Coral Reefs
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REPORT

Eukaryotic plankton communities across reef environments in Bocas del Toro Archipelago, Panamá

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Abstract Variation in light and temperature can influence the genetic diversity and structure of marine plankton communities. While open-ocean plankton communities receive much scientific attention, little is known about how environmental variation affects plankton communities on tropical coral reefs. Here, we characterize eukaryotic plankton communities on coral reefs across the Bocas del Toro Archipelago, Panamá. Temperature loggers were deployed, and midday light levels were measured to quantify environmental differences across reefs at four inshore and four offshore sites (Inshore = Punta Donato, Smithsonian Tropical Research Institute (STRI) Point, Cristobal, Punta Laurel and Offshore = Drago Mar, Bastimentos North, Bastimentos South, and Cayo de Agua). Triplicate vertical plankton tows were collected midday, and high-throughput 18S ribosomal DNA metabarcoding was leveraged to investigate the relationship between eukaryotic plankton community structure and inshore/offshore reef environments. Plankton communities from STRI Point were additionally characterized in the morning (~ 08:00), midday (~ 12:00), and late-day (~ 16:00) to quantify temporal variation within a single site. We found that inshore reefs experienced higher average seawater temperatures, while offshore sites offered higher light levels, presumably associated with reduced water turbidity on reefs further from shore. These significant environmental differences between inshore and offshore reefs corresponded with overall plankton community differences. We also found that temporal variation played a structuring role within these plankton communities, and conclude that time of community sampling is an important consideration for future studies. Follow-up studies focusing on more intensive sampling efforts across space and time, coupled with techniques that can detect more subtle genetic differences between and within communities will more fully capture plankton dynamics in this region and beyond.

Keywords Coral reefs · Plankton · Reef zones · Phytoplankton · Zooplankton · Metabarcoding · 18S · Heterotrophy

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Andrea Rodas and Rachel Wright to be co-first author.

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Introduction

While open-ocean and coastal plankton communities are relatively well studied, plankton communities inhabiting oligotrophic tropical coral reefs have received far less attention, even though these reefs experience

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environmental variations that are likely to structure these communities across space and time. The diversity and abundance of marine plankton communities are well known to be affected by environmental variation including, but not limited to temperature, nutrients, and light (Andersson et al. 1994; D'Croz et al. 2005). For example, organisms inhabiting inshore or offshore reefs experience strongly divergent environmental conditions (Varela et al. 2001; Castillo et al. 2011; Siegel et al. 2013). Inshore reef sites generally experience greater environmental variation associated with changing tidal cycles, increased mean temperatures driven by more restricted flow and shallower reef extent, and reduced salinities and increased turbidity associated with freshwater input from rivers and runoff (Lirman and Fong 2007). Offshore reefs are buffered by the open ocean and thus exhibit less turbid seawater with more stable temperatures, resulting in enhanced light penetration generally favoring photosynthetic organisms (Boyer et al. 2015). These physical differences in water quality parameters might therefore be expected to influence the structure of plankton communities on coral reefs. First, because there are species-specific thermal optima for plankton survival (Mauchline 1998), temperature differences across inshore and offshore reefs may play a strong role in structuring plankton communities. Additionally, as light levels are a major factor affecting phytoplankton growth (Harrison and Turpin 1982; Edwards et al. 2016), spatial variation in light availability across reefs can have cascading food web effects that influence the entire ecosystem (Andersson et al. 1994; Barrera-Oro 2002).

Shifts in the structure of plankton communities are considered to be robust bioindicators of subtle environmental changes because species that comprise these communities have rapid life cycles that allow for quick responses to environmental perturbations (Hays et al. 2005; Richardson 2008). For example, shifts in plankton distributions associated with warming waters were documented in the northeast Atlantic from 1959 to 2000 (Lindley and Daykin 2005). Furthermore, storms and upwelling events affect local water chemistry by introducing nutrient runoff from land, which can rapidly change the distribution of plankton, ultimately impacting their behavior and growth (Dunstall et al. 1990; Richmond and Woodin 1996). Plankton are also fundamental to a healthy food web, as they provide energy to higher trophic-level organisms such as marine birds, fish, and corals (Fenchel 1988; Frederiksen et al. 2006). On coral reefs specifically, plankton are an important source of heterotrophic nutrition to corals themselves. Heterotrophy has been shown to increase coral survival and recovery after heat stress (Johannes et al. 1970; Ferrier-Pagès et al. 2010; Hughes and Grottoli 2013; Tremblay et al. 2016) and to mitigate temperature-induced coral bleaching (Grottoli et al. 2006; Aichelman et al. 2016). However, few studies have examined how these important tropical coral reef plankton communities are influenced by environmental variation across inshore and offshore reef sites (Chiba et al. 2018).

Plankton community surveys began in the early 1800s, when the first net suitable for sampling zooplankton was developed (Fraser 1968). Historically, plankton communities were characterized by microscopic examination of each microorganism (Johannes et al. 1970; Irigoien et al. 2004). This method relies on advanced taxonomic abilities of the observer to identify diverse species across different life stages as well as extensive time investment. Other methods for assessing plankton communities include measuring zooplankton organic biomass, which can offer insights into the overall biomass, but not the diversity or taxa-specific abundance of the sampled community (D'Croz et al. 2005). Recent technological advancements in next-generation sequencing have provided a robust and reliable method to identify and characterize the diversity and relative taxa abundances of plankton communities through high-throughput single-locus metabarcoding sequencing (Albaina et al. 2016; Bucklin et al. 2016; Abad et al. 2016). In this approach, a genomic locus homologous across all Eukaryota is amplified and sequenced and unique taxa are identified as amplicon sequence variants (ASVs) based on some threshold of similarity in DNA sequence (Eiler et al. 2013; Lindeque et al. 2013; Kermarrec et al. 2014). This high-throughput analytical method provides information about species presence or absence and relative abundance, based on the number of observed reads mapping to any particular taxa, without the need for morphological examination. The precision of ASV identification by next-generation sequencing continuously improves as the databases used to identify species grow (Quast et al. 2013).

For the purpose of this study, eight sites were categorized based on their distance from mainland Panamá, with lagoonal sites classified as 'inshore' and oceanic sites classified as 'offshore' (Fig. 1a; Table 1). Inshore sites are presumed to experience increased nutrient and freshwater runoff from the mainland and also experience less flow than sites located on the oceanic side of these islands (D'Angelo and Wiedenmann 2014). We performed plankton tows at each of these sites midday and then leveraged 18S ribosomal DNA metabarcoding to gain insights into how the environmental conditions experienced between inshore and offshore reefs influence plankton communities. Additionally, we assessed plankton communities at three time points (morning, midday, and late afternoon) at a single site to explore temporal variation in plankton communities. Overall, these data illuminate how potential heterotrophic opportunities and biogeochemical contributions on coral reefs might vary across space and time in this

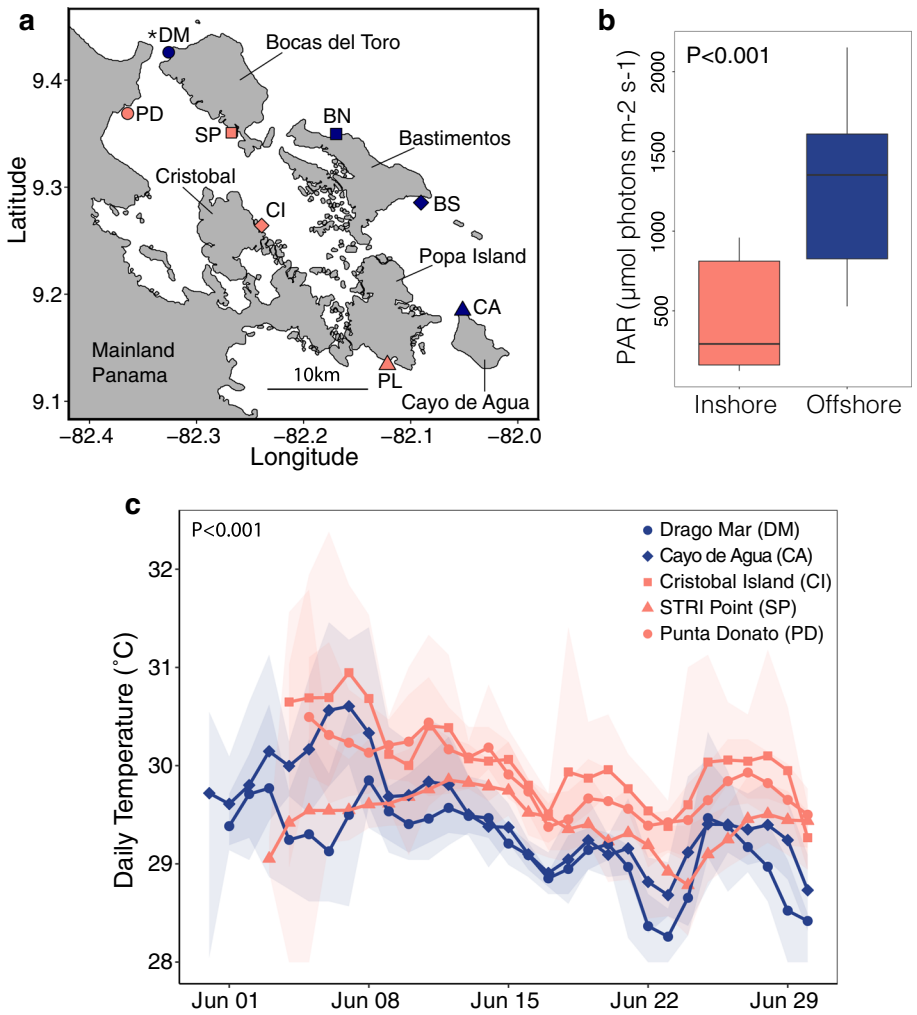

Fig. 1 Environmental conditions in Bocas del Toro. **a** Location of collection sites in Bocas del Toro, Panamá. Salmon symbols indicate inshore sites and offshore sites are in blue: PD: Punta Donato, SP: STRI Point, CI: Cristobal Island, PL: Punta Laurel, DM: Drago Mar, BN: Bastimentos North, BS: Bastimentos South, CA: Cayo de Agua. Drago Mar is additionally indicated with * to correspond with Fig. 2b. **b** Mean maximum daily photosynthetically active radiation (PAR; µmol photons m^{-2} s^{-1}) of the top 20 PAR values from inshore and offshore reef sites. PAR data were collected on the same day as plankton sampling. Error bars indicate the minimum and maximum PAR values. **c** Daily temperature ranges for each site (inshore = salmon, offshore = blue). Symbols represent mean temperatures for each date (28 May–30 June 2015) and shaded regions encompass the maximum and minimum values for each site on that particular date. *p*-value indicates that inshore reef sites are significantly warmer than offshore reef sites. It is also important to note that plankton sampling at STRI Point (SP) occurred on 5/28/15, while temperature data are not available until 6/3/15

region, which can ultimately affect food web dynamics in these marine environments.

Materials and methods

Abiotic environmental conditions in Bocas del Toro

To assess environmental differences across inshore and offshore reef sites, we characterized thermal and light profiles at four inshore sites (Punta Donato (PD), Smithsonian Tropical Research Institute [STRI] Point (SP), Cristobal Island (CI), and Punta Laurel (PL)) and four offshore sites (Drago Mar (DM), Bastimentos North (BN), Bastimentos South (BS), and Cayo de Agua (CA)) within the Bocas del Toro Archipelago reef complex in Panamá (Fig. 1a). These eight sites were categorized based on their location to mainland Panamá relative to its neighboring islands. Lagoonal sites located between mainland Panamá and the islands of Bocas del Toro, Bastimentos, Popa Island, and Cayo de Agua were classified as 'inshore,' and those on the oceanic sides of these islands were classified as 'offshore' (Fig. 1a; Table 1).

Temperature conditions in situ were quantified by deploying data loggers (HOBO Pendant, Onset Computer Corporation) at each sampling site for approximately 1 yr,

Table 1 Plankton sampling sites on the Bocas Del Toro Archipelago, Panamá including reef environment: time of day, latitude, longitude, date of collection, and depth of vertical plankton tows

Site	Reef	Lat (°N)	Long (°W)	Date	Depth (ft)
Punta Donato (PD)	Inshore	9.369240	82.36404	6/5/15	9
STRI Point (SP)	Inshore: midday	9.352434	82.26453	5/28/15	15
	Early (8:30)			6/3/15	17
	Late (15:00)			6/3/15	19
	Early (8:30)			6/4/15	19
	Late (15:30)			6/4/15	19
Cristobal Island (CI)	Inshore: midday	9.265449	82.24351	6/4/15	7
Punta Laurel (PL)	Inshore: midday	9.132796	82.119555	6/3/15	8
Bastimentos North (BN)	Offshore: midday	9.348495	82.17651	5/29/15	15
Bastimentos South (BS)	Offshore: midday	9.287438	82.09231	5/30/15	6
Cayo de Agua (CA)	Offshore: midday	9.193858	82.05395	5/31/15	10
Drago Mar DM)	Offshore: midday	9.424539	82.32479	6/1/15	12

Sunrise and sunset throughout the sampling period occurred at approximately 6:00 and 18:35, respectively

and temperature data were recorded every 15 min at each site for the duration of deployment. Logger deployment began at the end of May (Cayo de Agua) or early June (STRI Point, Punta Donato, Cristobal Island, and Drago Mar) in 2015, and loggers were retrieved in August 2016. Temperature loggers were deployed and affixed at depth (Table 2) immediately next to a large bouldering coral colony of the species *Siderastrea siderea* or *Pseudodiploria strigosa.* Therefore, temperatures described in this study are in situ temperatures experienced on the reef at depth, not throughout the water column or at the surface. Loggers from Cristobal Island, Punta Donato, STRI Point, Drago Mar, and Cayo de Agua were retrieved; however, loggers from Punta Laurel, Bastimentos North, and Bastimentos South were not located and presumed lost. For the loggers that were retrieved, temperature maximum, minimum, and daily mean were averaged between 28 May and 30 June 2015 and these data were used to characterize differences between thermal environments between sites near to the time of plankton community sampling.

An underwater 2π Quantum Sensor (LI-192, LI-COR Inc.) was used to measure photosynthetically active radiation (PAR) for all sites at the time of plankton sample collections with the exception of Cristobal due to consistently overcast conditions. For the remaining sites, PAR levels were measured every 30 s between the hours of approximately 10:00 and 14:00 on sampling days. To account for variations in daily cloud cover, only the maximum twenty PAR measurements collected from each site were used to compare differences between inshore and offshore reefs and between reef sites (Table 2). A one-way ANOVA (R Development Core Team 2018) was used to

Table 2 Mean photosynthetically active radiation (PAR) values ± SE (top 20 PAR values; µmol photons m^{-2} s^{-1}) and associated depths of light logger deployment (feet), mean daily temperature ± SE (°C) on the closest available date to plankton collection (Fig. 1; Table 1), mean daily maximum temperature ± SE from 28 May to 30 June 2015 and associated depths of temperature logger deployment (feet)

Site	Mean PAR	Light logger depth	Mean temp	Max temp	Temp logger depth
Punta Donato (PD)	326 ± 15	10	30.5 ± 0.04	29.9 ± 0.06	8
STRI Point (SP)	151 ± 6	17	29.1 ± 0.01*	29.7 ± 0.05	19
Cristobal Island (CI)	–	n/a	30.6 ± 0.06	30.4 ± 0.14	5
Punta Laurel (PL)	838 ± 8	8	–	–	n/a
Bastimentos North (BN)	562 ± 8	15	–	–	n/a
Bastimentos South (BS)	1673 ± 45	6	–	–	n/a
Cayo de Agua (CA)	1578 ± 48	6	29.7 ± 0.03	29.5 ± 0.08	6
Drago Mar (DM)	1040 ± 39	10	29.4 ± 0.02	29.2 ± 0.12	10

Cristobal Island (CI) does not have light values because it was too cloudy on the day of sampling to get an accurate reading. HOBO loggers for Punta Laurel (PL), Bastimentos North (BN), and Bastimentos South (BS) were not recovered so no temperature data are available for these sites. *Denotes the only site (STRI Point (SP)) whose temperature data were not available on the actual date of plankton sampling (6/3/15 instead of 5/28/15)

test for differences in mean light level and daily maximum temperature across the inshore and offshore reef sites (Fig. 1c, b).

Plankton community collections and 18S metabarcoding preparations

Vertical plankton tows were conducted in triplicate at each site midday by snorkeling. Each site was sampled on a different day between 28 May and 5 June (Table 1). During each plankton tow, the plankton net was deployed immediately next to a large bouldering coral (*Siderastrea siderea* or *Pseudodiploria strigosa*) on the reef. The snorkeler swam directly toward the surface, thus capturing all plankton in the water column above the reef. Sites varied in depth (6–19 ft; Table 1). Midday sampling depths ranged from 6 to 15 ft across the eight sites. While depth may play a structuring role in plankton communities, average sampling depths (± standard error) at inshore sites (9.8 ± 0.94 ft) did not differ significantly from average sampling depths at offshore sites (11.2 ± 0.97 ft; *T* test p value = 0.3; ESM Fig. S2A). Time between replicates was < 10 min, which was the time it took to process samples at the surface. All tows were conducted from the same position on the reef, and the same sampling strategy was used to collect tows from STRI Point at the early (triplicate tows at 08:30 on 3 June and 4 June) and late (triplicate tows at 15:00 on 3 June and 15:30 on 4 June) time points. Sunrise and sunset throughout the sampling period occurred at approximately 06:00 and 18:35, respectively. Due to field logistics and time constraints, we were able to collect more replicate tows across multiple days at STRI Point during the early and late hours than we were at midday. However, a minimum of three tows were collected at each time point. We account for the unbalanced groups in the statistical tests described below. Plankton tows were conducted using a plankton net with 0.5 m diameter and 60-µm mesh. Filtered water was then passed through an additional 60-µm filter to concentrate collections, and samples were preserved in 50 mL of 200 proof ethanol. Samples were transported to the laboratory at the University of North Carolina at Chapel Hill and maintained at − 20 °C until DNA isolation.

Two replicate DNA isolations were completed for each plankton tow following the extraction method described in Davies et al. (2013). A subset of each well-mixed plankton sample (1.5 mL) was centrifuged to pellet plankton, after which ethanol was decanted. Plankton were then immersed in DNA digest buffer (100 mM NaCl, 10 mM Tris–Cl pH 8.0, 25 mM EDTA pH 8.0, 0.5% SDS 5 µL Proteinase-K) for 1 h at 42 °C, followed by a standard phenol–chloroform extraction procedure. In brief, an equal volume of 25:24:1 buffer-saturated phenol/chloroform/isoamyl alcohol (PCA) was added to the sample, centrifuged, and the resulting aqueous layer was separated. PCA separation was repeated two additional times to further clean the sample and reduce PCR inhibition downstream. DNA was precipitated using 100% ethanol and 3 M NaOAc, rinsed with 80% ethanol, and then resuspended in 50 µL Milli-Q water. DNA concentrations were quantified using a NanoDrop (model ND1000, Thermo Scientific), and all extracts were visualized on 1% agarose gel to assess DNA integrity.

The V4 region of 18S rRNA was targeted in each plankton community using original primers from Stoeck et al. (2010), which were then modified for compatibility with Illumina MiSeq. While the V4 region of the 18S rRNA targeted in this study is among the most suitable markers for assaying diverse eukaryotic communities (Hadziavdic et al. 2014), a recent study found that V4 amplicons failed to capture every member of a known mock community (Bradley et al. 2016). Given these known issues, future studies could include additional 18S rRNA markers (e.g., V2 and V9 regions) to more completely examine the eukaryotic communities present on reefs given that incorporation of multiple loci may detect taxa that were missed by the V4 primers used here. The forward primer sequence was 5′-TCTCGGCGCTC*AGATGTGTA-TAAGAGACAGNNNN***CCAGC ASCYGCGGTAATT CC**-3′, and the reverse primer sequence was GTCTCGT GGGCTCGG*AGA TGTGTATAAGAGACAGNNNN***ACTT TCGTTCTTGAT**-3′ where the text in bold is the 18S target, italics represents linker sequence, and underlined text represents Illumina adapter linker sequences, which bind to Illumina adapters during the second PCR (ESM Fig. 1). Each 20-µL polymerase chain reaction (PCR) mixture contained 0.2 mM dNTP mix, 0.5 U *Ex taq* polymerase (Takara Biotechnology), 2.0 µL 10X *Ex taq* buffer, 100 ng of DNA template, 0.1 µM forward and reverse primer mix, and 12.4 µL Milli-Q water. PCR amplification was performed using the following profile: 95 °C for 5 min, followed by 20 cycles of 95 °C for 40 s, 59 °C for 2 min, and 72 °C for 1 min, and then an extension period of 7 min at 72 °C. To avoid PCR biases, samples were cycle checked as per Quigley et al. (2014) to ensure that all samples were amplified to an equivalent intensity when visualized on a 2% agarose gel. Samples that failed to amplify were diluted 10× with Milli-Q water, which yielded successful amplification in all cases. PCR products were purified using a GeneJET PCR purification kit (Fermentas Life Sciences). A second PCR was then performed to incorporate unique barcodes and Illumina adapters into each sample for Illumina MiSeq sequencing following Baumann et al. (2017). The PCR thermal profile for this barcode reaction was the same as that described above; however, only four cycles were used. All samples

were then visualized together on the same agarose gel, and differing volumes of each barcoded sample were pooled based on band intensities. The resulting pooled library was run on a 1.5% agarose gel, and the band was excised and soaked in 30 µL Milli-Q overnight at 4 °C. The liquid eluate was sequenced at University of North Carolina at Chapel Hill's High-Throughput Sequencing Facility using Illumina MiSeq paired-end 300 base pair (bp) sequencing. All raw reads are archived in the National Center for Biotechnology Information (NCBI) Short Read Archive (SRA) under accession number PRJNA507270. Illumina sequencing returned a total of 10,079,556 reads with an average of 130,903 reads per sample (Supplemental Table S1).

Plankton community analysis

The R statistical environment (R Development Core Team 2018) was used for all data analyses. Scripts for all environmental and sequencing analyses and all environmental data can be accessed at https://github.com/rachelwright8/planktonCommunities. We implemented the *dada2* package to characterize plankton community genetic diversity and structure (Callahan et al. 2016). First, FASTQ files were trimmed for sequence lengths of 250 bp for forward reads and 200 bp for reverse reads based on quality of reads. The first 24 bp from forward reads and 19 bp from reverse reads (representing primer sequence) and all base pairs with quality scores less than or equal to twenty were truncated from all reads. Identical reads were dereplicated, and then, matching forward and reverse reads were merged. Merged sequences with lengths outside the 365–386 bp range were removed from the analysis as likely products of non-specific primer binding. Chimeric sequences were also removed, resulting in a total of 39% of the original reads remaining (ESM Table 1; Supplemental Files 1 and 2), which were then assigned taxonomy from the Silva database (version 123; https://www.arb-silva.de) using the assignTaxonomy function in *dada2*, with minimum bootstrap confidences of 5 for assigning a taxonomic level. Minimum bootstrap confidences of 50 were also tested, and we observed identical results at both taxonomic levels (taxonomic identities can be found in Supplemental File 3). All downstream analyses here are reported on the bootstrap confidence of 5.

The package *phyloseq* was used to create an amplicon sequence variant (ASV) per sample counts table (McMurdie and Holmes 2013). Counts from technical replicates (i.e., 1.5 mL aliquots from the same tow) were summed. The ASV file was then separated for all midday samples and STRI Point time course samples for two separate sets of analyses. The R package *MCMC.OTU* was used to purge rare ASVs that appeared in fewer than 1% of all samples per Green et al. (2014). ASV count data were then log-normalized, and principal coordinate analyses (PCoA) were used to compare plankton communities between inshore and offshore reef types, sites, and time of day based on Manhattan distances using the R package *vegan* (Oksanen et al. 2018). The *adonis* function was used to test for differences in plankton communities across these factors. We report complimentary measures of species richness and evenness via the Shannon index values and a measure of species dominance via Simpson's index. Simpson and Shannon diversities for each plankton sample were calculated using the 'diversity' function in *vegan.* This function returns Simpson diversity (D) as 1-D, so that diversity increases with increasing values. Differences in diversity between inshore and offshore reefs, sites, and time of day were compared using ANOVA and Tukey's HSD tests.

Variation in specific plankton taxa

Differential abundance analyses were performed on ASV counts using DESeq 2 (Love et al. 2016). Two negative binomial models were fit to test for differentially abundant ASVs by reef type (inshore/offshore) and time of day using the models ASV count $\sim$ reef type and ASV count $\sim$ time, respectively. Raw ASV counts are available in Supplementary Files 1 (reef type) and 2 (time of day). Counts were normalized for size factor differences, and a pairwise contrast was computed for the comparison between inshore and offshore reefs and between all three pairwise comparisons for time of day. An FDR adjusted $p < 0.10$ (Benjamini and Hochberg 1995) represents significantly different abundances. To visualize these differences, raw counts were rlog normalized and heatmaps with hierarchical clustering of abundance profiles were created with the *pheatmap* package (Kolde 2018). Taxonomic assignments of all reference ASVs are reported at the level of order due to the limitations in taxonomic information. Because of these limitations, we identified multiple members within the same order that cannot be confidently assigned to more specific taxonomic groups and are therefore represented as distinct members of an order as separate rows in our differential abundance heatmaps. DESeq 2 results are available in Supplemental Files 4 (inshore versus offshore) and 5 (time of day), and taxonomic assignment results based on SILVA can be found in Supplemental File 3. We also used NCBI BLAST (Altschul et al. 1990) to search the standard nucleotide collection using the sequences associated with significantly differentially abundant ASVs identified by DESeq 2. Supplemental Files 4 and 5 contain the NCBI BLAST results these ASVs.

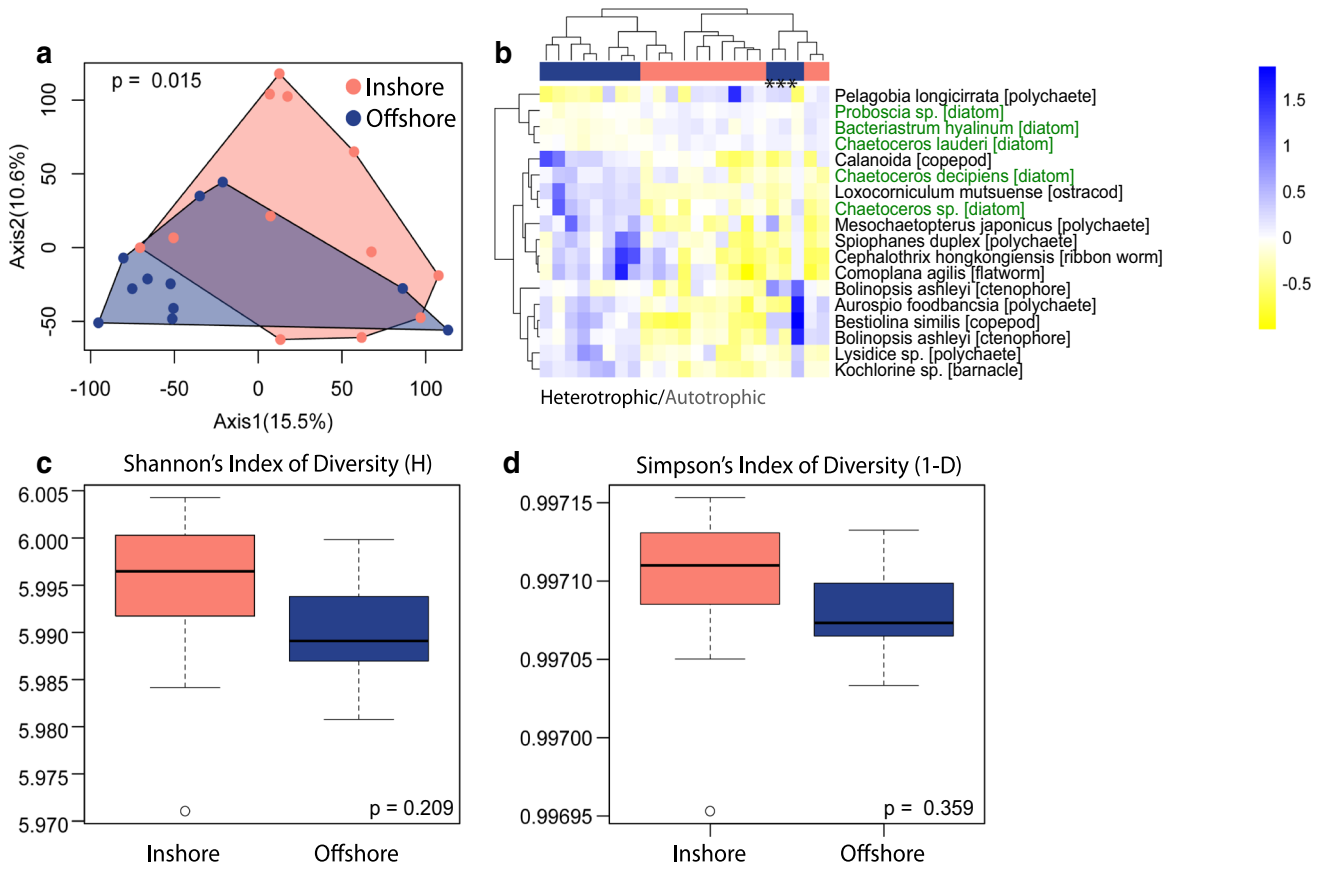

Fig. 2 Variation in midday plankton samples across inshore and offshore reefs. **a** Principal coordinate analysis of plankton communities clustered by inshore and offshore reefs. Percentages on each axis indicate the amount of variation explained by each axis (inshore = salmon, offshore = blue). *p*-value indicates results from the *Adonis* test demonstrating that overall plankton communities were significantly different between reef types. **b** Heatmap of the most differentially abundant taxa across inshore and offshore reefs. Coral and blue blocks indicate that libraries originated from inshore and offshore plankton communities, respectively. Columns represent unique plankton tows and rows represent differentially abundant taxa. *Symbols indicate libraries originating from Drago Mar (DM). Taxa listed in black are heterotrophic, whereas taxa listed in green are autotrophic. The color scale is in log2 (blue: more abundant, yellow: less abundant) and taxa and samples are clustered hierarchically based on Pearson's correlation of their relative abundance across samples. **c** Mean Shannon and **d** Simpson diversity of plankton communities based on inshore and offshore reefs. *p* values demonstrate that there were no statistical differences in diversity across inshore and offshore reefs

Results

Divergent environmental conditions across inshore and offshore reefs on Bocas del Toro

Temperature loggers were retrieved from five of eight sites. Maximum daily temperatures over the first two weeks of deployment were significantly higher at inshore sites than offshore sites ($p < 0.001$; Fig. 1c). Average temperature and standard error for the first 2 weeks of deployment at inshore sites were 30.02 ± 0.07 °C, while the offshore sites had an average of 29.37 ± 0.08 °C. The top twenty PAR values (µmol photons m^{-2} s^{-1}) recorded at each site show that in situ light levels at inshore sites were significantly lower (438 ± 38 µmol photons m^{-2} s^{-1}) than offshore sites (1213 ± 54 µmol photons m^{-2} s^{-1}) ($p < 0.001$; Fig. 1b). Overall, inshore sites are warmer and experience lower light levels when compared to offshore sites on Bocas del Toro reefs.

Plankton communities differ between inshore and offshore reef sites

Principal coordinate analysis (PCoA) revealed that sampled (> 60 µm) plankton communities were significantly different between inshore and offshore reefs ($p = 0.015$, Fig. 2a). Inshore and offshore reefs did not differ in mean Shannon's Index of Diversity (H) ($p = 0.209$) or mean Simpson's Index of Diversity (1-D) ($p = 0.359$) (Fig. 2b, c). Eighteen individual taxa displayed significant differences in abundance between inshore and offshore sites (FDR = 10%) (Fig. 2b). In particular, three out of the four

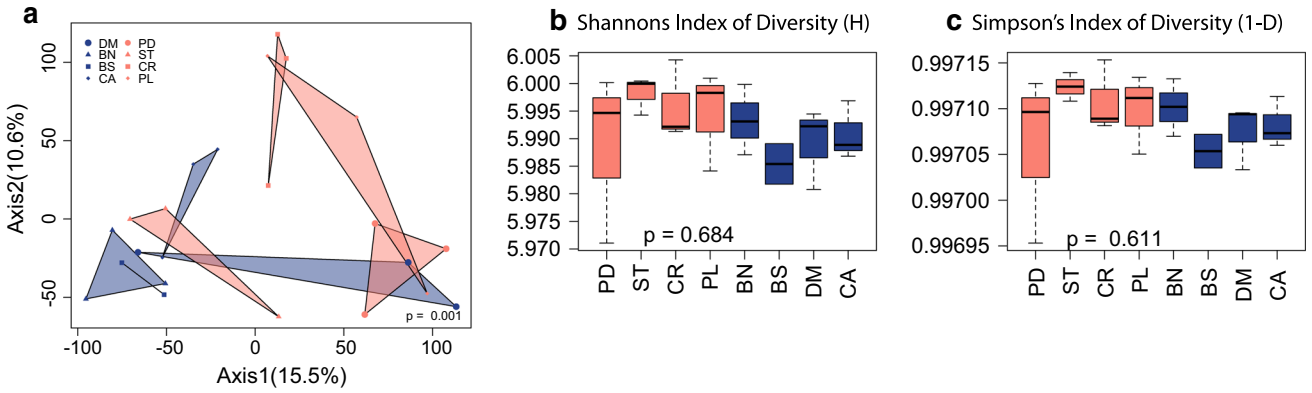

Fig. 3 Variation in plankton samples across sites collected midday. **a** Principal coordinate analysis of plankton communities by individual sites. Percentages on each axis indicate the amount of variation explained by each axis. Inshore sites = salmon, Offshore sites = blue. *Adonis* *p*-value demonstrates that there was a significant statistical difference in community composition across reef sites. **b** Mean Shannon and **c** Simpson diversity of the plankton communities across individual sites. *p*-values demonstrate that there were no statistical differences in diversity across sites and error bars represent the minimum and maximum indices of diversity. PD = Punta Donato. ST = Smithsonian Tropical Research Institute. CR = Cristobal. PL = Punta Laurel. DM = Drago Mar. BN = Bastimentos North. BS = Bastimentos South. CA = Cayo de Agua

more abundant taxa on inshore sites were photosynthetic organisms (highlighted in green text; Fig. 2b). Calanoid copepods are often the most abundant zooplankton taxa in the Caribbean ocean (Franco-Herrera et al. 2006) and, as expected, we found that copepods were also the most abundant taxa in this study. Copepod taxa represented approximately 35% of the total sequences, ranging from about 25–40% in each sample. Most of the Calanoida-associated ASVs were highly abundant; however, they were not differentially abundant between inshore and offshore reefs. It is also worth noting that Drago Mar exhibited taxa abundance profiles more similar to inshore sites (starred samples; Fig. 2b), which is interesting given its relative proximity to shore relative to other offshore sites (Fig. 1a).

Different sampling depths may drive differences in observed plankton diversity, as deeper tows sample large volumes of water, potentially increasing the chance of collecting novel taxa. While sampling depths in this study varied across sites at the midday collection time point (6–15 ft; Table 1), we did not find a significant association between sampling depth and observed Shannon diversity (ANOVA $p = 0.389$; ESM Fig. S2B).

Reef site-specific differences in plankton communities

PCoA grouped by individual reef sites indicated significant differences in plankton communities across individual sites ($p = 0.001$; Fig. 3a). We did not observe significant differences in diversity between sites based on mean Shannon's Index of Diversity ($p = 0.684$; Fig. 3b) or mean Simpson's Index of Diversity ($p = 0.611$; Fig. 3c). For diversity indices, means for most sites ranged from 5.98 to 6.0 for Shannon and 0.99700–0.99715 for Simpson (Fig. 3b, c). The top four most abundant ASVs were shared among all eight sites. The most abundant ASV was affiliated with a copepod (ASV1: *Paracalanus parvus*, *E*-value = 0, percent identity = 100%). The second-most abundant ASV was not characterized in the SILVA database, but shares some sequence homology with a nematode (ASV2: *Halicephalobus sp.*, *E*-value = 2e−31, percent identity = 80%). The third-most abundant species across all sites was a hydrozoan (ASV3: *Muggiaea sp.*, *E*-value = 0, percent identity = 99%), and a diatom was the fourth-most abundant ASV across all eight sites (ASV4: *Chaetoceros rotosporus*, *E*-value = 0, percent identity = 99%).

Twenty-seven ASVs were uniquely identified at only a single site. The three ASVs unique to Cayo de Agua all lacked taxonomic assignment (ASV400, ASV420, and ASV247), suggesting that this site may harbor understudied planktonic species. Punta Laurel had the greatest number of unique ASVs (6/27), including those with significant homology to a diatom (ASV571: *Chaetoceros protuberans*, *E*-value = 0, percent identity = 99%), a hydrozoan (ASV401: *Eudendrium carneum*, *E*-value = 0, percent identity = 99%), and a dinoflagellate (ASV493: *Tripos furca*, *E*-value = 8e−179, percent identity = 97%).

Time of day significantly influenced plankton community composition

PCoA partitioned by time of day revealed that there were significant shifts in plankton community structure across different times of day at STRI Point (early, midday, and late; $p = 0.001$; ESM Fig. S3A). These time course

differences were driven by midday plankton communities, which were distinct from plankton communities observed at early (approximately 08:00) and late (approximately 15:00) times of day (ESM Fig. S3A). Plankton samples collected midday exhibited the least variation in community composition between its three replicate tows (ESM Fig. S3A). It is important to note that this temporal variation could be due to variation from sampling at early and late time points on the same days ($n = 3$ tows each on 4 June and 5 June), whereas only one set of plankton tows ($n = 3$ on 28 May) was conducted for midday samples and these samples were taken on a different day. Interestingly, these differences in overall plankton community were not the result of changes in diversity given that neither the Shannon's Index of Diversity ($p = 0.489$) nor the Simpson's Index of Diversity ($p = 0.530$) showed significant differences in plankton community diversity across time of day (ESM Fig. S3B, C).

Heatmaps of the most differentially abundant ASVs highlight the taxonomic orders driving the observed overall community shifts between sampling time points (ESM Fig. S4A, B). We observe several ASVs associated with different Bacillariophyta diatoms that are more abundant midday compared to early or late (e.g., *Chaetoceros decipiens*), while other species are more abundant in the early and late time points (e.g., *Chaetoceros contortus*), highlighting the complexity of daily temporal variation observed on these reefs.

Discussion

Environmental differences across inshore and offshore reefs

It has been well established that inshore and offshore reefs differ in their environmental conditions across space and time. Inshore reef sites experience increased turbidity, sedimentation, nutrients, and temperature variation, while offshore reef sites are characterized by more moderate temperatures and lower turbidity as they are buffered by the open ocean (Lirman and Fong 2007; Lirman et al. 2011; Boyer et al. 2015). Here, we show expected environmental differences across inshore and offshore sites on coral reefs in the Bocas del Toro Archipelago, Panamá, with inshore reefs exhibiting lower light levels and warmer temperatures compared to offshore reef sites (Fig. 1). Warmer inshore waters and higher turbidity (i.e., reduced light) are also consistent with in situ data measured on other Caribbean reef tracts, including Belize (Castillo et al. 2012; Baumann et al. 2017) and Florida (Kenkel and Matz 2017; Rippe et al. 2018). These differences in mean midday light values and temperature are expected to drive niche specialization across marine environments, with specific taxa exhibiting preferences for distinct reef environments (Andersson et al. 1994; Takasuka et al. 2005; Edwards et al. 2016).

Plankton community differences across inshore and offshore reefs

Our observed significant differences in light and temperature across inshore and offshore reefs (Fig. 1) corresponded with overall differences in plankton communities (Fig. 2). This result is perhaps not surprising given that it has been estimated that variation in sea-surface temperature explains roughly 90% of the geographic variation in plankton diversity throughout the Atlantic Ocean (Rutherford et al. 1999), and it has been shown that plankton communities can be affected by even finer-scale environmental variations including depth, temperature, and trophic state of the water (i.e., particulate concentration, nutrients, and chlorophyll-*a*) (Owen 1989). In this study, replicate tows ($n = 3$) were collected at the same time of day (approximately noon) at each site across different days. Thus, these results should be interpreted with the consideration that sampling day may also confound observed differences in plankton communities across sites. Indeed, significant site-specific differences in plankton communities were observed across our eight sites regardless of whether the reef was inshore or offshore (Fig. 3a). These differences were driven by presence or absence of rarer taxa: 27 ASVs, which were unique to only one site (Supplemental Data 1). The most abundant taxa were similarly abundant across all eight sites, and most abundant species were affiliated with copepod and diatom species. However, the second-most abundant ASV was uncharacterized in the SILVA database, and the best BLAST match shared only 80% sequence homology with a nematode. We urge future studies to employ multiple research teams to simultaneously collect samples at different geographical locations or collect from each site across multiple dates to account for day-to-day variation.

While we found overall differences in community compositions between inshore and offshore reefs, the grand majority of taxa were shared across these environments. For example, no differences in Shannon diversity, a measure of species richness and evenness, or Simpson diversity, a measure of species dominance, were observed between reef types (Fig. 2c, d). Given that previous work has shown that kinetic properties of water influence planktonic organization (Mackenzie and Leggett 1991) and marine plankton communities can be more dispersed in high-energy, turbulent environments (Haury et al. 1990), it is possible that weather-related influences during the days of sampling, such as wind or rainfall (D'Croz et al. 2005),

could have homogenized the sampled plankton communities across sites. While previous work has described the biophysical processes governing this body of water (Robertson et al. 1999; Kaufmann and Thompson 2005), these circulation patterns have not been well resolved to the scale that we are investigating here (several kms) so we are unable to speculate about how these wind and current patterns might influence plankton dynamics on these reefs or how these dynamics might vary across seasons.

Another consideration is that our environmental metrics (temperature and light) were measured at depths ranging from 5 to 19 ft (Table 2) on reef substrate beside coral colonies; however, plankton communities were vertically sampled throughout the entire water column above the reef. While we did observe significant differences in overall plankton communities between reefs with different light and temperature regimes (i.e., inshore and offshore), we cannot rule out the possibility that additional variation may also exist between these sites at specific depths. It is also possible that samples taken across different seasons, as in the study by Huang et al. (2004), could yield different results. Furthermore, our collections were also conducted using a 60-μm net, which excludes the sampling of smaller organisms, so it is also possible that further community differences exist at smaller size fractions that were outside of the scope of this study. Lastly, we only measured temperature and light to assess environmental differences across inshore and offshore reefs. Other physical and biochemical properties that were not measured here, like nutrient runoff from the mainland (D'Croz et al. 2005) and long-term climate change (De Stasio et al. 1996), could also be strong influencers of tropical coastal plankton communities.

Lastly, sequencing plankton communities introduce its own set of caveats, including the fact that rDNA copy number per cell varies by orders of magnitude across unicellular eukaryotes (e.g., dinoflagellates and ciliates) (Weider et al. 2005; Gong et al. 2013). Therefore, caution must be exercised when interpreting organism abundance based on rDNA sequence abundance. Variation in rDNA copy number can even occur within a species, and a recent single-cell sequencing study found rDNA and rRNA copy number scaled with cell size in two ciliate species (Fu and Gong 2017). Therefore, variation in plankton size, which was not measured here, may have influenced relative plankton abundances. Equally plausible is that plankton community transcription differs across these sites, which has been previously observed in diatoms in response to iron availability (Cohen et al. 2017) and in dinoflagellates in response to light environment (Davies et al. 2018). We also leveraged 18S rDNA sequencing, which is known to be highly conserved across taxa, but this single-locus approach likely overlooks within-species population differences at other genetic loci that may also exist between reefs (Rodríguez et al. 2005; Martiny et al. 2009).

Given these caveats, we propose that future studies should couple more traditional microscopy techniques with 18S rDNA sequencing and perhaps consider a multidisciplinary approach incorporating metatranscriptomics or population genetics of specific taxa of interest in order to capture potential ecological and functional differences between plankton communities across inshore and offshore reefs.

Time of day played a role in structuring plankton communities

Sunrise and sunset at STRI Point occurred at approximately 06:00 and 18:35, respectively, throughout the sampling period. We collected early samples at approximately 08:00, midday samples at approximately 12:00, and late-day samples at approximately 15:00. We observed significant community differences. We observed significant overall differences in plankton communities between the eight individual sites regardless of whether the reef was inshore across sampling time points within the STRI Point site, with differences in overall community structure between midday samples when compared to early and late samples (ESM Fig. S3A). Our single midday sampling (n = 3 tows) occurred on 28 May 2015. Two early and late sampling efforts (n = 3 tows each) were conducted about a week later on 3 June and 4 June. Thus, we treat these results as preliminary evidence to motivate future studies to conduct more consistent replication in sampling for each time point across multiple days within the same site.

We observed several differentially abundant taxa between different time points (ESM Fig. S4A, B). Temporal variation in plankton and fish abundance is common as organisms move vertically in the water column during different times of day. For zooplankton, these movements are most commonly (but not always) up to the surface at dusk and back to the deeper waters at dawn (Lampert 1989; Ohman 1990; Brierley 2014) in order to avoid predation pressures (Ohman 1988; Lampert 1989). Planktivorous fishes are visual hunters, and most species inhabiting nearshore environments have been found to feed during the day, thus exerting a diurnal predation pressure on plankton (Morgan 1990; Motro et al. 2005). Predation pressure of planktivorous fishes on zooplankton is also strong on coral reefs (Hamner et al. 1988) and has been shown to drive vertical patterns of zooplankton in these habitats (Motro et al. 2005). Specifically in Bocas del Toro, Kerr et al. (2014) demonstrated that predation risk is higher during the day than at night for *Artemia franciscana* nauplii. However, the temporal gradient in planktonic predation risk was dependent on prey life history stage (i.e., size), as adult *A.*

franciscana did not show predation differences across the diurnal cycle (Kerr et al. 2014). While zooplankton migration is commonly considered to be ascending in the evening and descending in the morning, examples of reversed migrations are also common (Lampert 1989; Ohman 1990), with migration patterns varying by whether predation pressure is from visually hunting planktivorous fishes or nocturnally feeding zooplankton (Ohman 1990). Here, we were unable to describe how plankton communities differed between day and night; however, our results do highlight that these communities vary on even shorter timescales (i.e., early, midday, and late).

We also found evidence of phytoplankton temporal variation in Bocas del Toro, as some taxa were more abundant at midday, while others were more abundant in either the morning or evening. Temporal variation in phytoplankton abundance is generally understood as a mechanism for these organisms to optimize light and nutrient gradients, therefore moving into shallower waters during the day to photosynthesize (Raven and Richardson 1984; Ault 2000). However, photosynthetic characteristics of phytoplankton vary, and optimum depth and migration patterns might depend on underwater light patterns and the organism being considered (Ault 2000). As with zooplankton, we found evidence of temporal variation in phytoplankton, with some diatom ASVs exhibiting increased abundance midday (e.g., *Chaetoceros decipiens*), while other species (e.g., *Chaetoceros contortus*) were more abundant in the morning/evening (Fig. 5). This pattern of increased abundance during early and late time points is potential evidence of these organisms avoiding high noon-time irradiance in order to escape photoinhibition (Anderson and Stolzenbach 1985; Kingston 1999; Flynn and Fasham 2002).

These species identifications are based on homology to previously identify species at a single genetic locus. We assigned taxonomy using the SILVA database and NCBI BLAST nucleotide database. In almost every case, the BLAST and SILVA taxonomic assignments agreed, though the BLAST provided a more specific rank. In one instance (ASV 50), SILVA classified the ASV as Arachnida, but BLAST classified the sequence as Kochlorine sp. [barnacle] (*E*-value = 0.0; percent identity = 100%). In this instance, we deferred to the perfectly matched BLAST identification. Full taxonomic classifications for all ASVs based on SILVA results can be found in Supplementary Data 3, and counts for all ASVs for the inshore/offshore and time of day datasets can be found in Supplemental Data 1 and 2, respectively. The higher-level taxonomic classifications achieved in this study serve the overall purpose of identifying community differences between reefs and sampling time points. Future studies could implement finer-scale DNA genotyping methods at many loci to better distinguish between community members and identify variants within populations.

Future investigations into the relationships between microeukaryotes and corals

Nutrient provision through heterotrophy can improve coral outcomes during thermal stress and bleaching (Johannes et al. 1970; Ferrier-Pagès et al. 2010; Hughes and Grottoli 2013; Aichelman et al. 2016; Tremblay et al. 2016). Predictions about what heterotrophic opportunities are available for corals should take into account prey abundances and what prey local coral species can capture efficiently. For example, a study in the Gulf of Panamá quantified prey captured in feeding trials for three local coral species: *Pocillopora damicornis*, *Pavona clavus*, and *Pavona gigantea* (Palardy et al. 2006). This study found that less motile prey (e.g., crustacean larvae and polychaetes) were preferentially ingested relative to faster swimming prey taxa (e.g., isopods) and smaller taxa (e.g., copepods). Thus, despite the overall abundance of copepods and observed differential abundance of some copepod types between inshore and offshore reefs (Fig. 2) and sampling time points in this study (ESM Fig. 4), these differences may have limited impact on a coral's diet. Similarly, our observed differential abundance of some polychaete taxa (Fig. 2, ESM Fig. S4) may represent a greater change in the effective prey availability for corals on these reefs. Future studies may consider identifying prey captured in situ, in corals and other planktivores, to better explore the relationship between heterotrophic opportunities and coral reef ecosystem dynamics.

Concluding thoughts

Tracking plankton community composition through space and time is critical as climate change progresses. Types of zooplankton present on a reef may also affect nitrogen availability in the water column, as certain plankton species, such as some copepods (Order Calanoida), are known to associate with N_2-fixing bacteria (Azimuddin et al. 2016). While we did not directly test for biogeochemical processes at these sites, future work incorporating the bacterial component of the plankton along with water column chemistry would be interesting to integrate in this reef system. For example, a recent study investigating bacterial and archaea communities near coral colonies in the U.S. Virgin Islands found increased community diversity during the day that corresponded with diel fluctuations in inorganic nutrients (Weber and Apprill 2020). Correlating the environmental conditions experienced in Bocas del Toro to the plankton communities across these sites builds a baseline dataset upon which future studies

can build in order to assess how changing environments are influencing these communities.

Current estimates suggest that the oceans have warmed by ca. 0.6 °C over the past 100 yr (IPCC 2014) and have absorbed almost 50% of all the anthropogenic CO_2 emitted over the last 250 yr (Sabine et al. 2004). As the oceans continue to change, the need to characterize baseline community structure is critical. Given that the plankton tows conducted here were vertical tows, the plankton communities characterized in this study represent all heterotrophic opportunities throughout the sampled water column (from 6 to 19 ft up to the surface). The vertical sampling technique employed here masks zonation that may exist vertically in the water column. For example, other studies have reported diel fluctuations in near-bottom (Yahel et al. 2005) and near-surface (Alldredge and King 2009) zooplankton on coral reefs. While our study found differences in plankton communities between inshore and offshore reefs, future studies could implement a depth-stratified approach (Heidelberg et al. 2010) to resolve community structure throughout the water column at inshore and offshore reefs. These data would better inform how interactions between depth and reef environment may play a role in heterotrophic opportunities for sedentary organisms, such as corals. Plankton not only play a central and critical role in the health and productivity of the oceans, but can also serve as sensitive indicators of climate change. As plankton communities shift in response to climate change, the availability of energy for other trophic levels will also shift, which will undoubtedly modulate food web dynamics. Therefore, a more comprehensive description of baseline plankton communities provided by studies like the one presented here is needed before we can make accurate projections of what impacts these climate-mediated shifts in plankton communities will have on future reefs.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding authors state that there are no conflicts of interest.

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