



Characterization of marine fungal communities using next generation sequencing techniques



Helga Bårdsdatter Kristiansen

Master Thesis

Supervisors

Håvard Kauserud (UiO), Marie L. Davey (UiO), Thomas Haverkamp (UiO) and Tove M. Gabrielsen (UNIS)

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Front page photo: The view over Adventfjorden and Isfjorden, looking at the sampling area for ISA station, taken at Hotellneset by the author.

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1. Summary

As molecular and bioinformatics techniques are used more frequently and new marine habitats are analysed, a constant increase of marine fungal diversity has been reported, suggesting that marine fungi are a diverse but somewhat ignored organism group. The overall objective of this thesis was to further increase our knowledge about fungal diversity in marine environments by (1) investigating the fungal communities in the pelagic at different depths and dates using an Illumina amplicon sequencing approach, and (2) investigating the fungal communities in sediments using a 454 shotgun metagenomic pyrosequencing dataset. From the arctic marine environment only 14.3% of the DNA amplified was of fungal origin. This demonstrated that the primer pair ITS1F-ITS2 is not fungal specific in a marine environment. In the marine sediments, fungi accounted for, on average, 13.7% of the eukaryotic reads. Yeasts dominated in the sediments samples and are probably adapted to life in anoxic marine sediments. Mortierellales (Zygomycota), which includes known marine fungi, dominated the pelagic arctic fungal communities. What may be terrestrial fungi were detected in both habitats, but it is not clear whether they were deposited and dormant in the marine environment or if they were actively growing and adapted to marine environments. The pelagic fungal communities were not structured according to location or depth, but there seemed to be a seasonal fluctuation in the pelagic communities. Lack of taxonomic coverage of fungal taxa in the reference databases proved to be one of the major obstacles in determining the fungal diversity in both studies. While my study provides insight into the fungal communities in sediments and the pelagic, it also highlights the need for improved databases and further studies with proper sampling techniques and replicates in order to determine what factors actually structures these communities and what role fungi play in the marine system.

2. Introduction

”Invariably when new substrata are surveyed for fungi, a wide range of new genera and species are encountered” (Jones 2011b).

Fungi comprise a major component of terrestrial environments, act as degraders, parasites and symbionts (Jones 2011b; Kendrick 2000), and are ecologically important in chemical cycles and food webs (Gadd 2004). A large proportion of the total microbial diversity and biomass in terrestrial environments is fungal microbes (Lawley et al. 2004; Richards & Bass 2005). Similarly, in marine ecosystems and in the marine food web, fungi play a vital role (Jones 2011b). Even so, marine fungi are among the least studied of all marine organisms (Hyde 2002) and are understudied in comparison to their terrestrial counterparts. However there is increasing evidence showing the active role fungi play in the marine environment by participating in biogeochemical processes (Edgcomb et al., 2011). Molecular diversity estimates suggest a much more diverse range of fungi are present in marine environments than previously thought, which also points to their probable importance in particular ecological niches in the marine environment (Richards et al., 2012).

Definition and the main groups of marine fungi

While most organisms are defined by taxonomy, marine fungi form a group defined by physiology and ecology (Hyde & Pointing 2000). Historically marine fungi have been subdivided by taxonomy into “Filamentous fungi” and “Zoosporic fungi” (Jones & Pang 2012). Marine fungi can also be classified by their ecology as facultative or obligate.

Facultative marine fungi have physiologically adapted to the marine environment and can grow and most likely sporulate in seawater, yet may originate from terrestrial and freshwater habitats. By contrast, obligate marine fungi originate from seawater and are also restricted to it, as their growth and sporulation occurs solely in seawater (Kohlmeyer & Kohlmeyer 1979).

The majority of marine fungi are microscopic and represented mostly by yeast and lower zoosporic fungi (Jones & Pang 2012). Yeasts are defined by their unicellular growth form, and occur in the major fungal lineages; Basidiomycota and Ascomycota. Yeast is one of the dominant groups found in the deep sea and sediments (Bass et al. 2007; Kutty & Philip 2008). A wide range of yeast diversity is often affiliated with benthic animals in the deep sea (Nagahama et al. 2003), while sediments seem to act as a reservoir for yeast species (Singh et

al. 2011). Bass and colleagues (2007) showed that fungal diversity in deep-sea surficial sediments is dominated by basidiomycetes and ascomycetes; further, fungi with yeast growth forms appeared to be the dominant and most successful fungal form in the deep seas. Many new species of yeast have been described and current estimates include 1500 species in the marine environment (Fell et al. 2010).

Filamentous fungi also occur in the marine habitats, but the rough environment of the ocean does not allow large, fleshy fruiting bodies to develop. Abrasion by particles, grains, waves and currents all hinder these structures growth and persistence. However fruiting bodies of smaller size fractions (2 – 4 mm) can be found. The deep sea, anchored wood and sheltered areas are some of the places such small fungal fruiting bodies have been observed. The second largest source of marine fungi isolated from the ocean are connected to algae as endophytes, epiphytes or parasites and of all known higher filamentous marine fungi one third are associated with algae, seaweeds and sea fans (Bugni & Ireland 2004; Alker et al. 2001; Zuccaro et al. 2004; Alva et al. 2002).

The distribution of marine fungi seems to be limited more by temperature and oxygen deficiency (favouring yeast growth forms) than salinity (Kohlmeyer & Kohlmeyer 1979). Nevertheless, there is concern that the majority of the diversity of fungal attributes in the marine environment is overlooked (Richards et al. 2012) by focusing only on fungi able to grow in culture with distinguishing morphological features, and excluding taxa only detectable by sequencing or other methods (Jones 2011a; Jones 2011b; Jones 2011).

Number of marine fungi

The total species richness of fungi is unknown and there is not a good estimate for how many marine fungi there are in the world. Even so total fungal diversity has been estimated at 1.5 million species (Hawksworth 1991). However, recent studies indicate that a more reasonable number may be ten times higher (O'Brien et al. 2005), given that fungi affiliated with animals, freshwater and marine ecosystems or sediments, as well as cryptic species, were not included in the 1.5 million estimate and it was only based on morphological observations (Richards et al. 2012; Hawksworth 2001; Hawksworth 1991).

In the early years of this century, there were in total 444 accepted and 800 reported obligate marine fungi species (Kohlmeyer & Volkmann-Kohlmeyer 1991; Hawksworth 2001; Hyde & Pointing 2000). Among these 800, the largest fraction belongs to Ascomycota and a small fraction to Basidiomycota. Kis-Papo (2005) reported that 467 isolates of cultureable fungi of 244 genera have been collected from marine environments. These relatively small

numbers have traditionally led to the conclusion that marine fungi are of low abundance and diversity (Burgaud et al. 2009; Le Calvez et al. 2009).

Advances in molecular methods have recently revealed a wealth of previously unknown unculturable fungi and indicate that the estimate for all fungi worldwide could be raised from 1.5 to 3.5 – 5.1 million species (O'Brien et al. 2005). As these techniques, including high throughput sequencing (HTS) (see below), are used more frequently, and new marine habitats are analysed, a constant increase of known marine fungal diversity is reported, suggesting that marine fungi are indeed a diverse group (Jones 2011b).

History

The first extensive study of marine fungi was conducted early in the 20th century focusing on fungi growing on macro algae (Cotton 1909; Sutherland 1915). In the years to follow the main research on marine fungi has generally been on those associated with substrata like mangrove trees, sea grass, sea weed, algae, drift wood, decomposing wood and plant detritus from coastal waters, corals, calcareous tubes of molluscs and intestines of crustaceans (Hyde et al. 1998; Cuomo et al. 1985; Jones 2011b; Zuccaro et al. 2008; Kohlmeyer & Kohlmeyer 1979; Barghoorn & Linder 1944; Raghukumar et al. 1992; Mouton et al. 2012).

The study of marine fungi has also expanded into deep sea, pelagic, benthic and hydrothermal habitats, particularly in the last decade (Kohlmeyer & Kohlmeyer 1979; Mouton et al. 2012; Bass et al. 2007; Le Calvez et al. 2009; López-García et al. 2003; Jobard et al. 2010; Lai et al. 2007). Here the fungi again prove to be one of the most ecologically adaptable eukaryotic lineages, and are even found at the great depths of the Mariana trench, which exceeds 10 km (Takami et al. 1997; López-García et al. 2003). The small amount of data representing fungi isolated from the deep sea provides little information about their importance and role. The knowledge of fungi isolated from marine sediments is also poor and it is uncertain if the fungi isolated are dormant spores or active fungi (Jones 2011b).

Studies of marine fungi have predominantly been made in USA and Europe, but in the last decade, additional research in Asia has increased our knowledge of the flora in that area. However, investigations of marine fungi in Arctic and Antarctic regions are few in number (Bahnweg & Sparrow 1974; Loque et al. 2009; Pang et al. 2008; Pang et al. 2009; Pang et al. 2011; Rämä et al. 2014). Pang et al. (2008, 2009, 2011) and Rämä et al. (2014) conducted a few studies on Svalbard and in Northern Norway in relation to fungi connected to drift wood.

However, to my knowledge there have been no HTS studies done on marine fungi in the Arctic.

Detection and classification of marine fungi

The study of marine fungi has mainly focused on the morphology and ultrastructure of cultivated fungi found on marine substrates and has primarily provided taxonomic data (Jones 2011b; Kohlmeyer & Kohlmeyer 1979). Most studies have failed to demonstrate with certainty that the obtained isolates can grow and sporulate in the marine environment, calling into question their status as obligate or facultative marine fungi (Kohlmeyer & Kohlmeyer 1979).

In the last decades, environmental gene libraries targeting specific areas in the ribosomal RNA (rRNA) region have been used successfully to identify fungi in marine environments (Richards et al. 2012). Two main regions have been targeted; (1) SSU rDNA (small ribosomal subunit) and the (2) ITS (internal transcribed spacer) region. The ITS region consists of two parts; ITS1 which is located in the rRNA genes between the 18S (SSU) and the 5.8S and ITS 2; located between the 5.8S and the 28S LSU (large subunit). The ribosomal subunits are highly conserved areas while the ITS is a very variable region. The SSU region therefore provides good resolution for identifying fungi at higher levels of taxonomy and is poor at distinguishing fungi at species and genus levels while the ITS region gives increased resolution and accuracy within well sampled groups when assigning to genus and species level (Gardes & Bruns 1993; Horton & Bruns 2001). Well sampled sequence databases are thus necessary to provide good match results (Buchan et al. 2002; O'Brien et al. 2005). ITS divergence variation between taxonomic groups can be an obstacle using these sequences (Nilsson et al. 2008; Vilgalys 2003; Nilsson et al. 2006) and databases have high rates of erroneous classification of sequences (Vilgalys 2003), creating added challenges to using molecular methods to detect fungi in marine environments. Nevertheless, molecular methods present a promising approach to further increase our knowledge of fungi in marine environments.

High throughput sequencing (HTS)

Next Generation Sequencing (NGS) sequencing has advanced microbial ecology by allowing unprecedented detection of unculturable organisms and profiling of microbial communities, both fungal and bacterial. In the last decades, NGS has facilitated fundamental knowledge acquisition about the fungal communities in the environment by providing

massive amounts of data on microbial community composition (Sun et al. 2010; Heidelberg et al. 2010; Richards et al. 2012). Illumina and 454 (see below) are both commonly used high throughput NGS techniques for environmental sequencing. Both approaches greatly increase the sensitivity of environmental analyses because they allow high sequencing depth and can potentially capture and allow detection of template (DNA) that is present in only very small quantities, for example detecting a single yeast cell of a specific species (Gadd 2004; Orellana 2013; Logares et al. 2012). While Sanger sequencing, typically generates 10^2 sequences of 600 – 900 base pairs (bp) length in one run while (NGS) provides 10^6 – 10^9 sequences with 100 – 700 bp in a single run and up to 800 bp with the upgraded version, for a fraction of the cost per sequence (Lawley et al. 2004; Glenn 2011; Richards & Bass 2005; Scholz et al. 2012; Lanzén et al. 2012).

HTS techniques can be used to perform both shotgun sequencing of fragmented environmental or genomic DNA, and amplicon sequencing, where a specific region is targeted by PCR and these amplicons are then sequenced (Logares et al. 2012). There are several platforms for NGS and Roche 454 and Illumina are two of the most widely used platforms for environmental sequencing. Other platforms include e.g. SOLiD from Applied Biosystems/Life-Technologies and IonTorrent PGM by Life Technologies, but both of these are rarely used for ecological studies (Hyde 2002; Logares et al. 2012; Iverson et al. 2012; Mardis 2008). Illumina and Roche 454 were used in this project and only these platforms will be described further. They are both high throughput and involve massively parallel sequencing (Hyde & Pointing 2000; Glenn 2011).

The 454 pyrosequencing platform uses a single nucleotide addition where the template is fixed on beads and amplified during an emulsion PCR step. In a fixed order, the different nucleotides are flowed over wells and are incorporated when the complementary bases are available at the next position of the template DNA. A flash of light gives the signal when the incorporation is complete and the strength of this signal defines how many nucleotides were attached to the strand. The machine's capability to distinguish between the flash of light decreases as the number of nucleotides incorporated increases. Therefore, homopolymer length is the main sequencing error associated with the 454 platform (Logares et al. 2012). In Illumina all four nucleotides, each with its own fluorophore, are flushed over glass cells where the template with the adapter has been attached to the surface and amplified by a so-called "bridge amplification". This approach incorporates only one nucleotide at a time. Illumina can be run singly or paired end, where both ends of a template molecule are sequenced for a fixed, overlapping length, resulting in a pair of sequences that can be

assembled to give longer reads (Metzker 2009; Logares et al. 2012; Bartram et al. 2011). The major difference between the two approaches is total read length, error profiles and cost. The error profile is higher with 454, but the read length greater. While 454 provide sequences up to 800 bp the paired-end Illumina can generate sequences with 500 bp (2 x 250). The cost of running the platforms is quite different as well, since Illumina is considerably cheaper to run compared to a 454 platform (Logares et al. 2012; Glenn 2011).

Aim of the study

The overall objective of this thesis was to increase our knowledge about fungal diversity in marine environments. More specifically, the aims of my master's project were to; (1) investigate the fungal communities in the pelagic at different depths and dates using an Illumina amplicon sequencing approach and (2) investigate the fungal communities in sediments using a 454 pyrosequencing metagenomic dataset. Exploring this diversity of marine fungi using these two different approaches is intended to provide information about the fungal groups that are present in a pelagic fjord system in the Arctic (1) as well as the marine sediments around oil and methane seeps (2). The thesis is organized into two different parts addressing (1) and (2), respectively, with a common summary, introduction and conclusion.

3. Pelagic marine fungi in an arctic fjord

3.1 Materials and methods



Figure 1. Map of Svalbard showing the location of the ISA sampling station marked with a star at the junction of Isfjorden and Adventfjorden. Map modified from Sørensen et al. (2012).

Study site and sample collection

Samples were collected close to the Isfjorden-Adventfjorden (ISA) mooring located at $78^{\circ} 15' 6.0012''$ N and $15^{\circ} 32' 4.9200''$ E (Fig. 1) on the west coast of Spitsbergen, the largest island in the Svalbard archipelago. The Svalbard archipelago is situated between 76° N and 81° N and 10° E and 34° E and lies at the borders of the Arctic Ocean in the north, the Barents Sea in the south and east and the Fram Strait in the west. Spitsbergen has numerous fjords, the largest of which is Isfjorden, which is oriented in a SW ($78^{\circ} 7' N$) – NE ($78^{\circ} 27' N$) direction.

The main basin of Isfjorden is 24 km wide and 7 km long and the total system contains four smaller fjord systems. The ISA sampling station was located at the mouth of one of these smaller systems, Adventfjorden. The inlet from Isfjorden into Adventfjorden is 100 m deep and 3.4 km wide, providing easy water exchange between the two. Adventfjorden itself has a main depth between 50 and 80 m. Two currents run parallel to one another along the west coast of Spitsbergen. Closest to the coast is the East Spitsbergen current (ESC), which arrives from the east of Spitsbergen carrying arctic waters with low salinity and low temperature. The West Spitsbergen current (WSC) arrives from the Atlantic Ocean in the south, carrying warm Atlantic water with high salinity. When the WSC is strong it penetrates through the ESC and enters the Isfjorden system (Nagahama et al. 2003; Wassmann et al. 2006; Nilsen et al. 2008). In addition to influences from the ESC and WSC, the Adventfjorden system is supplied by freshwater from two rivers; Advent River and Longyear River. The Advent River runs for 35 km through Adventdalen before entering the fjord (Dobrzyn et al. 2005), while the Longyear River runs from the Longyear Glacier and Lars Glacier before entering the fjord after 4 km.

They are both braided rivers and supply a huge amount of sediment to the fjord (Zajączkowski et al. 2009; Kane & Yang 2004).

As part of an on going project, water samples and environmental data are collected regularly at ISA (Fig. 1) at predetermined depths (5, 15, 25, 60 m). For this study, 10 samples from 25 m were analysed representing the winter/spring/summer season of 2011 – 2012 (Table 1). An additional 9 samples were analysed to provide information from all depths (5, 15, 25, and 60 m) on three of the sampling dates (Table 1). All samples were collected and pre-filtered on site through a 10 µm mesh. Four litres of the filtrate were filtered again through a 0.45µm filter (Millipore, HVLP04700) in the laboratory. Chlorophyll *a* was measured from both filters using a 10-AU-005-CE Fluorometer (Turner Designs, Sunnyvale, USA) (Thomson 2014, unpublished data).

DNA extraction, amplification, and sequencing

DNA extracted from the 0.45µm filters using the DNeasy Plant Mini Kit (Qiagen 69106) was made available to this project. Illumina amplicon library preparation was conducted by Courtney Nadeau in the UNIS Arctic Biology labs. The primers ITS1F (Gardes & Bruns 1993) and ITS2 (White et al. 1990) were used to target the ITS1 region. The PCR reaction mixture contained 1X Dreamtaq buffer, 0.5 µM forward primer, 0.5 µM reverse primer, 1 mM dNTPs (0.25mM of each dNTP), 1.625 U enzyme and 0.5 µg BSA. The total volume of the reaction was 25µl whereas the template was 4µl (approximately 4% of the total template extraction). PCR conditions used were: 2min at 95°C, followed by twenty four cycles of 30s at 95°C, 30s at 55°C, and 1min at 72°C), followed by 30min at 72°C and a final holding stage at 10°C. Illumina adaptors including a 4-8 bp barcode were ligated to each sample to allow downstream identification of the samples. Then, a second SPRI purification was conducted before qPCR was used to determine the optimal number of PCR cycles, (C_T) for library enrichment. Enrichment PCR was carried out to amplify the DNA fragments that had an adaptor attached at both sides. An additional SPRI-treatment was performed in order to remove the excess of salts, primers, dNTPs from the PCRs. Illumina libraries were then pooled in equimolar amounts and sent to Source Bioscience (Nottingham) for paired end 250bp sequencing on an Illumina MiSeq version 2.

Bioinformatics

Paired reads were merged into full length ITS1 sequences using Flash v.1.2.8 and demultiplexed by barcode. Chimera checking, quality filtering, and trimming of the sequences in

the dataset were performed in Mothur (Schloss et al. 2009) using the Abel computing cluster at UiO (www.hpc.uio.no). De novo chimera checking was performed on each sample using the uchime algorithm (Edgar et al. 2011) with the minimum divergence parameter set to 1, removing sequences as chimeric only when the minimum divergence between the two identified parental sequences is greater than 1% (those sequences with identified parental sequences that are less than 1% divergent from each other were considered non-chimeric). The parental sequences were assumed to be at least twice as abundant as the resulting chimera; therefore the abundance skew was set to 2. Sequences with ambiguous bases were removed, together with sequences shorter than 200 bases and longer than 450 bases. The minimum average quality score for sequences retained in the dataset was set to 35.9, which, assuming high quality scores to have PHRED quality scores >35 and low quality positions <5, allows for 1-2 low quality base pairs within a single sequence. Filtered and chimera-checked sequences were then clustered using the uclust algorithm (Edgar 2010) as implemented in Qiime (Caporaso et al. 2010) with a 97% similarity threshold. The most abundant sequence variant was selected to represent the different OTUs and global singletons were removed as presumed sequencing errors.

The representative sequences of each OTU were compared to the GenBank non-redundant nucleotide database using BLASTn on Lifeportal (www.lifeportal.uio.no). The E-value parameter in the BLAST algorithm (Altschul et al. 1990) provides an index where a low E-value indicates a lower risk of the hits occurring by random chance. The standard cut off E-value of $1.0E^{-03}$ at Lifeportal (www.lifeportal.uio.no) was used. The retrieved BLAST output was analysed in MEGAN (version 5.1.0) (Huson et al. 2007). MEGAN uses the Lowest Common Ancestor (LCA)-algorithm to parse BLAST match output and extract the most parsimonious NCBI taxonomy for each sequence. Taxonomic assignments in MEGAN therefore depend on the parameters set. In this analysis, top hits were set to five, allowing only the five highest hits per sequence to be considered in MEGAN (if there were four or less hits these will be pushed back up in the taxonomy to “higher nodes”). The minimum support value was set to one, so only one read assigned is needed for a taxon to appear in the results. The minimum score value was set to 50; ignoring hits in the input dataset with bit scores were less than this threshold. A maximum threshold was set for the expected value of hits, ignoring any hit in the input data, which exceeded the value 0.01. The top percentage provides a threshold for a maximum percentage a hit may fall below with its best score for a given read and still be considered in the dataset: this was set to 10%. Minimum complexity was used to identify low complexity reads at 0.44, which were then placed on a special low complexity

node in the tree. This prevents low complexity sequences that perform badly in BLAST from being incorrectly assigned because they pull out a huge number of sequences unrelated to one another. Based on these settings, any OTU identified as fungal by its representative sequence was extracted from the dataset. All 190,528 sequences belonging to the OTUs that had been identified as fungal in this analysis were extracted from the original dataset using Qiime. These were then run on BLASTn with the previous mentioned settings through Lifeportal. The complete fungal dataset was then imported to MEGAN for visualization on the NCBI taxonomic tree with the settings: minimum score value at 70, max expected at 0.001 and the minimum support at 1. The MEGAN tree was summarized to show taxonomy at the order level. Order level was chosen as the best resolution to present the taxonomy due to few species- and genus-level BLAST hits (Fig. 3).

Community richness and composition

The OTU data was split into two subsets, one consisting of 10 samples taken at 25 meters depth on different dates throughout the season and the other consisting of samples from four different depths on three separate dates (Table 1). All analyses were conducted on both the subsets. To estimate how deeply the different samples were sequenced, rarefaction curves were calculated in Qiime (Edgar 2010) version 1.5 through the Abel cluster (www.hpc.uio.no) by averaging the number of OTUs recovered from 10 random resamplings of the data at increasing sequencing depths in intervals of 500 sequences (Fig. 4 and 7). The Shannon Wiener index (Fig. 5 and 8) was calculated for each sample in R (R Team 2012) using the diversity function in the package “vegan” (Oksanen et al., 2006). The main changes in community composition were visualized through ordination plots run in R, utilizing the vegan package. A Detrended Correspondence Analysis (DCA) was run with default settings with a Bray Curtis distance matrix. A general non-metric multidimensional scaling (GNMDS) was run for two dimensions with default settings. Environmental factors were related to the observed structure of community composition in each ordination using the envfit function in R. The proportional abundance for individual OTUs versus dates and depth was plotted in R.

3.2 Results

Data characteristics

A total of 1,315,767 full-length ITS1 reads were generated. Of the 1,251,178 that passed quality filtering and chimera checking, only 179,465 sequences were at the end identified as belonging to fungi, although a presumed fungal specific primer (ITS1F) was

used. The remaining sequences were identified as marine animals, algae, protists and alveolates. In general, fungal sequences accounted for only about 14.3% of the total sequences (See table 1 for more information). The exception is during the spring bloom (April), when a drop occurs in the percentage of fungal sequences and OTUs recovered (Fig. 2). The fungal order Mortierellales dominated greatly (Fig. 3). In addition, a number of reads were assigned to Basidiomycota (particularly Agaricomycetes), Ascomycota (particularly Hypocreales), and Chytridiomycota (Fig. 3). BLAST hits were generally of poor quality with high similarity over only short fragments in the conserved SSU and 5.8 regions or low similarity (average of 96% over a total average length of 80) over longer stretches within the ITS1 region.

Table 1. Sequence and sample information for pelagic samples taken at the ISA station during 2011-2012.

Sample	Sampling Date	Julian Date	Sampling Depth (m)	Total Sequences	Fungal Sequences	% Fungal Sequences	Total OTUs	Fungal OTUs	% Fungal OTUs
Sample1	14.12.11	-17	5	62149	15242	25	928	204	22
Sample2	14.12.12	-17	15	57588	13749	24	921	195	21
Sample3	14.12.12	-17	25	47508	10144	21	892	183	21
Sample4	14.12.12	-17	60	72057	16934	24	952	190	20
Sample5	17.01.12	17	5	32923	4734	14	944	177	19
Sample6	17.01.12	17	15	39905	6616	17	1077	196	18
Sample7	17.01.12	17	25	70642	12607	18	1297	242	19
Sample8	17.01.12	17	60	94340	14122	15	1414	236	17
Sample9	28.01.12	28	5	68717	10233	15	1197	212	18
Sample10	28.01.12	28	15	68182	11715	17	1264	228	18
Sample11	28.01.12	28	25	90017	14410	16	1339	249	19
Sample 12	28.01.12	28	60	62202	10576	17	1172	217	18
Sample13	16.02.12	47	25	62907	9989	16	973	188	19
Sample14	08.03.12	67	25	57959	7426	13	1000	204	20
Sample15	22.03.12	81	25	73651	7422	10	881	154	17
Sample16	11.04.12	101	25	127233	9177	7	1004	190	19
Sample17	26.04.12	116	25	92582	1449	2	433	57	13
Sample18	10.05.12	130	25	68335	836	1	217	33	15
Sample19	06.07.12	157	25	66870	12660	19	337	64	19

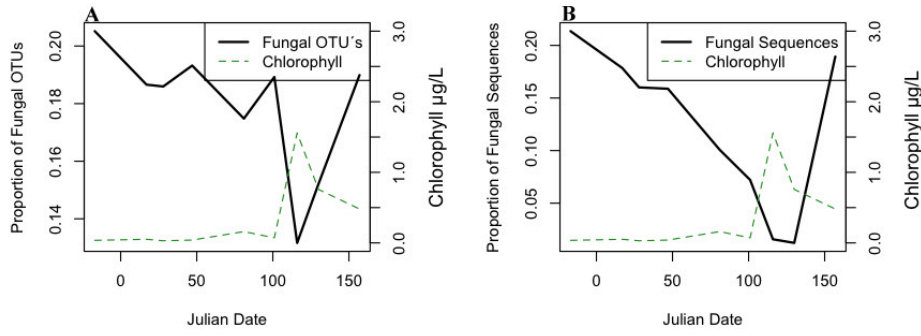


Figure 2. Chlorophyll a fluorescence levels and fungal OTU richness recovered at the ISA station during a single winter-summer cycle. A: Proportion of the total number of OTUs recovered that belonged to fungi, co-plotted with measurements of chlorophyll a fluorescence through the 2011-2012 winter-summer cycle. B. Proportion of the total amount of sequences belonging to fungi co-plotted with measurements of chlorophyll a fluorescence through the 2011-2012 winter-summer cycles.

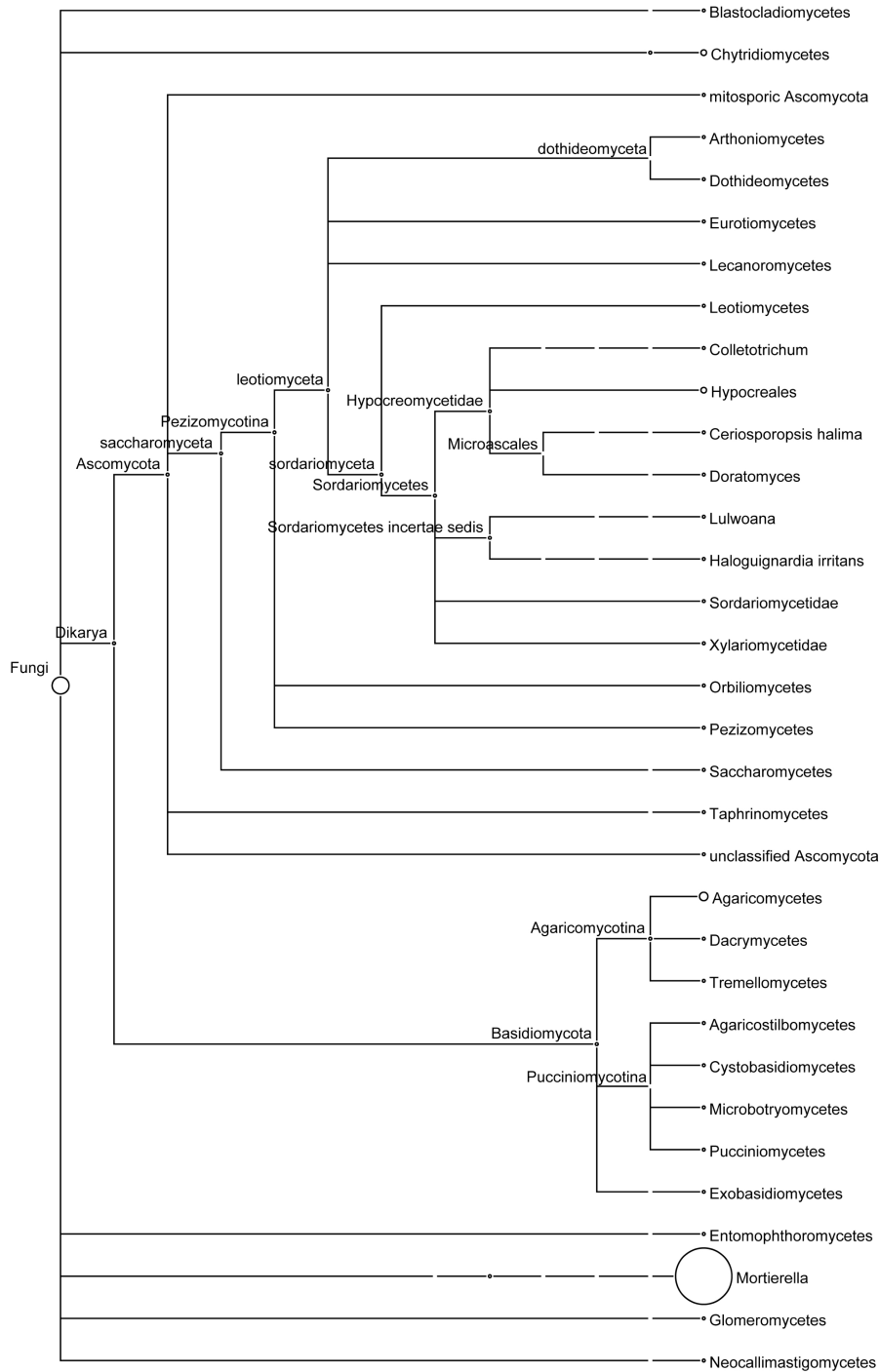


Figure 3. Taxonomic classification output from MEGAN assigning sequences from the ISA station to the backbone NCBI taxonomy. Assignments were based on the top 5 BLAST matches and all taxa receiving one or more hits are displayed. Number of hits assigned proportional to circle size.

Community changes at different depths

More than 10,000 fungal sequences were generated from most of the samples and while they showed signs of levelling off, none of them approached an asymptote, as can be seen from the accumulation curves (Fig. 4). There seems to be little variation in the total number of fungal OTUs recovered at different depths on each date (Fig 4). The Shannon Wiener index (Fig 5), shows the same trend of constant diversity across the four depths. The changes in proportional abundance of the ten most abundant OTUs across different depths were very small (Fig. S1). Community composition was primarily structured by date, and there was little structure in the GNMDS ordination by depth on any given date (Fig. 6). The first axis separates the two sample dates 17th and 28th of January (2012) from 14th of December (2011), while the second axis reveals less variation. Julian date was significantly correlated to the community structure recovered (Table 2). Salinity and chlorophyll also showed significant fitting, but changes in salinity and chlorophyll did not occur between depths, instead primarily between dates.

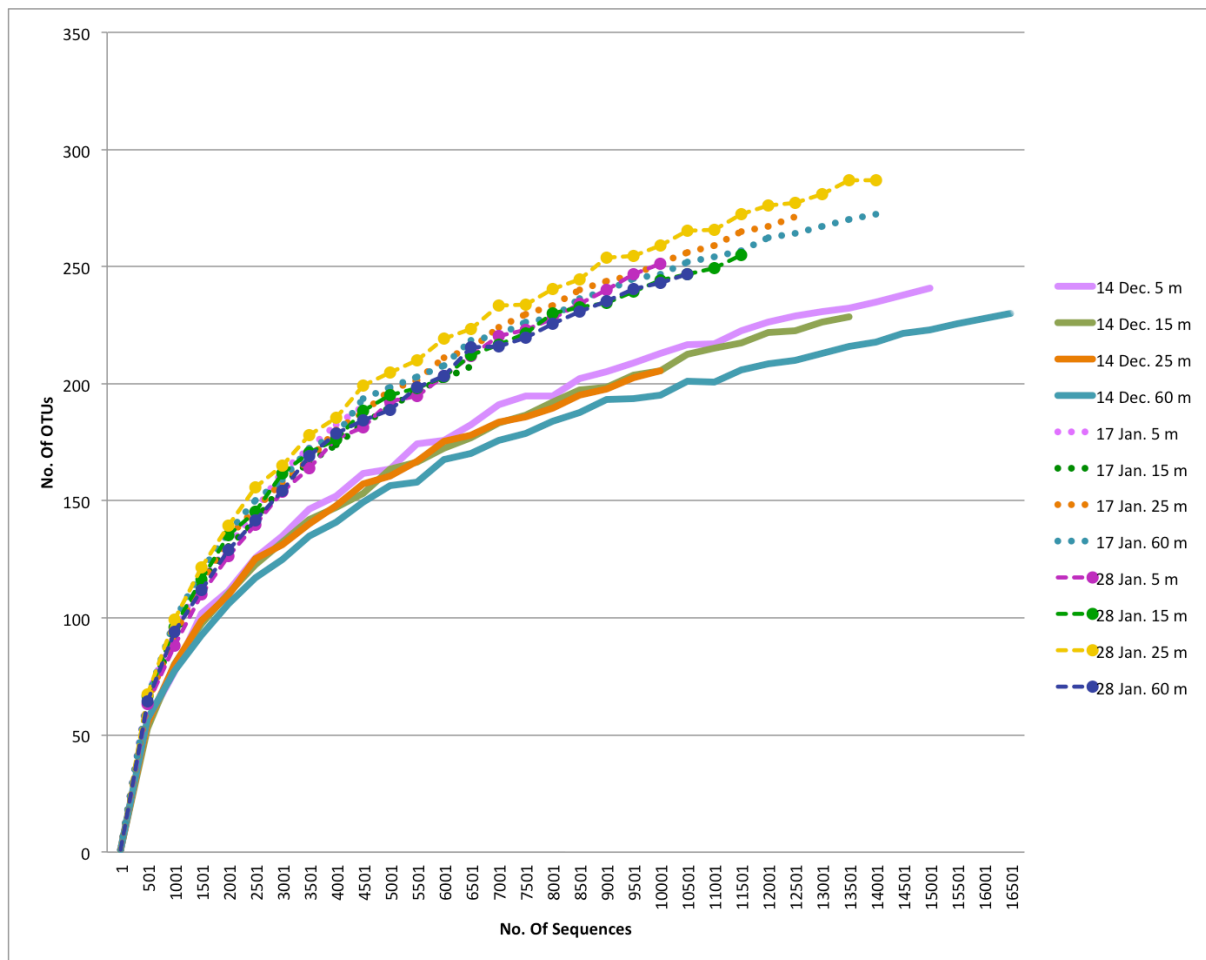


Figure 4. Rarefaction curves showing the sequencing depth for twelve samples from different depths at the ISA station. Number of OTUs represents an average of 10 resampling of sequencing depths in steps of 500.

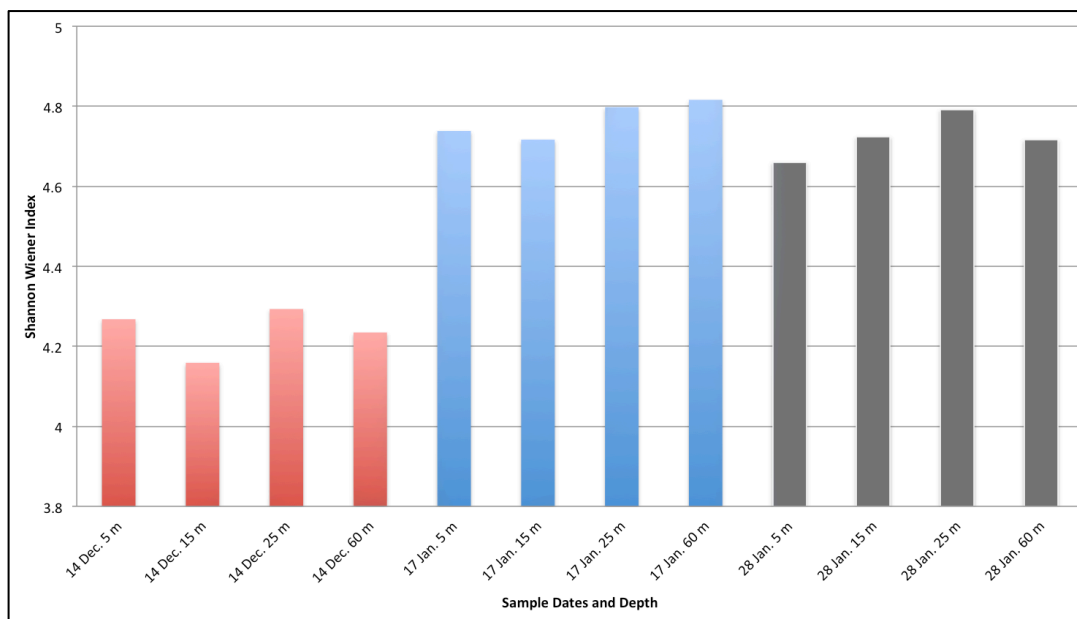


Figure 5. Shannon Wiener index of alpha diversity at ISA Station over three days at 4 different depth.

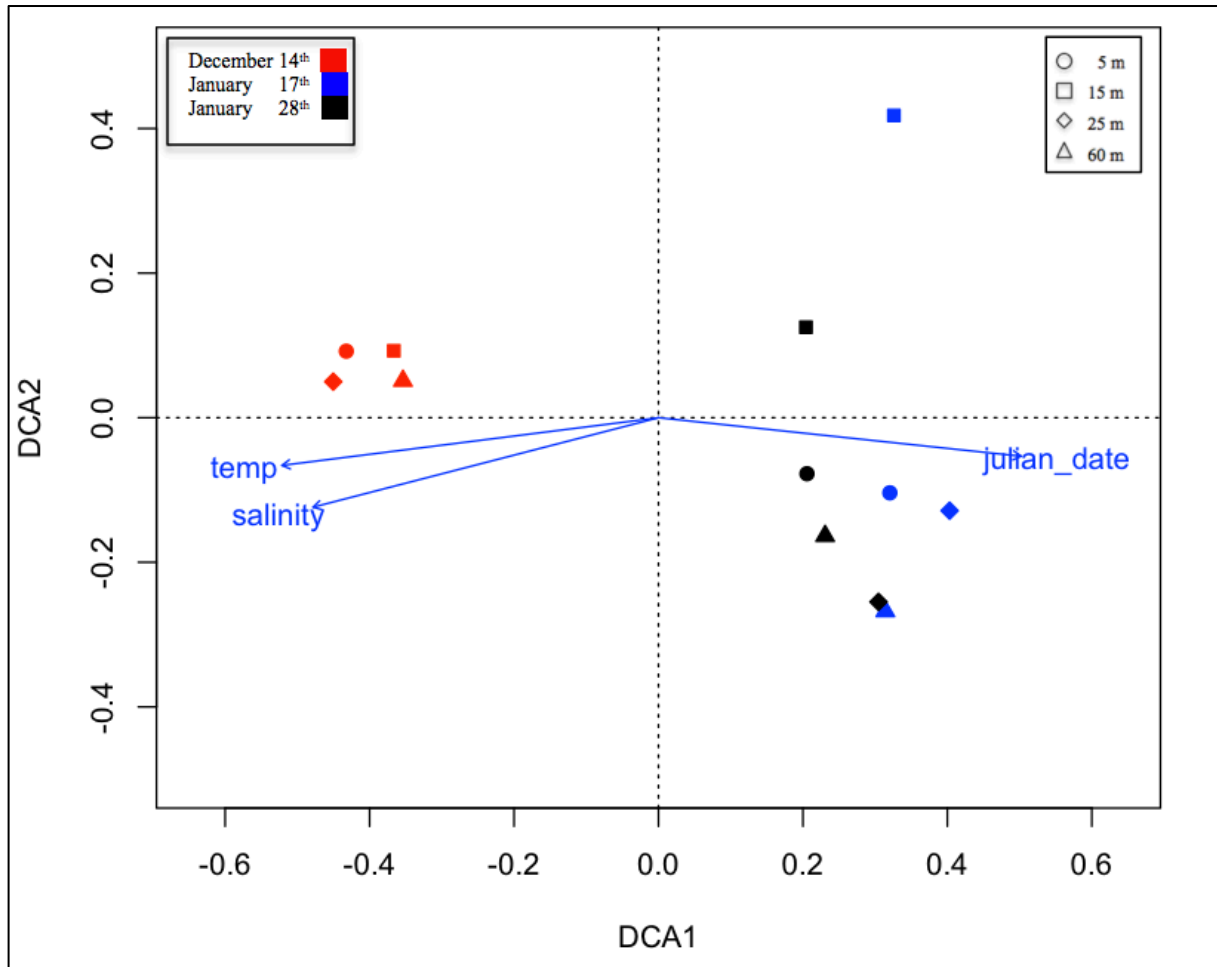


Figure 6. Detrended Component Analysis (DCA) of the twelve samples from the ISA station sampled at different depths and days. Sampling dates are represented by colour, while depths are represented by shape.

VECTORS:	GNMDS r2	GNMDS Pr(>r)	DCA r2	DCA Pr(>r)
julian_date	0.9451	0.001	0.9569	0.001
chlorophyll	0.5072	0.036	0.8457	0.003
salinity	0.9004	0.001	0.4322	0.08
temp	0.9590	0.001	0.1497	0.509

Table 2. Significance values from the fitted environmental vectors for the GNMDS and DCA ordinations of the 12 samples from the ISA station sampled at different depths and days.

Community changes across season

Most of the samples were analysed with high sequencing depth, and the rarefaction curves begin to level off but did not reach an asymptote (Fig. 7). There were two exceptions; 26th of April and 10th of May (Sample 17 and 18), which had low sequencing depths (Fig 2) and an abrupt stop in the rarefaction curves (Fig. 7), implying they were not sequenced adequately. The number of OTUs detected was mostly constant throughout the samples, with the exception of the three late spring and summer samples; April 26th, May 10th and July 6th,

where a distinct reduction in numbers was detected. There was also a reduction in the Shannon Wiener diversity index (Fig. 8) during April to July in comparison to the rest of the year.

When it comes to richness of OTUs, 6th of July (Sample 19) was substantially lower than the other samples although the rarefaction curve levels off, indicating that it was well sampled and had good sequencing depth (Fig. 2). Proportional abundance of the most abundant fungal OTUs did vary with date (Fig. 9). While some OTUs seemed to fluctuate more or less randomly, two patterns were observed in multiple OTUs: (1) an increase in proportional abundance in July and (2) a decrease in proportional abundance after April. Community composition also changed across dates (Fig. 10). The first axis of the DCA separates sample 17 and 18 from the others, the second axis separates sample 19. Julian date was the only significant vector fitted (Table 2B). According to the collected chlorophyll data the spring bloom occurs in the end of April (Fig. 2).

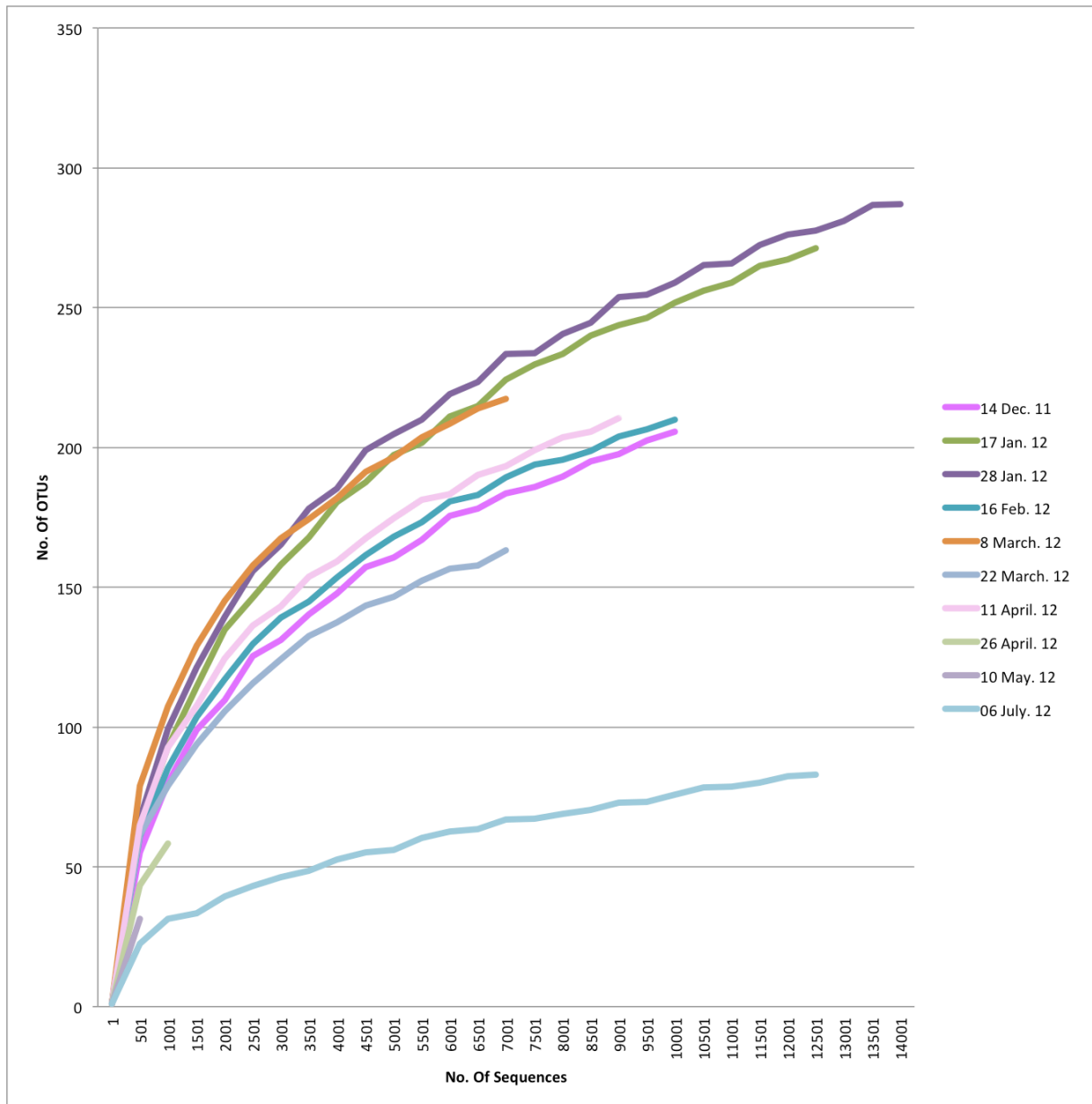


Figure 7. Rarefaction curves showing the sequencing depth for ten samples from different dates at the ISA station. Number of OTUs represents an average of 10 resampling of sequencing depths in steps of 500 sequences.

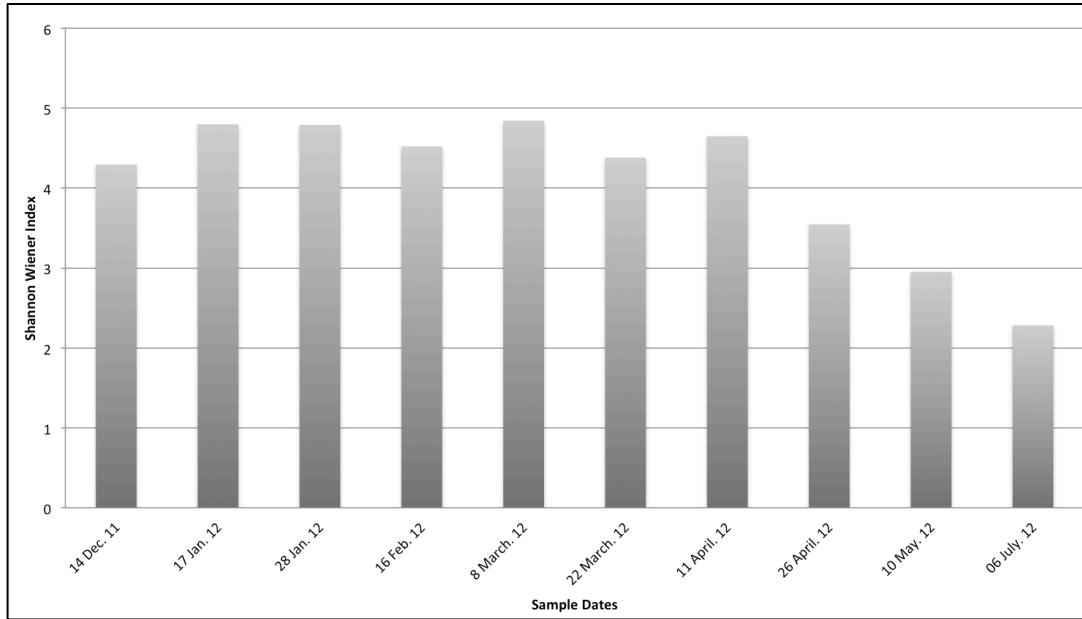


Figure 8. Shannon Wiener index of alpha diversity at the ISA Station over one winter-summer cycle in 2011-2012.

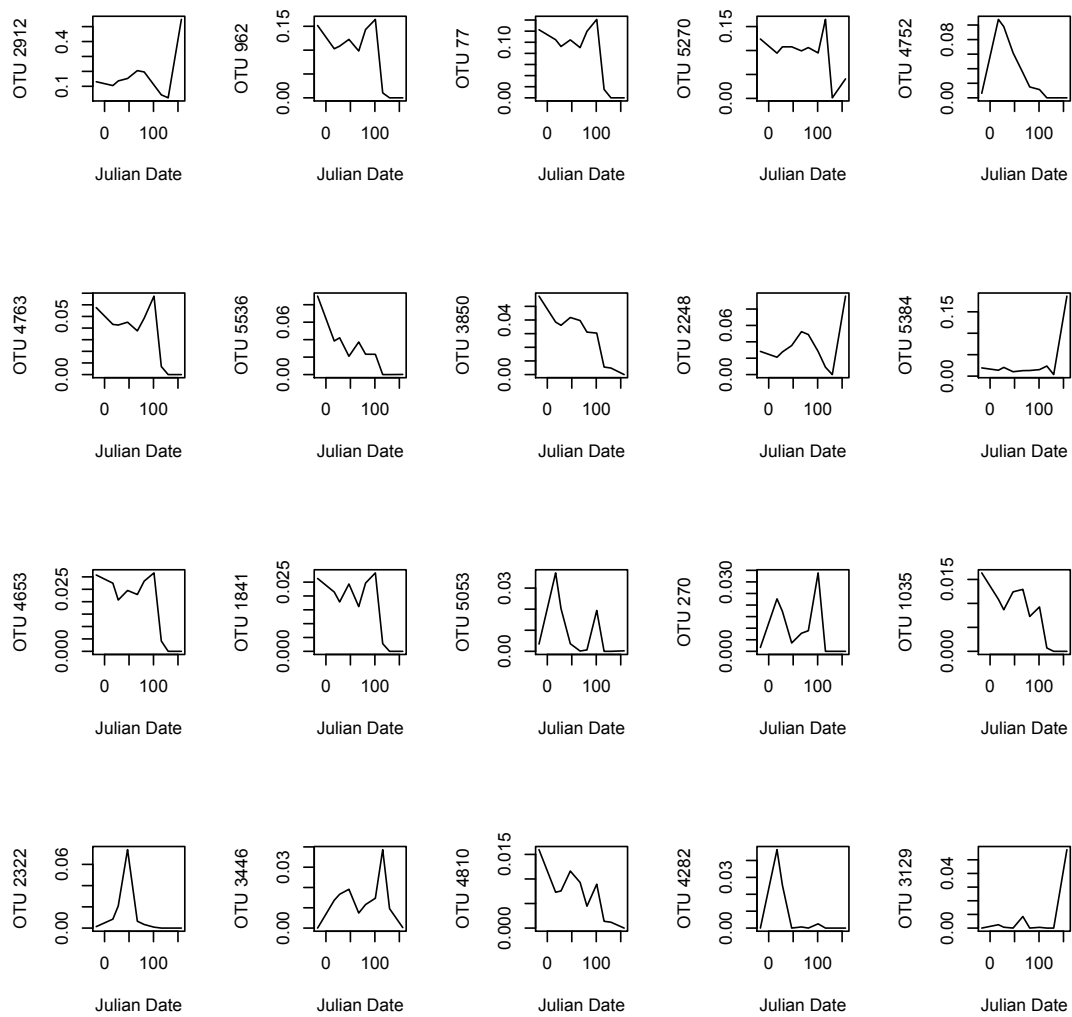


Figure 9. Proportional abundance of the most abundant fungal OTUs throughout a single winter-summer cycle at the ISA station.

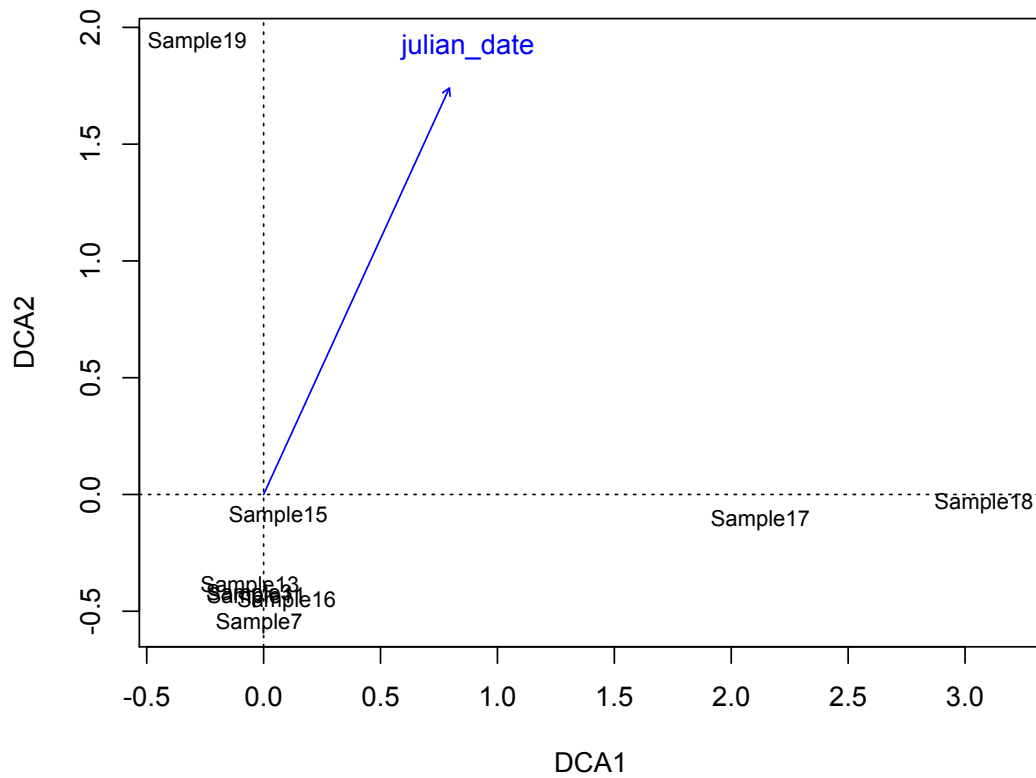


Figure 10. Detrended Component Analysis of samples 1, 3, 7, 11, 13 – 19 from the ISA station. Julian date is the only significant parameters affecting the compositional variation.

VECTORS:	GNMDS r ²	GNMDS Pr(>r)	DCA r ²	DCA Pr(>r)
julian_date	0.5603	0.073	0.8223	0.001
chlorophyll	0.6245	0.13	0.5987	0.123
salinity	0.1323	0.702	0.0797	0.743
temp	0.1518	0.663	0.4341	0.105

Table 3. Significance values from the fitted environmental vectors in the GNMDS and DCA ordination dataset by date (Sample 1, 3, 7, 11, 13 – 19) from ISA station. The only fitted value significantly correlated to the compositional variation was Julian dates.

3.3 Discussion

Usage of the ITS marker in marine environments

The ITS region has been selected as the DNA barcode region for fungi (Schoch et al. 2012; Bellemain et al. 2010; Seifert 2009) and is known to be useful for determining species diversity using molecular approaches (Köljalg et al. 2013; Blaalid et al. 2011; Davey et al. 2013). When targeting well-defined taxonomic groups where ITS primers are known to work well, the ITS approach can be used for ecosystem comparisons. However, for inferring higher-level phylogenetic relationships and identifying novel groups of fungi, it is of more limited use, as the region becomes more-or-less unalignable making it not useful for resolving phylogenetic relationships among deeper branches in the Fungi (Richards et al. 2012; Horton & Bruns 2001). In particular, the fungal specific primer ITS1F (Gardes & Bruns 1993) is known to be able to separate plants from fungi in terrestrial environments (Bellemain et al. 2010; Blaalid et al. 2011). However, when the ITS1F fungal specific primer was used to detect fungi in marine sediments, only 50% of the sequences recovered were fungal, while the rest represented bacteria and chimeric sequences (Singh et al. 2012). Conversely, when I attempted to target the pelagic fungal community using the same fungal specific primer (ITS1F), only 14.3% of the DNA amplified from the arctic marine environment was of fungal origin, while the remaining 85.7% was crustaceans, algae and protists. This suggests that the ITS1F-ITS2 primer combination is not specific to the fungal community when the environment includes significant proportions of protists and animal DNA. Hence, the ITS1F primer is not ideal for targeting fungi in marine environments. The lack of specificity observed here may be because fungi are more closely related to animals than plants, and are recognized as a monophyletic sister clade to the animal kingdom (Seifert 2009). In order to work with the fungal ITS in a marine environment, there is a need to either test or design more specific primers that exclude e.g. animals. Since the chosen ITS primer set amplifies more than just fungi, my data must be interpreted with caution, as the effective sampling effort for fungi may be changing under certain conditions, e.g. during the spring bloom. Almost no fungal sequences were recovered in April, which according to the chlorophyll data, coincides with the spring bloom. This may be because the fungal DNA is overwhelmed by a huge increase in the population of other crustaceans, algae, and protists present at this time. This reinforces the need to test out other fungal specific primers, especially if the aim is to track fungal populations over time in a marine environment.

Taxonomic diversity

Fungal diversity determination in terrestrial environments is a difficult task, as highlighted in several reviews (Kowalchuk 1999; Bridge & Spooner 2001; Anderson & Cairney 2004). Considering that terrestrial environments are much better known than marine ones, limitations connected to identification, isolation and quantification of fungi from marine environments pose a major challenge. There is also the additional complication of distinguishing between obligate, facultative and deposited terrestrial forms among the fungi recovered from marine environments.

Although a large number of fungal sequences were recovered in this study, most had poor BLAST matches to the GenBank non-redundant nucleotide database. Incomplete reference databases are a major problem when working with the ITS marker and fungal sequences. No match to a known sequence in a reference data base may indicate recovery of a known organism that has not been sequenced before, or may actually represent novel lineages (Richards et al. 2012). Reference databases are mostly compiled of already known and cultivable fungi or partly from “uncultured fungi” recovered from environmental sequencing efforts (Köljalg et al. 2013). Relying on databases primarily composed of cultivable fungi to determine marine diversity is difficult since many marine fungi are not cultivable, and do not sporulate well on their substrate or in culture (Jones & Hyde 2002). The data presented here, based on consensus between the top 5 BLAST hits, assigns sequences to the major orders and classes with no species level taxonomy, which reflects the difficulties of classifying marine fungi at species level due to the already highlighted problems above.

The order Mortierellales is one of the most prevalent groups detected (Fig. 3). Mortierellales has been placed under the phylum Zygomycota (White et al. 2006) and have seven accepted genera, some of which are known to be marine (<http://www.gbif.org/species/115556757>). While Bass et al. (2007) did not recover any Zygomycota in the deep sea; they are frequently recovered here in an arctic fjord. While Bass et al. (2007) argues that the deep sea might not be the best habitat for the Zygomycota to thrive, the fjord system may be a better location for the Zygomycota due to lower pressure (depth), less salinity because of fresh water and nutrients added to the fjord by the rivers. On the other hand, both Bass and co-workers and myself found yeasts and chytrids. Yeasts are known to be drivers in the marine environment and have been given their own focus by several marine mycologists (Jones & Pang 2012; Kohlmeyer & Kohlmeyer 1979). The unicellular yeast growth form is thought to give the group a benefit in the marine environment compared to fungal hyphae (Jones & Pang 2012) since a single cell is less affected by

currents, waves and particles causing abrasion in the harsh marine environment than a multicellular hypha or even fruiting bodies. However, the sampling techniques used (filtration to the 0.45-10 µm fraction) may have been biased towards yeasts due to their small size compared to multicellular hyphal forms. Therefore more yeast may be recovered in these samples providing a false image of the true diversity. It is somewhat surprising that the marine order Microascales only was recovered in a small fraction. Rämä et al. (2014) found that the OTUs having poor BLAST matches across the ITS region were primarily dominated by Halosphaeriaceae (Microascales) and Lulworthiales. This could also explain the low abundance of these groups in the data presented, as poor BLAST matches would result in their classification at higher nodes in the tree.

Terrestrial fungi recovered in the samples may be true facultative marine fungi, but are more likely spores deposited from wind and input from the rivers “Adventelva” and “Longyearlva”. Contamination from the equipment used to sample the water and in the lab could be another explanation for the terrestrial fungi detected. Although the Nansen-bottle is known to be easily contaminated by yeasts (Kutty & Philip 2008), it is not known if the niskin-bottles used to collect the samples in this experiment have the same bias since they are only rinsed with fresh water and not sterilized before usage.

Community composition at different depths

I observed no change in community diversity or composition across the four different depths on three different sampling dates. This is most likely due to a stable, well-mixed water column (supplementary 2A, 2B). While chytrids are known to move towards carbohydrates/amino acids (Muehlstein et al. 1988) other fungi are non-motile and consequently planktonic in marine environments. They are therefore forced to move with the water masses. Although I observe no change in community composition and diversity at different depths, this study addresses a very specific habitat during a short time frame (well mixed waters during the winter). It is unlikely that similar results would be obtained in other situations. For instance, in the summer (July), there is more clear stratification of the water column, which could lead to changes in community composition and diversity (supplementary figure 2A, 2B). Since marine fungi are known to parasitize and live in close relation with animals and micro- and macro-algae, as well as to decompose sunken drift wood (Hyde et al. 1998; Cuomo et al. 1985; Jones 2011b; Zuccaro et al. 2008; Kohlmeyer & Kohlmeyer 1979; Barghoorn & Linder 1944; Raghukumar et al. 1992; Mouton et al. 2012; Kagami et al. 2007),

it is easy to suspect the community composition would be more diverse if the benthos was compared to the pelagic.

Community composition across dates

A drop in OTU richness is observed in the samples from April, May, and July. In April and May this may be an artefact due to insufficient sequencing depth, while the rapid decrease in number of OTUs and estimated diversity in July may reflect a true trend and change in the community, since the sequencing depth is well saturated for July. Due to melt water arriving into the fjords from the rivers in July, the water column becomes layered with higher temperatures from around 40 meters and up (S. Fig. 2A), and less saline water around 20 meters and above (S. Fig. 2B). This may be one of the reasons why there is an observed lower abundance in July. The physical barrier between different water layers, created by density differences, tends to work as a lid that seals off the layers and prevents interactions between them (Cottier et al. 2010). These water layers may result in only a small fraction of the total community being detectable when samples are taken only at a single depth. It is reasonable to think that different water layers could affect the fungal community, since this is already known to affect the composition of other planktonic species (Sverdrup 1953; Hamilton et al. 2008).

In addition to low diversity in July, a number of abundant OTUs show a reduction in proportional abundance from April to July. However, it is not clear whether this reflects an actual population crash or simply changes in the abundance of other organisms. The reduced diversity might be due to a negative effect of the spring bloom resulting in high fungal mortality. Reduced diversity might also be due to reduced fungal signal, if organisms involved in the spring bloom are being preferentially amplified, i.e. reduced signal from the fungal component of the community due to a higher abundance of other organisms that can be amplified by the ITS1F/ITS2 primers. Since the marker successfully amplified organisms other than fungi, the spring bloom could possibly be responsible for the low sequencing effort seen in April in May. However, since the decline in the Shannon Wiener index for 26th of April and 10th of May could be due to low sampling effort and low sequencing depth, it is difficult to say anything reliable on community structure on these two dates.

A number of OTUs are present in relatively low proportional abundance throughout the year until July, when a drastic increase in abundance is observed. A crash in the fungal community would explain why some OTUs have a spike in their proportional abundances in July. If these OTUs are unaffected or less affected by a crash, they would suddenly account

for a larger proportion of the community, although there may be no change in their actual abundance. Another explanation to the sudden abundance of some OTUs in July may be the increased temperature in July and influx of nutrients and organic matter from melt water deposits from the two rivers. This may induce or intensify growth and reproduction, thereby allowing these OTUs to flourish.

The drastic decrease in proportional abundance of OTUs from 26th of April observed as pattern 2 (see results), could also be explained by a crash in the fungal community. OTUs that decrease after April and remain low throughout July may be more severely affected by a crash. However, it is not clear whether this is due to a population crash, or is simply an artefact of insufficient sequencing depth. The fact that decreased abundance persists into July, where sequencing depth was adequate suggests that a true crash occurs. Additional analysis of more samples with improved sequencing depth is needed in order to determine more precisely when and why these shifts in proportional abundance of specific OTUs occur.

Changes in community composition

The fungal community composition was primarily structured by Julian date, with the samples from April, May and July segregating away from a core group of winter samples. The fact that July is sampled to saturation and has fewer OTUs than the other samples suggests that the observed change is actually a true change in community composition and not a methodological artefact. Since the fungal communities from the April 26th and May 10th samples are very under-sequenced, they may differ from the other communities simply due to random chance and insufficient sampling effort. Nevertheless, there is a possibility that a population crash occurs in April and the community does not recover before July. Changes in temperature and salinity between the December and the January dates structures these factors as significant, but their significance just reflects the fact that the sampling was done on different days when there were small changes in the temperature and salinity, which did not change in the water column (S. Fig. 2A, 2B). Both the phytoplankton (Degerlund & Eilertsen 2010) and heterotrophic community such as dinoflagellates (Levinsen & Nielsen 2002) and nanoplankton (Wassmann et al. 2006) have a seasonal pattern in the Arctic regions. The study conducted by Thomson (2014, un published) from ISA on alveolates does show a decrease in abundance concurrent with the spring bloom as seen on the fungal data in my study.

Future perspectives

Improved methods for molecular capture of the fungal community would need to be combined with well-designed sampling methods that aim to detect marine fungi in order to provide better characterization of marine fungal communities. Sampling biases must be considered in experimental design. This study was initially based on sampling for other organismal groups before being screened for fungi: the pelagic samples were pre-filtered at 10 μm and filtered at 0.45 μm probably excluding larger spores and hypha that could have been detected. Additionally, all of the studies presented here could have benefitted from additional sampling. The lack of stratification of the fungal community by depth was observed in a well-mixed water column and the community may look completely different in a water column consisting of several layers. Similarly, observing the fungal community over a longer time line across a whole year and at several locations would be preferable. This could allow generalizations about seasonal fluctuations in marine fungal communities. Additional analyses of more samples with improved sequencing depth are needed to draw firmer conclusions.

4. Fungi in marine sediments detected by metagenomics pyrosequencing

4.1 Materials and methods



Figure 1. Sample location for the metagenomic samples www.google.com/maps.

Datasets

The metagenomic datasets were obtained through the project “High throughput sequencing of deep sea metagenomes”. Collection and preparation of the samples, generation of the metagenomic datasets, and quality control of the data were conducted by Håvelsrud et al. and are described in the following papers; Håvelsrud et al. 2011; Håvelsrud et al. 2012; Håvelsrud et al. nd. Sediment samples were collected from areas with hydrocarbon influx: The Troll oil field, the California coal oil seep and the Håkon Mosby mud volcano (HMMV). In addition two samples was collected as controls in the Oslofjord in an area with no known influx of hydrocarbons. In total 10 metagenomic datasets were generated (Table 1). Two sediment cores were taken at the California coal oil seep location, one from 0 – 4 cm sub seafloor and a second at 10 – 15 cm sub seafloor while the rest of the samples represent one core obtained from between 5 – 20 cm sub seafloor. DNA was isolated from the sediment cores and shotgun sequencing was performed using 454 pyrosequencing. With the exception of the HMMV sample that was sequences using the 454 GS system, which provides shorter read length, all samples were sequenced with 454 GS-FLX and typically had read lengths over 400 bp. The detailed descriptions of sample sequencing can be found in the Håvelsrud

papers cited above. General statistics, including GC content and length, were obtained for each dataset using the Emboss program Infseq (Rice et al. 2000).

Taxonomic classification

The ten metagenomic datasets were compared to the NCBI non-redundant Protein Database using BLASTx v.2.2.25 with the standard settings and the cut off e-value: $1.0E^{-03}$ at Bioportal (www.biportal.uio.no). The retrieved BLAST output files were analysed in MEtaGenome ANalyzer (MEGAN) v. 4.64.2 (Huson et al. 2007). The software provides visual representations of the taxa detected based on the BLAST comparison of the 454 shotgun sequences to a reference database. The default NCBI taxonomy provided with MEGAN was used as a backbone tree for assigning the 100 top reads from the BLAST output with these settings for the Lowest Common Ancestor (LCA) algorithm: Min Score: 35, Top Percent: 10.0, Min Support: 5 and Max expected 0.01. A detailed description of the LCA algorithm and settings in MEGAN can be found in section 2.1

Two subsets were extracted from the reads from all 10 datasets to reduce the size of the original metagenomic datasets; (1) “eukaryotic only” and (2) “fungal only”. These two subsets were then uploaded and run separately on Lifeportal using BLASTx with the same settings as above and imported into MEGAN based on the top 10 hits from blast with the same parameters as for the entire dataset above. In (1) Reads that did not assign to eukaryotic kingdoms or to undetermined levels of the eukaryotic taxonomy were excluded from further investigation. The entire “California 04” sample was removed from Fig. 2 because no sequences classified to fungi at levels lower than kingdom (Table 1). However due to several hits to fungi when only the fungal subset was re-blasted, California 04 is retained in Fig. 3 and Table 2. In (2) reads that did not assign to fungal kingdoms or to undetermined levels of the fungal taxonomy were excluded from further investigation. The entire “Troll 6” sample was removed from the subset due to few reads assigned and low resolution. Presented in the results is a chart of all reads that were identified eukaryotic based on the subset “eukaryotic only” (Fig. 2) and a taxonomic tree for fungal hits (Fig. 3) together with a table providing the overview of fungal orders in each sample (Table 2) based on the “fungal only” subset.

4.2 Results

Data characteristics

The total dataset included 6,871,458 reads, out of which 95,056 were extracted to the “eukaryotic only” and 5,930 were extracted to the “fungal only” subsets using MEGAN. The average read length was between 220 and 416 (Table 1) and the GC content was between 43 – 53 % in the main dataset.

Table 1. Sample and sequence information for sediments samples from the Eukaryotic subset. *HMMV was sequenced on a 454 GS system (resulting in shorter read lengths) while all the other samples was sequenced on a 454 GS-FLX. California samples were collected in a known seep area and therefore marked with a “+” for Oil components.

Sample Name	Position: Latitude (N)	Longitude (E)	Description	Oil components	Water Depth (m)	Number of Sequences	Number of fungal hits	Number of Eukaryotic hits	% of sequences that were fungal	Average Length	GC Content
1 Oslo	59.594333	10.633267	Oslo fjord	Low	212	914076	927	5739	16.15	373.69	48.94
2 Oslo	59.6238	10.626483	Oslo fjord	Low	200	918989	902	5753	15.68	377.9	47.45
1 Troll	60.63132	3.789782	Troll oil field	High	304.7	850039	833	6894	12.08	348.82	53.89
2 Troll	60.63132	3.789782	Troll oil field	High	315	663131	597	3935	15.17	360.6	49.9
4 Troll	60.63132	3.789782	Troll oil field	High	315	1227131	1114	11236	9.91	345.98	50.65
6 Troll	60.63132	3.789782	Troll oil field	High	311	607557	626	4163	15.04	343	49.32
7 Troll	60.63132	3.789782	Troll oil field	High	310.5	898796	693	4443	15.6	336.49	49.76
California 04	34.411944	-119.878056	California coal oil seep	+	-	278665	0	56	0	406.72	44.68
California 1015	34.411944	-119.878056	California coal oil seep	+	-	203854	102	650	15.69	413.98	44.99
HMMV	72.003	14.4350	Håkon Mosby mud vulcano	High	1317	309219	89	544	16.36	* 220.69	43.96

Taxonomy

Metazoa (animals) was the most frequently recovered kingdom (Fig. 2). Fungi accounted for on average 13.7% of the eukaryotic reads across samples from the eukaryotic subset, ranging from 0% in “California 04” to 16.3 % in the “7 Troll” sample (Table 1, Fig. 2). The number of fungal reads per sample ranged from 0, at the lowest in “California 04”, till 1,114 in “4 Troll” (Table 1). At a class level the taxonomic profiles within the fungal

kingdom were highly similar across samples (Fig. 3). Dominant classes found were: Sordariomycetes, Saccharomycetes, Eurotiomycetes, Dothideomycetes and Agaricomycetes. Dominant orders included the true yeasts (Saccaromycetales), Eurotiales, Sordariales, Hypocreales, Onygenales and Agaricales (Table 2). A number of basidiomycete groups were also recovered, including groups known to have dimorphic life cycles (producing a yeast phase, i.e. Ustilaginomycetes, Tremellales, and Pucciniomycetes) as well as filamentous fungi i.e Agaricales.

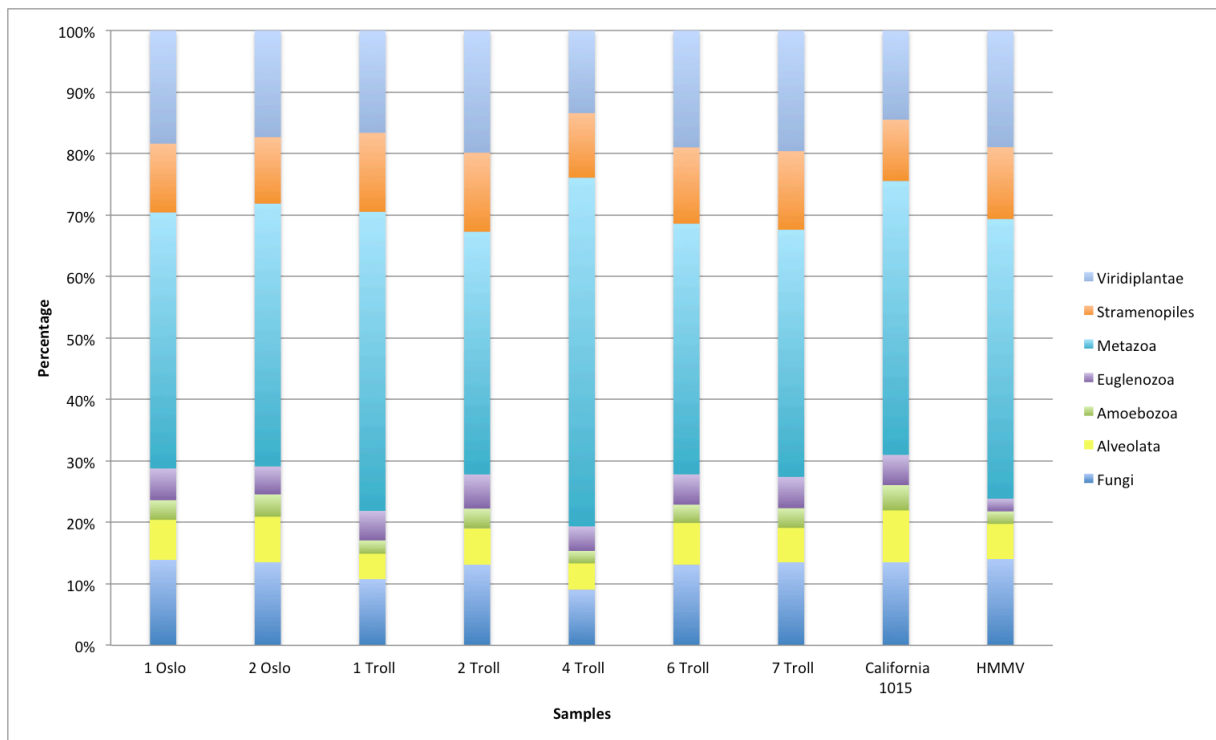


Figure 2. Proportional distribution of Eukaryotic kingdoms across samples based on Lowest Common Ancestor analysis of the top 10 BLAST hits in MEGAN.

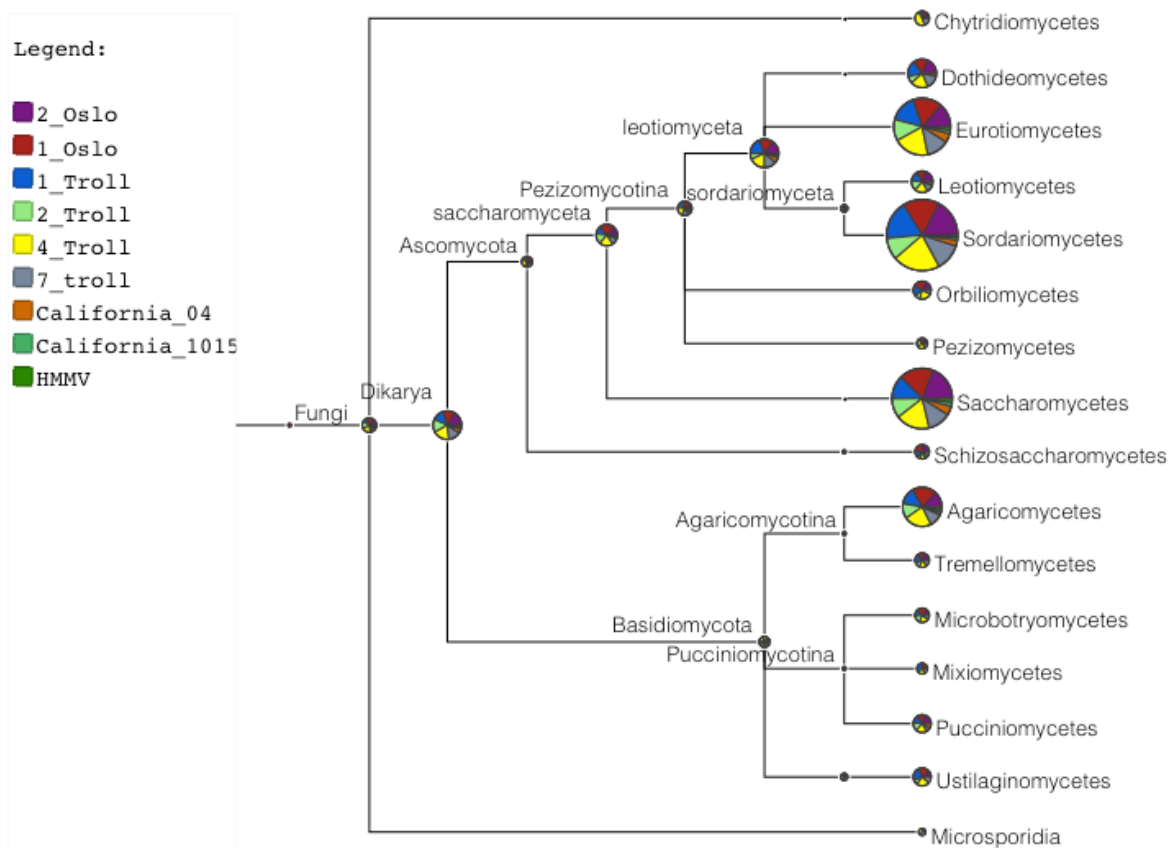


Figure 3. A schematic tree of the fungal kingdom collapsed at class level, based on GenBank taxonomy. Using MEGAN all detected fungal reads were mapped to the tree based on their top 10 BLAST match results. Each sample is represented by its own colour (legend at the top left) and number of hits to a specific node found in each sample is represented by the size of the wedge in the pie chart.

Table 2. Percentage of reads assigned to fungal orders based on Lowest Common Ancestor analysis of the top 10 BLAST hits in MEGAN from the fungal subset.

#Datasets	1 Oslo	2 Oslo	1 Troll	2 Troll	4 Troll	7 troll	California 04	California 1015	HMMV	Average
Agaricales	5.70	4.07	4.68	6.82	5.79	4.13	0.00	6.32	0.00	4.17
Boletales	0.89	0.70	1.23	0.87	0.93	0.92	0.00	0.00	0.00	0.62
Capnodiales	0.56	0.70	0.99	0.00	0.84	1.22	0.00	0.00	0.00	0.48
Eurotiales	9.16	8.15	9.98	10.66	10.27	10.86	11.88	11.58	9.52	10.23
Glomerellales	3.35	3.73	3.94	2.80	4.11	3.82	0.00	0.00	0.00	2.42
Helotiales	1.68	2.91	2.22	3.32	2.24	1.99	0.00	0.00	8.33	2.52
Hypocreales	7.82	10.01	9.48	8.22	9.71	8.10	7.92	6.32	5.95	8.17
Magnaporthales	1.23	1.28	1.23	1.40	1.31	1.53	0.00	0.00	0.00	0.89
Mixiales	0.89	0.00	1.35	0.00	0.65	0.76	0.00	0.00	0.00	0.41
Onygenales	6.93	5.47	6.40	7.52	5.79	6.12	10.40	6.32	9.52	7.16
Ophiostomatales	0.67	0.81	1.23	0.00	0.75	1.07	0.00	0.00	0.00	0.50
Orbiliiales	2.12	1.86	1.97	1.40	1.68	0.92	0.00	0.00	0.00	1.11
Pezizales	0.78	0.58	0.00	1.22	0.75	0.76	0.00	0.00	0.00	0.46
Pleosporales	3.69	4.42	4.31	2.80	3.55	4.13	2.97	0.00	0.00	2.87
Polyporales	0.67	0.00	0.00	0.00	0.75	0.00	0.00	0.00	0.00	0.16
Pucciniales	1.23	2.10	1.48	2.27	1.59	1.07	3.47	0.00	0.00	1.47
Rhizophydiales	0.89	1.05	0.74	0.00	2.89	1.99	0.00	0.00	0.00	0.84
Saccharomycetales	18.88	20.84	14.53	16.78	15.87	17.43	21.78	21.05	22.62	18.87
Schizosaccharomycetales	1.79	1.86	1.23	0.87	0.65	0.92	0.00	6.32	0.00	1.52
Sebacinales	1.68	1.16	0.00	0.00	0.93	1.38	0.00	0.00	0.00	0.57
Sordariales	8.83	8.15	9.85	8.04	8.31	7.65	5.94	0.00	7.14	7.10
Sporidiobolales	1.56	1.05	1.11	1.92	1.21	0.92	0.00	0.00	0.00	0.86
Tremellales	1.23	1.51	1.60	1.40	1.03	1.38	0.00	0.00	0.00	0.91
Ustilaginales	2.12	1.16	2.83	1.40	1.96	1.68	0.00	0.00	0.00	1.24

4.3 Discussion

Fungal DNA sequences were found in the analysed sediments across all sample areas and comprised, on average, 13.7 % of the eukaryotic community. In another study where fungi were detected in sediments across a variety of locations, the fungal part of the eukaryotic fraction was at a similar level; 10% (Quaiser et al. 2010). On the contrary, in a study by Edgcomb et al. (2011), a much higher percentage of fungi appeared in the sediments (2-100%, average 67%). It is not clear to what degree the differences in abundances across studies represent a true difference or are due to methodological biases, including sampling bias. Primer bias towards fungi as seen by Edgcomb et al. (2011) may also be a major part of the differences observed between this study and amplicon based studies. Moreover, because the fungi recovered in this study are from shotgun sequencing of environmental DNA, there is no way to prove if they are active, dormant or dead fungi. In several studies, fungi have been

shown to be an important part of the organismal community on the sea floor and some species may be important in utilization and recycling of nutrients (Thaler et al. 2012; Orsi et al. 2013; Edgcomb et al. 2011).

Taxonomy

Both active and dormant obligate and facultative marine fungi are likely present in our samples, in addition to fungi that are a part of marine snow, where fungal spores and hyphae of both active and dormant species sink to the seafloor and are preserved. This marine fungal snow can also include leaves and wood that have blown out to sea and sunk (Damare et al. 2006; Raghukumar et al. 2004)

Saccharomycetes, the true yeasts, was one of the predominant taxonomic groups recovered from the samples (19% of the reads), together with a number of groups known to include dimorphic stages, i.e. possessing a yeast stage in their lifecycle in addition to a filamentous hyphal form. Dimorphic groups detected here include Capnodiales, Onygenales, Sporidiobolales, Tremellales, and Ustilaginales. Yeast clades have been found to be both successful and dominant in the deep seas (Bass et al. 2007). Most likely they are abundant in sediments due to their capability of utilizing fermentation. Fermentation provides a major advantage in oxygen deprived environments such as sea floor sediments (Thaler et al. 2012); allowing the fungi to perform metabolic activity without oxygen, which is one of the main processes of re-mineralization. These findings indicate that fungi could have a very important role in the sea floor community (Orcutt et al. 2011). Since yeasts are known to be active in the sediments and are the major group recovered in the present survey, they likely represent an active community of fungi, rather than dead cells that have sedimented out of the water column.

Considering that Agaricales are mostly known to be terrestrial fungi, it is surprising that a relatively large proportion of the sequences were affiliated to this group (4%). There may be three alternative explanations for this; (1) they represent sedimented spores or hyphae from marine snow, (2) methodological biases, or (3) that some lineages within Agaricales are adapted to marine conditions. Notably, there is a distinctly higher abundance of Agaricales in the Oslofjord and Troll samples than in the California and Håkon Mosby samples. The narrow Oslofjord may have a higher influx of aerial spread material from terrestrial sources, while the Troll oil platform in theory could be influenced by human activities and ballast water from tankers involved in oil drilling. This may enhance the spore deposit of Agaricales in these sediments. Alternatively, methodological biases may account for the high abundance of

Agaricales. Relatively few fungi have been genome-sequenced and are available in reference databases (Richards et al. 2012), and of these, many represent ecologically important terrestrial agaricalean fungi. Notably, most of the species level matches to members of the Agaricales are to species with their genomes sequenced (data not shown). Hence, the sparse taxonomic coverage in the reference databases may be resulting in ‘false matches’ to Agaricales. When more genomes are added to databases one might see a shift in the taxonomic profiles in metagenomics datasets like the ones analysed here. Another possibility may be that marine fungi in fact are present within Agaricales. The species *Mycaureola dilseae* was shown to parasitize red algae (Binder et al. 2006) and Gao et al. (2008) identified agaricalean fungi in marine sponges, suggesting that this group may include other poorly known marine members.

High proportions of Eurotiales (10.23%), Hypocreales (8.17%) and Sordariales (7.10%), which are known to have marine members, were recovered. Surprisingly, none of the well-known, obligate marine fungal orders, Lulworthiales and Microascales, were recovered from any of the samples. However, these fungi are mostly known from wood, algae and seaweed in more shallow waters and simply may not be present in sediments (Kohlmeyer et al. 2000; Campbell et al. 2005). Most likely, the Troll field and Håkon Mosby may be too far out for the spores to be found, but the Oslo fjord and the California sample should expectably include these fungal orders. However, the obligate marine fungi in Lulworthiales and Microascales often have spores that are adapted to stay afloat until they reach suitable substrates (Rees 1980). Due to their floating ability it may be that they to a little extent sediment, especially farther out at sea. Also, they often produce mucilage to help them attach to substrate they come in contact with (Rees 1980), which might mean they are very efficient in attaching to a substrate and do not end up as part of the marine snow.

Compositional differences between samples

There were no distinct compositional differences in higher order taxonomy between the samples, despite their origins from sediments in different parts of the world. Likewise, a similar proportion of fungal reads among the eukaryotic fraction was recovered across samples. This may indicate that fungi in general make up a certain proportion of the eukaryotic communities in anoxic sediments. The Oslofjord samples were chosen to be controls since they have no hydrocarbon influence, and surprisingly this does not affect the compositions of fungal groups recovered. The consistency of the community compositions across the samples may also be a result of methodological bias or insufficiency. The datasets

were generated using a bacteria-targeted protocol and very few Eukaryotic reads were recovered (Håvelsrud et al. 2011; Håvelsrud et al. n.d.; Håvelsrud et al. 2012). Alternatively, the databases available are poor on fungal taxa and may result in huge bias by assigning the name from one known reference sequence to all those that are phylogenetically near, which could explain the lack of a geographic pattern we observe here.

Future perspectives

For further research on fungi residing in sea floor sediments, a more targeted approach using specific barcode regions such as rDNA ITS would possibly provide better taxonomic resolution and to a higher extent detect taxonomic differences between the communities. However, amplicon based approaches are known to cause more extensive compositional biases, preferentially amplifying the more abundant community members (Logares et al. 2013). If a metagenomic approach is to be used, far deeper sequencing would be required, enabled by e.g. Illumina HiSeq analyses (Logares et al. 2013). Moreover, by conducting a deeper sequencing, specific regions such as rDNA may be mined from a metagenomic dataset and used further to obtain a better taxonomic resolution (Logares et al. 2013).

5. Conclusion

A diverse group of fungi was recovered from marine sediments and the pelagic. Sediments across a worldwide sampling area were dominated by yeast, but no differences were found in these samples connected to geography. Zygomycota, a group known to contain marine members, dominated the pelagic in the arctic fjord system. In addition, the known terrestrial group Agaricomycetes was found in both studies. It is not known if these fungi actually play an active role or if they are deposited in to the system from the terrestrial environment. The only factor identified structuring fungal communities was seasonal and there was no structure by depth or hydrocarbon influences. Hence, it would seem that the major structuring factors influencing the fungal communities are most likely related to seasonal fluctuations in factors like temperature, nutrition and salinity. However further studies with proper sampling design and replications are needed to determine what factors actually structure these communities. In both studies there were large numbers of reads that could not be assigned to genus, order or even class level taxonomy and sparse taxonomic coverage in the reference databases proved to be one of the major obstacles to determining the fungal diversity. In addition to improved reference material, further research needs to incorporate better primer choice or design since the assumed fungal specific primer ITS1F targeted more than just the fungal community and were found less suitable for marker based studies in marine environments than terrestrial environments

Our understanding of the marine fungal community is still, after all these years, in its beginner phase. Fungi are present and thriving in all marine habitats and my thesis provides a tantalizing glimpse of the fungal community present in sediments across global areas and in an arctic pelagic fjord system. We know that fungi play a necessary and important role in terrestrial habitats and more research on the fungal community of the seas and oceans is needed to determine the role of fungi there. The continual development of new molecular and bioinformatics tools provides huge opportunities for further research into this poorly studied organism group. Now is the time for increased research on the role fungi play in the marine realm!

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7. References

- Alker, A.P., et al., 2001. Characterization of *Aspergillus sydowii* (Thom et Church), a fungal pathogen of Caribbean sea fan corals. *Hydrobiologia*, 460(1-3), pp.105–111.
- Altschul, S.F. et al., 1990. Basic local alignment search tool. *Journal of molecular biology*, 215(3), pp.403–410.
- Alva, P., et al. 2002. Do sea grasses harbour endophytes. *Fungal Divers Res Series*, 7, 167-178.
- Anderson, I.C. & Cairney, J.W.G., 2004. Diversity and ecology of soil fungal communities: increased understanding through the application of molecular techniques. *Environmental Microbiology*, 6(8), pp.769–779.
- Bahnweg, G. & Sparrow, F.K., 1974. Four new species of *Thraustochytrium* from Antarctic regions, with notes on the distribution of zoosporic fungi in the Antarctic marine ecosystems. *American Journal of Botany*, 754-766.

- Barghoorn, E.S. & Linder, D.H., 1944. Marine fungi: their taxonomy and biology. *Farlowia*, 1, 395-467.
- Bartram, A.K. et al., 2011. Generation of multimillion-Sequence 16S rRNA gene libraries from complex microbial communities by assembling paired-end illumina reads. *Applied and Environmental Microbiology*, 77(11), pp.3846–3852.
- Bass, D. et al., 2007. Yeast forms dominate fungal diversity in the deep oceans. *Proceedings of the Royal Society B: Biological Sciences*, 274(1629), pp.3069–3077.
- Bellemain, E. et al., 2010. ITS as an environmental DNA barcode for fungi: an *in silico* approach reveals potential PCR biases. *BMC Microbiology*, 10(1), p.189.
- Binder, M.M. et al., 2006. Evolutionary relationships of *Mycaureola dilseae* (Agaricales), a basidiomycete pathogen of a subtidal rhodophyte. *American Journal of Botany*, 93(4), pp.547–556.
- Blaalid, R. et al., 2011. Changes in the root-associated fungal communities along a primary succession gradient analysed by 454 pyrosequencing. *Molecular Ecology*, 21(8), pp.1897–1908.
- Bridge, P. & Spooner, B., 2001. Soil fungi: diversity and detection. *Plant and Soil*, 232(1-2), pp.147–154.
- Buchan, A. et al., 2002. Analysis of internal transcribed spacer (ITS) regions of rRNA genes in fungal communities in a southeastern U.S. salt marsh. *Microbial Ecology*, 43(3), pp.329–340.
- Bugni, T.S.T. & Ireland, C.M.C., 2004. Marine-derived fungi: a chemically and biologically diverse group of microorganisms. *Natural Product Reports*, 21(1), pp.143–163.
- Burgaud, G. et al., 2009. Diversity of culturable marine filamentous fungi from deep-sea hydrothermal vents. *Environmental Microbiology*, 11(6), pp.1588–1600.
- Campbell, J. et al., 2005. A re-evaluation of Lulworthiales: relationships based on 18S and 28S rDNA. *Mycological Research*, 109(5), pp.556–568.
- Caporaso, J.G. et al., 2010. QIIME allows analysis of high-throughput community sequencing data. *Nature Publishing Group*, 7(5), pp.335–336.
- Cottier, F.R. et al., 2010. Arctic fjords: a review of the oceanographic environment and dominant physical processes. *Geological Society, London, Special Publications*, 344(1), pp.35–50.
- Cotton., 1909. *Notes on marine Pyrenomycetes*. Transactions of the British Mycological Society 3: 92-99.
- Cuomo, V., et al., 1985. Fungal flora of *Posidonia oceanica* and its ecological significance. *Transactions of the British Mycological Society* 84.1: 35-40.

- Damare, S., et al., 2006. Fungi in deep-sea sediments of the Central Indian Basin. *Deep Sea Research Part I: Oceanographic Research Papers*, 53(1), pp.14–27.
- Davey, M.L. et al., 2013. Host- and tissue-specificity of moss-associated *Galerina* and *Mycena* determined from amplicon pyrosequencing data. *Fungal Ecology*, 6(3), pp.179–186.
- Degerlund, M. & Eilertsen, H.C., 2010. Main species characteristics of phytoplankton spring blooms in NE Atlantic and Arctic waters (68–80° N). *Estuaries and coasts*, 33(2), pp.242–269.
- Dobrzyn, P., et al., 2005. Sedimentation of chlorophylls in an Arctic fjord under freshwater discharge. *Hydrobiologia*, 532, pp.1–8.
- Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. *Journal of Gerontology*, 26(19), pp.2460–2461.
- Edgar, R.C. et al., 2011. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics*, 27(16), pp.2194–2200.
- Edgcomb, V.P. et al., 2011. Marine subsurface eukaryotes: the fungal majority. *Environmental Microbiology*, 13(1), pp.172–183.
- Fell, J.W. et al., 2010. Five new species of yeasts from fresh water and marine habitats in the Florida Everglades. *Antonie van Leeuwenhoek*, 99(3), pp.533–549.
- Gadd, Geoffrey. 2004. *Fungi in the environment*. Richmond: the British Mycological Society
- Gao, Z. et al., 2008. Molecular detection of fungal communities in the Hawaiian marine sponges *Suberites zeteki* and *Mycale armata*. *Applied and Environmental Microbiology*, 74(19), pp.6091–6101.
- Gardes, M. & Bruns, T.D., 1993. ITS primers with enhanced specificity for basidiomycetes-- application to the identification of mycorrhizae and rusts. *Molecular Ecology*, 2(2), pp.113–118.
- Glenn, T.C., 2011. Field guide to next-generation DNA sequencers. *Molecular Ecology Resources*, 11(5), pp.759–769.
- Hamilton, A.K. et al., 2008. Water masses and biogeography of picoeukaryote assemblages in a cold hydrographically complex system. *Limnology and Oceanography*, 53(3), pp.922–935.
- Hawksworth, D.L., 1991. The fungal dimension of biodiversity: magnitude, significance, and conservation. *Mycological Research*, 95(6), pp.641–655.
- Hawksworth, D.L., 2001. The magnitude of fungal diversity: the 1.5 million species estimate revisited. *Mycological Research*, 105(12), pp.1422–1432.
- Håvelsrud, O.E. et al., 2011. A metagenomic study of methanotrophic microorganisms in Coal Oil Point seep sediments. *BMC Microbiology*, 11(1), p.221.

- Håvelsrud, O.E. et al., Annotation of taxonomically binned sequences to understand metabolic potential in a highly diverse metagenome. *Unpublished*, pp.1–38.
- Håvelsrud, O.E. et al., 2012. Metagenomic and geochemical characterization of pockmarked sediments overlaying the Troll petroleum reservoir in the North Sea. *BMC Microbiology*, 12(1), p.203. Available at:
- Heidelberg, K.B., et al., 2010. Marine genomics: at the interface of marine microbial ecology and biodiscovery. *Microbial Biotechnology*, 3(5), pp.531–543.
- Horton, T.R. & Bruns, T.D., 2001. The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. *Molecular Ecology*, 10(8), pp.1855–1871.
- Huson, D.H.D. et al., 2007. MEGAN analysis of metagenomic data. *Genes & Development*, 17(3), pp.377–386.
- Hyde, Kevin D. 2002. *Fungi in marine environments*. Hong Kong: Fungal Diversity Press.
- Hyde, Kevin D., Stephen B. Pointing. 2000. *Marine mycology: a practical approach*. Hong Kong: Fungal Diversity Press.
- Hyde, Kevin D. et al., 1998. Role of fungi in marine ecosystems. *Biodiversity and Conservation*, 7(9), pp.1147–1161.
- Iverson, V. et al., 2012. Untangling genomes from metagenomes: revealing an uncultured class of marine Euryarchaeota. *Science*, 335(6068), 587-590.
- Jobard, M., et al., 2010. Diversity and functions of microscopic fungi: a missing component in pelagic food webs. *Aquatic Sciences*, 72(3), pp.255–268.
- Jones, E. & Hyde, K.D., 2002. Succession: where do we go from here? *Fungal Diversity*, 10, pp.241–253.
- Jones, E.B.G., 2011a. Are there more marine fungi to be described? *Botanica Marina*, 54(4).
- Jones, E.B.G., 2011b. Fifty years of marine mycology. *Fungal Diversity*, 50(1), pp.73–112.
- Jones, E.B.G. & Pang, K.-L., 2012. *Marine Fungi*, Walter de Gruyter.
- Jones, M.D.M. et al., 2011. Discovery of novel intermediate forms redefines the fungal tree of life. *Nature*, 474(7350), pp.200–203.
- Kagami, M. et al., 2007. Parasitic chytrids: their effects on phytoplankton communities and food-web dynamics. *Hydrobiologia*, 578(1), pp.113–129.
- Kane, Douglas L., and Daqing Yang. 2004. *Northern research basins water balance*. Wallingford, Oxfordshire, UK: International Association of Hydrological Science.
- Kendrick, B., 2000. *The Fifth Kingdom* Third, Focus Pub R Pullins & Company.
- Kis-Papo, T., 2005. Marine fungal communities. *Mycologist series*, 23, p.61.

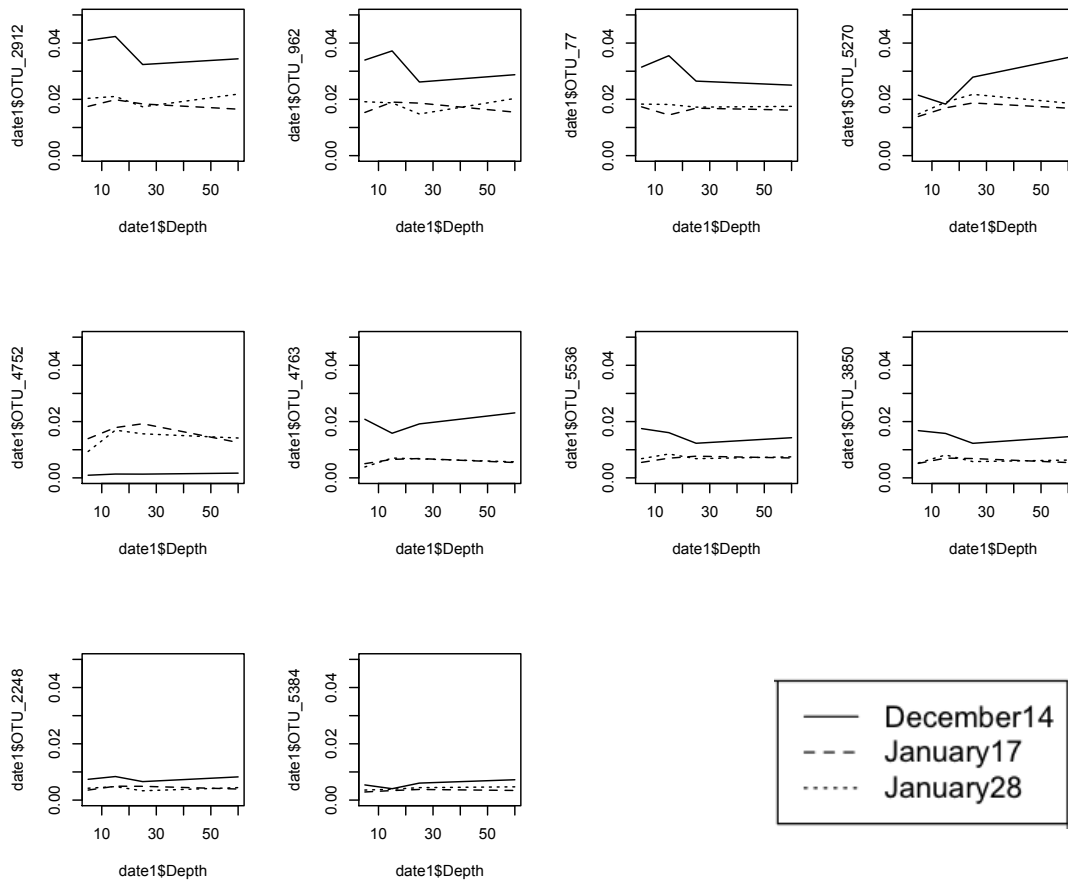
- Kohlmeyer, Jan, and Erika Kohlmeyer. 1979. *Marine mycology: the higher fungi*. New York: Academic Press.
- Kohlmeyer, J., & Volkmann-Kohlmeyer, B. 1991. Illustrated key to the filamentous higher marine fungi. *Botanica Marina*, 34(1), 1-61.
- Kohlmeyer, J., Spatafora, J.W. & Volkmann-Kohlmeyer, B., 2000. Lulworthiales, a new order of marine Ascomycota. *Mycologia*, 92(3), pp.453–458.
- Kowalchuk, G., 1999. New perspectives towards analysing fungal communities in terrestrial environments. *Current Opinion in Biotechnology*, 10(3), pp.247–251.
- Kõljalg, U. et al., 2013. Towards a unified paradigm for sequence-based identification of fungi. *Molecular Ecology*, 22(21), pp.5271–5277.
- Kutty, S.N. & Philip, R., 2008. Marine yeasts—a review. *Yeast*, 25(7), pp.465–483.
- Lai, X. et al., 2007. Fungal communities from methane hydrate-bearing deep-sea marine sediments in South China Sea. *The ISME Journal*, 1(8), pp.756–762.
- Lanzén, A. et al., 2012. CREST – Classification resources for environmental sequence tags L. Orlando, ed. *PLoS ONE*, 7(11), p.e49334.
- Lawley, B. et al., 2004. Molecular analysis of geographic patterns of eukaryotic diversity in Antarctic Soils. *Applied and Environmental Microbiology*, 70(10), pp.5963–5972.
- Le Calvez, T. et al., 2009. Fungal diversity in deep-sea hydrothermal ecosystems. *Applied and Environmental Microbiology*, 75(20), pp.6415–6421.
- Levinsen, H. & Nielsen, T.G., 2002. The trophic role of marine pelagic ciliates and heterotrophic dinoflagellates in arctic and temperate coastal ecosystems: A cross-latitude comparison. *Limnology and Oceanography*, 47(2), pp.427–439.
- Logares, R. et al., 2012. Environmental microbiology through the lens of high-throughput DNA sequencing: Synopsis of current platforms and bioinformatics approaches. *Journal of Microbiological Methods*, 91(1), pp.106–113.
- Logares, R. et al., 2013. Metagenomic 16S rDNA Illumina tags are a powerful alternative to amplicon sequencing to explore diversity and structure of microbial communities. *Environmental Microbiology*, pp.n/a–n/a.
- Loque, C.P. et al., 2009. Fungal community associated with marine macroalgae from Antarctica. *Polar Biology*, 33(5), pp.641–648.
- López-García, P. et al., 2003. Autochthonous eukaryotic diversity in hydrothermal sediment and experimental microcolonizers at the Mid-Atlantic Ridge. *Proceedings of the National Academy of Sciences*, 100(2), p.697.
- Mardis, E.R., 2008. Next-generation DNA sequencing methods. *Annual Review of Genomics and Human Genetics*, 9(1), pp.387–402.

- Metzker, M.L., 2009. Sequencing technologies — the next generation. *Nature Reviews Genetics*, 11(1), pp.31–46.
- Mouton, M. et al., 2012. Diversity and characterization of culturable fungi from marine sediment collected from St. Helena Bay, South Africa. *Microbial ecology*, 64(2), 311–319.
- Muehlstein, L.K., Amon, J.P. & Leffler, D.L., 1988. Chemotaxis in the marine fungus *Rhizophydium littoreum*. *Applied and Environmental Microbiology*, 54(7), pp.1668–1672.
- Nagahama, T.T. et al., 2003. *Rhodotorula benthica* sp. nov. and *Rhodotorula calyptogenae* sp. nov., novel yeast species from animals collected from the deep-sea floor, and *Rhodotorula lysiniphila* sp. nov., which is related phylogenetically. *International Journal of Systematic and Evolutionary Microbiology*, 53(Pt 3), pp.897–903.
- Nilsen, F. et al., 2008. Fjord– shelf exchanges controlled by ice and brine production: The interannual variation of Atlantic Water in Isfjorden, Svalbard. pp.1–16.
- Nilsson, R.H. et al., 2008. Intraspecific ITS variability in the kingdom fungi as expressed in the international sequence databases and its implications for molecular species identification. *Evolutionary Bioinformatics*, 4, pp.193–201.
- Nilsson, R.H. et al., 2006. Taxonomic reliability of DNA sequences in public sequence databases: a fungal perspective C. Fairhead, ed. *PLoS ONE*, 1(1), p.e59.
- O'Brien, H.E.H. et al., 2005. Fungal community analysis by large-scale sequencing of environmental samples. *Applied and Environmental Microbiology*, 71(9), pp.5544–5550.
- Oksanen, J., et al. 2007. The vegan package. *Community ecology package*.
- Orcutt, B.N. et al., 2011. Microbial ecology of the dark ocean above, at, and below the seafloor. *Microbiology and Molecular Biology Reviews*, 75(2), pp.361–422.
- Orellana, S.C., 2013. Assessment of fungal diversity in the environment using metagenomics: a decade in review. *Fungal Genomics & Biology*, 03(02).
- Orsi, W., et al. 2013. Deep sequencing of subseafloor eukaryotic rRNA reveals active fungi across marine subsurface provinces. *PLoS ONE*, 8(2), pp.–e56335.
- Pang, K.-L. et al., 2011. Diversity and physiology of marine lignicolous fungi in Arctic waters: a preliminary account. *Polar Research*, 30(0), p.83.
- Pang, K.-L., et al. 2008. *Havispora longyearbyenensis* gen. et sp. nov.: an arctic marine fungus from Svalbard, Norway. *Mycologia*, pp.291–295.
- Pang, K. L., et al. 2009. *Remispora spitsbergenensis* sp. nov., a marine lignicolous ascomycete from Svalbard, Norway. *Mycologia*, 101(4), pp.531–534.
- Quaiser, A. et al., 2010. Comparative metagenomics of bathypelagic plankton and bottom sediment from the Sea of Marmara. *The ISME Journal*, 5(2), pp.285–304.

- Raghukumar, C. et al., 2004. Buried in time: culturable fungi in a deep-sea sediment core from the Chagos Trench, Indian Ocean. *Deep Sea Research Part I: Oceanographic Research Papers*, 51(11), pp.1759–1768.
- Raghukumar, C., et al., 1992. Association of thraustochytrids and fungi with living marine algae. *Mycological Research*, 96(7), pp.542–546.
- Rämä, T. et al., 2014. Fungi ahoy! Diversity on marine wooden substrata in the high North. *Fungal Ecology*, 8(C), pp.46–58.
- Rees, G. 1980. Factors affecting the sedimentation rate of marine fungal spores. *Botanica marina*.
- Rice, P.P., et al., 2000. EMBOSS: the European molecular biology open software suite. *Trends in Genetics*, 16(6), pp.276–277.
- Richards, T.A. & Bass, D., 2005. Molecular screening of free-living microbial eukaryotes: diversity and distribution using a meta-analysis. *Current Opinion in Microbiology*, 8(3), pp.240–252.
- Richards, T.A. et al., 2012. Marine fungi: Their ecology and molecular diversity. *Annual Review of Marine Science*, 4(1), pp.495–522.
- Schloss, P.D. et al., 2009. Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology*, 75(23), pp.7537–7541.
- Schoch, C.L. et al., 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences*, 109(16), pp.6241–6246.
- Scholz, B.M., et al., 2012. Next generation sequencing and bioinformatic bottlenecks: the current state of metagenomic data analysis. *Current Opinion in Biotechnology*, 23(1), pp.9–15.
- Seifert, K., 2009. Progress towards DNA barcoding of fungi. *Molecular Ecology Resources*, 9(s1), 83–89.
- Singh, P. et al., 2011. Fungal community analysis in the deep-sea sediments of the Central Indian Basin by culture-independent approach. *Microbial Ecology*, 61(3), pp.507–517.
- Singh, P.P. et al., 2012. Assessment of fungal diversity in deep-sea sediments by multiple primer approach. *World Journal of Microbiology & Biotechnology*, 28(2), pp.659–667.
- Sun, S. et al., 2010. Community cyberinfrastructure for advanced microbial ecology research and analysis: the CAMERA resource. *Nucleic Acids Research*, 39(Database), pp.D546–D551.
- Sutherland, G., 1915. Additional notes on marine Pyrenomycetes. *New Phytologist*, 14(6–7), pp.183–193.

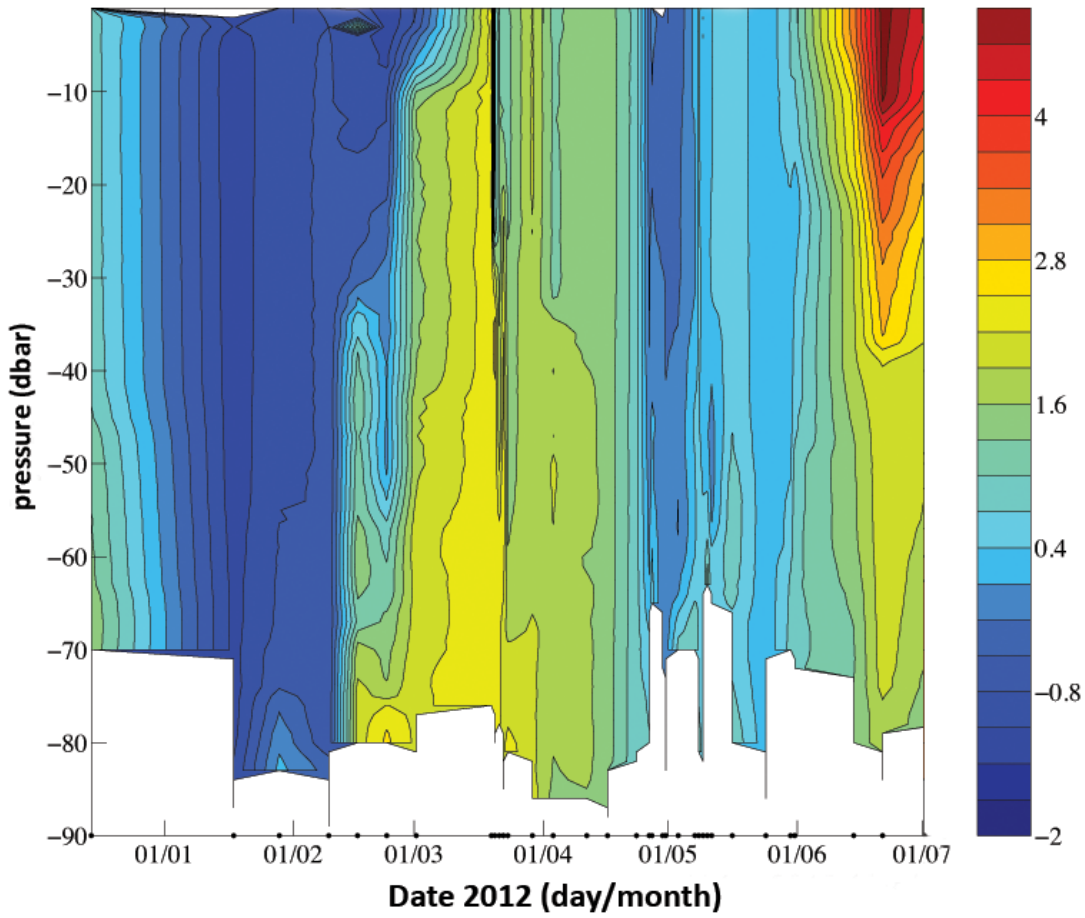
- Sverdrup, H.U., 1953. On conditions for the vernal blooming of phytoplankton. *ICES Journal of Marine Science: Journal du Conseil*, 18(3), pp.287–295.
- Sørensen, N., et al., 2012. Molecular diversity and temporal variation of picoeukaryotes in two Arctic fjords, Svalbard. *Polar Biology*, 35(4), pp.519–533.
- Takami, H. et al., 1997. Microbial flora in the deepest sea mud of the Mariana Trench. *FEMS microbiology letters*, 152(2), pp.279–285.
- Team, R.D.C., 2012. R: A language and environment for statistical computing. *R Foundation for Statistical Computing, Vienna, Austria*.
- Thaler, A.D., et al., 2012. Ascomycete phylotypes recovered from a Gulf of Mexico methane seep are identical to an uncultured deep-sea fungal clade from the Pacific. *Fungal Ecology*, 5(2), pp.4–4.
- Thomson, S., 2014 "Community structure for alveolates throughout the year". Master thesis, UiT/UNIS. Un published.
- Vilgalys, R., 2003. Taxonomic misidentification in public DNA databases. *New Phytologist*, 160(1), pp.4–5.
- Wassmann, P. et al., 2006. Food webs and carbon flux in the Barents Sea. *Progress in Oceanography* 71 (2006) 232–287, pp.1–56.
- White, M.M. et al., 2006. Phylogeny of the Zygomycota based on nuclear ribosomal sequence data. *Mycologia*, 98(6), pp.872–884.
- White, T.J. et al., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: a guide to methods and applications*, 18, pp.315–322.
- Zajaczkowski, M. et al., 2009. Vertical flux of particulate matter in an Arctic fjord: the case of lack of the sea-ice cover in Adventfjorden 2006–2007. *Polar Biology*, 33(2), pp.223–239.
- Zuccaro, A. et al., 2004. A new *Acremonium* species associated with *Fucus* spp., and its affinity with a phylogenetically distinct marine *Emericellopsis* clade. *Stud Mycol*, 50, pp.283–297.
- Zuccaro, A. et al., 2008. Detection and identification of fungi intimately associated with the brown seaweed *Fucus serratus*. *Applied and Environmental Microbiology*, 74(4), pp.931–941.

8. Supplementary

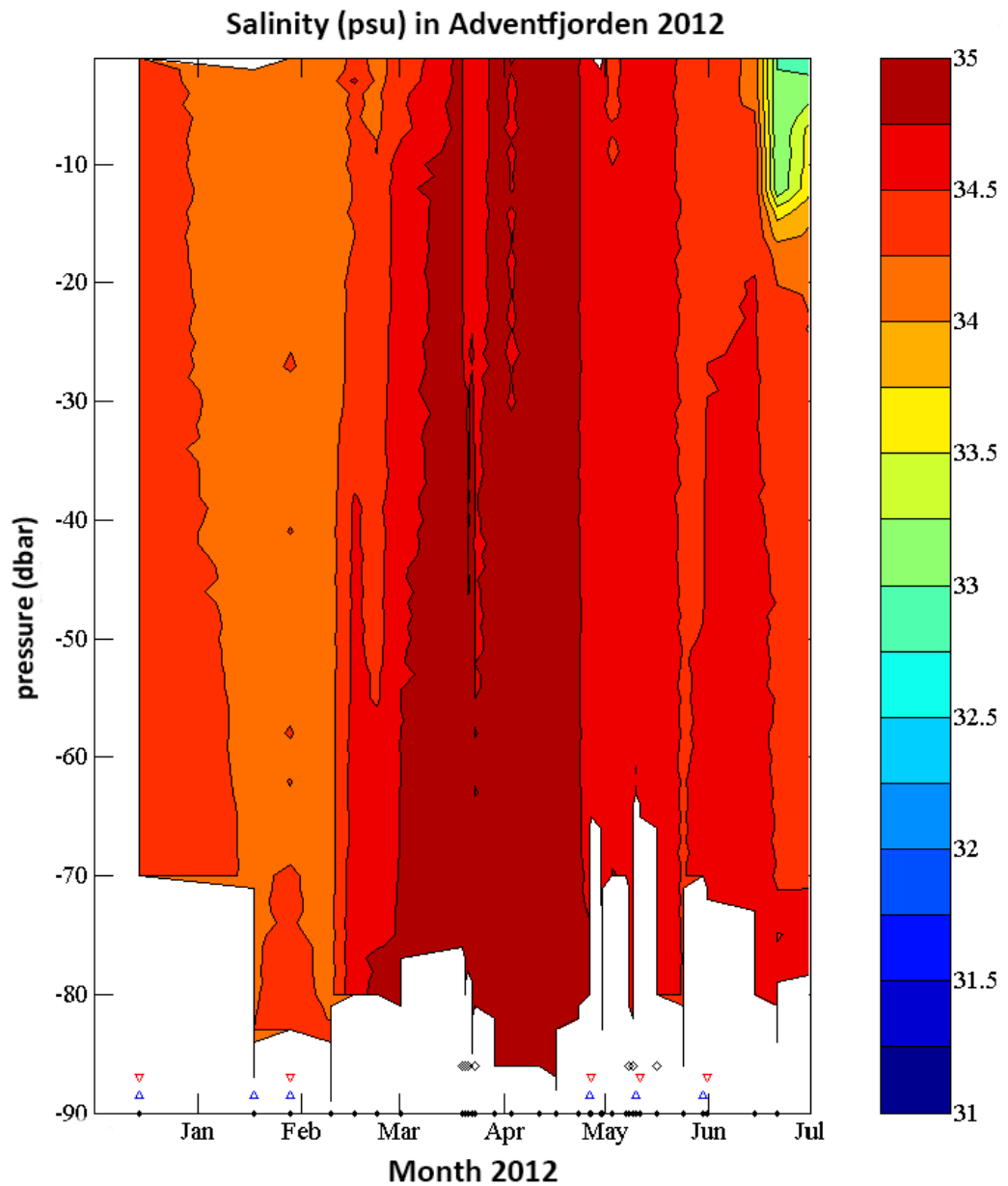


Supplementary figure 1. Proportional abundance of the most abundant fungal OTUs by depth sampled at ISA station.

Sea temperature (°C) in Adventfjorden 2012



Supplementary figure 2A. Temperature profile of ISA station across dates. Provided by Ragnheid Skogseth, department of Geophysics, UNIS. Bar at the right indicates temperature for the different colours represented in the plot while pressure at the left is approximately depth in meters.



Supplementary figure 2B. Salinity profile of ISA station across dates. Provided by Ragnheid Skogseth, department of Geophysics, UNIS. Bar at the right indicates salinity measure for the different colours represented in the plot while pressure at the left is approximately depth in meters.