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Large-scale eDNA metabarcoding survey reveals marine biogeographic break and transitions over tropical north-western Australia

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Abstract

Aim: Environmental DNA (eDNA) metabarcoding has demonstrated its applicability as a highly sensitive biomonitoring tool across small spatial and temporal scales in marine ecosystems. However, it has rarely been tested across large spatial scales or biogeographical barriers. Here, we scale up marine eDNA metabarcoding, test its ability to detect a major marine biogeographic break and evaluate its use as a regional biomonitoring tool in Australia.

Location: North-western Australia (NWA).

Methods: We applied metabarcoding assays targeting the mitochondrial 16S rRNA and CO1 genes to 284 surface seawater eDNA samples collected from 71 mid-shelf, inshore, coastal and nearshore estuarine sites over 700 km of the NWA coastline.

Results: Metabarcoding detected a wide range of bony fish (404 taxa), elasmobranchs (44) and aquatic reptiles (5). We detected bioregional and depth differentiation within inshore bony fish communities. These findings support the presence of a marine biogeographic break, which is purported to occur in the vicinity of Cape Leveque, demarcating the border between the Kimberley and Canning bioregions. Inshore bony fish and elasmobranch communities, as well as coastal bony fish assemblages, were additionally found to differ between the South and North Kimberley regions suggesting previously unrecognized subregional differentiation amongst these taxa. The overall compositional data have been used to update distribution information for a number of endangered, elusive and data-deficient taxa, including sawfish (family: Pristidae), northern river shark (*Glyphis garricki*) and wedgefish (genus: *Rhynchobatus*).

Main conclusions: eDNA metabarcoding demonstrated a high level of sensitivity that was able to discern fine-scale patterns across the large-scale, remote and oceanographically complex region of North-western Australia. Importantly, this study highlights the potential of integrating broad-scale eDNA metabarcoding alongside other baseline surveys and long-term monitoring approaches, which are crucial for

the sustainable management and conservation of marine biodiversity in this unique marine region.

1 | INTRODUCTION

Broad-scale biomonitoring of marine environments is integral for the detection of biological changes, stressors and shifting baselines over large spatial and temporal scales (Dafforn et al., 2016). Typically, these approaches utilize rapid assessment methods, such as underwater visual census (UVC), marine manta tow and baited remote underwater video (BRUV) surveys (Ellis et al., 2011; Gaertner et al., 2013; Piacenza et al., 2015) that provide information to distinguish broad-scale indicators and subsequently direct further research efforts to areas of interest. However, the application of these techniques is not suitable in marine environments with limited visibility and other safety hazards to divers, for example the presence of saltwater crocodiles (*Crocodylus porosus*). The advent of environmental DNA (eDNA) metabarcoding coupled with next-generation sequencing (NGS) has enabled the genetic detection and profiling of a wide range of biota present in environmental samples (e.g. water, scat and soil etc.). Environmental DNA metabarcoding has the potential to be utilized as a sensitive, cost-effective, and rapid broad-scale biomonitoring tool and is particularly well suited to marine environments (Thomsen et al., 2012; Thomsen & Willerslev, 2015; Valentini et al., 2016). Importantly, the collection of surface water (or at depth with a water sampler) for eDNA analyses bypasses logistical and safety hazards associated with visual surveillance work in turbid and dangerous marine environments. Furthermore, eDNA-derived compositional data can provide greater biological coverage to distinguish spatial and habitat variation, identify network associations, trophic structure, biological invasions and the presence of critically endangered species (Valentini et al., 2016). Whilst eDNA metabarcoding has demonstrated its applicability across small, yet highly sensitive, spatial (Jeunen et al., 2019; O'Donnell et al., 2017; Port et al., 2016; West et al., 2020) and temporal scales (Berry et al., 2019) in marine ecosystems, it is in a preliminary stage of being scaled up and tested across broader regional scales (Aglieri et al., 2020; Fraija-Fernández et al., 2020).

The extensive coastline of north-western Australia (NWA) supports a diverse array of tropical marine habitats and biota, extending from offshore coral reefs on the edge of the continental shelf, to coastal intertidal sand, rock and reef habitats, constituting 12 distinct bioregions (Wilson, 2014). A profound change in the underlying geomorphology of the Canning and Kimberley basins, from Cretaceous-Cainozoic sedimentary (largely sandstone) rocks to Proterozoic metasedimentary, metamorphic and igneous rocks, has shaped various coastal marine habitats in these bioregions

(Wilson, 2014). The Canning bioregion comprises coastlines typified by benthic soft substrates, such as intertidal sand and mudflat habitats with very little coral reefs, whilst the Kimberley bioregion is dominated by rocky, intertidal platforms, fringing coral and offshore coral reefs, and substantial mangrove habitat (Richards et al., 2018; Wilson, 2014). Environmental conditions and connectivity patterns across these bioregions are additionally shaped by various oceanic currents, immense tidal systems (macrotides ranging up to 11 m in the Kimberley), seasonal discharge and extreme turbidity from major rivers (Semeniuk, 1993; Thackway & Cresswell, 1998). Temperature varies between the bioregions and also across subregions, semi-arid in the Canning, sub-humid in the southern Kimberley, humid in the northern Kimberley and sub-humid in the northeast Kimberley (Cresswell & Semeniuk, 2011).

This environmental variation is purported to contribute to a major biogeographic break at Cape Leveque – the tip of the Dampier Peninsula, demarcating the border between the Kimberley and Canning bioregions – see Figure 1 (Travers et al., 2010; Wilson, 2014). A significant change in the fish assemblage composition across Cape Leveque (Hutchins, 2001a) likely reflects the latitudinal transition in benthic substrates, overlaid on a strong bioregional effect reflecting various habitat, tidal and riverine discharge influences (Travers et al., 2006, 2010). Population connectivity studies in bony fish (stripey snapper; *Lutjanus carponotatus* and blackspotted croaker; *Protonibea diacanthus*) and corals (*Isopora brueggemanni* and *Acropora aspera*) further revealed a genetic transition zone across Cape Leveque, with dispersal and gene flow likely constricted by extreme tidal flushing at the head of King Sound (DiBattista et al., 2017; Taillebois et al., 2017; Underwood et al., 2017).

The aim of this study was to conduct a broad-scale multi-marker eDNA metabarcoding survey across the extensive coastline of NWA in order to: (a) detect the purported biogeographic break across Cape Leveque using eDNA-derived bony fish, shark and ray and aquatic reptile taxonomic compositional data, (b) update distributional information for endangered elasmobranchs, such as sawfish (family Pristidae) and the northern river shark (*Glyphis garricki*), marine turtles (superfamily Chelonioidea) and for data-deficient taxa such as sea snakes (subfamily Hydrophiinae), and (c) evaluate the overall strengths and weaknesses of eDNA metabarcoding as a biomonitoring tool when used across a broad geographic region. Given the remoteness of NWA, long-term monitoring programmes are sparse, particularly for species that are not of commercial value (Evans et al., 2017). As such, there is a great potential to integrate eDNA metabarcoding as a long-term, large-scale biomonitoring tool
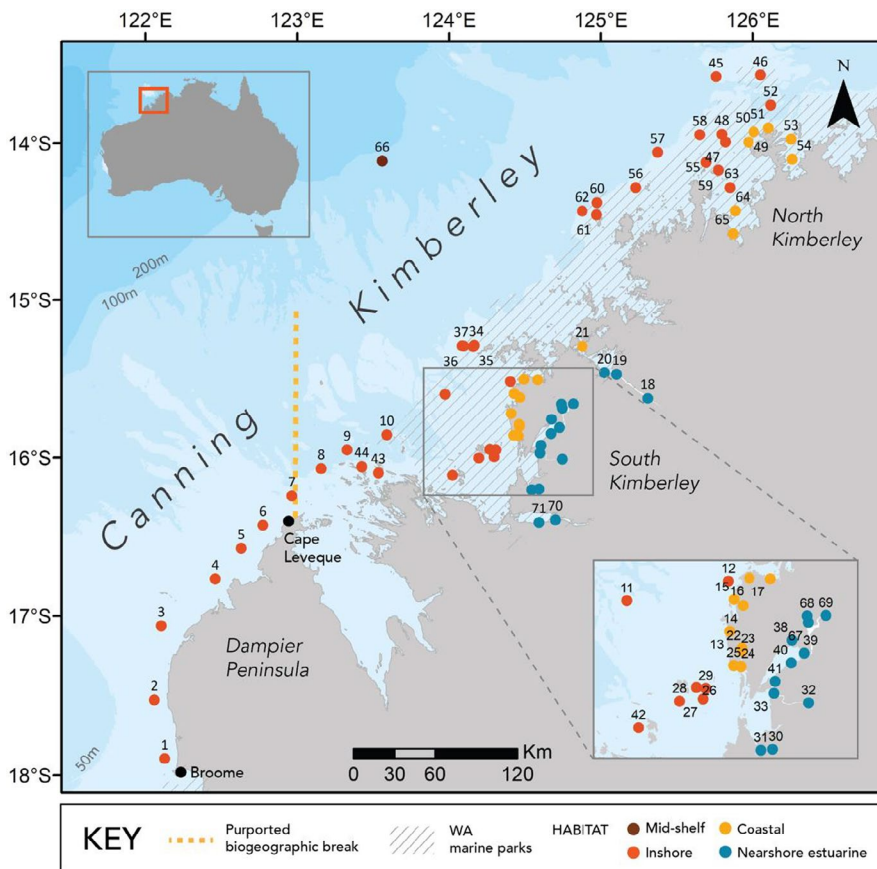
FIGURE 1 Location of sampling sites across the Canning and Kimberley bioregions in north-western Australia (NWA). The Canning sites (1–7; latitude 16.1°S-17.5°S) located within the Dampier Peninsula subregion extend northwards from Broome to the purported biogeographic break line off Cape Leveque. The Kimberley sites (8–71) located north-west of the purported biogeographic break are additionally categorized into the subregions, South Kimberley (sites 8–44 and 68–71; latitude 15.1°S-16.4°S) and North Kimberley (sites 45–66; latitude 13.6°S-14.6°S). Bathymetry data were sourced from Geoscience Australia (Whiteway, 2009)

in NWA, capable of providing distribution information on a wide variety of taxa.

2 | METHODS

2.1 | Field sampling

Four one-litre water replicates were sampled from 71 sites across the Canning/Kimberley bioregions (Figure 1; Table S1) in September 2017 and July/September 2018, totalling 284 samples over 700 km of coastline. Samples were taken on a transect line traversing the purported biogeographic break and more widely across the Kimberley region in mid-shelf, inshore, coastal and nearshore estuarine habitats. Surface water was collected using bleach sterilized 1L Nalgene bottles attached to an extended pole, to avoid close encounters with saltwater crocodiles (*C. porosus*). Samples were immediately stored on ice and were individually filtered across Pall 0.45μm Supor® polyethersulphone membranes using a Pall Sentino® Microbiology pump (Pall Corporation) within five hours of collection. Filtration equipment was cleaned using 10% bleach (4% chlorine) – a one-litre sample of this was taken at the end of each sampling day as a filtration control to test for any carry over contamination. Membranes with filtrate were immediately frozen at −20°C, prior to their transportation to the Trace & Environmental DNA (TrEnD) Laboratory in Perth, Western Australia, where they were stored at −80°C

until extraction – all eDNA was isolated within two months of collection.

2.2 | DNA extraction

DNA was extracted from half of the membrane using a DNeasy Blood and Tissue Kit (Qiagen) with the following modifications: 540 μl of ATL lysis buffer, 60 μl of Proteinase K and a 3-hr digestion at 56°C. Extracts were eluted in 100 μl of Buffer EB. This was completed within four weeks of collection. Extraction blank controls were processed in parallel with all samples to detect any cross-contamination. Genomic DNA extracts were then stored at −20°C.

2.3 | Metabarcoding assay design, amplification and library sequencing

Three PCR metabarcoding assays were employed: 16S Fish, COI Elasmobranch and 16S Reptile (Table 1) to amplify bony fish, elasmobranchs and aquatic reptiles, respectively, from mixed environmental samples. The 16S Fish and COI Elasmobranch PCR assays have been optimized and successfully applied in previous eDNA metabarcoding studies examining fish and shark diversity and trophic interactions (Bakker et al., 2017; Berry et al., 2017; Boussarie et al., 2018; Stat et al., 2017, 2019; West et al., 2020).

TABLE 1 PCR assay information for marine eDNA metabarcoding across the Canning/Kimberley bioregions

PCR assay	Target taxa	Primer name	Oligonucleotide sequence (5'-3')	Target length (bp)	Annealing temp (°C)	Primer reference
16S Fish	Bony Fish	16SF/D	GACCCTATGGAGCTTTAGAC	178–228	54	Berry et al. (2017)
		16S2R-degenerate	CGCTGTTATCCCTADRGTAACT			Deagle et al. (2007)
COI Elasmobranch	Sharks, Skates & Rays	FishF1-degenerate	ACCAACCACAAAGANATNGGCAC	110–241	52	Original: Ward et al. (2005) Degenerate: West et al. (2020)
		FishF2-degenerate	TCNACNAATCATAAAGATATCGGCAC			
		Shark COI-MINIR- degenerate	GATTATTACNAAAGCNTGGGC			Original: Fields et al. (2015) Degenerate: West et al. (2020)
16S Reptile	Aquatic Reptiles	AqReptileF-degenerate	AGACNAGAAGACCCTGTG	211–277	52	West et al. (2021)
		AqReptileR	CCTGATCCAACATCGAGG			

Note: Three primer sets: 16S Fish, 16S Reptile and COI Elasmobranch corresponding to the mitochondrial 16S rDNA and COI regions were applied to all collected seawater samples. In the primer name, "F" refers to the forward primer and "R" to the reverse primer.

The 16S Reptile assay has been recently designed to amplify northern Australian aquatic reptiles, such as sea snakes, turtles and crocodiles (West et al., 2021).

Following quantitative PCR-based (qPCR) quantification to optimize levels of input DNA (Murray et al., 2015), final qPCR was performed in a single step using fusion tagged primer architecture consisting of Illumina compatible sequencing adaptors, a unique index (6-8bp in length) and a respective primer sequence for each assay. All qPCR reactions were prepared in dedicated clean room facilities at the TrEnD Laboratory, Curtin University, and are described in detail in Section S1. Quantitative PCR amplicons were pooled at equimolar ratios based on qPCR ΔRn values and size-selected using a Pippin-Prep (Sage Science, Beverly, USA) to remove any off-target amplicons. Size-selected libraries were then purified using the Qiaquick PCR Purification Kit (Qiagen), quantified using a Qubit 4.0 Fluorometer (Invitrogen) and diluted to 2 nM for loading onto a 300 cycle MiSeq® V2 Standard Flow Cell. Sequencing was conducted on an Illumina MiSeq platform (Illumina), housed in the TrEnD Laboratory at Curtin University, Western Australia.

2.4 | Bioinformatics

Sequencing reads were demultiplexed and quality filtered in OBITools (v1.2.9; Boyer et al., 2014) and in R (v3.5.3; R Core Team, 2015) using the DADA2 (v1.10.1) bioinformatics package (Callahan et al., 2016; see Section S2 for bioinformatic parameter details). Resulting amplicon sequence variants (ASVs) were queried against NCBI's GenBank nucleotide database (accessed in 2018/19 for different assays; Benson et al., 2005) using BLASTn and also against an in-house 16S rRNA Western Australian fish database consisting of 306 species (Nester et al., 2020). Taxonomic assignments of ASVs were made using a lowest common ancestor approach (https://github.com/mahsa-mousavi/eDNAFlow/tree/master/LCA_taxonomyAssignment_scripts, Mousavi-Derazmahalleh et al., unpublished data; see Section S2). All taxonomic assignments required 100% query coverage, with a species-level assignment requiring at minimum a 98% identity match to a reference sequence. Consolidated taxa assignments (at the lowest possible taxonomic level) were then additionally categorized based on habitat association and biogeographic distribution information obtained from Kimberley biodiversity checklists (Moore et al., 2014), FishBase (Froese & Pauly, 2019) and the World Register of Marine Species (WoRMS; Horton et al., 2018). Nomenclature was reviewed and, if necessary, updated using the Australian Faunal Directory (AFD; ABRS, 2009).

In order to normalize the dataset, we determined appropriate subsampling depths for each assay (see Section S2 for more detail) and conducted consecutive rounds of subsampling using the multiple_rarefactions function in QIIME (v1.7.0; Caporaso et al., 2010). ASVs detected in filtration and/or extraction blanks were entirely removed, prior to ASVs being merged by taxonomy using the phyloseq (v1.24.2) "tax_glom" function (McMurdie & Holmes, 2013) in RStudio. We then consolidated our assay data into three taxonomic-based

datasets: Actinopterygii (bony fish), Elasmobranchii (elasmobranchs, i.e. sharks and rays) and Reptilia (reptiles), which allowed us to examine community composition by discrete taxonomic group, rather than by individual metabarcoding assays which contained some overlap in taxonomic detections.

2.5 | Statistics

Community composition variation was analysed across the study region, with a particular focus across the purported biogeographic break at Cape Leveque. In order to control for the effect of differing habitats, variation was tested between sites within inshore, coastal and nearshore estuarine habitats independently. Presence–absence data for each taxonomic dataset (bony fish, elasmobranchs and reptiles) were converted to Jaccard similarity matrices and tested for compositional variation by a distance-based linear routine (DistLM) using a step-wise selection procedure and adjusted R^2 criterion in the PERMANOVA + add-on (Anderson et al., 2008) of PRIMER v7 (Clarke & Gorley, 2015). Normalized spatial predictor variables included bioregion (Canning and Kimberley), subregion (Dampier Peninsula [Canning], South Kimberley and North Kimberley), latitude and site depth (Table S1); longitude was omitted due to collinearity with latitude. Temporal variation in sampling was not included, given earlier research indicated that season only has a small influence on inshore reef fish composition in this region (Travers et al., 2006). DistLM analyses are capable of handling unbalanced designs (Anderson et al., 2008), in this case, where there are an unequal number of sites in the spatial predictor variable groups. We did, however, run an additional DistLM routine on a subset (Sites 1–11 and 42–44) of the data assemblages to assess the bioregional influence across 14 sites (seven sites directly on either side of the purported biogeographic break). Site variation was visualized by principal coordinate analysis (PCO) using the stats function "cmdscale" and predictor variables overlaid using the vegan "ordisurf" function (Oksanen et al., 2019) in R Studio (v1.1.423; R Core Team, 2015). Observed taxonomic richness at each site was tested for significance between bioregions and subregions using ANOVA and graphed in ggplot2 (Wickham, 2016) in RStudio. Additionally, similarity percentage analyses (SIMPER) were conducted in PRIMER to identify the top inshore and coastal taxa that contribute to pairwise dissimilarity between bioregions and subregions, where significant in the DistLM analyses. This elucidated whether variation in community composition between the bioregions and subregions is driven by uneven taxonomic richness and/or variation in compositional diversity.

3 | RESULTS

3.1 | Sampling and sequencing statistics

The three eDNA metabarcoding assays yielded a total of 57,311,878 sequencing reads. The mean number of filtered sequences

(post-quality, denoizing and chimera filtering) was 75,800 ± 41,071 per replicate sample (303,202 ± 143,648 per site) for the 16S Fish assay; 15,838 ± 21,799 per replicate sample (61,347 ± 58,418 per site) for the COI Elasmobranch assay; and 32,569 ± 42,241 per replicate sample (130,278 ± 114,036 per site) for the 16S Reptile assay (Table S2). ASV accumulation curves based on the addition of each sampling replicate per site indicated that four one-litre water replicates (selected a priori to sampling) were just shy of maximizing ASV richness for each of the three assays (Figures S1–S3). On fitting a polynomial curve to the median accumulation curve for each assay, it was extrapolated that an average of 6.9, 6.1 and 6.1 one-litre water replicates would be required to maximize ASV richness for 16S Fish, COI Elasmobranch and 16S Reptile assays, respectively. The rarefaction analyses determined suitable subsampling cut-offs (after pooling of the four replicates per site) of 30,977 reads for the 16S Fish assay (Figures S4–S6), 4,000 reads for the COI Elasmobranch assay (Figures S7–S9) and 4,000 reads for the 16S Reptile assay (Figures S10–S12).

Potential cross-contaminant ASVs that were detected in filtration and/or extraction blanks were removed from subsequent analyses. This included ASVs that produced detection hits for giant trevally (*Caranx ignobilis*; 7 ASVs, 6,837 total reads), snapper (genus: *Lutjanus*; 4 ASVs, 55,199 total reads), blue threadfin (*Eleutheronema tetradactylum*; 6 ASVs, 7,868 total reads), barramundi (*Lates calcarifer*; 43 ASVs, 22,866 total reads) and pilchard (genus: *Sardinops*; 38 ASVs, 40,820 total reads). These species were targeted for fisheries and/or commercial research on the sampling vessels with the exception of pilchards, which were utilized as bait for BRUV deployments. Only compromised ASVs were removed from subsequent analyses. For example, we retained 15 barramundi ASVs that were not detected in filtration and/or extraction blanks. We also detected salmon (genus: *Salmo*; 1 ASV, 9 total reads), which has previously been detected as a sporadic reagent contamination in both our workflows and other laboratories (Thomsen et al., 2016), and as such was entirely removed. We also omitted all ASVs that produced detection hits for taxa outside of our targeted taxonomic groups of bony fish, elasmobranchs and reptiles. This included humans (*Homo sapiens*), chicken (*Gallus gallus*) and horse (*Equus caballus*).

3.2 | Overall diversity

A total of 310 taxa (ranging from family to species-level assignments; 4.9 ± 9.9 ASVs per taxa) were detected by the 16S Fish assay, 139 taxa (1.3 ± 1.0 ASVs per taxa) by the COI Elasmobranch assay and 181 taxa (2.9 ± 5.2 ASVs per taxa) by the 16S Reptile assay, prior to subsampling (Figure 2). Collectively, the three metabarcoding assays yielded 453 identifiable taxa, representing 96 families within 41 orders of bony fish, elasmobranchs and aquatic reptiles (Table S3). Of these taxa, 63.7% are widely distributed across the Indo-West Pacific, 12.4% circumglobal and 8.6% endemic to the Australian region (including Indo-Australian, Northern Australian and Western Australian bioregions). The majority of detected taxa are associated

FIGURE 2 Order level dendrogram of bony fish, elasmobranch and reptile diversity detected across the Canning and Kimberley bioregions using eDNA metabarcoding. Three metabarcoding assays were applied (16S Reptile, 16S Fish and COI Elasmobranch) and produced 181, 310 and 139 unique taxonomic assignments, respectively. As depicted in the *circlize* plot, there were cross-amplification of taxonomic groups between the three assays
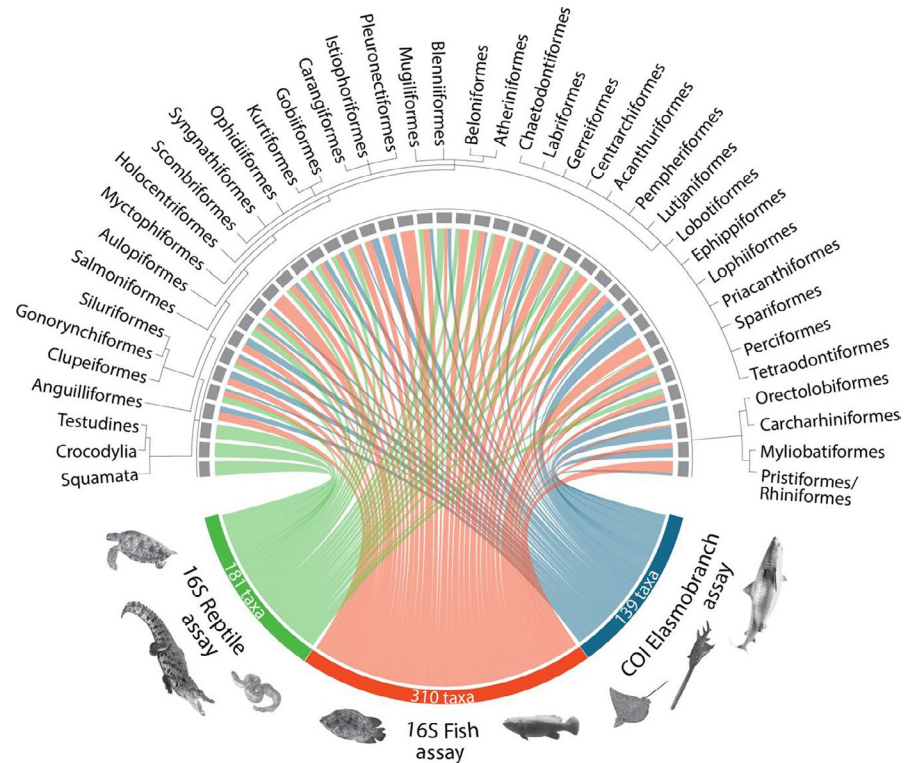
with hard substrate habitats (54.0%), followed by soft substrate (38.6%), estuarine (33.6%), pelagic (28%), freshwater (12.4%), mangrove (9.7%) and seagrass (2.6%) habitats (Table S3).

Four hundred and four bony fish taxa (class: Actinopterygii) were detected (15 at family level only, 136 at genus level only and 253 at a species level) from 80 families within 34 orders (Table S3). Predominant bony fish families included Gobiidae (gobies; 39 taxa), Labridae (wrasse; 33), Carangidae (jacks and pompanos; 26) and Lutjanidae (snapper; 20), which is in line with the most speciose families in tropical marine environments (Blaber, 2008; Mora, 2015). Bony fish of conservation, cultural, recreation and/or commercial importance in the Canning and Kimberley bioregions are presented in Table 2. This includes the protected Queensland groper (*Epinephelus lanceolatus)* and the highly prized barramundi (*Lates calcarifer*). We also report 21 putative new fish occurrence records in the Canning and Kimberley (Table S3); however, these cannot be fully validated in our study. To verify a new occurrence record based on eDNA metabarcoding, we required all congeneric taxa to have been barcoded for the targeted gene regions (in this case 16S and COI). However, as this criterion was not fulfilled, we cannot rule out the possibility that our new occurrence records represent a closely related (not yet barcoded) taxon.

Forty-four elasmobranch taxa (class: Chondrichthyes, subclass: Elasmobranchii) were detected from 11 families within four orders (Table S3). The two most speciose elasmobranch families were the Carcharhinidae (requiem sharks; 15 taxa) and Dasyatidae (stingrays; 14), which collectively comprised over half of the total detected shark and ray taxa (Table S3). We detected five elasmobranchs that are listed as either "Endangered" or "Critically Endangered" on the IUCN Red List and are under various national and state protection

management (see Table 2); these taxa were the largetooth sawfish (*Pristis pristis*), the dwarf sawfish (*Pristis clavata*), the knifetooth sawfish (*Anoxypristis cuspidate*), wedgefishes (genus: *Rhynchobatus*) and the northern river shark (*Glyphis garricki*). Other rare and uncommon taxa include the Australian weasel shark (*Hemigaleus australiensis*), the snaggletooth shark (*Hemipristis elongata*) and the pigeye shark (*Carcharhinus amboinensis*).

Only five reptile taxa (class: Reptilia) were detected from five families within three orders (Table S3): the saltwater crocodile (*Crocodylus porosus*), the black-headed python (*Aspidites melanocephalus*), Stokes's sea snake (*Hydrophis stokesii*), the white-bellied mangrove snake (*Fordonia leucobalia*) and the green turtle (*Chelonia mydas*). Given the low frequency of detection of these taxa, reptiles were excluded from all multivariate analyses.

3.3 | Community composition

3.3.1 | Bony fish composition

Bony fish composition was examined independently in each habitat type (inshore, coastal and nearshore estuarine), excluding the mid-shelf habitat which was only comprised of one site. In regard to inshore bony fish compositions, a distance-based linear model (DistLM) routine across all sites indicated that bioregion (i.e. the Canning and Kimberley bioregions) explained the highest proportion of fitted variance, followed by site depth and subregion (Table 3; Table S4). This result was additionally validated by the subset analysis (restricted to several sites either side of the break),

TABLE 2 Selected taxa of conservation, cultural, recreation and/or commercial importance detected via eDNA

Common name	Scientific name	Importance	Site/s detected	Assay	Associated habitat	Distribution
Bony fish						
Queensland groper	*Epinephelus lanceolatus*	Is a protected species in Western Australia, Queensland and New South Wales. Listed as "Data-Deficient" by the IUCN.	11, 14, 17, 21–23, 25, 31–33, 38, 40, 41, 64, 67–69	16S Fish, 16S Reptile	Hard substrate	Indo-West Pacific
Barramundi	*Lates calcarifer*	Is of cultural, recreational and commercial fishing importance.	1, 7–9, 14, 19–23, 25–27, 30, 32–36, 38–44, 46, 47, 53, 59, 61–71	16S Fish, COI Elasmobranch	Pelagic, Estuarine	Indo-West Pacific
Elasmobranchs						
Largetooth sawfish	*Pristis pristis*	Is a protected species across Australia. Listed as "Critically Endangered" by the IUCN.	62, 68	16S Fish	Soft substrate, Estuarine, Freshwater	Circumglobal
Dwarf sawfish	*Pristis clavata*	Is a protected species across Australia. Listed as "Endangered" by the IUCN.	34, 38, 39	16S Fish	Soft substrate, Estuarine, Mangrove	Northern Australia
Knifetooth sawfish	*Anoxypristis cuspidata*	Is a protected species across Australia. Listed as "Endangered" by the IUCN.	51–54	16S Fish, COI Elasmobranch	Soft substrate, Estuarine, Freshwater	Indo-West Pacific
Wedgefishes	*Rhynchobatus*	Are heavily targeted in the international fin trade. All species in the *Rhynchobatus* genus are listed as "Critically Endangered" by the IUCN.	17, 27, 31, 54, 59, 67	16S Fish	Soft substrate	Indo-West Pacific, Atlantic
Northern river shark	*Glyphis garricki*	Is a protected species in Western Australia and the Northern Territory. Listed as "Critically Endangered" by the IUCN.	68, 71	COI Elasmobranch	Soft substrate, Estuarine, Freshwater	Indo-Australian
Scalloped hammerhead	*Sphyrna lewini*	Listed as "Critically Endangered" by the IUCN.	41, 43, 51, 53, 54	COI Elasmobranch	Pelagic	Circumglobal
Great hammerhead	*Sphyrna mokarran*	Listed as "Critically Endangered" by the IUCN.	16, 17, 22, 23, 25, 26, 30, 35, 38, 40, 43, 51, 65	COI Elasmobranch	Hard substrate, Pelagic	Circumglobal
Ornate eagle ray	*Aetomylaeus vespertilio*	Listed as "Endangered" by the IUCN.	15, 34, 39, 59	COI Elasmobranch	Hard substrate, Soft substrate	Indo-Pacific

TABLE 3 Summary table of the distance-based linear model (DistLM) analyses for bony fish

Sites	Predictor	Pseudo-F	Proportion	Cumulative proportion	p
Inshore					
All sites	**Bioregion**	3.302	0.086	0.086	.000***
	Depth	3.005	0.074	0.160	.000***
	Subregion	1.837	0.044	0.205	.000***
Dampier Peninsula and South Kimberley	**Subregion**	1.993	0.160	0.160	.000***
	Depth	2.729	0.101	0.261	.000***
South and North Kimberley	**Depth**	3.087	0.099	0.099	.000***
	Latitude	1.943	0.060	0.159	.000***
	Subregion	1.349	0.041	0.201	.029*
Dampier Peninsula and North Kimberley	**Subregion**	3.537	0.157	0.157	.001**
	Depth	1.516	0.066	0.223	.015*
	Latitude	1.011	0.044	0.267	.452
Coastal					
South and North Kimberley	**Subregion**	3.280	0.179	0.179	.000***
	Depth	1.298	0.070	0.249	.087
	Latitude	1.244	0.066	0.315	.148
Nearshore estuarine					
South Kimberley	**Depth**	1.903	0.120	0.120	.034*
	Latitude	1.017	0.064	0.184	.368

Note: These were constructed using a sequential step-wise selection procedure and adjusted R^2 criterion. Significant codes are as follows: 0 < 0.001 "***," 0.001 < 0.01 "**"and 0.01 < 0.05 "*." The predictor variables highlighted in bold are significant ($p < .05$). Full DistLM results, including marginal tests and best solutions, are provided in Tables S4, S7 and S8.

which again indicated that bioregion had the highest significant influence on inshore bony fish compositions across the purported biogeographic break (Table S4). Subsequent pairwise DistLM analyses between each of the subregions (i.e. Dampier Peninsula [Canning], South Kimberley and North Kimberley) identified a highly significant subregional influence on the inshore bony fish compositions between the Dampier Peninsula and adjacent South Kimberley subregions, and between the Dampier Peninsula and North Kimberley subregions. This reflects the broader biogeographic boundary between the Canning and Kimberley bioregions. Inshore bony fish compositions between the South Kimberley and North Kimberley subregions were largely found to transition based on site depth and latitudinal gradients; however, there was also a smaller subregional influence. Bony fish composition across all inshore sites is visualized in a PCO in Figure 3a. Cumulatively, the formed models explained between 20.1% and 26.7% of total fitted variance between inshore fish assemblages (Table 3; Table S4).

Taxonomic richness of inshore bony fish did not significantly differ between the two bioregions (Table S5; Figure S13). This indicates that the detected bioregional variation was not influenced by uneven taxonomic richness, but solely compositional variation. Similarity percentage analysis (SIMPER) was used to identify prominent inshore bony fish taxa contributing most to pairwise

dissimilarity between the Canning and Kimberley bioregions (Table S6). This indicated a higher detection rate of sardinella (genus: *Amblygaster*), purple tuskfish (*Choerodon cephalotes*) and chub mackerels (genus: *Rastrelliger*) in the Canning bioregion, whilst the Kimberley region had a higher detection rate of Spanish mackerel (genus: *Scomberomorus*), giant trevally (*Caranx ignobilis*) and blue tuskfish (*Choerodon cyanodus*).

In examining coastal fish assemblages (those restricted to the South and North Kimberley subregions), a DistLM analysis indicated that subregion was a highly significant predictor variable, explaining 17.9% of fitted variance (Table 3; Table S7; Figure 3b). For bony fish composition in nearshore estuarine sites (only surveyed in the South Kimberley), depth was the only significant predictor variable, explaining 12% of the fitted variance (Table S8; Figure 3c).

3.3.2 | Elasmobranch composition

Elasmobranch composition across all inshore sites was found to be driven by subregion and depth, explaining 24.3% of fitted variance (Table 4; Figure 4a; Table S9). Pairwise DistLM analyses revealed a subregional effect between all three of the subregions being the Dampier Peninsula (Canning), the South Kimberley and
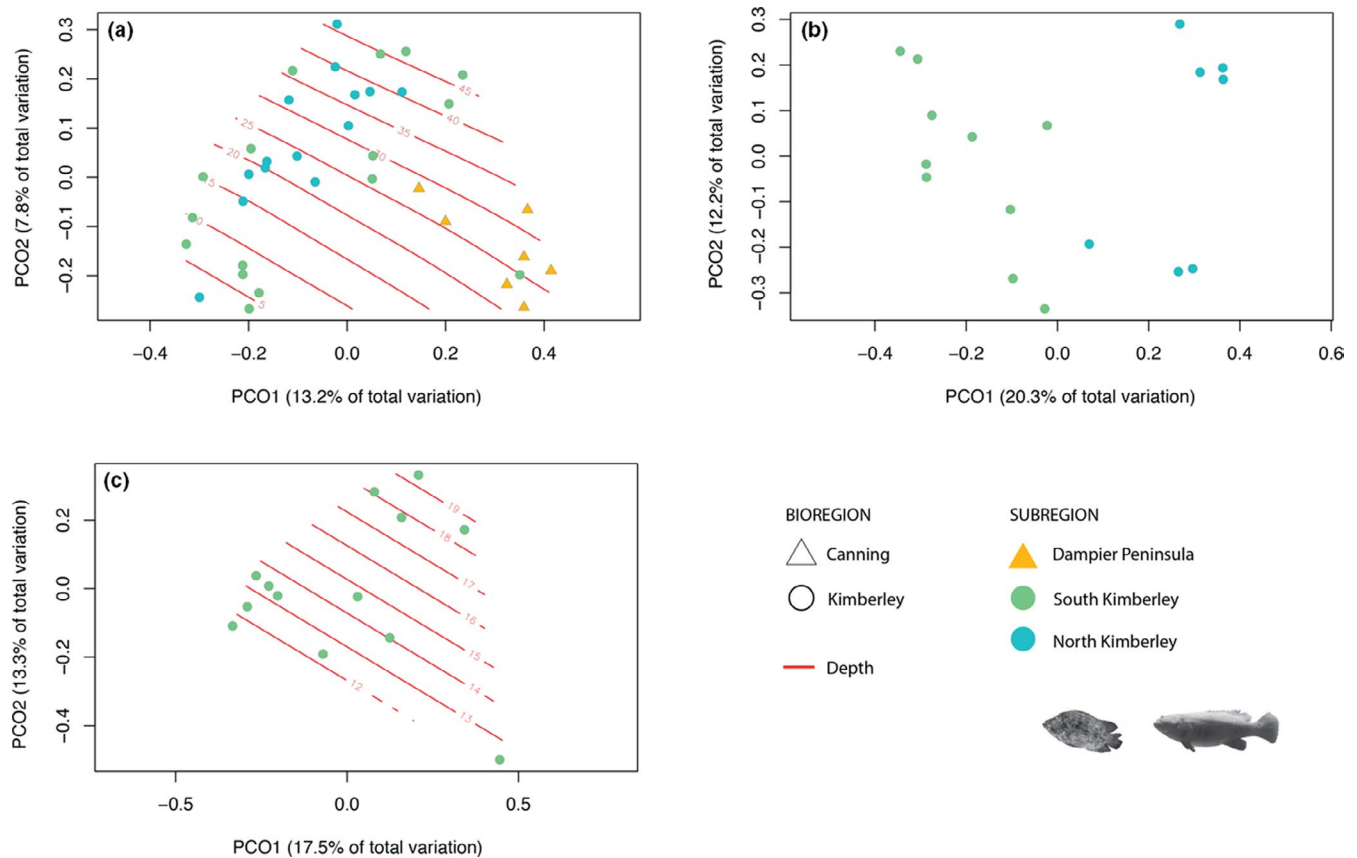
FIGURE 3 Principal coordinate analysis (PCO) of bony fish composition in (a) inshore, (b) coastal and (c) nearshore estuarine sites. Depth gradients are plotted if they are a significant predictor variable in the corresponding DistLM analyses. The proportion of variation explained by each axis is shown on the axis labels

the North Kimberley (Table 4; Table S9). Cumulatively, the significant spatial predictor variables that formed models explained between 23.6% and 34.3% of total fitted variance between inshore sites (Table 4). Taxonomic richness of inshore elasmobranchs did not differ significantly between the three subregions (Table S10; Figure S14); however, within the Dampier Peninsula only two sites (post-subsampling) had detectable traces of elasmobranch taxa. SIMPER analysis was used to identify prominent inshore elasmobranchs contributing most to pairwise dissimilarity between the three subregions (Table S11). Bluespotted maskray (*Neotrygon kuhlii*) dominated detections in the Dampier Peninsula, mangrove whipray (*Himantura granulate*), bluespotted ribbontail ray (*Taeniura lymma*) and brownbanded bamboo shark (*Chiloscyllium punctatum*) in the South Kimberley subregion, whilst the North Kimberley had a higher detection of requiem sharks such as grey reef shark (*Carcharhinus amblyrhynchos*), bull shark (*Carcharhinus leucas*), blacktip reef shark (*Carcharhinus melanopterus*) and spot-tail shark (*Carcharhinus sorrah*).

In examining elasmobranch taxa composition within coastal sites (South and North Kimberley only), site depth was the only significant predictor variable, explaining 13.3% of the total fitted variance (Table 4; Table S12; Figure 4b). For the nearshore estuarine sites, there were no significant tested predictor variables that could explain the fitted variance (Table 4; Table S13; Figure 4c).

4 | DISCUSSION

4.1 | Bony fish compositional transitions across NWA

The Canning and Kimberley bioregions have some of the least impacted marine and coastal ecosystems in the world (Halpern et al., 2008) with over 1,500 reported species of bony fish (Fox & Beckley, 2005; Moore et al., 2014, 2020). This synthesis is the result of numerous surveys and museum records since the 1880s (Hutchins, 2001b; Moore et al., 2014, 2020; Paxton et al., 2006). This singular eDNA study detected 404 bony fish taxa from 80 families across 71 sites, which comprises nearly a third of the total fish assemblage composition known from this region. This detection rate is comparable to a previous trawling and trapping survey of inshore fish fauna (up to 361 species from 85 families), revealing faunal transitions over NWA (Travers et al., 2010). With a similar high detection rate, we expected the eDNA site assemblages to be representative of the overall fish composition and reveal fine-scale changes across the Canning and Kimberley bioregions. Potential confounding effects of water movement on eDNA profiles between our sites (>4 km apart) are expected to be minimal, given a growing body of evidence indicating the localization of eDNA over small spatial scales (<1 km) (Jeunen et al., 2019; Koziol et al., 2019; Murakami

TABLE 4 Summary table of the distance-based linear model (DistLM) analyses for elasmobranchs

Sites	Predictor	Pseudo-F	Proportion	Cumulative proportion	p
Inshore					
All sites	**Subregion**	3.822	0.184	0.184	.000***
	Depth	2.578	0.059	0.243	.007**
Dampier Peninsula and South Kimberley	**Subregion**	3.288	0.135	0.135	.013*
	Depth	3.395	0.125	0.260	.005**
South and North Kimberley	**Subregion**	1.954	0.122	0.122	.003**
	Depth	2.428	0.072	0.194	.008**
	Latitude	1.414	0.042	0.236	.133
Dampier Peninsula and North Kimberley	**Subregion**	5.989	0.240	0.240	.000***
	Depth	1.675	0.065	0.304	.076
	Latitude	1.037	0.040	0.344	.417
Coastal					
South and North Kimberley	**Depth**	2.299	0.133	0.133	.013*
	Subregion	1.060	0.061	0.194	.404
	Latitude	1.237	0.070	0.264	.254

Note: These were constructed using a sequential step-wise selection procedure and adjusted R^2 criterion. Significant codes are as follows: 0 < 0.001 "***," 0.001 < 0.01 "**"and 0.01 < 0.05 "*." The predictor variables highlighted in bold are significant ($p < .05$). Full DistLM results, including marginal tests and best solutions, are provided in Tables S9 and S12.

et al., 2019; Stat et al., 2019; West et al., 2020). Variation in DNA persistence between sites is also expected to be minimal, given that sea surface temperatures only vary by 1–2°C across our study region (Bureau of Meteorology, 2020).

The profound change in the underlying geomorphology and ensuing offshore, inshore and coastal habitats of the Canning and Kimberley bioregions corresponds to documented transitions in the marine faunal composition across Cape Leveque (Wilson, 2013). Studies of inshore soft substrate and reef fish fauna across NWA (Travers et al., 2006, 2010, 2012) revealed a latitudinal transition overlaid on a strong bioregional influence, presumably reflecting the very different environmental characteristics of the Kimberley and Canning marine bioregions, such as tidal regime, turbidity and the distribution of mangrove forests and seagrass meadows which provide nursery habitats for certain fish species (Travers et al., 2010). This built upon earlier meta-analyses and visual surveys of fish distributions across Western Australia which identified a distinction between the Kimberley and the north-west shelf region (Fox & Beckley, 2005; Hutchins, 1997, 2001a). In our study, which examined the inshore bony fish compositions that traverse the purported biogeographic break across Cape Leveque, we demonstrated that site dissimilarity is driven by a bioregional effect (in addition to depth and subregion) across the Canning and Kimberley. This provides new evidence to support the existence of the biogeographic break on inshore fish composition. It also reveals the ability of eDNA to detect fine-scale changes across large-scale regions, even with a suboptimal level of replication to maximize the observed species richness.

The coastal bony fish assemblages, surveyed in this study across the South and North Kimberley regions, were shown to be influenced by a subregional effect. Previous research by Hutchins (1997, 2001a), identified discrete nearshore fish assemblages on either side of Cape Leveque and in the northeast Kimberley (east of Cape Londonderry), the latter typifying species found in the Northern Territory. Hutchins (1997) attributes the bioregional breaks in nearshore fish fauna to varying environmental conditions, such as increased turbidity in the King Sound area, which may prevent species from ranging further northwards. Unfortunately, the coastal sites surveyed in this study did not extend beyond the South and North Kimberley subregions; therefore, we could not examine whether this is a possible bioregional break in coastal fish between the Canning and Kimberley regions, and a subregional break between the North and Northeast Kimberley. However, this is the first report of a potential subregional break between coastal fish in the South Kimberley and North Kimberley regions.

4.2 | Elasmobranch compositional transitions across NWA

Detailed shark and ray fauna compositional data are underrepresented in NWA marine surveys, with scant information on populations and species compositions across the bioregion. Many elasmobranchs are not efficiently sampled by trawl surveys (Travers et al., 2012) and can elude visual observations in NWA, particularly taxa such as sawfish which are benthic in nature and prefer turbid
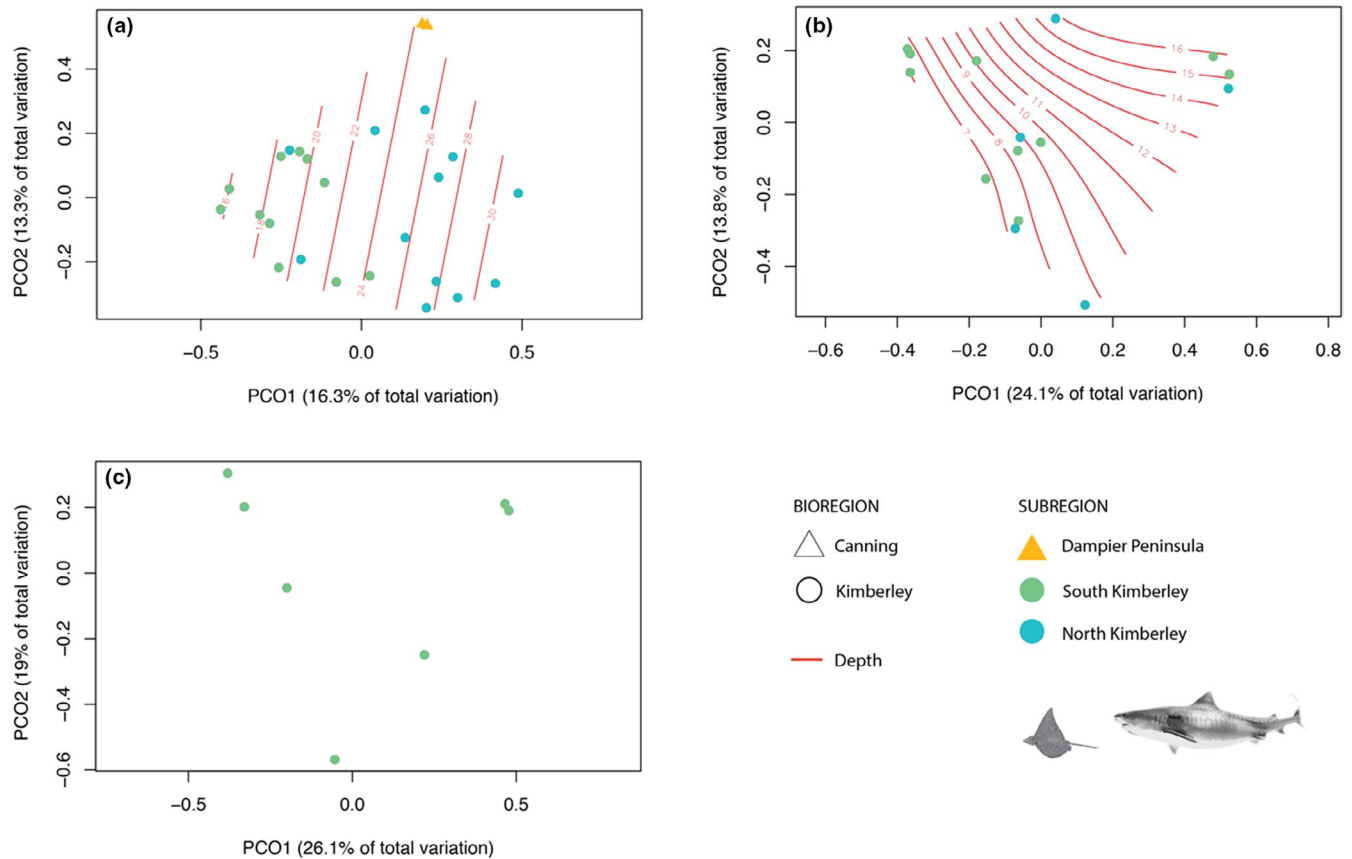
FIGURE 4 Principal coordinate analysis (PCO) of elasmobranch composition in (a) inshore, (b) coastal and (c) nearshore estuarine sites. Depth gradients are plotted if they are a significant predictor variable in the corresponding DistLM analyses. The proportion of variation explained by each axis is shown on the axis labels

environments (Simpfendorfer et al., 2016). With the exception of reef-associated species (MacNeil et al., 2020), existing compositional data from coastal species are based on limited, and invariably biased, observations from commercial fisheries (Braccini & Taylor, 2016; Field et al., 2012; McAuley et al., 2005). In examining shark and ray eDNA-derived compositional data across the Canning and Kimberley regions, we detected a significant subregional influence on inshore species. This reflected variation across the purported biogeographic break between the Dampier Peninsula (Canning) and South Kimberley and also between the South and North Kimberley regions.

This widespread subregional influence on inshore shark and ray composition across NWA was consistent with observations from elsewhere across northern Australia where composition and relative abundance have been shown to vary markedly at a range of spatial and temporal scales (Espinoza et al., 2014; Harry et al., 2011; Taylor & Bennett, 2013; White & Potter, 2004; Yates, Heupel, Tobin, Moore, et al., 2015; Yates et al., 2015a). Northern Australia has a comparatively high elasmobranch biodiversity that includes many large-bodied and highly mobile species (Last & Stevens, 2009). Variability in species composition is not only influenced by regional conditions, but also reflects the complex life-history strategies of many elasmobranchs that includes behaviour such as inshore nursery usage (Simpfendorfer &

Milward, 1993; Yates, Heupel, Tobin, Moore, et al., 2015; Yates et al., 2015b), partitioning by size and sex (Knip et al., 2012; Yates, Heupel, Tobin, Moore, et al., 2015), and seasonal migration between tropical and temperate waters (Braccini et al., 2018; Heupel et al., 2015). Disentangling such patterns is beyond the capability of presence–absence data alone; however, these data can nonetheless assist in corroborating existing patterns in composition as well as identify new ones.

The identification of subregional breaks (which additionally alludes to a biogeographical break) in inshore species across the Canning and Kimberley regions is consistent with observations from commercial shark fisheries in NWA, where historically there has been a shift in the main target species from blacktip (*C. tilstoni/C. limbatus*) and spot-tail sharks (*C. sorrah*) in the North Kimberley and Northern Territory, to sandbar sharks (*C. plumbeus*) in the South Kimberley, Canning, Pilbara and Gascoyne (Field et al., 2012; McAuley et al., 2007). Such observations are limited by confounding factors such as gear type and are also biased towards commercially valuable species (intermediate- to large-bodied sharks) (Bensley et al., 2010). The eDNA-derived compositional data lend support to the existence of multiple subregional breaks and suggest it may also extend to a broad range of shark and ray taxa. Given that we only detected two elasmobranch species in the Canning region however (post-subsampling), we do recommend that further in-depth

sampling is conducted to provide more definitive evidence of the purported biogeographic break on inshore elasmobranch species.

Interestingly, the coastal shark and ray assemblages in the South Kimberley and North Kimberley regions were solely influenced by site depth, unlike the inshore assemblage which exhibited subregional variation. The coastal elasmobranchs detected in this study are associated with a wide range of inhabited depths; some are commonly found in shallow coral and sandy areas, for example the blacktip reef shark (*C. melanopterus*), bull shark (*C. leucas*) and tawny nurse shark (*Nebrius ferrugineus*); others are found in deeper coral reef slopes, for example grey reef shark (*C. amblyrhynchos*) and whitetip reef shark (*Triaenodon obesus*), whilst others prefer neritic waters, for example the Australian sharpnose shark (*Rhizoprionodon taylori*), great hammerhead (*Sphyrna mokarran*) and scalloped hammerhead (*Sphyrna lewini*).

4.3 | A new approach for surveying endangered, elusive and data-deficient taxa in NWA

The coastline of NWA exhibits high turbidity resulting from immense tidal action and seasonal discharge from major rivers (Semeniuk, 1993; Thackway & Cresswell, 1998). This limits visibility and subsequently the application of visual surveillance techniques, such as UVCs, BRUVs and marine manta tows. Additionally, the widespread presence of saltwater crocodiles restricts diving even in inshore coral reef areas. Environmental DNA metabarcoding has thus provided an alternative biomonitoring approach which circumvents many of the logistical and safety limitations of working in this marine region. Our multi-marker eDNA metabarcoding survey has successfully amplified a wide range of bony fish and elasmobranchs, including 21 putative new occurrence records (requiring further validation), and a number of endangered, elusive and data-deficient taxa. Aquatic reptiles were under-detected in this study based on low detection rates, which was unexpected given the widespread distribution of saltwater crocodiles (*C. porosus*) and marine turtles (superfamily: Chelonioidea) across the Canning and Kimberley regions; this may reflect an emerging challenge in regard to the shedding of reptilian skin cells and subsequent detection with eDNA (Adams et al., 2019; see Section S3 for further discussion). Despite a low detection rate, this is the first study to our knowledge, to have detected crocodiles and sea snakes using an eDNA approach under field conditions.

A species-specific eDNA assay has previously been developed to detect the largetooth sawfish (*P. pristis*) across northern Australia (Simpfendorfer et al., 2016). A significant finding in our study was the detection of three out of the four globally endangered sawfish taxa (family: Pristidae) found in Australia using a metabarcoding approach. The largetooth sawfish is a euryhaline elasmobranch species that was once globally distributed in tropical marine, estuarine and freshwater environments of the Eastern and Western Atlantic, Eastern Pacific and Indo-West Pacific; however, population declines and extirpation have led to significant range contractions (Kyne, Carlson, et al., 2013). It is currently listed as Critically Endangered on the IUCN Red List of Threatened Species and is a protected species in Australia; Northern Australia may be the last viable stronghold for the Indo-Pacific population and likely comprises a large proportion of the remaining global population (Kyne, Carlson, et al., 2013; Last & Stevens, 2009). In Western Australia, this species has been identified in the King Sound, Fitzroy, Durack, Robinson and Ord Rivers, with eDNA detections this distribution now extends to the Gairdner River in the Southern Kimberley, and Robroy Reef, an inshore site in the North Kimberley.

The dwarf sawfish (*P. clavata*) and the knifetooth sawfish (*A. cuspidate*) are both currently listed as Endangered on the IUCN Red List and like their confamiliars have undergone significant, yet largely unquantified declines inside and outside of Australia (D'Anastasi et al., 2013; Kyne, Rigby, et al., 2013). Scattered records of the dwarf sawfish across the Indo-West Pacific indicate that it may have been widely distributed; however, there has been a lack of confirmed records outside of Australia since the 1800s (Kyne, Rigby, et al., 2013). Within Australia, the Kimberley and northern Pilbara regions represent significant strongholds for the dwarf sawfish; these include sites in the King Sound, Fitzroy, May and Robinson Rivers, Hall Point and Cape Kerauden (Stevens et al., 2008; Thorburn et al., 2008). Environmental DNA detection extends the distribution of dwarf sawfish to Wildcat Reef, George Water and the Glenelg River in the South Kimberley. The knifetooth sawfish distribution extends across the Indo-West Pacific from the Persian Gulf to Japan and the central coasts of western and eastern Australia (Last & Stevens, 2009); eDNA of knifetooth sawfish was detected in Cape Bougainville, Troughton Island, Freshwater Bay and Vansittart Bay in the North Kimberley.

The northern river shark (*G. garricki*) is considered a rare species with limited distribution information and population estimates available (Field et al., 2013). All identified populations are considered to be of high conservation value and as such, recreational fishing of the species is banned under Australian federal law. The detection of the northern river shark in this study extends its current known distribution in Western Australia from scattered sightings in the King Sound (Compagno et al., 2008; Thorburn & Morgan, 2004, 2005), Ord River, King River and Joseph Bonaparte Gulf (Pillans et al., 2009) to the Gairdner River and the Walcott River in the South Kimberley region. These additional distribution records of endangered elasmobranchs will contribute to recovery plans and management arrangements and are already contributing to locations where additional surveys will be undertaken.

5 | CONCLUSION

This large-scale eDNA metabarcoding study across the coastlines of North-western Australia was able to detect a purported marine biogeographic break between the Canning and Kimberley bioregions. This demonstrates that eDNA metabarcoding is a highly sensitive detection tool, capable of producing large amounts of high-resolution (e.g. to a species level) presence–absence data that can discern fine-scale patterns across large geographic regions. Further broad-scale applications of this technique could be used to potentially reveal marine biogeographic breaks in other regions. For example, significant phylogeographic structure in mantis shrimp and seahorses in south-east Asia is claimed to reflect historical oceanographic divisions; the former exhibits divergence along a sharp genetic break between the

Indian and Pacific Ocean regions (previously separated during the Last Glacial Maximum), whilst the latter is separated into east and west lineages reminiscent of the terrestrial Wallace's Line (Barber et al., 2000; Lourie & Vincent, 2004). Environmental DNA metabarcoding could be used to assess whether these phylogeographic breaks reflect wider biogeographic partitioning in marine community compositions.

The eDNA samples resulting from this study will be archived and available for further assay applications extending beyond the taxonomic groups targeted in this study. Additionally, our sequencing data can be retrospectively analysed with expanding databases to resolve ambiguous taxonomic assignments. Our georeferenced sites herein will be used as a baseline for future eDNA biomonitoring and notably will direct targeted surveying for the critically endangered elasmobranchs across NWA. We anticipate that eDNA metabarcoding will be integrated into broad-scale monitoring tool kits, particularly in northern Australia, where it circumvents many of the logistical and safety limitations of visual surveillance. At present, this technique is limited in its ability to provide quantitative data in relation to population sizes and biomass. However, its ability to produce multi-taxon and potentially even whole-ecosystem data, without the need for taxonomic expertise, is both time- and cost-efficient. Amidst global population declines and resource limitations, innovative approaches to whole-ecosystem and biodiversity surveying are required to underpin the best practice management of fisheries, tourism and commercial interests in this remote region of Australia. We advocate that eDNA offers a promising demand-driven solution that is fast gaining traction when planning and executing biodiversity surveys.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/ddi.13228.

DATA AVAILABILITY STATEMENT

Demultiplexed (unfiltered) metabarcoding sequencing data and taxonomic (read abundance) matrices are available for download on Dryad Digital Repository (https://doi.org/10.5061/dryad.8kprr4xmm).

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