Received: 24 April 2023 | Revised: 25 November 2023 | Accepted: 28 December 2023

DOI: 10.1002/edn3.512

Environmental DNA
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WILEY

ORIGINAL ARTICLE

Environmental DNA captures diurnal fluctuations of surface eukaryotes on a tropical coral reef

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Funding information
Research England; Bertarelli Program in Marine Sciences; Natural Environment Research Council, Grant/Award Number: NE/S007415/1

Abstract

Environmental DNA (eDNA) metabarcoding has been widely employed to describe biological communities in the marine environment and to compare the richness and diversity of sites across large spatial scales. However, fine-scale temporal eDNA dynamics are poorly understood and the time of eDNA sample collection is rarely reported in publications. Here, we collected surface eDNA samples every 6 h, for 3 days, at two coral reef sites to assess fine-scale changes in the eukaryotic communities detected. Distinct eukaryotic communities were detected at two sites within the same lagoon. Sampling time was found to have a significant effect on ESV and class richness, both peaking during the 1 p.m. sampling time at both sites. Sampling time also had a significant effect on the detection of eukaryotic taxa, with relative read frequency showing clear diurnal patterns in line with the migratory behavior of planktonic groups. Other groups of organisms showed considerable variation in read frequency, highlighting the dynamic nature of marine eukaryotic communities and potential stochasticity of eDNA detections. For eukaryotic communities, eDNA samples can provide a "snapshot" of contemporary biodiversity and provide information on short-term community dynamics on hyperdiverse coral reefs. However, our findings add to growing evidence that sampling time should be clearly considered and reported in marine eDNA studies and that multiple samples from the same site are needed to facilitate more robust comparisons across sites.

KEYWORDS
diurnal migration, eDNA methods, environmental DNA, metabarcoding, temporal variability

1 | INTRODUCTION

Tropical coral reefs support vast levels of biodiversity and provide valuable ecosystem services to millions of people (Eddy et al., 2021; Fisher et al., 2015; Mora et al., 2011) but are increasingly threatened by global climate change and direct anthropogenic disturbances (Williams, Graham, et al., 2019). In the face of these threats, reefs require effective methods of ecosystem monitoring to support their management and conservation (Fisher et al., 2015; Nichols & Williams, 2006).

A promising tool for characterizing and monitoring the diversity of broad taxonomic groups on coral reefs is the sampling of environmental DNA (eDNA). eDNA comprises trace genetic material in the environment originating from sources including gametes, mucus,

feces, and sloughed cells from macroscopic organisms (Kamoroff & Goldberg, 2018; Sassoubre et al., 2016; Stewart, 2019), and whole microorganisms (Pawlowski et al., 2020). An advantage of eDNA is that sampling is based on the collection of easily accessible environmental media such as water or sediments. This facilitates sampling in physically challenging, remote, and inaccessible settings that are beyond the logistical, visual, and taxonomic limits of traditional surveys (Rourke et al., 2021). eDNA can be used for single species detection via quantitative PCR (qPCR), digital droplet PCR (ddPCR), and CRISPR (Kutti et al., 2020; Thalinger et al., 2021; Williams, O'Grady, et al., 2019) or assessment of multispecies communities via next-generation sequencing (NGS), referred to as metabarcoding (Taberlet et al., 2012). eDNA can also complement established biomonitoring methods by alleviating the bias against small and cryptic organisms that often constitute the majority of biodiversity (DiBattista et al., 2017; Nichols et al., 2021; Pearman et al., 2018). Within the marine realm, eDNA is commonly utilized to characterize the diversity of sharks (Bakker et al., 2017; Dunn et al., 2022; Mariani et al., 2021), fish (Goldid et al., 2021; Jensen et al., 2022), corals (Dugal et al., 2022; Nichols et al., 2021), plankton (de Vargas et al., 2015; Djurhuus et al., 2018), and microbial communities (Bakker et al., 2019; Djurhuus et al., 2020). Further uses include population genetic analyses (Dugal et al., 2021; Weitemier et al., 2021), dietary analysis (Nalley et al., 2021), and monitoring ecological events, such as spawning (Ip et al., 2022; Tsuji & Shibata, 2020). Demonstrated by the above examples, the inference of presence/absence of species using eDNA approaches has been well documented across multiple taxonomic groups. Moving beyond this, several laboratory studies have found positive correlations between species abundance/biomass and eDNA particle concentration or relative read abundance (Hilário et al., 2023; Jo & Yamanaka, 2022). However, in the natural environment, these relationships have been found to be weaker, most likely due to more dilute DNA concentrations and stochasticity in environmental parameters and organism behavior that are known to impact the release, transport, and degradation of eDNA (Rourke et al., 2021; Yates et al., 2019). With careful consideration of key environmental parameters however, there is a promising outlook for answering quantitative questions in natural environments using eDNA (Morrison et al., 2023; Yates et al., 2023).

Coral reef surveys reporting community composition and diversity from eDNA found in the water column have been conducted on regional (e.g., the Caribbean; Bakker et al., 2019), national (e.g., Indonesia; Madduppa et al., 2021) and local (e.g., within a single reef system; DiBattista et al., 2019; Oka et al., 2020) spatial scales. The rapid degradation of in situ genetic material and limited transport of eDNA is thought to able to provide a high-resolution tool for monitoring these marine communities (Collins et al., 2018). Documented eDNA degradation rates in tropical marine environments concur that higher water temperatures decrease eDNA half-lives, with estimated half-lives of between 2 and 15 h at 28°C (Kwong et al., 2021; Tsuji et al., 2017). However, all current rates are calculated from laboratory or mesocosm studies and may not fully represent the natural environment. Several studies have also highlighted the detection of stable communities over multiple tidal cycles (Kelly et al., 2018; Lafferty et al., 2021) suggesting that the transport of eDNA into a given location is unlikely to influence the overall community detected. Evidence for fast degradation and limited eDNA transport is growing, with a number of studies reporting the detection of fine-scale habitat association and local differentiation (60 m to 5 km) of communities with eDNA metabarcoding (Jeunen et al., 2019; O'Donnell et al., 2017; Port et al., 2016; West et al., 2020; Wilms et al., 2022). However, it is still unknown as to whether single eDNA samples, as are often collected in biomonitoring campaigns, provide a complete picture of diversity and community structure, or whether such "snapshots" may be influenced by fine-scale temporal changes in eDNA. More studies are necessary to understand these dynamics and the implications for large spatial surveys (Jensen et al., 2022; Kelly et al., 2018).

With a few exceptions (Ely et al., 2021; Jensen et al., 2022; O'Rorke et al., 2022; Pawlowski et al., 2018), community variation introduced by time of sampling is rarely reported in eDNA studies (Jensen et al., 2022). Variation in sampling time could lead to inaccurate comparisons between sites and taking single "snapshots" of communities could lead to an incomplete picture of community structure and diversity. The diurnal cycle influences the behavior, reproduction, and habitat uses of many marine organisms (Tessmar-Raible et al., 2011). Foraging and predator–prey interactions are likely significant sources of eDNA and have been shown to occur on predictable diurnal cycles in many taxa (Bosiger & McCormick, 2014; Fox & Bellwood, 2011; Meyer et al., 2007; Shulman, 2020). Diurnal variation in organism abundance may also result from movement, most notably due to the diurnal vertical migration (DVM). DVM behavior is observed in a taxonomically diverse group of organisms, but most commonly refers to the movement of zooplankton to surface layers at dusk to feed and to deeper layers during the day to avoid predation (Brierley, 2014). To date, some diurnal patterns in the detection of copepods and fish events have been observed using eDNA, indicating potentially short signal persistence also in temperate marine ecosystems (Jensen et al., 2022; Suter et al., 2020; Tsuji & Shibata, 2020). Potential diurnal partitioning of spawning corals has also been documented with eDNA signals from both day and night spawning corals observed in samples taken at corresponding times (Ip et al., 2022). This indicates that time of sampling may be an important consideration in eDNA studies for varying taxa, with inconsistent sampling potentially introducing detection biases unaccounted for in analysis of eDNA communities.

Here, we investigate diurnal changes in community composition on a tropical coral reef recovered from eDNA metabarcoding. Surface water samples were collected every 6 h for 3 days at two sites to explore the fine-scale temporal dynamics exhibited by the eukaryotic community. We hypothesize that the communities detected from eDNA samples will differ between sampling times and sampling days, reflecting both expected diurnal abundances and stochastic detections of ecologically important reef taxa. A better understanding of fine-scale temporal variation in marine eDNA is important to ensure that the application and analysis of these methods on broad spatial and temporal scales are appropriate in highly diverse and dynamic environments, such as coral reefs.

2 | METHODS

2.1 | Study site and sampling

We collected surface water samples to assess community composition using eDNA detection from two sites in the Peros Banhos atoll, Chagos Archipelago, between the 26th of April and 1st of May 2021 (Figure 1). The two study sites are lagoonal coral reefs found at ~30 m depth and at least 700 m from the nearest land. The two sites were 20.4 km apart. Samples were collected every 6 h at 1 a.m., 7 a.m., 1 p.m., and 7 p.m. at each site. These times were chosen to coincide with sunrise (07:11) and sunset (19:02) and provide even sampling throughout the day. The site in northwest Peros Banhos (5.262056° S, 71.769917° E) was sampled for 2.5 days, from 1 a.m. on the 26th of April to 7 a.m. of the 28th of April, while the southwest site (5.446583° S, 71.77° E) was sampled for 3 days from 1 p.m. on the 28th of April to 7 a.m. on the 1st of May (Table S1). At each sampling site, water temperature was recorded and was between 29.3°C and 30°C during the whole sampling period. A previous study at a nearby site in the southwest of the lagoon described this area to have a low and stable flow velocity of ~0.2 m/s and wave height of ~0.25 H_s (m) (Steyaert et al., 2022). Conditions during this study were likely to be similar, and there were no significant weather events during the sampling period. There was a full moon on April 26th, the first night of sampling.

A water sample was collected from 1 m below the surface every 6 h using a 5 L Niskin bottle and was processed immediately. Three one-liter subsamples were drawn through three 0.22 µm Sterivex-GV filters (Merck Life Sciences) using a vacuum pump, before the Sterivex filters were run dry and 2.5 mL of Longmire's buffer (Renshaw et al., 2015) was added to preserve the DNA. Sterivex filters were then capped and stored at room temperature for 2 months until extraction. All sampling equipment was washed with 20% Clorox bleach solution and rinsed thoroughly with sterile water between uses. Field blanks consisting of sterile water (500 mL) were processed through the filtration system on the first day of sampling at each location (April 26th and 28th) to assess contamination from the filtration equipment during sample processing. The same filtration system was used at both sites.

2.2 | DNA metabarcoding

2.2.1 | Extractions

DNA was extracted following the protocol described by Spens et al. (2017) using the DNEasy Blood and Tissue kit (Qiagen). All DNA extractions were carried out in a dedicated pre-PCR room. Extraction blanks were performed using nuclease-free water. Extracted DNA was eluted in 100 µL nuclease-free water and

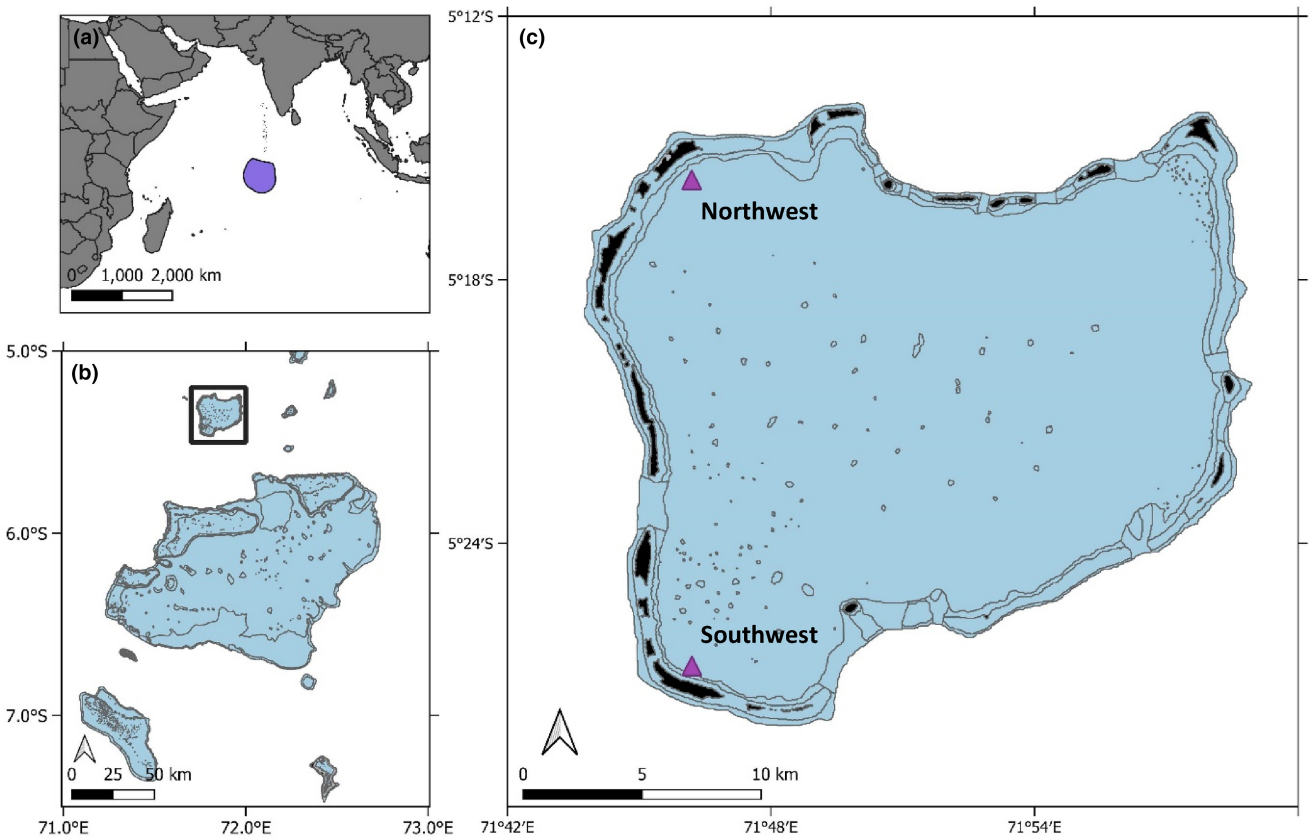

FIGURE 1 Map of (a) the wider Indian Ocean region with the British Indian Ocean Territory marine protected area highlighted in purple and (b) the Chagos Archipelago with (c) inset showing study sites in the Peros Banhos atoll. The two study sites are denoted by purple triangles. Islands within the atoll are shown in black.

quantified using a Qubit flex fluorometer (Life Technologies). Extracted DNA was stored at −20°C. DNA extracts were amplified using primer sets targeting a 130-bp region of the V9 18S rDNA: 1389F 5′-TTGTACACACCGCCC-3′, 1510R, 5′ CCTTCYGCAGGTTC ACCTAC 3′ (Amaral-Zettler et al., 2009). Triplicate PCR replicates were performed for each sample in 25 μL reactions, including the extraction blanks. Each PCR reaction included: 5 μL HOT FIREPol 5× MasterMix, 0.5 μL forward and reverse primers (10 μM), 5 ng of template DNA, and made up to 25 μL with PCR grade water. The thermocycler conditions were an initial denaturation at 94°C for 12 min, followed by 30 cycles of 10 s at 94°C, 30 s at 57°C, 30 s at 72°C, and a final 10-min extension at 72°C. A negative control with no DNA template was included in all PCR runs. 1% agarose gels stained with SYBR safe (Invitrogen) were run to confirm success of the PCRs, before PCR replicates were pooled.

To prepare libraries for sequencing, PCR products were purified with AppMag PCR cleanup beads (Appleton Woods) and the purified DNA was used as templates for the index PCR. A second 50 μL PCR reaction to add Nextera XT indexes (Illumina) was performed. The reaction comprised 10 μL HOT FIREPol 5× MasterMix, 5 μL of each index primer, 5 μL of purified PCR product, and 25 μL of PCR grade water. The PCR conditions were as follows: 95°C for 15 min, followed by 8 cycles of 30s at 95°C, 1 min at 55°C, 1 min at 72°C, and a final 10-min extension at 72°C. Indexed PCR products were purified as above and verified using an Agilent 4200 TapeStation. Libraries were sequenced on an Illumina MiSeq platform using V2 chemistry (2 × 250 bp paired end reads).

2.3 | Data analysis

Sequences were automatically demultiplexed into individual library fastq files using the Illumina MiSeq Reporter software. Demultiplexed sequences were then trimmed, denoised, and merged using the DADA2 pipeline in R to produce exact sequence variants (ESVs) (Callahan et al., 2016). The default parameters of MaxN = 0 and MaxEE = 2 were applied. Taxonomic assignment of ESVs was performed using IDTaxa and the SILVA (v138) reference database, with the assignment confidence threshold set at 40% (moderate) (Murali et al., 2018; Quast et al., 2013). Potential contaminant sequences were removed using decontam package in R (Davis et al., 2018) with a stringent threshold of 0.5, where ESVs more prevalent in field negatives than positive samples were identified as contaminants and removed from the dataset. Samples were rarefied to the minimum library size, with replacement, 100 times as advised by Cameron et al. (2021) to allow for robust diversity analysis using samples of equal library size but accounting for potential data loss during rarefaction.

Alpha diversity measures (ESV richness, Shannon, and Simpsons indices) and Jaccards dissimilarity (presence/absence) scores for non-metric multidimensional scaling (NMDS) plots were calculated using the Phyloseq package (McMurdie & Holmes, 2013) and metaMDS functions of the R-package vegan (v.2.6–4) (Oksanen et al., 2022), respectively. Analysis of variance (ANOVA) and post hoc tests were run to test the effect of site, sampling time (1 a.m., 7 a.m., 1 p.m., and 7 p.m.), and sampling day (1–6) on alpha diversity. Betadisper tests were used to test for homogeneity and determine community dispersion (Anderson et al., 2006) within site, sampling time, and sampling day. The ADONIS function of the vegan package was used to conduct a permutational multivariate analysis of variance (PERMANOVA) to test the effect of site, sampling time, and sampling day on community composition, calculated using Jaccard similarity. We examined diurnal patterns in relative read frequency of key eukaryotic phyla throughout the sampling period. ESVs assigned to taxa of interest were subset and two-way ANOVA and post hoc tests were used to test if site and sampling time influenced ESV abundance and richness. Cube root transformation was applied to data, if needed, to meet model assumptions of normality and variance.

3 | RESULTS

18S rDNA metabarcoding obtained a total of 2,722,305 reads in total from 22 samples, with read counts per sample ranging from 94,862 to 184,246. After quality filtering, merging, and chimera elimination, a total of 2,275,611 reads remained with individual samples ranging from 75,525 to 141,966 reads (average of 103,437 reads per sample) (Table S2). One hundred and ninety-four ESVs (48,988 reads), representing 1.97% of reads across all samples, were identified as contaminants and removed. The identities of all ESVs removed as contaminants can be found in the supplementary information; however, the most abundant contaminants were predominantly non-marine and included ESVs in the classes Embryophyta, Alphaproteobacteria, Maxillopoda, Insecta, and Mammalia. ESVs unassigned or not assigned as eukaryotic at the domain level were also removed, leaving a total of 4097 ESVs for further analysis. Rarefication curves (Figure S1) were examined to determine 72, 000 reads as an appropriate library size for normalization.

3.1 | Alpha and beta diversity metrics

Eukaryotic richness varied from 1011 to 1529 ESVs, with the lowest richness observed at 1 a.m. in the southwest site and the highest richness observed at 1 p.m. in the southwest site (Figure 2). ESV richness was significantly different between sampling times (ANOVA, median $p = 0.022$) with significantly higher ESV richness observed at 1 p.m. compared to 1 a.m. (TukeyHSD, adj $p = 0.017$). The highest richness of eukaryotic classes was 92, observed during 1 p.m. sampling at the northwest site (Figure S2). Sampling time also had a significant effect on eukaryotic class richness (ANOVA, median $p = 0.050$). There were no significant differences in ESV, or class richness observed between sites and sampling days. There was also no significant difference in the richness of genera or orders and no significant difference in Simpson's or Shannon's alpha diversity

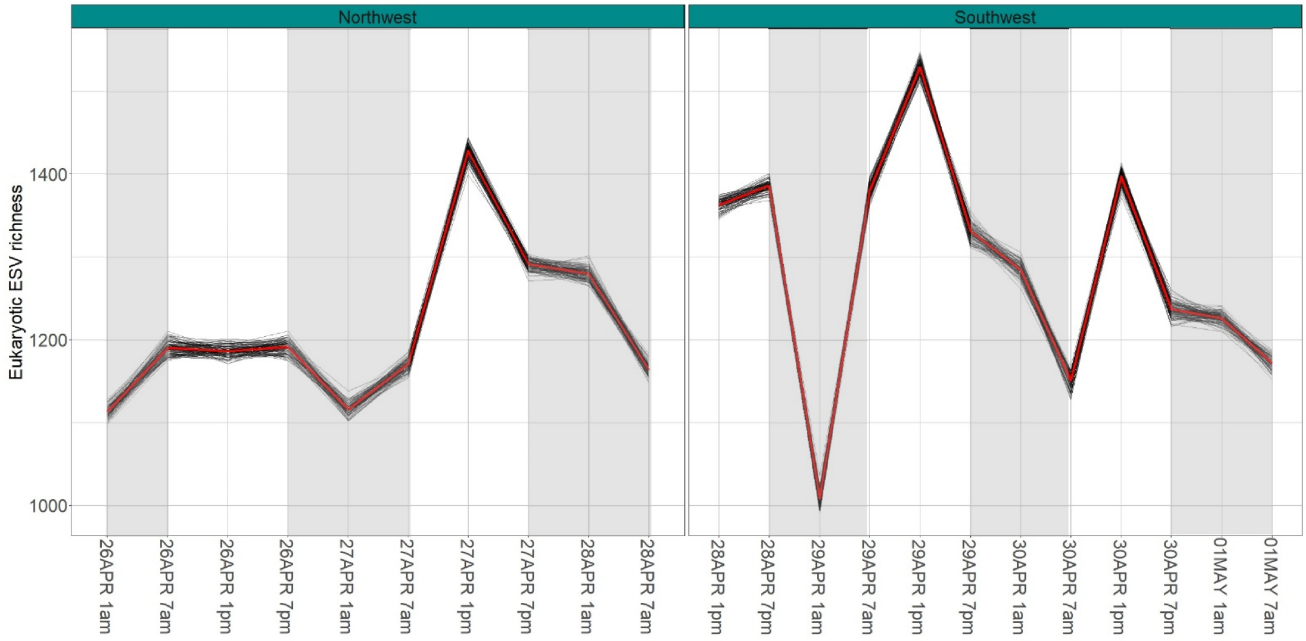

FIGURE 2 Eukaryotic ESV richness throughout the sampling period at both the northwest (left) and southwest (right) sites. Libraries have been rarefied 100 times to 72,000 reads with the average class richness of all normalized libraries plotted in red. Shaded areas indicate night hours.

TABLE 1 Table of results of permutational multivariate analysis of variance (PERMANOVA) performed on repeatedly rarefied libraries to explore community changes in relation to site, sampling time, and sampling day.

	df	SS	R^2	F	p
Site	1	0.331	0.139	3.300	0.001**
Time	3	0.324	0.136	1.080	0.214
Day	1	0.138	0.058	1.380	0.035*
Site*Time	3	0.289	0.121	0.965	0.600

Note: Median values of 100 PERMANOVAs are reported. Results are reported for both presence/absence (Jaccard's coefficient) data. Significant values are indicated with *where $p < 0.05$ and **where $p < 0.005$.

measures calculated with ESV data between sites, sampling times, and sampling days (Table S1).

PERMANOVA analyses carried out using presence/absence (Jaccard's similarity) to investigate the influence of site, sampling time, and sampling day on community composition, showed that site and sampling day had a significant effect on the eukaryotic community detected (Table 1). NMDS plots created using Jaccard's similarity index indicate that samples primarily split by the two sampling sites on the x-axis (Figure 3). There was no clear clustering at any sampling time (denoted by colors in the plot) at either site, with 1 a.m. samples spanning the greatest ordination space, indicating the highest compositional variability. Communities from the southwest site show greater variation than those in the northwest site. Higher dispersion was recorded in the southwest site with a median distance to group centroid of 0.4513 compared to 0.3889 in the northwest site.

3.2 | Diurnal patterns in ecologically important taxa

The eukaryotic phyla (and respective classes) with the highest number of reads were Dinoflagellata (Dinophyceae), Cnidaria (Hydrozoa), Arthropoda (Maxillopoda), Tunicata (Appendicularia), and Protalveolata (Syndiniales) (Figure S3). ESVs assigned to the above five classes were found in all samples, across all sampling times and sites. However, the proportion of reads assigned to some classes varied between samples and within each sampling time. For example, Appendicularia accounted for 38% of reads at 7 a.m. on April 30th but decreased to only 5% of reads at the subsequent 1 p.m. sampling.

From the four most abundant phyla detected (Dinoflagellata, Cnidaria, Arthropoda, and Tunicata), particular taxa were chosen that represent ecologically important planktonic and benthic groups and comprise useful indicators of environmental variation (Frederiksen et al., 2006; McQuatters-Gollop et al., 2019). In total, three phyla (Dinoflagellata, Tunicata, and Porifera), two classes (Hydrozoa and Anthozoa), and one subclass (Copepoda) were chosen. The classes Hydrozoa and Anthozoa were looked at separately within the phylum Cnidaria due to large differences in ecology and Copepoda was chosen as the most abundant subclass in the phylum Arthropoda. Porifera was also investigated as a further benthic group and was the 12th most abundant phylum.

Six classes of Copepoda were detected, of which Calanoida had the most ESVs ($n = 43$) (Appendix S2). The relative frequency of reads assigned to Copepoda showed a clear diurnal cycle, peaking at 1 a.m. and declining during daytime hours (Figure 4a). Copepoda reads

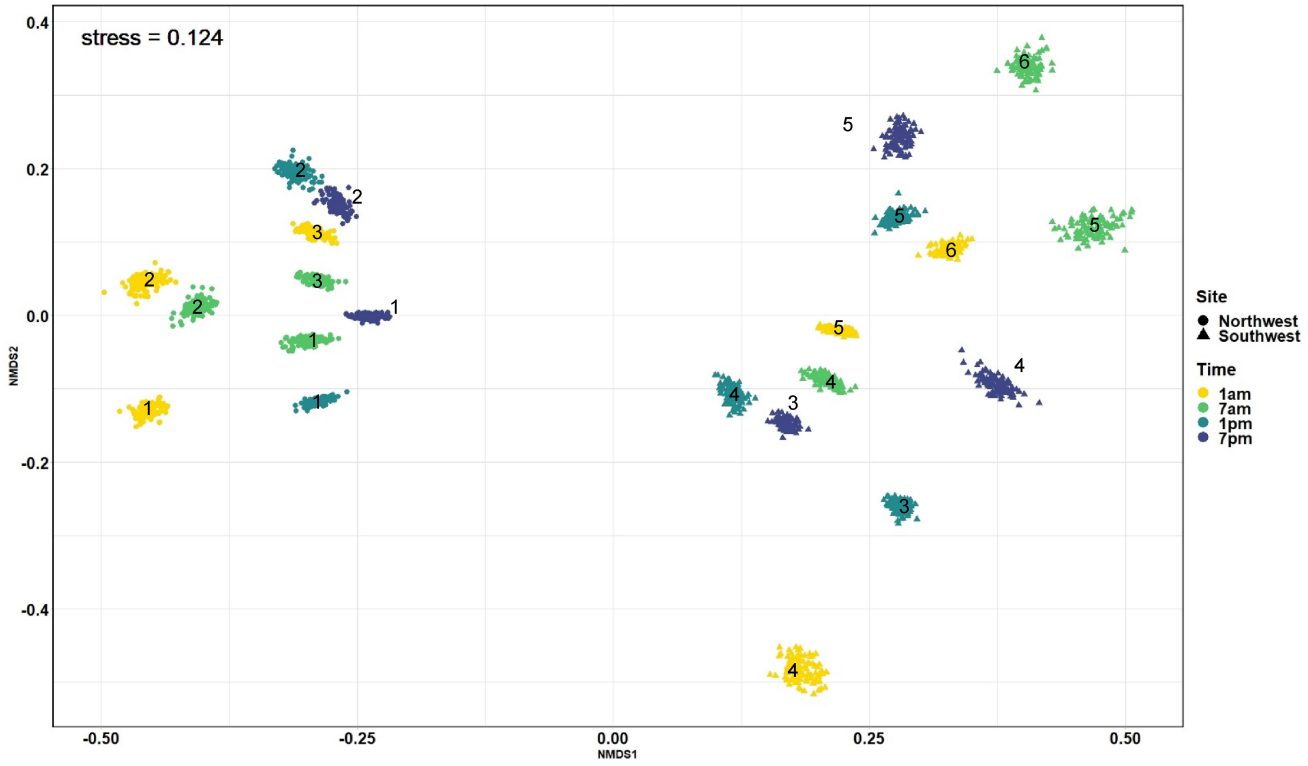

FIGURE 3 Non-metric multidimensional scaling (NMDS) plot of Jaccard's (presence/absence) distances between samples from both sites and all four sampling times. Libraries have been rarefied 100 times and all communities included in the nMDS. Sites are denoted by shape (circle = northwest site, tringles = southwest sites) and sampling time by color. Clusters are annotated by a number indicating the day of sampling (1–6).

were significantly different across sampling times (ANOVA, median $p = 0.019$), were more frequent at 1 a.m. than 7 p.m. (TukeyHSD, $p_{adj} = 0.0186$), and were not significantly different between sites. High read frequency was not driven by higher Copepoda ESV richness, with no significant difference between sampling times and sites. Read frequency of Dinoflagellata (Figure 4b) demonstrated the reverse pattern to Copepoda, with significant differences found across sampling times (ANOVA, median $p = 0.041$), and higher read frequency at 1 p.m. compared to 1 a.m. (TukeyHSD, $p_{adj} = 0.0447$) and 7 a.m. (TukeyHSD, $p_{adj} = 0.00432$). There was no significant effect of site on Dinoflagellata read frequency, and no significant effect of sampling time and site on ESV richness.

Sampling time did not have a significant effect on the relative read frequency assigned to Tunicata (Figure 4c). The high frequency of Tunicata reads appears to be driven by large numbers of reads assigned to Copelata, a free-swimming tunicate, from three non-consecutive samples across both sites. Hydrozoa were among the most abundant taxa observed. Both site (ANOVA, median $p = 0.003$) and sampling time (ANOVA, median $p = 0.033$) had a significant effect on read frequency of Hydrozoa, with increased read frequency detected during 7 p.m. sampling events and in the northwest site (TukeyHSD, $p_{adj} = 0.034$) (Figure S4).

There was a roughly 10-fold decrease in the frequency of reads assigned to Porifera and Anthozoa, compared to the taxa discussed above (Figure S5). However, these were the two most abundant benthic groups detected across all samples. Read frequency of both groups was more variable during the 1 a.m. and 7 a.m. sampling times, with up to 8% of reads assigned to Porifera in one 1 a.m. sample. Although due to stochastic detection, median read frequency remained close to 0 for both groups at most time points. Sampling time and site had no significant effect on the read frequency and ESV richness of either of these groups.

4 | DISCUSSION

Here, we show that eDNA metabarcoding from surface water samples not only detects distinct communities between coral reef sites but can also detect diurnal patterns in planktonic taxa by considering the change in relative read frequency. However, we also show that individual water samples only provide a partial view of the biodiversity present on coral reefs and that it is important to consider time of sampling to avoid making inaccurate comparisons between sites.

Sampling eDNA at different times of day has been shown to influence the detection of organisms, primarily fish, due to daily changes in behavior and habitat use (Ely et al., 2021; Jensen et al., 2022; Suter et al., 2020). Copepods are known to make diurnal migrations vertically in the water column to avoid predation at depth during the day, moving to the surface at night to feed (Olsson & Granéli, 1991). Here, this expected movement of copepod biomass was mirrored in

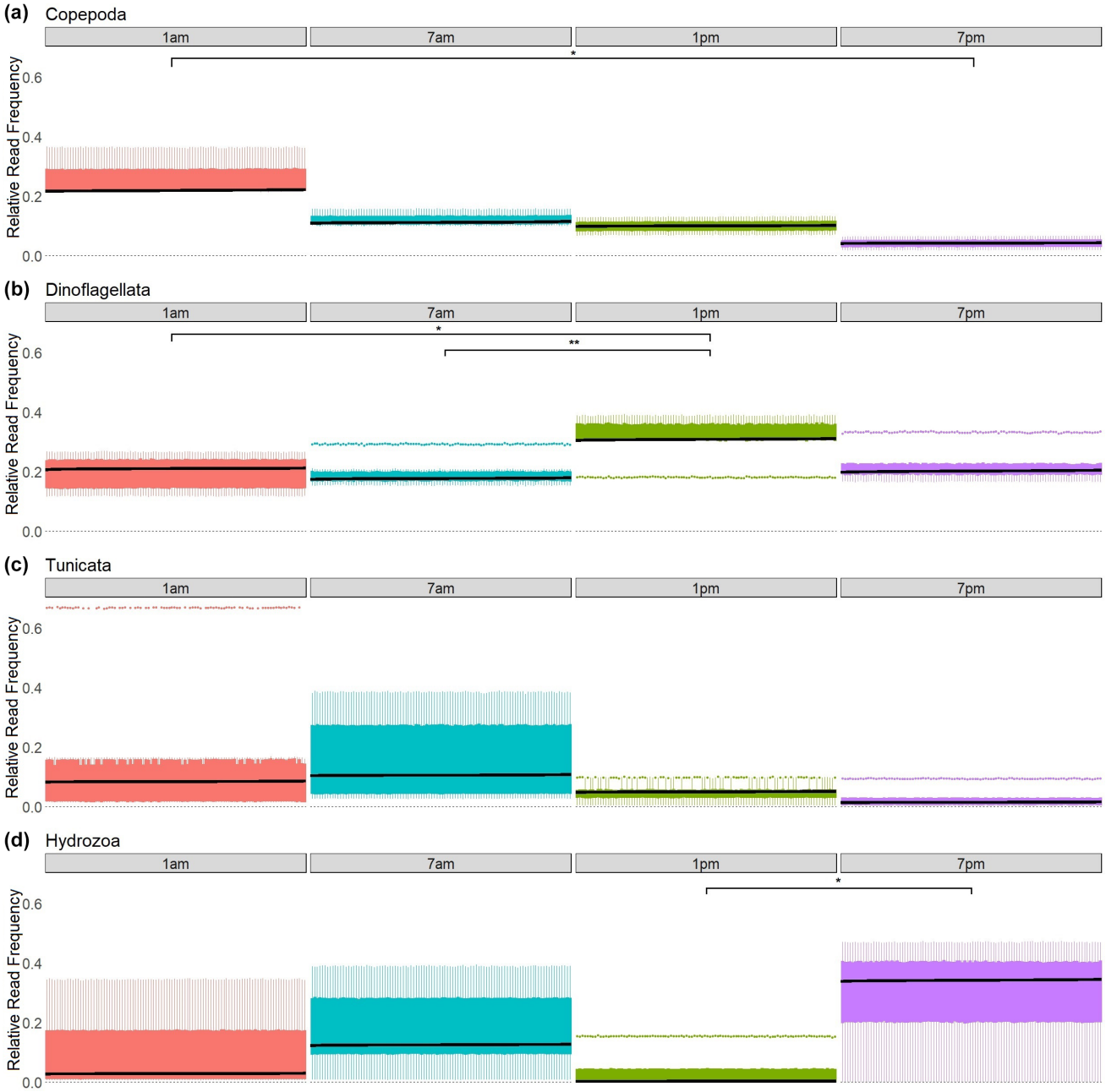

FIGURE 4 Box plots showing the relative read frequency of pelagic taxa at each sampling time point for (a) Copepoda, (b) Dinoflagellata, (c) Tunicata, and (d) Hydrozoa. Each time facet contains 100 consecutive boxplots, each representing one rarefied library. The median read frequency is shown for each library by the black line.

the frequency of reads assigned to this class during diurnal eDNA sampling. The opposite pattern was observed in dinoflagellates with decreased detection of reads during night and morning sampling events. Dinoflagellates have also been observed to exhibit diurnal vertical migrations (Bollens et al., 2012), migrating to the surface to photosynthesize during the day (Kamykowski, 1981). The reduction in these phytoplanktonic groups at the surface during the night could also be driven by predation from zooplankton (Casey et al., 2019). These findings suggest that these groups were detected most prominently when they were present in the immediate vicinity of sampling, and that eDNA samples can be used to investigate the diurnal movements of planktonic organisms. Although the relationship between read frequency and organism abundance or biomass is yet to be fully tested, there is considerable evidence for correlation in multiple taxa (Bourque et al., 2022; Rourke et al., 2021). Repeating this experiment with the collection of eDNA samples at multiple depths would consolidate these findings and provide further evidence for the feasibility of eDNA metabarcoding to track such behaviors.

When applying molecular methods to assess the abundance of planktonic organisms, such as copepods and dinoflagellates, a consideration of the source of genetic material is also required. Reported densities of zooplankton and phytoplankton at similar Indian Ocean

sites vary significantly, with estimates of up to 50×10^6 and 15×10^6 cells L^{-1}, respectively, as well as average copepod densities of 3700 individuals/100 m^3 (Radhakrishnan et al., 2020; Shaama et al., 2020; Soondur et al., 2022). With a total volume of 3 L filtered per sample, it is certain that whole microplankton would have been processed and contribute to relative read frequency recorded here. However, with larger taxa such as copepods, we detected multiple ESVs from different classes within each sample, which if each derived from a whole organism would most likely dominate the sample and be reflected with much higher single-species read frequencies than we observed. In this study, it is likely that the eDNA signal is derived from both the capture of whole organisms and the release of extra-organismal material due to increased movement and predator–prey interactions at these times. Further work investigating the source of organismal DNA is required to confidently infer these read frequency to abundance relationships.

Not all taxa investigated here exhibit clear diurnal patterns, and the eukaryotic community detected by eDNA does appear to be more dynamic than similar studies investigating fish (Ely et al., 2021; Jensen et al., 2022). In a few samples, reads assigned to tunicata, predominantly from the class Appendicularia, accounted for large proportions of the total reads, and over 60% of reads at 1 a.m. on 29th of April at the Southwest site. Appendicularia, more commonly referred to as larvaceans, are free-swimming, gelatinous members of the zooplankton community that can bloom in response to changes in phytoplankton abundance and composition (Sordino et al., 2019). The spikes in read abundance seen here are most likely due to the capture of whole organisms and the discharge of mucus for their temporary secreted houses (Galt & Sykes, 1983; Robison et al., 2005). Benthic taxa, such as sponges (Porifera) and corals (Anthozoa), were also only detected sporadically. This adds evidence to conclusions that surface water samples are unlikely to be the most effective way to target benthic groups on coral reefs outside of spawning events (Ip et al., 2022).

However, eDNA from water samples can consistently detected distinct communities at two sites that are found within the same lagoon and have comparable water depth and distance to land. The eukaryotic community detected in the northwest site was found to be more stable across both sampling times and sampling days. It is likely that this lagoon is subject to recurrent flushing of oceanic water due to tides and currents (Sheehan et al., 2019) and surface currents and winds are predominantly westward in the region during the sampling period in April (Nyadjro et al., 2020). Therefore, there may be higher water movement expected over the southwest site due to fewer islands and less rim reef providing shelter, potentially driving the decreased stability of detected eukaryotic communities compared to the more sheltered northwest site. This indicates the importance of understanding the role of hydrodynamics in the stability of eDNA signals in the marine environment.

In this environment, it is likely that eDNA signals are highly dynamic and influenced by variable eDNA release, fast degradation, water movement, and species behavior. Sampling time, and not site or day, was found to affect the alpha diversity (ESV richness) of the eukaryotic community, and less than half of the total diversity of Copepoda ESVs were detected in any one sample. In the PERMANOVA analysis, however, communities were found to be significantly different between sites and sampling day, indicating that eDNA samples reflect both diurnal signals and stochastic species presence over the sampling period. Recent developments including automated (Formel et al., 2021; Hendricks et al., 2022) and natural samplers, such as sponges (Cai et al., 2022; Mariani et al., 2019; Turon et al., 2020), may begin to alleviate some of the difficulties with taking multiple samples at individual sites (e.g., time, cost, and resources), by sampling larger volumes of water, and over a longer time period.

Overall, we conclude that eDNA metabarcoding of water samples can be a useful tool to examine surface eukaryotic communities on a tropical coral reef. Utilizing eDNA metabarcoding and relative read frequency could further provide the ability to investigate behavior in the surface ocean. However, a single water sample is likely insufficient to fully describe the community present, with temporal replicates needed to provide a comprehensive picture of species richness. Finally, a consistent time of sampling is likely important to appropriately compare eDNA samples across spatial scales. We strongly recommend that time of sampling be reported in available metadata to facilitate accurate data comparisons.

AUTHOR CONTRIBUTIONS

RD and ND conceptualized the study and carried out the sampling and laboratory work. JW performed the bioinformatics and assisted RD with the data analysis. RD wrote the manuscript with substantial input from all authors. RD, CH, ER, and CY acquired the funding for this work and ER, CH, and CY supervised the work.

ACKNOWLEDGMENTS

This work was funded by the Bertarelli Programme in Marine Science and the UK Natural Environment Research Council. Staff at ZSL (CY and CH) and their research are supported by funding from Research England. We would also like to thank the captain and crew of the research vessel for their logistical support.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Intermediary files and R scripts are available at https://zenodo.org/record/8370667.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Dowell, R., Dunn, N., Head, C., Yesson, C., Williams, J., & Ransome, E. (2024). Environmental DNA captures diurnal fluctuations of surface eukaryotes on a tropical coral reef. *Environmental DNA*, *6*, e512. https://doi.org/10.1002/edn3.512