Marine Environmental Research 192 (2023) 106239

Contents lists available at ScienceDirect

Marine Environmental Research

journal homepage: www.elsevier.com/locate/marenvrev

A large scale temporal and spatial environmental DNA biodiversity survey of marine vertebrates in Brazil following the Fundão tailings dam failure☆

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ARTICLE INFO

Keywords:
eDNA
Metabarcoding
Conservation
Rio Doce
Biodiversity
Endangered species
Environmental impact

ABSTRACT

Seawater contains a wealth of genetic information, representing the biodiversity of numerous species residing within a particular marine habitat. Environmental DNA (eDNA) metabarcoding offers a cost effective, non-destructive method for large scale monitoring of environments, as diverse taxonomic groups are detected using metabarcoding assays. A large-scale eDNA monitoring program of marine vertebrates was conducted across three sampling seasons (Spring 2018, Autumn 2019; Spring 2019) in coastal waters of Brazil. The program was designed to investigate eDNA as a testing method for long term monitoring of marine vertebrates following the Fundão tailings dam failure in November 2015. While no baseline samples were available prior to the dam failure there is still value in profiling the taxa that use the impacted area and the trajectory of recovery. A total of 40 sites were sampled around the mouths of eight river systems, covering approximately 500 km of coastline. Metabarcoding assays targeting the mitochondrial genes 16S rRNA and COI were used to detect fish, marine mammals and elasmobranchs. We detected temporal differences between seasons and spatial differences between rivers/estuaries sampled. Overall, the largest eDNA survey in Brazil to date revealed 69 families from Class Actinopterygii (fish), 15 species from Class Chondrichthyes (sharks and rays), 4 species of marine and estuarine mammals and 23 species of conservation significance including 2 species of endangered dolphin. Our large-scale study reinforces the value eDNA metabarcoding can bring when monitoring the biodiversity of coastal environments and demonstrates the importance of collection of time-stamped environmental samples to better understand the impacts of anthropogenic activities.

1. Introduction

Monitoring of aquatic environments has benefitted from the development of molecular tools that reveal the presence of broad taxonomic groups through analysis of environmental DNA (eDNA) (Ruppert et al., 2019). Additionally, DNA metabarcoding technologies have allowed the high throughput processing of environmental samples to reveal organisms present in the environment (Taberlet, et al., 2012; Thomsen and Willerslev, 2015). Aquatic organisms usually shed DNA into their surrounding environments via the loss of skin, hair or scales and through excretion of mucus, urine and faeces (Taberlet et al., 2012). Thus, much of the biodiversity found within an environment can be ascertained by the detection and analysis of eDNA from substrates such as water (Ficetola et al., 2008; Thomsen and Willerslev 2015), sediment (Taberlet

☆ Field Study Permissions: Field sampling was approved by the ICMBio (sampling license 64238-2).

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https://doi.org/10.1016/j.marenvres.2023.106239
Received 1 July 2023; Received in revised form 19 October 2023; Accepted 22 October 2023
Available online 28 October 2023

et al., 2012; Thomsen and Willerslev 2015) and biofoul (Koziol et al., 2019). Furthermore, ongoing eDNA biomonitoring can provide useful information on the health of ecosystems where industries such as fishing, (Stat et al., 2017), and mining (Fernandes et al., 2018) occur. eDNA biomonitoring also has the ability to track seasonal and climatic changes within an environment (Berry et al., 2019), to assess anthropogenic pressures on marine environments (Bernardino et al., 2019; DiBattista et al., 2020; Coppo et al., 2023), and to provide data on the remediation and restoration of soil environments following mining activities (van der Heyde et al., 2020). Molecular methods have the sensitivity to detect organisms from trace amounts of DNA, and have the ability to identify cryptic or juvenile species without the need to trap or observe individuals (Ficetola et al., 2008). The technology has been applied to monitor rare and endangered marine mammals (Hunter et al., 2018; Harper et al., 2019), elasmobranchs (Weltz et al., 2017; Lehman et al., 2020), and the marine turtle *Chelonia mydas* (Harper et al., 2020).

The Fundão mine tailings dam in Brazil failed in 2015 resulting in extensive environmental impact downstream on both the Rio Doce and its human settlements (Carmo et al., 2017; Gomes et al., 2017; Gabriel et al., 2020). When the dam failed, an estimated 39 million m^3 of iron ore tailings were released into the Rio Gualaxo do Norte in the state of Minas Gerais (Magris et al., 2019). The tailings plume flowed into the Rio do Carmo and eventually the Rio Doce, one of the largest rivers in south-eastern Brazil. After travelling approximately 650 km (km), the plume reached the Atlantic Ocean on 22 November 2015 (Marta-Almeida et al., 2016; Magris et al., 2019; Richard et al., 2020) (see map in Fig. 1).

It is estimated that about 80% of the tailings were deposited along the 100 km of waterways between the Fundão dam and the Risoleta Neves (Candonga) Hydroelectric dam (Samarco, 2018). The plume reached the Rio Doce river mouth and potentially extended into marine protected areas, but with acute pollution closer to the coast (Richard et al., 2020). Extensive programs have been put in place to monitor for any potential impacts of the affected ecosystems (Carmo et al., 2017; Gabriel et al., 2020; Queiroz et al., 2021; Condini et al., 2022; Vilar et al., 2022). Here we undertake a study of environmental DNA (eDNA) extracted from seawater samples taken from the Rio Doce river mouth ecosystem, the Rio Jequitinhonha river mouth 500 km to the north, and along the coastline in between. As the Rio Doce River may continually release sediment particles to the nearshore ecosystems (Sá et al., 2021), we hypothesized that the expected plume from the Rio Doce river mouth would exert an influence in aquatic species composition and diversity in periods of high river discharge. We aimed to answer this hypothesis by comparing temporal patterns of aquatic biodiversity between two regions – the Rio Doce shelf and the Jequitinhonha river mouth to the North, allied with an expanded spatial eDNA assessment over a three season period (2018–2019) along the Brazilian coastline.

2. Materials & methods

2.1. Sampling sites and seawater collection

This eDNA study spanned approximately 500 km of Brazil's south-eastern coastline, ranging from the southern-most site at the Rio Piraquê-Açu-Mirim river mouth in the State of Espírito Santo (Lat: −19.96413, Long: −40.09159), to the northernmost site at the Rio Jequitinhonha river mouth in the State of Bahia (Lat: −15.6711, Long: −38.8852) and including the river mouths of the Rio Doce, Rio Riacho, Rio São Mateus, Rio Mucuri, Rio Peixoto, and the Rio Alcobaça in between. These sites were additionally included to offer an integrated spatial biodiversity assessment of the coastal region, and are therefore discussed separately. For the Rio Doce and Rio Jequitinhonha study areas, sampling sites were distributed along distance gradients radiating outwards from the river mouth. For the other river sites, field samples were collected from sites both within estuarine waters and approximately 5 km offshore (Fig. 2, Table S1).

Field sampling was approved by the Instituto Chico Mendes de Conservação da Biodiversidade – ICMBio (sampling license 64238-2).

The initial survey conducted between 24th September and 1st October 2018 (Spring 2018) comprised twenty-two sites from around the mouths of the impacted Rio Doce and the Rio Jequitinhonha (Fig. 3, Table S1), with five 1L field replicates collected at each site. Sample collection was repeated between 4th May and 22nd May 2019 (Autumn 2019) with the addition of seven sites and thirty-five samples in the Rio Doce extension area as part of a complimentary survey that extended along the coastline. The final survey between 23rd September and 10th

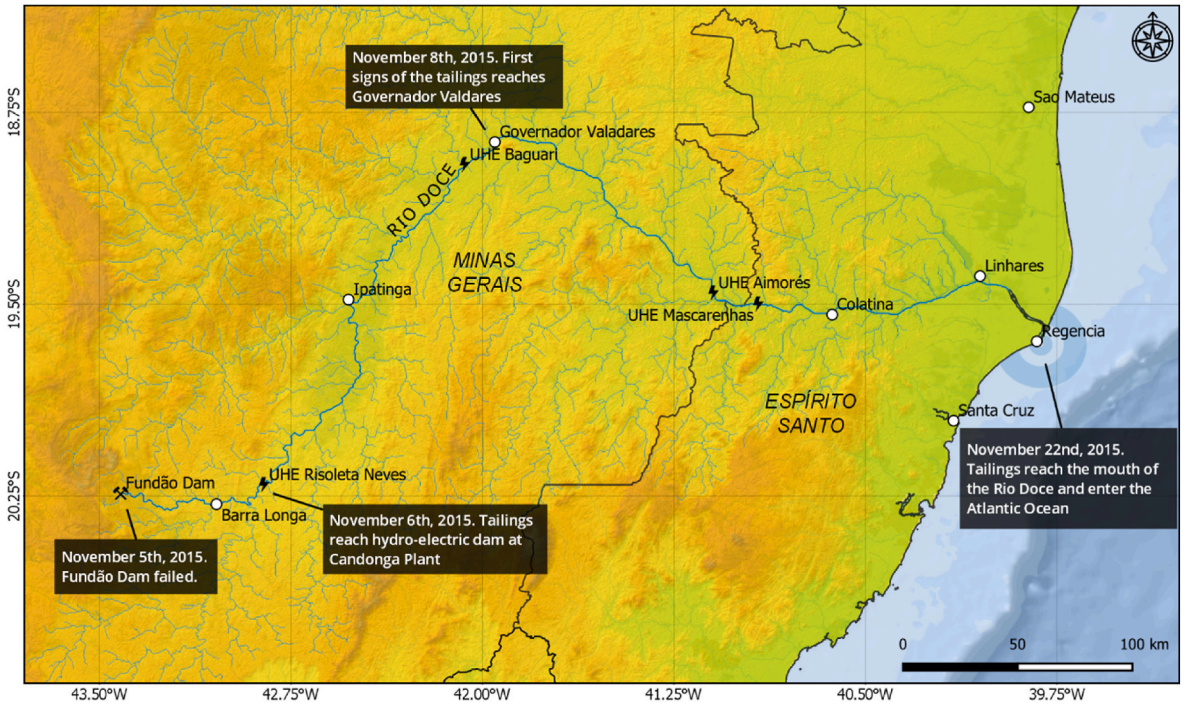

Fig. 1. Location of the Fundão dam failure in relation to the input of tailings to the marine environment.

Fig. 2. Overview of coastal sampling for eDNA undertaken at the Rio Doce, Rio Doce extension area and Rio Jequitinhonha in 2018 and 2019. Inset shows location of study areas relative to Brazil.

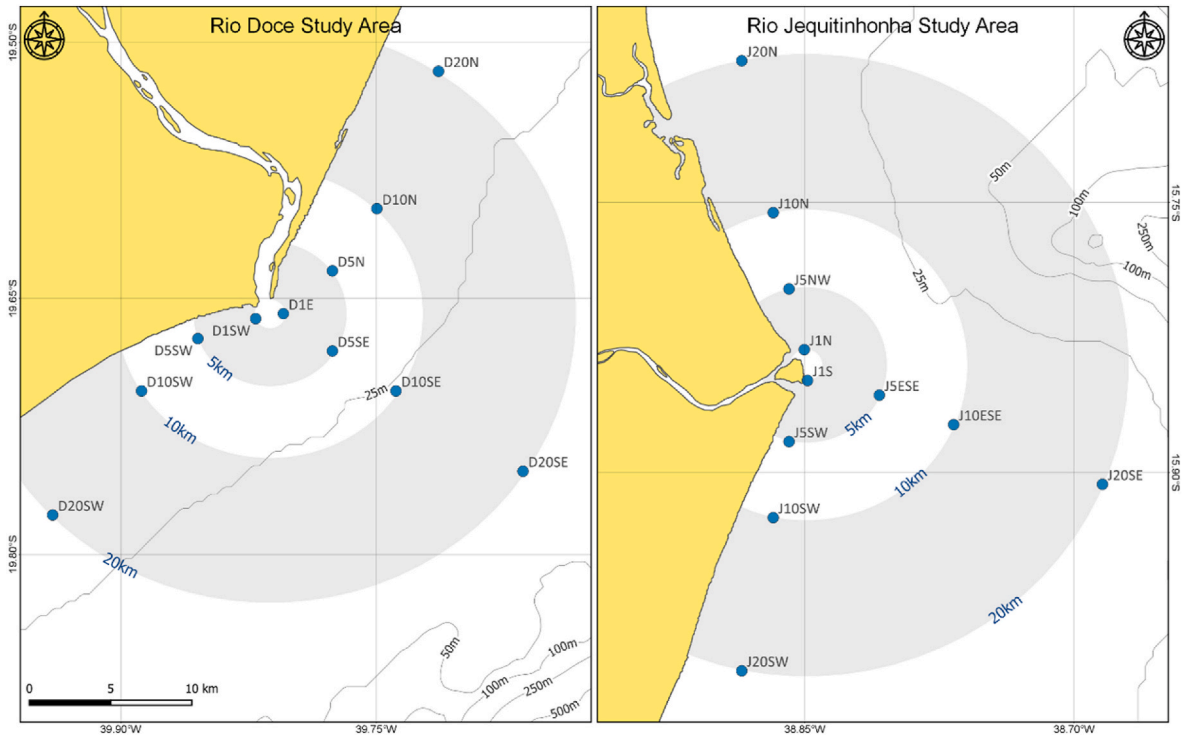

Fig. 3. Sampling sites at Rio Doce and Rio Jequitinhonha study areas, as used in statistical analyses.

October 2019 (Spring 2019) added an extra ten sites and fifty samples (Fig. 2, Table S1). In total, 445 field samples were collected from around eight river mouths across the three surveys. Rio Doce sites D1E and D1S were sampled interchangeably between 2018 and 2019 due to navigational limitations at the time of the survey. These sites were located less than 1 km apart, and have thus been considered as the same site for the purpose of this study (referred to as D1E in Fig. 3).

In the first survey, seawater samples were collected at a depth of approximately 30 cm from eleven sites around the mouth of the Rio Doce and eleven sites around the mouth of the Rio Jequitinhonha, with the sites placed along radial transects located at increasing distances from the river mouth: 1 km, 5 km, 10 km and 20 km in Northerly (N), Northwesterly (NW) Southeasterly (SE), Southwesterly (SW) directions (Fig. 3). Physical water quality parameters including turbidity (Tu; NTU), temperature (Tm; °C), dissolved oxygen (DO; mg/L), chlorophyll-a fluorescence (Ch; µg/L, salinity (S; PSU), conductivity (Co; mS/cm) and photosynthetically active radiation (PAR; µMol/m^2/s) were measured near the surface using a physical water quality profiler configured to sample at a rate of 6 Hz (XR Data Logger, RBR XRX-620). This instrument (also referred to as a CTD profiler) was deployed over the non-shaded side of the boat, lowered to approximately 1 m below the surface and left for a period of at least 30 s to allow the sensor readings to stabilise before lowering through the water column to collect a profile. The data were later checked for quality, and a surface value for each parameter was calculated by using the mean (average) value of the measurements captured between 1 and 2 m below the surface (i.e. a blanking distance was applied to the top 1 m). These surface values were calculated for each of the eleven coastal sampling sites at the Rio Doce and Rio Jequitinhonha study areas.

Five 1L field replicates of seawater were collected at each of the 22 sites and filtered onto individual 0.8 µm polyethersulfone membranes (Pall Life Sciences, NY, USA) using a peristaltic Sentino microbiology pump (Pall Life Sciences). Filter cups and forceps used to handle the membranes were sterilized using a 10% bleach solution for a minimum of 15 min in between processing each water sample, before rinsing in Milli-Q laboratory water. A sample of the rinse water was filtered at the conclusion of each filtering day to control for any incomplete sterilization of filtration equipment. Membranes were frozen and shipped to Curtin University, Australia for DNA extraction, metabarcoding, sequencing and analysis.

In the second survey, the sites were repeated with the exception of Site D1S which was replaced with site D1E due to difficulty in navigating shallow waters during the Autumn 2019 sampling season. An additional seven sites in the Rio Doce extension region, which occurred around and north of the Rio Doce were added to the second survey (Fig. 2, Table S1). These sites included freshwater samples from inside river mouths, estuarine, and marine samples. Filtration membrane and water volume was the same as used previously, with a modification of the filtration method to include the eDNA Sampler Backpack (SmithRoot, Vancouver, WA, USA) for some samples. Sampling was standardised by utilising the same membrane type as previously and measuring a 1L volume of water through the backpack.

In the third survey, all previous sites were repeated, and an additional fifty samples from ten sites were included from the Abrolhos region and around four additional river mouths (Fig. 2). Sample volumes and membrane types were as used previously.

2.1.1. DNA extraction

Half of each filter membrane (n = 445) was processed to extract DNA, with the remaining half preserved at −20 °C. Membranes were cut into strips and incubated overnight at 56 °C with 60 µL Proteinase K and 540 µL Qiagen ATL Buffer. DNA was extracted using a DNeasy Blood and Tissue kit (Qiagen, Venlo, Netherlands) on a QIACube extraction system (Qiagen) and eluted into 100 µL. Extraction controls were included and treated as samples through to sequencing and analysis.

2.1.2. PCR amplification

Two PCR assays were chosen for this study, that broadly target fish, marine mammals and elasmobranchs; the 16S rRNA primer set Fish_Sygnathid_Short (Nester et al., 2020) to detect fish and marine mammals and the COI Elasmobranch multiplex (West et al., 2020) to detect sharks and rays.

To optimise input DNA and detect the co-purification of PCR-inhibitory compounds (Murray et al., 2015) an initial quantification PCR (Neat and 1:10) was performed in duplicate on 2 μL of each DNA extraction using qPCR. The DNA dilution that showed optimal amplification was used in the subsequent single step MID-tag PCR reactions.

All PCR mastermix reactions were set up in an ultraclean laboratory separate from DNA extracts and post PCR workflows to prevent contamination. Each PCR mastermix (25 μL) comprised: 2.5 mM $MgCl_2$ (Applied Biosystems, USA), 10x PCR Gold buffer (Applied Biosystems), 0.25 mM dNTPs (Astral Scientific, Australia), 0.4 mg/ml bovine serum albumin (Fisher Biotec, Australia), 0.4 μmol/L forward and reverse primers, 0.6 μl of a 1:10,000 solution of SYBR Green dye (Life Technologies, USA) and 1 U AmpliTaq Gold DNA polymerase (Applied Biosystems) and 2 μL template DNA. PCR cycling (StepOne: Applied Biosystems) conditions consisted of denaturation at 95 °C for 5 min, followed by 50 cycles of: 95 °C for 30s, 52 °C (COI) or 55 °C (16S) for 30s, 72 °C for 45s, and a final extension stage at 72 °C for 10 min.

DNA samples derived from all 445 field replicates were assigned unique combinations of 6-8bp multiplex identifier tagged (MID-tag) primers in a single-step fusion tagging PCR reaction as described (see Koziol et al., 2019; van der Heyde et al., 2020) using 2 μL of template DNA at the dilution determined by the previous PCR. Duplicate PCR reactions were amplified using the same MID-tag combination. MID-tag combinations assigned to samples are only used once, and are never reassigned in the laboratory workflow to reduce cross-contamination (Murray et al., 2015, Bohmann et al., 2021). MID-tag PCR reactions were prepared in duplicate using a Qiagility instrument (Qiagen) in a designated ultra-clean laboratory to reduce the potential for MID-tag cross contamination, using the same master mix and PCR conditions as described. Following the MID-tagging PCR reaction, duplicate PCR reactions were pooled to reduced PCR stochasticity. Negative, extraction and PCR positive controls were included and treated as samples through to sequencing and anlaysis.

2.1.3. Preparation of sequencing libraries

To minimise primer index bias (O'Donnell et al., 2016) the libraries were blended in accordance with the sample PCR results. For each library, fusion tagged amplicons were mini-pooled with samples that had similar PCR amplification (delta Rn) results. These mini-pools were then quantified (QIAxcel; Qiagen) and proportionally blended together to form approximately equimolar sequencing libraries. Libraries were size selected using a Pippin instrument (Sage Sciences, USA), quantified on a Qubit (Thermofisher) and diluted to 2 nM. Libraries were sequenced on an Illumina MiSeq instrument using unidirectional 300 cycle V2 kits and custom sequencing primers.

2.1.4. Bioinformatics and taxonomic assignments

Bioinformatic analysis was performed in accordance with Mousavi-Derazmahalleh et al. (2021), as follows. First, multiple sequencing libraries from the 16S and COI assays were quality filtered (phred scores$\leq$20), then OBITools (Boyer et al., 2016) was applied to demultiplex samples and remove sequences smaller than 50 base pairs (bp) from the 16S sequence data and 100 bp from the COI sequence data. Subsequently, all sequence files from the same assay were concatenated together and changed to a format suitable for USEARCH (Edgar, 2016). Creation of zero-radius operational taxonomic unit (ZOTU) sequences and their relative abundance table was achieved by applying the unoise3 algorithm of USEARCH using –minsize of two, followed by post-clustering curation with LULU (Frøslev et al., 2017). The ZOTU sequences were aligned to the nucleotide database of Genbank using BLASTN (Altschul et al., 1990). ZOTUs were then assigned to their lowest common ancestor using the LCA script (Mousavi-Derazmahalleh et al., 2021), using qCov 100, percentage identity 97 and Diff 1. Further curation of the data was undertaken to collate duplicate taxa assigned to different ZOTUs, and verify the distribution of the taxa to ensure it occurred in the Southern Atlantic region (GBIF.org, 2020; WoRMS). Where a species was detected by both assays it was included only once in the analysis. Presence of taxa in each of the five field replicates was collated and reported at the site level.

2.1.5. Statistical analysis

Multivariate analyses and regression analyses were performed in PRIMER-e (6.1.1.13) with the PERMANOVA add-on (v1.03; Clarke and Gorley, 2006). ZOTU data was transformed to a presence absence matrix and a Jaccard similarity matrix (+1 dummy variable) was generated. Permutational analysis of variance (PERMANOVA) was used to assess if taxon assemblage was significantly different within and between the Doce and Jequitinhonha study areas. The pseudo-F value, and the probability value (P(perm)) were computed by the PERMANOVA routine with a maximum of 9,999 permutations under a reduced model and sum of squares is Type III (partial) for all tests.

The relationship between marine species data and environmental variables was analysed using distance-based analysis on a linear model (distLM) with marginal tests used to assess the importance of each variable separately. In the sequential tests, a stepwise test was used to find the optimal based on AICc (proportion of explained variation for the model) by sequentially adding environmental variables. The pseudo-F statistic was used to the general hypothesis of no relationship, in which the P-value provides the significance level and the percentage of the variance explained is shown per environmental variable. The data were visualised with distance based redundancy analyses plots (dbRDA), which are generally used to perform an ordination of fitted values from a given model. In a dbRDA plot the first two axes are shown which represent the highest percentage of explained variation out of the fitted model and the total variation. Percentage of fitted variation specifies the variability in the original data explained by the fitted model and percentage of total variation specifies the variation in the fitted matrix. Vector overlays using the environmental data and marine vertebrate species data separately as predictor variables (drawn as multiple partial correlations) were applied to visualize the effect, strength, and direction of the different variables in the ordination plots. To show seasonal and site-based changes, we used nonMetric MultiDimensional Scaling (nMDS) in the R (R Core Team, 2020) package vegan (Oksanen et al., 2020).

3. Results

All seawater samples yielded metabarcoding data. The PCR assay Fish_Sygnathid_Short detects a broad range of vertebrates. Accordingly, in addition to fish, terrestrial vertebrates native to Brazil including *Hydrochoerus hydrochaeris* (Capybara), *Callithrix* sp. (Marmosets), and the parrot *Amazona aestiva* that live along the river systems were detected. Sequences detected opportunistically from terrestrial vertebrates were not included in the analyses as the focus of this study was on marine vertebrate taxa.

3.1. Temporal monitoring of the Rio Doce and Rio Jequitinhonha study areas

Metabarcoding analysis of samples collected at the Rio Doce and Rio Jequitinhonha study areas in Spring 2018, Autumn 2019; Spring 2019, resulted in a total of 39,731,262 and 8,470,876 sequencing reads in 16S and COI assays respectively, of which 1,510,979 and 835,259 were unique. After denoising, and removal of 238 and 196 chimeras a total of 6,196 and 10,392 ZOTUs were generated for the 16S and COI assays respectively, out of which 5,728 and 9,329 remained after curation with LULU. Of the total number of non-curated ZOTUs 9.31% of 16S and 4.68% of COI had at least one match to the nucleotide database of GenBank, which enabled assignment of 386 (16S) and 238 (COI) curated ZOTUs to their lowest common ancestor, of which 57.7% and 32.7% were assigned to fish in 16S and COI assignments respectively. Following data curation to collapse duplicate taxa and verify taxa

distribution, we detected 58 families assigned to Class Actinopterygii, three families assigned to Class Chondrichthyes and three families to Class Mammalia from the Rio Doce and Rio Jequitinhonha study areas (Table S2). No taxa were detected in DNA extraction controls or PCR negative controls. Low level DNA contamination (determined by qPCR) of the Rio Doce rinse water in the Spring 2018 occurred, with eight ZOTUs detected that represented five families of fish taxa (Family Carangidae: *Seriola* sp.; Family Engraulidae: *Anchoa spinifer*; Family Lutjanidae: *Lutjanus purpureus*; Family Sciaenidae: *Macrodon* sp., *Stellifer brasiliensis, Stellifer rastrifer*, *Epinephelus morio*; Family Cynoglossidae: *Symphurus tessellatus*). For precaution, these species were subsequently removed from all samples in the Spring 2018 Rio Doce data set despite the fact that the taxa were not found in all samples.

Analysis of sequence data obtained from water samples collected at the Rio Doce and Rio Jequitinhonha study areas over the three sampling periods, revealed that the species richness was higher in Spring 2018 compared with Autumn 2019; Spring 2019 (Fig. 4).

A seasonal trend was observed with higher species richness during Spring followed by a decline in Autumn and a slight increase in the following Spring; with this pattern observed in both the Rio Jequitinhonha and Rio Doce study areas (Fig. 4). Non-metric multidimensional scaling analysis of spatial patterns in the species assemblages between sampling seasons and both rivers' study areas were statistically significant (stress = 0.12, k = 3 & $p \leq 0.001$) (Fig. 5).

PERMANOVA analysis of (i) Season, (ii) River, (iii) Site (River), (iv) Season*River and (v) Season*Site(River) showed that there was a highly significant difference ($p = 0.0001$) in the species composition for each factor, with strongest seasonal effects and its interaction with river basins ($F_{1,2} = 14.7$, $p < 0.0005$; Table 1).

DistLM on Jaccard similarities of the marine vertebrate presence/absence data, including the environmental factors only explained 11.1% of the total variation in the fitted model (Fig. 6). The marginal and sequential tests were significant for all 8 variables (Table 2).

Analysis at the taxonomic level showed that Class Actinopterygii had the highest representation in Spring 2018 for both study areas, followed by a decline in Autumn 2019 (Table 3). Fish taxa increased in Spring 2019 although not to previous levels (Table 3). Taxa in Class Chondrichthyes and Class Mammalia followed the same pattern with the highest number of taxa in these classes detected in the Spring 2018 season (Table 3).

3.2. Extended coastline survey results

Additional sites sampled during the complimentary survey that extended from south of the Rio Doce river mouth northwards to the Abrolhos region, detected 151 Species from 54 Families in Class Actinopterygii, 12 species from six families in Class Chondrichthyes, and three species of marine mammals (Table S2). These sites were along the coast and inside river mouths and so included freshwater and estuarine environments, resulting in the detection of freshwater species as well as marine species (Table S2).

3.3. Species with conservation significance

Twenty-three species of the marine vertebrates identified by eDNA metabarcoding in this study, are classified from Near Threatened to Critically Endangered on the IUCN red list (IUCN 2021) (Fig. 7). Additional reference for these species was made against the National Red List for Brazil (ICMBio, 2022). These species were detected during the three sampling seasons across all study areas: Rio Doce, Rio Jequitinhonha,

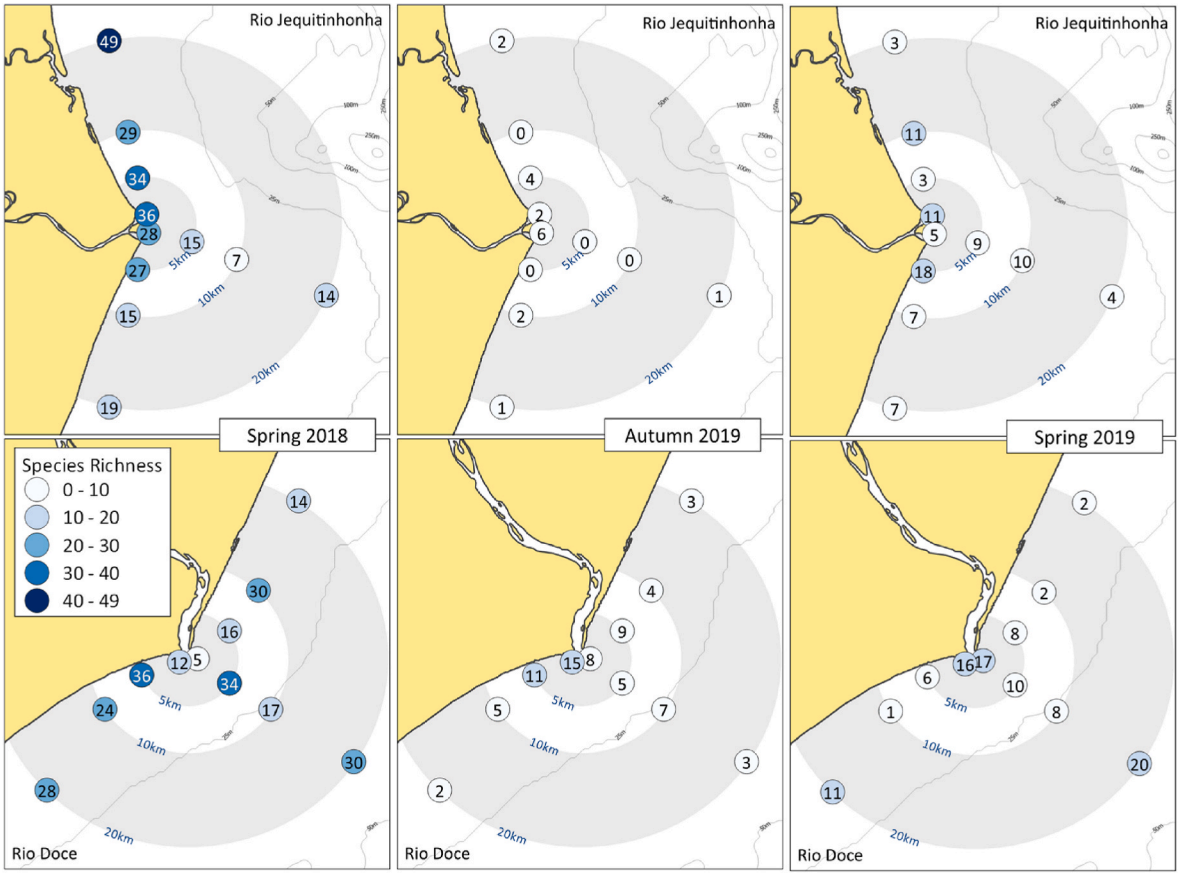

Fig. 4. The species richness distribution of marine vertebrates identified by eDNA metabarcoding in both Rio Doce and Rio Jequitinhonha during Spring 2018, Autumn 2019; Spring 2019 sampling seasons.

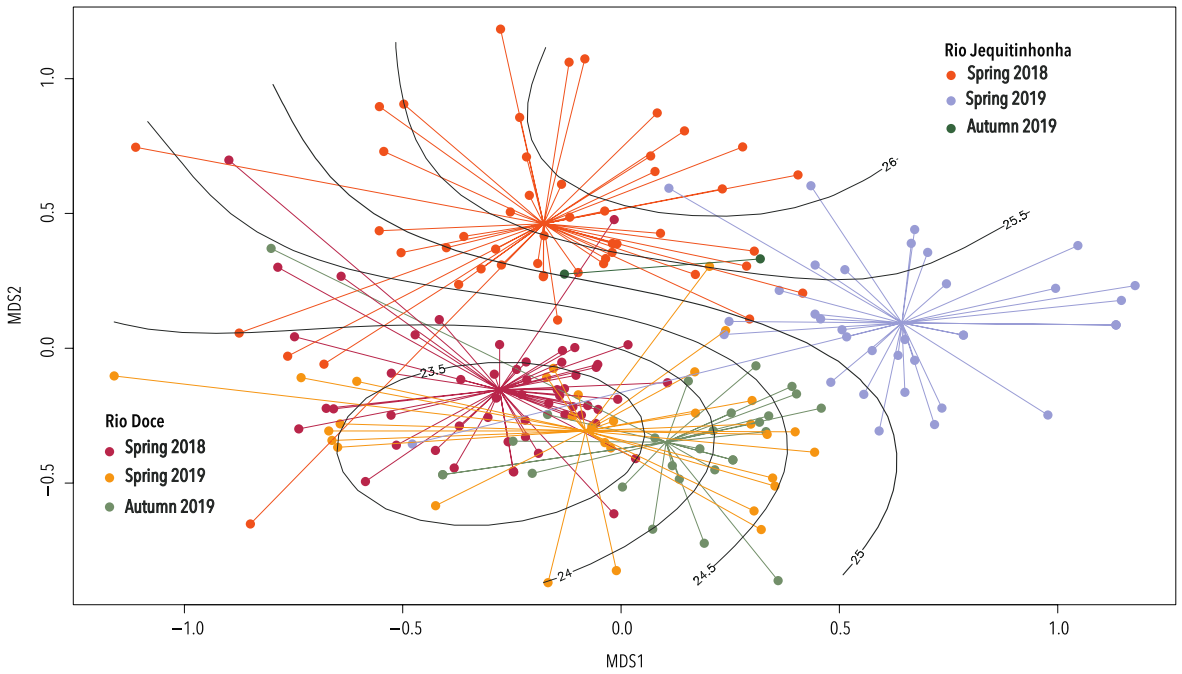

Fig. 5. Non-metric multidimensional scaling plot demonstrating the spatial pattern in the species assemblages between sampling seasons and both rivers (stress = 0.12, k = 3 & p ≤ 0.001). Clusters of the same colour represent seasonal samples from each river (ordispider: vegan (Oksanen et al., 2020)) and approximated sampling temperatures for each sample are overlaid on the arcs (ordisurf: vegan (Oksanen et al., 2020)). The junction of the coloured lines indicate the centroids for the seasonal samples from each river.

Table 1
PERMANOVA analysis on Jaccard to test for the fixed effect of Season (Spring 2018, Autumn 2019, Spring 2019) at Rio Doce and Rio Jequitinhonha sites on marine vertebrate species.

Source	df	SS	R2	Pseudo-F	P (perm)	Unique perms
Season	2	28577	0.87	14.656	**0.0001**	9929
River	1	7143.1	0.97	6.0306	**0.0001**	9899
Site(River)	21	18107	0.92	2.3455	**0.0001**	9518
SeasonxRiver	2	10271	0.95	4.7578	**0.0001**	9904
SeasonxSite (River)	39	34289	0.85	1.9596	**0.0001**	9421

and the Rio Doce extension study area. In Spring 2018, all sites in the Rio Doce study area and the Rio Jequitinhonha study area had a species of conservation significance present (Table S2). In Autumn 2019 only one site each at the Rio Doce and Rio Jequitinhonha had a species of conservation significance. There were ten species from five families in Class Actinopterygii, eleven species from four families in Class Chondrichthyes and two species from two families in Class Mammalia (Fig. 7).

4. Discussion

4.1. eDNA data

Here we report an extensive eDNA dataset obtained from seawater samples along the coastline of Brazil over a three-season period. Multivariate and regression analyses applied to marine vertebrate species assemblages (presence/absence) determined the effects of both environmental factors (season, rainfall, water depth, salinity, temperature etc.) and survey (year, study area, site), demonstrating that analysis of eDNA from seawater samples can be used to detect differences in ecosystems. Our hypothesis of a significant influence of river discharge on coastal marine biodiversity near the Rio Doce and Jequitinhonha rivers was then supported by the significant interactions between rainfall and species richness detected within both basins. In addition to the riverine influence, the combined effect of water mass properties including salinity, temperature, and chlorophyll to the seasonal biodiversity dynamics at both Rio Doce and Jequitinhonha basins suggest that the presence of large-scale water masses have a major influence on species occurrence in the region. The seasonal variability in water masses at this marine ecoregion, which vary from a dominance of tropical to subtropical water masses across seasons, have been shown to exert a major influence on coastal marine biodiversity (Mazzuco and Bernardino, 2022). Our survey then likely captured this seasonal variability in coastal biodiversity in periods of lower riverine discharge at both the Rio Doce and Jequitinhonha river basins.

In our study, eDNA metabarcoding determined seasonal patterns in species assemblages at both the Rio Doce and at the Rio Jequitinhonha, 500 km to the north. Strong seasonal weather effects, in particular rainfall (National Institute of Meteorology) (Table S3), may have influenced the observed reduction in marine vertebrate diversity when in Autumn 2019 when rainfall was low. Rainfall in Spring 2019 was also lower than in the previous year, which we suggest could have an effect on the species richness at both the Rio Doce and Rio Jequitinhonha study areas. Higher rainfall leads to increased discharge from the rivers into the surrounding ocean which are expected to influence both the input of DNA and of tailings to the coastal zone (Magris et al., 2019). The Rio Doce has one of the largest riverine discharges in Brazil (approx. 800 m^3 s^{-1}; Lana and Bernardino, 2018), which can explain the detection of numerous freshwater species in seawater samples, for example *Lepomis* sp. was detected at Site D10N, which is 10 km from the Rio Doce river mouth.

Seasonal effects on species composition have been reported previously in eDNA studies (de Souza et al., 2016; Berry et al., 2019; Postaire et al., 2020, Sales et al., 2021). Bonecker et al. (2019) also found that larval fish assemblages collected at the Rio Doce varied significantly due to seasonal effects, and Sales et al. (2021) used freshwater eDNA metabarcoding in Rio Jequitinhonha to report significant short-term variability in fish assemblages due to a large rainfall event.

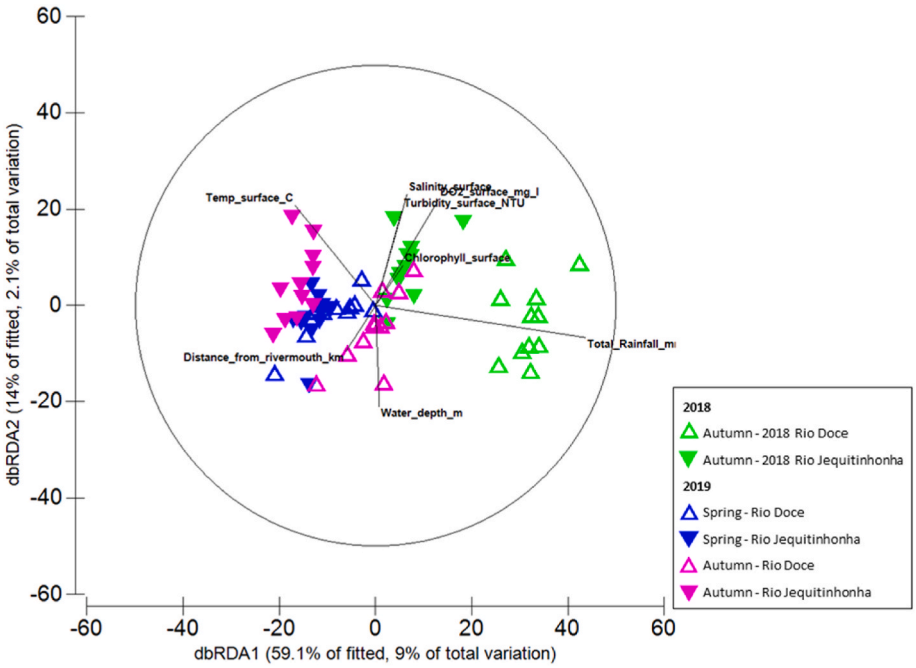

Fig. 6. DistLM analysis (PRIMER-e, v1.03; Clarke and Gorley, 2006) of marine vertebrate species matrix (using Jaccard) and environmental data shows the spatial distribution and variation (11.1%) in the fitted for sampling sites in both Rio Doce and Rio Jequitinhonha during the three sampling seasons: Spring 2018, Autumn 2019; Spring 2019.

Table 2
DistLM analysis on the marine vertebrate species matrix (Jaccard) and sequentially adding the environmental variables using a stepwise test based on AICc. Marine vertebrate species were Presence/Absence transformed. The level of significance was set at 0.05, AICc was 2616.5 and R^2 was 0.1518.

Variable	AICc	SS(trace)	Pseudo-F	P	Prop.	Cumul.	res.df
+Total_Rainfall_mm	2628.2	83464	29.198	**0.001**	0.082	0.082	328
+DO2_surface_mg_l	2625.4	13509	4.7803	**0.001**	0.013	0.095	327
+Water_depth_m	2622.6	13624	4.8781	**0.001**	0.013	0.108	326
+Salinity_surface	2620.4	11707	4.2335	**0.001**	0.011	0.120	325
+Temp_surface_C	2618.4	11012	4.0188	**0.001**	0.011	0.131	324
+Turbidity_surface_NTU	2616.7	10159	3.7392	**0.001**	0.010	0.141	323
+Chlorophyll_surface	2616.7	5604.8	2.0697	**0.013**	0.005	0.146	322
+Distance_from_rivermouth_km	2616.5	5947.1	2.2043	**0.003**	0.006	0.152	321

Table 3
The number of taxa in Class Actinopterygii, Class Chondrichthyes and Class Mammalia detected at the Rio Doce and Rio Jequitinhonha study areas for the three sampling seasons.

	SPRING 2018		AUTUMN 2019		SPRING 2019	
	Rio Doce	Rio Jequitinhonha	Rio Doce	Rio Jequitinhonha	Rio Doce	Rio Jequitinhonha
Actinopterygii	**78**	**95**	**32**	**14**	**48**	**33**
Chondrichthyes	**8**	**4**	**1**	**1**	**2**	**0**
Mammalia	**4**	**3**	**2**	**0**	**2**	**1**

The families of fish identified in Bonecker et al. (2019), from their collection of fish larvae using neustonic and bongo plankton nets, are similar to the families identified by eDNA results of this study and our results are also in accordance with the results presented from other field studies that use bottom trawling (Bevitório et al. (2022), Condini et al. (2022), Vilar et al. (2022) (Table S4). Despite these assurances, further work is needed to validate eDNA sampling of surface waters against, for example, demersal trawling to ascertain the distance at which signals in species assemblage can be detected. Ideally this work could be accomplished using a targeted, depth integrated eDNA survey run concurrently with scientific trawl surveys.

Accurate reporting of species detected in bulk environmental samples using metabarcoding relies on both a robust bioinformatic pipeline and a comprehensive reference database to assign taxa to sequences detected. eDNAFlow (Mousavi-Derazmahalleh et al., 2021), removes chimeras, low quality reads and reads below a user set length threshold. Of the 6,196 and 10,392 number of ZOTUs generated from 16S and COI, only 9.31% of 16S and 4.68% COI had at least one match to the nucleotide database of GenBank, and therefore were able to be assigned to the lowest common ancestor. Absence of many species in the reference database GenBank can result in underreporting of true species diversity. The use of eDNA metabarcoding for monitoring biota is mostly limited to presence/absence, with recent studies exploring comparison of DNA reads with biomass (Di Muri et al., 2020; Stoeckle et al., 2021). However, the high throughput capacity of the technology allows hundreds of water samples to be processed in a few days. Moreover the

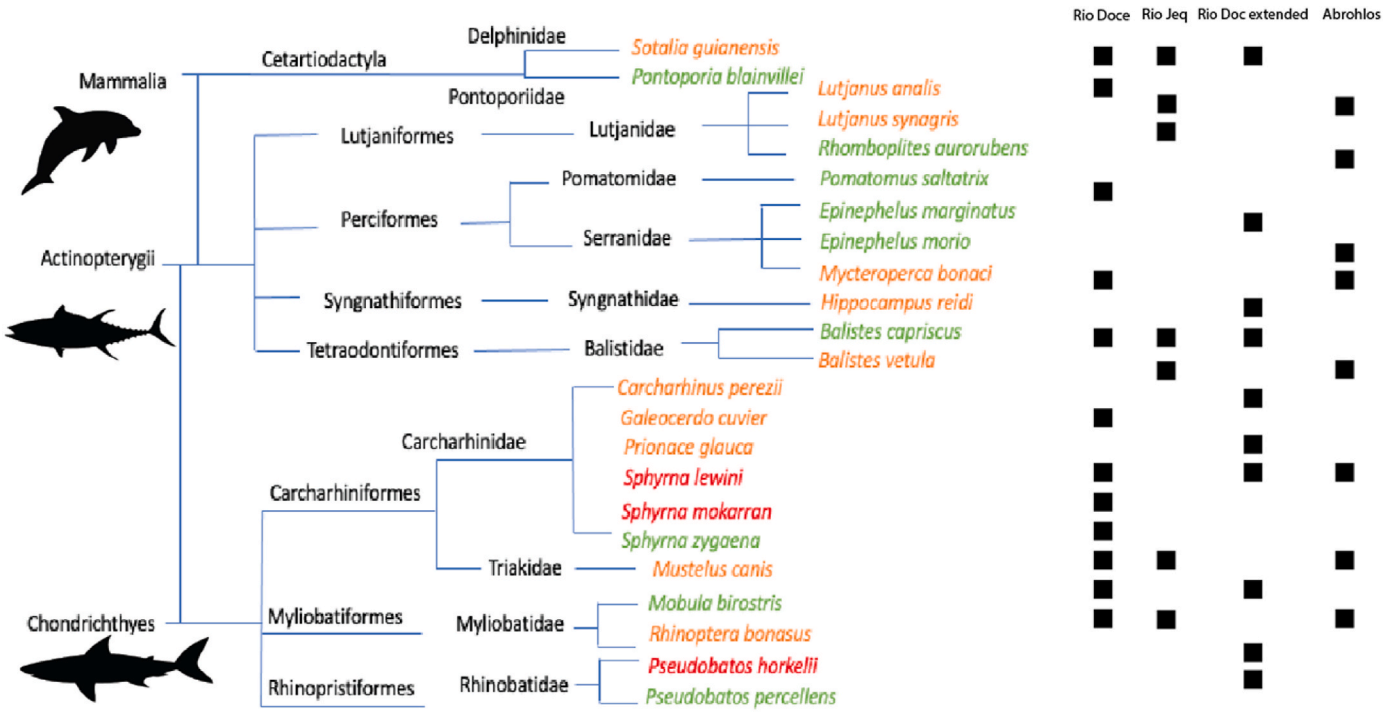

Fig. 7. Vulnerable (green), Threatened (orange) and Critically Endangered (red) marine vertebrate species detected in the Rio Doce, Rio Doce extended sites, Rio Jequitinhonha and Abrohlos sites during the sampling seasons Spring 2018, Autumn 2019; Spring 2019.

ability of eDNA metabarcoding to detect cryptic species and species in the larval stage makes the technology attractive for use as a monitoring tool as a repeated, seasonal monitoring program extending over several years should detect any obvious signs of stress in the system based on systemic changes in marine vertebrate species assemblages.

4.2. Extended coastline survey

eDNA metabarcoding detected marine coastal, marine neritic, marine oceanic and marine deep benthic species in the three estuarine sites; Rio São Mateus, Rio Mucuri, and the Rio Alcobaça, and included species of conservation significance. Due to the presence of local fishing ports and villages it is possible that the presence of these species may be due to processing of fish catches.

Site A0W had the highest diversity of species in the extended coastline survey. This site is situated in the river at the town of Alcobaça, where local fishing and fish processing is present. The town lies to the North of the Reserva Extrativista Marinha do Corumbau, which was created in the year 2000 to help protect the local fishing economy (ICMBio, 2016). This region includes coral reefs and rich marine biodiversity. Local fisherman, permitted to catch fish along the coastline, process fish catches in the town. Water samples for eDNA analysis were collected in the river adjacent to these activities. In the Autumn 2019 extended coastline survey, site MP02 had the highest species richness of all sites in the survey with forty-nine taxa detected at this site. Site MP02 was in close proximity to the local port of Rio Riacho where local fisherman unload and clean marine fish. It is therefore possible that detection of these taxa is as a result of the presence of DNA from species cleaned at the site.

4.3. Species of conservation significance

The detection of species of conservation significance at all sites in Spring 2018 reflected the high number of species detected in all sites. These species were detected at only one site each at the Rio Doce and Rio Jequitinhonha study area in Autumn 2019, which aligned with the overall reduction in species diversity during this season. The seasonal dynamics in the presence/absence of species of conservation significance on the coastal zone of the Rio Doce and Jequitinhonha suggests that both areas exhibit similar temporal patterns in the use of habitat by fishes and mammals. In all, this study detected 23 species of conservation significance, including *Hippocampus reidi* (Longsnout seahorse), which was also reported north of the Rio Doce based on direct sampling by Bonecker et al. (2019). Vulnerable mammal species detected by our survey are also observed in the study region by direct sampling or monitoring, including the dolphins *Pontopora blainvillei* and *Sotalia guianensis* (Pinheiro et al., 2019). This is the first report of this many species of conservation significance in this region using eDNA analysis and our results demonstrate the potential of eDNA metabarcoding of marine environments to monitor rare or endangered species.

5. Conclusions

eDNA analysis has become a viable and valuable source of information for monitoring programs, and is increasingly being used as a source of baseline information for many companies in the resources sector to assess environments prior to any development being undertaken. This study, representing one of the larger eDNA studies in the region, has demonstrated the value of the eDNA toolkit for characterising temporal and spatial patterns in marine biota, and provides a rich source of data for future comparative studies. To draw conclusions about the fluctuations seen in marine vertebrate diversity along the coastline of Brazil, will require long term studies to account for seasonal variation as a confounding factor. The call for environmental archiving (or biobanking see Berry et al., 2019) is supported in cases such as these, where the collection of water samples prior to the impact event, would have enabled a far more rigorous evaluation of marine impacts in the year following the discharge of tailings into the Rio Doce.

Continuation of monitoring at these sites will provide key information on recovery trajectories of the local environment following an impact such as occurred in Rio Doce, and will provide valuable data to inform management decisions. Moreover, extracted DNA can be tested

with other metabarcoding assays to further explore biodiversity patterns with other taxonomic groups from phytoplankton to bacteria.

Role of the funding source

The work was commissioned and funded by BHP Brazil as part of a larger routine marine environmental monitoring program in coastal waters of Espírito Santo and Bahia. The study was designed by Mr Philip Whittle and Dr James Keating (Hydrobiology), Professor Michael Bunce (Curtin University) and Professor Angelo Bernardino (Universidade Federal do Espirito Santo) in conjunction with Dr Alice Taysom (BHP). Samples were processed and data was generated, analysed and interpreted at Curtin University. The funding source did not have any input into the data analysis or interpretation of results. The original manuscript was written by Dr Rose Lines (Curtin University), with critical review from all authors.

Data archiving

The data that support the findings of this study are openly available in data dryad at https://doi.org/10.5061/dryad.sqv9s4n3t.

CRediT authorship contribution statement

RL: Investigation, Formal analysis, Data Curation, Writing – Original draft preparation.

MJ: Investigation, Formal analysis, Visualization, Writing - review and editing.

GP: Investigation, Validation, Writing - review and editing.

JK: Methodology, Investigation, Project Administration, Writing - review and editing.

TS: Investigation, Visualization, Writing - review and editing.

MD: Software, Data curation, Writing - review and editing.

MB: Methodology, Supervision of Curtin University researchers, Writing - review and editing.

TB: Visualization, Writing - review and editing.

AT: Conceptualization, Writing - review and editing.

AFB: Conceptualization, Methodology, Writing - review and editing.

PW: Conceptualization, Methodology, Funding acquisition, Supervision of Hydrobiology researchers, Writing - review and editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: All authors reports financial support was provided by BHP Brazil. Alice Taysom reports a relationship with BHP Brazil that includes: employment.

Data availability

Data is available at datadryad.org. The link is included in the manuscript.

Acknowledgements

The work was commissioned and funded by BHP Brazil as part of a larger routine marine environmental monitoring program in coastal waters of Espírito Santo and Bahia.

This work was supported by resources provided by the Pawsey Supercomputing Centre with funding from the Australian Government and the Government of Western Australia.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2023.106239.

References

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. J. Mol. Biol. 215 (3), 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2.

Bernardino, A.F., Pais, F.S., Oliveira, L.S., Gabriel, F.A., Ferreira, T.O., Queiroz, H.M., Mazzuco, A.C., 2019. Chronic trace metals effects of mine tailings on estuarine assemblages revealed by environmental DNA. PeerJ 7, e8042. https://doi.org/10.7717/peerj.8042.

Berry, T.E., Saunders, B.J., Coghlan, M.L., Stat, M., Jarman, S., Richardson, A.J., Davies, C.H., Berry, O., Harvey, E.S., Bunce, M., 2019. Marine environmental DNA biomonitoring reveals seasonal patterns in biodiversity and identifies ecosystem responses to anomalous climatic events. PLoS Genet. 15 (2), e1007943 https://doi.org/10.1371/journal.pgen.1007943.

Bevitório, L.Z., da Silva, N.G., Pirovani, J.C.M., Marques, J.A., Vieira, C.E.D., Zebral, Y. D., Damasceno, E.M., Lopes, L.L.R., de Sant'Ana, L.P., Marube, L.C., da Costa, S.R., De Martinez, C., Martins, M., Sandrini, J.Z., Souza, M.M., Bianchini, A., do Vale-Oliveira, M., 2022. Impacts of tailings of Fundão dam (Brazil) rupture on marine fish: metals bioaccumulation and physiological responses. Mar. Pollut. Bull. 177 https://doi.org/10.1016/j.marpolbul.2022.113511.

Bohmann, K., Elbrecht, V., Carøe, C., Bista, I., Leese, F., Bunce, M., Yu, D.W., Seymour, M., Dumbrell, A.J., Creer, S., 2021. Strategies for sample labelling and library preparation in DNA metabarcoding studies. Molecul. Ecol. Resour. 22, 1231–1246. https://doi.org/10.1111/1755-0998.13512.

Bonecker, A.C.T., Castro, M.S., Gomes Costa, P., Bianchini, A., Bonecker, S.L.C., 2019. Larval fish assemblages of the coastal area affected by the tailings of the collapsed dam in southeast Brazil. Reg. Stud. Marine Sci. 32 https://doi.org/10.1016/j.rsma.2019.100848.

Boyer, F., Mercier, C., Bonin, A., Le Bras, Y., Taberlet, P., Coissac, E., 2016. Obitools: a unix-inspired software package for DNA metabarcoding. Molecul. Ecol. Resour. 16 (1), 176–182.

Carmo, F., Kamino, L., Junior, R., Campos, I., Carmo, F., Silvino, G., Castro, K., Mauro, M., Rodrigues, N., Miranda, M., Pinto, C., 2017. Fundão tailings dam failures: the environment tragedy of the largest technological disaster of Brazilian mining in global context. Perspect. Ecol. Conservat. 15 (3), 145–151. https://doi.org/10.1016/j.pecon.2017.06.002.

Clarke, K.R., Gorley, R.N., 2006. PRIMER V6: User Manual/Tutorial (Plymouth Routines in Multivariate Ecological Research). PRIMER-E, Plymouth.

Condini, M.V., Pichler, H.A., de Oliveira-Filho, R.R., Cattani, A.P., Andrades, R., Vilar, C. C., Joyeux, J.C., Soeth, M., De Biasi, J.B., Eggertsen, L., Dias, R., Hackradt, C.W., Félix-Hackradt, F.C., Chiquieri, J., Garcia, A.M., Hostim-Silva, M., 2022. Marine fish assemblages of Eastern Brazil: an update after the world's largest mining disaster and suggestions of functional groups for biomonitoring long-lasting effects. Sci. Total Environ. 807 (2) https://doi.org/10.1016/j.scitotenv.2021.150987.

Coppo, G.C., Pais, F.S., Ferreira, T.O., Halanych, K.M., Donnelly, K., Mazzuco, A.C.A., Bernardino, A.F., 2023. Transition of an estuarine benthic meiofauna assemblage 1.7 and 2.8 years after a mining disaster. PeerJ 11, e14992.

De Souza, L.S., Godwin, J.C., Renshaw, M.A., Larson, E., 2016. Environmental DNA (eDNA) detection probability is influenced by seasonal activity of organisms. PLoS One 11 (10), e0165273. https://doi.org/10.1371/journal.pone.0165273.

Di Muri, C., Lawson Handley, L., Bean, C.W., Li, J., Peirson, G., Sellers, G.S., Walsh, K., Watson, H.V., Winfield, I.J., Hänfling, B., 2020. Read counts from environmental DNA (eDNA) metabarcoding reflect fish abundance and biomass in drained ponds. Metabarcod. Metagenom. 4, e56959 https://doi.org/10.3897/mbmg.4.56959.

DiBattista, J.D., Reimer, J.D., Stat, M., Masucci, G.D., Biondi, P., De Brauwer, M., Wilkinson, S., Chariton, A., Bunce, M., 2020. Environmental DNA can act as a biodiversity barometer of anthropogenic pressures in coastal ecosystems. Sci. Rep. 10, 8365. https://doi.org/10.1038/s41598-020-64858-9.

Edgar, R.C., 2016. UNOISE2: Improved Error-Correction for Illumina 16S and ITS Amplicon Sequencing. bioRxiv.

Fernandes, K., van der Heyde, M., Bunce, M., Dixon, K., Harris, R.J., Wardell-Johnson, G., Nevill, P.G., 2018. DNA metabarcoding—a new approach to fauna monitoring in mine site restoration. Restor. Ecol. 26, 1098–1107. https://doi.org/10.1111/rec.12868.

Ficetola, G.F., Miaud, C., Pompanon, F., Taberlet, P., 2008. Species detection using environmental DNA from water samples. Biol. Lett. 4, 423–425. https://doi.org/10.1098/rsbl.2008.0118.

Frøslev, T.G., Kjøller, R., Bruun, H.H., Ejrnæs, R., Brunbjerg, A.K., Pietroni, C., Hansen, A. J., 2017. Algorithm for post-clustering curation of DNA amplicon data yields reliable biodiversity estimates. Nat. Commun. 8 (1), 1188. https://doi.org/10.1038/s41467-017-01312-x.

Gabriel, F.A., Silva, A.G., Queiroz, H.M., Ferreira, T.O., Hauser-Davis, R.A., Bernardino, A.F., 2020. Ecological risks of metal and metalloid contamination in the Rio Doce estuary. Integrated Environ. Assess. Manag. 16 (5), 655–660. https://doi.org/10.1002/ieam.4250.

GBIF.org, 2020. GBIF home page. Available from: https://www.gbif.org. April 2020.

Gomes, L.E., Correa, L.B., Sa, F., Neto, R.R., Bernardino, A.F., 2017. The impacts of the Samarco mine tailing spill on the Rio Doce estuary, Eastern Brazil. Mar. Pollut. Bull. 120, 28–36. https://doi.org/10.1016/j.marpolbul.2017.04.056.

Harper, L.R., Lawson, L., Handley, Carpenter, A.I., Ghazali, M., Di Muri, C., Macgregor, C.J., Logan, T.W., Law, A., Breithaupt, T., Read, D.S., McDevitt, A.D., Hänfling, B., 2019. Biol. Conserv. 238, 108225 https://doi.org/10.1016/j.biocon.2019.108225.

Harper, K.J., Goodwin, K.D., Harper, L.R., LaCasella, E.L., Frey, A., Dutton, P.H., 2020. Finding crush: environmental DNA analysis as a tool for tracking the green sea turtle *Chelonia mydas* in a marine estuary. Front. Mar. Sci. https://doi.org/10.3389/fmars.2019.00810.

Hunter, M.E., Meigs-Friend, G., Ferrante, J.A., Kamla, A., Dorazio, R.M., Keith-Diagne, L., Luna, F., Lanyon, J.M., Reid, J.P., 2018. Surveys of environmental DNA (eDNA): a new approach to estimate occurrence in Vulnerable manatee populations. Endanger. Species Res. 35, 101–111. https://doi.org/10.3354/esr00880.

ICMBio, 2016. Reserva Extrativista Marinha do Corumbau - BA. Plano de Utilização (primeira fase do plano de manejo). 21 de Setembro de 2002 (com alterações até 19 de abril de 2016). https://www.gov.br/icmbio/pt-br/assuntos/biodiversidade/unidade-de-conservacao/unidades-de-biomas/marinho/lista-de-ucs/resex-marinha-corumbau/arquivos/dcom_icmbio_plano_de_utilizacao_resex_corumbau_abril2016.pdf. (Accessed 29 October 2021).

ICMBio, 2022. Brazilian Red List. https://www.icmbio.gov.br/portal/faunabrasileira. (Accessed 20 March 2022). https://www.nationalredlist.org. and.

IUCN, 2021. The IUCN Red List of Threatened Species. Version 2021-3. https://www.iucnredlist.org. (Accessed 20 March 2022).

Koziol, A.A., Stat, M., Simpson, T., Jarman, S., DiBattista, J.D., Harvey, E.S., Marnane, M., McDonald, J., Bunce, M., 2019. Environmental DNA metabarcoding studies are critically affected by substrate selection. Molecul. Ecol. Resour. 19, 366–376. https://doi.org/10.1111/1755-0998.12971.

Lana, P.C., Bernardino, A.F., 2018. Brazilian Estuaries: a Benthic Perspective - Brazilian Marine Biodiversity Series. Springer International Publishing, p. 212. ISSN: 2520-1085.

Lehman, R.N., Poulakis, G.R., Scharer, R.M., Schweiss, K.E., Hendon, J.M., Phillips, N.M., 2020. An environmental DNA tool for monitoring the status of the Critically Endangered Smalltooth Sawfish, *Pristis pectinata*, in the western Atlantic. Conservation Genet. Resour. 12, 621–629. https://doi.org/10.1007/s12686-020-01149-5.

Magris, R.A., Marta-Almeida, M., Monteiro, J.A.F., Ban, N.C., 2019. A modelling approach to assess the impact of land mining on marine biodiversity: assessment in coastal catchments experiencing catastrophic events (SW Brazil). Sci. Total Environ. 659, 828–840. https://doi.org/10.1016/j.scitotenv.2018.12.238.

Marta-Almeida, M., Mendes, R., Amorim, F.N., Cirano, M., Dias, J.M., 2016. Fundao dam collapse: oceanic dispersion of River Doce after the greatest Brazilian environmental accident. Mar. Pollut. Bull. 112, 359–364. https://doi.org/10.1016/j.marpolbul.2016.07.039.

Mazzuco, A.C.A., Bernardino, A.F., 2022. Reef larval recruitment in resposnse to seascape dynamics in the SW Atlantic. Sci. Rep. 12, 7750. https://doi.org/10.1038/s41598-022-11809-1.

Mousavi-Derazmahalleh, M., Stott, A., Lines, R., Peverley, G., Nester, G., Simpson, T., Zawierta, M., De La Pierre, M., Bunce, M., Christophersen, C.T., 2021. eDNAFlow, an automated, reproducible and scalable workflow for analysis of environmental DNA (eDNA) sequences exploiting Nextflow and Singularity. Molecul. Ecol. Resour. https://doi.org/10.1111/1755-0998.13356.

Murray, D.C., Coghlan, M.L., Bunce, M., 2015. From benchtop to desktop: important considerations when designing amplicon sequencing workflows. PLoS One 10 (4), e0124671. https://doi.org/10.1371/journal.pone.0124671.

National Institute of Meteorology. (n.d.). Registered conditions. from https://tempo.inmet.gov.br/CondicoesRegistradas (Retrieved August 20, 2020)..

Nester, G.M., De Brauwer, M., Koziol, A., West, K.M., DiBattista, J.D., White, N.E., Power, M., Heydenrych, M., Bunce, M., 2020. Development and Evaluation of Fish eDNA Metabarcoding Assays Facilitate the Detection of Cryptic Seahorse Taxa (Family: Syngnathidae). Environmental DNA. https://doi.org/10.1002/edn3.93.

Oksanen, F., Blanchet, G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R.B., Simpson, G.L., Solymos, P., Henry, M., Stevens, H., Szoecs, E., Wagner, H., 2020. vegan: Community Ecology Package. R package version 2.5-7. https://CRAN.R-project.org/package=vegan.

Pinheiro, F.C.F., Pinheiro, H.T., Teixeira, J.B., Martins, A.S., Cremer, M.J., 2019. Opportunistic development and environmental disaster threat Franciscana dolphin in the Southeast Brazil. Trop. Conserv. Sci. 12, 1–7. https://doi.org/10.1177/1940082919847886.

Postaire, B.D., Bakker, J., Gardiner, J., Wiley, T., Chapman, D., 2020. Environmental DNA detection tracks established seasonal occurrence of blacktip sharks (*Carcharhinus limbatus*) in a semi-enclosed subtropical bay. Sci. Rep. 10, 11847 https://doi.org/10.1038/s41598-020-68843-0.

Queiroz, H.M., Ying, S.C., Bernardino, A.F., Barcellos, D., Nobrega, G.N., Otero, X.L., Ferreira, T.O., 2021. Role of Fe dynamic in release of metals at the Rio Doce estuary: unfolding of a mining disaster. Mar. Pollut. Bull. 166, 112267 https://doi.org/10.1016/j.marpollbul.2021.112267.

R Core Team, 2020. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. URL. https://www.R-project.org/.

Richard, E.C., Estrada, G.C.D., Bechtold, J.P., Duarte Jr., H.A., Maioli, B.G., Freitas, A.H. A., Warner, K.E., Figueiredo, L.H.M., 2020. Water and sediment quality in the coastal zone around the mouth of the Doce River after the Fundão tailings dam failure. Integrated Environ. Assess. Manag. 16 (5), 643–654. https://doi.org/10.1002/ieam.4309.

Ruppert, K.M., Kline, R.J., Rahman, M.S., 2019. Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: a systematic review in methods, monitoring, and applications of global eDNA. Global Ecol. Conservat. 17, e00547 https://doi.org/10.1016/j.gecco.2019.e00547.

Sá, F., Longhini, C.M., Costa, E.S., Silva, C.A., Cagnin, R.C., Gomes, L.E., Lima, A.T., Bernardino, A.F., Neto, R.R., 2021. Time-sequence development of metal(loid) following the 2015 dam failure in the Doce river estuary, Brazil. Sci. Total Environ. 769, 144532 https://doi.org/10.1016/j.scitotenv.2020.144532.

Sales, N.G., Wagensteen, O.S., Carvalho, D.C., Deiner, K., Praebel, K., Coscia, I., McDevitt, A.D., Mariani, S., 2021. Space-time dynamics in monitoring neotropical fish communities using eDNA metabarcoding. Sci. Total Environ. 754, 142096 https://doi.org/10.1016/j.scitotenv.2020.142096.

Samarco, 2018. Fundão dam failure - samarco. Available at: https://www.samarco.com/en/rompimento-da-barragem-de-fundao/. (Accessed 6 July 2019).

Stat, M.M., Huggett, M.J., Bernasconi, R., DiBattista, J.D., Berry, T.E., Newman, S.J., Harvey, E.S., Bunce, M., 2017. Ecosystem biomonitoring with eDNA: metabarcoding across the tree of life in a tropical marine environment. Sci. Rep. 7, 12240 https://doi.org/10.1038/s41598-017-12501-5.

Stoeckle, M.Y., Adolf, J., Charlop-Powers, Z., Dunton, K.J., Hinks, G., VanMorter, S.M., 2021. Trawl and eDNA assessment of marine fish diversity, seasonality, and relative abundance in coastal New Jersey, USA. ICES (Int. Counc. Explor. Sea) J. Mar. Sci. 78 (1), 293–304. https://doi.org/10.1093/icesjms/fsaa225.

Taberlet, P., Coissac, E., Pompanon, F., Brochmann, C., Willerslev, E., 2012. Towards next-generation biodiversity assessment using DNA metabarcoding. Mol. Ecol. 21, 2045–2050.

Thomsen, P.F., Willerslev, E., 2015. Environmental DNA – an emerging tool in conservation for monitoring past and present biodiversity. Biol. Conserv. 183, 4–18. https://doi.org/10.1016/j.biocon.2014.11.019.

Van der Heyde, M., Bunce, M., Dixon, K., Wardell-Johnson, G., White, N., Nevill, P., 2020. Changes in soil microbial communities in post mine ecological restoration: implications for monitoring using high throughput DNA sequencing. Sci. Total Environ. 749, 142262 https://doi.org/10.1016/j.scitotenv.2020.142262.

Vilar, C.C., Andrades, R., Szablak, F.T., Guabiroba, H.C., Pichler, H.A., Bastos, K.V., de Lima, L.R.S., Bastos, P.G.P., Martins, R.F., Vitor Rodrigues, L.A., Hostim-Silva, M., Joyeux, J.C., 2022. Variability in nearshore fish biodiversity indicators after a mining disaster in eastern Brazil. Mar. Environ. Res. 175 https://doi.org/10.1016/j.marenvres.2022.105565.

Weltz, K., Lyle, J.M., Ovenden, J., Morgan, J.A.T., Moreno, D.A., Semmens, J.M., 2017. Application of environmental DNA to detect an endangered marine skate species in the wild. PLoS One 12 (6), e0178124. https://doi.org/10.1371/journal.pone.0178124.

West, K.M., Stat, M., Harvey, E.S., Skepper, C.L., DiBattista, J.D., Richards, J.T., Travers, M.J., Newman, S.J., Bunce, M., 2020. eDNA metabarcoding survey reveals fine-scale coral reef community variation across a remote, tropical island ecosystem. Mol. Ecol. 29 (6), 1069–1086. https://doi.org/10.1111/mec.15382.

WoRMS Editorial Board, 2021. World register of marine species. Available from. http://www.marinespecies.org. at VLIZ. Accessed April 2020.