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SPECIAL ISSUE Environmental DNA (eDNA) and Environmental RNA (eRNA)
in Ecotoxicology Studies and Environmental Risk Assessments

Environmental DNA 
Dedicated to the study and use of environmental DNA for basic and applied sciences

ORIGINAL ARTICLE 

Unveiling Structure of Tropical Estuarine Communities Through eDNA and Implications for Biomonitoring

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ABSTRACT

Tropical estuaries are hyper-diverse ecosystems, hosting essential habitats for freshwater, euryhaline, and marine life. Understanding how biological communities are distributed in these systems has long been a challenge because of their inherent dynamic nature and the diversity of interacting natural pressures and anthropogenic stressors they are subjected to. In addition, most studies focus on a single taxonomic group, hindering a comprehensive understanding of the interactive effects of natural and human-driven environmental variations on the different components of tropical estuarine biodiversity. In this study, we used environmental DNA (eDNA) metabarcoding to examine the structure of multi-taxonomic communities, from diatoms to fish, and their relationships with environmental drivers in three differentially impacted locations facing the Great Barrier Reef in Central Queensland (Australia). We first demonstrated that eDNA signals from sediment and water matrices provide complementary information and that both should be monitored for a more holistic understanding of community trajectories in anthropogenically impacted aquatic environments. We also observed that, independently of the taxonomic group considered, communities were primarily structured by the ecological conditions of the estuary. A within-estuary differentiation along an upstream–downstream gradient was detected but only for small-bodied organisms, which further adds credence to eDNA approaches as an ecologically relevant tool for monitoring fine-scale biodiversity patterns even in profoundly dynamic environments. Finally, the different communities exhibited contrasting response patterns, in terms of diversity, composition, and uniqueness, to the anthropogenic gradient. Hence, our findings emphasize the need for multi-taxonomic assessments, for which eDNA is well suited, to better understand the impacts of multiple stressors on biodiversity and thereby assist decision makers in the protection and management of tropical estuaries.

F. Viard, S.L. Simpson, and A.A. Chariton should be considered as joint senior author.

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1 | Introduction

Because of their unique location along the land-sea continuum in hyper-diverse regions, tropical estuaries host an extraordinary biodiversity, from freshwater to marine species (Twilley et al. 1996; Pearson et al. 2021). These highly dynamic systems provide many essential functions and services to humans, e.g., water purification, carbon storage, habitats, fish nurseries (Levin et al. 2001; Pearson et al. 2021). Globally, estuaries are facing increasing threats from the cumulative impacts of climate change and local anthropogenic pressures (O'Brien et al. 2019) that might have a wide spectrum of intertwined impacts on aquatic biodiversity and ecosystem processes (Johnston and Roberts 2009; O'Meara et al. 2017).

The naturally dynamic and transitional physico-chemical properties of estuaries, coupled with a potentially wide range of anthropogenic stressors, make assessing their biodiversity and condition particularly challenging. Tropical regions are indeed characterized by pronounced seasonal differences in precipitation, with variations in freshwater inputs playing a key role in structuring estuarine communities (Getz and Eckert 2022; Venarsky et al. 2022). Biological communities in the water column are typically distributed along an upstream–downstream gradient and driven by variations in the physicochemical properties of the water, especially salinity (Mariano and Barros 2015; Sheaves 2015). Whilst also affected by salinity and its correlates (e.g., pH), the composition of benthic biota is also driven by sediment composition (e.g., particle size and carbon content) (Chariton et al. 2010), as well as flow, especially during larval phases (Snelgrove and Butman 1994). Interacting with these natural pressures, local human activities are sources of multiple pressures, including vessel traffic, fishing, urbanization, and species invasion. However, from agriculture, industry, and urban development, a large range of contaminants are input to estuaries by land-based run-offs, and these stressors are frequently considered the most important threat (e.g., Halpern et al. 2008). In urban areas, contaminants can include complex mixtures of metals, nutrients, sewage, and organic chemicals, while in agricultural regions, pesticides, nutrients, and suspended sediments often constitute the dominant contaminant inputs impacting estuaries (Inglis and Kross 2000; Brodie et al. 2012). These contaminants affect the abundance, the diversity, and the distribution of estuarine biota, resulting in a strong anthropogenic imprint on community composition at the watershed level (Chariton et al. 2015). The ecotoxicological effects of metals and organic contaminants (e.g., pesticides) are numerous and diverse, being expressed from the sub-cellular to the ecosystem level (Tlili and Mouneyrac 2021). At the individual level, direct toxic effects depend on the taxonomic group and the chemical exposure (Stronkhorst et al. 2004; Campana et al. 2012). At a larger scale, they can have repercussions on population dynamics and ecological interactions between species, leading to long-lasting alteration of community composition and ecosystem functioning (Iliiff et al. 2019; DiBattista et al. 2020; Lewis et al. 2021). Similarly, eutrophication, related to increased nutrient concentration, and excessive sediment loads both increase turbidity and modify light availability to photosynthetic organisms, with bottom-up consequences on the entire food web (Lunt and Smee 2014). Algae blooms in eutrophic waters can have further widespread effects by dramatically decreasing

the dissolved oxygen available to heterotrophic organisms and by producing toxins that can harm other taxa (Paerl et al. 2016; Wurtsbaugh et al. 2019). Because of the complexity of these interacting processes, their combined impacts on aquatic biodiversity are difficult to predict and hardly generalizable.

In this context, routine biomonitoring is essential for assessing ecosystem health, understanding how biotic assemblages respond to human-induced stressors, and evaluating the ecological implications of on-ground management actions to restore the quality of estuarine habitats. Yet, biomonitoring in tropical estuarine ecosystems remains challenging, and a large fraction of the eukaryotic biodiversity, with the exception of fish, remains poorly described, even in well-studied hotspots (Mugnai et al. 2021). Hence, biomonitoring has traditionally been restricted to a few easily identifiable taxonomic groups that only capture a small fraction of the total biodiversity; despite the fact that different taxa and their associated communities respond differently to the same set of stressors (Kessler et al. 2009; Heino 2010; Donald et al. 2021). For example, diatoms are increasingly used as indicators of herbicide toxicity in aquatic ecosystems, but indirect impacts on higher trophic levels are highly context-dependent (Mensens et al. 2018; Fleeger 2020). Similarly, using a replicated whole-ecosystem experiment, Edge et al. (2020) showed that direct negative effects of a glyphosate-based herbicide on amphibian and invertebrate assemblages can be offset by indirect effects related to the reduction in the macrophyte cover caused by the herbicide applications that positively affect these animal communities. Such knowledge gaps on direct and indirect effects of stressors at the multi-taxonomic community level and potential inconsistencies in cross-taxon responses to environmental variations can lead to discordance in management decision making. This highlights the need for more holistic biomonitoring approaches in order to fully understand the ecological ramifications of multiple stressors on aquatic biodiversity (Westgate et al. 2014).

To address these limitations, a complementary tool to classical biomonitoring, metabarcoding based on environmental DNA (eDNA), emerged in the last decade for the multi-taxonomic assessment of biodiversity (Taberlet et al. 2012; Stat et al. 2017) and ecosystem health (Chariton et al. 2010, 2015). eDNA metabarcoding potentially allows the simultaneous characterization of all living assemblages, from microbes to fish, with the capacity to assist large-scale management actions and restoration efforts by increasing sampling efficiency and taxonomic coverage at a reduced cost (Nagarajan et al. 2022). As a side benefit, these comprehensive biodiversity eDNA-surveys can also help to track emerging disease (Thomas et al. 2022) and detect invasive species at early stages of the invasion process (Couton et al. 2022; Pearman et al. 2021). However, the dynamic hydrology of estuaries poses significant challenges for eDNA-based biomonitoring (Sanches and Schreier 2020). Freshwater flow and tides can indeed transport eDNA from its source (Kelly et al. 2018; Schwentner et al. 2021). eDNA retention time and dissemination also depend on the environmental matrix sampled. As such, several studies conducted in the marine realm have shown that taxa detections inferred from different matrices (typically sediment and water) are generally distinct and complement each other (Kozioł et al. 2019; Antich et al. 2021; Brandt et al. 2021). However, such comparisons between water

and sediment matrices have never been carried out in tropical estuaries, which are subjected to higher and more variable turbidity than most freshwater and marine environments, leading to a potentially higher signal congruency between matrices than previously reported (Nagarajan et al. 2022).

In this study, we used eDNA metabarcoding, derived from both sediments and water, for conducting a multi-taxonomic assessment of three tropical estuarine systems of varying ecological integrity adjacent to the Great Barrier Reef Marine Park (GBRMP) in Central Queensland, Australia. In that region, inputs of contaminants from land run-off, along with climate change, are considered to be the main threats for estuarine biodiversity and the coral reef conditions (Brodie et al. 2012; Kroon et al. 2020). We selected three estuaries as locations that receive low (Gregory River), medium (St Helen/Murray Creek) and high (Sandy Creek) inputs of anthropogenic runoff. We targeted all eukaryotes as well as three taxonomic groups that were selected for their distinct positions along the trophic network (diatoms, crustacea and fish). This design was used to evaluate three overarching hypotheses pertaining to the structure of estuarine communities and their drivers at different scales, the consistency of patterns among taxonomic groups, and the suitability of eDNA-based approaches for routine biomonitoring of tropical estuaries:

H1. *We assessed the complementarity of sedimentary eDNA and water eDNA signals for monitoring biodiversity. Water column communities might indeed respond more quickly to environmental changes than benthic ones. However, in these coastal waters subjected to high tides, mixing at the water/sediment interface is important, so we hypothesized that sampling one of two matrices might serve as a surrogate for monitoring the biodiversity of the other.*

H2. *We investigated the spatial structure of communities among and within estuaries, and their relationships with natural and anthropogenic drivers. Tropical estuaries are subjected to high seasonality with massive contaminant inputs during the wet season, but legacy effects of contamination can be long-lasting and shape communities all year long. Therefore, we hypothesized that, at the scale of the study area, community composition should be primarily structured by estuaries, mirroring the different levels of anthropogenic pressure these estuaries are subjected to (i.e., their ecological conditions).*

H3. *We evaluated the consistency of eDNA-based biodiversity metrics across taxonomic groups. We hypothesized that the different communities would exhibit similar trends in terms of alpha-, beta-diversity and uniqueness patterns being members of an interacting biotic network.*

2 | Material and Methods

2.1 | Study Sites

This study was conducted in three tropical estuaries in the Mackay-Whitsunday region of Central eastern Queensland, Australia, respectively subjected to low, medium, and high levels of human disturbances: Gregory River, St Helens/Murray

Creek, and Sandy Creek (Figure 1, Data S1). In the Mackay-Whitsunday region, sugarcane production, along with cattle grazing and urban development, delivers high loads of pesticides, nutrients, and sediments to coastal waters, and this pollution is considered the second main threat to the Great Barrier Reef (Brodie et al. 2012; GBRMPA 2019). The mean annual rainfall is ~1540 mm with high seasonal variability. Most of the precipitation (> 75%) and contaminant runoff from catchment to coastal waters occur from December to March (summer–spring period; Australian Bureau of Meteorology 2022). Sample collection was conducted during the late dry season from November 26 to 30, 2018, and the accumulated rainfall over the 30 days prior to sampling was unseasonably low, ranging between 7 and 16 mm.

The estuaries were located ~50 km apart and capture a range of ecological conditions, reflecting differences in catchment land uses and associated contaminant run-off profiles (Figure 1). Two of the estuaries, Gregory River and St Helens/Murray Creek, are located within the boundaries of the Great Barrier Reef Coast Marine Park, a State marine park that runs the full length of the Great Barrier Reef and provides protection for Queensland tidal lands and tidal waters. Long-term monitoring of estuarine conditions has shown that the Gregory River (low-impacted site) has historically had the highest water quality of all the estuaries monitored in the Mackay-Whitsunday region, categorized as having both good water and habitat quality and limited contaminant run-off (Mackay-Whitsunday-Isaac Healthy Rivers to Reef Partnership 2020). Most of the Gregory River estuary is bordered by low-intensity grazing, with a former prawn farm near the mouth. The St. Helens/Murray Creek estuary (medium-impacted site) is categorized as a moderately healthy ecosystem, in which nutrient concentrations, especially nitrogen, often exceed national guidelines, but concentrations of other monitored contaminants are limited (Mackay-Whitsunday-Isaac Healthy Rivers to Reef Partnership 2020). In contrast, elevated concentrations of nutrients and pesticides are regularly monitored in the third estuary, considered highly impacted, Sandy Creek (Mackay-Whitsunday-Isaac Healthy Rivers to Reef Partnership 2020). This system has consistently the highest concentrations of pesticides of any monitored catchment draining into the Great Barrier Reef (Smith et al. 2012; Wallace et al. 2016; Warne et al. 2020).

2.2 | eDNA Sample Collection

Withing each estuary, 10 sites, each located ~1 km apart, were sampled from the mouth in an upstream direction (total n sites = 30; Figure 1c). At the time of sampling, during the dry season, all the sites were classified as having marine conditions, with salinity ~35 PSU. At each site, water samples were first collected in triplicate for eDNA analyses, and three sediment cores were sampled afterwards. For water eDNA analyses, three 2-L water samples were collected in sterile Whirl-Pak bags (Nasco, America) and immediately placed on ice for transportation. Sediment samples were collected using a corer or grab sampling device, depending on the sediment granularity ($n = 90$). The equipment was sterilized (bleached and rinsed) between sites to avoid cross-contamination (Chariton et al. 2015; Zinger et al. 2019). The surface layer (~5 cm), the most biologically active

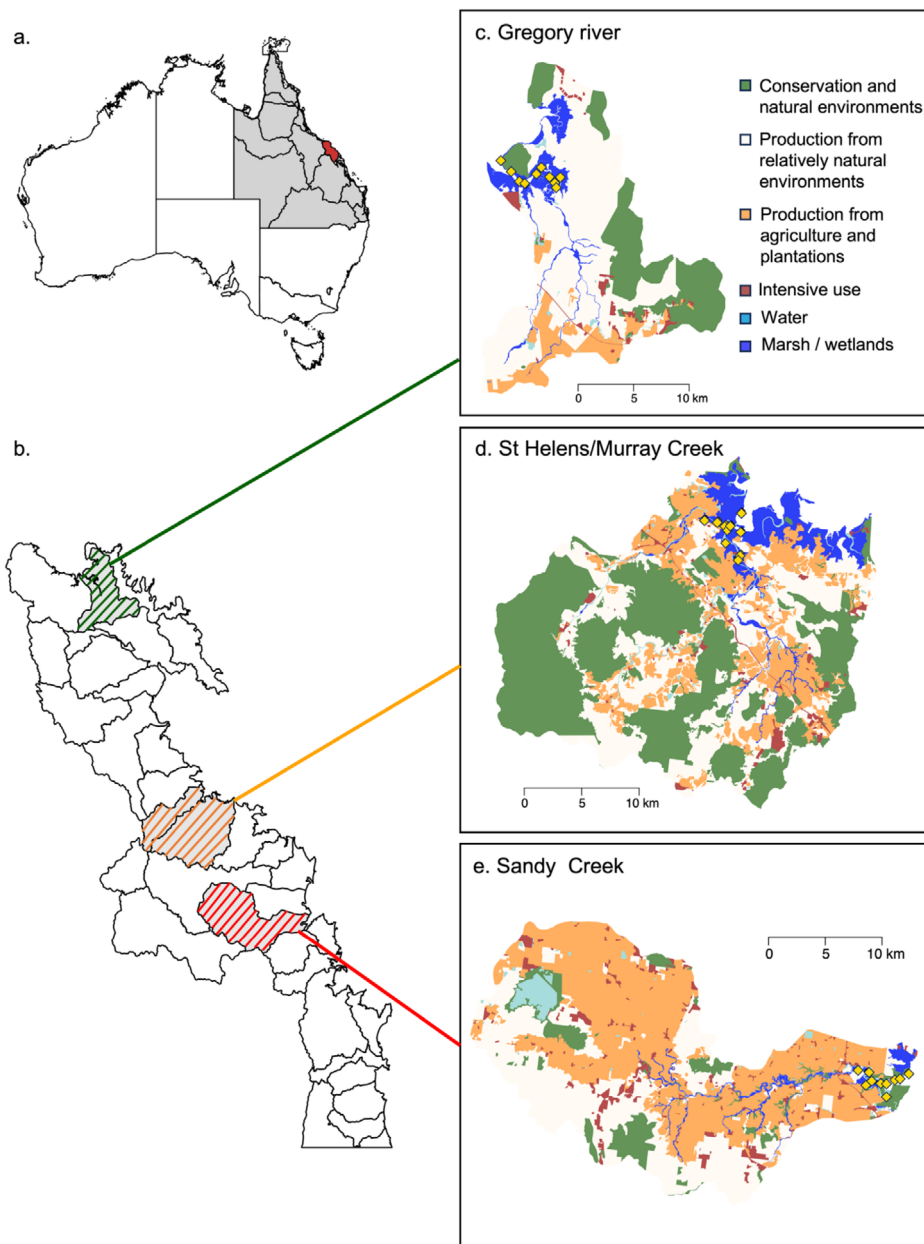


FIGURE 1 | Study area and sampling locations. (a) Location of the Mackay–Whitsunday natural resource management (NRM) region, Queensland, Australia. (b) River catchments within the Mackay–Whitsunday NRM region, catchments corresponding to estuaries investigated in this study are colored in green (Gregory River), orange (St Helens/Murray Creek) and red (Sandy Creek). (c–e) Land use in the catchment of each estuary investigated: Gregory River (c), St Helens/Murray Creek (d) and Sandy Creek (e); golden diamonds correspond to sampling points on the waterways (10 sites per estuary). At each site, three 2-L-samples of surface water and three surface sediment samples (5–10 cm) were collected.

part of the sediment (Simpson and Batley 2016), was scraped with sterile tools and transferred into a clean 50-mL falcon tube. All field samples were kept refrigerated until further processing.

On the same day as sampling, upon return to the field laboratory, each sediment sample to be used for eDNA analyses was homogenized, sub-sampled into 5-mL tubes, and kept frozen until DNA extraction. One liter of each water sample (i.e., a total of 3 L per site) was filtered on two 0.45 μm pore-size cellulose nitrate membranes (Djurhuus et al. 2017; Hinlo et al. 2017; Kumar et al. 2022) using a peristaltic pump (Sentino microbiology pump, Pall Life Sciences, MI, USA) within 24 h of collection (Stat et al. 2017). All materials were bleached (0.5% solution)

and rinsed with deionized water prior to filtering and between samples from each site to avoid cross-contamination (Zinger et al. 2019). A minimum of two blanks per estuary was included to serve as controls for monitoring potential contaminations during this procedure by filtering DNA-free molecular-grade water. Membranes were then placed into sterilized 2-mL tubes and frozen until DNA extraction.

Additional samples and measurements were collected for determining physico-chemical characteristics of water and sediment (hereafter called “environmental parameters”) and for conducting contaminant analyses (“anthropogenic stressors,” i.e., nutrients, pesticides and metals; see below).

2.3 | Physico-Chemical Measurements and Contaminant Analyses

Environmental parameters of the water column (including salinity, pH, dissolved oxygen, turbidity, chlorophyll-a and temperature) were measured using a calibrated EXO2 YSI multiparameter sonde (YSI, Yellow Springs, OH, USA). The sonde (probe) was drowned slowly in the water column until reaching the bottom; values were recorded every 5 s and then averaged per site. After collecting water samples for eDNA analyses, standard sampling protocols (Queensland Government Department of Environment and Science [DES]) were followed for determining contaminant (nutrient and pesticide) concentrations in the water column (DES 2018): (i) a 250-mL HDPE bottle was filled with water after three successive rinses with the sample water for total nutrient analysis; (ii) a 60-mL portion of water taken from this bottle was filtered through a sterilized 0.45- μ m Minisart cellulose acetate syringe filter (Sartorius, Germany) into a 125-mL HDPE bottle for dissolved nutrient analysis; (iii) a 200-mL hexane-washed amber bottle was filled with surface water for pesticide analyses. Water samples for nutrient and pesticide analyses were placed on ice in the field and immediately refrigerated (pesticide) or frozen (nutrients) upon returning to the field laboratory until analysis.

At each site, a composite sediment sample was prepared from sub-samples of the three cores used for eDNA analyses (see above), and this composite sample was transported on ice to the laboratory for sediment physico-chemical and contaminant analyses. The sediment composite samples were then split into three portions for further analyses: (i) one was kept refrigerated for determining total organic carbon content and particle size distribution (granulometry), (ii) a second one was placed into an amber jar and frozen at -20°C for pesticide analysis, and (iii) a third one was placed into a polycarbonate jar and kept refrigerated for metal analyses.

Total organic carbon analyses were performed using a high temperature total organic carbon analyzer (Dohrmann DC-190, Teledyne Tekmar, Mason, OH, USA; Chariton et al. 2010) and particle size analysis was conducted by successive sieving through 500-, 180-, and 63- μ m meshes and gravimetry. Four fraction sizes were defined to characterize sediment grain size: $< 63\ \mu\text{m}$, 63–180 μm , 180–500 μm and $> 500\ \mu\text{m}$.

Metal concentrations in sediments were determined at CSIRO using a dilute-acid (1M HCl) extractable metals method (Chariton et al. 2010). Pesticide analyses in both water and sediment samples were performed by the Queensland Government, Forensic and Scientific Services laboratory. Pesticides in water samples were analyzed by direct injection via liquid chromatography with tandem mass spectrometry (LC–MS–MS) while pesticides in sediment samples underwent a solvent extraction followed by QuEChERS solid phase cleanup prior to the LC–MS–MS analysis. Analysis of nutrients in water was carried out by the DES Chemistry Centre laboratory. Total and dissolved organic carbon were measured using a non-dispersive infrared sensor (NDIS). Total Kjeldahl values for nitrogen and phosphorus were measured through low-level atomic absorption (AA) analyses. All analyses were accompanied by quality assurance measures that included regular analyses of sample blanks,

duplicates, surrogate spike recoveries, and certified reference material where available (Chariton et al. 2010).

Metal concentrations in sediments were either below the detection limit or at least one order of magnitude below the guideline values (ANZG 2018) and therefore not included in subsequent analyses (Data S1). Similarly, pesticide concentrations in sediments and surface waters were excluded because they were all under the limit of detection threshold, except in a few sites—mostly in Sandy Creek—where traces of atrazine, diuron, and hexazinone were detected in the water but at concentrations considered not to be harmful for aquatic wildlife (ANZG 2018). Mean \pm SEM (standard error to the mean) values of environmental parameters and anthropogenic stressors per estuary are provided in Table S1.

2.4 | DNA Extraction, PCR Amplification and Sequencing

Environmental DNA analyses were performed in a dedicated facility (Environmental DNA and Biomonitoring Lab) at Macquarie University, Sydney. Water eDNA was extracted from filter papers using PowerWater kits (Qiagen, Hilden, Germany; Djurhuus et al. 2017; Lear et al. 2018; Kumar et al. 2022). Before starting, filter papers were cut into small pieces using sterile tools; the extraction was then carried out following the manufacturer's instructions with the following exception: samples were initially vortexed into the PowerWater DNA Bead Tube with Solution PW1 for 10 min instead of 5 min, and the tubes were rotated every 2.5 min to ensure adequate mixing. Sediment DNA was extracted from ~ 0.5 g of sediment using DNeasy PowerSoil kits (Qiagen; Lear et al. 2018; Pearman et al. 2020), following the manufacturer's instructions. Multiple extraction controls were included (one per batch of ~ 15 extractions) using DNA-free water. DNA extracts were then stored at -20°C .

Different primer pairs were employed to PCR-amplify taxonomically informative DNA markers. One primer pair was used to get a broad picture over uni- and multi-cellular eukaryotes (18S nuclear rRNA V7; Hardy et al. 2010), and three to specifically target diatoms (18S nuclear rDNA V4; this study, see also Taberlet et al. 2018), crustaceans (16S rRNA; Berry et al. 2017) and fish (12S mitochondrial rDNA; Valentini et al. 2016). Primer sequences and characteristics of these markers are provided in Table 1. Eight base pair (bp) tags, each differing by at least 5 nucleotides, were added to the 5' end of each primer to enable the multiplexing of multiple PCR products into the same library before high-throughput sequencing (Binladen et al. 2007; Valentini et al. 2009). PCR amplifications were performed in triplicate to monitor reproducibility of results and stochasticity of the PCR and sequencing processes (Ficetola et al. 2015; Zinger et al. 2019). PCR reactions were carried out in a 15- μ L reaction volume containing 1 \times of AmpliTaq Gold 360 MasterMix (Thermo Fisher Scientific, Waltham, MA, USA), 0.4 μM of each primer, and 2 μL of template DNA. Thermocycling conditions were adjusted for each primer pair as described in Table 1. Along with field and extraction blanks, numerous PCR controls (using DNA-free water instead of DNA) and positive controls were amplified and later sequenced. The positive controls comprised mock

TABLE 1 | Primer pairs employed in this study and their characteristics.

Taxonomic group	Marker name	Gene region	Primers sequence	Mean amplicon length (bp)	References	Initial denaturation	nb. of cycles	Thermocycling conditions			
								Denaturation	Hybridation	Elongation	Final elongation
Eukaryota	Euka01	18S rDNA (V7)	F: 5'-TGGTCATGGCGTTCTTAGT-3' R: 5'-CATCTAAGGGCATCACAGACC-3'	~161	Hardy et al. (2010)	95°C for 10 min	×40	94°C for 1 min	50°C for 30 s	72°C for 1 min 1 min 30 s	72°C for 10 min
Bacillariophyta	Baci01_mod	18S nuclear rDNA (V4)	F: 5'-TCCAGCTCCAATAGCCGTA-3' R: 5'-AACACTCTAATTTTTCACAGTA-3'	~150	This study ^a	95°C for 10 min	×40	95°C for 30 s	58°C for 30 s	72°C for 1 min	72°C for 7 min
Crustacea	Crust16S	16 rRNA	F: 5'-GGGACGATAAGACCCCTATA-3' R: 5'-ATTACGCTGTATCCCTAAAG-3'	~170	Berry et al. (2017)	95°C for 10 min	×40	95°C for 30 s	51°C for 30 s	72°C for 45 s	72°C for 10 min
Teleostei	Tele01	12S mitochondrial rDNA	F: 5'-ACACCCCGCTCACTCT-3' R: 5'-CTTCCGGTACACTTACCATG-3'	~64	Valentini et al. (2016)	95°C for 10 min	×50	95°C for 30 s	55°C for 30 s	72°C for 45 s	72°C for 10 min

^aThese primers overlap the Baci01 primers in Taberlet et al. (2018).

communities made from various concentrations of the same set of 12 species spanning a wide range of taxonomic groups, except diatoms (Table S2). These mock communities have been later used to control for PCR amplification success and validate the bioinformatic filtering process (Table S2).

The concentration of eukaryotes amplicons was determined using Quant-iT PicoGreen dsDNA Reagent assays (Thermo Fisher Scientific) on a PHERAstar FSX plate reader before pooling PCR products in equimolarity (Harris et al. 2010); for low concentrated samples and blanks, a volume of 10 µL was added. For other markers, PCR products were pooled by mixing an equal volume for all samples and blanks. Pooled amplicons were purified with AMPure XP beads (Beckman-Coulter, Brea, CA, USA). One library per marker was constructed using a TruSeq DNA PCR-free protocol. Library preparation and paired-end sequencing were performed at the Ramaciotti Centre for Genomics (University of New South Wales, Sydney, Australia). The eukaryote library was sequenced separately on an Illumina MiSeq platform (2×250 bp paired-end) while those for other groups were sequenced collectively on an Illumina NextSeq 100 (2×150 bp paired-end).

2.5 | Data Processing

Raw sequencing data were first curated using OBITools v.1.2 (Boyer et al. 2016). Paired-end reads were merged using the *illumina-paired-end* command, and sequences with a low alignment-quality score (< 40, the value corresponding to perfect alignment between the last 10 bases of each read) were discarded. Consensus sequences were then assigned to their original samples from the 8-bp tags attached to the primers using the *ngsfilter* command (with default parameters allowing zero errors on tags and a maximum of two errors on primers). We then discarded sequences with ambiguous nucleotides and those with a size outside the expected length range (i.e., 40–440 bp for eukaryotes, 100–240 bp for diatoms, 100–600 bp for crustaceans, 40–100 bp for fish) using *obigrep*. Identical sequences were merged with the *obiuniq* command, which retains information about their occurrence in each sample, and sequences with < 10 reads over the entire dataset were removed. Taxonomic assignment was performed using the *ecotag* command against *in silico* reference databases specific to each marker, generated with the *ecoPCR* program (Ficetola et al. 2010) from the ENA (European Nucleotide Archive) database (release r140), hosted at EMBL-EBI. Sequences not assigned to the correct taxonomic group to which they should belong (i.e., either eukaryote, bacillariophyta, crustacea or teleost depending on the marker) were discarded using *obigrep*. Molecular Operational Taxonomic Units (mOTUs) were created using the *Sumacust* program (Mercier et al. 2013) with a 97% similarity threshold (and the -R parameter set to 0.25). Fasta files were then converted into a sequence-by-sample matrix using the *obitab* command.

Additional filtering steps were performed in R version 4.0.3 using custom-made scripts (R Core Team 2022). We first discarded PCR products with low numbers of reads. For this, we compared the density distribution of the log-transformed number of reads in negative controls and in true samples

within each library, using the intersection of the two distributions as a threshold (Pansu et al. 2022). Because of differences in sequencing depths among libraries, this procedure led to the removal of samples with less than 13,324, 32,220, 741, and 3354 reads for eukaryote, diatom, crustacea, and fish, respectively. We also removed putative contaminants by discarding any sequence that had its maximal average relative read abundance (RRA) over the whole dataset in negative controls rather than in true samples (Zinger et al. 2021); so were those with a taxonomic assignment score < 70% which were considered likely to be chimaeras and/or highly degraded sequences. We then merged sequences into mOTUs based on *Sumacust* results and assigned to each mOTU the taxonomic assignment of the most common sequence composing it. Next, we discarded PCR replicates with non-reproducible results (i.e., with a too divergent composition in terms of mOTUs). For each library, we iteratively determined the density distributions of within- and between-sample Bray–Curtis distances (based on their reads composition) and discarded replicates that fell within the distribution of between-sample distances, the threshold being defined as the intersection of the two density distributions (Pansu et al. 2022). This process was iterated until no further replicate was removed. Then, we averaged the number of reads among replicates from the same sample and retained only sequences that represented at least 0.1% in at least one sample. Last, to reduce the impact of low-abundance false positives that can arise from tag-jumps during Illumina sequencing, we removed sequences representing < 0.1% of reads in each sample.

2.6 | Statistical Analyses

Prior to analysis, abundance data (i.e., reads counts) were converted into presence/absence because eDNA-based relative abundance data is heavily biased by differences in body size and biomass turnover among taxa as well as amplification biases (Chariton 2012); a pattern otherwise observed in our mock communities. However, for comparison purposes, analyses were also run using Hellinger-transformed data for groups hosting mostly micro-organisms (diatoms and eukaryotes) and provided qualitatively similar results (see Figures S5–S7).

Except for fish, taxonomic assignment provided reliable information only at a high taxonomic level. Since species identification was not the objective of this study, we performed all analyses at the mOTU level, unless otherwise specified. To assess the complementarity between water eDNA and sediment eDNA, for each taxonomic group (except fish, which were examined only through water eDNA analyses), we calculated the Sørensen compositional dissimilarity index, based on presence/absence data, between each pair of samples. Dissimilarity matrices were then ordinated using a principal coordinate analysis (PCoA). We further used perMANOVA in *vegan* (Oksanen et al. 2022) to test for significant differences among estuaries and sample matrices (i.e., sediment vs. water). mOTU sharing among matrices and estuaries was visualized using the *upset* tool in the *UpSetR* package, allowing us to represent intersections between more than 4 sets (i.e., an alternative to Venn diagram for 4+ sets; Conway et al. 2017). Finally, we used rarefaction curves implemented

in *vegan* to estimate the mOTU richness observed within each matrix per estuary according to the sampling effort (here, the number of collected samples).

At the regional scale, encompassing all three estuaries, we used distance-based RDA (db-RDA) for determining natural and anthropogenic drivers of community composition for water column and sediment communities, independently. Prior to this analysis, we calculated Sørensen distance matrices between pairs of samples for each group, separately. Values were then averaged by site for db-RDA analyses in *vegan*. Highly collinear variables (Variance Inflation Factor > 10) were identified using the *vif.cca* function implemented in *vegan*, leading us to discard total N, dissolved inorganic nitrogen (DIN) and chlorophyll concentrations from db-RDA analyses. For each taxonomic group and matrix, the best model was selected using a forward variable selection procedure, implemented in *vegan*, with 10,000 permutations. We then used nested perMANOVA analyses to estimate the proportion of variance explained by the variation between estuaries (inter-estuary variation) and the variation between sites within an estuary (intra-estuary variation), respectively. At the estuary scale, we further assessed if communities were structured along an upstream-downstream gradient that could reflect either within-estuary variation in environmental parameters and/or differentiation by distance. This analysis was also used to test for the ability of eDNA-based methods to capture small-scale variations even in highly dynamic environments. For this, we ran a distance-decay analysis per habitat for each community; Mantel tests were employed to assess the correlation between distances in community composition and geographical distances.

To characterize more precisely the differential responses of communities to environmental parameters and anthropogenic stressors, we calculated the contribution of each site to the beta-diversity ('LCBD' for 'local contribution to beta-diversity'; Legendre and De Cáceres 2013) for identifying sites in which communities exhibited high compositional uniqueness. LCBD values and their significance were calculated at the estuary-scale for each group and each habitat (sediment or water column) independently using the *beta.div* function in the *adespatial* R package (Dray et al. 2023) with 10,000 permutations. We then investigated to what extent local conditions could lead to congruent patterns of community uniqueness between taxonomic groups (i.e., if sites that host a community of high uniqueness for a given taxonomic group tend to also host unique communities for other groups). For this we assessed the correlation between LCBD values obtained for each taxonomic group in each habitat using Spearman rank correlation tests.

3 | Results

Filtered datasets contained 2052 mOTUs for eukaryotes, 792 for diatoms, 200 for fish, and 109 for crustaceans (Table S3; Figure S1). More than 95% of original samples passed the filtering procedure for eukaryotes and diatoms, but the percentage was much lower for crustaceans (43%) and fish (60%, only water samples; Table S3). For these two latter groups, most samples were discarded, as PCR replicates did not produce highly reproducible results according to our filtering procedure; note that

this filtering did not result in any specific bias towards a specific location of matrix types (except for fish, for which up to twice as many water samples were retained in St Helens/Murray than in other estuaries).

3.1 | Complementarity of Water and Sediment Matrices for eDNA-Based Assessment of Community Composition

The taxonomic composition of communities in both compartments is described in detail in Results and Discussion S1. We here focus on the comparison of these communities between the two matrices used (i.e., water vs. sediment). Interestingly, the study samples were primarily discriminated by the type of matrix from which the DNA was extracted (i.e., water or sediment) for both eukaryotes (perMANOVA, $\text{pseudo-}F_{1,169} = 72.65$, $r^2 = 0.30$, $p < 0.001$) and diatoms (perMANOVA, $\text{pseudo-}F_{1,167} = 104.31$, $r^2 = 0.38$, $p < 0.001$), and secondarily by the estuary of origin (perMANOVA, $\text{pseudo-}F_{2,168} = 11.88$, $r^2 = 0.12$, $p < 0.001$ for eukaryotes and $\text{pseudo-}F_{2,166} = 21.94$, $r^2 = 0.21$, $p < 0.001$ for diatoms; Figure 2a,b), invalidating our hypothesis H1. For both taxonomic groups, this strong differentiation in community composition between water and sediment samples is further illustrated by the low number of mOTUs they have in common (Figure S2a,b).

In contrast, crustacean communities were less differentiated according to the estuary of origin or the type of matrix, the latter playing a negligible role (perMANOVA, $\text{pseudo-}F_{1,74} = 4.78$, $r^2 = 0.06$, $p < 0.001$; Figure 2c). A relatively high number of mOTUs were actually unique to a given “estuary-matrix” combination, pointing towards sample specificities that cannot be explained by only one of these two factors (Figure S2c) and might reflect the fact that our sampling effort did not allow us to retrieve the whole diversity of crustacea in either of the two matrices (Figure S3c).

In terms of diversity, the number of eukaryotic mOTUs retrieved in sediments was twice that found in the water samples (Table S3 and Figures S2–S4). Similarly, diatom richness was four to five times greater in sediments than in water. In contrast, crustacean richness was marginally but consistently higher in water samples (see also Results and Discussion S1).

3.2 | Community Structure Among and Within Estuaries, and Their Relationships With Natural and Anthropogenic Drivers

When water column and sediment samples were analyzed independently, each taxonomic group was primarily discriminated by its estuary of origin in both compartments

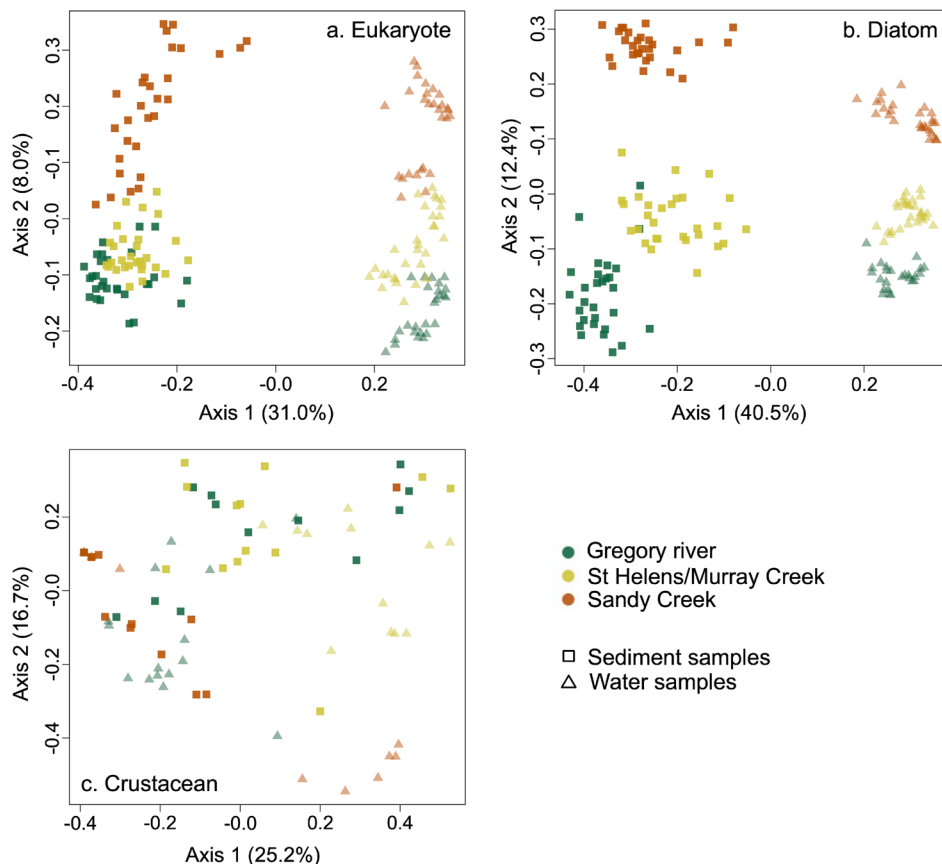


FIGURE 2 | (Dis-)similarity in mOTU composition between samples according to the sample type (water and sediment) and the estuary of origin for (a) eukaryotes, (b) diatoms and (c) crustaceans. Each ordination represents a principal coordinate analysis (PCoA) for a given taxonomic group, based on the Sorensen distance (presence/absence data) between samples. The shape of points corresponds to the type of sample (triangle for the water column, rectangle for sediment). Colors reflect the estuary of origin (green for Gregory River, yellow for St Helens/Murray Creek and brown for Sandy Creek). For both eukaryote and diatoms, the first axis of each PCoA discriminates water and sediment samples.

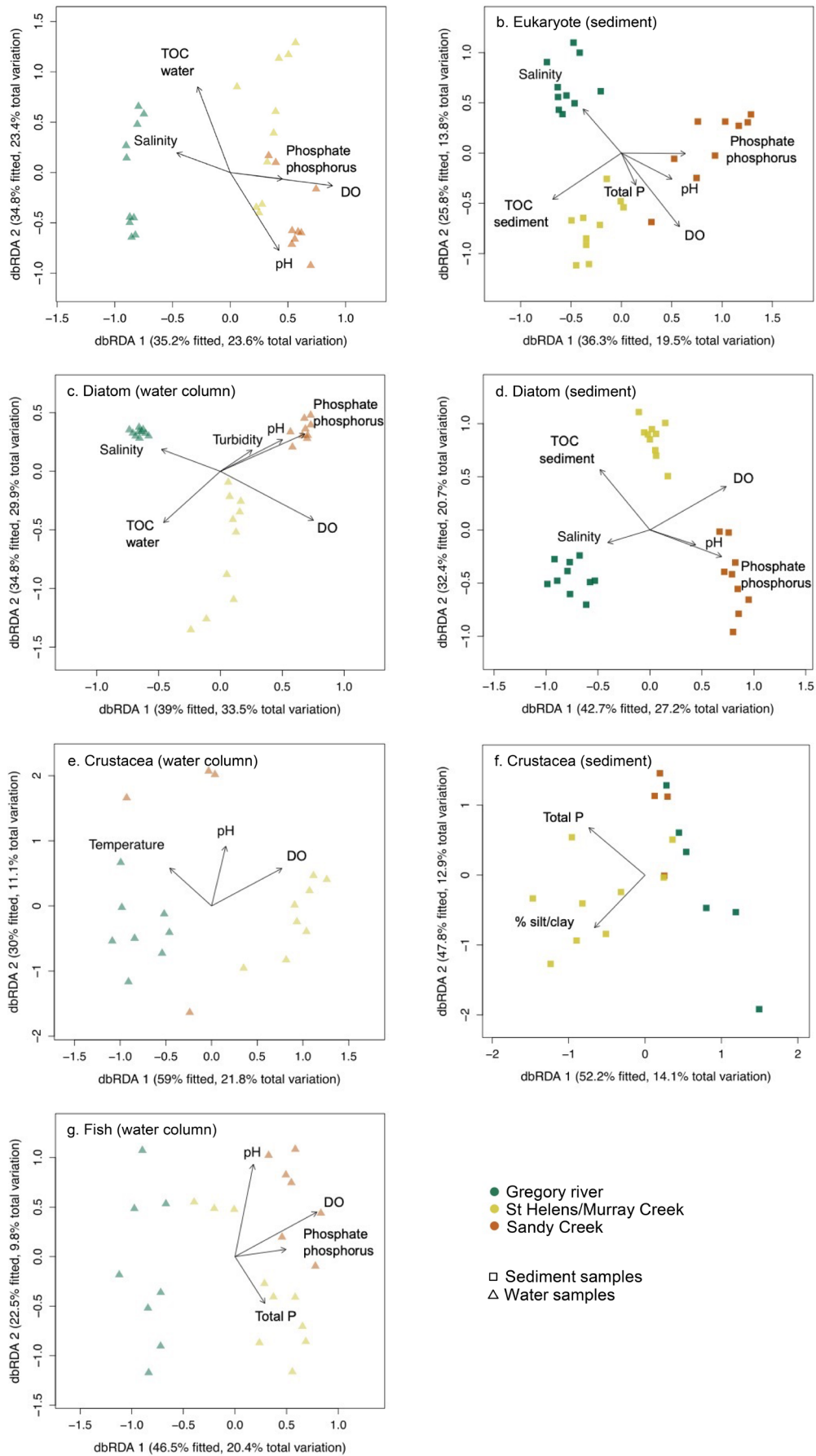


FIGURE 3 | Legend on next page.

FIGURE 3 | Communities within each habitat are structured per estuary, independently of the taxonomic group. Ordinations represent distance-based redundancy analysis (db-RDA) per habitat for each taxonomic group. db-RDA were computed from the average Bray–Curtis distance (based on presence-absence data) between sites for eukaryote (a, b), diatom (c, d), crustacean (e, f) and fish (g). Fish communities were determined from water samples only. Only significant environmental variables ($p < 0.05$) are represented: DO (dissolved oxygen, % saturation), pH, phosphate phosphorus, salinity, temperature, total P (total phosphorus), TOC water (total organic carbon in the water column), TOC sediment (total organic carbon in sediments), % silt/clay (percent of silt and clay in sediment, i.e., a proxy for the granulometry), CaCO_3 (calcium carbonate in sediment). For each taxonomic group and matrix, the best model was selected using a forward variable selection procedure.

TABLE 2 | Percentage of variance in community composition explained by the estuary and by sites within estuaries, respectively, determined by nested perMANOVA analyses on presence-absence data.

	df	Sum of squares	F	p	Variation explained (%)
Eukaryote					
Water column					
Estuary	2	0.059	10.624	0.001	38.5
Site	27	0.075	7.842	0.001	49.0
Residuals	54	0.019			12.5
Sediment					
Estuary	2	0.050	6.216	0.001	19.8
Site	27	0.108	2.453	0.001	43.1
Residuals	57	0.093			37.1
Diatom					
Water column					
Estuary	2	0.048	18.545	0.001	51.3
Site	27	0.035	6.465	0.001	37.4
Residuals	53	0.011			11.3
Sediment					
Estuary	2	0.073	13.998	0.001	39.0
Site	27	0.071	3.334	0.001	37.6
Residuals	56	0.044			23.4
Crustacea					
Water column					
Estuary	2	0.106	5.057	0.001	29.6
Site	18	0.188	2.463	0.001	52.6
Residuals	15	0.064			17.8
Sediment					
Estuary	2	0.064	3.859	0.001	21.5
Site	17	0.141	1.784	0.001	47.3
Residuals	20	0.093			31.2
Fish					
Water column					
Estuary	2	0.040	4.533	0.001	19.4
Site	23	0.101	1.846	0.001	49.3
Residuals	27	0.064			31.3

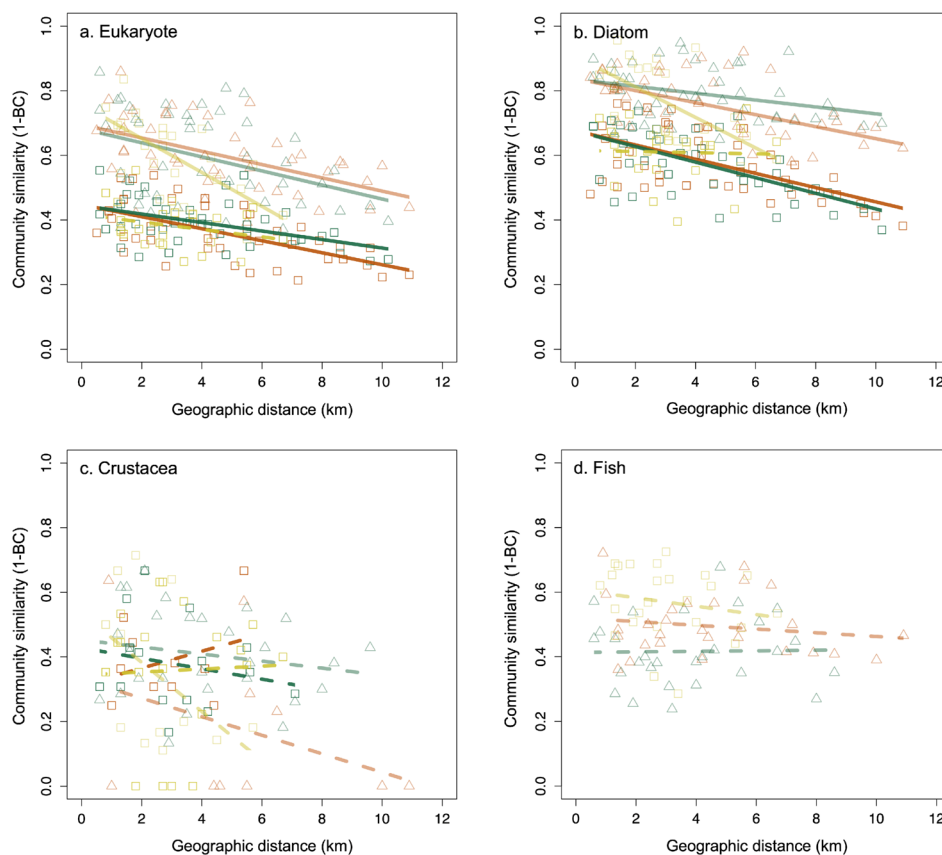


FIGURE 4 | Distance-decay in community composition similarity within estuaries. Community similarity decreased with geographic distance for (a) eukaryotes and (b) diatoms but not for (c) crustaceans and (d) fish. Community similarity was measured as 1-Bray–Curtis distance (presence-absence data) between pairs of sampling points within a single estuary. For St Helens/Murray Creek, only points belonging to the same river flow were plotted. The shape of points and line color shade corresponds to the habitat sampled (light-colored triangle for the water column and dark-colored rectangles for sediment) while colors reflect the estuary of origin as in previous figure. Plain and dotted lines represent significant ($p < 0.05$, Mantel test) and non-significant correlation between community composition dissimilarity and geographic distance matrices, respectively.

(Figure 3 and Figure S5), as expected in H2. The estuary of origin explained 39% and 51% of the variation in community composition for eukaryotes and diatoms in the water samples, respectively (vs. 49% and 37% for the location within estuary; nested perMANOVA $p < 0.001$; Table 2 and Table S5). This proportion was lower for sediment communities, ranging between 20% for eukaryotes and 39% for diatoms (vs. 43% and 38% for the location within estuary; nested perMANOVA $p < 0.001$). For crustaceans and fish communities, 19%–30% of the variation was explained by the estuary of origin (vs. 47%–53% for the location within estuary; nested perMANOVA $p < 0.001$; Table 2).

Although communities were primarily structured by estuaries, we observed a significant distance-decay for eukaryote and diatom communities within most estuaries (Mantel test $r = 0.33$ – 0.69 , $p < 0.05$; Figure 4 and Figure S6), indicating an upstream–downstream gradient in community composition. The only exceptions were eukaryote and diatom communities in sediments of St Helens/Murray ($p > 0.05$, Mantel test $r = 0.27$ and 0.03 for eukaryotes and diatoms). In general, water samples were more similar within estuaries than sediment samples for eukaryotes and diatoms. Conversely to small-bodied organisms, no significant distance-decay was observed for larger organisms (fish and crustaceans, Mantel test $p > 0.05$).

Community composition among and within estuaries was associated with a set of environmental characteristics, some being specific to the taxonomic group and/or matrix (Figure 3 and Figure S5). Water acidity (pH) and dissolved oxygen (DO) were correlated with the composition of all groups, in both the water column and sediments ($p < 0.05$ in db-RDA analyses), except for crustaceans in sediments. Similarly, the best models, determined individually for each group and matrix, showed that all communities (except crustacean ones) were associated with phosphate phosphorus (PP) concentration (Figure 3a–g). These three parameters, plus phosphate concentration, were the main determinants of fish assemblages (Figure 3g). Eukaryote and diatom communities in the sediments were associated with the almost same set of water parameters (pH, PP, DO and salinity, in addition to total P for eukaryote) and sediment characteristics (total organic carbon (TOC); Figure 3b,d). The same pattern was observed in the water column where a similar set of water characteristics (pH, PP, DO, TOC and salinity, in addition to turbidity for diatoms) influenced the composition of these two groups (Figure 3a,c). Finally, crustacean assemblages in the water column and sediment were influenced by a totally different set of environmental parameters (water pH, DO and TOC for crustacean living in the water column vs sediment granulometry and phosphate concentration for sedimentary ones; Figure 3e,f).

3.3 | Consistency of Alpha-, Beta-Diversity, and LCBD Patterns Among Taxonomic Groups

All communities showed consistent beta-diversity patterns at the regional scale, characterized by strong levels of inter-estuary variation (Figure 3); but they differ at the intra-estuary scale (Figure 4). These changes were, in general, mostly due to the turnover in community composition among locations rather than differences in mOTU richness (especially for small-bodied organisms, see Results and Discussion S1; Table S4) and were associated with different sets of environmental drivers (Figure 3).

When differences in richness among estuaries existed, they were neither uniform nor directional (Figure S3), contrary to the expectations from H3. For instance, the richness of diatoms in sediments was significantly higher in Gregory River, but that pattern was not observed in other taxonomic groups. Similarly, richness in the water column was higher in St Helens/Murray for most groups, except for Crustacea. At the estuary level, we observed a trend towards a higher eukaryotic richness in the water column in downstream sites, but not in sediments (Figure S4). This trend was also observed for pelagic diatoms in Saint Helens/Murray and to a lower extent in Sandy Creek. No noticeable difference was detected for fish and crustaceans. Finally, we used LCBD (local contribution to beta-diversity) to assess the uniqueness of each site. LCBD values measured at the estuary level showed a low congruence between taxonomic groups and matrices (Figure 5 and Figure S7), further invalidating H3. None of the sites exhibited significant LCBD values for all taxonomic groups simultaneously, and only three significant positive correlations in LCBD values among all “group-matrix” combinations were observed (Figure 5g). Among them, the two strongest correlations were between diatoms and eukaryotes in the water column on one side, and in the sediments on the other, which can partly be explained by the fact that diatoms represent between 17% and 19% of the eukaryote community (Figure S1). However, for both groups, LCBD values observed in sediment and water were negatively correlated.

4 | Discussion

4.1 | Sampled Matrices Provide Complementary Pictures of Community Composition

By conducting eDNA-based multi-taxon surveys (including all eukaryotes and three taxonomic groups at different trophic levels), we first demonstrated that eDNA from the water and sediment matrices each captured unique, but also complementary, perspectives of the biological communities within the region's estuaries. A vast majority of mOTUs were either unique to the water column or sediments (independently of the location), with few being shared among matrices (Figure S2). Contrary to our hypothesis H1, these results emphasize that in these shallow and highly dynamic systems which are subjected to high tide cycles and frequent particle resuspension, sampling of eDNA from the water column cannot be used as a surrogate for characterizing the sediment communities, and vice-versa. In that regard, our findings mirror observations from other marine ecosystems such as tropical reefs (Koziol et al. 2019), coastal hard substrate (Antich et al. 2021) and deep-sea environments

(Brandt et al. 2021). Nevertheless, because eDNA transport in aquatic systems includes both horizontal and vertical transport through particle resuspension (Harrison et al. 2019), we expect the observed strong differentiation between matrices to be less marked in the wet season when velocities and flows are extensively greater, increasing the mixing between the water and sediment matrices; a hypothesis to be tested in future studies.

The effect of the type of matrix sampled on community composition was particularly pronounced for diatoms and eukaryotes (Figure 2). The animal eukaryotic community in sediments was dominated by nematodes (roundworms) and, to a lesser extent, by annelids (segmented worms; Figure S1 and Results and Discussion S1), which generally reside in soft sediments and represented a negligible fraction of the eDNA in the water body (DiBattista et al. 2020). Conversely, most crustaceans are free living and highly mobile, and although some of them are sediment dwelling, the absence of strong differentiation according to the matrices (Figure 2) suggests that both compartments perform equally well in characterizing crustacean communities. However, Crustacea detection was relatively stochastic, which might be explained technically by primer biases and an incomplete sampling effort, and/or biologically by the molting cycle of crustaceans that largely influences DNA release in the environment (Chandler et al. 2024). In both water and sediment samples, crustaceans were dominated by decapods (order Decapoda) and copepods (order Calanoida; Figure S1 and Results and Discussion S1). Some taxa with a benthopelagic life cycle were, however, better detected in the water column (e.g., sessile crustacea from the order Sessilia), likely reflecting the larval dispersion of these species (Ip et al. 2021). Consequently, even when water eDNA fails to properly mirror soft-sediment communities, it may still have potential as a biomonitoring tool for hard-substrate species with a larval stage (Couton et al. 2022).

4.2 | All Communities Are Primarily Structured by Estuary, Reflecting Their Ecological State, but Small-Scale Within-Estuary Structure Emerges for Small-Bodied Organisms

All examined communities, from diatoms to fish, exhibited similar beta-diversity patterns at the regional scale and were primarily structured by estuary, supporting our hypothesis H2. This is especially remarkable in the sense that the markers used in this study have contrasted taxonomic resolutions; those with a large taxonomic coverage (e.g., eukaryotes or diatoms) tend to have a much lower taxonomic resolution than those focusing on a more restricted taxonomic group (e.g., teleosts) where resolution can go down to the species level. Nevertheless, we observed that inter-estuary variation was particularly important for smaller-bodied organisms (e.g., diatoms) and eukaryotes in general, suggesting that community variations not only occur at the species level but also at higher taxonomic levels and emphasizing the importance of sampling a wide breadth of taxonomic groups and life strategies.

From our data, it is not possible to decipher whether observed patterns were due to contaminants and/or fluctuations in natural abiotic variables (e.g., salinity and pH), which are seasonal and characteristic of tropical estuaries (Abrantes and

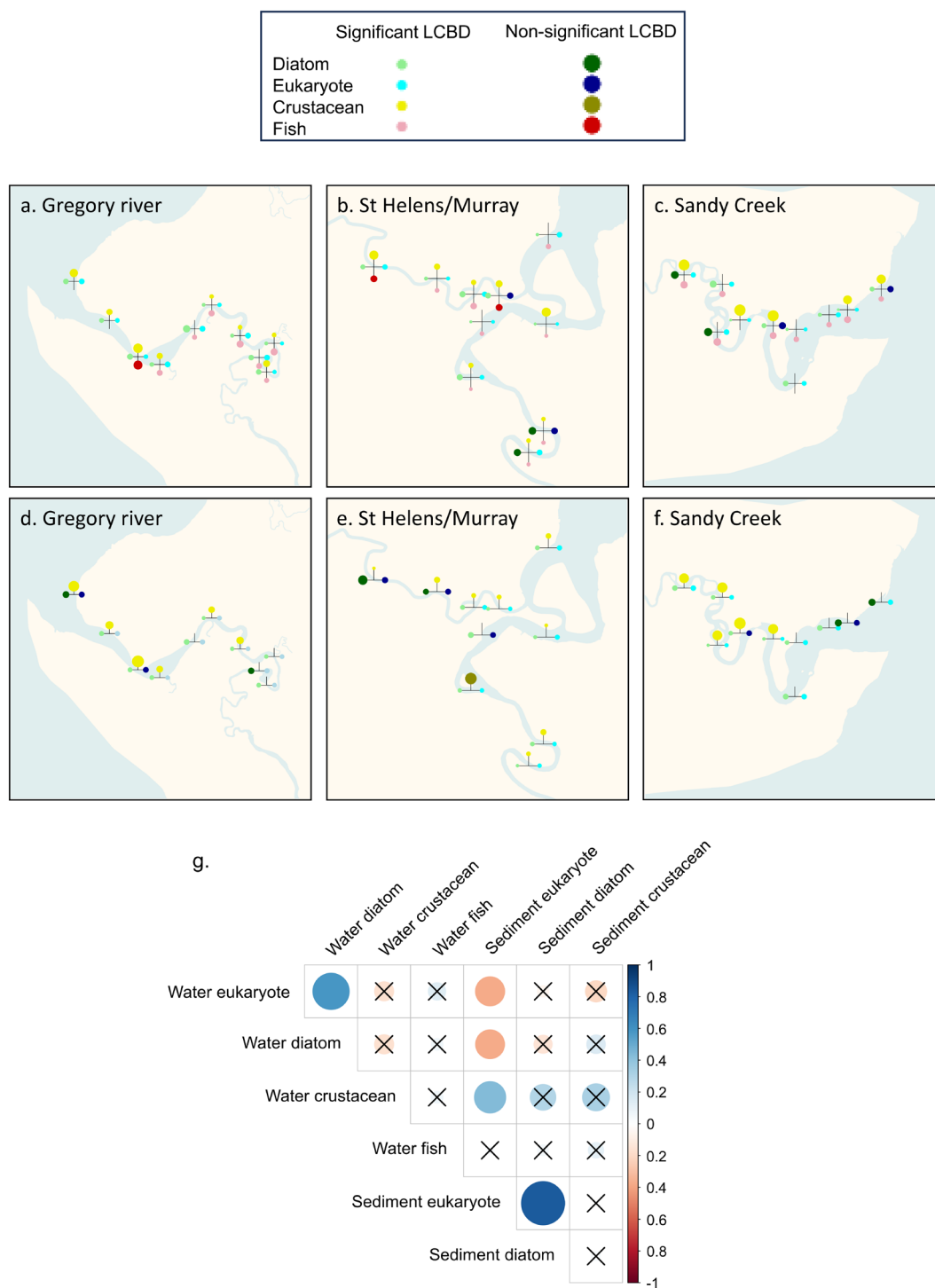


FIGURE 5 | LCBD (Local Contribution to Beta-Diversity) values at the estuary scale, reflecting the ‘uniqueness’ of diatom, eukaryote, crustacean and fish communities at each sampling point. LCBD values in the pelagic environment (i.e., water matrix) are represented in (a–c) while those measured in sediments are in (d–f). The size of points is proportional to the LCBD values, their colors correspond to the taxonomic group and their shade to the significance level (dark-colored points showing significant LCBD values at $p < 0.05$). Panel (g) shows correlations in LCBD values observed between pairs of taxonomic groups and according to the sampled matrix.

Sheaves 2010). At this broad scale, several abiotic characteristics of estuaries including pH, DO, salinity, sediment characteristics, and anthropogenically driven nutrient concentrations were associated with their community composition (Figure 3). This can reflect natural variation in environmental parameters among study sites. However, land use differences in catchments,

which result in strong disparities in nutrient concentrations reaching these estuaries (GBRMPA 2019), are also likely to have contributed to the observed biological variation. In addition, although only traces of pesticides have been detected at the time of sampling, inter-estuary variations in pesticide run-offs may also have played a role in this pattern. Contaminant run-offs

(including pesticide and nutrients) have been identified as one of the main drivers of spatio-temporal estuarine fish community composition along with salinity in the study area (Parikh et al. 2024). In these systems, frequent pulses of agricultural runoff occur over a period of up to 6 months (i.e., during the wet season) producing acute peaks in concentrations that tend to pass rapidly, but residuals of contaminated water may persist throughout the year, as observed in Sandy Creek (Warne et al. 2020; Pearson et al. 2021). These inputs of contaminants during the wet seasons may also induce year-long legacy effects. For example, reproduction of crustaceans and fish may be strongly influenced by contaminated freshwater flows (Yang et al. 2021). Similarly, herbicide or nutrient enrichment impacts on primary producers can reverberate through the entire food web, affecting the survival, biomass, and diversity of higher trophic levels (e.g., Breitburg et al. 2009; Warry et al. 2016; Starr et al. 2017). These changes can have long-lasting effects on community reassembly following oceanic inputs and the transition towards saltwater conditions typically observed in the dry season (Borja et al. 2010). Finally, although all three estuaries were located within the same biogeographical zone (Northeast Australian Shelf; Spalding et al. 2007) and shared the same oceanic input, we could not rule out that isolation by distance played a role in the observed community structure. This mechanism is hard to evaluate in this study, and the literature on connectivity between estuaries is heavily biased towards fish, but we expect it to be rather marginal in the observed pattern. For example, Sandy Creek and St Helens/Murray estuaries are much closer geographically to each other than to Gregory River estuary (Figure 1); yet, there is no evidence for a systematic stronger similarity in community composition between these two first locations (with the exception of fish; Figure 3), and in many instances, assemblages were more differentiated in Sandy Creek estuary.

At the estuary scale, an upstream-downstream community composition pattern was observed, albeit marginally, for diatoms and eukaryotes but not for fish and crustaceans (Figure 4). Such an upstream-downstream compositional gradient is typical of estuarine ecosystems and commonly associated with salinity (Sheaves 2015); yet, here, at the time of sampling (dry-season), even the most upstream sites were under hypersaline conditions (> 35 PSU; Ito 1958). Perennial freshwater flows lead to a salinity gradient that is typically maintained throughout the year (Godfrey et al. 2020), but in the absence of dry-season freshwater inputs, estuarine salinity may match normal seawater (Pearson et al. 2021). Fine-scale habitat variations and/or differentiation by distance might rather be a better explanation for the within-estuary distribution of diatom and eukaryote communities that encompass many passive dispersers (Ma et al. 2023). Although both groups, in both the water column and sediments, exhibited a distance-decay pattern in terms of composition at the estuary level, communities depicted from water eDNA were globally much more similar between them than sediment ones (Figure 4a). This may reflect the fact that small-bodied sedimentary communities inhabit a structurally more complex compartment with high habitat heterogeneity over a small spatial scale while organisms living in the water column can be more passively disseminated across the estuary with water flow and tides. In contrast, the absence of a strong within-estuary macro-faunal community structure reflects the

ability of these nekton species to move by themselves along the estuary during the dry season when the influence of the marine environment is predominant, as opposed to the wet season when these systems are mainly freshwater driven (Parikh et al. 2024).

Despite the rising popularity of eDNA for simultaneously describing a broad spectrum of taxonomic groups in a standardized way, several technical biases may induce variability in the detection probability among taxa (e.g., extraction and amplification biases), but also within taxa from different locations (e.g., due to a differential presence of PCR inhibitors), and thus affect the reliability of observed beta-diversity patterns (Zinger et al. 2019). In that regard, PCR biases are often considered the most important sources of variation, and the choice of highly conserved primers within the targeted taxonomic group is critical (Taberlet et al. 2018). In addition, the origin and fluxes of eDNA in coastal environments subjected to tides are still poorly understood (Harrison et al. 2019). This raises questions about effective sampling strategies to deploy for routine biomonitoring. Nevertheless, several empirical studies of marine communities have suggested a high site fidelity of eDNA to the source communities, with the ability to discriminate assemblages as close as 60 m apart (Port et al. 2016; Jeunen et al. 2019; Rey et al. 2023). In our study, sampling was conducted around high tide (i.e., at both rising and receding tide) to facilitate navigation and make it applicable to future biomonitoring programs; however, the eDNA signal was relatively insensitive to these water movements, as reflected by the distance-decay patterns observed along each estuary for planktonic groups, mirroring findings previously observed in the nearshore environment (Kelly et al. 2018). These estuaries receive only seasonal freshwater inputs, resulting in high water residence times and periodic hypersaline conditions during the dry season that limit eDNA dispersion. This property and our results suggest that eDNA-based investigations can provide relevant biological indications about the health state of the estuary, even outside periods of runoff.

4.3 | Inconsistent eDNA-Based Biodiversity Metrics Among Taxonomic Groups and Implications for Biomonitoring of Tropical Estuaries

One of the most salient observations of the study was the similar structuring of all communities according to their estuary of origin, reflecting the ecological status of these estuaries. This consistency of beta-diversity patterns at the regional scale among taxonomic groups expands on previous works suggesting that eDNA signal, from both water and sediments, reflects the ecological conditions of coastal environments and could be used as a barometer of human impacts (Chariton et al. 2015; DiBattista et al. 2020). However, in contradiction with hypothesis H3, our study highlighted the lack of consistency in both alpha-diversity and LCBD patterns among groups (Figure S3 and Figure 5). Although the pelagic and sedimentary communities were tightly linked to each other by biotic interactions, differences in physiology, evolutionary history, size, generation time, and habitat lead to variations in their sensitivity to the same stressors; consequently, communities may respond differently and at various scales (Heino 2010; Donald et al. 2021). We also showed that the community composition was not associated with the exact same set of environmental variables (Figure 3). Collectively, these

results emphasize the need for multi-taxonomic assessments in estuary biomonitoring to properly assess anthropogenic impacts along the land-to-sea continuum (Polidoro et al. 2021).

Alpha-diversity metrics are often used as indicators of environmental conditions, for instance for assessing marine sediment quality (Simpson and Batley 2016), with the assumption that local community diversity will decline with increasing levels of human impacts (Johnston and Roberts 2009). However, for at least some taxonomic groups, our findings were incongruent with this assumption. For example, Sandy Creek estuary-historically the most anthropogenically impacted location-exhibited as high, or even higher, alpha-diversity levels (i.e., mOTU richness) as other less impacted estuaries. This result is in line with previous studies that reported various trends in alpha-diversity metrics when comparing degraded streams with more pristine conditions, questioning the use of community richness/diversity estimates from eDNA samples as an indicator of ecological integrity (Mathers et al. 2024; Sinclair et al. 2024). This question is especially relevant in a multi-stressor context, where interactions among stressors may have antagonistic, rather than additive effects on local diversity (Bao et al. 2024). In contrast, indices based on community composition provide a good measure of how communities respond to environmental degradation (Hosokawa et al. 2021) and several eDNA-based ecological assessments of human impacts have demonstrated the robustness of these indices (e.g., Chariton et al. 2015; Tang et al. 2019; Gillmore et al. 2021). This point is supported by the fact that community changes, especially for small-bodied organisms, were more driven by turnover in composition rather than by species loss (Results and Discussion S1; Table S4), which further adds credence to the idea that biodiversity change at a broad scale can be uncoupled from trends in local species richness (Medeiros et al. 2016; Hillebrand et al. 2018).

5 | Conclusion

This study draws several conclusions pertaining to multi-group biodiversity patterns in tropical estuaries, with implications for eDNA biomonitoring of these systems. First, water and sediment matrices depicted complementary pictures of estuarine biodiversity. They thus should both be monitored simultaneously. Second, all taxonomic groups, independently of the sampled matrix, were primarily structured by estuary, reflecting their ecological status, even in the absence of recent runoff, making these ‘omics’ approaches relevant for routine biomonitoring in highly dynamic systems, such as tropical estuaries. Third, importantly, site-specific biodiversity metrics (such as alpha-diversity or LCBD) were highly variable from one taxonomic group to another, preventing the use of one taxonomic group as a surrogate for others. Rather, multi-taxonomic assessment at the landscape scale is required to improve modeling of coastal biodiversity trajectories in anthropogenic areas. Finally, it is emphasized that this study only provides ‘snapshots’ of the local diversity and may not reflect the long-term state of communities, especially in these areas with strong seasonality. The use of passive eDNA samplers in the water column might provide improved estimates of biodiversity over a longer time period. Such an approach could be coupled with field loggers and passive samplers for environmental contaminants to simultaneously monitor

ambient environmental conditions at study sites. Overall, this work illustrates how multi-taxon eDNA biomonitoring can assist the development of more integrated approaches to understand the response and resilience of biological communities in multi-stressor contexts, typical of estuarine systems.

Author Contributions

J.P., A.A.C., F.V., S.L.S., R.A.S. designed the study and contributed to funding acquisition. J.P., C.C., G.C., J.G., M.M.C., and AC collected samples. J.P. and C.C. conducted DNA analyses. J.P. processed the data and analyzed the results with advice from and discussion with F.V. and A.A.C. J.P. drafted the manuscript with contributions from all co-authors. All authors approved the submitted version.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Raw and filtered datasets, metadata, and scripts for analyses have been deposited in the Dryad depository: <https://doi.org/10.5061/dryad.qnk98sfvp>.

References

- Abrantes, K. G., and M. Sheaves. 2010. “Importance of Freshwater Flow in Terrestrial–Aquatic Energetic Connectivity in Intermittently Connected Estuaries of Tropical Australia.” *Marine Biology* 157: 2071–2086. <https://doi.org/10.1007/s00227-010-1475-8>.
- Antich, A., C. Palacín, E. Cebrian, R. Golo, O. S. Wangensteen, and X. Turon. 2021. “Marine Biomonitoring With eDNA: Can Metabarcoding of Water Samples Cut It as a Tool for Surveying Benthic Communities?” *Molecular Ecology* 30: 3175–3188. <https://doi.org/10.1111/mec.15641>.
- ANZG. 2018. “Australian and New Zealand Guidelines for Fresh and Marine Water Quality.” <https://www.waterquality.gov.au/anz-guide-lines>.
- Bao, S., J. Heino, H. Xiong, and J. Wang. 2024. “Disentangling the Effects of Multiple Stressors on Freshwater Macroinvertebrates: A Quantitative Analysis of Experimental Studies.” *Ecological Indicators* 169: 112880. <https://doi.org/10.1016/j.ecolind.2024.112880>.
- Berry, T. E., S. K. Osterrieder, D. C. Murray, et al. 2017. “DNA Metabarcoding for Diet Analysis and Biodiversity: A Case Study Using the Endangered Australian Sea Lion (*Neophoca cinerea*).” *Ecology and Evolution* 7: 5435–5453. <https://doi.org/10.1002/ece3.3123>.
- Binladen, J., M. T. P. Gilbert, J. P. Bollback, et al. 2007. “The Use of Coded PCR Primers Enables High-Throughput Sequencing of Multiple

- Homolog Amplification Products by 454 Parallel Sequencing." *PLoS One* 2: e197. <https://doi.org/10.1371/journal.pone.0000197>.
- Borja, Á., D. M. Dauer, M. Elliott, and C. A. Simenstad. 2010. "Medium- and Long-Term Recovery of Estuarine and Coastal Ecosystems: Patterns, Rates and Restoration Effectiveness." *Estuaries and Coasts* 33: 1249–1260. <https://doi.org/10.1007/s12237-010-9347-5>.
- Boyer, F., C. Mercier, A. Bonin, Y. Le Bras, P. Taberlet, and E. Coissac. 2016. "Obitools: A Unix-Inspired Software Package for DNA Metabarcoding." *Molecular Ecology Resources* 16: 176–182. <https://doi.org/10.1111/1755-0998.12428>.
- Brandt, M. I., F. Pradillon, B. Trouche, et al. 2021. "Evaluating Sediment and Water Sampling Methods for the Estimation of Deep-Sea Biodiversity Using Environmental DNA." *Scientific Reports* 11: 7856. <https://doi.org/10.1038/s41598-021-86396-8>.
- Breitburg, D. L., J. K. Craig, R. S. Fulford, et al. 2009. "Nutrient Enrichment and Fisheries Exploitation: Interactive Effects on Estuarine Living Resources and Their Management." *Hydrobiologia* 629: 31–47. <https://doi.org/10.1007/s10750-009-9762-4>.
- Brodie, J. E., F. J. Kroon, B. Schaffelke, et al. 2012. "Terrestrial Pollutant Runoff to the Great Barrier Reef: An Update of Issues, Priorities and Management Responses." *Marine Pollution Bulletin* 65: 81–100. <https://doi.org/10.1016/j.marpolbul.2011.12.012>.
- Bureau of Meteorology. 2022. "Average Annual, Seasonal and Monthly Rainfall." http://www.bom.gov.au/jsp/ncc/climate_averages/rainfall/index.jsp.
- Campana, O., D. A. Spadaro, J. Blasco, and S. L. Simpson. 2012. "Sublethal Effects of Copper to Benthic Invertebrates Explained by Changes in Sediment Properties and Dietary Exposure." *Environmental Science & Technology* 46: 6835–6842. <https://doi.org/10.1021/es2045844>.
- Chandler, L., A. J. Harford, G. C. Hose, et al. 2024. "Saline Mine Water Influences Eukaryote Life in Shallow Groundwater of a Tropical Sandy Stream." *Science of the Total Environment* 946: 174101. <https://doi.org/10.1016/j.scitotenv.2024.174101>.
- Chariton, A. A. 2012. "Short and Informative DNA Products to Indirectly Measure Vascular Plant Biodiversity." *Molecular Ecology* 21: 3637–3639. <https://doi.org/10.1111/j.1365-294X.2012.05633.x>.
- Chariton, A. A., A. C. Roach, S. L. Simpson, and G. E. Batley. 2010. "The Influence of the Choice of Physical and Chemistry Variables on Interpreting the Spatial Patterns of Sediment Contaminants and Their Relationships With Benthic Communities." *Marine and Freshwater Research* 61: 1109–1122. <https://doi.org/10.1071/MF092263>.
- Chariton, A. A., S. Stephenson, M. J. Morgan, et al. 2015. "Metabarcoding of Benthic Eukaryote Communities Predicts the Ecological Condition of Estuaries." *Environmental Pollution* 203: 165–174. <https://doi.org/10.1016/j.envpol.2015.03.047>.
- Conway, J. R., A. Lex, and N. Gehlenborg. 2017. "UpSetR: An R Package for the Visualization of Intersecting Sets and Their Properties." *Bioinformatics* 33: 2938–2940. <https://doi.org/10.1093/bioinformatics/btx364>.
- Couton, M., L. Lévêque, C. Daguin-Thiébaud, T. Comtet, and F. Viard. 2022. "Water eDNA Metabarcoding Is Effective in Detecting Non-Native Species in Marinas, but Detection Errors Still Hinder Its Use for Passive Monitoring." *Biofouling* 38: 367–383. <https://doi.org/10.1080/08927014.2022.2075739>.
- DES. 2018. *Monitoring and Sampling Manual: Environmental Protection (Water) Policy*. Department of Environment and Science Government. <https://environment.des.qld.gov.au/management/water/quality-guide/lines/sampling-manual>.
- DiBattista, J. D., J. D. Reimer, M. Stat, et al. 2020. "Environmental DNA Can Act as a Biodiversity Barometer of Anthropogenic Pressures in Coastal Ecosystems." *Scientific Reports* 10: 8365. <https://doi.org/10.1038/s41598-020-64858-9>.
- Djurhuus, A., J. Port, C. J. Closek, et al. 2017. "Evaluation of Filtration and DNA Extraction Methods for Environmental DNA Biodiversity Assessments Across Multiple Trophic Levels." *Frontiers in Marine Science* 4: 314. <https://doi.org/10.3389/fmars.2017.00314>.
- Donald, J., J. Murienne, J. Chave, et al. 2021. "Multi-Taxa Environmental DNA Inventories Reveal Distinct Taxonomic and Functional Diversity in Urban Tropical Forest Fragments." *Global Ecology and Conservation* 29: e01724. <https://doi.org/10.1016/j.gecco.2021.e01724>.
- Dray, S., D. Bauman, G. Blanchet, et al. 2023. "adespatial: Multivariate Multiscale Spatial Analysis." R package version 0.3-21. <https://CRAN.R-project.org/package=adespatial>.
- Edge, C. B., L. F. Baker, C. M. Lanctôt, et al. 2020. "Compensatory Indirect Effects of an Herbicide on Wetland Communities." *Science of the Total Environment* 718: 137254. <https://doi.org/10.1016/j.scitotenv.2020.137254>.
- Ficetola, G. F., E. Coissac, S. Zundel, et al. 2010. "An In Silico Approach for the Evaluation of DNA Barcodes." *BMC Genomics* 11: 434. <https://doi.org/10.1186/1471-2164-11-434>.
- Ficetola, G. F., J. Pansu, A. Bonin, et al. 2015. "Replication Levels, False Presences and the Estimation of the Presence/Absence From eDNA Metabarcoding Data." *Molecular Ecology Resources* 15: 543–556.
- Fleeger, J. W. 2020. "How Do Indirect Effects of Contaminants Inform Ecotoxicology? A Review." *PRO* 8: 1659. <https://doi.org/10.3390/pr8121659>.
- GBRMPA. 2019. "Great Barrier Reef Outlook Report 2019." Great Barrier Reef Marine Park Authority (GBRMPA). <https://elibrary.gbrmpa.gov.au/jspui/handle/11017/3474>.
- Getz, E., and C. Eckert. 2022. "Effects of Salinity on Species Richness and Community Composition in a Hypersaline Estuary." *Estuaries and Coasts* 46: 2175–2189. <https://doi.org/10.1007/s12237-022-01117-2>.
- Gillmore, M. L., L. A. Golding, A. A. Chariton, et al. 2021. "Metabarcoding Reveals Changes in Benthic Eukaryote and Prokaryote Community Composition Along a Tropical Marine Sediment Nickel Gradient." *Environmental Toxicology and Chemistry* 40: 1894–1907. <https://doi.org/10.1002/etc.5039>.
- Godfrey, P. C., R. G. Pearson, B. J. Pusey, et al. 2020. "Drivers of Zooplankton Dynamics in a Small Tropical Lowland River." *Marine and Freshwater Research* 72: 173–185. <https://doi.org/10.1071/MF20067>.
- Halpern, B. S., S. Walbridge, K. A. Selkoe, et al. 2008. "A Global Map of Human Impact on Marine Ecosystems." *Science* 319: 948–952. <https://doi.org/10.1126/science.1149345>.
- Hardy, C. M., E. S. Krull, D. M. Hartley, and R. L. Oliver. 2010. "Carbon Source Accounting for Fish Using Combined DNA and Stable Isotope Analyses in a Regulated Lowland River Weir Pool." *Molecular Ecology* 19: 197–212. <https://doi.org/10.1111/j.1365-294X.2009.04411.x>.
- Harris, J. K., J. W. Sahl, T. A. Castoe, B. D. Wagner, D. D. Pollock, and J. R. Spear. 2010. "Comparison of Normalization Methods for Construction of Large, Multiplex Amplicon Pools for Next-Generation Sequencing." *Applied and Environmental Microbiology* 76: 3863–3868. <https://doi.org/10.1128/AEM.02585-09>.
- Harrison, J. B., J. M. Sunday, and S. M. Rogers. 2019. "Predicting the Fate of eDNA in the Environment and Implications for Studying Biodiversity." *Proceedings of the Royal Society B: Biological Sciences* 286: 20191409. <https://doi.org/10.1098/rspb.2019.1409>.
- Heino, J. 2010. "Are Indicator Groups and Cross-Taxon Congruence Useful for Predicting Biodiversity in Aquatic Ecosystems?" *Ecological Indicators* 10: 112–117. <https://doi.org/10.1016/j.ecolind.2009.04.013>.
- Hillebrand, H., B. Blasius, E. T. Borer, et al. 2018. "Biodiversity Change Is Uncoupled From Species Richness Trends: Consequences for Conservation and Monitoring." *Journal of Applied Ecology* 55: 169–184. <https://doi.org/10.1111/1365-2664.12959>.

- Hinlo, R., D. Gleeson, M. Lintermans, and E. Furlan. 2017. "Methods to Maximise Recovery of Environmental DNA From Water Samples." *PLoS One* 12: e0179251. <https://doi.org/10.1371/journal.pone.0179251>.
- Hosokawa, S., K. Momota, A. A. Chariton, R. Naito, and Y. Nakamura. 2021. "The Use of Diversity Indices for Local Assessment of Marine Sediment Quality." *Scientific Reports* 11: 14991. <https://doi.org/10.1038/s41598-021-94636-0>.
- Iliff, S. M., R. J. Harris, and E. W. Stoner. 2019. "Effects of Chronic Pesticide Exposure on an Epibenthic Oyster Reef Community." *Marine Pollution Bulletin* 146: 502–508. <https://doi.org/10.1016/j.marpolbul.2019.06.060>.
- Inglis, G. J., and J. E. Kross. 2000. "Evidence for Systematic Changes in the Benthic Fauna of Tropical Estuaries as a Result of Urbanization." *Marine Pollution Bulletin* 41: 7–12. [https://doi.org/10.1016/S0025-326X\(00\)00093-X](https://doi.org/10.1016/S0025-326X(00)00093-X).
- Ip, Y. C. A., Y. C. Tay, J. J. M. Chang, et al. 2021. "Seeking Life in Sedimented Waters: Environmental DNA From Diverse Habitat Types Reveals Ecologically Significant Species in a Tropical Marine Environment." *Environmental DNA* 3: 654–668. <https://doi.org/10.1002/edn3.162>.
- Jeunen, G.-J., M. Knapp, H. G. Spencer, et al. 2019. "Environmental DNA (eDNA) Metabarcoding Reveals Strong Discrimination Among Diverse Marine Habitats Connected by Water Movement." *Molecular Ecology Resources* 19: 426–438. <https://doi.org/10.1111/1755-0998.12982>.
- Johnston, E. L., and D. A. Roberts. 2009. "Contaminants Reduce the Richness and Evenness of Marine Communities: A Review and Meta-Analysis." *Environmental Pollution* 157: 1745–1752. <https://doi.org/10.1016/j.envpol.2009.02.017>.
- Kelly, R. P., R. Gallego, and E. Jacobs-Palmer. 2018. "The Effect of Tides on Nearshore Environmental DNA." *PeerJ* 6: e4521. <https://doi.org/10.7717/peerj.4521>.
- Kessler, M., S. Abrahamczyk, M. Bos, et al. 2009. "Alpha and Beta Diversity of Plants and Animals Along a Tropical Land-Use Gradient." *Ecological Applications* 19: 2142–2156. <https://doi.org/10.1890/08-1074.1>.
- Kozioł, A., M. Stat, T. Simpson, et al. 2019. "Environmental DNA Metabarcoding Studies Are Critically Affected by Substrate Selection." *Molecular Ecology Resources* 19: 366–376. <https://doi.org/10.1111/1755-0998.12971>.
- Kroon, F. J., K. L. E. Berry, D. L. Brinkman, et al. 2020. "Sources, Presence and Potential Effects of Contaminants of Emerging Concern in the Marine Environments of the Great Barrier Reef and Torres Strait, Australia." *Science of the Total Environment* 719: 135140. <https://doi.org/10.1016/j.scitotenv.2019.135140>.
- Kumar, G., E. Farrell, A. M. Reaume, J. A. Eble, and M. R. Gaither. 2022. "One Size Does Not Fit All: Tuning eDNA Protocols for High-and Low-Turbidity Water Sampling." *Environmental DNA* 4: 167–180. <https://doi.org/10.1002/edn3.235>.
- Lear, G., I. Dickie, J. Banks, et al. 2018. "Methods for the Extraction, Storage, Amplification and Sequencing of DNA From Environmental Samples." *New Zealand Journal of Ecology* 42: 10–50. <https://www.jstor.org/stable/26538090>.
- Legendre, P., and M. De Cáceres. 2013. "Beta Diversity as the Variance of Community Data: Dissimilarity Coefficients and Partitioning." *Ecology Letters* 16: 951–963. <https://doi.org/10.1111/ele.12141>.
- Levin, L. A., D. F. Boesch, A. Covich, et al. 2001. "The Function of Marine Critical Transition Zones and the Importance of Sediment Biodiversity." *Ecosystems* 4: 430–451. <https://doi.org/10.1007/s10021-001-0021-4>.
- Lewis, J. L., G. Agostini, D. K. Jones, and R. A. Relyea. 2021. "Cascading Effects of Insecticides and Road Salt on Wetland Communities." *Environmental Pollution* 272: 116006. <https://doi.org/10.1016/j.envpol.2020.116006>.
- Lunt, J., and D. L. Smee. 2014. "Turbidity Influences Trophic Interactions in Estuaries." *Limnology and Oceanography* 59: 2002–2012. <https://doi.org/10.4319/lo.2014.59.6.2002>.
- Ma, C., R. Cui, Y. Duan, et al. 2023. "Differences in Biodiversity and Assembly Mechanisms Between Planktonic and Benthic Diatom Communities in Riverine Ecosystems: A Case Study in the Ashi River." *Ecological Indicators* 157: 111258. <https://doi.org/10.1016/j.ecolind.2023.111258>.
- Mackay-Whitsunday-Isaac Healthy Rivers to Reef Partnership. 2020. *Results for the MackayWhitsunday-Isaac 2019 Report Card Technical Report*. Mackay-Whitsunday-Isaac Healthy Rivers to Reef Partnership. <https://healthyriverstoreef.org.au/wp-content/uploads/2021/09/mwi-2019-20-report-card-results.pdf>.
- Mariano, D. L. S., and F. Barros. 2015. "Intertidal Benthic Macrofaunal Assemblages: Changes in Structure Along Entire Tropical Estuarine Salinity Gradients." *Journal of the Marine Biological Association of the United Kingdom* 95: 5–15. <https://doi.org/10.1017/S002531541400126X>.
- Mathers, K. L., C. T. Robinson, M. Hill, et al. 2024. "How Effective Are Ecological Metrics in Supporting Conservation and Management in Degraded Streams?" *Biodiversity and Conservation* 33: 3981–4002. <https://doi.org/10.1007/s10531-024-02933-7>.
- Medeiros, C. R., L. U. Hepp, J. Patrício, and J. Molozzi. 2016. "Tropical Estuarine Macrobenthic Communities Are Structured by Turnover Rather Than Nestedness." *PLoS One* 11: e0161082. <https://doi.org/10.1371/journal.pone.0161082>.
- Mensens, C., F. De Laender, C. R. Janssen, F. C. Rivera, K. Sabbe, and M. De Troch. 2018. "Selective and Context-Dependent Effects of Chemical Stress Across Trophic Levels at the Basis of Marine Food Webs." *Ecological Applications* 28: 1342–1353. <https://doi.org/10.1002/eap.1737>.
- Mercier, C., F. Boyer, A. Bonin, and E. Coissac. 2013. "SUMATRA and SUMACLUSt: Fast and Exact Comparison and Clustering of Sequences." In *Programs and Abstracts of the SeqBio 2013 Workshop*. Abstract, 27–29. <https://git.metabarcoding.org/obitools>.
- Mugnai, F., E. Megléc, M. Abbiati, et al. 2021. "Are Well-Studied Marine Biodiversity Hotspots Still Blackspots for Animal Barcoding?" *Global Ecology and Conservation* 32: e01909. <https://doi.org/10.1016/j.gecco.2021.e01909>.
- Nagarajan, R. P., M. Bedwell, A. E. Holmes, et al. 2022. "Environmental DNA Methods for Ecological Monitoring and Biodiversity Assessment in Estuaries." *Estuaries and Coasts* 45: 2254–2273. <https://doi.org/10.1007/s12237-022-01080-y>.
- O'Brien, A. L., K. A. Dafforn, A. A. Chariton, E. L. Johnston, and M. Mayer-Pinto. 2019. "After Decades of Stressor Research in Urban Estuarine Ecosystems the Focus Is Still on Single Stressors: A Systematic Literature Review and Meta-Analysis." *Science of the Total Environment* 20: 753–764. <https://doi.org/10.1016/j.scitotenv.2019.02.131>.
- Oksanen, J., G. Simpson, F. Blanchet, et al. 2022. "vegan: Community Ecology Package. R Package Version 2.6-4." <https://CRAN.R-project.org/package=vegan>.
- O'Meara, T. A., J. R. Hillman, and S. F. Trush. 2017. "Rising Tides, Cumulative Impacts and Cascading Changes to Estuarine Ecosystem Functions." *Scientific Reports* 7: 10218. <https://doi.org/10.1038/s41598-017-11058-7>.
- Paerl, H. W., W. S. Gardner, K. E. Havens, et al. 2016. "Mitigating Cyanobacterial Harmful Algal Blooms in Aquatic Ecosystems Impacted by Climate Change and Anthropogenic Nutrients." *Harmful Algae* 54: 213–222. <https://doi.org/10.1016/j.hal.2015.09.009>.
- Pansu, J., M. C. Hutchinson, T. M. Anderson, et al. 2022. "The Generality of Cryptic Dietary Niche Differences in Diverse Large-Herbivore Assemblages." *Proceedings of the National Academy of Sciences of the United States of America* 119: e2204400119. <https://doi.org/10.1073/pnas.2204400119>.

- Parikh, A., J. Pansu, A. Stow, et al. 2024. "Environmental DNA Highlights the Influence of Salinity and Agricultural Run-Off on Coastal Fish Assemblages in the Great Barrier Reef Region." *Environmental Pollution* 349: 123954. <https://doi.org/10.1016/j.envpol.2024.123954>.
- Pearman, J. K., N. B. Keeley, S. A. Wood, et al. 2020. "Comparing Sediment DNA Extraction Methods for Assessing Organic Enrichment Associated With Marine Aquaculture." *PeerJ* 8: e10231. <https://doi.org/10.7717/peerj.10231>.
- Pearman, J. K., U. von Ammon, O. Laroche, et al. 2021. "Metabarcoding as a Tool to Enhance Marine Surveillance of Nonindigenous Species in Tropical Harbors: A Case Study in Tahiti." *Environmental DNA* 3: 173–189. <https://doi.org/10.1002/edn3.154>.
- Pearson, R. G., N. M. Connolly, A. M. Davis, and J. E. Brodie. 2021. "Fresh Waters and Estuaries of the Great Barrier Reef Catchment: Effects and Management of Anthropogenic Disturbance on Biodiversity, Ecology and Connectivity." *Marine Pollution Bulletin* 166: 112194. <https://doi.org/10.1016/j.marpolbul.2021.112194>.
- Polidoro, B., C. W. Matson, M. A. Ottinger, et al. 2021. "A Multi-Taxonomic Framework for Assessing Relative Petrochemical Vulnerability of Marine Biodiversity in the Gulf of Mexico." *Science of the Total Environment* 763: 142986. <https://doi.org/10.1016/j.scitotenv.2020.142986>.
- Port, J. A., J. L. O'Donnell, O. C. Romero-Maraccini, et al. 2016. "Assessing Vertebrate Biodiversity in a Kelp Forest Ecosystem Using Environmental DNA." *Molecular Ecology* 25: 527–541. <https://doi.org/10.1111/mec.13481>.
- R Core Team. 2022. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. <https://www.R-project.org/>.
- Rey, A., F. Viard, A. Lizé, E. Corre, A. Valentini, and P. Thiriet. 2023. "Coastal Rocky Reef Fish Monitoring in the Context of the Marine Strategy Framework Directive: Environmental DNA Metabarcoding Complements Underwater Visual Census." *Ocean and Coastal Management* 241: 106625. <https://doi.org/10.1016/j.ocecoaman.2023.106625>.
- Sanches, T. M., and A. D. Schreier. 2020. "Optimizing an eDNA Protocol for Estuarine Environments: Balancing Sensitivity, Cost and Time." *PLoS One* 15: e0233522. <https://doi.org/10.1371/journal.pone.0233522>.
- Schwentner, M., R. Zahiri, S. Yamamoto, M. Husemann, B. Kullmann, and R. Thiel. 2021. "eDNA as a Tool for Non-Invasive Monitoring of the Fauna of a Turbid, Well-Mixed System, the Elbe Estuary in Germany." *PLoS One* 16: e0250452. <https://doi.org/10.1371/journal.pone.0250452>.
- Sheaves, J. 2015. "Influence of Seasonal Variability and Salinity Gradients on Benthic Invertebrate Assemblages in Tropical and Subtropical Australian Estuaries." PhD Thesis, James Cook University. <https://doi.org/10.25903/pcw9-mq19>.
- Simpson, S. L., and G. E. Batley. 2016. *Sediment Quality Assessment: A Practical Guide*. CSIRO Publishing. <http://hdl.handle.net/102.100.100/90767?index=1>.
- Sinclair, J. S., E. A. Welti, F. Altermatt, et al. 2024. "Multi-Decadal Improvements in the Ecological Quality of European Rivers Are Not Consistently Reflected in Biodiversity Metrics." *Nature Ecology & Evolution* 8: 430–441. <https://doi.org/10.1038/s41559-023-02305-4>.
- Smith, R., R. Middlebrook, R. Turner, R. Huggins, S. Vardy, and M. Warne. 2012. "Large-Scale Pesticide Monitoring Across Great Barrier Reef Catchments – Paddock to Reef Integrated Monitoring, Modelling and Reporting Program." *Marine Pollution Bulletin* 65: 117–127. <https://doi.org/10.1016/j.marpolbul.2011.08.010>.
- Snelgrove, P. V. R., and C. A. Butman. 1994. "Animal-Sediment Relationships Revisited: Cause Versus Effect." *Oceanography and Marine Biology* 32: 111–177.
- Spalding, M. D., H. E. Fox, G. R. Allen, et al. 2007. "Marine Ecoregions of the World: A Bioregionalization of Coastal and Shelf Areas." *Bioscience* 57: 573–583. <https://doi.org/10.1641/B570707>.
- Starr, A. V., S. Bargu, K. Maiti, and R. D. DeLaune. 2017. "The Effect of Atrazine on Louisiana Gulf Coast Estuarine Phytoplankton." *Archives of Environmental Contamination and Toxicology* 72: 178–188. <https://doi.org/10.1007/s00244-016-0335-z>.
- Stat, M., M. J. Huggett, R. Bernasconi, et al. 2017. "Ecosystem Biomonitoring With eDNA: Metabarcoding Across the Tree of Life in a Tropical Marine Environment." *Scientific Reports* 7: 12240. <https://doi.org/10.1038/s41598-017-12501-5>.
- Stronkhorst, J., S. Ciarelli, C. A. Schipper, et al. 2004. "Inter-Laboratory Comparison of Five Marine Bioassays for Evaluating the Toxicity of Dredged Material." *Aquatic Ecosystem Health and Management* 7: 147–159. <https://doi.org/10.1080/14634980490281579>.
- Taberlet, P., A. Bonin, L. Zinger, and E. Coissac. 2018. *Environmental DNA: For Biodiversity Research and Monitoring*. Oxford University Press. <https://doi.org/10.1093/oso/9780198767220.001.0001>.
- Taberlet, P., E. Coissac, F. Pompanon, C. Brochmann, and E. Willerslev. 2012. "Towards Next-Generation Biodiversity Assessment Using DNA Metabarcoding." *Molecular Ecology* 21: 2045–2050. <https://doi.org/10.1111/j.1365-294X.2012.05470.x>.
- Tang, J., J. Zhang, L. Ren, et al. 2019. "Diagnosis of Soil Contamination Using Microbiological Indices: A Review on Heavy Metal Pollution." *Journal of Environmental Management* 242: 121–130. <https://doi.org/10.1016/j.jenvman.2019.04.061>.
- Thomas, L. J., M. Milotic, F. Vaux, and R. Poulin. 2022. "Lurking in the Water: Testing eDNA Metabarcoding as a Tool for Ecosystem-Wide Parasite Detection." *Parasitology* 149: 261–269. <https://doi.org/10.1017/S0031182021001840>.
- Tlili, S., and C. Mouneyrac. 2021. "New Challenges of Marine Ecotoxicology in a Global Change Context." *Marine Pollution Bulletin* 166: 112242. <https://doi.org/10.1016/j.marpolbul.2021.112242>.
- Twilley, R. R., S. C. Snedaker, A. Yáñez-Arancibia, and E. Medina. 1996. "Biodiversity and Ecosystem Processes in Tropical Estuaries: Perspectives of Mangrove Ecosystems." In *Functional Roles of Biodiversity: a Global Perspective*, edited by J. H. Mooney, E. Cushman, E. Medina, O. E. Sala, and E. D. Schulze, 327–370. John Wiley and Sons.
- Valentini, A., C. Miquel, M. A. Nawaz, et al. 2009. "New Perspectives in Diet Analysis Based on DNA Barcoding and Parallel Pyrosequencing: The trnL Approach." *Molecular Ecology Resources* 9: 51–60. <https://doi.org/10.1111/j.1755-0998.2008.02352.x>.
- Valentini, A., P. Taberlet, C. Miaud, et al. 2016. "Next-Generation Monitoring of Aquatic Biodiversity Using Environmental DNA Metabarcoding." *Molecular Ecology* 25: 929–942. <https://doi.org/10.1111/mec.13428>.
- Venarsky, M. P., V. Lowe, C. L. J. Frid, and M. A. Burford. 2022. "Flow Regimes Among Rivers Influences Benthic Biota Biodiversity, but Not Abundance or Biomass, in Intertidal Mudflats and Sandflats in Wet-Dry Tropical Estuaries." *Estuarine, Coastal and Shelf Science* 271: 107858. <https://doi.org/10.1016/j.ecss.2022.107858>.
- Wallace, R., R. Huggins, O. King, et al. 2016. *Total Suspended Solids, Nutrient and Pesticide Loads (2014–2015) for Rivers that Discharge to the Great Barrier Reef—Great Barrier Reef Catchment Loads Monitoring Program*. Department of Science, Information Technology and Innovation. https://www.reefplan.qld.gov.au/__data/assets/pdf_file/0035/45989/2014-2015-gbr-catchment-loads-technical-report.pdf.
- Warne, M. S. J., R. A. Smith, and R. D. R. Turner. 2020. "Analysis of Pesticide Mixtures Discharged to the Lagoon of the Great Barrier Reef, Australia." *Environmental Pollution* 265: 114088. <https://doi.org/10.1016/j.envpol.2020.114088>.
- Warry, F. Y., P. Reich, P. L. M. Cook, R. Mac Nally, J. R. Thomson, and R. J. Woodland. 2016. "Nitrogen Loads Influence Trophic Organization of Estuarine Fish Assemblages." *Functional Ecology* 30: 1723–1733. <https://doi.org/10.1111/1365-2435.12647>.

Westgate, M., P. Barton, P. Lane, et al. 2014. “Global Meta-Analysis Reveals Low Consistency of Biodiversity Congruence Relationships.” *Nature Communications* 5: 3899. <https://doi.org/10.1038/ncomms4899>.

Wurtsbaugh, W. A., H. W. Paerl, and W. K. Dodds. 2019. “Nutrients, Eutrophication and Harmful Algal Blooms Along the Freshwater to Marine Continuum.” *WIREs Water* 6: e1373. <https://doi.org/10.1002/wat2.1373>.

Yang, C., W. Lim, and G. Song. 2021. “Reproductive Toxicity due to Herbicide Exposure in Freshwater Organisms.” *Comparative Biochemistry and Physiology, Part C* 248: 109103. <https://doi.org/10.1016/j.cbpc.2021.109103>.

Zinger, L., A. Bonin, I. G. Alsos, et al. 2019. “DNA Metabarcoding—Need for Robust Experimental Designs to Draw Sound Ecological Conclusions.” *Molecular Ecology* 28: 1857–1862. <https://doi.org/10.1111/mec.15060>.

Zinger, L., C. Lionnet, A. S. Benoiston, J. Donald, C. Mercier, and F. Boyer. 2021. “metabaR: An R Package for the Evaluation and Improvement of DNA Metabarcoding Data Quality.” *Methods in Ecology and Evolution* 12: 586–592. <https://doi.org/10.1111/2041-210X.13552>.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Data S1:** edn370180-sup-0001-Supinfo1.docx. **Data S2:** edn370180-sup-0002-Supinfo2.xlsx.