separated by distance to Korshavn havbruk aquaculture facility



Figur 11: Box plot showing the bacterial community diversity at taxonomic rank 4 (order) in all benthos samples separated by distance. "Within" are samples from Korshavn havbruk aquaculture facility outside Langøy, "Close" are samples from Leirsholmen and Skarvøyflaket, and "Far" are samples from MFS. Sample-id on the x-axis. Legend is provided in Appendix 7.1.

Table 7: Percentage coverage of the dominating orders in benthos samples from the categories "Within", "Close" and "Far" and compared with each other. Corresponds to Figure 10.

	Order	Within	Close	Far
		% of total reads		
Gammaproteobacteria	Cellvibrionales	15,0 %	8,6 %	7,0 %
Gammaproteobacteria	Burkholderiales	X	0,8 %	0,9 %
Gammaproteobacteria	Alteromonadales	4,4 %	2,4 %	1,4 %
Gammaproteobacteria	Thiomicrospirales	4,0 %	X	X
Gammaproteobacteria	Steroidobacterales	X	4,0 %	4,4 %
	Desulfobulbales	30,3 %	11,5 %	10,4 %
	Desulfobacterales	11,5 %	9,3 %	9,4 %
	Syntrophobacterales	X	5,4 %	6,5 %
	SVA_1033	X	3,6 %	4,1 %
	Thermoanaerobaculales	1,7 %	6,2 %	6,1 %
	Verrucomicrobiales	1,1 %	1,6 %	1,5 %
	Polyangiales	1,1 %	10,0 %	10,2 %
	Other	42,3 %	45, 5%	47,4 %

4.5 Alpha diversity analysis

Shannon Index ranges from 1-10 indicating the degree of bacterioplankton community diversity where 1 is low and 10 is high (abundance and evenness accounted for). Diversity in benthos samples were higher than in all other layers (Figure 9). Benthos samples have a mean Shannon index of 7,5 and 50 % of the data are within the 7,0-7,5 index range (Figure 12). The mean of both “Surface”, “Intermediate” and “Deep” all are within each other’s interquartile ranges with a Shannon index of around 5,0 (Figure 12). Thus, benthos samples are more diverse than water samples, this is supported by the Kruskal-Wallis analysis with p-values > 0.05 for all samples when compared with “Benthos” (Table 8). Observed ASVs in benthos layer are higher than all other layers, and the interquartile ranges from 375-450 observed ASVs with a median of just above 400 for benthos samples (Figure 13).

Table 8: Kruskal-Wallis results from Shannon diversity and Observed ASVs analysis. Group A = “Layer”, group B = “Benthos distance” and C = “Water distance”. To avoid Type I error (false positive) the p-value is corrected with the Benjamini-Hochberg procedure (q-value), and this is used to determine a significant result (α = 0.05, indicated by “x”).

			Shannon diversity				Observed ASVs			
	Group 1	**Group 2**	**H-statistic**	**p-value**	**q-value**	**Sig. result**	**H-statistic**	**p-value**	**q-value**	**Sig. result**
A	Surface (n=3)	Intermediate (n=8)	0.375	0.540	0.545	-	0.666	0.414	0.414	-
A	Surface (n=3)	Deep (n=13)	0.367	0.545	0.545	-	1.308	0.253	0.303	-
A	Surface (n=3)	Benthos (n=10)	6.429	0.011	0.022	X	6.428	0.1120	0.017	X
A	Intermediate (n=8)	Deep (n=13)	3.823	0.051	0.076	-	10.621	0.001	0.002	X
A	Intermediate (n=8)	Benthos (n=10)	12.632	0.001	0.001	X	12.632	0.001	0.001	X
A	Deep (n=13)	Benthos (n=10)	16.250	0.000	0.001	X	16.250	0.001	0.001	X
B	Close (n=5)	Far (n=2)	0.0	1.000	1.000	-	0.0	1.000	1.000	-
B	Close (n=5)	Within (n=3)	5.0	0.025	0.076	-	5.0	0.025	0.076	-
B	Far (n=2)	Within (n=3)	3.0	0.083	0.125	-	3.0	0.083	0.125	-
C	Within (n=14)	Far (n=10)	0.086	0.777	0.777	-	0.219	0.639	0.639	-

For “Surface” and “Deep” the mean is around 200 ASVs and 100 for “Intermediate” with an interquartile range of 100-225 for all water samples (Figure 13).

The benthos samples with regards to distance from Korshavn Havbruk, the lowest diversity is “Within”, with a Shannon index just above 7 (Figure 14). The number of observed ASVs are estimated to be between 350 and 370 with a mean of 360 ASVs (Figure 15). Both “Close” and “Far” have their mean focussed on a Shannon index of 7,6. Number of observed ASVs for “Close” is between 400 and 470, with a mean count of 410 ASVs, while “Far” only have two retained samples with counts of 387 and 514 (Table 1), which gives a mean of 450 ASVs.

The mean of both the “Within” and “Far” water samples receive a Shannon index of 5, and both means are within each other’s interquartile ranges (Figure 16). Both categories have around 170 as

their mean count of observed ASVs (Figure 17). The interquartile for “Within” ranges from 110 to 210 observations, and 130-190 for “Far” samples.

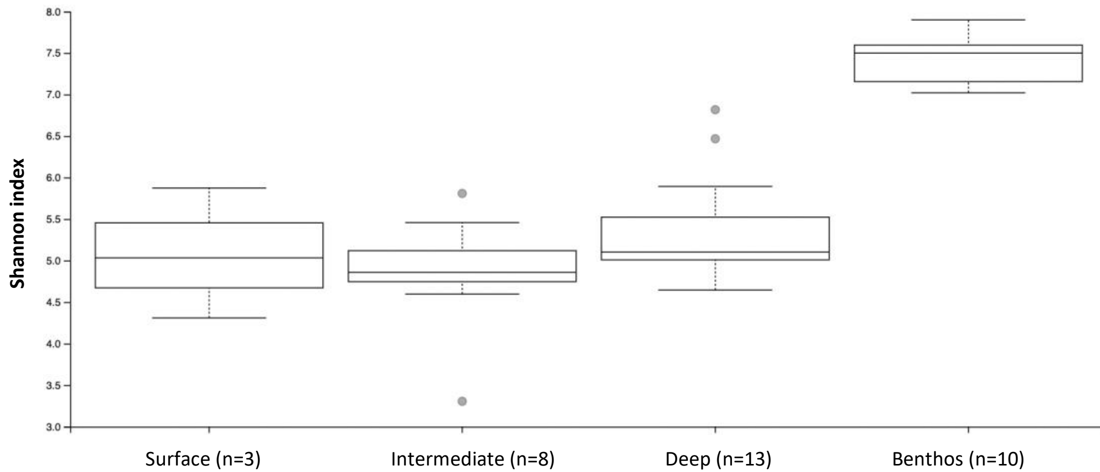


Figure 12: Box plot (with mean and standard error) showing the Shannon diversity in all samples when categorized by layer. “Surface” = 0-2m, “Intermediate” = 5-15m, “Deep” = 10 meters above sea floor, and lastly “Benthos” which is sediment samples. The dots are outliers, and Shannon index is shown on the y-axis.

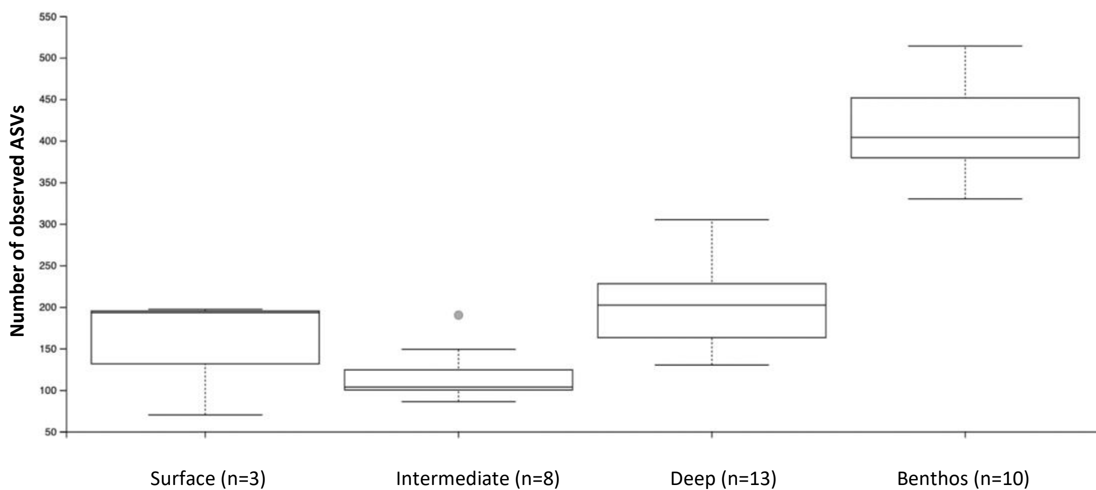


Figure: 13: Box plot showing number of observed ASVs in all samples when categorized by layer. “Surface” = 0-2m, “Intermediate” = 5-15m, “Deep” = 10 meters above sea floor, and lastly “Benthos” which is sediment samples. The dots are outliers, and number of observed ASVs is shown on the y-axis.

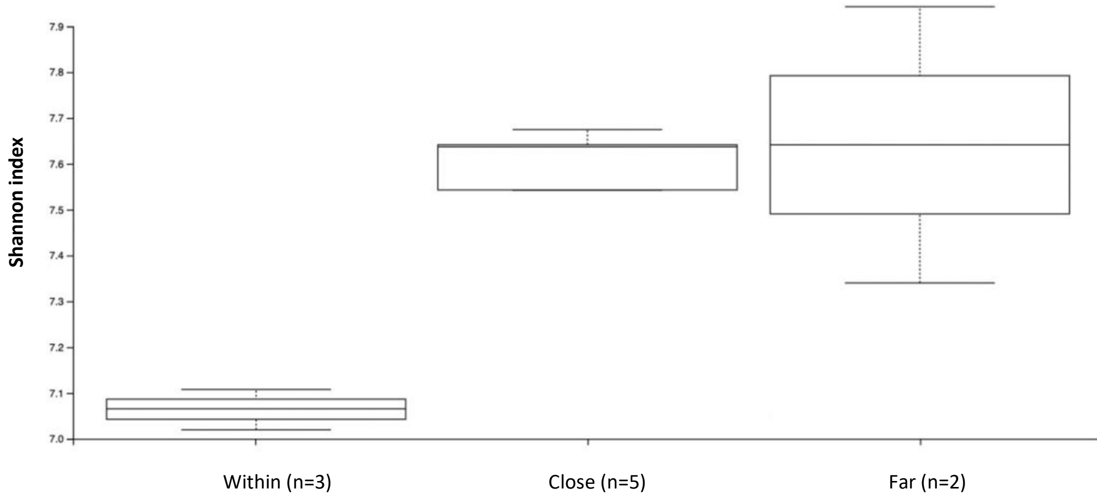


Figure 14: Box plot showing the Shannon diversity in benthos samples categorized by distance. "Within" = Korshavn Havbruk, "Close" = Leirsholmen and Skarvøyflaket, "Far" = MFS station. Y-axis show the Shannon index value.

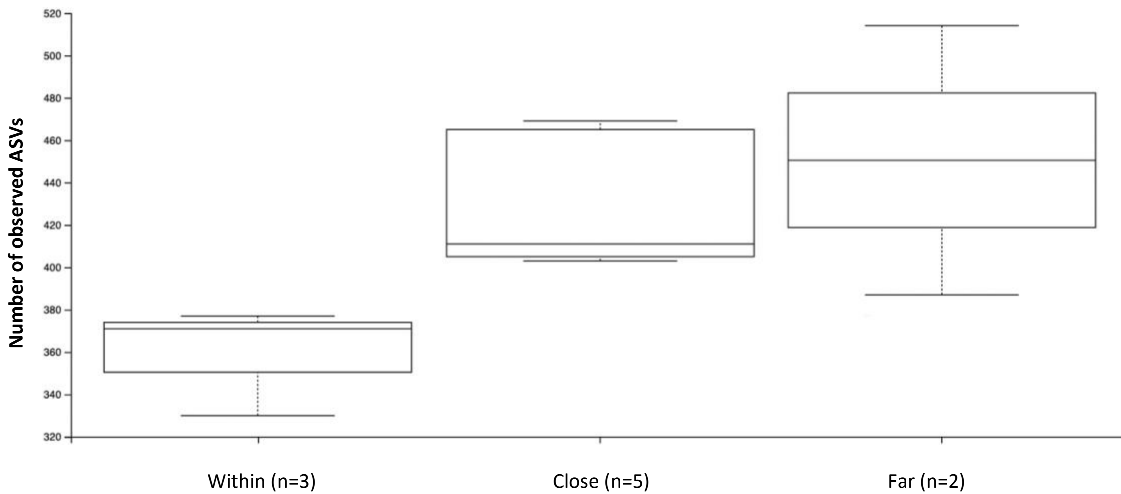


Figure 15: Box plot showing number of observed ASVs with mean in all samples when categorized by distance. "Within" = Korshavn Havbruk, "Close" = Leirsholmen and Skarvøyflaket, "Far" = MFS station. The number of observed ASVs is shown on the y-axis.

Shannon alfa diversity within water samples separated by distance

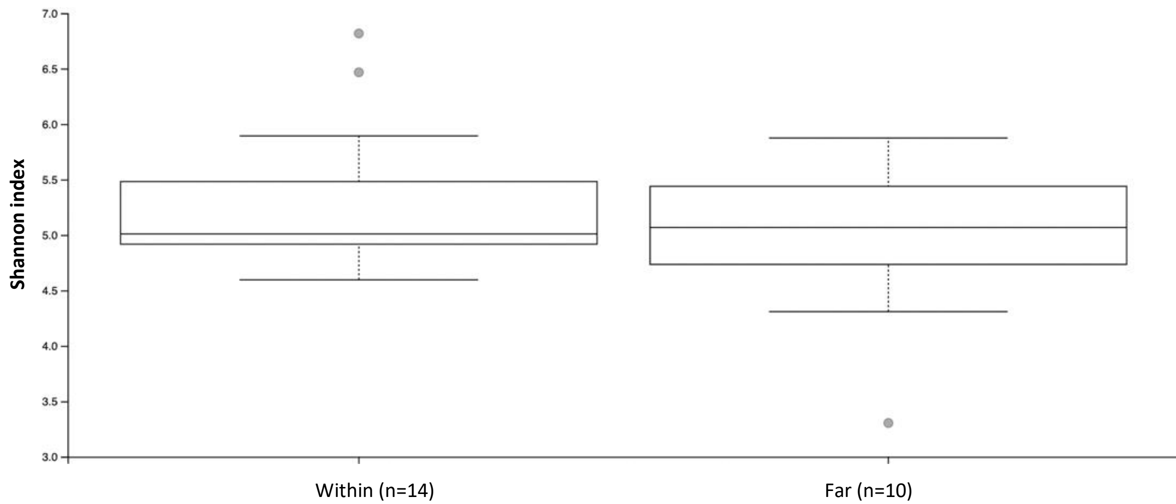


Figure 16: Box plot showing the Shannon diversity in all water samples when categorized by distance. "Within" = Korshavn Havbruk, "Far" = MFS station. Mean and outliers also included.

Observed ASVs within water samples separated by distance

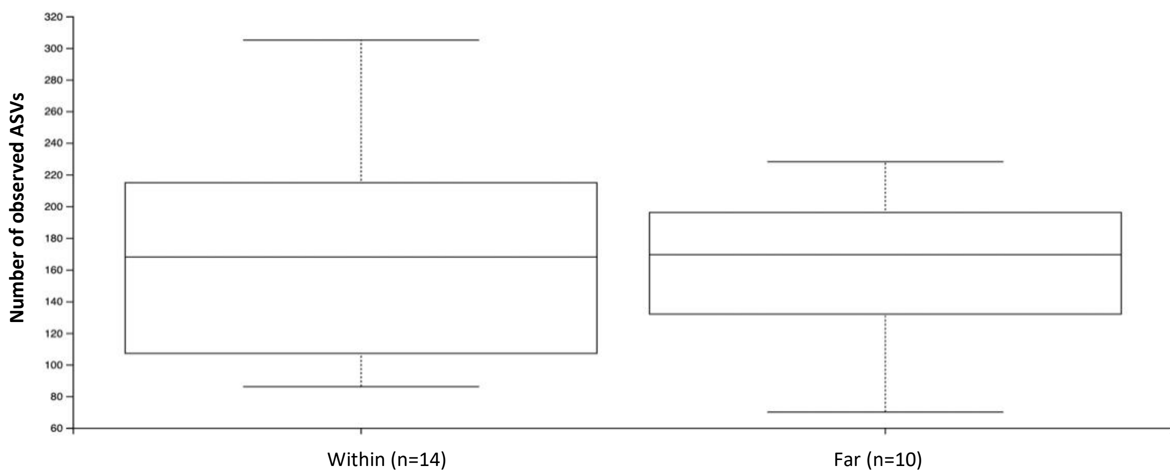


Figure 17: Box plot showing number of observed ASVs with mean in all water samples categorized by distance. "Within" = Korshavn Havbruk, "Far" = MFS station. The number of observed ASVs is shown on the y-axis.

4.6 Beta diversity metrics

Bray-Curtis analysis between samples within each layer (Surface = red, Intermediate = blue, Deep = orange and Benthos = green) shows the overall change in community profiles of bacteria. Figure 18 show which layers are more similar to each other. Benthos layer have a strong separation compared to all other layers. Bray-Curtis analysis also shows that the three benthos samples "Within" (green cones) clustered together separately from other benthos samples.

All water samples in the category "Deep" are clustered together and have a strong separation from the other layers. The exceptions are the three encircled samples which all are "bv" samples (water collected from grab). The "Intermediate" layer samples are also clustered together except for three

EMPeror beta diversity plot

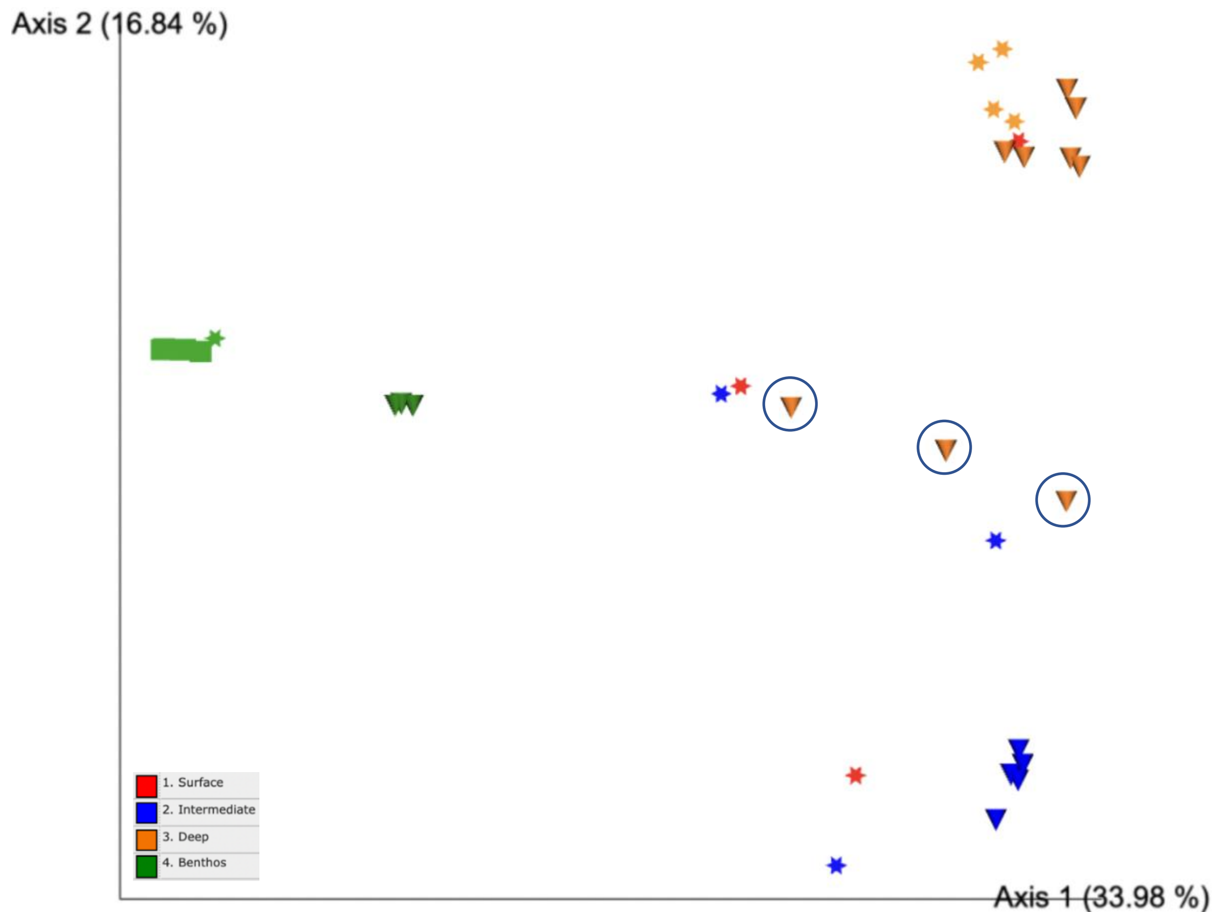


Figure 18: EMPeror plot with all samples (n=34). Layers are color-separated. Distances are separated with symbols: Cone = "Within", Square = "Close", Star = "Far". The three encircled benthos samples are the three "bv" samples (water samples retrieved from empty grab).

outliers showing weak similarity with the other samples. "Surface" layer samples are scattered and show no correlation to each other.

Pairwise PERMANOVA derived from the Bray-Curtis matrix (Table 9) confirm most of the clustering seen in the EMPeror plot. The community profile in benthos samples is statistically different to all other layers at $\alpha = 0.05$ with q-values 0.011 for "Surface" and 0.002 for both "Intermediate" and "Deep". Further, it also confirms the separate clustering of benthos samples collected from "Within" compared to samples from the two other distances with a q-value 0.048.

The layers "Intermediate" and "Deep" shows a statistically difference in community profiles with a q-value of 0.002. Three outliers in the "Deep" layer are positioned in the middle of "Intermediate" and "Deep". These samples are taken from empty grab.

The distance between water samples retrieved from "Far" and "Within" are not separated in figure 18, but the pairwise PERMANOVA (Table 9) show there is a significant dissimilarity between the two groups (q-value 0.005).

Table 9: Pairwise PERMANOVA derived from Bray-Curtis distance matrix. To avoid Type I error (false positive) the p-value is corrected with the Benjamini-Hochberg procedure (q-value), and this is used to determine a significant result (α = 0.05, indicated by "X"). Results for dissimilarity between "Layers", benthos measured by "Distance" and water measured by "Distance.

	Group 1	**Group 2**	**Sample size**	**p-value**	**q-value**	**Sig. result**
Layers	Surface	Intermediate	11	0.098	0.098	-
	Surface	Deep	16	0.018	0.022	X
	Surface	Benthos	13	0.007	0.011	X
	Intermediate	Deep	21	0.001	0.002	X
	Intermediate	Benthos	18	0.001	0.002	X
	Deep	Benthos	23	0.001	0.002	X
Benthos «Distance»	Close	Far	7	0.093	0.093	-
	Close	Within	8	0.016	0.048	X
	Far	Within	5	0.086	0.093	-
Water «Distance»	Within	Far	24	0.005	0.005	X

5. Discussion

The impacts of aquaculture breeding systems include nutrient enrichment that stems from waste food, fecal matter and other excretory products, and this can result in an increase of anoxic conditions in benthos and surrounding water masses (Keeley et al., 2013, Buschmann et al., 2006). The environmental impact assessments are traditionally performed by gathering taxonomical macrofaunal data and comparing the presence and abundance with other ecological data such as community composition of benthos (Hansen et al., 2001a, Ervik et al., 1997). One aim of this thesis was to investigate how benthic bacteria and bacterioplankton diversity change with distance to Korshavn havbruk fish farm facilities, and how this information can be used to evaluate the ecological status.

5.1 Benthic bacterial diversity variation by distance

The benthos samples from “Within” was dominated by the orders Desulfobulbales and Desulfobacterales with 30 % and 11,5 % coverage, respectively (Table 7). These orders include anaerobic sulphate reducing bacteria (SRB) that have been reported to prevail within organically enriched environments such as below and in close vicinity of fish farms (Bissett et al., 2006). Similar to our findings, a recent study by Stoeck et al. (2018) showed that SRB were found to be the dominating bacterial group in immediate proximity to fish farms. At sample stations further away from the fish farm, the dominance of SRB decreased (Table 7), an observation also in concordance with the findings from Stoeck et al. (2018). Hence, the dominance of these bacterial groups within the fish farm may be an indication of elevated levels of organic compounds originating from fish farming activity. Sulphate reducing bacteria have been identified to function as very good environmental indicators and are associated with “moderate” or “poor” conditions according to the microgAMBI index (Aylagas et al., 2017). As such, the findings in this study supports the notion that benthic bacterial diversity can provide valuable insight that informs about the environmental conditions related to fish farm activities.

5.2 Bacterioplankton diversity variation by distance

Only a few studies have reported on the variation of bacterial diversity in relation to a fish farm, but these have focused on diversity within the sediment (Bissett et al., 2006, Bissett et al., 2007, Dowle et al., 2015, Fodelianakis et al., 2015, Kawahara et al., 2009, Stoeck et al., 2018), and not in the water column. To our knowledge, no studies exist that have also investigated how the bacterioplankton diversity change in relation to fish farm activity. We found a statistically significant variation in the bacterial diversity between water samples collected from within the facility

compared to samples collected at a site further away (Table 9). Alteromonadales, an order within phylum Gammaproteobacteria, showed a higher presence within compared to outside the fish farm (Table 6). Several species within this order perform ecologically important functions, such as sulphur cycling (Gralnick et al., 2006). This order have been found in relation to fish farms in previous studies with a similar trend to decrease in abundance with distance (Stoeck et al., 2018). This could be an indication that the organic material inputs caused by fish farm activity promotes a heighted sulphate reduction caused by members of Alteromonadales. On the contrary, the opposite was found by Dowle et al. (2015) where Alteromonadales presence increased with distance. Nevertheless, this is an interesting find that deserves further investigation. The station outside had a high presence of Burkholderiales and Verrucomicrobiales. Burkholderiales have been found to dominate during wet seasons with prevalent rainfall (Angly et al., 2016) and also in rivers (Cottrell et al., 2005). Thus, the freshwater input from the river "Lygna" may explain the strong presence of this order.

5.3 Bacterial/Bacterioplankton diversity variation in different layers

In this study the benthos showed a higher bacterial diversity than the other layers investigated (Table 8). The benthos receives an influx of organic matter from upper water layers, and the seafloor also provides a varied and complex environment with several different surfaces for microbes to grow (Wang et al., 2012), and several studies have reported a higher diversity and bacterial biomass in the benthos compared to pelagic habitats (Jørgensen and Boetius, 2007, Zinger et al., 2011) As such, our results was expected.

From the 19 samples categorized as "Deep" layer, 9 samples were retrieved from the MFS station (106 meters deep and protected by several sills that are around 30 meters) and 4 samples were "bv" samples (water collected from empty grab). In this study, the order Thiomicrospirales were found to dominate the "Deep" layer. It has previously been reported that this order has been found in habitats with low oxygen (Pajares et al., 2020), but the oxygen levels close to the ocean floor at the MFS station did not indicate anoxic conditions (appendix 7.6). The samples retrieved from MFS station also covers a timeseries over two months, and the occurrence of Thiomicrospirales remain stable throughout the period (Figure 9). The order, mostly consisting of sulphur-oxidicing bacteria (Boden et al., 2017), may have the potential for being a viable environmental indicator representing the deep waters in and around fish farms, but more research ideally covering the entire fish farm production cycle is needed to better understand this finding.

Not much is known about the factors driving the distribution of Verrucomicrobiales, but it has been shown a potential to be ubiquitous in marine waters and favors high-light environments (Freitas et al., 2012). Cellvibrionales belongs to a group of hydrocarbon-degrading bacteria, and as such is

associated with the upper layers in the water column (Yakimov et al., 2019). Both these orders were present to a large degree in both “Intermediate” and “Surface” layer (Table 5). Moreover, it is also apparent that the bacterioplankton diversity composition also varies with seasonal changes (Figure 9). This variation might be due to changes in phytoplankton populations which usually takes place in the early spring with raising temperatures and more light availability (Buchan et al., 2014, Bunse and Pinhassi, 2017), but it could also be a result from this being samples collected from two different seasons (Table 1).

The dominating class found across all water samples were Gammaproteobacteria with a representation of almost 80 % (Table 6). This finding is expected as Gammaproteobacteria is among the most abundant class found across all oceans (Cottrell and Kirchman, 2000), and have been well documented in analysis on large datasets (Zinger et al., 2011).

Surprisingly, Alfaproteobacteria was nearly absent in both benthos and water samples. This class generally is recognized as one of the dominating classes of Proteobacteria, especially the orders SAR11 and Rhodobacterales which together are believed to represent over 40 % of all bacteria present in surface waters (Giovannoni, 2017). Low diversity of Alphaproteobacteria in the sediment related to fish farms have been reported in similar studies (Kawahara et al., 2009, Stoeck et al., 2018), but both these studies reported higher diversity as distance to the farm sites increased. In contrast, Bissett et al. (2006) reported a high presence of Alphaproteobacteria in close proximity to two fish farms. It remains unclear as to why only a negligible fraction of Alfaproteobacteria was identified in this study, but we suspect it could be a primer issue. Part of the dataset in this study was also used by Weeraman (2019), and they reported approximately 50 % of all Proteobacteria to be Alfaproteobacteria, with both SAR11 and Rhodobacterales in high abundances. They also reported high abundances of the phyla Bacteroidetes and Planctomycetes, but these phyla were also nearly absent in this study. The reason for this could be related to the primers used by Weeraman (2019): 515F- GTG**Y**CAGCMGCCGCGGTAA (Parada et al., 2016) and 806R- CCGYCAATTYMTTTRAGTTT (Caporaso et al., 2012). This study used Bakt_341F (CCTACGGGNGGCWGCAG) and Bakt_805R (GACTACHVGGGTATCTAATCC) (Herlemann et al., 2011).

5.4 Bacteria as bioindicators

Our results resonated well with findings from other studies performed under similar circumstances and from different parts of the world (Dowle et al., 2015, Kawahara et al., 2009, Stoeck et al., 2018). It also underlines the importance of using the same set of primers in order to retrieve consistent data. This study has shown that bacterial diversity composition may have the capability to function as bioindicators in relation to fish farm activity.

5.5 DNA isolation from water samples

DNA from water samples was extracted and isolated using DNEasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany) following the manufacturer's SOP. DNA was also isolated from the filters, after they had been washed. The SOP did not provide a step-by-step procedure to this operation. The handling of the filters was delicate and time consuming with an added risk of contamination. Comparing the results from filters and eluate does not show any significant difference (P-value 0.48, α=0.05) between them when comparing number of identified ASVs (Table 3). Visually inspection of the taxonomic analysis also shows a high degree of similarity (Figure 7). These results suggests that isolating DNA from the filters might not add significant richness to the complete dataset. Rather, the fact that the filters still contain DNA might suggest that the washing of the filters was not adequate. One solution to this could be to make an adjustment to the SOP step 2 and repeat the washing procedure. In general, we conclude that including the filters in future analysis will not affect the results.

5.6 Sample purity and DNA concentration

Absorbance provides an indication on sample purity for DNA-samples. Absorbance 260/280 was between 1.42 and 2.00 for all samples (not included are the samples from the 2019 dataset) with a mean of 1.78 and σ 0.14. Optimal value for A260/280 is 1.80, so this is just slightly below optimal. Absorbance 260/230 was between -2.19 and 60.09 with a mean of 4.00 and σ 12.41. Two samples gave very high values (MFS-A-5m and St1-M5-A-45m with 37.81 and 60.09 respectively), which skew the mean value in a negative direction. If these two samples are disregarded when calculating the mean, it would be 0.90 with a σ 1.32. It is not clear what caused some samples to absorb at A230, but we got no indication that it affected the results. Optimal values for A260/230 are between 2.0-2.2. Optimal values are expected to a greater extent when samples are collected directly from the source (from tissue or blood). This study has collected benthic and water samples, and such environments may contain several contaminating particles, for example humus acids from degraded organic material. This may have influenced the A260/230 values.

DNA concentration (ng/µl) indicates how much DNA that has been isolated from each sample. This varied from 5.6 to 182.4 ng/µl with a mean of 43.2 and σ 39.0, which is in the normal range. We got no indication that DNA concentration might have affected the results.

6. Conclusions

6.1 Concluding remarks

This study aimed to investigate the usefulness of benthic bacterial and bacterioplankton community variations as possible bioindicators in relation to a Norwegian salmon fish farm. To do so, water and sediment samples were sequenced with the 16S rRNA gene metabarcoding approach.

Strong indications were found that bacterial community diversity and richness are higher in the benthos than in the water column. The water column was mainly dominated by Gammaproteobacteria, while the benthos showed a stronger affiliation with Desulfobacterota.

Bacterial community diversity did not change significantly within benthos samples with distance considered. However, Bray-Curtis beta diversity analysis showed a clear separation between the benthos samples from “within” and “close”, indicating that there might be an impact originating from the fish farm activity. The same separation was identified between “within” and “far” as well, but not with statistical significance.

The bacterioplankton diversity between the water samples were also affected by distance. Samples collected from “within” was dominated by the sulphur reducing Thiomicrospirales order, indicating an impact from the fish farm activity.

6.2 Limitations and future studies

Due to limited access to suitable transport, all sediment and water sampling from within Korshavn havbruk was performed on the same day. During one breeding cycle, the activity in the fish cages vary which assumingly could influence the benthic and pelagic bacterial composition. Optimally, a complete sample set would include samples covering each step in the breeding cycle. Data covering the local waterflow regime and bathymetry should also be included in future studies to get a more complete picture of the influencing factors.

Future studies should also seek to compare their findings with macrofaunal data from the regularly performed, standardized MOM-tests, similar to what Stoeck et al. (2018) did in their study. Such a comparison would provide a clearer picture on how bacterial diversity analysis perform compared to the current traditional methodology employed in this field today. We would also recommend testing the primer set used in this study and compare the performance with the primers used in the study by Weeraman (2019) as they produced diverging results, especially in the identification of Alphaproteobacteria, Bacteroidetes and Planctomycetes.

7. Appendix

7.1 Legend to taxonomical bar charts at taxonomic rank 4 (order)

d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Cellvibrionales
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Thiomicrospirales
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__SAR86_clade
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales
d__Bacteria;p__Verrucomicrobiota;c__Verrucomicrobiae;o__Verrucomicrobiales
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Burkholderiales
d__Bacteria;p__Desulfobacterota;c__Desulfobulbia;o__Desulfobulbales
d__Bacteria;p__Desulfobacterota;c__Desulfobacteria;o__Desulfobacterales
d__Bacteria;p__Marinimicrobia_(SAR406_clade);c__Marinimicrobia_(SAR406_clade);o__Marinimicrobia_(SAR406_clade)
d__Bacteria;p__Myxococcota;c__Polyangia;o__Polyangiales
d__Bacteria;p__Acidobacteriota;c__Thermoanaerobaculia;o__Thermoanaerobaculales
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;__
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Steroidobacterales
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__KI89A_clade
d__Bacteria;p__Desulfobacterota;c__Syntrophobacteria;o__Syntrophobacterales
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__B2M28
d__Bacteria;p__Spirochaetota;c__Spirochaetia;o__Spirochaetales
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__UBA10353_marine_group
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Ectothiorhodospirales
d__Bacteria;p__Bdellovibrionota;c__Bdellovibrionia;o__Bacteriovoracales
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Chromatiales
d__Bacteria;p__Desulfobacterota;c__Desulfuromonadia;o__Sva1033
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__uncultured
d__Bacteria;p__Planctomycetota;c__OM190;o__OM190
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Milano-WF1B-44
d__Bacteria;p__Planctomycetota;c__Pla3_lineage;o__Pla3_lineage
d__Bacteria;p__NB1-j;c__NB1-j;o__NB1-j
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Vibrionales
d__Bacteria;p__Sva0485;c__Sva0485;o__Sva0485
d__Bacteria;p__Acidobacteriota;c__Subgroup_22;o__Subgroup_22
d__Bacteria;p__Latescibacterota;c__Latescibacterota;o__Latescibacterota
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Gammaproteobacteria_Incertae_Sedis
d__Bacteria;p__Gemmatimonadota;c__BD2-11_terrestrial_group;o__BD2-11_terrestrial_group
d__Bacteria;p__Acidobacteriota;c__Subgroup_26;o__Subgroup_26
d__Bacteria;p__Zixibacteria;c__Zixibacteria;o__Zixibacteria
d__Bacteria;p__Desulfobacterota;c__Desulfuromonadia;o__Desulfuromonadia
d__Bacteria;p__Acidobacteriota;__;__
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Ga0077536
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Legionellales
d__Bacteria;p__Dependentiae;c__Babeliae;o__Babeliales
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__MBAE14
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Coxiellales
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__JTB23
d__Bacteria;p__Firmicutes;c__Negativicutes;o__Acidaminococcales
d__Bacteria;p__Desulfobacterota;c__Desulfovibrionia;o__Desulfovibrionales
d__Bacteria;p__Myxococcota;c__Polyangia;o__PS-B29
d__Bacteria;p__Campilobacterota;c__Campylobacteria;o__Campylobacterales
d__Bacteria;p__Bdellovibrionota;c__Oligoflexia;o__0319-6G20
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Beggiatoales
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Francisellales
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__pltb-vmat-80
d__Bacteria;p__PAUC34f;c__PAUC34f;o__PAUC34f
d__Bacteria;p__Planctomycetota;c__BD7-11;o__BD7-11
d__Bacteria;p__Firmicutes;c__Bacilli;o__Mycoplasmatales
d__Bacteria;p__Fibrobacterota;c__Chitinivibrionia;o__Chitinivibrionales
d__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Granulosicoccales
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacterales
d__Bacteria;p__Dadabacteria;c__Dadabacteriia;o__Dadabacteriales
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__OM182_clade
d__Bacteria;p__Verrucomicrobiota;c__Verrucomicrobiae;o__Opitutales
d__Bacteria;p__Verrucomicrobiota;c__Chlamydiae;o__Chlamydiales
d__Bacteria;p__Planctomycetota;c__Planctomycetes;o__Planctomycetales
d__Bacteria;p__MBNT15;c__MBNT15;o__MBNT15
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__CH2b56
d__Bacteria;p__Actinobacteriota;c__WCHB1-81;o__WCHB1-81
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Piscirickettsiales
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__HOC36
d__Bacteria;p__Desulfobacterota;c__Desulfobacteria;o__Desulfatiglandales
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Thiotrichales
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Arenicellales
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Nitrosococcales
d__Bacteria;p__Gemmatimonadota;c__PAUC43f_marine_benthic_group;o__PAUC43f_marine_benthic_group
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__BD7-8
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales
d__Bacteria;p__Latescibacterota;c__Latescibacteria;o__Latescibacterales
d__Bacteria;p__Verrucomicrobiota;c__Kiritimatiellae;o__Kiritimatiellales
d__Bacteria;p__Modulibacteria;c__Moduliflexia;o__Moduliflexales
d__Bacteria;p__Myxococcota;c__Myxococcia;o__Myxococcales
d__Bacteria;p__Nitrospinota;c__P9X2b3D02;o__P9X2b3D02
d__Bacteria;p__Bdellovibrionota;c__Oligoflexia;o__Oligoflexales
d__Bacteria;p__Firmicutes;c__Bacilli;o__Izemoplasmatales
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__EPR3968-O8a-Bc78
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__AT-s2-59
d__Bacteria;p__Myxococcota;c__Polyangia;o__MidBa8
d__Bacteria;p__Acidobacteriota;c__Vicinamibacteria;o__Subgroup_17
d__Bacteria;p__Calditrichota;c__Calditrichia;o__Calditrichales
d__Bacteria;p__Schekmanbacteria;c__Schekmanbacteria;o__Schekmanbacteria
d__Bacteria;p__Actinobacteriota;c__Thermoleophilia;o__Solirubrobacterales
d__Bacteria;p__Desulfobacterota;c__Desulfuromonadia;o__Bradymonadales
d__Bacteria;p__Cloacimonadota;c__Cloacimonadia;o__Cloacimonadales
d__Bacteria;p__Acidobacteriota;c__Vicinamibacteria;o__Vicinamibacterales
d__Bacteria;p__Bdellovibrionota;c__Bdellovibrionia;o__Bdellovibrionales
d__Bacteria;p__Hydrogenedentes;c__Hydrogenedentia;o__Hydrogenedentiales
d__Bacteria;p__Verrucomicrobiota;c__Verrucomicrobiae;o__Pedosphaerales
d__Bacteria;p__Myxococcota;c__Polyangia;o__UASB-TL25
d__Bacteria;p__Desulfobacterota;c__uncultured;o__uncultured
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Thiohalorhabdales
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__SS1-B-07-19
d__Bacteria;p__Acidobacteriota;c__Vicinamibacteria;o__Subgroup_9
d__Bacteria;p__Gemmatimonadota;c__Gemmatimonadetes;o__Gemmatimonadales
d__Bacteria;p__Myxococcota;c__Polyangia;o__Haliangiales
d__Bacteria;p__Desulfobacterota;c__Desulfuromonadia;__
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__211ds20
d__Bacteria;p__Firmicutes;c__Bacilli;o__Erysipelotrichales
d__Bacteria;p__Desulfobacterota;c__Desulfobaccia;o__Desulfobaccales
d__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales
d__Bacteria;p__Acidobacteriota;c__Holophagae;o__Acanthopleuribacterales
d__Bacteria;p__Acidobacteriota;c__Holophagae;o__Subgroup_7
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Methylococcales
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__EC3
d__Bacteria;p__Acidobacteriota;c__Aminicenantia;o__Aminicenantales
d__Bacteria;p__Verrucomicrobiota;c__Kiritimatiellae;o__WCHB1-41
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__R7C24
d__Bacteria;p__Desulfobacterota;c__Syntrophia;o__Syntrophales
d__Bacteria;p__Myxococcota;c__Polyangia;o__mle1-27
d__Bacteria;p__FCPU426;c__FCPU426;o__FCPU426
d__Bacteria;p__Acidobacteriota;c__AT-s3-28;o__AT-s3-28
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__SZB50
d__Bacteria;p__WPS-2;c__WPS-2;o__WPS-2
d__Bacteria;p__Spirochaetota;c__MVP-15;o__MVP-15
d__Bacteria;p__Spirochaetota;c__Brevinematia;o__Brevinematales
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Run-SP154
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__HgCo23
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__EV818SWSAP88
d__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Diplorickettsiales
d__Bacteria;p__Planctomycetota;c__Planctomycetes;o__Pirellulales
d__Bacteria;p__Firmicutes;c__Negativicutes;o__Veillonellales-Selenomonadales
d__Bacteria;p__Firmicutes;c__Bacilli;o__RF39
d__Bacteria;p__Fermentibacterota;c__Fermentibacteria;o__Fermentibacterales
d__Bacteria;p__Desulfobacterota;c__Desulfuromonadia;o__PB19
d__Bacteria;p__Bacteroidota;c__Ignavibacteria;o__Ignavibacteriales
d__Bacteria;p__Bacteroidota;c__Bacteroidia;__
d__Bacteria;p__Acidobacteriota;c__Holophagae;o__Holophagales

Appendix figure 1: Complete legend to taxonomical bar chart at phylogenetic level 4

7.2 Feature table overview

Appendix table 1: Overview of all samples with read count during the trimming process, deblur and final filtering after filtering out contaminations and noise based on taxonomic levels.

Sample-ID	FastQC		Treatment				
			Trimmed	**Trim_joined**	**Trim_joined_filtered**	**Deblur**	**Final**
Leirs-A	30470		29588	22989	22986	4364	3835
Leirs-B	14055		13563	10696	10696	1799	1345
Leirs-C	14757		14310	11319	11317	2019	1591
Leirs-D	14804		14345	10635	10635	1949	1667
MFS-A	27263		26555	20055	20055	4222	3389
MFS-B	19423		18869	14391	14391	3089	2465
MFS-C	20213		19651	14788	14787	3033	2574
MFS-D	13120		12628	9170	9170	1756	1420
Skarvoy-A	43939		42719	32379	32378	6577	5709
Skarvoy-B	20178		19538	15315	15314	3339	2958
Skarvoy-C	18702		18159	14152	14151	3148	2534
Skarvoy-D	20383		19820	15009	15009	3255	2574
St1-M5-A	25886		25171	20258	20258	4181	3673
St1-M5-B	29849		28980	23227	23225	4969	4539
St1-M5-C	19178		18667	15145	15145	2689	2471
St1-M5-D	33213		32308	25926	25924	5202	4659
St5-M4-A	16554		16147	13233	13233	1967	1721
St5-M4-B	20608		20043	16168	16167	2319	1983
St5-M4-C	23065		22492	18464	18462	2641	2251
St5-M4-D	16807		16369	13092	13092	2307	2118
MFS-A-2m	39526		38309	28641	28641	4565	4194
MFS-A-5m	22776		22024	18035	18033	3653	3530
MFS-A-90m	15281		14835	12345	12342	2645	2568
MFS-Fi-2m	41179		40059	31102	31100	3390	3000
MFS-Fi-5m	24842		24078	20191	20189	1580	1539
MFS-Fi-90m	17486		16970	14275	14275	3099	3050
St1-M5-A-5m	68512		66550	53314	53312	9439	9321
St1-M5-A-45m	26066		25310	21459	21457	5193	5097
St1-M5-A-bv	21968		21405	18013	18012	3435	3216
St1-M5-Fi-5m	43027		41880	33932	33930	3346	3271
St1-M5-Fi-45m	30998		30098	25529	25529	5811	5747
St1-M5-Fi-bv	44156		42931	35792	35792	6000	5897
St5-M4-5m	16467		15950	13341	13339	2036	2011
St5-M4-A-45m	32770		31846	26495	26494	6259	6199
St5-M4-Fi-5m	34194		33217	26462	26462	2725	2686
St5-M4-Fi-45m	158521		154603	130100	130091	29838	29398
St6-M3-A-bv	21544		20902	17535	17534	3172	2885
St6-M3-Fi-bv	15859		15386	12985	12985	1935	1812
St7-M2-A-5m	29314		28398	23601	23598	3829	3799
St7-M2-A-45m	63850		62196	53343	53341	7813	7592
St7-M2-Fi-5m	47090		45669	35894	35893	3451	3412
St7-M2-Fi-45m	34552		33543	29190	29187	3357	3252
31may-0m	40810		39489	33079	33078	6801	6607
31may-90m	39432		38201	32153	32146	6284	6076
26june-0m	39191		38166	31983	31982	5825	5699
12june-15m	35690		34641	29344	29343	4458	4335
12june-90m	29920		28966	24627	24626	4684	4510
26june-15m	16494		15865	12948	12948	1661	1581
12june-0m	15495		14755	11991	11990	1407	1324
29july-0m	14896		14492	12052	12052	1221	1204
12july-0m	14141		13638	11203	11203	1694	1668
26june-90m	13917		13461	11118	11118	2292	2267
31may-15m	12064		11396	9559	9558	1011	962
29aug-90m	10858		10453	8706	8705	1748	1671
29july-15m	10464		10128	8596	8596	1201	1163
29aug-0m	9217		8801	7241	7240	577	528
12aug-90m	8971		8687	7201	7201	1452	1401
12july-90m	7313		7048	5577	5577	981	944
12july-15m	5596		5402	4523	4523	651	646
29july-90m	4424		4191	3482	3482	714	681
12aug-15m	3826		3648	2986	2986	497	489

12aug-0m	3255		2929	2348	2348	177	142
No. of samples	62		62	62	62	62	62
Mean reads	26264		25480	20785	20784	3656	3431

7.3 Comparing A and B samples

Appendix table 2: The 10 most dominating orders that was the base for estimating similarity between the A and B samples. The percentage are the respected proportion of total reads.

MFS-A-2m	MFS-B-2m	Order	MFS-A-5m	MFS-B-5m	Order
4,76 %	4,99 %	Thiomicrospirales	23,79 %	24,50 %	Cellvibrionales
3,97 %	3,10 %	Cellvibrionales	1,41 %	1,51 %	Alteromonadales
3,59 %	3,82 %	Alteromonadales	60,51 %	57,98 %	Verrucomicrobiales
5,25 %	5,12 %	SAR86 clade	3,55 %	4,47 %	SAR86 clade
9,96 %	9,48 %	Oceanospirillales	3,97 %	4,34 %	Oceanospirillales
57,20 %	56,75 %	Burkholderiales	3,16 %	3,73 %	Burkholderiales
2,64 %	2,43 %	Steroidobacterales	1,61 %	0,94 %	KI89A clade
1,69 %	1,17 %	UBA10353	0,25 %	0,34 %	Arenicellalis
1,19 %	1,59 %	Verrocomicrobiales	0,28 %	0,24 %	Granulosicoccales
2,50 %	3,10 %	SAR406 clade	0,45 %	0,43 %	Pseudomonadales

MFS-A-90m	MFS-B-90m	Order	St1-M5-A-5m	St1-M5-B-5m	Order
33,31 %	35,85 %	Thiomicrospirales	27,31 %	26,26 %	Cellvibrionales
14,81 %	13,91 %	Cellvibrionales	1,77 %	1,68 %	Alteromonadales
5,33 %	4,03 %	Alteromonadales	34,17 %	30,86 %	Verrucomicrobiales
4,63 %	3,19 %	Verrocomicrobiales	11,46 %	13,88 %	SAR86 clade
7,50 %	8,11 %	SAR86 clade	10,68 %	10,15 %	Oceanospirillales
6,53 %	6,65 %	Oceanospirillales	3,20 %	4,21 %	Burkholderiales
1,40 %	1,09 %	Burkholderiales	2,90 %	3,42 %	KI89A clade
1,83 %	2,46 %	SAR406 clade	1,08 %	1,31 %	OM90 clade
3,65 %	3,02 %	Bacteriavoracales	1,29 %	1,53 %	Vibrionales
7,66 %	6,25 %	OM190	0,28 %	0,17 %	Arenicellales

St1-M5-A-45m	St1-M5-B-45m	Order	St5-M4-A-45m	St5-M4-B-45m	Order
24,57 %	23,74 %	Thiomicrospirales	34,24 %	32,95 %	Thiomicrospirales
13,32 %	14,09 %	Cellvibrionales	12,42 %	12,31 %	Cellvibrionales
20,71 %	21,90 %	Alteromonadales	15,66 %	15,35 %	Alteromonadales
7,51 %	6,21 %	Verrocomicrobiales	4,19 %	5,20 %	Verrocomicrobiales
8,57 %	9,05 %	SAR86 clade	10,35 %	10,42 %	SAR86 clade
12,34 %	11,61 %	Oceanospirillales	8,81 %	9,64 %	Oceanospirillales
1,63 %	1,83 %	Burkholderiales	2,52 %	2,35 %	Burkholderiales
4,10 %	4,21 %	SAR406 clade	3,90 %	3,92 %	SAR406 clade
0,59 %	0,50 %	UBA10353	0,44 %	0,59 %	OM190
0,39 %	0,26 %	Ectothiorhodospirales	0,44 %	0,35 %	Ectothiorhodospirales

St7-M2-A-5m	St7-M2-B-5m	Order	St7-M2-A-45m	St7-M2-B-45m	Order
3,18 %	3,01 %	Thiomicrospirales	16,67 %	14,47 %	Thiomicrospirales
33,33 %	32,27 %	Cellvibrionales	8,36 %	7,40 %	Cellvibrionales
1,18 %	1,59 %	Alteromonadales	42,90 %	43,14 %	Alteromonadales
28,86 %	29,37 %	Verrucomicrobiales	3,87 %	5,43 %	Verrocomicrobiales
12,31 %	13,86 %	SAR86 clade	5,29 %	5,58 %	SAR86 clade
9,87 %	9,29 %	Oceanospirillales	6,03 %	6,78 %	Oceanospirillales
3,84 %	3,18 %	Burkholderiales	1,10 %	1,13 %	Burkholderiales
0,82 %	0,81 %	SAR406 clade	1,80 %	2,19 %	Desulfobulbales
2,53 %	2,85 %	KI89A clade	2,53 %	2,35 %	SAR 406 clade
1,08 %	1,34 %	OM190	2,26 %	2,36 %	Spirochaetales

7.4 QIIME2 input

```
#!/bin/bash
#SBATCH --account=nn9776k
#SBATCH --job-name=StianTest
#SBATCH --time=1-06:00:00
#SBATCH --mem-per-cpu=3G
#SBATCH --ntasks=16
set -o errexit
set -o nounset
module load FastQC/0.11.9-Java-11
export LC_ALL=en_US.utf-8
export LANG=en_US.utf-8
NCORES=1

mkdir fastqc_out
fastqc -t $NCORES raw_data/*.fastq.gz
         -o fastqc_out

module load QIIME2/2020.11
export LC_ALL=en_US.utf-8
export LANG=en_US.utf-8
NCORES=1

qiime tools import \
   --type SampleData[PairedEndSequencesWithQuality] \
   --input-path raw_data \
   --output-path reads.qza \
   --input-format CasavaOneEightSingleLanePerSampleDirFmt

qiime cutadapt trim-paired \
   --i-demultiplexed-sequences reads.qza \
   --p-front-f CCTACGGGNGGCWGCAG \
   --p-front-r GACTACHVGGGTATCTAATCC \
   --p-discard-untrimmed \
   --p-no-indels \
   --o-trimmed-sequences reads_trimmed.qza
```

```
qiime demux summarize \
   --i-data reads_trimmed.qza \
   --o-visualization reads_trimmed_summary.qzv

qiime vsearch join-pairs \
   --i-demultiplexed-seqs reads_trimmed.qza \
   --o-joined-sequences reads_trimmed_joined.qza

qiime demux summarize \
   --i-data reads_trimmed_joined.qza \
   --o-visualization reads_trimmed_joined_summary.qzv

qiime quality-filter q-score \
   --i-demux reads_trimmed_joined.qza \
   --o-filter-stats filt_stats.qza \
   --o-filtered-sequences reads_trimmed_joined_filtered.qza

qiime deblur denoise-16S \
   --i-demultiplexed-seqs reads_trimmed_joined_filtered.qza \
   --p-trim-length 427 \
   --p-sample-stats \
   --p-jobs-to-start 4 \
   --p-min-reads 1 \
   --output-dir deblur_output

qiime feature-table summarize \
   --i-table deblur_output/table.qza \
   --o-visualization deblur_output/deblur_table_summary.qzv

#SBATCH --mem-per-cpu=6G
#SBATCH --gres=localscratch:100G
export TMPDIR=$LOCALSCRATCH
export LC_ALL=en_US.utf-8
export LANG=en_US.utf-8
NCORES=1

qiime feature-classifier classify-sklearn \
   --i-reads deblur_output/representative_sequences.qza \
```

```
   --i-classifier silva-138-99-nb-classifier.qza \
   --p-n-jobs $NCORES \
   --output-dir taxa

qiime tools export \
   --input-path taxa/classification.qza \
   --output-path taxa

#!/bin/bash
#SBATCH --account=nn9776k
#SBATCH --job-name=StianTest
#SBATCH --time=1-06:00:00
#SBATCH --mem-per-cpu=3G
#SBATCH --ntasks=16

set -o errexit
set -o nounset
module load QIIME2/2020.11

export LC_ALL=en_US.utf-8
export LANG=en_US.utf-8
NCORES=1

qiime feature-table filter-features \
   --i-table deblur_output/table.qza \
   --p-min-frequency 4 \
   --p-min-samples 1 \
   --o-filtered-table deblur_output/deblur_table_filt.qza
qiime taxa filter-table \
   --i-table deblur_output/deblur_table_filt.qza \
   --i-taxonomy taxa/classification.qza \
   --p-include p__ \
   --p-exclude mitochondria,chloroplast \
   --o-filtered-table deblur_output/deblur_table_filt_contam.qza

qiime feature-table summarize \
   --i-table deblur_output/deblur_table_filt_contam.qza \
   --o-visualization deblur_output/deblur_table_filt_contam_summary.qzv
```

```
qiime diversity alpha-rarefaction \
   --i-table deblur_output/deblur_table_filt_contam.qza \
   --p-max-depth 29437 \
   --p-steps 20 \
   --p-metrics 'observed_features' \
   --o-visualization rarefaction_curves_test.qzv

qiime feature-table filter-samples \
   --i-table deblur_output/deblur_table_filt_contam.qza \
   --p-min-frequency 1 \
   --o-filtered-table deblur_output/deblur_table_final_all.qza

qiime feature-table summarize \
   --i-table deblur_output/deblur_table_final_all.qza \
   --o-visualization deblur_output/deblur_table_final_all_summary.qzv

qiime feature-table filter-samples \
   --i-table deblur_table_filt_contam.qza \
   --p-min-frequency 2800 \
   --o-filtered-table deblur_output/deblur_table_final_reduced.qza

qiime feature-table summarize \
   --i-table deblur_table_final_reduced.qza \
   --o-visualization deblur_output/deblur_table_final_reduced_summary.qzv

qiime feature-table filter-seqs \
   --i-data deblur_output/representative_sequences.qza \
   --i-table deblur_output/deblur_table_final_reduced.qza \
   --o-filtered-data deblur_output/rep_seqs_final_reduced.qza

qiime fragment-insertion sepp \
   --i-representative-sequences deblur_output/rep_seqs_final_reduced.qza \
   --i-reference-database /cluster/home/stiabh14/sepp-refs-gg-13-8.qza \
   --o-tree taxa/asvs-tree_reduced.qza \
   --o-placements taxa/insertion-placements_reduced.qza \
   --p-threads $NCORES
```

```
qiime taxa barplot \
   --i-table deblur_output/deblur_table_final_all.qza \
   --i-taxonomy taxa/classification.qza \
   --m-metadata-file mapfile.tsv \
   --o-visualization taxa/taxa_barplot_all.qzv

qiime diversity core-metrics-phylogenetic \
   --i-table deblur_output/deblur_table_final_reduced.qza \
   --i-phylogeny taxa/asvs-tree_reduced.qza \
   --p-sampling-depth 2800 \
   --m-metadata-file mapfile_stian.tsv \
   --p-n-jobs-or-threads $NCORES \
   --output-dir diversity

qiime diversity alpha-group-significance \
   --i-alpha-diversity diversity/shannon_vector.qza \
   --m-metadata-file mapfile.tsv \
   --o-visualization diversity/shannon_compare_groups.qzv

qiime diversity alpha-group-significance \
   --i-alpha-diversity observed_features_vector.qza \
   --m-metadata-file mapfile.tsv \
   --o-visualization observed_features.qzv
```

7.5 Overview of all sample sites and results from nanodrop analysis

Appendix table 3: From each station, four subsamples were taken from the same grab (one from each of the lids on the grab). 150 mg benthos from each subsample was used for DNA extraction.

STATION	COORDINATES	DEPTH	DNA (NG/µL)	A260/280	A260/230
Leirsholmen					
Subsample A	58°03.288 N	63,5 m	17,60	1,69	0,13
Subsample B	6°53.446 Ø		36,70	1,84	0,36
Subsample C			36,50	1,79	0,45
Subsample D			32,00	1,77	0,42
Skarvøyflaket					
Subsample A	58°04.093 N	63,5 m	36,40	1,84	0,36
Subsample B	6°53.631 Ø		26,80	1,83	0,14
Subsample C			53,10	1,83	0,40
Subsample D			50,50	1,82	0,74

St. 1, merd 5					
Subsample A	58°03.679 N	50,3 m	67,40	1,82	1,24
Subsample B	6°53.188 Ø		29,00	1,85	0,25
Subsample C			63,70	1,84	1,29
Subsample D			45,90	1,86	0,98

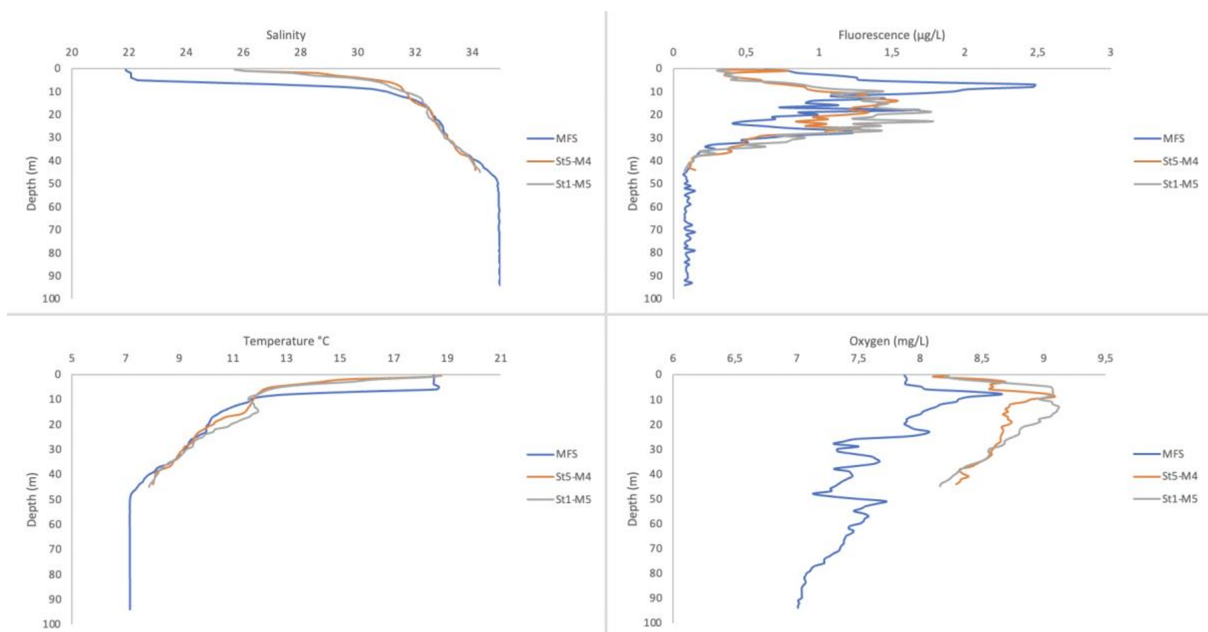
MFS					
Subsample A	58°05.137 N	103 m	22,10	1,79	1,47
Subsample B	6°49.842 Ø		20,80	1,78	0,73
Subsample C			14,70	1,70	0,07
Subsample D			21,40	1,72	1,26

St.5, merd 4					
Subsample A	58°03.553 N	55,1 m	98,30	1,86	1,09
Subsample B	6°53.131 Ø		110,70	1,85	0,70
Subsample C			182,40	1,82	0,54
Subsample D			122,70	1,84	2,32

STATION	COORDINATES	FILTERED	DEPTH	DNA (NG/µL)	A260/280	A260/230
St. 5 merd 4	58°03.553 N 6°53.131 Ø					
Water		1000 mL	5 m	29,6	1,94	2,81
Filter				15,1	1,80	2,14
Negativ				7,2	1,61	2,03
St 5, merd 4	58°03.553 N 6°53.131 Ø					
Water (1)		1000 mL	45 m	6,7	1,42	-1,86
Water (2)				6,7	1,59	-1,40
Filter				1,6	1,11	-0,25
Negativ						
St. 7 merd 2	58°03.625 N 6°53.188 Ø					
Water (1)		930 mL	5 m	29,7	1,91	2,27
Water (2)				26,5	1,91	2,90
Filter				9,9	1,95	6,43
Negativ				5,7	1,94	-12,87
St 7 merd 2	58°03.625 N 6°53.188 Ø					
Water (1)		1020 mL	45 m	20,0	2,00	3,15
Water (2)				16,9	2,04	4,14
Filter				9,1	1,98	4,13

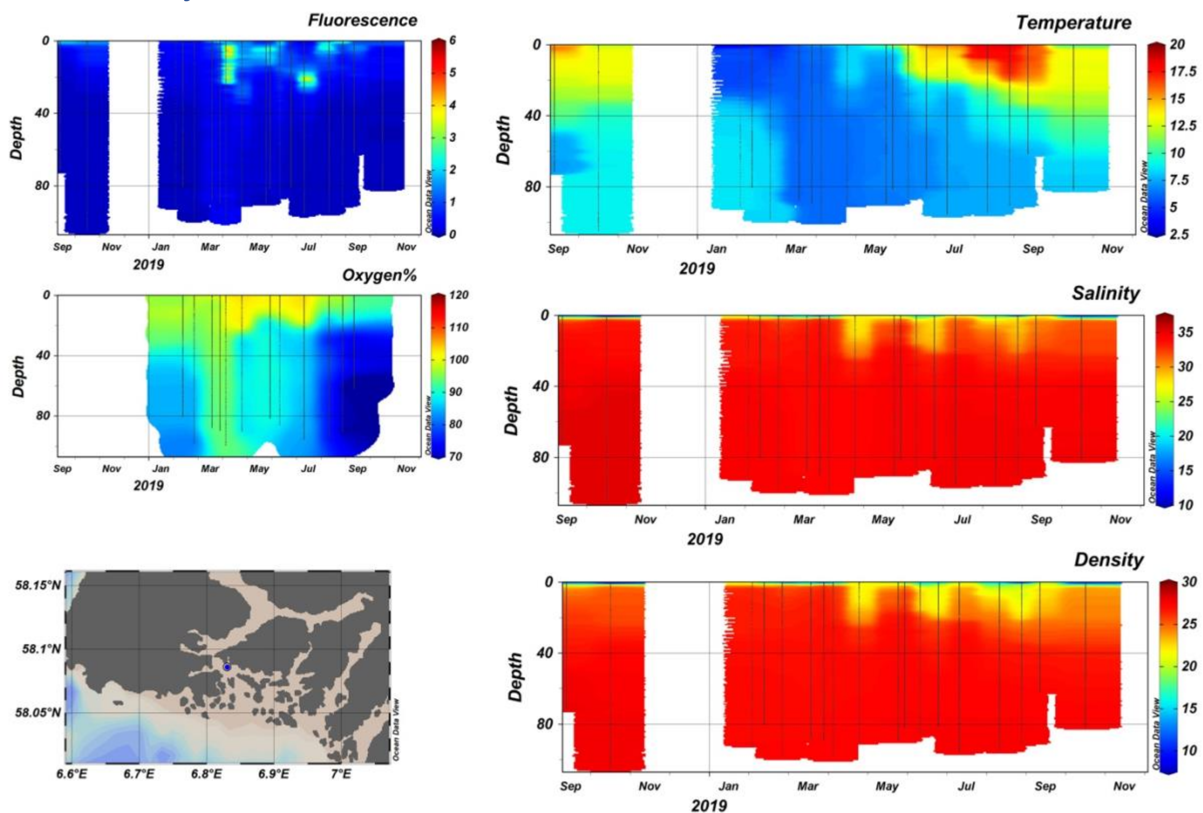
Negativ				7,2	1,91	1,97
St 1, merd 5	58°03.679 N 6°53.188 Ø					
Water (1)		1005 mL	5 m	29,0	1,92	1,73
Water (2)				29,0	1,94	2,37
Filter				12,6	1,76	3,39
Negativ						
St 1 merd 5 (1)	58°03.679 N 6°53.188 Ø					
Water (1)		990 mL	45 m	11,7	1,58	60,09
Water (2)				9,9	1,51	-3,56
Filter				4,7	1,23	-1,08
Negativ				4,9	1,15	-2,09
St. 1, merd 5	58°03.679 N 6°53.188 Ø					
St. 1, merd 5 (1)		1015 mL	50 m	39,4	1,88	3,58
St. 1, merd 5 (2)				35,0	1,88	3,36
Filter				10,3	1,66	5,75
Negativ				2,10	1,29	-0,53
St. 6, merd 3	58°03.698 N 6°53.153 Ø					
Water (1)		1050 mL	52 m	62,2	1,94	2,69
Water (2)				53,1	1,91	2,68
Filter				13,0	1,61	4,82
Negativ				3,6	1,08	-1,11
MFS	58°05.137 N 6°49.842 Ø					
MFS (1)		1000 mL	2 m	7,4	1,48	-2,19
MFS (2)				6,4	1,48	-1,76
Filter				4,1	1,61	-0,90
Negativ				1,0	1,24	-0,20
MFS	58°05.137 N 6°49.842 Ø	1000 mL	5 m	18,8	1,66	1,87
Water (1)				9,8	1,58	37,81
Water (2)				4,6	1,37	-1,52
Filter				3,8	1,13	-2,58
Negativ						
MFS	58°05.137 N 6°49.842 Ø					
Water (1)		1000 mL	90 m	5,6	1,54	-1,12
Water (2)				5,5	1,75	-1,52
Filter				3,7	1,32	-1,02
Negativ				2,0	1,01	-0,45

7.6 Retrieved CTD data 2020



Appendix figure 2: Retrived CTD data from St5-M4, St1-M5 and MFS.

7.7 CTD data from MFS station 2019



Appendix figure 3: CTD data from MFS station 2019. Unpublished data, TM Gabrielsen.

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