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SPECIAL ISSUE ARTICLE

Environmental DNA reveals a multi-taxa biogeographic break across the Arabian Sea and Sea of Oman

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Abstract

Environmental DNA (eDNA) is increasingly being used to assess community composition in marine ecosystems. Applying eDNA approaches across broad spatial scales now provide the potential to inform biogeographic analyses. However, to date, few studies have employed this technique to assess broad biogeographic patterns across multiple taxonomic groups. Here, we compare eDNA-derived communities of bony fishes and invertebrates, including corals and sponges, from 15 locations spanning the entire length of the Omani coast. This survey includes a variety of habitats, including coral and rocky reefs, and covers three distinct marine ecoregions. Our data support a known biogeographic break in fish communities between the north and the south of Oman; however, the eDNA data highlight that this faunal break is mostly reflected in schooling baitfish species (e.g., sardines and anchovies), whereas reef-associated fish communities appear more homogeneous along this coastline. Furthermore, our data provide indications that these biogeographic breaks also affect invertebrate

communities, which includes corals, sponges, and broader eukaryotic groups. The observed community shifts were correlated with local environmental and anthropogenic differences characteristic of this coastline, particularly for the eDNA-derived bony fish communities. Overall, this study provides compelling support that eDNA sequencing and associated analyses may serve as powerful tools to detect community differences across biogeographic breaks and ecoregions, particularly in places where there is significant variation in oceanographic conditions or anthropogenic impacts.

KEYWORDS
biodiversity, environmental DNA, genomics, habitats, metabarcoding, Oman

1 | INTRODUCTION

The latest wave of the molecular revolution has enabled biologists to isolate and amplify trace genetic material shed by multicellular organisms into their surrounding environment, often referred to as environmental DNA or simply eDNA (Deagle et al., 2018; Taberlet et al., 2012). In marine environments, metabarcoding of eukaryotic eDNA sourced from a number of substrata (Koziol et al., 2019) now allows scientists to address questions on ecosystem biodiversity across multiple trophic and taxonomic levels (Kelly et al., 2017; Stat et al., 2017), although these studies have generally been limited to relatively small spatial scales (Jeunen et al., 2019; O'Donnell et al., 2017; Sawaya et al., 2019; West et al., 2020). Few studies have "scaled up" eDNA surveys to sample across bioregions or to address biogeographic questions across multiple taxa (but see Kume et al., 2021; Pitz et al., 2020; West et al., 2021).

Biogeographic breaks in the marine environment represent the range limits for large numbers of species, boundaries that delimit genetic and phenotypic variation at the intraspecific level (Avise et al., 1987), and can influence demographics of populations (e.g., Broitman et al., 2001). New molecular approaches that can identify faunal shifts, such as eDNA metabarcoding, are therefore relevant for improved marine management. Moreover, biogeographic breaks are often not restricted to a single taxonomic group across the kingdoms of life. For example, Holman et al. (2021) found that biogeographic breaks were shared between animals, protists, and bacteria. This taxonomic overlap means that molecular tools that can rapidly detect a broad array of organisms may facilitate further refinements of existing boundaries.

The coastline of the Sultanate of Oman extends from the Strait of Hormuz in the north to the Yemeni border in the southwest and supports a wide array of marine biota and habitats, including offshore coral reefs, as well as coastal sand, rock, and reef (for an overview see Claereboudt, 2019). This region, therefore, provides an ideal environment to scale up eDNA approaches. Oman also borders several marginal gulfs and seas, namely the Arabian Sea, Sea of Oman, and the Arabian/Persian Gulf, which includes at least three distinct marine ecoregions (*sensu* Spalding et al., 2007). Notably, there is extreme variability in sea surface temperature and nutrient load across these bodies of water, including a "pseudo-high latitude effect" (Sheppard et al., 1992). For example, Oman's southern-most governorate, Dhofar, is influenced by southwest (SW) summer monsoon winds, known locally as the "Khareef", which trigger seasonal Ekman upwelling in the Arabian Sea (Elliott & Savidge, 1990; Shi et al., 2000) and result in dramatic drops in sea surface temperature down to 16–17°C (Claereboudt, 2019), as well as significant increases in nutrients (five fold increase; Savidge et al., 1990) and phytoplankton (10 fold increase; Brock & McClain, 1992). In contrast, the temperature in the north-western part of the Sea of Oman, including the northern-most governorate of Musandam, remains more stable, being only marginally affected by the SW summer monsoon winds. Between these two extremes, the Ras Al-Hadd Jet, a current that flows seaward from the eastern tip of Oman, is maintained by cyclonic and anticyclonic eddies in the Sea of Oman and the northern Arabian Sea, respectively (Al Shaqsi, 2017; Tang et al., 2002). This current creates an additional environmental gradient with potential to impede dispersal of marine organisms along this coastline.

This marked environmental variation across the Omani coastline is purported to contribute to a major biogeographic break between the southern Dhofar region in the Arabian Sea and the northern Sea of Oman into the Arabian/Persian Gulf. This break is supported by shifts in reef fish community assemblages along this coastline (Burt et al., 2011), as well as the restricted distribution of individual fish species (Priest et al., 2016; Simpson et al., 2014; Torquato et al., 2019), with some notable southern Oman endemics constrained to the Dhofar region (Randall & Hoover, 1995). Although there is clear evidence that these biogeographic breaks may apply to taxonomic groups other than fish, such as habitat-forming corals (Claereboudt, 2019; Salm, 1993), macroalgae (Schils & Wilson, 2006), and a suite of other marine invertebrates (e.g., Omani abalone, *Haliotis mariae*; Waal et al., 2013), these regional differences have yet to be formally tested across multiple trophic levels due to the challenges of comprehensively surveying marine communities across this coastline.

Significant socio-economic transformation across the Gulf Cooperation Council (GCC) states (Bahrain, Kuwait, Oman, Qatar, Saudi Arabia, and the United Arab Emirates) has resulted in large-scale mega projects and rapid coastal urbanization along the Arabian

Peninsula (Burt, 2014). Recent urban growth, land reclamation, and coastal degradation have put increased pressures on marine ecosystems and coral reefs adjacent to coastal settlements (van Lavieren et al., 2011). Portions of the Omani coastline have also undergone significant development in recent years related to population growth and increased use of the marine environment (Mansour, 2020; Mansour et al., 2017). Harbors and resort developments have been established along the coastline, along with numerous artificial reef structures. Previous biogeographic research in the region often precedes these developments, and so their impacts on patterns of marine biodiversity along the Omani coast are largely unknown. Given that anthropogenic stressors are repeatedly being shown to alter biogeographic patterns across the kingdoms of life (Holman et al., 2021), it is therefore critical to assess the biological, physical, and anthropogenic drivers of species distributions simultaneously. On coral reefs in particular, human activity has been shown to disrupt the distribution and function of entire groups of fishes (Bellwood et al., 2012), which may be associated with downstream effects to the rest of the ecosystem.

The aim of this study was therefore to conduct a multi-marker eDNA metabarcoding survey across the Omani coastline to:

- assess whether eDNA-derived fish communities reflect the known biogeographic break between the north and the south of Oman,
- assess whether the biogeographic break is apparent in eDNA data for taxonomic groups other than fish,
- determine whether and how the composition of eDNA correlates with environmental variables associated with this biogeographic break, and
- determine whether eDNA data from the developed northern coastline of Oman are indicative of anthropogenic pressures and discuss its potential to inform future biomonitoring in the region.

2 | MATERIALS AND METHODS

2.1 | Sample collection

Sampling was conducted between January and December 2018 at 15 sites in Oman, totaling 120 samples across 1,850 kilometers of coastline (Figure 1; also see Table S1). Sampling sites were broadly grouped into four regions: (1) coral reefs along the rocky shores of the Musandam peninsula near the Strait of Hormuz, (2) the rocky coast and islands around the capital, Muscat, which included artificial reef structures deployed at Al Sohar, Al Batinah (Al Hasani, 2008; Al Ismaili, 2017), and mangrove reserves at Qurum Nature Park, (3) sandy beach with exposed coral reef outcrops (Fins White Beach) near Sur, a major port city northwest of Ras Al-Hadd, and (4) the rocky reefs of the southern Dhofar region, including an intertidal rock pool (Rock Pools). Sampling at sites in between Sur and Dhofar, including the offshore Khuriya Muriya Islands, was not possible for this study due to logistic constraints and limited accessibility.

At each sampling site, eight replicate 750 ml seawater samples were collected 30 cm below the surface using bleach sterilized Nalgene bottles, which were immediately stored on ice. All water samples were filtered in the field or in the laboratory within 8 h. Each water sample was filtered through 47 mm polyethersulfone filters with a 0.2 μm pore size (Pall Life Sciences) using a Pall Sentino® Microbiology pump (Pall Life Sciences). Between samples, the filtration apparatus was cleaned by soaking in 10% bleach for at least 15 min. After filtration, the filter membranes were immediately frozen and stored at −20°C prior to their transportation to a quarantine facility within the Trace & Environmental DNA (TrEnD) Laboratory in Perth, Western Australia.

2.2 | DNA extraction

DNA bound to half of each filter membrane was extracted using Qiagen DNeasy Blood and Tissue kits (Qiagen Inc.) with the following modifications: 540 μl of ATL lysis buffer and 60 μl of Proteinase K were used during the 3-hour digestion step at 56°C, 400 μl of this digest was aliquoted into a sterile tube, and double the manufacturers recommended volume of AL lysis buffer and 100% ethanol were used to process this aliquot. All remaining steps followed the manufacturer protocol. DNA extraction controls containing no sample were carried out for every set of extractions (labeled DIG CONT). The other half of each filter membrane was vouchered at −80°C for long-term storage.

2.3 | Fusion-tag qPCR

In this study, we used previously published primers to amplify bony fish, corals, and sponges, as well as most other marine eukaryotes from mixed environmental samples. The four applied assays are hereafter referred to as "18Suni" targeting 18S rRNA in most eukaryotes (V1-3 hypervariable region; 18S_uni_1F: 5' – GCCAGTAGTCATATGCTTGTCT – 3'; 18S_uni_400R: 5' – GCCTGCTGCCTTCCTT – 3'; Pochon et al., 2013), "16SFish" targeting 16S rRNA in mostly bony fish (16SF/D: 5' – GACCCTATGGAGCTTTAGAC – 3'; 16S2R-degenerate: 5' – CGCTGTTATCCCTADRGTAACT – 3'; Berry et al., 2017; Deagle et al., 2007), "CP1" targeting ITS2 in basal metazoans such as corals and sponges (SCL5.8S_F: 5' – GARTCTTTGAACGCAAATGGC – 3'; SCL28S_R: 5' – GCTTATTAATATGCTTAAATTCAGCG – 3'; Brian et al., 2019), and "CP2" targeting a modified fragment of ITS2 more appropriate for the additional detection of Acroporid corals (SCL5.8S_F: 5' – GARTCTTTGAACGCAAATGGC – 3'; Acro874_R: 5' – TCGCCGTTACTGAGGGAATC – 3'; Alexander et al., 2020). Quantitative PCR (qPCR) experiments were set up in a separate ultra-clean laboratory at Curtin University designed for trace DNA work using a QIAgility robotics platform (Qiagen Inc.). All qPCR reactions were performed in duplicate on a StepOnePlus Real-Time PCR System (Applied Biosystems). PCR reagents included 10X

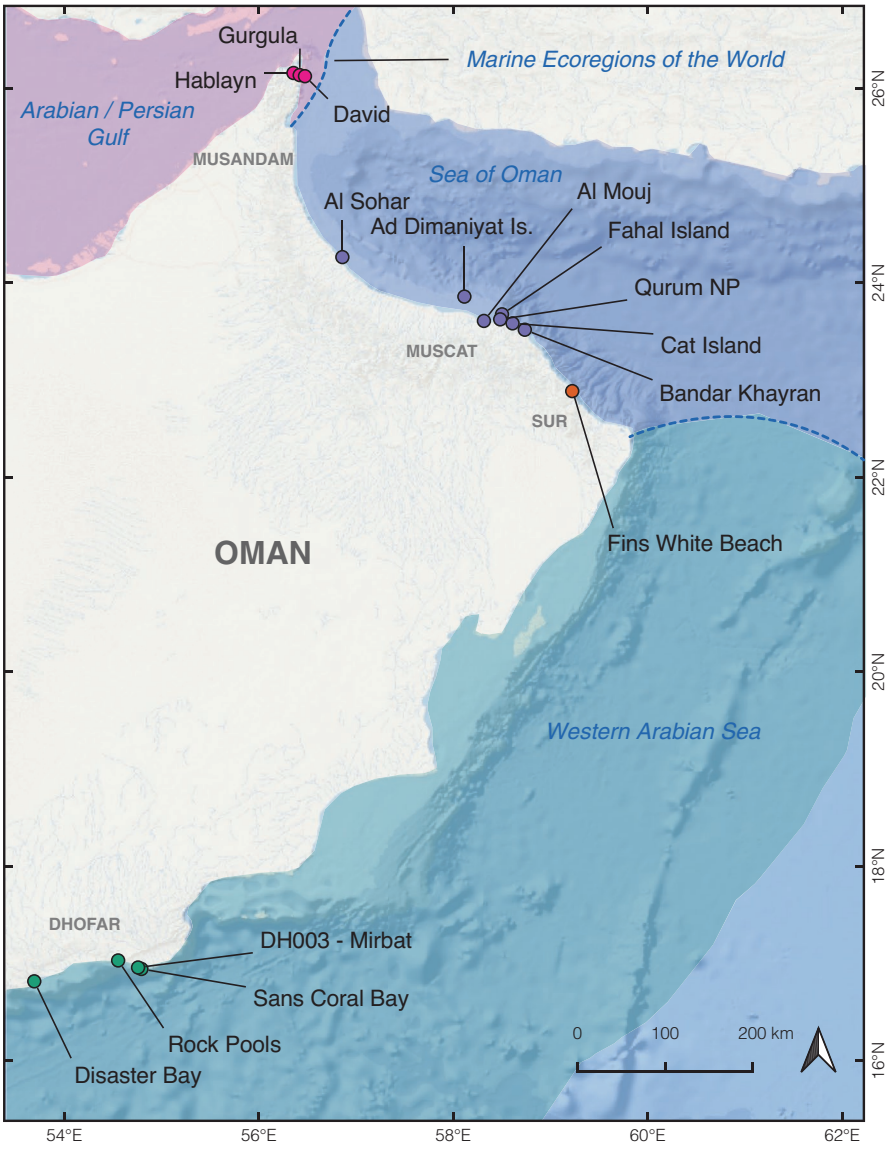

FIGURE 1 Seawater samples collected at 15 sites off the coast of Oman. The different colors for each circle represent the different sampling regions. For reference, we have overlaid the Marine Ecoregions of the World (MEOWs) from Spalding et al. (2007) relevant to our sampling: Arabian/Persian Gulf (Ecoregion 90), Sea of Oman (Ecoregion 91), and the Western Arabian Sea (Ecoregion 92)

AmpliTaq Gold® PCR Buffer (Applied Biosystems), 2 mM $MgCl_2$, 0.25 mM dNTPs, 0.4 mg/ml BSA (Fisher Biotec), 0.4 µmol/l of each primer (Integrated DNA Technologies), 0.12X SYBR® Green (Life Technologies), one Unit AmpliTaq Gold DNA polymerase (Applied Biosystems), 2 µl of DNA, and Ultrapure™ Distilled Water (Life Technologies) to make the solution to 25 µl total volume. Assay-specific annealing temperatures and cycle number are as follows: 18Suni, 52°C for 45 cycles; 16SFish, 54°C for 45 cycles; CP1, 55°C for 50 cycles; CP2, 55°C for 50 cycles (for more details see DiBattista et al., 2019). To check for contamination, non-template control (labeled as NTC) PCR reactions were run alongside the template PCR reactions, which only contained master mix including the assay primers.

Duplicate PCRs for each assay amplified from the same eDNA template were combined to control for amplification stochasticity and then pooled into a library with all amplicons at equimolar ratios based on amplification CT and ΔRn values. Each library was size selected using a Pippin Prep (Sage Science), retaining amplicons between 160 and 600 bp for 18Suni, CP1, and CP2 and between 160 and 400 bp for 16SFish, which were then purified using a QIAquick PCR Purification Kit (Qiagen Inc.). Final libraries were quantified using a Qubit 4.0 Fluorometer (Invitrogen, Carlsbad, USA) and if necessary, diluted to 2nM, prior to loading on either a 300 cycle (for unidirectional sequencing; 16SFish) or 500 cycles (for paired-end sequencing; 18Suni, CP1, and CP2) MiSeq® V2 Standard Flow Cell on an Illumina MiSeq platform.

2.4 | Quality filtering

All sequence data were quality filtered prior to taxonomic assignment using loose and conservative thresholds, except for 18Suni, where only conservative thresholds were used given the generality of this assay. In brief, sequences were merged (for paired-end sequencing only), demultiplexed based on their unique indexes, and quality filtered using the following tools: (1) AdapterRemoval (v2.2.0; Schubert et al., 2016) for trimming low quality reads from the 5′/3′ end (conservative quality score: 30; loose quality score: 20),

removing adapters, and merging the overlapping files (conservative minimum overlap: 20 bp; loose minimum overlap: 12 bp), (2) OBITools (v1.2.11; Boyer et al., 2014) for demultiplexing and filtering out reads below a minimum length (200 bp for 18Suni, and 100 bp for 16SFish, CP1, and CP2), and (3) USEARCH (v11.0.667; Edgar, 2010) for dereplication into unique sequences, removing singletons (i.e., minimum threshold of read abundance = 2 for all assays), removing chimeric sequences, and generating a ZOTU (Zero-radius Operational Taxonomic Unit) table based on the UNOISE algorithm, which performs denoising (error-correction) of the amplicon reads.

2.5 | Taxonomic assignment

Zero-radius Operational Taxonomic Units were queried against the National Centre for Biotechnology Information's (NCBI) GenBank nucleotide database (accessed in 2019/2020) using BLASTn with the following settings: percentage identity (18Suni: conservative = 90, loose = n/a; 16SFish, CP1, and CP2: conservative = 95, loose = 90), query coverage: (18Suni: conservative = 100, loose = n/a; 16SFish, CP1, and CP2: conservative = 100, loose = 95), best hit score edge of 0.05, best hit overhang of 0.25, and an E-value of $1e^{-3}$. LULU (Frøslev et al., 2017) was then run to curate the assignments and eliminate any remaining redundant sequences with the default parameters: minimum_ratio_type = min, minimum_ratio = 1, minimum_match = 84, minimum_relative_cooccurrence = 0.95. LULU identifies errors by simultaneously assessing sequence similarity and their co-occurrence patterns. This entire process was completed on the Zeus SGI cluster based at the Pawsey Supercomputing Centre in Kensington, Western Australia, using an in-house script developed by Mousavi-Derazmahalleh et al. (2021). All ZOTUs that were detected from extraction controls were removed from further analyses. This included ZOTU39 (*Apogon semilineatus*) for 16Fish quality filtered loose, ZOTU295 and ZOTU606 (*Ephydatia fluviatilis*) as well as ZOTU69 (uncultured eukaryote) for CP1 quality filtered conservative, ZOTU295 and ZOTU599 (*Ephydatia fluviatilis*) as well as ZOTU69 (uncultured eukaryote) for CP1 quality filtered loose, ZOTU3 (unknown Eukaryota) for CP2 quality filtered conservative, and ZOTU13 (*Halichondria okadai*), ZOTU1, ZOTU3, and ZOTU17 (unknown Eukaryota) for CP2 quality filtered loose.

2.6 | Environmental data and anthropogenic disturbance scale

The sampling sites that we selected along the coast of Oman experience a range of environmental conditions (Figure 2; also see Table S2) and are differentially impacted by local anthropogenic disturbances. For environmental conditions, sea surface temperature (SST) and chlorophyll a concentration (CHL), the latter a common proxy for nutrient load, were estimated based on 10 years of remote sensing data captured between January 1, 2009, and December 31, 2018. These data were median averaged for each day of the year to obtain seasonal cycles. The remote sensing data were obtained through the Ocean Products Portal of the Copernicus – marine environment monitoring service (https://resources.marine.copernicus.eu/products). SST and CHL originate from the OSTIA (Worsfold et al., 2020) and OCEANCOLOUR (Garnesson et al., 2019) data sets, respectively. Both are level four satellite products available as daily means at approximately 5 × 5 km spatial resolution.

We additionally calculated an index of anthropogenic effects across the Sea of Oman (Musandam to Sur) to assess the effects of human pressures on this developed coastline (Mansour, 2020; Mansour et al., 2017). Six physical variables, identified as major anthropogenic pressures, were considered: population settlement, hotels, cruise ports, commercial ports, oil and gas infrastructure, and marine overfishing. For each sampling site, the Euclidean distances to each of the six physical variables were considered using a GIS platform. For example, the distance of Musandam coral reefs

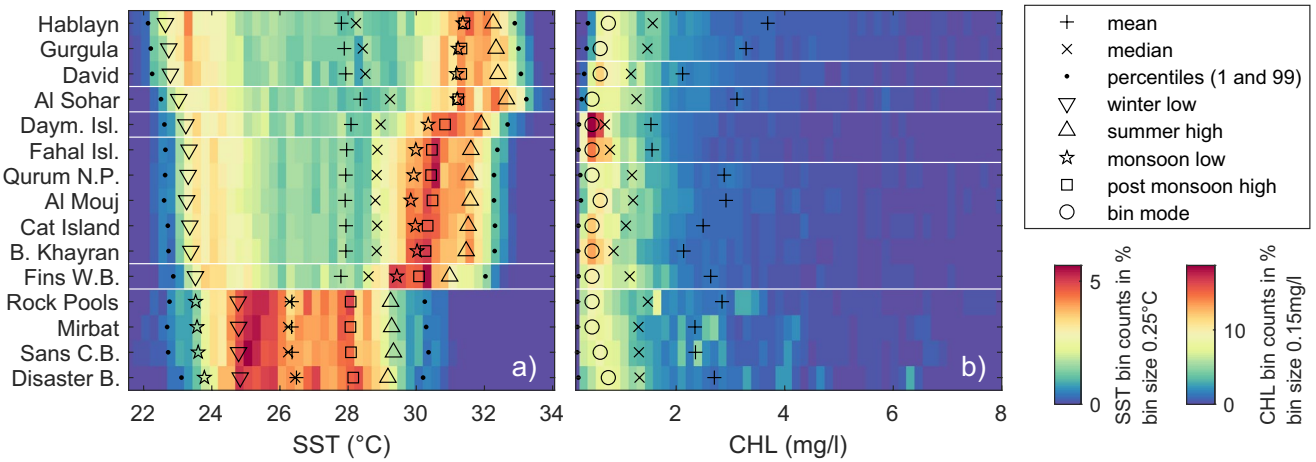

FIGURE 2 Average annual variation in sea surface temperature (SST; a) and chlorophyll a concentration (CHL; b) for each of the 15 sampling sites off the coast of Oman based on 10 years of remote sensing data captured between January 1, 2009, and December 31, 2018

to the nearest oil and gas infrastructure (330–360 km) is two orders of magnitude higher than coral reefs in the Muscat region (~3.5 km).

Given that the impact of each anthropogenic pressure varied, a weight was computed for each variable utilizing Analytical Hierarchal Process developed as a multicriteria process by Saaty (1980). This method provides a template for calculating a weight for each criterion in the decision process, particularly when modeling their effects on coral reef ecosystems (Mansour, 2020). Assigning a weight to each variable is necessary assuming that the impacts of variables vary based on the nature and mechanism of each stressor. For example, a short distance from oil infrastructure is assumed to be a high threat and thus risky compared to equivalent short distances from hotels and resorts. This method also relies on pairwise comparisons between the variables, which is computed by creating a matrix of all variables and comparing two criteria at a time. The process of pairwise comparison is implemented using a scale of 1–9, where one is assigned when the two variables are equal in their importance, whereas nine is assigned to the variable as an indicator of extreme importance (Saaty, 2000). The vector of weights for each variable was calculated according to Saaty's (2000) eigenvector method and by implementing normalization of the pairwise comparison matrix. This was followed by justifying the consistency of comparison scores via the consistency relationship (Saaty, 2008). Based on this, each site was therefore assigned to one of five anthropogenic pressure categories: Very Low, Low, Medium, High, or Very High.

2.7 | Statistical analyses

Zero-radius Operational Taxonomic Units for all four datasets were transformed to presence/absence format prior to further analyses. Regional differences, the role of environmental parameters (SST and CHL), and the role of anthropogenic pressures in structuring biological assemblages were tested using PRIMER v 7.013 (Clarke & Gorley, 2015). We ran PERMANOVA (9999 permutations) using a Jaccard similarity matrix to test differences between regions, with follow-up pairwise PERMANOVA tests to identify significantly different assemblages. Patterns in regional differences were visualized using principal coordinate analyses (PCO). PCO plots were overlayed with the ZOTUs most strongly correlated to the different regions (Pearson's correlation ≥0.6). We additionally used the Indicspecies package v 1.7.8 in RStudio v 1.2.5033 (Cáceres & Legendre, 2009; R Development Core Team, 2015) separately for each presence/absence transformed data set to find regionally indicative species. PERMANOVA outcomes of the QF conservative and QF loose datasets were no different from each other, and so only QF conservative results are presented here (but see Suppoting information for all ZOTU outputs).

The effects of environmental and anthropogenic variables were tested using distance-based linear models (DistLM) and plotted with distance-based redundancy analysis (dbRDA). Strongly correlated variables (Pearson's correlation ≥0.8) were removed prior to analyses. Models were selected based on the Akaike Information Criterion (AIC), using the "best" selection procedure in PRIMER v 7.013.

3 | RESULTS

The mean number of sequences generated by the combined MiSeq runs prior to quality filtering was 53,615 ± 2,925 SEM per replicate sample for 18Suni, 78,353 ± 4,547 per replicate sample for 16SFish, 86,771 ± 10,523 per replicate sample for CP1, and 73,858 ± 8,957 per replicate sample for CP2 (Table S1).

3.1 | Aim 1 – eDNA detections for fishes

We first assessed whether eDNA-derived fish communities reflected the known biogeographic break between the north and south of Oman. We found that the composition of ZOTUs for the 16SFish assay was different between regions (Pseudo-F_3: 2.146, $p < 0.001$; Figure 3c,d), with significant differences between Dhofar and Muscat, as well as Dhofar and Musandam (Table S3). Indicator species analysis showed schooling baitfish such as anchovies, sardines, and silversides to be indicators for Musandam (*Encrasicholina punctifer*), Muscat (*Sardinella longiceps*), and Sur (*Atherinomorus lacunosus* and *Herklotsichthys quadrimaculatus*) (Table 1). Other indicators in Sur included sand- or silt-associated species of goatfish (*Parupeneus* sp., *Mulloidichthys vanicolensis*) and mullet (*Plicomugil labiosus*) (Table 1 and Suppoting information). In Dhofar, indicator taxa were typically rocky reef-associated species that included blennies (*Blenniiformes*, *Istiblennius edentulous*), butterflyfishes (*Chaetodon* sp.), sweepers (*Pempheris mangula*), snappers (*Lutjanus* sp.), rabbitfishes (*Siganus* sp.), and sweetlips (*Plectorhinchus playfairi*) (Table 1). Other assays are not presented here based on uninformative taxonomic assignments (see "IndVal Outputs" in Suppoting Information).

3.2 | Aim 2 – eDNA detections for all other taxa

We next assessed whether this biogeographic break was apparent for taxonomic groups other than fish. Here, we found that the

FIGURE 3 Canonical analysis of principal coordinates (CAP) ordination plots of the presence/absence of eukaryotic Zero-radius Operational Taxonomic Units (ZOTUs) detected based on seawater samples collected at 15 sites in Oman using the 18Suni (a), 16SFish (c), CP1 (e), and CP2 (g) assays. For reference, 18Suni targets 18S rRNA in most eukaryotes, 16SFish targets 16S rRNA in mostly bony fish, and CP1 and CP2 target ITS2 in basal metazoans such as corals and sponges. The relationship of eukaryotic community assemblages identified in each sample using a Jaccard's coefficient for the factor "Region" is shown by the legend. We additionally include distance-based redundancy analyses (dbRDA) based on 18Suni (b), 16SFish (d), CP1 (f), and CP2 (h) assays; the proportion of fitted and total variation is indicated on each axis. Vectors indicate environmental variables and anthropogenic pressures correlated to the different regions (Pearson's correlation ≥0.6)

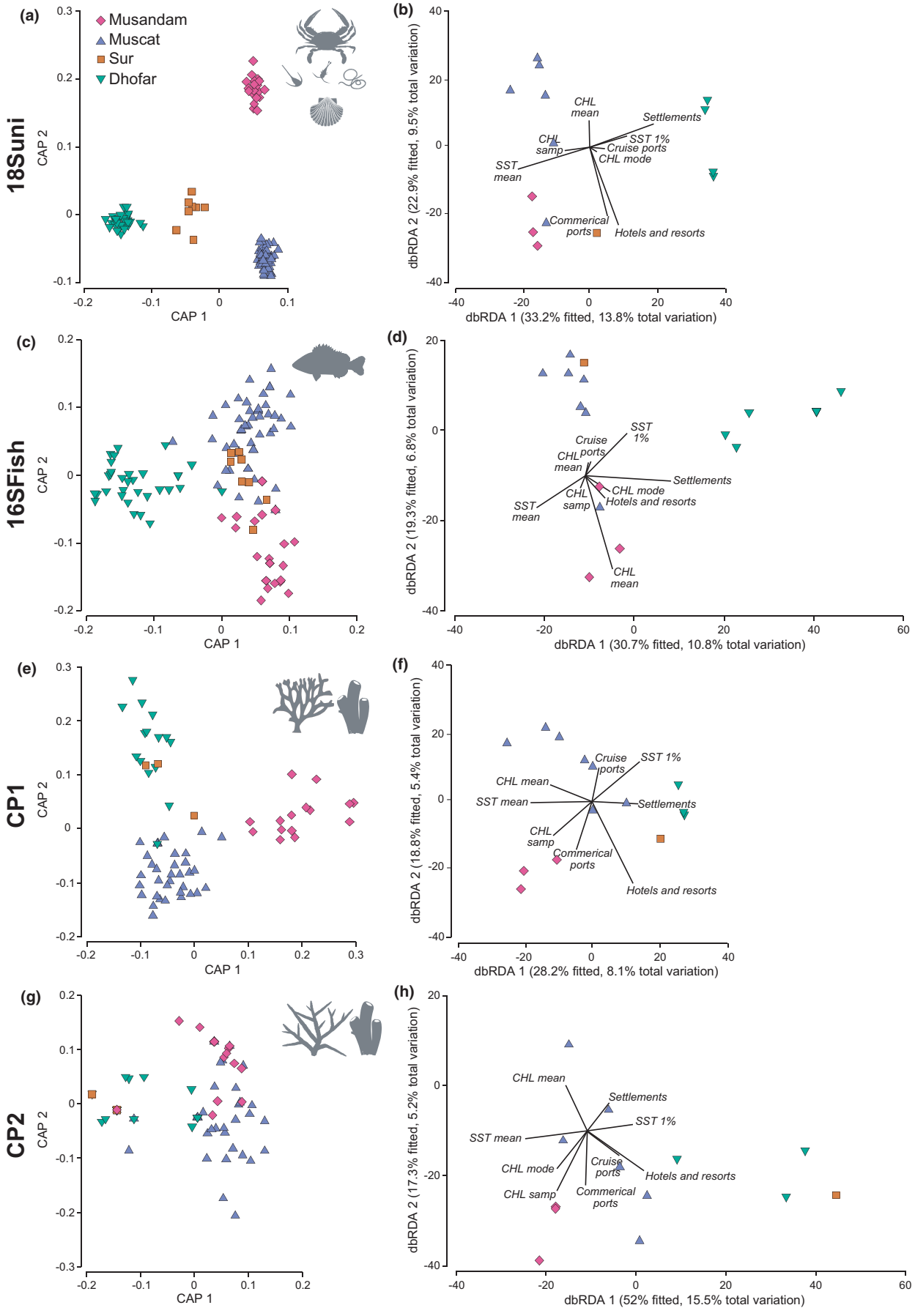

TABLE 1 Indicator value (IndVal) analyses based on seawater samples collected at 15 sites off the coast of Oman for the 16SFish metabarcoding assay, which targets 16S rRNA in mostly bony fish

Musandam			Muscat			Sur			Dhofar		
Taxon	Stat	*p* value	Taxon	Stat	*p* value	Taxon	Stat	*p* value	Taxon	Stat	*p* value
Encrasicholina punctifer	0.577	0.002	*Sardinella longiceps*	0.656	0.001	*Atherinomorus* sp. *lacunosus*–1	0.588	0.003	Blenniiformes	0.530	0.002
						Herklotsichthys quadrimaculatus	0.769	0.001	*Chaetodon*	0.685	0.001
						Herklotsichthys quadrimaculatus	0.738	0.001	*Istiblennius edentulus*	0.637	0.001
						Parupeneus	0.463	0.007	*Lutjanus*	0.500	0.007
						Plicomugil labiosus	0.574	0.001	*Pempheris mangula*	0.637	0.001
						Mulloidichthys vanicolensis	0.755	0.001	*Plectorhinchus playfairi*	0.433	0.005
						Mulloidichthys vanicolensis	0.680	0.001	*Siganus*	0.758	0.001

p values were selected at a 0.01 significance level. Other assays are not presented here based on uninformative taxonomic assignments (see Supporting Information).

18Suni assay, which targets all eukaryotes, identified different marine biological communities among regions (Pseudo-F_3: 2.9242, $p < 0.001$; Figure 3a, b), with differences between Muscat and Dhofar, Muscat, and Musandam, as well as Dhofar and Musandam (Table S3). For the CP1 and CP2 assays that primarily target corals and sponges, we detected significant differences between regions (CP1, Pseudo-F_3: 1.947, $p = 0.001$, Figure 3e, f; CP2, Pseudo-F_3: 2.459, $p = 0.017$, Figure 3g, h). We found apparent shifts in the mean proportion of positive replicates per site for all order-level detections (Figure 4a, b), as well as the family-level detections within the order Scleractinia (stony corals; Figure 4c, d). Notably, Sur had no scleractinian detections, Musandam was dominated by scleractinian detections (mostly from the family Poritidae), and Muscat and Dhofar displayed a diverse mix of soft corals (order Alcyonacea), black corals (order Antipatharia), anemones (order Actiniaria as well as subclass Ceriantharia), sponges (orders Clionaida, Dictyoceratida, Poecilosclerida, Suberitida, and Verongiida), and zoanthids (order Zoantharia) in addition to scleractinians (Figure 4). Although we did detect a diversity of invertebrate taxa using our CP1 and CP2 targeted assays, ZOTU richness plots per assay, per region, or for all regions combined suggest that further sampling might have uncovered an even greater diversity of taxa, particularly for the order Scleractinia given that none of the lines reached an asymptote (Figure S1).

3.3 | Aims 3 and 4 – Environmental data and anthropogenic disturbances

SST and CHL differed between the sampling sites based on mean seasonal variation between 2009 and 2018 (Figure 2). Average temperatures ranged from approximately 28°C in the Sea of Oman to 26.3°C in Dhofar. Near Salalah and Mirbat (i.e., Dhofar), SST was most frequently between 24°C and 29°C. The distribution of SST in the Sea of Oman, on the other hand, spans a larger range resulting in a distinctly bimodal frequency distribution; summer temperatures and winter lows generally increase and decrease, respectively, from east to west. The SW summer monsoon winds strongly affect conditions in the Arabian Sea where SST during the summer upwelling falls well below the winter low and CHL increases between June and October. In the Sea of Oman, the SW summer monsoon winds reduce summer SST up to Muscat and the Ad Dimaniyat Islands, whereas further north this influence is diminished.

To determine distinct environmental zones for further testing, we evaluated the clustering of long-term SST and CHL characteristics at the 15 sample locations. We used k-means and hierarchical algorithms and the Davies–Bouldin criterion to determine the optimal number of distinct zones and the corresponding station clusters. We detected two distinct clusters (Sea of Oman and Arabian Sea) for both SST and CHL, reflecting the obvious system differences between the two clusters in terms of SW summer monsoon winds impacts (Figure 2). Within the Sea of Oman, we found the

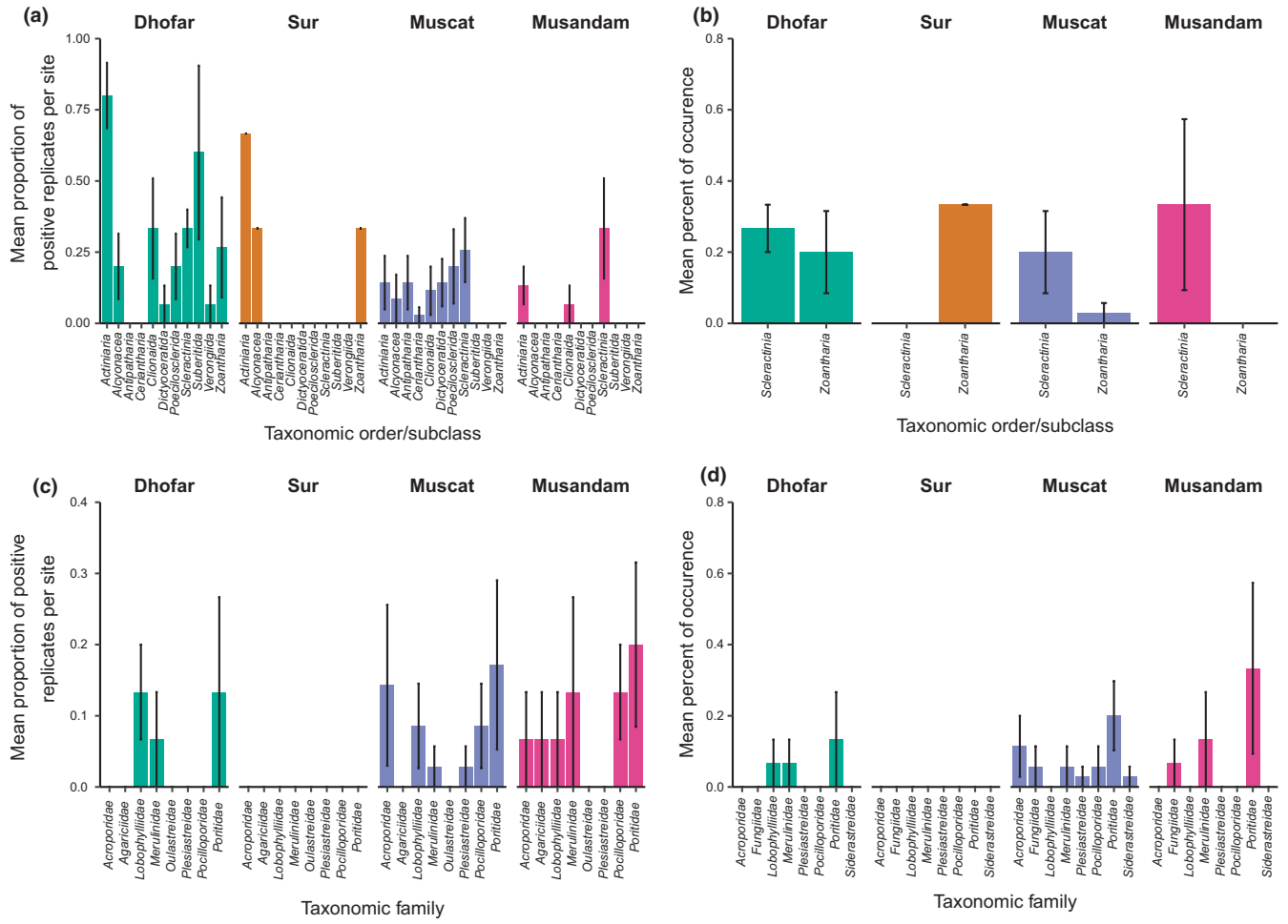

FIGURE 4 Proportion of positive replicates within a site averaged by region for each taxonomic order/subclass (a, b) and taxonomic family within the order Scleractinia (c, d) for CP1 (a, c) and CP2 (b, d) assays by region. For reference, these two assays target ITS2 in basal metazoans such as corals and sponges. Error bars represent the standard error of the mean (SEM)

same number of sub-clusters (5) for SST and CHL, albeit covering slightly different zones (horizontal lines in Figure 2), which illustrates the non-linear relationship between SST and CHL. Generally, exposed locations (e.g., David Reef in Musandam) and islands (e.g., Ad Dimaniyat Islands and Fahal Island in Muscat) had lower CHL compared to the inshore stations. The highest CHL in winter and highest SST in summer were detected at Al Sohar, which sits on the broad continental shelf. Here, a shallow thermocline combined with potentially high terrestrial nutrient influx may contribute to these bounding values.

With this information, we next tested whether the composition of eDNA correlated with environmental and anthropogenic variables estimated across the coastline of Oman (see Tables S2 and S4). We found that between 19.12% (CP2) and 42.67% (18Suni) of the DistlM variance was explained by environmental variables and anthropogenic pressures (Table 2). The environmental factor explaining most of the variation was mean SST (between 6.4% for CP1 and 13.5% for 18Suni), whereas the anthropogenic factor explaining the most overall variation was "settlements" (between 1.0% for 16SFish and 13.4% for 18Suni). Mean CHL explained the least variation for 16SFish (2.0%), whereas the mode of CHL explained the least variation for all the other assays (e.g., 2.7% for CP2 to 3.6% for CP1). Anthropogenic pressures appeared to have a lesser impact than environmental factors across all assays. For example, "settlements" explained the lowest variation for 16SFish but the highest variation for 18Suni (Table 2).

4 | DISCUSSION

4.1 | Aim 1 – eDNA detections for fishes

In this study, we tested the generality of a known biogeographic break in marine communities between the north and south of Oman using eDNA. We found that a biogeographic break in the fish community between the north and south of Oman was related to the detection of schooling baitfish as well as sand- or silt-associated taxa in the north (Musandam, Muscat, and Sur) versus rocky reef-associated taxa in the south (Dhofar). For example, the Indian oil sardine *Sardinella longiceps*, one of the indicator taxa apparent in our IndVal analysis, is abundant in the Sea of Oman (and elsewhere in the Indian Ocean) but observed less frequently further south

TABLE 2 Contribution of different environmental variables and anthropogenic pressures to ZOTU diversity based on seawater samples collected at 15 sites off the coast of Oman

		Environmental					Anthropogenic			
Assay	Statistic	SST 1%	SST mean	CHL sample	CHL mean	CHL mode	Hotels and resorts	Commercial ports	Cruise ports	Settlements
18Suni	SS(trace)	32,229	57,085	23,652	13,637	13,272	39,985	29,591	17,504	56,692
R^2 = 42.67%	Pseudo-F	9.368	17.715	6.727	3.784	3.68	11.856	8.543	4.904	17.574
	p	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
	Proportion	7.59%	13.45%	5.57%	3.21%	3.13%	9.42%	6.97%	4.12%	13.36%
16SFish	SS(trace)	25,312	36,685	14,947	7,702	12,133	18,835	24,003	11,557	38,981
R^2 = 35.05%	Pseudo-F	7.803	11.674	4.48	2.265	3.609	5.704	7.373	3.432	12.486
	p	0.001	0.001	0.001	0.0002	0.001	0.001		0.001	0.001
	Proportion	6.51%	9.44%	3.85%	1.98%	3.12%	4.85%	6.18%	2.97%	1.00%
CP1	SS(trace)	17,063	16,860	15,269	11,936	9,643	13,439	11,526	6,729	16,441
R^2 = 28.52%	Pseudo-F	4.551	4.494	4.044	3.119	2.497	3.533	3.007	1.723	4.375
	p	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.025	0.001
	Proportion	6.45%	6.37%	5.77%	4.51%	3.65%	5.08%	4.36%	2.54%	6.22%
CP2	SS(trace)	13,719	15,708	11,295	5,613	4,906	13,637	5,771	9,201	14,886
R^2 = 19.12%	Pseudo-F	5.324	6.168	4.322	2.079	1.81	5.289	2.139	3.478	5.817
	p	0.002	0.001	0.001	0.037	0.071	0.001	0.037	0.008	0.001
	Proportion	7.46%	8.55%	6.15%	3.05%	2.67%	7.42%	3.14%	5.00%	8.10%

Values estimated using Distance based Linear Models in Primer v 7. SST 1%: lowest 1% sea surface temperature; SST mean: mean sea surface temperature; CHL sample: chlorophyll a value at sampling location on sampling date; CHL mean: mean chlorophyll a value; CHL mode: mode chlorophyll a value. For reference, 18Suni targets 18S rRNA in most eukaryotes, 16SFish targets 16S rRNA in mostly bony fish, and CP1 and CP2 target ITS2 in basal metazoans such as corals and sponges.

along the Omani coastline. Conversely, the whitebarred rubberlip *Plectorhinchus playfairi*, another species identified in our IndVal analysis, is abundant in the south of Oman (and elsewhere in the Indo-West Pacific) but observed less frequently further north along the Omani coastline. Our result suggests that fish-specific eDNA assays, such as 16SFish, may prove useful to address broad-scale biogeographic hypotheses related to fish community assemblages, despite many coral-associated fish species going undetected or remaining taxonomically unresolved (Atta et al., 2019).

The ability to detect fishes using these sensitive approaches may be important in managing the significant demersal and pelagic fishery in Oman, which consists of more than 120,000 artisanal and industrial vessels (i.e., trawlers, dhows, and fiberglass boats) working across an extensive coastline (>3,000 km) and large exclusive economic zone (~300,000 km^2; Siddeek et al., 1999). Indeed, establishing monitoring programs at a scale broad enough to track marine fish populations is often considered a stumbling block to effective fisheries management (Pattengill-Semmens & Semmens, 2003). Although many high-value demersal fishery targets were represented in our eDNA detections, such as groupers (family Serranidae), snappers (family Lutjanidae), sea bream (family Sparidae), rabbitfish (family Siganidae), and emperors (family Lethrinidae), the most valued pelagic fish species ("kingfish", known as Spanish mackerel *Scomberomorus commerson* elsewhere) in Oman was notably absent. Given detections of other valued pelagic fishes in our study (e.g., family Carangidae) and detections of Spanish mackerel in other studies using the same 16SFish assay (e.g., West et al., 2021), we suggest that this absence may instead be related to PCR stochasticity due to low concentrations of species-specific eDNA in individual samples. Although only a fraction of the Omani ichthyofauna was detected in our study (Al-Jufaili et al., 2010; Fouda et al., 1998; Randall, 1995), the two most speciose fish families in Omani waters (Gobiidae with 69 species and Labridae with 59 species) were represented, and the eDNA fish community composition was sufficient to parse the northern from the southern region. Southern Oman endemic fish species such as the Oman anemonefish *Amphiprion omanensis*, the Dhofar parrotfish *Scarus zufar*, and the Dhofar cardinalfish *Apogon dhofar* may be nested within our eDNA detections, but we cannot confirm this owing to several genetic assignments at the genus rather than species level. That said, monitoring needs to be conducted at the population-scale to ensure that most demographic processes such as recruitment, mortality, immigration, and emigration are accounted for (Begg et al., 1999; Reiss et al., 2009). eDNA detections used to infer population-level fish information is currently possible but requires the development of species-specific primer sets (Sigsgaard et al., 2020), target capture approaches that enrich mitochondrial or nuclear DNA (Jensen et al., 2020), the use of complementary DNA sequence denoizing algorithms (Tsuji et al., 2020), or a means for validation of individual-level haplotypes (Dugal et al., 2021). It would thus be prudent to prioritize investment into the development of species-specific eDNA assays for high-value fishery species versus metabarcoding approaches that target multiple species for marine management in Oman.

4.2 | Aim 2 – eDNA detections for all other taxa

The biogeographic break between the north and south of Oman was also apparent based on eDNA detections from two coral and sponge assays (CP1 and CP2) and, to a greater degree, in a general eukaryotic assay (18Suni). We found shifts in the mean frequency of occurrence for all order-level detections using CP1 and CP2, with the northern regions of Musandam and Muscat dominated by stony corals (order Scleractinia), the one sandy reef site sampled in Sur dominated by sea anemones (order Actiniaria), soft corals (order Alcyonacea), and zoanthids (order Zoantharia) with stony corals absent, and the rocky reefs of the southern Dhofar region dominated by sea anemones and sponges (orders Clionaida and Suberitida) with some stony corals present. At the family level within the order Scleractinia, Musandam was dominated by detections in the family Poritidae and to a lesser extent Acroporidae (staghorn corals), Muscat was dominated by detections in the families Acroporidae, Lobophyliidae, and Poritidae, and Dhofar had detections from the families Lobophyliidae, Merulinidae, and Poritidae. This partitioning of families is consistent with known coral diversity in these different regions (Claereboudt, 2019; Salm, 1993), with some notable omissions. From a biogeographic perspective, the coral fauna in Oman is considered a complex mixture of regional endemic species (e.g., *Acanthastrea maxima*, *Porites decasepta*, *Porites fontanesii*, *Psammocora albopicta*), some species shared with the Arabian Gulf (e.g., *Acropora downingi*, *Porites harrisoni*), some species shared with the Red Sea (e.g., *Stylophora danae*), western Indian Ocean distributed species (e.g., *Anomastraea irregularis*, *Favites peresi*), and a significant proportion of species distributed throughout the Indo-West Pacific (e.g., *Pocillopora damicornis*, *Porites lutea*, *Platygyra daedalea*, *Stylophora pistillata*) (Claereboudt, 2019).

Porites is considered the dominant builder of framework reefs throughout Oman (Salm, 1993), and so it is not surprising that it was detected in a significant proportion of replicates and sites for three out of the four regions sampled in our study. The Musandam region is characterized by substantial reef development along its rocky slopes and hosts 41 coral genera, the Muscat region has 42 coral genera scattered in patches or dense assemblages, and Dhofar hosts 48 coral genera that displays extreme variability in live coral cover (0.5%–99%; Salm, 1993). Notably, we did not detect the family Dendrophylliidae (five genera known from both Musandam and Muscat, four genera known from Dhofar) nor the Faviidae (seven genera known from both Musandam and Muscat, 10 genera known from Dhofar). Our accumulation plots for both coral assays using ZOTU richness as a metric suggests that this family "drop out" effect may have been an artifact of sampling, given that further sampling is forecasted to uncover a greater diversity of scleractinian taxa in each region. Moreover, some of these coral species have previously been detected with these same assays in Australia (Alexander et al., 2020). We do note, however, that the two coral assays we used had different sensitivities where some families were detected by one assay but not the other, a result consistent with previous work focused on optimizing these primer sets (Alexander et al.,

2020). For example, stony corals from the family Agariciidae and the Oulastreidae were only detected using the CP1 assay. In contrast, stony corals from the family Fungiidae and the Siderastreidae were only detected using the CP2 assay. Thus, a combination of these two assays and greater sampling effort may provide the best opportunity for detection of coral diversity.

4.3 | Aims 3 and 4 – Environmental data and anthropogenic disturbances

The difference in fish, coral, and the remaining eukaryotic assemblage along this extensive coastline is often attributed to fixed variation in environmental and oceanographic conditions. For example, strong winds and currents in the Sea of Oman can cause irregular short, cold-water upwelling events in the summer. This contrasts with the Dhofar region, which experiences persistent upwelling conditions for up to 5 months during the SW summer monsoon winds. This upwelling of colder, highly productive waters causes rapid macroalgal (*Ecklonia*, *Nizamuddinia*, *Sargassum*, and *Ulva*) growth on all rocky coasts (Barratt et al., 1984; Claereboudt, 2019), which attract, feed, and host a unique suite of organisms. Despite this seasonal change in temperature and nutrients in the Dhofar region that aligns with the SW summer monsoon winds, dense coral communities remain year-round, which may explain why the predominately motile fish eDNA detections were more apparent when comparing between regions versus the coral eDNA detections.

Estimating 10-year averages of remotely sensed SST and CHL data revealed two highly distinct clusters corresponding to the northern and southern regions. DistLM analyses further highlighted that SST and CHL may be important drivers of the observed differences in eDNA community assemblages between the regions. These shifts in communities may reflect persistent differences versus the transient differences associated with the SW summer monsoon winds, however, we indirectly control for seasonal effects by sampling all sites between November and January. Indeed, three of our four sampling regions, including the two most geographically distant ones (Dhofar and Musandam), were sampled over a 7-day period. That said, the amount of variation in communities explained by these metrics was dependent on the genetic assay used. 18Suni eDNA detections demonstrated the greatest separation between sampling regions, although this came at a cost of fewer taxonomic assignments at the species, genus, and even the family level. Moreover, some of the variation may be explained by the different habitats sampled (e.g., coral reefs, mangroves, sandy beaches, rocky reefs, and an intertidal rock pool), which we discuss in more detail below. For example, previous studies have shown that community assemblages detected with seawater eDNA metabarcoding often vary with the sampled habitat and can even be different at the scale of tens of meters (e.g., Bakker et al., 2019; Nguyen et al., 2020).

The coastal development along the northern Omani shoreline (Musandam to Sur), particularly construction "megaprojects", pose one of the greatest risks to local coral ecosystems (Al-Jufaili et al.,1999; Mansour et al., 2017), but then so do overfishing, dive boat anchoring, and increasing natural threats in the form of tropical cyclones (Burt et al., 2016; Salm, 1993). Globally, the Sea of Oman is considered one of the most important arteries for exporting oil and gas (Mansour, 2020), which puts the coral reefs here at greatest risk to oil spills (Coles & Al-Riyami, 1996). Based on our results, distance to settlements had the largest effect on the eukaryotic marine community assemblage detected with eDNA, although the explained variation was again dependent on the genetic assay used.

4.4 | Methodological caveats

Due to the sensitivity of the eDNA approach, there are some caveats to consider when interpreting data of this kind. The primary caveat is that the molecular assignments can only be as good as the DNA reference database on which they rely for species identification (Ip et al., 2021; Leray et al., 2019). In general, rare, regional endemic taxa are less well-represented than more common and widespread taxa, with marginal or insular seas often the least represented in DNA reference databases (Ip et al., 2021; Leray et al., 2019). In our case, this deficiency in 16S reference sequences from Omani fishes available on GenBank may have led to some erroneous taxonomic assignments to closely related taxa that were better represented. For example, *Gymnothorax fimbriatus* (ZOTU56) was detected in one seawater replicate from Dhofar and two replicates from Musandam. Given that this species is known only from the Seychelles, Madagascar, and Mascarenes east to the Mariana Islands, this may represent a new record of this species in the north-western Indian Ocean, but more likely its more common congeners lacked 16S reference sequences on GenBank. Similarly, *Chaetodon selene*, a western Pacific butterflyfish species, was detected in two seawater replicates from Dhofar and three replicates from Muscat, but its two closest relatives previously recorded from Oman (*Chaetodon gardineri* and *Chaetodon leucopleura*) lacked 16S reference sequences on GenBank. Another important caveat is that sequence length can also influence taxonomic resolution irrespective of the completeness of the DNA reference database (Coissac et al., 2012; Zhang et al., 2020), with 16SFish ranging between 138 bp and 227 bp, which may not be sufficient for species-level assignments within some genera or families. The remaining marine eukaryotic fraction is even more troublesome in terms of their cryptic nature, a lack of reference sequences, lower resolution of the 18S marker applied here based on sequence length and variability, and the uncertain taxonomy of entire groups (Bucklin et al., 2016; Leray & Knowlton, 2016). Coral taxonomy is particularly unstable in that regard given frequent species, genus, and even family name reclassifications (Quattrini et al., 2020). While some of our coral eDNA detections may represent new records for Oman, these would require further confirmation. Additional assigned taxa not known from waters surrounding Oman are highlighted in the Supporting Information.

5 | CONCLUSIONS

Overall, our study suggests that eDNA sequencing may serve as a powerful tool to detect community differences across broad geographic scales, particularly in places where there is significant variation in habitat, oceanographic conditions, or anthropogenic pressures. Moving forward, the improvement of regional DNA reference databases through directed collection initiatives, international collaborations, or increased public access to voucher sequences will provide more robust taxonomic assignments. With this improvement, sensitive sequencing tools can help to overcome the challenges associated with working in regions where habitats are patchy, poor visibility prevents in situ surveys, and diving logistics are prohibitive, particularly when rapid presence/absence information is essential. By reducing the need for taxonomic specialists to lead sample collection initiatives, this tool lends itself to a broader cache of stakeholders.

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CONFLICT OF INTEREST

All co-authors confirm no conflict of interest.

AUTHOR CONTRIBUTIONS

JDD and AM conceptualized the study and were involved in project administration. JDD, MB, and AM designed the methodology. JDD, MAP, MD, DJC, THS, AH, MLB, CHRG, and AM performed sampling. JDD, MD, GB, and SM were involved in formal analysis. JDD was involved in data curation and writing – original draft preparation. All co-authors were involved in writing – review and editing. JDD, MD, GB, SM, and AM visualized the study. JDD, AH, MB, MLB, and AM were involved in funding acquisition. All authors have read and agreed to the published version of the manuscript.

DATA AVAILABILITY STATEMENT

All raw sequencing data needed to replicate the study are available from Dryad Digital Repository https://doi.org/10.5061/dryad.tdz08kpxx.

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