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A Biogeographical Assessment of Arctic Marine Fungi

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A BIOGEOGRAPHICAL ASSESSMENT OF ARCTIC MARINE FUNGI

by

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ABSTRACT

Marine fungi play a crucial role in recycling nutrients and channeling energy to higher trophic levels in the world oceans. Despite their critical role, their distributions and community composition, particularly in the Arctic, are largely unknown. This study reveals depth-related trends of abundance, diversity, and community composition of Arctic marine fungi through analysis of data obtained in the Tara Oceans expedition. With samples from surface (0-50 m), deep chlorophyll max (50-200 m), and mesopelagic (200-1000 m) depths, relative abundance, operational taxonomic unit (OTU) richness, and diversity were found to increase as a function of depth. Basidiomycota and Ascomycota were found to to be the most dominant OTUs in Arctic water samples. This study provides potential causes for depth-related trends and yields insight into the biogeographic distribution of Arctic marine fungi.

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INTRODUCTION

The study of marine fungi is a confluence of three fields: marine science, microbiology, and mycology. Within the marine science community, the most well-known microplankton are a few varieties of phytoplankton such as diatoms, coccolithophores, dinoflagellates, and cyanobacteria and a few varieties of zooplankton such as foraminiferans, radiolarians, and copepods. Fungi, however, have been largely overlooked and are rarely discussed in classroom settings. Approximately 20 years ago, chytrids were isolated and identified as a fungal parasite in aquatic and marine environments (Longcore, 1999). Since then, this group of zoosporic fungi has gained notoriety, but the global distribution and composition of fungal communities remains largely unexplored. Whereas mycology has historically been limited to terrestrial ecosystems, marine ecosystems have begun to garner attention only recently. Thanks to developments in eDNA recovery and DNA sequencing technologies, researchers have adopted metagenomic- and metabarcoding-based approaches to resolving fungal diversity independent of isolating cultures.

One such metabarcoding study is the Tara Oceans expedition, a geographically expansive study which analyzed water samples from the Arctic Ocean. The Arctic was of interest to the present study because it is a region warming twice as fast as any other region on the planet (NOAA, 2017). Therefore, establishing a baseline of fungal biogeography to determine how it changes over time is critical. Additionally, the massive fluvial input and stratified input from other world oceans lead to an environment where mycoplankton from distinct bodies of water can mix within one ecosystem. Furthermore, the nutrient

limitations of the Arctic Ocean create an environment where the cycling of nutrients from dead phytoplankton to higher trophic levels via fungal plankton is of increased importance for providing nutrients to zooplankton. This upcycling of otherwise exported energy into higher trophic levels has been termed the "mycoloop" (Amend et al., 2019).

The mycoloop is driven by fungi having diverse functional roles. Initially, marine fungi were thought to be primarily limited to parasitizing algae, but recent studies have found them in every ocean setting ranging from sea ice to sediments (Hassett & Gradinger, 2016) and everywhere in between (Amend et al., 2019). Fungi in marine ecosystems can be either unicellular or filamentous and can feed as saprotrophs, parasites, or mutualist. Some species are generalist. Others have been found to be very specific in their selection of host cells (Grossart et al., 2016).

The primary goals of my study were to identify the most universally distributed fungal OTUs of the Arctic and ascertain trends of OTU richness as they relate to depth. A paper overviewing the state of the field (Amend et al., 2019), highlighted most marine fungi identified to date are Dikarya (Basidiomycota or Ascomycota). Many studies (Bass et al., 2007; Richards et al., 2012; Morales et al., 2019; Li et al., 2019) reported Ascomycota and Basidiomycota as the most dominant fungal taxa. Modern DNA-sequencing methods have allowed for the detection of many poorly resolved and uncultured early-diverging fungi (Picard, 2017), often referred to as "dark matter fungi" (Grossart et al. 2015). Due to the dominance of Dikarya in genomic databases and the high recovery rate of Dikarya in other studies, it was hypothesized Ascomycota and Basidiomycota would account for the majority of sequences recovered from the Tara Ocean expedition's sampling of the Arctic Ocean.

Furthermore, it was hypothesized fungal community composition would display dramatic shifts between the epipelagic and mesopelagic layers; similar to trends seen in bacterial communities (Giovannoni and Stingl, 2005). This hypothesis was principally predicated on first principles pertaining to the stratification of Arctic waters. Whereas the epipelagic is a layer where energy is gained though photoautotrophy and the mesopelagic is a layer where energy is channeled to higher trophic levels via feeding on sinking particles, the fundamental ecologic strategies change between these two layers. The presence of distinct layers of water from the Atlantic and Pacific within the mesopelagic layer was suspected to further increase diversity and richness in comparison to the Polar layer at the surface.

METHODS

Tara Oceans Expedition

The data used in my study were collected and partially processed as part of the Tara Oceans expedition. This section provides an overview of the methods used in the Tara Oceans expedition as outlined in Pesant and coworkers (2015). The Tara Oceans expedition was a global survey of planktonic ecosystems in the world's oceans. From 2009 to 2013, more than 35,000 water samples from nine ocean regions were collected to study the ecology, genomics, and functional diversity of viruses, prokaryotic, and eukaryotic plankton in the world oceans. The survey was conducted on a 35-meter schooner (*SV Tara*) with a rotating team of five sailors and six scientists: one chief scientist, one optics engineer, two oceanographic engineers, and two biology engineers. The ship was equipped with state-of-the-art sampling technology including a 2,400-meter winched cable for deploying instruments, a CTD Rosette, Niskin bottles, optics equipment, peristaltic and vacuum pumps, and dry and wet labs.

The Arctic region sample set consisted of 19 stations from which water samples were collected (*Figure 1*). The samples from these stations were obtained from the surface (SRF, 3m-15m), deep chlorophyll max (DCM, 15m-200m), and mesopelagic (MES, 200m-1000m) depths. Water samples from these depths were collected in Niskin bottles and subsequently fractionated by filtration (0.8-2000 μm, 0.22-3 μm, 3-20 μm, 20-180 μm, and 180-2000 μm).

The details pertaining to nucleotide extraction and sequencing are outlined by Alberti and coworkers (2017). An overview of the relevant portions of that paper are

provided here. All filter samples were preserved in liquid nitrogen on board *SV Tara* and sent to the European Molecular Biology Laboratory approximately every 6 weeks. There, the samples underwent a cryogenic grinding process followed by RNA and DNA extraction with NucleoSpin RNA kits and DNA Elution buffer kits (Macherey-Nagel). The optimization of these RNA and DNA extraction methods were validated at the de Vargas laboratory at the Station Biologique de Roscoff.

To reveal the taxonomic assignments of eukaryotic sequences, the hypervariable V9 loop of the small sub-unit rRNA 18s gene was targeted as an amplicon for PCR. This gene was chosen due to its conserved length across diverse taxa, simple secondary structure, the presence of both highly conserved and variable regions, as well as prevalence in reference databases. Following PCR, the DNA was processed with an Illumina sequencer. After performing quality controls, the metabarcodes were assigned a eukaryotic taxonomy as determined by an in-house database. This in-house database was formed via global similarity analysis with around 80,000 reference sequences.

Data Analysis

The laboratory of Colomban de Vargas (Roscoff) shared a data set of all fungi metabarcodes from the Tara Oceans Expedition. Subsequent analysis included writing Matlab codes to read the data and extract the Arctic samples from the global data set. This reduced the number of samples from 1191 to 169. From a collection of files, I extracted the following variables for each sample: sample ID, date of sample, sample name, ocean region, latitude, longitude, OTU-list, average depth, minimum depth, maximum depth,

water density, temperature, salinity, oxygen concentration, chlorophyll concentration, and total number of reads.

I then used the list of OTUs to build a matrix with each row corresponding to a sample and each column corresponding to an OTU. The taxa from the OTU-list were grouped into one of twelve broader OTUs: unidentified core-fungi, unidentified Dikarya, unidentified Ascomycota, Pezizomycotina, Saccharomycotina, Taphrinomycotina, unidentified Basidiomycota, Agaricomycotina, Pucciniomycotina, Ustilaginomycotina, Chytridiomycota, and Microsporidia. To yield the OTU composition of each sample I counted the number occurrence for each OTU in the sample and divided by the total number of OTUs in the sample. This normalized the data by displaying each OTU as a percentage of the total number of OTUs present in the sample.

The 169 samples were further placed into subgroups based on depth and size fraction to generate community composition graphs. Furthermore, I used these groupings to generate box and whisker plots displaying OTU richness, total reads per sample, and Shannon index (where applicable). Shannon index values were calculated in Matlab using element operators on a larger matrix comprised of the relevant samples data.

RESULTS

Within the Arctic subset of data, the total number of reads per sample ranged from 0 to 82,330. The five samples with zero reads were omitted from community composition analysis but were included in all other portions of data analysis. Additionally, several samples registered one read resulting in those community compositions being represented solely by one OTU. The decision was made to include samples registering an arbitrarily low number of reads as long as that number was not zero. Although the maximum number of reads was 82,330, 168 of 169 samples had fewer than 1600 reads. The mean and median number of reads per sample increased with depth. Excluding the outlier of 82,330, the mean number of reads for the SRF, DCM, and MES samples were 30.21, 59.92, and 889.03, respectively. The medians were 10, 13, and 176, respectively. This trend was seen while grouping samples by depth; however, more nuanced patterns emerged when samples were further grouped by filter size.

Specifically, the median number of reads per sample increased as a function of depth for samples obtained with the 0.8-2000 μm and 3-20 μm size fraction. The median number of reads were highest at the DCM for the 0.22-3 μm size fraction. For filter sizes where only SRF and DCM measurements were taken (20-180 μm and 180-2000 μm), no conclusive trend was revealed. Boxplots were generated to provide a visual distribution of the total number of reads for each size fraction at each depth (*Figure 2*). In the figure, each depth is shown as a subplot with the number of reads per sample for each filter being shown as its own box and whisker plot. Although finer details are difficult to see, a standard value of 1600 was chosen for the y-axis to clearly show changes across depth. The 0.22-3 μm

and 3-20 μm size fractions are shown to consistently have the highest median reads for each depth (*Figure 2)*.

OTU richness displayed trends similar to the total number of reads. Boxplots showing OTU richness for various filter sizes and depths are shown in *Figure 3.* Across all depths, more OTUs were detected with the 0.22-3 μm and 3-20 μm filters than any other filter sizes. Again, these values increased as a function of depth, indicating a positive correlation between reads per sample and OTU richness. The number of OTUs detected ranged from 0 to 63. OTU richness changed more dramatically between the DCM and MES layers than between the SRF and DCM layers. This is reflected in the mean number of OTUs detected at each depth: 7.5, 9.8, and 29.0 at the SRF, DCM, and MES respectively.

Although 0.8-2000 μm, 0.22-3 μm, and 3-20 μm filters were used across all three depths, only two stations utilized the same filter size in all three depths. Between those two stations, were five occurrences where SRF, DCM, and MES samples were processed with the same filter size across all depths. Specifically, only TARA 158 (3-20 μm) and TARA 201 (0.8-2000 μm, 0.22-3 μm and 3-20 μm) obtained measurements from all three depths. The Shannon diversity index was calculated for each of these series (*Figure 4)*. For four of the five scenarios, the Shannon diversity index was found to increase as a function of depth with the more pronounced change occurring between the SRF and DCM layer as opposed to the DCM and MES layer.

Further analysis was conducted to determine the most pan-Arctic OTU. Of the total 2059 OTUs registered, the three most dominant were associated with Pucciniomycotina, Pezizomycotina, and Saccharomycotina. More broadly, Basidiomycota, Ascomycota, and unidentified Dikarya were the three most dominant OTUs. To account for the imbalance

in the number of samples per depth, the most prevalent OTU for each depth and filter size was calculated individually. This helped to elucidate bias resulting among different filter sizes fractions. The next section of results explores the trends and findings of each depth individually including the calculation mentioned in this paragraph.

Beginning with the SRF samples, the community composition for each sample is shown in *Figure 5.* Among these samples, is a qualitatively high degree of inter-sample variance, a direct result of the low number of reads per sample as shown in *Figure 2.* Note that 0.22-3 μm has the highest median reads per sample. For SRF samples, the rates of detection are shown in *Table 1.* Pucciniomycotina is the most prevalent OTU across all filter size fractions. For 0.22-3 μm, Saccharomycotina and unknown Dikarya were the second most prevalent OTU. For 3-20 μm the second most prevalent OTU was Saccharomycotina, though the detection rates of unknown Dikarya were considerably lower. Unknown Basidiomycota had the lowest prevalence among all samples with 0% in all filter size fractions.

For DCM samples, the inter-sample variance of community composition *(Figure 6)* was distinctly lower than that of SRF stations. Ustilaginomycotina's contributions to community composition in DCM samples was 21% of detected OTUs on average, the highest of any depth. The increased inter-sample consistency among DCM samples is matched with an increase in the number of reads per sample which are shown in *Figure 2.* Again, the 0.22-3 μm filter fraction size shows the highest median reads per sample for this depth. Looking at prevalence of OTUs in DCM samples *(Table 2)*, Pucciniomycotina is the most prevalent OTU for all filter size fractions except for 0.22-3 μm which had 100%

occurrence for unknown Dikarya, Pezizomycotina, and Agaricomycotina. Pezizomycotina prevalence decreases as a function of filter size fraction.

The MES stations display the lowest degree of inter-sample variability with regards to the community composition as is evident in *Figure 7.* With the consistent community composition of the MES samples, a typical distribution of OTUs in the water samples emerges. Mesopelagic samples were primarily comprised of two subgroups of Ascomycota (Pezizomycotina and Saccharomycotina) and two subgroups of Basidiomycota (Agaricomycotina and Pucciniomycotina). These 4 OTUs account for more than 83% of OTUs in each MES sample regardless of filter size. In tandem with this increased inter-sample consistency, the MES stations yield the highest number of reads per sample of any group, as shown in *Figure 2.* Although the 0.22-3 μm filter returned the highest number of reads per sample in SRF and DCM samples, the 3-20 μm filter had a median number of reads approximately 3 times higher than the 0.22-3 μm filter in MES samples. In looking at the prevalence of occurrence of each OTU in the MES stations (*Table 3)*, Pezizomycotina and Pucciniomycotina were found in all samples. Saccharomycotina and Agaricomycotina were found in all samples from the 0.22-3 μm and 3-20 μm size fractions, but a markedly smaller proportion were found in 0.8-2000 μm size fractions. Unidentified Dikarya, unidentified Ascomycota, and Taphrinomycotina all showed a distinct increase in occurrence frequency as a function of increasing filter size fraction.

Some of the most apparent trends emerge from looking at the community composition graphs (*Figure 5, Figure 6, Figure 7)* together. The most apparent trend seen in comparing these three figures is the consistency of community composition obtained with the 0.22-3 μm size fraction in all three depths. In contrast, samples obtained with the

0.8-2000 μm filter size fraction reveal largely different community compositions as a function of depth.

Chytrids, which were previously reported as seasonally abundant in the Arctic Ocean (Hassett & Gradinger, 2016) were found in only a few stations. TARA 188, 193, 194 and 196 found chytrids with multiple size fractions. TARA 158 (MES 0.22-3 μm), 173 (DCM 3-20 μm), 206 (MES 3-20 μm), and 209 (MES 3-20 μm) found chytrids with one size fraction. TARA 193, 194, and 196 are the three stations nearest the Bering Strait as shown in *Figure 1.* Microsporidia were found even more rarely. This OTU was only found in SRF samples from TARA 180.

DISCUSSION

Reads Per Sample

The high number of reads in the 0.22-3 μm and 3-20 μm filter samples draws attention to the potential variance in fungal cell sizes. Despite the narrow size range, these filters performed better than the broad range (0.8-2000 μm) filter. One explanation for the success of the smaller filter sizes is that the average fungal cell falls within the range of 0.22-20 μm. Yeast typically range from 2.5 μm to 6.0 μm (Maldonado, 2011). Fungi that were detected with larger filter size fractions were likely fixed on larger particles, such as larger phytoplankton cells or fragments of animal detritus which exceeded 20 μm, as opposed to free floating cells in the water column. The decreased detection of unidentified Dikarya and unidentified Ascomycota for 20-180 μm and 180-2000 μm filters suggest smaller filters are more likely to yield discoveries of novel species in the future. Little research has been done on the discrimination of varying cell sizes by filter size fractions; however, most of the marine fungal studies referenced in this study which size-fractionated water samples cited using 0.22 μm filters.

Across all filter sizes, the increasing number of reads per sample as a function of depth is indicative of an increasing abundance of fungi. Claims should not be made about the absolute abundance of groups of fungi due to limitations in the methodology; however, the uniform PCR procedures applied in the Tara Oceans expedition multiplies amplicons evenly across samples, and therefore, can be used as a proxy to determine which samples had the highest initial concentrations of DNA. PCR multiplies DNA exponentially within each cycle, so small differences in initial DNA concentration become more pronounced by

the time the sequences are processed with an Illumina Sequencer. Nonetheless, it is clear the MES layer had higher abundances of fungi as shown by the high number of reads.

The increase in relative abundance of fungi as a function of depth is likely due to the decreased health of phytoplankton at depth and transition away from photosynthetic energy acquisition below the photic zone. The SRF and DCM layers are epipelagic zones with net positive primary productivity. The MES layer, by definition, begins at the depth where photosynthetically active radiation (PAR) is 1% of the initial incident light and ends where light is no longer detected (Del Giorgio & Daurte, 2002). This has become commonly approximated at 200m-1000m. The depth of 1% incident light approximates the compensation depth, the depth at which the rate of photosynthesis is equal to the rate of respiration for phytoplankton. Therefore, the MES layer begins roughly at the depth where a cell is expending more energy than it can generate. After a short period of time, these cells die, and the opportunity for degradation increases. As one study (Kagami et al., 2007) points out, the impact of host population growth rates is inconclusive. Numerous studies suggest fungal epidemics arise in phytoplankton populations exposed to ideal growth parameters, but a comparable number of studies suggest these infections occur more easily when the phytoplankton are in a stress-inducing environment, such as below the compensation depth. Nonetheless, there is consensus that infections occur most readily in situations where the parasite growth rates outpace those of the host cell (Kagami et al., 2007), which would certainly be the case in the MES layer.

OTU Richness and Diversity

The patterns for OTU richness closely followed patterns seen in the number of reads per sample: an increase with depth with the most dramatic changes occurring between DCM and MES layers. Similarly, dramatic shifts in prokaryotic community composition between the DCM and MES have been reported (Giovannoni & Stingl, 2005). The dramatic increase in OTU richness in the MES layer is likely due to the limited geographic dispersion of fungi in the SRF layer. The Arctic Ocean is highly stratified, with discrete layers usually ordered (from top to bottom) as Polar mixed layer, Pacific waters, Atlantic waters, Norwegian and Greenland Sea deep waters (Macdonald et al., 2005). In some regions of the Arctic, the MES layer contains bands of Pacific, Atlantic, and Deep Arctic waters -- in other regions, only Atlantic waters. Thus, fungi in the MES layer come from a great geographic range and are presented with as many as three distinct physicochemical environments to grow in. Additionally, these layers are more homogenized than the cold and fresh Polar layer, being that the deeper layers originate in foreign oceans.

The increased richness and diversity of fungi as a function of depth was further supported by calculating the Shannon index. A group of researchers (Li et al., 2019) sampled waters from the epi-, meso-, bathy-, and abyssopelagic depths in the Western Pacific Ocean, but later categorized their samples as “upper” (5-500 m) and “deeper” (below 500 m). Their study found the Shannon index decreased as a function of depth in the upper layer but showed no correlation to depth in the deeper layer. Their findings conflict with the results of the Tara Oceans expedition, suggesting fungal diversity in the Arctic may display patterns of OTU richness with depth different from those in other oceans. This contrast may be reconciled with the reasoning laid out earlier.

Dominant OTUs

Data from the Tara Oceans expedition supported numerous studies which suggest Basidiomycota and Ascomycota are the most dominant fungal groups in the world oceans. A study which specifically targeted fungi with AU2 and AU4 fungal-specific primers in combination with the hypervariable V4 region reports these two groups as the dominant OTU in deep-sea samples (Bass et al., 2007). Similarly, the dominance of Basidiomycota and Ascomycota, regardless of depth or region, was supported by a recent paper (Morales et al., 2019) which implemented methods more akin to the Tara Oceans expedition: 0.22 μm filters, Illumina sequencing. Not only were their methods similar to the Tara Oceans expedition, but also the study reviewed forty-two metagenomes from the Tara Oceans expedition. This somewhat invalidates independence of data but suggests the subsequent analysis of data is in agreement. Furthermore, the Western Pacific Ocean study *(*Li et al., 2019) found Ascomycota and Basidiomycota were the most dominant OTUs.

Despite the support for these findings, chytrids are known to be the seasonally dominant fungal group in the Arctic sea-ice, sediments, and, to a lesser degree, the water column. (Hassett & Gradinger, 2016). Chytrid presence usually follows seasonal diatom blooms. Because the status of diatom blooms is unknown for the stations sampled in Tara Oceans study, the presence of chytrids cannot be conclusively linked to the presence of diatoms. Furthermore, even when using chlorophyll-A as a proxy for phytoplankton abundance, no clear trend emerged linking the detection of chytrids and abundance of phytoplankton. The previously mentioned Western Pacific study, which identified Basidiomycota and Ascomycota as the two most dominant OTUs, cited Chytrids as the third most dominant group (Li et al., 2019). That study and others (Morales et al., 2019)

suspected this group may be underrepresented in studies due to the lack of chytrid genomes in reference databases. Interestingly, three of the four stations with repeated recovery of chytrid sequences were nearest the Bering Strait, implying Pacific waters may have introduced chytrids in those samples.

Bias and Blind-spots

Comparing the Tara Oceans expedition to other studies is hindered by the lack of standardized methodologies across marine fungal studies and to a large extent, a lack of detail surrounding those methodologies. Across 75 metabarcoding studies reviewed in one study, 95% of studies utilized "subjective sampling methods and inappropriate field methods and/or failed to provide critical methodological information" (Dickie et al., 2017).

Furthermore, there are few papers regarding the community composition of Arctic marine fungi; thus, this present study is largely explorative. Studies by Brandon T. Hassett and associates stand out as reliable backdrops for comparing findings in the western Arctic and sub-Arctic (Hassett et al., 2017) with special attention to the seasonality of chytrids (Hassett & Gradinger, 2016). While Hassett does an excellent job detailing methods, many other studies gave cause for concern.

For instance, some studies *(*Morales et al., 2019) use the MoBio Powersoil DNA extraction kit. The Powersoil handbook (MoBio) states, "the kit is intended for use with environmental samples containing a high humic acid content including difficult soil types such as compost, sediment, and manure." In contrast, other studies took advantage of the MoBio Powerwater Sterivex DNA extraction kit (Hassett & Gradinger, 2016) or, in the case of the Tara Oceans expedition, used a general extraction kit such as the NucleoSpin

RNA kit (Alberti et al., 2017). The use of DNA extraction kits intended specifically for soil samples as recently as 2019 highlights the need for methods to be specialized for the marine environment.

Methodologies among studies further diverge in the application of PCR. Small changes to PCR protocols can dramatically alter the number of reads per sample as PCR doubles the number of sequences with each cycle. The Tara Oceans expedition purposely conducted fewer PCR cycles (25) than recommended by the kit to reduce the formation of chimeras (Alberti et al., 2017). Other studies utilized 34 cycles (Li et al., 2019), 29 cycles (Hassett et al., 2017), or do not directly clarify their PCR protocol (Morales et al., 2019). With 34 cycles of PCR, samples returned a minimum of 17,788 reads (Li et al., 2019). Despite yielding a lower number of reads per sample than the aforementioned studies, this adaptation by the Tara Oceans team was likely wise, as the later cycles of PCR are known to have higher rates of bias and error (Kagawana, 2003).

Perhaps even more important than the number of PCR cycles is the selection of a target gene and primers. The Tara Oceans expedition targeted the eukaryotes with the 18s gene of the SSU rRNA with 1389F and 1510R primers. Other studies utilized 18s and 28s genes with LR0R and LR5 primers (Hassett et al., 2017), ITS3 and ITS4 primers (Li et al., 2019), or make no mention of primers (Morales et al., 2019). While the strengths and weaknesses of each gene and primer are too nuanced for the purposes of this paper, studies focused on unifying the field suggest utilizing a multiple-marker approach is a much-needed standardization in order to reduce bias towards terrestrial fungi lineages (Reich & Labes, 2017).

CONCLUSION

This study provides a glimpse into the diversity and community composition of Arctic marine fungi. Analysis of the data obtained via the Tara Oceans expedition indicates the wide-spread prevalence of Basidiomycota and Ascomycota in Arctic waters and displays depth-related trends. The increased diversity and abundance of fungi in the MES layer is supported by the trends of OTU richness and reads per sample as a function of depth. The depth-related trends revealed in this study are believed to be a result of the change in trophic strategy between epipelagic and mesopelagic depths as well as the stratification of Arctic waters from the Pacific and Atlantic oceans. Future studies of this nature should focus on unifying methods aimed towards eliminating bias and blind-spots inherent to metabarcoding studies.

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APPENDIX

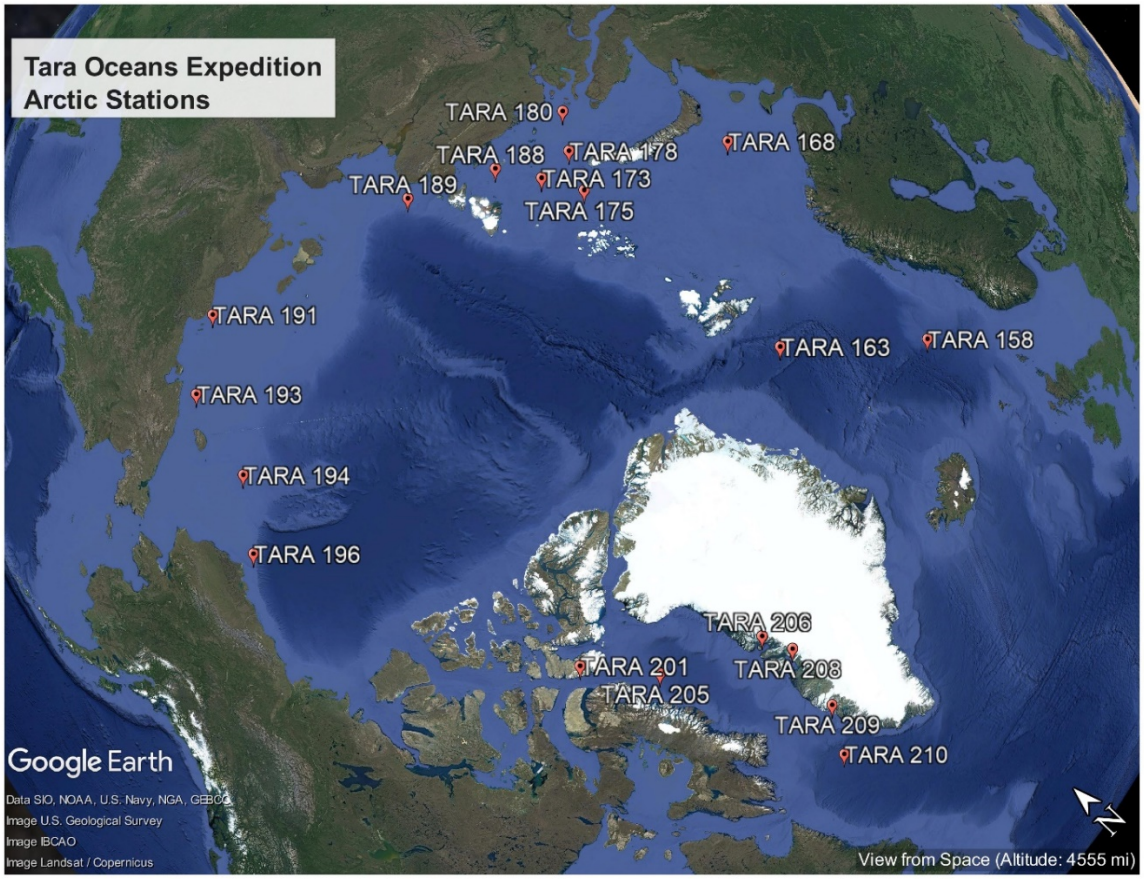

Figure 1. The 19 stations where samples were taken during the Arctic portion of the Tara Oceans expedition.

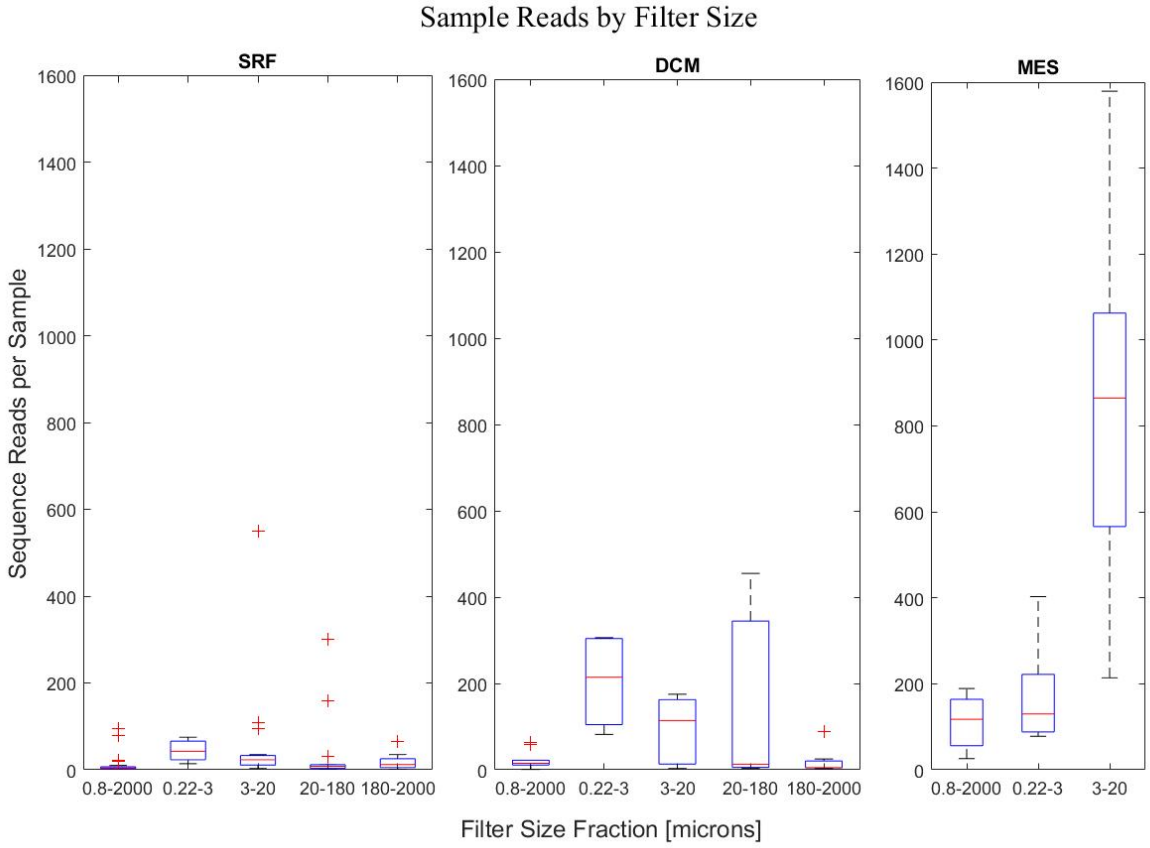

Figure 2. A box and whisker plot of the total number of reads per sample. Red lines represent the median value and box size represents the interquartile range. The size of the box corresponds with the 25 and 75 percentiles. Whiskers represent the maximum and minimum values. Outliers are shown as red crosshairs. One outlier of 82,330 reads is not shown in the SRF 180-200 size fraction plot.

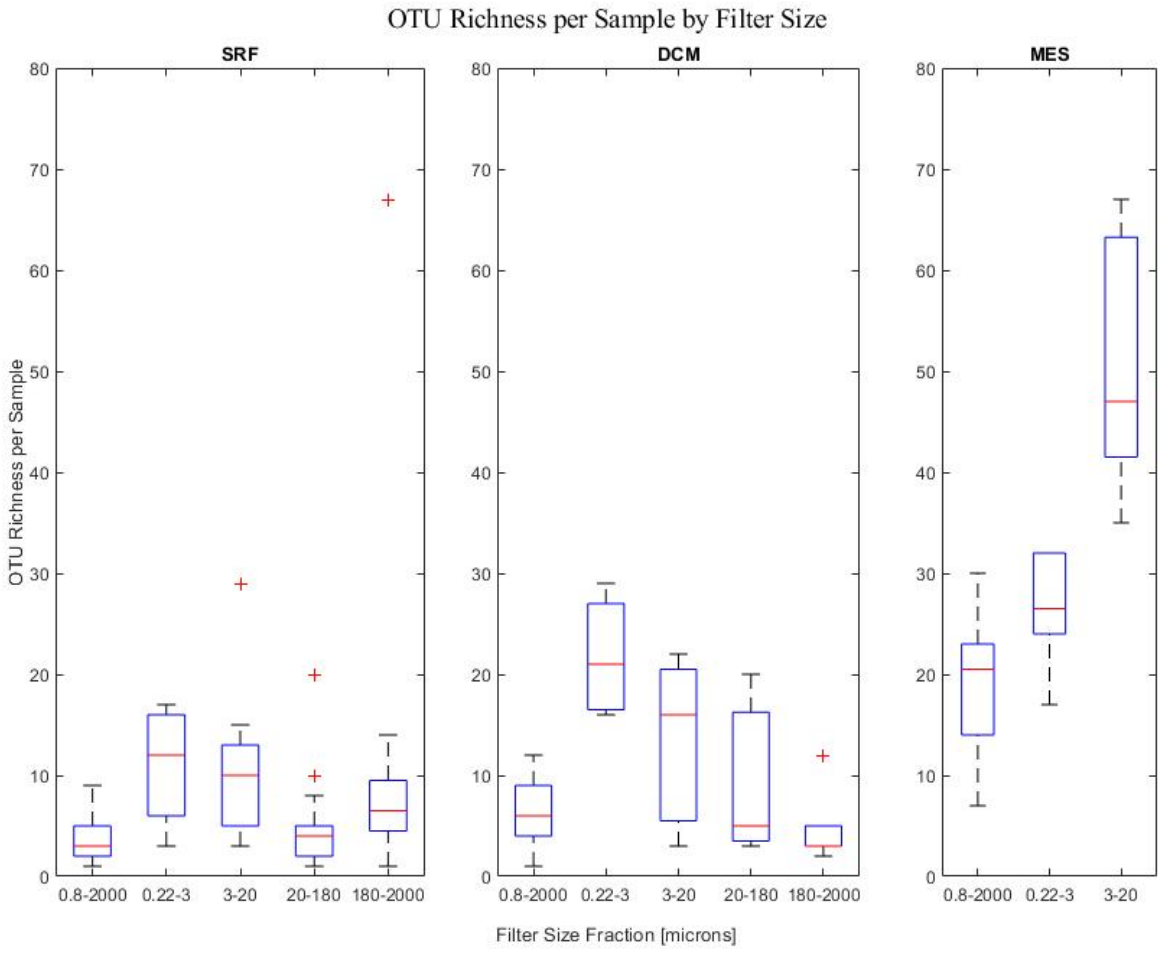

Figure 3. A whisker plot of OTU richness. Red lines represent the median value. Boxes represent the interquartile range (25 and 75 percentiles). Whiskers represent the maximum and minimum values. Outliers are shown as red crosshairs.

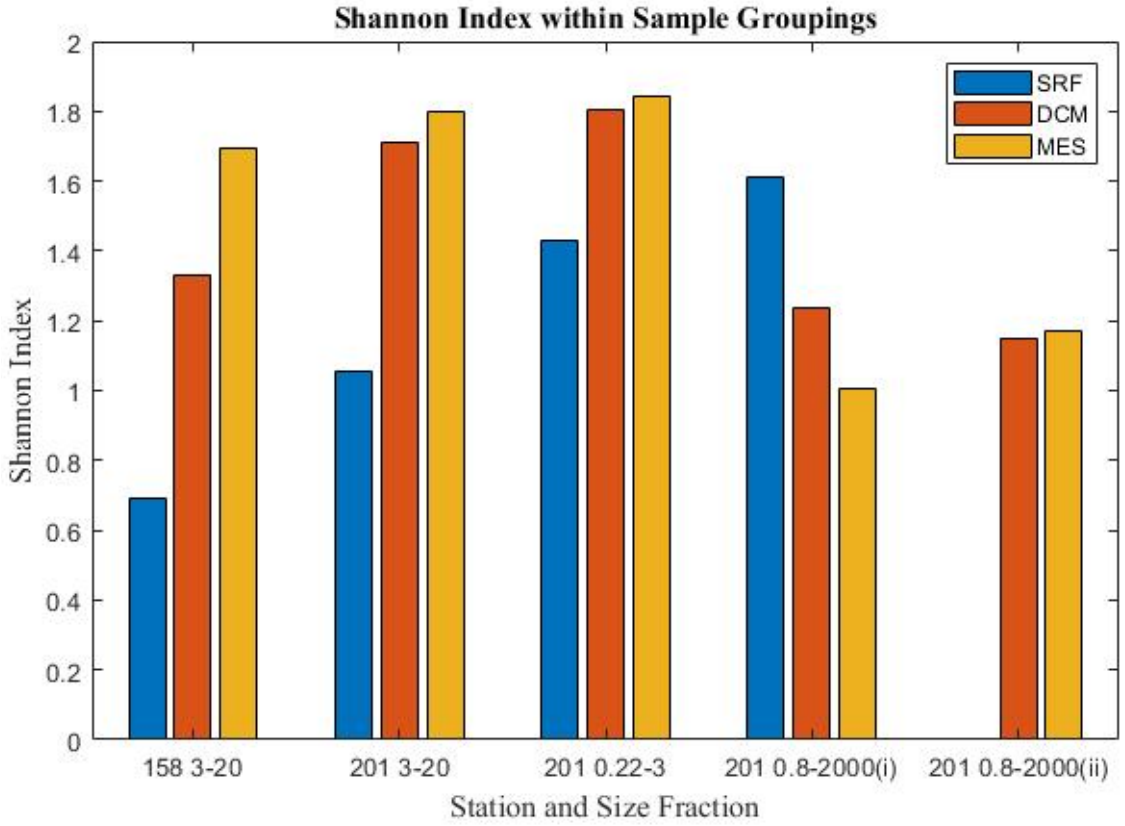

Figure 4. Grouped bar graphs showing the Shannon Index of various groupings. Bars are grouped by filter size. Each color corresponds to a different depth. These were the only sample groupings for which a sample was taken at each depth with the same filter size.

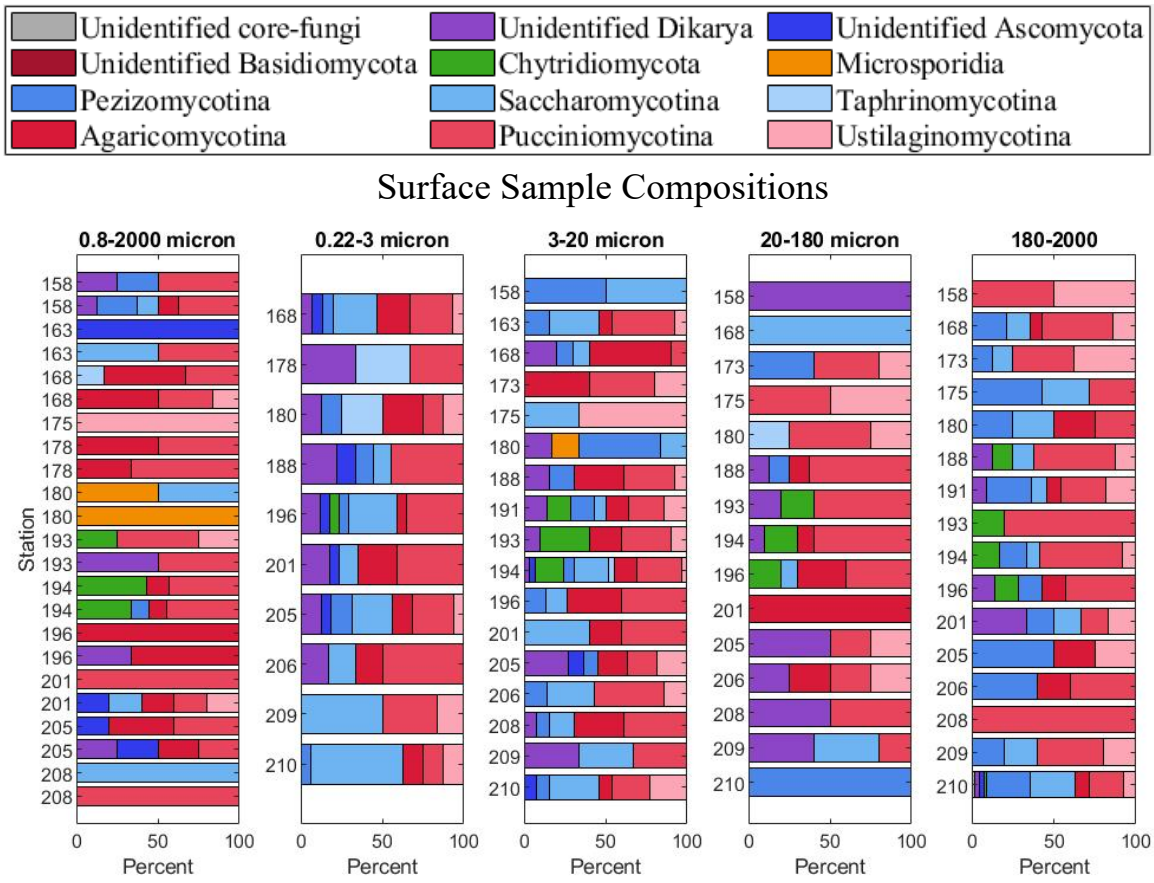

Figure 5. Stacked bar graphs showing the community composition of Surface Samples. Station numbers are shown on the y-axis. Each color corresponds to a different OTU.

OTU/Filter	Core-Fungi	Unk. Dikarya	Unk. Asco.	Unk. Basidio.	Chytrid	Micro.	Pezizo.	Saccharo.	Taphrino.	Agarico	Puccinio	Ustiligano.
0.8-2000	0	22	17	0	13	9	13	22	4	52	70	17
0.22-3	0	80	50	0	10	0	60	80	20	70	100	50
3-20	0	53	18	0	18	6	71	76	6	71	82	59
20-180	0	53	0	0	20	0	20	20	7	33	73	33
180-2000	6	31	6	0	31	0	75	63	0	44	95	63
Average	1	48	18	0	18	3	48	52	7	54	84	44

Table 1. The percent of SRF samples each OTU shows up in for various size fractions.

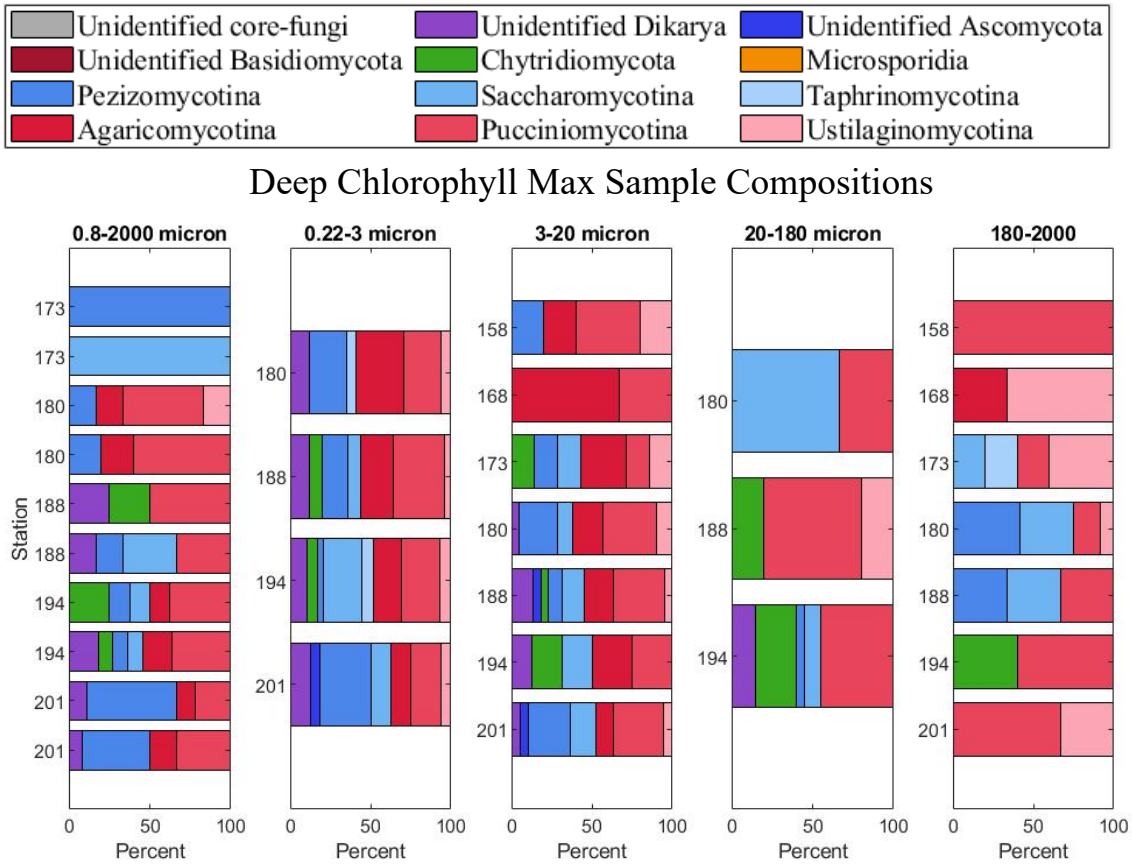

Figure 6. Stacked bar graphs showing the community composition of Deep Chlorophyll Max samples. Station numbers are shown on the y-axis. Each color corresponds to a different OTU.

OTU/Filter	Core-Fungi	Unk. Dikarya	Unk. Asco.	Unk. Basidio.	Chytrid	Micro.	Pezizo.	Saccharo.	Taphrino.	Agarico.	Puccinio	Ustiligano.
0.8-2000	0	50	0	0	30	0	80	40	0	60	80	10
0.22-3	0	100	25	0	50	0	100	75	50	100	80	10
3-20	0	57	29	0	43	0	71	71	0	100	100	71
20-180	0	33	0	0	67	0	33	67	0	0	100	33
180-2000	0	14	0	0	29	0	14	29	0	0	95	63
Average	0	51	11	0	44	0	60	56	10	52	85	46

Table 2. The percent of DCM samples each OTU shows up in for various size fractions.

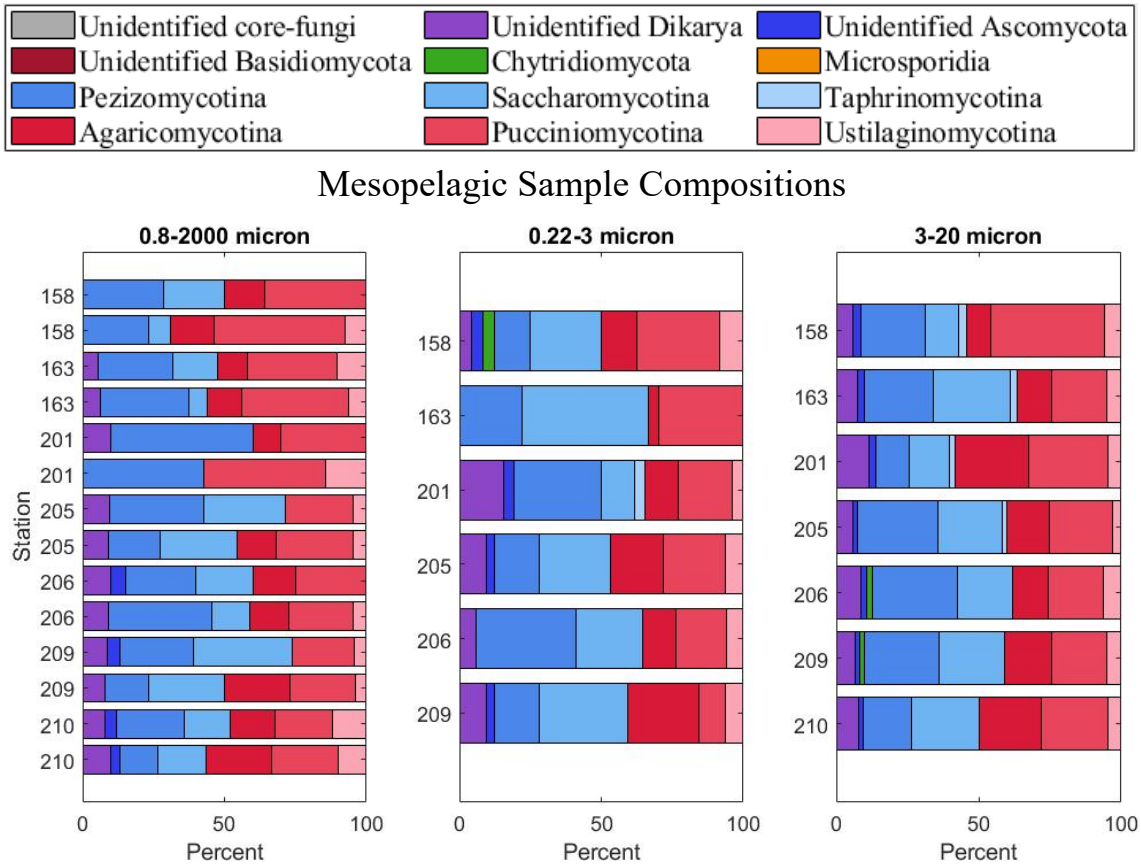

Figure 7. Stacked bar graphs showing the community composition of Surface Samples. Station numbers are shown on the y-axis. Each color corresponds to a different OTU.

OTU/Filter	Core-Fungi	Unk. Dikarya	Unk. Asco.	Unk. Basidio.	Chytrid	Micro.	Pezizo.	Saccharo.	Taphrino.	Agarico	Puccinio	Ustiligano.
0.8-2000	0	79	29	0	0	0	100	86	0	79	100	79
0.22-3	0	83	67	0	17	0	100	100	17	100	100	83
3-20	0	100	100	0	29	0	100	100	57	100	100	100
Average	0	87	65	0	15	0	100	95	25	93	100	87

Table 3. The percent of MES samples each OTU shows up in for various size fractions.

AUTHOR'S BIOGRAPHY

Bentley E. Simpson majored in Marine Science with a concentration in Oceanography. During his time at the University, Bentley interned for NOAA in summer 2017 and submitted cruise data to NASA's seaBass database in summer 2019. In his 2019 employment under Dr. Emmanuel Boss, Bentley was exposed to Matlab. That exposure inspired him to utilize coding in his thesis as a way to continue learning about the software. Bentley has further enriched his learning at the University though four years of serving in the Undergraduate Student Government. Bentley was President of the Student body during the COVID-19 outbreak of 2020, a trying time for our campus. Following graduation, Bentley hopes to realize a long-term career serving in NOAA's Commissioned Officer program, where he can continue to explore the oceans and push the boundaries of scientific knowledge.