Sharp transitions in phytoplankton communities across estuarine to open ocean waters of the tropical Pacific

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Running Title: Spatiotemporal shifts in phytoplankton

Abstract

Islands in the tropical Pacific supply elevated nutrients to nearshore waters that enhance phytoplankton biomass and create hotspots of productivity in otherwise nutrient-poor oceans. Despite the importance of these hotspots in supporting nearshore food webs, the fine-scale spatial and temporal variability of phytoplankton enhancement and changes in the underlying phytoplankton communities across nearshore to open ocean systems remain poorly understood. In this study, a combination of flow cytometry, pigment analyses, 16S rRNA gene amplicons, and metagenomic sequencing provide a synoptic view of phytoplankton dynamics over a four-year, near-monthly time-series across coastal Kāneʻohe Bay, Hawaiʻi, spanning from an estuarine Indigenous aquaculture system to the adjacent offshore environment. Through comparisons with measurements taken at Station ALOHA located in the oligotrophic North Pacific Subtropical Gyre, we elucidated a sharp and persistent transition between picocyanobacterial communities, from *Synechococcus* abundant in the nearshore to *Prochlorococcus* proliferating in offshore and open ocean waters. In comparison to immediately adjacent offshore waters and the surrounding open ocean, phytoplankton biomass within Kānʻeohe Bay was dramatically elevated. While phytoplankton community composition revealed strong seasonal patterns, phytoplankton biomass positively correlated with wind speeds, rainfall, and wind direction, and not water temperatures. These findings reveal sharp transitions in ocean biogeochemistry and phytoplankton dynamics across estuarine to open ocean waters in the tropical Pacific and provide a foundation for quantifying deviations from baseline conditions due to ongoing climate change.

Introduction

In marine ecosystems, phytoplankton play a crucial role by forming the base of the aquatic food web, where their productivity, abundance, cell size, and community composition are greatly influenced by light and nutrient availability (Azam et al. 1983). Surrounded by oligotrophic, open ocean waters, the coastal waters of remote islands in the tropical Pacific harbor a sharp increase in nutrients through physical oceanographic, biological, geological, and anthropogenic processes that results in increased phytoplankton biomass, cell size, and productivity (i.e. the Island Mass Effect, or IME; Doty and Oguri 1956; Gove et al. 2016). The enhanced primary productivity in turn promotes secondary productivity, supporting regional fisheries (Stock et al. 2017), increased biodiversity (Messié et al. 2022), and other marine resources relied upon by island communities.

Given the importance of elevated phytoplankton biomass from near-island coastal waters to maintaining healthy and productive coastal food webs, understanding the fine-scale variability of phytoplankton communities across coastal to open ocean systems adjacent to island masses can inform both the management of local marine environments and larger ecosystem models. Currently, phytoplankton biomass and productivity in the open oceans are rapidly changing. One result of increasing sea surface temperatures due to ongoing global climate change is an increase in the intensity of water column stratification of the open ocean, which can trap nutrients at depths below where phytoplankton at the ocean's surface can access them (Li et al. 2020). This has led to an expansion of nutrient-poor "ocean deserts" in the open ocean gyres and a decline in global phytoplankton biomass and primary productivity (Kwiatkowski et al. 2018). Declines in primary productivity will likely be amplified across the trophic food web in the near future, with an expected 14% decline in zooplankton biomass as soon as 2100 (Kwiatkowski et al. 2018) and a 20% decline in global fisheries by 2300 (Moore et al. 2018).

Small phytoplankton such as the two most abundant phytoplankton globally, *Prochlorococcus* and *Synechococccus*, are expected to increase in abundance under ocean warming conditions and decreased nutrient availability at the expense of larger sized phytoplankton groups (Flombaum et al. 2020). Small phytoplankton are often too small to be effectively grazed by metazoans, and are consumed by intermediary microzooplankton grazers who are then fed upon by larger zooplankton (Calbet and Landry 1999). Systems dominated by small phytoplankton have longer food chains and potentially a reduced energy transfer efficiency to higher trophic levels (Eddy et al. 2021). In contrast, large phytoplankton like diatoms can be consumed directly by zooplankton grazers (Calbet and Landry 1999) such as copepods, so that forage fish are only one trophic level apart from phytoplankton. Thus, changes in phytoplankton size structure and community composition also have profound implications for food web dynamics.

Unfortunately, the effects of increased open ocean stratification on food webs and biological productivity in adjacent coastal environments is uncertain. In part, this is due to studies predominantly focusing on coastal or oceanic systems in isolation (Xenopoulos et al. 2017), but also because the satellite-based methods that have led to an extensive understanding of primary productivity in the global open ocean have not yet been developed for shallow coastal waters (Carswell et al. 2017). Importantly, coastal marine food webs of islands situated in oligotrophic waters may be particularly vulnerable to the impacts of open ocean stratification.

Defining how nutrient availability and phytoplankton community composition and biomass vary with space and time in near-island and adjacent open ocean environments can provide a foundation for quantifying deviations from baseline conditions and predicting food web shifts because of climate change. To illuminate the factors influencing phytoplankton

communities across near-island to open ocean environments in the tropical Pacific, this study examined the effect of spatial and temporal variability in biogeochemical conditions on phytoplankton communities across multiple habitats that link the coastal environment of O‘ahu, Hawai‘i, with the offshore. These habitats span a tidally-influenced, estuarine environment within an Indigenous aquaculture system, through the interior of coastal Kāne‘ohe Bay, and to the offshore ocean environment surrounding Kāne‘ohe Bay. We also made comparisons to data collected by the Hawaii Ocean Time-series (HOT), a 30+ year time-series initiative measuring temporal trends of the adjacent ultraoligotrophic North Pacific Subtropical Gyre (NPSG; Karl and Church 2014). Together, this extensive spatial and temporal coverage revealed dramatic nearshore enhancement of phytoplankton biomass, pronounced seasonality in nearshore biogeochemistry and phytoplankton biomass and composition, and distinct transitions in phytoplankton communities spanning <6 km to >100 km across Kāne‘ohe Bay to the NPSG.

Materials and Methods

Study location

The Hawaiian archipelago within the oligotrophic NPSG is the world’s most remote island chain. Kāne‘ohe Bay, located on the windward side of the island of O‘ahu (21° 28′ N, 157° 48′ W), is a well-studied, coral-reef-dominated embayment (**Fig. 1a**). The bay has a total surface area of 41.4 km^2 and is approximately 4.3 km wide, 12.8 km in length, and 10 m deep on average (Jokiel 1991). Sharp nearshore to offshore gradients in biogeochemical parameters occur over a short distance (<6 km) along with a diverse topography due to patch, fringing, and barrier reefs (Jokiel 1991; Tucker et al. 2021). Localized freshwater input from streams contribute to episodic spatial variability in environmental conditions, including salinity and inorganic nutrient concentrations (Cox et al. 2006; Yeo et al. 2013; Tucker et al. 2021). Water residence time

within the bay varies from less than a day to over one month (Lowe et al. 2009), with the highest residence times in the sheltered southern lobe. Oceanic water, primarily driven by wave action, flows into the bay over a large barrier reef located in the central bay (Lowe et al. 2009). Water is generally transported out of the bay through two nearly-parallel channels positioned in the southern and northern portions of the bay. For most of the year, the bay is well mixed by tradewinds. However, periods of high temperatures and low wind speeds can cause vertical stratification in the water column (Smith 1981).

Collaboratively developed research

At the mouth of He‘eia Stream in the southern section of Kāne‘ohe Bay is an ~800-year-old, 0.356 km^2 Indigenous aquaculture system known contemporarily as He‘eia Fishpond, but anciently as Pihi Loko I‘a (Kelly 1973). Indigenous aquaculture systems in Hawai‘i engaged in trophic engineering to promote primary productivity that sustained the population through abundant food fish and reef fish (Winter et al. 2020a). A 2.5 km basalt rock wall filled with coral rubble encompasses He‘eia Fishpond. The wall is equipped with multiple sluice gates that increase water residence time while still allowing for exchange between coastal Kāne‘ohe Bay and He‘eia stream waters. The sluice gate (mākāhā) system allows juvenile fish to flourish within the high nutrient environment of the fishpond, while protecting them from predators. While it has been understood that the fishpond provided the perfect nursery habitat for prized food fish, little is known about the exchange of organisms from within the fishpond to the rest of Kāne‘ohe Bay, and beyond. With this interest expressed by the Indigenous stewards of He‘eia Fishpond, we sought to establish a current, baseline understanding of phytoplankton biomass and community composition from within the estuarine fishpond environment out to open ocean

waters adjacent to Oʻahu. The methods employed in this study were collaboratively developed with Heʻeia Fishpond stewards and the Heʻeia National Estuarine Research Reserve (NERR; Winter et al. 2020b). All individuals who collaboratively developed the methods also collaborated on interpreting the data and are listed as co-authors on this paper. Sampling campaigns were conducted with permission from Paepae o Heʻeia, the stewards of Heʻeia Fishpond, and the private landowner, Kamehameha Schools.

Sample collection and environmental parameters

Between August 2017 and June 2021, seawater was collected from a depth of 2 m at 10 sites in Kāneʻohe Bay and the adjacent offshore waters on a near-monthly basis (36 sampling events over 46 months) as part of the Kāneʻohe Bay Time-series (KByT) using previously described methods (**Fig. 1a, Supporting Information Table S1**; Tucker et al. 2021). Between September 2020 and June 2021, two additional stations within Heʻeia Fishpond were also sampled at a quarterly interval **(Fig. 1a**): Station Wai2 (now known as Waimāʻama), located in the northwestern corner of the Fishpond within the Heʻeia Stream mouth (known as muliwai) is a highly turbid and brackish water environment with tidal fluctuations resulting in salinity ranges of 10-35 ppt. Station Kahoʻokele (Kaho) is located at a sluice gate facing the ocean and receives high exchange with coastal Kāneʻohe Bay (~30-35 ppt, Möhlenkamp et al. 2019). At all stations, seawater samples for biogeochemical analyses and nucleic acids were collected, as were *in situ* measurements of seawater temperature, pH, and salinity with a YSI 6600 or ProDSS multi-parameter sonde (YSI Incorporated, Yellow Springs, OH, USA). Approximately one liter of seawater was prefiltered with 85-µm Nitex mesh and subsequently filtered through a 25-mm diameter, 0.1-µm pore-sized polyethersulfone (PES) filter membrane (Supor-100, Pall Gelman

158 Inc., Ann Arbor, MI, USA) to collect microbial cells for DNA isolation. The filters were

159 subsequently submerged in DNA lysis buffer (Suzuki et al. 2001; Yeo et al. 2013) and stored in

160 −80°C until further processing.

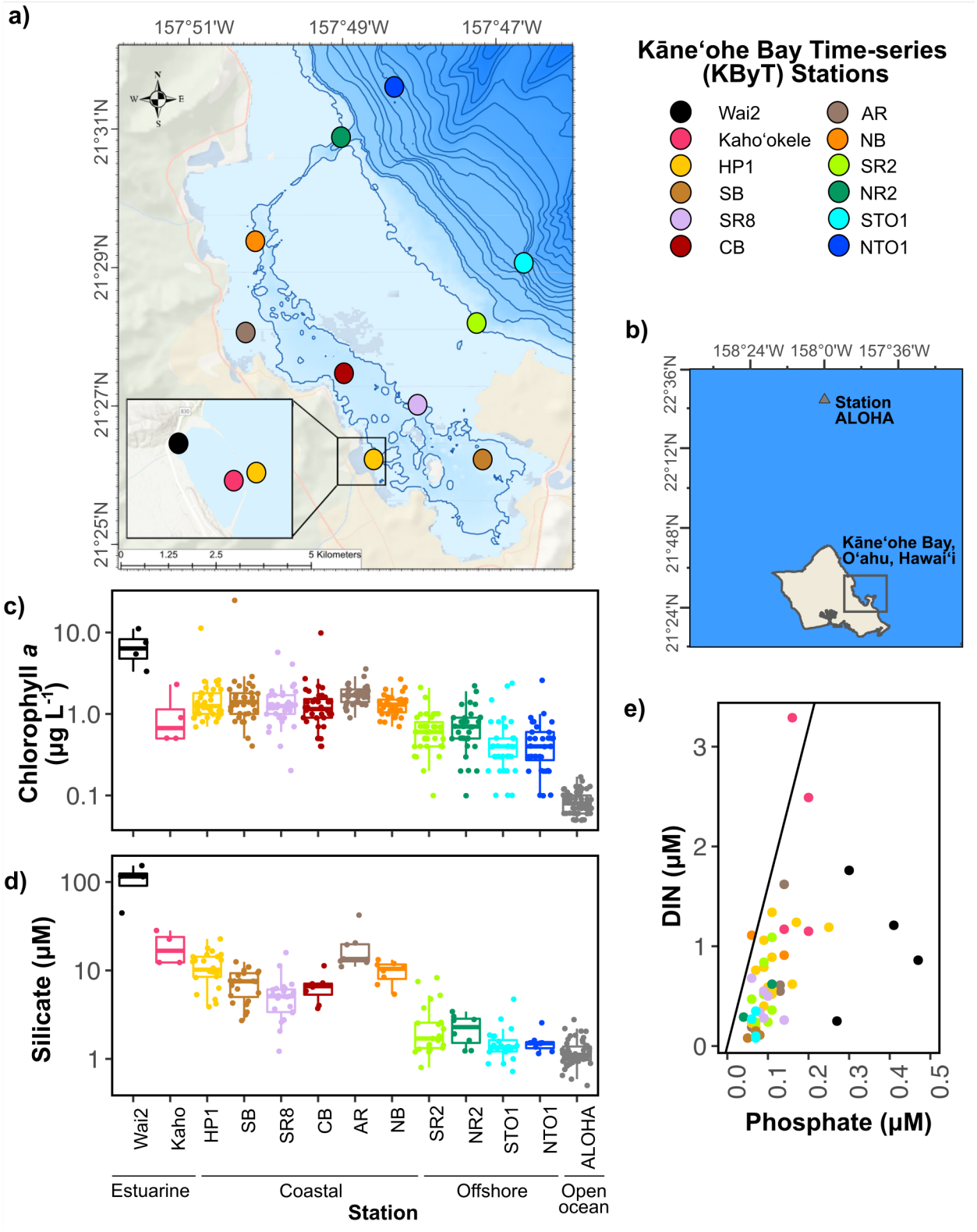

Fig. 1. a) Map of sampling stations located on the windward side of the island of O'ahu Hawai'i. Inset shows two stations within He'eia Fishpond and one immediately adjacent to the fishpond.

Contour lines mark every ten meters up until 50 m and then 50 m intervals for depths >50 m. b) Location of Kāneʻohe Bay on Oʻahu, Hawaiʻi and the position of Station ALOHA, a sampling station of the Hawaii Ocean Time-series (HOT) program. c) Chlorophyll *a* and d) silicate concentrations (both plotted on a log scale) from the estuarine to open ocean environments examined in this study. e) Ratios of dissolved inorganic nitrogen (DIN):Phosphate. Diagonal line denotes Redfield ratio of 16:1 suggesting nitrogen limitation is characteristic of the system.

Seawater subsamples for fluorometric chlorophyll *a* concentrations (125 mL) and photosynthetic pigments via high-performance liquid chromatography (HPLC; 2 L) were collected on 25-mm diameter GF/F glass microfiber filters (Whatman, GE Healthcare Life Sciences, Chicago, IL, USA) and stored in aluminum foil at −80°C until extraction. The collection of phytoplankton pigments on the GF/F glass microfiber filters allow for comparisons with the Hawaii Ocean Time-series data. However, because the filters have a pore size of 0.7µm, we acknowledge that most small cyanobacteria were likely missed. Chlorophyll *a* was extracted with 100% acetone and measured with a Turner 10-AU fluorometer (Turner Designs, Sunnyvale, CA, USA) following standard techniques (Welschmeyer 1994). Photosynthetic pigments measured via high performance liquid chromatography were extracted in 100% acetone and analyzed on a Waters 2690 separations module equipped with a C18 column and full spectrum photodiode array detector, following (Mantoura and Llewellyn 1983) and modified according to (Bidigare et al. 1989). Chlorophyll *a* concentrations measured via the fluorometer are herein referred to chlorophyll *a* (Chla), while chlorophyll *a* concentrations measured by high performance liquid chromatography are specified as total chlorophyll *a* (TChla).

For cellular enumeration, seawater was preserved in 2 mL aliquots in a final concentration of 0.95% (v:v) paraformaldehyde (Electron Microscopy Services, Hatfield, PA, USA) at −80 °C until analyzed via flow cytometry. Cellular enumeration of cyanobacterial picophytoplankton (*Synechococcus* and *Prochlorococcus*), eukaryotic picophytoplankton, and non-cyanobacterial (presumably heterotrophic) bacteria and archaea (hereafter referred to as heterotrophic bacteria) was performed on a Beckman Coulter CytoFLEX S, following the method of (Monger and Landry 1993). Inorganic nutrients were measured using a Seal Analytical AA3 HR Nutrient Autoanalyzer (detection limits: $NO_2^- + NO_3^-$, 0.009 μM; SiO_4, 0.09 μM; PO_4^{3-}, 0.009 μM; NH_4, 0.03 μM).

Rainfall, wind speed, and wind direction were monitored using data collected at a meteorological station located at the Hawai‘i Institute of Marine Biology (HIMB) on Moku o Lo‘e in Kāne‘ohe Bay (http://www.pacioos.hawaii.edu/weather/obs-mokuoloe/). Either maximum (e.g., rainfall, wind speed) or average (wind direction) values were taken across 1- to 7-day windows leading up to the sampling event, depending on data availability from the station. One sampling event (February 5, 2021) had no data for the 7 days prior to sampling and so the data for a 30-day window were used. Metadata from Station ALOHA (22° 45’N, 158° 00’W, ~5 m depth, August 2017 to December 2020), a sampling station of the HOT program (Karl and Church 2014), were downloaded from https://hahana.soest.hawaii.edu/hot/hot-dogs/ (accessed on 9/12/2022).

Spatiotemporal comparisons of environmental variables, cellular abundances, and phytoplankton pigments were conducted using the R package ‘multcomp’ (Hothorn et al. 2008) with one-way ANOVAs testing for multiple comparisons of means with Holm correction and Tukey contrasts. Summer (28 June through 28 September) and winter (27 December through 29

March) seasons were defined using harmonic regression analyses of surface seawater temperature collected hourly between 2010–2019 at NOAA station MOKH1 in Kāneʻohe Bay (https://www.ndbc.noaa.gov/station_page.php?station=mokh1; Tucker et al. 2021). Spatiotemporal variation in cellular abundances and phytoplankton pigments were visualized using 'mba.surf' from MBA (Finley et al. 2017) to interpolate data over the KByT sampling events and stations.

The map of Kāneʻohe Bay was plotted in ArcGIS Pro v2.9. Contour lines were drawn using bathymetry metadata (http://www.soest.hawaii.edu/hmrg/multibeam/bathymetry.php). The map of Oʻahu was plotted in R with 'geom_sf' from ggplot2 (Wickham 2016), using shape file from the Hawaii Statewide GIS Program (https://prod-histategis.opendata.arcgis.com/maps/HiStateGIS::coastline).

DNA extraction, 16S rRNA gene amplicon sequencing, & metagenome sequencing

DNA extraction and 16S rRNA gene sequencing followed previously published methods (Tucker et al. 2021). Briefly, amplicon libraries were made from polymerase chain reactions of the 16S rRNA gene using barcoded 515F and 926R universal primers (Parada et al. 2016) and paired-end sequenced with MiSeq v2 2x250 technology (Illumina, San Diego, CA, USA). Genomic DNA from a subset of 32 of the 368 total samples collected between 2017-2021 were used for metagenomic sequencing. This included samples from four sampling events between 2017 and 2019 at 6-10 stations. Libraries were constructed from approximately 100 ng of genomic DNA using the Kappa HyperPrep Kit (Roche, Pleasanton, CA, USA) with mechanical shearing (Covaris, Woburn, MA, USA) and paired-end sequenced on a single lane of the NovaSeq 6000 SP 150 (Illumina, San Diego, CA, USA).

232 **Sequence analysis**

233 Amplicon sequence data generated from KByT sampling between July 2019 and June
234 2021 were analyzed in conjunction with previously published amplicon data spanning August
235 2017 to June 2019 (PRJNA706753; (Tucker et al. 2021). For each of the two sequencing runs,
236 samples were demultiplexed and quality controlled using Qiime2 v2.4 (Bolyen et al. 2019). Full
237 length forward reads (251 base pairs) were denoised using DADA2 (Callahan et al. 2019) to
238 delineate amplicon sequencing variants (ASVs). Reverse reads were not used because of
239 inconsistent quality. ASVs were assigned taxonomy using SILVA v138 as a reference database
240 (Quast et al. 2012) and the two runs were subsequently merged in Qiime2. ASVs that contained
241 at least 10 reads in at least two samples were retained.

242 ASVs classified by the SILVA v138 database as Eukaryota, unassigned at the domain
243 level, or classified as chloroplast at the order level were re-classified using the PR2 v 4.14.0
244 database (Guillou et al. 2013) in the DECIPHER R package (Wright 2016) using a 60%
245 confidence threshold cut off. Sequences classified as Bacteria, Archaea, or chloroplast at the
246 order-level were retained for further analyses, while those unclassified at the domain level or
247 classified as Eukaryota were excluded from further analyses. In the context of amplicon
248 sequence data, "phytoplankton" herein refers to ASVs classified as cyanobacteria and eukaryotic
249 plastid sequences, although we recognize that mixotrophic and phagotrophic lifestyles may be
250 included in this broad definition.

251 Statistical analyses were conducted using the R packages phyloseq (McMurdie and
252 Holmes 2013), ggplot2 (Wickham 2016), pheatmap (Kolde 2019), and microbiome (Lahti and
253 Shetty 2017). An Aitchison distance (Aitchison 1982), the Euclidean distance between centered
254 log-ratio (clr)-transformed compositions, was used on the entire quality-controlled dataset of

255 phytoplankton raw read counts using 'transform' in the microbiome package (Lahti and Shetty
256 2017). Ward D2 hierarchical clustering using 'hclust ( )' in the stats base package of R was
257 applied to this matrix to cluster samples with amplicon data into groups and visualized with
258 dendextend (Galili 2015). DESeq2 (Love et al. 2014) was used to model differential abundance
259 patterns of amplicon data across environmental clusters using Wald Tests and Bonferroni
260 correction for multiple comparisons (alpha cutoff < 0.05). Divnet (Willis and Martin 2020) was
261 used to estimate differences in alpha diversity and test for significance between spatiotemporal
262 groupings. Pearson's correlation analyses were conducted in corrplot (Wei and Simko 2021).

263 Lomb Scargle Periodograms (LSP) in the lomb package (Ruf 1999) were used to define
264 seasonality among phytoplankton genera by determining the spectrum of frequencies in a
265 dataset: this approach can account for unevenly sampled time-series data and has been
266 previously applied to microbiome time-series analyses (Auladell et al. 2021). Only genera with
267 annual intervals (peak frequency = 1±0.25, $p<0.01$) as their most significant periodic trend were
268 considered as having seasonality. A starting frequency of 0.16 was used so as to not include
269 periodic components between two consecutive months. An inverse hyperbolic sine
270 transformation (asinh) was conducted on sequence data prior to LSP. Significance (q-
271 values<0.05) was corrected for multiple-testing using data randomization for LSP analyses using
272 fdrtools (Strimmer 2008).

273 **Metagenomic read recruitment**

274 To investigate the dominant cyanobacteria within and surrounding Kāne'ohe Bay, we
275 conducted metagenomic read recruitment of 32 metagenomes from KByT and 12 previously
276 published from the open ocean Station ALOHA (PRJNA352737; Mende et al. 2017) to 56
277 cyanobacterial genomes from *Prochlorococcus* (six minor clades) and the three major lineages of

278 the marine *Synecchococcus*/*Cyanobium* lineage [SC 5.1 (14 minor clades), SC 5.2, and SC 5.3]
279 (**Supporting Information Table S2**). SC 5.2 is the only clade with both *Synechococcus* and
280 *Cyanobium* members (Doré et al. 2020).

281 A contig database of the 56 cyanobacteria isolate genomes was constructed using anvi'o
282 v 8.0 (Eren et al. 2021) following previously described pipelines (Delmont and Eren 2018).
283 Briefly, Prodigal v2.6.3 (Hyatt et al. 2010) was used to identify open reading frames (ORFs)
284 from the contigs and an anvi'o database was created using 'anvi-gen-contigs-db'. Metagenomic
285 reads were first quality filtered using an Illumina-utils library v1.4.1 called 'iu-filterquality-
286 minoche' (Eren et al. 2013) that uses quality filtering parameters described previously (Minoche
287 et al. 2011). Quality filtered metagenomic reads were competitively mapped with Bowtie2 v2.3.5
288 (Langmead and Salzberg 2012) to an anvi'o contig database of cyanobacterial isolate genomes.
289 The 'anvi-profile' function stored coverage and detection statistics of each cyanobacterial
290 genomes found in the KByT and Station ALOHA metagenomic samples.

291 To evaluate the distribution of individual genomes, a "detection" metric, the proportion
292 of the nucleotides in a given sequence that are covered by at least one short read, was used to
293 evaluate if a population was present in a metagenomic sample. A detection value of at least 0.25
294 was used as a criterion to eliminate false positives, when an isolate genome was falsely found
295 within a sample (Utter et al. 2020). Mean coverage Q2Q3, which refers to the average depth of
296 coverage excluding nucleotide positions with coverages in the 1st and 4th quartiles, was mapped
297 for each genome. Mean coverage Q2Q3 was summed across all cyanobacterial genomes per
298 sample and then the genome (or all genomes in a clade) was divided by this sum to determine a
299 relative abundance of a genome (or a clade) in each sample. Average nucleotide identity (ANI)
300 was calculated using pyANI (Pritchard et al. 2015). A phylogenomic tree was estimated from the

56 cyanobacterial isolates and an outgroup using GTotree v1.4.16 (Lee 2019) with cyanobacterial single-copy genes and visualized in FigTree v 1.4.4 (http://tree.bio.ed.ac.uk/).

Data & code availability

Sequencing data are available in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under BioProject number PRJNA706753 as well as PRJNA971314. Environmental data were submitted to BCO-DMO under https://www.bco-dmo.org/project/663665. Code used in the analysis is available at https://github.com/tucker4/Tucker_Phytoplankton_KByT_HeNERR.

Results

Biogeochemical parameters

Along the nearshore to open ocean waters of the tropical Pacific, biogeochemical parameters sharply declined across both small spatial scales and vast stretches of ocean (**Supporting Information Table S1**). On average, chlorophyll *a* concentrations at stations in the coastal waters of Kāneʻohe Bay increased 18-fold (1.6±1.9 *vs.* 0.09±0.03 μg L^{-1}, mean±sd) from the open ocean and 3-fold (1.6±1.9 *vs.* 0.6±0.4 μg L^{-1}) from the immediately adjacent offshore waters. The estuarine waters of Heʻeia Fishpond harbored higher chlorophyll *a* concentrations compared to coastal stations (3.9±3.8 *vs.* 1.6±1.9 μg L^{-1}, **Fig. 1c**). Mean chlorophyll *a* concentrations increased 43-fold between the estuarine waters of Heʻeia Fishpond and the open ocean (3.9±3.8 *vs.* 0.09±0.03 μg L^{-1}, **Supporting Information Table S1**) and 7-fold over the <6 km distance covering the interior and surrounding waters of Kāneʻohe Bay (3.9±3.8 *vs.* 0.6±0.4 μg L^{-1}; **Fig. 1c**, **Supporting Information Table S1**). Stations offshore from Kāneʻohe Bay had elevated concentrations of chlorophyll *a* compared to the open ocean (7-fold increase; 0.6±0.4 *vs.* 0.09±0.03 μg L^{-1}). Elevated phytoplankton biomass was a persistent feature within Kāneʻohe

Bay, with increased chlorophyll *a* concentrations detected in at least one of the stations positioned in the coastal environment compared to the stations offshore during all 36 sampling events (**Supporting Information Fig. S1**).

Elevated concentrations of inorganic nutrients (silicate, nitrate+nitrite, phosphate, ammonia) were also found in the nearshore waters of Kāneʻohe Bay compared to offshore and open ocean stations (**Supporting Information Table S1 & S3**). Mean silicate concentrations at Wai2 of the estuarine stations were 107.3 µM, compared to 42.2 µM in the coastal stations. Across all KByT stations, phosphate and silicate concentrations differed significantly but were positively correlated with increasing chlorophyll *a* concentrations. However, nitrate+nitrite concentrations did not correlate with chlorophyll *a* concentrations (**Supporting Information Fig. S2**). Despite the overall increase of inorganic nutrients in the estuarine and coastal stations, all stations were below 16:1 N:P ratios using dissolved inorganic nitrogen (nitrate+nitrite plus ammonia) and phosphate (**Fig. 1e**, (Redfield 1960).

Microbial cell counts and phytoplankton pigments

In contrast to coastal stations where *Synechococcus* cellular abundance was high, *Prochlorococcus* cellular abundance was elevated in the stations positioned in the offshore waters surrounding Kāneʻohe Bay and at Station ALOHA (**Fig. 2a, Supporting Information Table S1 & S3**). Cellular abundances of heterotrophic bacteria and eukaryotic picophytoplankton were also greater in the coastal stations compared to offshore (**Fig. 2a, Supporting Information Table S1 & S3**). In coastal Kāneʻohe Bay, ratios of fucoxanthin, peridinin, and alloxanthin to total chlorophyll *a* (Tchla) concentrations were higher than in the offshore, indicating an increase in diatoms, dinoflagellates, and cryptophytes (respectively)

closer to shore (**Fig. 2b, Supporting Information Table S4**). In contrast, pigments relative to Tchla for photosynthetic pigments diagnostic of prymnesiophytes (19′-hexanoyloxyfucoxanthin), pelagophytes (19′-butanoyloxyfucoxanthin), cyanobacteria (zeaxanthin), and *Prochlorococcus* (divinyl chlorophyll *a*) were higher in the offshore stations compared to the coastal environment (**Fig. 2b**, **Supporting Information Table S4**).

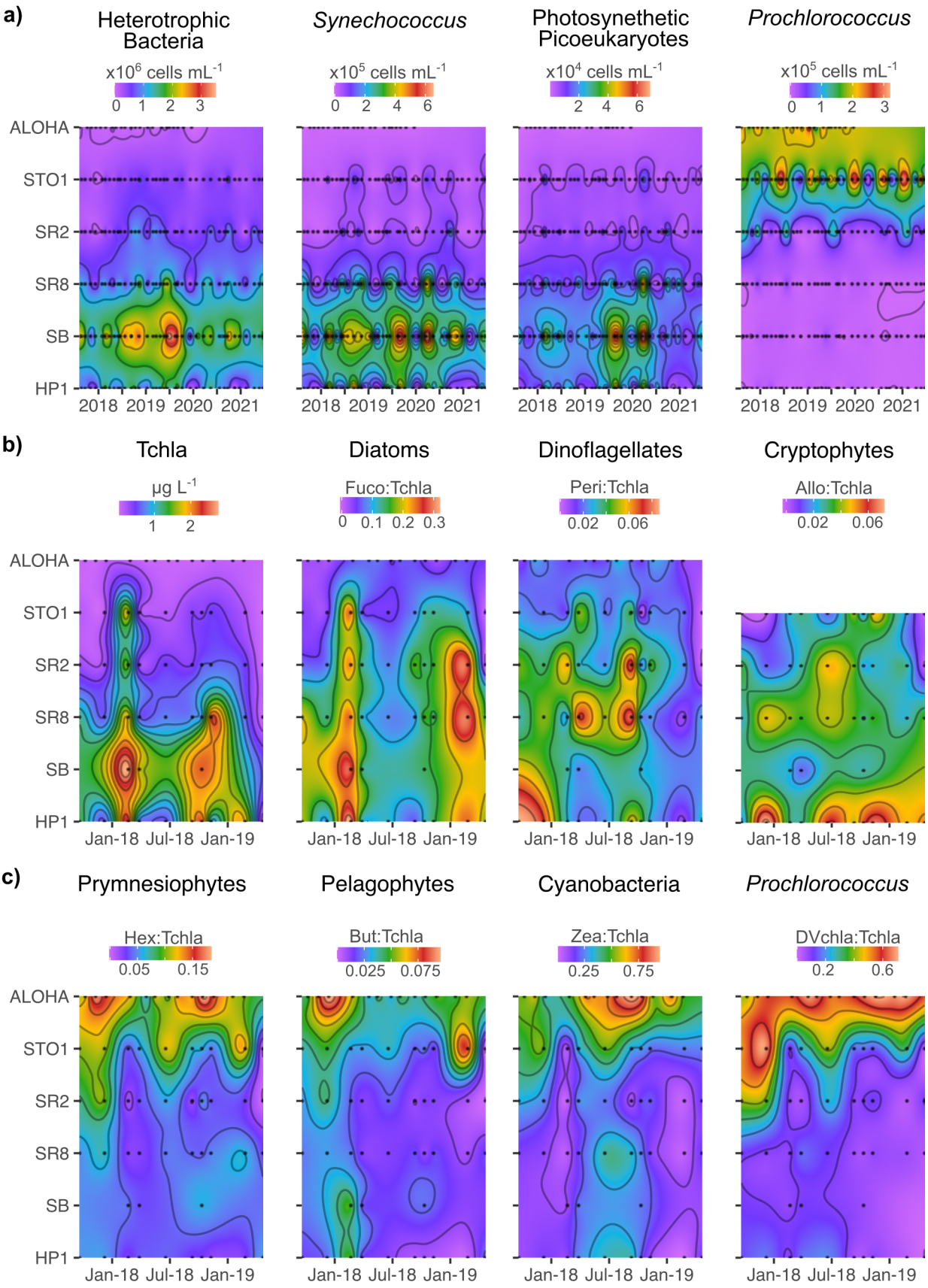
a)
Heterotrophic Bacteria
x10^6 cells mL^-1
0 1 2 3
Synechococcus
x10^5 cells mL^-1
0 2 4 6
Photosynethetic Picoeukaryotes
x10^4 cells mL^-1
2 4 6
Prochlorococcus
x10^5 cells mL^-1
0 1 2 3
ALOHA
STO1
SR2
SR8
SB
HP1
2018 2019 2020 2021
b)
Tchla
µg L^-1
1 2
Diatoms
Fuco:Tchla
0 0.1 0.2 0.3
Dinoflagellates
Peri:Tchla
0.02 0.06
Cryptophytes
Allo:Tchla
0.02 0.06
Jan-18 Jul-18 Jan-19
c)
Prymnesiophytes
Hex:Tchla
0.05 0.15
Pelagophytes
But:Tchla
0.025 0.075
Cyanobacteria
Zea:Tchla
0.25 0.75
Prochlorococcus
DVchla:Tchla
0.2 0.6

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Fig. 2. Microbial cellular abundances and pigment concentration vary through time and space across stations from the southern sector of Kāneʻohe Bay (HP1, SB, SR8), offshore stations (SR2 and STO1), and open ocean Station ALOHA: a) Cellular abundances (cells mL^{-1}) of heterotrophic bacteria, *Synechococcus*, photosynthetic picoeukaryotes, and *Prochlorococcus*; b) Total chlorophyll *a* (Tchla) concentrations (μg L^{-1}) and ratios of phytoplankton pigments indicative of specific phytoplankton groups relative to Tchla. Note, alloxanthin was below detection levels for Station ALOHA and not presented here. Abbreviations: Fuco: Fucoxanthin, Peri: Peridinin; Allo: Alloxanthin; Hex: 19′-hexanoyloxyfucoxanthin; But: 19′-butanoyloxyfucoxanthin; Zea: zeaxanthin; Dvchla: divinyl chlorophyll *a*.

Phytoplankton community composition through 16S rRNA gene sequencing

We delineated 505 phytoplankton ASVs across 366 samples, including 66 from cyanobacteria and 439 from eukaryotic plastids. Examining the distribution of phytoplankton ASVs across samples revealed that phytoplankton communities clustered into three major community types that coincide with spatial differences in biogeochemistry (**Fig. 3**, **Supporting Information Table S5**). We categorized the three major community types as nearshore, transition, and offshore because of their distinct biogeochemical characteristics and geographic location (**Supporting Information Table S5, Fig. S3**). The nearshore cluster of 229 samples included all samples collected from six stations found most closely located to land (Wai2, Kahoʻokele, SB, CB, HP1, AR) and at least one sample from the six remaining stations. The transition cluster of 85 samples consisted of samples collected from stations not immediately next to land (NB, SR8, NR2, SR2, STO1, NTO1), while the offshore cluster encompassed 52

samples collected exclusively from the four stations located the furthest distance from land (SR2, NR2, STO1, NTO1).

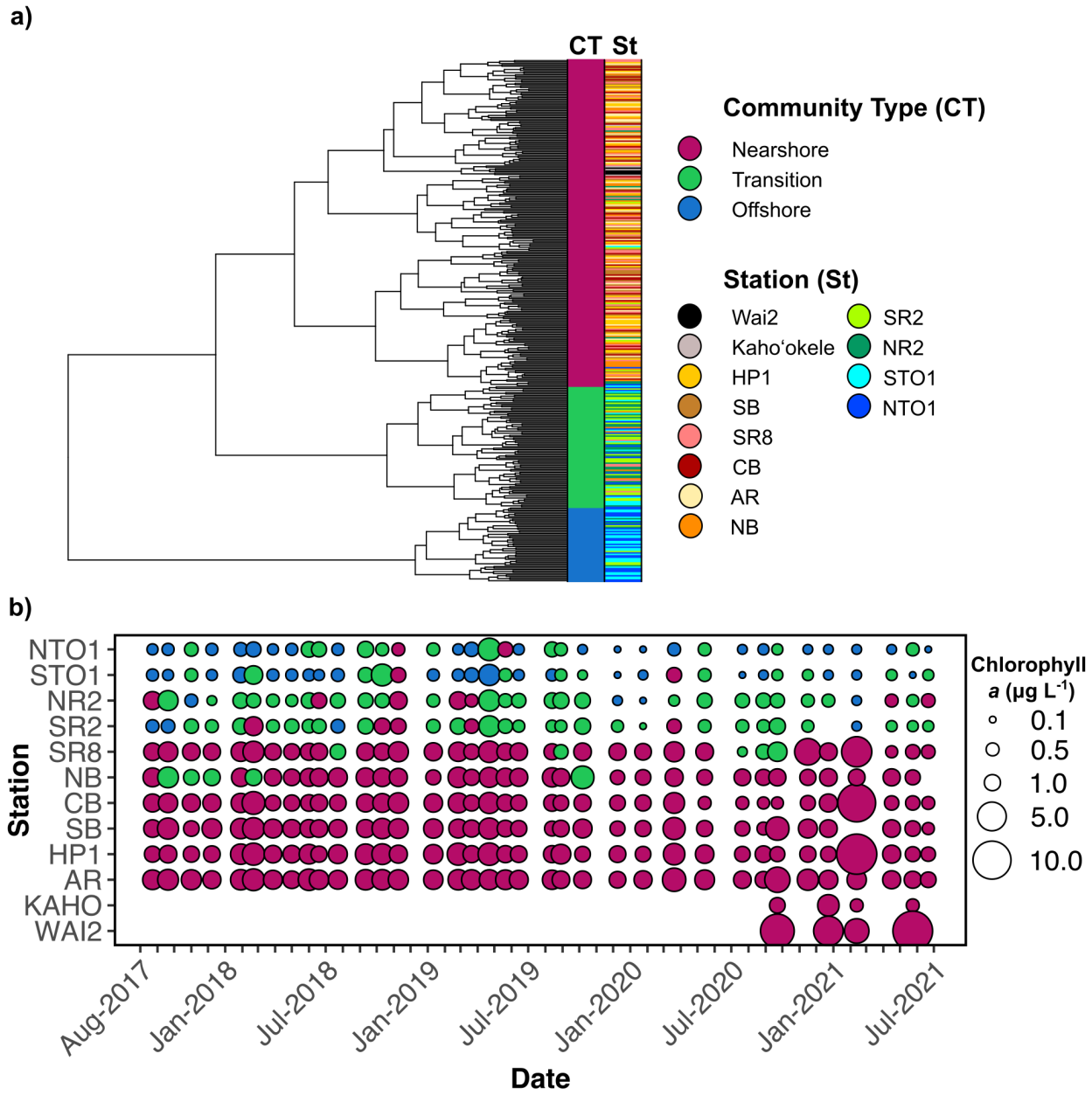


Figure 3. a) Hierarchical clustering of phytoplankton communities from 366 samples collected at 12 sampling stations form three major groups, hereafter referred to as community types:

nearshore, transition, and offshore. b) The distribution of samples defined as nearshore, transition, and offshore community types across stations and sampling events (36 total between 2017-2021) shows spatial and temporal persistence in the distinct community types. The size of the circle represents the chlorophyll *a* concentration during the time of sampling and the color of the circle represents the community type: nearshore, transition, and offshore.

ASV richness was the highest in the nearshore, while the transition community group had the highest Shannon's diversity estimate (**Supporting Information Table S6**). Phytoplankton relative abundance was dominated by a few highly abundant groups, including *Synechococcus*, *Prochlorococcus*, Mamiellophyceae (green algae), and Bacillariophyta (diatoms) (**Fig. 4a)**. Using DESeq2 variance stabilized abundances, 20 classes (**Supporting Information Table S7**) and 33 genera of phytoplankton (or groups unclassified at genus-level but classified at the family-level; **Fig. 4b**, **Supporting Information Table S8**) differed significantly in abundance across the three community types.

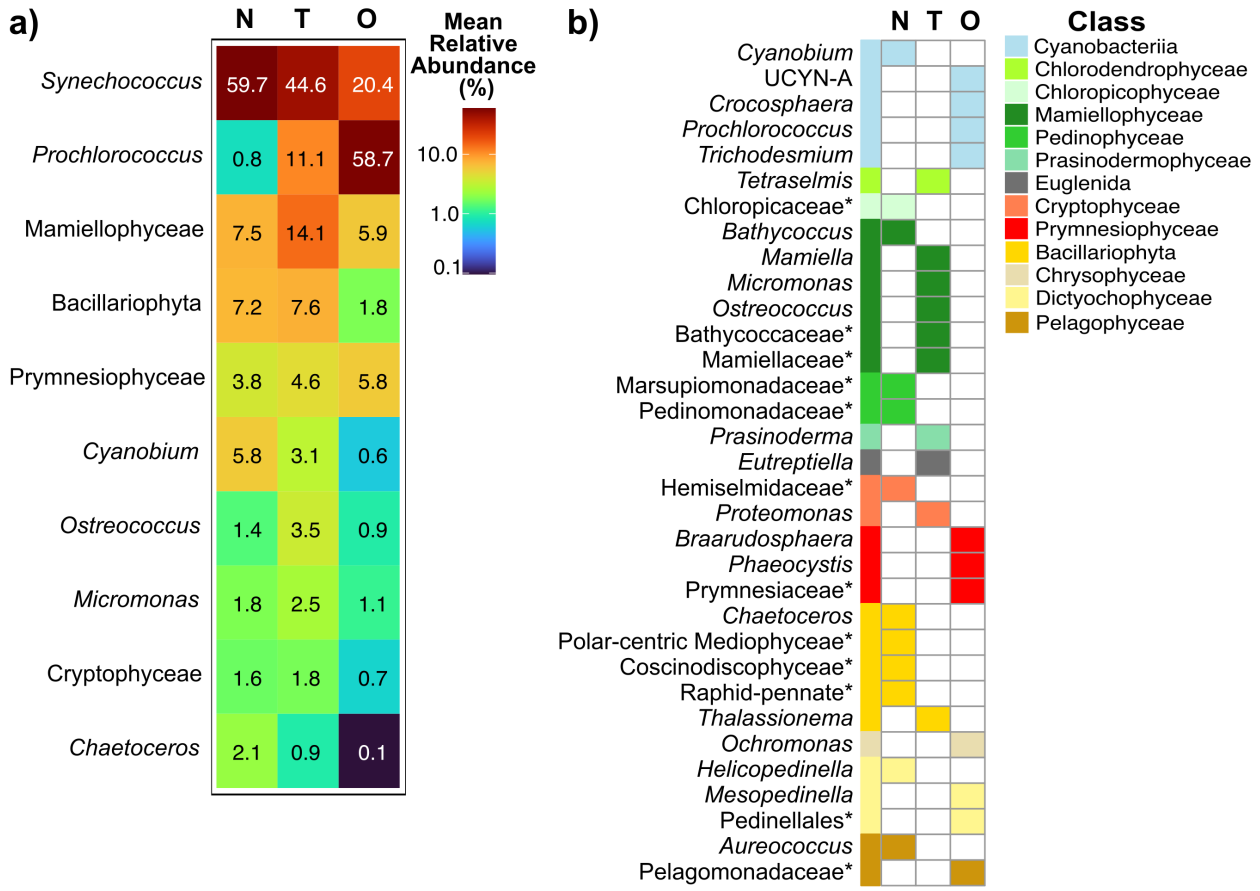


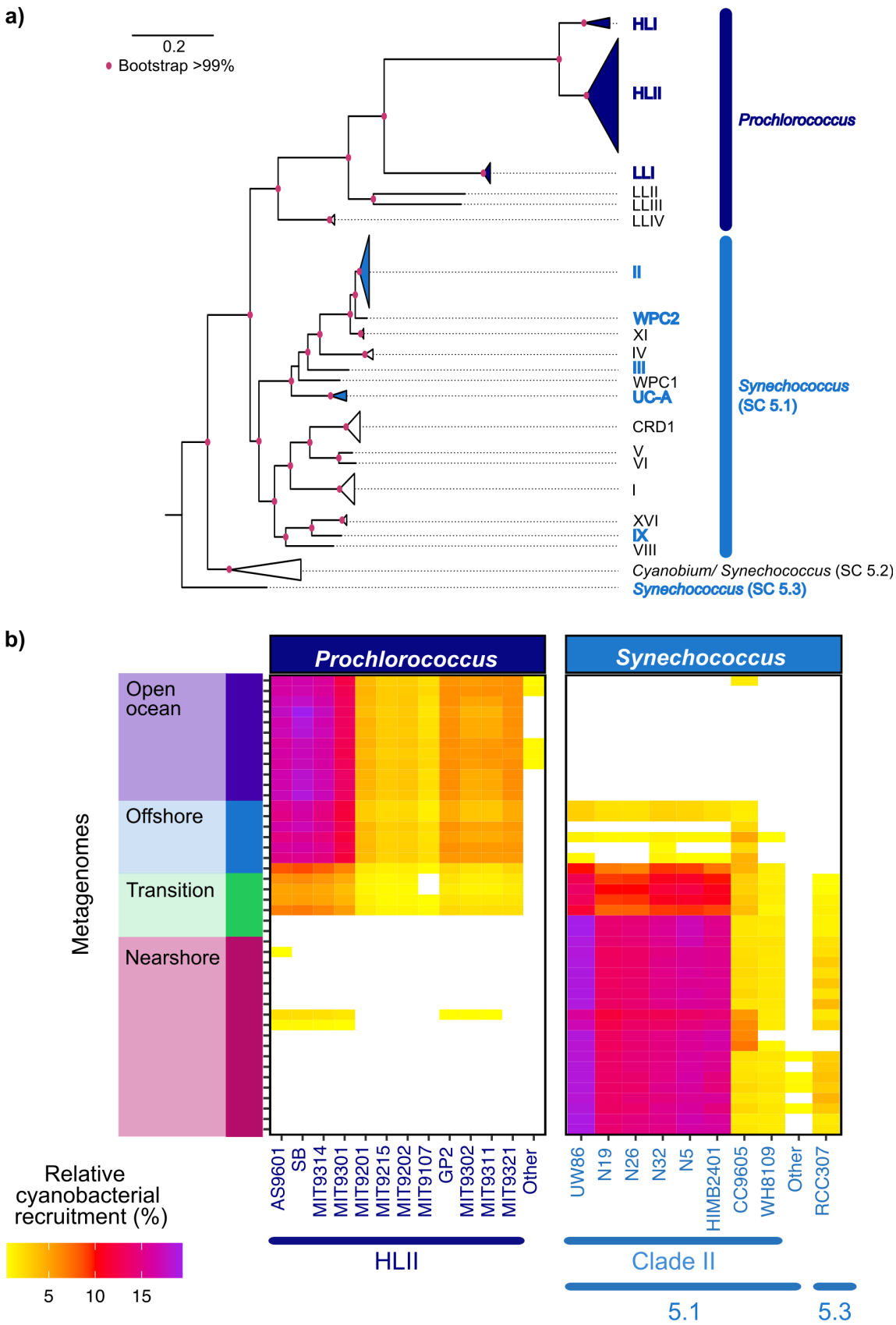
Fig. 4. a) Mean relative abundance of the top 10 phytoplankton groups across the nearshore (N), transition (T), and offshore (O) environments. b) Phytoplankton genera with significantly different distributions across the three environments. Colored boxes denote the peak in abundance for each genus. Phytoplankton groups that were classified at the family-level but unidentified at the genus-level are denoted with an asterisk.

Cyanobacterial population structure

Metagenomic read recruitment to 56 genomes of the cyanobacterial genera *Prochlorococcus*, *Synechococcus*, and *Cyanobium* showed that *Prochlorococcus* HLI, HLII, and LLI, and *Synechococcus* SC 5.1 II, WPC2, III, UC-A, and IX and SC 5.3 were detected in

metagenomes from the Kāneʻohe Bay Time Series and surface ocean samples from Station ALOHA (**Fig. 5a**). Although *Cyanobium* 16S rRNA gene ASVs were detected in the amplicon data, no *Cyanobium* representatives (SC 5.2) were detected in our metagenomic read recruitment. Genomes from *Synechococcus* SC 5.1 II and SC 5.3 and *Prochlorococcus* HLII were among the most abundant representatives within our samples (**Fig. 5b**). *Prochlorococcus* HLII comprised 98.9±1.0% (mean±sd) of cyanobacterial relative abundance in the open ocean and 83.1±17.4% of the cyanobacterial relative abundance in the offshore. *Prochlorococcus* HLII recruited only a small proportion of reads from a handful of nearshore samples, where it made up 1.1±2.4% of the cyanobacterial relative abundance. *Synechococcus* clade II comprised 96.3±2.5% of the cyanobacterial relative abundance in the nearshore Kāneʻohe Bay community type. *Synechococcus* SC 5.3 also recruited some metagenomic reads, but only from coastal Kāneʻohe Bay samples and at low relative cyanobacterial abundance (2.4±1.2%) (**Fig. 5b**).

Within the *Prochlorococcus* HLII and *Synechococcus* II clades, read recruitment varied between closely related genomes. Read recruitment was substantially higher in *Synechococcus* clade II isolate UW86 compared to all other clade II genomes, despite sharing >95% ANI with most other clade II genomes (**Supporting Information Fig. S4**). Within *Prochlorococcus* HLII, isolate genomes AS9601, SB, MIT9314, and to a lesser extent MIT9301, recruited a substantially greater proportion of reads than other members of this clade. AS9601, SB, MIT9314, and MIT9301 shared 94% ANI, which is higher than what was shared with other HLII isolates (<93%), with the exclusion of MIT9215 and MIT9202 who share 97% ANI with each other (**Supporting Information Fig. S4**).


a)
0.2
Bootstrap >99%
HLI
HLII
LLI
LLII
LLIII
LLIV
Prochlorococcus
II
WPC2
XI
IV
III
WPC1
UC-A
CRD1
V
VI
I
XVI
IX
VIII
Synechococcus (SC 5.1)
Cyanobium/ Synechococcus (SC 5.2)
Synechococcus (SC 5.3)
b)
Prochlorococcus
Synechococcus
Metagenomes
Open ocean
Offshore
Transition
Nearshore
AS9601
SB
MIT9314
MIT9301
MIT9201
MIT9215
MIT9202
MIT9107
GP2
MIT9302
MIT9311
MIT9321
Other
UW86
N19
N26
N32
N5
HIMB2401
CC9605
WH8109
Other
RCC307
HLII
Clade II
5.1
5.3
Relative cyanobacterial recruitment (%)
5
10
15

430

431 **Fig. 5.** a) Phylogenomic tree based on cyanobacterial marker genes found in 56
432 *Prochlorococcus*, *Synechococcus*, and *Cyanobium* isolate genomes and outgroup (*Gloeobacter*
433 *violaceus*- not shown). Clades detected in surface ocean metagenomic samples from the
434 Kāneʻohe Bay Time-series (KByT) and Station ALOHA are colored and bolded. b) The relative
435 abundance of cyanobacterial genomes across KByT and Station ALOHA metagenomes was
436 dominated by *Prochlorococcus* HLII, *Synechococcus* II (SC 5.1), and *Synechococcus* SC 5.3.
437 Recruitment of <0.5% not shown.
438

439 **Seasonality in biogeochemistry and community composition**

440 Surface seawater temperatures were significantly cooler in the winter than summer in
441 nearshore Kāneʻohe Bay and the adjacent transition and offshore waters (**Fig. 6a, Supporting**
442 **Information Table S9**). Cellular concentrations of *Synechococcus* and heterotrophic bacteria
443 were higher in the summer than the winter in the nearshore, transition, and offshore (**Fig.6a,**
444 **Supporting Information Table S9**). Photosynthetic picoeukaryote cellular concentrations
445 increased during the summer in coastal and transition environments (coastal: $p=0.004$, transition:
446 $p<0.001$, **Fig. 6a, Supporting Information Table S9**), but did not vary seasonally in the
447 offshore. *Prochlorococcus* cell concentrations only varied seasonally in the nearshore where it
448 increased in the winter ($p<0.001$, **Fig. 6a, Supporting Information Table S9**).

449 Chlorophyll *a* concentrations increased in the winter compared to the summer in the
450 nearshore (mean±sd; winter: 2.3±3.4 μg L^{-1}, summer: 0.8±0.5 μg L^{-1}; $p=0.031$; **Fig. 6a,**
451 **Supporting Information Table S9**), but not in the transition or offshore waters. Importantly,
452 given that the collection method for chlorophyll *a* concentrations likely missed most small
453 cyanobacteria, it is possible that seasonality has been underestimated. Nearshore chlorophyll *a*

concentrations increased with wind speed, wind direction, and rainfall, but not with seawater temperature (**Fig. 6b**). Three sampling events with elevated chlorophyll *a* concentrations in the nearshore occurred during anomalously high wind speeds or rainfall events (**Supporting Information Fig. S5**). Chlorophyll *a* concentrations in the transition and offshore did not correlate with seawater temperatures, but did positively correlate with rainfall (**Supporting Information Fig. S6**). Phosphate concentrations increased in the summer compared to the winter in the nearshore and transition clusters, while silicate concentrations increased in the summer compared to the winter in the nearshore cluster (**Supporting Information Table S9**).

Ratios of phytoplankton pigments to Tchla representing cyanobacteria, diatoms, and pelagophytes showed correlations with seawater temperature in the nearshore community type, suggestive of seasonality in the abundance of major phytoplankton groups (**Supporting Information Fig. S7**). Correlations between seawater temperature and pigment to Tchla ratios were not detected in the transition and offshore cluster (**Supporting Information Fig. S7**).

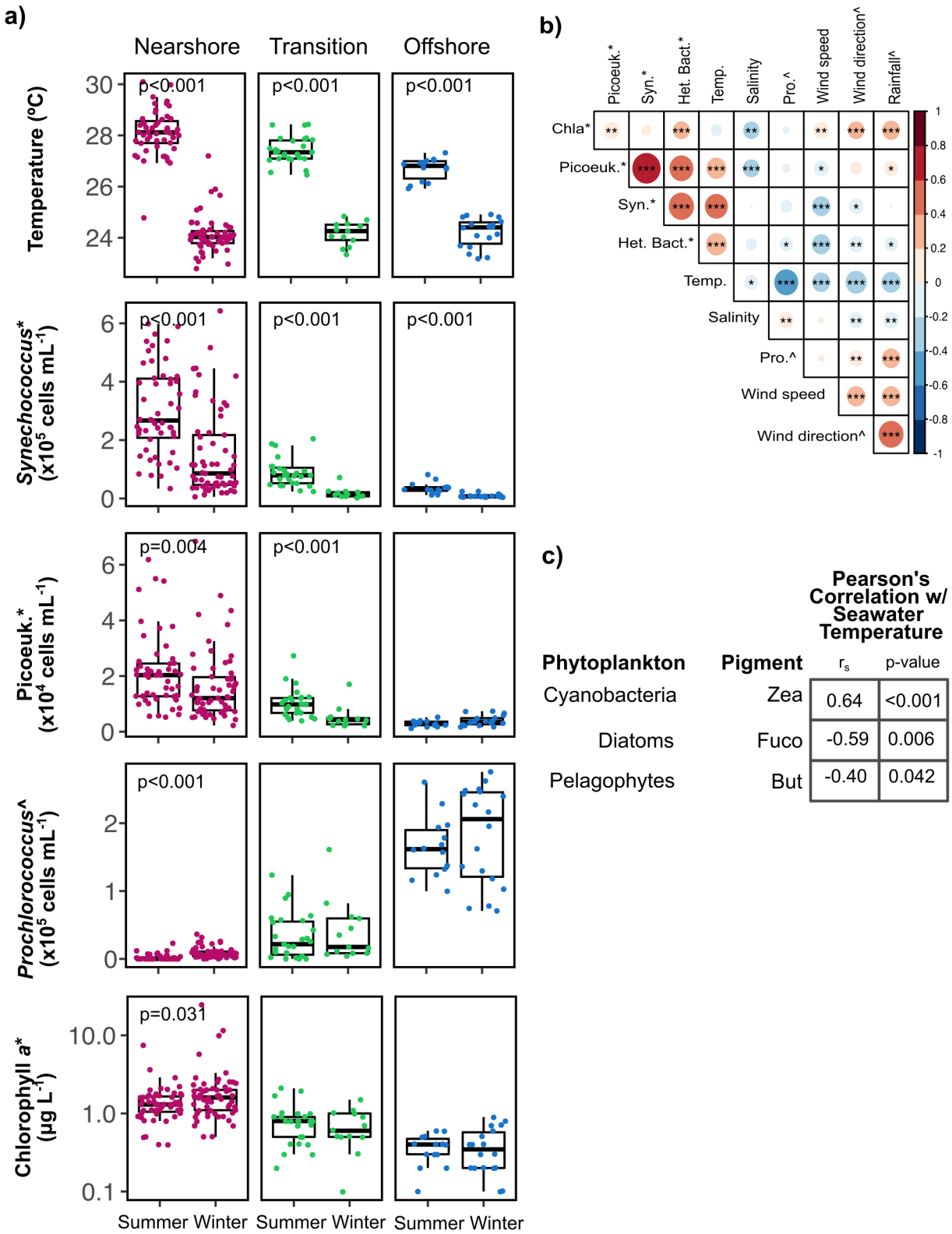
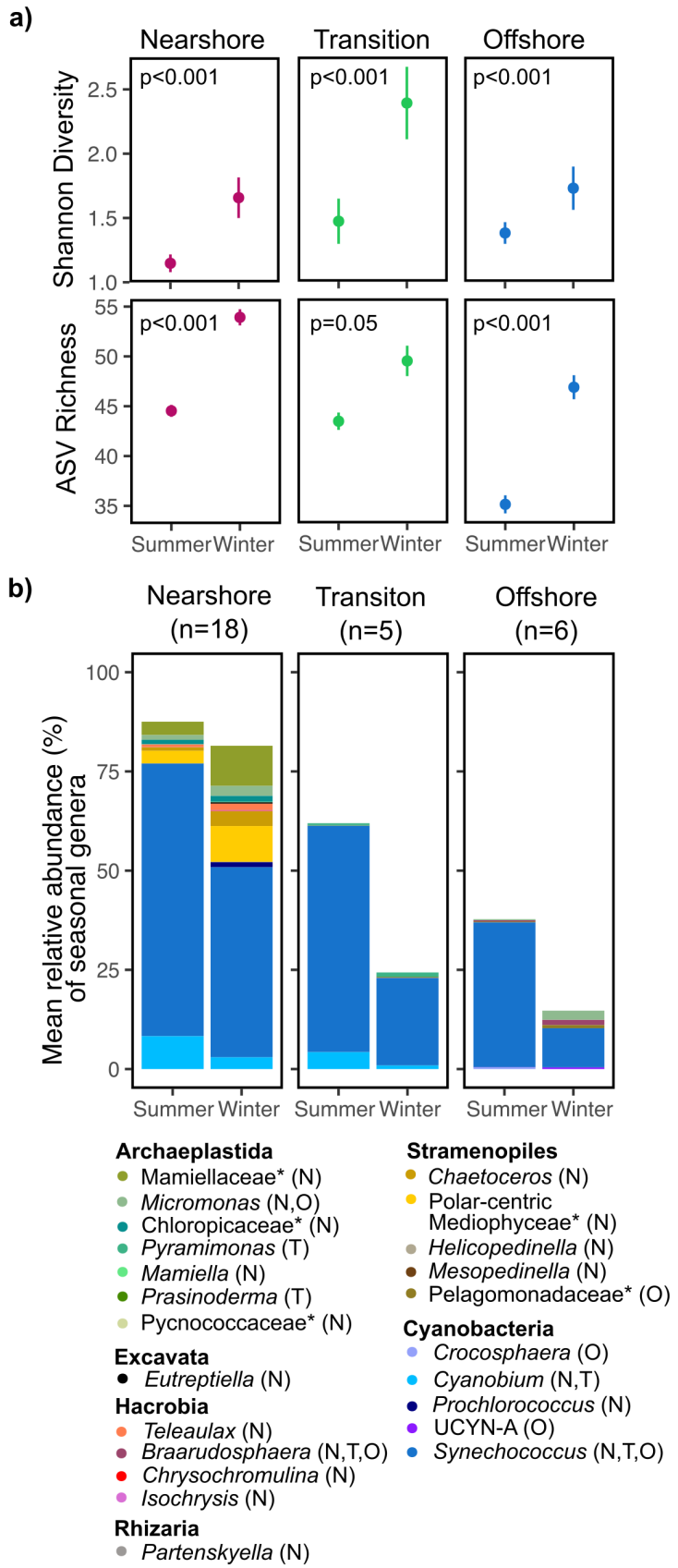


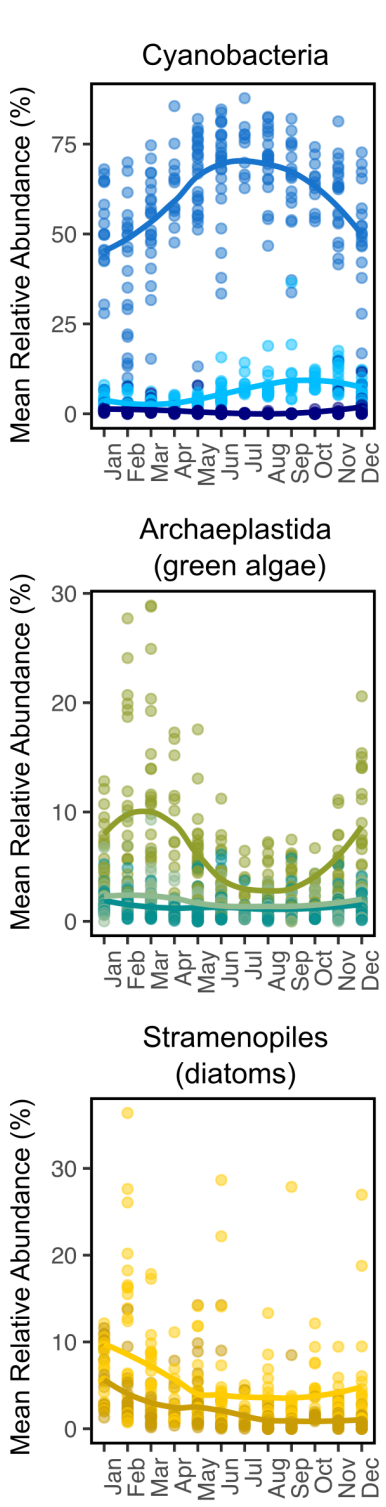
Fig. 6. a) Changes in seawater temperature, cell counts of *Synechococcus*, photosynthetic

picoeukaryotes, *Prochlorococcus*, and chlorophyll *a* across seasons and environments. b) Correlations between chlorophyll *a* concentrations and other environmental parameters in the nearshore. c) Significant correlations between phytoplankton pigment:tchla ratios and seawater temperature in the nearshore. An asterisk (*) denotes variables with log transformations, while a carrot (^) denotes variables with log+1 transformations. Het.Bac: heterotrophic bacteria (cells mL^{-1}); Syn: *Synechococcus* (cells mL^{-1}); Pro: *Prochlorococcus* (cells mL^{-1}); Picoeuk: Photosynthetic picoeukaryotes (cells mL^{-1}); Temp: Seawater temperature (°C); Chla: Chlorophyll *a* (µg L^{-1}); Salinity (ppt); Wind direction (degrees); Wind Speed (ms^{-1}); Rainfall (mm); Zea: Zeaxanthin; Fuco: Fucoxanthin; But: 19′-butanoyloxyfucoxanthin.

Seasonal differences were found in phytoplankton alpha diversity and the relative abundance of phytoplankton genera across the nearshore, transition, and offshore. ASV richness and Shannon diversity increased in the winter compared to the summer in all three community types (**Fig. 7a**, **Supporting Information Table S10**). Seasonality was observed among 23 phytoplankton genera (or groups unclassified at the genus-level, but classified at the family-level) within each of the clusters, including 18 nearshore, 5 in the transition, and 6 offshore (**Fig.7b, Supporting Information Table S11**). Seasonal genera accounted for 84.9±7.4, 48.6±18.9, and 23.2±13.1% of the relative abundance of the community on average in the nearshore, transition, and offshore clusters, respectively. *Synechococcus* was the most abundant seasonal genus, increasing in relative abundance in the summer months across all community type clusters (**Fig.7b,c**). *Cyanobium*, *Crocosphaera*, and unidentified Pycnococcaceae also increased in abundance in the summer months (**Fig. 7b**). Seasonal genera more often increased in relative abundance during the winter (n=18) including those belonging to diatoms (e.g.

Chaetoceros and unclassified polar-centric Mediophyceae), green algae (e.g. unclassified Mamiellophyceae, *Micromonas*, and *Mamiella*), prymnesiophytes (e.g. *Isochrysis*, *Chrysochromulina*, and *Braarudosphaera*), and Dictyochophyceae (*Helicopedinella*, *Mesopedinella*) (**Fig. 7b**). In the nearshore, the dominant phytoplankton in the winter remained Cyanobacteria followed by Mamiellophyceae and then diatoms, although at times both Mamiellophyceae and diatoms exceeded >30% of the total phytoplankton relative abundance (**Fig. 7c**).


a)
Nearshore
Transition
Offshore
Shannon Diversity
ASV Richness
p<0.001
p<0.001
p<0.001
p<0.001
p=0.05
p<0.001
Summer Winter
b)
Nearshore (n=18)
Transiton (n=5)
Offshore (n=6)
Mean relative abundance (%) of seasonal genera
Archaeplastida
Mamiellaceae* (N)
Micromonas (N,O)
Chloropicaceae* (N)
Pyramimonas (T)
Mamiella (N)
Prasinoderma (T)
Pycnococcaceae* (N)
Excavata
Eutreptiella (N)
Hacrobia
Teleaulax (N)
Braarudosphaera (N,T,O)
Chrysochromulina (N)
Isochrysis (N)
Rhizaria
Partenskyella (N)
Stramenopiles
Chaetoceros (N)
Polar-centric Mediophyceae* (N)
Helicopedinella (N)
Mesopedinella (N)
Pelagomonadaceae* (O)
Cyanobacteria
Crocosphaera (O)
Cyanobium (N,T)
Prochlorococcus (N)
UCYN-A (O)
Synechococcus (N,T,O)


c)
Cyanobacteria
Archaeplastida (green algae)
Stramenopiles (diatoms)
Mean Relative Abundance (%)
Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec

499

Fig. 7. a) ASV richness and Shannon's diversity across all three community types differ during the summer and winter seasons. b) Mean relative abundances of the 29 significantly seasonal genera across the three community types for summer and winter seasons. c) Nearshore seasonal phytoplankton genera from Cyanobacteria, Stramenopiles, and Archaeplastida with relative abundances >0.5% on average. A local polynomial regression fit line is shown for each genus. Phytoplankton groups that were classified at the family-level but unidentified at the genus-level are denoted with an asterisk.

Discussion

The activity and distribution of phytoplankton has important implications for food web dynamics and biocultural restoration and management of near-island waters of the tropical Pacific, especially under predicted climate change conditions that are expected to limit nutrient availability, phytoplankton size, productivity, and biomass in open oceans. Ecosystem-level time-series analyses spanning estuarine waters within Kāneʻohe Bay of the Hawaiian island of Oʻahu to the North Pacific Subtropical Gyre revealed that surface ocean biogeochemistry, phytoplankton biomass, and phytoplankton community structure varied dramatically across both broad (e.g. nearshore Kāneʻohe Bay to the NPSG) and narrow (e.g. nearshore Kāneʻohe Bay to adjacent offshore waters) spatial scales. Through investigations of these spatiotemporal dynamics, we gained insight into the ecology and phenology of phytoplankton communities of surface oceans in the tropical Pacific and identified indicators to help evaluate deviations from current conditions.

Spatially distinct phytoplankton and biogeochemical regimes within and adjacent to Kāneʻohe Bay

Our sampling approach provides an increased resolution upon satellite-based studies (Gove et al. 2016; Messié et al. 2022), which are typically not reliable within ~5 km to shore due to heavy cloud cover, along with a synoptic view of the biogeochemistry and phytoplankton communities. Compared to offshore and open ocean waters, nearshore Kāneʻohe Bay had elevated chlorophyll *a* concentrations- a 3-fold increase in phytoplankton biomass within 6 km from coastal Kānʻeohe Bay and an 18-fold increase over a roughly 100 km distance. Inorganic nutrient and chlorophyll *a* concentrations increased with decreasing salinity, suggesting that freshwater input from streams is an important driver of nutrient delivery and subsequent phytoplankton enhancement within Heʻeia Fishpond and Kāneʻohe Bay. Previous studies within Kāneʻohe Bay have also revealed stream-delivered nutrient input as an important driver of phytoplankton enhancement (Yeo et al. 2013), as well as additional processes including submarine groundwater discharge (McKenzie et al. 2019) and human-driven pollution (Ringuet and Mackenzie 2005). Transport from offshore, subsurface nutrient-rich waters to nearshore waters via internal waves is likely an important process contributing to phytoplankton enhancement of surface waters in the larger Hawaiian archipelago (Gove et al., 2016), although its prevalence and impact specifically within Kāneʻohe Bay remains unknown.

Across Kāneʻohe Bay and the adjacent open ocean, phytoplankton communities resolved into three distinct groups that coincide with spatial differences in biogeochemistry. Pigments indicative of diatoms and cyanobacteria revealed that these groups were the main phytoplankton contributing to the enhanced chlorophyll *a* concentrations in the nearshore environment of

Kāneʻohe Bay. Some phytoplankton that were more abundant closer to shore, such as *Teleaulax* (Cryptophyta), *Pyamimonas* (Chlorophyta), *Tetraselmis* (Chlorophyta), Chlorarachniophytes (Rhizaria), and dinoflagellates, are associated with mixotrophic lifestyles (Stoecker et al. 2016). Mixotrophy provides a crucial trophic link in planktonic food webs by supplementing primary production via heterotrophy, increasing carbon transfer to high trophic levels, and serving as a source of nutrients (Stoecker et al. 2016). While establishing the relative importance of mixotrophy across the nearshore to the adjacent offshore environment requires further investigation, these initial insights show distinctions in food web dynamics, phytoplankton ecologies, and contributors to primary productivity across the nearshore to open ocean waters of the tropical Pacific.

Drivers of seasonality in nearshore chlorophyll a *concentrations*

While seasonality within transition and offshore phytoplankton communities was muted, nearshore phytoplankton biomass was significantly elevated in winter, and members with the highest relative abundance in the nearshore phytoplankton community showed high seasonality. Chlorophyll *a* concentrations varied seasonally in nearshore Kāneʻohe Bay, where it increased with wind speed, rainfall, and wind direction. In Hawaiʻi, storm events generally increase during the winter months where storm-associated rainfall and wind may serve to elevate inorganic nutrient concentrations through increased stream outflow, leading to short-term enhancement of phytoplankton biomass in nearshore Kāneʻohe Bay (Ringuet and Mackenzie 2005; Cox et al. 2006; Yeo et al. 2013). Periods of intense rainfall have strong impacts on the food web dynamics of Kāneʻohe Bay, with wet periods decreasing trophic complexity and increasing total community biomass and the transfer of production to metazoans (Selph et al. 2018). Because

phytoplankton growth and loss processes can occur over short time scales of a day to a week, the near-monthly sampling interval employed in KByT is unlikely to fully capture the rapid fluctuations of chlorophyll *a* concentrations that occur in response to storm events. Thus, our observations likely underestimate the episodic variability of phytoplankton biomass in the nearshore (Yeo et al. 2013).

Distinct growth and nutrient uptake strategies between *Synechococcus* and diatoms, along with changes in the environment that might favor one over the other, likely produced the dynamic seasonal patterns observed in nearshore Kāneʻohe Bay: summer months showed a near doubling of the cellular and relative abundance of *Synechococcus*, while winter months were marked by sharp increases in the relative abundances of diatoms such as *Chaetoceros* and unidentified polar-centric Mediophyceae. To understand competition outcomes under different environmental conditions, resource competition theory characterizes trade-offs between slow-growing nutrient specialists with high affinity for uptake but low maximum growth (e.g. *Prochlorococcus* and *Synechococcus*) and fast-growing nutrient opportunists with high maximum growth but low affinity for uptake (e.g. flagellates and diatoms) (Dutkiewicz et al. 2009). Due to their small size and high uptake capacity, *Synechococcus* are likely less limited by the typically low nitrogen concentrations within Kāneʻohe Bay than other phytoplankton (Burson et al. 2018). In addition, seawater temperatures have been shown to positively correlate with *Synechococcus* cellular abundances, likely because seasonal increases in temperature positively impact *Synechococcus* division rates (Hunter-Cevera et al. 2016). Thus, in the summer under high-light, warm-water temperatures and limited nutrients, *Synechococcus* dominated Kāneʻohe Bay. In contrast, despite higher nutrient requirements due to their larger cell sizes, diatoms have high rates of growth allowing them to outcompete other phytoplankton under periods of elevated

nutrient availability (Laws 1975). Pulses of nutrients from the more frequent storm events during the winter could have led to the observed fluxes in diatom relative abundances.

Phytoplankton indicators of climate change impacts

Phytoplankton biodiversity (e.g. ASV richness, Shannon's diversity) in Kāneʻohe Bay was significantly elevated in comparison to the adjacent offshore. Near-island environments can export diversity offshore and, importantly, biodiversity can offer functional redundancy and ecological stability in times of environmental perturbation (Messié et al. 2022). In a recent study that modeled changes to phytoplankton biodiversity under climate change conditions, some tropical regions were found to face up to 30% of phytoplankton types becoming locally extirpated (Henson et al. 2021). Importantly, phytoplankton of higher size classes, predominantly diatoms, are expected to be lost due to increased nutrient limitation (Flombaum et al. 2020). Diatoms serve as an important part of the diet of herbivorous fish grown in Hawaiian aquaculture systems (Hiatt 1947), and contribute significantly to primary productivity in nearshore systems broadly. Diatoms also contribute a large portion of the total chlorophyll *a* in the nearshore environment of Kāneʻohe Bay. Shifts or reduction in diatom abundance within the Heʻeia Fishpond and Kāneʻohe Bay when compared the baseline knowledge characterized here might thus help to identify impacts of ocean warming and stratification in advance of shifts in the food web structure.

Synechococcus and *Prochlorococcus*, the small cyanobacteria that dominated the total phytoplankton relative abundance in our study, may also provide valuable bioindicators of environmental change within Kāneʻohe Bay under climate change scenarios. *Prochlorococcus* and *Synechococcus* are responsible for roughly 25% of the ocean's net primary production

615 (Flombaum et al. 2013) and will likely further increase in abundance with projected climate
616 change conditions (Flombaum et al. 2020). These marine cyanobacteria encompass fine-scale
617 genetic diversity that distinguishes their ecologies, metabolisms, and biogeochemical roles at the
618 level of major and minor clades (Berube et al. 2019). These clade identities are often difficult to
619 resolve with the use of single gene markers, and thus metagenomic read recruitment can increase
620 genetic resolution.

621 Across the open ocean NPSG to nearshore Kāneʻohe Bay, *Prochlorococcus* Clade HLII
622 and *Synechococcus* Clade II (SC 5.1) were the most abundant, consistent with previous reports
623 from oligotrophic oceans (Delmont and Eren 2018; Lee et al. 2019). *Prochlorococcus* Clade
624 HLII from the offshore waters adjacent to Kāneʻohe Bay and Station ALOHA showed high
625 similarity in population structure and thus may represent continuous populations with ongoing
626 gene flow responding to similar environmental parameters in both environments. Continued
627 examination of population structure, as well as cellular and relative abundances of these
628 cyanobacteria, could identify eventual expansions of ultraoligotrophic waters into Kāneʻohe Bay,
629 shifts in gene-flow, and selection for clades with unique ecological adaptations. Given the high
630 seasonality of *Synechococcus* cellular and relative abundance in the nearshore environment,
631 changes in the magnitude and timing of these metrics could also be used to identify alterations in
632 seasonality and associated food-web dynamics within Kāneʻohe Bay.

633

634 *Implications for biocultural restoration*

635 The enhancement of phytoplankton biomass in the estuarine Heʻeia Fishpond and coastal
636 Kāneʻohe Bay provides critical ecosystem services and is an important consideration for
637 biocultural restoration activities, community-based research efforts, and resource management.

Within He‘eia and across the islands of Hawai‘i, biocultural practitioners are undertaking restoration projects to maximize primary and secondary production of the estuarine environment, including removing invasive mangrove, managing stream use to ensure adequate flow and water quality, and engineering water exchange through repairs and updates to fishpond walls (Möhlenkamp et al. 2019). The baseline understanding of phytoplankton communities provided by this study helps to inform biocultural stewards of He‘eia and Kāne‘ohe Bay of the conditions that promote phytoplankton growth and subsequently herbivorous fish growth.

High-resolution sampling was key to identifying the magnitude of chlorophyll *a* enhancement across the system, areas of high localized phytoplankton biomass enhancement such as He‘eia Fishpond, and drivers of chlorophyll *a* enhancement including freshwater input and increased storm conditions and nutrient concentrations. Despite fine-scale biogeochemical differences within Kāne‘ohe Bay, phytoplankton communities sampled from the He‘eia Fishpond and across the northern, central, and southern sections of the bay grouped as one nearshore community type. The similar phytoplankton communities found within the estuarine fishpond and the nearshore environment of the bay emphasizes the connectedness of these estuarine and coastal systems and highlights the need to manage them in close coordination.

The particularly wet conditions during the winter months appears to play a substantial role in determining the variability of phytoplankton biomass and restructuring of the coastal food web within the coastal environment studied here. Growing this baseline understanding of phytoplankton cycling to relate to the timing and conditions documented in Hawaiian knowledge systems like *kaulana mahina*, the Hawaiian lunar calendar (Nu‘uhiwa 2019), and in regard to life cycles of bioculturally relevant species like ‘ama‘ama (*Mugil cephalus*) that feeds on

microphytoplankton at juvenile stages (Hiatt 1947), would further advance this area of study and utility for management within Hawaiian aquaculture systems.

Despite a poor understanding of the outcomes, near-island food webs will likely shift with ongoing climate change impacts on phytoplankton communities and biomass. Collaborating across diverse knowledge systems could improve the ability for local people to document and adapt to changes in near-island marine resources (Winter et al. 2020b). Our collaboratively developed study reveals distinct spatial and seasonal dynamics that define phytoplankton communities and biogeochemical conditions from an estuarine aquaculture system on the coast of O‘ahu, Hawai‘i, to the open ocean of the North Pacific Subtropical Gyre. Understanding the seasonal and spatial dynamics underlying phytoplankton communities and biogeochemistry of near-island environments in the tropical Pacific provides the necessary knowledge to further co-develop capacities to model and track changes to the marine food web, and to build resilience now and in the future.

Acknowledgements

We thank Paepae o He‘eia and the He‘eia National Estuarine Research Reserve for their involvement in and perspectives on the directions on this research. We thank Jason Jones, Ciara Ratum, Hanako Mochimaru, Oscar Ramfelt, Amber Boettiger, Clarisse E.S. Sullivan, Elizabeth A. Monaghan, and Evan Freel for their assistance collecting samples, Gus Robertson for assistance with sonde calibration, Andrew McGowan for his help with the creation of maps, and Karen Selph for flow cytometry measurements. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation or the National Oceanic and Atmospheric Administration (NOAA). This research was funded by the National Oceanic and Atmospheric Administration (NOAA) Margaret A. Davidson Fellowship (No. NA20NOS4200123), National Science Foundation Graduate Research Fellowship Program under Grant No. 1842402, the Colonel Willys E. Lord, DVM and Sadina L. Lord Scholarship, and the University of Hawai‘i at Mānoa Ecology, Evolution, and Conservation Biology’s Maybelle Roth ARCS Award to SJ Tucker and NSF grants OCE-1538628 and OCE- 2149128 to MS Rappé. This is SOEST contribution XXX and HIMB contribution XXX.

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Data availability statement

Data supporting the results within the manuscript are available within the main text (See Materials & Methods). Sequencing data are available in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under BioProject number PRJNA706753 as well as PRJNA971314. Environmental data were submitted to BCO-DMO under https://www.bco-dmo.org/project/663665. Code used in the analysis is available is available at https://github.com/tucker4/Tucker_Phytoplankton_KByT_HeNERR.

Author contribution statement

SJT led the formal analyses and wrote the initial manuscript draft. SJT, YMR, KFC, MSR collected the samples with assistance from AHK and KK. SJT, KFC, YMR, and MSR processed samples. All authors were involved in the conceptualization, development of methodology, interpretation of results, and providing input to the manuscript draft and revisions.