Exploring the diversity of microeukaryotic communities in New England tide pools

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ABSTRACT: Though historically understudied, due in large part to most species being uncultivable, microbial eukaryotes (i.e. protists) are abundant and widespread across diverse habitats. Recent advances in molecular techniques, including metabarcoding, allow for the characterization of poorly-known protist lineages. This study surveys the diversity of SAR (Stramenopila, Alveolata, and Rhizaria), a major eukaryotic clade that is estimated to represent about half of all eukaryotic diversity. SAR lineages use varied metabolic strategies like mixotrophy in dinoflagellates (Alveolata), parasitism in apicomplexans (Alveolata) and labyrinthulids (Stramenopila), and life cycle stages that included encystment and attachment (e.g. in ciliates (Alveolata)) to survive in highly dynamic habitats. Using metabarcoding primers designed specifically to target a portion of the 18S SSU-rRNA gene of SAR lineages, we compare protist community composition from tide pools in Acadia National Park, Maine (USA). We characterize

over 500 lineages, here operational taxonomic units (OTUs), many of which are found abundant in the tide pool environment. We also find that communities vary by month sampled and exhibit patterns by size (i.e. macro-, micro-, and nanosized). Taken together, these data allow us to further catalog protist diversity in extreme environments (e.g. subject to extreme fluctuations in temperature and salinity during tidal cycles). Such data are critical in the explorations of biodiversity patterns among microorganisms on our rapidly changing planet.

KEY WORDS: Protist, Metabarcoding, Community analysis, 18S rRNA, Stramenopila, Alveolata, Rhizaria

Data availability: All data are publicly available at BioProject number PRJNA952215 on NCBI.

1. INTRODUCTION

Tide pools in the rocky intertidal zone are dynamic environments subject to the continuous ebbing and flooding of tides, and as a result can experience substantial fluctuations in abiotic conditions (e.g. temperature, salinity, pH) over relatively short periods of time (reviewed in Metaxas & Scheibling 1993, Miller et al. 2009, Leong et al. 2018). Although considered extreme environments, tide pools foster immense biodiversity at the 'macrobial' level (e.g. corals, sea stars, sea urchins, crustaceans, bivalves, fish, macroalgae; Metaxas & Scheibling 1993, Bégin & Scheibling 2003, Arakaki & Tokeshi 2006, Miller et al. 2009, Mishra et al. 2010, Firth et al. 2014, Mendonça et al. 2018). Ecological surveys offer insight into community structure and tolerance in the environment, describing predator/prey interactions, identifying keystone species, heterogeneity among tide pools in the same location, and observing spatial biodiversity patterns (McQuaid et al. 1985, reviewed in Metaxas & Scheibling 1993, Nielsen 2001, Mouritsen & Poulin 2002, Martins et al. 2007). Communities located in the upper intertidal zone are most affected by shifting abiotic factors as they spend the majority of the time isolated from the tide, while communities in the lower intertidal zone are most affected by biotic interactions as they are nearly always inundated by the tide (Noël et al. 2009).

Given that microeukaryotes (i.e. protists) and bacteria account for two-thirds of the ocean's biomass (de Vargas et al. 2015, Sunagawa et al. 2015, Bar-on & Milo 2019), the continuous flooding and mixing of coastal ocean waters with tide pools is likely to have a profound effect on the microbial communities present in these environments. Yet patterns of biodiversity in tide pools are poorly understood. With a

focus on protist communities, studies of distinct lineages (e.g. ciliates, foraminifera, diatoms) have largely depended on morphological observations (e.g. reviewed in Metaxas & Scheibling 1993, Montagnes et al. 2002, Esteban & Finlay 2007, Chao et al. 2013, Weinmann & Langer 2017, Zidarova et al. 2022). These analyses have found an abundance of diatoms, abiotically tolerant Foraminifera, high diversity and richness of ciliates, and unique strategies of persistence among ciliates. Badger and colleagues (2017) further explored this diversity using molecular techniques to describe ciliate diversity in tide pools, identifying a ciliate lineage (*Epiclintes* sp.) specific to tide pools. Another study indicates that there is a high level of diversity among ciliate morphospecies in tide pool environments (Katz et al. 2005). To our knowledge, there are no studies that describe broad protist diversity in tide pools using molecular techniques.

To compare the diversity of protists among tide pools, we deployed a metabarcoding analysis of the major clade SAR (Stramenopila, Alveolata, and Rhizaria) developed in our lab (Sisson et al. 2018, Sleith & Katz 2022). The lineages that make up SAR are estimated to represent ~50% of all eukaryotic diversity with some ~61.000 named species (reviewed in Grattepanche et al. 2018). SAR includes organisms that employ a variety of life strategies: photosynthetic (e.g. diatoms), parasitic (e.g. oomycetes), heterotrophic (e.g. Cercozoa), mixotrophic (e.g. chrysophytes), among many other diverse lineages (Sisson et al. 2018, Flynn et al. 2019, Grattepanche & Katz 2020). Organisms within SAR play essential roles in the biogeochemical cycling of marine environments, particularly in the carbon and nitrogen cycles (Arrigo 2005, Hutchins et al. 2009, Pajares & Ramos 2019). In this study, we examine overall SAR

diversity captured using a metabarcoding approach that allows us to make comparisons across samples, describes how tide pool protist communities differ by size, and highlights the differing patterns of abundance dependent on time and location of sampling.

2. MATERIALS & METHODS

2.1 Sampling site and collection

Samples were collected from tide pools and the adjacent coastal ocean in Acadia National Park (ANP; Permit: ACAD-2019-SCI-0034), Maine (44°13.7' N, 68°18.75' W) in May and October 2019. Tide pools were labeled either low, middle, or high elevation based on their proximity to the coastal ocean along the intertidal gradient and the timing in which they became isolated as tides ebbed. Samples were taken over two days from three tide pools depending on their accessibility and the constraints on the fieldwork schedule (Table 1). Samples were also taken from the adjacent ocean waters during each sampling period. For each sample, 500 mL of water was collected and pre-filtered through a 300 µm mesh filter. We then serially filtered each sample through an 80, 10, and 2 µm Nylon Net or Isopore™ PC Membrane Filter (Millipore, MA, USA) using vacuum filtration. Here, we use the terms nanosized, microsized, and macrosized to define the 2-10, 10-80, and 80-300 µm communities respectively. Filters were placed in 1.5 mL microcentrifuge tubes prepared with 500 µL of Buffer RLT (Qiagen) and stored at -80°C prior to DNA and RNA extraction. Temperature, salinity, and pH were recorded for each sample.

106 **2.2 Sample preparation**

107 We extracted community RNA using the RNeasy Mini Kit (Qiagen) following the
108 manufacturer's protocol. RNA was processed further to remove DNA using the TURBO
109 DNA-*free*™ Kit (Invitrogen, CA, USA) followed by the SuperScript™ III First-Strand
110 Synthesis System (Invitrogen, CA, USA) with Random Hexamers (ThermoFisher
111 Scientific, MA, USA) to create single-stranded cDNA as described in Sisson et al.
112 (2018). Community DNA was extracted using the ZR *Quick*-DNA™ Fecal/Soil Microbe
113 Miniprep Kit (Zymo Research, CA, USA) following the manufacturer's protocol. cDNA
114 (from here on referred to as RNA and active) and DNA were stored at -80°C prior to
115 PCR amplification.

116

117 **2.3 Amplification and sequencing**

118 We used SAR-targeted primers as developed in Sisson et al (2018) to amplify
119 the ~150 bp hypervariable V3 region of the small subunit ribosomal RNA (SSU-rRNA)
120 gene. We ran PCRs in triplicate and pooled them together to reduce PCR bias (Lahr &
121 Katz 2009, Jung et al. 2012, Sisson et al. 2018). Sequence libraries were prepared by
122 the University of Rhode Island RI INBRE Molecular Informatics Core where Illumina
123 MiSeq High Throughput Sequencing (HTS) was performed.

124

125 **2.4 Data curation and analysis**

126 We curated HTS data to create operational taxonomic unit (OTU) libraries using
127 the bioinformatic pipeline outlined in Sisson et al. (2018). We initially included 37
128 samples from another New England location, which contributed to our ability to identify

OTUs; however, here, we only compare samples from ANP (n=204 uncurated samples). To summarize the pipeline from Sisson et al. (2018), raw reads were merged into paired-end reads using PEAR (Zhang et al. 2012), OTUs were clustered using a default SWARM of 1 (Mahé et al. 2015), and non-SAR OTUs were removed using a phylogenetic approach (Sisson et al. 2018, Sleith & Katz 2022). OTUs were assigned taxonomy using a phylogenetic approach based on a curated full-length SSU-rRNA eukaryotic database with 3.810 GenBank reference sequences (3,484 SAR sequences and 326 non-SAR outgroup sequences). Phylogenetic trees were built from MAFFT alignments (Katoh & Standley 2013) using RAxML with the GTR GAMMA phylogenetic model (Stamatakis 2014). After removing outgroup OTUs, we rarefied samples at 20.000 reads and created an ingroup OTU table (Supplemental file S1).

To further authenticate OTU taxonomy using a phylogenetic approach, after the identification of multiple long-branch OTUs and missing sister reference sequences, we added an additional 92 unique full-length ingroup (i.e. SAR; n=84) and outgroup (n=8) sequences from GenBank to the initial reference database described above. We then rebuilt the phylogenetic tree using the updated reference database which resulted in some OTUs falling among the expanded outgroup (i.e. Amoebozoa, Opisthokonta, Excavata, Archaeplastida). Through an iterative process, a total of 37 OTUs that consistently fell among the outgroup across four different iterations taking into account different alignment gap masking parameters were removed from further analysis.

We then curated the data further to remove samples with fewer than 4.000 reads and remove OTUs with fewer than 100 reads and fewer than five occurrences across the entire study. We removed adjacent ocean samples (n=39) from community analyses

to ensure we were focused on describing the tide pool communities; however, we did use the adjacent ocean sample information to calculate tide pool specific (TPS) OTUs, defined as OTUs with 95% or more of their reads in tide pool samples. All analyses and figures include curated OTUs that had at least 100 reads, unless otherwise stated. Dissimilarity matrices were calculated with weighted UniFrac distances and Principal Coordinate Analyses (PCoAs) were made using the R package phyloseq (McMurdie & Holmes 2013).

For the TPS OTUs, we chose to analyze only the RNA from tide pool samples (n=92) to focus on describing the community that was active at the time of sampling. To account for highly-abundant sequences that 'contaminate' other samples during the analysis (also referred to as bleedthrough), we added a curation step as we evaluated the relative abundance of the top 30 TPS OTUs as follows: for any abundant OTU (defined here as an OTU with >1K reads), read numbers were replaced with a zero for any given sample with fewer than 20 reads. To discuss any OTU identity, we relied on BLAST (Basic Local Alignment Search Tool; NCBI) in the following manner: 1) an OTU was assigned to a genus or species if the BLAST percent identity was >98% over 100% of the sequence length (if the species could not be determined we added "sp." and if the genus could not be determined we added "-like"); 2) an OTU was assigned to a genus if the percent identity was >95% over 100% of the sequence length (if the genus could not be determined, we added "-like"); and 3) if the percent identity was <95% over 100% of the sequence length, we added "-like", but if the length coverage fell below 100%, we indicated this as "Unknown" and added the class name.

3. RESULTS

3.1 Data summary

We sampled from tide pools of varying heights (low, middle, high) at ANP on two days in May and two days in October 2019, generating a total of 148 tide pool and 36 adjacent ocean samples after curation (Table 1). The ranges of salinity, pH, and temperature were 16-35, 7.4-9.2, and 6.9-18.1 in May and 35-38, 7.4-8.3, and 11.6-16.6 in October, respectively (Table 1). Using SAR-targeted primers (Sisson et al. 2018) that allow for comparison between samples, we obtained 654 OTUs (Supplemental file S2) represented by a total of 4.444.471 reads. After data curation (i.e. removal of poorly performing samples and samples from Southern Maine that are not included in analyses here (see methods)), we focused community analyses on 148 tide pool samples and 519 OTUs totaling 2.783.515 reads (Supplemental files S3 & S4). To exemplify the diversity we captured in our samples, we chose a subset of the most abundant OTUs removing those with fewer than 300 reads; these 346 OTUs represent 98.9% of the subsetted curated reads (2.752.732) in the study (Fig. 1). We also define a subset of OTUs as TPS taxa if 95% or more of their reads were in tide pool samples (when compared to the adjacent ocean samples; Fig. 1).

Phylogenetic analysis including the 519 OTUs from 148 tide pool samples reveal that alveolates, particularly ciliates, dominated the tide pools sampled (Supplemental file S5). 219 OTUs fall among ciliate reference sequences representing just under half of all the OTUs in this study. When looking at the overall diversity of the 346 most abundant OTUs (Fig. 1), we observe 97 Stramenopila (28.0%), 185 Alveolata (53.5%), and 64 Rhizaria (18.5%). Again, a majority of the Alveolata OTUs are ciliates (n=158), which

represent 45.7% of all 346 OTUs and 85.4% of alveolate-only OTUs. The Stramenopila OTUs are dominated by diatoms (n=51), comprising 52.6% of stramenopile OTUs. Within the Stramenopila clade, there are 3 oomycete (i.e. “water molds”; OTUs 235, 72, and 354) and 3 labyrinthulid (i.e. “slime nets”; OTUs 230, 368, and 548) OTUs, which are both groups known to contain pathogenic lineages (Hyde et al. 1998, Scholz et al. 2016, Buaya et al. 2022). Lastly, the 64 Rhizaria OTUs are mostly composed of Cercozoa, likely heterotrophic flagellates (Zamora-Terol et al. 2020).

3.2 SAR communities within tide pools vary by size

We found no distinct clustering patterns among the high, middle, and low tide pool or adjacent ocean samples (Supplemental Fig. 1); however, principal coordinate analysis (PCoA) of all 148 tide pool samples and 519 OTUs shows distinct clustering patterns by size fraction and SAR clade (Fig. 2). The nanosized community (defined here as 2-10 μm) clusters distinctly from the micro- (10-80 μm) and some macrosized (80-300 μm) communities, which show some overlap (Fig. 2A). There is the greatest dispersion among the macrosized samples across PCo1, which explains 31.6% of the variation among these samples (Fig. 2A). Exploration of the taxonomic identity underlying these patterns plotted with the same ordination space shows that Rhizaria OTUs cluster in the upper right corner of the PCoA (Fig. 2B), suggesting that the nanosized samples are influenced by small rhizarians. Stramenopiles are clustered in the lower right region of the plot, driving the similarity between the microsized and some of the macrosized communities (Fig. 2B). Alveolate OTUs occupy the largest ordination space in the plot

(Fig. 2B) and are responsible for the differences seen among the macrosized communities (Fig. 2A).

To examine the influence of temporal sampling, we evaluated patterns within each size class in samples from the spring (May) and fall (October) of 2019 (Fig. 3). Here, the PCoA of all 148 tide pool samples partitioned by size shows that the month of sampling has a substantial effect only on the microsized communities but not the macro- or nanosized communities (Fig. 3 top row). In each of the OTU PCoAs, plotted with the same ordination space, for the respective size class, alveolates spread across the most ordination space (Fig. 3 bottom row) and are driving certain samples to be distinct from the rest of the communities. We did not find any distinct pattern among the communities in regards to location of pool (high, middle, low), though we do find that some individual OTUs show different patterns of abundance depending on the height of pool in the intertidal zone (Fig. 4).

3.3 Patterns among the top TPS OTUs by height of pool and time

We identified 121 TPS OTUs as any OTU with at least 300 reads and 95% or more of its reads in tide pool samples versus adjacent ocean samples. These 121 OTUs represent 426.869 (15.3%) reads out of all curated reads and include 22 Stramenopila, 87 Alveolata, and 12 Rhizaria based on phylogeny (Supplemental file S5). We then focused on the top 30 most abundant OTUs in 92 RNA (i.e. active) samples that were defined as tide pool specific; here we refer to each OTU either by species, genus, or genus-like depending on hand-curated analyses of BLAST hits as

described in the methods (Fig. 4). Of these OTUs, the majority (22 out of 30 OTUs) are Alveolata, all of which are ciliates (Fig. 4).

Within the top 30 TPS OTUs, we found four OTUs abundant in only May and nine OTUs abundant in only October (Fig. 4). Of the four May OTUs, they are identified as ciliates in the genera *Zoothamnium*, *Chlamydonella*, *Pleuronema,* and *Pseudochilodonopsis* while of those in October, two are stramenopiles in the genera *Bilabrum* and *Licmophora*, one is a rhizarian of the genus *Minorisa*, and the remainder are ciliates belonging to *Condylostoma*, *Scyphidia*, *Chromidina*, *Strombidinopsis*, and an unknown Scuticociliate (Fig. 4). OTUs that belong to Stramenopila and Rhizaria lineages are seen in a greater abundance in October relative to May (Fig. 4). Aside from differences between May and October, some OTUs exhibit abundance patterns dependent on pool location along the intertidal gradient.

We observed several OTUs that were restricted to certain tide pool locations (either high, middle, or low) in the intertidal space. For example, OTUs 38, 77, and 145 are only detected in middle tide pools. OTU145, a *Zoothamnium* ciliate, was only found in the middle tide pool sampled in May. In addition, OTUs 38 and 77, both in the ciliate genus *Condylostoma*, were only found in the same middle tide pool in October at approximately the same relative abundance. OTU193, a ciliate in the genus *Chromidina*, is only abundant in October, especially in high tide pools. OTU114 is another October-abundant ciliate in the genus *Scyphidia* that dominates the low tide pool along with OTU61, a *Strombidinopsis* ciliate.

4. DISCUSSION

We assessed microbial diversity in tide pools, environments subject to continuous and at times extreme changes in abiotic factors (e.g. temperature, salinity) that result from the constant ebbing and flooding of the tide. By evaluating the biodiversity of SAR lineages across tide pools sampled in ANP, we find that: 1) tide pools harbor a diverse community of SAR lineages; 2) the nanosized (define here as 2-10 μm) communities are distinct from the rest of the communities, while the macrosized (100-300 μm) communities are more variable (with variability often driven by large ciliates); 3) there are numerous TPS SAR lineages whose presence may be driven by height of pool, time of year (e.g. May vs. October), and potentially season; and 4) the TPS lineages include ciliate genera known to have adapted to life in these turbulent environments as well as lineages that are yet-to-be characterized.

4.1 SAR diversity within tide pools

Using primers designed to target the V3 region of the 18S rRNA gene (Sisson et al. 2018), we successfully characterized the SAR community from 148 tide pool samples taken during two sampling periods in May and October of 2019. The majority of all OTUs characterized in this study are ciliates (Fig. 1), which may be representative of biases common in metabarcoding studies (Heywood et al. 2011) such as the size of organisms. The potential overrepresentation of ciliates may also reflect the dynamic nature of their genomes, which includes hyperpolyploidy of somatic chromosomes (Maurer-Alcalá et al. 2018, Rzeszutek et al. 2020, Ahsan et al. 2022) that may cause overrepresentation in PCR (Lavrinienko et al. 2021). However, high ciliate diversity is

consistent with the ubiquity of ciliates in marine settings (Weisse & Montagnes 2022), which we've likely captured in our sampling.

Alongside the abundance of ciliates, we captured a diversity of photosynthetic stramenopiles as well as a number of cercozoans (Rhizaria). We also acknowledge the biases in these SAR primers consistent with other studies that demonstrate challenges in using universal or clade-specific primers (e.g. Hadziavdic et al. 2014, Piñol et al. 2018, Stern et al. 2018, Burki et al. 2021). For example, the SAR primers we used are incompatible with foraminifera rDNAs, which have large insertions in otherwise highly conserved regions (Pawlowski et al. 1999, Pawlowski & Lecroq 2010). We also detect only a few members of apicomplexans (Alveolata) and in this marine setting we found these primers provided limited taxonomic resolution for dinoflagellates (Alveolata), differing from Sisson et al. (2018) findings in freshwater vernal pools.

We identified a number of OTUs in this study that have differing lifestyles that may allow them to survive in such harsh environments like tide pools. One example of such a lifestyle is pathogenicity, which we found prevalent in this study from each of the SAR clades. For example, OTU72 is an *Olpidiopsis*-like oomycete (Stramenopila) that is a known parasite of red algae (Klochkova et al. 2016). OTU368 is an *Oblongichytrium* sp. of labyrinthulid (Stramenopila) a causative agent of sea star wasting disease (FioRito et al. 2016). OTU114 is a *Scyphidia*-like ciliate (Alveolata) that has been found parasitizing *Littorina* snails, fish, and copepods (Fish & Goodwin 1976, Abowei et al. 2011, Pane et al. 2014). OTU193 is a *Chromidina*-like ciliate (Alveolata), an organism that has been found to parasitize cephalopods (Souidenne et al. 2016). OTU496 is *Marinomyxa marina*, a rhizarian that parasitizes macroalgae (Kolátková et al. 2021).

Pathogenicity is a strategy deployed by many microeukaryotes across the tree of life (Leander 2020), playing an important role in food web interactions in the ocean (Bjorbækmo et al. 2020). Other lifestyles such as suspension feeding (e.g. OTU220, a ciliate *Pseduovorticella* sp.) and mixotrophy (i.e. auto and heterotrophic; e.g. OTU98, a dinoflagellate like *Karlodinium*) may be advantageous in the harsh intertidal zone where conditions fluctuate from favorable to unfavorable often.

4.2 SAR communities are structured by size and sampling date

We used serial filtration to understand the different size classes of organisms within tide pools, as previously done by others in diverse systems (Deiner et al. 2018, Grattepanche & Katz 2020, Ruggiero et al. 2020, Egge et al. 2021). Though these methods are imperfect as cells may rupture or pass through filters depending on their orientation and their presence in biofilms or other larger matter, they still provide a point of comparison among varying communities. We find that the nanosized communities (2-10 μm) were distinct from the larger communities (10-300 μm) found in tide pools sampled from ANP (Fig. 2A), suggesting that the serial filtration successfully selected for at least a portion of smaller species. The clustering based on taxonomy indicates that nanosized communities are driven by Rhizaria in a similar ordination space (Fig. 2B), a finding consistent with observation of many small heterotrophic rhizarian flagellates in marine plankton communities (e.g. Boenigk & Arndt 2002, Massana et al. 2006, Obiol et al. 2021). The greatest variability is seen among macrosized communities (Fig. 2B) driven by alveolates (Fig. 3 macrosize), most of which are ciliates. The larger cell size of ciliates and their patchy distribution among tide pools

(Grattepanche et al. 2016) along with differences in abundance may explain the variability found within these macrosized samples (Fig. 4).

We also find a distinct partitioning in the microsized communities, but not the other two communities, between the May and October samples (Fig. 3 microsized communities). Some rhizarian and stramenopile clustering in the lower portion of ordination space is driving the October samples to be different from the May samples where more alveolates are influencing these samples (Fig. 3 microsized OTUs by SAR clade). The difference in the microsized community in May and October is consistent with an effect of oceanic spring blooms and fall upwelling (Lindemann & St. John 2014), though it is unclear why only communities at one size class varied temporally.

4.3 Tide pool specific SAR patterns across time and pools

We identified 121 TPS OTUs in this study, defined here as OTUs that have more than 95% of their reads in tide pool samples when compared to the adjacent ocean "control" samples (see methods). We focus on the top 30 of 121 active (i.e. RNA) TPS OTUs, which represent 81.0% of all TPS active reads (Fig. 4). Of particular interest is OTU56, *Epiclintes auricularis*, a spirotrich ciliate; using the same set of primers as this study, *E. auricularis* was abundant in tide pools along the coast of Portland, Maine (Badger et al. 2017), while it was not abundant in several studies of non-tide pool samples from New England waters (Doherty et al. 2010, Grattepanche et al. 2016, Santoferrara et al. 2016, Badger et al. 2017). We find only 77 reads from two ocean samples that represent *E. auricularis,* in contrast to 16.986 reads from 42 tide pool samples in which this taxon was abundant (excluding samples for which this OTU had

fewer than 20 reads as this could represent either rarity or bleedthrough; supplemental file S6). In addition, we find that *E. auricularis* is abundant in our October samples. Together, these data suggest that *E. auricularis* is a specialist for tide pools and might be worth in-depth study to elucidate mechanisms for survival in these turbulent environments.

In addition to *E. auricularis*, we found 12 other TPS OTUs that were also abundant in only one of the two time periods we sampled, a pattern that may be linked to differences in biological productivity (e.g. host and prey abundance) that fluctuates seasonally (Lindemann & St. John 2014, Caracciolo et al. 2022). For example, the ciliate *Zoothamnium* (similar to OTUs 21, 145, and 197) has been shown to exhibit seasonal patterns in abundance relative to its host copepod abundance in the Chesapeake Bay (Safi et al. 2022). Along the French Atlantic coast, Hernández Fariñas and colleagues (2017) found the diatom *Licmophora* (similar to OTUs 84 and 109, prevalent only in October) to be a summer specific taxon. In the Sargasso Sea, Blanco-Bercial and colleagues (2022) found the heterotrophic rhizarian *Minorisa* (similar to OTU 179) peaks in abundance near the end of summer, consistent with our finding of *Minorisa* as abundant in our October samples. In sum, our metabarcoding approach provides evidence of numerous lineages that may exhibit both ecological and temporal preference (May vs. October), and provides a first glimpse at what might be seasonal patterns.

4.4 Lifestyles that allow for persistence in tide pools

For organisms to persist over time in tide pools, they must be adapted to surviving in such harsh environments of intense abiotic stress and wave action. In our study, OTU62 was identified as *Strombidium apolatum*, a close relative of the well-studied *Strombidium oculatum* that has a cyclical lifestyle in tide pools referred to as 'circatidal' behavior (Montagnes et al. 2002). *Strombidium oculatum* is able to persist in tide pools as it encysts on the rocky substrate 30 minutes before tidal flooding, after which it remains encysted for nearly 20 hours before excysting 30 minutes after the pool becomes isolated from the tide (Montagnes et al. 2002). Both *S. oculatum* and *S. apolatum* are considered cryptic species inhabiting the same environment that potentially carry out the same behavior (McManus et al. 2010), and we speculate that OTU62 may also encyst and have a cyclical behavior.

Many OTUs in this study were identified as having particular morphologies and lifestyles that help with persistence in tide pools. *Licmophora* (OTUs 84 and 109) are colonial diatoms that attach to substrates (e.g. rocks or algae in their environment; Hernández Fariñas et al. 2017). *Zoothamnium* (OTUs 21, 145, and 197) are large colonial ciliates with a stalk that attaches to substrates (Sergeeva et al. 2022). *Rhabdostyla commensali*s (OTU154) and *Scyphidia* (like OTU114) are epibiotic ciliates that live on other organisms (Dias et al. 2007). *Epicarchesium* (OTU141) is another stalked ciliate that attaches to substrates (Leitner & Foissner 1997) while *Chromidina* (OTU193) is a genus of ciliates known to be parasitic (Souidenne et al. 2016). These 10 OTUs highlight the diversity of life strategies among protists in tide pools and exemplify the methods that may facilitate survival under different tide pool conditions.

5. SYNTHESIS

Tide pools experience extreme fluctuations in abiotic factors, such as temperature and salinity, making them ideal habitats to study patterns and drivers of diversity. While macrobes in tide pools are relatively well-studied, protist tide pool communities are largely unexplored. Molecular characterization of Stramenopila, Alveolata, and Rhizaria sampled from tide pools located on the coast of Acadia National Park, ME reveals a diversity of tide pool specific lineages. We find over 500 unique SAR lineages in the tide pools sampled that reflect a diversity of life histories and we identify how size, time of sampling, and pool type influence community and organismal structure among these samples. Overall, these data illuminate the biodiversity of understudied protists in extreme environments such as tide pools.

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Table 1. Summary of samples collected from tide pools and the adjacent coastal waters (labeled “Ocean” here) in Acadia National Park, ME (44°13.7’ N, 68°18.75’ W) in 2019. Ranges are shown for abiotic factors when applicable. Multiple samples for each pool is the number of successful PCR reactions from serial-filtered samples taken throughout the tidal cycle (see supplemental file S4 for detail). The low pool on 10/14 was inundated by the tide and, therefore, could not be sampled. Times marked with an asterisk are estimated.

Date	Site	# of samples	First sample	Last sample	Tide	Salinity	pH	Temperature (°C)
05/20	Low pool	10	5:39 PM	6:26 PM	Ebbing	35	8	7.6
05/20	Middle pool	10	5:26 PM	6:19PM	Ebbing	30	7.8-8.1	8.3
05/20	High pool	9	5:49 PM*	6:35 PM	Ebbing	32	8	11.7
05/20	Ocean	12	6:06 PM	6:45 PM	Ebbing	NA	NA	NA
05/21	Low pool	5	7:15 AM	7:15AM	Flooding	35	7.6	7.7
05/21	Middle pool	10	7:25 AM	11:55 AM	Flooding	35	7.4-8	7.8-8.4
05/21	High pool	32	7:39 AM	1:41 PM	Flooding	16-34	7.5-9.2	9.5-18.1
05/21	Ocean	15	7:45 AM	12:10 PM*	Flooding	35	8-8.6	6.9-7.7
10/13	Low pool	15	5:00 PM	6:10 PM	Flooding	35-36	8	13.2-13.7
10/13	Middle pool	16	5:00 PM	6:10 PM	Flooding	36	8.2-8.3	14.6-14.9
10/13	High pool	13	5:00 PM	6:10 PM	Flooding	37	8.2-8.3	15.5-16.6
10/13	Ocean	-	-	-	-	-	-	-
10/14	Low pool	-	-	-	-	-	-	-
10/14	Middle pool	10	8:15 AM	9:20 AM	Flooding	35-38	7.5-7.6	11.6-12.4
10/14	High pool	18	8:15 AM	10:05 AM	Flooding	35-38	7.4-7.6	11.7-13.5
10/14	Ocean	9	8:15 AM	11:00 AM*	Flooding	36	7.6	12.2

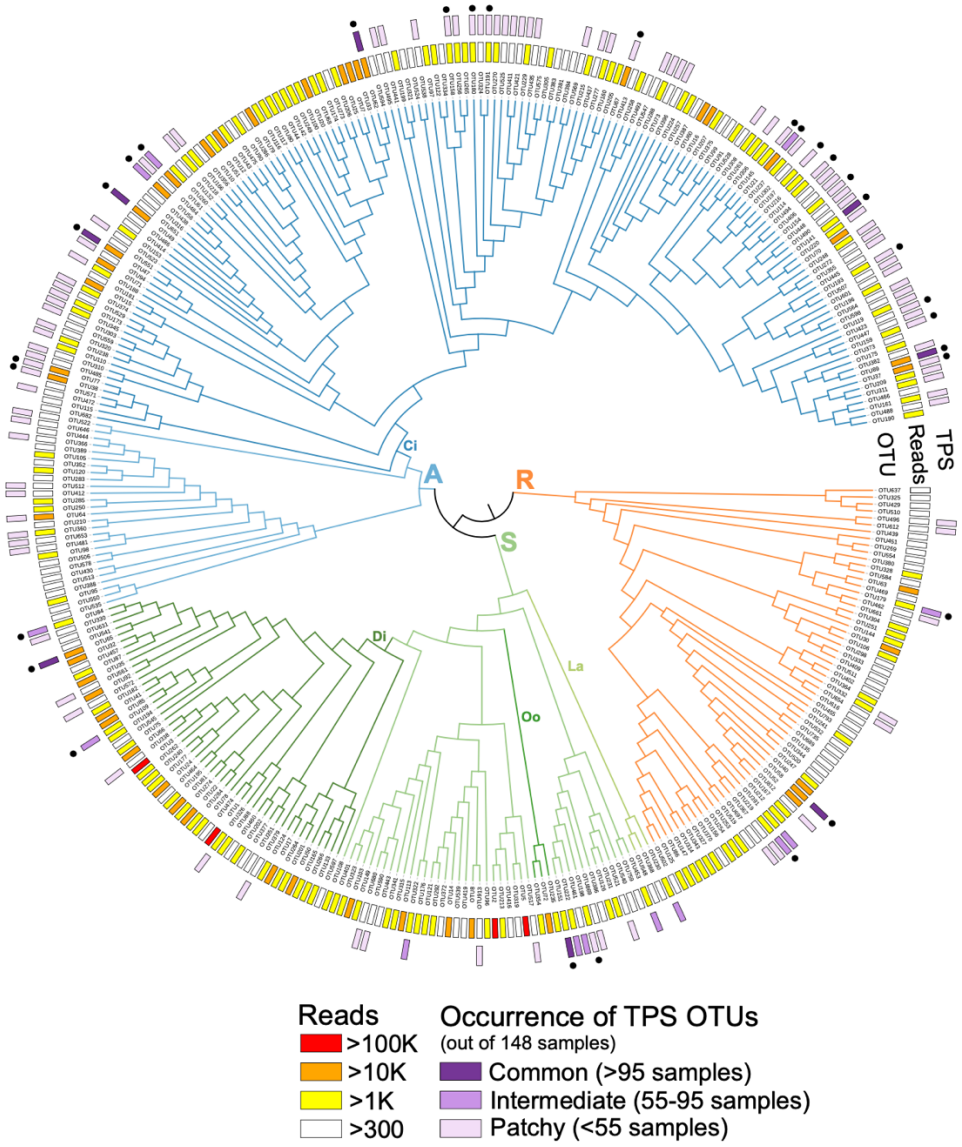
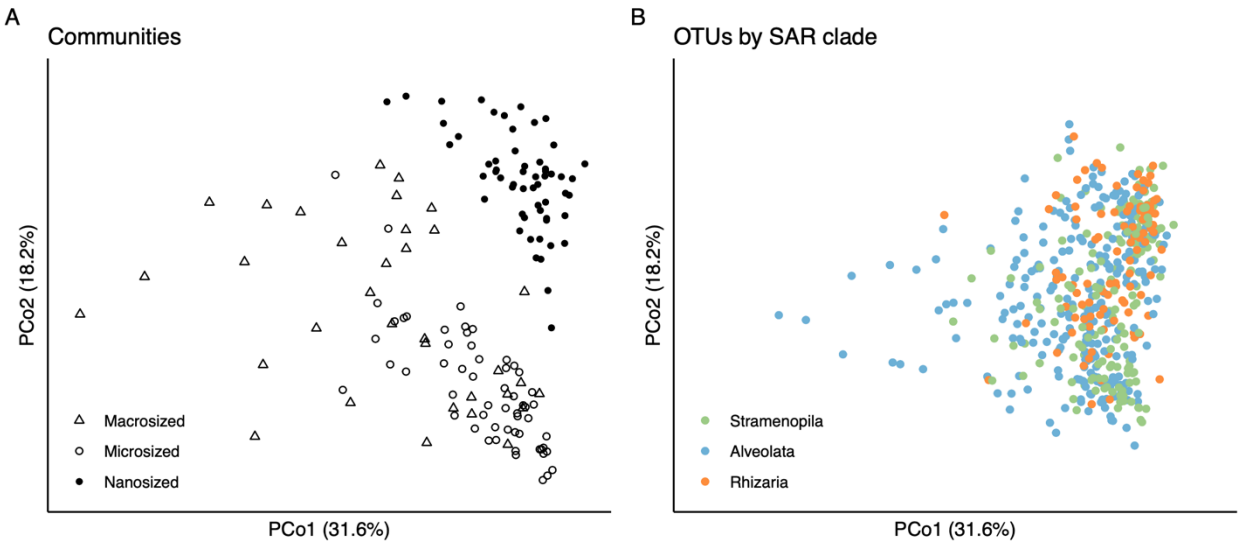


Fig. 1. To exemplify the biodiversity in our study, we depict the 346 most abundant operational taxonomic units (OTUs) colored by SAR clade: Stramenopila (S) = green, Alveolata (A) = blue, and Rhizaria (R) = orange. Reads (inner ring) and occurrence of tide pool specific (TPS) OTUs (outer ring) are shown for each of the 346 OTUs. Clades with labels are as follows: Ci = ciliates, Di = diatoms, Oo = oomycetes, La = labyrinthulids. Bubbles on the outermost ring indicate the TPS taxa of interest presented in Fig. 4. The details on each OTU and the full phylogeny are included in supplemental files S3 and S5.



687
688 Fig. 2. Principal coordinate analysis (PCoA) of (A) communities shows a distinction
689 between the nanosized (2-10 µm) communities and the macro- (80-300 µm) and
690 microsized (10-80 µm) communities, with the greatest variability among the macro-
691 communities. (B) The operational taxonomic units (OTUs) underlying this pattern (same
692 ordination space as 2A) indicate that Alveolata are driving the spread in macro-
693 communities while the nano-communities are enriched with Rhizaria.

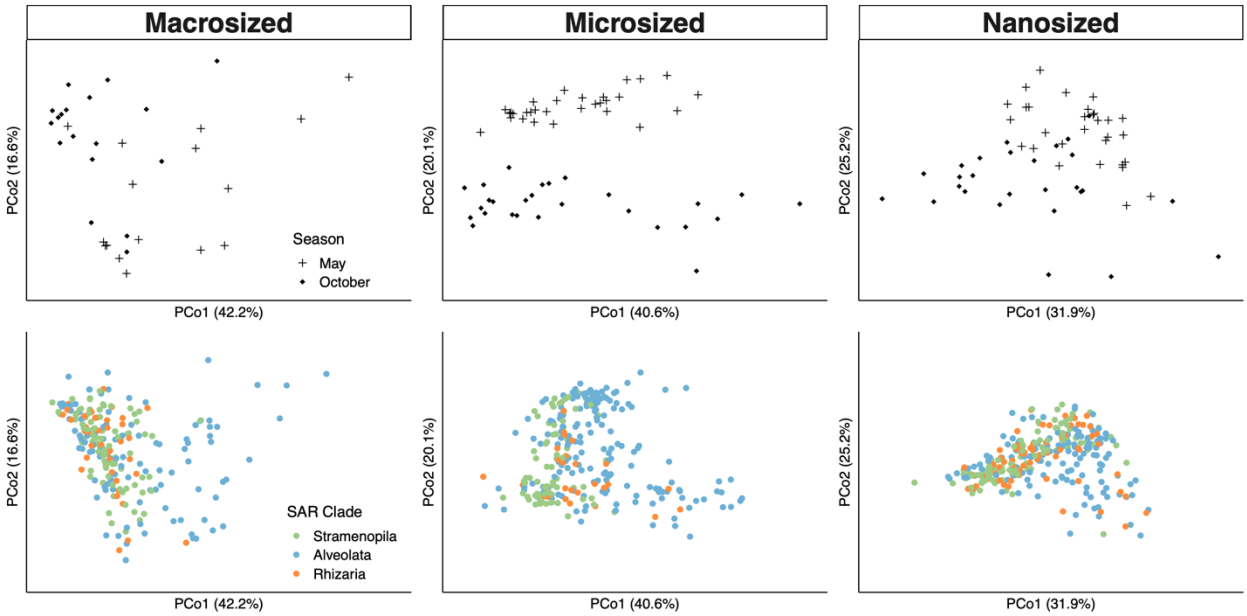


Fig. 3. Principal coordinate analyses separated by the macro- (80-300 µm), micro- (10-80 µm), and nanosized (2-10 µm) communities reveal that only the microsized communities are distinct between the two sampling periods and that Alveolata are main drivers of variability among macrosized communities. For each size class, the community samples (top row) and individual OTUs (bottom row) are plotted on the same ordination space.

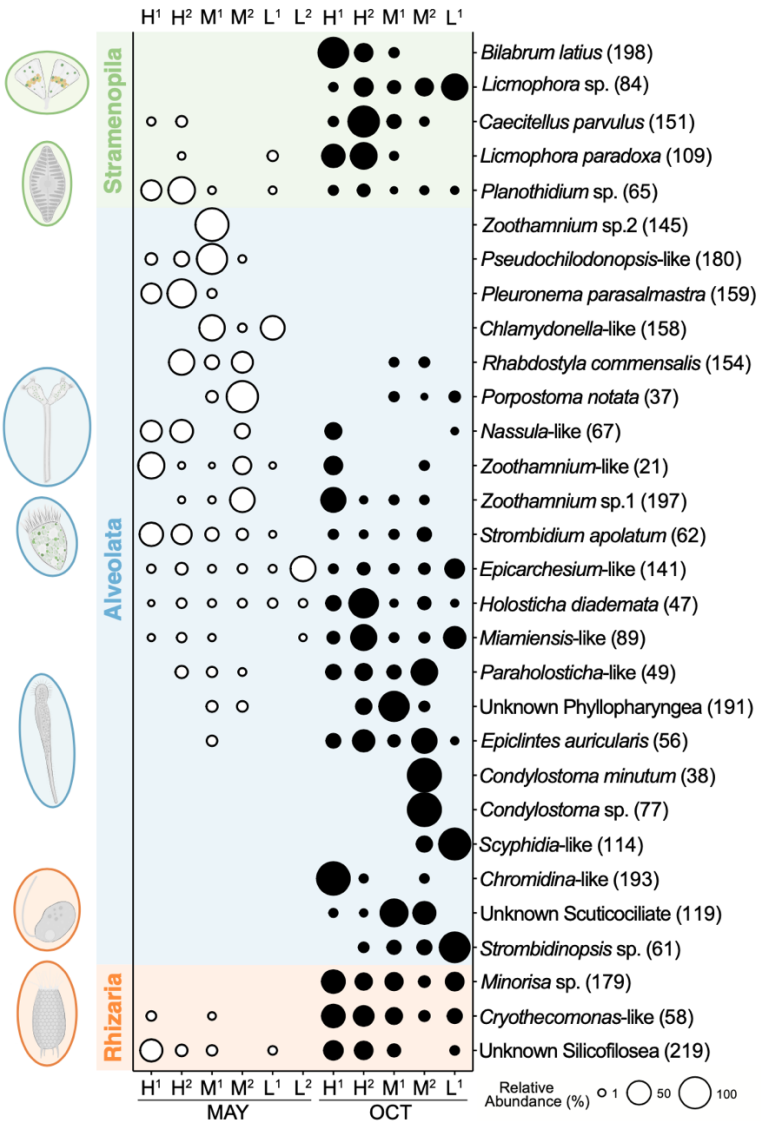


Fig. 4. Top 30 tide pool specific (TPS) lineages show varying patterns of abundance depending on the height of the pool and sampling period. Taxonomy was assigned based on visual inspection of BLAST against NR (GenBank) as described in the methods. Operational taxonomic unit (OTU) numbers are in parentheses after the taxa name. The superscript on the x-axis reflects either the first or second day of sampling given the month (see Table 1). Drawings in order from top to bottom represent the genera *Licmophora*, *Planothidium*, *Zoothamnium*, *Strombidium*, *Epiclintes*, *Minorisa*, and lastly an unknown Silicofilosea.