



REPORT

Distinct coral reef habitat communities characterized by environmental DNA metabarcoding

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Abstract Coral reefs are biodiversity hotspots, places of high endemism and provide essential services to billions of people globally. With increasing threats to these reefs worldwide, there is a need to implement faster, more efficient ways to monitor spatial and temporal patterns of biodiversity. Environmental DNA (eDNA) metabarcoding offers a promising tool to address this issue, as it has revolutionized our ability to monitor biodiversity from complex environmental samples such as seawater. However, the capacity for eDNA to resolve fine scale shifts in community composition across habitats in seascapes is yet to be fully explored. Here, we applied eDNA metabarcoding using the rRNA 18S Universal eukaryote assay to explore differences in community profiles between samples collected from the lagoon and reef slope habitats across more than 170 km of the Ningaloo Coast World Heritage Area in Western Australia. We recovered 2061 amplicon sequence variants that comprised of 401 taxa spanning 14 different metazoan phyla such as cnidarians, poriferans, molluscs, algae, worms, and echinoderms. Our results revealed strong clustering of samples by habitat type across the length of the reef. Community dissimilarity (beta diversity) between samples collected from the reef slope and lagoon habitats was high and was driven largely by a strong rate of spatial turnover, indicating a distinct set

of taxa representing each reef zone community. We also detected a strong pattern of isolation by distance within our slope samples, suggesting that communities are spatially stratified across the length of the reef. Despite high connectivity due to regular flushing of the lagoon environment, our results demonstrate that metabarcoding of seawater eDNA from different habitats can resolve fine scale community structure. By generating multi-trophic biodiversity data, our study also provided baseline data for Ningaloo from which future changes can be assessed.

Keywords Biodiversity · Coral reefs · Metazoan · Environmental DNA · Metabarcoding · Biomonitoring

Introduction

Coral reefs are experiencing increasingly frequent, and larger scale impacts due to a range of direct and indirect human activities (Hughes et al. 2018). Notably, marine heat-wave events induced by global warming have caused extensive coral bleaching and mass mortality across most tropical waters (Baker et al. 2008; Hughes et al. 2018). These widespread declines in both coral cover and diversity (Moore et al. 2012; Descombes et al. 2015) have been mirrored by declines in the diversity and abundance of associated biota such as reef fishes (Jones et al. 2004; Pratchett et al. 2011). The biodiversity value of coral reefs is profound as they support an estimated one million species globally, hence declines in coral cover and diversity threaten the health of coastal habitats and the species they sustain (Jones et al. 2004; Knowlton et al. 2010; Descombes et al. 2015). Corals and their holobiont form the calcium carbonate framework of reefs and fill significant roles related to the biogeochemical cycling of littoral zones (Rädecker et al. 2015; Gong

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et al. 2020). The species composition of corals drives key ecosystem function on reefs, and stressors that alter these configurations threaten the functioning and stability of such ecosystems (Richardson et al. 2020). Consequently, there is an urgent need for documenting baselines of biodiversity that can be used to assess the extent and severity of anthropogenic impacts, provide recovery targets, and increase reef resilience. Such baselines would ideally provide comprehensive data on all components of the reef community across trophic levels, rather than focussing on indicator or keystone species.

Traditional methods of coral reef monitoring (e.g. manta tows, underwater visual and photo benthic surveys, Automated Underwater Vehicles (AUVs), towed video systems, Baited Remote Underwater Stereo-Video Systems (BRUVS)) typically only focus on targeted components of reef communities and are generally time, cost, and labour intensive (Stat et al. 2017). The inconspicuous reef cryptobiome largely made up of small cryptic invertebrates, as well as nocturnal species, are underrepresented in traditional surveys due to difficulties of detection (Pearman et al. 2016; Carvalho et al. 2019). As a result, such monitoring techniques rarely survey holistically across all trophic levels, particularly when surveying large areas of reefs.

Environmental DNA (eDNA) metabarcoding, which enables multispecies detection from an environmental sample such as seawater, may promise greater resolution for whole-ecosystem surveying by increasing detectability of the biosphere that can go unnoticed using traditional methods (Stat et al. 2017; West et al. 2021). This method has become a rapid assessment tool for biodiversity, able to provide broad coverage across taxa. Although a multi-assay survey has been highlighted as the optimal approach (coined “Tree of Life”) to generate the highest taxonomic resolution, it significantly increases costs and time required, as well as technical complexity (Stat et al. 2017; Eble et al. 2020). As such, a rapid universal metabarcoding survey (RUMS; DiBattista et al. 2019) that employs a single broad-coverage assay allows managers to efficiently scale up monitoring to include a much wider range of taxa. Environmental DNA RUMS have been used to investigate shifts in community structure across a depth gradient on a coral reef (DiBattista et al. 2019), between Caribbean islands (Bakker et al. 2019), tropical habitats (Nguyen et al. 2020) or MPA zones (Gelis et al. 2021); however, there are limited applications of the technique to explore finer scale differences in community structure across reef habitat zones (West et al. 2021).

The World Heritage Ningaloo Coast stretches 280 km along the Western Australian coastline and is one of the world’s largest fringing coral reef systems. It is composed of a series of fringing reefs connecting shallow lagoons to the reef flat and outer reef slope broken by with reef channels that allow water flow out of the lagoon (Vanderklift

et al. 2020). Ningaloo Reef is thought to be less threatened than the Great Barrier Reef due to limited historical commercial fishing activity, minimal nutrient run-off, and low levels of human coastal development (Cassata and Collins 2008; Johansson et al. 2010). However, the reef has nonetheless experienced a recent increase in frequency of coral bleaching (Moore et al. 2012; Depczynski et al. 2013; Gilmour et al. 2019). Thermal anomalies such as marine heatwaves are predicted to increase in frequency and severity (Ateweberhan et al. 2013; Oliver et al. 2018) and will likely impact Ningaloo in the near future (Babcock et al. 2021). Therefore, a large-scale eDNA biodiversity assessment can provide important baseline data, with assessments of disturbance-related shifts in fine scale community structure that can be used to inform adaptive management actions. Here, we applied a RUMS approach using seawater eDNA to characterize the marine metazoan (and protozoan macroalgae) biodiversity along Ningaloo. We also explored the power of eDNA metabarcoding to resolve differences in community profiles between the sheltered lagoon and exposed reef slope environments. Our results contribute to growing evidence of the power of eDNA metabarcoding in teasing apart habitat-specific localized signals and its ability in generating comprehensive profiles of biodiversity.

Materials and methods

Field sampling

In May 2019, a total of 120 seawater samples were collected at 14 different localities along Ningaloo Reef, spanning approximately 172 km and 2 degrees of latitude along the Western Australian coastline (Fig. 1). We collected near-surface (1–2 m depth) seawater samples from two distinct habitats along the reef; the backreef lagoon and the exposed reef slope. For shallow lagoon samples, five replicates of 1 L samples were collected in Nalgene bottles by snorkellers (site depth < 6 m) from three different locations at five different sites across the reef ($n = 75$ samples total, 15 samples per lagoon site). The five replicate samples were collected at a single location within a 5 m radius of each other and at each site the substrate was categorized as intermittent coral bommies and structures separated by sand patches with moderate coral cover. The water bottles were kept on ice in a cooler during sampling and stored at ~ 4 °C until filtering. On the reef slope, five replicate samples were collected using a livewire CTD Niskin rosette system, repeated at nine sites approximately ~ 20 km (10 nautical miles) apart along the western edge of the outer reef at a site depth range of 11 to 22 m ($n = 45$ samples total) (Fig. 1). For each replicate sample collected via CTD Niskin rosettes, we used a small volume of the sample to thoroughly rinse out the individual

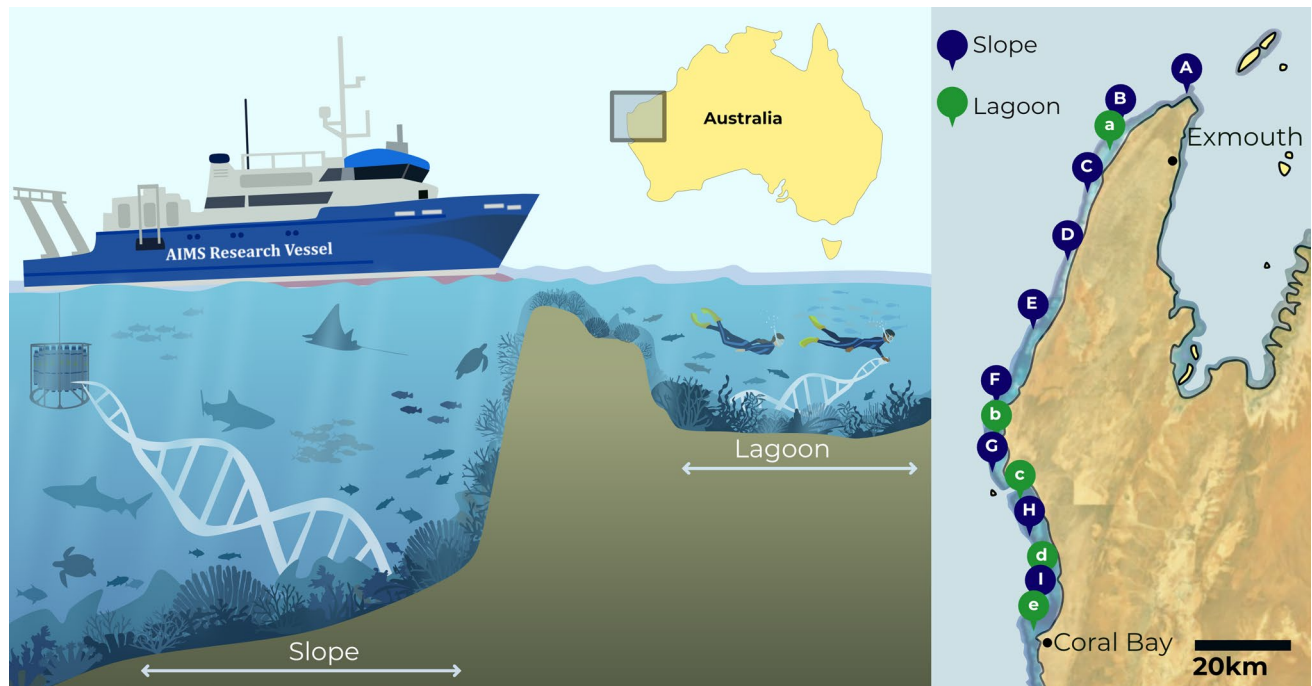


Fig. 1 Map of sampling locations and visual depiction of the sampling methodology for the lagoon and slope habitats. Slope sites are A: NW Cape, B: Tantabiddi, C: Milyering, D: Osprey, E: Widera-

bandi, F: Point Edgar, G: Point Cloates, H: Bateman Bay and I: Bru-boodjoo. Lagoon sites are a: Tantabiddi, b: Norwegian Bay, c: Point Cloates, d: Stanley Pool and e: Coral Bay (Table S1)

Nalgene bottles prior to transferring the sample into the bottle and storing at ~ 4 °C until filtering. To track contamination, five tap water controls were also taken by collecting a 1 L sample using the sample bottles post-bleach rinse bucket filled with freshwater originating from the vessel's desalination system. All samples were filtered using sterile single-use 250 ml funnel EZ-Fit filtration units with $0.45 \mu\text{m}$ mixed cellulose ester membranes and an EZ-Fit Manifold base (3-place) from Merck Millipore (Merck Group, Burlington, USA). Gloves were changed and the manifold base was sterilized with a 10% bleach solution between the filtering of samples from different sites. Following filtration, the filter papers were rolled and kept in cryogenic tubes at -20 °C until transport and storage at -80 °C. All subsequent laboratory work was conducted at the Trace and Environmental DNA (TrEnD) laboratory at Curtin University, Australia.

Sample processing and sequencing

DNA was extracted from half of the filter membranes using the Qiagen DNeasy Blood & Tissue kit with the following modification to the protocol: $360 \mu\text{L}$ of ATL buffer and $40 \mu\text{L}$ of proteinase K for an overnight incubation at 56 °C (Qiagen, Hilden, Germany). Negative extraction controls were included to check for cross contamination. The resulting supernatant was loaded into the QIAcube DNA extraction robot for automated DNA extraction (Qiagen).

Quantitative PCR (qPCR) employed the nuclear rRNA 18S Universal eukaryote assay (V1-3 hypervariable region; 18S_uni_1F: 5' – GCCAGTAGTCATATGCTTGTCT - 3' and 18S_uni_400R: 5' – GCCTGCTGCCTTCCTT - 3'; Pochon et al. 2013). Quantitative PCR amplification was performed on duplicates of each sample (seawater, extraction, negative (no template) and positive controls) and using fusion tagged primers; unique sample-specific tag combinations (6–8 bp) that allow separation of reads post-sequencing. Each qPCR reaction was made up to $25 \mu\text{L}$ containing: 1X AmpliTaq Gold PCR buffer (Life Technologies), 2mM MgCl_2 (Fisher Biotec, Wembley, Australia), $0.4 \mu\text{M}$ dNTPs (Astral Scientific, Caringbah, Australia), 0.1mg BSA (Fisher Biotec), $0.6 \mu\text{L}$ of 5X SYBR Green (Life Technologies), 1U AmpliTaq Gold DNA Polymerase (Life Technologies), $0.4 \mu\text{M}$ of forward and reverse fusion tags, $2 \mu\text{L}$ of eDNA template (at optimized dilution) and UltraPure Distilled Water (Life Technologies, Carlsbad, USA). Thermocycler conditions were as follows: initial denaturation at 95 °C for 5 min followed by 45 cycles of 95 °C for 30 s, 52 °C for 30 s, 72 °C for 45 s, 95 °C for 15 s, 60 °C for 1 min, 95 °C for 15 s and a final extension of 72 °C for 10 min. All pre-PCR and post-PCR steps were performed in separate laboratories using UV-sterilized cabinets at TrEnD laboratory, Curtin University. Amplicons were pooled to approximate equimolar ratios into Mini Pools (MPs) based on the samples qPCR ΔRn values, after which size distribution and DNA concentration

were verified using the QIAxcel Advanced System (Qia-gen). Based on the QIAxcel results, the MPs were blended into a final equimolar library, which was size selected to a size range of 175–600 bp using Pippin Prep (Sage Science, Beverly, USA). The size-selected library was then purified using the QIAquick PCR purification kit (Qiagen), before fluorometric quantitation using Qubit 4.0 (Thermo Fisher Scientific). The final library (2 nM) was sequenced on a MiSeq 500-cycle V2 chemistry paired-end run (Illumina, San Diego, USA) in a post-PCR laboratory at TrEnD, Curtin University, Australia.

Bioinformatics and data analyses

We used the Greenfield Hybrid Analysis Pipeline (GHAP) to demultiplex raw reads based on the unique sample-specific tags assigned during qPCR using the DemultiplexCustomBC function (Greenfield 2017) with only exact matches retained for further processing. Reads were quality filtered and clustered into ASVs using DADA2, which removes the arbitrary sequence similarity threshold component of OTU-based clustering (Callahan et al. 2016). The ASVs were then curated using the LULU algorithm with default parameters (Frøslev et al. 2017). The resulting ASVs were queried against NCBI's GenBank nucleotide databases (downloaded on 28/07/2021) using BLASTn on the KAYA HPC housed at the University of Western Australia. Taxonomic assignments were processed using the lowest common ancestor (LCA) step of the eDNAFlow pipeline (Mousavi-Derazmahalleh et al. 2021). All taxonomic assignments required a 100% query coverage and over 98% identity match to a reference sequence for a species-level assignment to be made. Genus-level assignments required a 97% identity match, family-level a 95% match, and order-level a 90% match. Taxonomic hits that were detected in the field tapwater and/or laboratory controls were deemed to be potential contaminants and were excluded from the datasets (Table S2).

All analyses used R Studio (v3.5.3) and figures were made using the *ggplot2* R package (Wickham 2016). All analyses used a binary-transformed (presence/absence) matrix, except for the ANCOM-BC analysis which used the raw eDNA counts matrix. We used the metaMDS function of the *vegan* R package to perform nonmetric multidimensional scaling (nMDS) plots using the Jaccard binary dissimilarity index (Oksanen et al. 2020). We used the *adonis* function from the *vegan* R package to conduct a permutational multivariate analysis of variance (PERMANOVA) of Jaccard dissimilarities using 9999 permutations (Oksanen et al. 2020). Homogeneity of variance was then tested using the *betadisper* and *permutest* functions to verify the effect of dispersion. To explore latitudinal patterns in our dataset, we focussed on a single habitat, the reef slope, and performed a Mantel test (*ade4* R package; Dray and Dufour 2007) to test

the relationship between community composition and the geographic distance between samples along the length of the reef. We also categorized the nine slope sites into Northern, Middle, and Southern sections, consisting of three sites each. To understand the differences in the eDNA communities recovered across the reef, we calculated the contributions of turnover (β_{ju}) and nestedness (β_{jnc}) components to the pairwise Jaccard's dissimilarity index (β_{jac}) using the *betapart* R package (Baselga & Orme 2012). A β_{jac} index (beta diversity) of 0 indicates that species composition is identical between groups, whereas an index value of 1 indicates complete dissimilarity. The turnover component indicates species replacement between sites or groups, whereas nestedness indicates that the extent to which a group is a subset of another (Baselga and Orme 2012). To assess whether our unbalanced sampling effort produced a bias, we randomly selected five sites per reef zones to set an equal sampling effort of 25 samples per habitat and recomputed our diversity analyses a total of 50 times. The relative proportion of each phylum was calculated by adding up all ASVs (taxa) corresponding to the different phyla in relation to the total ASVs (taxa) recovered in each site. To detect differentially abundant taxa across habitats, we performed an Analysis of Compositions of Microbiomes with Bias Correction (ANCOM-BC) which accounts for the compositional nature of eDNA metabarcoding data (Mandal et al. 2015). We used the *ancombc* R package (Lin & Peddada 2020) to test for differences in abundance of metazoan taxa with a significance of $p < 0.05$ adjusted with the default Bonferroni correction. We evaluated how comprehensively eDNA surveyed the communities using species accumulation curves from the *iNEXT* package (Chao et al. 2014; Hsieh et al. 2020) and estimated the total richness of the system using the Chao2 estimator in *SpadeR* (Chao and Chiu 2014).

Results

Sequencing results and overall diversity

Metabarcoding of the rRNA 18S Universal eukaryote assay yielded 7,072,787 demultiplexed sequences. A total of 4,177,383 sequences remained after quality control and error filtering with the DADA2 pipeline. The average number of reads passing filters was 25,317 ($\pm 11,693$) per sample. The DADA2 pipeline generated 7751 ASVs, which were filtered down to 2778 ASVs by LULU. We removed ASVs with less than five total reads, resulting in a final dataset of 2061 ASVs. Following BLAST and LCA, a total of 603 eukaryotic ASVs were taxonomically identified (Fig. 2), representing approximately 29% of all ASVs recovered. Approximately one third of eukaryotic sequences were assigned to the kingdoms Protozoa and Chromista, including

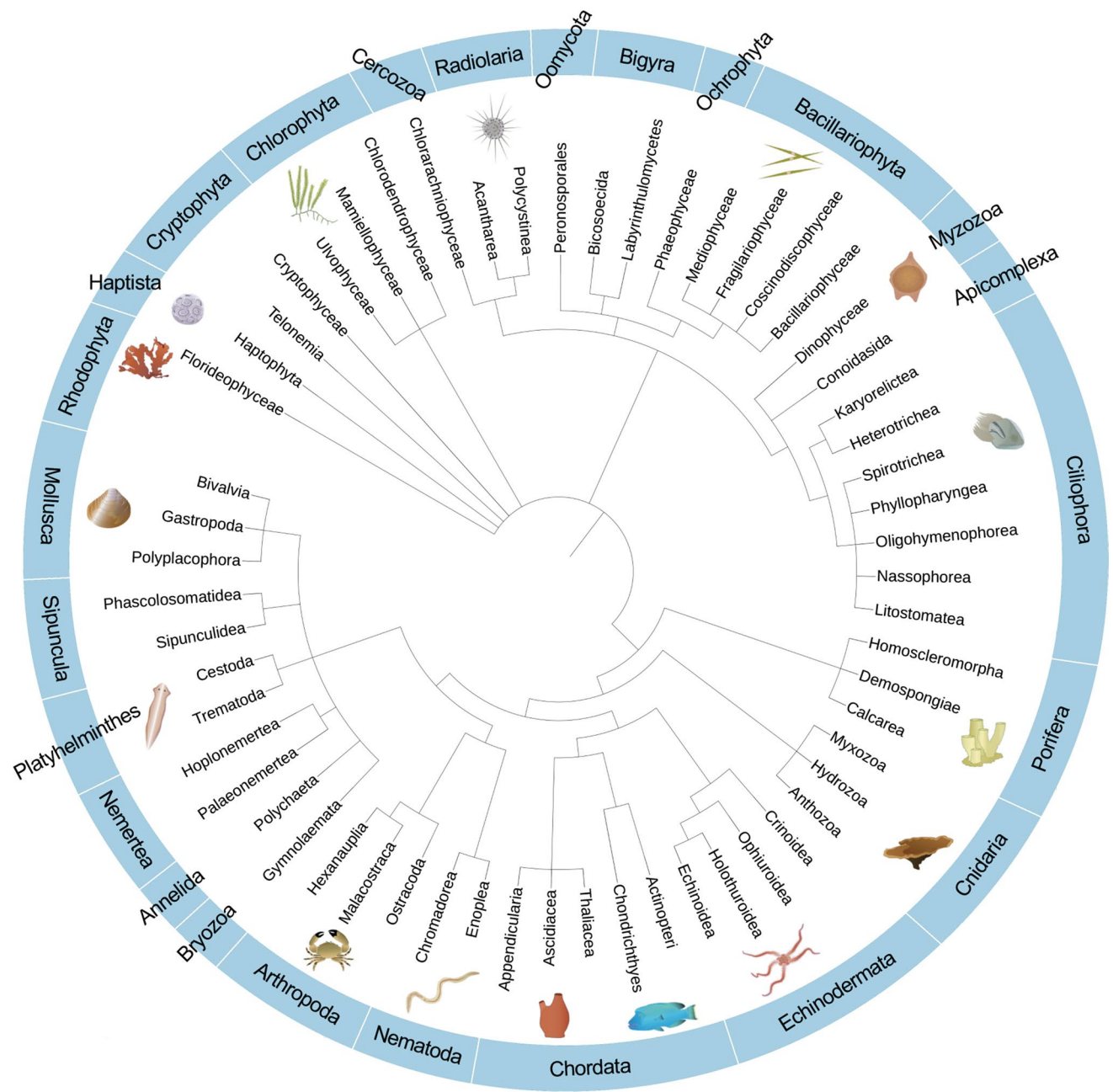


Fig. 2 Class-level dendrogram of all eukaryotic detections (603 taxa) recovered in the seawater samples at Ningaloo Reef

Bacillariophyta (diatoms), Apicomplexa, Radiolaria, Ciliophora (Fig. 2). However, for this study, we focused our analyses on metazoan taxa with the addition of red (Rhodophyta) and green (Chlorophyta) algae. The final taxonomic dataset included 401 taxa (ASVs) spanning 14 phyla, 35 classes, 84 orders, 112 families, 109 genera and 57 species detected in 118 seawater samples (two samples returned no taxonomic assignment).

Across all samples, Arthropoda was the most represented group constituting 16.7% of all taxa identified.

This was followed by Porifera (14.2%), Cnidaria (13%), Annelida (13%) and Mollusca (13%). Sequences belonging to Chordata (10.5%) were mostly derived from ascidian tunicates, whereas very few were assigned to fishes. Within Cnidaria, the reef-building coral genus *Acropora*, which is the most common genus of coral at Ningaloo, was widely detected across the reef. Some potential contaminants were detected in the field controls as well as across some of our seawater samples (12 metazoan ASVs). These hits were excluded from the analyses as they may

have originated from the ship's desalinated and UV-treated water which was used to rinse out the sampling bottles after the bleach sterilization step (Table S2).

Diversity signals across the reefscape

Levels of metazoan diversity in seawater samples collected from the lagoon were consistently greater than in samples collected from adjacent reef slope habitats. Lagoon alpha diversity was higher for both total ASVs and number of metazoan taxa identified (Fig. 3a, d). A total of 1683 ASVs were recovered in the lagoon, compared to 898 ASVs in the slope samples. A total of 520 ASVs (~25.2%) were shared between both habitats; 1163 ASVs were exclusively recovered in the lagoon, whereas 378 ASVs were only recovered on the slope (Fig. 3b). Metazoan alpha diversity was also higher in the lagoon (355 taxa) than on the slope (118 taxa). Of the 401 taxa detected, only 18% (72) were shared between reef zones. The remaining taxa had a unique signal corresponding to a specific habitat type, with 283 taxa only occurring in the lagoon and 46 taxa only found in the slope (Fig. 3e). As a result of this limited overlap, the level of eDNA assemblage dissimilarity between habitats was high (β -diversity=0.8205), and taxonomic compositional

differences were primarily due to a high rate of spatial turnover (0.5610), rather than nestedness (0.2595), indicating a pattern of species replacement when moving between habitats. Habitat-specific eDNA communities were apparent and clustering of samples based on reef zone was evident in the nMDS plots (of Jaccard dissimilarities) using both datasets (Fig. 3c, f). This was confirmed with the PERMANOVA which suggested significant differences in the eDNA communities detected among habitat types (ASV: $R^2=0.0988$, $p<0.001$, Taxa: $R^2=0.1167$, $p<0.001$). However, there was significant heterogeneity in dispersion (ASV: Pseudo- $F=22.649$, $p=0.001$, Taxa: Pseudo- $F=16.574$, $p=0.001$) which could impair the PERMANOVA results in an unbalanced design. While the PERMANOVA results should be interpreted with caution, the nMDS ordinations indicate strong divergence among eDNA communities across reef zones (Fig. 3c, f).

In addition to differences in eDNA communities across habitat types, we also detected a significant influence of geographic distance on community composition. The Mantel tests between the ecological (binary) and geographic (km) distances among samples revealed a significant positive correlation for both datasets (ASV: $r=0.3987$, $p=0.001$, Taxa: $r=0.3419$, $p=0.001$) (Fig. 4). Furthermore, PERMANOVA

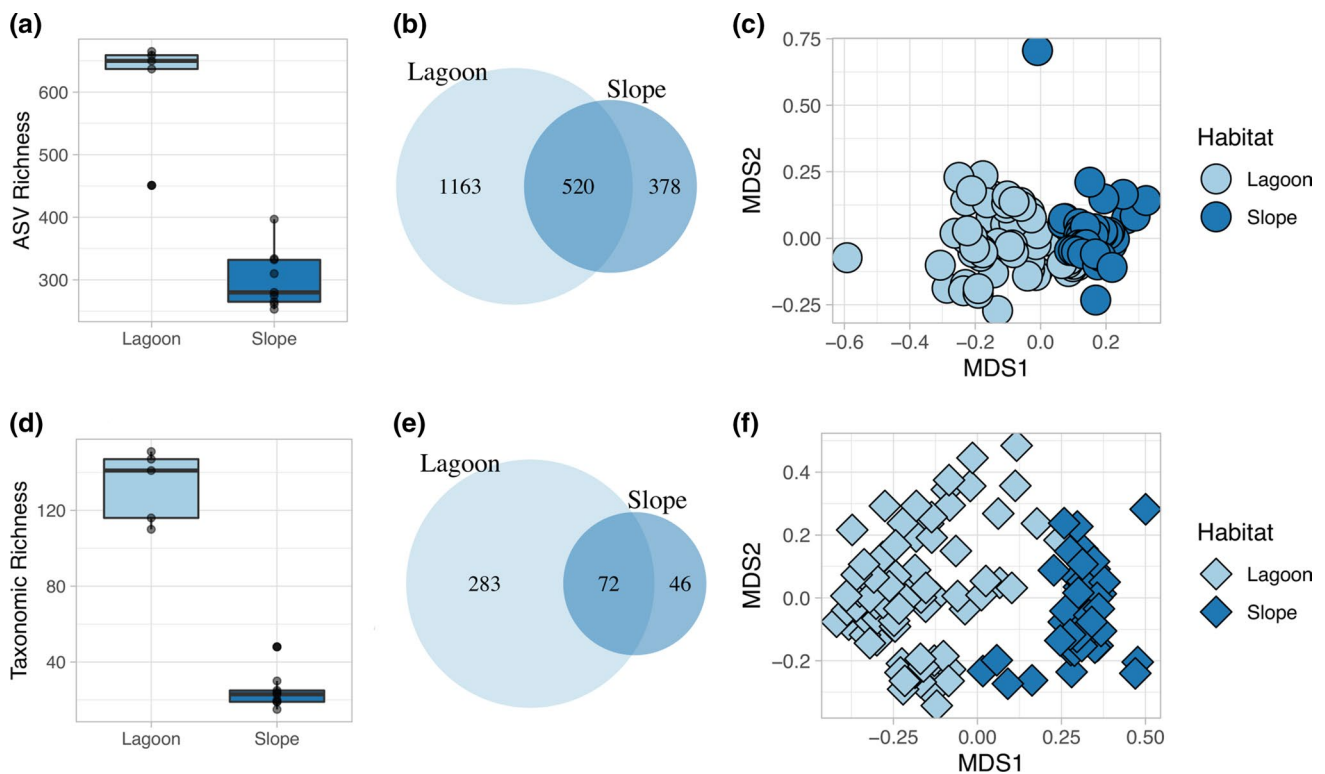
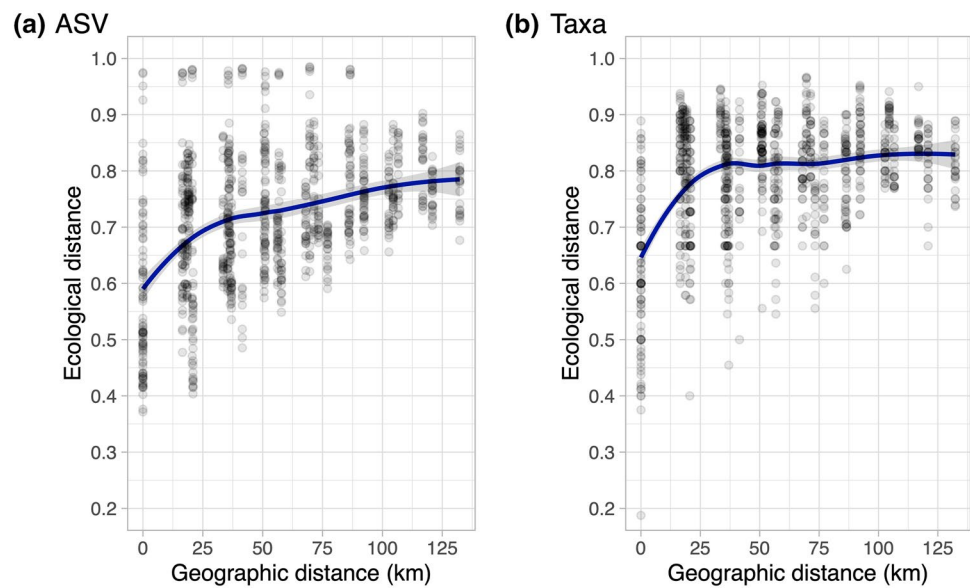


Fig. 3 Boxplots of alpha diversity between habitats for the number of **a** ASVs and **d** metazoan taxa recovered. Venn diagrams showing the unique and shared **b** ASVs and **e** metazoan taxa recovered across habitats. Nonmetric multidimensional scaling (nMDS) of Jaccard dis-

similarities with samples colour-coded by habitat type for the **c** ASV dataset and the **f** taxonomic dataset. Ordination stress values were 0.1426 and **f** 0.2088

Fig. 4 Results of the Mantel test showing a significant positive correlation between ecological distance (binary) plotted against geographic distance (km) with regression lines for the **A** ASV dataset and **B** metazoan taxonomic dataset. (ASV: $r=0.3987$, $p=0.001$, Taxa: $r=0.3419$, $p=0.001$)



tests revealed significant differences in assemblages between the Northern, Middle, and Southern areas of the Ningaloo reef (ASV: $R=0.1657$, $p<0.001$, Taxa: $R=0.1418$, $p<0.001$). We did not find any evidence for heterogeneity of dispersion (ASV: Pseudo- $F=1.2301$, $p=0.308$, Taxa: Pseudo- $F=0.8896$, $p=0.415$). Levels of alpha diversity were similar across the reef slope samples collected from the Northern, Middle, and Southern sections of Ningaloo, with 497 ASVs (56 taxa), 507 ASVs (41 taxa) and 533 ASVs (64 taxa) recovered, respectively. Latitudinal metazoan community dissimilarity was high (β -diversity=0.8314) and was almost entirely driven by spatial turnover (0.7943), indicating a pattern of species replacement when moving along the length of the reef.

Community composition between reef habitats

The relative proportion of each phylum differed substantially in samples collected from the two reef zones (Fig. 5a), and there were consistent sets of organisms that were more frequently detected in each habitat. For example, annelid worms, molluscs, and cnidarians were well represented in the lagoon samples, and the phyla Sipuncula, Rhodophyta, Nematoda and Bryozoa were unique to the lagoon zone. In contrast, green algae, arthropods, and chordates were more frequently detected on the reef slope. Taxa such as echinoderms and other worms (Nemertea and Platyhelminthes) were detected in low numbers regardless of habitat type. Interestingly, reef slope sites showed wide variation in community composition among sites (Fig. 5a). This pattern seemed less evident in the lagoon samples, where the relative proportions of phyla were more constant across replicates and sites (Fig. 5a). We also found that at the taxonomic

class level, only 51% (18) of classes were shared between habitats, with 15 classes found only in the lagoon samples (Fig. 5b). A total of 33 out of the 35 classes were found in the lagoon, whereas only 20 classes were found in the slope zone. This indicates that despite high levels of broadly distributed taxa, we also recovered localized eDNA signals associated solely in the lagoon samples. Overall, looking at the family level, 40.5% of all taxonomic families ($n=37$) detected in the reef slope samples could be categorized as pelagic or free-swimming in nature, whereas 59.5% were benthic-associated organisms. In contrast, the taxonomic families ($n=102$) detected in the lagoon samples were primarily (67.6%) benthic associated, whereas only 28.4% were categorized as pelagic, and 3.9% were parasitic organisms.

Key indicator taxa

Of the 401 total ASVs, 20 metazoan taxa were identified by ANCOM-BC as differentially abundant across reef zones (Fig. 6). The majority of these taxa (70%) were found to be more abundant in the lagoon samples, and these consisted primarily of habitat-forming benthic organisms such as sponges (order Haplosclerida), corals (orders Scleractinia and Anthoathecata), ascidians (order Stolidobranchia) and red algae (order Corallinales). Additional taxa also found to be of higher abundance included bivalves (order Mytiloidea and Ostreida), polychaetes (order Terebellida) and copepods (order Decapoda). Inversely, there were six taxa that were more abundant in the slope samples, and these consisted of copepods (order Calanoida), free swimming tunicates (order Copelata) as well as sponges (order Clionaida) (Table S3).

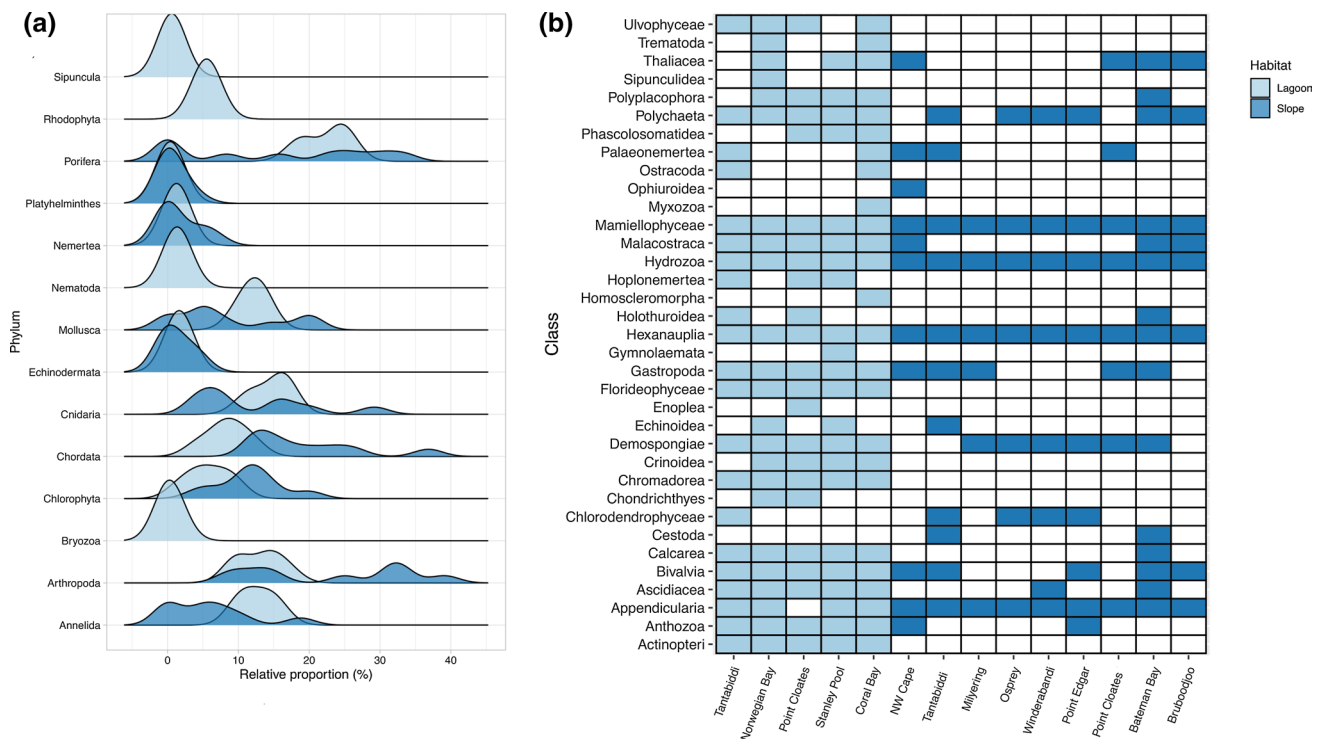


Fig. 5 **a** Ridgeline plot showing the relative proportion of the 14 phyla recovered in the two habitats sampled. The relative proportion of each phylum was calculated by adding up all ASVs (taxa) corresponding to the different phyla in relation to the total ASVs (taxa)

recovered in each site. **b** Heatmap of the presence and absence (P/A) of metazoan classes recovered with seawater eDNA. Classes are ordered alphabetically, and P/A was calculated at the site level

Sampling effort

Despite uncovering high biodiversity in our samples, our sampling effort did not completely capture the eukaryotic marine biodiversity of the Ningaloo region. ASV accumulation curves showed no plateauing of diversity across all sites, as well as within the reef slope and lagoon habitats, with estimates indicating more than 600 samples would be required to adequately characterise biodiversity (Fig. 7a). We captured approximately 46.6% of the total 4420 ASVs (SE 4010–4915) estimated by the Chao-2 index. Sample coverage curves across the lagoon sites indicated that only Stanley Pool approached sampling saturation (91.5%), although the sample coverage of the remaining sites was quite high (ranging from 82.1 to 85.8%), indicating near-sufficient sampling (Fig. 7b, Table S4). However, sample coverage across the slope sites was lower, ranging from 63.6% at Osprey to 87.6% at Point Edgar (Fig. 7c, Table S4). Our data suggest that to consistently reach over 80% in sample coverage, a minimum of 10 replicate 1 L samples need to be collected at each location to comprehensively census the gamma diversity of Ningaloo Reef.

Randomly selecting five sites per reef zone, resulting in an equal sampling effort of 25 samples per reef zone, we recomputed our analyses 50 times and found that patterns

of diversity and community composition were consistent (Table S5). Levels of metazoan diversity in seawater samples collected from the lagoon remained consistently greater than in samples collected from adjacent reef slope habitats. On average, 267 taxa were detected across the 10 sites, with 222 taxa in the lagoon and 80 taxa in the slope. Only approximately 13.2% ($\pm 3.1\%$) were shared across reef habitats. As a result of this limited overlap, the level of eDNA assemblage dissimilarity between habitats remained high (β -diversity $\sim 0.8680 \pm 0.0306$), and taxonomic compositional differences were always primarily due to a high rate of spatial turnover ($\sim 0.7184 \pm 0.0448$) rather than nestedness ($\sim 0.1492 \pm 0.0231$).

Discussion

We used a Rapid Universal Metabarcoding Survey (RUMS) approach targeting the 18S gene region of marine eukaryotes to catalogue a total of 401 metazoan taxa spanning 14 phyla. Seawater eDNA signals were heterogeneous across two distinct reef habitat zones and revealed pronounced differences in the community profiles of reef habitats that can be periodically connected by tidal and other water currents. This study adds to growing evidence of the utility of eDNA

Fig. 6 Analysis of composition of microbiomes with bias correction (ANCOM-BC) identified 20 taxa that were differentially abundant across reef zones. Figure shows effect size (log fold change) and 95% confidence intervals, Bonferoni adjusted. Negative log fold change values indicate an increase in abundance in the Lagoon (pale blue), whereas positive log fold change values indicate an increase in abundance in the Slope (dark blue)

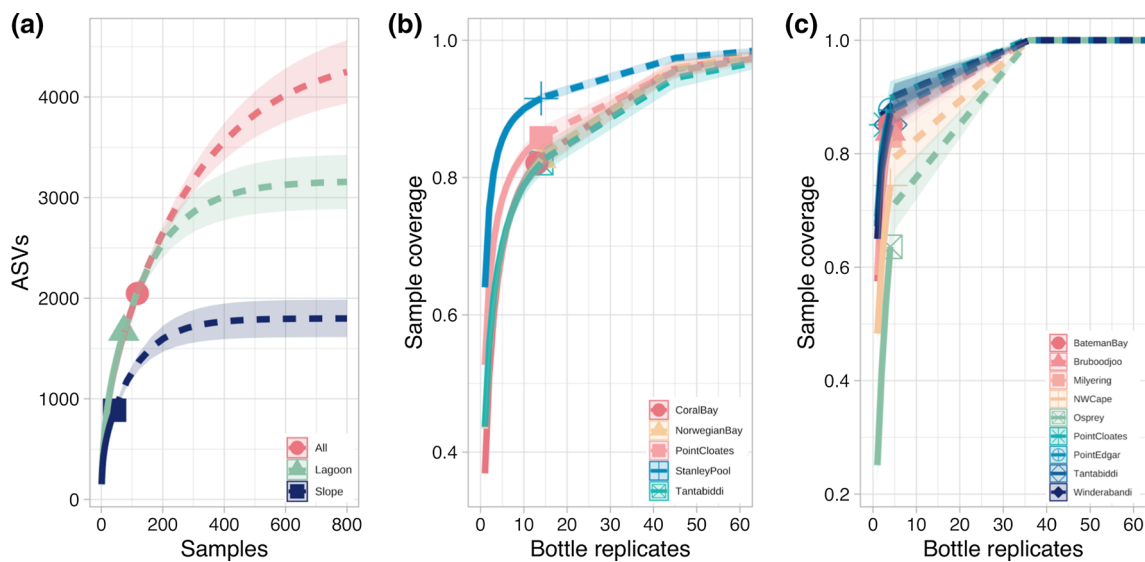
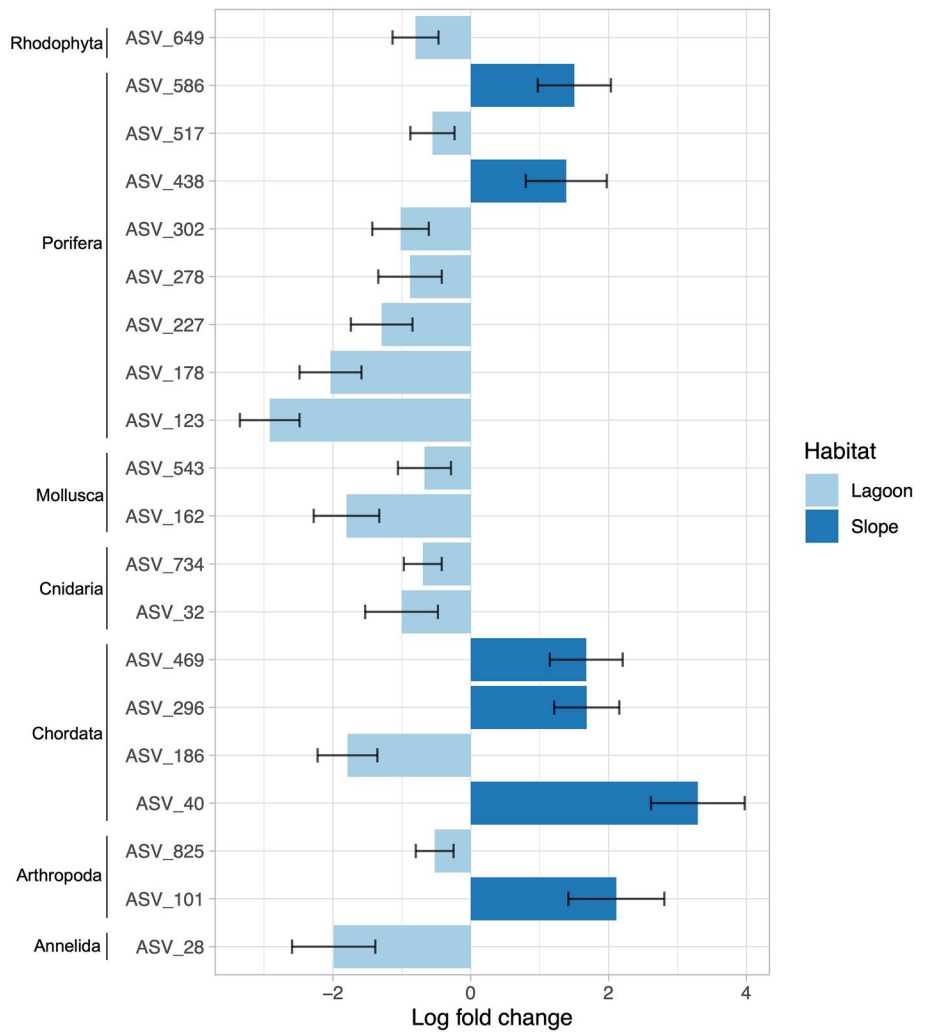


Fig. 7 Eukaryotic ASV accumulation curves across **a** all 1 L sample replicates (118), lagoon samples (74) and slope samples (44), as well as sample coverage estimates at the site-level for the **b** lagoon and **c** slope reef zones

for detecting fine-scale differences in community structure and provides novel insights into the spatial organization of eDNA within reef environments.

Community partitioning across the reefscape

eDNA metabarcoding offers the ability to detect localized signals of community composition corresponding to discrete marine habitat types (Jeunen et al. 2018; Oka et al. 2020; Ip et al. 2021). Most studies have compared eDNA signals across distinct and spatially separate environments such as coral reef, seagrass, mangroves, sandy shores, with few focusing on how more subtle signals can be structured across different reef zones (West et al. 2021). Our findings revealed that seawater samples collected from adjacent reef habitats returned distinct assemblages of metazoan diversity. The exposed shallow reef slope tends to be coral-dominated with relatively high cover, whereas the shallow lagoon consists largely of macroalgae meadows and sparse, patchy low-relief structures and coral bommies scattered across sand or rubble substrates (Cassata and Collins 2008; Kobryn et al. 2013; Vanderklift et al. 2020). As such, the lagoonal eDNA signal is expected to include macroalgae-associated organisms, which are unlikely to be represented in the outer reef slope samples. Furthermore, reef zones with different environmental gradients such as light levels, wave and temperature regimes can select for specific benthic assemblages that in turn attract a different suite of associated taxa (Thomson et al. 2020).

Beta diversity, the variance in species composition of biological assemblages among sites, can be partitioned into components of spatial turnover or nestedness (Baselga & Orme 2012). In this study, we observed a high β -diversity index of community dissimilarity (0.8205), which was primarily driven by the turnover component, indicating a pattern of species replacement when moving across habitat types. However, the nestedness component contributed a third of the dissimilarity index, which could indicate that the less diverse reef slope communities are a subset of the overall Ningaloo Reef communities recovered with eDNA. Indeed, few taxa were found to be unique to the reef slope samples (11.5%), whereas a large proportion of the diversity recovered was detected only in the lagoon samples (70.6%) or across both habitats (17.9%). Thus, the high eDNA-derived beta diversity observed at Ningaloo Reef was a result of both taxon replacement between habitats (the substitution of taxa in one site/habitat by different taxa in the other habitat), and taxon loss when moving from the lagoon to the reef slope (the less diverse assemblage resembling a subset of the richest one) (Baselga and Orme 2012). This signal was not biased by sample size as patterns of diversity and community composition described above remained the same after bootstrap resampling. Indeed, even with equal

sampling effort, the lagoon samples were consistently more diverse than samples collected from the adjacent reef slope, and beta diversity remained high and largely driven by turnover. Furthermore, we detected a strong pattern of isolation by distance within our reef slope samples, as well as high community dissimilarity when comparing the Northern, Middle, and Southern sites, suggesting that communities are spatially partitioned across the length of the reef. Together, these results revealed cross-shore as well as latitudinal variation in metazoan communities in a dynamic, exposed and connected ecosystem, highlighting the ability of eDNA to provide localized diversity snapshots. It is however unclear whether these patterns hold over time, and accurate assessments of biodiversity rely on an understanding of species abundance distributions and how these change over time and space (Seymour et al. 2021). A temporal eDNA sampling approach would allow the identification of core resident species that are typically abundant, versus ephemeral species (Magurran and Henderson 2003).

Vertical dispersal of eDNA in the marine environment is thought to be limited, leading to distinct communities throughout the water column (Jeunen et al. 2020; Monuki et al. 2021). In this study, seawater was collected close to the surface in both habitats; however, the lagoon sites were shallower (<6 m) than the reef slope sites (11–22 m). As a result, the slope-specific assemblages we recovered could be more representative of mid-water diversity, rather than benthic diversity which was probably well described in the lagoon samples. This could have potentially driven the divergence in alpha diversity we detected between reef zones. Indeed, our results reflected these patterns, and showed that worms (annelids, sipunculids, nematods) and benthic taxa were more dominant in the lagoon seawater samples. In contrast, free-swimming and pelagic species such as planktonic copepods and tunicates were well represented in the slope samples. Notable taxa missing from the slope samples included fishes, crinoids, and many types of worms. Sediment-dwelling worms can be harder to detect from seawater eDNA, and in our case were perhaps missed in the reef slope samples due to the larger distance between substrate and sampling depth (Kozioł et al. 2018; Antich et al. 2020). Further studies investigating how eDNA signals differ throughout the water column are necessary to understand vertical partitioning in shallow coral reef habitats, as well as to inform on the best sampling methodologies to adequately capture comprehensive inventories of biodiversity.

eDNA as a monitoring tool for coral reef ecosystems

Traditionally, monitoring of coral reefs focuses on well-studied macrofauna such as corals and/or fishes which are used as surrogates for gamma diversity (Plaisance et al. 2011; Mellin et al. 2011). However, this approach ignores crucial

components of reef-associated biota, such as cryptic species that live within the complex reef framework (Knowlton et al. 2010) or sediments. To remedy this issue, standardized 3D sampling devices called Autonomous Reef Monitoring Structures (ARMS) have been deployed globally; they enable the settlement of encrusting sessile species as well as mobile organisms that inhabit the stacks of plates, however they are long-term structures that require up to three years of deployment (Zimmerman and Martin 2004; Carvalho et al. 2019). Alternatively, eDNA metabarcoding can be used as a rapid biodiversity assessment tool to provide inventories of local species diversity. In this study, we used an eDNA RUMS approach and recovered 14 different metazoan phyla in a single survey conducted over five days. This highlights the potential of eDNA metabarcoding to characterize marine biodiversity across a broad taxonomic spectrum simultaneously, as well as capturing some of the reef cryptobiome that can be overlooked.

Limitations of the current approach

Despite the breadth of diversity recovered in this study using a single assay, our results only reflect a fraction of the total biodiversity of Ningaloo Reef. Indeed, a multi-assay approach has been shown to capture the most comprehensive overview of biodiversity (Stat et al. 2017; West et al. 2021). For example, a study at Coral Bay (Ningaloo Reef) calculated that the same 18S primer set used here detected only 44% of the total number of taxa recovered when combining 10 assays, highlighting the risk of severely underestimating the diversity of an area when only employing one universal assay (Stat et al. 2017). Our sampling recorded few fishes, and we were also unable to detect any megafauna, supporting the assertion that although universal markers provide great overall snapshots of biodiversity, their resolution within certain taxonomic groups can be poor (Stat et al. 2017). Indeed, fish eDNA studies have largely employed mitochondrial primers such as 12S or 16S because they allow for more specificity (Miya et al. 2015), whereas nuclear universal primers such as 18S often provide lower resolution but very wide taxonomic coverage (Deagle et al. 2014). For example, at Coral Bay, earlier work by Stat et al. (2017) identified only two operational taxonomic units (OTUs) corresponding to Actinopterygii fishes using 18S. For this reason, it is not unexpected that we also observed limited detectability of fishes. Additionally, DNA shedding rates vary not only among species but also across taxonomic groups, impacting the probability of detection of different taxa within an eDNA sample (Wood et al. 2020). Nonetheless, one of the key advantages of eDNA metabarcoding is that samples can be analysed again at a later date using a different suite of genetic markers, thus offering the possibility to retrospectively target specific taxa.

Inadequate sampling effort can critically influence the results obtained with eDNA metabarcoding (Juhel et al. 2020; Gold et al. 2021; Marwayana et al. 2021; West et al. 2021). ASV accumulation curves showed that our sampling effort was insufficient at capturing total estimated gamma diversity across Ningaloo Reef, and that overall, sample coverage was high but did not approach completeness. To achieve a more complete census, we sampled three sites within each lagoon location ($n = 15$), but still fell short of obtaining a complete picture of the estimated biodiversity for this reef zone. These results are typical of other eDNA metabarcoding surveys of species diversity in tropical ecosystems such as the Coral Triangle or Cocos Keeling Islands (Juhel et al. 2020; Marwayana et al. 2021; West et al. 2021). Since the majority of eDNA studies have been focussed on temperate marine ecosystems that typically harbour lower levels of species diversity (Port et al. 2016; Jeunen et al. 2018; Gold et al. 2021), it is likely that sampling protocols must be refined for highly diverse tropical ecosystems (Juhel et al. 2020; Marwayana et al. 2021; Stauffer et al. 2021). The tropical fringing reef ecosystem surveyed here will almost certainly contain assemblages of resident and transient species, and the transient component will complicate attempts to provide a comprehensive snapshot of diversity (Magurran and Henderson 2003). Transient, low-abundant species have low detectability, and their presence may even change over the timescale of an eDNA survey, making the complete sampling of gamma biodiversity very unlikely.

It can be beneficial to implement pilot studies to estimate optimal sampling intensity prior to undertaking large scale eDNA surveys, however that is not always possible. Another alternative to increased sampling (and higher costs) is to filter higher volumes of seawater since oligotrophic systems such as reefs do not usually pose problems related to the clogging of filters due to turbidity (Bessey et al. 2020; Marwayana et al. 2021). Given that replicated seawater samples can harbour distinct signals due to the patchiness of eDNA molecules, increasing sampling volumes will likely lead to better estimates of gamma diversity (Bessey et al. 2020; Stauffer et al. 2021).

Additionally, biodiversity estimates obtained from eDNA techniques are affected by the substrate selected for sampling and combining environmental sample types is recommended (Kozioł et al. 2018). For example, sediment samples can yield higher overall biotic richness than seawater and are particularly efficient at detecting benthic taxa such as polychaete worms (Kozioł et al. 2018). A recent study revealed that “ambient” seawater samples collected above the substrate contained different species assemblages than “crevice” seawater samples collected from within the reef matrix, and that an analysis focussed only on ambient seawater may not adequately characterize

the presence of cryptic reef species such as chitinous or calcifying organisms (Nichols et al. 2021). For this reason, if the main aim of an eDNA survey is to maximize the biodiversity recovered from coral reefs, it will likely require sampling across multiple habitat types and/or reef zones, at multiple depths, and include a variety of substrate types.

Conclusion

Surveying coral reef biodiversity across a broad taxonomic spectrum over time will allow a better understanding of how reefs and their associated biota will react to changing impacts and conditions. We conducted a biodiversity survey using eDNA RUMS and found that despite high physical connectivity among adjacent habitats, metabarcoding of seawater eDNA could reveal strong differences in community composition. The implementation of eDNA metabarcoding into monitoring schemes, using either universal or multi-assay approaches, offers managers with a non-invasive, effective, and synoptic way to monitor biodiversity across habitats and trophic levels. With increasing anthropogenic impacts to threatened marine ecosystems, eDNA metabarcoding can provide a way to rapidly monitor post-disturbance effects to inform adaptive management responses.

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Data availability Raw MiSeq sequence data (fastq files), demultiplex indices file, metadata and binary ASV and taxonomic matrices are available at Zenodo.org (<https://doi.org/10.5281/zenodo.5792468>).

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