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RESEARCH ARTICLE

Seasonal and spatial transitions in phytoplankton assemblages spanning estuarine to open ocean waters of the tropical Pacific

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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 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 provides a synoptic view of phytoplankton dynamics over a 4-yr, 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 observed a sharp and persistent transition between picocyanobacterial communities, from *Synechococcus* clade II abundant in the nearshore to *Prochlorococcus* high-light adapted clade II (HLII) proliferating in offshore and open ocean waters. In comparison to immediately adjacent offshore waters and the surrounding open ocean, phytoplankton biomass within Kāneʻohe Bay was dramatically elevated. Members of the phytoplankton community revealed strong seasonal patterns, while nearshore phytoplankton biomass positively correlated with wind speed, rainfall, and wind direction, and not water temperatures. These findings elucidate the spatiotemporal dynamics underlying 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.

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; Chisholm 1992; Dutkiewicz et al. 2020). Surrounded by oligotrophic, open ocean waters, the coastal waters of remote islands in the tropical Pacific harbor a sharp increase in nutrients through physical, biological, geological, and anthropogenic processes that result in increased phytoplankton biomass, cell size, and productivity (i.e., the Island Mass Effect; Doty and Oguri 1956; Gove et al. 2016). The enhanced primary productivity boosts marine biodiversity (Messié et al. 2022), promotes carbon sequestration (Falkowski et al. 1998), and increases secondary productivity, which in turn supports regional

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Data Availability Statement: Data supporting the results within the manuscript are available within the main text (*see* "Materials and Methods" section). Sequencing data are available in the National Center for Biotechnology Information Sequence Read Archive 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.

fisheries and other resources relied upon by island communities (Williams et al. 2015).

Elevated phytoplankton biomass from near-island coastal waters is important to maintaining healthy and productive coastal food webs, however these food webs may be vulnerable due to their close proximity to open ocean oligotrophic waters. One result of increasing sea surface temperatures from ongoing global climate change is that it has the potential to increase 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 is expected to lead to a number of potential ecosystem impacts: an expansion of nutrient-poor waters in the open ocean gyres (Irwin and Oliver 2009), a shift toward small phytoplankton classes (Flombaum et al. 2020), a decline in global phytoplankton biomass and primary productivity (Boyce et al. 2010), and an amplification of biomass declines across the trophic food web (Moore et al. 2018). However, recent studies suggest that genetic and physiological adaptations of phytoplankton may sustain primary productivity even under the lower nutrient conditions that may occur with increased ocean stratification (Kwon et al. 2022; Martiny et al. 2022).

An improved understanding of the spatiotemporal variability of biogeochemical conditions and phytoplankton assemblages across coastal to open ocean systems adjacent to island masses in the tropical Pacific could help inform management of local marine environments and larger ecosystem models. Nutrients are delivered to near-island waters by both land-based (e.g., stream discharge, groundwater, anthropogenic impacts; McKenzie et al. 2019) and ocean-based processes (e.g., upwelling, internal waves; Gove et al. 2016; Comfort et al. 2024). The exchanges of nutrients and phytoplankton across these adjacent environments have important implications for enhancing phytoplankton productivity and structuring of community composition (Tucker et al. 2021; Comstock et al. 2022). However, most studies examining phytoplankton dynamics in the tropical Pacific have sampled the offshore or the coastal environment in isolation (Rii et al. 2016; Selph et al. 2018) or sampled both environments but typically with limited taxonomic information or temporal coverage (James et al. 2020; Messié et al. 2022). Coastal-ocean gradients in biogeochemistry and phytoplankton communities are often modified by seasonal or episodic changes in the environment (Cox et al. 2006). On high islands in the tropical Pacific, storm events can be particularly impactful, with high stream discharge carrying a substantial proportion of the annual nutrient budget for coastal waters and invoking rapid phytoplankton responses (Ringuet and Mackenzie 2005). Furthermore, submarine groundwater discharge can contribute greater nutrient inputs than surface run-off (Dulai et al. 2016) and future climate change scenarios are estimated to substantially impact groundwater contributions to coastal systems (Ghazal et al. 2023).

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, a 30+ yr time-series initiative measuring temporal trends of the adjacent oligotrophic North Pacific Subtropical Gyre (Karl and Lukas 1996). Together, this extensive spatial and temporal coverage revealed nearshore enhancement of phytoplankton biomass, pronounced seasonality in nearshore biogeochemistry and phytoplankton biomass and composition, and distinct transitions in phytoplankton communities spanning < 6 to > 100 km across Kāne‘ohe Bay to the North Pacific Subtropical Gyre.

Materials and methods

Study location

The Hawaiian archipelago within the oligotrophic North Pacific Subtropical Gyre 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,b). 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 contributes 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), with non-trivial contributions in inorganic nitrogen and silica via submarine groundwater discharge (Dulai et al. 2016). Water residence time within the bay varies from less than a day to over 1 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.

At the mouth of He‘eia Stream in the southern section of Kāne‘ohe Bay is an ~ 800-yr-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

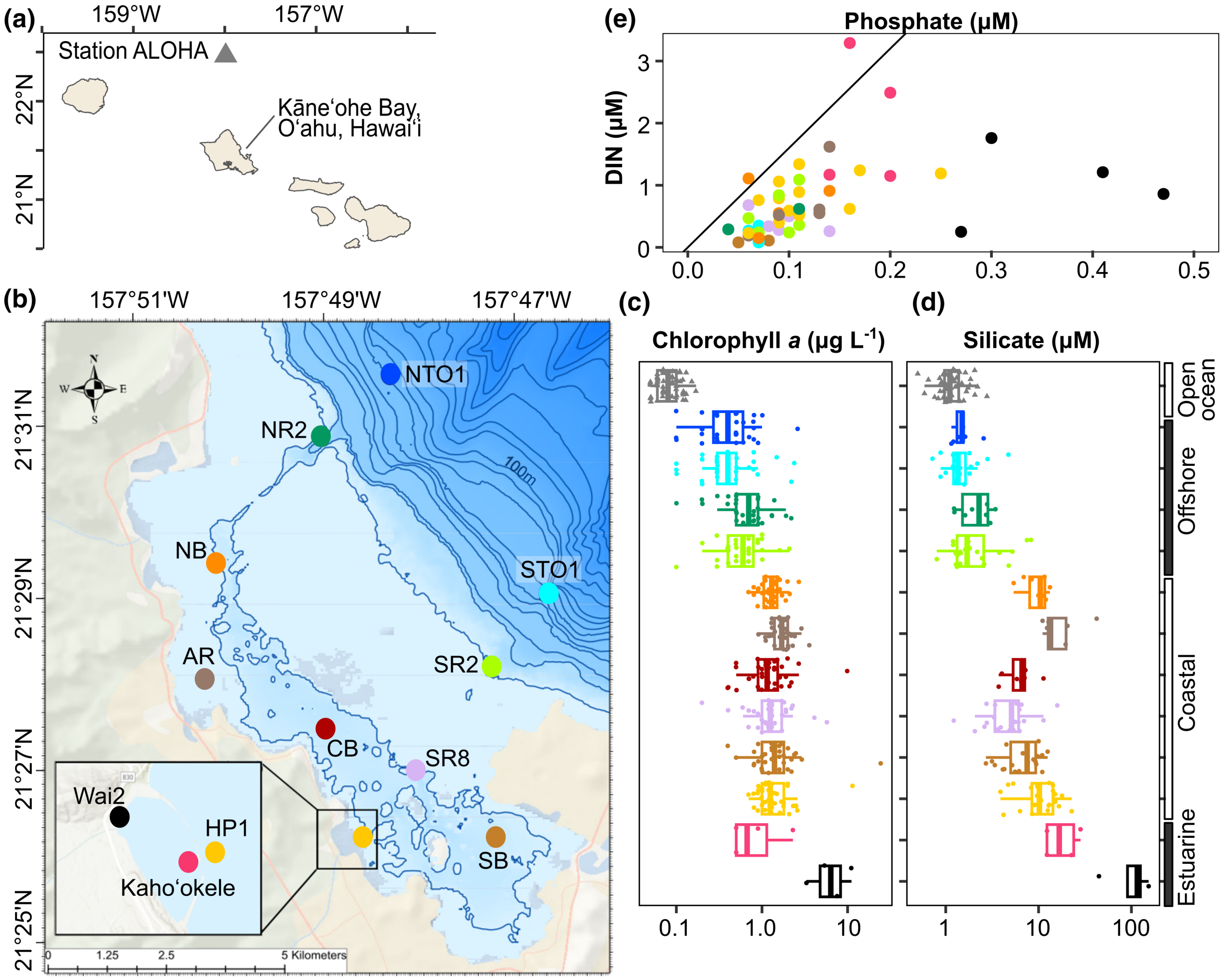


Fig. 1. (**a**) Location of Kāneʻohe Bay on Oʻahu, Hawaiʻi and the position of Station ALOHA (22°45′N, 158°W), a sampling station of the Hawaii Ocean Time-series program. (**b**) Map of sampling stations located on the windward side of the island of Oʻahu Hawaiʻi. Inset shows two stations within Heʻeia Fishpond (Wai2 and Kahoʻokele) and one immediately adjacent to the fishpond (HP1). Contour lines mark every 10 m up until 100 m and then 50 m intervals for depths > 100 m. (**c**) Fluorometric chlorophyll *a* (Chl *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.

primary productivity that sustained the population through abundant food fish and reef fish (Winter, Lincoln, et al. 2020). A 2.5-km basalt rock wall (kuapā) filled with coral rubble encompasses Heʻeia Fishpond. The wall is equipped with multiple sluice gates (mākāhā) that increase water residence time while still allowing for exchange between coastal Kāneʻohe Bay and Heʻeia stream waters. The mākāhā system size selects for juvenile fish exchange within the fishpond and Kāneʻohe Bay, allowing them 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 biogeochemically productive water and organisms from within the fishpond to the rest of Kāneʻohe Bay and beyond.

Collaboratively developed research

In following the collaborative research methodology practiced in the Heʻeia National Estuarine Research Reserve (Winter, Rii, et al. 2020; Kūlana Noiʻi Working Group 2021), and guided by interests expressed and knowledge possessed by the Indigenous (*Kānaka ʻŌiwi*, Native Hawaiian) stewards of Heʻeia 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. All individuals who 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 organization that stewards He‘eia Fishpond, and the private landowner, Kamehameha Schools and Bishop Estate.

Sample collection and environmental parameters

Between August 2017 and June 2021, seawater was collected from a depth of 2 m at 10 sites in Kāne‘ohe Bay and the adjacent offshore waters on a near-monthly basis (36 sampling events over 46 months) as part of the Kāne‘ohe Bay Time-series (KByT) using previously described methods (Fig. 1b; Supporting Information Table S1; Tucker et al. 2021). Between September 2020 and June 2021, two additional stations within He‘eia Fishpond were also sampled at a quarterly interval (Fig. 1b): Station Wai2 (now known as Waimā‘ama), located in the northwestern corner of the Fishpond within the He‘eia Stream mouth (known as muliwai) is a highly turbid and brackish water environment with tidal fluctuations resulting in salinity ranges of 10–35 ppt (Wai2, National Estuarine Research Reserve Centralized Data Management Office: https://cdmo.baruch.sc.edu/). Station Kaho‘okele (Kaho) is located at a sluice gate facing the ocean and receives high exchange with coastal Kāne‘ohe Bay (~ 25–35 ppt, National Estuarine Research Reserve Centralized Data Management Office: https://cdmo.baruch.sc.edu/; 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). Details on seawater filtration for nucleic acid isolation, subsampling for flow cytometry, high-performance liquid chromatography analysis, and measures of inorganic nutrient and chlorophyll *a* (Chl *a*) concentrations, the acquisition of data from Station ALOHA, and the curation of meteorological data collected from a station located at the Hawai‘i Institute of Marine Biology on Moku o Lo‘e in Kāne‘ohe Bay can be found in the Supporting Information "Sample collection and environmental parameters" section. Chl *a* concentrations measured fluorometrically are referred to as Chla, while Chl *a* concentrations measured by high-performance liquid chromatography are specified as total Chl *a* (TChla), totaling monovinyl and divinyl Chl *a*.

Spatiotemporal comparisons of environmental variables, cellular abundances, and phytoplankton pigments were conducted using the R package "multcomp" (Hothorn et al. 2008) with one-way ANOVA testing for multiple comparisons of means with Holm correction and Tukey contrasts. Summer (June 28 through September 28) and winter (December 27 through March 29) seasons were defined using harmonic regression analyses of surface seawater temperature collected hourly between 2010 and 2019 at NOAA station MOKH1 in Kāne‘ohe Bay (https://www.ndbc.noaa.gov/station_page.php?station=mokh1; Tucker et al. 2021). Spatiotemporal variation in cellular abundances and phytoplankton pigments was visualized using "mba.surf" from MBA (Finley et al. 2017) to interpolate data over the KByT sampling events and stations.

The map of Kāne‘ohe 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 O‘ahu was plotted in R v 4.4.1 (R Core Team 2023) with ggplot2 v3.4.3 (Wickham 2016), using a shape file from the Hawai‘i Statewide GIS Program (https://prod-histategis.opendata.arcgis.com/maps/HiStateGIS::coastline).

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

DNA extraction (Qiagen Blood and Tissue Kit, Qiagen Inc.) 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 complete with Illumina sequencing adapters, barcode, and index (Parada et al. 2016) and paired-end sequencing with MiSeq v2 2x250 technology (Illumina). Genomic DNA from 32 of the 368 total samples collected between 2017 and 2021 was 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) with mechanical shearing (Covaris) and paired-end sequenced on a single lane of the NovaSeq 6000 SP 150 (Illumina).

Sequence analysis

Amplicon sequence data generated from KByT sampling between July 2019 and June 2021 were analyzed in conjunction with previously published amplicon data spanning August 2017 to June 2019 (PRJNA706753; Tucker et al. 2021). For each of the two sequencing runs, samples were demultiplexed, quality controlled, and merged using Qiime2 v2.4 (Bolyen et al. 2019). Full-length forward reads (251 base pairs) were denoised and chimeras removed using DADA2 (Callahan et al. 2019) to delineate amplicon sequencing variants (ASVs). Reverse reads were not used because of inconsistent quality. Taxonomic databases SILVA v138 (Quast et al. 2012) and PR2 v 4.14.0 (Guillou et al. 2013) were used to assign taxonomy to bacterial and archaeal ASVs and Eukaryotic 18S rRNA and chloroplast ASVs, respectively. Eukaryota 18S rRNA sequences were removed from the study because, unlike 16S rRNA amplicons from the 515Y/926R primer sets, analyses of the 18S rRNA amplicons require overlap between forward and reverse reads for accurate resolution (Yeh et al. 2021), and our sequences were not able to be merged. See Supporting Information "Amplicon Sequence Analysis" section for additional details.

In the context of amplicon sequence data, "phytoplankton" herein refers to ASVs classified as cyanobacteria and eukaryotic plastids, although we recognize that mixotrophic and phagotrophic lifestyles may be included in this broad definition. Despite the utility and cost-effectiveness of amplicon sequencing, amplicon datasets present limitations, including biases during PCR that can miss certain groups, especially dinoflagellates, uneven copy numbers of the 16S rRNA genes across taxa, relatively shallow taxonomic resolution, and the compositional nature of amplicon sequencing data that requires unique statistical approaches (Decelle et al. 2015; Gloor et al. 2017).

Hierarchical clustering of the phytoplankton communities observed in each of the 366 samples was conducted using an Aitchison distance (Aitchison 1982) transformation on the entire quality-controlled dataset of phytoplankton raw read counts. ANCOM-BC v 2.6.0 (Lin and Peddada 2024) was used to assess differential abundance patterns of phytoplankton amplicon data across environmental clusters using linear regression models on a bias-corrected log abundance table with different pseudo-counts. Lomb Scargle Periodograms in the lomb package v 2.1.0 (Ruf 1999) were used to define seasonality among phytoplankton genera by determining the spectrum of frequencies in a dataset. Divnet v 0.4.0 (Willis and Martin 2020) was used to estimate differences in alpha diversity and test for significance between spatiotemporal groupings. Pearson's correlation analyses were conducted in corrplot v 0.92 (Wei and Simko 2021). Additional details on statistical analyses can be found in Supporting Information "Amplicon Sequence Analysis."

Metagenomic read recruitment

Metagenomic read recruitment to genome representatives can provide insights into the biogeography of cyanobacteria with high taxonomic resolution (Delmont and Eren 2018; Lee et al. 2019). To investigate the dominant cyanobacteria within and surrounding Kāne'ohe Bay, we conducted metagenomic read recruitment of 32 metagenomes from KByT and 12 previously published from the open ocean Station ALOHA (PRJNA352737; Mende et al. 2017) to 56 cyanobacterial genomes from *Prochlorococcus* (six minor clades) and the three major lineages of the marine *Synechococcus*/*Cyanobium* lineage (SC 5.1 [14 minor clades], SC 5.2, and SC 5.3) (Supporting Information Table S2). Following previously described pipelines (Delmont and Eren 2018), a contig database of the 56 cyanobacteria isolate genomes was constructed using anvi'o v 8.0 (Eren et al. 2021), metagenomic reads were recruited to the 56 picocyanobacterial genomes to assess the relative abundance of a genome (or a clade) in each metagenomic sample, and average nucleotide identity (ANI) calculated among genomes (*see* Supporting Information "Metagenomic Read Recruitment").

Data and code availability

Sequencing data are available in the National Center for Biotechnology Information Sequence Read Archive 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

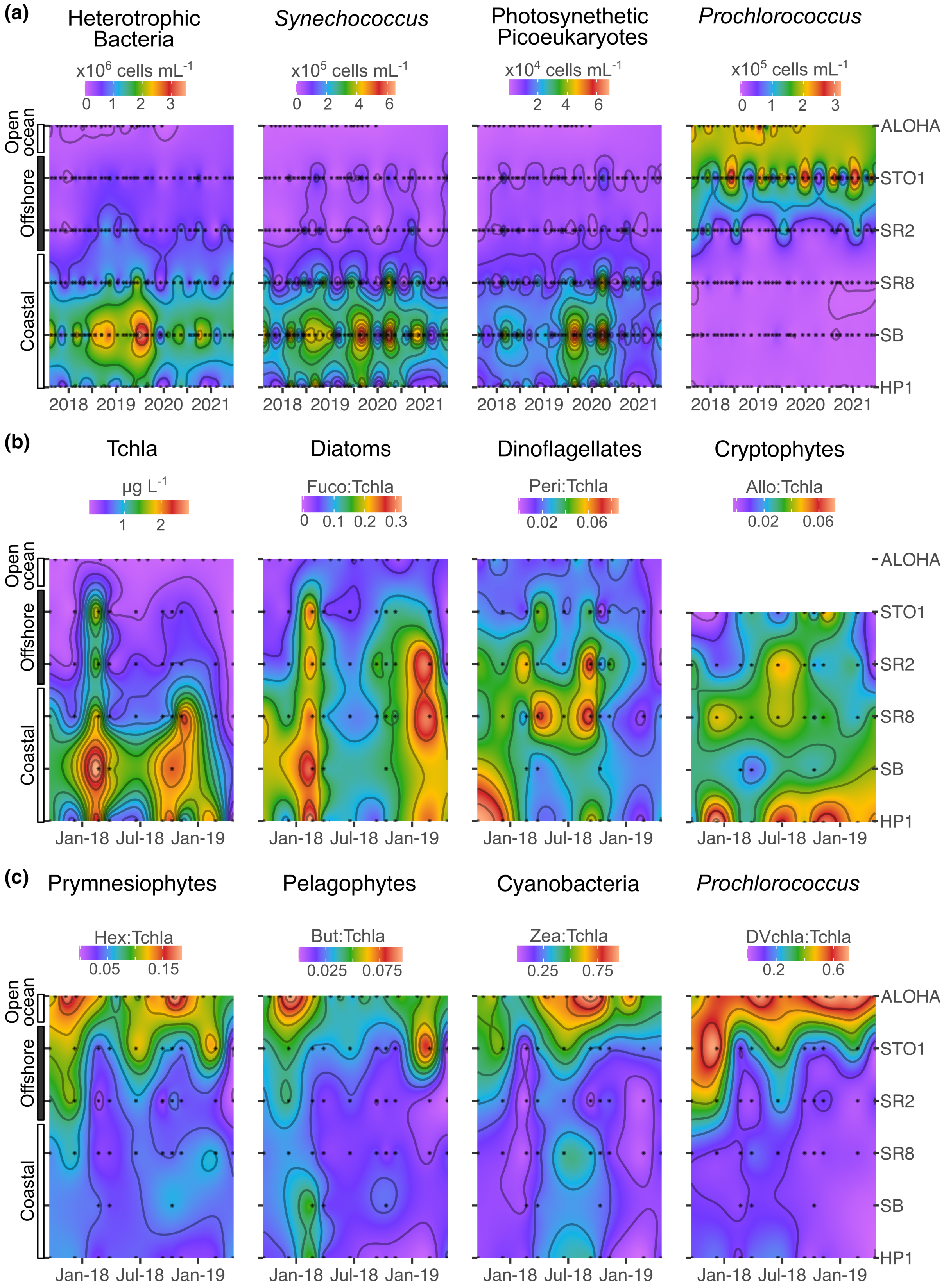
Biogeochemical parameters

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 across time, fluorometrically determined Chla concentrations at stations in the coastal waters of Kāne'ohe 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 He'eia Fishpond harbored higher Chla concentrations compared to coastal stations (3.9 ± 3.8 vs. 1.6 ± 1.9 μg L^{-1}; Fig. 1c). Stations offshore from Kāne'ohe Bay had elevated concentrations of Chla compared to the open ocean (0.6 ± 0.4 vs. 0.09 ± 0.03 μg L^{-1}). Elevated phytoplankton biomass was a persistent feature within Kāne'ohe Bay, with higher Chla 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 Kāne'ohe Bay compared to offshore and open ocean stations (Fig. 1d; Supporting Information Table S1). Mean silicate concentrations at Wai2 of the estuarine stations were 107.33 ± 45.38 μM, compared to 8.79 ± 5.58 μM in the coastal stations (Fig. 1d). Across all KByT stations, phosphate and silicate were positively correlated with increasing Chl *a* concentrations ($p < 0.001$, $p = 0.001$, respectively), while nitrate + nitrite concentrations did not correlate with Chl *a* concentrations ($p = 0.08$; Supporting Information Fig. S2). Importantly, both inorganic nutrients and Chl *a* concentrations increased with decreasing salinity (Supporting Information Fig. S2), suggesting that freshwater and brackish water input (e.g., surface stream and submarine groundwater discharge) may be an important driver of nutrient delivery and subsequent phytoplankton enhancement within He'eia Fishpond and Kāne'ohe Bay (McKenzie et al. 2019). 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 1958).

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 Kāne'ohe Bay and at the open ocean Station ALOHA (Fig. 2a; Supporting Information Table S1). Cellular abundances of heterotrophic bacteria and photosynthetic picoeukaryotes were also greater in the coastal stations compared to offshore and open ocean (Fig. 2a; Supporting Information Table S1). In



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coastal Kāneʻohe Bay, ratios of fucoxanthin, peridinin, and alloxanthin to total Chl *a* (Tchla) concentrations were higher than in the offshore and in the open ocean, indicating an increase in diatoms, dinoflagellates, and cryptophytes (respectively) closer to shore (Fig. 2b; Supporting Information Table S3). In contrast, pigments relative to Tchla for photosynthetic pigments diagnostic of prymnesiophytes (19′-hexanoyloxyfucoxanthin), pelagophytes (19′-butanoyloxyfucoxanthin), cyanobacteria (zeaxanthin), and *Prochlorococcus* (divinyl Chl *a*) were higher in the offshore and in the open ocean compared to the coastal environment (Fig. 2b; Supporting Information Table S3).

At stations within Kāneʻohe Bay, peaks in the cellular abundances of heterotrophic bacteria, *Synechococcus*, and photosynthetic picoeukaryotes coincide with summer sampling months (Fig. 2b; Supporting Information Fig. S3). In addition, increased rainfall and wind speeds from storms during three winter sampling events (February 21, 2018, March 3, 2020, and February 12, 2021) align with large changes in photosynthetic picoeukaryotes and *Synechococcus* cellular abundances and Chla concentrations (Fig. 2b; Supporting Information Fig. S3). Elevated Tchla and fucoxanthin (a pigment indicative of diatoms) were also measured during the February 2018 storm in both coastal Kāneʻohe Bay and the adjacent offshore (Fig. 2b), suggesting that episodic weather events can trigger phytoplankton responses at relatively large spatial scales.

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 we labeled as nearshore, transition, and offshore because of their distinct biogeochemical characteristics and geographic origins (Fig. 3a,b; Supporting Information Table S4; Fig. S4). 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; Fig. 3b). A small number of samples (4 of 72) collected from the two furthest offshore stations (STO1 & NTO1) resolved as a nearshore community type, suggesting that nearshore phytoplankton communities are rarely, but occasionally found outside of the bay (Fig. 3b). However, despite a high level of previously reported water exchange (Lowe et al. 2009), we do not observe offshore phytoplankton communities establishing at stations within Kāneʻohe Bay.

Amplicon sequencing variant richness was the highest in the nearshore, while the transition community group had the highest Shannon diversity estimate (Supporting Information Table S5). A large proportion of the ASVs were shared among all 3 community types ($n = 172$ of 505), while 126 ASVs were uniquely found in the nearshore, 29 ASVs were found only in the offshore, and only 9 ASVs were found solely within the transition community type (Supporting Information Fig. S5). Differential abundance analysis of amplicon data (Fig. 3c) was consistent with results from phytoplankton pigments (Fig. 2): the offshore phytoplankton community was enriched in *Prochlorococcus*, prymesiophytes (e.g., *Phaeocystis* and *Braarudosphaera*), and pelagophytes (e.g., Pelagomonadaceae, Sacrinochrysidaceae) relative to the nearshore, while the nearshore was enriched in diatoms (e.g., *Chaetoceros*, Polar-centric Mediophyceae) and cryptophytes (e.g., *Teleaulax*, Cryptomonadales) relative to the offshore (Fig. 3c; Supporting Information Table S6). The transition community was uniquely enriched in green algae (e.g., *Ostreococcus*, Mamiellophyceae) and Pinguiophyceae relative to the nearshore and the offshore. However, the transition also represented characteristics of both the nearshore (i.e., similar abundances of diatoms as the nearshore) and offshore communities (i.e., elevated abundances of *Prochlorococcus* compared to the nearshore; Fig. 3c).

Cyanobacterial population structure

Metagenomic read recruitment to 56 genomes of the cyanobacterial genera *Prochlorococcus*, *Synechococcus*, and *Cyanobium* showed that genomes from *Synechococcus* SC 5.1 II and SC 5.3 and *Prochlorococcus* high-light adapted clade II (HLII) were among the most abundant representatives within our samples (Fig. 4; Supporting Information "Cyanobacterial Population Structure" section). *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, but only 1.1% ± 2.4% of the cyanobacterial relative abundance in the nearshore. *Synechococcus* clade II comprised 96.3% ± 2.5% of the cyanobacterial relative abundance in the nearshore Kāneʻohe Bay community type. *Synechococcus* SC 5.3

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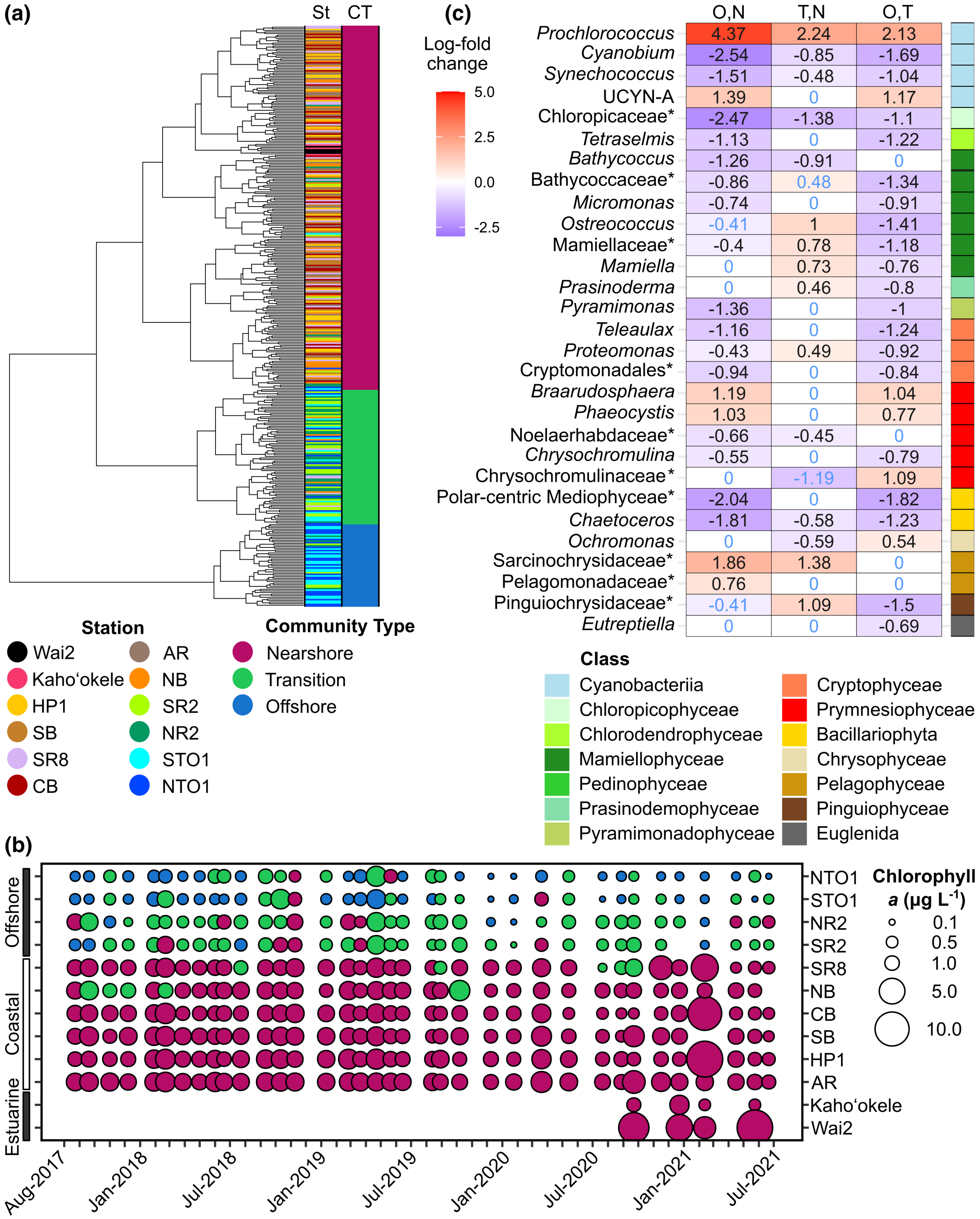
Fig. 2. Microbial cellular abundances and pigment concentration vary through time and space across stations from the southern sector of Kāneʻohe Bay (HP1, SB, SR8), offshore stations (SR2 and STO1), and open ocean Station ALOHA: (**a**) cellular abundances (cells mL^{-1}) of heterotrophic bacteria, *Synechococcus*, photosynthetic picoueukaryotes, 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. Allo, alloxanthin; But, 19′-butanoyloxyfucoxanthin; Dvchla, divinyl chlorophyll *a*; Fuco, fucoxanthin; Hex, 19′-hexanoyloxyfucoxanthin; Peri, peridinin; Zea, zeaxanthin.

also recruited some metagenomic reads, but only from coastal Kāneʻohe Bay samples and at low relative cyanobacterial abundance (2.4% ± 1.2%) (Fig. 4).

Within the *Prochlorococcus* HLII and *Synechococcus* II clades, read recruitment varied between closely related genomes. Read recruitment was substantially higher in *Synechococcus* clade II



	O,N	T,N	O,T
Prochlorococcus	4.37	2.24	2.13
Cyanobium	-2.54	-0.85	-1.69
Synechococcus	-1.51	-0.48	-1.04
UCYN-A	1.39	0	1.17
Chloropicaceae*	-2.47	-1.38	-1.1
Tetraselmis	-1.13	0	-1.22
Bathycoccus	-1.26	-0.91	0
Bathycoccaceae*	-0.86	0.48	-1.34
Micromonas	-0.74	0	-0.91
Ostreococcus	-0.41	1	-1.41
Mamiellaceae*	-0.4	0.78	-1.18
Mamiella	0	0.73	-0.76
Prasinoderma	0	0.46	-0.8
Pyramimonas	-1.36	0	-1
Teleaulax	-1.16	0	-1.24
Proteomonas	-0.43	0.49	-0.92
Cryptomonadales*	-0.94	0	-0.84
Braarudosphaera	1.19	0	1.04
Phaeocystis	1.03	0	0.77
Noelaerhabdaceae*	-0.66	-0.45	0
Chrysochromulina	-0.55	0	-0.79
Chrysochromulinaceae*	0	-1.19	1.09
Polar-centric Mediophyceae*	-2.04	0	-1.82
Chaetoceros	-1.81	-0.58	-1.23
Ochromonas	0	-0.59	0.54
Sarcinochrysidaceae*	1.86	1.38	0
Pelagomonadaceae*	0.76	0	0
Pinguiochrysidaceae*	-0.41	1.09	-1.5
Eutreptiella	0	0	-0.69

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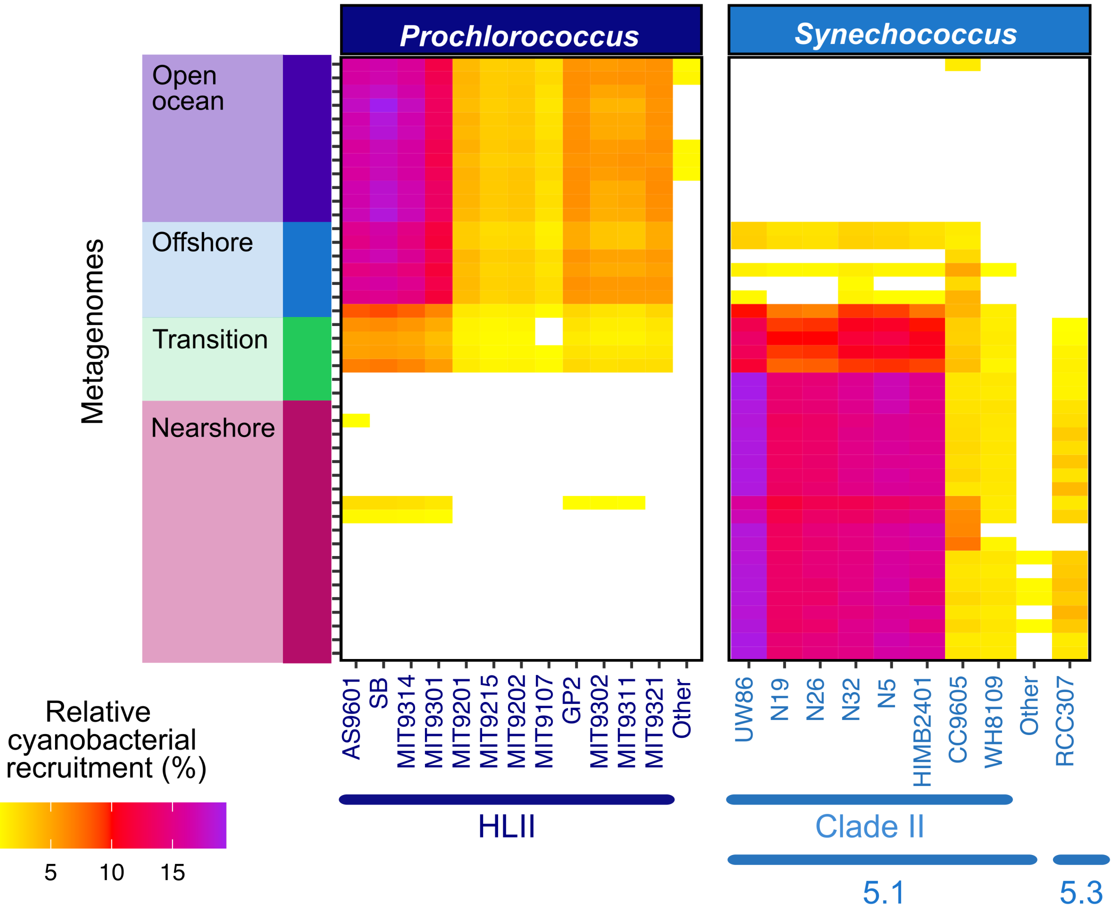

Fig. 4. The relative abundance of cyanobacterial genomes across Kāne'ohe Bay Time-series (KByT) and Station ALOHA metagenomes. *Prochlorococcus* high-light adapted clade II (HLII) had the highest abundance relative to the other cyanobacteria in the offshore and Station ALOHA metagenomes, while *Synechococcus* clade II (SC 5.1) dominated in the nearshore metagenomes. The other group within *Prochloroccocus* encompassed isolates with low relative abundance from the low-light adapted clade I (LLI) and the high-light adapted clade I (HLI). The other within *Synechococcus* 5.1 encompassed isolates with low relative abundance from WPC2, III, UC-A, and IX clades. Recruitment of < 0.5% not shown.

isolate UW86 compared to all other clade II genomes, despite sharing > 95% ANI with most other clade II genomes (Supporting Information Fig. S6). 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. S6).

Seasonality in biogeochemistry and community composition

We detected seasonal differences in biogeochemistry and phytoplankton community composition across nearshore Kāne'ohe Bay and the adjacent transition and offshore waters, although seasonality in the transition and offshore waters was more subtle than patterns in the nearshore. Cellular concentrations of *Synechococcus* and heterotrophic bacteria were higher in the summer than the winter in the nearshore, transition, and offshore (Fig. 5a; Supporting Information Table S7).

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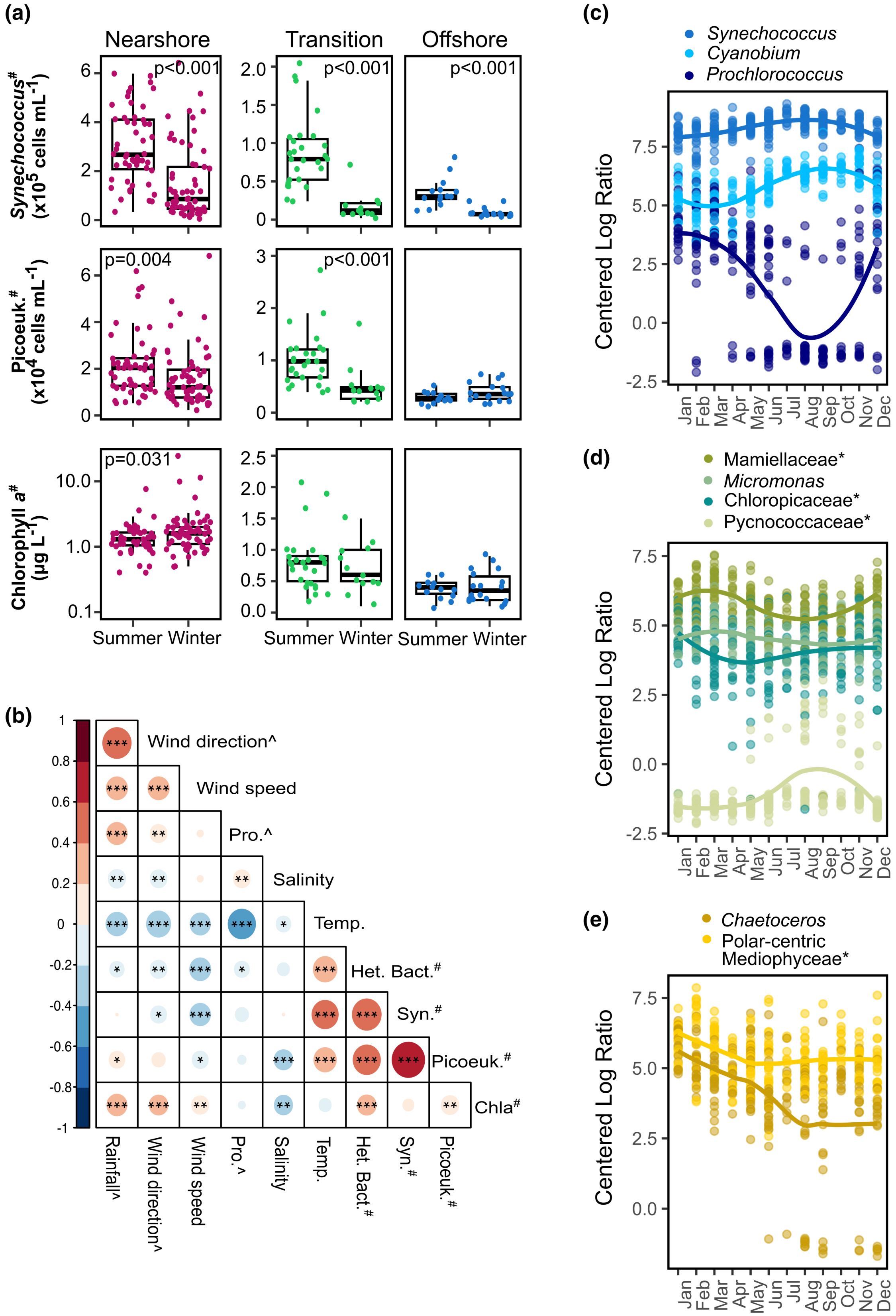
Fig. 3. (**a**) Hierarchical clustering of phytoplankton communities from 366 samples collected at 12 sampling stations form three major groups, hereafter referred to as community types: nearshore, transition, and offshore. (**b**) The distribution of samples defined as nearshore, transition, and offshore community types across stations and sampling events (36 total between 2017 and 2021) shows spatial and temporal persistence in the distinct community types. The size of the circle represents the fluorometric chlorophyll *a* concentration (Chla) during the time of sampling and the color of the circle represents the community type: nearshore, transition, and offshore. (**c**) Log-fold changes in phytoplankton genera with significantly different abundances between the offshore compared to the nearshore (O, N), transition compared to the nearshore (T, N), and the offshore compared to the transition (O, T). Log-fold changes in abundance are shown in text, with black text indicating the comparison passed a sensitivity analysis to assess the impact of pseudo-counts. Phytoplankton groups that were classified at the family-level but unidentified at the genus-level are denoted with an asterisk.



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Photosynthetic picoeukaryote cellular concentrations also increased during the summer in coastal and transition environments (coastal: $p = 0.004$, transition: $p < 0.001$, Fig. 5a; Supporting Information Table S7), but did not vary seasonally in the offshore. In contrast, *Prochlorococcus* cell concentrations only varied seasonally in the nearshore where it increased in the winter ($p < 0.001$; Supporting Information Table S7).

Chla concentrations increased in the winter compared to the summer in the nearshore (mean ± SD; winter: $2.3 \pm 3.4\ \mu\mathrm{g\ L}^{-1}$, summer: $0.8 \pm 0.5\ \mu\mathrm{g\ L}^{-1}$; $p = 0.031$; Fig. 5a; Supporting Information Table S7), but not in the transition or offshore waters. Importantly, given that the collection method for Chla concentrations likely missed most small cyanobacteria, it is possible that seasonality has been underestimated. Nearshore Chla concentrations increased with wind speed, wind direction, and rainfall, but not with seawater temperature (Fig. 5b). Ratios of phytoplankton pigments to Tchla representing diatoms and pelagophytes showed a negative correlation with seawater temperature in the nearshore community type, suggestive that these phytoplankton may be driving the winter increase in Chla concentrations in the nearshore (Supporting Information Fig. S7). In contrast, pigments indicative of cyanobacteria showed a significant positive relationship with seawater temperature in the nearshore (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). Chla concentrations in the transition and offshore also did not correlate with seawater temperatures, but did positively correlate with rainfall (Supporting Information Fig. S8).

Amplicon sequencing variant richness and Shannon diversity increased in the winter compared to the summer in all three community types (Supporting Information Table S8). 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 (Lomb Scargle Periodogram test, $q \leq 0.05$; Supporting Information Table S9; Fig. S9; "Seasonality in Biogeochemistry and Community Composition" section). In the nearshore, dominant phytoplankton groups with seasonal patterns include cyanobacteria, green algae, and diatoms (Fig. 5c–e). While the two diatom groups are both enriched in the winter months relative to the summer months, the seasonality of members belonging to green algae and cyanobacteria differs by genus in the nearshore (Fig. 5c–e). The distinct seasonal patterns among the cyanobacteria, such that *Prochlorococcus* increases in the winter in the nearshore, while *Synechococcus* increases in the summer, is further supported by cellular abundance data (Supporting Information Table S7). As water sample collection included a pre-filtering step with 85-μm mesh to exclude zooplankton, our measures may have resulted in an underestimation of diatom species larger than 85 μm.

Discussion

The activities and distributions of phytoplankton have important implications for food web dynamics, biocultural restoration, and adaptive management of near-island waters of the tropical Pacific, especially under predicted climate change conditions that are expected to shift nutrient availability, phytoplankton size, productivity, and biomass in open oceans (Boyce et al. 2010; Flombaum et al. 2020; Li et al. 2020). 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 North Pacific Subtropical Gyre) 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.

Distinct phytoplankton and biogeochemical regimes within and adjacent to Kāneʻohe Bay

In line with previous work, our results show that the elevated phytoplankton biomass in nearshore Kāneʻohe Bay is predominantly driven by the freshwater input of land-derived and anthropogenic nutrients delivered via surface streams to coastal waters (Cox et al. 2006; Yeo et al. 2013) and submarine groundwater-derived inputs (Dulai et al. 2016; Ghazal et al. 2023), which reduces the prevailing nitrogen limitation and enhances phytoplankton growth (Ringuet and Mackenzie 2005). Although not examined in this study, sewage and agriculture (McKenzie et al. 2019), ocean-driven internal waves (Gove et al. 2016; Comfort et al. 2024), and coral-reef processes (James et al. 2020) are other likely important mechanisms for nutrient delivery into coastal Kāneʻohe Bay. Over the 4-yr time-series, inorganic nutrients and phytoplankton

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Fig. 5. (**a**) Changes in cell counts of *Synechococcus* and photosynthetic picoeukaryotes and fluorometric chlorophyll *a* concentrations (Chla) across seasons and environments. Note that Chla concentrations are presented on a log scale for the nearshore, but not for the transition and offshore environments. (**b**) Correlations between Chla and other environmental parameters in the nearshore. (**c–e**) Genera belonging to cyanobacteria (**c**), archaeplastida (green algae) (**d**), and stramenopiles (diatoms) (**e**) show seasonal changes in abundance in the nearshore. 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. A pound sign (#) denotes variables with log transformations, while a carrot (^) denotes variables with log + 1 transformations. Salinity (ppt); wind direction (degrees); wind speed ($\mathrm{m\ s}^{-1}$); rainfall (mm); Chla, chlorophyll *a* concentration ($\mu\mathrm{g\ L}^{-1}$); Het.Bac, heterotrophic bacteria (cells mL^{-1}); Picoeuk, photosynthetic picoeukaryotes (cells mL^{-1}); Pro, *Prochlorococcus* (cells mL^{-1}); Syn, *Synechococcus* (cells mL^{-1}); Temp, seawater temperature (°C).

biomass were persistently elevated within Kāneʻohe Bay relative to offshore stations, with enhanced phytoplankton biomass primarily attributed to *Synechococcus* and diatoms. Members of phytoplankton groups with larger cell sizes were enriched in the nearshore relative to the offshore, including diatoms, dinoflagellates, and cryptophytes, following previous work that has observed increased phytoplankton cell sizes with increased nutrient availability (Chisholm 1992). Offshore phytoplankton communities were enriched in relatively smaller phytoplankton groups, including *Prochlorococcus*, prymnesiophytes, and pelagophytes, which have been reported to reach high abundances in warm oligotrophic oceans (Li et al. 2013; Rii et al. 2016).

Despite a constant input of oceanic seawater, phytoplankton communities within Kāneʻohe Bay were structured into distinct communities that varied in composition, diversity, and potentially lifestyles. Limited dispersal alone cannot explain this strong structuring, as there is a high overlap in ASVs shared across the community types and most of our sampling stations in the bay receive advection of oceanic-sourced waters (Lowe et al. 2009). Instead, community structure most likely reflects a high degree of environmental selection for locally adapted populations across this sharp environmental gradient (Yeo et al. 2013; Tucker et al. 2021). One potential outcome of environmental selection and resource competition is the enrichment of phytoplankton associated with mixotrophic lifestyles closer to shore, including *Teleaulax* (Cryptophyta), *Pyramimonas* (Chlorophyta), *Tetraselmis* (Chlorophyta), and dinoflagellates (Stoecker et al. 2016). By supplementing primary production with heterotrophy, mixotrophy serves as a source of inorganic and organic nutrients, increases carbon transfer to high trophic levels, and shifts the community toward larger plankton size classes (Stoecker et al. 2016; Ward and Follows 2016). While establishing the relative importance of mixotrophy across nearshore Kāneʻohe Bay to the adjacent offshore environment requires further investigation, the abundant bacterial prey items and limited inorganic nutrients of coastal Kāneʻohe Bay may favor mixotrophy over autotrophy (Edwards 2019). These initial insights highlight important implications for the structuring of phytoplankton communities across the nearshore to open ocean waters of the tropical Pacific, including distinctions in food web dynamics, phytoplankton ecologies, and contributors to primary productivity.

Drivers of seasonality in nearshore Chl **a** *concentrations*

In Hawaiʻi, storm events increase during the winter months, leading to higher stream discharge, increased nutrient input, and phytoplankton growth (Ringuet and Mackenzie 2005; Cox et al. 2006; Yeo et al. 2013). We observed three distinct storm events within the winter months that likely contributed to the observed increase in phytoplankton biomass during the wet (winter) months in the nearshore. Previous work has shown that the wet (winter) season has strong impacts on the food web dynamics of Kāneʻohe Bay: increased diatom biomass decreases the number of trophic levels between autotrophs and metazoan larvae and nauplii and leads to an increase in total community biomass and in the transfer of production to metazoans (Selph et al. 2018). Periods of high nutrient availability favor diatoms because they are fast-growing nutrient opportunists with high maximum growth but low affinity for uptake (Laws 1975; Inomura et al. 2023). In contrast, in the summer under high light, warm-water temperatures and limited nutrients, the high affinity for uptake but low maximum growth of *Synechococcus* is expected to be favored (Agawin et al. 2000). These trade-offs between growth and nutrient uptake strategies, along with changes in the environment that favor one strategy over another (Edwards et al. 2012), likely produced the dynamic seasonal patterns in the dominant phytoplankton observed in nearshore Kāneʻohe Bay.

Phytoplankton indicators of climate change impacts

Biodiversity within phytoplankton groups can modulate the impacts of variable temperature and nutrient regimes on growth, and thus may provide good bioindicators of resilience to ongoing climate impacts (Martiny et al. 2022). Within *Prochlorococcus* and *Synechococcus*, small gene gain and loss events have allowed for local adaptations of major and minor clades to variable nutrient concentrations and substrates (Doré et al. 2023). We find the dominance of *Synechococcus* clade II and *Prochlorococcus* HLII in coastal and offshore environments of the tropical Pacific, respectively, as well as clear population-level differences within these two clades. Using read recruitment from global surface and subsurface ocean metagenomes, Delmont and Eren (2018) found distinct distribution patterns among similar subgroups of HLII isolates as those reported here and associated such differences in fitness with fine-scale differences in gene content. *Prochlorococcus* HLII from the offshore waters adjacent to Kāneʻohe Bay and Station ALOHA showed high similarity in population structure and thus likely represent continuous populations with ongoing gene flow and adaptations driven by similar environmental pressures. Continued examination of population structure, as well as cellular and relative abundances of these cyanobacteria, could identify eventual expansions of ultraoligotrophic waters into Kāneʻohe Bay, shifts in gene flow, gains or losses of genes related to nutrient acquisition or temperature stress, and selection for clades with unique ecological adaptations.

In addition, deviations in the magnitude and timing of seasonal changes in *Synechococcus* and diatom abundances could also be used to identify variable responses to warming temperatures and changing nutrient availability. Temperature has been shown to positively impact *Synechococcus* division rates (Hunter-Cevera et al. 2016), suggesting that under warmer seawater temperatures *Synechococcus* may further dominate the nearshore phytoplankton community. In contrast, while some diatom species appear to have the capacity to adapt physiologically and genetically to increased temperatures (Hattich et al. 2024), temperature adaptation may be limited under the low nitrogen concentrations that are characteristic of Kāneʻohe Bay (Aranguren-Gassis et al. 2019). The magnitude and frequency of storm events on windward Oʻahu are expected to change with ongoing climate change (Fandrich et al. 2022), suggesting that it is possible that under wetter conditions nearshore diatoms may keep pace with increasing temperatures, but under drier conditions increased nitrogen limitation may lead to reduced diversity, seasonality, and/or overall abundances of diatoms. It has also been observed that high-island environments of Hawaiian Islands promote elevated delivery of submarine groundwater discharge into nearshore regions during king tides (McKenzie et al. 2021), thus further impacting phytoplankton community composition under combined scenarios of increased storms, stratification, and sea-level rise.

Implications for biocultural restoration and adaptive management

Within Heʻeia and across the islands of Hawaiʻi, Indigenous People and Local Communities are undertaking biocultural restoration projects of Indigenous aquaculture systems 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 Heʻeia and Kāneʻohe Bay of the conditions that promote phytoplankton growth and subsequently herbivorous fish growth. Despite differences in biogeochemistry across nearshore stations, phytoplankton communities sampled from the Heʻeia Fishpond and across the bay grouped as one nearshore community type. This emphasizes the connectedness of these estuarine and coastal systems and highlights the need to manage them in close coordination. Further growing this baseline understanding of phytoplankton assemblages to relate to the timing and conditions documented in Hawaiian knowledge systems, and in regard to life cycles of bioculturally relevant species like ʻamaʻama (*Mugil cephalus*) which are grown in the fishpond and feed on diatoms and other microphytoplankton at juvenile stages (Hiatt 1947), would advance this area of study and utility for management within these Indigenous aquaculture systems. Our study also provides opportunities to refine hydrological models conducted in social-ecological systems (Ghazal et al. 2023) to further inform biocultural restoration toward adaptive management.

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 to co-produce new knowledge could improve the ability of local people to document and adapt to changes in near-island marine resources (Winter, Rii, et al. 2020). 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 Oʻahu, Hawaiʻi, 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.

Author Contributions

Sarah J. Tucker led the formal analyses and wrote the initial manuscript draft. Sarah J. Tucker, Yoshimi M. Rii, Kelle C. Freel, and Michael S. Rappé collected the samples with assistance from A. Hiʻilei Kawelo and Keliʻiahonui Kotubetey. Sarah J. Tucker, Kelle C. Freel, Yoshimi M. Rii, and Michael S. Rappé processed the samples. All authors were involved in the conceptualization, development of methodology, interpretation of results, and providing input to the manuscript draft and revisions.

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Conflicts of Interest

None declared.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

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