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Diversity of Marine Eukaryotic Picophytoplankton Communities with Emphasis on Mamiellophyceae in Northwestern Philippines

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Eukaryotic picophytoplankton (EPP) play vital roles in primary productivity and biogeochemical cycling in the marine environment. In this study, we explored the diversity of EPP communities in two different embayments and the shifts in their community structuring during monsoonal reversal in the northwestern Philippines. Water samples were collected weekly from late northeast (NE) monsoon to intermonsoon (IM) or summer periods (February–April 2019) in Bolinao, Pangasinan, and once in January in Masinloc, Zambales. EPP community profiling was done through targeted sequencing of the V4 region of the 18S rRNA gene. Grouping of samples based on physicochemical parameters was consistent with that of community beta diversity, suggesting strong clustering between late NE and IM periods. This exhibits short-term community shifts of EPPs possibly associated with the monsoonal transition. Specifically, overall EPP alpha diversity increased towards summer coupled with increased temperature and lower nutrient concentrations. NE monsoon samples from Bolinao and Masinloc were dominated by Chlorophyta and Stramenopiles, while Prymnesiophyta, Rhizaria, and Picozoa dominated the IM period samples in Bolinao. Specifically, the prasinophytes (Chlorophyta) *Ostreococcus* and *Nannochloris* distinguished the late NE communities of Masinloc and Bolinao, respectively. Phylogenetic analysis of dominant photosynthetic EPP further revealed the presence of Clades B5 and A1 of *Micromonas*, as well as Clades B and E of *Ostreococcus*. Tree topology of *Ostreococcus* diversity suggests the presence of a clade distinct from other established clades, possibly indicating novel diversity in the West Philippine Sea. This is the first report of these major picophytoplankton in Philippine waters, suggesting their significance and potential "hidden" diversity, which warrants further studies.

Keywords: monsoonal reversal, 18S rRNA gene, picophytoplankton, *Micromonas*, *Ostreococcus*, West Philippine Sea

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INTRODUCTION

Picophytoplankton are very small photosynthetic microorganisms, which are < 3 µm in diameter (Li *et al.* 1983). Although minute in size, these organisms are thought to contribute 26–56% to the global phytoplankton biomass (Buitenhuis *et al.* 2013) and play vital roles in primary production and major biogeochemical cycles (Campbell and Vaulot 1993; Bonachela *et al.* 2015). As an energy resource, they are the prey of most nano- and microzooplankton due to their size and high abundance. Picophytoplankton are ubiquitous and found in almost all types of aquatic environments, particularly in nutrient-limited or oligotrophic conditions such as in the open oceans (Zhao *et al.* 2010), newly opened waters of the central Arctic (Zhang *et al.* 2015), and towards summer or at the end of spring blooms in coastal waters (Onda *et al.* 2017).

This size class of eukaryotic phytoplankton is composed of taxonomically and functionally diverse organisms (Vaulot *et al.* 2008). Taxonomic distribution, however, may vary depending on geographic location and the environment. To date, many picophytoplanktonic groups have been demonstrated to exhibit distinct biogeographical distributions, indicating either environmental filtering, niche partitioning, or dispersal limitation (Chust *et al.* 2013). Prasinophyceae tends to be the most distributed and abundant group of green algae in the marine environment (dos Santos *et al.* 2017). In Mamiellales alone, several lineages have been observed to exhibit distinct clades with unique distributions. Specifically, *Micromonas* under Mamiellophyceae has seven distinct clades that are associated with the latitudinal distribution or temperature range based on the 18S rRNA gene or its V4 region (Tragin and Vaulot 2019). Since their discovery in the late 1970s, *Micromonas* remains understudied because of its fine size, lack of unique morphological features, and the limitations of the classical morphology- or culture-based tools. It was only around the year 2000 when picophytoplankton diversity was further revealed using molecular techniques such as PCR coupled with cloning, DGGE, direct sequencing, and metagenomics (*e.g.,* Díez *et al.* 2001; López-García *et al.* 2001). Since then, global and regional surveys of picophytoplankton revealed extensive information regarding their distribution, functionality, and diversity. Vaulot *et al.* (2008) reported that among the 3,561 unique 18S rRNA gene sequences from published datasets generated from marine waters, the most abundant photosynthetic groups are the green algae (specifically Prasinophyceae), followed by dinoflagellates, cryptophytes, prymnesiophytes, and stramenopiles (*e.g.* diatoms) – where their abundances are greatly driven by different factors including water temperature (Morán *et al.* 2010), light gradient (Stawiarski *et al.* 2018), predation (Perez *et al.* 1996), nutrient levels (Behrenfeld *et al.* 2006), as well as seasonality (Romari and Vaulot 2004). Among the members of Prasinophyceae, *Micromonas, Ostreococcus*, and *Bathycoccus* cover more than 90% of the total available sequences and are mostly found in temperate coastal waters (Vaulot *et al.* 2008).

Oceanic or planetary scale expeditions to probe and explore microbial diversity in the marine environment that generated large sequence datasets heavily focused in North Atlantic, South and East Pacific, Mediterranean, Arctic, Antarctic, and North Indian basins (Buitenhuis *et al.* 2012; De Vargas *et al.* 2015). In smaller but known to be diverse regions such as the coastal waters of the South China Sea, only a few studies have been done assessing picophytoplankton diversity and abundance (Wu *et al.* 2014a, b; Lin *et al.* 2017a) and mostly using microscopy (Pan *et al.* 2005; Zhang *et al.* 2013). Meanwhile, in the Philippines, picophytoplankton occurrence and diversity have not yet been explored and remains underappreciated (see Onda *et al.* 2019). The structuring, diversity, and factors influencing the occurrence of picophytoplankton communities in a tropical setting remain little understood. Here, we investigated EPP diversity in the tropical coastal waters using high throughput sequencing (HTS) to generate 18S rRNA gene profiles from fractionated samples. Samples were collected during the transition from the NE to IM periods (January–April) in two geographic sites (open and semi-enclosed bays) associated with varying environmental conditions in the northwestern Philippines. In addition, we further focused on the members of Prasinophyceae (*Ostreococcus* and *Micromonas*) that dominated the dataset. This study is the first to provide information on the diversity and abundance of EPP communities in the tropical coastal waters of the West Philippine Sea within the South China Sea.

MATERIALS AND METHODS

Study Sites and Sample Collection

In this study, diversity and community structuring of major picophytoplankton groups were investigated by collecting samples from two separate embayments on the west coast of Luzon – namely Bolinao, Pangasinan (BOL) and Masinloc, Zambales (MZ) (Figure 1). Both sites are mariculture-impacted areas with reported occurrences of harmful algal blooms (Azanza and Taylor 2001; Albelda *et al.* 2019). To determine possible short-term temporal shifts in the community structuring of major picophytoplankton groups, samples were collected every week from a single station in Guiguiwanen channel in Bolinao, Pangasinan (GUI10; Albelda *et al.* 2019) from February to late April 2019 mainly capturing the transition period from the late NE to IM period. To generate a snapshot of potential

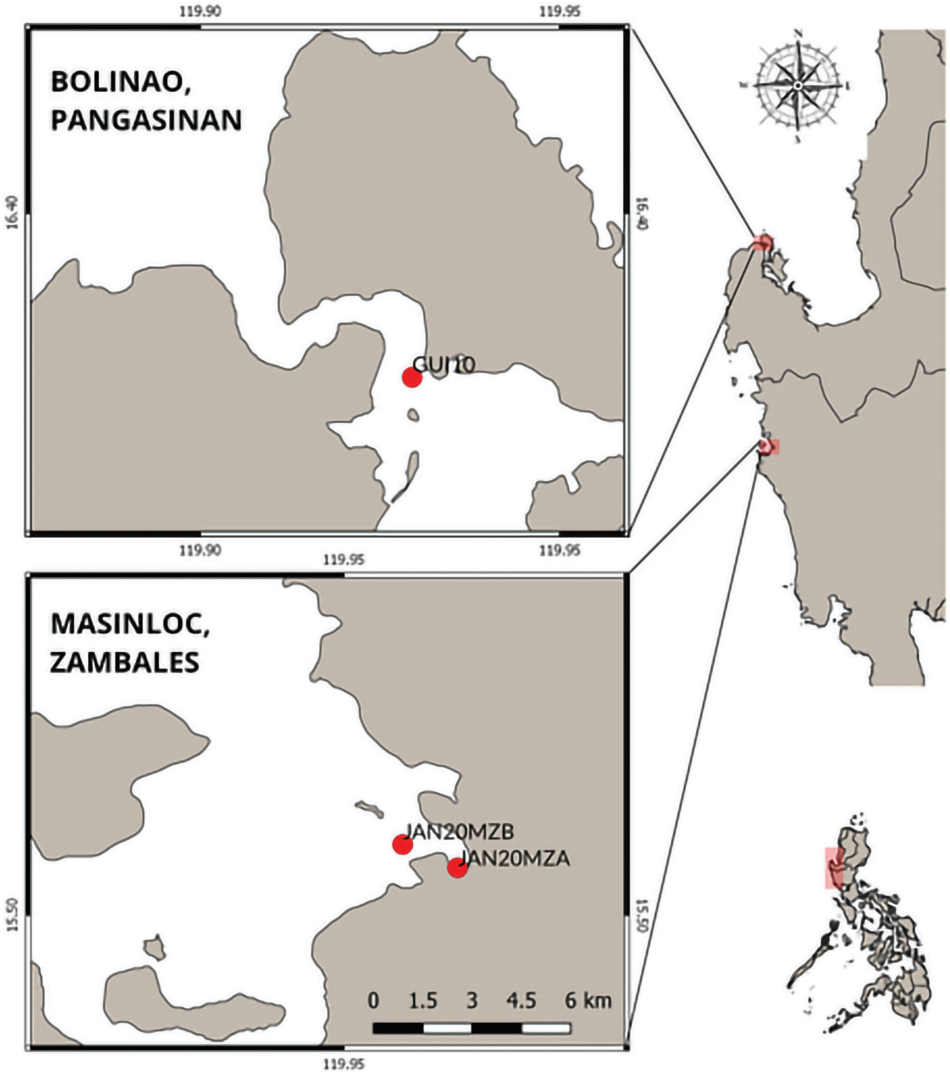

Figure 1. Map of Bolinao, Pangasinan and Masinloc, Zambales. Red dots represent the sampling stations.

biogeographic differences, another set of samples were collected from two stations (A and B) in Masinloc, Zambales in January 2019, which was approximately 97 km away from Bolinao.

Seawater samples were obtained from the surface (ca. 1 m below) using a Niskin-type bottle sampler. A total of 2 L of water were directly collected and pre-filtered immediately through a 120-µm sieve to remove zooplankton and other larger debris before being transferred into acid-washed 1 L Nalgene bottles. All samples were stored in ambient seawater temperature until sample processing. Separate water samples were collected at each site for chlorophyll *a* (Chl *a*) analysis and stored in an icebox until they reached the laboratory. Environmental data including temperature, salinity, conductivity, and dissolved oxygen (DO) values were recorded at each site during the time of sampling using a multiparameter (Hanna HI9829 and YSI Pro 2030).

In the laboratory, the pre-filtered 2 L of seawater were then serially filtered through a 42-mm polycarbonate (PC) 3-µm pore size filter to collect the nano-microplankton fractions, and lastly through a 0.2-µm Sterivex filter (Millipore), corresponding to the picoplankton fractions. A total of 50 mL of serially filtered seawater sample was collected and stored in separate 10-mL conical tubes, which were later used to measure the macronutrients. The PC filters were transferred to 2-mL sterile microcentrifuge tubes, and both filters were added with at least 1.6 mL RNALater (Ambio). Samples were stored at -20 °C until DNA extraction.

Seawater samples for Chl *a* analysis were filtered through Whatman GF/F filters, wrapped in aluminum foil, and kept frozen until analysis. Chl *a* was extracted from the filters with 90% acetone and subsequently analyzed using a Trilogy laboratory fluorometer following the method of

Parsons *et al.* (1984). Nutrient samples were separately analyzed for ammonium (NH_4^+), silicate (SiO_4^{4-}), and phosphate (PO_4^{3-}) using spectrophotometry following the methods based on Parsons *et al.* (1984). However, due to logistical limitations during transport, some of the corresponding samples for nitrate analysis were deemed not useful to be analyzed and, thus, were not included in this study.

DNA Extraction and HTS

Total genomic DNA was extracted from the filters using the DNeasy PowerSoil Kit (Qiagen, Germany) following the manufacturer's protocol with few modifications. To focus on the EPP communities, only the 0.2 µm fraction (0.2–3.0 µm) was used in this study. In brief, Sterivex filters were purged and washed with 1 mL ultrapure water (Nanopure) prior to opening. Half of the filter was then prepared for extraction by cutting into small pieces to enhance cell lysis. The samples were then placed inside the PowerBead tube provided by the kit and vortexed gently. Subsequently, 60 µL of Solution C1 was added to the tube as prescribed by the kit. The tubes were then vortexed at 3,200 RPM for 15 min to disrupt the cells. This was then followed by the procedures prescribed by the kit's manufacturer. DNA yield and integrity were checked using spectrophotometry (Nanodrop) and agarose gel electrophoresis. Moreover, random PCR using the universal eukaryotic primer pair 4616F and 4618R (Logares *et al.* 2007) targeting the 18S rRNA gene was performed as a quality check. The samples were sent for Illumina multiplex HTS to the Integrated Microbiome Resource in Dalhousie University, Canada using E572F and E1009R forward and reverse primers, respectively – targeting only the V4 region of the 18S rRNA gene (Comeau *et al.* 2011, 2017).

Bioinformatics Processing

Bioinformatics processing and quality filtering were carried out in QIIME (Quantitative Insights into Microbial Ecology) platform v.1.0 (Caporaso *et al.* 2010). Raw reads were assembled with at least 25 bp overlap using the join paired-ends function (Aronesty 2013). Reads were quality-filtered and chimeric sequences were identified using USEARCH v.6.1 (Edgar 2010). The identified chimeras were subsequently removed, and the resulting dataset was processed for *de novo* operational taxonomic unit (OTU) picking at 98% similarity (Caron *et al.* 2009). The assignment of taxonomic identity was performed using "mothur" with a 0.8 confidence threshold (-c) against the revised Nordicana Reference Database v.1.0 (Lovejoy *et al.* 2016). This was derived from the Silva database but with added curated references from the tropics, temperate, and the Arctic and has a modified taxonomic ranking for some groups of dinoflagellates, ciliates, and mamiellophytes (Onda *et al.* 2017) – making it useful for this study. Singletons as well as metazoa and fungi-related reads were excluded from the analysis. To make the diversity indices more comparable, the dataset was rarefied at 3,087 reads per library using single rarefaction based on the sample with the lowest reads or counts. The final dataset was then used for subsequent statistical, ecological, and phylogenetic analyses. All raw reads have been deposited into the NCBI SRA database under accession code PRJNA656691.

Phylogenetic Analysis

Due to their dominance in the dataset, phylogenetic analysis of the HTS-generated sequences focused on chlorophytes, particularly those under Mamiellophyceae (*Ostreococcus* and *Micromonas*), was done to better ascertain their phylogenetic identities. Reference sequences were obtained for each clade of *Ostreococcus* and *Micromonas* as described in Tragin and Vaulot (2019). In the case of *Ostreococcus*, a sequence cited as having 100% similarity to a Clade "E" assigned sequence was downloaded from NCBI GenBank to represent this clade (accession number MH008654) (Tragin and Vaulot 2019). The Mamiellophyceae HTS-generated sequences from the collected samples were then aligned with the reference sequences in MAFFT (Multiple Alignment using Fast Fourier Transform) v.7.450 (Katoh and Standley 2013), as implemented in Geneious Prime v.2019.0.4 (Kearse *et al.* 2012). Mismatches were then identified between the HTS-generated and reference sequences based on the positions described by Tragin and Vaulot (2019). Maximum likelihood tree construction was subsequently using FastTree ver 2.1.11 (Price 2010). Similar clustering was observed when trees were generated using the Bayesian approach. Reference trees based on nearly full-length 18S rRNA gene sequences downloaded from GenBank were generated and compared with the topology of the V4 fragment-derived phylogenetic trees to verify that placement did not significantly vary between the two trees. Topologies and consistency in clustering in the newly generated phylogenetic trees were then compared with those of Tragin and Vaulot (2019). This allowed careful re-examination of the possible clades in the samples based on mismatches found in the sequences.

Ecological and Statistical Analyses

To assess differences in community composition within a site (alpha diversity), Faith's PD, Observed OTUs, and Chao1 indices were calculated from the rarefied OTU table as implemented also in QIIME, focusing on the EPP OTUs. Differences in community composition between sites or samples (beta diversity) were then assessed by calculating the Unweighted UniFrac values in QIIME 1 (Chen *et al.* 2012) and plotted using principal coordinate

analysis (PCoA). Bray-Curtis dissimilarity, which looks at both presence and abundance based on the rarefied OTU table, was visualized using non-metric multidimensional scaling (NMDS). Analysis of similarity (ANOSIM) was done to test for significant differences in community dissimilarity between sites and seasons, while analysis of similarity percentages (SIMPER) determined the taxa contributing the most to variances observed across samples. Correlations between taxa and environmental parameters were explored using Spearman's Rank Correlation (rho) as implemented in the R package "Hmisc" (Harrell 2020). Possible drivers of clustering were then determined using linear regression of principal coordinates (PC1 and PC2 of PCoA) with the measured environmental parameters. Statistical analyses, heatmaps of OTU abundance, and visualizations were done in R ver 3.6.0 using the packages "ggplot2," "vegan," and "pheatmap" (Wickham 2016; Kolde 2019; Oksanen *et al.* 2019).

RESULTS AND DISCUSSIONS

Environmental Characteristics of the Sampling Sites

Clustering of the samples from Bolinao (hereafter referred to as BOL) based on physicochemical parameters (Figure 2A) revealed distinct grouping by periods, namely late NE monsoon (February to early March, or NE BOL) and early IM or summer (late March to April, IM BOL). The clustering was strongly correlated with the changing temperature within GUI10 from January–April. The temperature was significantly higher in IM than NE monsoon (t-test, $p < 0.01$; Figure 2B), consistent with previous reports in the channel (Baula *et al.* 2011). The GUI10 station studied here was located in the inner part of the Guiguiwanen channel, where the build-up of warmer waters due to inhibited outflow by the fish cages has been previously reported towards summer (San Diego-McGlone *et al.* 2008; Albelda *et al.* 2019). This is usually accompanied by increased Chl *a* (NE BOL: 12.74 ± 7.76 mg m^{-3}; IM BOL: 14.72 ± 9.65 mg m^{-3}), possibly pertaining to the intermonsoon bloom commonly observed in tropical aquatic ecosystems (Wang and Tang 2014) or accumulation of biomass due to inhibited flow and nutrient accumulation (Albelda *et al.* 2019). The same pattern has also been reported in Panguil Bay also in the Philippines towards the end of February, mainly driven by changes in temperature and increased availability of nutrients (Canini *et al.* 2013). Although the Philippines has only two recognized monsoon seasons (wet and dry), the intermonsoon periods have also been shown to be characterized by significantly different water column profiles mainly driven by the weakened wind conditions during monsoonal reversal (Udarbe-Walker and Villanoy 2001). This could affect vertical mixing, horizontal transport, sea surface temperature, and nutrient availability; therefore, it would also have significant effects on the more sensitive phytoplankton communities (Udarbe-Walker and Villanoy 2001; Wang and Tang 2014).

Masinloc, Zambales (MZ) samples also formed a distinct cluster apart from the BOL samples, indicating significant differences in environmental conditions between the two sites, which could either be related to geographic or seasonal differences since the samples were collected in January. Particularly, MZ stations were characterized by lower nutrients such as P and Si (MZ PO_4^{3-} = 0.317 ± 0.041 µM, BOL PO_4^{3-} = 1.862 ± 1.01 µM; MZ SiO_4^{4-} = 2.206 ± 3.120 µM, BOL SiO_4^{4-} = 3.626 ± 2.683 µM) and DO (MZ = 5.04 ± 0.01 ppm, BOL = 5.422 ± 142 ppm) but

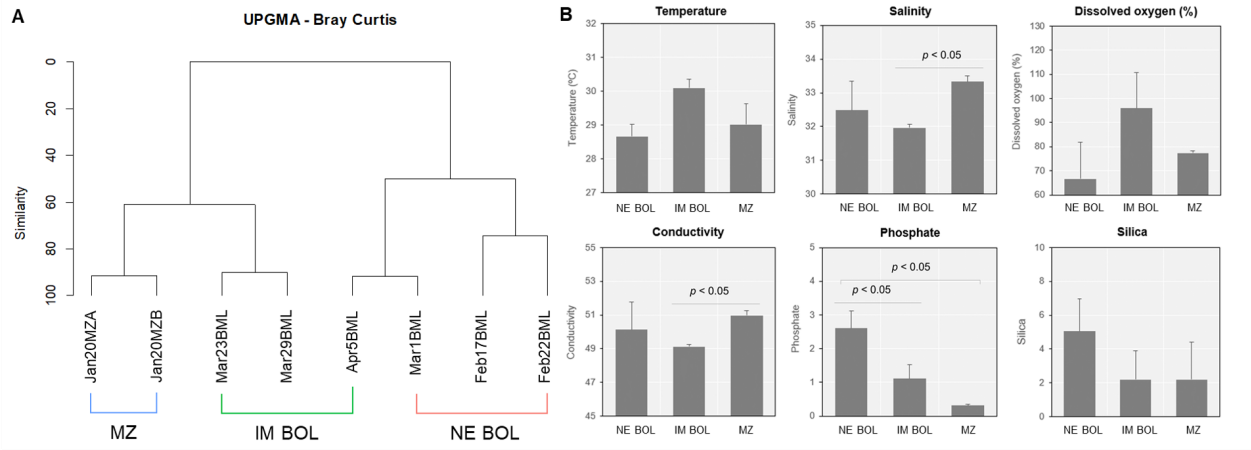

Figure 2. (A) UPGMA clustering of samples based on measured physicochemical parameters. (B) Bar graphs of physicochemical parameters (temperature, salinity, DO %, conductivity, phosphate, and silica).

higher salinity (MZ = 33.33 ± 0.26, BOL = 32.23 ± 0.78) (Figure 2B). However, the temperature during the January sampling was also in the same range as those observed in Bolinao from February–March (28.40–29.64 ºC), suggesting that it was still in the late NE monsoon period. The striking differences between the two sites could be attributed to their hydrography and bay topology. For example, unlike the GUI10 station – which was located within the Guiguiwanen channel and restricted by land masses and fish cages – Masinloc stations featured a wider opening and larger exposed area to the open sea waves (Figure 1). This allows more frequent flushing, thereby affecting the buildup of nutrients along the coast. Faster current flow rate aids the deposition of nutrient pore water to increase, which in turn assists a shorter residence period of nutrients in the water column (Serpetti *et al.* 2016). This was supported by the higher salinity of the Masinloc stations (Figure 2B, MZ: 33.3 ± 0.26 *vs.* BOL: 31.92 ± 0.78), possibly indicating a more-saline influenced water column than the GUI10 station inside the Guiguiwanen channel. The possible influence of submarine groundwater discharge has also been reported around Santiago Island (Senal *et al.* 2011). These differences between Masinloc and Bolinao could also drive variability in the patterns of occurrences of the core picophytoplankton communities with different adaptation mechanisms (Figure 3A; Appendix Figure I).

EPP Diversity and Community Structuring

Different alpha diversity indices (*i.e.* PD, Chao1, Observed OTUs) consistently indicated that the mean diversity of the total eukaryotic picoplankton was higher towards summer (mean PD = 8.32 ± 1.21; OTUs = 377 ± 33; Chao1 = 739.96 ± 17.50) than the late NE monsoon (mean PD = 2.66 ± 0.69; OTUs = 294 ± 49; Chao1 = 587.27 ± 84.03) (Figure 3B) within Bolinao. This was consistent with the observed clustering based on physicochemical parameters indicating short-term transitional change during monsoonal reversal (Figure 2).

Interestingly, although only sampled once in January, MZ samples had higher within site diversity than any of the Bolinao samples (Figure 3B), specifically for observed and rare OTUs (Chao1). However, MZ also had lower PD values than IM BOL samples despite having higher observed diversity. Unlike the first two indices, which do not usually consider the distinction between species, PD considers all the phylogenetic differences between the species based on the generated phylogenetic tree. This indicates that there were more OTUs that

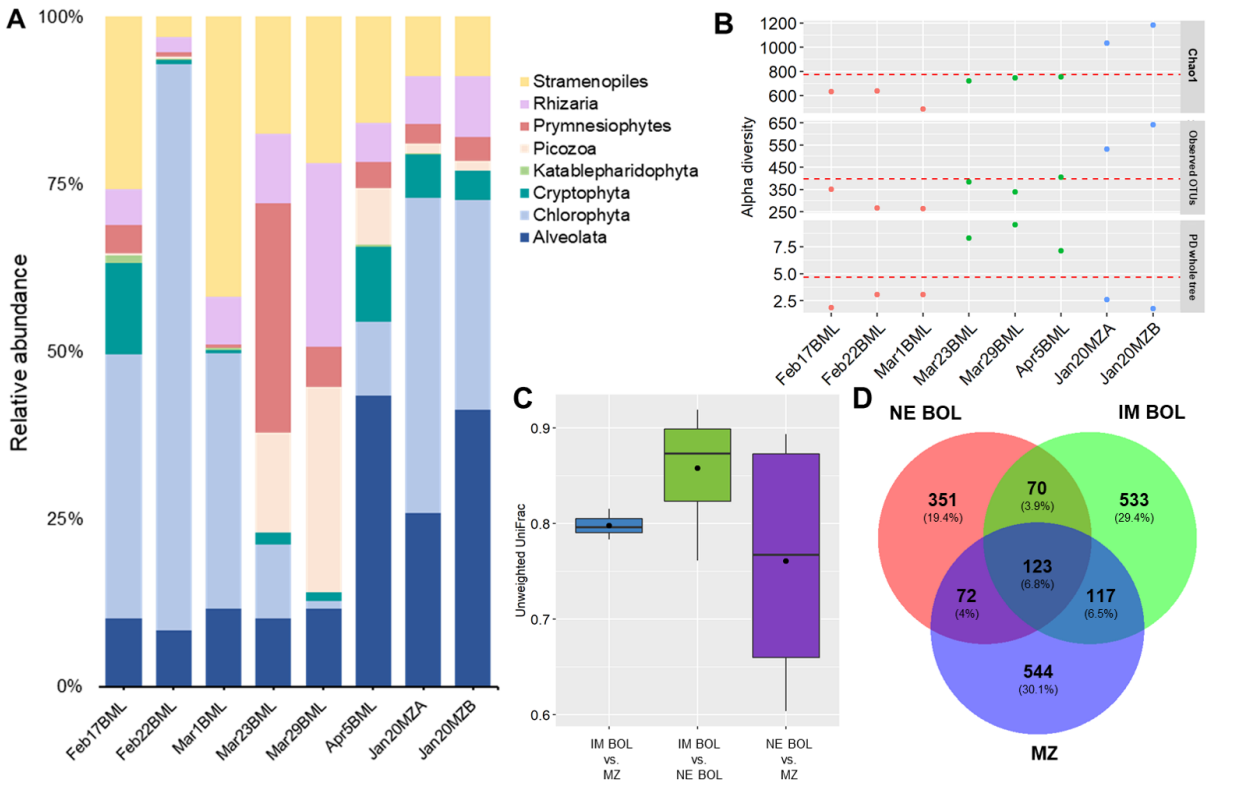

Figure 3. (A) Relative abundance of taxa per sampling period and sampling site; (B) alpha diversity (Faith's PD, observed OTUs, and Chao1) per site; (C) box plot for beta diversity (unweighted Unifrac) and (D) Venn diagram representing the shared and unique OTUs per sampling period and sampling site.

phylogenetically clustered together, which occurs during the environmental selection of favorable traits present in related taxa (Cavender-Bares *et al.* 2009), suggesting community structuring with similar adaptive traits as a response towards certain environmental factors.

At the community level (based on all picoplanktonic OTUs), NMDS ordination based on Bray-Curtis dissimilarity showed clustering of late NE BOL apart from IM BOL and MZ samples (Figure 4). Interestingly, no significant difference was observed between IM BOL and MZ (ANOSIM r = -0.031, $p > 0.05$) but a significant difference (ANOSIM r = 0.81, $p < 0.05$) was seen between sampling periods (NE *vs.* IM) in Bolinao, indicating distinct communities. Further, beta diversity using unweighted UniFrac (Figure 3C) strongly corroborated previous observations implying that the dissimilarity of eukaryotic picoplankton communities increased between these sampling periods, suggesting strong turnover with the change in seasons. Indeed, only ca. 6.8% of OTUs were shared among the samples (Figure 3D), consistent with the alpha diversity values across sampling points. These indices showed that late NE BOL was the least diverse as implied by the lowest phylogenetic relatedness (Faith's PD), rareness (chao1), and richness (observed OTUs) (Figure 3B). Taxonomic community composition with OTU frequencies of at least 50 also revealed the most represented picoplankton classes to be Chlorophyta, Prymnesiophytes, Alveolata, Cryptophyta, Picozoa, Rhizaria, Stramenopiles, and Katablepharidophyta (Figure 3A). The detection of known larger species of diatoms, dinoflagellates, ciliates, and rhizarians in the EPP fraction could be attributed to cell breakage during filtration (Goldman and Dennett 1985).

Potential Drivers of Community Structuring

Multiple linear regression showed temperature (R^2 = 0.63, p = 0.019) to be the only significantly negatively correlated physical driver with PC1 based on unweighted UniFrac, which also represents the strongest structuring of the community. Combined PC1 and PC2, however, only contributed to around ~ 43% of the variance observed. The temperature has also been cited to be a significant factor in seasonal shifts in microphytoplankton (Grover and Chrzanowski 2006; Barrera-Alba *et al.* 2019) and picoeukaryote communities (Wang *et al.* 2019; Jiang and Sun 2020). This was consistent with previous observations, where decreasing dissimilarity was accompanied by increasing temperature, signifying that the EPP community homogenizes as the temperature becomes warmer (Canini *et al.* 2013; Canini and Metillo 2017). Here, the increase in temperature towards summer was associated with lower Chlorophyta abundance

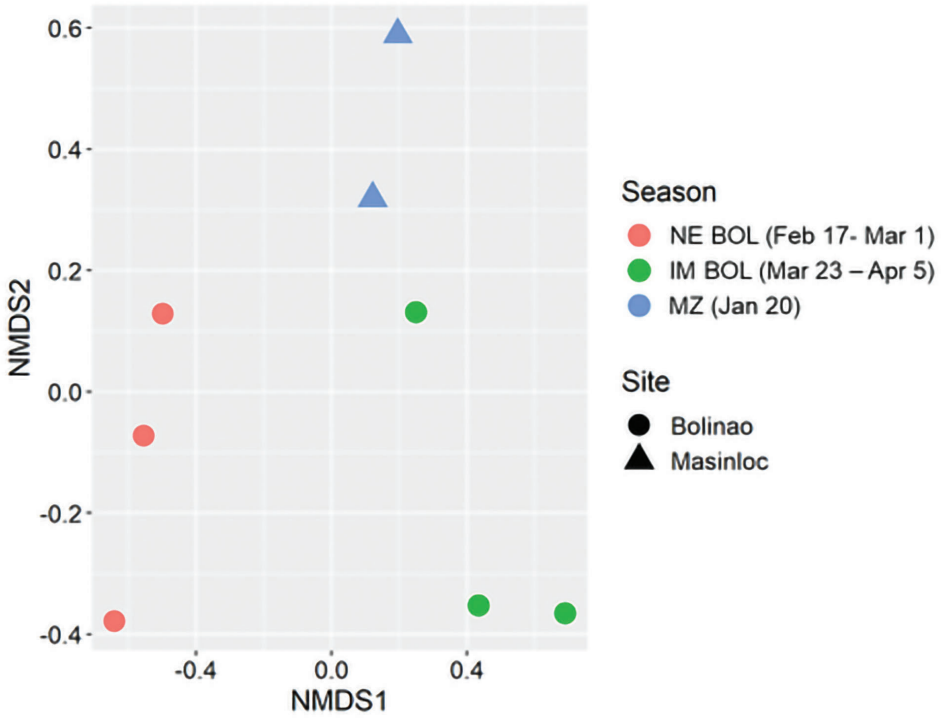

Figure 4. NMDS of Bray-Curtis dissimilarity of OTU abundances showing clustering of samples according to season (stress = 0.029).

(Spearman's rho: -0.72, $p < 0.05$; Appendix Figure II). However, the significant correlation with temperature might also indicate its effects on other parameters that are directly affecting different phytoplankton groups but were not measured in this study, such as nutrient availability and other physical processes.

Nutrients (P, Si) in the channel remain relatively high during the study period because of mariculture activities. Although not measured here, nitrate is also available for most of the year (~ 2–11 μM), though its concentration varies seasonally, with summer being reported to be usually low (Ferrera *et al.* 2016; Albelda *et al.* 2019). Beringuela *et al.* (2020) also reported that nitrate levels in Bolinao decreased from late NE monsoon (13.025 μM, February) to intermonsoon (5.183 μM, April). SiO_4^{4-} (5.04 ± 2.6 to 2.21±2.2 μM) and PO_4^{3-} (2.61 ± 0.7 to 1.11 ± 0.5 μM) also decreased significantly from late NE to IM (t-test, $p < 0.05$; Figure 2B) but were never depleted. In fact, P has always been in excess in the Guiguiwanen channel due to its continued release from the P-rich feeds being given to the cultured fishes in the pens. When left unconsumed, the P from the feeds is dissolved and released back into the environment (Ferrera *et al.* 2016). Since nitrogen (N) and P are consumed in a constant ratio of 16:1, excess in P leads to N limitation, which then also limits primary production towards the end of summer (Albelda *et al.* 2019; Ferrera *et al.* 2016). EPPs during this period still have an advantage because of their large surface area: volume ratio (Massana 2011), allowing them to still proliferate. However, the strong variability in resources and competition with other taxa during summer could drive high dissimilarity in the communities.

Other factors such as the prevailing wind conditions, solar radiation, and their implications to the water column might also affect EPP composition. Udarbe-Walker and Villanoy (2001) and Ferrera *et al.* (2016) specifically showed significant differences in the wind speed between late NE and IM periods in the northwestern Philippines. The higher wind speed in late NE monsoon (~ 1–2 m s^{-1}) could result in mixing that might not be favorable for larger species such as dinoflagellates (Berdalet 1992). This could have favored the dominance of a few photosynthetic taxa such as Stramenopiles and Chlorophyta (Figure 3A; Appendix Figure I), which accounted for the observed high richness or lower diversity during the NE monsoon. Towards IM, the lack of wind movement (~ 0 m s^{-1}) coupled with increased solar radiation (Ferrera *et al.* 2016) could have resulted in the growth of more taxa leading to an overall increase in diversity but lower richness during the summer period (Figures 2A and 3) similar to the patterns observed in Panquil Bay (Canini *et al.* 2013) and Sulu Sea (Miki *et al.* 2008) in the Philippines.

Biological interactions (*e.g.* predation, grazing, and competitive exclusion) can also influence picoplankton abundance and, thus, its community structuring (Zhao *et al.* 2016). As shown in Figure 3A, heterotrophic Picozoa (0.16% of total reads), Alveolates (9.58% of total reads), and Rhizaria (4.70% of total reads) were lower during late NE monsoon, with the community being significantly dominated by photosynthetic Chlorophyta (51.21% of the total reads) and Stramenopiles (22.39%). However, towards IM, other groups became more abundant (Picozoa – 16.96%, Alveolates – 20.39%, Rhizaria – 13.77% of total reads) than the chlorophytes (7.22% of total reads) but with increased abundance of Prymnesiophyta (13.79%), when nutrients were also lower. The increased prymnesiophytes during this period (IM BOL) is typical as they tend to dominate stratified conditions (Cabello *et al.* 2016), favoring their growth over larger species. These dominant OTUs may represent *K*-selected mixotrophic species that are competitive in nutrient-limited conditions during summer due to their capacity to utilize organic nutrients or grazing (Egge *et al.* 2015). The abundance of the other heterotrophic groups may also have led to the decline of Chlorophyta towards summer. The role of grazing in the shift of the community towards summer is demonstrated by the negative correlations of Chlorophyta with Picozoa (Spearman's rho: –0.73, $p < 0.05$) and Prymnesiophyta (Spearman's rho: –0.80, $p < 0.05$; Appendix Figure II), which are associated with parasitism and grazing (Unrein *et al.* 2014). Overall, shifts in these groups suggest community succession observed during the transition period from the late NE monsoon to IM in Bolinao.

Phylogenetic Placement and Genetic Diversity of Dominant Picophytoplankton

SIMPER analysis (Table 1) further revealed that Chlorophyta largely contributed to the differences observed between the two periods (NE *vs.* IM). Differences of NE BOL to other samples were highly driven by *Nannochloris* and diatoms, which were both dominant during the colder months. In contrast, *Ostreococcus* was shown to be the discriminating taxon in the MZ samples. In IM BOL samples, Picozoa, Alveolata, and Mamiellophyceae contributed greatly to differences between the two clustered periods. The dominance of Mamiellophyceae in the picoplankton fraction has been often observed (Not *et al.* 2008; Massana 2015). Here, we further focused on members of Mamiellophyceae (Chlorophyta) since although widely reported, these taxa have not yet been studied in the Philippine waters either using conventional imaging or molecular-based methods resulting in the limited global understanding of the species.

Table 1. Results of SIMPER analysis showing the ten most influential OTUs contributing to pairwise dissimilarity between NE BOL, IM BOL, and MZ samples.

Taxon	Most resolved taxonomy	Percent contribution to pairwise dissimilarity (overall dissimilarity)			Average abundance (reads)		
		NE BOL / IM BOL (88.19%)	NE BOL / MZ (80.07%)	MZ/ IM BOL (81.24%)	NE BOL	IM BOL	MZ
Chlorophyta	*Nannochloris*	14.82	15.42	0.94	1000.67	85.33	48.5
Chlorophyta	*Ostreococcus*	3.34	9.55	12.84	209.33	6	799
Bacillariophyta	*Arcocellulus*	8.48	8.33	0.20	534	10.67	19.5
Picozoa	Uncultured picozoa clone, NW617.02	3.44	0.20	3.24	1.67	214.33	14
Alveolata	MALV I	2.97	1.33	2.53	162	157.67	80
Picozoa	Uncultured picozoa clone, NW617.02	2.44	0.05	2.39	0.67	151.67	4
Alveolata	*Gyrodinium fusiforme*	0.83	1.47	1.07	7	57	97.5
Alveolata	MALV I	1.34	0.39	1.25	28.67	78.33	11
Prymnesiophyta	*Chrysochromulina*	1.34	0.22	1.27	1	83	14.5
Cercozoa	*Bigelowiella*	1.25	0.27	1.16	0.67	77.67	17.5

Results showed that *Nannochloris*, a member of Trebouxiophyceae, was the most abundant OTU (15.1% of total reads). It was found in all samples but largely dominant during the early intermonsoon period. Recent studies have revealed their potential to dominate coastal subtropical areas (Nelson *et al.* 2019) as well as phytoplankton blooms (Olsen and Mahoney 2001). Sequence data on Trebouxiophyceae from the Ocean Sampling Day project did not show any environmental preferences, but it was reported that this group alternatively replaces Mamiellophyceae as the dominant group in some coastal stations (Tragin and Vaulot 2018). However, the taxonomy of this genus remains to be resolved, and global data on their abundance in marine waters are still unavailable (Henley 2004; Vaulot 2008).

The second most abundant OTUs present were from *Ostreococcus,* another member of the group Mamiellophyceae. Five clades have been identified, including those belonging to the newly established *O. mediterraneus, O. tauri*, and *O. luciminarus* (formerly Clade A), as well as Clades B and E, which are yet to be formally described (Tragin and Vaulot 2019). Representative Clade E sequences, as described by Tragin and Vaulot (2019) from the Ocean Sampling Day (OSD) dataset, and a sequence from GenBank (accession number MH008654) reported as 100% similar to the Clade E ASV were included as representatives of this clade for this study. There was a total of 169 *de novo* sequences in the original OTU table with the assigned taxonomy of *Ostreococcus*. However, only 17 had more than 20 reads, which were then included in the phylogenetic analysis. The resulting phylogenetic tree shows the clustering of *de novo* OTUs with Clade B and Clade E reference sequences (Figure 5A). *Ostreococcus* was highly dominant, representing 84.8% of the total Mamiellophyceae reads. The genus appeared mostly during NE monsoon, as few reads were present in the IM BOL samples (Figure 5B). In our dataset, Clade B represented at least 8% of total Mamiellophyceae reads, while Clade E accounted for at least 72%. The most abundant *Ostreococcus* OTU (denovo1), appearing in both Bolinao and Masinloc samples, was assigned to Clade E. This clade was first identified by Tragin and Vaulot (2019) as a distinct sequence having two bp difference from Clade B. Clade E is described to dominate in coastal warm temperate regions, although its presence in the dataset suggests that it can also dominate in coastal tropical regions. Clade B on the other hand was first described as a low-light adapted clade (Rodriguez *et al.* 2005), present in warm oligotrophic sites.

Further, the alignment of *Ostreococcus* V4 region with the main signatures (Figure 5C) revealed some novel point mutations that were not present in the reference sequences. The representative sequences of OTUs from this study featured several mismatches with many of the reference sequences. These OTUs were present in both Bolinao and Masinloc samples, suggesting that these were not just sequenced artifacts as they were occurring in more than one site and sample. In the generated ML tree, the generated sequences also formed a separate cluster (Figure 5) with 91% bootstrap support. This may suggest a different clade altogether apart from Clades B and E present in the West Philippine Sea, here referred

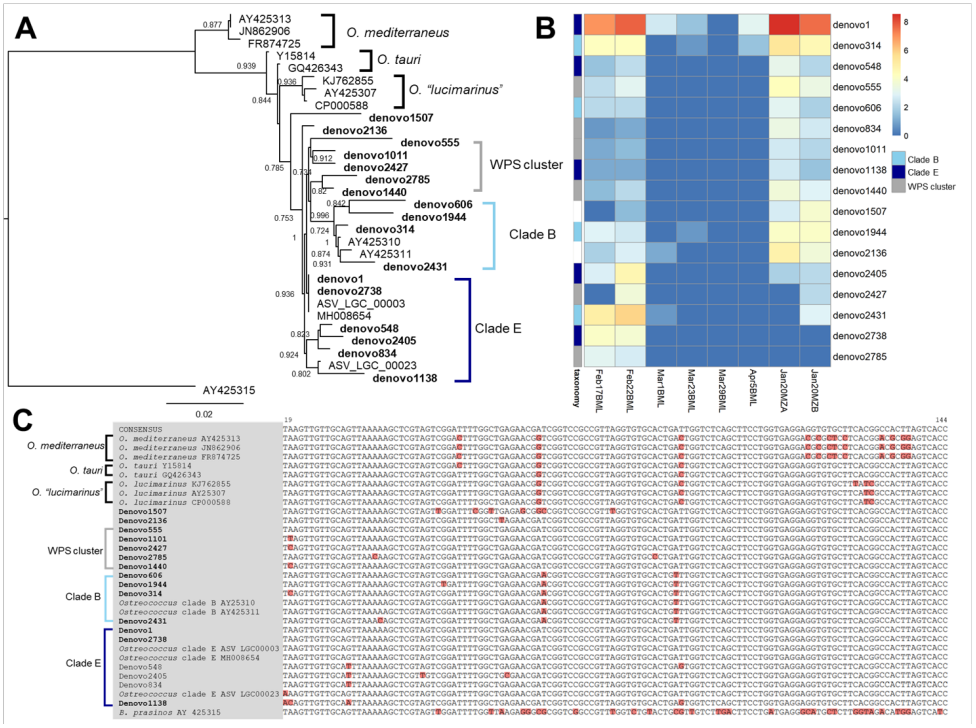

Figure 5. (A) Maximum likelihood analysis of *Ostreococcus* sequences derived from this study (shown in boldface) as well as reference sequences (accession numbers are shown) using FastTree. The tree is rooted in the outgroup *Bathycoccus prasinos*. Bootstrap values less than 70% are not shown. (B) Heatmap of *Ostreococcus* OTU occurrence across samples. (C) Segment of the sequence alignment (342 bp) of *Ostreococcus* sequences.

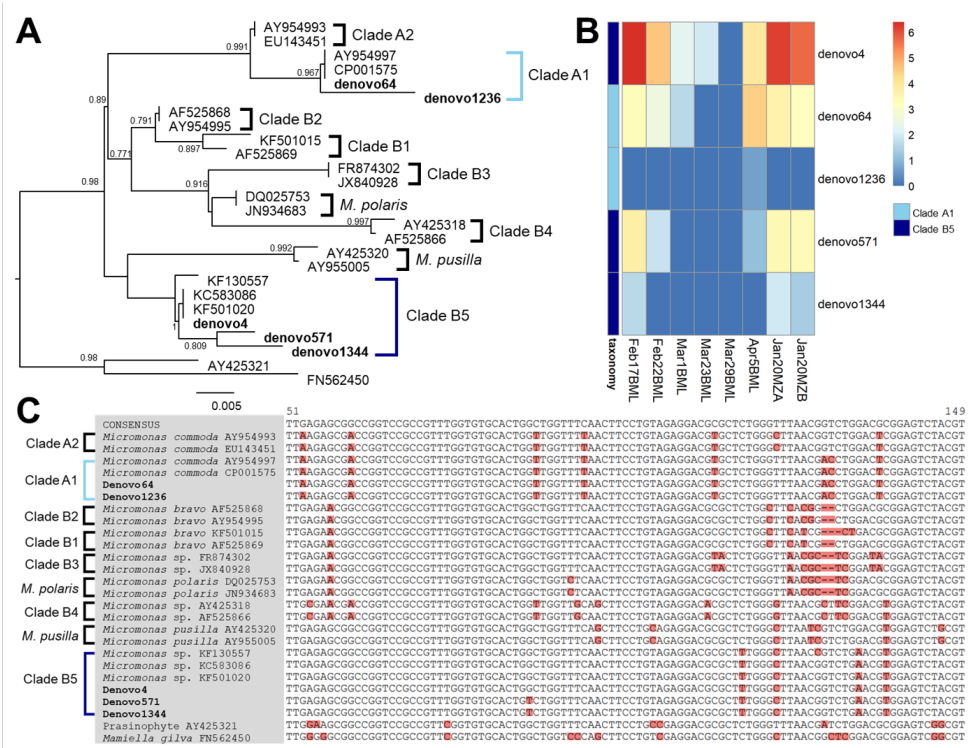

Figure 6. (A) Maximum likelihood analysis of *Micromonas* sequences derived from this study (shown in boldface) as well as reference sequences (accession numbers are shown) using FastTree. The tree is rooted in the outgroup *Mamiella gilva*. Bootstrap values less than 70% are not shown. (B) Heatmap of OTU occurrence across samples. (C) Sequence alignment (326 bp) of *Micromonas* sequences.

to as the "WPS Cluster." The OTUs denovo1507 and denovo2136 also have distinct branching patterns. To the best of our knowledge, this is the first report of a sequence-based *Ostreococcus* diversity in the Philippines. Further sequence studies coupled with microscopy and single-cell isolation techniques or culturing of *Ostreococcus* in the Philippines may help delineate its taxonomy and validate observations of this study.

Another member of the class Mamiellophyceae, *Micromonas*, was present in both sites. Five OTUs were identified as *Micromonas* through taxonomic assignment and sequence similarity search (Figure 6A). Phylogenetic placement showed the assignment of the OTUs as Clade A1 (*Micromonas commoda)* and *Micromonas sp.* Clade B5 (Figure 6A). *M. commoda* Clade A1 is known to be well-distributed in tropical and subtropical waters, although it may also be described as ubiquitous (Tragin and Vaulot 2019). *Micromonas* sp. Clade B5, on the other hand, is notably distributed in tropical and warm waters and has been detected off Singapore and Taiwan, both in the South China Sea (Wu *et al.* 2014b; Lin *et al.* 2017b; Tragin and Vaulot 2019; Chénard *et al.* 2019). In our dataset, *Micromonas* was dominant in Masinloc samples as well as in late NE BOL samples. An OTU designated as Clade B5 (denovo4) was locally dominant in one NE BOL sample (Feb17BML), as well as the MZ samples (Figure 6B). This clade accounted for 11% of the total Mamiellophyceae reads in the dataset.

Our results point to Chlorophyta as a major component of the picophyoplankton community of Masinloc and Bolinao during late NE to summer periods. In particular, the prasinophytes *Micromonas* and *Ostreococcus* were found to be highly dominant in both sites. Interestingly, class Mamiellophyceae was less abundant in early intermonsoon samples, which had higher recorded temperatures (29.8–30.5 °C) compared to NE BOL and Masinloc samples (28.3–29.6 °C). In one sample (IM BOL), no OTUs from Mamiellophyceae were detected. This agrees with the global distribution pattern described by Demir-Hilton *et al.* (2011), wherein seawater temperatures were significantly higher (26 ± 3 °C) in sites with no detection of *Ostreococcus*, compared to those with detected presence (22 ± 3 °C). Studies of the diversity of chlorophytes reveal geographic patterns and distinct ecotypes that may help us understand their roles in the marine ecosystem.

CONCLUSIONS AND RECOMMENDATIONS

Our results showed that picophytoplankton possibly exhibit short-term temporal shifts, similar to the well-studied microphytoplankton groups. Specifically, in the West Philippine Sea, a significant shift in EPP community structuring was observed possibly associated with the changes in conditions during the transition from the NE to IM periods. Our results also showed that species belonging to Mamiellophyceae contributed the most to the observed short-term changes. Phylogenetic analysis further revealed the presence of distinct clades of the dominant chlorophytes *Ostreococcus* Clades B and E in both Bolinao and Masinloc samples. Further, we observed a potential novel clade (WPS Cluster) that is distinct from the previously reported clades, although further studies are needed to validate this claim. Similarly, *Micromonas* Clades A1 and B5 were detected in all the sampling sites. To the best of our knowledge, this is the first report of these major picophytoplankton in Philippine waters, suggesting their underappreciated significance and unexplored potential "hidden" diversity, which warrants further studies.

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REFERENCES

ALBELDA R, PURGANAN DJ, GOMEZ NC, NARVARTE BC, CALALANG PC, GENOVIA TG, GERNATO EG, BONDOC KG, SAN DIEGO-MCGLONE ML, ONDA DFL. 2019. Summer phytoplankton community structure and distribution in a mariculture-affected coastal environment. Philippine Science Letters 12(2): 157–166.

ARONESTY E. 2013. Comparison of sequencing utility programs. The Open Bioinformatics Journal 7(1).

AZANZA RV, TAYLOR FM. 2001. Are *Pyrodinium* blooms in the Southeast Asian region recurring and

spreading? A view at the end of the millennium. AMBIO: A Journal of the Human Environment 30(6): 356–364.

BARRERA-ALBA JJ, ABREU PC, TENENBAUM DR. 2019. Seasonal and inter-annual variability in phytoplankton over a 22-year period in a tropical coastal region in the southwestern Atlantic Ocean. Continental Shelf Research 176: 51–63.

BAULA IU, AZANZA RV, FUKUYO Y, SIRINGAN FP. 2011. Dinoflagellate cyst composition, abundance and horizontal distribution in Bolinao, Pangasinan, Northern Philippines. Harmful Algae 11: 33–44.

BEHRENFELD MJ, WORTHINGTON K, SHERRELL RM, CHAVEZ FP, STRUTTON P, MCPHADEN M, SHEA DM. 2006. Controls on tropical Pacific Ocean productivity revealed through nutrient stress diagnostics. Nature 442(7106): 1025–1028.

BERDALET E. 1992. Effects of turbulence on the marine dinoflagellate *Gymnodinium nelsonii* 1. Journal of Phycology 28(3): 267–272.

BERINGUELA RT, PURGANAN DJE, AZANZA RV, ONDA DFL. 2020. Spatio-temporal variation and association of epiphytic dinoflagellate-diatom assemblages on selected red algae (Rhodophyceae). The European Journal of Phycology DOI: 10.1080/09670262.2020.1740797

BONACHELA JA, KLAUSMEIER CA, EDWARDS KF, LITCHMAN E, LEVIN SA. 2015. The role of phytoplankton diversity in the emergent oceanic stoichiometry. Journal of Plankton Research 38(4): 1021–1035. doi:10.1093/plankt/fbv087

BUITENHUIS ET, VOGT M, MORIARTY R, SWAN C, BEDNARSEK N, DONEY S, ..., O'BRIEN T. 2013. MAREDAT: towards a world ocean atlas of marine ecosystem data. Earth System Science Data Discussions 5(2): 1077–1106.

BUITENHUIS ET, LI WK, VAULOT D, LOMAS MW, LANDRY M, PARTENSKY F, ..., LANTOINE F. 2012. Picophytoplankton biomass distribution in the global ocean. 1foldr Import 2019-10-08 Batch 6.

CABELLO AM, LATASA M, FORN I, MORÁN XAG, MASSANA R. 2016. Vertical distribution of major photosynthetic picoeukaryotic groups in stratified marine waters. Environmental Microbiology 18(5): 1578–1590. doi:10.1111/1462-2920.13285

CAMPBELL L, VAULOT D. 1993. Photosynthetic picoplankton community structure in the subtropical North Pacific Ocean near Hawaii (station ALOHA). Deep Sea Research Part I: Oceanographic Research Papers 40(10): 2043–2060.doi:10.1016/0967-0637(93)90044-4

CANINI ND, METILLO EB, AZANZA RV. 2013. Monsoon-influenced phytoplankton community structure in a Philippine mangrove estuary. Tropical Ecology 54(3): 331–343.

CANINI ND, METILLO EB. 2017. Temporal changes in the community structure of phytoplankton in Panguil Bay, Philippine mangrove estuary. Aquaculture, Aquarium, Conservation & Legislation 10(2): 410–420.

CAPORASO JG, KUCZYNSKI J, STOMBAUGH J, BITTINGER K, BUSHMAN FD, COSTELLO EK, ..., HUTTLEY GA. 2010. QIIME allows analysis of high-throughput community sequencing data. Nature Methods 7(5): 335–336.

CARON DA, COUNTWAY PD, SAVAI P, GAST RJ, SCHNETZER A, MOORTHI SD, ..., JONES AC. 2009. Defining DNA-based operational taxonomic units for microbial-eukaryote ecology. Appl Environ Microbiol 75(18): 5797–5808.

CAVENDER-BARES J, KOZAK KH, FINE PV, KEMBEL SW. 2009. The merging of community ecology and phylogenetic biology. Ecology Letters 12(7): 693–715.

CHEN J, BITTINGER K, CHARLSON ES, HOFFMANN C, LEWIS J, WU GD *et al.* 2012. Associating microbiome composition with environmental covariates using generalized UniFrac distances. Bioinformatics 28: 2106–2113. doi: 10.1093/bioinformatics/bts342

CHÉNARD C, WIJAYA W, VAULOT D, DOS SANTOS AL, MARTIN P, KAUR A, LAURO FM. 2019. Temporal and spatial dynamics of Bacteria, Archaea and protists in equatorial coastal waters. Scientific Reports 9(1): 1–13.

CHUST G, IRIGOIEN X, CHAVE J, HARRIS RP. 2013. Latitudinal phytoplankton distribution and the neutral theory of biodiversity. Global Ecology and Biogeography 22(5): 531–543.

COMEAU AM, LI WK, TREMBLAY JÉ, CARMACK EC, LOVEJOY C. 2011. Arctic Ocean microbial community structure before and after the 2007 record sea ice minimum. PLOS ONE 6(11).

COMEAU AM, DOUGLAS GM, LANGILLE MGI. 2017. Microbiome Helper: a custom and streamlined workflow for microbiome research. mSystems 2: e00127-16. doi:10.1128/mSystems.00127-16

DÍEZ B, PEDRÓS-ALIÓ C, MARSH TL, MASSANA R. 2001. Application of denaturing gradient gel electrophoresis (DGGE) to study the diversity of marine picoeukaryotic assemblages and comparison of DGGE with other molecular techniques. Applied and Environmental Microbiology 67(7): 2942–2951.

DEMIR-HILTON E, SUDEK S, CUVELIER ML, GENTEMANN CL, ZEHR JP, WORDEN AZ. 2011. Global distribution patterns of distinct clades of the photosynthetic picoeukaryote Ostreococcus. The ISME Journal 5(7): 1095–1107.

DE VARGAS C, AUDIC S, HENRY N, DECELLE J, MAHÉ F, LOGARES R, ..., CARMICHAEL M. 2015. Eukaryotic plankton diversity in the sunlit ocean. Science 348(6237): 1261605.

DOS SANTOS AL, GOURVIL P, TRAGIN M, NOËL MH, DECELLE J, ROMAC S, VAULOT D. 2017. Diversity and oceanic distribution of prasinophytes clade VII, the dominant group of green algae in oceanic waters. The ISME Journal 11(2): 512–528.

EDGAR RC. 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26(19): 2460–2461.

EGGE ES, JOHANNESSEN TV, ANDERSEN T, EIKREM W, BITTNER L, LARSEN A, ..., EDVARDSEN B. 2015. Seasonal diversity and dynamics of haptophytes in the Skagerrak, Norway, explored by high-throughput sequencing. Molecular Ecology 24(12): 3026–3042.

FERRERA CM, WATANABE A, MIYAJIMA T, SAN DIEGO-MCGLONE ML, MORIMOTO N, UMEZAWA Y, NADAOKA K. 2016. Phosphorus as a driver of nitrogen limitation and sustained eutrophic conditions in Bolinao and Anda, Philippines, a mariculture-impacted tropical coastal area. Marine Pollution Bulletin 105(1): 237–248.

GOLDMAN JC, DENNETT MR. 1985. Susceptibility of some marine phytoplankton species to cell breakage during filtration and post-filtration rinsing. Journal of Experimental Marine Biology and Ecology 86(1): 47–58.

GROVER JP, CHRZANOWSKI TH. 2006. Seasonal dynamics of phytoplankton in two warm temperate reservoirs: association of taxonomic composition with temperature. Journal of Plankton Research 28(1): 1–17.

HARRELL FE. 2020. with contributions from Charles Dupont and many others (2017). "Hmisc: Harrell Miscellaneous". R package version 4.0-1.

HENLEY WJ, HIRONAKA JL, GUILLOU L, BUCHHEIM MA, BUCHHEIM JA, FAWLEY MW, FAWLEY KP. 2004. Phylogenetic analysis of the 'Nannochloris-like'algae and diagnoses of *Picochlorum oklahomensis* gen. et sp. nov. (Trebouxiophyceae, Chlorophyta). Phycologia 43(6): 641–652.

JIANG ZY, SUN FL. 2020. Diversity and biogeography of picoplankton communities from the Straits of Malacca to the South China Sea. Oceanological and Hydrobiological Studies 49(1): 23–33.

KATOH K, STANDLEY DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution 30(4): 772–780.

KEARSE M, MOIR R, WILSON A, STONES-HAVAS S, CHEUNG M, STURROCK S, THIERER T. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28(12): 1647–1649.

KOLDE R. 2019. pheatmap: Pretty Heatmaps. R package version 1.0.12. https://CRAN.R-project.org/package=pheatmap

LI WKW, RAO DVS, HARRISON WG, SMITH JC, CULLEN JJ, IRWIN B, PLATT T. 1983. Autotrophic Picoplankton in the Tropical Ocean. Science 219(4582): 292–295. doi:10.1126/science.219.4582.292

LIN YC, CHIANG KP, KANG LK. 2017a. Community composition of picoeukaryotes in the South China Sea during winter. Continental Shelf Research 143: 91–100.

LIN YC, CHUNG CC, CHEN LY, GONG GC, HUANG CY, CHIANG KP. 2017b. Community composition of photosynthetic picoeukaryotes in a subtropical coastal ecosystem, with particular emphasis on Micromonas. Journal of Eukaryotic Microbiology 64(3): 349–359.

LOGARES R, SHALCHIAN-TABRIZI K, BOLTOVSKOY A, RENGEFORS K. 2007. Extensive dinoflagellate phylogenies indicate infrequent marine–freshwater transitions. Mol Phylo Evol 45: 887–903.

LÓPEZ-GARCÍA P, RODRIGUEZ-VALERA F, PEDRÓS-ALIÓ C, MOREIRA D. 2001. Unexpected diversity of small eukaryotes in deep-sea Antarctic plankton. Nature 409(6820): 603–607.

LOVEJOY C, COMEAU A, THALER M. 2016. Curated reference database of SSU rRNA for northern marine and freshwater communities of archaea, bacteria and microbial eukaryotes, v. 1.1. Nordicana D23. doi: 10.5885/45409XD-79A199B76BCC4110

MASSANA R. 2015. Protistan diversity in environmental molecular surveys. In: Marine Protists. Tokyo: Springer. p. 3–21.

MASSANA R. 2011. Eukaryotic picoplankton in surface oceans. Annual Review of Microbiology 65: 91–110.

MIKI M, RAMAIAH N, TAKEDA S, FURUYA K. 2008. Phytoplankton dynamics associated with the monsoon in the Sulu Sea as revealed by pigment signature. Journal of Oceanography 64(5): 663–673.

MORÁN XAG, CALVO-DÍAZ A, DUCKLOW HW. 2010. Total and phytoplankton mediated bottom-up control of bacterioplankton change with temperature

in NE Atlantic shelf waters. Aquatic Microbial Ecology 58(3): 229–239.

NELSON DR, CHAIBOONCHOE A, FU W, HAZZOURI KM, HUANG Z, JAISWAL A, ..., SALEHIASHTIANI K. 2019. Potential for Heightened Sulfur-Metabolic Capacity in Coastal Subtropical Microalgae. iScience 11: 450–465.

NOT F, LATASA M, SCHAREK R, VIPREY M, KARLESKIND P, BALAGUÉ V, ONTORIA-OVIEDO I, CUMINO A, GOETZE E, VAULOT D, MASSANA R. 2008. Protistan assemblages across the Indian Ocean, with a specific emphasis on the picoeukaryotes. Deep Sea Research Part I: Oceanographic Research Papers 55(11): 1456–1473. doi:10.1016/j.dsr.2008.06.007

OKSANEN JF, BLANCHET G, FRIENDLY M, KINDT R, LEGENDRE P, MCGLINN D, MINCHIN PR, O'HARA RB, SIMPSON GL, SOLYMOS P, STEVENS H, SZOECS E, WAGNER H. 2019. vegan: Community Ecology Package. R package version 2.5-6. https://CRAN.R-project.org/package=vegan

OLSEN PS, MAHONEY JB. 2001. Phytoplankton in the Barnegat Bay—Little Egg Harbor estuarine system: species composition and picoplankton bloom development. Journal of Coastal Research 115–143.

ONDA DF, MEDRINAL E, COMEAU AM, THALER M, BABIN M, LOVEJOY C. 2017. Seasonal and interannual changes in ciliate and dinoflagellate species assemblages in the Arctic Ocean (Amundsen Gulf, Beaufort Sea, Canada). Frontiers in Marine Science 4: 16.

ONDA DFL, SANTOS MAG, DELA CRUZ-PAPA D, YNIGUEZ A, SIRINGAN MA, AZANZA RV. 2019. Microbial oceanography studies in the context of climate change in the Philippines. Philippine Science Letters 12(1): 9–23.

PAN LA, ZHANG LH, ZHANG J, GASOL JM, CHAO M. 2005. On-board flow cytometric observation of picoplankton community structure in the East China Sea during the fall of different years. FEMS Microbiology Ecology 52(2): 243–253. doi:10.1016/j.femsec.2004.11.019

PARSONS TR, MAITA Y, LALLI CMI. 1984. A manual of chemical and biological methods for seawater analysis. Oxford, UK: Pergamon Press. p. 1–73.

PEREZ MT, DOLAN JR, RASSOULZADEGAN F, MOSTAJIR B. 1996. Predation on marine picoplankton populations examined with an 'add-in'approach. Journal of Plankton Research 18(4): 635–641.

PRICE MN, DEHAL PS, ARKIN AP. 2010. FastTree 2 – Approximately Maximum Likelihood Trees for Large Alignments. PLoS ONE 5(3): e9490. doi:10.1371/journal.pone.0009490

RODRIGUEZ F, DERELLE E, GUILLOU L, LE GALL F, VAULOT D, MOREAU H. 2005. Ecotype diversity in the marine picoeukaryote *Ostreococcus* (Chlorophyta, Prasinophyceae. Environmental Microbiology 7(6): 853–859.

ROMARI K, VAULOT D. 2004. Composition and temporal variability of picoeukaryote communities at a coastal site of the English Channel from 18S rDNA sequences. Limnology and Oceanography 49(3): 784–798.

SAN DIEGO-MCGLONE ML, AZANZA RV, VILLANOY CL, JACINTO GS. 2008. Eutrophic waters, algal bloom and fish kill in fish farming areas in Bolinao, Pangasinan, Philippines. Marine Pollution Bulletin 57(6–12): 295–301.

SENAL MIS, JACINTO GS, SAN DIEGO-MCGLONE ML, SIRINGAN F, ZAMORA P, SORIA L, ..., CABRERA O. 2011. Nutrient inputs from submarine groundwater discharge on the Santiago reef flat, Bolinao, Northwestern Philippines. Marine Pollution Bulletin 63(5–12): 195–200.

SERPETTI N, WITTE UFM, HEATH MR. 2016. Statistical modeling of variability in sediment-water nutrient and oxygen fluxes. Frontier Earth Science 4:65. doi: 10.3389/feart.2016.00065

STAWIARSKI B, BUITENHUIS ET, FALLENS M. 2018. The physiological response of seven strains of picophytoplankton to light, and its representation in a dynamic photosynthesis model. Limnology and Oceanography 63(S1): S367–S380.

TRAGIN M, VAULOT D. 2018. Green microalgae in marine coastal waters: The Ocean Sampling Day (OSD) dataset. Scientific Reports 8(1): 1–12.

TRAGIN M, VAULOT D. 2019. Novel diversity within marine Mamiellophyceae (Chlorophyta) unveiled by metabarcoding. Scientific Reports 9(1): 5190.

UDARBE-WALKER MJB, VILLANOY CL. 2001. Structure of potential upwelling areas in the Philippines. Deep Sea Research Part I: Oceanographic Research Papers 48(6): 1499–1518.

UNREIN F, GASOL JM, NOT F, FORN I, MASSANA R. 2014. Mixotrophic haptophytes are key bacterial grazers in oligotrophic coastal waters. The ISME Journal 8(1): 164–176.

VAULOT D, EIKREM W, VIPREY M, MOREAU H. 2008. The diversity of small eukaryotic phytoplankton (≤ 3 μm) in marine ecosystems. FEMS Microbiol-

ogy Reviews 32(5): 795–820. doi:10.1111/j.1574-6976.2008.00121.x

WANG JJ, TANG DL. 2014. Phytoplankton patchiness during spring intermonsoon in western coast of South China Sea. Deep Sea Research Part II: Topical Studies in Oceanography 101: 120–128.

WANG F, XIE Y, WU W, SUN P, WANG L, HUANG B. 2019. Picoeukaryotic diversity and activity in the northwestern Pacific Ocean based on rDNA and rRNA high-throughput sequencing. Frontiers in Microbiology 9(3259). https://doi.org/10.3389/fmicb.2018.03259

WICKHAM H. 2016. ggplot2: Elegant Graphics for Data Analysis. New York: Springer-Verlag.

WU W, HUANG B, ZHONG C. 2014a. Photosynthetic picoeukaryote assemblages in the South China Sea from the Pearl River estuary to the SEATS station. Aquatic Microbial Ecology 71(3): 271–284. doi:10.3354/ame01681

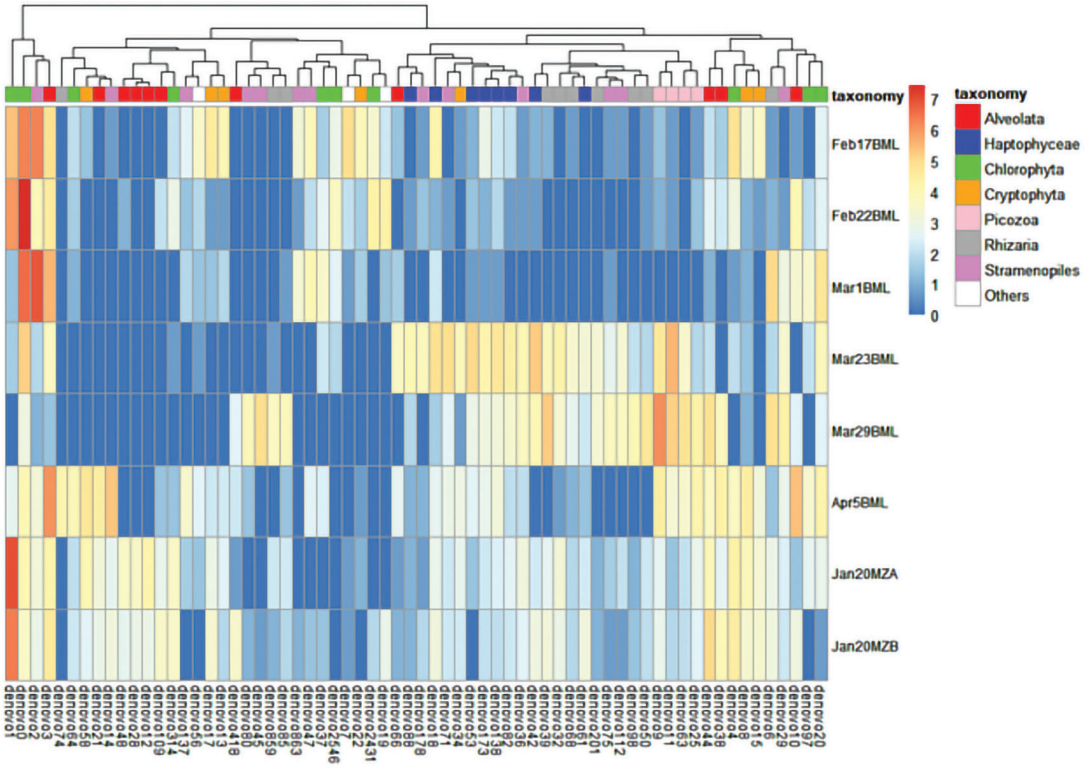
WU W, HUANG B, LIAO Y, SUN P. 2014b. Picoeukaryotic diversity and distribution in the subtropical-tropical South China Sea. FEMS Microbiology Ecology 89(3): 563–579.

ZHANG F, HE J, LIN L, JIN H. 2015. Dominance of picophytoplankton in the newly open surface water of the central Arctic Ocean. Polar Biology 38(7): 1081–1089. doi:10.1007/s00300-015-1662-7

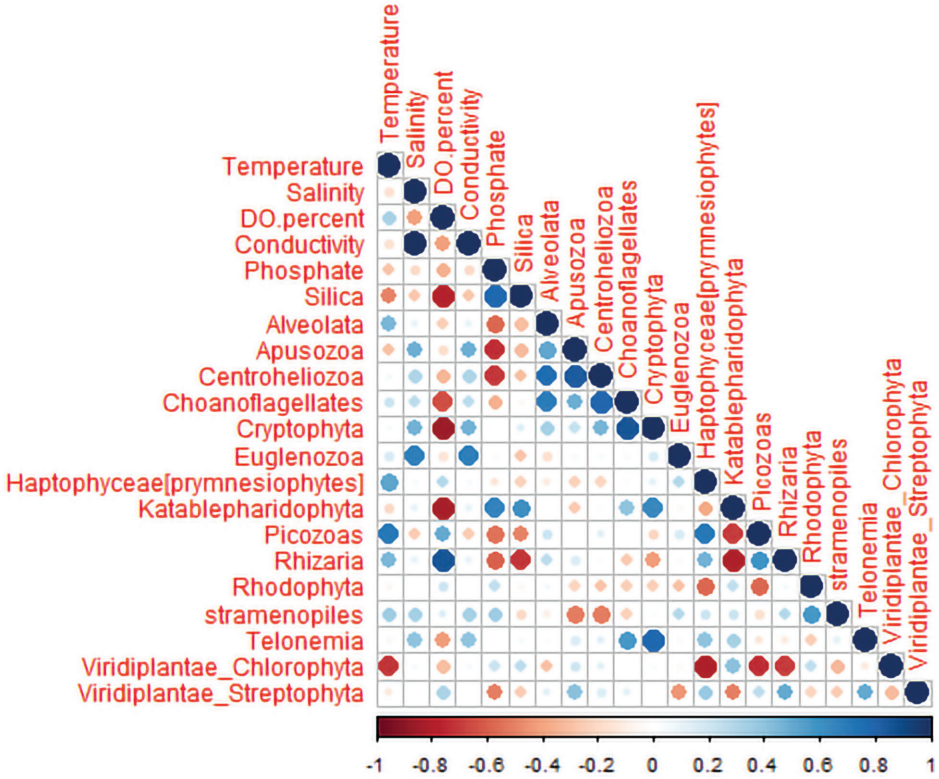
ZHANG X, SHI Z, YE F, ZENG Y, HUANG X. 2013. Picophytoplankton abundance and distribution in three contrasting periods in the Pearl River Estuary, South China. Marine and Freshwater Research 64(8): 692. doi:10.1071/mf12303

ZHAO S, WEI J, YUE H, XIAO T. 2010. Picophytoplankton abundance and community structure in the Philippine Sea, western Pacific. Chinese Journal of Oceanology and Limnology 28(1): 88–95.

ZHAO L, ZHAO Y, XU J, ZHANG W, HUANG L, JIANG Z, FANG J, XIAO T. 2016. Distribution and seasonal variation of picoplankton in Sanggou Bay, China. Aquaculture Environment Interactions 8: 261–271.



Appendix Figure I. Heatmap of the top OTU reads among the samples. Taxonomic annotation is shown.



Appendix Figure II. Spearman's correlation between environmental parameters and taxa.